ISSN: 1308-7576
e-ISSN: 1308-7584

YUZUNCU YIL UNIVERSITY JOURNAL OF AGRICULTURAL SCIENCES

Volume 34 **Number** 1
March 2024

YYU J AGR SCI

Van Yuzuncu Yıl University
Faculty of Agriculture, Van-Türkiye
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YUZUNCU YIL UNIVERSITY JOURNAL OF AGRICULTURAL SCIENCES

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YÜZÜNCÜ YIL ÜNİVERSİTESİ TARIM BİLİMLERİ DERGİSİ

YYÜ TAR BİL DERG

ISSN 1308-7576
e-ISSN 1308-7584

VAN – TÜRKİYE

Volume: 34	Issue: 1	March 2024

Yuzuncu Yil University
Journal of Agricultural Sciences
Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi
https://dergipark.org.tr/en/pub/yyutbd

YUZUNCU YIL UNIVERSITY JOURNAL OF AGRICULTURAL SCIENCES
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)

Volume: 34	**Issue: 1**	**March 2024**

ISSN: 1308-7576, **e-ISSN:** 1308-7584

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Press and Date: Efe Kırtasiye, March 2024, Van

Yuzuncu Yil University Journal of Agricultural Sciences is the continuation of the previously published **Yuzuncu Yil University, Agriculture Faculty Journal of Agriculture Sciences** and **Yuzuncu Yil University, Journal of Agriculture Faculty.**

Yuzuncu Yil University
Journal of Agricultural Sciences
Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi
https://dergipark.org.tr/en/pub/yyutbd

CONTENTS

Yuzuncu Yil University
Journal of Agricultural Sciences
Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi
https://dergipark.org.tr/en/pub/yyutbd

Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

ISSN: 1308-7576 e-ISSN: 1308-7584

Research Article

Ameliorative Effect of Jamaican Cherry (*Muntingia calabura* L.) Leaf Extract Toward Glucose Control and Immune Cells Modulation in High Fat Diet-Administrated Mice

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Article Info

Received: 22.07.2023
Accepted: 28.10.2023
Online published: 15.03.2024
DOI: 10.29133/yyutbd.1331257

Keywords

Blood sugar level,
High fat diet,
Hyperglycemia,
Immune system,
M. calabura

Abstract: Hyperglycemia is a dangerous condition in which too much glucose circulates in the blood plasma and is the leading cause of diabetes mellitus. It is a complex condition with varying degrees that can change over time, mainly owing to metabolic factors that reduce insulin secretion, decrease glucose use, and increase glucose production. This study aims to evaluate *Muntingia calabura* leaf extract's effect on glucose control and immune cell modulation in high-fat diet-administrated mice. According to the result, we found that *M. calabura* leaf extract significantly reduced the fasting blood sugar. Importantly, *M. calabura* leaf extract exerts immunomodulation effects by suppressing the relative number of regulatory T cells in the hypoglycemic mice model. Finally, this study showed *M. calabura* leaf extract exerts ameliorative potency against hyperglycemia by lowering the blood sugar level and suppressing the regulatory T cells. These results suggested that *M. calabura* leaf extract could develop into complementary and alternative medicine.

To Cite: Putra, W E, Shofiyah, I N, Rahim, A R, Hidayatullah, A, Rifa'i, M, 2023. Ameliorative Effect of Jamaican Cherry (*Muntingia calabura* L.) Leaf Extract Toward Glucose Control and Immune Cells Modulation in High Fat Diet-Administrated Mice. *Yuzuncu Yil University Journal of Agricultural Sciences*, 34(1): 1-13. DOI: https://doi.org/10.29133/yyutbd.1331257

1. Introduction

Hyperglycemia, or high blood sugar, is a dangerous condition in which too much glucose circulates in the blood plasma (Chaudhury et al., 2017; Wanrooy et al., 2018). It is a complicated disease with multiple degrees that can alter over time, primarily due to metabolic variables that diminish insulin secretion, decrease glucose utilization, and increase glucose generation (Ormazabal et al., 2018; Garcia et al., 2020). Specifically, hyperglycemia is defined as a condition in which the blood sugar level is consistently between 5.6 and 7.0 mmol L^{-1} (100-126 mg dl^{-1}), whereas diabetes is defined as a condition in which the blood sugar level is greater than 7.0 mmol L^{-1} (126 mg dl^{-1}) (American Diabetes Association Professional Practice Committee 2021). Endocrine abnormalities, end-stage terminal disease, significant

operations, and a prolonged unhealthy lifestyle such as excessive high-calorie eating, being overweight, and high amounts of stress are all common risk factors for acquiring this syndrome (Abideen et al., 2017; Escott et al., 2021; Riddle et al., 2021; Zhang et al., 2021).

Diabetes and hyperglycemia are frequently referred to as the same thing and used interchangeably. In fact, hyperglycemia is the most commonly diagnosed cause of diabetes, both type I and type II. Type I diabetes is characterized by decreased insulin production by pancreatic β-cells due to an autoimmune response driven by B cells and particular antibodies such as anti-islet cells, anti-GAD, and anti-insulin antibodies. It dramatically reduces insulin levels recognized by the insulin receptor in cells and downregulates the expression of glucose transporter protein in membrane cells, lowering glucose absorption by cells and increasing glucose concentration in the plasma, commonly known as hyperglycemia (Barnett, 2018; Basu et al., 2020). Type II diabetes, on the other hand, is characterized by insulin resistance associated with metabolic syndrome, resulting in irresponsive insulin receptors and their respective pathways, decreased expression of glucose transporters, and significantly reduced glucose intake by cells, leaving a high amount of glucose in the blood plasma. This hyperglycemic condition stimulates the pancreatic β-cells to produce more elevated insulin, resulting in hyperinsulinemia and, over time, causing high stress to the pancreatic β-cells by releasing amyloid in the extracellular matrix, a condition known as amyloid deposition, which can damage the β-cells and lower insulin production (Kanatsuka et al., 2018; Galicia-Garcia et al., 2020).

Treatment of diabetes is essential for hyperglycemia treatment. Acute hyperglycemia caused by type I diabetes may usually be managed with early insulin injection; however, persistent hyperglycemia can be controlled with oral hypoglycemic medicine and healthy lifestyle changes (Silver et al., 2018). However, the medications utilized are commonly pharmaceuticals such as metformin, thiazolidinediones, sulfonylureas, and meglitinides, which can cause lactic acidosis, edema, hypoglycemia, and diarrhea in patients (Wang et al., 2017; Wu et al., 2017; Harsch et al., 2018; Salvatore et al., 2019). As a result, people are beginning to recognize the potential of medicinal herbs in curing ailments with few side effects. For people who are aware that long-term use of pharmaceutical medications may have serious adverse effects, medicinal plants are a great therapeutic choice (Putra and Rifa'i, 2019; Putra et al., 2021; Rahayu et al., 2022; Nurcholis et al., 2023). In developing nations, traditional medicine is the primary therapy for around 80% of the population. Plant extract accounts for around 85 percent of traditional medicine (Ahn, 2017; Jamshidi-Kia et al., 2018). Herbal medicine development is progressing in tandem with public knowledge of contemporary pharmaceuticals' health dangers and toxicity. Until recently, several plants were investigated for their potential medicinal advantages for various ailments. According to some studies, bioactive compounds such as thymoquinone from *Nigella sativa*, curcumin from *Curcuma longa*, and (S)-[8]-gingerol from *Zingiber officinale* can increase insulin levels, increase pancreatic islet immunoreactivity, reduce oxidative stress by acting as free radical scavengers, and upregulate glucose transporter 4 (GLUT4) protein, increasing glucose transport activity (Noipha and Ninla-Aesong, 2018; Abdelkader et al., 2020; Den Hartogh et al., 2020).

One of the species gaining popularity as a therapeutic herb is *M. calabura* (Elaecarpaceae), sometimes known as cherry. In many places, cherry plants are employed as herbal treatments. In Peru, for example, flowers and tree trunks are used as antiseptics, while the leaves are cooked and used to treat prostate gland enlargement (Sarojini and Mounika, 2018). Cherry leaf treats stomach problems, acne, and chickenpox in the Philippines (Tantengco et al., 2018). Cherry leaves have been examined for quite some time in Indonesia as a traditional remedy for treating diabetes. Based on chromatography examination, it contains various bioactive components that may contribute to its anti-hyperglycemic activity, including geniposide, luteolin, daidzein, quercetin, kaempferol, formononetin, 6-hydroxy flavone, gallic acid, kaempferide, genistein, and chrysin (Zakaria et al., 2019; Zolkeflee et al., 2022). However, little study has been undertaken to determine the specific effect of cherry leaf on hyperglycemia or diabetes. Therefore, more research into the advantages of cherry leaf on these entities is required. Thus, this study aims to evaluate the effect of *M. calabura* leaf extract on glucose control and immune cell modulation in high fat diet-administrated mice.

2. Material and Methods

2.1. *M. calabura* leaf extraction procedure

Dry powder of cherry leaf was obtained from Materia Medica, Batu, East Java. Decoction with freeze-drying methods was used for cherry leaf extraction. The cherry leaf powder was then cooked in distilled water (80°C) for about two hours with a leaf: aquadest ratio of 1: 10 (gr: ml) until the final volume was half the original volume, then filtered with a thin and clean cloth. The filtrate was then placed in a freezer set to -70 °C before freeze-dried to eliminate moisture.

2.2. Experimental treatments

Approximately 20 three-week-old male BALB/c mice were used. These pathogen-free mice were obtained from Gadjah Mada University, Yogyakarta. The mice were split into five groups with four repetitions, including vehicle group (aquadest), hyperglycemic group (12 weeks of HFD), MCE420 (12 weeks of HFD + 2 weeks of cherry leaf extract with 420 mg kg^{-1} BW), MCE700 (12 weeks of HFD + 2 weeks of cherry leaf extract with 700 mg kg^{-1} BW), and MCE2800 (12 weeks of HFD + 2 weeks of cherry leaf extract with 2800 mg kg^{-1} BW). The variation of doses used in this study was based on our preliminary study. HFD feed consists of 35% Hi-Gro 551, 10% high protein flour, 30% liquid fructose, 8% duck egg yolk, and 17% oil generated from beef fat (Saravanan and Pari, 2015). All ingredients are mixed and formed into a biscuit, baked at 100 °C for 10 minutes. The glucose levels of all mice were assessed on the tenth week, and then they were administered with *M. calabura* leaf extract (MCE) when the blood sugar levels had reached higher than 140 mg dL^{-1} after underwent fasting for 12 hours for two weeks. This research has been evaluated and received approval by the Research Ethics Committee of Brawijaya University, Malang with no. 670-KEP-UB for conducting animal model experiment.

2.3. Blood sugar test and evaluation

Blood sugar levels were measured using the GlucoDr AGM-2100 after the mice were fasting for 12 hours, and blood was drawn from the tail end and dripped on the stick of the blood sugar check instrument. Hyperglycemia occurs when fasting blood sugar levels exceed 140 mg dL^{-1} (Maffettone et al., 2018; Goyal et al., 2020). Blood sugar levels were measured twice after the week-10 of HFD administration and once after week-12 of therapy.

2.4. Splenocytes isolation

After two weeks of oral administration of *M. calabura* leaf extract, the mice were sacrificed to isolate the splenocytes. The spleen was then washed with PBS and homogenized in PBS using a syringe in a clockwise motion. After that, the homogenate was placed in a propylene tube and replenished with PBS until the amount reached 10 ml. The sample was then placed in a propylene tube and kept in an icebox until further analysis. The spleen homogenate was then centrifuged at 2500 rpm for 5 minutes at 10 °C. The pellets were collected and resuspended in 1 ml of PBS.

2.5. Flow cytometry analysis

In this study, we employed the FACS procedures and analyses according to our previous study (Putra et al., 2015; Putra et al., 2016). About 50 µl of pellet suspension of splenocytes was placed in a microtube containing 500 µl of PBS and centrifuged at 2500 rpm for 5 minutes at 4 °C. Extracellular staining was performed by adding 50 µl of extracellular antibody to the resultant pellet and incubating it at 4 °C for 20 minutes. It was then resuspended in 300 µl of PBS and put in a cuvette for flow cytometry analysis. After extracellular labeling, intracellular staining was performed using a suspension of cytofix-cytoferm and incubated for 20 minutes at 4 °C. Washperm was then applied in up to 500 µl and centrifuged for 5 minutes at 2500 rpm at 10 °C. The pellets were then resuspended in 300 µl of PBS after being treated with 50 µl of intracellular dye. The suspension is then placed within the cuvette. The FACS Calibur™ Flow cytometer (BD Bioscience) was used for the analysis. The extracellular dyes used were FITC-labeled rat anti-mouse CD4, PE-labeled rat anti-mouse CD25, and PE/Cy5-labeled rat anti-mouse TGF-β.

2.6. Compounds and Protein-Protein Interactions Prediction

About three compounds that are widely found in *M. Calabura,* including kaempferide, genistein, and gallic acid were evaluated in this study. In our previous study, the STITCH webserver (http://stitch.embl.de/) was used to predict the chemical association network toward the proteins and its specific biological activity (Putra et al., 2017; Putra et al., 2023).

2.7. Statistical analysis

The data were analyzed with the normality and homogeneity tests of variance. The CellQuest™ (BD Bioscience) and SPSS 20.0 for Windows were used to examine the data, then assessed using an ANOVA test with a 95% confidence interval, followed by a Tukey's honest significant difference test.

3. Results and Discussion

3.1. *M. calabura* leaf extract reduces blood sugar

In this study, we evaluate the effect of MCE on the blood sugar level and immune modulation in HFD-administrated mice (Figure 1). Following a high sugar and fat meal, glucose levels in mice's blood, and body weight increased dramatically compared to normal mice (Figure 2). According to those findings, these mice groups displayed signs of obesity, indicating excessive lipid buildup in adipose tissue. Overnutrition causes adipocyte hypertrophy and hyperplasia, which leads to cellular stress, which triggers inflammatory responses in adipose tissue (Longo et al., 2019).

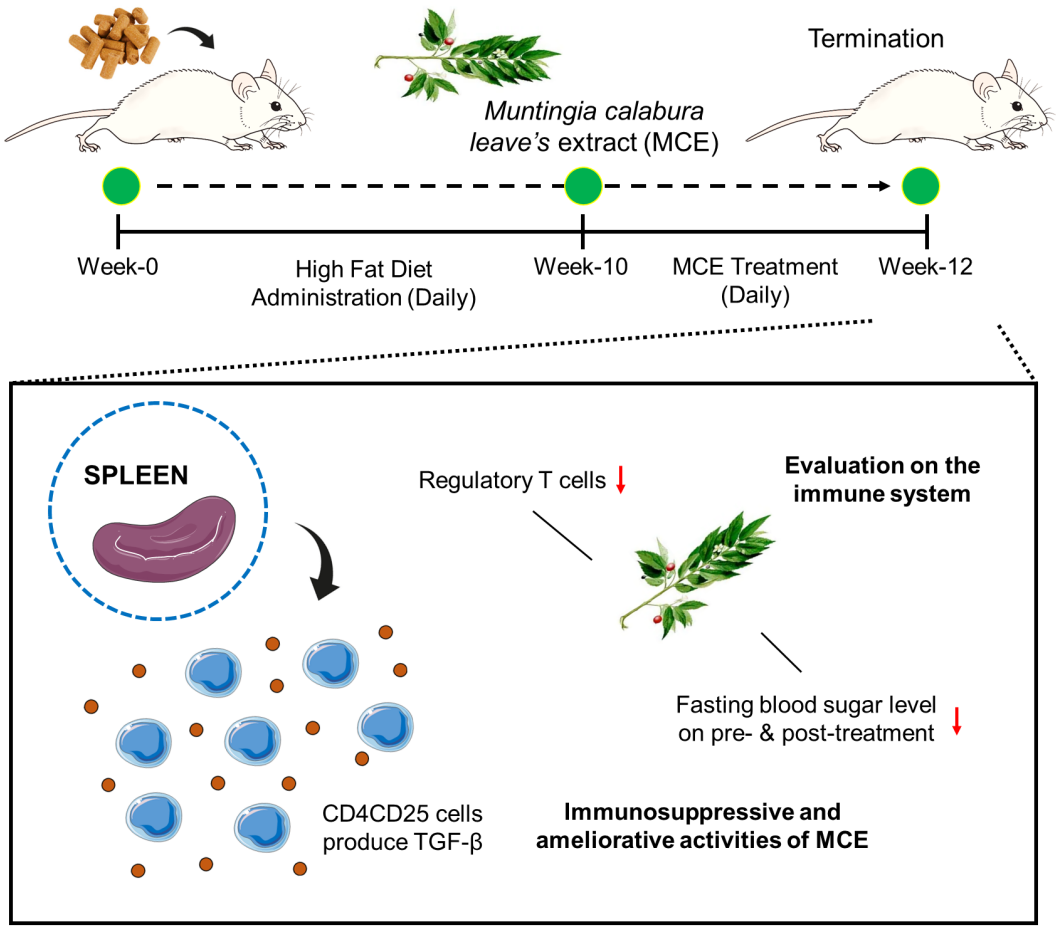


Figure 1. Schematic picture showed how *M. calabura* leaf extract suppresses the regulatory T cells and fasting blood sugar of high-fat diet-administrated mice to normal conditions.

Inflammatory reactions in adipose tissues self-generate, resulting in elevated local and systemic levels of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and IL-1 (Ellulu et al., 2017). These pro-inflammatory cytokines connect to cell-surface receptors and activate kinases such as JNK, which phosphorylate IRS-1 on serine residues, limiting its action. Thus, inflammatory cytokines suppress the insulin signal, resulting in insulin resistance, since irresponsive insulin receptors and their corresponding pathways reduce the expression of glucose transporters and significantly reduce glucose absorption by cells, resulting in hyperglycemia. TLR2 and TLR4 activate the kinase proteins JNK and IKK-β, boosting the production and release of pro-inflammatory cytokines and blocking the insulin signaling pathway, resulting in hyperglycemia (Chen et al., 2015; Yung and Giacca, 2020).

Hyperglycemia promotes glycolysis and tricarboxylic acid cycle fluxes, which raise NADH/NAD^+ ratios in the cell's cytosol and mitochondria. This increases electron disposal at the electron transport chain, producing reactive oxygen species (ROS) (Ola, 2021). Increased ROS levels in the cells would activate the JNK pathway while simultaneously inhibiting the insulin signaling cascade, resulting in high glucose accumulation in the blood because the cells do not respond appropriately to insulin communication signals and cannot easily take glucose from the blood (Volpe et al., 2018). It has also been proposed that high concentrations of IL-1β, IFN-γ, and TNF-α might interfere with insulin sensitivity (Tao et al., 2019; Wondmkun, 2020) and that increasing high oxidative stress in pancreatic β-cells and cell death by activation of the caspase-9 pathway could further impair insulin levels (Eguchi et al., 2021). High-fat content in HFD may also activate TLR4-mediated pro-inflammatory signaling pathways in pancreatic cells by activating NF-κB and translocating into nuclei, upregulating TNF-β expression, and initiating the activation of receptor-interacting protein 3 (RIP3) kinase, which marks the activation of programmed necrosis (Meng et al., 2015; Hong et al., 2020).

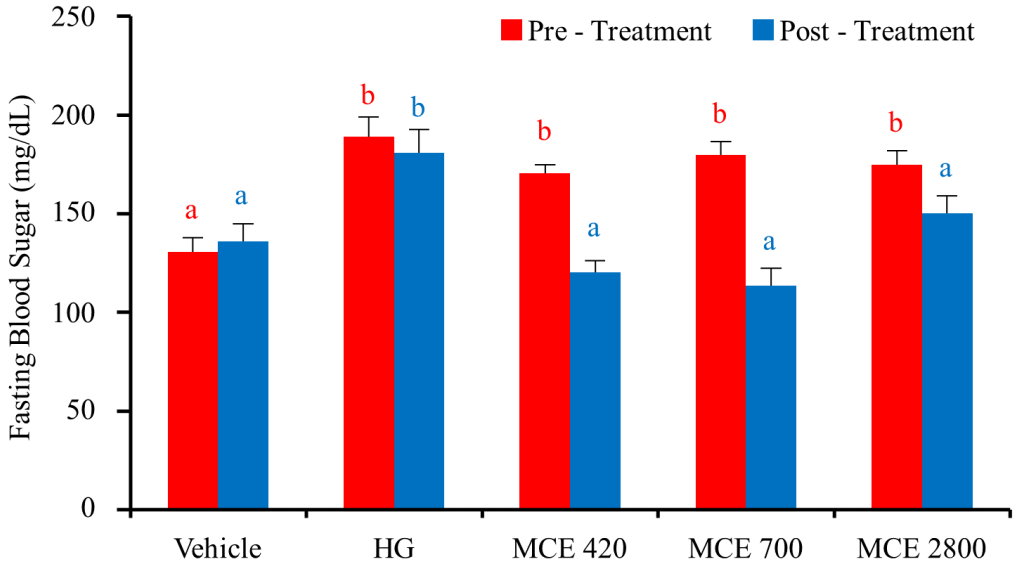


Figure 2. Fasting blood sugar level on pre- and post-treatment of *M. calabura* extract in high fat diet-administrated mice. The p value < 0.05 was considered significant. The graph shows considerable differences using alphabetic characters. Hyperglycemic group (HG); *M. calabura* leaf extract (MCE).

Additionally, to validate our findings, we predicted the main compounds of *M. calabura* leaf such as genistein, kaempferide, and gallic acid. According to the prediction, these compounds are included in many biological processes, including cellular response to lipids, glucose homeostasis, positive regulation of fat cell differentiation, and positive regulation of nitric oxide biosynthetic process (Figure 3). Several investigations indicate that *M. calabura* leaf extract has a high quantity of geniposide, pinostrobin, genistein, daidzein, quercetin, kaempferol, formononetin, and gallic acid (Zakaria et al., 2016; Zakaria et al., 2019; Zolkeflee et al., 2022). Geniposide reduces hyperglycemia-induced oxidative stress and inflammation by upregulating the Nuclear factor erythroid 2-related factor 2 (NRF2) pathway, inhibiting ROS buildup and decreasing NF-κB activation and the consequent inflammatory response

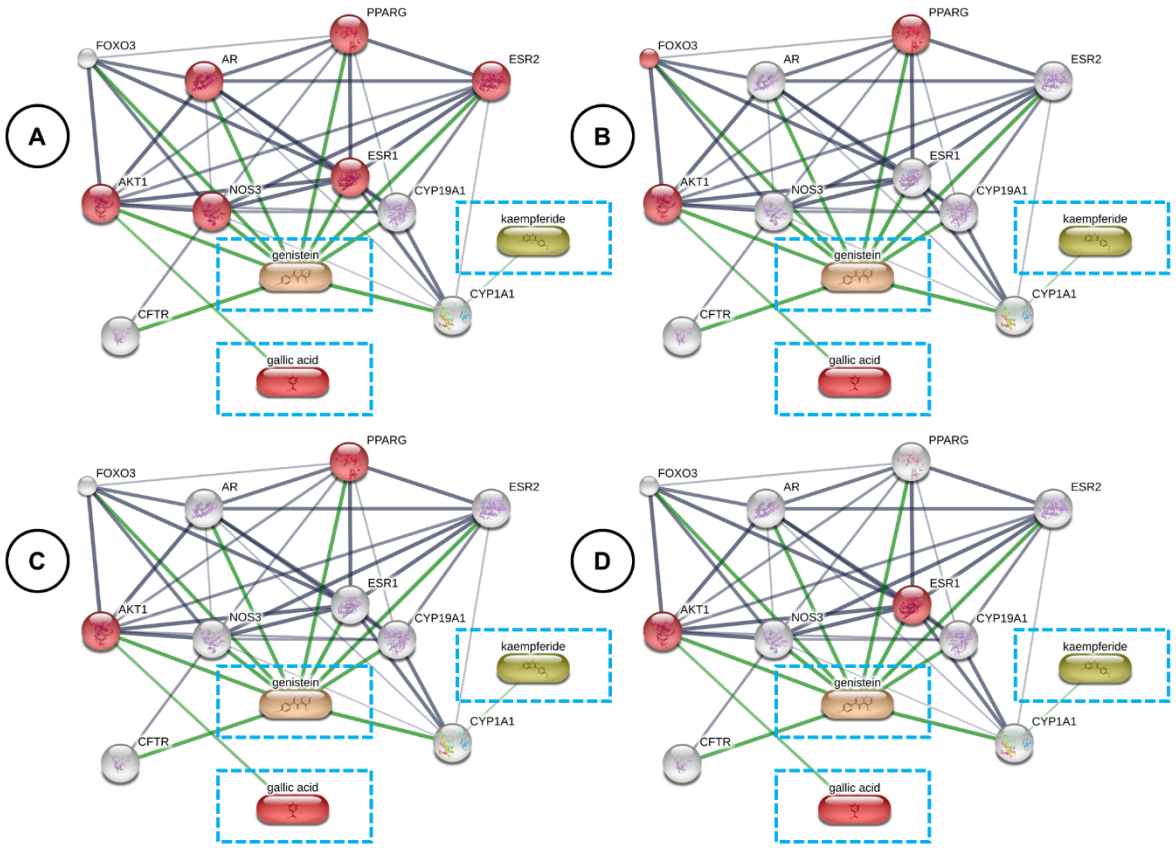
(Tu et al., 2021). It is also thought to suppress the transcriptional activity of Forkhead box protein O1 (FOXO1) by activating the phosphorylation of protein kinase B (PKB) (Yang et al., 2018), preventing more glucose from passing through the blood plasma. Daidzein helps to increase the mRNA level in β-cells to exert more insulin production (Zolkeflee et al., 2022), increasing the ratio of GLUT4 to Na^+/K^+ ATPase in the plasma membrane, which increases glucose uptake (Das et al., 2018), upregulating the gene expression of Peroxisome proliferator-activated receptor gamma (PPARγ) and adiponectin and downregulating the monocyte chemoattractant protein-1 (MCP-1) and TNF-α gene expression in adipose (Sakamoto et al., 2014). Quercetin protects the intact-cells of the islets of Langerhans by preventing lipid peroxidation and scavenging free radicals, hence maintaining appropriate insulin concentration (Abdelkader et al., 2020). Genistein reduced the levels and mRNA expression of pro-inflammatory cytokines IL-1, IL-6, and TNF-α, and when combined with gallic acid, it suppressed the ROS/Akt/NF-κB pathway and increased adenosine monophosphate protein kinase (AMPK) activation, enhancing insulin sensitivity (Xu et al., 2021; Goh et al., 2022).

3.2. *M. calabura* leaf extract suppresses the relative number of $CD4^+CD25^+$ T Cells

Regulatory T cells (Tregs) are a subpopulation of $CD4^+$ T cells that secrete TGF-β, IL-10, and IL-4 to prevent autoimmune and pro-inflammatory responses to preserve peripheral tolerance and decrease antigen-specific immune responses carried out by $CD8^+$ T cells (Qiao et al., 2016; Zhou et al., 2021). In hyperglycemic mice, the relative number of $CD4^+CD25^+$ Tregs rose considerably compared to normal mice (Figure 4). It is suggested that diabetic mice's spleen $CD4^+CD25^+$ Tregs have defective immunosuppressive capability despite having a higher relative number, which is thought to correlate with decreasing insulin levels because low insulin levels in hyperglycemic mice impacted thymic $CD4^+CD25^+$ Treg development. Furthermore, Treg cells are dominated by a subpopulation that lacks the CD62L protein (Putra and Rifa'i, 2020). They exhibit high levels of CD44 and CTLA-4 expression simultaneously, which weakens their suppressive action (Zhen et al., 2012; Hyun et al., 2019), hinders continuous cell activation, and boosts pro-inflammatory cytokine production, which is predominantly carried out by T regulatory type 1 cells (Hull et al., 2017).

Under hyperglycemic conditions, increased production of pro-inflammatory cytokines such as IL-2 by adipocytes, monocytes, and macrophages might occur due to high blood glucose levels and decreased insulin expression, which leads to increased CD25 expression in the $CD4^+$ T cell population (Kochumon et al., 2020). As previously demonstrated, high glucose levels can enhance the production of pro-inflammatory cytokines via many pathways, including the NF-κB pathway (Meng et al., 2015; Hong et al., 2020). Fatty acids can alter T cell functions such as proliferation and cytokine production, hence increasing inflammation. Low-dose fatty acids, in contrast to large dosages, can enhance T cell growth and alter the production of cytokines such as TNF-α, IL-6, IL-8, IL-1, IL-2, IL-10, and IFN-γ (Heintzman et al., 2022). We anticipated that a highly inflammatory milieu, namely a high concentration of IL-2, might convert naive $CD4^+$ cells to $CD4^+CD25^+$ Treg cells, which then counteract those highly inflammatory circumstances by releasing large quantities of anti-inflammatory cytokines, including TGF-β, creating a positive loop that promotes additional Treg cells, increasing their relative proportion.

The administration of cherry water extract to hyperglycemic mice at all experimental doses lowered the relative number of $CD4^+CD25^+$ regulatory T cells to near-normal levels. All doses had no significant change (Figure 4), with all three significantly reducing the relative number of regulatory T cells. The decrease in the close number of $CD4^+CD25^+$ T cells may be related to a reduction in CD25 expression in the $CD4^+$ T cell population induced by a high content of quercetin (Kobori et al., 2016), which is consistent with the results of (Leyva-López et al., 2016), who discovered that flavonoids like quercetin could reduce the activity of NF-κB, a common transcription factor for pro-inflammatory cytokines that are activated by ROS, resulting in suppression of pro-inflammatory Furthermore, the extract's kaempferol may boost and maintain forkhead box P3 (FOXP3) expression in Treg cells while decreasing proto-oncogene serine/threonine-protein kinase (PIM-1)-mediated FOXP3 phosphorylation, improving their suppressive action on effector T cells and pro-inflammatory helper T cells (Lin et al., 2015).



Symbol	Biological Process	False Discovery Rate	Protein Involved
A	Cellular response to lipid	6.74E-05	AKT1, AR, ESR1, ESR2, NOS3, PPARG
B	Glucose homeostasis	0.0199	AKT1, FOXO3, PPARG
C	Positive regulation of fat cell differentiation	0.0449	AKT1, PPARG
D	Positive regulation of nitric oxide biosynthetic process	0.0283	AKT1, ESR1

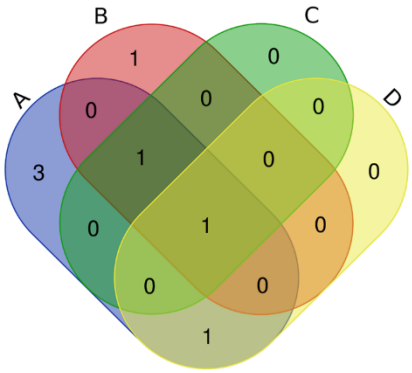


Figure 3. The *M. calabura* compounds-proteins interaction network analysis. (A). Cellular response to lipid; (B). Glucose homeostasis; (C). Positive regulation of fat cell differentiation; (D). Positive regulation of nitric oxide biosynthetic process.

It has been proposed that enhanced circumstances in the cherry treatment groups may function as a negative feedback mechanism. Fewer Treg cells are required to limit and stabilize lower pro-inflammatory cytokine expression and population pro-inflammatory immune cells in adipose tissue and the pancreas. Furthermore, in the untreated hyperglycemic group, a lower number of Tregs with highly suppressive characteristics is preferable to a higher number of Tregs with low suppressive action because these highly suppressive Tregs are more capable of alleviating tissue-damaging inflammatory responses than their less-suppressive counterparts. According to the previous explanation, numerous cherry extract components enhance the targeted tissues' inflammatory milieu and considerably ameliorate the hyperglycemic condition. Thus, lowering the oxidative stress reduces the Tregs needed to control the inflammation caused by hyperglycemia.

3.3. *M. calabura* leaf extract suppresses the relative number of TGF-β-expressing $CD4^+CD25^+$ T Cells

The number of $CD4^+CD25^+$ T lymphocytes expressing TGF-β rose considerably in hyperglycemic mice compared to normal mice because hyperglycemia increases the production of TGF-β and its receptors to counteract the buildup of ROS as well as chronic inflammation (Budi et al., 2015; Chen et al., 2020). Increased blood sugar levels mediate these effects via various pathways, including the polyol activation pathway, protein kinase C, and severe oxidative stress (Jha et al., 2016; Dhanya, 2022). As previously noted, hyperglycemia may increase ROS levels in mouse tissue due to greater blood glucose levels and reduced insulin synthesis by pancreatic β-cells.

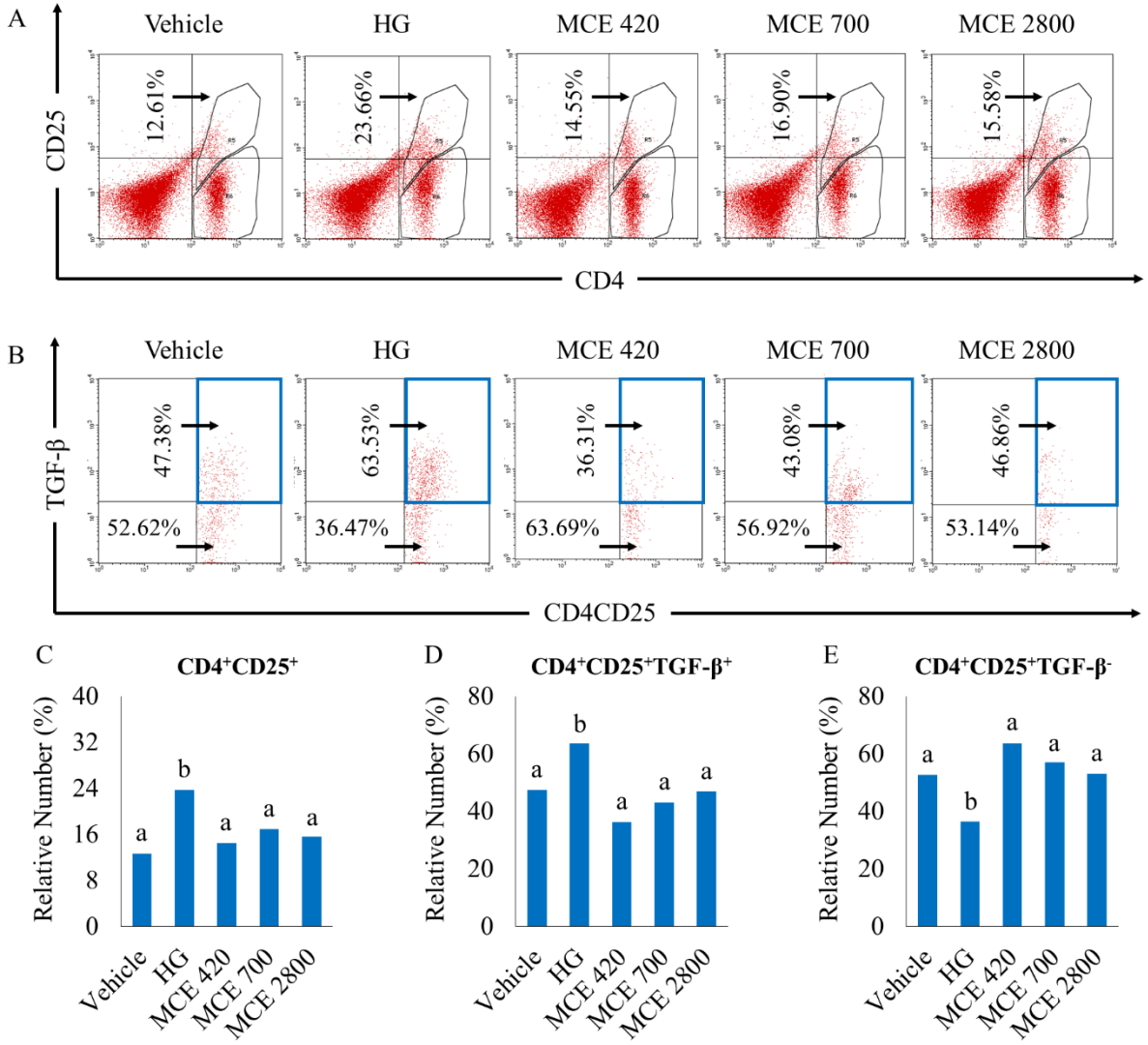


Figure 4. Immunosuppressive effects of *M. calabura* extract toward regulatory T cells high-fat diet-administrated mice. The p-value < 0.05 was considered significant. The graph shows considerable differences using alphabetic characters. Hyperglycemic group (HG); *M. calabura* leaf extract (MCE).

Furthermore, increasing fatty acid levels might increase ROS generation and decrease catalase activity in T cells, resulting in cell death (De Jong et al., 2014). These assertions are consistent with the findings, which reveal a direct relationship between mouse body weight and a rise in $CD4^+CD25^+TGF\text{-}\beta^+$ T cells. Higher oxidative stress imposed by hyperglycemia-induced inflammation in pancreatic β-cells might cause cell death, as discussed in the preceding chapter, via endoplasmic reticulum stress and mitochondrial malfunction. Another critical issue is that pancreatic β-cells have decreased antioxidant activity, rendering them more vulnerable to oxidative stress and necrosis (Eguchi et al., 2021). If not mediated by the insulin promoter, TGF-β might increase β-cell death and pancreatic fibrosis via the

TGF-β/Smad3 signaling pathway (Lee et al., 2021). Higher TGF-β expression in the untreated hyperglycemia group might alternatively be interpreted as an attempt to increase insulin production from damaged β-cells, putting additional strain on this already harmed tissue (Dhawan et al., 2016).

We discovered that all three dosages significantly lowered the quantity of TGF-β generated by regulatory T cells (Figure 4). The three doses provided did not differ much since they were labeled the same way, yet all three dramatically lowered cytokine levels compared to untreated hyperglycemic mice. Aside from the previously mentioned impact, we believe bioactive chemicals in cherry leaf extract may block TGF-β production in Treg cells. To the best of our knowledge, bioactive substances like quercetin, kaempferol, and genistein might block PKC activation and its signal transduction pathway, hence suppressing TGF-β expression in Treg cells (Kanazawa et al., 2017; Alam et al., 2020; Salehi et al., 2020; El-Far et al., 2022). TGF-β expression, on the other hand, appears to be strongly related to the relative amount of Treg cells and insulin production. As previously indicated, TGF-β upregulation is preceded by hyperactivation of its synthesis pathway, which is driven by hyperglycemia. Higher insulin levels in the treated groups may also help ameliorate hyperglycemia by reducing glucose levels and suppressing TGF-β synthesis and its response pathways.

Conclusion

This study showed *M. calabura* leaf extract exerts ameliorative potency against hyperglycemia by lowering the blood sugar level and suppressing the regulatory T cells. These results suggested that *M. calabura* leaf extract could develop into complementary and alternative medicine. However, more research on the effects of *M. calabura* leaf extract on various immune cells is required.

Acknowledgements

All authors thank Brawijaya University for facilitating and funding (M.R.) this study.

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Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

ISSN: 1308-7576 e-ISSN: 1308-7584

Research Article

Determination of The Fatty Acid Composition of Some Taxon of The Apiaceae Family

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Article Info

Received: 05.05.2023
Accepted: 07.11.2023
Online published: 15.03.2024
DOI: 10.29133/yyutbd.1292849

Keywords

Apiaceae,
Fatty acid components,
Fatty oil rate,
Petroselinic acid

Abstract: The aim of the study was to determine the fatty oil ratio and fatty acid components of 19 taxa, of which 6 are endemic and belong to the Apiaceae family, naturally distributed in the Isparta and Burdur provinces. In the study, the fruits of *the Ammi visnaga, Angelica sylvestris, Bifora radians, Echinophora tournefortii, Echinophora tenuifolia* subsp *sibthorpiana, Echinophora trichophylla* (endemic), *Foeniculum vulgare, Ferulago cassia, Ferulago pauciradiata* (endemic), *Glaucosciadium cordifolium, Heracleum platytaenium* (endemic), *Hippomarathrum cristatum, Hippomarathrum microcarpum, Laser trilobum, Opopanax hispidus, Pastinaca sativa* subsp *urens, Peucedanum chryseum* (endemic), *Prangos platychlaena* (endemic), and *Prangos uechtritzii* (endemic) were used as materials. The fatty oil ratios of the species were determined by NMR, and the fatty acid components were determined by the GC/FID instrument. In the study, the fatty oil ratios of the species varied from 4.0% to 27.6%. A total of 43 different fatty acids were identified, mainly palmitic acid, stearic acid, petroselinic acid, oleic acid, and *cisvaccinic* acid fatty acids. Most of the fatty oils of the taxa consisted of oleic acid, linoleic acid, and petroselinic fatty acids. Therefore the taxa investigated in the study are believed to be rich in fatty oil content and unsaturated fatty acids and can be used as an oil source in the future.

To Cite: Tosun, B, Karadoğan, T, 2023. Determination of The Fatty Acid Composition of Some Taxon of The Apiaceae Family. *Yuzuncu Yil University Journal of Agricultural Sciences*, 34(1): 14-23. DOI: https://.doi.org/10.29133/yyutbd.1292849

Footnote: This study is derived from Ph.D. thesis named "Determination of Economic Value And Cultivation Potantials of Some Plant Species Belongıng to Apiaceae Family Growing In Lakes Region".

1. Introduction

Depending on the increase in the world population, there may be difficulties in the future in the supply of vegetable oils, which are essential for human nutrition. This will require a, higher oil yield per area, or alternative sources of vegetable oil will be sought. Apiaceae is one of the genus groups with the greatest economic importance in the world. Various plant species belonging to this family have been used as medicine and spice for many years. Worldwide, the family is represented by 455 genera and 3600-3751 species (Pimenov and Leonov, 1993; Pimenov and Leonov, 2004), while in Türkiye, it consists of 101 genera and 485 species, with an endemism percentage of 37.3% (Guner et al., 2000). Cumin, anise, fennel, and coriander species belonging to this family have been cultivated in our country

for many years. The species grown in this country are generally used as spices or essential oils in foods. In addition, the fatty oils contained in the species of the family are used. The previous study showed that especially the fruits contain fatty oil and high amounts of unsaturated fatty acids. The majority of the fatty acids are petroselinic fatty acids (18:1, *cis*-6), which are rare in other oil plants. This offers the possibility of using fatty oils as both edible and industrial oil (Bayrak and Korkut 1995; Reiter et al. 1998a; Bayrak, 2006; Tosun, 2021). In addition, the herbicidal activity of fatty acids, and some of them are currently used commercially as herbicides (Dayan et al., 2009). The objective of this study was to determine the fatty oil content and fatty acid components of the fruits of 19 taxa of the Apiaceae family, which are thought to be a source of vegetable oil.

2. Material and Methods

The research was carried out in laboratories of Isparta University of Applied Sciences, Faculty of Agriculture, Field Crops during 2017–2019. Fruit samples were collected at the full maturity period in the study using the location information of the species whose distribution areas were determined in the TUBITAK 113O284 project. Taxa used as material are listed in Table 1 with GUL Herbarium codes and location information.

Table 1. Herbarium codes and locality data of the taxa

Species	GULHerbarium Codes	Loctite/Altitude
Ammi visnaga (L.) Lam	GUL 63.58.1.1.	Burdur: Bucak/ 790 m
Angelica sylvestris L.	GUL 63.62.1.1	Isparta: Yenişarbademli/ 1600 m
Bifora radians Bieb.	GUL 63.16.2.5	Isparta:Merkez/ 1000 m
Echinophora tournefortii Jaub. & Spach.	GUL 63.7.2.5	Burdur: Yeşilova/ 1236 m
Echinophora tenuifolia L. subsp *sibthorpiana* (Guss) Tutin	GUL 63.7.2.1.2	Isparta: Merkez/ 1010 m
Echinophora trichophylla J.E.Smith (Endemic)	GUL 63.7.4.1	Isparta: Sav kasabası/ 981 m
Ferulago cassia Boiss.	GUL 63.69.11.1	Burdur: Ağlasun/1047 m
Ferulago pauciradiata Boiss. & Heldr. (Endemic)	GUL 63.69.8.1	Isparta: Eğirdir/ 1574 m
Foeniculum vulgare Miller	GUL 63.34.1.6	Isparta: Eğirdir/ 924 m
Glaucosciadium cordifolium (Boiss.) Burtt & Davis	GUL 63.83.1.5	Isparta- Antalya karayolu/ 360m
Heracleum platytaenium Boiss. (Endemic)	GUL 63.75.3.2	Isparta: Eğirdir/ 1235 m
Hippomarathrum cristatum (DC.) Boiss.	GUL 64.44.2.1	Burdur: Yeşilova/1159 m
Hippomarathrum microcarpum (Bieb.) Fedtsch.	GUL 64.44.1.1	Isparta: Eğirdir/ 946 m
Laser trilobum (L.) Borkh.	GUL 63.83.1.1	Isparta: Eğirdir/ 1575 m
Opopanax hispidus (Friv.) Gris.	GUL 63.70.2.2	Isparta: Eğirdir/ 940 m
Pastinaca sativa L. subsp *urens* (Req. Ex Gordon) Celak	GUL 63.73.1.1.2	Isparta: Eğirdir/ 932 m
Peucedanum chryseum (Boiss. & Heldr.) Chamb. (Endemic)	GUL 63.72.4.1	Isparta: Şarkikaraağaç/ 1385 m
Prangos platychlaena Boiss. ex Tchihat (Endemic)	GUL 63.42.8.1	Burdur: Bucak/ 800
Prangos uechtritzii Boiss & Hausskn (Endemic)	GUL 63.42.10.1	Isparta: Eğirdir/ 1576 m

2.1. Determination of fatty oil rate

Samples were placed in a measuring cup of the nuclear magnetic resonance ce (NMR, Bruker mqone) device measuring cup and read at five replicates, and the average oil content was calculated (Baydar and Erbas, 2014).

2.2. Fatty acid components

The ground fruit samples (5 g) were mixed with 10 ml of n-hexane to extract the crude oil. It was then filtered and dried at 45 °C to remove the solvent from the filtrate. A 25 µL of extracted oil was mixed in 750 µL 0.5% sodium methylate (NaoMe), followed by 1 ml n-hexane, and shaken to prepare the fatty acid methyl esters (FAME). A 1 µL of the upper phase (FAME) was withdrawn and subjected to gas chromatography (Shimadzu GC-2025). The operating conditions of the GC device were set as follows: column 100 m × 0.25 mm, 0.20 µm (Technochroma TR-CN100), injector temperature 250 °C, detector temperature 250 °C, flow rate 10 psi, carrier gas N (40 ml/min), injector capacity 1 µL. The initial temperature was maintained at 140 °C for 10 minutes, followed by an increase of 3 °C per minute until 240 °C was reached for 10 minutes. The peaks in the chromatograms were compared against the standard F.A.M.E. mix (Supelco® 37 Component FAME Mix, Sigma).

3. Results

The values of the fatty oil ratio and components of all species included in the study are given in Table 2.

Table 2. Fatty acid components and rate of the taxa (%)

Retention Times		Components	Av	As	Br	Et	Eteni	Etri	Fc	Fp	Fv
14.396			-	7.88	-	-	-	-	-	-	-
15.362	$C_{6:0}$	Coproic	0.13	0.21	-	0.05	-	-	2.91	9.20	0.07
19.153	$C_{8:0}$	Caprylic	0.01	0.02	-	0.02	0.01	-	0.11	0.25	-
22.937			-	-	-	-	-	-	-	-	5.21
24.349	$C_{10:0}$	Capric	<0.01	-	0.04	0.03	<0.01	-	0.06	0.04	-
27.599	$C_{11:0}$	Undecanoic	0.01	0.10	-	-	0.02	-	0.50	0.10	-
30.291	$C_{12:0}$	Lauric	0.02	0.09	0.02	0.08	0.08	-	0.15	0.04	11.79
33.289	C 13:0	Tridecanoic	-	0.03	<0.01	-	-	-	0.06	0.04	
36.137	$C_{14:0}$	Myristic	0.15	0.07	0.03	0.31	0.17	0.10	0.94	0.14	0.11
38.393	C14:1	Myristoleic	-	-	-	-	-	-	-	0.01	0.03
38.918	$C_{15:0}$	Pentadecanoic	0.03	0.02	0.02	0.11	0.04	-	0.49	0.05	-
40.916	$C_{15:1c10}$	*cis*-Pentadecanoic	<0.01	0.09	-	-	-	-	0.37	0.02	-
41.640	$C_{16:0}$	Palmitic	3.86	3.46	3.02	9.78	4.65	4.60	6.38	4.19	3.62
43.297	$C_{16:1}$	Palmitoleic	0.18	0.09	0.10	0.26	0.13	-	9.93	0.17	0.05
44.170	$C_{17:0}$	Heptadecanoic	0.03	0.06	0.03	0.10	0.04	-	0.49	0.03	0.03
45.689	$C_{17:1c10}$	cis-Heptadecanoic	0.03	0.03	0.03	0.70	0.06	-	0.47	0.27	0.01
46.816	$C_{18:0}$	Stearic	0.86	0.94	0.05	2.85	1.55	1.45	2.28	1.41	1.02
48.235	$C_{18:1n6c}$	Petroselinic	72.61	31.21	81.00	45.24	58.45	56.82	31.05	55.58	64.70
48.287	$C_{18:1n9c}$	Oleic	3.91	7.42	2.82	10.08	8.33	14.58	11.80	6.19	2.57
48.378	$C_{18:1n7c}$	*cis*-Vassenic	0.61	0.48	0.52	1.08	0.47	0.65	0.55	0.69	0.11
49.718	$C_{18:2n6t}$	Linolelaidic	0.11	0.11	0.05	0.55	-	-	0.11	0.04	-
50.252	$C_{18:2n6c}$	Linoleic	13.09	16.97	11.06	24.48	24.40	21.37	28.50	18.39	9.88
51.260	$C_{20:0}$	Arasidic	0.09	0.06	0.06	0.27	0.15	-	-	-	0.24
51.728	$C_{18:3n6}$	Ɣ-linoleic	-	0.06	-	-	0.15	-	0.55	-	0.04
52.636	$C_{20:1c1}$	Leicosenoic	0.33	0.39	0.27	1.83	0.82	0.40	0.38	0.40	0.23
54.108	$C_{18:3n3}$	Linoleic	1.23	-	0.02	-	0.03	-	0.23	0.02	0.02
55.271	$C_{21:0}$	Heneicosanoic	0.20	-	0.22	-	0.06	-	0.63	2.26	0.05
55.828	$C_{20:2\ c11.14}$	Eicosadienoic	0.06	0.10	0.02	-	0.11	-	-	0.11	0.07
57.463	$C_{20:3n3c11.14.17}$	Eicosatrienoic	2.26	-	-	0.99	-	-	-	0.02	-
57.920	$C_{20:4n6}$	Aracidonic	-	0.10	-	0.24	0.11	-	-	0.03	0.03
58.188	$C_{23:0}$	Tricosanoic	0.02	1.68	-	0.14	0.05	-	1.07	0.06	0.04
60.037	$C_{22:2c13.16}$	Docosadienoic	0.05	-	0.02	-	-	-	-	0.11	-
60.642	$C_{24:0}$	Lingoseric	0.06	0.08	-	0.48	0.12	-	-	0.12	0.07
61.263	$C_{24:1}$	Nervonic	0.04	-	-	0.36	-	-	-	-	-
65.420	$C_{22:6n3}$	Docosahexaenoic	-	28.24	-	-	-	-	-	-	-
	ΣSFA		5.48	6.82	3.49	14.20	6.94	6.14	16.07	17.94	17.06
	ΣMUFA		77.71	39.72	84.74	59.19	68.25	72.45	54.54	63.34	67.68
	ΣPUFA		16.80	45.58	11.17	26.61	24.81	21.37	29.38	18.72	10.05
	Other		-	7.88	-	-	-	-	-	-	5.21
	Oil Content (%)		23.7 ± 0.49	17.5 ± 0.53	16.6 ± 0.38	2.1 ± 0.44	9.3 ± 0.28	4.1 ± 0.18	18.8 ± 0.26	14.0 ± 1.06	20.0 ± 0.10

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids, Av: *Ammi visnaga*, As: *Angelica sylvestris*, Br: *Bifora radians*, Et: *Echinophora tournefortii*, Eteni: *Echinophora tenuifolia* L. subsp *sibthorpiana*, Etri: *Echinophora trichophylla*, Fc: *Ferulago cassia*, Fv: *Foeniculum vulgare*.

Table 2. Fatty acid components and rate of taxa (%) (continued)

Retention Times		Components	Gc	Hc	Hm	Hp	Lt	Op	Ps	Pc	Pp	Pu
14.396			-	-	-	-	3.54	-	-	-	17.45	-
14.491			-	-	-	-	11.80	-	-	-	-	-
15.362	$C_{6:0}$	Coproic	-	0.13	0.48	1.43	0.07	-	0.10	-	0.45	-
19.153	$C_{8:0}$	Caprylic	-	-	0.04	0.34	-	0.04	-	-	0.02	0.37
22.652			-	-	-	-	-	-	-	-	-	0.83
22.937			-	-	-	11.39	-	-	3.58	-	-	1.74
24.349	$C_{10:0}$	Capric	-	-	0.06	0.02	0.02	0.02	0.37	0.08	-	6.92
26.709			-	-	-	-	-	-	-	-	-	0.97
27.599	$C_{11:0}$	Undecanoic	-	-	0.26	-	0.05	-	-	0.38	0.30	1.56
28.850			-	-	-	-	-	-	-	-	-	2.80
30.291	$C_{12:0}$	Lauric	-	0.04	0.03	0.24	0.16	0.08	0.01	0.07	0.02	0.89
33.289	C 13:0	Tridecanoic	-	-	-	-	-	-	-	22.66	-	-
36.137	$C_{14:0}$	Myristic	-	0.18	0.07	0.08	4.10	0.29	0.03	0.10	0.09	0.11
38.393	C14:1	Myristoleic	-	-	0.02		0.20	-	-	-	-	-
38.918	$C_{15:0}$	Pentadecanoic	-	0.06	0.05	0.05	0.09	0.10	0.04	0.09	0.11	0.05
40.916	$C_{15:1c10}$	*cis*-Pentadecanoic	-	-	0.01	-	0.06	-	-	-	-	0.28
41.640	$C_{16:0}$	Palmitic	8.39	4.84	4.44	4.49	7.09	7.75	5.21	5.41	3.59	4.50
43.297	$C_{16:1}$	Palmitoleic	0.16	0.22	0.30	0.14	0.15	0.20	0.16	0.33	0.24	0.27
44.170	$C_{17:0}$	Heptadecanoic	-	0.04	0.51	0.05	0.08	0.07	0.02	-	0.04	0.60
45.689	$C_{17:1c10}$	cis-Heptadecanoic	1.95	0.03	0.06	0.04	0.15	0.07	0.01	-	0.02	0.07
46.816	$C_{18:0}$	Stearic	1.39	1.36	0.70	0.97	2.49	2.22	1.08	1.10	1.16	1.07
48.235	$C_{18:1n6c}$	Petroselinic	61.19	69.43	71.47	53.73	34.96	58.94	63.93	17.29	61.35	50.80
48.287	$C_{18:1n9c}$	Oleic	4.36	6.51	4.82	7.13	10.40	0.92	4.90	21.35	2.81	5.94
48.378	$C_{18:1n7c}$	*cis*-Vassenic	0.30	0.52	0.84	0.58	0.38	-	0.38	0.81	0.59	0.60
49.718	$C_{18:2n6t}$	Linolelaidic	1.58	0.15	0.03	0.04	-	-	-	-	-	0.16
50.252	$C_{18:2n6c}$	Linoleic	17.24	15.46	15.14	18.42	21.08	26.16	19.51	27.31	10.93	18.42
51.260	$C_{20:0}$	Arasidic	-	0.11	0.07	0.12		0.27	0.12	0.14	-	0.11
51.728	$C_{18:3n6}$	Ɣ-linoleic	-	-	-	-	0.22	-	-	-	-	0.11
52.636	$C_{20:1c1}$	Leicosenoic	1.38	0.24	0.25	-	1.78	0.64	0.27	0.35	0.43	0.29
54.108	$C_{18:3n3}$	Linoleic	-	0.02	0.02	-	0.18	0.24	0.03	-	-	-
55.271	$C_{21:0}$	Heneicosanoic	-	0.31	0.10	-	0.19	1.34	0.07	0.45	0.13	-
55.828	$C_{20:2\ c11.14}$	eicosadienoic	-	0.04	0.05	0.05	-	0.16	0.05	0.50	0.06	-
56.114	C22:0	Behenic	1.02	-	-	-	-	-	-	-	-	-
57.463	$C_{20:3n3c11.14.17}$	Eicosatrienoic	-	-	-	-	-	-	-	0.23	-	-
57.920	$C_{20:4n6}$	Arachidonic	0.07	0.07	0.02	0.04	0.04	0.16	0.02	0.35	0.09	-
58.188	$C_{23:0}$	Tricosanoic	0.28	0.06	0.05	0.06	0.09	0.19	0.04	-	0.09	0.36
60.037	$C_{22:2c13.16}$	Docosadienoic	0.52	0.04	-	0.04	-	0.05	0.01	0.23	-	-
60.642	$C_{24:0}$	Lingoseric	-	0.12	0.11	0.08	0.64	0.10	0.05	0.51	0.04	0.16
65.420	$C_{22:6n3}$	Docosahexaenoic	0.15	-	-	0.07	-	-	0.03	0.25	-	-
	ΣSFA		11.61	7.27	6.96	8.13	14.40	12.47	7.13	30.99	6.03	16.72
	ΣMUFA		69.34	76.95	77.78	61.82	48.08	60.77	69.94	40.14	65.44	58.26
	ΣPUFA		19.05	15.79	15.26	18.65	22.17	26.76	19.64	28.87	11.07	18.68
	Other					11.39	15.34		3.58		17.45	6.34
	Oil content (%)		15.3 ± 0.38	7.4 ± 0.36	20.6 ± 0.41	23.1 ± 0.57	12.6 ± 0.37	11.4 ± 0.75	27.6 ± 0.33	17.2 ± 0.26	11.5 ± 0.43	16.2 ± 0.24

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids, Gc: *Glaucosciadium cordifolium;* Hp: *Heracleum platytaenium;* Hc: *Hippomaranthum cristatum* Hm: *Hippomarathum microcarpum* Lt: *Laser trilobum* Oh: *Opopanax hispitus*; Ps: *Pastinica sativa subsp urens*; Pc: *Peucedonum chyrseum*, Pp: *Prangos platychlaena*; Pu*: Prangos uechtrizii*.

The fruits of *A. visnaga* species contained 23.7±0.49% fatty oil and consisted of 28 different fatty acids. The fatty oils consisted of 5.52% saturated fatty acids, 77.68% monounsaturated fatty acids, and 16.80% polyunsaturated fatty acids. The fatty oil of *A. visnaga* fruits had high levels of petroselinic acid (72.61%), linoleic acid (13.09%), oleic acid (3.91%), and palmitic acid (3.86%).

The fruits of *A. sylvestris* had 17.5±0.53% fatty oil, containing 27 different fatty acids. While 6.82% of the fruit oil was saturated, 39.72% was monounsaturated, 45.58% polyunsaturated fatty acids, and 7.88% was unidentified. The main fatty oil components of the fruits of the species were determined as palmitic acid (3.46%), petroselinic acid (31.21%), oleic acid (7.42%), linoleic acid (16.97%), and docosahexaenoic acid (16.97%).

The fruits of the species *B. radians* contained 16.6±0.38% fatty oil, which consisted of 21 different fatty acid components. The crude oil of the species was composed of 84.74 % monounsaturated, 11.17% polyunsaturated, and 3.49% saturated fatty acids. The main components of

the fatty oil of the species were petroselinic acid (81.0%), linoleic acid (11.06%), and palmitic acid (3.02%).

The fruits of the species *E. tournefortii* had an oil content of 2.1±0.44%, and the composition of the fatty oil consisted of 23 components. The fatty oil of the species consisted of 14.20% saturated, 59.19% monounsaturated, and 26.61% polyunsaturated fatty acids. The main components of the fatty acid composition of *E. tournefortii* fruits were petroselinic acid (45.24%), linoleic acid (24.48%), oleic acid (10.09%), and palmitic acid (9.78%).

The fruits of *E. tenuifolia* subsp *sibthorpiana* subspecies consisted of 9.3±0.28% oil and 24 different fatty acid components. The oil composition of the species comprised 6.94% saturated, 68.25% monounsaturated, and 24.81% polyunsaturated fatty acids. The major components of the fatty oil of the species consisted of 58.45% petroselinic acid, 24.40% linoleic acid, 8.33% oleic acid, and 4.65% palmitic acid.

The fruits of the species *E. trichophylla* contained 4.10±0.18% oil and 8 different fatty acids. The fatty oil of the fruits of the species consisted of 6.14% saturated, 72.45% monounsaturated, and 21.37% polyunsaturated fatty acids. The major fatty acids in the fruits of the species were petroselinic acid (56.28%), linoleic acid (21.37%), oleic acid (14.58%), and palmitic acid (4.60%).

Fruits of the genus *F. cassia* contained 18.8±0.26% oil and consisted of various fatty acids. The total fat content of the species was 16.07% saturated, 54.54% monounsaturated, and 29.38% polyunsaturated fatty acids. The major fatty acid components of the species were petroselinic acid (31.05%), linoleic acid (28.50%), oleic acid (11.80%), palmitoleic acid (9.93%), and palmitic acid (6.38%).

The fruits of the species *F. pauciradiata* contained 14.0±1.06% fatty oil and consisted of 29 different components. The fatty oil of the species consisted of 17.94% saturated, 63.34% monounsaturated, and 18.72% polyunsaturated fatty acids. The main components of *F. pauciradiata* were petroselinic acid (55.58%), linoleic acid (18.39%), caproic acid (9.20%), oleic acid (6.19%), and palmitic acid (4.19%).

The fruits of *F. vulgare* contained 20±0.10% oil and 23 different components. The fatty oil of the species consisted of 17.06% saturated, 67.68% monounsaturated, and 10.05% polyunsaturated fatty acids. The main fatty acids of the fruits of *F. vulgare* are 64.70% petroselinic acid, 11.79% lauric acid, 9.88% linoleic acid, and 3.62% palmitic acid, while the fatty acid with a retention time of 22.917 with a retention time of 5.21% was not identified.

The fruits of *G. cordifolium* contained 15.3±0.38% oil and consisted of 15 different fatty acids. The fixed oil of the species had 11.61% saturated, 69.34% monounsaturated, and 19.05% polyunsaturated fatty acids. The main fatty acid components of the species' fatty oil were petroselinic acid (61.19%), linoleic acid (17.24%), palmitic acid (8.39%), and oleic acid(4.36%).

The fruits of the species *H. cristatum* had an oil content of 7.4±0.36% and contained 23 different fatty acids. The fatty oil of the species consisted of 7.27% saturated, 76.95% monounsaturated, and 15.79% polyunsaturated fatty acids. Petroselinic acid (69.43%), linoleic acid (15.46%), oleic acid (6.51%), and palmitic acid (4.84%) were determined as the main components of the fixed oil of *H. cristatum* species.

The fruits of *H. microcarpum* species contained 20.6±0.41% oil and had 27 different fatty acids. The oil contained 6.96% saturated, 77.78% monounsaturated, and 15.26% polyunsaturated fatty acids. The fruits of *H. microcarpum* species contained a high percentage of petroselinic acid (71.47%) in oil, the other important fatty acids were linoleic acid (15.14%), oleic acid (4.82%) and palmitic acid (4.44%), which made up approximately 95% of the fatty oil of the species.

The fruits of *H. platytaenium* were 23.1±0.57% oil and consisted of 24 different fatty acids. The fatty oil of the species constituted 8.13% saturated, 61.82% monounsaturated, and 18.65% polyunsaturated fatty acids. The fatty oil of the species was found to be 53.73% petroselinic acid, 18.42% linoleic acid, 7.13% oleic acid, and 4.49% palmitic acid. However, the part that had a retention time of 20.095 and a rate of 11.39% could not be detected.

The fruits of the species *L. trilobum* contained 12.6±0.37% oil and 26 different oil components. The fatty oil of the species consisted of 14.40% saturated, 48.08% monounsaturated, and 22.17% polyunsaturated fatty acids. The oil of the fruits of the species contained 34.96% petroselinic acid, 21.09% linoleic acid, 10.40% oleic acid, 7.09% palmitic acid, and 4.10% myristic acid. The fatty acid, that appeared at 14.491 retention time and constitutes 11.80% of oil, could not be identified.

In the fruits of *O. hispidus* species, 11.4±0.75% oil and 22 different fatty acids were determined. In the fatty oil of the species, there were 12.47% saturated, 60.77% monounsaturated, and 26.76 polyunsaturated fatty acids were present. The fatty oil of the species was mainly petroselinic acid (58.94%), linoleic acid (26.16%), and palmitic acid (7.75%).

The fruits of *P. sativa* subsp *urens* contained 27.6±0.33% oil and were composed of 25 different components. The oil of the species was saturated 7.13%, monounsaturated 69.64%, and polyunsaturated 19.64% fatty acids. The major components of the fatty acid composition of *P. sativa* subsp. *urens* fruits were petroselinic acid (63.93%), linoleic acid (19.51%), palmitic acid (5.22%), and oleic acid (4.90%).

The fruits of *P. chryseum* species contained 17.2±0.26% oil and 22 different fatty acids. The fatty oil was determined to be 30.99% saturated, 40.14% monounsaturated, and 28.87% polyunsaturated fatty acids. The main components of the fatty oil of the species are linoleic acid (27.31%), tridecanoic acid (22.66%), oleic acid (21.35%), petroselinic acid (17.29%), and palmitic acid (5.41%).

The fruits of species *P. platychlaena* contained 11.5±0.43% fatty oil and 22 different fatty acids. The oil of the species had 65.44% monounsaturated, 11.07% polyunsaturated, and 6.03% saturated fatty acids. Petroselinic acid (61.35%) and linoleic acid (10.93%) were the major fatty acid components in the oil of the fruits of the species. However, the fatty acid which accounts for 17.45% of the oil and has a retention time of 14.295, wasn't identified.

The fruits of the species *P. uechtritzii* had a fatty oil content of 16.2±0.24% and consisted of 26 different components. The oil of the fruits contained monounsaturated 58.26% monounsaturated, 16.72% saturated, and 18.68% polyunsaturated fatty acids. The main components of the fatty oil of the species were petroselinic acid (50.80%), linoleic acid (18.42%), capric acid(6.92%), oleic acid (5.95%), and palmitic acid (4.50%).

4. Discussion

According to the Red Data Book of Turkish Plants, the genera *Angelica, Ferulago,* and *Prangos* are used for medicinal purposes. This is the first study describing the crude fat content and fatty acid composition of the fruits of the taxa *E. tournefortii, E. tenuifolia* subsp *sibthorpiana, E. trichophylla*, *F. cassia, F. pauciradiata*, and *G. cordifolium*. Endemic taxa also include *E. trichophylla, F. cassia, F. pauciradiata, H. platytaenium, P. chryseum, P. platychlaena,* and *P. uechtritzii.*

In an earlier study the percentage of fatty oil in the fruits of *A. visnaga* species was determined to be 12.6% containing 74.95% petroselinic acid, 16.68% linoleic acid, and 4.92% palmitic acid (Nguyen et al. 2015). Another study reported that the fruits of *A. visnaga* contain 7.2% fatty oil, composed mainly of petroselinic acid (76.10%), linoleic acid (15.60%), and palmitic acid (4.58%) (Houachri et al., 2017). Grindley (1950) stated that the fatty oil of *A. visnaga* fruits contains 5% palmitic acid, 50% petroselinic acid, 32% oleic acid, and 13% linoleic acid. The fatty oil ratio of *A. visnaga* fruits, in our study, is higher than the values compared to the previous studies, while the fatty acid composition is similar to the values available in the literature.

While the oil ratio of *A. sylvestris* was similar to Placek (1963) (17.3%), Kleiman and Spencer (1982) reported that the fruits of the species contained 32.2% fatty oil. As previously reported by other researchers, the major oil components of the species were determined as petroselinic acid (19.6-42.1%), oleic acid (32.8-18.6), and linoleic acid (33.2%) (Placek, 1963; Kleiman and Spencer, 1982). In contrast to the researchers, palmitic acid (3.46%) and docosahexaenoic acid (16.97%) were among the major components of fatty oil in our study.

Kleiman and Spencer (1982) determined the fatty oil rate of the species to be 41.5% in their study of *B. radians*. Comparison of our study with that of Kleiman and Spencer (1982) revealed a high rate of petroselinic and oleic fatty acids and a low rate of oleic and palmitic fatty acids.

Bagci (2007) reported that the fruits of the species *F. pauciradiata* contain 19.7% oil, and the major components of the oil are petroselinic acid (44.0%), linoleic acid (26.1%) and palmitic acid (7.13%). On the one hand, in contrast to the research, caproic (9.20%) and oleic (6.19%) fatty acids were determined in our study; and on the other hand, the results were similar to Bagci's study.

The oil content of *F. vulgare* fruits has been reported to vary from 9.2 to 23% (Placek, 1963; Kleiman and Spencer, 1982; Bahmani et. al., 2021). In terms of the oil ratio, our study was within the range found in previous studies. Similar to previous studies on the components of the oil of the species, the main components were petroselinic acid (62.08-89.8%) (Placek, 1963; Kleiman and Spencer, 1982;

Charvet et al., 1991; Agarwal et al., 2018), palmitic acid (0.3-4.1%) (Kleiman and Spencer, 1982; Bahmani et al., 2021), and linoleic acid (3.6-39%) (Placek, 1963; Kleiman and Spencer, 1982; Agarwal et al., 2018; Bahmani et al., 2021). In contrast to our findings, Bahmani et al. (2021) found that the main components of oleic acid (52-64%), stearic acid (1.3-2.4%), myristic acid (0.35-1.07%), and according to Agarwal et al., (2018) 10-Nonadecanone (4.70-22.80%). Contrary to the researchers, the in present study, the fruits of the species were found to be 11.79% lauric acid.

Previous studies reported oil content of *H. platytaenium* ranged from 11.2 to 19.0% (Placek, 1963; Kleiman and Spencer, 1982; Bagci, 2007; Kucukkboyaci et al., 2016). The results of our study were partially higher than those of the researchers. The difference may be due to the filling rate of the seeds of the species as well as ecological conditions. Our results on the main components of the oil were similar to previous studies reported by different researchers, which consisted of petroselinic, linoleic, and palmitic acids (Placek, 1963; Kleiman and Spencer, 1982; Bagci, 2007; Kucukboyaci et al., 2016).

While Keiman and Spencer (1982) determined the oil ratio of the species as 8.6% in studies as interest in *H. cristatum*, Kucukboyaci et al. (2016) reported that the fruits of the species contain 15.8% fatty oil. Similar to our results with the researchers, the studies on the oil content of the species found palmitic acid (4.3-7.1%), linoleic acid (13.2-19.8%), oleic acid (4.2-74.84%), and petroselinic acid (54.4-72.2) as important components of the oil (Kleiman & Spencer, 1982; Ozturk et al., 2014; Kucukboyaci et al., 2016).

Kleiman and Spencer (1982) determined the oil rate of *H. microcarpum* as 14.1% and reported that the main components were 15.0% palmitic acid, 6.5% petroselinic acid, 20.5% oleic acid, 37.8% linoleic acid. Another research reported that the *H. microcarpum* species' fruits had an oil content of 9.0%, with the main fatty acid constituents being palmitic acid 13.5%, stearic acid 8.4%, petroselenic acid 7.5%, oleic acid 26.5%, and linoleic acid 36.9% (Kucukboyaci et al. 2016). Although the fatty acid content of the species in our research was partially similar to other studies, the oil ratio was higher than the values reported by other researchers.

According to Parlatan et al. (2009), fatty acids of *L. trilobum* fruits consist of caproic acid (59.01-69.27%), myristic acid (1.77-3.16%), lignoceric acid (1.75-8.83%), and myristoleic acid (3-01-31.09%). The results of our study are very different from those of Parlatan et al. (2009). While Kleiman and Spencer (1982) determined the oil ratio of fruits of the *O. hispidus* taxon as 15.6%, the main components of the oil were 6.1% palmitic acid, 40% petroselinic acid, 16% oleic acid, and 35.2% linoleic acid. The results of our study are in agreement with those of Kleiman and Spencer (1982).

In the fruits of *P. sativa* subsp. *urens* oil content was determined to be 18.1% according to Kleiman and Spencer (1982) and 17.3% by Placek (1963). In our study, the fat ratio was higher than in the study of the researchers. The studies conducted on oil components mainly contained high levels of petroselinic fatty acid (46.0-60.1%), similar to our results. In addition, palmitic, oleic, and linoleic fatty acids are among the important components of fatty acids (Placek, 1963; Kleiman and Spencer, 1982).

As reported by Akpinar et al. (2012), *P. chryseum* species occurred at 16.0% palmitic acid, 50.89% oleic acid, and 27.79% linoleic acid. The oil contained 14.82% saturated, 52.58% unsaturated, and 31.66% polyunsaturated fatty acids. In addition, the fruits of the species in our study contained tetradecanoic acid and petroselinic fatty acids.

Bagci (2007) reported that the fruits of *P. platychlaena* species contain 18.7% oil as well as the main components of petroselinic and linoleic acids. Our results were similar to the results of the researcher.

This species reported that the oil content of *P. uechtritzii* varies from 10.11 to 22.8% (Bagci, 2007; Ghafoor et al., 2019). Similar to our results in studies on fatty acids of the species, the main components were palmitic acid, petroselinic acid, oleic acid, and linoleic acid (Bagcıcı, 2007; Ghafoor et al., 2019). Our study was similar to the results of these researchers.

When the study was evaluated, 19 different taxa belonging to the Apiaceae family were significantly rich in unsaturated fatty acids. It was determined that the fatty acid contents of the taxa were different; however, petroselinic fatty acid, oleic, and linoleic fatty acids were the main fatty acid components. Researchers have reported that the major fatty acid of Umbelliferae seeds is *cis* 6-octadecenoic or petroselinic fatty acid (Kleiman and Spencer, 1982; Reiter et al. 1998b; Bagci 2007). Petrocelinic fatty acid (18:1 *cis*6) differs from oleic acid (18:1 *cis* 9) in the way that the double bond is attached in terms of position (Cahoon and Ohlrogge 1994). The degradation of petroselinic fatty acid (C18:1 (6c)), has yielded lauric and adipic acids as important oleochemical raw materials (Reiter et al.

1998b). Moreover, petroselinic fatty acid, oleic (C18:1 (9c)), and *cis*-vaccenic fatty acids (C18:1 (11c)) were found as isomers in the fruits of Umbelliferae. Reiter et al. (1998b) reported that petroselenic fatty acids in fennel, coriander, and cumin fruits contained stearic, petroselinic, oleic, and *cis*-vaccenic fatty acids, respectively. As a matter of fact, similar to Reiter et al. (1998b), the fatty acid order of all species in our study was determined by stearic, petroselinic, oleic, and *cis*-vaccenic fatty acids. Oil plants' fatty acid composition is not steady and can change under the influence of various physiological, ecological, and cultural factors. The fatty acid composition changes depending on the species and is open to various internal and external effects (Baydar and Turgut 1988). The fatty acids in many oil plants are sensitive to various climatic conditions, especially temperature. Besides, the position of fruit formations on the plant causes a large variation in terms of fatty acids within the plant. Furthermore, after fertilization, there may be a continuous variation in fatty acids during the different developmental periods of the seed. In particular, the composition of C18 fatty acids is highly sensitive to environmental effects, especially with the temperature (Pleines and Friedt 1989); also been stated that there were cytoplasmic and maternal effects other than the effects of the nuclear genes of the embryo on genetic control (Pleines and Friedt 1989). The differences between the research results and the species compared with other studies are probably due to the light, temperature, soil type, and plant nutrients (Rahmatalla et al., 1998) as well as the differences in the climatic and geographical conditions in which the plants are grown.

5. Conclusion

In the analysis of the taxa included in the study, it was grıp found that the fatty acid ratio and composition were. However, in general, the taxa were found to have a high proportion of unsaturated fatty acids. The fatty acid consisted of fatty acids of the C18 group. Consequently, considering the species considered in the study can be used as oil sources in various fields in the coming years.

Acknowledgements

The authors are grateful to Prof. Dr. Hasan ÖZÇELİK from Süleyman Demirel University Biology Department for identifying the plants, to TUBİTAK (Türkiye Bilimsel ve Teknolojik Araştırma Kurumu, grant number: 113O284) for material support.

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Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

ISSN: 1308-7576 e-ISSN: 1308-7584

Research Article

Characteristic Features of Kolludere Valley (Bitlis-Hizan) Honey

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Article Info

Received: 30.05.2023
Accepted: 13.12.2023
Online published: 15.03.2024
DOI: 10.29133/yyutbd.1306890

Keywords

Bee flora,
Beekeeping,
Hizan,
Honey,
Pollen

Abstract: This study was carried out to reveal the characteristic features of honey produced in the Kolludere Valley, which is located within the borders of the Hizan district of Bitlis province. This area is isolated as there are no seasonal migratory beekeepers. In 2022, a flora study was carried out in the area and 133 plant taxa belonging to 19 families from which bees receive nectar or pollen were identified. 23 of these taxa are endemic. Content analysis of honey samples taken from the study area was carried out. Proline value, which is an important parameter of honey quality, was determined as 809.41 mg kg^{-1}, diastase number 28.9, HMF 2.9 mg kg^{-1}, and sucrose 0.2 g/100g. All other parameters (humidity, acidity, pH, fructose+glucose, fructose/glucose, saccharose, maltose, electrical conductivity) were also met standart according to the values of the Food Codex Honey Communiqué and European Union Standards. In addition, pollen analysis of honey samples was made and the data were compared with the flora. In honey samples, pollen is generally minor or rare, and pollen of a dominant taxon was found in only one sample. For this reason, most of the honey produced in the Kolludere Valley was evaluated as flower honey.

To Cite: Demir, I, Keser, A M, Altunoğlu, M K, Akpınar, S, Akdoğan, G E, 2024. Characteristic Features of Kolludere Valley (Bitlis-Hizan) Honey. *Yuzuncu Yil University Journal of Agricultural Sciences*, 34(1): 24-35. DOI: https://doi.org/10.29133/yyutbd.1306890

1. Introduction

With the rapidly increasing world population, it is getting harder to reach clean and safe food. Basic foods contaminated with toxic compounds (pesticides, artificial fertilizers, food additives, etc.) are among the most important factors threatening human health (Demir and Ayaz, 2022). One of the foods that has an important place in human nutrition is honey. Honey, with its vitamins, minerals, organic acids, amino acids, enzymes, and compounds such as flavonoids, is an important food that is nutritious, easily digestible, and has protective properties against various diseases (Ozmen and Akalın, 2006; Mutlu et al., 2017). Sugar, moisture, elements, HMF, enzyme, organic acids, vitamins, etc. content, glucose and fructose ratios, pH, acidity, electrical conductivity, etc. parameters determine the value of honey (Acquarone et al., 2007). One of the factors affecting honey quality is plant diversity.

The producers of natural products are decreasing day by day. Therefore, the sustainability of these products is important. Hizan district of Bitlis is one of the best examples of traditional crop cultivation. Honey is an important product for the province of Bitlis. In terms of its geographical structure and vegetation, Hizan is a district that has an advantageous position in terms of beekeeping activities (Ozdemir et al., 2016). Hizan's geographical location and rich plant diversity provide advantages for honey production.

The aim of this study is to reveal the quality of the Kolludere Valley (Hizan) honey with a scientific approach so that it can get the value it deserves. For this purpose, honey flora of the region, pollen analysis, and honey content analysis were revealed.

2. Material and Methods

2.1. Research Area

Kolludere Valley is located in the northeast of Hizan and the west of Gevaş district (Figure 1).

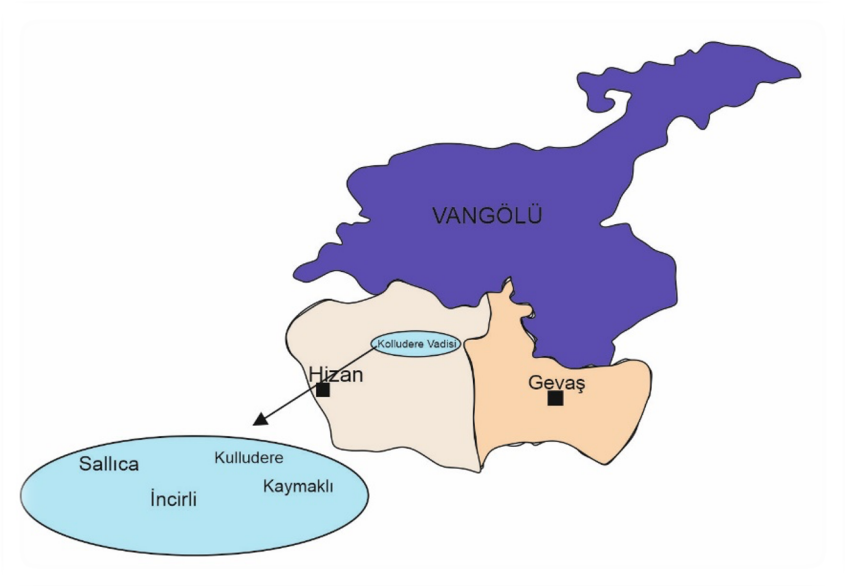

Figure 1. Map of Research area.

2.2. Distinctive Features of Kolludere Valley Honey

Kolludere Valley is a geographically isolated place surrounded by high mountains. In addition, it has a rich plant diversity as it has different habitats such as deep valleys, streams, forest areas, and steppe areas. Because of these properties, it is very suitable for honey production (Behçet, 1994). Beekeeping activity starts in April in the spring and continues until the end of August. Beekeeping starts in June, as the flowering period is a little late in high places. However, the natural comb honey harvest is expected until October.

Beekeepers carry out all their beekeeping activities in Hizan. It is not possible to move it to another place depending on the season. It is also an isolated area as there is no entrance for itinerant beekeepers. Traditional honeycomb is mostly preferred for honey production. (Figure 2). Caucasian honey bees are preferred in Hizan.

Figure 2. An image from the research area.

2.3. Flora study

Between May and August of 2022, a total of 12 field studies were carried out and plant samples visited by bees were collected. The collected samples were identified and preserved in the Herbarium of the Biology Department of the Faculty of Arts and Sciences at Bitlis Eren University. Flora of Türkiye (Davis, 1965 and 1985; Davis et al., 1988) was used to define plant taxa. Families are listed alphabetically, and species according to their importance. The phrase "**END**" has been added to the end of the endemic taxa.

2.4. Honey analysis

Ten honey samples were taken from different locations in the research area. Honey analyses were carried out at Ordu Province Apiculture Research Institute, Food Technology Department Laboratory. The following parameters were determined for each sample.

- Proline (mg kg^{-1})
- Fructose+Glucose (g $100g^{-1}$)
- Fructose/Glucose
- Saccharose (g $100g^{-1}$)
- Maltose (g $100g^{-1}$)
- Humidity %
- pH
- Acidity (meq kg^{-1})
- Electrical conductivity (mS cm^{-1})
- Diastase
- HMF (mg kg^{-1})

Biochemical analyses of honey samples (Proline, electrical conductivity, pH, moisture, acidity, HMF, diastase, fructose, glucose, and maltose) were performed according to the methods specified by Bogdanov et al. (2002).

2.5. Pollen analysis

Pollen analysis is generally used to determine the botanical source of honey (Almeida-Muradian et al., 2005; Bastos et al., 2004). The following method was used for pollen analysis in honey (Sorkun 2008). 10 g of honey is poured into a test tube and diluted with 20 ml of pure water. For this process, a water bath with a temperature of 45 °C is used for 10-15 m. After the mixture is homogenized, the test tube is centrifuged at 3500 rpm for 45 m. After this stage, the particle at the bottom of the tube is transferred to a slide with the help of a needle.

The prepared slides were examined with an Olympus CX21 light microscope. In the diagnosis of pollen, reference pollen microphotographs from various sources (Sawyer, 1981; Pehlivan, 1995;

Sorkun 2008), the pollen slides of the plants collection of Kafkas University Biology Department, and plants collected from the research area were used.

The results obtained from the examinations of the preparations were evaluated according to the criteria used by Louveaux et al. (1978). According to these criteria; 45% or more pollen are dominant, 16-44% secondary, 3-15% minor, and 3% or less trace pollen were accepted. Pollen analyses were carried out by pollen experts from the Department of Biology of Kafkas University.

3. Results and Discussion

3.1. Bee flora of the research area

In the plant samples collected from the research area, 133 taxa belonging to 19 families were determined. 23 of these taxa were endemic (Table 1).

Table 1. Bee plants determined in the research area

Family	Taxon	
Apiaceae	*Eryngium billardieri* F.Delaroche	*Eryngium giganteum* M.Bieb.
	Prangos pabularia Lindl	*Heptaptera anatolica* (Boiss.) Tutin
	E. campestre L.	*Eryngium thyrsoideum* Boiss.
	Ferula orientalis L.	*Heracleum crenatifolium* Boiss. (END)
	Eryngium falcatum F.Delaroche	*Heracleum persicum* Desf
	Ferulago setifolia K.Koch	
Apocynaceae	*Vincetoxicum tmoleum* Boiss	*Vincetoxicum canescens* (Willd.) Decne.
	Vincetoxicum fuscatum subsp. *boissieri* (Kusn.) Browicz	
Asparagaceae	*Muscari comosum* (L.) Mill.	*Ornithogalum narbonense* L.
	M. caucasicum (Griseb.) Baker	*Ornithogalum persicum* Hausskn. ex Bornm
Asteraceae	*Cota wiedemanniana* (Fisch. & C.A.Mey.) Holub	*Cota tinctoria* (L.) J.Gay
	Senecio vernalis Waldst. & Kit	*Gundelia colemerikensis* Fırat (END)
	Echinops orientalis Trautv	*Gundelia tournefortii* var. *armata* Freyn & Sint. (END)
	Echinops ritro L.	*Scorzonera semicana* DC. ((END)
	Onopordum carduchorum Bornm. & Beauverd	*Tanacetum zahlbruckneri* (Náb.) Grierson (END)
	Arctium tomentosum Mill	*Taraxacum kurdiciforme* G.E.Haglund.
	Carlina oligocephala Boiss. & Kotschy	
Boraginaceae	*Anchusa azurea* Mill.	*Onosma armena* DC. (END)
	A. leptophylla subsp. *incana* (Ledeb.) D.F.Chamb. (END)	*Onosma alborosea* subsp. *sanguinolenta* (Vatke) Bornm
	Anchusa leptophylla subsp. *tomentosa* (Boiss.) D.F.Chamb. (END)	*Onosma affinis* Hausskn. ex Riedl (END)
	Alkanna froedinii Rech.f. (END)	*Onosma isaurica* Boiss. & Heldr. (END)
		Onosma rechingeri Riedl (END)
Brassicaceae	*Lepidium latifolium* L.	*Aethionema grandiflorum* Boiss. & Hohen.
	Lepidium draba L.	
Caryophyllaceae	*Gypsophila pallida* Stapf	
Euphorbiaceae	*Euphorbia macroclada* Boiss	*Euphorbia esula* subsp. *tommasiniana* (Bertol.) Kuzmanov
	Euphorbia denticulata Lam	
Fabaceae	*Trifolium badium* subsp. *rytidosemium* var. *rivulare* (Boiss. & Balansa) Hossain	*Astragalus robustus* Bunge
		Astragalus onobrychioides M.Bieb.
	Trifolium hybridum L	*Astragalus amblolepis* Fisch.
	Trifolium nigrescens Viv.	*Astragalus gummifer* Labill
	Trifolium alpestre L	*Astragalus aureus* Willd.
	Trifolium ambiguum M.Bieb.	*Astragalus caspicus* M.Bieb
	Trifolium argutum Sol.	*Astragalus pycnocephalus* Fisch.
	Trifolium arvense L	*Astragalus eriocephalus* Willd
	Trifolium ochroleucum Huds	*Astragalus zahlbruckneri* Hand.-Mazz. (END)
	Trifolium pratense L.	*Astragalus brachycalyx* Fisch. ex Boiss
	Vicia alpestris subsp. *hypoleuca* (Boiss.) P.H.Davis (END)	*Medicago sativa* L.
		Lotus gebelia Vent
	Vicia alpestris Steven	*Onobrychis hajastana* Grossh.
	Ononis spinosa L.	*Onobrychis fallax* Freyn & Sint. ex Freyn

Table 1. Bee plants determined in the research area (contunied)

Family	Taxon	
Fabaceae	*Vicia noeana* Boiss.& Reut. ex Boiss *Vicia sepium* L. *Vicia canescens* Labill. *Vicia cracca* L. *Vicia sativa* L.	*Onobrychis sulphurea* Boiss. & Balansa (END) *Onobrychis montana* DC *Pisum sativum* L
Fagaceae	*Quercus brantii* Lindl *Quercus infectoria* Oliv	*Quercus petraea* subsp. *pinnatiloba* (K.Koch) Menitsky (END)
Hypericaceae	*Hypericum triquetrifolium* Turra *Hypericum lysimachioides* Boiss.& Noë	*Hypericum scabrum* L.
Lamiaceae	*Thymus fedtschenkoi* Ronniger *Thymus kotschyanus* Boiss. & Hohen. *Thymus praecox* Opiz *Thymus haussknechtii* Velen. (END) *Salvia nemorosa* L. *Salvia verticillata* L. *Salvia macrochlamys* Bois	*Stachys annua* (L.) L. *Stachys cretica* L. *Ziziphora capitata* L. *Nepeta italica* L. *Nepeta nuda* L. Lallemantia iberica (M.Bieb.) Fisch. & C.A.Mey *Origanum vulgare* L.
Malvaceae	*Alcea apterocarpa* (Fenzl) Boiss *Alcea kurdica* (Schltdl.) Alef	*Alcea remotiflora* (Boiss. & Heldr.) Alef
Papaveraceae	*Fumaria asepala* Boiss	*Fumaria officinalis* subsp. *cilicica* (Hausskn.) Lidén
Plantaginaceae	*Plantago lanceolata* L.	
Resedaceae	*Reseda lutea* L.	
Rosaceae	*Crataegus pseudoheterophylla* Pojark *Crataegus azarolus* L. *Crataegus orientalis* Pall. ex M.Bieb *Crataegus heterophylloides* Pojark. ex K.I.Chr. (END) *Crataegus meyeri* Pojark. *Crataegus* x *sinaica* Boiss *Crataegus monogyna* Jacq *Rosa hemisphaerica* J. Herrm *Rosa foetida* J.Herrm	*Prunus divaricata* Ledeb. *Rosa canina* L. *Rubus caesius* L. *Cotoneaster nummularius* Fisch. & C.A.Mey *Potentilla anatolica* Peşmen (END) *Potentilla anserina* L. *Potentilla armeniaca* Siegfr. ex Th.Wolf (END) *Potentilla meyeri* Boiss *Potentilla recta* L.
Rubiaceae	*Asperula glomerata* (M.Bieb.) Griseb.	
Scrophulariaceae	*Verbascum orientale* (L.) All *Verbascum oreophilum* K.Koch *Verbascum murbeckianum* Hub.-Mor. (END) *Verbascum kurdicum* Hub.-Mor	*Verbascum agrimoniifolium* (K.Koch) Hub.-Mor *Verbascum songaricum* subsp. *subdecurrens* Hub.-Mor. (END) *Verbascum orientale* (L.) All *Verbascum oreophilum* K.Koch

3.2. Honey Analysis

The amino acid profile can give an idea about the botanical origin of honey samples (Anklam, 1998). Besides proline, honey contains 26 amino acids and their amount depends on the source of the honey (nectar or honey extract). The proline content of honey constantly decreases during storage, so proline is an indicator of honey maturity (Von der Ohe et al., 1991). A minimum value of 180 mg kg^{-1} for proline is internationally accepted (Hermosín et al., 2003).

Many parameters such as proline and HMF content, electrical conductivity, and enzyme activities are important in sugar-added honey. Proline has been proposed as a quality criterion for honey in terms of sugar adulteration (Bogdanov and Martin, 2002). The proline results of the samples taken from different locations were well above the standards. Proline values were in the range of 366-1286, with an average proline value of 809 mg kg^{-1}.

Table 2. Turkish Food Codex Honey Communiqué (Official newspaper; Communiquity No: 2020/7) and European Union standards (Codex Alimentarius, 2001)

Analysis	Limit
Proline (mg kg^{-1})	Minimum 300
Fructose+Glucose (g $100g^{-1}$)	Minimum 60
Fructose/Glucose	0,9-1,4
Saccharose (g $100g^{-1}$)	Maximum 5
Maltose (g $100g^{-1}$)	Maximum 4
Humidity %	Maximum 20
pH	-
Acidity (meq kg^{-1})	Maximum 50
Electrical conductivity (mS/cm)	Maximum 0,8
Diastase	Minimum 8
HMF (mg kg^{-1})	Maximum 40

Table 3. Analysis results of honey samples taken from the research area

Locations	Proline	Fruktoz+Glukoz	Fruktoz/Glukoz	Saccharose	Maltose	Humidity	PH	Acidity	Electrical conductivity	Diastase	HMF
1.Kolludere	640.8	66.6	1.3	N.D*	1.6	15.1	3.8	17.0	0.25	18.5	3.5
2.Sallıca	1048.0	65.7	1.3	N.D*	1.2	17.9	4.2	28.0	0.61	35.0	1.0
3.Kaymaklı	638.5	70.3	1.3	N.D*	1.9	14.4	3.8	18.0	0.24	25.9	2.9
4.İncirli	876.3	70.3	1.3	N.D*	2.0	14.4	3.8	24.0	0.33	36.0	4.5
5.İncirli 2nd station	1286.2	72.8	1.3	N.D*	2.0	13.5	3.8	28.0	0.41	44.1	2.4
6.Kolludere 2nd station	366.7	66.6	1.2	0.9	2.0	16.9	3.8	14.0	0.18	14.3	3.1
Overall Average	809.41	68.7	1.3	0.2	1.8	15.3	3.8	21.5	0.33	28.9	2.9

N.D*: not detected.

In the study, fructose + glucose values of flower honey ranged between 65.7 and 72.8 and it was determined that it was 68.7% on average. Fructose+glucose values were expected to be at least 60 (g $100g^{-1}$). The fructose/glucose ratio was determined as 1.3 on average. The expected value in this parameter should be between 0.9 and 1.4. As the fructose/glucose ratio increases, the tendency to crystallize in honey decreases. Another sugar component value examined was sucrose. Very low sucrose was detected in only two of the samples. The average sucrose was 0.2. This value was found below 5%, which is the highest value determined by the Turkish Food Codex Honey (Official newspaper; Communiquity No: 2020/7) Communique and European Union Standards (Bogdanov et al., 2002). Maltose average was 1.8. The moisture content of honey is the most important criterion in evaluating the maturity and shelf life of honey. The moisture average detected in the samples was 15.3. The standard water ratio should be less than 20%.

The sum of free acids, lactones, and esters determines the total acidity in honey (Kahraman et al., 2010). Free acidity contributes to flavor, provides resistance to microorganisms, increases chemical reactions, antibacterial and antioxidant properties, and also gives some information about the source of honey. The amount of free acid should not be more than 50 meq in 1000 g honey according to the standards. In this study, the average free acid value was 21.5 meq.

Enzyme content is one of the quality criteria of honey. The enzyme invertase, which converts nectar into honey, converts sucrose into glucose and fructose. Diastase enzyme converts starch into small sugars. Although there are different levels in honey depending on the plant source and flora, the diastase rate being more or less than the expected level can give clues during the quality determination in honey. However, the diastase activity may differ depending on the protein amount of the pollen in honey and other substances (Artık, 2004). According to the analysis results, the average diastase number of honey samples was 29. This rate is far above the standards.

The average value of hydroxymethylfurfural (HMF) was 2.9 mg kg^{-1}. HMF is a substance that is formed as a result of heating carbohydrates in honey or storing them in unsuitable environments in terms of heat and is unsuitable for human health. HMF ratio in honey is a maximum 40 mg kg^{-1}. The fact that the HMF value is above this value indicates that the honey may have been stored in a hot environment or subjected to heat treatment and that honey with this feature cannot be sold legally. 5-HMF may be formed by dehydration of sugar at low temperatures under acidic circumstances (Lee and Nagy, 1990). Its concentration rises dramatically as the temperature of the thermal treatment and storage rises (Capuano and Fogliano, 2011). According to Turhan et al. (2008), there is no direct association between the 5-HMF level of honey and its composition.

3.3. Pollen Analysis

As a result, pollens of 43 genera belonging to 20 families were determined. One out of six honey samples were defined as unifloral and five honey samples were defined as polyfloral honey. In a previous study on Bitlis honey, five honey samples were investigated and all of them were found to be multifloral (Kızılpınar Temizer et al., 2020). As a result of pollen analysis of 24 honey samples collected from Siirt province, it was determined that eight of them were unifloral (Gürbüz et al., 2019b). According to honey pollen analysis conducted in Şırnak province, which is one of the regions close to Bitlis province, two of 23 honey samples were determined to be unifloral (Gürbüz et al., 2019a). Unifloral honey was determined at the fifth station. The dominant pollen taxa of fifth station honey is *Taraxacum. Taraxacum* was the most densely pollinated genus. It was present in all samples. It was secondary in the first, second, fourth locations, minor in the sixth location, and a trace in the second location. Especially *Taraxacum kurdiciforme* was the most common species in the region. Pollen analysis was carried out on 67 different honey samples in Hakkari province, and it was determined that *Taraxacum* pollen was dominant in one sample and mostly in minor and trace amounts in the other samples (Sarısu, 2011). According to melissopalynological examinations made on 100 honey samples of Kars province and its district, *Taraxacum* pollen was detected as minor and trace in eight honey samples (Gençay Çelemli et al., 2018). *Taraxacum* (dandelion) honey is a honey produced and characterized in Europe (Oddo and Piro, 2004). Rapid complete granulation with finely ordered crystals, cream to yellow color sometimes with a grayish tint, and an intense pungent ammonia persistent odor and taste are other typical characteristics of Italian dandelion honey (Oddo et al., 1995). Dandelion honey, which is a new record in terms of Turkish monofloral honey, was obtained from Bingöl province, and melissopalynological and chemical content analyses of the honey were performed (Ozenirler et al., 2018). In Bingöl province, one of the eight honey samples was determined to be unifloral and this honey was determined to be *Quercus* honey (Soyer, 2018). The pollens in polyfloral honey were rarely secondary (*Astragalus, Hypericum, Plantago,* and *Taraxacum*), mostly in minor or trace amounts. *Eryngium*, *Taraxacum*, *Astragalus*, *Rosa*, and *Verbascum* were observed in all samples, *Prangos, Arctium, Tragopogon, Vicia, Mentha, Salvia, and Rumex* were observed in five samples, varying in dominant, secondary, minor or trace rates. These results were compatible with the results obtained in the flora determination. According to another study conducted on Bitlis Hizan honey, Rosaceae, Fabaceae, Boraginaceae, and Brassicaceae taxa were observed in all honey samples (Kılıç et al., 2016).

Hypericum was the group that was intensely detected in the research area and preferred by bees. Especially *Hypericum triquetrifolium* was concentrated in the research area. *Hypericum* pollens were detected as secondary in third and sixth locations, and as minor or trace pollen in other locations. In honey research conducted in Hakkari province, *Hypericum* pollen was determined to be dominant in one honey sample (Sarısu, 2011). According to the research conducted by Tosunoğlu et al. (2023) on 44 different honey samples in Gümüşhane province, *Hypericum* pollen was determined as an indicator for altitudes above 1500 m and was also detected as trace, minor, and secondary pollen taxon in 24 pollen samples.

Arctium tomentosum was among the plants that bees showed great interest in the research area. The pollen of this plant was detected in five of the six locations sampled. It was mostly in the minor or trace pollen group. Asteraceae pollen is one of the most abundant pollen taxa in honey samples (Sarısu, 2011; Bakoğlu et al., 2014; Gençay Çelemli et al., 2018; Gürbüz et al., 2019b). Asteraceae pollen also was determined as an indicator for altitudes above 1500 m (Tosunoglu et al., 2023). *Astragalus* (geven) was one of the leading plants in beekeeping. In fact, the name of the honey found in most of Anatolia is

associated with this plant. It was one of the dominant plants of the steppe areas in the search area. *Astragalus* pollen was found in all honey samples. Secondary pollen was detected in two samples (first and third), minor in two of the other locations, and trace pollen in two of them. This means that Kolludere Valley honey was not "geven" honey. It was determined dominantly in honey samples from most places in the Eastern Anatolia region. In the research conducted on five honey samples in Bingöl province, it was determined that two honey samples contained *Astragalus* honey. The taxon whose pollen is most frequently found in Hakkari honey is *Astragalus*, one of the natural plants of the region, and was determined to be the main nectar and pollen source for the local honey (Sarısu, 2011). *Astragalus* honey has antioxidant and antimicrobial properties. The *Astragalus* honey sample from Erzurum has the best antioxidant activity (Küçükaydın et al., 2023).

Verbascum is one of the genus with the most species in the Flora of Türkiye. There are more than 340 species, most of which are endemic in our country, with approximately 360 species worldwide. Endemic species are mostly found in Eastern, Southern, and Central Anatolian regions. Pollen of *Verbascum* was detected in all honey samples taken in the search area. *Verbascum murbeckianum* Hub.-Mor, and *Verbascum songaricum subsp. subdecurrens* Hub.-Purple. are endemic taxa, and also, the type specimen was collected from Bitlis. Therefore, this group has an important place in search area honey. Among the honey samples obtained from Diyarbakır and Bingöl regions which are close to the study area, it was determined that *Verbascum* pollen was dominant at a rate of 97% in mullein honey, while other pollen types (Asteraceae and *Campanula*) were found in trace amounts below 3% (Ozkök, 2019).

Prangos pabularia is one of the plant groups that are found in wide areas in Hizan. According to the statement of beekeepers, it is one of the plants most preferred by bees. Pollens of this plant were detected in five of six samples of Hizan honey. It is generally in the minor or trace pollen group.
Brassicaceae pollen was found in all locations except the fifth location. Most members of this family bloom in early spring and bees do not benefit much in short-term flowering. However, especially the *Lepidium latifolium* is very dense and remains flowering for a long time.

Lamiaceae is one of the most important plant families in terms of beekeeping. However, the pollen of the members of this family was not found in Hizan honey. *Salvia* and *Mentha* pollen were detected in trace or minor amounts in five locations. Ozler (2018) claimed that Fabaceae, Rosaceae, *Eucalyptus*, and *Centaurea* were determined melliferous plants (Ozler, 2018). Sorkun et al. (1989) determined that pollen grains belonging to the families of Asteraceae, Fabaceae, Fagaceae, Myrtaceae, Malvaceae, Brassicaceae, Scrophulariaceae, Lamiaceae, and Oleaceae are the important source of Turkish flower honey (Sorkun et al., 1989).

In the samples from all locations, it was defined as *Taraxacum* honey, because *Taraxacum* was dominant in the fifth sample, and because there was no dominant pollen in the other samples (generally below 45%), the honeys were determined as polyfloral honey (multifloral origin) (Table 4). This shows that the plant biodiversity is high in Hizan, so bees collect pollen from a large number of plants. Since single dominant pollen cannot be determined in polyfloral honey, these honeys are generally named according to the geographical region where they are obtained and offered for sale in this way. Especially in our country, this practice is one of the most important criteria in determining the price of honey.

Honey pollen analysis can be used to determine the botanical origin of honey. This study, which was carried out in Hizan, was also carried out for this purpose. Pollen analysis is also an important parameter in determining the quality of honey. Evaluation of pollen together with other parameters (Flora, proline, HMF, etc.) reveals the characteristic features of Hizan honey.

Honey is classified by pollen analysis. Which plant has the most pollen in honey, is called by the name of that plant (Sorkun, 1985). According to the results of the pollen analysis performed on honey samples from the Kemaliye-Erzincan region, only one of 29 samples was identified as unifloral (Yurtsever, 2004). As a result of the pollen analysis study conducted in the Antalya region, Apiaceae, *Raphanus raphanistrum*, *Cirsium*, *Eucalyptus*, *Plantago,* and *Ulmus* pollens were determined as dominant (Silici, 1995). In their pollen analysis in the Rize region, they found *Castanea sativa* pollen to be dominant (Sorkun et. al., 1989). In the study conducted in Bingöl province, 46.14% of the pollen was composed of *Astragalus,* and the others were composed of *Thymus*, *Tribulus*, and *Lamium* (Bakoğlu et al., 2004).

Table 4. Pollen analysis results of honey samples

		Stations					
		1	2	3	4	5	6
Family	Genus	200	200	200	200	200	200
Amaryllidaceae	*Colchicum*				1		
Apiaceae	*Daucus*	1		2	1		
	Eryngium	9	3	10	5	1	3
	Peucedanum			1	1		
	Prangos	3	20	10		9	12
Asparagaceae	*Muscari*			1			
Asteraceae	*Anthemis*				2		
	Artemisia				1		
	Arctium		15	3	23	8	19
	Bellis		1	1	7		4
	Carduus				6	2	7
	Cichorium	2			1	1	
	Echinops		5	1			
	Scorzonera		9		14		
	Taraxacum	40	36	3	44	120	14
	Tragopogon	8	15		11	8	1
Betulaceae	*Corylus*				5		
Boraginaceae	*Echium*			1		1	
	Onosma	2		1			
Brassicaceae	*Brassica*	4	3	15	3		14
Campanulaceae	*Campanula*		4		2	1	
Euphorbiaceae	*Euphorbia*						1
Fabaceae	*Astragalus*	57	1	40	16	3	13
	Melilotus	5		9	10	1	
	Medicago		16			2	22
	Onobrychis		3	9			
	Vicia	10	1	2	2		1
	Trifolium	1	7		4		1
Hypericaceae	*Hypericum*		13	42	6		45
Lamiaceae	*Mentha*		3	2	1	2	3
	Lamium	1				1	4
	Thymus			6			
	Salvia		4	6	10	3	7
Papaveraceae	*Papaver*				3		
Plantaginaceae	*Plantago*	4	24			32	7
Poaceae	*Poa*	1		1	1		
Poligonaceae	*Rumex*	1	5	8	2		1
Ranunculaceae	*Thalictrum*		4				
Rosaceae	*Rosa*	5	3	13	3	1	2
	Malus		3			1	12
	Potentilla	20			1		2
Scrophulariaceae	*Verbascum*	25	2	12	10	3	5
Urticaceae	*Urtica*	1		1	4		

Conclusion

Field studies and honey analyses (chemical, pollen) conducted in the Kolludere Valley, where intensive beekeeping activities are carried out, show that this place has suitable conditions in terms of beekeeping. The rich flora affects the quality of honey in a very important way (Karakaya et al., 2023). The suitability of values such as proline, diastase, and HMF, which determine the quality of honey, reveals the honey quality of the region. When the results obtained are compared with the results of the provinces that obtained geographical indication certificates in the Eastern Anatolia Region, it was determined that better results were obtained. Thus, the results obtained in this scientific study support the necessity of applying for geographical indication of Hizan Honey and its usability for situations such as the promotion and marketing of honey.

Acknowledgements

We would like to thank DAKA (Eastern Anatolia Development Agency) for financially supporting this study.

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Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

ISSN: 1308-7576 e-ISSN: 1308-7584

Research Article

Pomological and Biochemical Characteristics of Local Pomegranate Genotypes of Kahta (Adıyaman) Region

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Article Info

Received: 07.06.2023
Accepted: 08.12.2023
Online published: 15.04.2024
DOI: 10.29133/yyutbd.1311018

Keywords

Acidity,
Fruit size,
Phenolic compounds,
SSC

Abstract: In the study carried out to determine the pomological characteristics of local pomegranate genotypes grown in Kahta district center and Bostanli, Eceler, Balli, Kilisk, Sarica, and Narince villages of Adıyaman province, one orchard belonging to a grower in each region and 1 genotype in each orchard were determined. 10 fruits in each genotype were harvested, and pomological measurements and biochemical analyses were performed. The largest fruit was obtained with the Sarıca genotype and Narince was the genotype with the smallest fruit. In genotypes, the fruit weight was between 196.300-328.909 g, the fruit length 61.528-72.801 mm, and the fruit width between 73.047-86.613 mm. Total aril weight was between 94.144-203.567 g and the fruit volume was between 188.333-327.000. The Sarıca genotype had the highest juice volume and the lowest juice ratio was recorded in the Eceler genotype. Calyx length was longer in the Sarıca genotype and the highest values in terms of calyx radius were recorded with the Kilisk genotype. The Eceler genotype had thicker shells and the Narince genotype had thinner shells. The number of chambers in the genotypes was between 5 and 6. There were significant differences between genotypes in terms of fruit skin and aril color. The soluble solids content (SSC) in genotypes was determined between 12.011-17.267, pH was 3.583-4.073 and total acidity (TA) was 0.736-1.489%. Phenolic compounds such as protocatechuic acid, rutin, gallic acid, chlorogenic acid, epicatechin, ferulic acid, floridzin, vanillic acid, hydroxycinnamic acid, catechin, caffeic acid, syringic acid, and *p*-coumaric acid were detected in pomegranate fruit, and rutin was phenolic compound with the highest concentration.

To Cite Alan, D, Ağlar, E, Küçüker, E, Tekin, O, Akbal, R, 2023. Pomological and Biochemical Characteristics of Local Pomegranate Genotypes of Kahta (Adıyaman) Region. *Yuzuncu Yil University Journal of Agricultural Sciences*, 34(1): 36-43.
DOI: https://doi.org/10.29133/yyutbd.1311018

1. Introduction

The changing climatic conditions, the nutritional problem of the increasing world population, and the problems related to food supply reveal the fact that the most important natural resource of the current century is genetic resources and make it necessary to preserve these resources and turn them into benefits. Türkiye is one of the few countries in the world in terms of plant genetic resources, which allows the cultivation of most horticultural crops due to favorable ecological conditions, being on trade

routes and hosting many civilizations since the first ages of history (Agaoglu et al., 1995). Improving the use of plant genetic resources for food and agriculture can be achieved by determining all the properties of the material and bringing those with superior characteristics into breeding (Sehirali and Ozgen, 1987). The selection of the most suitable types and varieties considering the production purpose with selection breeding is one of the requirements of rational fruit growing (Guleryuz, 1977). Pomegranate cultivation has been carried out for more than 7 thousand years in Anatolia (Ozbek, 1977), which is among the homeland of pomegranate and has extremely suitable ecological conditions for its cultivation (Kaygisiz, 2009).

Pomegranate, which is known as a "super fruit" in the global functional food industry due to its medicinal uses (Mertens et al., 2006) has been grown as a hedge plant and border tree in Türkiye for many years while providing a good profit to its producer (Onur and Kaska, 1979; Onur, 1983) and the understanding of its health benefits (Lansky et al., 1998; Al-Maiman and Ahmad, 2002; Fischer et al., 2011) has increased day by day cultivation in the form of orchard. With this increase, Türkiye has become one of the leading countries in world pomegranate production. Pomegranate production in Türkiye is concentrated in the Mediterranean, Aegean, and Southeastern Anatolia regions (Anonymous, 2018).

The southeastern Anatolia Region, where continental climate prevails, realizes 10% of Türkiye's pomegranate production with pomegranate cultivation in microclimatic areas. Adıyaman province, which enables the cultivation of subtropical climate fruits such as pistachio, olive, fig, persimmon, and pomegranate, as well as many temperate climate fruit species with its suitable climate characteristics, ranks 11th in pomegranate production in Türkiye. In the region, which has an important pomegranate potential, cultivation has been carried out with traditional habits and local varieties (Katırbaşı) until recent years, but with the increase in the added value of pomegranate to the people of the region, more modern cultivation has started with standard varieties. However, it should not be ignored that local varieties that have been cultivated since ancient times are genetically important. There are many varieties and types in Türkiye, which is one of the homelands of pomegranate. Many studies have been carried out in different parts of the country to reveal the characteristics of these varieties and types and to benefit genetically. As a result of these selection studies, many pomegranate genotypes were determined. However, no study has been performed in terms of pomegranate selection in the Adıyaman region. Our study, it was aimed to determine the pomological and biochemical characteristics of local pomegranate genotypes grown in and to select the promising ones among these genotypes. The study is important as it is a guide presented to researchers and producers in the process of standardizing promising genotypes and expanding their commercial production.

2. Materials and Methods

As plant material in the study that was carried out in 2022, the genotypes of local pomegranate cultivars grown in Adıyaman province, Kahta district center, and Bostanlı, Eceler, Ballı, Kilisk, Sarıca, and Narince villages were used. Within the scope of the study, an orchard belonging to a grower was determined in each region. In line with the statements of the owner of the orchard, one genotype was determined by considering the fruit quality characteristics and the fact that the orchard was established with a single variety and it is a local variety that has been grown in the region for years. During the harvest period, 10 fruits of each genotype were harvested from the trees and transferred to the Van Yuzuncu Yil University, Horticulture Department laboratory, and pomological measurements and biochemical analyses were performed with the following methods.

2.1. Fruit physical characteristics

Fruit weight and peel weight were determined by weighing 5 randomly selected fruits with a scale sensitive to 0.01 grams and taking their averages. Fruit width was determined by measuring the diameter of the equatorial region in 5 fruits, and the fruit length was determined by measuring the distance between the stem part and the lower part of the calyx with a 0.01 mm digital caliper. The calyx length, calyx radius, and shell thickness of the fruit were determined by measuring with a digital caliper sensitive to 0.01 mm and averaging them (Onur, 1983). The juice volume and pulp were determined by removing the juice from 5 fruits and putting them in the measuring cylinder, the juice volume in ml, and the remaining fruit pulp was weighed with a scale sensitive to 0.01 g, and the pulp weights were

determined as g. The arils of five fruit were removed and each of them was weighed separately and the total aril weight was determined by taking the average. The number of upper and lower chambers was determined by counting the upper and lower chambers separately in 5 fruits. The ease of the husking was determined as easy, medium, and hard by husking. Fruit skin color and aril color were determined in terms of L^*, a^*, and b^*. It was determined in 5 fruits and their arils by measuring by means of a colorimeter (Minolta, model CR-400, Tokyo, Japan) from points determined at 2 opposite poles of the equatorial part of the fruit and the arils. According to the prepared scale, the a^* value is expressed as redness-greenness, and the b^* value is expressed as yellowness-blueness. The chroma value = (a^*2+b^*2)1/2 and the hue angle value will be determined by the formula $h^o= \tan^{-1} \times b^*/a^*$ (McGuire, 1992).

2.2. Biochemical characteristics

SSC, titratable acidity, and pH: the fruit juices were obtained by extracting the arils of the five fruits, squeezing them with a blender, and passing them through cheesecloth. By taking enough of the obtained fruit juice sample, SSC was determined by digital refractometer (PAL-1, Atago, USA) and expressed as %. To determine the titratable acidity, the obtained juice was taken from the sample, 10 mL of the sample was diluted with 10 mL of distilled water and titrated with 0.1 mol L^{-1} (N) sodium hydroxide (NaOH) until the pH reached 8.1, and the amount of NaOH consumed in the titration was taken. It was expressed in terms of citric acid (g citric acid 100 mL^{-1}) based on. The pH was determined in the juice obtained by measuring with a pH meter.

Individual phenolic compounds: Individual phenolic compounds were analyzed as follows. Homogeneously selected fresh fruit samples were weighed as 1 gram and extracted with methyl alcohol (5 mL) in a test tube for 6 hours. The extract was analyzed by high-pressure liquid chromatography (HPLC) (Perkin-Elmer series 200, Norwalk, USA). The HPLC system was equipped with a UV detector (Series 200, UV/Vis detector) and a quaternary solvent dispersion system (Series 200, analytical pump) and used at 280 nm. Analytes were separated with a Phenomenex Kromasil (Phenomenex, Torrance, USA) 100A C18 (250 mm x 4.60 mm, 5 µm) column. The clone temperature was maintained at 26°C using a water bath (Wisebath, WB-22, Daihan Scientific, Seoul, Korea). The mobile phase was formed from acetonitrile (A) containing water and 2.5% formic acid (B). The mobile phase flow rate was maintained at 1 mL per minute, and the 20 µL of sample was injected and the results of the peak areas obtained were expressed as mg 100 g^{-1}.

2.3. Statistical analysis

The data obtained in the study were evaluated according to the significance level of $p<0.05$ by analysis of variance according to the randomized plot design. Statistics; Expressed as mean±SH. Duncan multiple comparison test was used to determine the differences between genotypes. “IBM SPSS v23.0” statistical package program was used in the calculations (SPSS. 2023).

3. Results and Discussion

3.1. Pomological characteristics

There were very significant differences between genotypes in fruit size. The largest fruit was obtained with the Sarıca genotype, and Narince was the genotype with the smallest fruit. In genotypes, fruit weight was between 196.300-328.909 g, fruit length was 61.528-72.801 mm and fruit width was between 73.047-86.613 mm in proportion to fruit size while total aril weight was 94.144-203.567, fruit volume was between 188.333-327.000 (Table 1). When compared with similar studies, it will be seen that the genotypes have medium-sized fruit with fruit weights varying between 196.300 and 328.909 g. Gundogdu (2006), obtained similar findings (fruit weight: 197-328 g) in his thesis study he conducted to determine the characteristics of local pomegranate genotypes in the Pervari (Siirt) region. In studies conducted with local varieties, it has been reported that the fruit weight of pomegranate was 208-553 g (Ercan et al., 1992), 250-461 g (Polat et al., 1999), 192-388 g (Yildiz et al., 2003), 131-337 g (Muradoglu et al., 2006), 157.4-402.3 g (Ak et al., 2006) and 161.45-302.35 g (Gundogdu et al., 2010) and in

standard varieties, the fruit size was 374.9 g (Turkmen and Eksi, 2010) and 251.01-530.25 g (Gundogdu et al., 2015).

Sarıca genotype with the largest fruits had the highest juice volume, and the lowest fruit juice ratio was recorded in the Eceler genotype. Significant differences occurred between genotypes in terms of calyx sizes. While the calyx length was longer in the Sarıca genotype, which had the largest fruits, the highest values in terms of calyx radius were recorded with the Kilisk genotype. The significant differences were detected in terms of shell thickness and shell weight. The Eceler genotype had the thickest shells, it was observed that the shells were thinner in the Narince genotype. The shell weight was higher with the Bostanlı genotype, and the lowest shell weight was recorded with the Narince genotype. The number of chambers in the genotypes varied between 5 and 6, and the seed had a hard and medium hard structure (Table 1). Gundogdu (2006), determined that in his thesis study conducted with the local pomegranate genotypes of the Pervari (Siirt) region, the amount of the juice was 76-170 ml, the fruit density was 0.78-2.05 g cm^{-3}, the total arils weight was 31.7-52.6 g, aril yield was 51.6-66.4%, calyx length was 20.1-24.8 mm, calyx radius was between 11.2 and 15.3 mm, shell thickness was between 2.2 and 4.5 mm. In the study conducted by Kılıc (2014), it was determined that the fruit volume was 275.00-731.67 ml, juice amount was 81-98 ml, fruit density was 0.868-0.974 g cm^{-3}, total arils weight was 141.33-361.33 g, calyx length was 13.47-22.49 mm and calyx radius was 10.19-17.03 mm. Gundogdu et al. (2015) who determined the characteristics of standard pomegranate cultivars, reported that in pomegranate varieties such as Hicaznarı, Silifke aşısı, Katırbaşı, 33N23-Çevlik, 01N04, fellahyemez, 33N34, İzmir26, İzmir23, İzmir1513, 33N24 and Kuşnarı, the fruit volume was 230.00-542.50 cm^3, the fruit juice amount was 106.66-186.00 ml and the fruit density was between 0.92-1.19 g cm^{-3} values. In another study (Ozturk et al., 2019) determined that total arils weight was 84-400 g, 100 arils weight was 25.3-49.5 g, aril yield was 40.5-78.4%, juice amount was 78-296 ml, calyx length was 12.1-17.9 mm and calyx radius was between 9.15 and 22.50 mm.

Table 1. Pomological characteristics of pomegranate genotypes

Genotype	Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	Total arils weight (g)	Fruit volume	Juice volume
Kahta	267.632±41.991^{ab}	68.522±3.405^{ab}	83.298±3.599^{ab}	119.203±11.955^{b}	277.444±50.651^{ab}	67.889±6.918^{bcd}
Bostanlı	291.668±11.239^{ab}	70.697±0.641^{a}	83.896±3.060^{ab}	139.704±8.415^{b}	308.444±23.865^{a}	80.333±8.720^{abc}
Eceler	212.012±26.257^{b}	65.747±1.683^{ab}	76.121±1.680^{ab}	91.957±16.173^{b}	220.000±23.540^{ab}	43.111±12.010^{d}
Ballı	248.262±19.409^{ab}	66.100±2.646^{ab}	79.530±1.716^{ab}	154.538±14.704^{ab}	252.000±19.313^{ab}	96.444±10.819^{ab}
Kilisk	214.060±41.730^{b}	64.700±4.386^{ab}	74.139±7.271^{ab}	94.144±28.416^{b}	222.333±44.108^{ab}	53.778±15.602^{cd}
Sarıca	328.909±37.553^{a}	72.801±2.279^{a}	86.613±3.412^{a}	203.567±28.525^{a}	327.000±36.226^{a}	112.111±13.132^{a}
Narince	196.300±11.827^{b}	61.528±1.671^{b}	73.047±2.291^{b}	119.448±10.202^{b}	188.333±13.132^{b}	68.333±5.667^{bcd}
Genotype	**Calyx length (mm)**	**Calyx diameter (mm)**	**Peel thickness (mm)**	**Peel weight (g)**	**Top cubby number**	**Base cubby number**
Kahta	22.126±2.569^{ab}	22.688±2.470^{ab}	4.670±0.476^{a}	131.786±31.789^{ab}	5.778±0.111^{ab}	5.778±0.111^{ab}
Bostanlı	21.251±1.663^{ab}	21.214±1.313^{abc}	4.424±0.612^{a}	141.363±13.910^{a}	5.667±0.333^{ab}	5.667±0.333^{ab}
Eceler	18.067±1.590^{b}	20.366±0.656^{abc}	4.889±0.248^{a}	102.046±15.264^{ab}c	5.222±0.294^{b}	5.222±0.294^{b}
Ballı	18.888±1.041^{b}	17.183±0.480^{cd}	3.093±0.141^{b}	80.747±5.608^{bc}	6.556±0.484^{a}	6.556±0.484^{a}
Kilisk	20.974±0.834^{ab}	23.211±0.702^{a}	4.382±0.499^{a}	109.980±15.034^{abc}	5.222±0.401^{b}	5.222±0.401^{b}
Sarıca	25.179±2.589^{a}	18.870±1.252^{bcd}	3.129±0.157^{b}	115.267±12.596^{abc}	6.556±0.401^{a}	6.556±0.401^{a}
Narince	20.742±1.527^{ab}	16.056±0.994^{d}	2.723±0.436^{b}	67.461±4.723^{c}	5.444±0.401^{ab}	5.444±0.401^{ab}

Means in columns with the same letter do not differ P<0.05.

Significant differences were detected between genotypes in terms of fruit skin and aril color. When the fruit peel L^*, a^*, and b^* values were examined, a^* and b^* values were higher in the Kahta Genotype while the Kilisk genotype had the highest L^* value. The smallest values in L^* a^* and b^* color values were recorded with Narince, Sarıca, and Narince genotypes, respectively. The highest values in terms of hue angle were determined in the Eceler genotype, Bostanli genotype had lower values. The highest chroma values were obtained with the Kilisk Genotype while the lowest values were recorded with the Narince genotype. It was observed that the aril color of the genotypes was very different. Considering a value, which expresses the red color, it can be said that the arils are redder in the Eceler genotype, and the arils have a lighter color in the Narince and Sarıca genotypes (Table 2).

Table 2. Fruit and aril color (L^*, a^*, b^*, chroma, and hue angle) characteristics of pomegranate genotypes

Genotype	Fruit Color				
	L^*	a^*	b^*	Chroma	Hue angle
Kahta	60.268±1.543^{cd}	16.590±3.842^{a}	33.248±1.455^{a}	39.014±0.883^{ab}	64.714±5.475^{a}
Bostanli	63.117±2.844^{bcd}	19.026±9.779^{a}	29.898±4.340^{a}	38.966±1.769^{ab}	58.371±15.780^{a}
Eceler	58.263±0.847^{d}	25.489±1.646^{a}	31.196±0.274^{a}	42.561±1.056^{a}	52.213±2.028^{a}
Balli	67.454±3.639^{abc}	19.852±5.341^{a}	31.063±2.269^{a}	39.130±0.630^{ab}	56.089±6.381^{a}
Kilisk	60.884±1.882^{cd}	13.409±5.520^{a}	32.733±1.086^{a}	36.920±2.334^{b}	68.803±7.748^{a}
Sarıca	70.812±2.102^{a}	9.939±3.044^{a}	36.070±1.518^{a}	38.746±0.420^{ab}	74.278±5.238^{a}
Narince	69.286±2.304^{ab}	9.143±2.328^{a}	36.096±0.586^{a}	40.363±0.845^{ab}	75.899±3.215^{a}
Genotype	**Fruit Arils Color**				
	L^*	a^*	b^*	Chroma	Hue angle
Kahta	45.239±2.903^{ab}	9.606±4.766^{ab}	16.123±0.744^{a}	20.231±2.081^{ab}	62.268±12.217^{abc}
Bostanli	44.061±0.184^{ab}	6.331±2.002^{ab}	14.868±0.728^{ab}	16.489±1.070^{b}	67.639±6.166^{ab}
Eceler	46.127±1.492^{a}	14.092±0.973^{ab}	15.921±1.130^{a}	21.677±0.344^{a}	49.137±3.709^{abc}
Balli	38.213±0.526^{bc}	15.044±1.857^{a}	12.954±0.472^{bc}	20.199±1.072^{ab}	41.376±4.651^{bc}
Kilisk	48.277±2.191^{a}	5.342±3.011^{b}	15.487±0.885^{ab}	17.094±0.614^{b}	71.451±9.868^{a}
Sarıca	41.498±0.786^{abc}	13.910±1.776^{ab}	14.031±0.361^{abc}	20.566±0.986^{ab}	46.614±4.524^{abc}
Narince	35.698±4.084^{c}	15.360±3.650^{a}	11.882±1.235^{c}	20.126±1.811^{ab}	39.650±10.313^{c}

Means in columns with the same letter do not differ P<0.05.

3.2. SSC, TA, and pH

SSC and pH contents showed significant differences between genotypes. The SSC amount in genotypes varied between 12.011 (Kilisk) and 17.267 (Balli and Narince), and the pH was determined between 3.583 (Kilisk) - 4.073 (Bostanlı). There was no difference between genotypes in terms of titratable acidity content. When the studies are examined, it will be seen that the SSC, titratable acidity, and pH contents vary and the cultivar and region used in the studies are effective in the emergence of these results. Generally, it can be said that the SSC ratio in pomegranates varies between 9-19% and the SSC ratio of genotypes in our study was at normal levels (Table 3). In his thesis study carried out by Gundogdu (2006) to determine the characteristics of the local pomegranate genotypes of the Pervari (Siirt) region, the SSC amount was determined as 12.4%-14.9%, pH was 3.60-4.40% and total acidity was 0.55-2.99%. Muradoglu et al. (2006) reported that pH value varied between 2.6-3.8 and total acidity was between 1.5-2.9% in pomegranates in the Hakkari region. In the study conducted by Kılıc (2014) in order to determine the characteristics of the local pomegranate genotypes of the Siverek (Şanlıurfa) region, the SSC amount was determined between 12.64-16.68%, pH was 2.84-3.31%, and total acidity was 0.55-2.99%. In the study conducted by using varieties such as Hicaznarı, Silifke aşısı, Katırbaşı, 33N23-Çevlik, 01N04, fellahyemez, 33N34, İzmir26, İzmir23, İzmir1513, 33N24 and Kuşnarı in order to determine the physicochemical properties of pomegranate cultivars and genotypes, it was determined that SSC amount was between 11.50-14.60%, pH was 3.45-4.71, total acidity was 0.19-1.17% (Gundogdu et al., 2015). In the Artuklu and Kızıltepe districts of Mardin province, the SSC amount in local pomegranates varied between 15.00-18.00%, pH was 2.38-3.49% and total acidity was 0.06-0.69% (Ozturk et al., 2019).

Table 3. SSC, pH, and titratable acid content of pomegranate genotypes

Genotype	SSC (%)	pH	Titratable Acidity (%)
Kahta	16.022±1.626^{ab}	3.731±0.048^{ab}	1.312±0.256^{a}
Bostanlı	14.656±0.323^{b}	4.073±0.247^{a}	0.736±0.330^{a}
Eceler	17.022±0.426^{a}	3.663±0.098^{b}	1.422±0.225^{a}
Ballı	17.256±0.349^{a}	3.620±0.041^{b}	1.604±0.088^{a}
Kilisk	12.011±0.400^{c}	3.583±0.045^{b}	1.489±0.164^{a}
Sarıca	17.000±0.306^{a}	3.636±0.060^{b}	1.387±0.164^{a}
Narince	17.267±0.133^{a}	3.730±0.130^{ab}	1.402±0.412^{a}

Means in columns with the same letter do not differ P<0.05.

3.4. Individual phenolic compounds

Fruits are acceptable as a natural source of antioxidants such as anthocyanins and polyphenols, which can reduce the risk of cancer, heart disease, and stroke (Gilgun-Sherki et al., 2002) and prevent cardiovascular diseases (Cuzzocrea et al., 2001) and asthma (Kirkham and Rahman, 2006). The phenolic compounds such as protocatechuic acid, rutin, gallic acid, chlorogenic acid, epicatechin, ferulic acid, fluoroidzin, vanillic acid, hydroxycinnamic acid, catechin, caffeic acid, syringic acid, and *p*-coumaric acid were detected in pomegranate fruit. However, some of them were not given numerically because they were in very trace amounts. The phenolic compound content generally did not change depending on the genotype, only the protocatechuic acid and gallic acid content changed. Rutin is the phenolic compound with the highest concentration, followed by protocatechuic acid, gallic acid, chlorogenic acid, floridzin, ferrulic acid, and epicatechin, respectively (Table 4). Turgut and Seydim (2013) reported that there were similar phenolic compounds in pomegranate juice, in their study, it was determined 3 hydroxybenzoic acids (gallic, vanillic, and syringic acids), 2 flavanols (epicatechin, catechin), 1 hydroxycinnamic acid (chlorogenic acid), 1 flavanone (floridzin), and 1 flavonol (rutin). In the same study was reported that epicatechin was the dominant phenolic component in all pomegranate juice samples. However, in our study, this phenolic compound had a very low concentration. Poyrazoglu et al. (2002) determined that in raw pomegranate juice, gallic acid was 0.34-30.86 g L^{-1}, protocatechuic acid was 0.12-2.09 g L^{-1}, catechin was 0.13-8.44 g L^{-1}, chlorogenic acid was 0.09-4.72 g L^{-1}, caffeic acid was 0.09-2.89 g L^{-1}, *p*-coumaric acid was 0.04-0.15 g L^{-1}, ferulic acid was 0.01-0.06 g L^{-1}, *q*-coumaric acid was 0.07-0.30 g L^{-1}, floridzin was 0.06-4.93 g L^{-1} and quercetin was 0.23-5.30 g L^{-1}. Pande and Akoh (2009) stated that caffeic acid was 12.3-14.4 mg 100 g^{-1}, *p*-coumaric acid was 6.6-8.1 mg 100 g^{-1}, ferulic acid was 1.3-2.0 mg 100 g^{-1}, catechin was 82.7-101.2 mg 100 g^{-1}, epicatechin was 9.6 -11.7 mg 100 g^{-1} quercetin was 66.7-77.1 mg 100 g^{-1} in pomegranate juice. Swatsitang et al. (1999) reported that the amounts of phenolic compounds found in pomegranate juice are 3.49 mg 100 g^{-1} was gallic acid, 0.39 mg 100 g^{-1} was protocatechuic acid, 4.23 mg 100 g^{-1} was p-hydroxybenzoic acid, 2.16 mg 100 g^{-1} was vanillic acid, 0.24 mg 100 g^{-1} was caffeic acid, 10.01 mg 100 g^{-1} was *p*-coumaric acid 13.95 mg 100 g^{-1} was ferulic acid. On the other hand, Kelebek and Canbas (2010) reported that there were phenolic compounds such as gallic acid (13.95 mg mL^{-1}), protocatesic (4.98 mg mL^{-1}), caffeic acid (6.39 mg mL^{-1}), vanillic acid (2.33 mg mL^{-1}) and *p*-coumaric acid (16.62 mg mL^{-1}) in pomegranate. It is thought that the differences in the results of the study are due to factors such as genetic, environmental, and climatic factors, cultural practices and analysis methods used.

Table 4. Content of individual phenolic compounds of pomegranate genotypes

Phenolic Compounds (mg kg^{-1})	Genotype						
	Kahta	**Bostanli**	**Eceler**	**Balli**	**Kilisk**	**Sarica**	**Narince**
Protocatechuic Acid	1.8097± 0.9106^{a}	0.6520± 0.2176^{ab}	0.8126± 0.1891^{ab}	1.0187± 0.3722^{ab}	0.2184± 0.0514^{b}	0.8335± 0.2327^{ab}	0.7385± 0.4654^{ab}
Rutin	1.8599± 1.5195^{a}	0.0578± 0.0203^{a}	0.6359± 0.3746^{a}	1.3201± 0.4715^{a}	0.1910± 0.0669^{a}	0.6879± 0.2541^{a}	0.7183± 0.5623^{a}
Gallic Acid	0.0532± 0.0210^{b}	0.0935± 0.0329^{ab}	0.2096± 0.0735^{a}	0.0086± 0.0010^{b}	0.0499± 0.0174^{b}	0.0285± 0.0071^{b}	0.1847± 0.0612^{a}
Chlorogenic Acid	0.0097± 0.0051^{a}	0.0035± 0.0026^{a}	0.0169± 0.0123^{a}	0.0260± 0.0115^{a}	0.0048± 0.0022^{a}	0.0097± 0.0047^{a}	0.0022± 0.0006^{a}
Epicatechin	0.0006± 0.0004^{a}	0.0005± 0.0003^{a}	0.0002± 0.0001^{a}	0.0003± 0.0002^{a}	0.0006± 0.0004^{a}	0.0001± 0.0001^{a}	0.0010± 0.0003^{a}
Ferulic Acid	0.0037± 0.0030^{a}	0.0008± 0.0003^{a}	0.0186± 0.0079^{a}	0.0450± 0.0362^{a}	0.0001± 0.0001^{a}	0.0285± 0.0208^{a}	0.0013± 0.0010^{a}
Floridzin	0.0074± 0.0038^{a}	0.0006± 0.0003^{a}	0.0152± 0.0140^{a}	0.0116± 0.0070^{a}	0.0003± 0.0002^{a}	0.0122± 0.0039^{a}	0.0006± 0.0001^{a}

Means in columns with the same letter do not differ P<0.05.

4. Conclusion

Fruit size varied depending on genotype. Larger fruits were harvested with the Sarıca genotype. Narince was the genotype with the smallest fruits. Sarıca genotype had the highest fruit juice volume, and the lowest fruit juice rate was recorded in the Eceler genotype. The Eceler genotype had the thickest shells, but the Narince genotype had thinner shells. The number of chambers in the genotypes varied between 5 and 6, and the nuclei had a hard and medium hard structure. While the a* and b* values were

higher in the Kahta genotype, the Kilis genotype had the highest L* value. The amount of SSCM in the genotypes was between 12.01 (Kilisk) and 17.25 (Ballı and Narince), and the pH was between 2.71 (Narince) and 4.38 (Kilisk). No difference was found between genotypes in terms of titratable acidity content. Phenolic compounds such as protocatechuic acid, routine, gallic acid, chlorogenic acid, epicatechin, ferulic acid, phloridzin, vanillic acid, hydroxycinnamic acid, catechin, caffeic acid, shincinic acid, and p-coumaric acid were detected in pomegranate fruit.

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Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

ISSN: 1308-7576 e-ISSN: 1308-7584

Research Article

A Comparative Study of Soil Fertility in Organic, Semi-Organic, and Conventional Rice Field Farming Systems (Case Study: Nguntoronadi District, Wonogiri, Indonesia)

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Article Info

Received: 18.09.2023
Accepted: 05.11.2023
Online published: 15.03.2024
DOI: 10.29133/yyutbd.1357044

Keywords

Irrigated rice field,
Organic matter,
Soil fertility factor,
Soil fertility index,
Sustainability farming

Abstract: The soil fertility of rice fields is closely related to rice crop production. The research aims to identify soil fertility under different rice field farming systems, find the key factor of soil fertility, and recommend strategies to improve soil fertility based on the key factor. The research was conducted in Nguntoronadi District, Indonesia, on conventional, semi-organic, and organic rice fields. The research was an exploratory descriptive survey through a field survey approach and soil chemistry and physics analysis. Soil sampling was conducted in 12 Land Map Units (LMUs) with three replicates using purposive sampling methods. Observation indicators include soil pH, organic C, total N, C/N ratio, available P, available K, exchangeable Ca, exchangeable Mg, Cation Exchangeable Capacity (CEC), Base Saturation (BS), Aluminum saturation, soil texture, and worm population density representing soil chemical, physical, and biological properties. Soil fertility is determined using Principal Component Analysis (PCA) and scoring based on the category. The research results show that the level of soil fertility under various rice field farming systems was included in the moderate with ranges of 0.53-0.70, and organic farming has the highest soil fertility. The key factors of soil fertility include pH, organic C, available P, available K, Ca-dd, CEC, and Aluminum saturation. The appropriate management direction is the addition of organic fertilizer in the planting period.

To Cite: Mujiyo, M, Naaifah, M I, Suntoro, S, Maro'ah, S M, 2023. A Comparative Study of Soil Fertility in Organic, Semi-Organic, and Conventional Rice Field Farming Systems (Case Study: Nguntoronadi District, Wonogiri, Indonesia). *Yuzuncu Yil University Journal of Agricultural Sciences*, 34(1): 44-61. DOI: https://doi.org/10.29133/yyutbd.1357044

1. Introduction

Soil fertility is essential for sustainable agriculture and ecosystem health. It directly influences plant growth, nutrient availability, water retention, and environmental sustainability. According to Chirila et al.(2013), soil fertility is a complex process in the constant nutrient cycle between organic and inorganic forms, such as plant and animal residues as waste that enter and release nutrients into the soil and then act as fertilizer and a source of energy for the soil. Soil fertility is achieved through the presence of soil organic matter and the contribution of soil macro and microorganisms (Sofo et al., 2020). In terms of rice production, soil fertility is closely related to the productivity of rice fields. Rice production continues to increase to avoid food insecurity and realize national food security (Soegoto and Sumarauw, 2014; Rohman and Maharani, 2017) because the demand for food needs, especially rice, continues to

increase in line with Indonesia's population growth. Good soil fertility will produce good rice for various heritage food product (Azkiyah et al., 2021; Pinandoyo et al., 2023). Increasing agricultural production activities (intensive farming). Adekiya et al. (2018) stated that this is inappropriate because it promotes land degradation. Hailu et al. (2015) stated that continuous management, nutrient loss due to crop transport and erosion events, and leaching are the primary causes of land degradation.

Rice fields are the most significant component of rice production (Arlius et al., 2017; Nurmegawati et al., 2019) and can experience soil fertility degradation due to excessive use of chemical fertilizers and pesticides (Mujiyo et al., 2022). In the research area, farmers and stakeholders use NPK chemical fertilizers such as Urea, TSP, and Phonska to increase essential macronutrients that support the growth and production of rice plants. Based on the data by Pahalvi et al. (2021), Indonesia is one of 13 countries in the world with NPK chemical fertilizer used reaching up to ≥100 kg ha^{-1}. The use of chemical fertilizers tends to have a negative impact on ecological balance (Yolci and Tunçtürk, 2022). Conventional farming uses chemicals during planting, such as fertilizers and pesticides to eradicate pests. The results of Rahman et al. (2020) illustrate that the long-term use of pesticides and chemical fertilizers in the soil in conventional rice field farming showed negative effects. the chemical properties of the soils, such as nitrate, ammonia, SOC, and total N and C compositions, were also significantly decreased. This suggests that the intensive use of pesticides and chemical fertilizers can degrade the biochemical and chemical properties of the soil. Additionally, chemical fertilizers and pesticides produce residue after their use. The resulting residue settles on the ground, evaporates into the air, and is carried away by water flows in irrigation canals. These chemical residues have an impact on contamination, pollute the environment, and increase the potential for soil degradation. The level of land degradation indicates a decrease in soil fertility in various land management systems (Kagabo et al., 2013). Low-energy and low-degradation engineering innovations are needed to increase productivity (Jeon et al., 2021). Appropriate innovations also support the realization of the second goal of the Sustainable Development Goals (SDGs) related to zero hunger by ending hunger, attaining food security, enhancing nutrition, and promoting sustainable agriculture.

Nguntoronadi District, Wonogiri Regency has 1 488 ha of rice fields (Central Bureau of Statistics, 2022) that are managed conventionally, semi-organically, and organically. In the last 5 years based on the data from the Central Bureau of Statistics in 2023, Nguntoronadi District had a harvest area of 2 635.74 ha with a production of 13 803.49 tons, so the average land productivity achieved was around 5.24 tons ha^{-1}. The diverse farming systems of rice fields will affect the level of soil fertility (Sukristiyonubowo et al., 2019), also affect plant secondary metabolites (Khoerunnisa et al., 2022) as an important part of the plant defense system and are currently used as medicine ingredients and food additives and culinary purposes (Azkiyah et al., 2021; Mahendradatta et al., 2021), so an assessment of the soil fertility index is needed. Sukristiyonubowo et al. (2019) stated that organic cultivation has a better soil fertility level than conventional and semi-organic rice fields, especially in the parameters of pH, organic C, total N, available P, and available K with organic rice paddy yields increasing 61% from the previous year. Information on soil fertility status is key to investigating nutrient status, predicting relative soil responses to fertilizer application, and adopting appropriate management strategies (Aytenew and Kibret, 2016). Soil fertility assessment or evaluation is based on nitrogen, phosphorus, and potassium elements, and is affected by soil factors such as soil pH, cation exchangeable capacity, and organic matter content (FAO, 1988; Daksina et al., 2021).

A high level of soil fertility is crucial in rice field farming to improve productivity and strengthen food security, especially in the Nguntoronadi District. Research on soil fertility assessment on a wide range of rice field farming systems in Nguntoronadi District is still limited, so further research is needed to provide information on soil fertility levels in the area with more complete research parameters. The purpose of this research is to measure the degree of soil fertility in various rice field farming systems and to comprehend the impact of land management techniques on soil fertility. Another very important objective is to increase soil fertility at the research site with appropriate land management recommendations based on the key factors of soil fertility. Proper rice field farming advice can then be utilized as a reference for stakeholders to improve farmers' welfare through higher land production and support sustainable integrated agriculture.

2. Material and Methods

2.1. Study area and soil sampling

The research was conducted on conventional, semi-organic, and organic rice fields in Nguntoronadi District, Wonogiri Regency, Central Java Province, Indonesia (Figure 1). The research area has an area of 60.96 km^2 which is geographically located between 7°51'8.71"-7°58'55.90" LS and 110°53'56.59"-111°2'58.78" BT at an altitude of 173 - 410 meters above sea level with regional characteristics in the form of hills and mountains. Land use in Nguntoronadi District includes 1 488 ha of rice fields, 2 213 ha of moorland, 634 ha of state forest, 90 ha of smallholder plantations, 3 379 ha of settlements, and 237 ha of other land uses (Central Bureau of Statistics, 2022).

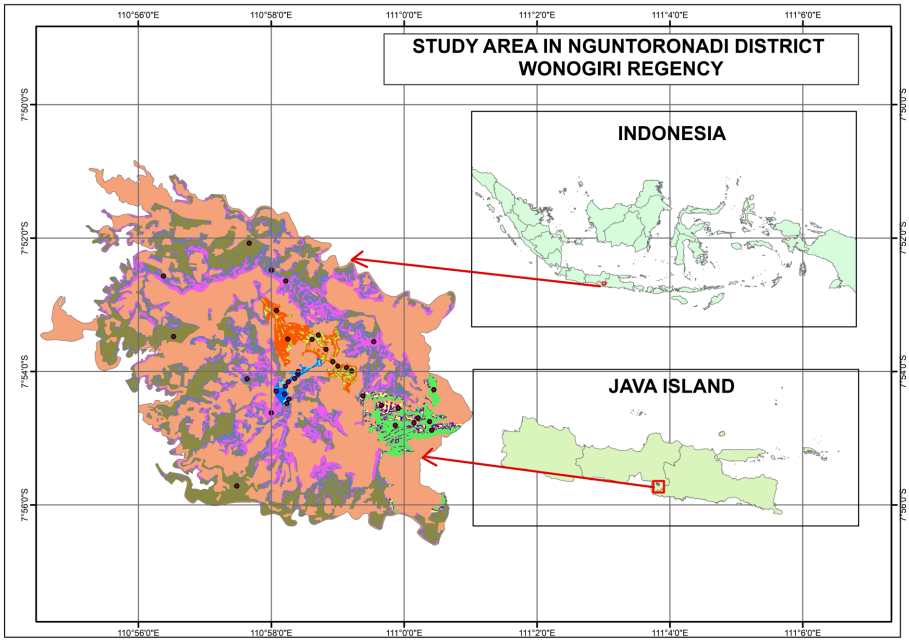

Figure 1. Study area.

The farming system of rice fields in the research location consists of three farming systems, including conventional rice fields, semi-organic rice fields, and organic rice fields. In the field survey stage, based on the information we got from farmers and local rice field stakeholders, it was discovered that fertilization in conventional rice fields is urea fertilizer 100-150 kg ha^{-1}, phonska fertilizer 100-150 kg ha^{-1}, and phosphorus fertilization with TSP fertilizer 50 kg ha^{-1}. Fertilizers used in semi-organic rice fields are organic fertilizer of 1 ton ha^{-1} as a base fertilizer before planting, urea fertilizer of 100 kg ha^{-1}, and liquid organic fertilizer (LOF) of 10 L ha^{-1}. Fertilization in organic rice fields is 3-6 ton ha^{-1} of organic fertilizer and liquid organic fertilizer (LOF) 15 L ha^{-1}. The farming of organic rice fields has been started since 2014 under the auspices of Gapoktan Beji Makmur, which passed the organic certification test in 2017 and was recertified in 2020 for the scope of rice, crops, and fertilizers with the basic reference of SNI 6729: 2016 by Lembaga Sertifikasi Organik Seloliman (LeSOS).

The research was conducted using an exploratory descriptive survey method through a field survey approach and the results of laboratory analysis of soil chemistry and physics. Soil sampling was conducted based on purposive sampling (Lenaini, 2021) in a composite manner at a depth of 0-30 cm. Soil sampling points were based on land map units (LMUs) obtained from overlaying the Indonesian landform map (RBI) of Nguntoronadi District, Wonogiri Regency, and thematic maps. The thematic map represents the diversity of the research location, including a map of rice field farming systems, a soil map, a slope map, and a rainfall map. Soil types at the research site are mostly Inceptisols and Entisols in the eastern part with the geological formation of Qvu. The slope is about 0-8%, 8-15%, 15-25%, and 25-45%. The average rainfall is about 2250 mm per year. The survey area consists of 12 LMUs with 3 replicates, so the total sample points are 36 as shown in Figure 2.

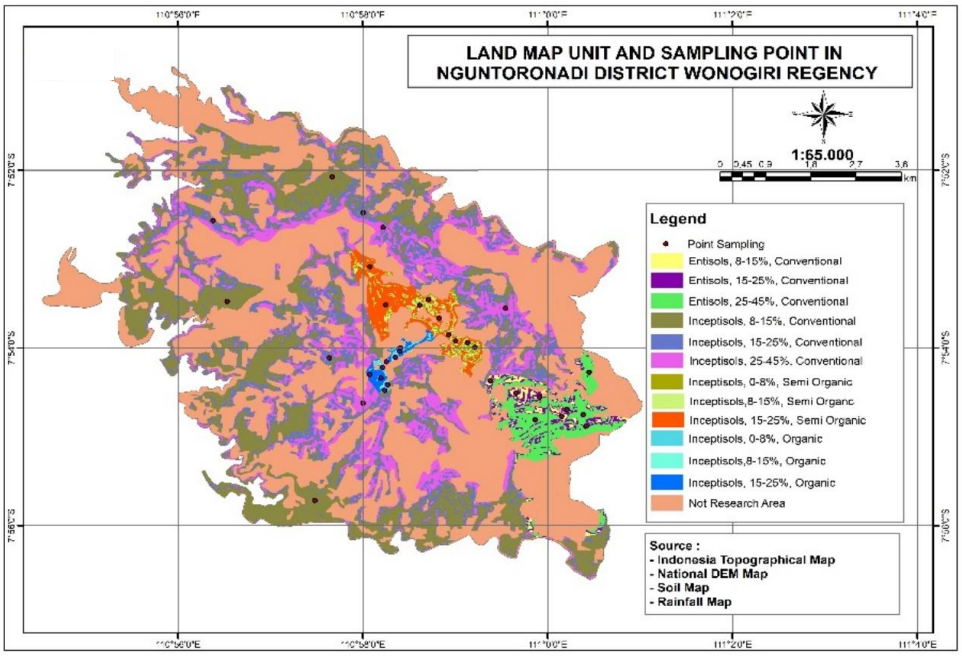

Figure 2. Land map unit and sampling point.

2.2. Soil analysis

Soil sample analyses were conducted at the Chemistry and Soil Fertility Laboratory and Soil Conservation and Physics Laboratory, Faculty of Agriculture, Sebelas Maret University. Soil chemical analysis included soil pH (electrometric method), organic C (Walkley and Black method), total N (Kjeldahl method), C/N ratio, available P (Olsen method), available K (extraction NH_4OAc 1N), exchangeable Ca (extraction NH_4OAc 1N), exchangeable Mg (extraction NH_4OAc 1N), Cation Exchangeable Capacity (extraction NH_4OAc 1N), base saturation (extraction NH_4OAc 1N), and Aluminium saturation (KCl saturation) (Soil Research Institute, 2009). Analysis of soil physics is soil texture (pipette method) (Center for Research and Development of Agricultural Land Resources, 2007). Worm density population observation (PVC ring sample) was conducted directly in the field (Center for Research and Development of Agricultural Land Resources, 2006).

2.3. Data analysis

Data analysis consists of determining the soil fertility index and key factor indicators of soil fertility in this research. The soil fertility index is determined by calculating the score, PCA, and index. Meanwhile, the key factor indicator is determined using a statistical test, namely Pearson's correlation.

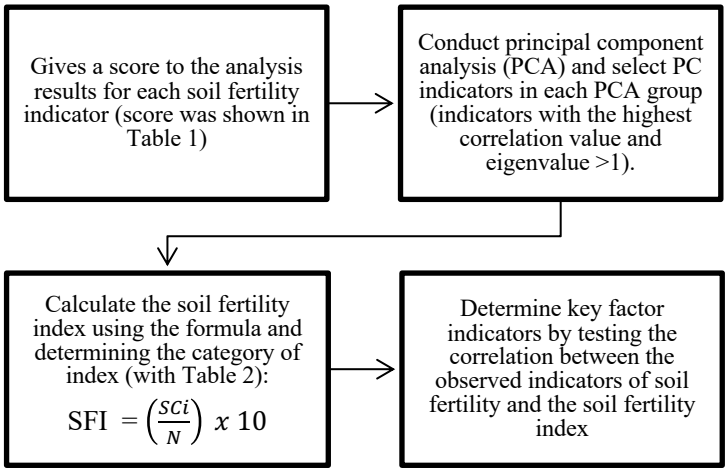

Figure 3. Data analysis stages.

2.3.1. Soil fertility index (SFI)

The soil fertility index was determined utilizing the Statistical Package for the Social Sciences (SPSS) and Minitab software. The soil fertility index was determined based on Pearson Correlation Analysis followed by Principal Component Analysis (PCA) to produce in Minimum Soil Fertility Indicator (MSFI). Minimum Soil Fertility Indicator (MSFI) is the outcome of Principal Component Analysis (PCA), where the major component or Principal Component (PC) employed has the highest eigenvalue >1 and is correlated. The indicators with the highest value and are correlated with the highest value are selected as MSFI indicators, except Aluminum is chosen from the lowest value (Mukashema, 2007). MSFI that met the criteria were then scored based on the results of the score of the Soil Research Center (2009) as shown in Table 1.

Table 1. Score index of soil fertility index (SFI)

Indicators	Score				
	1 (VL)	**2 (L)**	**3 (M)**	**4 (H)**	**5 (VH)**
Texture	C, S	LS, SiC	CL, SL	SiL, Si, SiCL	L
pH	<5.5 and >7.5	5.5-6.0	6.0-6.5	6.5-7.5	7.0-7.5
Organik C (%)	<1	1-2	2-3	3-5	>5
Total N (%)	<0.1	0.1-0.2	0.21-0.5	0.51-0.75	>0.75
C/N	<5	5-10	11-15	16-25	>25
Available P (ppm)	<5	5-10	11-15	16-20	>20
Available K (me $100g^{-1}$)	<0.1	0.1-0.3	0.4-0.5	0.6-1.0	>1
Exchangeable Ca (me $100g^{-1}$)	<2	2-5	6-10	11-20	>20
Exchangeable Mg (me $100g^{-1}$)	<0.3	0.4-1	1.1-2.0	2.1-8.0	>8
CEC (me $100g^{-1}$)	<5	5-16	17-24	25-40	>40
BS (%)	<20	20-40	41-60	61-80	>80
Al Saturation (%)	<5	5-10	10-20	20-40	>40

Source: Lal (1994), Soil Research Institute (2009), Mukashema, (2007).
Remark: VL (Very Low), L (Low), M (Moderate), H (High), VH (Very High), C (Clay), S (Sandy), LS (Loamy Sand), SiC (Silty Clay), CL (Clay Loam), SL (Sandy Loam), SiL (Silty Loam), Si (Silt), SiCL (Silty Clay Loam), and L (Loam).

The scoring results are then applied to the calculation of the soil fertility index using the following formula (Mukashema, 2007).

$$\text{SFI} = \left(\frac{SCi}{N}\right) x\ 10 \quad (1)$$

Remark :

$$SC_i = c_j\, x\, p_c \quad (2)$$

$$C_j = W_i\, x\, S_i \quad (3)$$

$$p_c = \frac{1}{nc} \quad (4)$$

Where; SFI is the soil fertility index, SCi represents the scoring indicator, N is the number of indicator MSFI, c_j is the class number which varies from 1 to j, p_c represents the probability of the class, n_c is the number of classes, Wi is weight index and Si is scoring index. The results of the SFI assessment are classified based on Table 2.

Table 2. Classification of the soil fertility index

Fertility İndex Value	Class
0.00-0.25	Very Low
0.25-0.50	Low
0.50-0.75	Moderate
0.75-0.90	High
0.90-1.00	Very High

Source: Bagherzadeh et al. (2018).

2.3.2. Key factors of soil fertility index (SFI)

Analysis of Variance (ANOVA) statistical testing was utilized to assess the effect of diversity sources such as rice field farming systems, slopes, soil types, and rainfall on soil fertility index values. If it has a real influence, Duncan's Multiple Range Test (DMRT) is used to assess the true difference in influence. Key factors of the soil fertility index were obtained from Pearson Correlation Analysis between indicators and soil fertility index values that were significantly correlated. Key factors are used as the basis for the direction of improvement or recommendations for proper management of rice fields to improve the status of soil fertility.

3. Results and Discussion

3.1. Study area and soil sampling

Soil fertility indicators are parameters that can be utilized in defining soil fertility indices. In addition to soil chemical properties, physical, and biological properties are also linked to soil fertility. The results of laboratory analysis (Table 3) show that rice field soils in the study area have a pH between 6.4-6.87 including in the category of slightly acidic (5.6-6.5) to neutral (6.6-7.5) (Soil Research Institute, 2009). The average pH value in conventional rice fields tends to be slightly acidic at 6.51 compared to semi-organic and organic rice fields which have neutral pH of 6.8 and 6.84. The application of urea fertilizer can contribute to soil acidity because dissolved urea reacts with water to produce carbonic acid (H_2CO_3). Furthermore, ammonium applied to soil undergoes nitrification, producing nitrites and nitrates that release H^+ ions and lower pH (Yamsil et al., 2022). Increased soil pH increases the availability of basic cations such as K, Ca, Mg, and Na. Meanwhile, Al, Fe, and Mn levels, which frequently bind basic cations, will decrease (Fitria and Soemarno, 2022).

Organic rice fields have the highest average organic C content of 2.75%, ranging from 2.42 to 3.18%, which is influenced by incorporating organic matter into the soil in the form of compost and straw harvest wastes. The addition of organic fertilizer (compost, manure, and green manure) improves organic C (Fitria and Soemarno, 2022; Syamsiyah et al., 2023). The range value of organic C in conventional rice fields was 0.84 to 1.85% and in semi-organic rice fields, it was 1.31%. The low organic C is due to the lack of organic matter returning to the soil such as straw left over from harvesting, which is often burned or used as animal feed. Meanwhile, the total N content in various rice field farming systems is low, with values between 0.12-0.2%. Nitrogen in the soil is one of the factors that improve plant productivity (Suminto et al., 2023). Conventional rice fields have more total N than organic rice fields because N from inorganic fertilizers can deliver N directly to plants, whereas N from organic fertilizers is released slowly, therefore the response is slower (Herdiansyah et al., 2022). The C/N ratio in conventional and semi-organic rice fields has a C/N value ranging from 6.10-10.02 which is classified as a low category, while organic rice fields have the highest C/N ratio value of 16.23-21.1 which is ideal in the decomposition process. Organic fertilizers increase soil qualities in terms of physical, chemical, and microbial activity (Kipcakbitik and Sensoy, 2023). Research by Ostrowska and Porębska (2014) showed that the C/N ratio in addition to being related to organic C is also related to soil N content where too high N input, especially from fertilizers, causes a lower C/N ratio compared to organic farming with abundant organic matter input.

Organic rice fields have higher P (6.18 ppm), K (0.63 me $100g^{-1}$), Ca (12.96 me $100g^{-1}$), and Mg (1.87 me $100g^{-1}$) than semi-organic rice field farming systems (P (5.34 ppm), K (0.55 me $100g^{-1}$), Ca (10.62 me $100g^{-1}$), and Mg (1.86 me $100g^{-1}$)), and conventional rice fields (P (2.38 ppm), K (0.53 me $100g^{-1}$), Ca (10.66 me $100g^{-1}$) and Mg (1.86 me $100g^{-1}$)). Organic matter sources such as compost, straw,

and legume green manure crops will improve soil chemical properties such as macronutrients N, P, K, Ca, Mg, and S because of their ability to release P fixation by Al, Fe or Mn (Sukristiyonubowo et al., 2019). Analysis of BS in various rice field farming systems is included in the moderate level with an average value of BS in conventional rice fields at 43.35%, semi-organic rice fields at 43.76%, and organic rice fields at 43.60%. This is related to the leaching of base cations supported by the research of Aytenew and Kibret (2016), which states that the loss of base cations due to runoff causes increased acidity and decreased soil fertility.

The CEC value in various land farming systems is included in the high value because it is in Inceptisols and Entisols soils, classified as young soils and dominated by clay textures. Soil that is still young and supported by a relatively neutral pH and clay-dominated soil texture will increase the CEC value (Pinatih et al., 2015). However, the highest CEC value is found in organic rice fields at 39.87 me $100g^{-1}$ due to the colloidal content of organic matter that can contribute a negative charge to soil colloids. The role of organic matter as a colloid can increase the capacity of absorption and cation exchange (Prasetiyo et al., 2015), which also increases the concentration of K in the soil (Roy et al., 2016). Al saturation at the research site is considered very low ranging from 3.20-5.27% with organic rice fields having the lowest average Al saturation value of 3.57%. The low Al saturation in the organic rice field farming system is due to the high content of organic matter that in decomposition, will release fulvic acid, humic acid, and organic acids that bind Al through the mechanism of binding Al-monomer (Al^{3+}) to a stable chelate complex (Muzaiyanah and Subandi, 2016).

Texture as an indicator of soil physics at the research site is dominated by clay and clay loam textures. According to Islam et al. (2021), soil texture affects the available water capacity of soil in rice farming because of its ability to hold and absorb water so that water can be available to plants. Worm population density as a supporting bioindicator showed the highest density in organic rice fields at 0.11 L^{-1} because organic matter content affects the metabolic activity of soil organisms (Supriyadi et al., 2020). According to Lou et al. (2022), soil organic matter is the most abundant organic carbon source and has an ecological impact on soil fauna, particularly earthworms that mineralize soil organic matter components.

3.2. Soil fertility index

The soil fertility index is a functional indicator in soil fertility assessment that provides information and appropriate management recommendations for sustainable agriculture. The results of PCA analysis (Table 4) show that the principal components (PC) that meet the requirements are PC 1 to PC 4. Zhang et al. (2018) stated that PCs that become the Minimum Soil Fertility Indicator (MSFI) have an eigenvalue ≥1 or a cumulative percentage of at least 60%. The four PCs have a cumulative presentation of 80.1%, showing the main components' confidence levels. Indicators with the highest value and correlated with the highest value are selected as MSFI indicators, except Aluminum is selected from the lowest value (Mukashema, 2007). Indicators selected as MSFI are pH, organic C, C/N, exchangeable Ca, CEC, Al saturation, BS, total N, exchangeable Mg, and available K.

The level of soil fertility at the study site (Table 5) based on the classifications of Bagherzadeh et al. (2018) was included in the moderate category with a value ranging from 0.53 to 0.70. LMU 1, 2, and 3 have an average SFI of 0.54 which is conventional rice fields farming on Entisols soil, while LMU 4, 5, and 6 with conventional rice field farming on Inceptisol soil have an average SFI of 0.56. SFI in both soil types with conventional rice field farming systems has a value with a slight difference. This could occur because Entisols and Inceptisols soils have similarities, namely undeveloped soils with diverse parent materials (Helmi et al., 2016) so the weathering process runs slowly.

Table 3. Analysis of soil fertility ındicators in various farming systems of rice fields in the study area

Soil type	Farming Systems	LMU	pH	Org-C (%)	Total N (%)	C/N	Available P (ppm)	Available K (ppm)	Exc-Ca (me $100g^{-1}$)	Exc-Mg (me $100g^{-1}$)	CEC (me $100g^{-1}$)	BS (%)	Al Saturation (%)	Texture	Worm population density (individuals L^{-1})
Entisols	Conventional	1	6.41±0.11	1.85±0.07	0.19±0.03	9.93±1.58	2.17±0.30	0.48±0.05	10.31±1.01	1.81±0.10	31.75±2.36	42.07±0.52	4.23±0.59	CL	0.00±0.00
		2	6.40±0.55	1.14±0.05	0.16±0.15	6.95±0.59	0.47±0.15	0.55±0.05	10.36±0.15	1.87±0.15	33.04±0.34	41.57±1.30	4.63±0.47	C	0.05±0.09
		3	6.56±0.08	0.84±0.08	0.19±0.28	4.52±0.37	2.33±0.28	0.51±0.05	9.88±0.19	1.86±0.21	31.04±0.18	45. 90±0.56	5.27±0.19	C	0.05±0.09
Inceptisols	Conventional	4	6.59±0.05	1.26±0.13	0.18±0.60	7.07±1.09	6.38±0.60	0.54±0.05	11.99±1.66	1.73±0.21	33.14±2.39	44.18±1.57	4.95±0.66	C	0.11±0.09
		5	6.53±0.18	1.17±0.02	0.12±0.30	10.02±2.12	2.20±0.30	0.51±0.03	10.54±0.66	1.74±0.11	32.99±1.14	41.36±1.25	4.26±0.33	CL	0.05±0.00
		6	6.56±0.25	0.87±0.08	0.15±0.10	6.10±1.64	0.74±0.10	0.58±0.04	10.88±1.05	2.16±0.33	35.05±1.95	45.04±1.20	3.61±0.07	C	0.11±0.09
	Semi-organic	7	6.85±0.05	1.08±0.01	0.14±0.37	7.84±0.90	5.38±0.37	0.57±0.03	10.91±1.26	1.70±0.14	34.37±1.76	44.33±2.49	4.57±0.66	C	0.05±0.09
		8	6.71±0.06	1.43±0.10	0.19±0.76	7.60±0.39	5.43±0.76	0.57±0.07	10.58±1.62	1.83±0.22	34.31±3.04	45.80±1.95	4.88±0.81	C	0.11±0.09
		9	6.84±0.03	1.43±0.12	0.20±0.65	7.25±0.62	5.20±0.65	0.52±0.02	10.39±0.28	2.04±0.16	33.13±0.45	41.15±0.24	4.53±0.05	CL	0.11±0.09
	Organic	10	6.79±0.03	2.42±0.34	0.15±0.53	16.23±1.56	6.31±0.53	0.62±0.02	12.88±0.70	1.73±0.18	37.42±1.13	44.92±0.47	3.20±0.11	C	0.11±0.18
		11	6.87±0.04	2.64±0.19	0.14±1.46	19.25±3.48	6.37±1.46	0.66±0.01	13.12±0.46	1.70±0.21	41.53±2.50	42.65±0.60	3.62±0.44	C	0.05±0.09
		12	6.86±0.04	3.18±0.17	0.16±0.16	21.10± 4.39	5.87±0.16	0.62±0.03	12.87±1.13	2.17±0.28	40.65±2.81	43.24± 1.95	3.89±0.56	C	0.16±0.16

Table 4. Results of PCA to determine MSFI

Variable	PC1	PC2	PC3	PC4
Eigenvalue	5.3629	1.5538	1.4058	1.2936
Proportion	0.447	0.129	0.117	0.108
Cumulative	0.447	0.576	0.694	0.801
pH	0.268	0.047	-0.265	-0.209
Organic C	0.349	-0.319	0.127	-0.206
Total N	-0.167	0.138	0.274	-0.664
C/N	0.366	-0.344	0.01	0.037
Available P	0.261	-0.043	-0.343	-0.536
Available K	0.371	0.255	0.073	0.069
Exchangeable Ca	0.389	0.148	0.007	-0.019
Exchangeable Mg	-0.008	0.115	0.751	-0.104
CEC	0.409	0.023	0.155	0.037
BS	0.066	0.674	-0.029	-0.144
Al saturation	-0.316	0.007	-0.304	-0.271
Texture	-0.124	-0.449	0.187	-0.269

Remark: the number written in bold is selected as the PC in each PC group.

Table 5. Results of soil fertility index calculation

Farming System	LMU	Point	Indicator Scoring										cj	nc	pc	SCI	N	SFI	SFI Average	Class
			pH	Org-C	C/N	Ca	CEC	Al	BS	N	Mg	K								
Conventional	1	1	3	2	2	3	4	1	3	3	3	3	2.72	5	0.2	0.54	10	0.54	0.55	Moderate
		2	3	2	3	3	4	1	3	2	3	3	2.74	5	0.2	0.55	10	0.55		
		3	4	2	2	4	4	1	3	2	3	3	2.83	5	0.2	0.57	10	0.57		
		4	4	2	2	3	4	1	3	2	3	3	2.74	5	0.2	0.55	10	0.55		
	2	5	4	2	2	3	4	1	3	2	3	3	2.74	5	0.2	0.55	10	0.55		
		6	2	2	2	3	4	2	3	2	3	3	2.65	5	0.2	0.53	10	0.53		
		7	3	1	1	3	4	2	3	3	3	3	2.63	5	0.2	0.53	10	0.53		
	3	8	4	1	1	3	4	2	3	2	3	3	2.65	5	0.2	0.53	10	0.53		
		9	4	1	1	3	4	2	3	2	3	3	2.65	5	0.2	0.53	10	0.53		
		10	4	2	2	4	4	1	3	2	3	3	2.83	5	0.2	0.57	10	0.57		
	4	11	4	2	2	3	4	2	3	3	3	3	2.91	5	0.2	0.58	10	0.58		
		12	4	2	2	4	4	2	3	2	3	3	2.93	5	0.2	0.59	10	0.59		
		13	3	2	2	3	4	1	3	2	3	3	2.65	5	0.2	0.53	10	0.53		
	5	14	3	2	3	3	4	1	3	1	3	3	2.67	5	0.2	0.53	10	0.53		
		15	4	2	2	4	4	1	3	2	3	3	2.83	5	0.2	0.57	10	0.57		
		16	3	1	1	4	4	1	3	2	4	3	2.63	5	0.2	0.53	10	0.53		
	6	17	4	1	2	4	4	1	3	2	3	4	2.88	5	0.2	0.58	10	0.58		
		18	3	1	2	3	4	1	3	2	4	3	2.63	5	0.2	0.53	10	0.53		
Semi-organic	7	19	4	2	2	4	4	1	3	2	3	4	2.97	5	0.2	0.59	10	0.59	0.57	Moderate
		20	4	2	2	3	4	1	3	2	3	3	2.74	5	0.2	0.55	10	0.55		
		21	4	2	2	3	4	2	3	2	3	3	2.83	5	0.2	0.57	10	0.57		
		22	4	2	2	3	4	2	3	2	3	3	2.83	5	0.2	0.57	10	0.57		
	8	23	4	2	2	3	4	2	3	2	3	3	2.83	5	0.2	0.57	10	0.57		
		24	4	2	2	4	4	1	3	2	3	4	2.97	5	0.2	0.59	10	0.59		
		25	4	2	2	3	4	1	3	3	4	3	2.89	5	0.2	0.58	10	0.58		
	9	26	4	2	2	3	4	1	3	2	4	3	2.81	5	0.2	0.56	10	0.56		
		27	4	2	2	3	4	1	3	2	3	3	2.74	5	0.2	0.55	10	0.55		
Organic	10	28	4	3	4	4	4	1	3	2	3	3	3.11	5	0.2	0.62	10	0.62	0.66	Moderate
		29	4	3	4	4	4	1	3	2	3	4	3.25	5	0.2	0.65	10	0.65		
		30	4	3	4	4	4	1	3	2	3	4	3.25	5	0.2	0.65	10	0.65		
		31	4	3	4	4	5	1	3	2	3	4	3.34	5	0.2	0.67	10	0.67		
	11	32	4	3	4	4	4	1	3	2	3	4	3.25	5	0.2	0.65	10	0.65		
		33	4	3	4	4	5	1	3	2	3	4	3.34	5	0.2	0.67	10	0.67		
		34	4	4	4	4	5	1	3	2	4	4	3.51	5	0.2	0.70	10	0.70		
	12	35	4	4	4	4	4	1	3	2	3	3	3.21	5	0.2	0.64	10	0.64		
		36	4	4	4	4	5	1	3	2	4	4	3.51	5	0.2	0.70	10	0.70		

Organic rice fields (LMU 10, 11, and 12) have the highest average soil fertility index value of 0.66 compared to semi-organic rice field farming systems (LMU 7, 8, and 9) of 0.57 and conventional (LMU 1, 2, 3, 4, 5, and 6) of 0.55. Map of soil fertility index rice fields in Nguntoronadi District, Wonogiri Regency as shown in Figure 2. The high value of the soil fertility index (SFI) in organic rice fields indicates that the provision of organic inputs will improve soil fertility status. This is corroborated by El-Mogy et al. (2020) assertion that organic farming will restore, preserve, and improve soil physiochemistry and biology, thereby increasing crop production. The higher the organic matter in the soil, the more fertile it will be. Conversely, the lower the organic matter content, the lower the soil fertility (Hanafiah, 2013). The application of organic materials can increase optimal soil fertility for crop management with sustainable agricultural yields and better production quality (Mutammimah et al., 2020). Conventional agriculture dependent on fertilizers and pesticides for crop production reduces fertility due to nutrient loss from erosion and leaching (Roy et al., 2016). Using chemical fertilizers in disproportionately high concentrations can also lead to nutrient imbalances in the soil that can cause other nutrient deficiencies (Mujiyo et al., 2022).

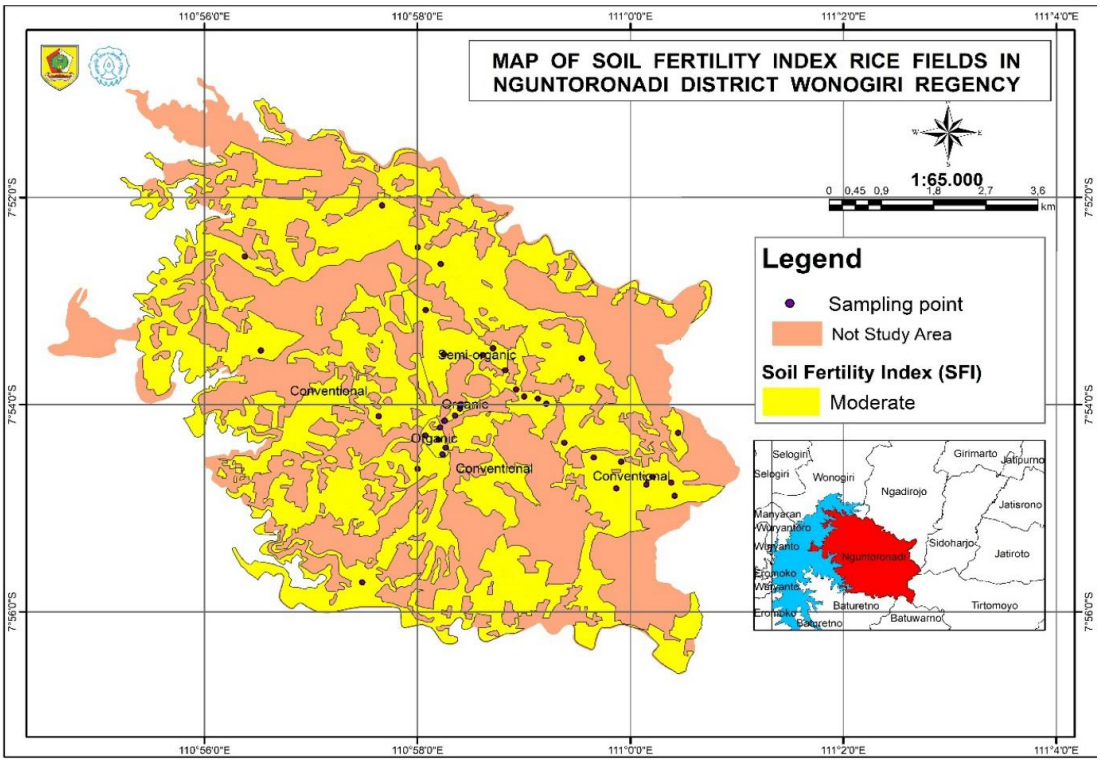

Figure 4. Map of soil fertility index.

3.3. The distribution and effects of different rice fields farming systems on SFI

The results of the ANOVA analysis showed that the farming system of rice fields had a very significant effect on soil fertility (p-value<0.01). According to Sukristiyonubowo et al. (2019), organic, semi-organic, and conventional farming systems affect soil chemical and physical fertility. Furthermore, Duncan's Multiple Range Test (DMRT) analysis was performed in Figure 5. Based on Figure 5. shows that the three rice field farming systems (conventional, semi-organic, and organic) have significant differences (not followed by the same letter) with each other. The organic rice field farming system has the highest value and differs from conventional and semi-organic farming systems. In contrast, conventional rice fields have the lowest SFI value and are significantly different from semi-organic and organic rice fields. Although conventional, semi-organic, and organic rice field farming systems are still in the same SFI class moderate, organic rice fields have a considerable difference in SFI values from other farming systems due to organic rice fields that have been cultivated for 8 years so that they affect higher soil fertility levels. This is similar to the findings of Das et al. (2017) that organic farming practices will gradually improve soil properties by increasing carbon storage in the soil and long-term

N, P, and K availability (Pambayun et al., 2023). According to Reeve et al. (2016), organic systems have been proven to increase chelate microelements, buffer soil pH, and increase cation exchangeable capacity, influencing the availability of adequate plant nutrients and reducing leaching potential.

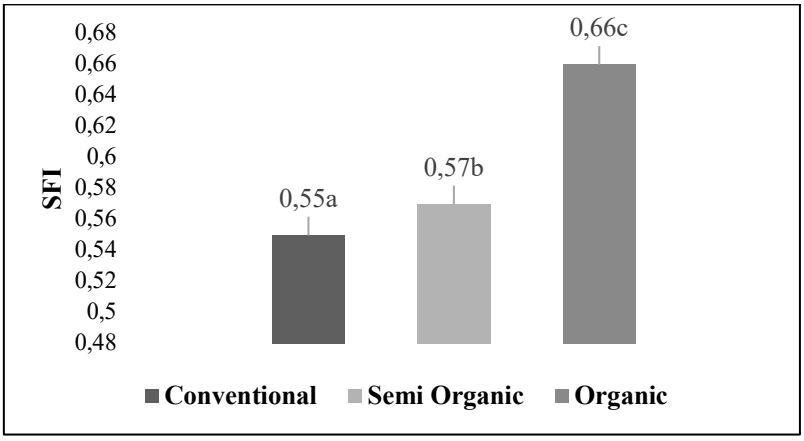

Figure 5. Soil fertility index (SFI) under different rice field farming systems.

3.4. Key factor indicators

Key factors of soil fertility are indicators that are significantly correlated with the results of the soil fertility index. The key factors become the basis for appropriate recommendations to improve soil fertility in rice fields of the study area. Indicators selected as key factors include pH (r=0.59, P-value<0.01, N=36), organic C (r=0.878, P-value<0.01, N=36), available P (r=0.651, P-value<0.01, N=36), available K (r=0.732, P-value<0.01, N=36), Exchangable-Ca (r=0.831, P-value<0.01, N=36), CEC (r=0.875, P-value<0.01, N=36), and Aluminum saturation (r=-0.57, P-value<0.01, N=36). The research results show that key factor indicators of soil fertility found in the research area are positively correlated with soil fertility. This value explains that the higher the key factor indicator value includes organic C (Figure 6), CEC (Figure 7), Exchangable Ca (Figure 8), Available K (Figure 9), Available P (Figure 10), pH (Figure 11), and Aluminum saturation (Figure 12), the higher the soil fertility level.

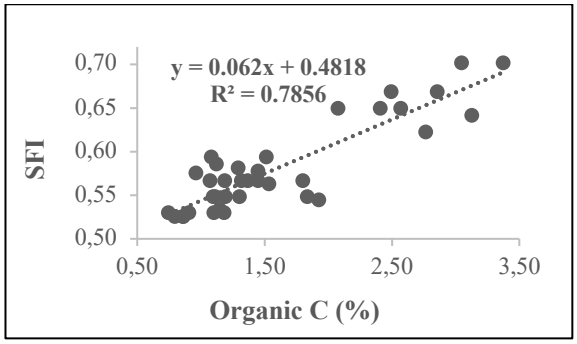

Figure 6. The correlation of organic carbon and soil fertility.

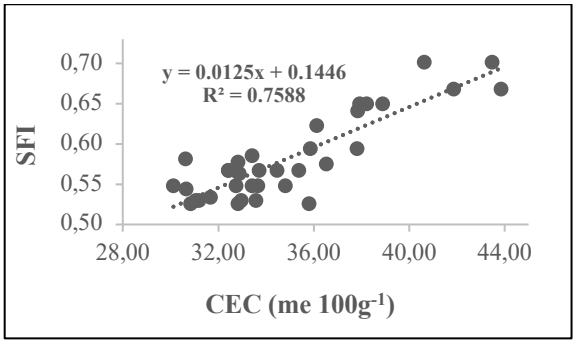

Figure 7. The correlation of CEC and soil fertility.

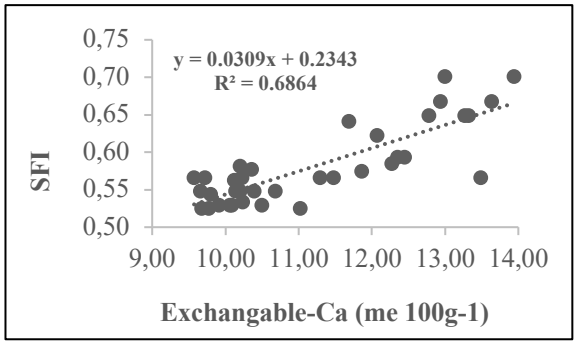

Figure 8. The correlation exchangable-Ca and soil fertility.

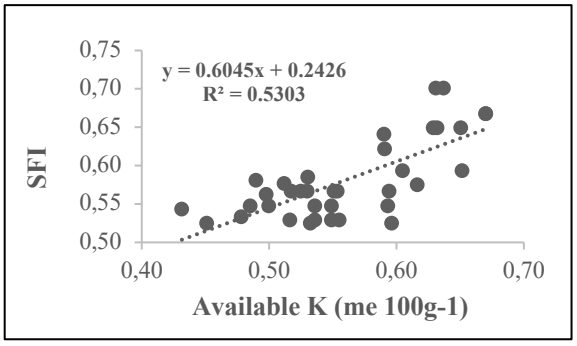

Figure 9. The correlation available K and soil fertility.

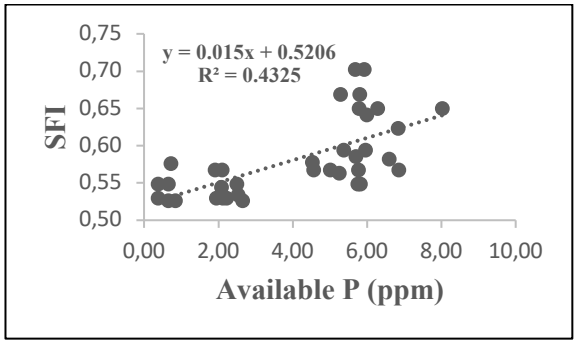

Figure 10. The correlation of available P and SFI.

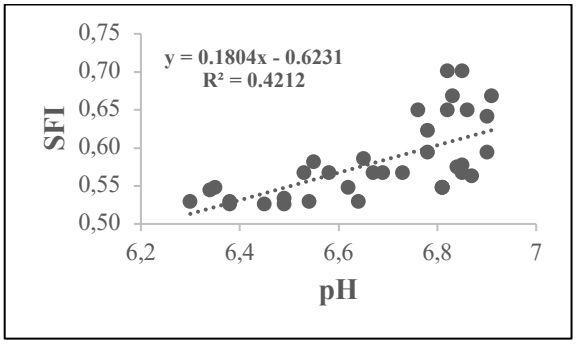

Figure 11. The correlation of pH and soil fertility.

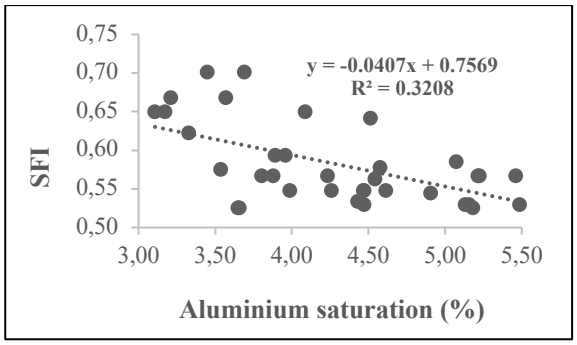

Figure 12. The correlation of Aluminium saturation and soil fertility.

The content of organic C will increase with the addition of organic matter, the increase in organic C will be directly proportional to the increase in CEC (r= 0.750), available P (r=0.559), and pH (0.392). Organic C content in conventional rice fields is very low to low due to the absence of added organic matter, semi-organic rice fields are low due to the provision of organic matter that is not optimal, and organic C content in organic rice fields is moderate. Organic matter requires the addition of microbes to facilitate the decomposition or release of minerals, thus making nutrients more available to roots in an early stage for sustainable plant growth (Ossai et al., 2022). Soil pH is very crucial in soil fertility because it involves the availability of other nutrients. The optimal pH range is 5.5 – 6.5 ensures that the nutrients in the soil are available for uptake by the rice plants. If the pH deviates from this range, it can lead to nutrient deficiencies or toxicities, affecting the overall fertility of the soil (Johnson et al., 2019).CEC is determined by organic matter content. Soils with higher organic matter content generally have higher CEC values (Sihi et al., 2017). The lack of soil organic matter results in a low pH so that H^+ ions are firmly bound to active groups and positively charged groups (-$COOH^{2+}$ and -OH^{2+}), as a consequence negatively charged colloids become low and CEC decreases. Conversely, in high pH conditions, OH^- dissolves and binds H^+ released from organic groups increasing negative charges (-COO^- dan -O^-) and CEC (Irawan et al., 2021).

The increase in organic C will reduce Aluminum saturation so that soil acidity decreases and increases the soil fertility index. Aluminum saturation negatively affects soil fertility by causing multiple nutrient deficiencies (Zhao and Shen, 2018). Aluminum saturation in organic rice fields is lower than in conventional and semi-organic rice fields. Organic matter undergoing decomposition produces organic acid compounds that can bind and reduce Al metal cations in acidic soils and can increase soil fertility and pH (Farrasati et al., 2019). In organic rice fields, the CEC is high due to the humification of organic material so that soil colloids increase and soil fertility also increases (Jawang, 2021). In addition, increasing soil CEC will increase the availability of K and Ca and protect against leaching. Organic rice fields have the highest CEC compared to other rice field farming systems so the quantity of available K and exchangeable Ca is also higher. The increase in available K and Ca-dd in the soil is due to the soil's ability to bind K and the clay content in the soil. High clay content has a large surface area, so the CEC becomes larger, increasing the ability to hold K and Ca from leaching (Jawang, 2021). High soil fertility is related to the availability of K^+ which plays an important role in crop yields. Organic farming will increase the efficiency of P availability (Adamtey et al., 2016) and increase the fertility of agricultural land. Organic C with the mechanism of inhibiting P binding by metal ions (Fe and Al) through the production of organic acids, humic acids, fulvic acids, and organic leachates (Li et al., 2021) can increase P availability.

3.5. Land management recommendation as a strategy to maintain soil fertility

Proper management can enhance soil properties so that fertility increases (Mutiara and Bolly, 2019) which is an important factor in determining plant growth and yield (Pinatih et al., 2015). Organic agriculture systems are recommended for rice field management in the study area because they have a higher soil fertility index than conventional and semi-organic rice fields. This is linked to the availability of organic matter inputs, which will improve the soil fertility index. Integrated soil fertility management considers site-specific conditions biotic and physio-chemical factors, and administrative aspects (Abukari and Abukari, 2020). In addition, organic farming can be the main road to socio-economic and ecologically sustainable development by avoiding or excluding synthetic inputs and utilizing crop rotation, crop residues, manure, organic waste, natural rock minerals, and crop protection.

Sources of organic matter that can be applied in rice field management are compost, liquid organic fertilizer (LOF), manure, biochar, green fertilizer, and biofertilizer. Compost comes from crop residues and animal waste that undergo a biological decomposition process by microorganisms with or without bio-activators such as EM4 (effective microorganism 4) technology, which accelerates the composting process (Dahlianah, 2015) and increases the total N, available P, and K (Viandari et al., 2022) and enhanced soil quality recovery (Kurniawan et al., 2023). Compost is generally in the form of solid organic fertilizer, while liquid organic fertilizer is commonly referred to as liquid organic fertilizer (LOF). The advantages of liquid organic fertilizer are that it has the potential to improve soil fertility because the nutrients are easily decomposed and quickly available to plants, reduce farming costs, and save on environmental problems (Arfarita et al., 2020).

Using organic fertilizers such as manure and vermicompost can raise the relative water content by up to 75%, aid in plant water absorption, and increase nutrition, resulting in optimal plant vegetative growth (Rahimi et al., 2023). Manure not only contains macronutrients needed by plants but can also maintain the balance of nutrients in the soil. Animal manure contains complete nutrients and is relatively available to plants because organic matter has gone through a complete transformation quickly. Animal manure contains complete and relatively available nutrients for plants consisting of 26.2 kg ton^{-1} N, 4.5 kg ton^{-1} P, 13 kg ton^{-1} K, 2.2-13.6 kg ton^{-1} S, 5.3-16.28 kg ton^{-1} Ca, and 3.5-12.8 kg ton^{-1} Mg (Suntoro et al., 2018). Biochar is a carbon product derived from biomass pyrolysis in an anaerobic environment that is beneficial for soil fertility by decreasing soil acidity, increasing CEC, and nutrient availability (Diatta et al., 2020). Green fertilizers are green plants that can increase the physical and biochemical structure of the soil, reduce nutrient losses due to leaching, increase water holding capacity, increase carbon absorption, increase nitrogen fixation, and increase organic matter content (Iderawumı and Kamal, 2022). Organic fertilizers combined with biological agents can produce high-quality fertilizers that can increase soil fertility and support soil productivity (Mujiyo et al., 2022). Organic farming also helps to reduce greenhouse gas emissions, ensuring a more sustainable environment for the future (Angon et al., 2022; Suwardi et al., 2023)

Conclusion

Intensive farming of rice fields to meet food needs with the addition of chemical fertilizers and pesticides causes land degradation. This land degradation indicates a decrease in soil fertility in rice fields. In fact, rice field soil fertility is required for rice plant productivity. Thus, research is necessary to analyze soil fertility to determine the soil fertility level in various rice field farming systems used for rice production and management recommendations based on key soil fertility factors. The research results show that the soil fertility level in the research area is moderate, with an index value range of 0.55 to 0.66. Differences in rice field farming systems affect the level of soil fertility. Organic farming has the highest soil fertility with an index of 0.66, and conventional farming has the lowest fertility compared to the others with an index of 0.55. The key indicators of soil fertility are pH (R^2=0.4212), organic C (R^2=0.7856), available P (R^2=0.4325), available K (R^2=0.5303), exchangeable Ca (R^2=0.6864), CEC (R^2=0.7588), and Aluminum saturation (R^2=0.3208). Suitable management recommendations for rice fields in the area are through the implementation of organic farming systems with the addition of organic materials such as compost, liquid organic fertilizer (LOF), manure, biochar, green fertilizer, and biological fertilizer. In addition, organic farming recommendations support the implementation of sustainable integrated agriculture by increasing soil fertility. Increased soil fertility has a clear impact on enhancing rice crop productivity and achieving food security.

Acknowledgments

The authors would be thanked for P2M research grant of Universitas Sebelas Maret. Also thank you to the PPOWW, M Rizky R, Viviana I, Nanda Mei, Tiara Hardian, Akas Anggita, and Khalyfah Hasanah for the paper elaboration.

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Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

ISSN: 1308-7576 e-ISSN: 1308-7584

Research Article

Acceptability of Different Concentrations of *Chlorella* sp. in Filipino Delicacy Puto as Coloring Agent

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Article Info

Received: 28.08.2023
Accepted: 20.12.2023
Online published: 15.03.2024
DOI: 10.29133/yyutbd.1351176

Keywords

Chlorella sp.,
Colorant,
Pigment,
Puto,
Sensory properties

Abstract: Natural colorants play a crucial role in food product development and improvement of health. Microalga *Chlorella* sp. is one of the sources of natural colorant. In this study, different concentrations of microalga *Chlorella* sp. (0.5, 1, and 2%) were added to Puto as coloring agents to evaluate its sensory properties. Pigments such as chlorophyll *a* and total carotenoid quantities of *Chlorella* powder and the experimental group were also investigated. It was found that the natural colorant *Chlorella* sp. at all levels of concentrations did not affect the color properties ($p \geq 0.05$) of the Puto products. However, the smell and texture of Puto differed significantly ($p \leq 0.05$) when 2% *Chlorella* sp. was incorporated. The study also found that the 0.5% and 1% amounts of *Chlorella* sp. component did not significantly affect ($p \geq 0.05$) the Puto's taste and overall acceptability. However, the 2% level of *Chlorella* sp. significantly decreased both overall acceptability and taste attributes. Moreover, *Chlorella* sp. powder constituted 4004.79±119.1 µg g^{-1} chlorophyll *a* and 1442.67±74.41 µg g^{-1} total carotenoids. Chlorophyll *a* amounts in experimental groups varied from 14.34±0.49 µg g^{-1} to 54.06±1.71 µg g^{-1} while total carotenoids amounts were found ranging from 5.59±0.37 µg g^{-1} and 18.06±0.66 µg g^{-1}. Puto used these biomasses at a concentration of 0.5%, 1%, and 2% as natural green colorants. However, chlorophyll *a* and carotenoid pigments level at 2% *Chlorella* sp. were not tolerable for the production of Puto. Hence, the *Chlorella* sp. biomass at 0.5% and 1% would be suitable for use as a natural colorant in the Filipino delicacy Puto.

To Cite: Sarri, J, Erbil, G Ç, Elp, M, Kadak, A E, 2024. Acceptability of Different Concentrations of *Chlorella* sp. in Filipino Delicacy Puto as Coloring Agent. *Yuzuncu Yil University Journal of Agricultural Sciences*, 34(1): 62-73. DOI: https://doi.org/10.29133/yyutbd.1351176

1. Introduction

Puto is a Filipino delicacy from the Philippines, also known as a rice cake, that is generally made from flour, usually cooked by steaming, and includes rice-free varieties. It is actually less sweet and fluffy and makes a great base for different toppings and flavors, such as cheese, ube (purple yarn), pandan (screwpine), or salted egg, which gives it different colors. It is believed that the color of food is one of the most crucial factors affecting its safety and quality since the color of food influences one's perception of its taste and safety. Colors used in food can be categorized into natural and synthetic colors (Mortensen, 2006). It is normally possible to use synthetic food colors in foods without further processing since they are usually water-soluble chemicals manufactured in factories (Sadar et al., 2017). Unlike natural colors, which are less stable and more expensive, synthetic colorants can be trusted and are both economical and reliable (Saleem and Umar, 2013). However, human health was adversely affected by synthetic food colorants. There is evidence that synthetic food color additives can sometimes be harmful to the liver, kidneys, and testes (Van Bever et al., 1989; Mahmoud, 2006).

There are a variety of natural food coloring sources, such as fruits, seeds, vegetables, insects, and microbes that do not require chemical treatment (Sadar et al., 2017). Renewable resources are used to produce natural food colorants. Typically, plant material is used as a source of colorants; however, insects, fungi, algae, and cyanobacteria are also employed (Mortensen, 2006). In today's society, the benefits of natural colorants, such as anthocyanins and carotenoids, are further reinforced by scientific results. *Hibiscus* sp. has historically been used in the reduction of liver dysfunction and hypertension due to its presence of 2.5% (dry weight) anthocyanin (Da-Costa-Rocha et al., 2014; Abdallah, 2016; Mushtaq et al., 2016). Natural colors containing carotenoids, such as β-carotene and pro-vitamin A, offer a variety of health benefits (Britton and Khachik, 2009; Bohn, 2012; De Andrade and De Andrade, 2017). For example, lycopene protects against prostate cancer (Aghajanpour et al., 2017; Li et al., 2019), and Lutein reduces cataract incidence (Akhtar and Bryan, 2008; Jia et al., 2017; Zhao et al., 2017). Consequently, Colorants and pigments derived from natural sources can provide important aspects for food product development. According to Durmaz and Bandarra (2017), microalgae are an extremely valuable source of natural pigments.

Due to the fact that microalgae contain a variety of macro- and micronutrients and are increasingly being studied since they can potentially provide humans with health benefits such as antioxidation, anticarcinogenesis, and antihypertensive effects (Pulz and Gross, 2004; Durmaz and Bandarra, 2017; Koyande et al., 2019). Microalgae's natural pigments may have numerous benefits, such as enhancing not only the visual qualities of a product but also its antioxidant capacity and preservation function (Durmaz and Bandarra 2017; Sun et al., 2023).

Biomass from microalgae can be utilized to make a wide range of foods. White chocolate with *Nannochloropsis oculata* (Genc Polat et al., 2020); yogurt with *Spirulina platensis* (Barkallah et al., 2017); pasta with *Isochrysis galbana* and *Diacronema vlkianum* (Fradique et al., 2013); yogurt with *Pavlova lutheri* (Robertson et al., 2016); *Nannochloropsis gaditana, Isochrysis galbana, Tetraselmis suecica, Scenedesmus almeriensis,* and *Isochrysis galbana* in bread (Garcia Segovia et al., 2017); chewing gum with *Isochrysis galbana* and *Nannochloropsis oculata* (Palabiyik et al., 2017); and pasta with *Chlorella vulgaris* and *Spirulina maxima* (Fradique et al., 2010), currently being evaluated.

Chlorella is a eukaryotic green microalga with a spherical shape whose importance has increased commercially and scientifically (Liu and Chen, 2014; De Andrade and De Andrade, 2017). Moreover, *Chlorella* has been used as a source of food and animal feed, as well as a protein source, and can also be used as a natural fuel since it contains a high oil content (Liu and Chen, 2014). Although microalgae have been studied in the literature for their potential use in food products, however, the use of microalgae *Chlorella* sp. as a natural colorant in Filipino delicacies has not been studied. Thus, the main goal of this study was to examine the effects of the natural pigment source *Chlorella* on sensory evaluation and acceptability in the Filipino delicacy Puto as a coloring agent.

2. Material and Methods

2.1. Preparation of chlorella powder

Chlorella sp. biomass was produced at the Faculty of Fisheries, Kastamonu University. The culture procedure was given by Erbil et al. (2021). After harvesting microalgae from the

photobioreactor, biomass was separated from water and then dried in an oven at 50 °C for 4 hours. Dried biomass was ground in a coffee grinder.

2.2. Preparation of Puto Filipino delicacy

Puto was made of mixtures of flour, sugar, baking powder, vanilla, egg, milk, and water. The formulation of ingredients is given in Table 1. Three different *Chlorella* sp. concentrations were determined after pre-trials. Each treatment with three replicates was placed into a *Puto* molder and then steamed over medium heat to prevent overcooking or drying out of Puto. The lid of the steamer was covered with a soft cloth to absorb the moisture. After cooking, the Puto was cooled for 5 minutes before removing it from the Puto molder.

Table 1. Composition of product

Ingredients	Group A (0% control)	Group B (0.5% *Chlorella* sp.)	Group C (1% *Chlorella* sp.)	Group D (2% *Chlorella* sp.)
Flour (g)	100	100	100	100
Sugar (g)	50	50	50	50
Baking powder (g)	10	10	10	10
Vanilla (g)	5	5	5	5
Egg (g)	55	55	55	55
Milk (ml)	70	70	70	70
Water (ml)	50	50	50	50
***Chlorella* sp. (g)**	-	1.71	3.43	6.94

2.3. Color measurements

The color values of the Puto products in the control group and incorporated with *Chlorella* sp. were measured with a HunterLab color measurements system (2°-10° observer angle, 10 nm wavelength interval, 400-700 nm wavelength range). A standard white plate (L^*=86.6, a^*=-0.5, b^*=0.5) was used to calibrate the colorimeter. Triplicate measurements for three different parts of the top and bottom of Puto products. L^*, a^*, and b^* were determined, where L^* is the brightness coefficient from dark (0) to bright (100), a^* is the coefficient from red (+) to green (-), and b^* is the coefficient from yellow (+) to blue (-). *Chroma* indicates the intensity of color, whereas *hue* angle is an indication of red versus yellow color. Chroma (C*; Eq. 1), *hue* (h^*; Eq. 2), whiteness (W^*; Eq. 3), and total color difference (*TCD*; Eq. 4) were obtained by following equations:

$$Chroma = \sqrt{(a^2 + b^2)} \quad (1)$$

$$Hue = arctan\frac{b}{a} \quad (2)$$

$$Whiteness = 100 - \sqrt{(100 - L^2) + a^2 + b^2} \quad (3)$$

$$TCD = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2} \quad (4)$$

2.4. pH measurements

The pH values of the Puto products in the control group and incorporated with *Chlorella* sp. were determined by a pH-meter (IsoLab, METERS" tabletop," Microprocessor pH-meter, Germany). All pH analyses of products were carried out in triplicate.

2.5. Sensory evaluation of Puto

The newly prepared *Puto* delicacies were evaluated by 32 panelists composed of consumers from two nationalities, Filipinos, and Turkish. Following steaming, a 5-point Hedonic scale was used to evaluate the products, varying from extremely like (5) to extremely dislike (1) for appearance/color, flavor/aroma, mouthfeel or texture, taste, and overall acceptability (Blase and Labay, 2017). A random product of three replications was given to each panelist for each Puto treatment. One treatment was served at a time to each panelist in random order. The evaluators were provided with bottled mineral water to rinse their mouths before receiving another set of Puto products every one to two minutes. The

researchers were instructed to evaluate each product using a 5-point Hedonic scale in order to eliminate any bias.

2.6. Pigment analysis

Pigment analysis was performed both for *Chlorella* sp. powder and for the Puto experimental groups. A total of 5 ml of methanol was added to the products in glass tubes. Products underwent 10 minutes of ultrasound at 65 °C after 30 seconds of vortexing. Following this, products were vortexed again for 30 seconds, then centrifuged for 10 minutes at 5000 rpm. Supernatants were read in a spectrophotometer, and pigment amounts were calculated by equations 5-6 (Zou and Richmond, 2000; Macıas-Sánchez et al., 2005) given below:

$$\text{Chlorophyll } a \text{ (µg/ml)} = 13.9\ A_{666} \quad (5)$$

$$\text{Total carotenoids (µg/ml)} = 4.5\ A_{475} \quad (6)$$

2.7. Statistical analysis

SPSS software version 22.0 was used to analyze the data of experimental results of incorporated *Chlorella* sp. in Puto, including untreated control, by using analysis of variance (ANOVA) at the $p \leq 0.05$ significance level. All experimental values are at least the mean of triplicate determination. Duncan's multiple range test method was applied to rank the mean, and Levene's Test was used to test for homogeneity of variance. The following ranges were used to interpret the mean scores for appearance/color, odor/aroma, mouthfeel/texture, flavor/taste, and general acceptability:

4.50 – 5.00	Extremely like
3.50 – 4.49	Very much like
2.50 – 3.49	Neither like nor dislike
1.50 – 2.49	Very much dislike
0.50 – 1.49	Extremely dislike

3. Results

3.1. Color properties and pH measurement of Puto product

Color properties were measured on the top and bottom parts of the Puto. Table 2 shows the color measurement of the Puto product. On the top part of Puto, the L^* values of group A (66.28±0.36) were significantly ($p \leq 0.05$) different from those of group B, group C, and group D, while on the bottom part of Puto, the L^* values in group A and B were significantly different from those of group C and D ($p \leq 0.05$). In terms of a^* and b^* values, group A was significantly different from groups B, C, and D for both the top and bottom parts of Puto. Moreover, the chroma (C^*), hue (h^*), whiteness (W^*), and total color difference (TCD) values were calculated based on the average results of L^*, a^*, and b^* values, which are frequently used to determine how a food product's color tone and saturation can be defined. Table 3 shows the calculated color measurement of the Puto product. According to the results, C^* values ranged from 15.28 – 27.56 on the top part of the Puto, while 14.92 – 27.40 were recorded on the bottom part. The h^* values on the top part of Puto varied from -1.55 to 1.56, while the h^* values on the bottom part of Puto ranged from -1.53 to 1.50. Additionally, W^* values ranged from 38.16 – 62.98 on the top part of the Puto, while W^* values (31.85) of group D is lower than 39.79 in group A at the bottom part of the Puto product. The TCD values on the top part of Puto varied from 25.16 to 49.93, while the TCD values on the bottom part of Puto ranged from 31.52 to 55.66. Furthermore, Figure 1 shows the pH values of the Puto products incorporated with *Chlorella* sp. Based on the result of the present study, groups A, B, C, and D obtained pHs of 8.97, 9.03, 8.96, and 8.87, respectively.

Table 2. Color measurement of Puto

Color parameters	TOP				BOTTOM			
	Group A (0%)	Group B (0.5%)	Group C (1%)	Group D (2%)	Group A (0%)	Group B (0.5%)	Group C (1%)	Group D (2%)
L*	66.28±0.36[a]	59.85±0.83[b]	49.65±0.45[c]	44.64±1.25[d]	58.60±1.90[a]	55.07±1.67[a]	46.39±1.23[b]	36.51±1.47[c]
a*	1.29±0.24[a]	0.39±0.07[b]	-0.61±0.09[c]	-0.47±0.16[c]	1.08±0.06[a]	-0.81±0.59[b]	-1.19±0.34[b]	-0.98±0.11[b]
b*	15.23±0.89[a]	25.66±0.96[b]	27.29±0.72[c]	27.55±0.05[c]	14.88±0.08[a]	26.93±1.68[b]	27.38±1.14[b]	24.75±2.11[b]

Values followed by the different letters in the same row are significantly differences (P≤0.05). Means (±SE) are based on triplicate analyses. L=lightness (black to white), a=red(+) to green(-) and b=yellow(+) to blue(-). Different letters (a,b,c,d,…) indicate significant differences among the same color parameter in different groups.

Table 3. Calculated color measurement of Puto

Color parameters	TOP				BOTTOM			
	Group A (0%)	Group B (0.5%)	Group C (1%)	Group D (2%)	Group A (0%)	Group B (0.5%)	Group C (1%)	Group D (2%)
Chroma	15.28	25.66	27.3	27.56	14.92	26.94	27.4	24.77
Hue	1.49	1.56	-1.55	-1.55	1.5	-1.54	-1.53	-1.53
Whiteness	62.98	52.35	42.73	38.16	55.99	47.61	39.79	31.85
TCD	25.16	36.74	45.64	49.93	31.52	41.14	48.37	55.66

Chroma (*C**), hue (*h**), whiteness (*W**), and total color difference (*TCD*) values were calculated based on the average results of *L**, *a**, and *b** values.

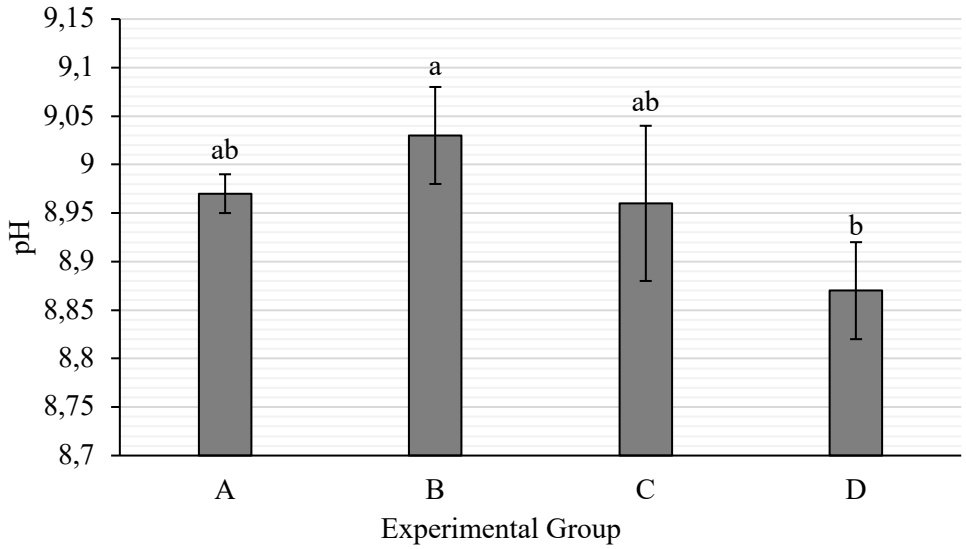

Figure 1. pH measurement of Puto. Group A (0% *Chlorella* sp.), group B (0% *Chlorella* sp.), group C (0% *Chlorella* sp.), and group D (0% *Chlorella* sp.).

3.2. Sensory evaluation of Puto

Sensory evaluation results of the Puto product containing *Chlorella* sp. at different concentrations are presented in Table 4 and Figure 2. The panelists' preferences were not influenced by the color of the product; hence, the appearance/color of the Puto did not differ significantly (p≥0.05). The slightly sweet aroma in group A and group B, with mean scores of 3.66 ± 0.18 and 3.38 ± 0.21, respectively, were significantly higher (p≤0.05) than in group C and group D, with mean scores of 2.97 ± 0.21 and 2.88 ± 0.22, respectively. In terms of mouthfeel or texture of the Puto products, group D and C with a mean score of 3.31 ± 0.17 and 3.53 ± 0.19, respectively, were statistically lower (p≤0.05) than group A, and group B. In addition, the taste or flavor of group A, group B, group C, group D, with mean scores of 3.69 ± 0.18, 3.16 ± 0.22, 3.13 ± 0.24, and 2.84 ± 0.22, respectively, where group A significantly different (p≤0.05) than group D. For overall acceptability scores, group A and group B were significantly different (p≤0.05) from group D, while group C did not significantly different (p≥0.05) from the group D.

Table 4. Sensory mean scores of Puto with different concentrations of *Chlorella* sp.

Treatments	Color / Appearance	Aroma / Odor	Texture / Mouthfeel	Taste / Flavor	Overall Acceptability
Group A	3.94 ± 0.21^{a}	3.66 ± 0.18^{a}	4.19 ± 0.12^{a}	3.69 ± 0.18^{a}	3.72 ± 0.17^{a}
Group B	3.69 ± 0.17^{a}	3.38 ± 0.21^{ab}	3.88 ± 0.16^{ab}	3.16 ± 0.22^{ab}	3.44 ± 0.19^{a}
Group C	3.94 ± 0.18^{a}	2.97 ± 0.21^{b}	3.53 ± 0.19^{bc}	3.13 ± 0.24^{ab}	3.25 ± 0.21^{ab}
Group D	3.69 ± 0.21^{a}	2.88 ± 0.22^{b}	3.31 ± 0.17^{c}	2.84 ± 0.22^{b}	2.78 ± 0.216^{b}

Columns with the same letters are not significantly different ($p \geq 0.05$). Values in SEM (standard error mean), n=32.

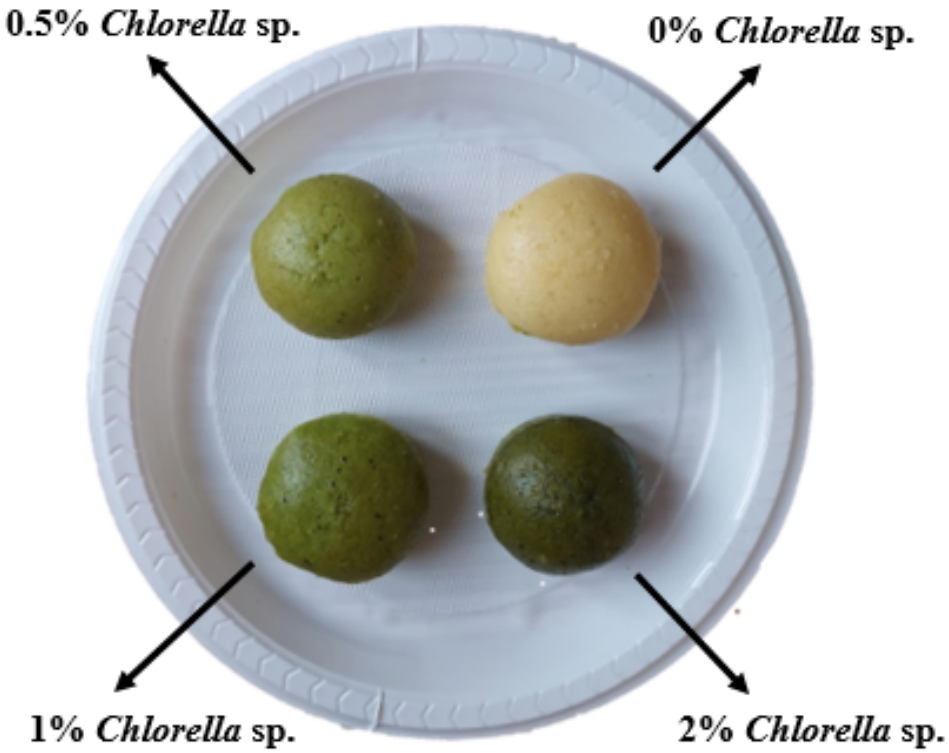

Figure 2. Products of Puto prepared with different concentrations of *Chlorella* sp. (Photograph using Samsung Galaxy A72). Group A (0% *Chlorella* sp.), group B (0% *Chlorella* sp.), group C (0% *Chlorella* sp.), and group D (0% *Chlorella* sp.).

3.3. Pigment analysis

Chlorophyll *a* and total carotenoid amounts of crude *Chlorella* sp. powder and experimental groups were measured. *Chlorella* sp. powder was constituted of 4004.79±119.1 µg g^{-1} chlorophyll *a* and 1442.67±74.41 µg g^{-1} total carotenoids according to pigment analysis. Chlorophyll *a* amounts in experimental groups varied from 14.34±0.49 µg g^{-1} to 54.06±1.71 µg g^{-1}. Also, total carotenoid amounts were found between 5.59±0.37 µg g^{-1} and 18.06±0.66 µg g^{-1} (Table 5).

Table 5. Pigment amounts of crude *Chlorella* sp. and Puto experimental groups

Pigments	Crude *Chlorella* sp.	Puto with 0.5% *Chlorella* sp.	Puto with 1% *Chlorella* sp.	Puto with 2% *Chlorella* sp.
Chlorophyll *a* (µg g^{-1})	4004.79±119.1	14.34±0.49	27.51±1.09	54.06±1.71
Total carotenoids (µg g^{-1})	1442.67±74.41	5.59±0.37	10.28±0.85	18.06±0.66

4. Discussion

Color is a very important aspect of a person's perception of food, serving as a quality indicator that can promote or hinder their acceptance of a food product (Chranioti et al., 2015). Colorants are widely used in food products for coloring, but their toxicological potential on humans is a controversial issue in the food industry (Mizutani, 2009). The color properties of the Puto product incorporated with *Chlorella* sp. were investigated in the present study. The *C**, *h**, and *W** values for each Puto product were calculated based on the average values for the *a**, *b**, and *L** parameters. The values of total color difference (*TCD*) were also calculated based on the *a**, *b**, and *L** values. As a result of the present study, increasing concentrations of *Chlorella* sp. in Puto decreased the color parameters of *L*, a*, h**, and *W**. Similar to another study, increasing the concentration of spray-dried *Nannochloropsis oculata* in white chocolate decreased the color parameters of *L*, h**, and *W** (Genc Polat et al., 2020). In addition, there is considered to be a noticeable color change when the *TCD* value of the product exceeds 3.00 (Witzel et al., 1973; Periche et al., 2015). Thus, products with *TCD* values below 3.00 indicate color stability under the relevant circumstances. Consequently, as *Chlorella* sp. concentration in Puto increases, a total color difference can be easily perceived by the human eye. Moreover, a pH measurement was also conducted on the Puto products. Based on the result of the present study, *Chlorella* sp. incorporated into Puto obtained a pH measurement ranging from 8.87 – 9.03. As a result, it is lower than the study conducted by García-Segovia et al. (2017), in which microalgae such as *Isochrysis galbana*, *Scenedesmus almeriensis*, *Tetraselmis suecica*, *Nannochloropsis gaditana* were incorporated into wheat bread obtained at pH values that ranged from 10.10 to 10.50, which is still considered suitable for human consumption.

As a traditional product of the Philippines, Puto is consumed daily as a snack food, dessert, or even breakfast, served during family gatherings, and can be purchased at almost any grocery store. During Ramadan, Muslims in the southern Philippines also consume Puto with hot coffee or cold beverages as part of their iftar (breaking the fast). Puto is usually flavored or topped with ingredients such as ube, cheese, and salted eggs. However, to ensure that the organoleptic properties of the experimental product were not affected, no other flavors or toppings were used in the present study.

Consumer acceptance of a product is influenced heavily by the color or appearance, and the choice of food can affect consumer perception of subsequent flavors and acceptability of the food (Imram, 1999; Silva et al., 2022). Hence, it is vital to evaluate the sensory properties in developing the new product, optimization process, and re-formulation studies. In this study, a sensory analysis was performed to quantify the appearance, odor, mouthfeel, taste, and overall acceptability properties.

Nowadays, due to food coloring pigments being unstable and deteriorating during processing, colorants are added to food products worldwide to maintain or restore product color uniformity (Silva et al., 2022; Nabi et al., 2023). In order to determine a product's overall appearance, three factors must be considered: its optical properties, its shapes or physical form, and the way it is presented (Hutchings, 1977). Natural colorants are said to be more expensive than synthetic colors; however, microalgae might be a solution to this problem since they can be produced almost everywhere, like non-arable areas. Green food is perceived as healthier, especially among health-conscious consumers (Schuldt, 2013). Trends in the food industry include functional food and green-colored foods, which have more potential health benefits than normal nutrition (Villaró et al., 2021). In microalgae, green pigments provide health benefits for humans, including anticarcinogenesis, antihypersion, and antioxidative effects (Koyande et al., 2019). Typically, a Puto is white in color, though variations may occur due to the flavorings such as pandan (screwpine) and ube (purple yarn). However, *Chlorella* sp. was incorporated at different concentrations (0.5, 1, and 2%) in this experiment, resulting in a change in color. Results found that the color/appearance of Puto products did not affect the preferences of the panelists; hence, Puto's appearance/color did not differ significantly ($p \geq 0.05$) based on the level of the microalgal component. Genc Polat et al. (2020) also stated that using different concentrations (0.125, 0.25, 0.5, and 0.75%) of dried encapsulated microalga *Nannochloropsis oculata* in white chocolate did not affect the appearance of the panelist's references.

The way a person perceives texture is based on their oral responses to touch; auditory stimulation may also determine the deep responses of the masseter muscle (Rustagi, 2020). In addition to the food characteristics, such as its composition and nature, the deformation rate in the mouth can influence texture perception (Mathoniere et al., 2000). Odors can be evaluated based on their hedonic

value by our sense of smell (Puleo et al., 2021). Humans share a sense of smell with many animal species that was crucial to their survival and evolution. In reality, it provides information about our surroundings, stimulates our emotional states, allows us to socialize, protects us from risks and stresses (Ludvigson and Rottman, 1989), and helps us avoid food hazards (Stevenson, 2010). It is important to consider the textural and odor attributes when developing new products to ensure their acceptance by consumers (Fradique et al., 2013). Puto is often soft or fluffy in texture with various odors depending on the flavor, such as ube (purple yarn), pandan (screwpine), or salted egg. As the panelists evaluated, adding 0.5% and 1% of *Chlorella* sp. to Puto had no significant effect, as demonstrated in the present study. However, Puto's mouthfeel and odor decreased dramatically with 2% *Chlorella* sp. enriched. It is parallel to the study of Genc Polat et al. (2020), where dried encapsulated *Nannochloropsis oculata* was not found to affect the texture and odor of white chocolate. As far as microalgae biomass for food applications is concerned, they are generally green and have volatile compounds that can cause musty, muddy, and fishy odor (Persson, 1980; Fradique et al., 2013; Andrade et al., 2018; Villaró et al., 2021). In the present study, Puto enriched with 2% *Chlorella* sp. smells slightly seaweedy, which may explain its low acceptance in terms of smell.

Taste is used to identify essential nutrients and toxic compounds. The human tongue can discern five primary flavors: sweet, umami (the taste of amino acids), sour, bitter, and salty (Briand and Salles, 2016). Puto made in this study is actually less sweet and serves as a great base for flavors such as microalgae *Chlorella* sp. The 0.5% and 1% amounts of *Chlorella* sp. component in Puto were not found to affect the significant level of this difference in taste and overall acceptability. However, Barkallah et al. (2017) mentioned that 0.75% and 1% of microalga *Spirulina platensis* concentrations added to yogurt possessed lower sensory properties of flavor and overall acceptability. *Spirulina* supplements produce an off-flavor due to their ability to oxidize lipids and minerals, which act as both pro-oxidants as well as produce metallic compounds (Shimamatsu, 2004). In addition to being strong-colored (generally) and strong smell, microalgae biomass is also disadvantageous for food applications due to its "fishy" taste (Villaró et al., 2021). 2% level of *Chlorella sp.,* in the present study, decreased overall acceptability and taste, according to the panelists. Thus, higher concentrations of *Chlorella* sp. may lead to an undesirable preference among evaluators.

Pigments play a key role in algae photosynthetic activity and have many beneficial biological properties, such as anti-inflammatory, anti-oxidant, anti-angiogenic, anti-obesity, neuroprotective, and anti-cancer properties (Guedes et al., 2011; Ciccone et al., 2013). Carotenoids and chlorophylls found in microalgae are widely used in industries like food, nutraceuticals, pharmaceuticals, cosmetics, and aquaculture (Begum et al., 2016). In photosynthesis, a plant's light processes are supported by chemical energy converted by chlorophyll (Barsanti and Gualtieri, 2005; Oo et al., 2017). The majority of microalgae contain chlorophyll *a*, but Dinophyta have chlorophyll *b* and *c* (Barsanti and Gualtieri, 2005).

In this study, chlorophyll *a* pigment levels were determined in the Puto products. Chlorophyll *a* levels varied from 14.34 to 54.06 µg g^{-1} in the Puto containing 0.5%, 1%, and 2% of *Chlorella*. In white chocolate products containing 0.125, 0.250, 0.500, and 0.750% of spray-dried microalga *Nannochloropsis oculata*, the amount of chlorophyll *a* ranged from 9.60 to 20.5 µg g^{-1}, indicating that the effects of the white chocolate production process on this pigment are tolerable (Genc Polat et al., 2020). In addition, chewing gum was determined to have chlorophyll *a* levels of 7.775-11.377 µg g^{-1} in *Isochrysis galbana* and 1.141-1.836 µg g^{-1} in *Nannochloropsis oculata* biomass (0.5% and 1%) respectively are also acceptable in the chewing gum production (Palabiyik et al., 2018). However, Puto that contains 0.5% and 1% *Chlorella*, with a chlorophyll *a* content ranging from 14.34 to 27.51 µg g^{-1}, is acceptable in the production of Puto. The encapsulation and spray drying of microalgae could be used to increase pigment concentration (Palabiyik et al., 2018; Genc Polat et al., 2020). However, the *Chlorella* sp. used in the current study was powdered using the conventional processing method, which may be the cause of the unsuitable pigment at 2% *Chlorella* sp. concentration.

Pigments called carotenoids, found in microalgae as natural sources of carotenoids, provide powerful antioxidants that prevent many human diseases and maintain good health (Britton and Khachik, 2009; De Andrade and De Andrade, 2017). Generally, they prevent the oxidation of lipids and enhance the stability and functionality of the cells' photosynthetic machinery by shielding them from reactive radicals (Grobbelaar, 2004). Palabiyik et al. (2018) mentioned that chewing gum with 0.5% and 1% biomass of microalgae *Isochrysis galbana* and *Nannochloropsis oculaa* has total carotenoids

ranging from 1.984–3.373 µg g^{-1} and 0.378–0.077 µg g^{-1} respectively. Comparatively, it is lower than the present study where Puto enriched with 0.5, 1, 2% of *Chlorella* sp. has total carotenoid pigments varied from 5.59 – 18.06 µg g^{-1}. In general, humans need carotenoids in their diets of between 10 - 20 mg daily, either from b-carotene, lutein, lycopene, a-carotene, or b-cryptoxanthin (O'Neill et al., 2001; Bohn, 2012). Hence, a puto may supply 10-20% of a person's daily carotenoid requirement.

Conclusion

Microalgae offers the opportunity to create bioactive ingredients for the food industry. Natural colorants may be incorporated into a wide variety of foods by using microalgae sources. In this study, 2% of *Chlorella* sp. did not meet the sensory expectations of the panelists due to the slightly seaweedy smell and fishy taste. Thus, higher concentrations of *Chlorella* sp. may lead to an undesirable preference among consumers. However, 0.5% and 1% *Chlorella* sp. biomass had been found to have potential as a colorant for the Filipino delicacy Puto. Hence, incorporating lower concentrations of natural colorant microalga *Chlorella* sp. into Puto is beneficial for human health, as it contains pigments like chlorophyll *a* and carotenoids that facilitate disease protection and immunity enhancement (Liu and Hu, 2013). Consequently, it has been found that *Chlorella* sp. is a potential healthy natural food coloring agent for daily products such as Puto. However, further investigation needs to be done for optimization of the *Chlorella* sp. in the Filipino delicacy Puto.

Acknowledgments

The abstract of this study was presented at the International Symposium on Fisheries and Aquatic Sciences (SOFAS 2022) in Trabzon, Türkiye, on October 25-27, 2022. The authors are also indebted to Iannie P. Maribao from the Food Engineering Department, Kastamonu University, Türkiye, for her valuable contribution to the paper.

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Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

ISSN: 1308-7576 e-ISSN: 1308-7584

Research Article

Determination of Pomological and Molecular Characteristics of Some Pomegranate (*Punica granatum* L.) Cultivars and Selected Genotypes

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Article Info

Received: 22.11.2023
Accepted: 09.01.2024
Online published: 15.03.2024
DOI: 10.29133/yyutbd.1394287

Keywords

Antioxidant,
Correlation,
Genotypes,
ISSR,
Selection

Abstract: In this study, it was aimed to determine the quality, phytochemical contents and molecular characterization of the best thirteen pomegranate genotypes selected as a result of the selection study carried out between 2019-2020 in the İnhisar district of Bilecik province. As a result of the research, the fruit weight of the selected promising genotypes and cultivars was between 208.00 and 601.3 g, fruit width was 74.54-103.47 mm, fruit length was 63.08-92.32 mm, hundred-aril weight was 28.00-66.25 g, aril yield was determined as 35.48-85.00%. The amount of soluble solid was determined between 14.33 and 18.77%, while pH values were between 3.22 and 4.36% and titratable acidity was between 0.23 and 1.72%. The total antioxidant capacity, which was determined with the TEAC method, was 3.28-8.48 µmol TE g^{-1}, while the total amount of phenolic substances was 956.10-2116.10 g GAE kg^{-1}, and the total amount of anthocyanins was 45.50-344 µg Plg-3-glu/g. Seven UBC-ISSR primers were employed to conduct molecular analyses aiming to determine polymorphism levels among the selected thirteen genotypes, along with the comparative Fellahyemez, Katırbaşı, and Hicaznar varieties. The resulting dendrogram is divided into two main clusters at a 25% dissimilarity level, one smaller and the other larger. All local genotypes clustered within the larger group, with Genotype 9 and Genotype 10 exhibiting the closest similarity. When the criteria determined as a result of the study were examined, it was determined that among the selected pomegranate genotypes, there were individuals that could be registered as table and industrial.

To Cite: Koş, N, Öztürk Erdem, S, 2024. Determination of Pomological and Molecular Characteristics of Some Pomegranate (*Punica granatum* L.) Cultivars and Selected Genotypes. *Yuzuncu Yil University Journal of Agricultural Sciences*, 34(1): 74-86.
DOI: https://doi.org/10.29133/yyutbd.1394287

Footnote: This study is derived from the first author's master thesis.

1. Introduction

Pomegranate (*Punica granatum* L.), the most important species of the *Punicaceae* family of the *Myrtales* order, is one of the very old fruit species with subtropical and tropical climate characteristics. It is known that the pomegranate fruit, whose history goes back about seven thousand years, is used by people for food and medicine. In recent years, as a result of the studies carried out in the fields of fruit

growing and breeding techniques, food technology, transportation, and storage, its production and consumption have increased every year (Kahramanoglu, 2019).

The country with the highest pomegranate production worldwide is India and it is followed by Iran, Türkiye, USA, and Iraq. With the establishment of closed pomegranate orchards in Türkiye in the last two decades, production has increased continuously, and in 2022, 681.460 tons of pomegranate have been produced in 58 provinces (TURKSTAT, 2022).

The reason for this increase is that pomegranate is rich in nutritional content, taste, aroma, antioxidants, vitamin C, and phenolic compounds. Studies have shown that pomegranate regulates the digestive system, prevents allergies, cardiovascular, cancer, and diabetes, and reduces blood pressure. It has been reported that it contains a natural source of bioactive components (Teixeira da Silva et al., 2013). It is known that the products with high antioxidant content among the vegetables and fruits that are recommended to be consumed regularly are preferred by consumers, so it has gained importance to determine the phytochemical contents in the selection studies (Montefusco et al., 2021).

It is known that pomegranate cultivars are obtained by selection studies instead of planned breeding studies in countries where there are selection studies for many fruit cultivars cultivated in the world and where pomegranate production is the highest.

Since Türkiye is one of the countries of origin of the pomegranate, there are many cultivars and types of pomegranates. In many regions of Türkiye, pomegranate cultivation is carried out with local cultivars. Various studies have been carried out on some pomegranate cultivars and genotypes in different regions such as the Aegean, Mediterranean region, and Siirt, Hatay, Tokat, Artvin, Hakkari, Bitlis, Şanlıurfa, and Diyarbakır provinces in Türkiye (Caliskan and Bayazit, 2013; Gercekcioglu et al., 2015; İkinci and Kilic, 2016; Akbel, 2017; Öztürk et al., 2019; Kos, 2022).

In recent years, biotechnological applications have also been integrated into breeding studies, known for shortening the breeding duration and enabling the selection of important traits through this method (Simsek and Etik, 2022). Until today, morphological, biochemical, and molecular markers have been extensively employed to define and assess genetic diversity in pomegranates. In many studies, molecular marker techniques such as RAPD, SSR (Orhan et al., 2014; Caliskan et al., 2018), and ISSR have been utilized (Jbir et al., 2014). The ISSR molecular marker technique has been found useful for examining genetic relationships among pomegranate genotypes and varieties, mainly due to its ability to generate a higher number of polymorphic bands (Ismail et al., 2014; Heidari et al., 2016; Almiahy and Jum'a, 2017; Hajiyeva et al., 2018; Al Mousa et al., 2019; Karapetsi et al., 2021).

This study aimed to determine the quality and phytochemical contents, as well as the molecular characterization, of the top 13 genotypes selected using the weighted grading method from among 33 naturally grown pomegranate genotypes in the Inhisar district of Bilecik province, which are well adapted to local climatic conditions.

2. Material and Methods

In 2020, in line with the information received from the Inhisar District Directorate of Agriculture and Forestry in Bilecik, the villages where pomegranate cultivation is carried out were visited and 33 genotypes, which are at the forefront in terms of yield and quality, were determined with the information obtained from the producers. Bilecik Şeyh Edebali University Agricultural Application and Research Center laboratory conducted the determination of pomological and phytochemical characteristics by obtaining three replications from each genotype, with five fruit samples taken in each replication (Akbel, 2017; Cicek et al., 2019; Ozturk et al., 2019).

The 33 selected genotypes were evaluated according to the weighted grading method. In the weighted grading method, 20% points were given for fruit weight and fruit taste, 12% for aril yield, ease of graining, and soluble solids, 10% for juice yield, 9% for seed hardness, and 5% for titratable acid. Genotypes were evaluated accordingly, out of a total of 100 points. As a result of this evaluation, 13 genotypes, which were superior and constituted the material of this study, were determined (Table 1). The standard pomegranate cultivars (Hicaznar, Fellahyemez, Katırbaşı) used for comparison were obtained from the Yalova Horticultural Research Institute.

Table 1. The genotypes selected as a result of the weighted grading method and the location where they were selected

	Genotype/Cultivar	Total score	Latitude	Longitude
1	Genotype 3	735	40°03'35.7"N	30°23'18.3"E
2	Genotype 4	795	40°03'36.7"N	30°23'21.7"E
3	Genotype 5	735	40°03'38.2"N	30°23'20.1"E
4	Genotype 8	718	40°02'33.4"N	30°25'38.7"E
5	Genotype 9	724	40°02'34.0"N	30°25'39.4"E
6	Genotype 10	706	40°02'34.6"N	30°25'41.9"E
7	Genotype 14	747	40°02'44.0"N	30°25'14.4"E
8	Genotype 19	720	40°02'45.1"N	30°25'14.1"E
9	Genotype 20	605	40°03'33.5"N	30°22'54.8"E
10	Genotype 22	848	40°03'33.7"N	30°22'54.0"E
11	Genotype 24	813	40°03'29.0"N	30°22'46.7"E
12	Genotype 27	742	40°03'26.4"N	30°22'44.7"E
13	Genotype 33	718	40°03'54.1"N	30°23'02.7"E
14	Hicaznar	Yalova Horticultural Research Institute		
15	Fellahyemez	Yalova Horticultural Research Institute		
16	Katırbaşı	Yalova Horticultural Research Institute		

Fruit width (mm), fruit length (mm), calyx diameter and length (mm), and peel thickness (mm) were measured with a digital caliper (OEM KMP200 Digital caliper) and expressed in millimeters (mm). Fruit weight (g), hundred-aril weight (g), and seed weight (g) were determined by weighing with a precision balance (Kern PNS600) sensitive to 0.01 g. The total soluble solids content of arils was measured using a digital handheld refractometer and expressed as degrees Brix. Titratable acidity was determined by titration of 5 mL of fruit juice with 0.1 N NaOH and expressed as a percentage of citric acid content (Simsek and Etik, 2022). Peel and aril color were determined with the CR 400 Model Minolta Colorimeter (Konica Minolta, CR400) device. pH was measured by a ph-meter (Hanna edge pH parameter).

Total antioxidant capacity was determined according to the TEAC method, which is frequently used for plant materials, according to Özgen et al. (2008). The amount of total phenol was determined according to the method of Singleton and Rossi (1965) using Folin-Ciocalteu's chemical and the results were calculated as µg gallic acid equivalent/g fresh fruit in gallic acid. The total anthocyanin amount was determined according to the pH difference method according to Giusti and Wrolstad (2005), and the values were calculated as µg anthocyanin / g dry matter.

DNA isolation was performed according to the protocol of Doyle and Doyle (1987). DNA samples were quantified using a spectrophotometer, and their concentration was diluted to 10 ng μl^{-1}. In the PCR application, it was initially planned to use 15 primers that had previously successfully amplified in pomegranate. However, due to the inability to achieve amplification with eight of these primers, the study was conducted using seven primers (UBC807; 808; 811; 826; 835; 889; 891) (Jbir et al., 2014; Almiahy and Jum'a, 2017; Amar and El-Zayat, 2017).

The ISSR PCR reaction was performed with a 23 µL reaction mixture of 2 µL DNA (10 ng μl^{-1}) (Kos, 2022). The PCR products were separated for approximately 2 hours at a constant voltage of 100V using 1X TBE buffer and a 2% agarose gel.

The mean minimum and maximum value analyses of the data obtained as a result of the research were made using the SPSS 16.0 package program. Moreover, biplot plots and correlation analysis were performed using the JMP (2020) program. In the molecular section, the data used in the statistical analysis were scored as one (1) in the presence of ISSR bands and zero (0) in their absence. Similarities and differences between genotypes were studied at the molecular level. Principal Coordinates Analysis (PCoA) was performed using similarity coefficients, and analysis was conducted using the Popgene32 version 1.32 (Population Genetic Analysis) and MEGA 5.0 (Molecular Evolutionary Genetic Analysis) software packages. A UPGMA (Unweighted Pair-Group Method with Arithmetic Average) dendrogram was constructed based on the UPGMA method to visualize the relationships among genotypes.

Statistical analysis was performed using the Minitab 19 package program. The data were submitted for variance analysis and the means were tested by the least significant difference ($p<0.05$).

3. Results and Discussions

Fruit weight (g), fruit width (mm), fruit length (mm), hundred-aril weight (g), aril yield (%), juice volume (%), calyx length (mm), calyx diameter (mm), calyx number, peel thickness (mm), flavour and seed hardness in fruits of promising pomegranate genotypes cultivars are given in Table 2. The mean fruit weight of genotypes and cultivars was determined as 332.1 g. While the lowest fruit weight was obtained in Genotype 3 with 208.00 g, the highest fruit weight was determined as 601.25 g in the Fellahyemez cultivar, and the highest fruit weight among genotypes was determined as 399.80 g in Genotype 8 (Table 2). In studies conducted on pomegranate cultivars and genotypes in different regions, fruit weights have been determined as 267.72-650.56 g by Kilic (2014), 251.01-530.25 g by Gundogdu et al. (2015), 267.72-650.56 g by Ikinci and Kilic (2016), 205.44-525.87 by Boguc (2018), 207.30-689.50 g by Ozturk et al. (2019), and 201.55-637.50 g by Dursun (2021).

As seen in Table 2, fruit width and length means were determined as 84.25 and 74.26 mm, respectively. When the genotypes were examined, it was determined that the width and length ratio of Genotype 9 had the highest value. When the previous studies were examined, the fruit width and length values in the present study were found to be similar to other studies (Okatan et al., 2015; Dursun, 2021; Simsek and Etik, 2022).

It was determined that the hundred-aril weight of the genotypes and cultivars was between 27.00-61.00 g, and the aril yield was between 35.48-85.00% (Table 2). In previous studies, while Akbel (2017) weighed hundred-aril with 30 pomegranate genotypes in the Central Sakarya Basin as 17.50-46.60 g, Özden et al. (2017) determined three pomegranate cultivars in Şanlıurfa province as 32.33-61.20 g, Boguc (2018) found as 36.98-61.81 g in their study in Şırnak province, and Simsek and Etik (2022) determined the hundred-aril weight as 19.77-35.07 g in their study in Diyarbakır province. When the hundred-aril weight results obtained in our study were compared with the previous studies, it was determined that they were in a similar value range. While in the study that was carried out on three pomegranate genotypes grown in the Adana region, Gercekcioglu et al. (2015) found the aril yield as 71.33%-81.17%, Ozturk et al. (2019) determined it as 40.50-78.40% in 18 pomegranate genotypes grown in Mardin districts, Dursun (2021) reported that they found the aril yield between 43.55% and 68.98% in the study they conducted with some pomegranate cultivars in Şanlıurfa province. As a result of our study, it was determined that the aril yield value was higher when compared to previous studies.

The mean fruit juice yield of the genotypes and cultivars included in the study was found to be 39.88%. The lowest juice yield was found in Genotype 27 (25.20%) among the genotypes, and in the Fellahyemez cultivar (24.62%) among the cultivars, while the highest juice yield was found in Genotype 3 with 62.12% among the genotypes (Table 2). In the previous studies, Ozturk et al. (2019) reported that the juice yield of local pomegranate genotypes varied between 32-66% in their study in Mardin province. In addition, the fruit juice yield results obtained in our study were found to be similar to previous studies.

The sensory-evaluated fruit flavours were determined as sourish, sweet-sour, and sweet, and the seed hardness was determined as hard, medium-hard, soft, and very soft.

While the lowest calyx length was measured in Genotype 3 with 12.85 mm, the lowest calyx diameter was measured in Genotype 4 with 19.83 mm. Moreover, the highest calyx length and diameter were determined in Genotype 20 with 16.49 mm and 34.78 mm in the genotypes and cultivars in the study, respectively (Table 2). The calyx length and diameter obtained were similar to those of other researchers (Akbel, 2017; Ozturk et al., 2019; Dursun, 2021).

Peel L, a, b, values of genotypes and cultivars are presented in Table 2. The L* value expressing the peel brightness of the fruit was 28.22-56.85, the a value expressing the change of the fruit peel from green to red colour was 6.39-32.51, and the b value expressing the change of the fruit peel from yellow to blue colour was determined between 16.76 and 29.67. Compared to previous studies, L and the values of the peel were similar (Yaman et al., 2015; Akbel, 2017). It is thought that this difference, in which the b value differs from previous studies, is due to the ecological conditions of the region where the fruits are grown (Akbel 2017; Toprak 2019). It was determined that while the L value of aril colour varied between 7.21 and 22.05, the a value was 13.91-24.62 and the b value was between 4.62 and 16.53 (Table 2). The findings obtained in the study were similar to other studies (Akbel 2017; Toprak 2019).

Table 2. Pomological characteristics of pomegranate genotypes and cultivars

Genotype	Fruit						Calyx		Peel							Aril	
	Weight (g)	Width (mm)	Length (mm)	Hundred-aril weight (g)	Aril yield (%)	Juice volume (%)	Diameter (mm)	Length (mm)	Thickness (mm)	L	a	b	L	a	b	Flavor	Seed hardness
	**	**	**	**	**	**	**	*	**	**	**	**	**	**	**		
Genotype 3	208.0^{i}	79.4^{efg}	71.5^{def}	33.0^{fg}	85.0^{a}	62.1^{f}	20.1^{g}	12.9^{c}	6.4^{bc}	53.5^{ab}	6.4^{g}	29.7^{a}	21.2^{a}	21.4^{cd}	10.7^{c}	Sweet-sour	Medium-hard
Genotype 4	255.0^{h}	77.0^{fg}	63.9^{g}	60.0^{ab}	78.0^{b}	58.4^{d}	19.8^{g}	13.1^{bc}	4.6^{cde}	43.6^{cd}	19.4^{c}	24.9^{bcd}	16.0^{d}	18.3^{ef}	11.4^{bc}	Sweet	Medium-hard
Genotype 5	209.0^{i}	75.3^{fg}	63.1^{g}	28.0^{g}	82.5^{a}	56.8^{g}	20.9^{fg}	12.9^{c}	4.4^{cde}	44.7^{cd}	32.5^{a}	24.4^{cd}	19.9^{ab}	24.0^{ab}	13.8^{ab}	Sweet-sour	Medium-hard
Genotype 8	399.8^{c}	79.6^{efg}	71.8^{def}	57.0^{abc}	43.3^{l}	32.3^{g}	23.4^{def}	15.4^{abc}	4.2^{de}	52.5^{b}	10.9^{f}	27.7^{ab}	22.1^{a}	14.9^{gh}	14.0^{ab}	Sweet	Soft
Genotype 9	338.0^{e}	88.0^{bc}	77.2^{c}	41.0^{ef}	51.1^{fgh}	33.3^{i}	33.5^{a}	14.5^{abc}	4.4^{cde}	43.4^{cd}	17.2^{c}	23.6^{d}	17.5^{bcd}	19.1^{def}	10.6^{c}	Sweet-sour	Medium-hard
Genotype 10	323.4^{f}	84.0^{b-e}	76.7^{c}	45.0^{de}	52.8^{f}	31.3^{h}	28.6^{b}	14.3^{abc}	5.1^{cde}	52.2^{b}	19.2^{c}	26.7^{abc}	16.0^{d}	20.1^{cde}	11.5^{bc}	Sweet	Hard
Genotype 14	275.6^{g}	81.7^{c-f}	73.1^{d}	48.0^{cde}	62.6^{e}	41.1^{g}	28.2^{b}	15.6^{ab}	5.1^{cde}	41.7^{d}	17.1^{cd}	23.0^{d}	13.1^{e}	21.0^{cd}	10.7^{c}	Sweet	Medium-hard
Genotype 19	214.8^{i}	74.5^{g}	68.9^{f}	43.0^{def}	48.6^{hij}	28.6^{l}	26.1^{bcd}	15.4^{abc}	4.6^{cde}	45.5^{c}	13.2^{ef}	24.3^{cd}	11.5^{e}	22.1^{abc}	10.4^{cd}	Sweet	Soft
Genotype 20	313.2^{f}	86.8^{bcd}	76.9^{c}	45.0^{de}	46.4^{jk}	36.8^{j}	34.8^{a}	16.5^{a}	5.9^{bcd}	56.9^{a}	24.1^{b}	28.3^{a}	17.3^{bcd}	21.2^{cd}	11.3^{bc}	Sweet-sour	Medium-hard
Genotype 22	246.6^{h}	79.3^{efg}	69.4^{ef}	42.0^{def}	74.8^{c}	53.0^{e}	26.5^{bc}	14.0^{abc}	4.8^{cde}	53.2^{b}	23.1^{b}	28.3^{a}	19.4^{abc}	15.5^{gh}	9.6^{cd}	Sweet	Soft
Genotype 24	269.0^{g}	79.8^{d-g}	72.4^{de}	44.0^{def}	66.0^{d}	59.0^{c}	22.4^{efg}	15.3^{abc}	4.4^{cde}	53.7^{ab}	7.5^{g}	27.5^{ab}	20.4^{a}	24.6^{a}	16.5^{a}	Sweet	Soft
Genotype 27	345.2^{de}	88.0^{bc}	74.8^{cd}	40.0^{ef}	51.4^{fg}	25.2^{g}	22.7^{efg}	13.5^{bc}	3.5^{e}	46.5^{c}	14.3^{de}	27.7^{ab}	17.2^{cd}	21.8^{bc}	10.7^{c}	Sweet	Soft
Genotype 33	354.0^{d}	79.7^{efg}	69.6^{ef}	53.0^{bcd}	35.5^{m}	27.4^{k}	25.0^{cde}	13.5^{bc}	4.4^{cde}	54.9^{e}	17.8^{c}	29.9^{d}	16.9^{bcd}	16.0^{h}	8.6^{d}	Sweet	Soft
Hicaz Nar	568.2^{b}	101.4^{a}	92.3^{a}	38.0^{efg}	49.4^{ghi}	36.5^{a}	24.9^{cde}	14.3^{abc}	7.8^{ab}	28.2^{f}	30.5^{a}	16.8^{e}	7.2^{f}	14.1^{h}	4.6^{e}	Sweet-sour	Medium-hard
Fellahyemez	601.3^{a}	103.5^{a}	91.6^{a}	66.0^{a}	46.9^{ij}	24.6^{b}	24.0^{cde}	13.1^{bc}	8.7^{a}	35.4^{e}	17.8^{c}	22.8^{d}	17.3^{bcd}	13.9^{h}	7.7^{d}	Sweet	Medium-hard
Katırbaşı	393.0^{c}	90.1^{b}	83.3^{b}	60.0^{ab}	44.1^{kl}	31.6^{hi}	28.1^{b}	16.3^{a}	9.8^{a}	46.0^{c}	10.8^{f}	29.1^{a}	21.3^{a}	16.7^{fg}	9.5^{cd}	Sweet	Soft
Mean	332.1	84.3	74.8	45.8	57.4	39.9	25.6	14.4	5.5	45.8	17.6	25.5	17.2	18.9	10.7		
Min.	208.0	74.5	63.1	27.0	35.5	23.6	19.8	12.9	3.5	28.2	6.4	16.8	7.2	13.9	4.6		
Max.	601.3	103.5	92.3	61.0	85.0	63.2	34.8	16.5	9.8	56.9	32.5	29.7	22.1	24.6	16.5		

*: Significant at the p<0.05 probability level, **: Significant at the p<0.01 probability level.

It is known that the amount of soluble solid is important in terms of quality criteria since it determines the amount of sugar in the fruit content and fruits with high sugar content are demanded by consumers. In the study, the amount of soluble solids varied between 14.33-18.77%, and while the lowest amount of soluble solids was determined in Genotype 33 (14.67%), the highest was observed in Genotype 22 (18.77%). Among the cultivars, the lowest amount of soluble solid was found in the Fellahyemez cultivar with 14.33%, and the highest amount of soluble solid was found in the Hicaznar cultivar with 15.7% (Table 3).

In the previous studies on the soluble solid value of pomegranate, while Akbel (2017) found the soluble solid value between 15.60-24.00% in the study conducted in the Central Sakarya Basin, Boguc (2018) determined the soluble solid value between 15.90-18.20% in the study conducted with pomegranate cultivars and genotypes in Sırnak province. Furthermore, while Dursun (2021) found the soluble solid value between 14.60-16.60% in a study conducted with different pomegranate cultivars in Sanlıurfa province, Cicek et al. (2019) found the soluble solid value of ten pomegranate genotypes between 15.00-21.00% in their study in the districts of Diyarbakır province, and Öztürk et al. (2019) found that the soluble solid values of 18 pomegranate genotypes between 15.00-18.00% in their study in the districts of Mardin province. The values we found are similar to previous studies.

Table 3. Amount of soluble solid (%) of selected promising genotypes and cultivars

Genotype	TSS	pH	TA	TAA	TMA	TPA
	**	ns	**	**	**	**
Genotype 3	15.9^{b-e}	3.48	0.47^{cde}	5.22^{c-f}	104.7^{f}	1917.8^{cd}
Genotype 4	16.4^{a-e}	3.37	0.50^{bcd}	5.60^{cd}	72.4^{g}	1927.8^{c}
Genotype 5	17.8^{abc}	3.38	0.47^{cde}	6.80^{b}	116.6^{f}	2116.1^{a}
Genotype 8	16.8^{a-e}	3.33	0.58^{bc}	4.36^{efg}	53.6^{h}	1663.6^{fg}
Genotype 9	17.3^{abc}	3.39	0.59^{bc}	5.38^{cde}	142.2^{de}	2014.5^{abc}
Genotype 10	16.7^{a-e}	3.27	0.61^{b}	5.20^{c-f}	160.7^{bc}	1779.5^{ef}
Genotype 14	18.4^{ab}	3.41	0.54^{bc}	8.48^{a}	108.7^{f}	2104.5^{a}
Genotype 19	16.9^{a-e}	3.35	0.58^{bc}	6.16^{bc}	131.0^{e}	1973.6^{bc}
Genotype 20	15.8^{b-e}	3.43	0.53^{bcd}	5.56^{cd}	152.5^{cd}	1802.0^{de}
Genotype 22	16.7^{a-e}	3.37	0.46^{cde}	5.19^{c-f}	172.7^{b}	1567.0^{g}
Genotype 24	17.0^{a-d}	3.37	0.54^{bc}	6.65^{b}	83.8^{g}	2079.5^{ab}
Genotype 27	18.8^{a}	3.98	0.40^{de}	4.27^{fgh}	75.5^{g}	1430.3^{h}
Genotype 33	14.7^{de}	3.44	0.52^{bcd}	4.63^{d-g}	81.7^{g}	1390.3^{h}
Katırbaşı	14.5^{de}	3.24	0.36^{ef}	3.28^{gh}	149.8^{a}	1119.5^{ef}
Fellahyemez	14.3^{e}	4.36	0.23^{f}	6.15^{bc}	47.5^{h}	956.1^{j}
Hicaznar	15.7^{cde}	3.22	1.72^{a}	3.61^{h}	344.6^{cd}	1729.5^{i}
Mean	16.5	3.46	0.57	5.41	124.9	1723.2
Min.	14.3	3.22	0.23	3.28	47.5	956.1
Max.	18.8	4.36	1.72	8.48	344.6	2116.1

*: Significant at the p<0.05 probability level, **: Significant at the p<0.01 probability level, ns: non-significant, TSS: Total soluble solid (%); pH; TA: Titratable acidity (%); TAA: Total antioxidant amount (µmol TE g^{-1}); TMA: Total anthocyanin content (µg Plg-3-glu g^{-1}); TPA: Total phenol amount (g GAE kg^{-1}).

In the study, pH values were found to be between 3.22 and 4.36. The lowest pH value was found in the Katırbaşı cultivar as 3.22, while the highest pH value was found in the Fellahyemez cultivar as 4.36 (Table 3). In the previous studies conducted on the pH value of pomegranates are examined, while Gündogdu et al. (2015) observed as 3.45-4.71 in the study they conducted with Silifke aşı and Hicaznar cultivar, and Boguc (2018) stated that the pH value of pomegranates cultivars between 3.57-3.96 in the study conducted with Hicaznar and four local cultivars.

Titratable acidity (TA) values of genotypes and cultivars were found to be between 0.23-1.72%. The lowest TA value was found in the Fellahyemez cultivar with 0.23%, while the highest value was found in the Katırbaşı cultivar with 1.72% (Table 3). In the previous studies on the titratable acidity value of pomegranate, Dursun (2021) observed between 0.67 and 2.74 in a study conducted with Hicaznar, Katırbaşı, Devedişi, Suruc, and Suruc Karası cultivars in Şanlıurfa province. The taste of

pomegranate juices is associated with titratable acidity values. The titratable acidity of pomegranate juice is known as Sweet Pomegranate with less than 1%, Sourish Pomegranate with 1-2%, and Sour Pomegranate with more than 2%. Accordingly, in the results we found, the taste of pomegranate genotype and cultivars can be evaluated as sweet.

The relationship analysis performed is given in Table 4. According to the analysis, there was a significant and positive relationship between weight and width (r=0.911**), length (r=0.885**), juice yield (r=0.648**), and peel thickness (r=0.599*). Besides, there was a negative and significant relationship between weight and aril yield (r=-0.606*), peel colour L value (r=-0.615*), peel colour b value (r=-0.512*), aril colour a value (r=-0.701**), aril colour b value (r=-0.593*), and total soluble solid (r=-0.605*) (Table 4). Furthermore, a significant and positive relationship was determined between peel thickness (PT) and weight (r=0.599*), width (r=0.692**), and length (r=0.760**). The priority feature of pomegranate in both table consumption and processing in the food industry is the juice yield (Gündogdu et al. 2015). A significant and positive relationship was found between juice yield, which was obtained by dividing juice weight by fruit weight, weight (r=0.648**), width (r=0.685**), and length (r=0.611*).

It is known that TSS, pH, and titratable acidity ratios can be caused by characteristics of the variety as well as being affected by climate, soil, and cultural practices. It was expected that there were differences between the cultivars and genotypes examined in the study. There was a significant and positive relationship between TSS and aril colour a value (r=0.679**) and aril colour b value (r=0.606*) while there was a significant and negative relationship between TSS and weight (r=-0.605**), width (r=-0.511**), length (r=-0.549*), and peel thickness (r=-0.658**).

When Table 4 is examined, there was significant and positive relationship between the peel colour L value (PCL) and the peel colour b value (r= 0.895**), the aril colour L value (r=0.623*), the aril colour a value (r= 0.511*) and aril colour b value (r= 0.721**) (Table 4).

When the total phenolic substance content of the genotypes and cultivars was examined, it was determined that it ranged from a minimum of 940.3 g GAE kg^{-1} to a maximum of 2205.30 g GAE kg^{-1}. While the lowest amount of phenolic substance was observed in Genotype 33 (1390 g GAE kg^{-1}) among the genotypes, it was observed in the Fellahyemez cultivar (956.11 g GAE kg^{-1}) among the cultivars. Moreover, the highest value was determined in Genotype 5 (2116.11 g GAE kg^{-1}). Ozgen et al. (2008) reported that the total phenolic content of six pomegranate cultivars grown in Türkiye was between 1245-2076 g GAE kg^{-1}, Akhavan et a. (2015) found between 943-2931 g GAE kg^{-1}, Okumus (2016) determined that the total phenolic substance content of Wonderful and Hicaznar cultivars was 1156.67-1428.1 g GAE kg^{-1}, and Akbel (2017) reported that the total phenolic content of pomegranate genotypes in the Central Sakarya Basin ranged from 551 to 3282 g GAE kg^{-1}.

Like other fruits, the physical and chemical properties of pomegranate, its phenolic content, and therefore its antioxidant activity can vary according to many factors such as variety, maturity, climate, growing region, and cultural practices. For these reasons, it is thought that the results obtained from the genotypes and cultivars in the study are partially similar as they are in the range of values in the literature.

In the study, the TEAC method was used to determine the antioxidant capacity of pomegranates. The total antioxidant capacity of genotypes and cultivars was determined at a mean of 5.40 µmol TE g^{-1}. The lowest antioxidant capacity was found in Katırbaşı (3.28 µmol TE g^{-1}) cultivar among the cultivars and in Genotype 27 (4.27 µmol TE g^{-1}) among the genotypes. Furthermore, the highest antioxidant capacity was determined in Genotype 14 (8.48 µmol TE g^{-1}).

The total anthocyanin content of the genotypes was determined as 124.88 µg Plg-3-glu g^{-1} ta between genotypes and cultivars. The lowest amount of anthocyanin among genotypes was observed in Genotype 8 (53.6 µg Plg-3-glu g^{-1} ta), and the highest amount of anthocyanin was in Genotype 22 (172.67 µg Plg-3-glu g^{-1} ta). Moreover, among cultivars, the Hicaznar cultivar (344.55 µg Plg-3-glu g^{-1} ta) was observed with the highest amount of anthocyanin (Table 3).

The classification of the traits examined in the study according to genotypes and the change of genotypes according to the traits are given in Figure 1. In the biplot graph, if the angle between the vectors is less than 90, it shows that the performance of that genotype is better than the mean, if the angle between the vectors is greater than 90, the performance of the genotype is lower than the mean, and if the angle is equal to 90, it is close to the mean (Yan and Tinker, 2006).

Table 4. Correlation coefficients and significance levels of the analyzes

	Wg	Wd	L	HAW	AY	JY	CD	CL	PT	PCL	PCA	PCB	ACL	ACA	ACB	TSS	pH
Wd	0.911**																
L	0.885**	0.963**															
HAW	0.480	0.290	0.287														
AY	-0.606*	-0.454	-0.519*	-0.472*													
JY	0.648**	0.685**	0.611*	0.192	0.168												
CD	0.110	0.246	0.308	0.041	-0.503*	-0.306											
CL	0.001	0.010	0.179	0.192	-0.467	-0.312	0.645**										
PT	0.599*	0.692**	0.760**	0.370	-0.214	0.468	0.128	0.165									
PCL	-0.615*	-0.531*	-0.457	-0.194	0.304	-0.372	0.131	0.317	-0.326								
PCA	0.168	0.198	0.076	-0.296	0.098	0.182	0.154	-0.212	0.008	-0.387							
PCB	-0.512*	-0.434	-0.384	0.007	0.236	-0.35	-0.007	0.202	-0.133	0.895**	-0.550						
ACL	-0.290	-0.325	-0.338	0.111	0.227	-0.122	-0.171	-0.049	-0.081	0.623*	-0.440	0.789**					
ACA	-0.701**	-0.498	-0.48	-0.579*	0.465	-0.322	-0.050	0.108	-0.425	0.511*	-0.146	0.356	0.119				
ACB	-0.593*	-0.629**	-0.604*	-0.153	0.362	-0.233	-0.168	0.190	-0.533*	0.721**	-0.346	0.585*	0.588*	0.677**			
TSS	-0.605*	-0.511*	-0.549*	-0.401	0.342	-0.333	-0.012	0.091	-0.658**	0.419	-0.111	0.324	0.147	0.679**	0.606*		
pH	0.427	0.477	0.336	0.288	-0.127	0.419	-0.191	-0.426	0.139	-0.228	-0.092	-0.043	0.069	-0.127	-0.194	-0.09	
TA	-0.056	-0.034	0.089	0.281	-0.230	-0.295	0.252	0.541*	0.449	0.164	-0.314	0.374	0.310	-0.042	0.055	-0.024	-0.428

*p<0.05 and **p<0.01 are significant.

Wg: weight (g); Wd: width (mm); L: length (mm); HAW: Hundred-aril weight (gr); AY: Aril yield (%); JY: Juice yield (%); CD: Calyx diameter (mm); CL: Calyx length (mm); PT: Peel thickness (mm); PCL: Peel color L; PCA: Peel color a; PCB: Peel color b; ACL: Aril color L; ACA: Aril color a; ACB: Aril color b; TSS: Total soluble solid (%);Ph (%); TA: Titratable acidity.

When Figure 1 is examined, it can be seen which genotypes have higher values in terms of the characteristics discussed, and whether these characteristics are positively or negatively related to each other. In Biplot analysis, Major component 1 was 37.2% and Major component 2 was 18.2%, constituting 55.4% of the variation in total (Figure 1).

Hicaznar and Fellahyemez cultivars, which are high in weight, came to the fore in terms of width, length, juice yield, pH, peel thickness, and hundred-aril weight. As seen in Figure 1, in the correlation analysis performed between the features in the same group, it was determined that the relationship between these features was significant and positive at the 1% and 5% levels (Table 4). A strong positive correlation was found between titratable acid amounts, total phenol amount, calyx diameter, and calyx length for the Katırbaşı cultivar included in the study. Besides, it was determined that there was a very strong positive correlation (<90) between total antioxidant amount and aril yield for Genotype 3, Genotype 4, and Genotype 5.

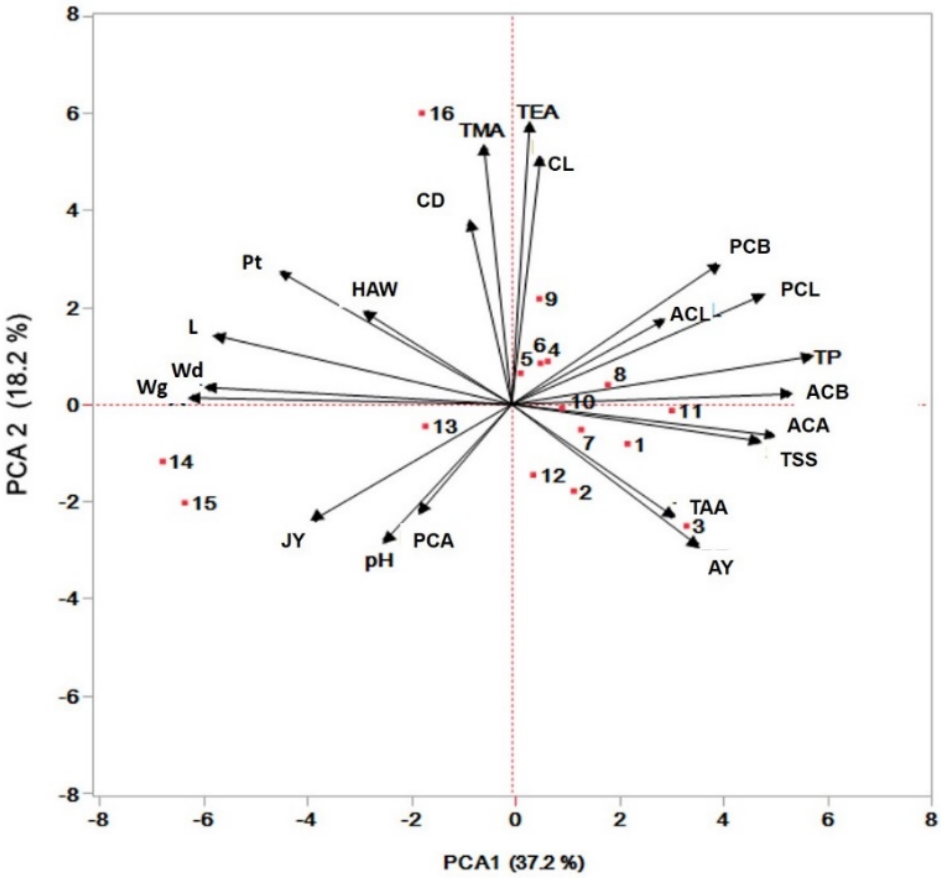

Figure 1. Grouping the examined traits with the biplot analysis method and the relationship of genotypes with the examined traits.

(Wg: weight (g); Wd: width (mm); L: length (mm); HAW: Hundred-aril weight (gr); AY: Aril yield (%); JY: Juice yield (%); CD: Calyx diameter (mm); CL: Calyx length (mm); PT: Peel thickness (mm); PCL: Peel color L; PCA: Peel color a; PCB: Peel color b; ACL: Aril color L; ACA: Aril color a; ACB: Aril color b; TSS: Total soluble solid (%); pH; TA: Titratable acidity (%); TAA: Total antioxidant amount; TMA: Total anthocyanin content; TPA: Total phenol amount).

In the PCR analysis, seven ISSR primers were utilized, resulting in the formation of 51 bands, of which 41 were found to be polymorphic. The number of bands obtained from the ISSR primers ranged from five to twelve, with an average of 7.29 bands and an average of 5.86 polymorphic bands. Among the used primers, the highest band count was obtained from primer 808 (12 bands), while the lowest band count was from primers 811 and 891 (5 bands each). The lowest polymorphism rate was determined to be 60.00% in primer 811. The average polymorphic band ratio among the seven primers was found to be 80.39%.

As seen in Figure 2, the dendrogram depicts two main clusters at a similarity level of 25%, one larger and the other smaller. The smaller cluster includes the varieties Hicaznar and Fellahyemez. The

larger cluster further divides into two subgroups at a 19% level of dissimilarity. Within these subgroups, the Katırbaşı variety forms one branch, while the other branch comprises various genotypes.

In the study, the closest similarity (2%) among the used genotypes was determined between Genotype 9 and Genotype 10. It was observed that these genotypes were selected from neighboring locations.

During the survey conducted in the İnhisar district, it was established that the grown pomegranates differed from one another. Pomegranates referred to by local farmers as Devedişi were designated as Genotypes 14, 19, and 20, and it was noted that these genotypes exhibited similarity in the dendrogram.

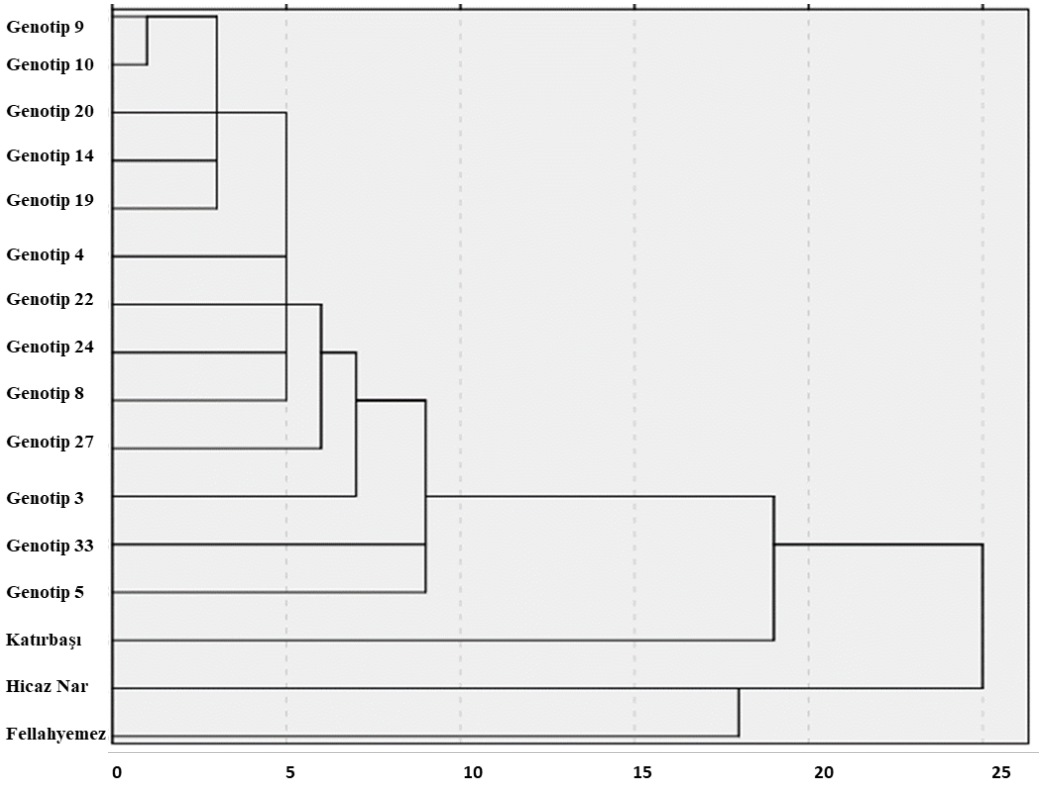

Figure 2. Dendogram obtained by cluster analysis.

Conclusion

It is known that many fruit cultivars cultivated in the world are found by selection studies and in countries where pomegranate production is most common, pomegranate cultivars are obtained by selection studies instead of planned breeding studies. In this study, it was aimed to determine the quality and phytochemical analyzes of selected pomegranate genotypes.

It is known that weight, taste, and seed hardness are important criteria in genotypes selected as promising in previous studies. When the fruit weights and sizes were examined in the study, it was determined that the standard pomegranate cultivars were heavier and more voluminous than the genotypes on the mean. Moreover, fruit weights and sizes are affected by environmental factors and cultural practices as well as depending on the cultivar.

Besides, Genotype 8, Genotype 33, and Genotype 27 can be considered promising table genotypes since they have higher fruit weights compared to other genotypes, as well as being sweet and having soft seed hardness.

Although the fruit sizes of the genotypes are generally smaller than the standard cultivars, the high fruit juice yields of the genotypes allow these types to be used effectively in the fruit juice processing industry. The priority feature of pomegranate in its industrial use is its fruit juice efficiency. As a result of the study, the aril and juice yields of Genotype 3, Genotype 4, and Genotype 5 were found

to be higher than the other genotypes. According to these results, the use of genotypes in the juice processing industry can be evaluated.

When the fruit taste and seed hardness of the genotypes and cultivars in the study are compared with the studies in the literature, it is thought that the fruit taste is between sweet and sweet-sour, the seed hardness is medium and soft, and it is suitable for both table consumption and fruit juice production.

The study is thought to be a guide for researchers and producers in the process of standardizing promising genotypes and expanding their commercial production.

Acknowledgements

This work has been supported by the Scientific Research Projects Coordination Unit of Bilecik Seyh Edebali University under the project number 2020-02.BŞEÜ.01-01.

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Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

ISSN: 1308-7576 e-ISSN: 1308-7584

Research Article

Low-Cost Classification of Close and Open Shell Antep Pistachio Nuts based on Image Analysis and Machine Learning

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Article Info

Received: 22.06.2023
Accepted: 29.02.2024
Online published: 15.03.2024
DOI: 10.29133/yyutbd.1318589

Keywords

Classification,
Decision Tree,
Gaziantep Pistachio,
Image Analysis,
Image Processing,
Random Forest

Abstract: The effectiveness of post-harvest industrial processes is critical to maintaining the economic worth of pistachio nuts, which play an essential role in the agricultural economy. To achieve this level of efficiency, updated applications and technology for pistachio separation and categorization are required. Different pistachio species target different markets, highlighting the need for pistachio species classification. This work aims to develop a classification model that is distinct from existing separation approaches, based on image processing and machine learning, and can provide the required categorization. A computer vision application was done to identify between three types of pistachios. A high-resolution camera was used to capture 385 images of these pistachios. The photos of the pistachio samples were processed using image processing techniques like segmentation and feature extraction. On the given dataset, an advanced classifier based on Decision Tree and Random Forest predictions was constructed, as well as a simple and successful classifier. In the research, an application with feature extraction based on the dimension and pixel measurement is proposed. The proposed approach attained a classification success rate of 100% at 70% train and 30% test, and also, 80% train and 20% test data rate with Random Forest prediction, according to the experimental data. The provided high-performance classification model fills an important demand for the separation of pistachio types while increasing the economic worth of the species.

To Cite: Beyaz, A, 2024. Low-Cost Classification of Close and Open Shell Antep Pistachio Nuts based on Image Analysis and Machine Learning. *Yuzuncu Yil University Journal of Agricultural Sciences*, 24(1): 87-105. DOI: https://doi.org/10.29133/yyutbd.1318589

1. Introduction

Pistachio has a high economic value in the world. The quality of the pistachio nuts produced has a significant impact on their popularity among consumers. When pistachios arrive at the processing plant, the following procedures are carried out (a) dehulling, which involves separating the soft hull from the pistachios; (b) trash and blank separation, which involves removing blank pistachios and trash such as small branches, remaining shells, and leaves; (c) unpeeled pistachios separation, which involves removing unpeeled and unripe nuts (Omid et al., 2009). The quality of pistachios is affected by factors that often come together as planting, human harvesting, transportation, storage, etc. (Brosnan and Sun, 2002).

Inspection and categorization of mixed pistachios into lots of uniform shapes and sizes is desirable to provide consumers with a more uniform product. Visual inspection is typically performed by human operators, and its output is influenced by a variety of factors, including operator age, concentration and motivation, fatigue and visual acuity, and room conditions (lighting, heating, ventilation, noise, and so on); for these reasons, automated systems are especially welcome (Omid et al., 2009).

The importance of optimizing post-harvest procedures cannot be overstated. Common practice involves categorizing pistachios as either open (with the shell split) or closed (with the shell intact). These subsets are processed independently later on. Pistachios are promoted as a snack food and are typically served as roasted nuts. Unsplit pistachios are unsuitable because they are difficult to open and may contain immature kernels, so they cannot be used for these purposes. Therefore, separating pistachios into open and closed shells is an essential part of the post-harvest procedure (Ghezelbash et al., 2013).

Pistachios have a lot of healthy nutrients. There are 560 calories in 100 g of it, and it's a good source of protein, fiber, minerals, and vitamins B, thiamine, and B6. Pistachios have many positive health effects, especially on the cardiovascular system (Kay et al., 2010; Ertürk et al., 2011; Dreher et al., 2012).

Pistachios are grown in 56 of Türkiye's province, making the country the world's third-largest producer. Pistachio production in Türkiye is increased by planting Kirmizi and Siirt species, which have larger fruits and less of a tendency toward periodicity (Ertürk et al., 2011).

Pistachios are typically categorized based on several factors, one of the most well-known being the nuts' quality. Close-head pistachios are particularly important from an economic, export, and marketing perspective because pistachios are one of the most expensive agricultural products, and their prices are based on their quality. A highly precise and user-friendly system is needed to prevent such losses. Several methods have been developed in recent years by scientists for accurately identifying agricultural products, most notably pistachios (Mahmoudi et al., 2006). Mechanical winnowing is impossible for pistachios because their kernel-close shell and hollow-close shell structures are so similar. Additionally, the carcinogen aflatoxin may be introduced to pistachios using floating techniques (Pearson et al., 1994).

Pistachios have been sorted using a wide variety of methods, including optical, mechanical, electrical, and acoustic approaches. Using machine vision, it is possible to identify pistachios that have been damaged or opened too soon (Pearson, 1996).

As an alternative to more conventional electro-optical and mechanical sorting devices, machine vision can be used to classify pistachio nuts. Interest in using machine vision for sorting and grading agricultural products has increased over the past two decades (Ghazanfari et al., 1998). Rapid growth is being seen in low-priced post-harvesting systems like those that use computer vision for sorting.

In their study, a computer-vision-based intelligent system is developed at a reasonable cost for sorting pistachios (Ghezelbash et al., 2013). The limitations of real-time applications make it challenging to implement many different methods, such as Fourier methods, spectral methods, or active contours. Thus, straightforward methods are appropriate and can benefit from careful offline analysis and refinement. This work aims to develop a classification model that is distinct from existing separation approaches, based on image processing and machine learning, and can provide the required categorization.

Different varieties and growing regions produce pistachios with varying sizes, hues, and flavors. Machine vision can play an important role in this context because of the size of the pistachio, which makes using human resources to do so impractical and a waste of time. (Anonymous, 2022). It is one of the crops that requires human resources to classify and count to assess crop quality based on whether the shell is open or closed. Pistachios are primarily classified depending on the shape of their shell, which can be open-mouth or close-mouth, and the price and worth of these two types differ (Rahimzadeh and Attar, 2022).

Various limits occur in real-time applications, and implementations of many approaches, such as Fourier methods, spectral methods, or active contours, are difficult to use. As a result, simple procedures are appropriate, and they should be thoroughly studied and optimized offline. Image threshold segmentation is a subset of high-speed algorithms used in real-time image processing applications. These are optimized and implemented in this work for a close pistachio sorting system. To

the best of our knowledge, minimal work on the installation of pistachio sorting systems employing low-cost and basic methods has been published. A low-cost camera is used, which is bound to produce images with significant noise and low quality. Furthermore, the camera's frame rate is limited, which causes major issues with the segmentation and evaluation of pistachio images in open or closed form. Image processing uses multilevel thresholding to address these issues.

2. Material and Methods

2.1. Material

Analyzed pistachios get their name from the Turkish city of Gaziantep, which has variants in numerous languages. The main reason why this fruit is referred to as Antep pistachio in Turkish literature is that the pistachio processing facilities in Türkiye have increased in density in Gaziantep, and production is focused on this location and distributed to other regions from here. In this context, pistachio production occurs in over 40 provinces throughout Türkiye. However, because of temperature and soil characteristics, the Southeastern Anatolia Region accounts for around 95% of output. Şanlıurfa, Gaziantep, Nizip, Siirt, Kahramanmaraş, Adıyaman, and Diyarbakır are among the first to produce in this region. While Gaziantep was the most famous pistachio-production region in Türkiye until 2014, new production areas have relocated to Şanlıurfa since then. Currently, Şanlıurfa and Gaziantep provinces account for around 80% of overall output (Coban et al, 2022). The pistachio market currently has four varieties: Antep, Siirt, Damascus, and Iranian pistachios. World production of the pistachios (first ten countries) in the shell for the 2021 year can be seen in Figure 1 (FAO, 2023).

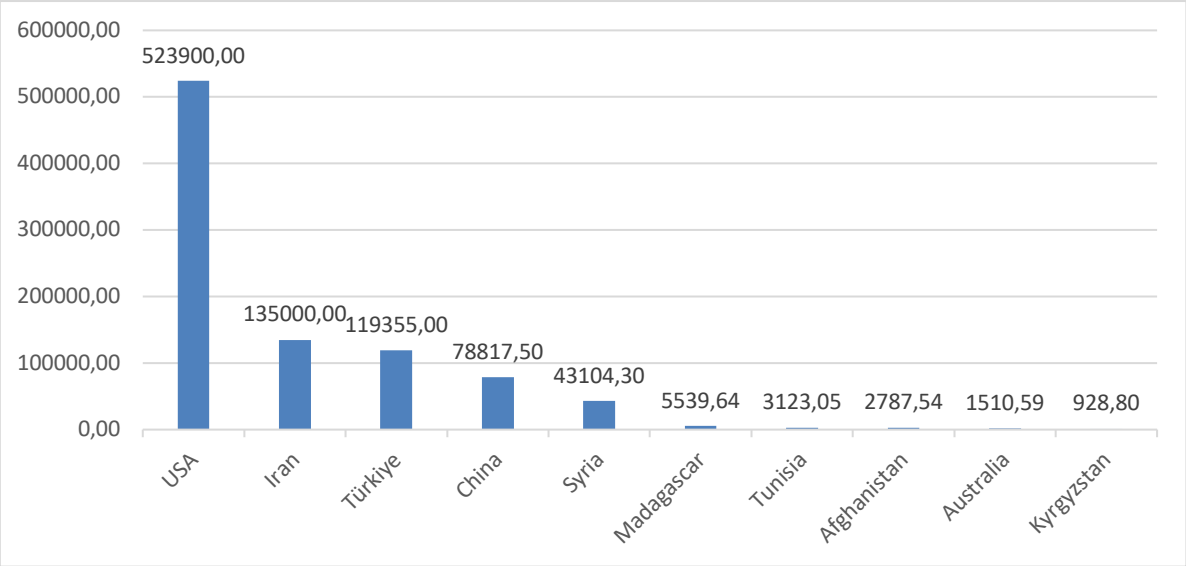

Figure 1. World production of the pistachios (first ten countries) in the shell for the 2021 year (FAO, 2023).

2.2. Method

2.2.1. Data collection and enhancement

The images were captured with a Nikon D800 camera from various situations in one lighting environment. Each scene's background was white, efficient detection of pistachios and to prevent unnecessary noise from anything scattered on it. Each image has a resolution of 5184 x 3456 and a varying number of items. The camera and the pistachio samples are 35 cm apart. Furthermore, black is employed (Figure 2). The objects are then localized using segmentation. A pistachio grain is depicted in Figure 2.

Figure 2. Antep pistachio nuts image capture set-up.

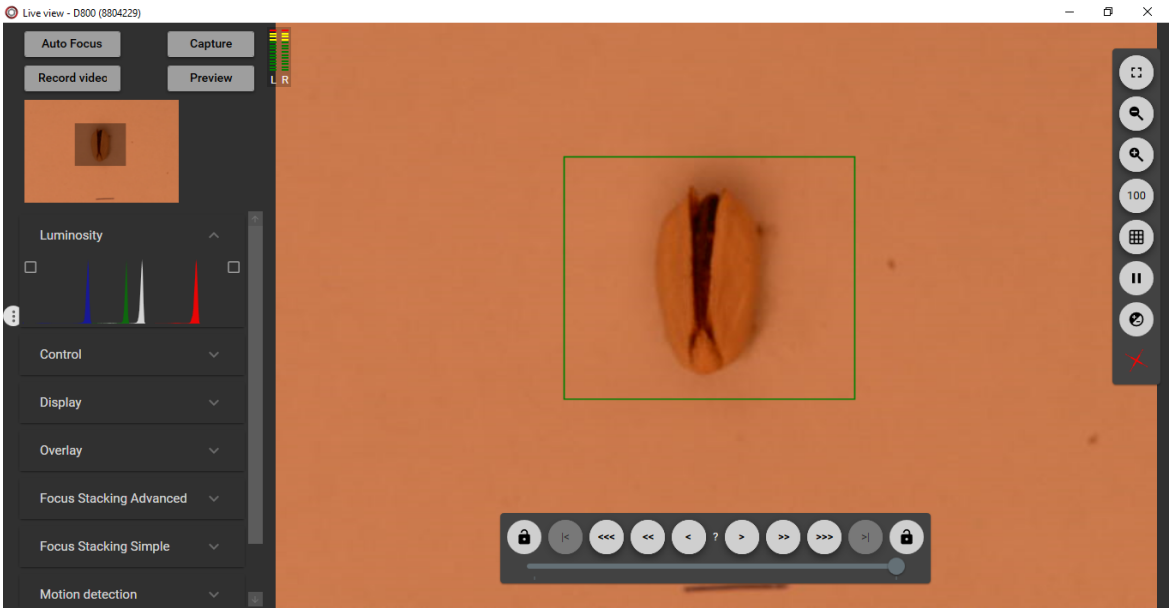

Figure 3. Live view of Antep pistachios on digiCamControl image capture software.

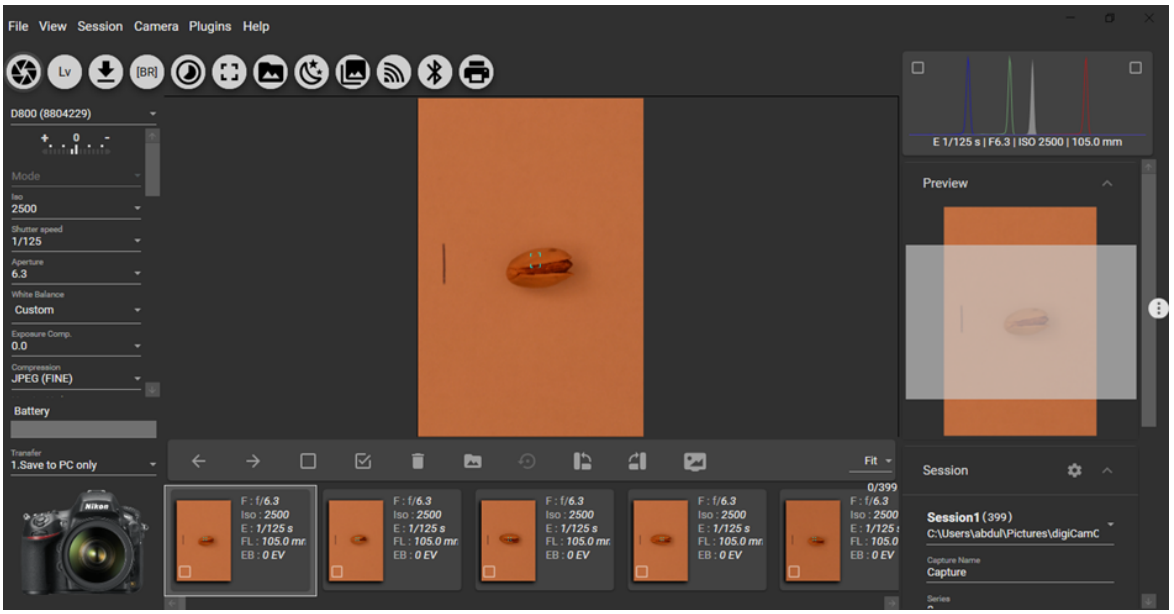

Figure 4. Captured view of Antep pistachios on digiCamControl image capture software.

The data set, as previously stated, is divided into three categories: desired open-shell pistachios, defective pistachios, and trash (chip woods mainly). It is worth noting that there are various sorts of defective pistachios: open-shell pistachios, close-shell pistachios, and a few or semi-open pistachios (Figure 5).

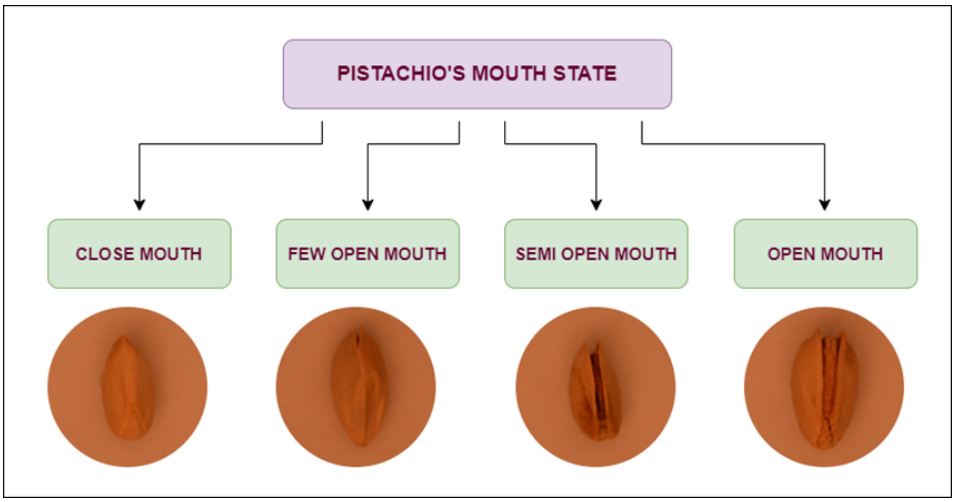

Figure 5. Classification of pistachios based on mouth states.

2.2.2. Image analysis

The images were analyzed and measured using ImageJ (Figure 6). This software is available both as a web-based applet and as a downloadable application, both of which work on any computer with a Java 1.4 or later virtual machine. Distribution packages are available for multiple platforms, including Windows, OS X, and Linux. Images in 8, 16, and 32-bit depths can be viewed, edited, analyzed, processed, saved, and printed. It is compatible with numerous image file formats, such as TIFF, GIF, JPEG, BMP, DICOM, FITS, and 'raw.' 'Stacks,' groups of related images that are displayed side by side, are supported. Time-consuming tasks, such as reading image files, can be completed alongside other tasks thanks to the multithreaded nature of the system. For the areas and pixel values that the user specifies, it can also generate statistics.

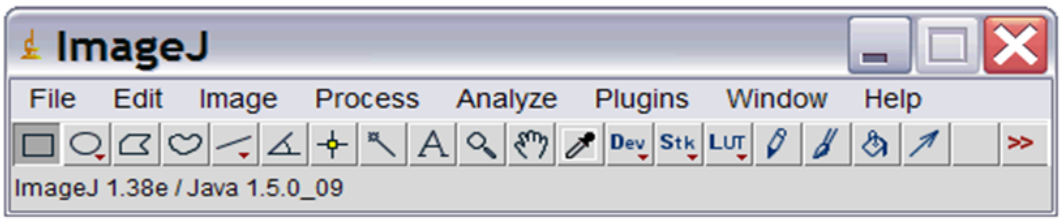

Figure 6. The interface of the ImageJ software.

In this research, first, the calibration process was done by using the 'set scale' command on the software with the help of a calibration object with known dimensions (Figure 7).

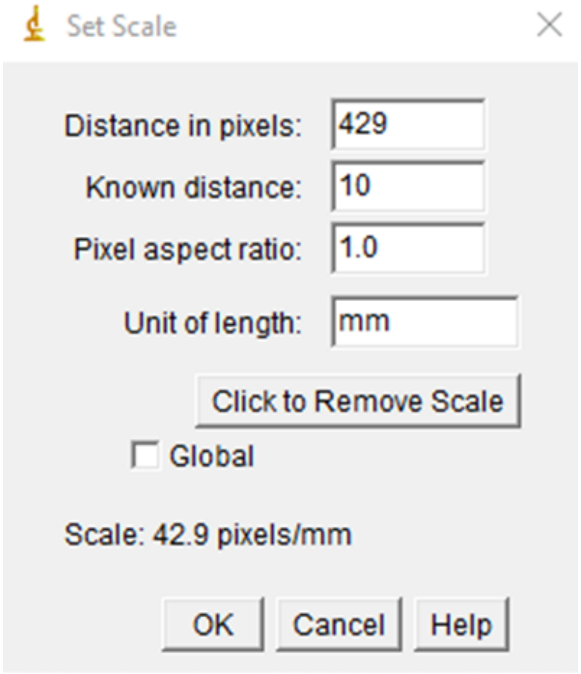

Figure 7. Set scale command interface.

Area statistics, line lengths, angles, and point coordinates are all calculated and displayed in a table, depending on the selection type. It is possible to enter the values for the measurements in the 'Set Measurements' dialogue box. Area statistics are calculated for the entire image, or a region defined by one of the 'Area Selection Tools' if no selection is made. All three types of line selections (straight, segmented, and freehand) have their lengths and angles (for straight lines only) computed automatically (see Line Selection Tools for details). For Point choices, both the X and Y coordinates are stored (see Point Tool and Multi-Point Tool). To derive results from RGB images, brightness values are used. The brightness values of RGB pixels are calculated. Statistics for intensity (average, mode, median, minimum, maximum, standard deviation, and integrated) (Figure 8).

Figure 8. Set scale command interface with a pistachio and calibration bar.

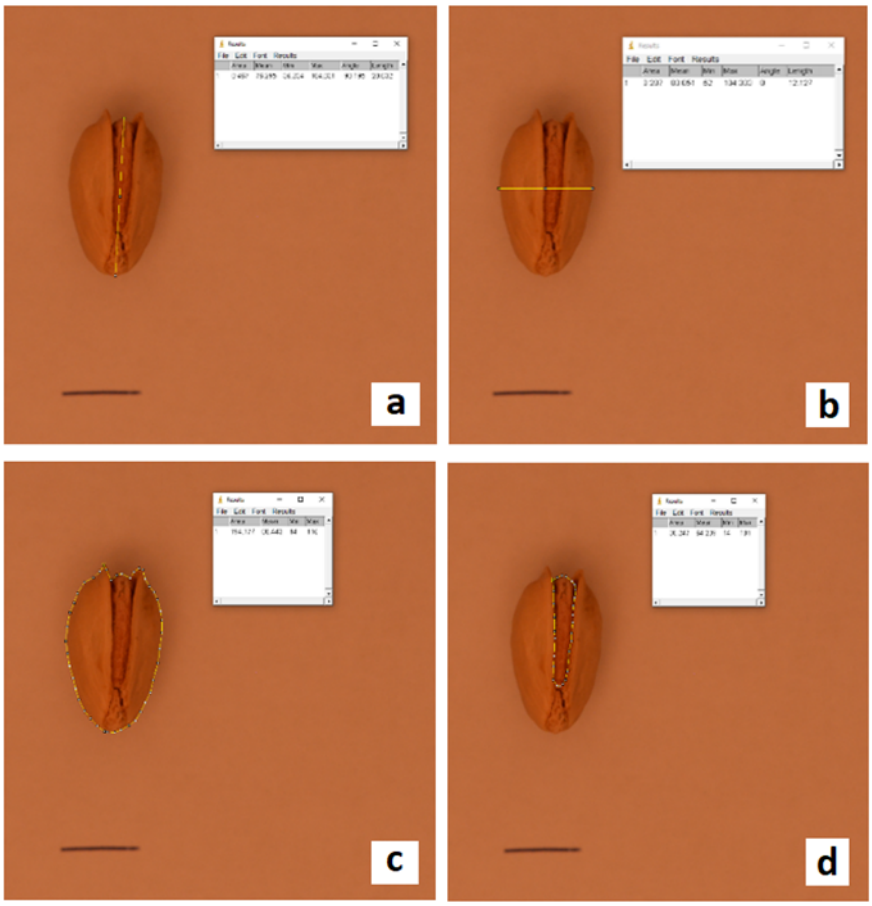

Figure 9. Pistachio length (a), pistachio width (b), pistachio area(c), and nut area(d) measurement with ImageJ software.

Figure 10. Mouth measurement of a pistachio with ImageJ software.

2.2.3. Classification

Decision Trees and Random Forests machine learning algorithms were used to predict which pistachios would be open-shelled and which would be trash. Metrics for success, including accuracy, sensitivity, specificity, precision, and F1-Score, are established so that the model can be tested and improved upon. Table 1 provides an in-depth look at the algorithms used to determine success. True positive (TP), false positive (FP), true negative (TN), and false negative (FN) stand in for these four possible outcomes. As a precaution against bias and high variance, the '10-fold cross-validation' method was used to evaluate pistachio classification algorithms. The 10-fold cross-validation approach divides the dataset into 10 equal parts, with the first part serving as a test set and the remaining 9 as a training set. After ten iterations, we get our result by averaging each test set's performance against our set of criteria. The best c value was selected for efficacy.

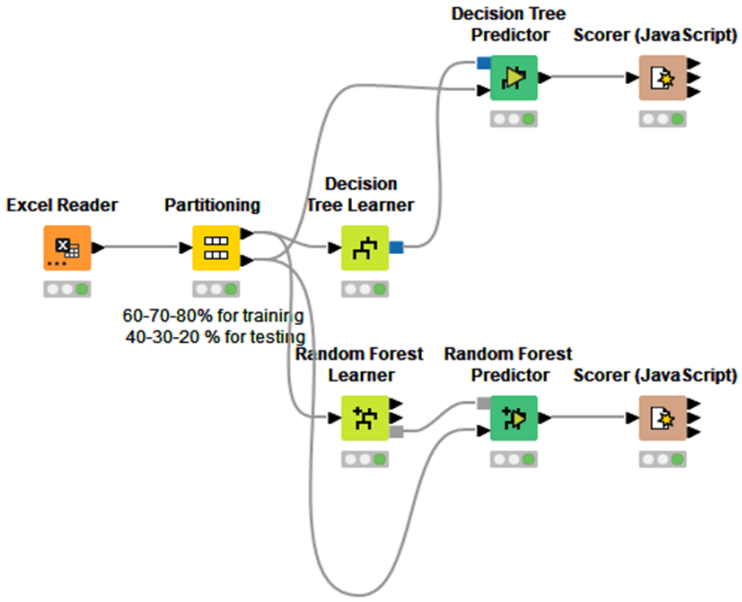

Figure 11. KNIME flowchart of Decision Tree and Random Forest machine learning algorithms.

3. Results

Some biological parameters of Pistachios were measured by using the image analysis technique and presented in Table 1. Also, Decision Tree and Random Forest confusion matrixes, class statistics, and overall statistics at 60-70-80% train and 40-30-20% test data rates were presented in Table 2-7. The proposed approach attained the best classification success rate of 100% at 70% train and 30% test, and, 80% train and 20% test data rate with Random Forest prediction, according to the experimental data.

Table 1. Some biological parameters of pistachios

Pistachio Mouth State	Pistachio Mouth Value (mm)	Pistachio Lenght (mm)	Pistachio Width (mm)	Pistachio Area (mm^2)	Pistahio Nut Area (mm^2)
Close mouth	0.00	18.93	9.11	131.84	0.00
Few open mouths	1.41	16.41	149.86	114.91	7.62
Semi-open mouth	3.09	16.80	9.35	127.19	15.31
Open mouth	4.81	18.64	10.46	155.30	26.48

Table 2. Decision tree confusion matrixes at 60% train and 40% test, 70% train and 30% test, and 80% train and 20% test data rate

Row ID	Close mouth	Few open mouths	Open mouth	Semi-open mouth	Data rate
Close mouth	56	0	0	0	
Few open mouths	0	22	0	0	60% train and 40%
Open mouth	0	0	22	0	test
Semi-open mouth	0	0	1	53	
Close mouth	42	0	0	0	
Few open mouths	0	15	0	2	70% train and 30%
Open mouth	0	0	16	0	test
Semi-open mouth	0	0	0	41	
Close mouth	28	0	0	0	
Few open mouths	2	9	0	0	80% train and 20%
Open mouth	0	0	11	0	test
Semi-open mouth	1	0	0	26	

Table 3. Decision tree class statistics table at 60% train and 40% test, 70% train and 30% test, and 80% train and 20% test data rate

Row ID	TP	FP	TN	FN	Recall	Precision	Sensitivity	Specificity	F-measure	Data rate
Close mouth	56	0	98	0	1	1	1	1	1	
Few open mouths	22	0	132	0	1	1	1	1	1	60% train
Open mouth	22	1	131	0	1	0.957	1	0.992	0.978	and 40% test
Semi-open mouth	53	0	100	1	0.981	1	0.981	1	0.991	
Close mouth	42	0	74	0	1	1	1	1	1	
Few open mouths	15	0	99	2	0.882	1	0.882	1	0.938	70% train
Open mouth	16	0	100	0	1	1	1	1	1	and 30% test
Semi-open mouth	41	2	73	0	1	0.953	1	0.973	0.976	
Close mouth	28	3	46	0	1	0.903	1	0.939	0.949	
Few open mouths	9	0	66	2	0.818	1	0.818	1	0.9	80% train
Open mouth	11	0	66	0	1	1	1	1	1	and 20% test
Semi-open mouth	26	0	50	1	0.963	1	0.963	1	0.981	

Decision tree overall accuracy at 60% train and 40% test data rate were found as 0.99% and Cohen's kappa value was 0.99, at 70% train and 30% test data rate was found as 0.98% and Cohen's kappa value was 0.97, at 80% train and 20% test data rate was found as 0.96% and Cohen's kappa value was 0.94 seen in Table 4.

Table 4. Decision tree overall statistics table at 60% train and 40% test, 70% train and 30% test, and 80% train and 20% test data rate

Row ID	Overall Accuracy	Overall Error	Cohen's kappa	Correctly Classified	Incorrectly Classified	Data rate
Overall	0.994	0.006	0.991	153	1	60% train and 40% test
Overall	0.983	0.017	0.975	114	2	70% train and 30% test
Overall	0.961	0.039	0.944	74	3	70% train and 20% test

Table 5. Random forest confusion matrix at 60% train and 40% test, 70% train and 30% test, and 80% train and 20% test data rate

Row ID	Close mouth	Few open mouths	Open mouth	Semi-open mouth	Data rate
Close mouth	56	0	0	0	
Few open mouths	0	22	0	0	60% train and 40%
Open mouth	0	0	22	0	test
Semi-open mouth	0	1	0	53	
Close mouth	42	0	0	0	
Few open mouths	0	17	0	0	70% train and 30%
Open mouth	0	0	16	0	test
Semi-open mouth	0	0	0	41	
Close mouth	28	0	0	0	
Few open mouths	0	11	0	0	80% train and 20%
Open mouth	0	0	11	0	test
Semi-open mouth	0	0	0	27	

Table 6. Random forest class statistics table at 60% train and 40% test, 70% train and 30% test, and 80% train and 20% test data rate

Row ID	TP	FP	TN	FN	Recall	Precision	Sensitivity	Specificity	F-measure	Data rate
Close mouth	56	0	98	0	1	1	1	1	1	
Few open mouths	22	1	131	0	1	0.957	1	0.992	0.978	60% train
Open mouth	22	0	132	0	1	1	1	1	1	and 40% test
Semi-open mouth	53	0	100	1	0.981	1	0.981	1	0.991	
Close mouth	42	0	74	0	1	1	1	1	1	
Few open mouths	17	0	99	0	1	1	1	1	1	70% train
Open mouth	16	0	100	0	1	1	1	1	1	and 30% test
Semi-open mouth	41	0	75	0	1	1	1	1	1	
Close mouth	28	0	49	0	1	1	1	1	1	
Few open mouths	11	0	66	0	1	1	1	1	1	80% train
Open mouth	11	0	66	0	1	1	1	1	1	and 20% test
Semi-open mouth	27	0	50	0	1	1	1	1	1	

Random forest overall accuracy at 60% train and 40% test data rates were found as 0.99% and Cohen's kappa value was 0.99, at 70% train and 30% test data rates were found as 100% and Cohen's kappa value was 1, at 80% train and 20% test data rates were found as 100% and Cohen's kappa value was 1 seen in Table 7.

Table 7. Random forest overall statistics table at 60% train and 40% test, 70% train and 30% test, and 80% train and 20% test data rate

Row ID	Overall Accuracy	Overall Error	Cohen's kappa	Correctly Classified	Incorrectly Classified	Data rate
Overall	0.994	0.006	0.991	153	1	60% train and 40% test
Overall	1	0	1	116	0	70% train and 30% test
Overall	1	0	1	77	0	80% train and 20% test

Figure 12-17 shows class statistics and overall statistics at 60-70-80% train and 40-30-20% test data rates, in addition to the Decision Tree and Random Forest confusion matrices.

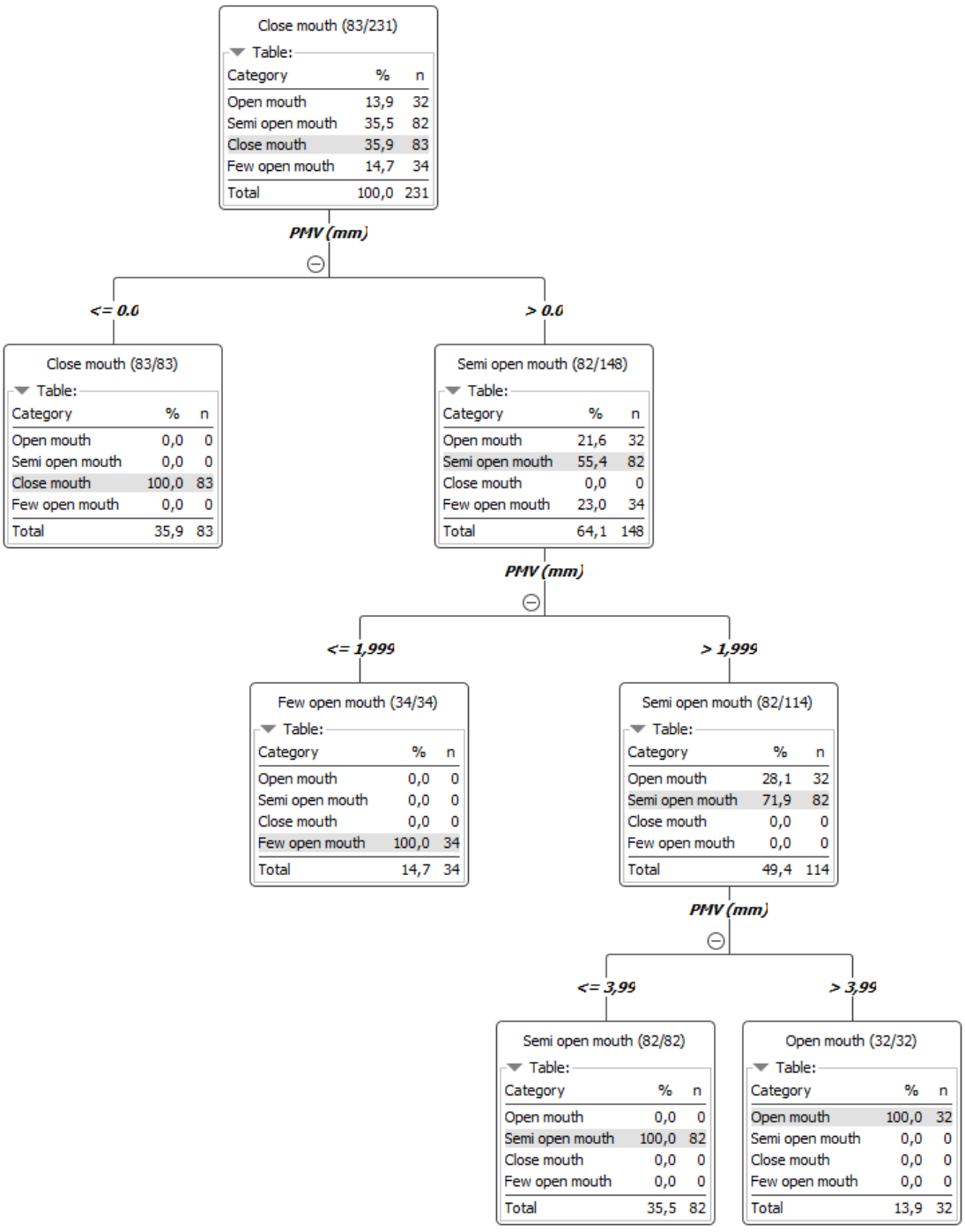

Figure 12. Decision tree view of pistachio mouth states at 60% train and 40% test data rate.

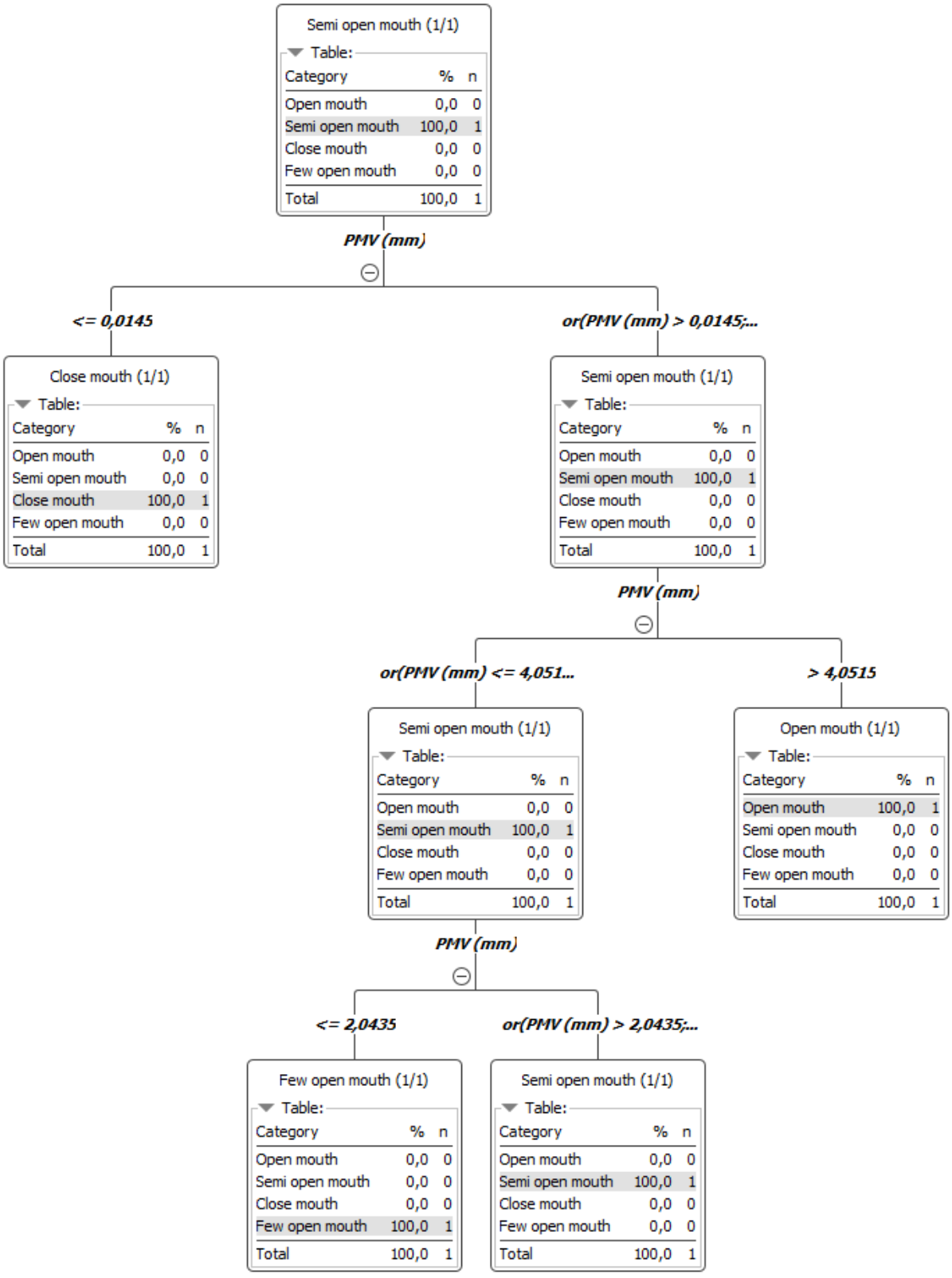

Figure 13. Random forest tree view of pistachio mouth states at 60% train and 40% test data rate.

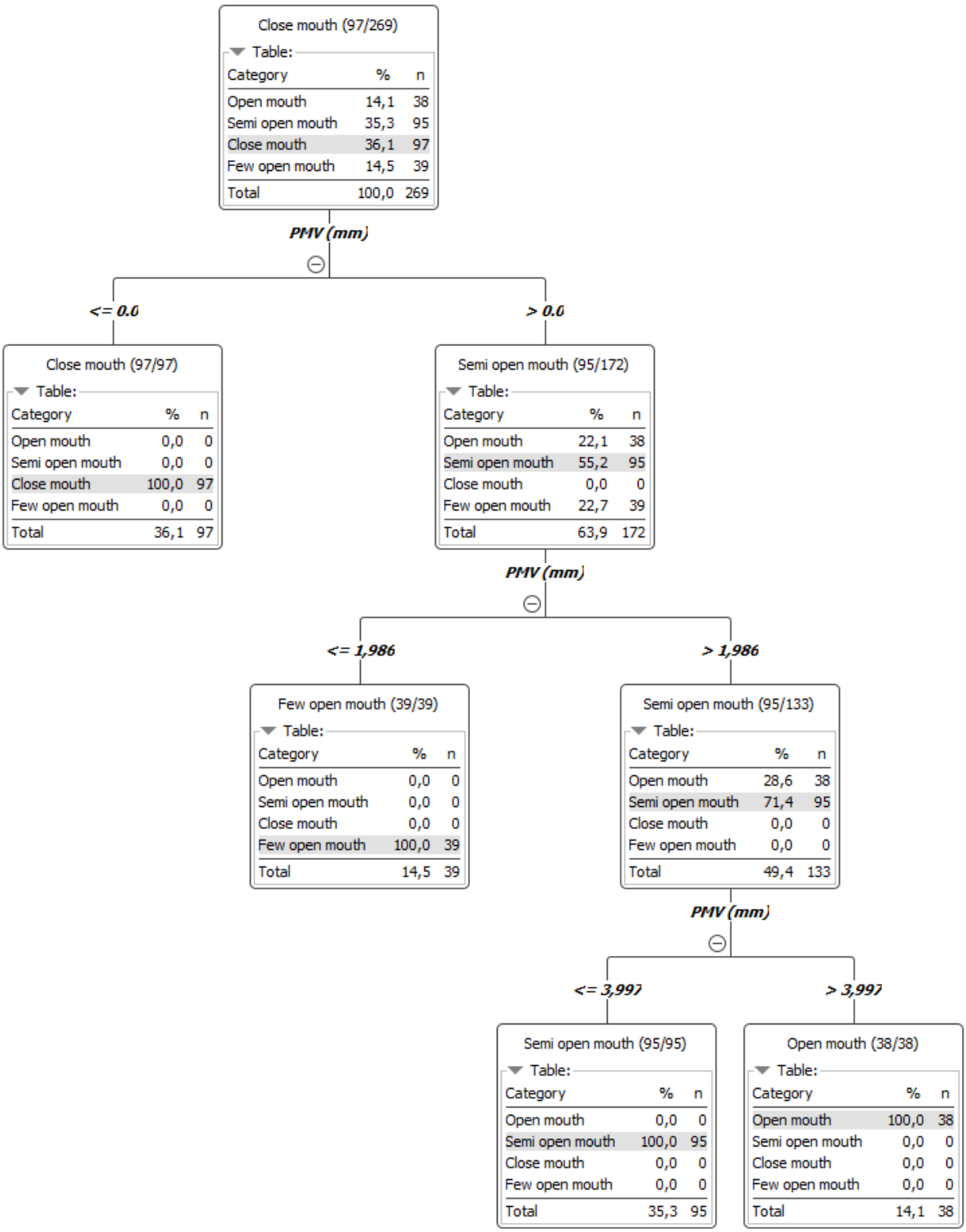

Figure 14. Decision tree view of pistachio mouth states at 70% train and 30% test data rate.

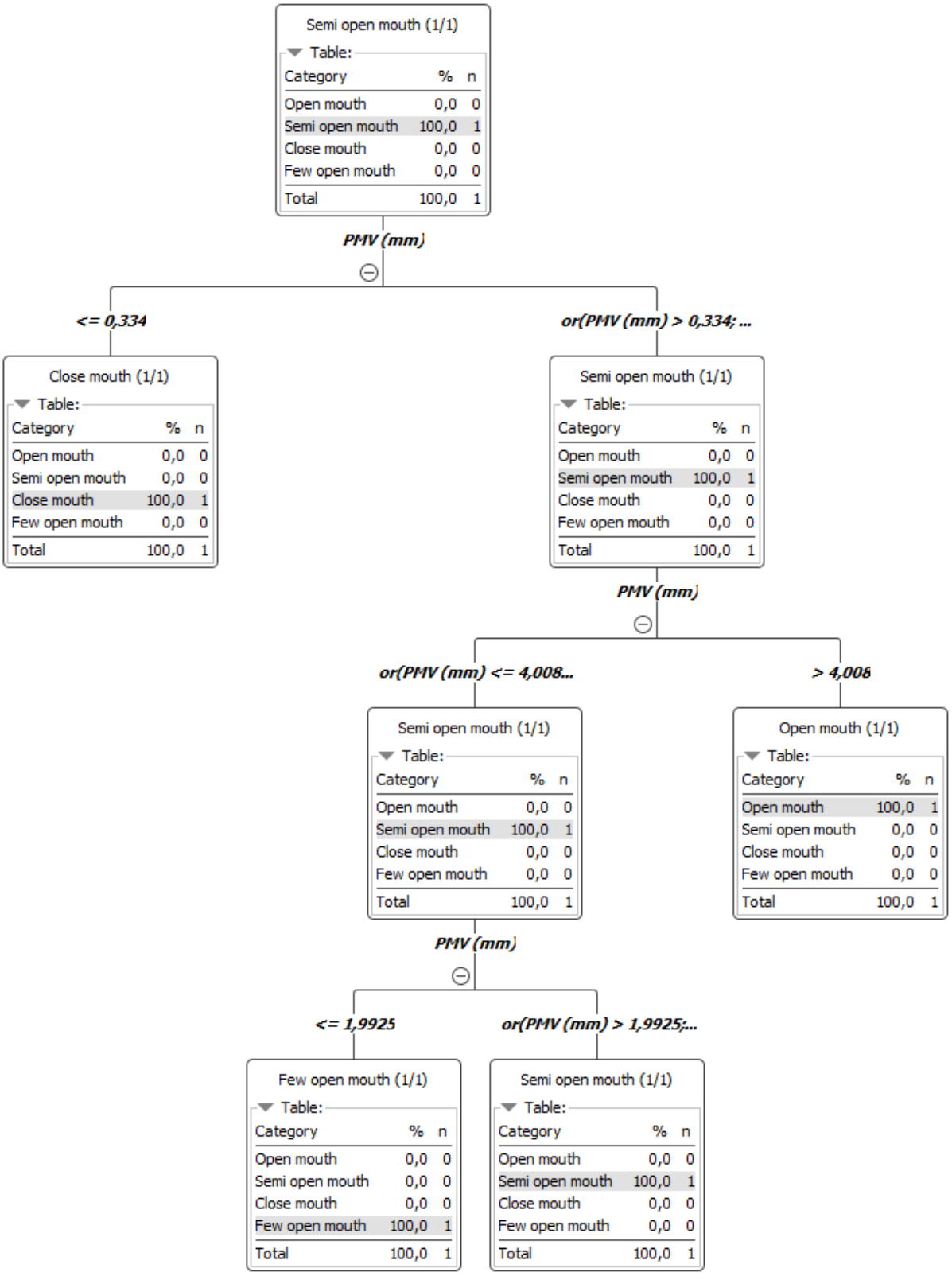

Figure 15. Random forest tree view of pistachio mouth states at 70% train and 30% test data rate.

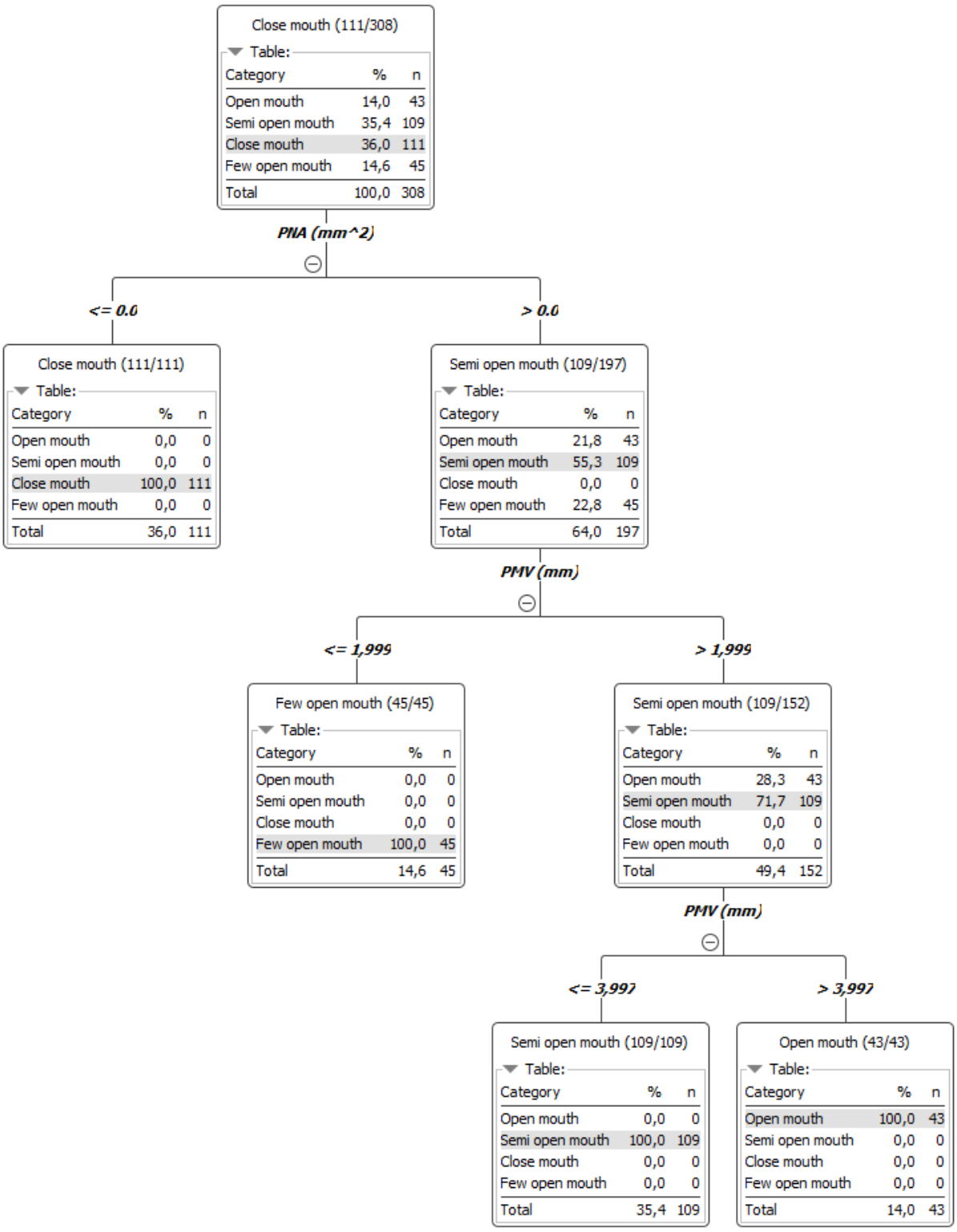

Figure 16. Decision tree view of pistachio mouth states at 80% train and 20% test data rate.

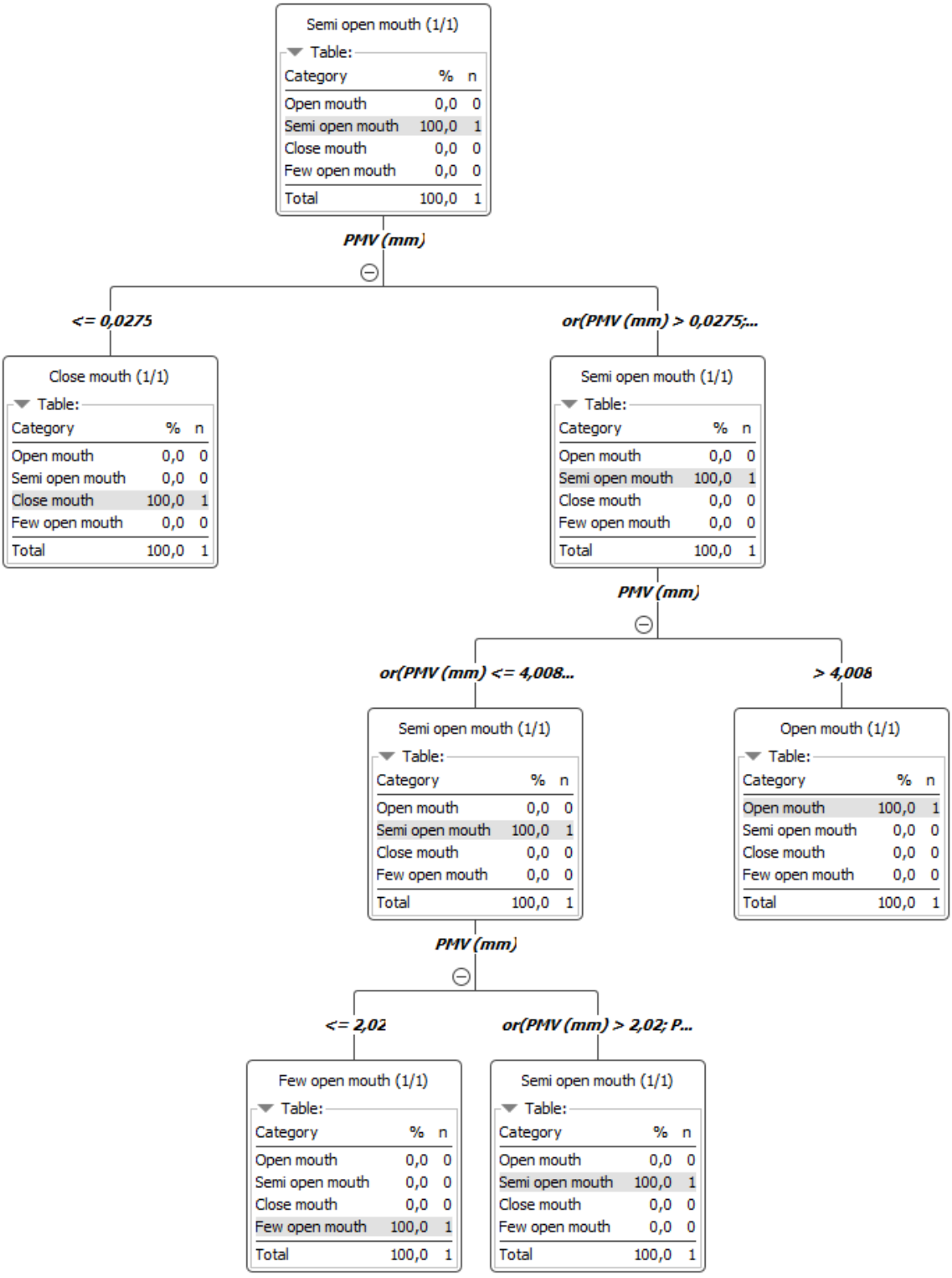

Figure 17. Random forest tree view of pistachio mouth states at 80% train and 20% test data rate.

4. Discussion

In the literature there have been some samples on this subject, Ghazanfari et al. (1997 and 1998), for instance, classified pistachio nuts into one of three USDA size grades or as having close shells by using Fourier descriptors and gray-level histogram features of two-dimensional images. Fourier descriptors require a great deal of processing time and are therefore unsuitable for real-time applications.

The method developed by Pearson and Toyofuku (2000) demonstrates how to distinguish between pistachios with open and closed shells from a photograph. Subsequently, an automated machine vision system was developed to detect and remove pistachio nuts with close shells during the processing stages. They said that in their research, the material handling apparatus of the system gently propels nuts past three high-speed line-scan cameras. Digital signal processing boards use camera signals to extract close and open shell pistachio-specific characteristics. This machine vision system can distinguish between open-shell and close-shell nuts with an accuracy of approximately 95% after two iterations.

Pearson and Slaughter (1996) have worked on the detection of early open pistachio nuts using machine vision. They stated that by integrating unhulled nut cross-sectional area with adjacent profile data, they accurately identified 100% of the early open nuts and 99% of the normal nuts out of a total of 180 nuts evaluated.

Ince et al. (2008) utilized a double tree un-decimated wavelet transform to classify close and open-shell (Turkish) pistachio nuts. Their proposed method utilized a small number of characteristics, yet still achieved 91.5% accuracy in their validation set. In addition, they emphasize that an earlier method based on maximum signal amplitude, absolute integration, and gradient characteristics achieved 82% classification accuracy on the same dataset. The results demonstrate the viability of classifying open and close-shell Turkish pistachios based on the time-frequency information extracted from impact acoustics.

Ghezelbash et al. (2013) developed and evaluated an inexpensive computer vision system for sorting pistachio nuts with close shells. To identify pistachios with close shells, their system captures three-dimensional images of the nuts using two flat mirrors and an inexpensive camera. In the three tests, the average removal accuracy for open pistachio nuts was 92.7%, while it was 86.7% for closed pistachio nuts.

Ozkan et al. (2021) developed an enhanced k-NN classifier to classify pistachio species. Using experimental data, the classification success rate of the proposed method was determined to be 94.18%. They provide a high-performance classification method that facilitates the economic benefits of pistachio species separation in response to a critical need in the industry.

Deep learning research has also been conducted on this topic; for instance, Farazi et al. (2017) used a convolutional neural network's transmitted mid-level picture representation to sort pistachios using machine vision. They discovered that across all test photos, their model with GoogleNet transferred weights achieved a final average accuracy of 99% for corrected classified items, implying faultless classification.

Research on pistachio species categorization and analysis using pre-trained deep-learning models was conducted by Singh et al. (2022). With the AlexNet model, they achieved a success rate of 94.42%, with the VGG16 model coming in at 98.54 %, and with the VGG19 model coming in at 98.14%.

Aktaş et al. (2022) examined the impact of different datasets on accuracy in pistachio deep learning categorization. They imply that the test accuracy was computed as 100% when training and testing the AlexNet structure with this desktop dataset.

Based on experimental data, the proposed method outperformed the research literature with a classification success rate of 100% using 70% train data and 30% test data, and 80% train data and 20% test data using Random Forest prediction.

Conclusion

Close-shell pistachios may by consumers because they are difficult to open and may contain immature kernels. Consequently, it is essential to differentiate them from open-shell pistachio nuts. To separate close-shell pistachios from open-shell pistachios, different systems are utilized (Ince et al., 2008). Moreover, according to Pearson (2001), mechanical devices incorrectly classify 5 to 10% of all open-shell U.S. pistachios as having a close shell, resulting in between $3.75 million and $7.50 million

in annual lost revenue. Therefore, the sector requires categorization systems with a high degree of precision.

Consequently, a low-cost technique for sorting closed pistachios was conceived and implemented considering the research literature. Machine learning algorithms were used for the evaluations. About this issue, Sharma and Dutta (2023) stress the importance of machine learning algorithms in the evaluations of agricultural applications. Throughout the training and testing phases, it was determined that the technology is particularly adaptable to different mouth types. Future work in this field can be accomplished by updating the entire subsystem, including the feeder, exposing, separator, lighting, and camera, as well as the optimization and image processing algorithms. Also, updating the optimization and image processing algorithms with further studies will give a chance for better image processing and analysis applications.

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Yüzüncü Yıl University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

ISSN: 1308-7576 e-ISSN: 1308-7584

Research Article

Phenotype Characteristics of Diiti Cattle in the Coastal Region of Tomini Bay-Gorontalo, Indonesia

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Article Info

Received: 01.09.2023
Accepted: 16.02.2024
Online published: 15.03.2024
DOI:10.29133/yyutbd.1353226

Keywords

Diiti cattle,
Phenotypes,
Qualitative,
Quantitative

Abstract: This study aims to determine the qualitative and quantitative traits of Diiti cattle in the coastal area of Tomini Bay, Gorontalo, Indonesia. A total of 201 phenotypes (qualitative and quantitative) of Diiti cattle were collected, consisting of 138 females and 63 males aged 4-5 years. Qualitative observations were focused on body color, face shape, horn, and dewlap. Quantitative traits were focused on the eight traits that were influenced by genetic and environmental factors. The methods used were descriptive and chi-square analysis. IBM Statistics SPSS 22 was used to analyze the data obtained. Female Diiti cattle had nine body colors, while the males had seven body colors. Based on the front view, male and female Diiti cattle have hexagonal, triangular, and perpendicular facial shapes of 7.94%, 49.20%, 42.68% and 36.95%, 5.80%, 57.25%, respectively. The body sizes of male and female Diiti cattle were different. The body size p-values of male and female Diiti cattle ranged between 0.00-0.063. Diiti cattle have various body colors. Female cattle were reddish white, whitish red, and white, while male cattle were black, brown, and white. Female cattle do not have a hump, and generally have a hexagonal face shape, flat face line, body size, and weight almost the same as Bali cattle, but smaller than PO cattle. Further characterization of Diiti cattle was required as basic information of Diiti cattle as Gorontalo local cattle.

To Cite: Dako, S, Laya, N K, Fathan, S, Gubali, S, Datau, F, Syahruddin, S, Pateda, S Y, 2024. Phenotype Characteristics of Diiti Cattle in the Coastal Region of Tomini Bay-Gorontalo, Indonesia. *Yuzuncu Yil University Journal of Agricultural Sciences*, 34(1): 106-114.
DOI: https://doi.org/10.29133/yyutbd.1353226

1. Introduction

Gorontalo is a province located on the Sulawesi island in Indonesia. The potential of beef cattle is 1.413% of the total cattle in Indonesia (BPS, 2021). The types of livestock that are mostly kept by farmers are Bali cattle, OngoleCross (PO), Local cattle, and exotic cattle. Dako et al., (2023) research using microsatellite DNA analysis shows that Gorontalo local cattle are closely related to PO cattle. This was explained such that these cattle underwent genetic drift and inbreeding a long time ago. In addition, there has also been crossbreeding with Bali cattle, so there's a separate cluster formed that is different from PO cattle and Bali cattle.

One of Gorontalo's local livestock is Diiti cattle, which are kept by many breeders. Known for a long time, Diiti cattle are closely related to PO cattle. These cattle experienced genetic decline due to long inbreeding, as well as livestock trade outside the area which caused a decrease in the quality. From the 2000s up to now, there has been a change in policy towards the development of Bali cattle from the start of the formation of the Province of Gorontalo. The problem arose during the Bali cattle policy period that the breeders crossed Ditti cattle with Bali cattle and PO cattle, without realizing that they had performed the simplest livestock breeding process at the community group level, and that caused uncontrolled inbreeding. The breeding process has been going on for a long time, to increase the numbers and reserves of livestock to be used. These transformations resulted in a decrease in productivity, body shape, and linear body size of the cattle.

The phenotype range of Diiti cattle is not clearly known, but the differences are clearly visible when compared with Bali and PO cattle. For this reason, important phenotypes must be known in these cattle. Based on the phenotype, each animal can be observed and measured, especially the phenotype which is influenced by genetics and the environment. To describe the characteristics of local cattle in Indonesia, some researchers have used color patterns (Azis et al., 2022), body size (Heryani et al., 2018; Laya et al., 2020), and body weight (Abdullah et al., 2006; Budiarto et al., 2013). Mustefa et al., (2020), used the same thing to describe local cattle from Ethiopia.

Breeding and development of Diiti cattle in Gorontalo is very beneficial for increasing the genetic quality, sustainability, and economic value of livestock. Realization of livestock development requires initial information about qualitative characters, size, shape, and phenotypic range that can be used as a basis for classifying, selecting, breeding, and standardizing Diiti cattle, as one of the local cattle breeds in Indonesia. The initial step was performed specifically on local cattle located in the coastal area of Tomini Bay, Gorontalo. This study aims to determine the qualitative and quantitative characteristics of Diiti cattle in the coastal area of Tomini Bay, Gorontalo, Indonesia.

2. Material and Methods

All procedures related to animal use in this study were approved by the Animal Care and Use Committee of Brawijaya University under regulation number 145-KEP-UB-2022. This research was carried out using the observation method and field observations in the Kabil Bone District (Bonebolango Regency), Batudaa Pantai subdistrict, and Biluhu subdistricts (Gorontalo Regency), with the consideration that they are located in the Tomini Bay area and have sufficient numbers of beef cattle. A total of 201 phenotypes of Diiti cattle were collected from November 2022 to May 2023 (qualitative and quantitative), each consisting of 138 females and 63 males aged 4-5 years. Phenotypic observations were focused on body color, face shape, and Dewlap, while quantitative traits were focused on the eight traits that are influenced by the environment, and the four traits that are influenced by genetics, namely body length (Bl), chestcircumference (cc), body height (Hb), hip height (Hh), hip width (Hw), Chest width (Cs), Inside Chest (Dc), Dewlap Length (Wl), Dewlap width (Dw), Head length (Hl), Head width (Hhi), and BodyWeight (Bb).

Measurements of qualitative traits were performed according to the directions of Aguantara et al. (2019), the quantitative phenotypes were performed according to the directions of Asmare et al. (2021); Ikhsanuddin et al. (2018); and Safriyanto et al. (2022). The dominant value of body skin color is tested by the Chi-square analysis.

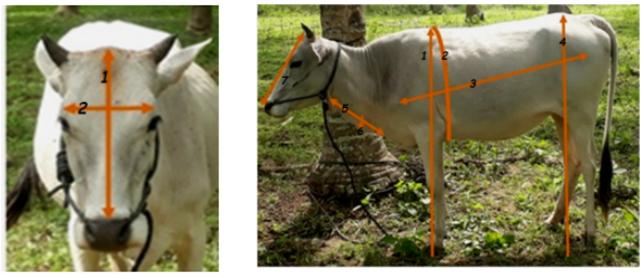

Figure 1. Body size measurement of Diiti cattle.

2.1. Statistical analysis

The descriptive analysis and chi-square analysis were used to test the qualitative traits, while descriptive analysis was used to test the quantitative traits. The data analysis is performed by IBM statistic SPSS 22 statistical package.

3. Results and Discussion

3.1 Qualitative traits

Local cattle in Indonesia are very diverse, especially in their phenotypic appearance. One of the local livestock of Gorontalo is Diiti cattle (Safriyanto et al., 2022). The phenotypic characteristics of Diiti cattle along the coast of Tomini Bay and Gorontalo are varied greatly based on the qualitative characteristics. According to FAO (2009), livestock body color is categorized into 2 parts, namely colored and colorless. Colorless/without pigment is white, while colored is a color other than white or has pigment. The color diversity of Diiti cattle is shown in Table 1.

Table 1. Diversity of qualitative traits in female and male Diiti cattle

	Qualitative trait	Female			Male		
		138	PF (%)	Chi-Square X_{count} X_{table}	63	PF (%)	Chi-Square X_{count} X_{table}
	Body Color						
1	White	39	28.26	6.94/36.64	16	25.4	3.808/32.67
2	Brown	7	5.07		12	19.05	
3	Brick red	15	10.87		3	4.76	
4	Black	6	4.35		12	19.05	
	Body color pattern						
1	Gray	13	9.42		4	6.35	
2	Black-white	16	11.59		6	9.52	
3	Dark brown	14	10.14		8	12.7	
4	Whitish brick red	27	19.57		0	0	
5	Cream	1	0.72		0	0	
6	Reddish-Black	0	0		2	3.17	
	Vaginal Color						
1	Black	110	80.43		-	-	-
2	Reddish	28	19.57		-	-	-
	Eyelids color						
1	Black	133	96.38	Dominant	63	100	Dominant
2	White	5	3.62		0	0	
	Eye hair color						
1	Redish brown	41	29.71		16	25.4	
2	White	72	52.17	Dominant	32	50.79	Dominant
3	Black	25	18.12		15	23.81	
	Tail hair						
1	Black	134	97.1	Dominant	63	100	Dominant
2	White	4	2.89		0	0	
	Dewlap						
1	Dewlaped	138	100	Dominant	63	100	
2	Dewlapless	0	0		0	0	
	Horn						
1	Hornless	0	0		16	25.4	
2	Horned	138	100		47	74.6	
	Horn direction						
1	V-shaped horn	71	51.45	Dominant	44	93.62	Dominant
2	U-shaped horn	48	34.78		2	4.26	
3	Horns straight to the side	6	4.35		1	2.13	
4	Horns Curved backwards	5	3.62		0	0	
5	Forward curved horns	8	5.8		0	0	

Table 1. Diversity of qualitative traits in female and male Diiti cattle (continued)

	Qualitative trait	Female			Male		
		138	PF (%)	Chi-Square xcount xtable	63	PF (%)	Chi-Square xcount xtable
	Shape face of Front view						
1	Hexagon	51	36.95		5	7.94	
2	Triangular	8	5.8		31	49.21	
3	Perpendicular	79	57.25	Dominant	27	42.86	
	Shape face of Side view						
1	Convex	35	25.36		42	66.66	Dominant
2	Flat	103	74.64	Dominant	21	33.34	
	Hump						
1	Humped	0	0		0	0	
2	Humpless	138	100	Dominant	63	100	Dominant

Note: PF: Phenotype Frequency.

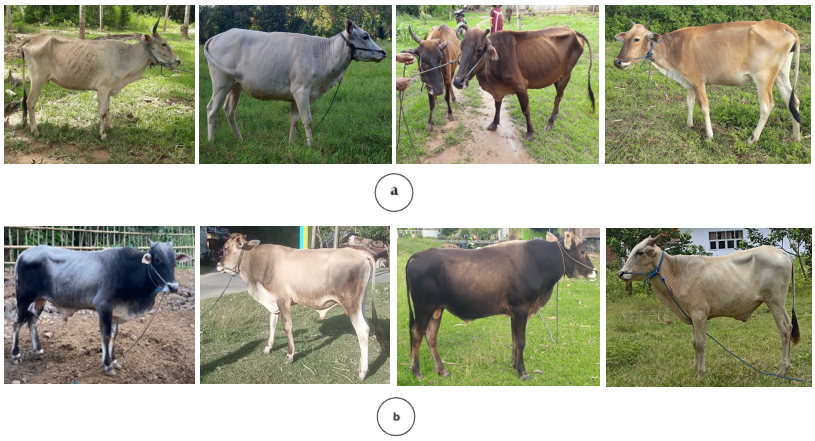

Figure 2. Diiti cattle (a. Female, and b. Male).

3.1.1 Body color profile of Diiti cattle

Table 1 shows that the female Diiti cattle had nine body colors, while the males had eight body colors. Chi-square analysis revealed that there was no dominant body color in male and female cattle. This shows that the body color of Diiti cattle varies and the distribution of skin color is even in the three research locations. The body color of Diiti cattle was mostly white, Whitish brick red, and blackish-white.

The body color diversity of local cattle in Indonesia was reported by Agustriadi et al. (2019). The color of Kuantan cattle was white (38%), brownish white, brick red (33%), and brownish red (16%). Kuantan cattle were white, gray, black, and brick red. Pasundan cattle have four body colors, namely brick red, cream, black, and brown (Naufal et al., 2016). Katingan cattle have nine body color variations (Utomo, 2016), and the dominant color was brownish white (Misrianti et al., 2018), while the body color of local cattle in the Kaur area had no dominant color in the population, and all cows have horns. The body colors of Aceh cattle were predominantly dark, including black, brown, black-brown, and brick red.

The body color and body color patterns of Diiti cattle were identified as having different thermal colors among the cattle in the population. This was due to gene coherence in the inbreeding of Bali, Ongole Cross (PO), and Diiti cattle, where the genes and alleles for body color complement each other between loci. Body pigmentation can be reduced in line with reduced melanocyte activity. White body color in cattle indicates that cows lack melamine, black and brown are regulated by eumelanin, and sorrel, red, and yellows are regulated by phaeomelanin (Joerg et al., 1996). Locus Extension (E) plays a role in the occurrence of body color variations in cattle.

3.1.2 Horn of Diiti cattle

Based on Table 1, male and female Diiti cattle were dominant for horns. Female cattle generally have horns. Bulls with horns were 74.60% and bulls without horns were 25.40%. There are four forms of horns found in male and female Diiti cattle including V-shaped horns, U-shaped horns, horns straight to the side, and horns curved backward. The most dominant horn directions found were V-shaped horns, respectively 93.62%(males) and 52.17% (females). The shape of the horns of Diiti cattle was different in the Kuantan cattle, Male Kuantan cattle from Benai Regency were generally 53% hornless (Misrianti et al., 2018). The dominant horn shape of Katingan cattle in West Kalimantan was curved forward (Utomo, 2016), while Aceh and PO cattle have horns in the form of a small hump (Abdullah et al., 2006). Female Bali cattle from Atinggola District, Gorontalo dominantly have downward curved horns (100%) (Gobel et al., 2021), while dominant males have upward curved horns (76.71%) (Domili et al., 2021).

3.1.3 Dewlap of Diiti cattle

Dewlap is the skin that hangs along the lower neck to the chest in cattle. Male and female Diiti cattle have dewlap (100%). The dewlap width in males was wider than in females. Local cattle in Indonesia generally have a dewlap, especially cattle produced from female cattle who have been inseminated with exotic cattle, for example, male and female Pasundan, Aceh cattle, PO cattle, and Madura cattle have Dewlap (Budiarto et al., 2013; Mukhtar et al., 2015; Utomo, 2016; Said et al., 2017; Misrianti et al., 2018; Putra et al., 2020; Domili et al., 2021; Gobel et al., 2021; Masduqi et al., 2021).

3.1.4 Face shape of Diiti cattle

Based on the side view, the face shape consisted of three shapes, namely concave, flat, and convex. According to researchers Gelaye et al. (2022); Mustefa et al. (2020), and Masduqi et al. (2021), the faces of cattle in Indonesia, Ethiopia, America, and Europe are convex and flat.

They investigated the cattle from the right or left side view, but there was no information about the shape of the cattle's face when viewed from the front. Based on the front view, male and female Diiti cattle have hexagonal, triangular, and perpendicular facial shapes of 7.94%, 49.20%, 42.68% (males) and 36.95%, 5.80%, 57.25% (females), respectively.

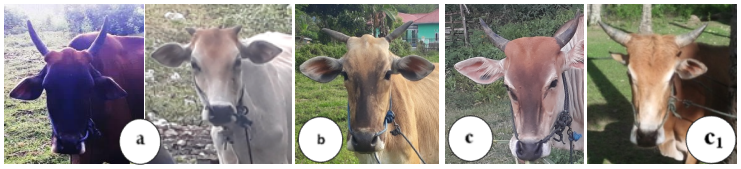

Figure 3. The Face shape of Diiti Cattles: a. Hexagon (female), b. Rectangular perpendicular (female), c.Triangular prism (female) and c_1.Triangular prism (male).

Figure 4. The Face shape of Diiti Cattles a. Convex (male), b.Flate (female).

Based on the side view, male and female Diiti cattle have flat and convex facial profiles of 33.34%, 66.66%, and 74.64%, 25.36%, respectively. The results of this study differed from Aceh cattle which have concave face shapes in males and females with percentages of 80.65% and 90.57% (Masduqi et al., 2021). The facial shapes of Rustaqi local cattle (Irak) were flat. The face shapes of Hill cattle were short and concave (Pundir et al., 2012). Face profiles of Kenana cattle were straight (83.7%). The facial profiles of the native Ethiopian bulls range from flat to slightly concave (Getaneh et al., 2019; Baye et al., 2022).

3.2 Quantitative traits

3.2.1 Body size profile of Diiti cattle

Characteristics of body shape and morphometrics are related to variations and changes in livestock size, including body size and body weight. The genetic existence of livestock in an area makes these livestock adapt to their environment to survive. The diversity ofDiiti cattle body sizes in the coastal area of Tomini Bay was explained in Table 2.

Table 2. Characteristics of body size of male and female Diiti cattle in the Tomini Bay area, Gorontalo, Indonesia

	Male			Female		
	Mean	**SD**	**SE**	**Mean**	**SD**	**SE**
Body length (Bl)	113.75	7.26	0.91	111.11	7.48	0.64
Chest circumference (Cc)	140.67	8.31	0.71	137.32	7.81	0.66
Height body (Hb)	112.15	5.23	0.66	109.26	5.90	0.50
Hip height (Hh)	112.78	4.64	0.59	110.95	4.60	0.39
Hip width (Hw)	33.06	4.46	0.56	34.45	3.05	0.26
Chest width (Cw)	27.99	3.45	0.44	25.72	3.42	0.29
Deep chest (Dc)	56.87	5.29	0.67	53.76	5.20	0.44
Dewlap length (Dl)	49.22	8.21	1.03	33.48	7.59	0.65
Dewlap width (Dw)	15.83	4.28	0.54	10.35	5.24	0.45
Head length (Hl)	41.61	3.10	0.39	40.59	2.85	0.24
Head width (Hhi)	19.61	2.84	0.36	18.34	3.00	0.26
Body weight (Bw)	210.86	35.61	4.49	193.15	31.76	2.70

SD: Standart Deviation, SE: Standart Error.

Descriptive analysis showed that the Bl of male and female Diiti cattle were 113.75±7.26 and 111.11±7.48, respectively. The Cc of male and female Diiti cattle were 140.67 ± 8.31 cm and 137.32 ± 7.81 cm, respectively. The Hb of male and female Diiti cattle was 112.15 ± 5.23 cm and 109.26 ± 5.90 cm, respectively. Hh of male and female Diiti cattle were 112.78±4.64 cm and 110.29±4.60 cm, respectively. The Dc male female Diiti cattle were 56.87±5.29 cm and 53.76±5.20 cm, respectively. Dl values of male and female Diiti cattle were 49.22±8.21 cm and 33.48±7.59 cm, respectively. The Dw of male and female Diiti cattle were 15.83±4.28 cm, and 10.35 ± 5.24 cm, respectively. Hl and Hhi of male and female Diiti cattle were 41.61±3.10 - 40.59±2.85 cm and 19.61±2.84 - 18.34±3.00, respectively. The Bw of male and female Diiti cattle in this study were 210.86 ± 35.61 and 193.15 ± 31.76 kg.

Male and female Diiti cattle have a smaller body length compared to Jabres, PO, and Rancah cattle. The body length of male and female Jabres cattle was 125.8 ± 5 cm, and 119.2 ± 5 cm (Utomo, 2016). PO female cattle 128.10 ± 66.7, while bulls 123.97 ± 11.58-156.1 ± 13.75 cm (Hilmawan et al., 2017; Laya et al., 2020), Rancah Catle 123.52 ± 35.81 cm. The body lengths of male and female Diiti cattle were longer than those of Aceh and Sumatra cattle. Local male cattle from West Sumatra have a body length of 112.40 and 110.7 cm. Male and female Aceh cattle have a body length of 103.60 and 102.90 cm.

According to Domili et al. (2021), Zulkarnaiin Gobel et al. (2021), and Margawati et al. (2019), the body length measurements of local cattle for male and female Bali Cattle were 98.73 ± 10.31 cm and 101.62 ± 6.04 cm. Meanwhile, Bali cattle and crosses between Bali cattle and limousines (Linbal and Sinbal) have body lengths of 110.95 ± 5.89, 132.48 ± 6.39, and 136.25 ± 10.73 cm, respectively (Baliarti et al., 2023).

The body height of the male and female Diiti cattle were 112.15 ± 5.23 cm and 109.26 ± 5.90 cm. The body height of local cattle in other areas of Indonesia was different from that of Diiti cattle. Several research results on the body height of Rancah, PO, Bali, Aceh, and Katingan cattle in Indonesia i.e. Rancah local cattle have a height of 115.21 ± 4.48 cm, while female PO cattle have a body height of 128.10 ± 6.77 cm and male PO cattle have a body height of 124.74 ± 6.70-148.90 ± 6.78 cm (Hilmawan et al., 2017; Laya et al., 2020). The body height of female Diiti cattle was the same size as Bali cattle. Female Bali cattle height was 110.00 ± 4.24 cm (Budiarto et al., 2013; Wilastra et al., 2021), 109.52 ± 5.94 cm (Gobel et al., 2021). Female Aceh cattle and Katingan cattle have a lower height than female Diiti cattle Aceh cattle height was 99.32 ± 4.59 cm (Sari et al., 2021), and Katingan cattle was 102.63 ±

4.90 cm (Utomo, 2016). The average final weight (kg) for fattened PO and Bali cattle were 285.96 and 274.44, respectively (Aditya et al., 2013)

The reason for the population difference was more due to genetic and environmental factors, where inbreeding was more common in cattle within one population, and between adjacent populations. Cattle body size is a descriptive picture of the genetic potential of cattle in a population. The growth and development of cattle bodies were influenced by genetics and the environment (Ciptadi, 2015; VMA Nurgiatiningsih, 2023). There are more interbreeding, and livestock-rearing techniques between populations. Livestock that have superior genetics that are reared in an unfavorable environment will be affected for the growth and development of these livestock, while livestock that have poor genetics will provide maximum growth and development if reared in a good environment. The phenotype of cattle is influenced by genetics and the environment (Hardjosubroto, 1994), while Putra (2020) stated that variations in the productivity of crossbreed cattle are caused by differences in mating parents, so according to Utomo et al., (2011) the offspring produced have different body sizes. Agung et al. (2019), and Priyanto et al. (2015) state that Indonesian local cattle belong to the Zebu cattle family, and have different body sizes and diversity values as a result of genetic mixing with other cattle.

Conclusion

Diiti cattle is one of the local Gorontalo cattle with phenotypes of various body colors in both males and females. Generally, female Diiticattles have white, reddish white, and brownish white colors, and male Diiti cattle have black, brown, white, and reddish brown colors. Male and female Diiti cattle have hexagonal, triangular, rectangular, and wavy face shapes. Therefore, further characterization of Diiti cattle in the study area and the whole Gorontalo area is required as basic information on Diiti cattle as Gorontalo local cattle.

Acknowledgements

The authors gratefully acknowledge the Chancellor of Gorontalo State University and the Dean of the Faculty of Agriculture of Gorontalo State University, for the research support funding through the 2023 UNG PNBP Fund.

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Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

ISSN: 1308-7576
e-ISSN: 1308-7584

Research Article

Extraction, Characterization and Antibacterial Potential Assessment of Polar Phytoconstituents of *Caesalpinia bonducella* Seeds Extract

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Article Info

Received: 19.09.2023
Accepted: 05.02.2024
Online published: 15.03.2024
DOI: 10.29133/yyutbd.1362736

Keywords

Antibacterial activity,
Caesalpinia bonducella,
Polar phytoconstituents

Abstract: Plant-based medicine has been utilized to cure ailments at a low cost all over the world, medicinal plants are the primary source of medicines and the healthcare system. Traditional medicine has long utilized the seeds of *Caesalpinia bonducella* to cure a variety of symptoms and afflictions, including malaria, colic, fever, edema, leprosy, and abdominal pain. The current investigation aimed to identify the polar phytoconstituents and their antibacterial activity in *Caesalpinia bonducella* seed extracts using polar solvents (methanol, ethanol). The extraction of the phytoconstituents of seed powder of *Caesalpinia bonducella* was carried out by using Soxhlation method. Then the extract was examined by FT-IR, RP-HPLC, and the traces were confirmed by using the GC-MS technique. Antibacterial studies of the extract showed that the active constituents present in the extract have considerable activities against microbes like *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger*, and *Salmonella typhi*. Perhaps it could serve as a substitute for the commercially available synthetic antibiotics. A microbial assay has been performed to assess the antibacterial potency of the identified phytochemicals.

To Cite: Khamkat, P, Chatterjee, B, Barik, V, Patra, D, Chakraborty, A, 2024. Extraction, Characterization and Antibacterial Potential Assessment of Polar Phytoconstituents of *Caesalpinia bonducella* Seeds Extract. *Yuzuncu Yil University Journal of Agricultural Sciences*, 34(1): 115-127. DOI: https://doi.org/10.29133/yyutbd.1362736

1. Introduction

Traditional medical practices are used to treat more than 64% of the population and 82% of those are in emerging countries, demonstrating the expanding acceptance of traditional medicine as it is accessible and affordable. Traditional medicine uses a wide range of plant parts, such as bark, roots, seeds, stems, fruits, and leaves for the treatment and prevention of various diseases. In Pune, India, flowering and fruiting take place between July–April and November–December. In Asia's tropical and subtropical regions, such as India, Sri Lanka, Bangladesh, Burma, Myanmar, China, and Vietnam, *C. bonducella* is a prickly shrub or woody vine. It can be found in wastelands, hills, plains, forests, seaside areas, and the Himalayas up to 1000 meters. After sowing, *C. bonducella* grows swiftly, reaching a height of 26 cm in 40 days can grow in a wide range of soil pH and loves full sun to mild shade. It scrambles onto low trees and bushes and grows well in grassy and herbaceous areas. Beach vegetation,

coastal dunes, and mangrove forests are examples of distributed areas where *C. bonducella* flourishes. To create a hedge, seeds are sown at 50 cm intervals after being soaked overnight at the start of the rainy season. After 3–4 weeks, the plants sprout, and in 2–2½ years, reach their optimum height. In order to induce complete germination when exposed to blue light, the naturally occurring plant might be treated with strong sulfuric acid for a duration of 30 to 90 minutes (Sasidharan et al., 2021). On the other hand, modern medicine uses active constituents that are isolated from various plant parts, with 80% of these active components being effective in treating particular diseases with their anticancer, hepatoprotective, antioxidant, antimalarial, antibacterial, antipyretic, antifertility, and anti-inflammatory qualities (Kannur et al., 2006; Moon et al., 2010). In India, the traditional Ayurvedic medical system is used by 76% of the rural population. With over 20 000 medicinal plants and 250 000 registered Ayurvedic practitioners, India is the world's greatest producer of medicinal plants. Traditional medical systems are used by more than 1.5 million practitioners, and more than 7800 industrial facilities produce natural health products and conventional plant-based medications (Ali et al., 2009; Subbiah et al., 2019). Herbal medicine can reduce the adverse effects of pharmaceutical medications by addressing the root cause of pain or discomfort. By switching from prescription medicines to natural remedies, patients can speed up their recovery and benefit from the help of naturopathic physicians (Ghosh and Khamkat, 2021). Herbal medications contain vitamins, antibodies, and other health-improving ingredients, addressing problems from the inside out (Mehra et al., 2015). Additionally, using herbal medications offers financial savings and better, more economical healthcare. *Caesalpinia bonducella*, also known as the bonduc bean or bonduc nut, is an Ayurvedic medicine plant with numerous beneficial effects on the human body (Kannur et al., 2012; Gadakh et al., 2020). Its seeds are used in Ayurvedic medicine, and the plant grows to 10 meters tall and has sharp spines (Billah et al., 2013). The leaves are around 30- to 60-cm-long bipinnate having thorny petioles, with small yellow flowers and clusters of leaflets. The calyx has lobes that are obovate-oblong and obtuse, and it is 6–8 mm long, fulvous, and hairy. The petals have declinate, flattened filaments at the base, and are yellow and oblanceolate. The pods of fruits with 10 seeds are hard and brown with a bitter taste. The treated seeds are extracted from 1–1.25 mm testa in a dry state and have a firm, glossy coat. Their colour is pale yellowish white, their texture is ridged, and their flavour is bitter (Sundare et al., 2007; Kakade et al., 2017). The common name of *Caesalpinia bonducella* plant seed is Bonduc nut, fever nut. It belongs to the family of Fabaceae/Caesalpiniaceae. Katkaliji, Gataran, Karanju, Gajaga, Gajjuga, and Heggejjuga are some of its common names in Hindi. It is also referred to as Gatchakai in Sanskrit and Lata Karanja in Telugu. It is referred to as Kazhanchikkuru Kalechikai, Kazharchikkaai in Tamil (Arindam et al., 2007). This Bonduc nut is useful to cure a variety of symptoms and afflictions, including diabetes mellitus, malaria, colic, fever, edema, leprosy, and abdominal pain. Although this plant has several medical properties, the major chemical constituents and the effects of its 'seeds (Bonduc nut)' have not yet been studied properly. The major phytoconstituents in the alcoholic extract (methanol and ethanol) and their potential for microbiological evaluation have been analyzed using a unique analytical approach in this work.

2. Materials and Methods

2.1. Materials

2.1.1. Plant material

Caesalpinia bonducella seeds were collected from an authentic shop in the local market of Barasat, Kolkata-700125. The seeds were identified and authenticated by Acharya Jagadish Chandra Bose Indian Botanical Garden, Shibpur, Howrah- 711103.

2.1.2. Chemicals

Here, the ingredients were used for the extraction and characterization of phytoconstituents of Caesalpinia seeds. Ethanol (Oxford Lab Fine Chem LLP), Methanol (Oxford Lab Fine Chem LLP), Petroleum ether (Oxford Lab Fine Chem LLP), Chloroform (Oxford Lab Fine Chem LLP), Ethyl acetate (Nice Chemical Pvt Ltd), Sulphuric acid (Nice Chemical Pvt Ltd), Formic acid (Nice Chemical Pvt Ltd), Toluene (Nice Chemical Pvt Ltd), Mayer's reagent (Universal Chemicals), Dragendroff's reagents (Universal Chemicals), Benedict's reagent (Stanbio Reagents Pvt Ltd), Ninhydrin reagent (Universal Chemicals), Fehling's A (Universal Chemicals), Fehling's B (Universal Chemicals), Iodine (Nice

Chemical Pvt Ltd), Potassium iodide (Nice Chemical Pvt Ltd), Ferric chloride (Oxford Lab Fine Chem LLP), Ammonia (Nice Chemical Pvt Ltd), Acetic acid (Loba Chemie Pvt Ltd), Anisaldehyde (Loba Chemie Pvt Ltd), Millon's reagent (Universal Chemicals), Olive oil (Nice Chemical Pvt Ltd) were obtained from Brainware University, Barasat.

2.1.3. Instruments

GC-MS manufactured by Perkin Elmer GC Clarus 680 MS Clarus 600 (EI), Electronic balance manufactured by Mettler Toledo ME204, Water bath manufactured by Vinayak Enterprise, pH meter manufactured by Mettler Toledo, Hot air oven manufactured by Vinayak Enterprise, UV spectroscopy manufactured by Shimadzu UV-1900I, RP-HPLC manufactured by Waters 1525 & 2998 PDA, IR spectroscopy manufactured by Bruker Alpha II, UV Cabinet manufactured by Vinayak Enterprise, Autoclave manufactured by Vinayak Enterprise, Laminar flow manufactured by Vinayak Enterprise, BOD Incubator manufactured by Vinayak Enterprise, were utilized in the research**.**

2.1.4. Microorganisms

Microorganisms like *Salmonella Typhi* (Gram Negative bacteria), *Escherichia coli* (Gram Negative bacteria), *Staphylococcus aureus* (Gram Positive bacteria), and *Aspergillus niger* (Fungi) were used for determining the inhibitory activity of antibacterial agents for the study.

2.2. Method

2.2.1. Preparation of the extract

In this study, the process of extraction and preservation of *C. bonducella* seed extracts was meticulously carried out to ensure the purity and potential therapeutic value of the obtained extracts. Initially, the seed kernels were separated from the outer seed shell using a mortar and pestle, allowing for precise isolation of the desired material. 50g of *C. bonducella* seeds were ground into a powder, and 500 ml of 95% methanol and ethanol (polar in nature) were used as the extracting solvent (Joshi et al., 2016; Sembiring et al., 2018). This method included maceration, percolation, and Soxhlet extraction. The extraction method, which lasted 16 hours, effectively dissolved the beneficial chemicals in the powdered seeds. Particulate debris and contaminants were then filtered out using Whatman filter papers. Evaporation was used to further concentrate the filtrate, producing a highly concentrated C. bonducella seed extract. Finally, to ensure the preservation of the extract's potency and quality, it was carefully stored in a sterile glass container at a temperature of 4°C, providing an optimal environment for future utilization and potential therapeutic applications (Kannur et al., 2006; Ali et al., 2009).

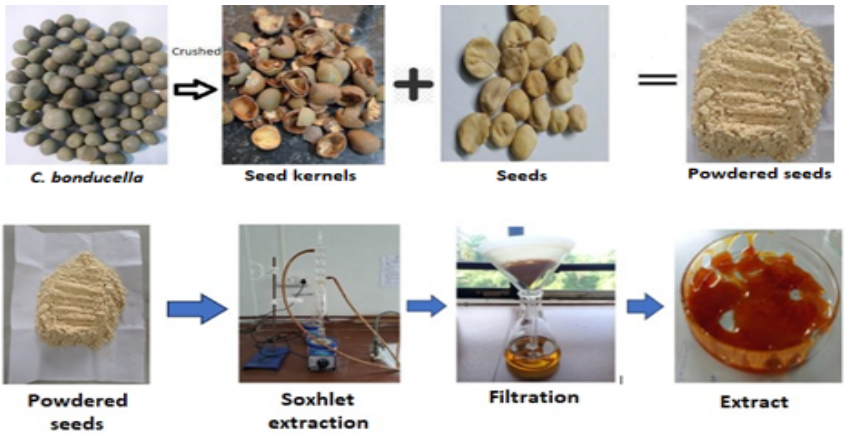

Figure 1. Extraction of *Caesalpinia bonducella* through Soxhlet apparatus.

2.2.2. Preparation and sterilization of Agar Plate

The ingredients used for the preparation of nutrient agar media are beef extract, peptone, sodium chloride, distilled water, and agar (Simin et al., 2001; Sabu et al., 2003).

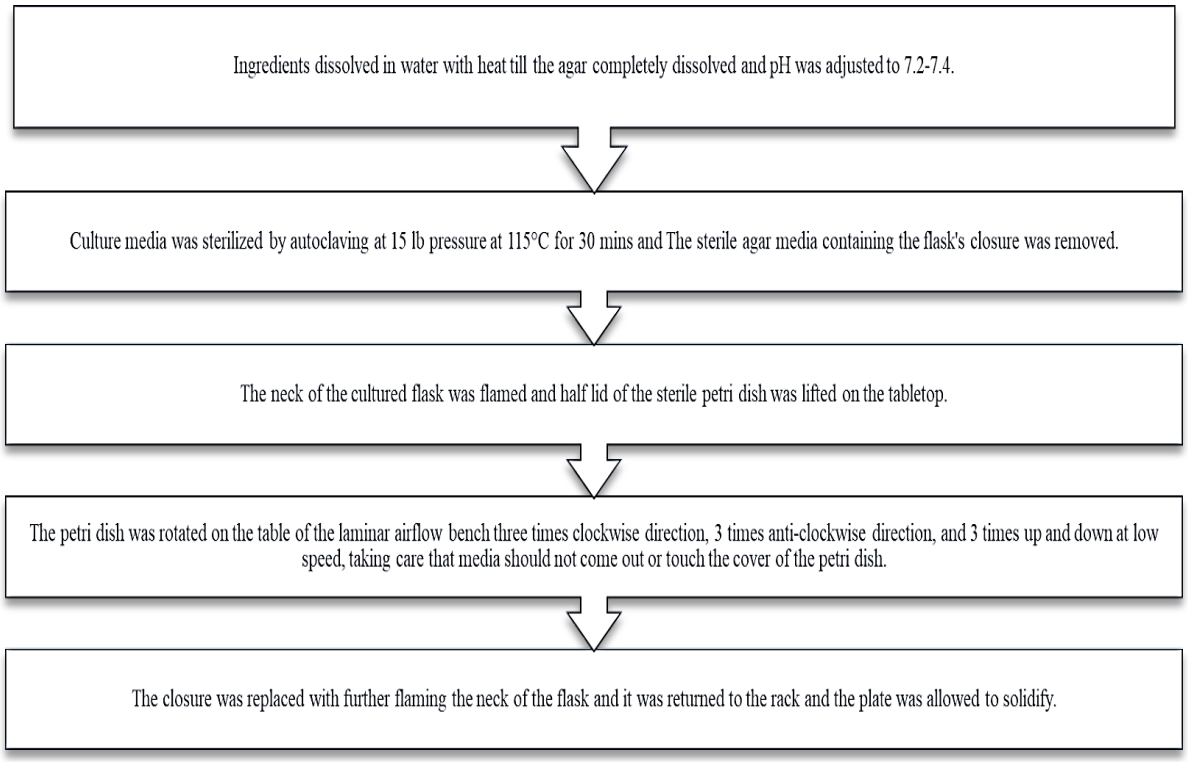

Figure 2. Sterilized agar plate preparation for microbial assay.

2.2.3. Characterization studies of seeds

Organoleptic properties like colour, odour, shape, size, and taste of *C. bonducella* seeds and extract have been assessed.

2.2.4. Chemical studies for identifying phytoconstituents

Standard techniques were applied when subjecting the methanolic extracts to a range of chemical analyses for the detection of phytoconstituents (Shalini and Ilango, 2021).

2.2.4.1. Test for alkaloids

Dragendroff's Test: 1 ml of Dragendroff's reagent was added to 2 ml of the extract along the test tube's edge. Alkaloids were present when an orange or orange reddish-brown precipitate formed.

Wagner's Test: Wagner's reagent (2 mL) was combined with 1 ml of crude extract. Alkaloids are indicated by reddish-brown precipitate, which is a marker of their presence. A solution of 2.5 g of iodine and 12.5 g of potassium iodide (KI_2) in 250 ml of water was created to create Wagner's reagent.

2.2.4.2. Test for cardiac glycosides

Keller-Kiliani Test: 2 ml of glacial acetic acid with a single drop of ferric chloride solution were added to 5 ml of extract. 1 ml of sulfuric acid that had been concentrated came next. The presence of carotenoids' deoxy sugar is suggested by the interface's rich brown colour. Under the brown ring, a violet ring may show up, and as the acetic acid layer steadily grows, a greenish ring may form.

2.2.4.3. Test for carbohydrates

Fehling's Test: Equal amounts of Fehling A and B reagents were added, and the mixture was then heated slightly before adding 2 ml of crude extract. Because a brick-red precipitate developed at the test tube's bottom, reducing sugars were discovered.

Benedict's Test: 1 ml of crude extract and 2 ml of Benedict's reagent were combined and heated. Carbohydrates were present because a reddish-brown precipitate formed (Manikandaselvi et al., 2016).

2.2.4.4. Test for flavonoids

Shinoda Test: 2 ml of the extract was mixed with 1 ml of a 1% ammonia solution. The easiest way to identify whether flavonoids are present visually is by the colour yellow.

2.2.4.5. Test for saponins

Foam Test: 2 ml of natural extract and 5 ml of distilled water were mixed and agitated vigorously in a test tube. Add some olive oil drops. The formation of steady foam was thought to indicate the presence of saponins.

2.2.4.6. Test for free amino acid

Millon's Test: 1 ml of crude extract and 2 ml of Millon's reagent are combined to generate a white precipitate. This precipitate turns red when it is slowly heated, signifying the presence of protein.

Ninhydrin Test: 1 ml of the natural extract and 2 ml of Ninhydrin 0.2% solution were combined and heated. The presence of proteins and amino acids was indicated by the violet precipitate that appeared.

2.2.4.7. Test for tannins

5% Ferric chloride Test: 0.5 ml of 5% ferric chloride was added to 5 mg of extract. Tannins are present when a dark bluish-black colour develops (Nakajima et al., 2005).

2.2.5. Quantitative estimation of phytoconstituents

2.2.5.1. Thin layer chromatographic study

For isolating, identifying, and quantifying plant components, chromatography techniques are essential. To establish the existence of early phytochemicals, thin-layer chromatography (TLC) methanol and chloroform in the ratios of 9:1, 8.8:1.2, and 9.2:0.8 served as the mobile phase. Compounds are separated and classified using TLC depending on how well they adhere to the stationary and mobile phases. A TLC plate is used to apply the prepared sample, and as the mobile phase passes through the stationary phase, different places on the plate represent various phytochemicals (Juvatkar and Jadhav, 2021). The relative polarity and other features of these spots can be learned from them. TLC is a useful approach for analyzing plant extracts and helps characterize and identify bioactive substances (Singh and Raghav, 2012).

2.2.5.2. Absorbance maxima determination

Regarding the domain of characterizing natural products, the utilization of ultraviolet-visible (UV-Vis) spectrophotometry to determine absorbance maxima has become a crucial technique. UV-Vis spectrophotometry precisely helped to identify what was performed. A TLC plate serves as the stationary phase, while a solvent combination includes and quantifies the absorbance maxima at specific wavelengths that correspond to the phytoconstituents like the presence of flavonoids (Pandey et al., 2018).

2.2.5.3. RP-HPLC analysis

Utilization of Reverse Phase HPLC for the comprehensive characterization of *Caesalpinia bonducella* seed extract was done. The RP-HPLC was carried out with the help of a Waters 1525 series chromatograph equipped with a gradient pump, and photodiode array detector (2998) and the sample injection volume of 20 µl. The Rhseodyne sample injector was utilized for the study. The analytical column diameter was 4.6 x 250 mm C18 (Waters, USA), and the particle size of the packed silica is 5 microns, it was 1 ml min^{-1} for the mobile phase flow rate. A binary pump gradient program was carried out in the process. Reservoir A contained acetonitrile, and Reservoir B contained water. Prior to injecting into the column, the extract is diluted twenty times and filtered through a 0.22 µm syringe filter. The gradient software utilized was as follows: 0–5 min 20% A; 5–8min: 30% A; 8–12 min: 40% A; 12–18 min: 50% A; 18–24 min: 60% A, 24-30 min: 70% A, 30-40 min: 80%. The total analysis time was 40 minutes. The peaks were recorded at 280nm. The scanning of the UV spectrum was performed from 200 to 800nm (Kumar et al., 2015; Mondal et al., 2023).

2.2.5.4. IR study

The methanolic extract has selective absorption in the infrared region, which makes it suitable for the structural investigation of its functional groups using infrared spectroscopy. (Khamkat et al., 2022).

2.2.5.5. GC-MS Study

Helium was used as a carrier gas at a constant flow rate of 1 ml min^{-1} to separate the components of a fused silica column packed with Elite-5MS (5% biphenyl, 95% dimethylpolysiloxane; 30 m × 0.25 mm ID × 250 µm df) that was utilized in the Clarus 680 GC analysis. During the chromatographic run, the injector temperature was set to 260 °C. The apparatus was filled with 1 µL of methanolic extract sample, and the oven temperature was set to 60 °C for two minutes, then 300 °C at a rate of 10 °C per minute; finally, 300 °C was maintained for six minutes. Conditions for the mass detector were a 240 °C transfer line, a 240 °C ion source, an ionization mode electron impact at 70 eV, a 0.2 s scan time, and a 0.1 s scan interval. The objects range was 40–600 Da. A database of component spectrums kept up to date in the GC-MS NIST (2008) library was compared to the component spectrums.

2.2.6. Microbial assay

Through uniformly circular zones of inhibition, the antimicrobial agents were permitted to spread into a plate (İnci et al., 2021). In order to assess the fungicidal and inhibitory concentrations' potency, the cup plate method was employed (Gupta et al., 2003; Ata et al., 2009).

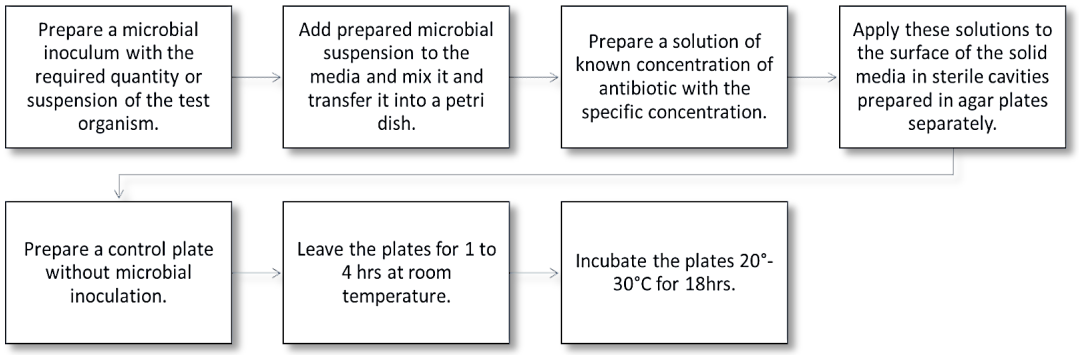

Figure 3. Steps involved in cup plate method for microbial assay.

3. Results

3.1. Characterization studies of seeds extract

Different extraction methods of *C. bonducella* seeds with different yield values are mentioned in Table 1.

Table 1. Different extraction methods by using different solvents

Sl no	Physical nature of seeds	Solvent	Method of extraction	Observation
1	Solid Powder	Methanol, ethanol	Soxhlet Extraction	More Yield
2	Solid Powder	Methanol, ethanol	Percolation	Least Yield
3	Solid Powder	Methanol, ethanol	Maceration	Moderate Yield

3.2. Organoleptic characters

The organoleptic properties of *C. bonducella* seeds and extract are described in Table 2.

Table 2. Observation of organoleptic properties of *C. bonducella* seeds and their powder form

Sl no	Properties of seeds		Sl no	Properties of powdered seeds	
1	Colour of seeds:	Off-white	1	Nature of powder:	Coarse powder
2	Taste:	Bitter	2	Colour of powder:	Off-white
3	Shape of seeds:	Globular	3	Colour of extract:	brown and dark
4	Size:	1-2 cm in diameter and 2.2-4 cm in length	4	Odour:	Characteristics
5	Odour:	Characteristics	5	Taste:	Bitter

3.3. Chemical studies for identifying phytoconstituents

Polar and nonpolar extract for the phytochemical tests. The polar extracts are ethanolic and methanolic extract and the nonpolar extract is the petroleum ether extract. The polar extracts have shown positive results in Table 3 for the chemical constituents like alkaloids, glycosides, tannins, flavonoids, etc.

Table 3. Qualitative analysis of phytochemicals presents in *Caesalpinia bonducella* seeds in different solvents

Sl no	Phytochemical tests	Methanolic extract	Ethanolic Extract
1	Alkaloids	+	+
2	Cardiac Glycosides	+	+
3	Flavonoids	+	+
4	Tannins	+	+
5	Saponins	-	-
6	Phenols	+	-
7	Steroids	-	-
8	Terpenoids	-	-
9	Quinones	-	-
10	Proteins	-	-

"+" indicates positive result and "-" indicates negative result.

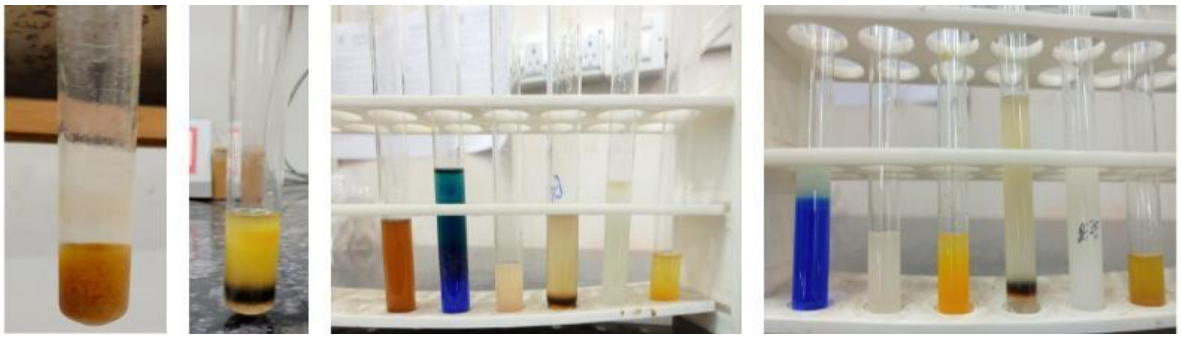

Figure 4. Identification tests of phytochemicals present in *Caesalpinia bonducella* seeds extract.

3.4. Thin layer chromatography

Methanolic and ethanolic extracts have undergone TLC. In Table 4, the methanolic extract has demonstrated the separation of eight distinct compounds, whereas the ethanolic extract has demonstrated the separation of two to three distinct compounds by displaying distinct colors on the TLC plate when viewed at 366 and 254 nm.

Table 4. Thin Layer Chromatography of methanolic and ethanolic extract from *Caesalpinia bonducella* seeds

Sl no	Chemical Constituent	Solvent System	Spraying Reagent	Observation	Retention Factor
1.	Methanolic extract	Formic acid: toluene: methanol: ethyl acetate: (5:4.5:4.5:1)	Aniline-Sulphuric acid	Showed the presence of 2-3 compounds	0.292,0.414,0.853
2.	Methanolic extract	Chloroform: methanol (9:1/8.8:1.2/ 9.2:0.8)	Anisaldehyde - Sulphuric acid	Showed the presence of 8 compounds	0.243,0.452,0.707,0.829,0.951
3.	Ethanolic extract	Methanol: acetic acid: ethyl acetate (6:4:1)	Anisaldehyde - Sulphuric acid	Showed the presence of 2 compounds	0.2, 0.709

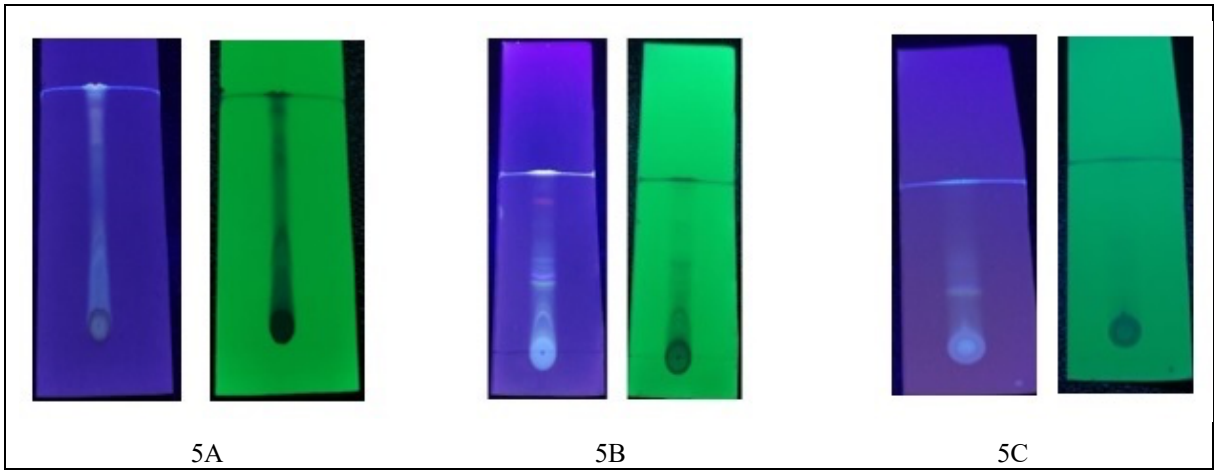

Figure 5. Separation of compounds visible at different wavelengths (366nm & 254nm) of methanolic extract using toluene, ethyl acetate methanol, and formic acid as solvent (5A), methanolic extract using chloroform and methanol as solvent (5B), ethanolic extract using ethyl acetate, methanol, and acetic acid as solvent (5C).

3.5. Infrared Spectroscopy

FT-IR Spectroscopy is done for functional group investigation of the present compounds. Here, the methanolic extract has been used, different functional groups of different compounds are shown at their peak in Figure 6A and interpretation of IR spectra is given in Table 5.

Table 5. Interpretation of IR spectra of methanolic extract of *Caesalpinia bonducella* seeds

Wavenumber(cm^{-1})	Functional Groups
3331.10	O-H Stretching
2914.97	C-H Stretching (Nearby Unsaturation point)
2833.41	C-H Stretching (Nearby saturation point)
1655.89	R-COOR´(Ester)

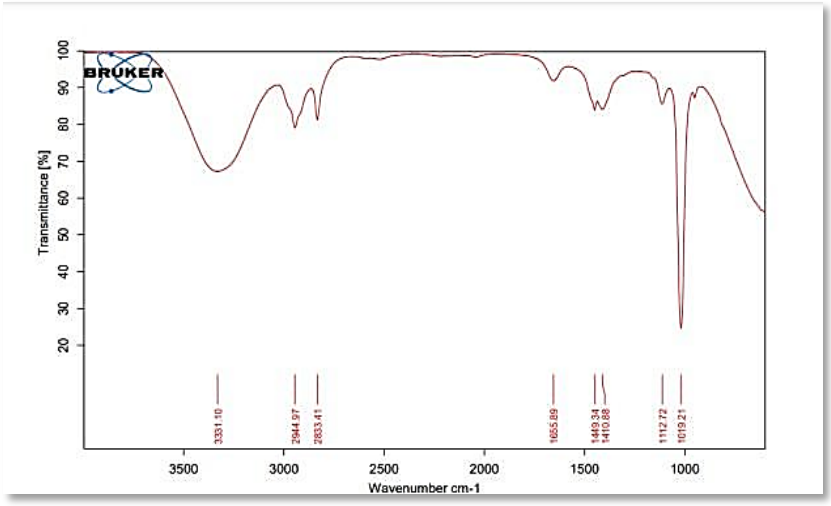

Figure 6. Graphical representation of specific absorbance of methanolic extract of *Caesalpinia bonducella* seeds in IR Spectroscopy.

3.6. RP-HPLC

The concentrated methanolic extract has been diluted 20 times with methanol and the dilute sample has been used in RP-HPLC for the analysis of different compounds and their concentrations are shown in Figure 8A and the data is given in Table 6.

Table 6. RP-HPLC chromatogram data of methanolic extract of *Caesalpinia bonducella*

Retention Time (min)	Area (µV*sec)	%Area	Height (µV)
2.011	4668353	27.65	212940
2.287	2981797	17.66	335308
2.444	4487020	26.58	224078
3.272	694613	4.11	188697
3.383	1537591	9.11	475799
3.812	754089	4.47	79587
4.083	250316	1.48	15139
4.426	327079	1.94	12293
12.915	399487	2.37	49358
21.087	170005	1.01	11584
26.824	165148	0.98	19226
27.187	243846	1.44	22640
27.895	204674	1.21	21567

3.7. GC-MS study

It helps to identify the number of components and the types of compounds present in the methanolic extract showed in Figure 8B. Table 7 and Figure 7 indicate the structural representation of compounds identified in the *Caesalpinia Bonducella* Seeds extract by GC-MS.

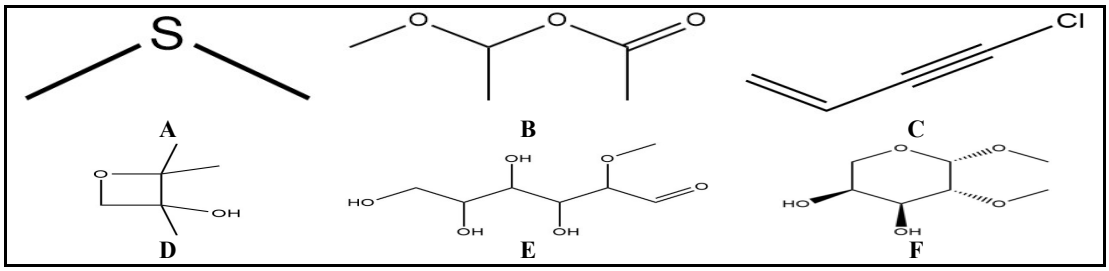

Figure 7. Compounds (A–F) found in the *Caesalpinia bonducella* seed extract and their structural representation using GC–MS.

Table 7. Data of GC-MS study of methanolic extract from *Caesalpinia bonducella* seeds

Compounds	Retention Time(min)	Peak Area %	Compound Name	Molecular formula	Molecular weight(m/z) (Dalton)	Compound nature
A	1.123	5.467	Dimethyl sulfide	C_2H_6S	62	Thioether
B	1.168	2.998	Ethanol, 1-methoxy-, acetate	$C_5H_{10}O_3$	118	Ester
C	1.188	8.373	4-chlorobuten-3-yne	C_4H_3Cl	86	Choloro alkyne
D	1.253	5.395	3-oxetanol, 2,2,3-trimethyl	$C_5H_{10}O$	116	Alcohol
E	16.875	56.294	2-o-methyl-d-mannopyranosa	$C_7H_{14}O_6$	194	Polyhydroxy alcohol
F	16.945	21.473	Methyl-2-o-methyl beta l-arabinopyranoside	$C_7H_{14}O_5$	178	Carbohydrate

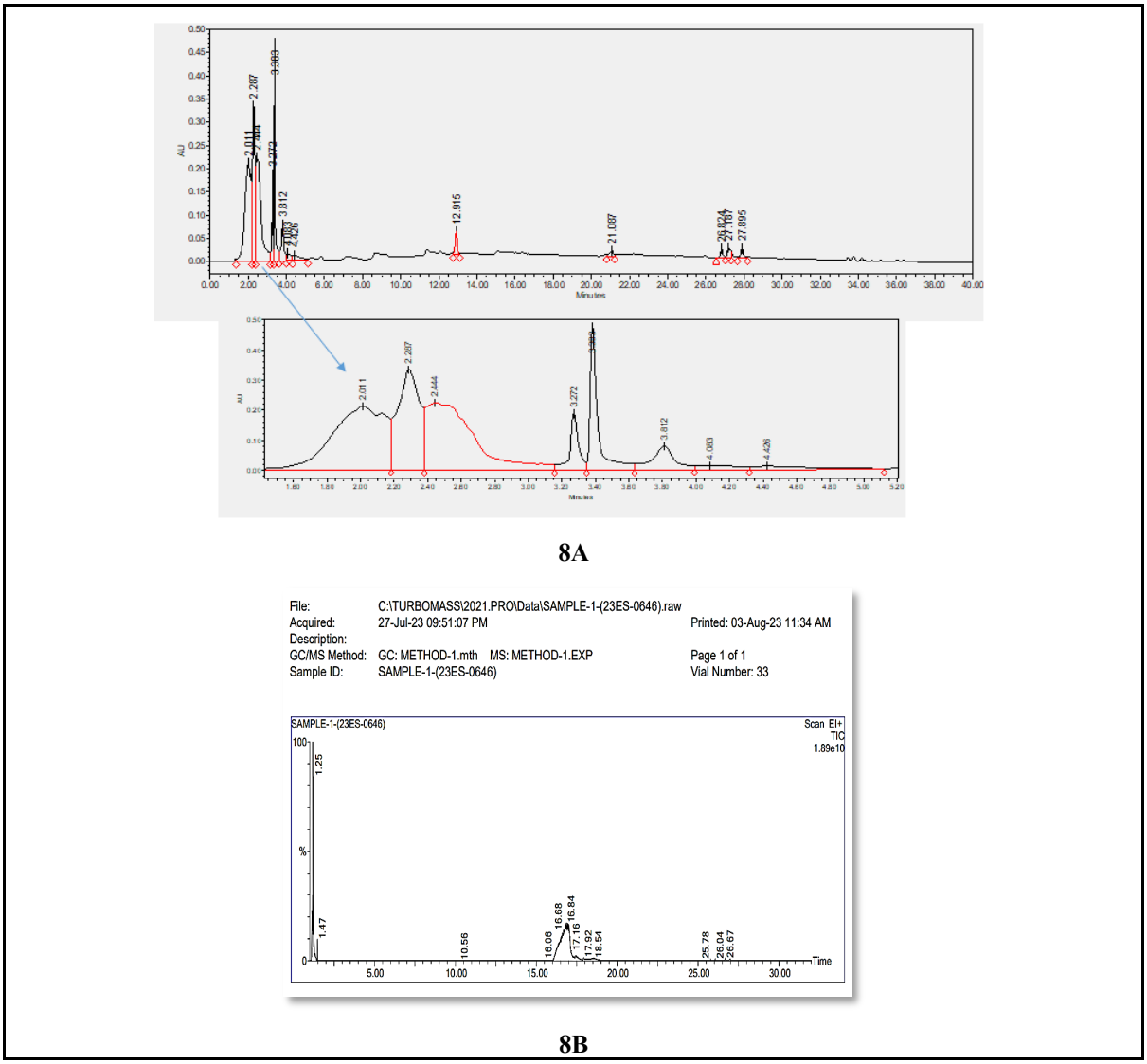

Figure 8. Methanolic extract of *Caesalpinia bonducella* seeds obtained by RP-HPLC (8A) and GC-MS chromatography (8B).

3.8. Microbial assay

Microbiological experiment has been carried out to identify the area where microorganisms undergo inhibition by the methanolic and Ethanolic extract using the Soxhlet extraction method. The

methanolic extract has the highest antibacterial activity shown in Table 8 and Figure 9 shows a zone of inhibition for determining the potency.

Table 8. Ethanolic and methanolic extract's Antibacterial Properties

Microbes	Number of tested isolates	Concentrations of ethanolic extract	Diameter of inhibition zone of Ethanolic extract	Concentrations of Methanolic extract	Diameter of inhibition zone of Methanolic extract
Salmonella typhi	4	100,150,200,250	12±0.53	100,150,200,250	28±8.0
E. coli	5	100,150,200,250	16±0.54	100,150,200,250	28±8.2
Staphylococcus aureus	5	100,150,200,250	12±0.54	100,150,200,250	30±7.9
Aspergillus niger	4	100,150,200,250	17±0.58	100,150,200,250	20±5.0

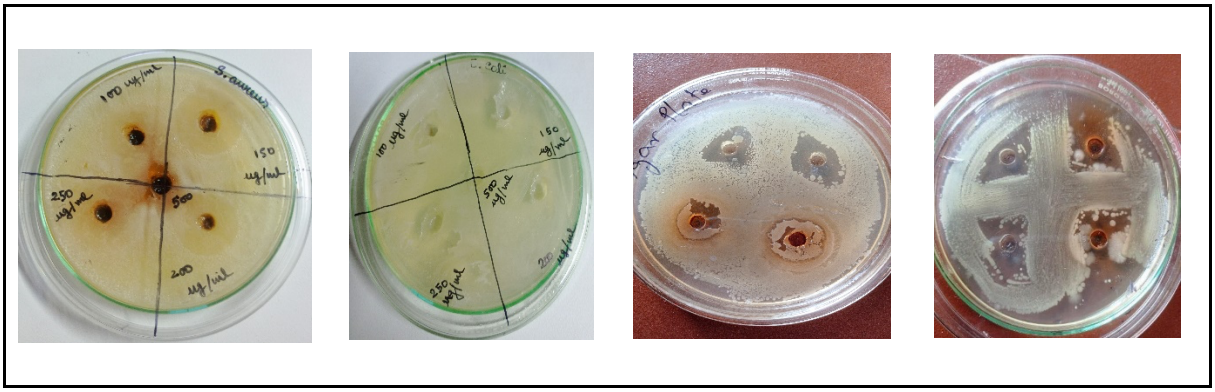

Figure 9. Zone of inhibition of antibacterial microorganisms against methanolic extract.

4. Discussion

The extraction process of the *Caesalpinia bonducella* seeds was carried out using several extraction techniques, including soxhlation, percolation, and maceration. Among all these techniques from the soxhlation process, the yield was the highest. When the methanolic and ethanolic extracts were examined chemically, the methanolic extract gave a superior result. Then further study is carried out with reference to methanolic extract. TLC for the methanolic extract showed eight significant spots, which provisionally indicates the presence of several polar phytoconstituents. Then further FTIR, HPLC, and GC-MS were carried out to determine those polar phytochemicals. The existence of "alcohol", "ether", and unsaturated "alkanes" has been detected in the IR spectroscopy report. Then RP-HPLC was also done to confirm more evidentially the presence of polar constituents in the extracts, and it showed a positive result when the peaks were recorded at 280nm in the investigation. To confirm it, again GC-MS study was carried out. In 2021 Sasidharan et al. reported about the antibacterial activity of *Caesalpinia bonducella* plant (Sasidharan et al., 2021). Finally, a microbiological assay was carried out for both the extracts (methanolic and ethanolic), and it showed a more significant area of inhibition for the methanolic extract.

Conclusion

Caesalpinia bonducella is a valuable therapeutic plant, as demonstrated by the phytochemical investigations done on its seeds. Percolation, maceration, and the Soxhlet apparatus were used to get the total methanolic extract. The extract from the Soxhlet apparatus was more suitable for TLC. Thin Layer Chromatography was performed to isolate compounds. By using UV spectroscopy to quantify phytoconstituents for phytochemical substances, the highest absorbance of two distinct molecules was discovered. at 271.0 and 210.0 nm. IR spectroscopy method has been carried out and it shows the presence of "Alcohol", "Ester" & "Unsaturated alkanes" and later we confirmed their presence with the GC-MS Study. As part of the examination, RP-HPLC was also used to prove the presence of major

chemical constituents. When peaks were recorded at 280 nm, the RP-HPLC test yielded a positive result, and a GC-MS analysis was used to confirm it. Numerous gram-positive and gram-negative bacteria as well as fungi have been used in microbial assays for ethanolic and methanolic extract. It can be said that *Caesalpinia bonducella* seeds containing different phytochemicals have antibacterial and antifungal activity which can be determined by the zone of inhibition. *Caesalpinia bonducella* can be used as an antibacterial agent alternative to synthetic compounds and methanolic extract showed better antibacterial activity. Cultivation of *Caesalpinia bonducella* should be increased in India to treat microbial infections.

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YuzuncuYil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

ISSN: 1308-7576 e-ISSN: 1308-7584

Research Article

Relationships Between Partial Milk Yield and Actual Milk Yield According to Parity in Buffaloes

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Article Info

Received: 10.10.2023
Accepted: 05.02.2024
Online published: 15.03.2024
DOI: 10.29133/yyutbd.1373681

Keywords

Anatolian Buffalo,
Lactation yield,
Partial milk yield,
Regression

Abstract: In this study, the relationships between 100-day of partial milk yield and 270-day of additive and total milk yield of buffaloes according to different parity were investigated. A total of 697 lactation records belonging to 135 heads of buffalo and seven lactations were used in the material. The relationships between the animals' 100-day partial milk yield for 270-day and total milk yield were analyzed for seven lactations. In the study, 100-day milk yield was taken as an independent variable, 270-day and total milk yields as dependent variables, and possible relationships were determined according to simple and multiple linear regression analysis methods. The average lactation period was 256.74 ± 2.61 days, the average 270-day additive milk yield was 2078 ± 65.26 litres and the average total milk yield was 1831 ± 89.57 litres. The daily average milk yields were 7.69 ± 0.11 and 7.08 ± 0.07 liters for 270-day and 100-day respectively. The correlation coefficients were calculated for each parity and calculated as 0.901 ($p < 0.01$) between the 100-day and 270-day additive yield for the general group. The simple and multiple linear regression equations were shown as [$V_{270} = 470.72 + 1.737\ V_{100}$ ($R^2 = 80.2\%$)], [$V_{270} = 966.23 + 0.645\ V_{100} + 0.001 V_{100}^2$ ($R^2 = 82.4\%$)] for the groups. As the parity of lactation increased, it was seen that the determination coefficients were increased. Finally, predicting the total lactation yield by using 100-day of partial milk yield has the highest accuracy.

To Cite: Soysal, M, Gürcan, E K, Özkan Ünal, E, 2024. Relationships Between Partial Milk Yield and Actual Milk Yield According to Parity in Buffaloes. *YuzuncuYil University Journal of Agricultural Sciences*, 34(1): 128-137. DOI: https://doi.org/10.29133/yyutbd.1373681

1. Introduction

There are some intensive buffalo farms in our country that carry out buffalo farming at the level of modern enterprises but in general terms, it is extensively maintained family sized small enterprise conditions. This situation causes the yield levels of our animals to be low. In recent years, with the activities of Community-Based Anatolian Buffalo Breeding Program of the General Directorate of Agricultural Research and Policies of The Ministry of Agriculture and Forestry and Buffalo Breeding Associations carried out projects in national dimensions and significant developments have been achieved in buffalo breeding for our country. The number of buffaloes in our country has increased over time. Accordingly, according to the statistics of 2023, our buffalo presence was 167 000 heads (Turkstat, 2023).

Lingna et al. (2018) showed that, monthly records as the partial yield from the data of the Dairy Herd Improvement Centre were used to estimate the 305-day milk yield with six models (Gaines, Sikka,

Nelder, Wood, Dhanoa, and Hayashi) and controlled with actual records from farms. These models can be used to predict the 305-day milk yield and further assist farm management decisions and for the genetic evaluation of cattle. Singh (2022) showed that the simple linear regression of the first lactation yield on each partial milk yield can be used to predict the whole first lactation milk yield of Murrah and Nilli Ravi buffaloes. In the case of cumulative milk production, the 90-day yield showed the highest magnitude of R^2 value for the prediction of first lactation production. In another study, Jafarabadi breeds were used to predict the first lactation at 305 days. A linear regression model was fitted by incorporating all 11 monthly test day milk yield records as a partial lactation yield with different combinations to predict the standard lactation yield as early as possible Analysis carried out revealed that when all the monthly test day records (1 to 11) were incorporated in an equation to predict first lactation 305 day or less milk yield, the accuracy (R^2 value) was maximum with 97.06% (Sharma et al., 2022).

In the other studies conducted on water buffaloes, Yilmaz et al. (2023) conducted a study with a questionnaire of 98 enterprise owners rearing Anatolian water buffalo in 2015 in Igdir city of Turkey. The average daily milk yield, annual milk yield, and sales price of buffaloes milk of the enterprises were determined as 5.48±0.07 kg, 1228.56±15.60 kg/lactation, and USD 1.06 (price/per kg), respectively. Non-native buffalo insemination should not be allowed. Otherwise, it is important to emphasize that the heads rearing in each region are adapted to the region they inhabit.

Chisowa (2023) showed the evaluation of the effect of parity on milk yield among water buffaloes and the main objective was to assess the effect of parity number on milk yield among water buffaloes which was provided at Livestock Development Trust Centre (LDTC) Agricultural Research Trust near Batoka in Southern Province of Zambia. The lactating water buffaloes were conducted to assess the effect of parity on Daily Milk Yield (DMY). The mean DMY's for the 1st, 2nd, and 3rd parities were (5.06 ± 2.33 L, 8.00 ±3.23 L, and 6.89±4.00 L, respectively) as significant ($p<0.05$). The study also indicates that milk yield is influenced by parity with cows in 2nd parity showing the highest milk yield of first three parities.

Zhigao et al. (2023) investigated that this study aimed to evaluate the difference between the parity of buffalo colostrum and mature milk. Any difference was not found in mature milk composition and yield by parity affected ($p>0.05$). Observation is provided as parity affected colostrum characteristics rather than mature milk and caused subtle variations in minerals in the colostrum and mature milk of buffaloes. The purpose of this study is to investigate the relationships between 100-day of partial milk yield and 270-day of additive and total milk yield of buffaloes according to different parity. If the total lactation milk yield that the animal can give during lactation from the partial milk yield determined at an early age could be estimated this would be useful for breeding practice.

Matera et al. (2022), aim to investigate those correlations using data collected at a commercial Italian buffalo farm. It can be concluded in buffalo, as in other species, there is a strong relationship between electrical conductivity and sometic cells.

Ayad et al. (2023), aim to estimate the effect of some environmental factors on the productive and reproductive traits of Egyptian buffaloes. 2149 data were collected from dairy Menoufi buffalo, covering the period between 1999 to 2018. Year's effect of calving was found significantly higher ($p<0.001$) on test milk yield, lactation length and dry period. Its effect also important by calving season, likewise, parity had a highly significant ($p<0.0001$) effect on test milk yield, lactation length, dry period. The highest milk production has been recorded at 4th and 5th parity and then decreased. The year of calving had a highly significant ($p<0.001$) effect on all reproductive traits.

Fathy et al. (2023), investigates the effects of four groups of buffalo breed and five levels of parity on lactation yield and lactation length as productive traits. On the other hand, calving interval, dry period and days open as reproductive traits, using data from a dairy buffalo farm in Ismailia governorate. The results shows that parity, breed and their interactions showed significant ($p<0.05$) effects on productive and reproductive traits. Considering the second parity was the highest across breeds for the production traits, but the first parity was the highest for reproductive traits. Buffaloes produce more milk have poor reproductive performance, animals after the fourth parity performed parity poorly in productive and reproductive activities.

In this study, the relationships between 100-day of partial milk yield and 270-day of additive and total milk yield of buffaloes according to different parity were investigated. Considering the investigation the results are given in the article.

2. Material and Methods

The animal material of the study consisted of a total of 697 lactation records belonging to a total of 135 heads of Anatolian buffalo and seven different parity raised in Istanbul. Buffaloes are cared for in a single enterprise under the same feeding and growing conditions. Especially, the buffalo feeding is provided by roughage and also concentrate supplemented feed (maize, wheat, barley, etc.)Animals are milked with a fully automated system twice a day. Milk yield records are automatically recorded daily. Since only animal yield records were used in this research, for this reason, ethics committee approval was not required.

The relationships between 100-day of partial milk yield and 270-day of additive and total milk yield of buffaloes according to different parity were analyzed by correlation coefficients and regression analysis. Furthermore, 270 days of lactation in buffaloes is accepted as the standard lactation day. In the study, 100-day milk yield was taken as an independent variable, 270-day and total milk yields as dependent variables, and possible relationships were determined according to simple and multiple linear regression analysis methods. For each lactation parity, the determination coefficients of the obtained regression equations and the correlation coefficients between the properties considered were calculated. The determination coefficients of the regression equations obtained in the study were used to choose best linear regression equation criteria (Soysal, 2012). All statistical analyses were performed in the SPSS package program(SPSS, 2018).

3. Results

A total of 135 head buffaloes and 697 lactation records were used in the study. The buffaloes are divided into seven parity as a number of lactations. From the milk yield records obtained from the buffaloes, 100-day of additive milk yield, 270-day of additive milk yield, total milk yield, and daily average milk yields were calculated and the number of milking days was determined. Descriptive statistics of the buffaloes in terms of characteristics considered in parity and the results of the Duncan multiple comparison test are presented in Table 1 and Table 2.

Table 1. Descriptive statistics and significance test results of the yield traits according to parity

Parity	100-day milk yield [L – (n)]	270-day milk yield [L –(n)]	Total milk yield [L –(n)]
1. Lactation	831.99±23.62^{c}(134)	2046.40±61.77^{b} (87)	2062.58±73.84^{a} (134)
2. Lactation	875.64±21.95^{bc}(132)	2092.13±62.95^{b}(51)	1894.43±58.15^{ab} (132)
3. Lactation	893.35±21.05^{bc}(134)	2021.57±58.94^{b}(52)	1794.18±56.87^{b}(134)
4. Lactation	874.86±22.41^{bc} (125)	1966.75±78.29^{b} (48)	1726.14±56.29^{b} (125)
5. Lactation	861.89±29.16^{bc}(93)	2230.24±96.30^{b}(33)	1669.39±75.36 ^{b} (93)
6. Lactation	937.37±39.27^{b} (55)	2223.75±172.05^{b}(16)	1743.47±95.95 ^{b} (55)
7. Lactation	1038.08±69.38^{a} (24)	2705.20±543.82^{a}(5)	1793.54±182.26^{b}(24)
General	879.14±10.11 (697)	2078.65±32.26 (292)	1831.89±27.57 (697)

Notice: Different letters in the same column constitute statistically different groups ($p < 0.05$).

Table 2. Descriptive statistic and significance test results of the yield traits according to parity

Parity	Lactation period [day – (n)]	Daily average milk yield (100-day) [L – (n)]	Daily average milk yield (270-day) [L – (n)]
1. Lactation	291.94±5.89^{a}(134)	6.98±0.17^{b} (134)	7.57±0.22^{b} (87)
2. Lactation	261.31±5.87^{b}(132)	7.19±0.14^{b}(134)	7.74±0.23^{b} (51)
3. Lactation	253.28±5.79^{bc}(134)	7.01±0.14^{b}(134)	7.48±0.21^{b}(52)
4. Lactation	249.24±5.52^{bc} (125)	6.88±0.16^{b} (125)	7.28±0.28^{b} (48)
5. Lactation	241.65±6.85^{bc}(93)	6.79±0.19^{b}(93)	8.26±0.35^{b} (33)
6. Lactation	232.41±9.14^{c} (55)	7.49±0.27^{b}(55)	8.23±0.63^{b} (16)
7. Lactation	207.79±12.91^{d} (24)	8.49±0.57^{a}(24)	10.01±2.01^{a}(24)
General	256.74±2.61 (697)	7.08±0.07 (697)	7.69±0.11 (292)

Notice: Different letters in the same column constitute statistically different groups ($p < 0.05$).

In the study, the total milk yield of buffaloes for 100-day was found as 879.14 ± 10.11, the mean total milk yield for 270-day was 2078.65 ± 32.26, and the total yield average was 1831 ± 89.27.57 liters. In addition, the average lactation period of the animals was 256.74 ± 2.61 days, the daily average milk yield of 100-day was 7.08 ± 0.07 and the daily average milk yield of 270-day was 7.69 ± 0.11 liters. When 100-day productivity of the animals was examined, it was seen that they were the seventh lactation highest (1038.08 ± 69.38) and the first lactation was the lowest (831.99 ± 23.62) in terms of parity of lactation.

When the daily and 270-day milk yield averages were compared in the study, the average of the animals during the seventh lactation was statistically different from the other number of lactation ($p < 0.05$). The correlation coefficients between the features discussed in the study were calculated separately for each parity and the results were calculated separately in Table 3- Table 6 is also presented.

Table 3. Correlation coefficients and importance test results between the traits considered according to one and second lactation

Parity 1-2	V_{100}	V_{270}	V_{Total}	LP	DAMY	$DAMY_{270}$
V_{100}	1.000	0.846**	0.710**	0.135	0.787**	0.846**
V_{270}	0.845**	1.000	0.887**	0.252*	0.949**	1.000**
V_{Total}	0.653**	0.868**	1.000	0.569**	0.852**	0.887**
LP	0.092	0.262	0.674**	1.000	0.075	0.252*
DAMY	0.823**	0.959**	0.762**	0.047	1.000	0.949**
$DAMY_{270}$	0.845**	1.000**	0.868**	0.262	0.959**	1.000

The upper side of the diagonal is the 1st lactation and the lower side is the 2nd lactation.
Notice: V100:100-day yield, V270: 270-day yield, VTotal: Total yield, LP: Lactation period (day), DAMY: Daily average milk yield, DAMY270: 270 Daily average milk yield, **: $p < 0.01$, *: $p < 0.05$.

Table 4. Correlation coefficients and significance test results between the traits considered according to third and fourth lactation

Parity 3-4	V_{100}	V_{270}	V_{Total}	LP	DAMY	$DAMY_{270}$
V_{100}	1.000	0.905**	0.759**	0.206	0.898**	0.905**
V_{270}	0.962**	1.000	0.918**	0.389**	0.952**	1.000**
V_{Toplam}	0.873**	0.940**	1.000	0.711**	0.775**	0.918**
LP	0.053	0.137	0.428**	1.000	0.118	0.389**
DAMY	0.947**	0.976**	0.891**	0.002	1.000	0.952**
$DAMY_{270}$	0.962**	1.000**	0.940**	0.137	0.976**	1.000

The upper side of the diagonal is the 3rd lactation and the lower side is the 4th lactation.
Notice: V100:100-day yield, V270: 270-day yield, VTotal: Total yield, LP: Lactation period (day), DAMY: Daily average milk yield, DAMY270: 270 Daily average milk yield, **: $p < 0.01$, *: $p < 0.05$.

Table 5. Correlation coefficients and significance test results between the traits considered according to the fifth and sixth lactation

Parity 5-6	V_{100}	V_{270}	V_{Total}	LP	DAMY	$DAMY_{270}$
V_{100}	1.000	0.969**	0.932**	0.156	0.962**	0.969**
V_{270}	0.976**	1.000	0.981**	0.259	0.979**	1.000**
V_{Toplam}	0.959**	0.989**	1.000	0.430*	0.930**	0.981**
LP	0.061	0.073	0.204	1.000	0.080	0.259
DAMY	0.960**	0.986**	0.957**	0.085	1.000	0.979**
$DAMY_{270}$	0.976**	1.000**	0.989**	0.073	0.986**	1.000

The upper side of the diagonal is the 5th lactation and the lower side is the 6th lactation.
Notice: V100:100-day yield, V270: 270-day yield, VTotal: Total yield, LP: Lactation period (day), DAMY: Daily average milk yield, DAMY270: 270 Daily average milk yield, **: $p < 0.01$, *: $p < 0.05$.

Table 6. Correlation coefficients and significance test results between the traits considered in seventh lactation and overall lactation

Parity 7-Overall	V_{100}	V_{270}	V_{Total}	LP	DAMY	$DAMY_{270}$
V_{100}	1.000	0.992**	0.991**	0.257	0.993**	0.992**
V_{270}	0.901**	1.000	1.000**	0.320	0.999**	1.000**
V_{Toplam}	0.749**	0.902**	1.000	0.325	0.999**	1.000**
LP	0.068	0.204**	0.541**	1.000	0.288	0.320
DAMY	0.864**	0.962**	0.847**	0.028	1.000	0.999**
$DAMY_{270}$	0.901**	1.000**	0.902**	0.204	0.962**	1.000

The upper side of the diagonal is the 7th lactation and the lower side is the overall lactation.
Notice: V100:100-day yield, V270: 270-day yield, VTotal: Total yield, LP: Lactation period (day), DAMY: Daily average milk yield, DAMY270: 270 daily average milk yield, **: $p < 0.01$, *: $p < 0.05$.

When the correlation coefficients were evaluated over the general data in the study, the correlation coefficients between 100-day yield and 270-day additive yield and total yield were found to be 0.901 and 0.749, respectively, and the relationship between 270-day yield and total yield was found to be 0.902. When all lactations were examined, it was seen that there was a high and significant relationship between 100-day of yield and subsequent yields.

In the study, regression equations were created that allow the estimation of 270-day of additive milk yield and total milk yield by linear regression analysis by using 100-day of yield recording. In addition to the regression equations, the concordance of the equations was also compared by giving the determination coefficients. The regression equations were calculated separately and eventually overall for each lactation number and presented in Table 7 and Table 8.

Table 7. Regression equations and determination coefficients showing the relationship between partial milk yield (100-day of milk yield) and 270-day milk yield for each parity

Parity	Simple linear regression equation (Y=a+bx) (R^2%)	Quadratic regression equation ($Y=a+b_1X+b_2X^2$) (R^2%)
1. Lactation	$V_{270}=447.91+2.22V_{100}$ (50.4	$V_{270}=2352.62-2.740V_{100}+0.003V_{100}^2$ (61.9)
2. Lactation	$V_{270}=781.62+1.46V_{100}$ (76.4)	$V_{270}=1103.68+0.339V_{100}+0.001V_{100}^2$ (80.3)
3. Lactation	$V_{270}=496.81+1.625V_{100}$ (81.8)	$V_{270}=1162.10+0.159V_{100}+0.001V_{100}^2$ (83.5)
4. Lactation	$V_{270}=256.35+1.829V_{100}$ (63.3)	$V_{270}=673.78+0.869V_{100}+0.001V_{100}^2$ (63.8)
5. Lactation	$V_{270}=187.19+1.952V_{100}$ (93.9)	$V_{270}=322.58+1.699V_{100}+0.0001V_{100}^2$ (93.9)
6. Lactation	$V_{270}=211.97+1.950V_{100}$ (95.3)	$V_{270}=442.42+1.525V_{100}+0.0001V_{100}^2$ (95.4)
7. Lactation	$V_{270}=-461.67+2.458V_{100}$ (98.5)	$V_{270}=2177.13-1.31V_{100}+0.001V_{100}^2$ (98.8)
Overall	$V_{270}=470.72+1.737V_{100}$ (80.2)	$V_{270}=966.23+0.645V_{100}+0.001V_{100}^2$ (82.4)

Notice: V100:100-day yield, V270: 270-day yield, Y: Dependent variable, X: Independent variable, b: Regression coefficient, a: Intercept, $Y=a+b_1X_1$: Regression equation, R^2: Coefficient of determination.

The animals' 100-day yield (V_{100}) and 270-day (V_{270}) additive milk yield were evaluated for each lactation number by simple and multiple linear regression equations. The general group was determined as [$V_{270}=470.72+1.737V_{100}$ (R^2 = 80.2%)] and [$V_{270}= 966.23 + 0.645\ V_{100} + 0.001V_{100}^2$ (R^2 = 82.4%)] regardless of parity. All possible relationships for each lactation number are presented in the form of tables.

As the lactation number of the animals increased, it was seen that the determination coefficients of the regression equations also increased. Between the two models used in regression equations, the determination coefficients of the quadratic model were found to be approximately 1-2% higher than the simple linear model.

Table 8. Regression equations and determination coefficients showing the relationship between partial milk yield (100-day of milk yield) and total milk yield for each parity

Parity	Simple linear regression equation (Y=a+bx) (R^2 %)	Quadratic regression equation ($Y=a+b_1X+b_2X^2$) (R^2%)
1- Lactation	V_{tot}=125.08+2.32V_{100} (52.5%)	V_{tot}=1556.43-1.512V_{100} + 0.002V_{100}^2 (59.0%)
2- Lactation	V_{tot}=331.38+1.78V_{100} (45.4%)	V_{tot}=1041.41-0.202V_{100} + 0.001V_{100}^2 (49.2%)
3- Lactation	V_{tot}=-130.25+2.15V_{100} (63.6%)	V_{tot}=-248.04+2.42V_{100} + 0.000V_{100}^2 (63.6%)
4- Lactation	V_{tot}=-100.51+2.08V_{100} (69.1%)	V_{tot}=-673.37+3.44V_{100} + 0.0001V_{100}^2 (70.2%)
5- Lactation	V_{tot}=-324.08+2.31V_{100} (80.1%)	V_{tot}=-151.06+1.91V_{100} + 0.0001V_{100}^2 (80.2%)
6- Lactation	V_{tot}=-214.17+2.08V_{100} (73.1%)	V_{tot}=-49.270+1.75V_{100} + 0.0001V_{100}^2 (73.2%)
7- Lactation	V_{tot}=-700.03+2.40V_{100} (83.6%)	V_{tot}=-69.603+1.00V_{100} + 0.001V_{100}^2 (85.7%)
Overall	V_{tot}=25.790+2.054V_{100} (56.8%)	V_{tot}=437.37+1.076V_{100} + 0.001V_{100}^2 (57.6%)

Notice: V100:100-day yield, Vtot: Total yield, Y: Dependent variable, X: Independent variable, b: Regression coefficient, a: Intercept, Y=a+b1X1: Regression equation, R2: Coefficient of determination.

The comparison of observed milk yield (270-day and total) with expected milk yields according to linear and quadratic models are presented in Figure 1 and Figure 2 according to parity for each animal respectively.

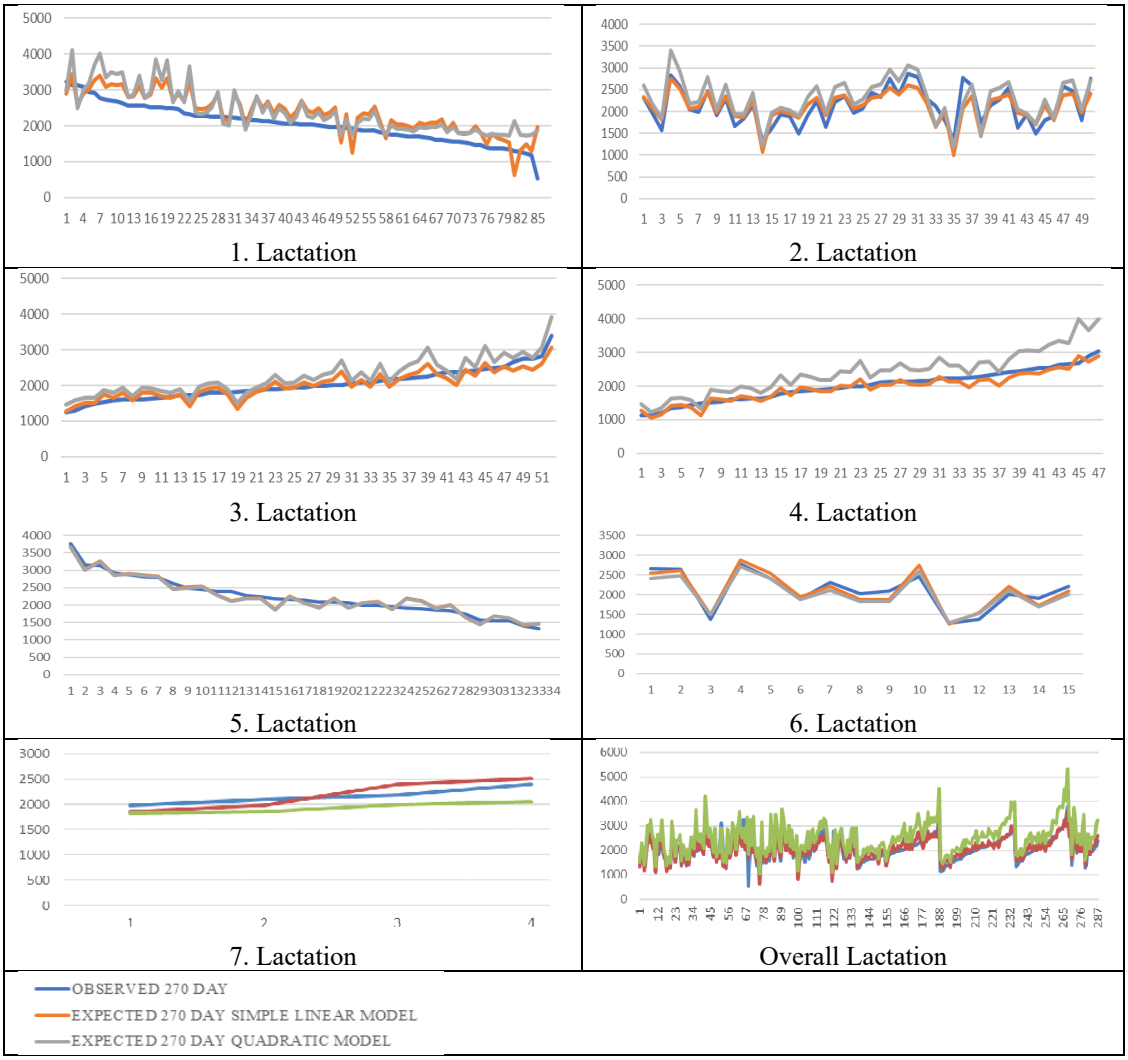

Figure 1. Comparison of 270-day observed milk yield and expected milk yield (estimated by 100-day partial yield as independent variable) according to simple linear and quadratic models according to parity for each animal. Notice: The numbers given on the x-axis are animal numbers and milk yields are given y-axis.

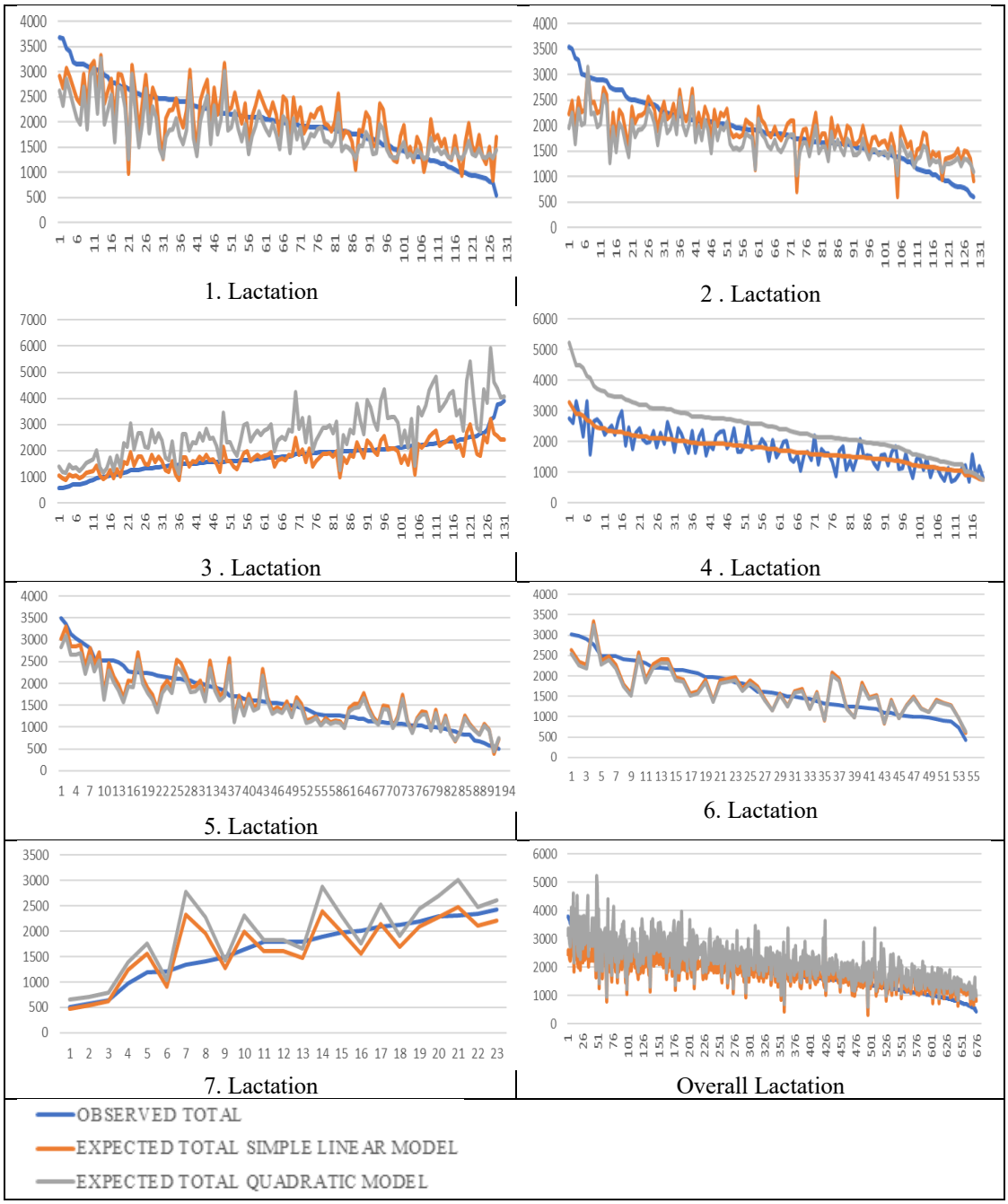

Figure 2. Comparison of observed total milk yield and expected total milk yield (estimated by 100-day partial yield as independent variable) according to simple linear and quadratic models according to parity for each animal. Notice: The numbers given on the x-axis are animal numbers and milk yields are given y-axis.

4. Discussion

In this study, the total milk yield of buffaloes for 100-day was found as 879.14 ± 10.11, the mean total milk yield for 270-day was 2078.65 ± 32.26, and the total yield average was 1831 ± 89.27.57 liters. In addition, the average lactation period of the animals was 256.74 ± 2.61 days, the daily average milk yield of 100-day was 7.08 ± 0.07 and the daily average milk yield of 270-day was 7.69 ± 0.11 liters. When the 100-day productivity of the animals was examined, it was seen that they were the seventh lactation highest (1038.08 ± 69.38) and the first lactation was the lowest (831.99 ± 23.62) in terms of parity of lactation.

In another study, Muhammad (2009) found the first lactation milk yields in Nili Ravi, Murrah, and Egyptian buffaloes as 1854, 1654, and 1185 kg respectively, and the 3rd lactation milk yields as 2396, 2056, and 1678 kg, respectively. Madad et al. reported that the total milk yield of buffaloes with 2327 first lactation records in buffaloes raised in Azerbaijan in 2013 was 1420 kg, the fat yield was 99.8 kg and the fat percentage was 7%.

Similarly, Garcia et al. (2013) reported that the lactation period of the buffaloes they studied in 2013 was 240 days and the lactation yield of 244 days was 864 kg. Malhado et al. (2013) reported that the lactation period of buffaloes was 252 days and the average yield was 1546 kg. In the studies carried out, it has been pointed out that the selection to be made according to the first lactation yields in general will be accurate and without waiting for subsequent lactations.

Soysal et al. (2015) reported that the average lactation length of Anatolian buffaloes raised in Istanbul in 2015 was 216.6 days, the average lactation yield was 1360 kg and the average daily milk yield was 6.26 kg.

In this study, the correlation coefficients were evaluated over the general data in the study, the correlation coefficients between 100-day yield and 270-day additive yield and total yield were found to be 0.901 and 0.749, respectively, and the relationship between 270-day yield and total yield was found to be 0.902.

In another study, Soysal et al. (2015) reported the correlation coefficient between 30-day additive yield and total yield ($r = 0.74$, $p<0.01$), the correlation coefficient between 90-day additive yield and total yield ($r = 0.88$, $p<0.01$), and the correlation coefficient between 180-day additive yield and total yield ($r = 0.94$, $p<0.01$). The researchers suggested that partial yield records could be used accurately to predict the total milk yield of buffaloes early.

In this study, regression equations were created that allow the estimation of 270-day of additive milk yield and total milk yield by linear regression analysis by using 100 day of yield recording. The animals' 100-day yield (V_{100}) and 270-day (V_{270}) additive milk yield were evaluated for each lactation number by simple and multiple linear regression equations. To the general group, the coefficient of determination was determined as($R^2 = 80.2\%$) and ($R^2 = 82.4\%$) regardless of parity.

Elmaghraby (2009) studied regression equations for predicting 305-day milk yield by simple linear equations using individual monthly milk yield and multiple linear regression equations using the maximum coefficient of determination (R^2) as criteria for choosing the best among variables. Among the equations of simple linear regression for predicting 305-day milk yield from individual monthly milk yield, the best single–month prediction model was for three-month partial milk yield ($R^2 = 0.49$). Predictability declined afterward with the advancing month of lactation.

Sahoo et al. (2019) examined the relationship between partial milk yield and of weekly test day period. The best single, two, three, and four test day combinations were selected for the prediction of 305-day milk yield based on adjusted coefficient of determination(R^2) values. It was concluded that the 305-day milk yield can be predicted as early as the 153rd day of lactation and this day can be used for early genetic evaluation of Murrah sires.

Rana et al. (2020) used the data of the first lactation bimonthly test day milk yield records of Murrah buffaloes. The study compared the conventional and computational methods for prediction of first lactation milk yield which could be used for early selection of the animals. The first lactation 305-day milk yield was observed to be best estimated by Multiple Linear Regression (MLR), followed by Artificial Neural Network (ANN) and Centering Date Method (CDM). Early prediction of 305 Day Milk yield using bimonthly test day milk yield (BTDY- 2 (65th day), BTDY-3 (125th day), and BTDY-4 (185th day) gave higher accuracy (85.29%). Evaluation of the sires and dam based on early test day yields would eventually result in reduced cost incurred on milk records, reduced generation interval, and increased response to selection. If selection is to be made for the milk yield of an animal in animal husbandry, the earlier the milk yield of that animal can be determined, the decision is made to improve milk yield. It is possible to make such a choice by taking advantage of partial yield records before the total lactation yields of the animals are completed with regression analysis methods.

Chaudhari et al (2022) studied predicting standard lactation milk yield by simple regression equation from weekly, fortnightly, and monthly, individual and cumulative part yield in Jafarabadi buffalo. They revealed that correlation coefficients between 305-day milk yield and different weekly (1st to 20th week), fortnightly (1st to 5th fortnight), and monthly (1st to 5th month), individual and

cumulative part lactations were positive and significant ($p<0.01$) and showed an increasing trend with the advancement of lactation.

Finally, all lactations were examined, and it was seen that there was a high and significant relationship between 100-day of yield and subsequent yields. As the lactation number of the animals increased, it was seen that the determination coefficients of the regression equations also increased. In addition to, the two models used in regression equations, the determination coefficients of the quadratic model were found to be approximately 1-2% higher than the simple linear model.

Conclusion

In this study, the relationships between 100-day of partial milk yield and 270-day of additive and total milk yield of buffaloes according to different lactation numbers were investigated. If the total lactation milk yield that the animal can give during lactation from the partial milk yield determined at an early age can be estimated, this will be useful for breeding practice. In general, as the lactation number of the buffaloes increased, the determination coefficients of the regression equations increased and between the two models used in the regression equations, As a result, it was seen that benefiting from 100 day of partial milk yield would be accurate and useful in predicting the milk yield of the animal before the animals finished their lactation period. As a result, it has been seen that partial yield records can be used to effectively estimate total lactation milk yield of the animal.

Acknowledgements

We thank the private enterprise for permission to use the yield records used in this study.

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Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

ISSN: 1308-7576 e-ISSN: 1308-7584

Research Article

Evaluating a Portable Method and Two Irrigation Drippers for Field Application of Entomopathogenic Nematodes

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Article Info

Received: 11.11.2023
Accepted: 01.02.2024
Online published: 15.03.2024
DOI: 10.29133/yyutbd.1389512

Keywords

Drip irrigation,
Emitter,
Fertigation,
Heterorhabditis,
Steinernema

Abstract: Entomopathogenic nematodes (EPNs) can be applied using drip irrigation systems. However, the choice of driplines and types of drippers significantly impacts the efficacy of field applications. This study investigated the performance of EPN applications using two common dripper types (katif and cylindrical drippers) under both pot and field conditions. The primary objective of the study was to optimize EPN applications and create a modular system in which driplines and drippers can be selected based on the target pest or plant. In our modular system, driplines were connected to a battery-powered backpack sprayer rather than an irrigation system. The efficacy of EPN applications was assessed on *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvae at a commercial dose of approximately 50 IJs cm^{-2}. The results revealed that only 60% of the nematodes were discharged from the cylindrical drippers, with 40% becoming trapped in the irrigation system. In contrast, over 90% of the nematodes were successfully discharged from the katif dripper. As a result, the katif dripper exhibited significantly higher larval mortality compared to all other application methods. These findings emphasize the substantial impact of the dripper type on EPN discharge, while also highlighting the applicability of the modular method for EPN applications.

To Cite: Erdoğan, H, Ulu, T C, 2024. Evaluating a Portable Method and Two Irrigation Drippers for Field Application of Entomopathogenic Nematodes. *Yuzuncu Yil University Journal of Agricultural Sciences*, 34(1): 138-150. DOI: https://doi.org/10.29133/yyutbd.1389512

1. Introduction

The use of biopesticides is increasing due to the adverse effects of chemical pesticides on the environment (Glare et al., 2012; Olson, 2015). Biocontrol agents, such as entomopathogenic nematodes (EPNs), contribute significantly to the biopesticide market. EPNs, soil-dwelling microscopic roundworms, are among the successfully employed organisms in biological control. They are obligate endoparasites that require an insect host to complete their life cycle (Kaya and Gaugler, 1993). Although there are other insect-parasitic species, *Heterorhabditis* and *Steinernema* are the foremost genera. Specialized free-living third-stage juveniles of EPNs, known as infective juveniles (IJs), are the only stage outside the host that can seek and infect a suitable host in the soil. As IJs enter the host, they release their symbiotic bacteria, which kill the host and turn it into a cadaver in a short period. After several

generations in the host, IJs emerge from the cadaver and seek new hosts in the soil (Gaugler, 2002). EPNs are effectively used against a broad host range of pests (Askar et al., 2023; Dede et al., 2023). They can be mass-produced in fermenters and on solid media, and they are potent alternatives to chemicals used for below ground pests, where pesticides mostly fail (Shapiro-Ilan et al., 2012; Devi, 2018; Şahin et al., 2018; Ulu and Susurluk, 2021).

As with other biological control agents, EPNs have some disadvantages. Mass production of EPNs requires high initial expenses; their shelf life is approximately 40 days (E-nema GmbH product info), they need a cold chain during transport, and they need to be applied as fresh products (Grewal, 2000; Guy et al., 2017; Kagimu and Malan, 2019). They also show inconsistent virulence in field applications (Jaffuel et al., 2019; Oliveira-Hofman et al., 2019). EPN products are generally not preferred because of their high prices and inconsistent field efficacy, which cannot compete with chemical products. For this reason, there is a tendency to optimize production in liquid culture medium, improve formulations, develop superior characteristics by genetic selection, increase resistance to adverse conditions, and apply using different techniques (Mukuka et al., 2010; Singh and Upadhyay, 2018; Nxitywa and Malan, 2021).

Many methods can be used in the field applications of EPNs, depending on the target pest or plant. These application methods include spray and fertilization equipment, irrigation systems, and specific application techniques, such as cadaver application (Wright et al., 2005; Garcia et al., 2008; Raja et al., 2015). New robotic techniques are also optimized for EPN applications (Erdoğan et al., 2021, 2023). Although EPNs can be applied with all these techniques in theory, the most preferred application method is drip irrigation because EPNs live belowground and need moisture to move through the soil. However, there are some obstacles in the application of EPN using drip irrigation. For instance, a heterogeneous EPN distribution was observed throughout driplines (Garcia et al., 2008; Campos-Herrera, 2015). The filters used in irrigation systems prevent the exit of nematodes (Łaczyński et al., 2007). There are many types of drippers, and their physical structures differ in such a way that they affect the application of EPNs (Erdoğan et al., 2020). For instance, a wider flow path or higher flow rate allows the EPNs to pass more easily. To overcome these obstacles, the drip irrigation system should also be adapted to EPN application, using suitable dripper types, irrigation pressure, filters, or formulation. However, it is unlikely to replace drip irrigation systems already installed in the field. In other words, if the irrigation system is not suitable for EPN applications, it is necessary to use external application equipment. Thus, EPNs can be applied more efficiently using a more modular irrigation technique.

Improper application of EPNs not only results in waste of commercial products and effort but also tarnishes the reputation of EPNs as effective biocontrol agents. To ensure successful field applications, it is essential to have a practical and adaptable technique. Therefore, we integrated driplines with a battery-powered backpack sprayer to create a modified portable irrigation system for the EPN application. Our goal was to develop a system that could be tailored to various conditions and offer a more controlled, precise, and practical method for applying EPNs in a wide range of agricultural settings, including crops, fields, and greenhouses.

2. Material and Methods

2.1. Entomopathogenic nematodes

The study involved three nematode species: *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, and *S. feltiae*. Commercial products for these EPN species were provided by a local distributor (Bioglobal A.Ş.). The selection of these different species was based on variations in their biology, host-seeking strategies (cruiser, ambusher, and intermediate, respectively), and physical characteristics such as body size (Poinar and Grewal, 2012). Specifically, *S. carpocapsae* and *H. bacteriophora* have relatively shorter body length (L) and diameter (D) (L: <600 µm, D: <25 µm), whereas *S. feltiae* is larger (L: >850 µm, D: >25 µm). To obtain populations of these EPNs, an *in vivo* method was employed, using the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae), as the host. Each larva was inoculated with 100 IJs in a mixture of silver sand and then kept in incubation at 24 °C ± 1. Subsequently, around 250 000 fresh IJs were harvested from the White trap and placed

into a 50 ml falcon tube containing tap water. One-week-old IJs and nematode batches with 99% viability were used in the experiments.

2.2. Modified application method

A 16-liter battery-powered backpack sprayer was utilized as both the container and the application device (Figure 1). The battery powers the pump, creating pressure to facilitate the spraying process. The backpack sprayer's outlet was connected to the driplines using a hose clamp. Two distinct types of drippers were selected for the study: the on-line katif dripper (Rivulis Eurodrip, India) positioned above the dripline, and the in-line cylindrical dripper (MGF Irrigation, Turkey) positioned inside the dripline. The katif dripper is designed to be pressure-compensated (PC) and features a simplified and wide flow path without a labyrinth, as depicted in Figure 2A. Conversely, the cylindrical dripper incorporates a distinctive narrow flow path labyrinth, as illustrated in Figure 2B, but it is non-pressure-compensated (non-PC). These drippers have different flow rates, with the katif dripper having a flow rate of 12 l h^{-1} and the cylindrical dripper having a flow rate of 4 l h^{-1}. Both drippers are equipped with inlet filters that have pore sizes larger than 500 µm, ensuring easy passage for the IJs, as depicted in Figure 2. It's worth noting that both types of drippers have widespread usage on a global scale.

Figure 1. Modular application method. Battery-powered backpack sprayer was combined with different types of driplines. Main body (a), coupled with the dripline (b), coupling part (c) and battery (d).

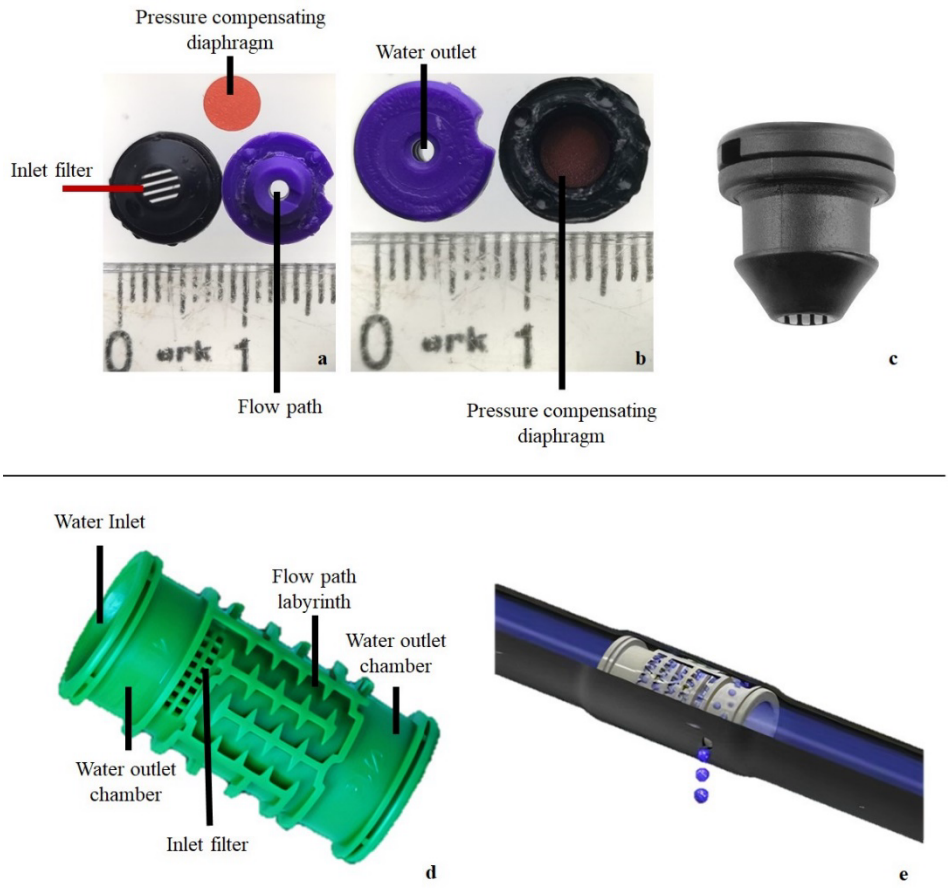

Figure 2. Illustrations of katif (upper part) and cylindrical (lower part) drippers. Parts of the disassembled katif dripper can be seen from the rear (a), above (b), and ready to use the katif dripper (c), the physical structure of the cylindrical dripper (d) and the water flow illustration of the cylindrical dripline (e).

The driplines featured consistent dimensions, with a diameter of 16 mm and a length of 10 meters. They were equipped with a total of 50 drippers, positioned at 20 cm intervals along the entire length of the dripline. Filters were omitted from the system as we relied on pre-filtered tap water for filling the sprayer tank. The backpack sprayer had an adjustable outlet pressure that allowed for pressure settings of up to 2 bars. Nonetheless, all applications were executed under a consistent 1-bar pressure setting, which was ideal for the non-pressure-compensating (non-PC) cylindrical dripper in use. Pressure within the system was measured using a pressure gauge filled with glycerin. Each application was systematically named by combining the initials of the EPN species and the specific dripper type. For example, "Hb Katif" served as the nomenclature for the application of *Heterorhabditis bacteriophora* via a katif-type dripper.

2.3. Evaluation of the drippers

The initial experiment aimed to assess the suitability of drippers for EPN application. The first step involved examining the uniformity of water discharge from the drippers prior to EPN applications. A 10-meter-long dripline was extended between two iron fences, suspended approximately 20 cm above the ground. A total of 300 ml glass containers were positioned beneath each dripper. The process began by filling the driplines with tap water to ensure uniformity. Subsequently, 5 liters of tap water were passed through the dripline, and the water discharge from all drippers was measured and recorded. In the second experiment, a solution of 250 000 IJs in 5 liters of tap water was administered to the glass containers, positioned under every 5th dripper (under the 1st, 5th, 10th, and so forth, up to the 50th dripper). Prior to the EPN application, the driplines were filled with tap water. Following the application, the number of IJs and their mortality rate within each container were quantified, allowing for the

determination of the total discharged IJs per container. This process was duplicated for both the driplines and the specific EPN species under examination. Each experiment was replicated three times to ensure accuracy and reliability.

2.4. Pot application

Plastic seedling pots with a diameter of 9 cm and a height of 12 cm (approximately 750 ml) were employed to evaluate the application's effectiveness. A mixture of sterilized silver sand and sandy soil at a 1:1 ratio was used to fill the pots halfway. Four caged *G. mellonella* larvae were positioned in the center of each pot, and the remaining space was filled with the same soil mixture. The moisture content of the pots was adjusted to 10% (w/w) using tap water after the application. Six pots were prepared and positioned under the 1st, 10th, 20th, 30th, 40th, and 50th drippers (one pot for every 10 drippers). This setup allowed for an assessment of nematode distribution through the dripline. Similar to previous experiments, the driplines were filled with tap water, and a suspension of 250 000 IJs in 5 liters was applied. Subsequently, the pots were placed in a dark climate chamber maintained at 25 °C ± 1, with 70% relative humidity for incubation. Four days post-incubation, dissection of the deceased larvae confirmed EPN infection. As a positive control, the same amount of IJs per pot was administered using a Pasteur pipette. In contrast, tap water was utilized for drip irrigation as a negative control. The application dose closely approximated the commercial dose of 50 IJs cm^{-2}. Each experiment was conducted three times.

2.5. Field application

Field experiments were conducted on a 1000 m^2 research plot. Prior to the application, the soil was tested for EPN abundance. For this purpose, soil samples from the field were analyzed using the insect bait method, and no EPNs were detected. The field received daily irrigation before the application. The same procedure used in the pot experiments was followed for the field applications. Initially, the driplines were filled with tap water, and 250 000 IJs were applied with 5 liters of water. After 24 hours, three different drippers along the dripline were randomly selected, and four caged *G. mellonella* larvae were buried at a 20 cm depth in the soil under each selected dripper. The buried larvae remained in the soil for 5 days without additional irrigation. Subsequently, the mesh cages were removed from the soil, and dead larvae were dissected to confirm EPN infection. The same amount of IJs per dripper was applied with a Pasteur pipette as a positive control, while tap water served as the negative control. Each experiment was replicated four times, and there was a 2 m buffer zone between replicates. An untreated plot was used for each replicate. Field applications were carried out at sunset, and the average soil temperature was 26 °C.

2.6. Data analysis

Statistical analyses were conducted using GraphPad Prism v9.4 software. Prior to analysis, all data underwent a normality check using the Shapiro–Wilk test. One-way ANOVA was performed to evaluate the results. Since positive control groups were included, Dunnett's multiple comparison post hoc test and Bonferroni correction were used to determine the significance between larval mortalities in pot and field trials, respectively. Tukey's HSD post-hoc test was employed to compare the means of the IJ discharge data. Additionally, correlation analysis was performed to evaluate the relation between discharged water volume and IJ numbers. All analyses were conducted with a significance level set at $p<0.05$.

3. Results

3.1. Evaluation of drippers

At a consistent pressure of 1 bar during the experiments, the water discharge rates through the drippers remained uniform (Figure 3). Upon evaluating the drippers' performance, it became evident that the katif dripper was notably superior and better suited for EPN application [$F(5, 60) = 18.54$; $p<0.001$]. The cylindrical dripper, on the other hand, consistently impeded IJ discharge for all EPN species (Figure 4). Despite the declining trends and variations in IJ discharge from the drippers located

further along the line (Figure 5), the distribution of IJs throughout the dripline had no impact on larval mortality. The IJ population's mortality remained below 1% both before and after application, signifying that the drippers did not adversely affect IJ survival.

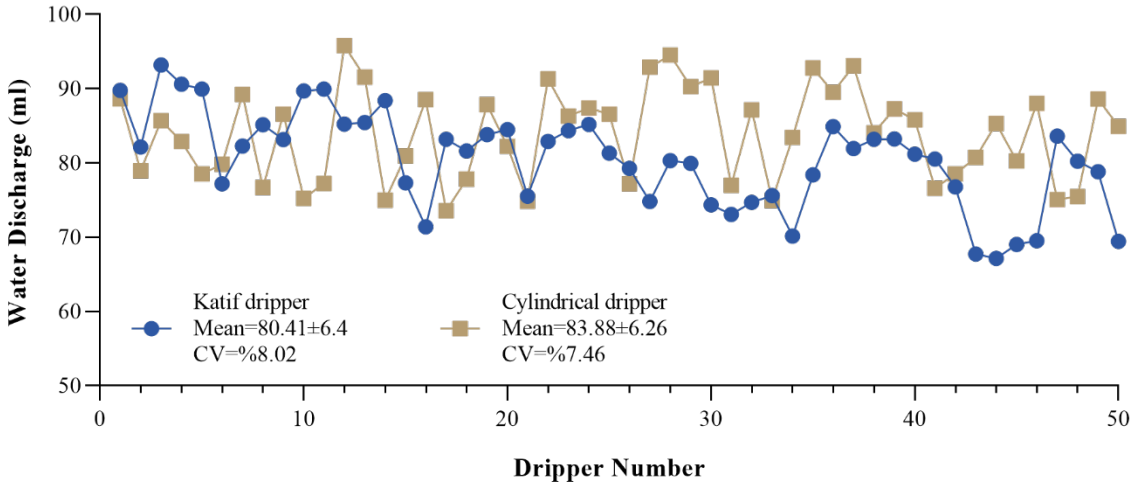

Figure 3. Water discharge of katif and cylindrical drippers from all 50 drippers throughout the driplines.

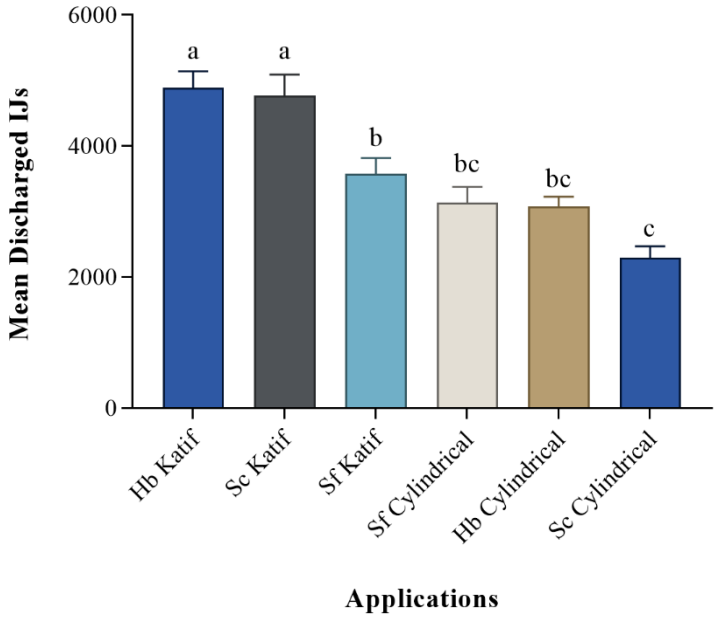

Figure 4. Comparison of mean discharged IJs of different species and dripper types. *H. bacteriophopra* (Hb) and *S. carpocapsae* (Sc) Katif dripper applications significantly differ from other applications. Different letters indicate significant differences according to Tukey's HSD test ($p<0.05$). Error bars represent standard error mean.

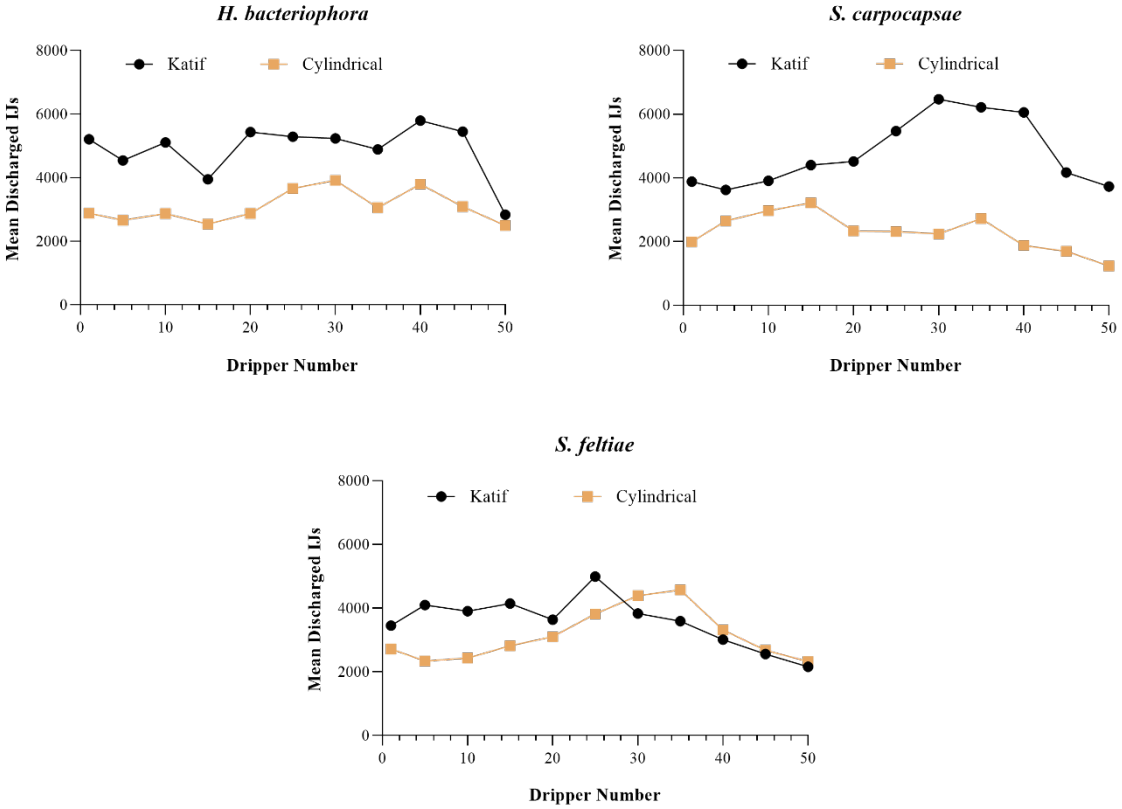

Figure 5. Discharged IJs throughout the driplines.

The correlation analysis revealed that the volume of water and IJ discharge from the drippers were unrelated. Correlation coefficients exhibited low values in nearly all applications and were determined to be statistically insignificant (Table 1). The sole exception was the Sc Katif application, which displayed a strong correlation.

Table 1. Correlation coefficient (r) and p-values (p) between water discharge (X) and IJ discharge of all applications (Y)

Water Discharge ***X***	**Hb Katif** ***Y1***	**Hb Cylindrical** ***Y2***	**Sc Katif** ***Y3***	**Sc Cylindrical** ***Y4***	**Sf Katif** ***Y5***	**Sf Cylindrical** ***Y6***
r	0.085	-0.072	0.810	0.419	-0.144	-0.270
p	0.804	0.834	0.0025	0.199	0.673	0.423

3.2. Pot applications

In all experiments, whether through dripper or positive control applications, all larvae within the pots were dead (Figure 6). The application dosage per pot was approximately 5000 IJs, slightly above the commercial dose (50 IJs cm^{-2}). A few larvae in the negative control group died due to excessive water. Notably, all applications exhibited significantly greater efficacy than the negative control, and there were no discernible distinctions between the applications and the positive control [$F(7, 40) = 3088.41$; $p<0.001$].

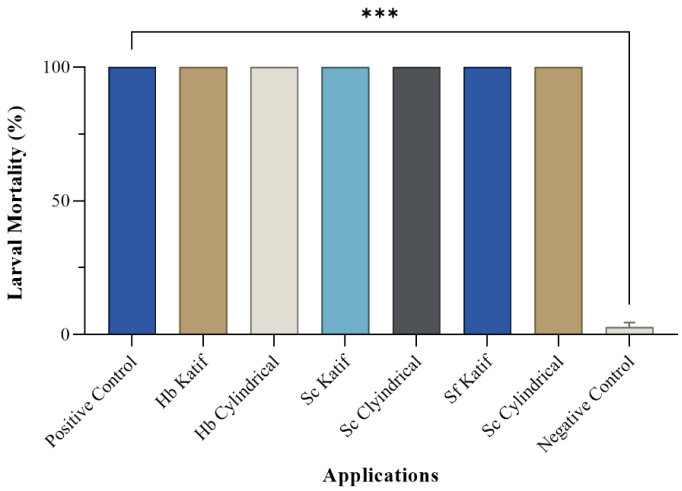

Figure 6. Larval mortality among pot applications. All applications except negative control remain significantly the same according to Dunnet's multiple comparisons post hoc test (p<0.05) (*** indicates p < 0.001). The error bar represents the standard error mean.

3.3. Field Applications

Field application results exhibited a notable reduction in larval mortality in contrast to pot applications, a result that was anticipated. However, some applications could be regarded as less effective. When compared to the positive control, all katif applications displayed notably similar levels of larval mortality (Figure 7). In contrast, larval mortality in all cylindrical applications remained below 20% [F(7, 16) = 34.26; p<0.001].

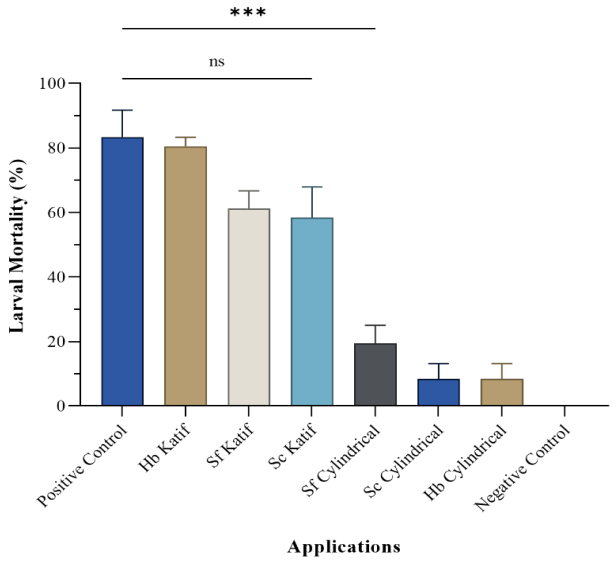

Figure 7. Comparison of the larval mortality in field applications. All katif applications showed no significance compared to positive control according to Bonferroni multiple comparisons post hoc test (p<0.05) (*** indicates p<0.001, ns: not significant). Error bars represent standard error mean.

4. Discussion

In this study, a portable method was used to perform the application of EPNs under field conditions. Instead of using an irrigation system, the driplines were connected to a battery-powered backpack sprayer. The aim was to provide a more precise, controlled, and modular way to apply EPNs.

The results of this study are promising and demonstrate that this portable technique can be used in EPN applications. We also determined that the two drippers with different physical structures had a significant impact on the EPN application. In field conditions, the larval mortality rate was higher in the katif dripper, while it remained low in the cylindrical dripper. Many studies have been conducted on the application of EPNs in drip irrigation (Curran and Patel, 1988; Reding et al., 2004; Arrington et al., 2016). However, few studies have examined the effects of drippers on EPN application (Erdoğan et al., 2020). In fact, we believe that in some studies comparing EPN efficacy with chemical pesticides, EPNs failed due to unsuitable dripper selection (Arrington et al., 2016). Our results indicate that the dripper type should be considered in EPN applications.

Three common EPNs species were used in this study. We aimed to determine whether the body size and foraging strategies of these species affect larval mortality. First, powder formulations were used in the initial tests to make the results of the study more realistic, but we had to produce IJs ourselves because of the difficulty in supplying commercial products. Wax moth larvae were used to evaluate the pathogenicity as they are easy to produce and susceptible to EPNs (Moreira et al., 2013). Similarly, we used wax moth larvae to determine the efficacy of the applications (Brusselman et al., 2012a; Dito et al., 2016). There were no differences between pot experiments (Figure 6). All larvae died because of the small pot volume, despite applying the recommended field dose (~50 IJ cm^{-2}). However, the results of the field trials have varied. The first notable result was that *S. feltiae* had a low discharge rate, even in the katif dripper (Figure 4). *S. feltiae* had the longest body length (~850µm) among the EPNs used (*S. carpocapsae* and *H. bacteriophora* had less than 600µm). Although the katif dripper does not have any flow labyrinth, we believe that the IJs of *S. feltiae* get stuck while passing through the dripper because of its long body (unpublished data). As expected, the field efficacies were in harmony with the discharged IJ amount (Figure 7).

EPNs have been applied in many spray and fertilizing equipment and irrigation systems, such as soil injectors, boom sprayers, hand sprayers, and spinning discs (Mason et al., 1998; Lara et al., 2008; Morton and García del Pino, 2008; Raja et al., 2015). Although nematodes can be applied to the soil using these methods, there are problems, such as the inability to adjust the nematode dose, lack of homogeneous distribution, and low application efficacy. The Dosatron injector is another popular technique for EPN applications. However, the EPNs tend to settle in the water; therefore, they must be mixed continuously in the tank (where Dosatron pulls up the EPN suspension) and during the application, which requires an additional power source. A Dosatron also contains moving parts that can damage EPNs. These problems mentioned above showed that drip irrigation (or fertigation) is the most suitable method and is recommended by commercial EPN producers (Wennemann et al., 2003; Wang et al., 2009; Arrington et al., 2016; Erdoğan et al., 2020). Although drip irrigation is the most preferred method, it also has problems in EPN application, as mentioned above (Conner et al., 1998).

Consequently, it is known that fewer EPNs emerge from further drippers in long driplines (Cabanillas and Raulston, 1996; Wennemann et al., 2003). EPNs are distributed more homogeneously throughout the dripline in high-flow drip irrigation systems. However, at the same time, these EPNs must also leave the system and reach the target for a successful application. We used katif and cylindrical drippers, which have completely different physical structures (Figure 2). While the katif dripper drips water in the dripline without hindrance, the cylindrical dripper drips water by passing it through a long flow labyrinth. According to the study results, the katif dripper statistically outperformed the cylindrical dripper in terms of both nematode discharge and larval mortality. Although the first thing that comes to mind is that the nematodes were trapped in the cylindrical dripper (inside the flow labyrinth), however, the pipes were split in half after experiments, and nematodes were also found inside the dripline. That means the flow rate of the dripper also affects the nematode discharge. EPNs are carried by water when they exit the irrigation system. For successful application, drippers must allow IJs to pass through the flow path and exit from the irrigation system. Although the water and IJ discharge from the drippers are closely related, it cannot simply be said that they are related to each other. As a matter of fact, correlation analysis between water and IJ discharge showed that there is no significant relation. Pressure-compensated drippers mostly have a uniformly distributed water discharge. However, non-PC drippers may have fluctuating data. Because we carried out all the experiments at constant pressure, the water discharge between the drippers did not vary significantly (Figure 3). However, using non-PC drippers may lead to an uneven distribution of EPN throughout the field. In addition to the physical properties of the dripper, pressure compensation increases the homogeneity and success of its application.

Many methods have been used to increase the success of EPNs. While EPNs are only applied against belowground pests, they can also be applied to aboveground parts owing to various supplementary chemicals and new-generation formulations (Shapiro-Ilan et al., 2012; Platt et al., 2018, 2020). The efficiency of EPNs can be increased by using spreader-adhesive chemicals during their application (Portman et al., 2016). It is also possible to increase its efficacy by changing the EPN behavior in the soil. For example, in a study conducted by Oliveira-Hofman et al. (2019), it was observed that the behavior of EPNs was changed by using pheromones. Although *S. carpocapsae* is an ambusher, its dispersal increases after pheromone application (Kaplan et al., 2020). Customized applications of EPNs can be developed to replace conventional systems. For instance, Erdoğan et al. (2021) designed a robotic system to apply IJs to the desired location. In their work, a customized mixing method was developed for EPNs, and a new peristaltic pump was used that did not damage EPNs during application. In another set of studies, pressure and nozzle types suitable for EPN application were researched, and the appropriate nozzle types were determined (Brusselman et al., 2011, 2012b). Furthermore, Erdoğan et al. (2020) investigated suitable dripper types for EPNs and found a significant difference in application success among different drippers, in parallel with this study. These studies showed that the efficacy of EPNs can be increased during and after application. Improvements at many stages, from production to field application of EPNs, will make significant contributions to environmental pest control in the future.

Although the use of pesticides is inevitable in agricultural production, the use of biological products is increasing (Çelik et al., 2023). EPNs are natural and safe alternatives to chemical control. The most critical disadvantage of EPNs is that their commercial formulations are expensive (Dunn et al., 2021). Other factors also reduce the effectiveness of EPNs under field conditions. Many microorganisms, natural enemies, stimulants, and odors in the soil affect the behavior of EPNs and reduce their success. As with other biological control agents, EPNs must be able to compete with pesticides. To make EPNs more attractive, products must be cheaper, more effective, and easier to apply. In addition to reducing production costs, studies are also carried out to increase the efficiency in field conditions and to increase the success of EPNs with new application methods (Wright et al., 2005; Beck et al., 2013; Kapranas et al., 2017; Dunn et al., 2020).

In summary, the study's objective was to assess a portable method applicable to various scales and product patterns. The modular approach allows adaptation to longer dripline configurations, different dripper types, microjets, or nozzles for specific target pests or crops. It was highlighted that the use of appropriate drippers is crucial for successful field applications. Additional research is essential to refine the effective application of EPNs in field conditions. The aspiration is that refined application methods will enhance the future efficacy of EPNs.

Acknowledgements

This study was financially supported by The Scientific and Technological Research Council of Turkey (Project number: TOVAG - 120O286). The authors would like to thank the Biotechnology Application and Research Centre for providing the facilities for laboratory assays. The authors also thank Edwin Lewis, Glen Stevens, Lucas Ripa, and Halil Ünal.

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Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

ISSN: 1308-7576 e-ISSN: 1308-7584

Research Article

Investigation of Heavy Metal Concentrations and Accumulation Capacities of Naturally Growing Species in Old Garbage Area

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Article Info

Received: 01.12.2023
Accepted: 03.02.2024
Online published: 15.03.2024
DOI: 10.29133/yyutbd.1399025

Keywords

Garbage leachate,
Heavy metal,
Hyperaccumulator plant,
Phytoremediation

Abstract: In underdeveloped and/or developing countries, garbage is often randomly piled up in open areas. This method has been used to dispose of garbage/solid waste in Turkey for many years. Although pollution is not at the forefront in Bingöl province, the area located in the city center of the city has been used as a wild garbage storage area for approximately 18 years. Since the garbage in the area poses a danger to people and the environment, this area has become inactive with the establishment of a new solid waste disposal facility in the city. There are plants that have adapted to this area, which has been empty for about ten years. In this study, it was tried to determine in what proportions and organs the plant species distributed in the area accumulate heavy metals that may have come from garbage leachate. Plants identified in the field; *Alyssum simplex*, *Cirsium libanoticum*, *Descurainia sophia*, *Fumaria asepala*, *Fumaria officinalis*, *Matricaria chamomilla*, *Papaver dubium*, *Scrophularia canina*, *Trifolium repens* and *Ziziphora capitata* species. Fe, Cr, As, Cd and Pb concentrations (mg kg^{-1}) of these species were measured in root, stem, leaf and flower organs and translocation factors (TF) were calculated for these species. In conclusion; *Alyssum simplex*, *Cirsium libanoticum* and *Fumaria asepala* for Fe, *Cirsium libanoticum*, *Fumaria asepala*, *Fumaria officinalis* and *Matricaria chamomilla* Cr and As, *Cirsium libanoticum*, *Papaver dubium* and *Scrophularia canina* for Cd and all other species except *Alyssum simplex* and *Scrophularia canina* for Pb translocation factors (TF) were found to be greater than 1 (TF>1). The accumulation potential of these species is thought to be promising so that they can be evaluated in phytoremediation.

To Cite: İnci, H Ş, Ahiskali, M, Macit, M, Çaçan, E, 2024. Investigation of Heavy Metal Concentrations and Accumulation Capacities of Naturally Growing Species in Old Garbage Area. *Yuzuncu Yil University Journal of Agricultural Sciences*, 34(1): 151-165.
DOI: https://doi.org/10.29133/yyutbd.1399025

1. Introduction

Today, one of the most important issues that the earth, nature, the world, and all humanity struggle with passively or actively is undoubtedly pollution. This is the main reason underlying environmental problems, health problems, and many other problems. Among the types of pollution,

heavy metal pollution, which can enter the food chain and threaten humans, is at the forefront. The term "heavy metal" is generally used for metals with a specific gravity of more than 5 g cm^{-3} (Holleman and Wiberg, 1985; Sharma and Agrawal, 2005). The heavy metals of most concern are the metalloids cadmium (Cd), mercury (Hg), lead (Pb) and arsenic (As). The uptake and accumulation of health-threatening toxic metals by plants are potential entry routes into human and animal food. Emissions of toxic heavy metals have greatly increased over the last 200 years (Clemens, 2006).

Heavy metal pollution is mainly caused by burning fossil fuels, municipal waste, sewage, pesticides, and smelting (Naila et al., 2019). Their high presence in the environment is due to anthropogenic activities, including the application of paint, batteries, metal scraps, motor oil, pesticide-herbicides, and fertilizers (Awokunmi, 2010). The development in industry, agriculture, and mining and the increase in their activity has led to increased heavy metal pollution (Kalay and Yasam 2000; Kuzu et al., 2018).

Plants that can take up more metals than other species from the same soils and above the metal concentrations determined in the soil are called hyperaccumulator plants (Kabata-Pendias, 2011). Phytoremediation is an effective, inexpensive, and environmentally friendly technique in which living green plants are used to transfer or stabilize heavy metals and environmental pollutants in contaminated soil or groundwater (Saleem et al., 2020a). Hyperaccumulator plants have the potential to accumulate high concentrations of heavy metals in their above-ground parts without showing signs of stress. They have been used in phytoremediation of metal contaminated areas with promising results (Wan et al. 2023; Doku et al. 2024). It is reported that there are nearly 400 plant species that accumulate metals in their above-ground organs. Important families with this feature are Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Fabaceae, Lamiaceae, Poaceae, Violaceae and Euphorbiaceae. Brassicaceae family is the largest family with 11 genera and 87 species (Ozbek, 2015; Boysan Canal et al., 2022).

The increase in the world's population in parallel with increasing industrial activities has led to the generation of large volumes of household and industrial waste (Lagerkvist and Dahlén, 2019). In underdeveloped and/or developing countries, garbage/solid waste is often deposited randomly in open areas (away from residential areas). For many years in Turkey, this method has been used for the disposal of garbage/solid waste (Gokce and Hasanoglu, 2015).

Today, with wild landfilling still in place, of the 32.3 million tons of waste collected by municipalities (waste service providers), 69.4% was disposed of in sanitary landfills, 17% in municipal dumps, 13.2% in recycling facilities and 0.4% by open burning, burial, dumping in streams or land (TUIK, 2020).

Rainwater inflow to landfills causes biochemical and physical breakdown of garbage/waste, resulting in the formation of highly polluted leachate (Ebin, 2004). The content of leachate varies according to the type of waste stored, and landfill leachate can contain high levels of organic and inorganic substances, ammonia-nitrogen, heavy metals, and chlorinated organic compounds. Heavy metals such as cadmium (Cd), lead (Pb), chromium (Cr), nickel (Ni), and copper (Cu) are commonly found in landfill leachate (Oksuz, 2019).

These landfills, which are known to be responsible for toxic leachate from waste, have been reported to significantly affect all forms of life. Such leachate is often found in surface water, groundwater, soils, and other biophysical components of the environment, causing adverse impacts on humans, aquatic organisms, plants, and animals (Agbeshie et al., 2020). However, most people use such sites without knowing the risk of plants taking up heavy metals found in soils. Therefore, risk assessment of heavy metal pollution in landfills is an important issue (Agbeshie et al., 2020).

The area located in the city center of Bingöl province was used as a wild landfill for about 18 years where both domestic and medical wastes were dumped. Due to the constant spontaneous combustion of garbage in the area and the danger it posed to people and the environment (Anonymous, 2013a), this area became inactive with the establishment of a new solid waste disposal facility in the city (Anonymous, 2013b).

The region is a place where some animal husbandry (grazing, beekeeping) activities continue in spring. There are many plants adapted to this area. Today, it is still unknown whether these plants contain the pollution materials and heavy metals emitted by these landfill leachates. This study aimed to identify the plant species adapted to the area, investigate their hyperaccumulatory properties, determine their potential for use in phytoremediation, and make suggestions and predictions for similar areas.

2. Material and Methods

2.1. Study area

The area in the auto industry zone of Bingöl province was used as a wild garbage storage area for approximately 18 years, from 1996 to 2013, and became inactive with the establishment of a new solid waste disposal facility in the city (Anonymous, 2013b). The size of the study area is approximately 11 ha (Figure 1).

Figure 1. Location of the Old Landfill in Bingöl Province.

In April, May, and June 2022, the vegetation was monitored and the species adapted to the region were collected during the development of roots, stems, leaves, and flowers. Visuals of the region are presented in Figure 2.

Figure 2. Residue Images from Bingöl Province Old Landfill (May 2022).

Plant material was sampled and 10 species were identified. Soil samples were taken from 4 different points of the area.

2.2. Plant species

Ten (10) plant species were collected from the landfill area and identified according to the 11-volume Flora of Turkey (Davis, 1965-1985; Davis et al., 1988; Guner et al., 2000). The altitude of the area where the samples were collected was 1225 m and the coordinates were 38° 54' 13" N-40° 32' 47" E. After recording the location, the general view of the area was photographed together with the general view of the plant and the habitat area.

During the collection of samples, an attempt was made to collect as many parts as possible for species identification, such as fruits, seeds, flowers, and basal leaves. Information about the study area and the collected samples, which may be important in identification and may change when dried or pressed (color, odor, shape), was also recorded in the field notebook. Scientific names and authors of the taxa were checked from the current Turkey Plants List book (Guner et al., 2012). The plant species identified as a result of the study are given in Table 1.

Table 1. List of identified plant species

	Species	**Family**
1	*Alyssum simplex* Rudolph	Brassicaceae
2	*Cirsium libanoticum* DC.	Asteraceae
3	*Descurainia sophia* (L.) Webb ex Prantl	Brassicaceae
4	*Fumaria asepala* Boiss	Papaveraceae
5	*Fumaria officinalis* L.	Papaveraceae
6	*Matricaria chamomilla* L.	Asteraceae
7	*Papaver dubium* L.	Papaveraceae
8	*Scrophularia canina* L.	Scrophulariaceae
9	*Trifolium repens* L.	Fabaceae
10	*Ziziphora capitata* L.	Lamiaceae

Two species of Brassicaceae family (*Alyssum simplex, Descurainia sophia*), 2 species from Asteraceae family (*Cirsium libanoticum, Matricaria chamomilla*), Papavereraceae family 3 species (*Fumaria asepala, Fumaria officinalis, Papaver dubium*) and Scrophulariaceae (*Scrophularia canina*) and 1 species of Lamiaceae family (*Ziziphora capitata*) was determined. Images of the species are presented in Figure 3.

Figure 3. a:*Cirsium libanoticum*, b:*Ziziphora capitata*, c: *Descurainia sophia*, d: *Scrophularia canina*, e:*Alyssum simplex*, f:*Fumaria asepala*, g:*Matricaria chamomilla*, h:*Fumaria officinalis*, ı:*Trifolium repens*, j:*Papaver dubium.*

Heavy metal (Fe, Cr, As, Cd and Pb) contents and pH levels of soil samples taken from the area are given in Table 2. The concentrations of Fe, Pb and Cr, except Cd and As, are similar to the results of Tas and Demir (2022) on heavy metals in the agricultural soils of Bingol plain.

Table 2. Fe, Cr, As, Cd and Pb contents and pH levels of soils

pH	**Fe (mg kg^{-1})**	**Cr (mg kg^{-1})**	**As (mg kg^{-1})**	**Cd (mg kg^{-1})**	**Pb (mg kg^{-1})**
6.58	3037.44	7.13	2.00	0.50	7.50

Permissible limit values for toxic heavy metal concentrations in soil and plants (WHO/FAO, 2007) are presented in Table 3.

Table 3. Permissible limit values for toxic heavy metal concentrations in soil and plants (WHO/FAO, 2007; Sonmez and Kılıc, 2021)

Metal	Soil (mg kg^{-1})	Plant (mg kg^{-1})
Fe	50.000	450
Cr	150	5.00
As	20	0.10
Cd	3	0.20
Pb	300	0.30

2.3. Heavy metal (Fe, Cr, As, Cd and Pb) analysis in plants and soil

Plants were collected from the field with all their organs present and separated into their organs as roots, stems, leaves, and flowers in the laboratory, then washed with water and dried in a drying oven at 70 °C. Dried plant parts were ground in a hand mill and made ready for analysis. The total combustion process in the microwave was applied according to the method described in the literatüre (Campbell and Plank, 1998; Kacar and Inan, 2008: Gurbuz et al., 2016). Then, filtration was done with filter paper and the volume of the tubes was completed to 50 mL with ultrapure water. Elemental readings were made in diluted samples with the ICP-MS device. Soil samples were sieved and wet digestion was performed. Then, filtration and dilution were performed in the same way and heavy metal concentrations were read on the ICP-MS device.

2.3.1. Translocation factor (TF)

It is the ratio of the heavy metal concentration in the shoot of the plant to the heavy metal concentration in the root and indicates the ability of heavy metals to be transported from the root to other organs of the plant. If the TF values of plants are greater than 1, they can be used as bioaccumulators in phytoremediation (Surmen et al., 2019).

$$\mathrm{TF} = \frac{\text{Heavy metal concentration in the aerial parts (mg kg}^{-1})}{\text{Heavy metal concentration in the roots (mg kg}^{-1})} \quad (1)$$

2.4. Statistical analysis

Analysis of variance was applied to the obtained data in the JMP program and the differences were compared with the Tukey test (JMP, 2018).

3. Results

3.1. Distribution of Fe, Cr, As, Cd and Pb concentrations in plant organs and translocation factor (TF) values of species

Concentrations of Fe, Cr, As, Cd, and Pb metals in organs (root, stem, leaf, and flower) and Translocation Factor values of plant species are shown in Table 4.

Alyssum simplex species accumulated chromium most in its roots (1.56 mg kg^{-1}) and stem (1.44 mg kg^{-1}), and arsenic and cadmium accumulated most in its roots, stems and leaves. Similar amounts of iron and lead accumulated in all organs of *Alyssum simplex*. Accumulation of Fe, Cr, As, Cd and Pb metals in the organs of *Cirsium libanoticum* species was not found to be statistically significant. *Descurainia sophia* accumulated chromium (1.78 mg kg^{-1}) and arsenic (0.21 mg kg^{-1}) mostly in its roots, and lead in its leaves (0.18 mg kg^{-1}) and flowers (0.16 mg kg^{-1}). *Fumaria asepala* accumulated iron (366.78 mg kg^{-1}) and arsenic (0.17 mg kg^{-1}) mostly in its leaves, while chromium (1.92 mg kg^{-1}) and cadmium (0.11 mg kg^{-1}) accumulated mostly in its stem. *Fumaria officinalis* accumulated iron (335.03 mg kg^{-1}) and arsenic (0.22 mg kg^{-1}) mostly in its leaves, chromium (1.53 mg kg^{-1}) mostly in its flower and cadmium (0.08 mg kg^{-1}) mostly in its root. The analysis results showed that the heavy metal

concentrations in the soil did not exceed the allowed limit values for toxic heavy metal concentrations in soil and plants (Table 2).

Matricaria chamomilla accumulated iron (327.29 mg kg^{-1}) and arsenic (0.20 mg kg^{-1}) mostly in its leaves, chromium mostly in its above-ground organs, cadmium (0.18 mg kg^{-1}) mostly in its roots and lead mostly in its flowers (0.12 mg kg^{-1}). *Papaver dubium* accumulated iron (222.46 mg kg^{-1}) and chromium mostly in its root (2.91 mg kg^{-1}). *Scrophularia canina* accumulated iron (302.79 mg kg^{-1}), chromium (0.88 mg kg^{-1}) and lead mostly in its roots (0.53 mg kg^{-1}), arsenic (0.09 mg kg^{-1}) mostly in its leaves and cadmium (0.25 mg kg^{-1}) mostly in its stems. *Trifolium repens* accumulated iron (331.65 mg kg^{-1}), chromium (0.68 mg kg^{-1}), arsenic (0.06 mg kg^{-1}) and lead (0.19 mg kg^{-1}) mostly in leaves and cadmium (0.02 mg kg^{-1}) mostly in roots. *Ziziphora capitata* accumulated iron, chromium and arsenic mostly in roots and leaves, cadmium mostly in roots and lead mostly in roots, leaves and flowers (Table 4).

3.2. Translocation factor (TF) values of species

Translocation factor (TF) is the ratio of heavy metal concentration in the shoot of the plant to the heavy metal concentration in the root and indicates the ability of heavy metals to be transported from the root to other organs of the plant. If the TF values of plants are greater than 1, they have the possibility of being used as bioaccumulators in phytoremediation (Surmen et al., 2019).

The translocation factor measures plant defense mechanisms that tend to limit inorganic pollutants to the roots to prevent the translocation of trace elements to the above-ground organs of the plant, especially seeds. Normally, plants exhibit TF<1 when under heavy metal stress. TF>1 indicates that plants not only tolerate but also utilize the contaminant, which is often seen as a general characteristic of hyperaccumulators. Thus, TF>1 is a determining factor in the classification of plant species for phytoremediation (Chanu and Gupta, 2016).

As seen in Table 4, TF>1 for Fe in *Alyssum simplex*, *Cirsium libanoticum*, *Fumaria asepala* species, TF>1 for Cr in *Cirsium libanoticum*, *Fumaria asepala*, *Fumaria officinalis* and *Matricaria chamomilla* species, TF>1 for As in *Cirsium libanoticum*, *Fumaria asepala*, *Fumaria officinalis*, *Matricaria chamomilla* and *Papaver dubium* species, TF>1 for Cd in *Cirsium libanoticum*, *Papaver dubium* and *Scrophularia canina* species, TF>1 for Pb in all species except *Alyssum simplex* and *Scrophularia canina* (Table 4).

Table 4. Distribution of Fe, Cr, As, Cd and Pb concentrations (mg kg^{-1}) in organs and TF values

	***Alyssum simplex* Rudolph.**				
	Fe	**Cr**	**As**	**Cd**	**Pb**
Root	79.88^{ns}	1.56a**	0.16ab*	0.40a**	0.27^{ns}
Stem	92.50	1.44a	0.14ab	0.34a	0.11
Leaf	52.65	1.27b	0.22a	0.36a	0.14
Flower	100.62	1.09c	0.10b	0.22b	0.07
TF	1.03	0.81	0.93	0.76	0.41
	***Cirsium libanoticum* DC.**				
	Fe	**Cr**	**As**	**Cd**	**Pb**
Root	132.13^{ns}	1.84^{ns}	0.14^{ns}	0.19^{ns}	0.18^{ns}
Stem	146.95	1.80	0.17	0.27	0.18
Leaf	197.81	1.95	0.22	0.26	0.24
Flower	233.50	1.75	0.23	0.18	0.38
TF	1.46	1.00	1.54	1.27	1.47
	***Descurainia sophia* (L.) W.ex P.**				
	Fe	**Cr**	**As**	**Cd**	**Pb**
Root	129.76^{ns}	1.78a**	0.21a*	0.33^{ns}	0.08b*
Stem	144.60	1.52b	0.13ab	0.26	0.08b
Leaf	96.28	1.55b	0.16ab	0.37	0.18a
Flower	130.45	1.34b	0.12b	0.27	0.16a
TF	0.95	0.83	0.63	0.90	1.86

*:p<0.05, **:p<0.01, ns: non significant.

Table 4. Distribution of Fe, Cr, As, Cd and Pb concentrations (mg kg^{-1}) in organs and TF values (continued)

			***Fumaria asepala* Boiss.**		
	Fe	**Cr**	**As**	**Cd**	**Pb**
Root	179.23c**	1.24c**	0.06d**	0.08ab*	0.11^{ns}
Stem	313.26b	1.92a	0.14b	0.11a	0.14
Leaf	366.78a	1.79ab	0.17a	0.08ab	0.17
Flower	222.56c	1.64b	0.10c	0.02b	0.14
TF	1.68	1.44	2.10	0.92	1.39
			***Fumaria officinalis* L.**		
	Fe	**Cr**	**As**	**Cd**	**Pb**
Root	240.91b**	1.00c**	0.11c**	0.08a**	0.05^{ns}
Stem	227.66b	1.32b	0.15b	0.04c	0.27
Leaf	335.03a	1.31b	0.22a	0.05b	0.12
Flower	93.36c	1.53a	0.09d	0.02d	0.05
TF	0.91	1.39	1.39	0.44	2.65
			***Matricaria chamomilla* L.**		
	Fe	**Cr**	**As**	**Cd**	**Pb**
Root	236.56b**	1.58b**	0.12b**	0.18a**	0.06b*
Stem	190.84bc	1.84a	0.10b	0.10b	0.08ab
Leaf	327.29a	1.88a	0.20a	0.09bc	0.10ab
Flower	160.92c	1.86a	0.09b	0.06c	0.12a
TF	0.96	1.18	1.09	0.46	1.58
			***Papaver dubium* L.**		
	Fe	**Cr**	**As**	**Cd**	**Pb**
Root	222.46a**	2.91a**	0.08^{ns}	0.07^{ns}	0.16^{ns}
Stem	50.39b	1.41b	0.19	0.28	0.30
Leaf	91.04b	1.49b	0.14	0.20	0.10
Flower	72.44b	1.16b	0.08	0.03	0.12
TF	0.32	0.47	1.81	2.31	1.10
			***Scrophularia canina* L.**		
	Fe	**Cr**	**As**	**Cd**	**Pb**
Root	302.79a**	0.88a**	0.08b**	0.14b**	0.53a*
Stem	139.41bc	0.42b	0.02c	0.25a	0.02b
Leaf	191.99b	0.75a	0.09a	0.10c	0.24ab
Flower	113.07c	0.30b	0.03c	0.09c	0.07ab
TF	0.49	0.56	0.61	1.03	0.21
			***Trifolium repens* L.**		
	Fe	**Cr**	**As**	**Cd**	**Pb**
Root	315.79b**	0.57b**	0.05b**	0.02a**	0.10ab*
Stem	185.72c	0.49bc	0.02c	0.01b	0.08b
Leaf	331.65a	0.68a	0.06a	0.00c	0.19a
Flower	156.46b	0.45c	0.02c	0.00c	0.09ab
TF	0.71	0.95	0.65	0.19	1.23
			***Ziziphora capitata* L.**		
	Fe	**Cr**	**As**	**Cd**	**Pb**
Root	279.36a**	0.75a**	0.07a**	0.05a**	0.06a**
Stem	132.28c	0.48b	0.02b	0.02ab	0.02b
Leaf	288.10a	0.69a	0.08a	0.01b	0.10a
Flower	166.37b	0.47b	0.03b	0.00c	0.08a
TF	0.70	0.73	0.57	0.17	1.00

*:p<0.05, **:p<0.01, ns: non significant.

3.3. Fe concentration of species (mg kg^{-1})

The distribution of Fe concentrations (mg kg^{-1}) in plant organs of plant species collected from the garbage area are shown in Table 5.

The values obtained in each organ and their averages were found to be statistically very significant ($p<0.01$). In all organs (root, stem, leaf, and flower), *Fumaria asepala* accumulated the highest (270.46 mg kg^{-1}) Fe, while *Alyssum simplex* accumulated the least (81.41 mg kg^{-1}). Among the species, *Scrophularia canina* and *Trifolium repens* accumulated the most iron (Fe) in roots, *Fumaria asepala* accumulated the most in stems, *Fumaria asepala, Fumaria officinalis*, *Trifolium repens* and *Matricaria chamomilla* accumulated the most in leaves and *Cirsium libanoticum* accumulated the most in flowers (Table 5).

Table 5. Fe concentrations in organs of species (mg kg^{-1})

	Fe Concentration (mg kg^{-1})					
	Species	**Root**	**Stem**	**Leaf**	**Flower**	**Mean**
1.	*Alyssum simplex*	79.88f**	92.50de**	52.65d**	100.62c**	81.41D**
2.	*Cirsium libanoticum*	132.13ef	146.95cd	197.81bc	233.50a	177.60BC
3.	*Descurainia sophia*	129.76ef	144.60cd	96.28cd	130.45bc	125.27CD
4.	*Fumaria asepala*	179.23de	313.26a	366.77a	222.56ab	270.46A
5.	*Fumaria officinalis*	240.91bc	227.66b	335.03a	93.36c	224.24AB
6.	*Matricaria chamomilla*	236.56bc	190.84bc	327.29a	160.92abc	228.90AB
7.	*Papaver dubium*	222.46cd	50.39e	91.04cd	72.44c	109.08CD
8.	*Scrophularia canina*	302.79a	139.41cd	191.99bc	113.07c	186.81BC
9.	*Trifolium repens*	315.79a	185.72bc	331.65a	156.46abc	247.41AB
10.	*Ziziphora capitata*	279.36ab	132.28cd	288.10ab	166.37abc	216.53AB
	Mean	211.89A**	162.36B	227.86A	144.97B	

**:$p<0.01$, level of significance; capital letters show significant differences between the average concentrations of species and the average concentrations of organs; small letters show significant differences between the concentrations in each organ.

When all species are considered together, it is observed that the most iron accumulated in roots (211.89 mg kg^{-1}) and leaves (227.86 mg kg^{-1}), followed by stems (162.36 mg kg^{-1}) and flowers (144.97 mg kg^{-1}) (Table 5).

3.4. Cr concentration of species (mg kg^{-1})

The distribution of Cr concentrations (mg kg^{-1}) in plant organs of plant species collected from the garbage area are shown in Table 6. The values obtained in each organ and their averages were found to be statistically very significant ($p<0.01$). *Cirsium libanoticum*, *Matricaria chamomilla*, and *Papaver dubium* accumulated the highest (1.84, 1.79, and 1.74 mg kg^{-1}) Cr in all organs, while *Scrophularia canina*, *Trifolium repens* and *Ziziphora capitata* accumulated the least (0.59, 0.55 and 0.59 mg kg^{-1}). Among the species, *Papaver dubium* accumulated the most chromium (Cr) in its roots, *Fumaria asepala*, *Matricaria chamomilla* and *Cirsium libanoticum* accumulated it in its stems, *Cirsium libanoticum*, *Fumaria asepala* and *Matricaria chamomilla* accumulated it in their leaves, while *Cirsium libanoticum* and *Matricaria chamomilla* accumulated it in their flowers (Table 6).

Table 6. Cr concentrations in organs of species (mg kg^{-1})

	Cr Concentration (mg kg^{-1})					
	Species	**Root**	**Stem**	**Leaf**	**Flower**	**Mean**
1.	*Alyssum simplex*	1.56c**	1.44b**	1.27b**	1.09b**	1.34B**
2.	*Cirsium libanoticum*	1.84b	1.80a	1.95a	1.75a	1.84A
3.	*Descurainia sophia*	1.78b	1.52b	1.55ab	1.34ab	1.55AB
4.	*Fumaria asepala*	1.24d	1.92a	1.79a	1.64ab	1.65AB
5.	*Fumaria officinalis*	1.00e	1.32b	1.31b	1.53ab	1.29B
6.	*Matricaria chamomilla*	1.58c	1.84a	1.88a	1.86a	1.79A
7.	*Papaver dubium*	2.91a	1.41b	1.49ab	1.16b	1.74A
8.	*Scrophularia canina*	0.88ef	0.42c	0.75c	0.30c	0.59C
9.	*Trifolium repens*	0.57fg	0.49c	0.68c	0.45c	0.55C
10.	*Ziziphora capitata*	0.75f	0.48c	0.69c	0.47c	0.59C
	Mean	1.41A**	1.26AB	1.34AB	1.16B	

**:$p<0.01$, level of significance; capital letters show significant differences between the average concentrations of species and the average concentrations of organs; small letters show significant differences between the concentrations in each organ.

When all species are considered together, it is observed that most chromium accumulated in the roots (1.41 mg kg^{-1}) followed by leaves (1.34 mg kg^{-1}) and stems (1.26 mg kg^{-1}) (Table 6).

3.5. As concentration of species (mg kg^{-1})

The distribution of As concentrations (mg kg^{-1}) in plant organs of plant species collected from the garbage area are shown in Table 7. The values obtained in each organ and their averages were found to be statistically very significant ($p<0.01$). *Cirsium libanoticum* accumulated the highest (0.19 mg kg^{-1}) As in all organs, while *Scrophularia canina*, *Trifolium repens* and *Ziziphora capitata* accumulated the least (0.06, 0.04 and 0.05 mg kg^{-1}). Among the species, *Descurainia sophia* accumulated the most arsenic (As) in its roots, *Papaver dubium* and *Cirsium libanoticum* in its stems, *Alyssum simplex*, *Cirsium libanoticum*, *Fumaria officinalis*, and *Matricaria chamomilla* in its leaves and *Cirsium libanoticum* in its flowers (Table 7).

Table 7. As concentrations in organs of species (mg kg^{-1})

As Concentration (mg kg^{-1})						
	Species	**Root**	**Stem**	**Leaf**	**Flower**	**Mean**
1.	*Alyssum simplex*	0.16b**	0.14ab**	0.22a*	0.10ab**	0.15AB**
2.	*Cirsium libanoticum*	0.14bc	0.17a	0.22a	0.23a	0.19A
3.	*Descurainia sophia*	0.21a	0.13ab	0.16abc	0.12ab	0.15AB
4.	*Fumaria asepala*	0.06f	0.14ab	0.17ab	0.10ab	0.12BC
5.	*Fumaria officinalis*	0.11cde	0.15ab	0.22a	0.09ab	0.14AB
6.	*Matricaria chamomilla*	0.12cd	0.10ab	0.20a	0.09ab	0.13AB
7.	*Papaver dubium*	0.08ef	0.19a	0.14abc	0.08b	0.12B
8.	*Scrophularia canina*	0.08def	0.02b	0.09bc	0.03b	0.06CD
9.	*Trifolium repens*	0.05f	0.02b	0.06c	0.02b	0.04D
10.	*Ziziphora capitata*	0.07ef	0.02b	0.08bc	0.03b	0.05D
	Mean	0.11B**	0.11B	0.16A	0.09B	

*:p<0.05, **:p<0.01, level of significance; capital letters show significant differences between the average concentrations of species and the average concentrations of organs; small letters show significant differences between the concentrations in each organ.

When all species are considered together, it is seen that the most arsenic is accumulated in the leaves (0.16 mg kg^{-1}) and that As is accumulated in other organs, although less than the leaves, at similar concentrations (0.09-0.11 mg kg^{-1}) (Table 7).

3.6. Cd concentration of species (mg kg^{-1})

The distribution of Cd concentrations (mg kg^{-1}) in plant organs of plant species collected from the garbage area are shown in Table 8.

Table 8. Cd concentrations in organs of species (mg kg^{-1})

Cd Concentration (mg kg^{-1})						
	Species	**Root**	**Stem**	**Leaf**	**Flower**	**Mean**
1.	*Alyssum simplex*	0.40a**	0.34a**	0.36a**	0.22b**	0.33A**
2.	*Cirsium libanoticum*	0.19b	0.27ab	0.26ab	0.18b	0.22B
3.	*Descurainia sophia*	0.33a	0.26ab	0.37a	0.27a	0.31A
4.	*Fumaria asepala*	0.08bcd	0.11ab	0.08d	0.02de	0.07CDE
5.	*Fumaria officinalis*	0.08bcd	0.04b	0.05d	0.02e	0.05DE
6.	*Matricaria chamomilla*	0.18b	0.10ab	0.09cd	0.06cd	0.11CD
7.	*Papaver dubium*	0.07bcd	0.28ab	0.20bc	0.03de	0.15BC
8.	*Scrophularia canina*	0.14bc	0.25ab	0.10cd	0.09c	0.15BC
9.	*Trifolium repens*	0.02d	0.01b	0.00d	0.00e	0.01E
10.	*Ziziphora capitata*	0.05cd	0.02b	0.01d	0.00e	0.02E
	Mean	0.16A**	0.17A	0.15A	0.09B	

**:p<0.01, level of significance; capital letters show significant differences between the average concentrations of species and the average concentrations of organs; small letters show significant differences between the concentrations in each organ.

The values obtained in each organ and their averages were found to be statistically very significant ($p<0.01$). *Alyssum simplex* and *Descurainia sophia* accumulated the highest (0.33 and 0.31 mg kg^{-1}) Cd in all organs, while *Trifolium repens* and *Ziziphora capitata* accumulated the least (0.01 and 0.02 mg kg^{-1}). Among the species, *Alyssum simplex* and *Descurainia sophia* accumulated the most arsenic (As) in roots, *Alyssum simplex* accumulated the most in stems, *Alyssum simplex* and *Descurainia sophia* accumulated the most in leaves, and *Descurainia sophia* accumulated the most in flowers (Table 8).

When all species are considered together, it is seen that the highest cadmium accumulates in similar concentrations in the roots, stems and leaves (0.15-0.17 mg kg^{-1}), and the least accumulates in the flowers (0.09 mg kg^{-1}) (Table 8).

3.7. Pb concentration of species (mg kg^{-1})

The distribution of Pb concentrations (mg kg^{-1}) in plant organs of plant species collected from the garbage area are shown in Table 9. The values obtained in each organ and their averages were found to be statistically very significant ($p<0.01$). The species that accumulated Pb in all organs was *Cirsium libanoticum*. Among the species, *Scrophularia canina* accumulated the most lead (Pb) in its roots, *Papaver dubium* accumulated it in its trunk, and *Cirsium libanoticum* accumulated it in its flowers. The concentration of Pb accumulated in leaves did not differ significantly between species (Table 9).

Table 9. Pb concentrations in organs of species (mg kg^{-1})

		Pb Concentration (mg kg^{-1})				
	Species	**Root**	**Stem**	**Leaf**	**Flower**	**Mean**
1.	*Alyssum simplex*	0.27ab**	0.11bc*	0.14^{ns}	0.07b**	0.15AB**
2.	*Cirsium libanoticum*	0.18b	0.18abc	0.24	0.38a	0.25A
3.	*Descurainia sophia*	0.08b	0.08c	0.18	0.16b	0.12AB
4.	*Fumaria asepala*	0.11b	0.14abc	0.17	0.14b	0.14AB
5.	*Fumaria officinalis*	0.05b	0.27ab	0.12	0.05b	0.12AB
6.	*Matricaria chamomilla*	0.06b	0.08c	0.10	0.12b	0.09B
7.	*Papaver dubium*	0.16b	0.30a	0.10	0.12b	0.17AB
8.	*Scrophularia canina*	0.53a	0.02c	0.24	0.07b	0.21AB
9.	*Trifolium repens*	0.10b	0.08c	0.19	0.09b	0.11AB
10.	*Ziziphora capitata*	0.06b	0.02c	0.10	0.08b	0.06B
	Mean	0.16^{ns}	0.13	0.16	0.13	

**:$p<0.01$, level of significance; ns: non significant, capital letters show significant differences between the average concentrations of species and the average concentrations of organs; small letters show significant differences between the concentrations in each organ.

When all species are considered together, it is seen that there is no statistically significant difference between plant organs for Pb accumulation.

4. Discussion

Among the plant species, *Alyssum simplex* accumulated the least iron (Fe) and the most cadmium (Cd) in all its organs. Plants such as *Alyssum, Thlaspi, Urtica*, and *Polygonum* have a high ability to accumulate heavy metals such as cadmium, copper, lead, nickel, and zinc (Ozay and Mammadov, 2013). It has been reported that some plant species such as *Alyssum murale, Thlaspi vaerulescens, Nicotiana tabacum, Zea mays, Salix viminalis, Helianthus annuus* and *Viola baoshanensis* are used for phytoremediation purposes (Kabata-Pendias, 2011). Similar to this study, in the study conducted by Celiktas (2020), *Alyssum oxycarpum* species accumulated iron in its roots, stems, and leaves at concentrations close to each other. Although there was no Pb pollution in the area, *Alyssum simplex* preferred to accumulate the available lead in its roots. Similarly, in a study conducted around Adana Cr mine, *Alyssum alyssoides* preferred to accumulate lead mostly in its roots (root: 4.86, stem: 1.22, leaf: 3.01, mg kg^{-1}) (Celiktas, 2020).

Cirsium libanoticum tended to accumulate Cr and As in its aboveground organs compared to other species. Dokmeci and Adiloglu (2020) reported that *Cirsium vulgare* offers the potential for use in the removal of chromium from the soil. In this study, TF>1 was found for Cr. Sajad et al. (2020)

reported a similar result as TF>1 for Cr in *Cirsium vulgare* plant in their study. In this study, Pb TF >1 was calculated for *Cirsium libanoticum*. Sajad et al. (2019) also reported TF >1 for Pb in *Cirsium vulgare* species in their study.

Descurainia sophia accumulated the most Cd in all organs compared to other plant species. While the TF value for Cd was 0.90, it was 1.86 for Pb. Moameri et al. (2017) found that the TF value for Pb (TF>1) was higher than 1 in *Descurainia sophia, Stachys lavandulifolia*, and *Echium amoenum* plants. They also reported Cd translocation factor value as TF>1 for *Brassica juncea, Scariola orientalis, Descurainia sophia, Achillea millefolium, Centaurea virgata* and *Stachys lavandulifolia* plants.

Fumaria asepala accumulated the most iron (276.46 mg kg^{-1} Fe) in all organs compared to other plant species. *Fumaria officinalis* accumulated an average of 224.24 mg kg^{-1} Fe in all plant organs. Zokaei et al. (2018) reported the Fe concentration of plants collected in Shiraz, including *Fumaria officinalis* species, as 187.24 mg kg^{-1}. The researchers also reported that the same species had Cd concentration in the range of 0.01-0.08 mg kg^{-1} and Pb concentration in the range of 0.02-0.3 mg kg^{-1}. These results are parallel for both *Fumaria* species examined in this study (Cd: 0.02-0.11 mg kg^{-1} and Pb: 0.05-0.3 mg kg^{-1}).

Matricaria chamomilla accumulated the most Cr in all organs compared to other species. In addition, in the evaluation within the organs, stem, leaves, and flowers accumulated more Cr than roots (root: 1.58 mg kg^{-1}, stem: 1.84 mg kg^{-1}, leaf: 1.88 mg kg^{-1}, and flower: 1.86 mg kg^{-1}). Glišić et al. (2021) reported that the leaves and stem of *Matricaria inodora* can be used in the phytoextraction of chromium (Cr). *Matricaria chamomilla* preferred to accumulate cadmium in its roots and this aspect weakened its usability in phytoremediation (root: 0.18 mg kg^{-1}, stem: 0.10 mg kg^{-1}, leaf: 0.09 mg kg^{-1} and flower: 0.06 mg kg^{-1}). Kováčik et al. (2006) reported that *Matricaria chamomilla* cannot be classified as a hyperaccumulator due to the preferential accumulation of Cd in the roots and is therefore not suitable for phytoremediation.

Papaver dubium preferred to accumulate iron and chromium in the roots than in the above-ground organs. The difference in the distribution of As, Pb and Cd in plant organs was not statistically significant.

Alizadeh et al. (2022) found that *Papaver dubium*, *Trifolium fragiferum* and *Achillea vermicularis* species collected from a mine and waste dumpsite did not exceed the hyperaccumulation thresholds for the relevant trace elements (As, Ca, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, Zn). The researchers also determined concentrations of Cr 0.72 mg kg^{-1}, Pb 4.39 mg kg^{-1}, and As 2.14 mg kg^{-1} for *Papaver dubium*. According to the results of Alizadeh et al. (2022), Cr was determined higher in our study, while As and Pb were determined lower. Ghaderian and Ravandi (2012) reported Pb concentration in the leaf dry matter of *Papaver dubium* collected from copper mining area as 11 mg kg^{-1}. The concentration in the plant collected from the mining area was higher than the plant collected from the garbage area, as expected.

It appears that the *Scrophularia canina* plant prefers to accumulate Pb and Fe elements in its roots. The TF of the plant for Pb was calculated to be TF<1, which limits the usability of the species for phytoremediation in Pb-polluted areas. Cd concentration in the *Scrophularia canina* plant was determined as 0.14 mg kg^{-1} in the roots, 0.25 mg kg^{-1} in the stem, 0.10 mg kg^{-1} in the leaf, and 0.09 mg kg^{-1} in the flower. Boularbah et al. (2006) found that the *Scrophularia canina* plant collected from the mining area had a Cd concentration of 0.25 mg kg^{-1}, which is similar to the study findings. Shallari et al. (1998) heavy metal contents (1 mg kg^{-1} Cd, 4 mg kg^{-1} Cr, and 8 mg kg^{-1} Pb) of the *Scrophularia canina* plant collected from serpentine areas are higher than our study findings.

In the *Trifolium repens* plant, TF<1 for Fe, Cr, As, and Cd, while TF>1 was determined only for Pb. In addition, it accumulated the elements Cr, Cd, and As in the least concentration in all its organs compared to other plant species. Wen et al. (2018) reported in their study that *Trifolium repens* and *R. nepalensis* plants could increase the phytoremediation efficiency of Pb-Zn-contaminated areas when planted together. Matanzas et al. (2021) found TF <1 for As (TF: 0.38) and Pb (TF: 0.31) in the *Trifolium repens* plant. TF <1 for arsenic was similar to the study findings.

Ziziphora capitata was the species that accumulated Cr, As, and Cd elements the least in all plant tissues compared to other species. The TF value was found to be 1.00 only for Pb, but it still did not exceed the concentration limit value allowed in plants. Cd and Pb were found to be higher than the permissible limit values in the *Ziziphora persica* plant (Alinia-Ahandani et al., 2021). Cd, Pb, and Cr were found to be <0.05 mg kg^{-1} in the *Ziziphora tenuior* plant (Hajihashemi et al., 2021).

When all species were considered together, it was observed that the most iron, chromium, and cadmium accumulated in the roots, arsenic was distributed in the leaves, and lead was distributed in all plant organs in similar proportions. Nouri et al. (2009) reported that metals accumulated by plants were mostly distributed in root tissues. It has been reported that in contaminated soils, cadmium is especially concentrated in the roots of plants (Kabata-Pendias, 2011).

Conclusion

It was determined that the heavy metal contents (Fe, Cr, As, Cd and Pb) of the soil samples taken from the study area (former garbage area) did not exceed the heavy metal limit values allowed in soils (WHO/FAO, 2007; Sonmez and Kılıc, 2021). The reason for this is that although the area has been used for garbage storage for approximately 18 years, it may not have encountered a new pollution factor in the last 10 years. It is thought that the existing pollution may have been removed by the effect of climatic factors such as rainfall. The potential of the plant species distributed and identified in the area to carry heavy metals to the above-ground organs was evaluated. It has been observed that *Alyssum simplex, Cirsium libanoticum, Descurainia sophia, Fumaria asepala, Fumaria officinalis, Matricaria chamomilla*, and *Papaver dubium* species accumulate arsenic (As) above the allowed limit values by WHO/FAO. It has been observed that cadmium (Cd) *Alyssum simplex, Cirsium libanoticum, Descurainia sophia, Papaver dubium*, and *Scrophularia canina* species accumulate, and lead (Pb) *Cirsium libanoticum* and *Papaver dubium* species accumulate above the limit values allowed in plants by WHO/FAO. It is not recommended to use these plants in the area for human and animal nutrition. When the use of phytoremediation purposes (TF>2) for soil with this pollution is evaluated, it can be said that *Fumaria asepala* for As, *Fumaria officinalis* for Pb, and *Papaver dubium* species for Cd have potential.

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Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

ISSN: 1308-7576 e-ISSN: 1308-7584

Research Article

In vitro Antifungal Activity of *Mentha piperita* and *Thymus vulgaris* Essential Oils against Ochratoxigenic *Aspergillus carbonarius* Isolated from Bozcaada Çavuş Grape

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Article Info

Received: 11.12.2023
Accepted: 14.02.2024
Online published: 15.03.2024
DOI: 10.29133/yyutbd.1403366

Keywords

Anti-ochratoxigenic,
Antifungal activity,
Aspergillus carbonarius,
Mentha piperita,
Thymus vulgaris

Abstract: In this study, the antifungal properties of *Mentha piperita* and *Thymus vulgaris* essential oils against an isolate of ochratoxin A producer, *Aspergillus carbonarius*, isolated from Bozcaada Çavuş grape, were evaluated in three steps. By GC-MS of *M. piperita* and *T. vulgaris* essential oils, the main components were determined to be menthol (39.911%) and carvacrol (49.042%). Antifungal activity was first evaluated by the agar well diffusion method, and it was determined that the tested essential oils completely inhibited the growth of *A. carbonarius* and were as effective as fluconazole antifungal. In the second step, the MIC and MFC values of the tested essential oils were determined; both values were 1 µL mL^{-1}. Finally, it was determined that *M. piperita* and *T. vulgaris* essential oils completely inhibited the radial growth of *A. carbonarius* at the MIC value. These results show that *M. piperita* and *T. vulgaris* essential oils may be a good strategy to control ochratoxigenic *A. carbonarius* contamination.

To Cite: Özcan Ateş, G, 2024 *In vitro* Antifungal Activity of *Mentha piperita* and *Thymus vulgaris* Essential Oils against Ochratoxigenic *Aspergillus carbonarius* Isolated from Bozcaada Çavuş Grape. *Yuzuncu Yil University Journal of Agricultural Sciences*, 34(1): 166-175. DOI: https://doi.org/10.29133/yyutbd.1403366

1. Introduction

Mycotoxins are harmful substances produced by certain moulds that grow in various agricultural products (Dammak et al., 2018; Nurtjahja et al., 2022). These toxins have been causing significant health and economic problems since their discovery. So far, around 300-400 different mycotoxins have been identified. *Aspergillus*, a genus of mould, produces several mycotoxins, such as aflatoxins, fumonisins, gliotoxin, ochratoxins, patulin, sterigmatocystin, etc., that can have adverse effects on human health (Pócsi et al., 2020; Ráduly et al., 2020). When humans or animals consume food or feed contaminated with mycotoxins, it can lead to acute and chronic toxicity. According to the Food and Agriculture Organization (FAO), approximately 25% of food products are contaminated by mycotoxins. The World Health Organization (WHO) and FAO have set guidelines and restrictions to address the problem of mycotoxin contamination in feeds and foods (Navale et al., 2021).

Recently, researchers have identified at least 20 analogues of ochratoxin, one of the most important mycotoxins. Ochratoxin A (OTA) is the most toxic and common among these analogues. OTA is immunosuppressive, immunotoxic, embryotoxic, genotoxic, neurotoxic, and teratogenic and is rated as a potential carcinogen (group 2B) by the International Agency for Research on Cancer (IARC, 1993). The European Union and China have recommended a maximum tolerable OTA level in cereal

grains of 5 µg kg^{-1}. OTA is produced by some species such as *Aspergillus niger, Aspergillus carbonarius, Aspergillus ochraceus, Aspergillus westerdijkiae, Penicillium verrucosum,* and *Penicillium nordicum*. *A. ochraceus* is responsible for producing OTA in rice, oats, wheat, coffee, and other beverages. On the other hand, *A. niger* and *A. carbonarius* also produce OTA in grapes, raisins, and wine (Chiotta et al., 2013; Hua et al., 2014; Pantelides et al., 2017). *A. carbonarius* is the primary ochratoxigenic fungus found on grapes, especially in Mediterranean countries, and contaminates grapes during ripening (Pantelides et al., 2017; Dammak et al., 2018; Özcan Ateş and Zorba, 2020).

With global climate change, the spread of mycotoxigenic *Aspergillus* species has increased and is expected to continue to do so, leading to a greater possibility of mycotoxin contamination in the food and feed supply chain. Currently, measures are taken to ensure food safety and prevent the development of moulds, mycotoxin accumulation, and mycotoxin formation in the food chain (Ráduly et al., 2020). Chemical-based control is a standard method used to minimize post-harvest contamination in different types of food. This involves using antifungal chemicals, for example, aromatic hydrocarbons, benzimidazoles, and sterol biosynthesis inhibitors. However, applying these chemicals enhances the risk of toxic residues in foods and can have side effects, such as carcinogenic and teratogenic effects and producing residual toxicity. Due to these concerns, researchers are exploring natural alternatives to synthetic fungicides. The aim of current research is to find effective natural preservatives to limit the need for chemical fungicides. One area of focus is the use of natural antibacterial and antifungal agents. Essential oils (EO) and their components are being extensively researched for their antifungal and anti-toxigenic effects (Hua et al., 2014; Thippeswamy et al., 2014; Boukaew et al., 2017; Dammak et al., 2018 and 2019; Kapetanakou et al., 2019; Rodrigues et al., 2019; Achar et al., 2020; Bouderba et al., 2020; Kalagatur et al., 2020; Nerilo et al., 2020; Laaziz et al., 2022). Therefore, the study evaluated the antifungal activity of *Mentha piperita* and *Thymus vulgaris* EOs against the *A. carbonarius* isolate, which is known to produce ochratoxin.

2. Material and Methods

2.1. Culture and essential oil

A. carbonarius PP264185 (NCBI gen bank number) (closely related species accession number MK778845.1), isolated from grape samples taken from the Çavuş vineyard in Bozcaada Çayır location in 2015, was used in the study (Özcan Ateş and Zorba, 2020; Özcan Ateş et al., 2024). It was determined through HPLC analysis that the isolate produced 49.448 ± 0.354 ppm of OTA in the medium (Özcan Ateş et al., 2024).

M. piperita (MPEO) and *T. vulgaris* (TVEO) EOs were obtained from Altın Toroslar A.Ş. (Adana, Türkiye).

2.2. Preparation of spore suspension

The stock culture was first revived in the Potato Dextrose Agar (PDA) medium (Biolife, Italy). Then, it was planted in Malt Extract Agar (MEA) (Oxoid, England) medium and incubated at 25°C for 7-10 days for sporulation. Sterilized Tween 80 (Merck, Germany) solution (0.1%, v v^{-1}) was added to the MEA, and spores were collected. The spore solution was vortexed for 15-30 seconds for a homogeneous mixture and adjusted to 0.5 McFarland density (Özcan Ateş, 2023).

2.3. Agar well diffusion method

To determine the antifungal activity of *MPEO* and *TVEO*, a modified version of the NCCLS M44-A method was used (NCCLS, 2004; Özcan Ateş, 2023). A spore solution was prepared to 0.5 McFarland density and inoculated on the PDA medium using the spreading plate method. A well with a diameter of 6 mm was created on a PDA medium using a cork borer set instead of a disc. Then, 20 µL of *MPEO* and *TVEO* were added to the well. Plates were incubated at 25°C for 3-5 days, and zone diameters were measured.

As a positive control, amphotericin B (AMB, 20U) (Bioanalyse, Türkiye) and fluconazole (FLU, 25 mcg) (SD232-5CT, Himedia, India) antifungal disks were used. Amphotericin B is a polyene produced by *Streptomyces nodosus* and binds to ergosterols in the fungal cell wall, disrupting wall permeability (Erdem et al., 2018). Conversely, fluconazole is effective against some species of the

Aspergillus genus and inhibits the synthesis of ergosterol in the fungal cell wall (Öncel and Keçeli, 2018).

2.4. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

The MIC value was determined with a minor modification by the method in NCCLS M38-A2 (NCCLS, 2008; Özcan Ateş, 2023). An RPMI 1640 medium (Himedia, India) containing 0.165 M MOPS was prepared to determine the MIC value. To ensure a homogeneous mixture of EOs in the medium, dimethylsulfoxide (DMSO) (Merck, Germany) was added to a final concentration of 3%. A double-content RPMI 1640 medium containing EO and DMSO was distributed as 100 µL into each well of 96-well U-bottom microplates, with decreasing concentrations in rows B and G. Then, 100 µL of the spore solution adjusted to 0.5 McFarland and diluted 1:10 was added to each well, and the microplates were incubated at 25°C for 24-48 hours. The final concentration of EOs in the study was 40, 20, 10, 5, 2.5, and 1 µl ml^{-1}. It was used as a positive control, and 200 µL of RPMI 1640 medium containing 40 µl ml^{-1} EO and 3% DMSO was added to row A of the microplates. In the H row of the microplates, 100 µL of RPMI 1640 medium containing 3% DMSO and 100 µL of culture were added and used as a negative control. After incubation, the MIC value was determined as the first well in which no growth was observed by visual evaluation. The well was defined as the MIC value, and the subsequent two wells were inoculated into PDA medium using the drip seeding method and incubated at 25°C for 24-48 hours. The MFC value was determined as the lowest concentration at which no growth was observed after incubation.

2.5. Effect of EOs on *A. carbonarius* mycelium radial growth

The effect of EOs on the radial growth of mould mycelium was carried out according to the method specified in Özcan Ateş (2023). PDA media were prepared to contain EO at different concentrations (1⁄(4) x MIC, 1⁄(2) x MIC, MIC, 2 x MIC, 4 x MIC) and 3% DMSO. A 7-10-day-old *A. carbonarius* isolate was grown in a PDA medium and inoculated in a single spot in the middle of the petri dish using a needle loop. A PDA medium containing 3% DMSO was used as a control. Plates then incubated at 25°C for 7 days. After incubation, the colony diameters were measured, and radial growth inhibition was calculated using the equation (1).

$$I\% = \frac{C - T}{C} * 100 \quad (1)$$

C: the growth diameter in the control petri dish (mm), T: the growth diameter in the petri dish containing EOs (mm), and I: inhibition (%).

2.6. Determination of *M. piperita* and *T. vulgaris* EOs volatile chemical composition by GC-MS

MPEO and *TVEO* volatile component compositions were determined according to Özcan Ateş and Kanbur (2023). In the study, a gas chromatograph 7890 A coupled to the mass spectrometer series MSD 5975 C (Agilent Technologies) was used, and the integrations were made with MSDCHEM software.

2.7. Statistical analysis

Microbiological analysis was carried out in three parallel; the results were evaluated using the SPSS (v23.0, IBM Corp., Armonk, NY, USA) program and presenting the results as mean (M) ± standard deviation (sd).

3. Results

The antifungal activity of *MPEO* and *TVEO* against the OTA producer *A. carbonarius* isolated from the Bozcaada Çavuş grape was first evaluated by the agar well diffusion method, and the results of the inhibition zone diameters are given in Table 1. It was determined that *MPEO* and *TVEO* were as effective as the positive control fluconazole, and the inhibition zone diameter was 90.00 ± 0.01.

Table 1. Inhibition zone diameters (in mm), MIC (µL mL^{-1}), MFC (µL mL^{-1}), and MFC/MIC ratio of MPEO and TVEO

	***Aspergillus carbonarius* PP264185**			
	Zone diameter (in mm)	**MIC**	**MFC**	**MFC/MIC**
MPEO	90.00 ± 0.01	1 µL mL^{-1}	1 µL mL^{-1}	1
TVEO	90.00 ± 0.01	1 µL mL^{-1}	1 µL mL^{-1}	1
FLU	90.00 ± 0.01	-*	-	-
AMP	14.72 ± 1.21	-	-	-

-*: not determined.

MIC and MFC methods were used to evaluate the fungistatic and fungicidal properties of *MPEO* and *TVEO* against the ochratoxigenic *A. carbonarius* isolate. The MIC and MFC values (Table 2) of *MPEO* and *TVEO* were determined to be 1 µL mL^{-1}. It was found that the tested EOs had an MFC/MIC ratio of 1; therefore, they were fungicidal (MFC/MIC ≤ 4) (Snoussi et al., 2018; Mseddi et al., 2020).

The effect of *MPEO* and *TVEO* on the radial growth of ochratoxigenic *A. carbonarius* was also evaluated. While determining the effect on radial growth, the radial growth inhibition of EOs at 1⁄(4) x MIC, 1⁄(2) x MIC, MIC, 2 x MIC, and 4 x MIC concentrations was examined. Results are given in Table 2. It was determined that *TVEO* completely inhibited the radial growth of the tested *A.carbonarius* isolate starting from 1⁄(2) x MIC concentration, and *MPEO* inhibited the radial growth of the isolate at the MIC value and all concentrations thereafter.

Table 2. *A. carbonarius* radial growth inhibition rate of tested EOs (in %)

EO Concentration	**MPEO**	**TVEO**
1⁄(4) x MIC	72.49 ± 3.72	100.00 ± 0.01
1⁄(2) x MIC	91.05 ± 0.10	100.00 ± 0.01
MIC	100.00 ± 0.01	100.00 ± 0.01
2 x MIC	100.00 ± 0.01	100.00 ± 0.01
4 x MIC	100.00 ± 0.01	100.00 ± 0.01

GC-MS analyses of *MPEO* and *TVEO* identified 51 and 41 different components, respectively, and the volatile chemical composition in the EOs is given in Table 3. The main components of *MPEO* are menthol (39.911%), menthone (17.933%), isomenthone (8.986%), neomenthol (5.942%) and limonene (5.644%), while *TVEO* is carvacrol (49.042%), p. -cymene (11.681%), gamma-terpinene (11.607%) and linalool (7.084%).

Table 3. Volatile chemical compositions of *MPEO* and *TVEO*

Components	**MPEO**		**TVEO**	
	Abundance %	**RT**	**Abundance %**	**RT**
1,3,8-p-Menthatriene	-	-	0.007	26.430
1,5,8-p-Menthatriene	-	-	0.038	34.327
1,8-Cineole	0.629	11.584	-	-
1-[1-Methyl-1-(4-methyl-cyclohex-3-enyl)-ethyl]-1H-pyrrole	-	-	0.065	27.451
1-C42Nonen-3-ol	0.034	23.178	-	-
1-okten-3-ol	-	-	0.324	23.12
1-terpinen-4-ol	-	-	0.804	31.512
2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, cis	-	-	0.041	32.914
2-Hexen-1-ol, (E)-	-	-	0.013	20.776
3-(1'Isopropenyl-2',2'-dimethyl cyclopentyl)-1-propanol	0.03	44.089	-	-

-*: not determined.

Table 3. Volatile chemical compositions of *MPEO* and *TVEO* (continued)

Components	MPEO		TVEO	
	Abundance %	RT	Abundance %	RT
3-Decyne	0.01	19.098	-	-
3-Octanol	0.884	20.064	-	-
3-Octanone	0.023	13.43	-	-
4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-, (R)-	0.54	35.738	-	-
Alcanfor	0.018	42.048	-	-
Alpha-amorphene	-	-	0.019	35.996
Alpha-cadinol	-	-	0.135	60.227
Alpha-humulene	-	-	0.031	34.877
Alpha-pinene	1.725	6.605	3.875	6.633
Alpha-terpinene	-	-	2.757	10.594
Alpha terpineol	0.974	36.783	0.121	39.731
Alpha-terpinolene	0.01	14.667	0.525	14.679
Amorphane	0.031	20.591	-	-
Benzene, methyl(1-methylethenyl)	-	-	0.022	22.416
Beta-bisabolene	-	-	0.616	38.194
Beta Bourbonene	0.116	26.714	0.033	26.685
Beta-myrcene	0.426	9.96	2.841	9.972
Beta-phellandrene	-	-	0.036	22.578
Beta-pinene	2.899	8.47	0.269	8.448
Bicyclo[4.1.0]heptane, 3,7,7-trimethyl- Carane	0.073	28.445	-	-
Borneol	-	-	1.414	36.919
Camphene	-	-	0.651	7.473
Camphor	-	-	0.015	26.572
Carane, trans	0.072	27.405	-	-
Carvacrol	-	-	49.042	62.72
Carvacrol methyl ether	-	-	0.599	31.638
Carveol	0.047	39.719	-	-
Caryophyllene	-	-	1.124	31.022
Caryophyllene oxide	0.077	51.027	-	-
Cis-2-alpha-methylbicyc[4.3.0]nonan-1 beta-ol	0.034	43.515	-	-
Cis-3a-methyl-3a,4,5,6,7,7a-hexahydro-1H-inden-1-one	0.016	21.000	-	-
Citronella	0.055 0.502	28.575 31.699	-	-
Cyclohexanol, 3-methyl0	0.009	22.316	-	-
Cyclohexanol, 3-methyl-2-(1-methylethyl)-, (1.alpha.,2.alpha.,3.alpha.)	0.603	32.339	-	-
Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1.alpha.,2.beta.,5.alpha.)-	1.035	33.018	-	-
Cyclohexanol, 5-methyl-2-(1-methylethyl)-, [1R-(1.alpha.,2.beta.,5.alpha.)]-	0.963	35.146	-	-
Cyclohexanone, 2-methyl-5-(1-methylethyl)-, trans	0.048	40.332	-	-
Cyclohexanone, 3-methyl-, (R)-	0.286	16.839	-	-
Delta-3-carene	0.016	13.252	0.156	9.58
Dihydro-neoclovene	0.027	20.78	-	-
Ethyl amyl carbinol	-	-	0.023	20.024
Gamma-terpinene	-	-	11.607	13.171
Isomenthone	8.986	25.431	-	-
Isopulegol	1.309	29.731	-	-
Isopulegone	0.295	30.474	-	-
Leaf alcohol	0.011	19.599	-	-
Ledene	-	-	0.027	36.331
Limonen-6-ol, pivalate	-	-	0.025	26.864

-*: not determined.

Table 3. Volatile chemical compositions of *MPEO* and *TVEO* (continued)

Components	MPEO		TVEO	
	Abundance %	RT	Abundance %	RT
Limonene	5.644	11.302	0.408 0.034	11.245 24.159
Linalool	-	-	7.084	28.67
Linalyl acetate	-	-	0.431	29.032
Menthol	39.911	33.872	-	-
Menthone	17.933	23.973	-	-
Menthyl acetate	3.727	29.318	-	-
Naphthalene, decohydro-2-methyl	0.015	41.788	-	-
Naphthalene, 1-isocyano	0.034	47.288	-	-
Neomenthol	5.942	31.312	-	-
p-Cymen-8-ol	0.028	44.875	-	-
p-Cymene	0.234	14.14	11.681	14.225
Pentane, 3-methyl-	0.019	17.949	-	-
Phellandrene	-	-	0.652	10.102
Piperitenone	0.083	48.158	-	-
Piperitone	0.705	38.127	-	-
Piperitone-oxide	0.039	50.025	-	-
Pulegone	2.245	34.258	-	-
Sabinene	-	-	0.508	11.608
Salvene	0.012	22.597	-	-
Spathulenol	-	-	0.124	58.015
Terpineol, Z-.beta.-	-	-	0.910	23.902
Trans-3-Methylcyclohexanol	0.041	23.286	-	-
Trans-Caryophyllene	0.422	31.005	-	-
Thymol	-	-	0.572	61.35
Zingiberene	-	-	0.056	25.266

-*: not determined.

4. Discussion

A. carbonarius contamination, found during the ripening process of grapes in vineyards in Mediterranean countries, is still a critical problem. Because *A. carbonarius* produces OTA, especially in grapes and products, it endangers human health and causes economic losses to producers. Synthetic pesticides with different chemical structures control this mould type and other OTA producers. However, these synthetic chemical fungicides negatively affect human and environmental health. Therefore, their use becomes limited (Dammak et al., 2019). In addition, fungal infection agents are increasing and becoming resistant to synthetic chemicals. This makes it difficult to control and treat. For this reason, researchers have focused on the antifungal activities of natural substances for various reasons, such as identifying natural and safe products as alternatives to synthetic chemical fungicides. EOs from plants have important biological (antibacterial and antifungal) activities and are a potentially helpful source of antifungal compounds (Moghaddam and Mehdizadeh, 2020). The study aimed to explore the potential of *MPEO* and *TVEO* as natural alternatives to synthetic fungicides for controlling *A. carbonarius*, a fungus that produces OTA.

The composition of EO is affected by numerous factors, such as the geographical conditions where the plant grows, abiotic and biotic factors during the growing phase, the age of the plant, the condition of the plant, which part of the plant is collected, the genotype of the plant, and the method of obtaining the EO. Therefore, before recommending an EO as a food preservative or alternative to drugs, issues such as the composition of EOs and the concentration of active ingredients need to be standardized. As a result, the chemical composition of the EO and the change in the concentration of the active ingredient will affect its biological activities. Thus, synergistic effects can be seen, as well as antagonistic effects (Dammak et al., 2019). In this study, *MPEO*'s main components were menthol (39.911%) and menthone (17.933%). In the literature, Moghaddam et al. (2013) reported that the main components of *MPEO* obtained from Tehran, Iran, were menthone (30.63%), menthol (25.16%), and

Beigi et al. (2018) also reported that the main components of MPEO obtained from Isfahan in Southwest Iran were found to be menthol (44.39%), menthone (15.36%). In addition, Camele et al. (2021) determined that the main components of *MPEO* obtained in Slovakia were menthol (70.08%) and menthone (14.49%). As a result, it was determined that the main components were the same as in the literature, but the percentage of presence changed. Also, the main component of *TVEO* was determined to be carvacrol (49.042%). It has a series of multicomponent chemotypes, including (1)linalool, (2)borneol, (3)geraniol, (4) sabinene hydrate, (5)thymol, (6)carvacrol based on the main components of *TVEO* (Satyal et al., 2016). Even Satyal et al. (2016) stated that *TVEO* has 20 different chemotypes through a cluster analysis based on 85 different TVEO compositions. Therefore, TVEO's main components vary widely.

Kostik et al. (2015) reported that MPEO, the main component of which is 34.3% menthol, gave 20 ± 2 and 28 ± 1 mm inhibition zones against *Aspergillus flavus* and *A. niger* isolates, and the MIC values were 115.4 ± 3.9 and 65.4 ± 3.1 µg ml^{-1}, respectively. Ambindei et al. (2017) reported that the main components of MPEO and TVEO were menthol (33.59%) and thymol (35.12%), respectively, and the MIC values against the *Aspergillus tamarii* isolate were 0.13 µl ml^{-1} and 0.33 µl ml^{-1}, respectively. Císarová et al. (2016) stated that TVEO, whose main component is 40±3% ρ-cymene, completely inhibited the growth of *A. niger* and *Aspergillus tubingensis* at a concentration of 0.625 µl cm^{-3}. The study determined that MPEO and TVEO gave an inhibition zone of 90.00 ± 0.01 mm for the ochratoxigenic *A.carbonarius* isolate, and the MIC and MFC values were 1 µl ml^{-1}. Additionally, MPEO and TVEO were found to completely inhibit radial growth at MIC, 2xMIC, and 4xMIC concentrations. Our study results are similar to the data in the literature. It is important to measure the antifungal activity of plant extracts, especially MIC. A lower MIC value means that a lower dose is needed to control the growth of foodborne fungi. This means that extracts with a lower MIC are more effective as antifungal agents. As a result, plant essential oil can be applied to both fields and stored crops to control fungal growth and mycotoxin production as a potential alternative to synthetic chemicals. Thus, food safety can be ensured to a large extent.

Conclusion

In conclusion, it is clearly seen from this study that the radial growth of the ochratoxigenic *A. carbonarius* isolate is completely inhibited at concentrations as low as 1 µl ml^{-1}, and accordingly, it provides inhibition of ochratoxin production. Mould species contaminated with foods and the mycotoxins they produce cause economic losses and endanger human and animal health. Therefore, it can be concluded that it can be used as an alternative and therapeutic agent to pesticides and synthetic food preservatives to limit mould contamination and mycotoxin formation in foods. In addition, there are a few limited studies in the literature on the growth of ochratoxigenic moulds and ochratoxin production inhibition. Therefore, the study will contribute to developing safer natural products and an effective strategy to control the growth of ochratoxigenic moulds and ochratoxin contamination, especially in foods.

Acknowledgment

In this study, the identification of the isolate was supported by Çanakkale Onsekiz Mart University Scientific Research Projects Coordination with the project number FBA-2019-3028 and the antifungal activity studies were supported by the project number THD-2021-3571. The analysis of the volatile chemical composition of essential oils was not supported. Regarding the use of culture, I would like to thank Dr. Nükhet Nilüfer ZORBA and Dr. Burhan ŞEN. I would like to thank Altın Toroslar A.Ş. (Adana, Türkiye) company for supplying *Mentha piperita* and *Thymus vulgaris* essential oils.

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Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

ISSN: 1308-7576 e-ISSN: 1308-7584

Review Article

Review of *Arnebia euchroma* as a Potential Medicinal Plant Based on Phytochemistry and Pharmacological Activity

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Article Info

Received: 29.05.2023
Accepted: 22.12.2023
Online published: 15.03.2024
DOI: 10.29133/yyutbd.1306035

Keywords

Chemical content,
Distribution of *A. euchroma,*
Pharmacological activities,
Traditional uses

Abstract: *Arnebia euchroma*, commonly known as "Pink Arnebia," is a plant from the Boraginaceae family found in Western and Central Asia. Traditionally, it has been used to treat respiratory, gastrointestinal, and dermatological ailments. Recent studies have highlighted its pharmacological properties and potential health advantages, resulting in increased interest in this plant. Pharmacognostic investigations have revealed the presence of various beneficial phytochemicals. Phytochemical studies have identified several bioactive compounds in *A. euchroma*, such as eugormoside A, eugormoside B, scopoletin, and β-sitosterol, which exhibit diverse biological activities like antioxidant, antimicrobial, anticancer, and anti-ulcer effects. The therapeutic potential of these bioactive compounds suggests that *A. euchroma* could be beneficial for a wide range of diseases. Pharmacological studies have validated the plant's healing properties, demonstrating its antimicrobial activity against various pathogens. Furthermore, *A. euchroma* extracts possess antioxidant and anti-inflammatory properties that can potentially mitigate oxidative stress and inflammation-related diseases. Other pharmacological actions of *A. euchroma* include wound healing, gastroprotective, hepatoprotective, and anti-diabetic effects. Overall, *A. euchroma* exhibits promise as a medicinal plant with significant health benefits. However, further research is required to identify the active compounds responsible for its pharmacological activity and elucidate their primary mechanisms of action. Additionally, clinical studies are necessary to assess its safety and efficacy when used therapeutically. The primary objective of this review is to showcase the phytochemical composition and traditional ethnopharmacological applications of *A. euchroma* worldwide. The study examines previous research concerning this plant, laying the foundation for a forward-looking perspective on the potential future of *A. euchroma.*

To Cite: Kazybekov, U, Kurmanbekova, G, Tornuk, F, 2024. Review of *Arnebia euchroma* as a Potential Medicinal Plant Based on Phytochemistry and Pharmacological Activity. *Yuzuncu Yil University Journal of Agricultural Sciences*, 34(1): 176-191.
DOI: https://doi.org/10.29133/yyutbd.1306035

1. Introduction

According to the fossil record, the relationship between humans and plants has lasted for centuries (Trüeb et al., 2020). Conventional medical systems are repositories of expertise in medicine, and their value is not limited to their historical aspects (Aldayarov et al., 2022). Plants play an indispensable role in these systems, and identifying their characteristics derived from old medical works could motivate us to develop innovative medicines (Fabricant et al., 2001; Kaya et al., 2019). *A. euchroma* is a perennial plant of the Boraginaceae group. It is widespread throughout the Mediterranean, Central Asia, and the Middle East (Shilov et al., 2022). For centuries, this herb has been used in conventional medicine to treat a variety of ailments, including respiratory issues, skin disorders, wounds, and inflammation (Jain et al., 2021). With the growing interest in natural products, *A. euchroma* is being explored by the scientific community as a prospective source of new drugs. The *A. euchroma* plant, or aerobic herb, has been demonstrated to treat burn wounds. According to surveys, naphthoquinone and additional substances are common in bivalve parents (Pirbalouti et al., 2014). These compounds, as well as their derivatives (alkanins and alkanes), exhibit a wide range of characteristics, such as moisturizing and antibacterial properties (Sasaki et al., 2002; Shen et al., 2002; Annan et al., 2008; Pirbalouti et al., 2014; Bali, 2015), antiviral (such as influenza and HIV), and anticancer (Nuorani et al., 2005; Zhang et al., 2017). Scientific studies have also shown that this plant possesses antibacterial and anti-inflammatory properties. Among the called naphthoquinones, shikonin serves as the primary active component (Papageorgiou et al., 1999; You et al., 2000; Singh et al., 2003; Malik et al., 2016). Shikonin and its derivatives exhibit substantial anticancer action, with the potential to inhibit cell growth and cause apoptosis in a variety of human cancers, including gastric cancer (Liang et al., 2016). In addition, it has antiviral, antioxidant, anti-inflammatory, and antifertility pharmacological properties and can be used in food supplements and cosmetics (Gao et al., 2011; Huang et al., 2018). The aforementioned dried roots of the plant are utilized in the pharmaceutical industry to extract natural chemicals. As a result of its medicinal properties, excessive consumption of this plant has led to a decline in its population and endangered status (Kala et al., 2000). Furthermore, it is not easy to cultivate such plants using conventional agricultural methods and thus cannot meet the increasing industrial demand (Gupta et al., 2014). Altogether, this article provides an up-to-date range of information on *A. euchroma* (taxonomy, plant characteristics, distribution, wide range of uses, bioactive composition, health effects, and technological potential) to enhance comprehension of the potential of this species across diverse domains. Biome and species conservation considerations will also be provided. This review aims to comprehensively describe and provide an up-to-date overview of *Arnebia euchroma* from multiple perspectives, including its taxonomy, plant characteristics, distribution, diverse uses, bioactive composition, health effects, and technological potential. Additionally, the review will address the conservation concerns related to the species and its habitat. The primary goal is to enhance understanding of the potential applications and significance of *A. euchroma* across various domains, spanning from traditional medicine to industrial applications.

2. Morphological Structure

Morphologically, *A. euchroma* has glabrous shoots, funnel-shaped corollas, terminal, subglobose inflorescences, and purple roots (Kumar et al., 2021). The roots of *A. euchroma* are thick and devoid of violet coloring. The stems of Euchroma are typically erect or spreading, having one or more branches. They have delicate hairs and are light yellow or white in hue. Leaves are linear, with long bristles, spiny to prevent the plant from being eaten. The flowers are multifloral, in rounded, racemose inflorescences with pinkish-purple tips that eventually become blackish-purple and have few stems. Blooms are heterogamous, bisexual, and insect-pollinated (Singh, 2010). Figure 1 shows the visual structure of the parts of the plant of *A. euchroma*. The calyx is thick and light golden in color, with both sides pubescent. The corolla is dark purple, bell-shaped, and often tinted with light yellow or red. Fruiting is infrequent due to self-sterility. The majority of seeds land around the plant's base and

germinate there, resulting in a thick clump of plants. Throughout June to August, flowers bloom, while seeds ripen from July to September.

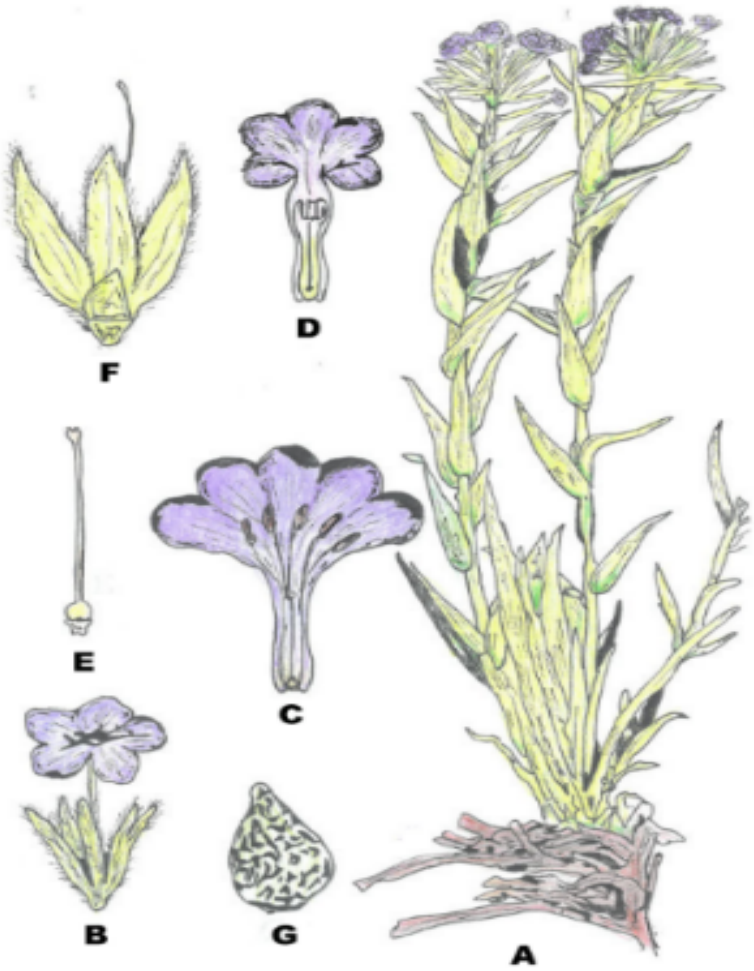

Figure 1. Illustration of the morphological description of *Arnebia euchroma*: (A. habit x 1/2, B. flower x 3, C. short styled flower 1.s x 3, D. long styled flower x 3, E. carpel x 6; F. fruit x 8, G. seed x 10).

3. Geographical Distribution of *A. euchroma*

A. euchroma, also known as "Red Root" or "Sappan Tree," is a plant species commonly found in the Himalayas and Naryn region, Kyrgyzstan. Its habitat preferences and distribution have been documented in several studies. According to a study published in the International Journal of Agriculture and Biology, *A. euchroma* grows in all regions of Kyrgyzstan, as well as in Naryn and Issyk-Kul oblasts on rocky slopes between 3 000 and 4 500 meters above sea level. It is also widespread in mountainous areas, including the Tien Shan and Pamir-Alai mountain ranges. It prefers well-drained soils with good sunlight exposure. The study also reports that this plant species is usually found in rocky and stony habitats, including slopes and grasslands (Li, 2015). Janibekov, (2017) reported that the plant is one of the medicinal plant species found in the flora of Kyrgyzstan. The study states that the plant species are found in different regions of Kyrgyzstan, including the Tien Shan and Alai mountain ranges. It is used in folk medicine for its anti-inflammatory and wound-healing properties (Janibekov, 2017). Akram, (2019) reported that it is commonly found in the subalpine and alpine regions of the Himalayas at an altitude of 2 000-4 500 meters above sea level. It prefers well-drained soils with good exposure to sunlight and is often found in rocky and rocky habitats, including slopes and grasslands. Another study published in the Journal of Ethnopharmacology reports that *A. euchroma* occurs wild in the northwestern region of Pakistan, where it grows on dry and rocky slopes in mountainous areas. The study also reports

that this plant species is commonly associated with other species of the same genus, such as *A. benthamii* and *A. hispidissima* (Bali, 2015).

In addition, a study published in the Journal of Medicinal Plant Research reports that *A. euchroma* is commonly found in the western Himalayas, including Afghanistan, India, Nepal, Pakistan, and Tibetan regions. It is found in various habitats, including forest edges, thickets, and grasslands (Hussain et al., 2012). Thus, *A. euchroma* is a plant species well adapted to rocky and scree habitats in the Himalayas and often found at high altitudes. Its distribution range extends to several countries and habitats in the region. In China, it is found in Xinjiang (East Turkistan) and the Sijiang region (autonomous region of Tibet). In India, the plant grows in Jammu and Kashmir, Himachal Pradesh, and Uttarakhand in the Transgimalaya region. In Pakistan, *A. euchroma* is found in the Karakorum mountain range, in the Batura Valley (mountain-steppe area) at altitudes between 2 600 and 3 900 meters above sea level, and in the Hindu Kush in the Swat region. In Iran, *A. euchroma* grows in rocky areas in the Zagros Mountains and the Caspian Mountains between 3 600 and 4 000-meters altitude (El-Keblawy et al., 2017). *A. euchroma* is also found in the Pamir mountains in Tajikistan, Afghanistan, Siberia (Russia), Kazakhstan, Nepal, Tibet (China), East Turkestan (Xinjiang, China), and Uzbekistan (Lee et al., 2017).

Figure 2. Distribution areas of *Arnebia euchroma* in the world.

4. Chemical Content

The most important and prevalent phytochemical components of *A. euchroma*, coumarins, shikonin, acetyl shikonin, iso-butyryl shikonin, -di-methyl acryl shikonin, isovaleryl shikonin, B-hydroxy isovaleryl shikonin, deoxy-shikonin, isobutyl-shikonin, arnebinone, arnebin-7, and stigma sterol are some examples, are recognized for their antibacterial properties, anticancer, and anti-immunodeficiency characteristics (Lin et al., 1980; Kashiwada et al., 1995; Jain et al., 2000; Samant et al., 2015). Additionally, Arnebia benthamii, a different species, is utilized in the treatment of cardiac and febrile illnesses (Dar et al., 2002; Ganie et al., 2012; Parray et al., 2015). Roots of *A. euchroma* contain various organic compounds such as naphthoquinone, arnebin-1, alkanin, ethyl-9-(2, 5-dihydroxyphenyl) nonanoate, iso-hexenyln-aphthazarin, octyl-ferulate, and butyryl-alkannin are anti-microbial, wound healing, and anti-tumor agents used in various medications (Liu et al., 2010; Ashkani-Esfahani et al., 2012; Hosseini et al., 2018; Cao et al., 2020).

Shikonin: The roots of the *A. euchroma* plant, which is extensively dispersed throughout South and Central Asia, are the primary source of the naphthoquinone chemical known as shikonin. Shikonin has been utilized in traditional medicine for millennia due to its wide range of medicinal characteristics, including its anti-inflammatory, antibacterial, and wound-healing capabilities. In-depth investigation of

shikonin and its derivatives' pharmacological properties has been done recently. By preventing the synthesis of cytokines that promote inflammation like TNF- and IL-6 and stimulating the production of nuclear factor-kappa B (NF-B), it has anti-inflammatory characteristics (Zhao et al., 2018). Shikonin also displays antimicrobial activity against various types of organisms, among them bacteria, fungi, and viruses (Zhou et al., 2017). Additionally, shikonin has been shown to have antitumor effects against a wide variety of cancer cell types, from lung cancer to breast and prostate cancer (Zhang, 2019). Research has also been conducted to explore the therapeutic potential of shikonin in addressing various skin conditions. Shikonin has been found to have significant wound-healing properties, promoting the growth and movement of skin cells, and increasing the production of collagen and other extracellular matrix components (Yin, 2017). Moreover, shikonin has been shown to exhibit therapeutic potential in the treatment of psoriasis, a chronic autoimmune disease characterized by skin inflammation and hyperproliferation. Shikonin has been shown to suppress the differentiation and proliferation of keratinocytes, which play a key role in the pathogenesis of psoriasis (Liu, 2019). The potential of shikonin in conjunction with other medicinal drugs has also been looked at in a number of research. For instance, it has been demonstrated that shikonin and chemotherapeutic drugs together can boost chemotherapy's anticancer effects (Huang et al., 2017). Shikonin has also been reported to increase the antibacterial action of several antibiotics, including erythromycin and tetracycline. Despite its pharmacological potential, the use of shikonin is limited as a result of its limited availability and low absorption. To overcome these limitations, various approaches, such as nanoparticle-based drug delivery systems, have been investigated to improve the therapeutic efficacy of shikonin (Zhou et al., 2017).

Organic acids: Studies have shown that *A. euchroma* contains several organic acids, including benzoic, cinnamic, caffeic, and ferulic acids. These acids are synthesized through the shikimate and phenylpropanoid pathways in the plant (Sing et al., 2015). Based on reports, the organic acids in *A. euchroma* exhibit a variety of pharmacological functions, which comprise antioxidative, antibacterial, anti-inflammatory, and anticancer actions. Benzoic acid is one of the most common organic acids found in *A. euchroma*. Studies have indicated its antimicrobial properties, anti-inflammatory, and antitumor activities (Zhang et al., 2014). In a study by Wanninger et al. (2018), benzoic acid has been shown to successfully stop the development of human cancerous cells in the intestines. Cinnamic acid is another organic acid found in *A. euchroma*, which has been reported to exhibit anti-inflammatory and antioxidant properties (Pandey et al., 2017). Caffeic acid, a well-known antioxidant and anti-inflammatory agent, has also been isolated from *A. euchroma*. It has been shown to possess anti-cancer properties against various types of cancer, such as lung, colon, and breast cancer (Singh et al., 2015; Zhang et al., 2014). Ferulic acid is another organic acid found in *A. euchroma*, which has been reported to exhibit antioxidant and anti-inflammatory properties (Pandey et al., 2017). In addition to their pharmacological properties, organic acids extracted from *A. euchroma* have also been used in the food and cosmetic industries. For example, benzoic acid is commonly used as a preservative in food products, while ferulic acid is used in cosmetic formulations due to its antioxidant properties (Singh et al., 2015).

Naphthoquinones: One of the major groups of compounds found in the plant are the naphthoquinones, which include alkannin, shikonin, acetylshikonin, and β,β-dimethylacrylshikonin. These compounds have received significant attention due to the possible pharmacological effects of shikonin encompass its potential to exhibit anti-inflammatory, antioxidative, antimicrobial, and anticancer characteristics. Alkannin and shikonin are two of the most extensively studied naphthoquinones in *A. euchroma*. Alkannin has been shown to exhibit anti-inflammatory and antioxidant activities, as well as wound-healing properties (Amanpour et al., 2015). In a study conducted on mice, alkannin was found to have significant anti-inflammatory effects, reducing the production of inflammatory cytokines and prostaglandins (Kim et al., 2019). Shikonin has also been shown to possess anti-inflammatory and antioxidant activities, as well as anticancer properties against various cancer cell lines (Liu et al., 2010). One study found that shikonin inhibited the proliferation of breast cancer cells by inducing cell cycle arrest and apoptosis (Sharma et al., 2020). Acetylshikonin and β,β-dimethylacrylshikonin are two other naphthoquinones that have received attention for their potential pharmacological activities. Acetylshikonin has been reported to have anti-inflammatory, antioxidant, and antiproliferative activities against cancer cells (Li, 2010 and 2015). β,β-dimethylacrylshikonin has been shown to have anticancer effects against lung cancer cells by inducing apoptosis (Lee et al., 2017). In addition to their pharmacological activities, naphthoquinones extracted from *A. euchroma* have also

been investigated for their potential use in various industries. Alkannin and shikonin have been used as natural dyes in the food, cosmetic, and textile industries (Zhao et al., 2018). Shikonin has also been used as a colorant in the pharmaceutical industry (Yang, 2016).

Phenolics: Numerous studies have demonstrated that *A. euchroma* contains several phenolic compounds, such as flavonoids, coumarins, and phenolic acids, which are synthesized via the phenylpropanoid pathway in the plant (Li, 2015). These phenolic compounds possess diverse pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and antitumor effects. Quercetin, kaempferol, and rutin are among the most abundant flavonoids found in *A. euchroma*, and they have been reported to exhibit antioxidant, anti-inflammatory, and antitumor properties (Hussain et al., 2019). Quercetin has been found to suppress breast cancer cell growth, while kaempferol has anti-tumor properties against different types of cancer, such as prostate, liver, and colon cancer (Choudhary et al., 2004). *A. euchroma* also contains coumarins such as scopoletin and esculetin, which exhibit antioxidant and anti-inflammatory properties (Li, 2015). Scopoletin has been demonstrated to have antimicrobial activity against various bacterial strains, including *Staphylococcus aureus* and *Escherichia coli* (Wang et al., 2019). Moreover, *A. euchroma* also contains phenolic acids such as gallic acid, caffeic acid, and ferulic acid, which possess antioxidant, anti-inflammatory, and antitumor activities (Hussain et al., 2019). Gallic acid has been found to inhibit breast cancer cell growth, while caffeic acid and ferulic acid have antitumor properties against various types of cancer. In addition to their pharmacological properties, phenolic compounds from *A. euchroma* have various applications in the food and cosmetic industries. For example, quercetin and kaempferol are commonly used as natural food colorants, while gallic acid and caffeic acid are employed in cosmetic formulations due to their antioxidant properties (Choudhary et al., 2004).

Table 1. Chemical compounds reported from *Arnebia euchroma*

№	Compound names	Part of plants	References
1	Shikonin	Roots	(Sankawa et al, 1977; Lin et al, 1980)
2	Acetyl-shikonin		Kashiwada et al, (1995)
3	β,β-Dimethylacrylshikonin		(Jain et al, 2000; Ganie et al, 2012)
4	Deoxyshikonin		
5	Isovalerylshikonin		(Andujar et al., 2002)
6	β-Hydroxyisovalerylshikonin		(Hosseini et al., 2018)
7	Arnebin-1		(Yang et al., 1992)
10	Arnebin-6		(Yuzbasioglu et al., 2020).
11	Isobutylshikonin		(Andujar et al., 2002; Parray et al., 2015).
12	Stigmasterol		(Liu et al., 2010)
13	Teracrylalkannin		(Yuzbasioglu et al., 2020)
14	Tormentic acid		
15	Triterpenic acids		
16	2α-Hydroxyursolic acid		
17	Naphthoquinone	Roots bark, flower and leaves	(Yang et al., 1992; Roeder et al., 1993; Srivastava et al., 1999; Singh et al., 2001)
18	Tormentic chemical		
19	Octylferulate		
20	Butyrylalkannin		
21	Isohexenylnaphthazarin		
22	O^7– Angeloylretronecine		
23	O^9– Angeloylretronecine		
26	Alkaloids	Roots bark, flower and leaves	(Liu et al., 2010; Ashkani-Esfahani et al., 2012; Sharma et al, 2020).
27	Pyrrolizidine		
28	2α-Hydroxyursolic acid		
29	O^7- Angeloylretronecine	Roots bark, flower and leaves	(Yang et al, 1992; Roeder et al, 1993; Srivastava et al, 1999; Singh et al, 2001)
30	Betulin, lupeol		
31	Pyrrolizidine		
33	β-Amyrin acetate		

Table 1. Chemical compounds reported from *Arnebia euchroma* (continued)

34	Butyryl-alkannin		
35	Octyl-ferulate		
36	Copsamine		
37	Ethyl 9-(2,5-dihydroxyphenyl) nonanoate		
38	Lycopsamine		
39	Tetrabenzoylalkannin		
40	β-Methylanthracene	Roots bark, flower and leaves	(Liu et al., 2010; Ashkani-Esfahani et al., 2012)
41	Tetrabromoalkannin		
42	Dimethoxyalkannin		
43	Dicarboxyalkannin	Roots bark, flower and leaves	(Ashkani-Esfahani et al., 2012)
44	Naphthoquinones, alkannin		
45	β-Acetoxyisovalerylalkannin	Roots bark, flower and leaves	(Liao et al., 2020)
46	Isovalerylalkannin dimer		
47	Isovalerylalkannin		
48	β-Acetoxyisovalerylalkannin dimer		
49	2,3-Secodiplopterol dioic acid	Roots	(Cao et al., 2020)
50	Shikometabolin H		

5. Traditional Uses

A. euchroma has been employed in Central Asia, as well as in Ayurvedic and herbal medicine for an extended duration. It is valuable in managing nausea and vomiting, specifically in cases of motion sickness and hyperemesis gravidarum.

Use in Kyrgyzstan: *Arnebia euchroma*, known by its common name "Boyochu Endik," has a long history of traditional use in Kyrgyzstan, where it holds both cultural and medicinal significance. Found in various parts of the country, this versatile plant has been an integral part of Kyrgyz traditional medicine for centuries, with its roots being a key component of many remedies. One prominent use of *A. euchroma* in traditional medicine is in the treatment of digestive ailments. Infusions or decoctions made from the plant's roots are commonly consumed to alleviate indigestion, bloating, and general discomfort after meals. It is also used to manage various stomach-related issues, including gastritis and gastralgia, as it is believed to have soothing properties for the gastrointestinal tract (Israili, 2009). In Kyrgyzstan's cold climate, respiratory ailments are common, and *A. euchroma* plays a role in addressing these health concerns. Traditional preparations often include *A. euchroma* to alleviate symptoms of common respiratory infections such as coughs and colds. This is attributed to its potential anti-inflammatory and antimicrobial properties that are beneficial for respiratory health. *A. euchroma* is highly regarded for its potential to treat a variety of skin issues in Kyrgyz traditional medicine, particularly skin conditions like eczema and dermatitis. The roots of the plant are prized for their skin-healing properties, and ointments and poultices made from these roots are applied topically to soothe irritated skin. Traditional healers also utilize *A. euchroma* to accelerate the healing process of minor wounds, cuts, and burns, given its effectiveness in reducing inflammation and promoting tissue regeneration (Uysal, 2018). Beyond its medicinal uses, *A. euchroma* finds its way into Kyrgyz cosmetics. The plant's soothing and healing properties make it a suitable ingredient in skincare products, including creams and lotions. These products are employed to maintain healthy and radiant skin. Moreover, one of the most renowned traditional uses of *A. euchroma* in Kyrgyzstan is its role as a natural dye. The roots of the plant are used to create natural dyes that impart a range of red and purple hues to textiles. This age-old dyeing tradition has left an indelible mark on the vibrant colors of traditional Kyrgyz clothing and textiles (Bazarbayev, 2020). Currently, some regions of Central Asia, including Kyrgyzstan, are experiencing excessive sales of rootstocks to neighboring countries and depletion of stocks. This raises concerns about the sustainability of plant utilization (Sujatha, 2016). To address these concerns, several efforts have been made to promote sustainable cultivation and conservation of *A. euchroma* in Kyrgyzstan. For example, a study conducted by scientists from Kyrgyzstan and Germany

investigated the optimal conditions for the cultivation of this plant in the country. Other initiatives aim to promote the use of alternative natural dyes to reduce the burden of *A. euchroma* (Baktybekov, 2017).

Use in Xinjiang, East Turkistan (China): *Arnebia euchroma,* renowned for its medicinal properties, is an integral component of Traditional Chinese Medicine (TCM), a holistic approach that harnesses natural substances for therapeutic purposes. TCM embraces the versatile capabilities of *A. euchroma,* particularly in the realms of skin health and wound care. It stands as a potent remedy for various skin conditions, effectively soothing irritations caused by allergies, insect bites, and contact dermatitis. The application of ointments or creams infused with *A. euchroma* extracts brings much-needed relief (Li, 2015). Moreover, *A. euchroma* extends its healing potential to the treatment of minor burns, with its anti-inflammatory attributes playing a pivotal role in reducing inflammation and promoting the healing process of burn wounds. Traditional practitioners also employ this botanical wonder to combat skin infections, including bacterial and fungal maladies, through topical applications on affected areas. Expanding its horizons, *A. euchroma* assumes a prominent role in addressing respiratory issues within the realm of TCM. Traditional preparations seamlessly incorporate the plant to alleviate the discomfort of common respiratory infections such as coughs and bronchitis. This therapeutic prowess is attributed to the plant's potential anti-inflammatory and antimicrobial properties, enhancing its effectiveness in respiratory care (Zhang, 2019). Beyond its pivotal role in Traditional Chinese Medicine, *A. euchroma* makes a remarkable entrance into the world of Chinese cosmetics. The plant's soothing and rejuvenating attributes make it a prized ingredient in skincare products like creams, lotions, and serums. These formulations harness *A. euchroma* extracts to amplify their efficacy, contributing to the attainment of healthy and radiant skin. In the tapestry of traditional Chinese crafts, *A. euchroma* weaves a vibrant thread into the fabric of textile and fabric dyeing traditions. The roots of this botanical gem serve as the source of natural dyes, bestowing textiles with rich hues of red and purple. This dyeing tradition, deeply entrenched in Chinese culture and craftsmanship, exerts a profound influence on the colors and intricate patterns adorning traditional Chinese clothing, particularly in regions like Tibet (Li, 2015). However, the unrelenting over-harvesting and depletion of *A. euchroma* in its natural habitat have cast a shadow of concern over its sustainability. In response, dedicated efforts have been undertaken to champion the cause of sustainable cultivation and conservation, seeking to redress the challenges stemming from over-harvesting and habitat loss (Wang, 2019).

Use in Iranian traditional medicine: Skin Conditions: *A. euchroma* has been traditionally used in Iran to treat various skin conditions, including eczema and psoriasis. A study published in the Journal of Ethnopharmacology found that *A. euchrom* root extract has potent anti-inflammatory and wound-healing properties that may support its traditional use in these conditions (Rustaiyan et al., 2001).

Respiratory ailments: *A. euchroma* has been traditionally used to treat respiratory diseases, including cough and asthma. A study published in the Journal of Ethnopharmacology found that plant root extract has significant bronchodilator activity, which may support its traditional use in these conditions (Monsef-Esfahani et al., 2004), Digestive Disorders: *A. euchroma* is traditionally used in Iran to treat digestive disorders such as diarrhea and dysentery. A study published in the Journal of Medicinal Plants Research found that *A. euchroma* root extract has significant anti-inflammatory and antispasmodic effects on the digestive system, which may support its traditional use in these conditions (Tavakoli et al., 2010). Pain Relief: *A. euchroma* has been traditionally used for pain relief, including joint pain and headache. A study published in the Journal of Medicinal Plants Research found that *A. euchroma* root extract has significant analgesic and anti-inflammatory effects that may support its traditional use for pain relief (Saeedi et al., 2011)

Use in traditional Indian medicine: *A. euchroma,* also known as "Lal Jari" in Hindi, has a long history of use in traditional Indian medicine, also known as Ayurveda. Here are some examples of its traditional use in Ayurveda and links to supporting studies: *A. euchroma* has been traditionally used in Ayurveda to treat various skin conditions, including wounds, burns, and eczema. A study published in the Journal of Ethnopharmacology found that *A. euchroma* root extract has potent wound healing and anti-inflammatory properties that may support its traditional use in these conditions (Saeedi et al., 2001). *A. euchroma* is also traditionally used in Ayurveda to treat respiratory ailments such as cough and asthma. A study published in the Journal of Ethnopharmacology found that *A. euchroma* root extract has significant bronchodilator activity, which may support its traditional use in these conditions

(Monsef-Esfahani et al., 2004). *A. euchroma* has traditionally been used in Ayurveda to treat gastrointestinal disorders such as diarrhea, dysentery, and stomach ulcers. A study published in the Journal of Ethnopharmacology found that *A. euchroma* root extract has significant anti-inflammatory and anti-ulcer effects on the digestive system, which may support its traditional use in these conditions (Husain et al., 2001). *A. euchroma* is also traditionally used as an antimicrobial agent in Ayurveda. A study published in the Journal of Ethnopharmacology showed that *A. euchroma* root extract has significant antimicrobial activity against various bacteria and fungi, which may support its traditional use for these purposes (Singh et al., 2006). *A. euchroma* is traditionally used as an anti-inflammatory agent in Ayurveda. A study published in the Journal of Ethnopharmacology found that *A. euchroma* root extract has significant anti-inflammatory effects that may support its traditional use in various inflammatory conditions. These studies suggest that *A. euchroma* has potential medicinal properties supporting its traditional Ayurveda use. However, further research is needed to fully understand the therapeutic effects of this plant and its active compounds.

6. Pharmacological Activities

A. euchroma is a medicinal plant that has been traditionally used for various ailments. It is known for its pharmacological activities such as anti-inflammatory, antioxidant, antibacterial, wound healing, antitumor, and hepatoprotective activities. *A. euchroma* contains several bioactive compounds such as shikonin, alkannin, phenolic compounds, and flavonoids, which contribute to its various pharmacological activities. These compounds have been studied in vitro and in animal models, and have shown promising results in treating inflammation, oxidative stress, bacterial infections, wound healing, cancer, and liver diseases.

Wound healing: *A. euchroma* has traditionally been used as a wound-healing agent. Studies have shown that *A. euchroma* root extract has pronounced wound-healing properties, accelerating wound-healing and promoting tissue regeneration (Yagn et al., 2021).

Anti-inflammatory and analgesic: *A. euchroma* has been reported to have anti-inflammatory and analgesic properties. Studies have shown that *A. euchroma* root extract has significant anti-inflammatory and analgesic effects (Ali, 2007).

Antibacterial: *A. euchroma* has potent antibacterial activity against a range of bacteria. The root extract is effective against Gram-positive and Gram-negative bacteria, making it a potential alternative to conventional antibiotics (Ali, 2007).

Antifungal activity: *A. euchroma* has been found to have antifungal activity against several fungal pathogens, including *Candida albicans* and *Aspergillus niger*. The root extract has been shown to inhibit the growth of these fungi, making it a potential alternative to conventional antifungal drugs (Mirage et al., 2019).

Antioxidant activity: *A. euchroma* is rich in phenolic compounds and is reported to have antioxidant properties. Studies have shown that *A. euchroma* root extract has significant antioxidant activity that may help prevent diseases associated with oxidative stress (Mirage et al., 2019).

Dermatological Uses: *A. euchroma* is used in traditional medicine to treat various skin conditions such as psoriasis, eczema, and dermatitis. The root extract is used in topical formulations to treat burns, wounds, and skin infections (Singh et al., 2003)

Anticancer activity: *A. euchroma* has been found to exhibit anticancer properties due to naphthoquinone compounds such as shikonin and alkannin. These compounds have been shown to inhibit the growth of various cancer cells, including breast, lung, and colon cancer cells (Yang, 2021).

Hepatoprotective: *A. euchroma* has been reported to have hepatoprotective properties and protect the liver from damage caused by various toxins and drugs. The root extract has been shown to reduce liver damage caused by carbon tetrachloride, paracetamol, and other hepatotoxic agents in animals (Mirage et al., 2019).

Neuroprotective: *A. euchroma* has been found to exhibit neuroprotective properties due to shikonin and other compounds. These compounds have been shown to protect neurons from damage caused by oxidative stress, inflammation, and other neurotoxic effects (Yang, 2021).

Anti-diabetic: *A. euchroma* is used in traditional medicine to treat diabetes. The root extract has been found to have significant anti-diabetic activity, lowering blood glucose levels and improving glucose tolerance in animal models (Khan, 2015).

Anti-ulcer properties: *A. euchroma* is reported to have anti-ulcer properties. The root extract has been shown to protect the gastric mucosa from ulceration caused by various factors such as alcohol, stress, and non-steroidal anti-inflammatory drugs (NSAIDs) (Sujhata et al., 2016).

Anti-arthritic activity: *A. euchroma* has been reported to have anti-arthritic properties. Studies have shown that *A. euchroma* root extract has significant anti-arthritic effects by reducing inflammation and joint destruction in animal models of arthritis (Yang, 2021).

Antitumor: *A. euchroma* has been found to have potential antitumor activity. Studies have shown that *A. euchroma* root extract has a cytotoxic effect on cancer cell lines, including lung, liver, and breast cancer (Yang, 2021).

Anti-aging properties: *A. euchroma* is reported to have anti-aging properties. Studies have shown that *A. euchroma* root extract has a significant anti-aging effect that reduces oxidative stress and improves skin elasticity (Mirage et al., 2019).

Gastrointestinal Disorders: *A. euchroma* is used in traditional medicine to treat gastrointestinal disorders such as diarrhea and dysentery. The root extract is used in oral preparations to treat these conditions (Singh et al., 2001).

Cardiovascular health: *A. euchroma* is reported to have heart-protective properties, including lowering blood pressure and cholesterol levels. The root extract has been shown to improve heart function and reduce oxidative stress in animal models of cardiovascular disease (Yang, 2021).

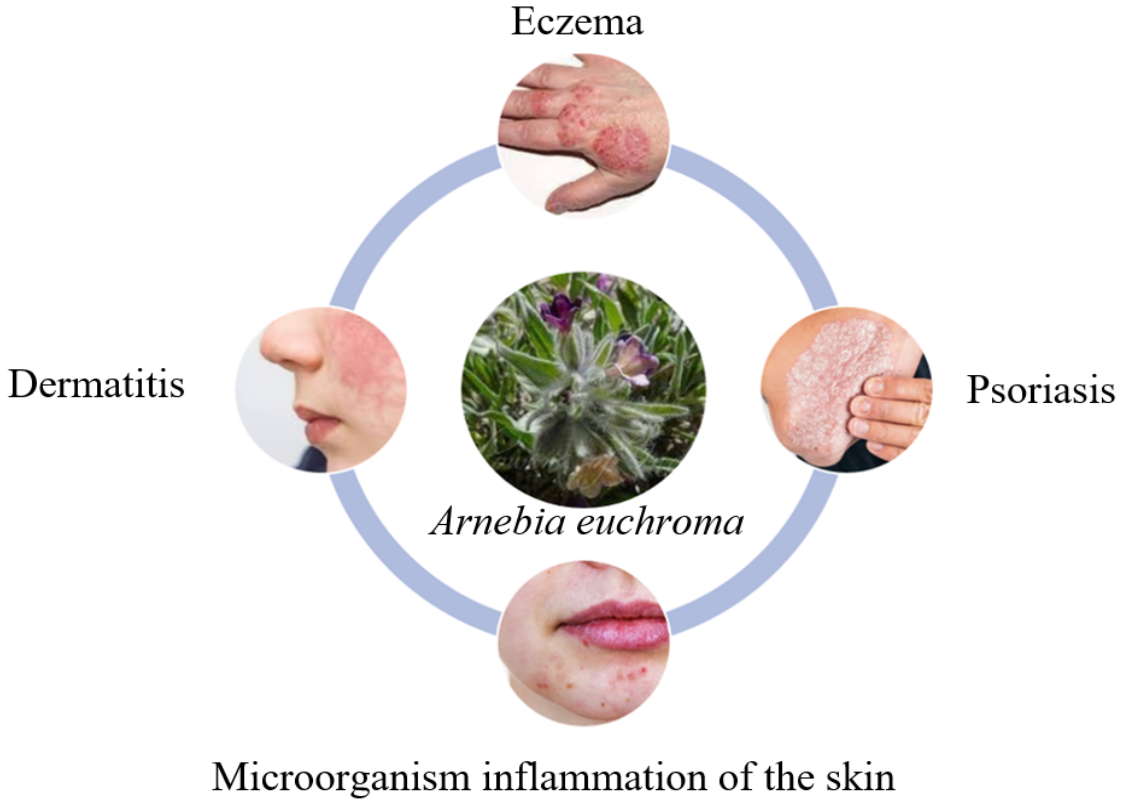

Figure 3. Relevance of the roots of *Arnebia euchroma* for the treatment of skin diseases.

7. Future Considerations

The current investigation aims to elucidate the inherited knowledge of *A. euchroma*. There is a need for extensive research and awareness in the Naryn region and neighboring regions in Kyrgyzstan. Although the foliage and roots of *A. euchroma* are frequently applied in ethno-medicine treatments, information on the formulations and usage of such medicinal plants is limited to books and a handful of regional practitioners, highlighting the urgent need to explore these traditional practices among the younger generation. Because root harvesting is a damaging method, it is critical to protect these therapeutic plants from over-exploitation. While some aspects of ethno-medicine science have been studied, more work needs to be done from various perspectives, such as corona and cancer treatment, dose administration, antioxidants, geo-tagging, metabolomics, bioinformatics, genomics, proteomics, and data-based studies. Studies on secondary metabolites, including phenolic compounds, need to be conducted using in vitro methods for animal issues. The majority of the study focused on the pharmacological and therapeutic applications of *A. euchroma*; however, the biotechnological features of the medicinal plant, such as cell culture, were not well examined. The natural surroundings of *A. euchroma* in Naryn are situated in the At-Bashy regions (Naryn), requiring care for commerce and numerous reasons. Using scientific tools and conventional methods, forest agencies, research institutions, and non-governmental organizations (NGOs) should collaborate to protect *A. euchroma* and its natural locations. To enhance the competitiveness of domestic *A. euchroma* production, it is crucial to strive for high-quality *A. euchroma* products with a high yield in the production-to-consumption chain. Working with wide genetic variation in breeding programs is effective in developing new varieties, and hybridization between different subspecies can create broad variation. Crossbreeding the *A. euchroma* plant with commercial varieties can produce high-yielding commercial varieties that are more resistant to disease and stress conditions such as cold tolerance. Preventing yield losses due to stressors of commercial varieties can increase the yield of *A. euchroma*, even at low levels, which can significantly increase the amount of product produced. This increase in yield will boost our country's exports abroad.

Conclusion

In conclusion, this literature review on the phytochemistry and pharmacological activity of *Arnebia euchroma* shows the profound importance of this plant in Central Asian countries. The traditional use of this plant for a wide range of health problems has been confirmed by scientific studies, which have revealed a whole clade of biologically active compounds with significant therapeutic potential. The significance of *A. euchroma* in Central Asian countries cannot be overstated. Its historical role as a remedy for respiratory, gastrointestinal, and dermatological ailments has been enriched by the discovery of phytochemicals such as flavonoids, tannins, alkaloids, saponins, and phenolic compounds. These compounds exhibit diverse pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, and even anticancer effects, aligning with the plant's traditional applications. *A. euchroma*'s relevance extends beyond its medicinal uses. It plays a crucial role in the cultural fabric of Central Asian societies and contributes to textile dyeing traditions, imparting vibrant shades of red and purple to fabrics. Its cultural significance, particularly in ceremonial and symbolic contexts, adds a layer of value to this botanical treasure. However, the over-harvesting and depletion of *A. euchroma* in its natural habitat raise serious concerns about its sustainability. To address these challenges, it is imperative to explore conservation measures employing biotechnological methods. Initiatives aimed at sustainable cultivation and preservation of the plant's genetic diversity are vital to ensure its continued availability for traditional practices and scientific research. Encouraging and promoting the sustainable cultivation of *A. euchroma* in controlled environments can help meet the demand for this valuable plant while reducing pressure on wild populations. Conservation efforts should prioritize the preservation of the plant's genetic diversity. This can be achieved through the establishment of germplasm banks and genetic resource management programs. Investing in biotechnological research can lead to the development of propagation methods, tissue culture techniques, and genetic improvement strategies that enhance the plant's resilience and productivity. Involving local communities in conservation efforts can create a sense of ownership and responsibility, fostering a collaborative approach to the sustainable management of *A. euchroma*. Enforcing and strengthening legislative protections for the plant and its

natural habitat is crucial to prevent over-exploitation and habitat loss. *A. euchroma* is not just a botanical species but a cultural and medicinal heritage deeply rooted in different parts of the world. Its traditional uses have found validation in modern scientific research, revealing a reservoir of potential therapeutic benefits. To ensure the continued availability of this precious resource, it is imperative to embrace conservation practices that combine traditional wisdom with biotechnological advancements. This holistic approach will safeguard *A. euchroma* for generations to come, preserving its rich legacy and offering hope for the development of novel medicinal remedies.

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Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

ISSN: 1308-7576 e-ISSN: 1308-7584

Research Article

Turkish Consumers' Purchase Motivation towards Erzurum Stuffed-Kadayif with Protected Geographical Indication (PGI) at the Dessert Retailers

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Article Info

Received: 20.07.2023
Accepted: 13.11.2023
Online published: 15.12.2023
DOI: 10.29133/yyutbd.1330521

Keywords

Cluster analysis,
Erzurum stuffed-kadayif,
Etnocentrism,
Exploratory factor analysis,
Purchase motivation

Abstract: It was planned to determine the main factors affecting Erzurum Stuffed-kadayif purchase motivation of Turkish consumers in the study. The main material of the research was obtained from 385 households residing in Erzurum, Türkiye. Exploratory Factor Analysis and Two-step Cluster Analysis were used to explore Turkish consumers' Erzurum Stuffed-kadayif purchase motivation at the dessert retailers. The results of the research highlighted that consumers consuming this product at the local restaurants were satisfied highly with the food images under cultural integration. On the other hand, those consuming this dessert at the local patisseries also attituded a big importance to the entrocentrism approach based on cultural integration. Similarly, consumers purchasing Erzurum Stuffed-kadayif as a ready-made local dessert from local manufacturer vendors tried to contribute considerably to sustainable food supply and consumption with an entrocentrism approach under cultural integration. It should be improved appropriate positioning and segmentation strategies according to the purchase motivation of each consumer segment, and then they should be implemented by policy makers.

To Cite: Topcu, Y, 2023. Turkish Consumers' Purchase Motivation towards Erzurum Stuffed-Kadayif with Protected Geographical Indication (PGI) at the Dessert Retailers. *Yuzuncu Yil University Journal of Agricultural Sciences*, 33(4): 717-728.
DOI: https://doi.org/10.29133/yyutbd.1330521

1. Introduction

In recent years, there has considerably maintained a change in consumers' food consumption preferences and purchase motivations under the negative effects of global climate change due to lower yield and quality attributes suffered in plant and livestock products, biodiversity losses, possible risk factors on food safety and security at food life cycle from the farms to the retailer shelves, negative consumer perceptions about emotional food quality attributes, as well as negative impacts on human health and the environment (Bernabeu et al., 2023; Bouranta et al., 2023; Mesias et al., 2023).

Under the impact of the Covid-19 pandemic and the Ukraine and Russia war along with the negative effects of climate change, the production of wheat being the main raw material of stuffed-kadayif has considerably decreased for the last few years in the world and Türkiye. As considered global wheat supply and demand trends, while global wheat production and stocks decreased from 764 and 284 million tons in 2019 to 769 and 271 million tons in 2022, wheat consumption increased from 741 million tons to 782 million tons (TEPGE, 2022). In response to the decreases in both global wheat production

and current stocks, a significant increase in global wheat consumption was also observed in view of the trend figures. Consequently, this situation has indicated the existence of a serious problem in meeting consumer demands of wheat supply worldwide and a large supply gap in the future if the necessary preventive and adaptation studies are not carried out to an adequate extent.

Wheat production in Türkiye was 19.00, 17.65, and 19.80 million tons in 2019, 2021, and 2022 respectively, whereas domestic wheat consumption was given as 20.00, 19.01, and 19.00 million tons (TEPGE, 2022). In particular, it abnormally caused product prices to increase with the effects of panic buying by narrowing the supplies of wheat and bakery products under the negative impacts of ongoing climate change and the Covid-19 pandemic hitting 2019 (Arafat et al., 2021). Indeed, while the average annual wheat price was ₺1.5 kg^{-1} in 2018, it increased to about ₺5.5 kg^{-1} in 2022 (PTB, 2022). The dramatic increases in wheat prices at commodity markets caused wheat flour prices to trade from ₺1.76 kg^{-1} in 2018 to increase by ₺7.7 kg^{-1} in 2022 (PTB, 2022a). Manufacturing cost increase resulting from excessive rises in the prices of Stuffed-kadayif ingredients such as sugar, walnuts, pistachios, and hazelnuts, along with the price of the flour being the main input of Erzurum Stuffed-kadayif, therefore, caused the price per kg to rise from ₺15 ($2) in 2019 to ₺140 ($7) in 2023.

On the other hand, besides the natural risk factors having a negative impact on agriculture and the agricultural food industry, when the macroeconomic data are taken into consideration for 2022-2023 years in Türkiye, the consumer price index (CPI) and food price increases (food inflation) were annually realized as 50.51 and 67.89% (TUIK, 2023). The annual increases in the producer price index (PPI) and food input prices were calculated as 62.45% and 88.38%, respectively (TUIK, 2023a). The pressures of these inflationary and natural risk factors have todays caused the food prices to increase dramatically with the contraction in the economy by increasing the production costs, and then the formation of social welfare losses created by the contraction in demand resulting from the real decline in consumer incomes. This situation has indeed caused an excessive increase in the share of consumer incomes allocated to mandatory food needs in the expenditure budget, and thus their consumption motivations have also changed considerably depending on the marketing mix.

It was reported that consumers' psychographic factors on their food consumption motivation had a much greater impact than their socioeconomic ones such as gender, age, education, and profession on their attitudes and behaviors patterns (Graham and Ambrahamse, 2017; Harguess et al., 2020). Consumers' individual factors, therefore, (attitude and value, knowledge and skill, emotion and cognitive level, taste and flavour, demographic factors), their sociocultural attributes (culture and belief, social norm and status), and the external factors (political and economic factors related to food marketing environments) must be assessed rationally how their food purchase motivations are impacted under current conditions (Chen and Antonelli, 2020; Harguess et al., 2020). Therefore, consumers trying to meet their food needs under the effects of climate change have rationally tried to shape their food choices and purchase motivations at retail levels by taking into account not only the hedonic and sensory food attributes but also the negative progressions in the Turkish economy in the last years.

It was reported in the prior researches that it was firstly attempted to determine consumers' purchase motivations by taking into account the hedonic food attributes, a part of the marketing mix focused on consumers' visual sense (price, brand, labeling, package weight, and size, geographical indications, purchase convenience, reaching to retailers, conformity and comfort at retail stores, health claims) (Edenbrandt and Nordström, 2023; Fakhreddine and Sanchez, 2023; Petrontino et al., 2023; Yeh and Hirsch, 2023; Zanchini et al., 2023; Zeng et al., 2023), and then the sensorial food attributes based on a variation of the nutritional composition at farming and manufacturing process (taste, aroma, flavor, colour, texture, appearance, sound, content or ingredient, juiciness, sweetness) (Bejaei and Xu, 2023; Fakreddine and Sanchez, 2023; Giannoutsos et al., 2023; Kleih et al. 2023; Lavui et al., 2023) impacting on their purchase models at retail levels.

Especially, when making consumers' food purchase decisions based on their hedonic experience perceptions, it was emphasized that they make purchasing decisions to a large extent by taking into account the marketing mix such as the region of origin and prices (Topcu and Çavdar, 2022; Bernabeu et al., 2023; Chaffee and Ross, 2023), the food brands and their communication tolls (Bernabeu et al., 2023), food packaging and label knowledge (Chaffee and Ross, 2023) and the retailers and their positioning strategies (Bytyqi et al., 2023; Curutchet et al., 2023; Seo and Kim, 2023).

In these studies based on consumers' food purchasing motivations, it was pointed out that the extrinsic/hedonic food attributes were the major determinants of their purchase motivation at the food

retailers, and also provided vital information about their socioeconomic attributes. Similarly, it was also reported that there were much stronger interactions between the intrinsic/sensory food attributes and hedonic/extrinsic ones on consumers' purchase motivations.

On the other hand, differentiated product types of traditional food products registered by PGI and manufactured by traditional production models are heavily preferred by target consumer mases. Because they are not exposed to an intensive manufacturing process and the chemical pollutants creating a negative impact on human health and the environment. Similarly, it was also reported that the factors such as the high sensory quality and core benefit attributes of the food products with PGI, the use of natural inputs free of chemical additives and preservatives with the region of origin, their traceability and sustainability at manufacturing process, the ethnocentrism approach contributing to regional and rural development affected positively consumers' purchase motivation (Sanchez-Bravo et al., 2020; Devia et al., 2021; Rahman et al., 2021; Topcu and Çavdar, 2022).

Within the scope of the current research, the extrinsic and intrinsic food motives impacting consumers' consumption preferences and purchase decisions towards Erzurum Stuffed-kadayif could considerably shape their purchase patterns. In this context, the aim of the study is to determine consumers' purchase motivations based on the intrinsic and extrinsic food motives for Erzurum Stuffed-kadayif with protected geographical indication bought from the food retailers in Erzurum and then to create customer-oriented marketing strategies for each consumer segment.

2. Material and Methods

2.1. Material

The main material of the study consisted of primary data obtained from face-to-face questionnaires conducted with the households in Erzurum; Yakutiye, Aziziye, and Palandöken Central Districts, consuming Erzurum Stuffed-kadayif with PGI in 2019 by taking into consideration the questionnaire form approved by Ataturk University Ethics Committee with 2021/14 number. In addition to primary data, secondary data were obtained from the data of various statistical institutions and organizations (TUIK, FAO, Erzurum Chamber of Commerce, Commodity Exchanges), as well as domestic and foreign scientific research project reports and article findings and results.

2.2. Methods

2.2.1. Method used to determine the sample size

In order to ensure the homogenous participation of the households consuming Erzurum Stuffed-kadayif in Erzurum, the city was divided into three central districts; Yakutiye, Aziziye, and Palandöken (44.325, 14.818, and 38.674 households), respectively and then the sample size in Equation 1 was calculated with the Simple Random Sampling Method (Malhotra, 1993).

$$n = \frac{Z^2 \cdot p \cdot (1-p)}{c^2} = \frac{1.96^2 \cdot 0.05 \cdot (0.05)}{0.05^2} = 385 \quad (1)$$

In Equation 1,
n: Sample size
Z: Standardized Z value (at 95% confidence interval, 1.96)
p: Erzurum Stuffed-kadayif consumption probability (0.50)
c: Error term (0.05 = ±5)

The survey numbers under the proportional techniques were calculated as 175 in Yakutiye, 58 in Aziziye, and 152 in Palandöken, and a total of 385 in Erzurum by taking into account the sample size and the number of households in each district.

2.2.2. Method used for preparation of questionnaire forms

In order to design the attitude scale related to the intrinsic and extrinsic food attributes that determine consumers' purchase motivation consuming Erzurum Stuffed-kadayif in Erzurum were utilized from the domestic and foreign studies related to the research scope and context. The scale was

firstly designed with 43 marketing mix attributes (product mix: 25 items, price mix: 6 items, communication mix: 3 items, distribution mix: 9 items) impacting on their Erzurum Stuffed-kadayif purchase decisions, it was asked consumers participated in the survey to mark each statement on the attitude scales with 5-point Likert Scale (1: no important, 3: neutral/undecided, 5: very important) allowing consumers' attitudes to be perceived more accurately at the scale dimension (Kotler and Amstrong, 2018).

2.2.3. Methods used in statistics analyses

In the first step, Explanatory Factor Analysis (EFA) due to having not applied any approved research scale was used to determine the main factors impacting their Erzurum Stuffed-kadayif purchase motivation (Hair et al., 2013). The EFA is a multivariate statistical dimension reduction technique trying to create a small number of unrelated, but conceptually meaningful new factors (Bursal, 2019; Civelek, 2020). Hierarchical steps for the EFA were followed to test the suitability of the data, determine the main factor number, perform the rotation (transformation) techniques, identify main factors, and calculate the explained and cumulative variances for each factor dimension, respectively.

In order to investigate the data suitability of the sample mass according to the main population for the EFA, Kaiser-Meyer-Olkin (KMO) and Bartlett's test of Sphericity were used in the research. KMO, the adequacy criterion of the sample size should be in an acceptable confidence interval (between 0.50 and 1.00). On the other hand, the correlation matrix should be different from the unit matrix in Bartlett's test of Sphericity explaining the relationship among the variables depending on the correlation matrix calculated between each pair of variables.

Whereas determining the main factor number with the EFA used Maximum Likelihood (ML) extraction method in the study, the factors with Eigenvalues greater than 1 or equal to 1 were statistically taken into consideration. The rotation technique was also used to be able to give the factor names and eliminate the variable overlaps in factor matrices. In the rotation process, the factors in the axes are rotated so that reducing the variable loads to optimal levels. Rotation could be applied in two groups as vertical (orthogonal) and oblique rotation. While it could be minimized the relationships among the factor dimensions at vertical rotation, it could be accepted the relative relations among them at oblique rotation. It is often used the varimax, quartimax, and equamax methods for vertical rotation techniques, however, it is generally used direct oblimin and promax methods for oblique rotation ones. In this study, therefore, it was applied the vertical rotation technique and its varimax method to minimize the relationships among the factors.

On the other hand, to retain and select the items under each factor dimension on a rotated component matrix in the EFA, the factor loads with a range of 0.30 and 0.50 scores are generally accepted for the cut-off threshold of the items depending on number of the items on scaling instrument and sample size reflecting main population (Quy and Ha, 2018; Bursal, 2019; Civelek, 2020). These authors suggested that, thus, if the sample size was more than 300 cases, the cut-off threshold of factor load was accepted as 0.30, also if the sample size was between 300 and 200 cases and between 200 and 150 cases, the cut-off thresholds of factor loads would be considered as 0.40 and 0.50, respectively.

In the second step, it was used the cluster analysis, a two-step cluster analysis, dividing a heterogenic target mass into two or more homogeneous segments by taking into account their attributes such as socioeconomic, psychological, and individual characteristics (Topcu and Baran, 2017; Karagöz, 2019). Two-step cluster analysis considering the ideal numbers of clusters and yielding the relationships between the main factors obtained and the consumption groups desired to be created is one of the most effective clustering techniques. In the present study, the main factors impacting the Turkish consumers' Erzurum Stuffed-kadayif purchase motivation were used in a two-step clustering analysis (CA) taking into consideration their retail selling points. It was thus segmented target consumers into three groups consuming at the restaurant (29.1% of overall consumers) and the patisserie (30.4% of those) and buying from the manufacturer stores (40.5% of those).

3. Results

3.1. Consumers' demographic and socioeconomic profiles

Participants' gender, age, life cycle, education and occupation status, monthly income, and expenditure groups at each cluster were presented in Table 1. The results of the study indicated that 59%

of the target consumer mass consisted of men, and the consumers with college graduates and white collars concentrated generally at each consumption segment of Erzurum Stuffed-kadayif.

Table 1. Consumers' various demographic and socioeconomic attributes

Consumers' attributes		Consumption segments of Erzurum Stuffed-kadayif						Overall consumers	
		Patisserie		Manufacturer		Restaurant			
		n	*%*	*n*	*%*	*n*	*%*	*n*	*%*
Gender	Male	77	66	85	55	65	58	227	59
	Female	40	44	71	45	47	42	158	41
	$(Pearson\ Chi-kare) = \chi^2_{(2603;2)} = 24{,}746$ *p=0.000*								
Education	Literate	4	4	9	6	3	3	16	4
	First school	20	17	39	25	31	28	90	23
	High school	45	39	39	25	35	31	119	30
	College	48	41	69	44	43	38	160	42
	$(Pearson\ Chi-kare) = \chi^2_{(2606;4)} = 77.378$ *p=0.000*								
Occupation	Businessman	11	9	27	17	13	12	51	13
	White-collar	50	43	49	31	42	38	141	37
	Blue-collar	18	15	12	8	17	15	47	12
	Retailers	27	23	40	36	26	24	93	24
	Pensioners	9	8	15	10	12	11	36	9
	Farmers	1	1	7	5	1	1	9	2
	Housewife	1	1	6	4	1	1	8	2
	Total	117	100	156	100	112	100	385	100
		$\bar{x}$	*n*	$\bar{x}$	*n*	$\bar{x}$	*n*	$\bar{x}$	*n*
Age group	+ < 30 years (young)	30.76	37	30.43	30	30.56	16	30.60	83
	30-50 years (mature)	42.23	69	43.33	84	42.59	64	42.76	217
	+ > 50 years (more mat.)	56.64	11	58.05	42	56.94	32	57.45	85
	Group means	39.96	117	44.81	156	44.97	112	43.38	385
	$F_{(382,2)} = 10.559$ *p=0.000*								
*Income**	+ < $400 (low-income)	321.43	14	360.00	24	365.00	12	350.40	50
	$400-1000 (middle-income)	416.04	91	751.56	109	750.91	88	740.14	288
	+ > $1000 (high-income)	1398.33	12	1421.74	23	1384.67	12	1406.30	47
	Group means	738.80	117	790.13	156	777.46	112	770.85	385
	$F_{(382,2)} = 0{,}903$ *p=0,406*								
*Expenditure**	+<$400 (low-expenditure)	320.00	21	318.60	43	310.00	19	316.99	83
	$400-700 (middle-expend)	591.94	62	582.90	69	596.56	61	590.16	192
	+>$700 (high-expenditure)	848.82	34	885.36	44	890.94	32	875.69	110
	Group means	617.78	117	595.36	156	632.05	112	612.85	385
	$F_{(382,2)} = 0.903$ *p=0.404*								
Family size	+< 4 person (core family)	2.55	56	2.63	40	2.68	25	2.60	121
	4-6 person (small family)	4.18	57	4.64	108	4.67	75	4.54	240
	+> 6 person (large family)	11.00	4	8.50	8	9.17	12	9.25	24
	Group means	3.63	117	4.32	156	4.71	112	4.22	385
	$F_{(382,2)} = 10.084$ *p=0.000*								

$\bar{x}$: arithmetic means, *n*: sample size, *%*: relative rate, *exchange rate is ₺ $^{-1}$ 5.75 on September 15, 2019.

On the other hand, the results also highlighted that the average age of overall consumers was 43.38 years, the family size consisted of 4.22 individuals, and the middle age group and large families showed intensity in each consumption segment. Similarly, the average income and expenditure levels for all consumption groups were $1406.30 and $875.69, and these economic indicators also were of the highest shares in each consumer segment.

3.2. Results of the EFA

The goodness fit statistics results and five factor dimensions that consider 31 items impacting the consumers' Erzurum Stuffed-kadayif purchase decisions in the EFA by being eliminated their load overlap and meaningless loads were given in Table 2. KMO that compares the observation and partial correlation coefficients in the EFA was calculated as a value of 0.911 ($p < 0.001$). The test score was acceptable at an excellent level due to much closer to the 0.99 threshold value, thus, providing the confirmation of sampling adequacy for the EFA. Bartlett's test of Sphericity statistics for the main factors related to consumers' purchase decisions, then, was calculated as $\chi^2_{(190;0.05)} = 6650.10$ ($p = 0.000$), and the unit matrix hypothesis was rejected ($p < 0.001$). Two statistics evaluating the data indicated that the data was at an excellent level for the EFA.

Table 2. The results of the EFA related to consumers' Erzurum Stuffed-kadayif purchase motives and their item loads

The items and factors interpretations	The factors and items loads*			
	F1	**F2**	**F3**	**F4**
Food image and value				
Price and quality relation	0.928			
Product quality	0.903			
Product brand and package label	0.860			
Product price	0.802			
Packaging appeal	0.750			
Package weight and size	0.690			
Advertisement impact	0.624			
Ready-made local dessert				
Serving at social meetings		0.835		
Preparing in a pratical way		0.765		
Being a light dessert		0.761		
Longer storage possibilities		0.746		
Offering along with tea		0.689		
Reference group impact		0.682		
Entrocentrism approach				
Contribution to the regional food retailers			0.897	
Contribution to the region economy			0.804	
Contribution to regional development			0.774	
Contribution to mitigate migration			0.634	
Cultural integration				
Being a part of the regional diet culture				0.862
Being a food with the region of origin				0.841
Being a part of cultural integration				0.640
Eigenvalues	*5.153*	*3.806*	*3.017*	*2.217*
Explained share of variance (%)	*25.764*	*19.028*	*15.085*	*11.085*
Cumulative share of variance (%)	*25.764*	*44.792*	*59.876*	*70.961*
KMO (Kaiser-Meyer-Olkin) statistic				*0.911*
Bartlett's test of sphericity	$[Chi-square\ (\chi^2_{190;\,0.05} = 6650.10\ (p = 0.000)]$			
Maximum Likelihood (goodness-of-fit test)	$[Chi-square\ (\chi^2_{116;\,0.05} = 494.860\ (p = 0.000)]$			

*It was suppressed the smaller *coefficients than 0.350.*

The results of the EFA indicated that the four-factors solution with Eigenvalue scores greater than 1.0 were derived from 20 items impacting the consumers' Erzurum Stuffed-kadayif purchase motivation in Table 2. The four factors were logically identified as the food image and value, ready-made local dessert, entrocentrism approach, and cultural integration, and their explained total variance was found as 70.96% (Quy and Ha, 2018). The first factor referring to the food image and value explained 25.76% of the total variance. It was thus assessed that the food image and value consisted of the loaded items measuring a wide range of real food images and value based on the relationships among

the product mix, price mix, and communication mix, which strengthened consumers' Erzurum Stuffed-kadayif purchase motivation.

Similarly, the second factor explained by 19.03% total variance identified as a ready-made local dessert that is often serviced practically along with meals or tea at social meetings under the effects reference groups. At the social meetings organized by consumers with Erzurum-originated, Erzurum Stuffed-kadayif is one of the most preferred desserts, and it has been also consumed by overall consumers with great satisfaction in diets. The third factor supported the first and second factors was named as entrocentrism approach (15.09% explain rate), that is, Erzurum-originated consumers consuming Erzurum Stuffed-kardayif have also tried to obstacle regional migration by orientating to the local food retailers to trigger regional economic development. On the other hand, the last factor was determined as the cultural enragration explaining 11.09% of the total variance. Erzurum Stuffed-kadayif, indeed, a crucial dessert of traditional culinary culture in Erzurum, created a cultural integration by being a part of the diets with protected geographical indication (PGI) under the region of origin.

3.3. Results of the CA

The main factors derived from the EFA, and shaping the purchase motivations of Turkish consumers who bought Erzurum Stuffed-kadayif from the local food manufacturer stores, patisseries, and restaurants were given in Table 3. The results of the CA indicated that consumers consuming Erzurum Stuffed-kadayif at the local restaurants focused on the food image and value representing a part of the regional cultural integration that triggered their purchase motivation. Especially, in order to maintain the regional culinary culture for Erzurum Stuffed-kadayif, consumers oriented consciously to these dessert images with the cultural consumption motives together with daily meals at the local restaurants.

On the other hand, it was analyzed that consumers buying Erzurum Stuffed-kadayif from the local food manufacturer stores also contributed meaningfully to the entrocentrism approach by serving the ready-made Erzurum Stuffed-kadayif at the social-cultural meeting strengthening the cultural integration on their purchase motivations. Similarly, it was assessed that consumers buying from or consuming Erzurum Stuffed-kadayif at the local patisseries also attributed a greater priority to entrocentrism approach triggering their purchase motivation through interactive cultural integration.

Table 3. The cluster center values related to the consumers' Erzurum Stuffed-kadayif purchase motives and the sample sizes in each cluster

The main factors	Consumer segments*					
	Restaurant		Manufacturer		Patisserie	
	$\bar{x}$	p	$\bar{x}$	p	$\bar{x}$	p
Food image and value	0.24	*0.002*	-0.05	*0.002*	-0.10	*0.002*
Ready-made local dessert	-0.17	*0.001*	0.18	*0.001*	-0.07	*0.001*
Entrocentrism approach	-0.30	*0.002*	0.14	*0.002*	0.18	*0.002*
Cultural integration	0.17	*0.001*	0.12	*0.001*	0.23	*0.001*
Number of total cases at each cluster (n)	*112*		*156*		*117*	
Population ratio for each cluster (%)	*29.1*		*40.5*		*30.4*	

*Bold values indicate the highest final cluster center scores in each segment.
**Total sample size (n): 385 households.

4. Discussion

The most effective factors on consumers' food purchase motivation during their purchase period are accepted as the drivers of the marketing mixes covering the product, price, communication, and buying convenience mixes (Kotler and Amstrong, 2018). Especially, the local food image and cultural values impacting the consumers' purchase decisions are shaped by appealing motives of the product, price, and communication mixes under cultural integration. Previous researches also informed that the food image and value under culinary culture linked firstly with the various combinations of the major extrinsic product motivation drivers on consumers' local food purchase decisions (Chong et al., 2022;

Khan and Pandey, 2022; Liu et al., 2022; Shi et al., 2022; Topcu, 2022; Giannoutsos et al., 2023; Kaçmaz et al., 2023; Kumar et al., 2023).

Of these studies related to the food image and value on consumers' local food purchase motivation, Kushwah et al. (2019), Akay (2021), Kadirhanoğulları et al. (2021), Shi et al. (2022), Khan and Pandey (2022), Apak and Gürbüz (2023), Huddleston et al. (2023), Kumar et al. (2023) and Perumal et al. (2023) highlighted that the cultural attitude, the region of origin, food brand, advertising, food packaging and labelling, optimum pricing, social media platform, consumption ethically and culinary culture, social responsibility consciousness affected positively consumer perceived local food image and cultural appreciation (subjective value) linking with the cultural integration on their local food buying intentions at the food stores or online platforms, and thus there was a strong correlation among local food image and cultural appreciation under the cultural integration, and it could be also provided a major contribution to sustainable food consumption with the local or the region of origin in the context of cultural integration.

Similarly, Fakreddine and Sanckez (2023), Magalhaes et al. (2023), Mesias et al. (2023), and Siddiqui et al. (2023) also emphasized that the brands, labels, and the region of origin information presented on food packages were generally considered as the important determinants on consumers' purchase decision and motivations, and thus local food image and cultural value judgments for consumers impacted directly on their repurchase decisions. In the current study, indeed, the Erzurum Stuffed-kadayif image and cultural value appreciation under the cultural integration were found to be the most impact stimuli on purchase motivation of consumers preferring the local diners.

On the other hand, Erzurum Stuffed-kadayif promoting to maintain cultural integration among Erzurum-origined consumers purchasing from the manufacturer vendors has still functioned as a ready-made local dessert and has also provided a fairly significant contribution to the entrocentrism approach at the research region. The results of the study, indeed, highlighted that Erzurum Stuffed-kadayif as a part of Erzurum culinary culture buying from the manufacturer stores was serviced more practically along with the daily meals or tea presentations as a ready-made local dessert at social meetings organized by Erzurum-origined consumers residing in all the provinces of Türkiye, and thus not only was it sufficient to ensure a stronger cultural integration among the younger generations, but the entrocentrism approach was also activated. Consequently, it was pointed out that there was a strong correlation among three factors impacting on buying motivation of the consumers purchasing the local dessert from the local manufacturer vendors.

Focused on the entrocentrism approach driving consumers' buying motivation and decision towards the local foods, Chen and Antonelli (2020), Migliore et al. (2021), Miguel et al. (2022), Maro et al. (2023), Siddiqui et al. (2023), Skalkos and Kalyva (2023) and Sundqvist (2023) pointed out that consumer entrocentrism was of a strong and positive relationship with trust in the local and organic foods, to their vendors, cultural integration and convenience food, and thus it was also found to be a vital motivator of their willingness to buy and consume the local foods due to instilling a pride sense that leads to an overestimation of local food appreciation belonging to their culinary culture or ethnicity and satisfiying through consumers' discriminatory sensitive buying behaviour towards the local food with the region of origin.

Attributed similarity to the trends of consumers' willingness to buy Erzurum Stuffed-kadayif from manufacturer stores, consumers adopted only the entrocentrism approach formed by cultural integration by consuming the local dessert at the local patisseries. The identify-based motivation (IBM) model based on the food consumption studies revealed that, indeed, it was differentiated with longitudinal cultural processes and situational activation by contextual cues, each with different implications for the availability and accessibility of ethnic cultural knowledge, and thus the motivation model also associated by consumers' cultural integration with a linear and positive correlation on their food consumption motivations (Aguirre-Rodrigez et al., 2022; Shi and Jiang, 2022; Apak and Günbüz, 2023; Hedriana et al., 2023).

As a result of the cultural integration based on Erzurum culinary culture, consumer entrocentrism promoted their purchase intention and motivation towards the local cultural foods. Erzurum-origined consumers' motivation to purchase Erzurum Stuffed-kadayif was reflected in their attitude towards loving and taking pride in unique local foods and cultures as compared with the others, and thus this phenomenon affected positively their purchase motivation. Dhewi and Oktaviani (2023), Maro et al. (2023), Siddiqui et al. (2023) and Sundqvist (2023) revealed that consumer entrocentrism

was of a positive and significant impact on buying attitude and motivation, and thus consumers entrocentrism was also accepted as a market segmentation tool in most developed countries.

Conclusion

The EFA results of the study revealed that the main factors impacting the Turkish consumers' Erzurum Stuffed-kadayif purchase intension and motivation were the food image and value, a ready-made dessert, entrocentrism approach, and cultural integration. The CA results also highlighted that while middle-income consumers consuming this traditional dessert at the local restaurants were satisfied fairly higher with the local food image under cultural integration, low-income consumers consuming Erzurum Stuffed-kadayif at the local patisseries also attributed big importance to the entrocentrism approach under cultural integration. On the other hand, high-income consumers purchasing Erzurum Stuffed-kadayif a ready-made traditional dessert from local manufacturer vendors tried to contribute considerably to sustainable food supply and consumption with an entrocentrism approach under cultural integration.

Therefore, it should be implemented differentiation and positioning strategies based on the food image along with the manufacturing and processing strategies strengthing cultural integration at the local restaurants for middle-income consumers, and the intensified multy-segment marketing strategies contributing to regional development by acting entrocentrism approach through cultural integration at the local patisseries for low-income consumers, respectively. Similarly, it should be applied the manufacturing and penetrating to new markets strategies focused on Erzurum Stuffed-kadayif as a ready-made dessert reflecting Erzurum culinary culture as a crucial tool of cultural integration and entrocentrism approach for high-income consumers purchasing this local dessert from local manufacturer vendors.

Although this study was one of the first research conducted on consumers' Erzurum Stuffed-kadayif purchase motivation in the economics literature, there were also some limitations. In the study, thus, these limitations could be addressed for the next research. Firstly, the study focused on only consumers in Erzurum due to funding and time constraints. The future researches, hence, could be planned for larger sample sizes accounting for consumers residing at more important trade and consumption centers. Secondly, it was applied the EFA as the research model in the study, but it could be utilized from Confirmatory Factor Analysis (CFA) for the next research, as well.

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