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Address	*: Ordu University, Faculty of Agriculture, Department of Horticulture, Altınordu-Ordu, Türkiye*
Contact	*: Burhan Ozturk* *E-mail: burhanozturk55@gmail.com or turjfas@gmail.com* *Phone: +905062036255*

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Review

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The impact of climate change on hazelnut cultivation

Ayhan AHMADOV [1*]

[1]Azerbaijan State Agricultural University, Faculty of Agronomy, Department of Horticulture, Ganja-Azerbaijan

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[*] CONTACT

ayhan.ehmedov@gmail.com

A B S T R A C T

Hazelnut (*Corylus avellana* L.) cultivation faces substantial challenges in the wake of climate change. This review synthesizes findings from various studies to examine the impacts of climate change on hazelnut cultivation, strategies for mitigating these impacts, and the potential role of hazelnut orchards as carbon sinks. I discuss the physiological responses of hazelnut trees to changing climatic conditions, explore management strategies to enhance resilience and productivity, and evaluate the carbon sequestration potential of hazelnut orchards. Additionally, I assess the role of fertilization, irrigation, and other agricultural practices in shaping hazelnut growth and yield under shifting climate scenarios. By integrating sustainable agricultural practices and leveraging precision agriculture technologies, hazelnut growers can improve environmental sustainability and economic viability. This review provides comprehensive insights and practical recommendations for sustaining hazelnut production in the face of climate change.

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1. Introduction

Hazelnut (*Corylus avellana* L.) cultivation stands at the intersection of agricultural productivity and environmental sustainability, facing significant challenges posed by climate change. As one of the most important perennial fruit crops, hazelnuts play a vital role in global food security and economic prosperity, particularly in regions with temperate climates. However, the increasing frequency and intensity of extreme weather events, shifts in precipitation patterns, rising temperatures, and elevated atmospheric CO_2 levels threaten the stability and productivity of hazelnut orchards worldwide.

Climate change poses multifaceted challenges to hazelnut cultivation, impacting various stages of growth and development, from floral differentiation and blossom to pollination and nut setting. The sensitivity of hazelnut trees to environmental conditions, particularly their reliance on chilling requirements, makes them highly susceptible to the effects of warming temperatures. Additionally, the projected increase in global temperatures is expected to alter the geographical distribution of suitable growing regions, potentially displacing hazelnut cultivation to new areas with more favorable climate conditions on long term (Cabo, 2020). In the face of these challenges, there is an urgent need to develop and implement strategies to mitigate the impacts of climate change on hazelnut production while ensuring the long-term sustainability of orchards. This necessitates a comprehensive understanding of the physiological responses of hazelnut trees to changing climatic conditions, as well as the development of adaptive management practices to enhance resilience and productivity.

Furthermore, hazelnut orchards have the potential to serve as important carbon sinks, sequestering atmospheric carbon dioxide through the process of photosynthesis and storing it in tree biomass and soil organic matter. Harnessing this carbon sequestration potential can contribute to climate change mitigation efforts while providing additional economic and environmental benefits to hazelnut growers (Granata et al., 2020).

In this context, this review aims to synthesize existing knowledge on the impacts of climate change on hazelnut cultivation, explore management strategies for climate resilience and productivity enhancement, and evaluate the carbon sequestration potential of hazelnut orchards. By examining the interplay between hazelnut cultivation, climate change, and sustainable agricultural practices, this review seeks to provide insights and recommendations for the future of hazelnut production in a changing climate landscape.

2. Physiological responses of hazelnut trees to climate change

Hazelnut trees exhibit complex physiological responses to changing climatic conditions, with temperature, precipitation, and atmospheric CO_2 levels playing key roles in shaping growth, development, and yield (Table 1). Understanding these responses is important for predicting the impact of climate change on hazelnut cultivation and developing adaptive strategies to mitigate its effects.

Table 1. Physiological responses of hazelnut trees to climate change

Climate factor	Response	Impact on growth/Yield	Source/Reference
Rising Temperatures	Disruption in chilling requirement, earlier phenology, a reduction in the duration of the flowering period, increasing degree of dichogamy	Reduced yield, smaller nut size, decreased kernel weight	Črepinšek et al., 2012; Asseng et al., 2015; Škvareninová, 2016; Cabo, 2020; Balık and Arif, 2023
Changes in Precipitation	Increased water stress	Reduced nut set, lower kernel quality, root health issues	Milosevic and Miosevi, 2012; Asseng et al., 2015; Tonkaz and Bostan, 2019
Elevated CO_2 Levels	Enhanced photosynthesis and growth	Potentially increased yield under optimal conditions	An et al., 2020
Extreme Cold Events	Frost damage, reduced pollination success	Significant yield losses, tree damage	Beyhan et al., 2007; Balik and Kayalak Balık, 2015; Anonymous, 2021

2.1. Effects of temperature, precipitation, and CO_2 levels on hazelnut growth and yield

The findings indicate that temperature is the most influential climatic factor affecting hazelnut cultivation (Ustaoğlu, 2009). The impact of rising temperatures on yields can vary depending on the different characteristics. However, in general, a significant increase in temperature could lead to water scarcity. This scenario suggests a negative correlation between rising temperatures and agricultural productivity. Increased precipitation might improve soil moisture in semi-arid areas, yet exacerbate issues in regions already facing water surplus especially flat areas. Conversely, a decrease in rainfall could yield opposite effects. In irrigated areas, the adverse effects of altered precipitation and higher temperatures are mitigated by access to irrigation water, rendering yields more resilient to climate fluctuations (Agovino et al., 2019). Even in the Black Sea Region, where hazelnut cultivation is most intense in Türkiye, some years there may be yield and quality losses due to insufficient and irregular rainfall (Anonymus, 2024).

The adverse effects of extreme cold temperatures on hazelnut orchards in spring, exacerbated by climate change, were notably exemplified in events from 2004, 2014 and 2021 in Türkiye, the world's main hazelnut producer. Following temperatures above seasonal norms in December and January of that year, a cold wave swept through the region, affecting hazelnut orchards (Figure 1). Subsequently, the region witnessed a frost damage, in Eastern Black Sea Region, due to effective snowfall in February and March. Detailed assessments conducted by producer associations revealed significant damage ranging from 30 to 90 percent in hazelnut orchards situated between altitudes of 200 and 600 meters (Balık and Kayalak Balık, 2015; Anonymus, 2021).

Figure 1. Impact of extreme cold temperatures on hazelnut orchards in Ordu, Türkiye (Anonymous, 2024)

Temperature exerts a significant influence on hazelnut growth and development, with both mean temperatures and temperature extremes affecting various physiological processes (An et al., 2020). The hazelnut trees have chilling requirements, which can affect critical phases of floral differentiation, blossom, and fruit nut setting. (Cabo, 2020). Changes in climatic conditions, especially temperatures, influence the initiation and progression of the phenological growth stages (Kasprzyk et al., 2004). Previous studies (Črepinšek et al., 2012; Škvareninová, 2016) conducted in Slovenia and Slovakia have demonstrated that rising temperatures lead to an earlier onset of phenological growth stages in hazelnuts and a reduction in the duration of the flowering period. In addition, higher temperatures may extend growing seasons and promote increased photosynthetic activity, potentially enhancing yield under certain conditions. Changes in precipitation patterns, including alterations in the frequency and intensity of rainfall events, also impact hazelnut growth and yield. Hazelnut trees require regular annual precipitation for efficient nut development, and drought conditions during critical growth stages lead to reduced yields and lower nut quality. Conversely, excessive precipitation can increase the risk of waterlogging and nutrient leaching, negatively affecting root health and overall tree vigor. In contrast to water stress, which can be alleviated through irrigation, the adverse impacts of rising temperatures on chill hours and leaf scorching are not easily remedied. Likewise, elevated temperatures can accelerate vegetative growth, shortening the time available for kernel development and decreasing kernel weight (Asseng et al., 2015).

A repercussion of climate alteration manifests as heightened occurrence and severity of hailstorms. Hailstorms represent a significant peril to hazelnut producers particularly in regions characterized by temperate climates, where hazelnut cultivation predominates and where the incidence of hail is notably elevated. Projections (Botzen et al., 2010) based on climate change models forecast a substantial rise in both the frequency and severity of hailstorms, thereby amplifying the economic challenges faced by the agriculture.

While floods may not exert as substantial an impact on hazelnut cultivation as they do on certain other crops, their occurrence remains a notable concern deserving careful consideration. Azerbaijan ranks among the principal global producers of hazelnuts, with its primary production hub located in the Zagatala district. In recent years, the incidence of hazelnut orchard inundations in this region has escalated, attributed to the impacts of climate change (Figure 2).

Figure 2. Flooding in hazelnut orchards in Zagatala District, Azerbaijan (Anonymous, 2022)

Similarly, in July 2023 (Anonymous, 2023), a flood disaster struck Duzce, Türkiye, inflicting significant damage on hazelnut orchards and underscoring the vulnerability of hazelnut cultivation to extreme weather events. With hazelnuts cultivated across 632 thousand decares, the inundation of floodwaters wreaked havoc on agricultural lands (Figure 3). The heavy rains not only submerged crops in gardens but also triggered landslides and strong winds in cultivated areas on slopes, leading to premature crop loss. The overarching assessment points to a considerable threat looming over agricultural productivity. With an estimated 430 thousand square kilometers of flood-prone cropland facing a doubling in flood frequency by 2050 under certain climate scenarios, the potential impact on crop yields and food security is profound (Arnell and Gosling, 2016).

Figure 3. Flooding in hazelnut orchards in Duzce, Türkiye (Anonymous, 2023)

These findings underscored the susceptibility of hazelnut cultivation to extreme weather events induced by climate change, highlighting the urgency for implementing adaptive strategies to ensure the resilience and sustainability of hazelnut production in affected regions.

Atmospheric CO_2 levels play a dual role in influencing hazelnut physiology, acting as both a substrate for photosynthesis and a driver of climate change. Since the onset of the Industrial Revolution, atmospheric CO_2 levels have surged from 280 parts per million (ppm) to surpassing 410 ppm (Ciais et al., 2013). Initially, a rise in soil CO_2 benefits plants by curbing evapotranspiration through biomass conversion fueled by water stored in leaves. Elevated CO_2 concentrations have the potential to stimulate photosynthetic rates and increase biomass production in hazelnut trees, leading to enhanced growth and potentially higher yields. Yet, as air temperatures climb, this early advantage may diminish (An et al., 2020). Heightened CO_2 levels are anticipated to boost leaf photosynthetic rates. However, the extent to which this enhancement will manifest remains uncertain, as CO_2-induced stimulation of photosynthesis hinges on factors such as leaf temperature, as well as the availability of water and nutrients (Chaves and Pereira, 1992; Leakey et al., 2009; Zhu et al., 2017).

2.2. Phenological shifts and implications for pollination, flowering, and nut development

Climate change is driving phenological shifts in hazelnut trees, altering the timing of critical developmental stages such as pollination, anthesis, fertilization, nut set and harvest time. Increased temperatures and altered precipitation patterns might result in the advancement or delay of phenological events (Table 2). Hazelnut pollens wind-pollinated. Successful pollination requires the synchrony of pollen release and female flower receptivity. However, dichogamy is common in most of the cultivars. Protandry is often seen. Phenological shifts can disrupt this synchrony, reducing pollination efficiency. For instance, asynchronous pollen release and female flower receptivity, or adverse weather conditions (e.g., heavy rainfall, lack of pollinizer) during the pollination period, can significantly diminish the likelihood of successful pollination.

Table 2. The key impacts of phenological shifts on hazelnut flowering, pollination, and nut development

Phenological phase	Impact of climate change	Implications
Pollination- Flowering	Disrupted timing of pollen release, flower receptivity and degree of dichogamy	Reduced pollination efficiency
Nut Development	Accelerated ripening due to higher temperatures, flower bud development (produce the next year's crop)	Smaller, lower quality nuts, kernels, yield deficiency
Overall Impact	Shift in growing season length and temperature patterns	Variable yield and quality, increased management costs

The research conducted on the Iberian Peninsula has documented notable phenological changes in early spring species, including *Corylus* L. (hazelnut trees), over the years. Specifically, an earlier onset of flowering was observed at most of the studied locations. Additionally, earlier nut ripening was recorded at all sampling sites, and earlier nut harvesting was noted at the majority of these locations (Hidalgo-Galvez et al., 2018).

3. Management strategies for climate resilience and productivity

As climate conditions evolve, there is the potential for changes in the suitability of certain regions for hazelnut cultivation over the long term. While immediate shifts may not be imminent, the gradual impact of changing temperatures, precipitation patterns, and environmental factors could eventually alter the landscape of hazelnut production. This dynamic underscores the importance of ongoing assessment and adaptation in hazelnut farming practices to ensure resilience in the face of future climate scenarios.

Mitigating the impacts of climate change on hazelnut cultivation requires the implementation of effective management strategies to enhance resilience and productivity. Research findings suggest several approaches to address climate challenges and optimize hazelnut orchard management.

One key strategy involves the utilization of preharvest foliar spray treatments. Studies (Cabo, 2020; Cabo et al., 2020) have demonstrated that treatments with compounds such as kaolin, natural biostimulants, and salicylic acid can mitigate heat and drought stresses, improve water use efficiency, and enhance physiological performance in hazelnut trees. These treatments have been associated with increased nut and kernel sizes, higher vitamin E and antioxidant activity levels, and improved biometric parameters. In addition to foliar spray treatments, leveraging hazelnut by-products for bioactive compounds presents an opportunity for sustainable agricultural practices. Hazelnut husks, a by-product of hazelnut cultivation, contain phenolic compounds with antioxidant properties. Valorizing hazelnut husks through extraction and purification processes can contribute to the development of bioactive molecules for various applications, further enhancing the sustainability of hazelnut production. Transitioning to sustainable agricultural practices is another crucial aspect of climate resilience and productivity enhancement. Organic farming and good farming techniques, reduced fertilizer use, and efficient irrigation management can promote soil health, conserve water resources, and minimize environmental impacts in hazelnut orchards. Additionally, regular maintenance practices such as pruning, based on soil and leaf analysis fertilizing, pest control to yield increase and orchard sustainability. Promoting genetic diversity and breeding resilient cultivars are essential components of climate-resilient hazelnut cultivation. Research on hybrid hazelnut trees has shown their potential for low-input, high-productivity systems capable of sequestering carbon. Breeding programs focused on developing cultivars resilient to climate stressors can further enhance orchard resilience and productivity. Continuous monitoring and adaptive management are critical for effectively addressing climate challenges in hazelnut cultivation. Integrated pest and disease management, weather monitoring systems, and precision agriculture technologies can help optimize resource use, minimize risks, and ensure orchard productivity under changing environmental conditions.

Crop adaptation to climate change heavily relies on the practice of breeding (Araus and Kefauver, 2018). Breeding new cultivars of hazelnut trees adapted to climate change is pivotal for sustaining hazelnut production amidst evolving environmental challenges. By selecting varieties tolerant to heat, drought, and other climatic stresses and incorporating genetic diversity, breeders enhance hazelnuts' resilience. Moreover, breeding for pest and disease resistance reduces dependence on pesticides. Although breeding programs increasingly prioritize climate resilience, there is growing evidence indicating the obstacles and complexities involved in creating crops prepared for climate change (Xiong et al., 2022). Collaborative efforts among researchers, geneticists, agronomists, and farmers are crucial for advancing these objectives and securing the future of hazelnut production systems.

Soil carbon sequestration emerges as a promising strategy to mitigate climate change effects on hazelnut cultivation. By restoring depleted soil organic carbon (SOC) through innovative land management practices like cover cropping, reduced tillage, and nutrient recycling, carbon emissions can be slowed, and soil fertility and resilience can be improved (Nazir et al., 2024). No-tillage (NT) practices have emerged as a scientifically supported method for mitigating climate change by significantly reducing CO_2 emissions from soils. Research conducted over a six-year period has consistently demonstrated the efficacy of NT in comparison to conventional tillage (CT) methods. NT, particularly when combined with surface mulch, has shown to reduce CO_2 emissions by an average of 51% compared to CT practices. These findings underscore the potential of NT as a sustainable agricultural approach that aligns with global efforts to combat climate change (Mühlbachová et al., 2023).

Hazelnuts, like many other tree crops, typically don't require tilling (USDA Climate Hubs, n.d.). This characteristic offers promising implications for climate change mitigation. By minimizing tillage, hazelnut orchards contribute to preserving soil organic matter and enhancing carbon sequestration potential, thus aligning with efforts to mitigate greenhouse gas emissions. Therefore, expanding hazelnut orchards or adopting minimal tillage practices in hazelnut cultivation can serve as effective strategies for promoting sustainable agriculture and combating climate change.

4. Carbon sequestration potential of hazelnut orchards

Hazelnut trees exhibit considerable potential for carbon sequestration, often ranking among the leading fruit tree species in terms of carbon storage capacity. Research has elucidated the amount of carbon dioxide (CO_2) sequestered by hazelnut orchards, particularly in the Mediterranean region, where woody agriculture predominates. Studies have quantified the carbon sequestration capacity of hazelnut orchards under routine horticultural care, revealing substantial CO_2 uptake rates. Hazelnut orchards have been found to sequester an average of 58.8 ± 9.1 Mg CO_2 ha^{-1} $year^{-1}$, with peak sequestration occurring during the growing season (Granata et al., 2020). Notably, hazelnut cultivation areas are expanding, indicating the increasing significance of these orchards as carbon sinks. Pacchiarelli et al. (2022) demonstrated that the cultivation of European hazelnut (*Corylus avellana* L.) initially leads to a decline in soil organic carbon stock, with a reduction ranging from 23% to 58% during the first 3-5 years after planting. This decrease is attributed to land preparation, frequent tillage operations, and the transition from grassland to orchard. However, despite this initial decline, the study highlights the potential for soil carbon stock recovery and the exponential increase in hazelnut orchards with optimal fertilization and management practices. The distribution of carbon sequestration within hazelnut orchards varies over time, with carbon allocated to different tissues evolving as trees mature. While the exact mechanisms underlying carbon allocation patterns require further investigation, it is evident that hazelnut orchards contribute to soil carbon storage over time.

Furthermore, the impact of fertilization on carbon sequestration in hazelnut orchards has been examined. While fertilization primarily enhances woody biomass production, hazelnut cultivation has been characterized as a low-input crop with significant potential for carbon storage. Comparisons with conventional commodity crops underscore the carbon sequestration potential of hazelnut orchards. Despite yielding slightly lower in-shell nut production compared to certain crops like soybeans, hazelnut orchards exhibit substantial woody biomass accumulation. Hazelnut orchards had stored an estimated 12 tonnes/hectare of woody biomass, highlighting their role in long-term carbon storage (Fireman, 2019).

Hazelnut orchards employing a single trunk training system have garnered popularity in recent years. But, emerging research (Granata et al., 2021) suggests that bush-like training systems offer heightened efficacy in carbon sequestration, crucial for mitigating greenhouse gas emissions. A recent study conducted in Italy's Piedmont region, a prominent hub for hazelnut production, compared the carbon sequestration capabilities of two orchard management approaches: single trunk and bush-like. Results unveiled a notable advantage for the bush-like system, showcasing significantly higher rates of carbon dioxide (CO_2) sequestration per plant and per unit area. This disparity was attributed to the bush-like system's ability to foster a greater leaf area index, facilitating enhanced carbon assimilation. Despite inherent CO_2 emissions associated with orchard management practices, such as diesel fuel usage and machinery operation, both orchard types were found to function as net carbon sinks over the study period (Granata et al., 2021). While the single trunk system is gaining popularity, the majority of global hazelnut orchards still utilize bush-like systems. Based on the findings presented above, enhancing the efficiency of these bush-like systems holds significant potential to improve both carbon sequestration and overall productivity on a global scale.

Although challenges such as cost-effective harvest methods persist, small-scale hazelnut orchards demonstrate strong potential as low-input, high-productivity systems that sequester carbon effectively. Continued research and innovation in hazelnut cultivation hold promise for maximizing carbon sequestration while ensuring sustainable food production and environmental stewardship.

5. Conclusion and Future Directions

This review underscores the substantial challenges that climate change poses to hazelnut cultivation, highlighting the necessity for adaptive management strategies to ensure sustainability. Physiological responses of hazelnut trees to climate change, including temperature, precipitation, and CO_2 levels, have profound implications for growth and productivity. The practical implications of this research are multifaceted, encompassing the need for improved agricultural practices, such as the application of preharvest foliar sprays to enhance plant resilience and the development of climate-resilient hazelnut cultivars through advanced breeding programs. For stakeholders, including farmers, agricultural policymakers, and researchers, the findings emphasize the importance of adopting integrated approaches that combine sustainable agricultural practices with innovative mitigation techniques.

Management strategies for climate resilience and productivity play a crucial role in mitigating the adverse effects of climate variability on hazelnut production. One of the key recommendations is the implementation of soil and water management practices that optimize resource use and enhance plant health. Additionally, the carbon sequestration potential of hazelnut orchards presents an opportunity for mitigating greenhouse gas emissions and enhancing environmental sustainability. Continued research on carbon dynamics and ecosystem services of hazelnut agroforestry systems is needed to quantify their contribution to climate change mitigation. Interdisciplinary research that integrates agronomic, ecological, and socio-economic perspectives will be crucial in developing holistic solutions.

Given the regional variability in climate impacts, examining the adaptations of hazelnut varieties according to different regions is critical. Specific adaptations may include selecting varieties that are better suited to local climate conditions, such as drought-resistant cultivars for semi-arid regions or cold-hardy varieties for areas prone to late frosts. This regional approach ensures that adaptation strategies are tailored to the unique environmental conditions and challenges faced by hazelnut growers in diverse geographic areas.

By addressing practical and research-oriented recommendations, such as continued innovation and adoption of sustainable management practices, the hazelnut industry can better navigate the challenges posed by climate change. This comprehensive approach, coupled with scientific research and extension efforts, will ensure the sustainability of production and contribute to broader environmental conservation efforts. It will not only benefit hazelnut growers but also support global endeavors to combat climate change and promote sustainable agriculture. Overall, these concerted efforts will be critical for ensuring the resilience, productivity, and sustainability of hazelnut cultivation in the face of climate change and global environmental challenges.

Compliance with Ethical Standards

Conflict of interest

The author declares no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Authors' contributions

The author carried out the concepting, designing, supervision, data collection and/or processing, data analysis and/or interpretation, literature search, writing, critical review, submission and revision, project management, funding acquisition.

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Effect of seasonal shift on the proximate nutritional composition of some plant materials from the aquaponics farming

Labaran IBRAHIM [1*]

[1] Department of Biochemistry, Federal University Dutse, Jigawa State, Nigeria

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* CONTACT

labaranibrahim80@gmail.com

A B S T R A C T

This study intends to evaluate the variations of the proximate nutritional makers of some plant samples [red chili (RCH) fruit, red tomato (RTO) fruit, green spinach (GSP) leaf, and green lettuce (GLE) leaf] from aquaponics farming in relation to seasons. The research was carried out for the duration of four (4) periods (winter, spring, summer, and autumn). The proximate nutritional parameters determined were total moisture (TMO), crude ash (CAS), crude fiber (CFI), total carbohydrate (TCA), and soluble protein (SPR). The CAS, CFI, TCA, and SPR levels of the RCH, RTO, GSP, and GLE plant materials are significantly ($P<0.05$) difference among seasons. The CAS, CFI, TCA, and SPR amounts of each experimental plant materials increases significantly ($P<0.05$) in the summer in comparison to winter, spring, and autumn. However, the TMO content of above plant samples was detected to increase significantly ($P<0.05$) in the winter season. The findings of this research indicated that shifts in the seasonal factors such as temperature, solar intensity, and daylight length have revealed differences in the proximate nutritional indices of the study plant materials from aquaponics farming.

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ORCID> 0000-0003-2618-5931 (LI)

1. Introduction

The aquaponics system is a sustainable farming method for the simultaneous production of fish and plants (Diver, 2006). Globally, the system is gaining recognition towards achieving sustainable food production to revert hunger and starvation, malnutrition, and poverty in urban and rural communities (Love et al., 2015). Hence, this farming method could be of great advantage to the African countries with limited resources for agricultural production (Mchunu, 2018). The technology is new to the African countries, with a few cited research articles on the theme (Obirikorang et al., 2021). From an international survey carried out in 2014 on this technology, responses were only received from South Africa and Ghana (Love et al., 2015). From the 15 African nations, the total published articles in aquaponics studies were 82. Egypt, South Africa, and Kenya appear to have widely adopted the technology, with 23, 20, and 14 published research papers, respectively. Nigeria had 9 articles, and the remaining countries had between 1 and 3 articles (Obirikorang et al., 2021).

The system could provide a solution to the majority of problems associated with food supply to feed the growing populace (Castro et al., 2006; Diver, 2006). The farming system can enable the production of more plants per square foot due to closer the proximity of planting compared with conventional practice that needs large tracts of land (Rakocy and Hargreaves, 1993). In addition, roots absorb required nutrients without competition with non-edible plants such as weeds in comparison to traditional agricultural method that requires fertilizer application (Savidov, 2004; Savidov and Hutchings, 2005). Also, the technology is usually set up in a greenhouse to limit the adverse effects of pests, and insects to promote rapid growth rates, high yields, and more nutritious products (Blidariu and Grozea, 2011; Brook, 2017). Furthermore, the system has the potential to offer a lasting solution to most of the issues of phosphorus, nitrogen runoff, and environmental pollution linked to traditional farming operation (Timmons et al., 2002; Flavius and Grozea, 2011).

The selection of the experimental plant materials (red chili fruit, red tomato fruit, green spinach leaf, and green lettuce leaf) is mainly because of their nutritional constituents. They are also among the most commonly grown and regularly consumed fruit and leafy plants globally.

Mudzengi et al. (2020) and Castro et al. (2021), reported the relationship between plants and seasonal variations in semi-arid zones. To another report, productivity and constituents of plants are determined by group of factors such as change in climate (Herrera et al., 2017). Additionally, Adeyeye (2005) cited that the growth and development of crops were often influenced by changes in the weather factors such as temperature and intensity of solar radiations. Therefore, this study intends to find out and describe the impacts of seasonal shifts on the proximate nutritional properties of red chili fruit, red tomato fruit, green spinach leaf, and green lettuce leaf plant materials from aquaponics farming.

2. Materials and methods

2.1. Study site, system setup, and operation

The experimental aquaponics system was located in Makhanda, Eastern Cape of South Africa. The study was conducted for consecutive four (4) seasons (winter, spring, summer, and autumn). The investigation commenced in the winter, from the 29th of June 2020 to the 31st of August 2020. The spring research was carried out from the 3rd of September 2020 to the 30th of November 2020. The summer experiment began from the 3rd of December 2020 to the 1st of March 2021. Finally, the autumn evaluation started on the 4th of March 2021 and ended on the 27th May 2021.

The aquaponics system setup was as a coupled commercial system enclosed in a greenhouse and only exposed to ambient sun light. It consisted of 4 × 1,500 L fish water tanks; 2-sump tanks (1 × 1,500 L; 1 × 500 L), each with an associated submersible pump (SOBO®, WP-7000, 105W, 5000L/H); 20 × 400 L flood-and-drain gravel stones media beds; and 24 × 900 L deep-water culture tanks. PVC pipes connected the system's components to form a closed loop. The fish tanks and sumps were placed in a separate housing unit within the aquaponics greenhouse.

The water from the 4-fish tanks first flowed to the single sump tank (1 × 1,500 L) by gravity, then pumped to gravel stones media beds. As the water volume in flood and drain media beds reached its highest level, it drained into deep-water hydroponics tanks by gravity through PVC pipe outlets. Water from the deep-water culture tanks was all fed directly into the second single sump (1 × 500 L), which was eventually delivered to fish rearing tanks, thus, completing a cycle. The flood-and-drain system was maintained by bell siphons installed in each gravel stones media bed.

2.2. Plant materials, supplementation, and water replacement

In this study, the plant materials used were Bird's eye red chili (*Capsicum frutescens* L.) fruit, red cherry tomato (*Solanum lycopersicum*) fruit, green Silver-beet spinach (*Spinacia oleracea*) leaf, and green Locarno lettuce (*Lactuca sativa* L.) leaf. The research plants were collected each season for nutritional composition assessment. The red chili fruit was obtained from gravel stone media-bed I and II, denoted by GMB-I and GMB-II. Besides, each red tomato fruit and green spinach leaf was collected from gravel stone media-bed III and IV (GMB-III and GMB-IV), respectively. Nevertheless, the green lettuce leaf was acquired from a polystyrene sheet on deep-water culture-1 (DWC-1).

The supplementation (iron-chelating, buffering, and manufactured organic food addition) and water replacement were made to the system over the study period. During the winter season, on the 3rd of July 2020, 13th of July 2020, and 17th of August 2020; iron chelate (500 g), calcium hydroxide (450 g), and potassium hydroxide (450 g) were added to the system through the sump, respectively. Furthermore, in the spring, on the 10th of September 2020, 10th of October 2020, and 22nd of October 2020; 2.0 L of Sea-Grow (manufactured organic plant food), 1000 L of fresh rainwater stored in the reservoir, and 2.0 L of Sea-Grow were added. In addition, during the summer period, 1000 L of fresh rainwater, 2.0 L each of Sea-Grow and Sea- Brix (manufactured organic plant foods), and 500 g of iron chelate were added to the system on the 5th of December 2020, 14th December 2020, and 22nd January 2021, respectively. Finally, in the autumn season, neither supplementation nor water replacement was made to the aquaponics system.

2.3. Chemical reagents and apparatus

The chemical reagents used were sodium hydroxide (Merck, Batch No. MB1M610352), concentrated sulfuric acid (Merck Chemicals, Batch No. 42980), HPLC-grade methanol (Merck, CAS-No. 67-56-1, Germany), glucose (Merck Chemicals, Germany), lead acetate (Sigma Aldrich, CAS Number: 546-67-8), bovine serum albumin (Sigma Aldric, CAS No. 9048-46-8), and Bradford reagent (Sigma Life Science, Lot No. SLBP3810V). The equipment included Benchtop laboratory oven (Labcon laboratory equipment, Krugersdorp, South Africa), muffling furnace (Gallenkamp muffle furnace, REX C900, England), refrigerated centrifuge (Heraeus Megafuge 1.0R, Germany), Epoch UV-vis microplate reader spectrophotometer (EPOCH2C, Bio-Tek Instruments, Inc., USA), analytical balance (RADWAG, 220 g × 0.1 mg, Model, AS/220/C/2, Poland), silica crucible dishes, Büchner funnel, moisture dishes, desiccator, and heating plate (Fied Electric, Model MI-4, Haifa, Israel). The consumables were Milli-Q water (EMD-Millipore machine Model 13681), Whatman filter paper (125 mm, 11 μm, Maidstone, England), and Eppendorf tubes.

2.4. Total moisture determination

2.4.1. Experimental procedure

The total moisture determination was carried out per the method of International Association of Official Analytical Chemists (AOAC, 2005). Moisture dishes were washed with Mili-Q water, oven-dried at 105 °C for 3 h, cooled in a desiccator for 30 min, and weighed (W1). Different weights (1, 2, and 3 g) of each plant material (red chili fruit, red tomato fruit, green spinach leaf, and green lettuce leaf) were added into moisture dishes and weighed (W2). The moisture content of each plant sample was obtained by constant weight drying for 12 h at 105 °C Each sample was then cooled in a desiccator for 30 min and weighed (W3). The percentage of total moisture was calculated using the following relations:

$$\% \text{ Total moisture} = \frac{W_2 - W_3}{W_{2-W_1}} \times 100$$

Where: W1 = initial weight of empty moisture dishes

W2 = weight of moisture dishes + samples before drying

W3 = Final weight of moisture dishes + samples after drying

2.5. Crude ash determination

2.5.1. Experimental procedure

The procedure was conducted as described by the International Association of Official Analytical Chemists' method (AOAC, 2005). Empty crucibles were weighed (W1). Various weights (1, 2, and 3 g) of each previously oven dried (105 °C for 3 h) plant material (red chili fruit, red tomato fruit, green spinach leaf, and green lettuce leaf) was added into crucibles and weighed (W2). They were incinerated in a muffle furnace at 550 °C for 6 h, allowed to cool in a desiccator for 1 h, and weighed (W3). The percentage of crude dry ash was determined as follows:

$$\% \text{ Crude Ash} = \frac{W_3 - W_1}{W_{2-w_1}} \times 100$$

Where: W1 = weight of empty crucibles

W2 = weight of crucibles + samples before incineration

W3 = weight of crucibles + samples after incineration

2.6. Crude fiber analysis

2.6.1. Experimental procedure

The crude fiber analysis was done as explained by the method of International Association of Official Analytical Chemists (AOAC, 2005). Different weights (1, 2, and 3 g) of each previously oven dried plant material (red chili fruit, red tomato fruit, green spinach leaf, and green lettuce leaf) were weigh out (W1) and placed in round bottom flasks. To each plant sample in the round bottom flask, 100 mL sulphuric acid solution (0.25 M) was added. Each mixture was boiled under reflux for 30 min with continuous swirling. Each mixture was then filtered under suction using a Buchner funnel. The insoluble fiber (residue) of each plant sample was washed thoroughly with boiling Milli-Q water until acid-free. The acid-free insoluble residue of each plant was transferred to a second round bottom flask and 100 mL of 0.30 M sodium hydroxide solution was added. Each mixture was boiled under reflux for 30 min with continuous swirling. Each mixture was then filtered with a Whatmann No. 1 filter paper under suction and washed with boiling Milli-Q water until base-free. Each insoluble residue was air-dried in an oven at 105 °C for 3 h, cooled in a desiccator for 30 min, and weighed (W2). Finally, each dried residue was incinerated at 550 °C for 6 h in a muffle furnace, cooled in a desiccator, and weighed (W3). The percentage crude fiber content was calculated using the formula:

$$\%\text{Crude Fiber} = \frac{W_2 - W_3}{W_1} \times 100$$

2.7. Total carbohydrate determination

2.7.1. Sample extraction

Each plant sample extraction was carried out as reported by Mannem et al. (2012), with modification in sample weight and volume of methanol added. To each 10 g of multiple dried sample, 50 mL of 80% HPLC-grade methanol was added. Each mixture was boiled on a heating plate for 5 min and allowed to stand for 30 min to complete extraction. Each extract was filtered through Whatman No. 1 filter paper. The various filtrates were oven dried at 70 °C to remove methanol. To generate a 1.0 mg mL^{-1} sample solution, 1 mg of each residue was dissolved in 1.0 mL of 0.1 M Lead acetate (clarifying agent) solution and vortexed. Each solution was then centrifuged at 2,000 RCF × g for 5 min. Lastly, the supernatant of each sample solution was diluted with Milli-Q water in a ratio of 1:25 and determined for total carbohydrate content.

2.7.2. Standard preparation

A glucose stock solution was prepared by dissolving 5.0 mg of glucose crystals in 1.0 mL of Milli-Q water. A working standard solution of 500 μg mL^{-1} was generated from the stock. Different concentration values of 25, 50, 100, 200, and 300 μg mL^{-1} were made from the working standard by dilution with Milli-Q water.

2.7.3. Experimental procedure

The procedure was conducted as reported by Albalasmeh et al. (2013). This procedure is analogous to the phenol–sulfuric acid method of DuBois et al. (1956). An aliquot of 250 μL of each sample solution was mixed with 50 μL of ice-cooled concentrated sulfuric acid (98.9%). Each reaction mixture was then vortexed for 30 s and incubated for 2 min at room temperature (25±5 °C). The absorbance of each reaction mixture was read at 315 nm against a reagent blank, using a 96-well plate reader.

2.8. Soluble protein determination

2.8.1. Sample extraction

Protein extraction from each plant material was done as reported by Barman et al. (2015), with modifications in sample weight and volume of phosphate buffer added. To 1.0 g of each multiple dried sample, 25 mL of a cold phosphate buffer (0.1 M, pH 7.4) was added. Each sample mixture was kept overnight at 4 °C to extract the protein completely. The various samples were centrifuged (4,000 RCF × g) at 4 °C for 20 min and filtered with Whatman No. 1 filter paper.

2.8.2 Standard preparation

To 5 mg of bovine serum albumin crystals, 1.0 mL of distilled water was added to produce a stock solution of 5 mg mL^{-1}. A standard working solution of 500 μg mL^{-1} was generated from the stock solution. Different concentration levels of 50, 100, 200, 300, 400, and 500 μg mL^{-1} were made from the working standard by dilution with Milli-Q water.

2.8.3. Experimental procedure

The assay was performed as described by Bradford (1976). To 200 μL of each sample extract, 1.20 mL of the reagent (Bradford reagent) was added and mixed. Each sample mixture was allowed to stand for 15 min at room temperature (25±5 °C). The absorbance of each mixture was read at a wavelength of 595 nm against the reagent blank using a 96 well plate reader.

2.9. Statistical analysis

All data obtained from this research were statistically evaluated with repeated-measures analysis of variance (RM ANOVA). The level of significance applied was 5%. As the RM ANOVA revealed a significant difference among seasons, a post-hoc test using unpaired student's t-test is performed to detect the position where the significant difference between seasons occurred.

3. Results and discussion

Tables 1 to 5 depict the results for the total moisture, crude ash, crude fiber, total carbohydrate, and soluble protein contents, respectively, of the plant materials for the comparative seasonal research.

3.1. Total moisture

In this research, the total moisture (TMO) level of the red chili (RCH) fruit (fresh weight basis) indicated no significant ($P>0.05$) different in the winter, spring, and autumn, however, statistically different in the summer period. (Table 1). There was a notable significant ($P<0.05$) variation in the TMO of the red tomato (RTO) fruit among the comparative four (4) seasons (Table 1). The TMO of the green spinach (GSP) leaf and green lettuce (GLE) leaf were significantly ($P<0.05$) varied among the winter, spring, and summer. The TMO of the GSP and GLE detected in autumn were similar to those obtained in the summer and spring (Table 1). In this research, the winter indicated the highest TMO for the RCH, RTO, GSP, and GLE plant materials (Table 1).

Table 1. Seasonal changes in the total moisture content

	Total moisture (%)			
	Plant materials (n = 3)			
Seasons	RCH, fw	RTO, fw	GSP, fw	GLE, fw
Winter	74.17±1.20^{a}	88.79±0.78^{a}	92.40±0.61^{a}	94.71±0.48^{a}
Spring	71.82±0.33^{a}	83.41±1.81^{b}	90.04±0.21^{b}	90.54±0.21^{b}
Summer	67.25±2.05^{b}	72.16±0.89^{d}	86.56±1.23^{c}	87.25±0.52^{c}
Autumn	73.82±1.94^{a}	79.52±1.02^{c}	88.18±0.49^{bc}	88.52±2.19^{bc}

fw = fresh weight, RCH = red chili fruit, RTO = red tomato fruit, GSP = green spinach leaf, GLE = green lettuce leaf, n = number of repeats of each plant sample. The results were expressed as mean±SD. Values with different superscript letters between seasons in a column are significantly (P<0.05) different from each other.

There were no reports on the influence of seasonal changes on the TMO level of the RCH, RTO, GSP, and GLE plant materials. Notwithstanding, the TMO level of the RCH fruit of the present work was lower than the finding (93.00±1.80%) of Dalhatu et al. (2018) of the Nigerian *Capsicum annum* from open and solar drying. Likewise, the observed TOM level for the RTO fruit was lower compared with the reported value (93.50±0.21%) by Hanif et al. (2006). The TMO content of the GSP leaf of this research was compatible with the cited value (94.20±0.04%) of Singh and Sehgal (2001). However, higher than the reported level (81.72±0.40%) from soil farming method by Sani et al. (2011). Nevertheless, the TMO of the GLE leaf of this study was lower than the amount reported for different cultivar from rainy (12.58±1.36) and dry (12.58±1.36) seasons (Birnin-Yauri et al., 2011).

The winter period and GLE leaf revealed the highest TMO level in this study (Table 1). The higher TMO revealed by the study plants (RCH, RTO, GSP, and GLE) in the winter could be linked to the characteristic low temperature and transpiration rate characteristics of the season. In this current research, seasonal changes have induced variations in the TOM levels of the RCH, RTO, GSP, and GLE plant materials.

3.2. Crude ash

A significant (P<0.05) difference in the crude ash (CAS) content (dry weight basis) of the red chili (RCH) fruit, red tomato (RTO) fruit, green spinach (GSP) leaf, and green lettuce (GLE) leaf was detected among the comparative seasons. The highest CAS level of the RCH, RTO, GSP, and GLE plant samples was obtained in the summer (Table 2). The RCH possessed the highest CAS content in this season (summer).

Table 2. Seasonal variations in the crude ash content

	Crude ash (%)			
	Plant materials (n = 3)			
Seasons	RCH, dw	RTO, dw	GSP, dw	GLE, dw
Winter	10.17±0.02^{d}	0.87±0.01^{d}	0.49±0.06^{d}	0.48±0.06^{d}
Spring	11.78±0.43^{c}	1.22±0.07^{c}	0.76±0.17^{c}	0.81±0.12^{c}
Summer	13.47±0.32^{a}	1.74±0.05^{a}	1.64±0.29^{a}	1.54±0.23^{a}
Autumn	12.16±0.09^{b}	1.39±0.05^{b}	1.26±0.09^{b}	1.13±0.27^{b}

dw = dry weight, RCH = red chili fruit, RTO = red tomato fruit, GSP = green spinach leaf, GLE = green lettuce leaf, n = number of repeats of each plant material. The results were resented as mean±SD. Values with different superscript letters between seasons in a column are significantly (P<0.05) different from each other.

The effect of seasonal variations on the CAS level was lacking in the existing literature. However, the CAS value of the RCH fruit obtained in this experiment was compatible with the amount (10.67±1.89%) reported by Dalhatu et al. (2018) for the open and solar drying *Capsicum annum* from Nigerian. But, higher than the finding (6.26±0.15%) of Sharma et al. (2017). The CAS percentage of the RTO fruit of the present research was higher compared with the amount (0.80±0.01%) reported by Hanif et al. (2006). Similarly, the CAS level of the GLE leaf of this work (summer and autumn periods) was higher than the reported amount (0.90±0.05%) of Hanif et al. (2006). However, lower than values obtained in the winter and spring. It is only in the summer and autumn that the GSP leaf indicated a higher CAS content in comparison to the finding (1.10±0.15%) of Hanif et al. (2006).

From finding (1.9±0.02%) of Bangash et al. (2011) and the cited value (1.71±0.09%) by Barman et al. (2015), the CAS level of the GSP leaf sourced from normal ground cultivation was not in support to the detected amount of this experiment. The elevated CAS amount observed during the summer season and the RCH fruit may be attributed to an augmented mineral composition, farming method, and or cultivar type (Nuri et al., 2014). In this study, seasonal differences have induced differences in the CAS value of the experimental plants.

3.3. Crude fibre

There was significant (P<0.05) difference in the crude fiber (CFI) amount (dry weight basis) of the red chili (RCH) among the experimental four (4) periods (Table 3). Although there was no significant (P>0.05) difference between spring and autumn values for the RTO and GLE plant materials and between summer and autumn values for the GSP, a significant (P<0.05) differences were found between other seasonal comparisons of the CFI values of these plants (RTO, GSP, and GLE) (Table 3).

Table 3. Seasonal differences in the crude fibre content

	Crude fiber (%)			
	Plant materials (n = 3)			
Seasons	RCH, dw	RTO, dw	GSP, dw	GLE, dw
Winter	19.87±0.28^{d}	9.28±0.29^{c}	4.89±0.08^{c}	10.29±0.2^{c}
Spring	23.71±1.05^{c}	16.90±0.79^{b}	6.46±0.62^{b}	11.70±0.36^{b}
Summer	38.21±2.90^{a}	19.29±1.14^{a}	9.78±0.64^{a}	13.84±0.33^{a}
Autumn	32.06±0.41^{b}	17.67±0.25^{b}	8.67±0.24^{a}	11.95±0.23^{b}

dw = dry weight, RCH = red chili fruit, RTO = red tomato fruit, GSP = green spinach leaf, GLE = green lettuce leaf, n = number of repeats of each vegetable plant. The results were expressed as mean±SD. Values with different superscript letters between seasons in a column are significantly (P<0.05) different from each other.

The highest CFI content for the RCH, RTO, GSP, and GLE plant samples was indicated in the summer (Table 3). The higher CFI content obtained in the summer could be due the observed increased in the biomass accumulation and cultivation practice differences (Nuri et al, 2014). The RCH fruit revealed the highest CFI value compared with RTO, GSP, and GLE plants materials (Table 3). The influence of seasonal impact on the percentage CFI of the RCH, RTO, GSP, and GLE plant materials was not detected in the previous studies. Notwithstanding, the CFI value of the RCH fruit in this research was higher than the finding (18.33±0.76%) of Dalhatu et al. (2018) of the Nigerian capsicum annum from open and solar drying. The CFI content for each RTO fruit, GSP leaf, and GLE leaf of this research was greater than the obtained levels of 0.3±0.07%, 0.6±0.01%, and 0.7±0.20% in tomato, spinach, and lettuce, respectively, by Hanif et al. (2006). In this experimental work, the CFI amounts of the evaluated plant samples have been affected by seasonal changes.

3.4. Total carbohydrate

In this study, the statistical analysis of the data revealed that the total carbohydrate (TCA) content of plant samples on a dry weight basis exhibited significant (P < 0.05) differences in different seasons (Table 4). However, there was no significant (P>0.05) difference between the values of the red chili (RCH) fruit observed in the winter and spring, between the values of the red tomato (RTO) fruit and green spinach (GSP) leaf observed in the spring and autumn, and between the values of the green lettuce (GLE) leaf obtained in the spring and summer. The summer revealed the highest TCA among the comparative seasons (Table 4).

Table 4. Seasonal variations in the total carbohydrate

	Total carbohydrate (%)			
	Plant materials (n = 3)			
Seasons	RCH, dw	RTO, dw	GSP, dw	GLE, dw
Winter	4.75±0.04^{c}	5.03±0.01^{c}	1.01±0.01^{c}	2.55±0.03^{c}
Spring	5.01±0.04^{c}	5.44±0.04^{b}	2.00±0.05^{b}	3.57±0.07^{a}
Summer	5.98±0.01^{a}	6.91±0.06^{a}	2.89±0.08^{a}	3.77±0.01^{a}
Autumn	5.74±0.08^{b}	5.45±0.05^{b}	2.05±0.06^{b}	2.92±0.23^{b}

dw = dry weight, RCH = red chili fruit, RTO = red tomato fruit, GSP = green spinach leaf, GLE = green lettuce leaf, n = number of repeats of each plant sample. The results were given as mean±SD. Values with different superscript letters between seasons in a column are significantly (P<0.05) different between seasons.

Among the experimental plant materials, it was observed that the RCH exhibited higher TCA values than the other samples in the autumn, while the RTO exhibited the highest values in all other seasons. The impact of seasonal variations on the TCA amounts of the RCH, RTO, GSP, and GLE plant samples in not found in the existing repots. Nevertheless, the percentage TCA for the RCH fruit obtained in this research was higher compared with finding of Ananthan et al. (2014) of six (6) different cultivars sourced from local markets, which range between 1.96±0.09% to 2.71 ± 0.06%. However, much lower than the reported value (66.40±1.3) of Dalhatu et al. (2018) from the Nigerian capsicum annum. The total TCA for RTO fruit of this study was higher in comparison to the value (3.90±0.10%) obtained by Hanif et al. (2016). The reported TCA amount of (2.92±0.05), (2.93±0.04%), and (4.00±0.12%) by Asaolu et al. (2012), Barman et al. (2015), and Hanif et al. (2016), respectively of a spinach from soil farming was higher than detected levels in the present work. Likewise, a greater than the level (3.90%) cited by Ranawade et al. (2017) from aquaponics spinach. Similarly, a relatively higher percentage (2.90%) was reported by Kumar et al. (2020) in comparison to the current study. Higher temperature and strong light intensity characteristics features of the summer season enhances sugar synthesis in plants (Lee and Kader, 2000; Caruso et al., 2011). However, not in support with the published research by Ferreyra et al. (2007), they detected that lower temperature which is linked to the winter period promotes sugar production in the alpine strawberry grown in a greenhouse. In this study, the TCA levels of the experimental plant materials were affected by the seasonal differences.

3.5. Soluble protein

The results of the statistical evaluation conducted for the soluble protein (SPR) content (Table 5) indicated no statistically significant differences (P>0.05) between the SPR values of the RCH on a dry matter basis in spring and autumn, and between the values of the RTO in winter and summer. However, there were statistically significant differences (P<0.05) between other seasonal comparison of the values of above samples (RTO and RCH). In addition, a statistically significant differences (P<0.05) existed between all seasonal comparison of values of the GSP and GLE plant samples (Table 5).

Table 5. Seasonal differences in the soluble protein

Seasons	Soluble protein (%)			
	Plant materials (n = 3)			
	RCH, dw	RTO, dw	GSP, dw	GLE, dw
Winter	0.29±0.02^{b}	0.28±0.03^{a}	0.34±0.01^{d}	025±0.01^{c}
Spring	0.24±0.01^{c}	0.23±0.01^{b}	0.79±0.01^{b}	0.30±0.01^{b}
Summer	0.30±0.03^{a}	0.29±0.03^{a}	0.83±0.00^{a}	0.35±0.03^{a}
Autumn	0.26±0.03^{c}	0.22±0.06^{c}	0.68±0.01^{c}	0.22±0.01^{d}

dw = dry weight, RCH = red chili fruit, RTO = red tomato fruit, GSP = green spinach leaf, GLE = green lettuce leaf, n = number of repeats of each experimental sample. The results were expressed as mean±SD. Values with different superscript letters between seasons in a column are significantly (P<0.05) different between seasons.

In this experiment, the highest SPR level for the RCH fruit, RTO fruit, GSP leaf, and GLE leaf was detected in the summer season (Table 5). The GSP leaf showed the highest percentage of SPR among the experimental plant samples.

There were no reports of the effect of seasonal variations on the SPR values of the investigated plant materials from the aquaponics farming. However, Ananthan et al. (2014) reported the SPR values between 0.91±0.02 to 1.98±0.10% from six (6) different cultivars of the red chilies obtained from local markets. Values obtained from the above report were higher than the amount detected in the present research. Hanif et al. (2006) reported a higher soluble protein from tomato fruit (0.9±0.06%) and lettuce (1.2±0.00%) in comparison with obtained amounts in this study. The percentage SPR observed from the GSP leaf in this finding was lower than values of 1.45±0.03%, 2.00%, and 2.70% reported by Barman et al. (2015), Kumar et al. (2020), and Ranawade et al. (2017), respectively. Variation in the SPR levels in the investigated plants in comparison to the above findings might be due to cultivar differences and or employed analytical methods. In the current research work, seasonal differences have induced changes in the level of SPR of the RCH, RTO,GSP, and GLE vegetable plants.

4. Conclusion

This study determined the impacts of seasonal variations on proximate nutritional makers of some vegetable plants derived from the aquaponics cultivation. The total moisture (TMO) content (fresh weight basis); the crude ash (CAS), crude fibre (CFI), total carbohydrate (TCA), and soluble protein (SPR) values (dry weight basis) of the red chili (RCH) fruit, red tomato (RTO) fruit, green spinach (GSP) leaf, and green lettuce (GLE) leaf were significantly ($P<0.05$) different among the comparative seasons. The highest CAS, CFI, TCA, and SPR contents of the RCH, RTO, GSP, GLE plants materials were detected in the summer period. However, the TMO content of the RCH, RTO, GSP, and GLE was detected significant ($P<0.05$) in the winter period. Therefore, the findings of this research indicated that aquaponics food farming and harvesting in the warmer periods could significantly increases the nutritional value composition in plants.

Compliance with Ethical Standards

Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contributions

The author carried out the methodology, investigation, conceptualization, data analysis, and curation. The author also write, review, edit, and validate the original draft of the research manuscript.

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Not applicable.

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Data availability

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Functional and pasting properties of starches isolated from unripe and ripe cultivars (two) of aerial yam bulbils

Adetunji Ismael LAWAL [1*], Rahman AKINOSO [2], Kazeem Koledoye OLATOYE [3], Folasade Omowumi AFOLABI [1]

[1] Department of Food Science and Technology, Faculty of Engineering and Technology, First Technical University, Ibadan, Nigeria
[2] Department of Food Technology, Faculty of Technology, University of Ibadan, Ibadan, Nigeria
[3] Department of Food Science and Technology, Faculty of Agriculture, Kwara State University, Malete, Ilorin, Kwara State, Nigeria

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* CONTACT

tunjawal@gmail.com

A B S T R A C T

The impact of the harvesting period (sixth, seventh and eighth month) on functional and pasting properties of starches from Tob2857 and Tob3059 cultivars of aerial yam was investigated. Matured yam bulbils were classified into two; matured ripe bulbils (MRB) and matured unripe bulbils (MUB) based on their peel colors at harvest. Starch content, amylose, resistant starch (RS), pasting and morphological characteristics of starches were evaluated using standard methods. RS (5.11-12.37%), amylose (14.88-20.15%) and solubility (1.16-3.98%) were higher in MUB than MRB starches and varied with the harvesting period. Pasting profiles revealed that peak, hot, break down, cold paste and set back viscosities, respectively ranged thus; (261.50-528.92 RVU), (269.00-458.38 RVU), (16.95-70.54 RVU), (304.14-576.79 RVU) and (71.42-118.41 RVU). Pasting time and temperature ranged from 4.68-5.92 min and 84.18-88.26 °C, respectively. The swelling power (0.53-9.85%) varied significantly between the cultivars and ripeness of the bulbils. The starch granules showed similar shapes (ovo-triangular-oblong) and granule size (16.80-32.34 μm) varied significantly with the harvesting period. The starch of good functionality was obtained from the MRB of both cultivars in the 8th month. The variation in functionality of aerial yam starch cultivars at different harvesting periods in this study could be exploited for the postharvest valorization of aerial yam starches.

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ORCID> 0000-0003-3097-8790 (AIL), ORCID> 0000-0002-2780-5760 (RA), ORCID> 0000-0003-4250-164X (KKO), ORCID> 0009-0009-7907-7724 (FOA)

1. Introduction

Yam, a significant staple crop in Africa, is mostly produced in Nigeria (>75% of global production) and is considered a luxury commodity for pounded yam production by traditional people (FAO, 2022). The rising price has required research into alternative lesser-known kinds, such as aerial yam. Aerial yam, *Dioscorea bulbifera* (L.) also known as potato yam is one of the five economically important species in the genus Dioscorea (Akinwande et al., 2008). It is a tropical and sub-tropical crop with vast strength for growing as a wild or cultivated variant and requires fewer pre-planting operations and capital compared to other yams (Olatoye and Arueya, 2019). This yam species had added value over other yams, because of its early maturity, rich sources of phytochemicals and essential nutrients (Lawal and Akinoso, 2019). Aerial yam contains about 77.76% carbohydrate (majorly starch), 6.39% crude protein, 1.50% crude fibre, 0.86% fat, 3.81% ash and 9.50% moisture (Olatoye and Arueya, 2019). Besides the utilization of yam as a staple food, the application of their isolated starch has also been reported by Abiodun and Akinoso (2015). Starches from aerial yam are now gaining research attention as a result of their resistant starch content, which can aid weight loss and act as an essential substrate for promoting the growth of beneficial microorganisms in the gut in addition to the thickening, gelling and stabilizing roles of starch in food industries (Libra et al., 2011). The starch contents like all other food components could exhibit variation based on inherent (cultivars), environmental (harvesting season) and cultural (harvesting period) factors. Attempts have been made by the International Institute of Tropical Agriculture (IITA) to promote aerial yam cultivation by developing new cultivars with better agronomical traits and yields, however, variation existed in their nutrient composition during growth (Lawal et al., 2023). Changes in aerial yam bulbils upon maturity (after 6th month of planting) are easily obvious via the peel color and could be an important parameter for the assessment of harvesting periods since the ripening of bulbils could influence the stability of starch (Lawal and Akinoso, 2019). According to Abiodun and Akinoso (2015), starch content increased with the harvesting period of trifoliate yam, but other studies reported variable levels of starch from different peel colors of aerial yam at a single harvest time (Princewill-Ogbonna and Ibeji, 2011; Libra et al., 2011). The greatest impediment to commercial aerial yam production in Nigeria and other West African nations is a lack of information on the ripening of aerial yam cultivars at various harvesting periods. To exploit the application of aerial yam starches in value-added products, there is a need to have adequate knowledge about their starches during growth and ripening. Thus, this work was carried out to evaluate the role of the ripening and harvesting period on the physicochemical attributes of the starches from two aerial yam cultivars.

2. Materials and methods

The aerial yam germplasms (Tob2857 and Tob3059) used in this study were obtained from the IITA, Ibadan and planted in April 2021, 2022, and 2023. Matured and healthy bulbils were harvested randomly at three different harvest regimes (6, 7 and 8 months after planting), classified into matured unripe bulbils (MUB) and matured ripe bulbils (MRB) based on the visual peel color at harvest (Figure 1).

Figure 1. Visual color of aerial yam bulbils at harvest (MUB, matured unripe bulbils; MRB, matured ripe bulbils)

2.1. Preparation of AYB starches

Starch extraction was done using water extraction due to its cost-effectiveness and minimal damage to starch granules as described by Abiodun and Akinoso (2015). Healthy bulbils were peeled, cut into cubes (5 mm) and washed with potable water. The AYB cubes were then blended (Kenwood BL380, Malaysia), mixed with excess water (1:3) and sieved through triple-layered cheese cloth. The filtrate (starch) was allowed to settle (16 h), and water was decanted and centrifuged. The residue starch which was scrapped into trays before oven drying (60 oC for 12 hours) was milled, sieved (600 μm), packaged in laminated packaging, and stored in a freezer until required for further analysis. AYB starch yield was estimated as the percentage ratio of starch mass to the peeled bulbil's mass.

2.2. Determination of amylose, amylopectin and resistant starch contents

The amylose contents of AYB starches were measured as previously described by Li et al. (2020). It is simply a colorimetric method that uses a spectrophotometer (U-1500; Hitachi, Japan) to measure starch-iodine color (blue) formed at pH 4.5-4.8 in acetate buffer (29±2 °C for 20 min in a dark room). Absorbance was measured (620 nm) and estimation of amylose content was done from a standard potato-amylose curve. Amylopectin was determined by deducting amylose content from 100%.

For the enzymatic determination of resistant starch content, paste aliquots of 100 mg starch were dispersed in 20 mL of phosphate buffer, pH 6.0 (55.6 mM), and 0.16 g of α-amylase (A-3176, Sigma-Aldrich, USA) added to each tube, incubated for 16 h at pH 4.5. About 0.4 mL of amyloglucosidase (A-7095, Sigma-Aldrich, USA) was added, incubated (60 °C for 30 min) and centrifuged (4,000 g for 15 min). The residue obtained was suspended in 20 mL of phosphate buffer, pH 7.5 (0.08 M), and 0.4 mL of protease (P-2143, Sigma-Aldrich, USA) and incubated for 4 h at 42 °C. The sample was centrifuged (6,000 × g for 15 min), dried (60 °C for 24 h) and weighed to determine resistant starch content.

2.3. Swelling power

The swelling power was carried out in glass tubes (with screw caps) containing samples (0.20 g) mixed with distilled water (18 g) and completed to 20 g (AACC, 2000, method 56-21.01). The tubes were constantly agitated in a water bath (60 - 90 °C at 10 °C intervals) for 30 min and centrifuged for 5 min (1700 g). The supernatant was removed carefully, and swelling power was determined as sediment weight divided by dry weight of flour (g/g).

2.4. Water binding capacity and solubility

Water binding capacity (WBC) and solubility of AYB starches were determined using centrifuge procedure 38-12-02 of AACC (AACC, 2000). The starch sample (0.5 g) mixed with distilled water (10 mL) was stirred for 1 min and centrifuged for 15 min at 500 rpm. The WBC was expressed as the weight of water bound by the dry flour from the residue. For solubility, the supernatant was decanted into a weighed evaporating dish and dried at 100 °C for 20 min. The difference in weight of the evaporating dish was used to estimate the starch solubility.

2.5. Wettability

The wettability was determined using the method of Akinoso et al. (2021). The AYB sample (1 g) in a graduated cylinder (25 mL) of 1 cm diameter was inverted with a finger placed over the open end and clamped at a height of 10 cm from the surface of a 600 mL beaker containing 500 mL distilled water. The finger was removed, and the sample fell freely into the beaker.

2.6. Pasting properties

Pasting characteristics were determined with a Rapid Visco Analyser (RVA), (RVA Superty, 2011, 2112582-S4A, Australia). The mixture sample (3 g) and distilled water were dispensed into the canister containing the sample to make a total weight of 28 g suspension, held at 50 °C for 1 min and later heated to 95 °C. It was held at 95 °C for 3 min before subsequently cooled to a constant temperature of 50 °C within 4 min period (Kaushal et al., 2012).

2.7. Morphological properties and granules diameter

Bulbil starch (0.5 mg) was dissolved into distilled water (10 mL) and agitated for 1 min in an ultrasonic bath. Then, a few drops of the starch suspension obtained were placed on a glass slide followed by two to three drops of potassium iodide (KI) stain. Light microscope image was acquired under high-power magnifications (x40 and x100). Starch grain diameters were measured with Image J software and classified according to their size (Singh et al., 2006).

2.8. Statistical analysis

All the experiments were carried out in triplicate and data were analyzed for conformance to normality distribution before analysis of variance (ANOVA) was conducted. Both analyses were carried out using SPSS package 16.0 and the values were expressed as means ± standard deviations and the statistical significance level was selected to be $p<0.05$.

3. Results and discussion

3.1. Starch yield, amylose, amylopectin and resistant starch contents

The starch yield of AYB varied significantly ($p<0.05$) between 8.23% (MUB of Tob3059) and 19.63% (MRB of Tob2857) during the 6th and 8th months harvesting period, respectively. The highest values at 8 months were higher than 10% reported for taro at 10th month of harvest (Abo-El-Fetoh, 2010), but lower than the range (39.62 - 57.26% and 17.28 – 35.37 .68%) reported for potato and cassava (Tsakama et al., 2010; Chisenga et al., 2019). The MRB of both cultivars showed a significantly higher yield of starch than MUB. However, despite the observed lowered starch contents of MUB, these values are within the values reported in trifoliate yam (5.09-12.07%) harvested between 8 and 10 month after planting (Abiodun and Akinoso, 2015). The component regarded as the most important part of starch is amylose since its content is a pointer to the usefulness of the starch (Addy et al., 2014). It ranged from 14.88 to 20.15% (Table 1).

Table 1. Effect of harvesting period on starch yield, amylose, amylopectin and resistant starch

Cultivar	Harvesting period (months)	Ripeness	Starch yield (%)	Amylose (%)	Amylopectin (%)	Resistant starch (%)
Tob2857	6	MUB	12.02 ± 0.15^{f}	18.64 ± 0.30^{b}	81.36 ± 0.51^{de}	10.62 ± 0.32^{c}
		MRB	15.76 ± 0.18^{c}	16.56 ± 0.14^{ef}	83.44 ± 0.07^{abc}	$7.67.44\pm 061^{f}$
	7	MUB	14.31 ± 0.32^{d}	19.85 ± 0.21^{a}	80.15 ± 0.08^{e}	11.84 ± 0.22^{b}
		MRB	17.62 ± 0.20^{b}	18.45 ± 0.19^{bc}	81.55 ± 0.32^{de}	6.61 ± 0.40^{g}
	8	MUB	13.94 ± 0.21^{de}	20.15 ± 0.06^{a}	79.85 ± 0.24^{e}	12.37 ± 0.50^{a}
		MRB	19.63 ± 0.18^{a}	17.44 ± 0.32^{cde}	82.56 ± 0.09^{bcd}	9.56 ± 0.17^{d}
Tob3059	6	MUB	8.23 ± 0.26^{g}	18.03 ± 0.27^{bcd}	81.97 ± 0.16^{c}	7.52 ± 0.30^{f}
		MRB	11.28 ± 0.14^{f}	14.88 ± 0.45^{f}	85.12 ± 0.25^{a}	5.11 ± 0.26^{h}
	7	MUB	9.27 ± 0.41^{g}	19.09 ± 0.32^{ab}	80.91 ± 0.07^{d}	8.80 ± 0.29^{e}
		MRB	12.58 ± 0.35^{ef}	17.37 ± 0.18^{de}	82.63 ± 0.56^{bcd}	6.87 ± 0.06^{g}
	8	MUB	9.57 ± 0.23^{g}	19.97 ± 0.12^{a}	80.03 ± 0.34^{e}	7.05 ± 0.27^{fg}
		MRB	14.59 ± 0.34^{cd}	15.94 ± 0.08^{f}	84.06 ± 0.02^{ab}	7.43 ± 0.41^{f}

Values in the same column with different superscripts are significantly different ($p < 0.05$)

There was an increase in the amylose content of both MRB between the 6th and 7th month and then declined in the 8th month, this could be attributed to the effect of genetics variability in the aerial yam cultivars. The MUB were significantly higher ($p<0.05$) than the MRB harvested in the same month. The observed increases were 12.56, 7.59 and 15.53% for Tob2857 and 21.17, 9.90 and 25.28% for Tob3059 harvested at the 6th, 7th and 8th months, respectively. The amylose content of MUB was in a range of 18.03-20.15%, which was within the values of 14.45-32.72% reported for other varieties of yam (Ezeocha and Okafor, 2016). High amylose content is also an indication of the lower swelling power of the starches, and such are reputable ingredients for the preparation of low glycemic index starches foods (Arici et al., 2016).

The range (79.85-85.12%) observed for amylopectin was within the range (67.28-85.45%) reported for trifoliate yam starches (Ezeocha and Okafor, 2016).

3.2. Starch granule morphology and swelling power

Starch granules of aerial yam cultivars were detected to be oval, triangular and oblong in shape (Figures 2-3) and the shapes were similar for both MUB and MRB irrespective of the harvesting period. These were consistent with shape classification for D. alata tubers (Tetchi et al., 2012). All the granules had sphericity values (0.48-0.66) that were less than one (Table 5) which is an indication of non-spherical granules. According to Akinoso and Lasisi (2013), sphericity values farther from 1 were regarded as non-spherical. The surfaces of the granules were generally smooth and lacked fissures. Although, fissure on the granules could aid starch hydrolysis, the granules of potato and other yam cultivars have been associated with granules smoothness (Arici et al., 2016). The granule size of aerial yam cultivars was between 20.87 and 27.39 μm. The granules of MRB had higher values than MUB harvested in the same month and the values were significantly different at all harvesting periods except Tob2857 harvested in the 7th month. The increase in sizes of starch granules with the harvesting period recorded in the two cultivars supported the report of Abiodun and Akinoso (2015) that the size of the starch granule increases with the harvesting period of storage organs. For swelling power, the starches of MRB (0.82-9.85 g/g) had higher swelling power than MUB (0.53-7.26 g/g) harvested in the same month, and the values were significantly different ($p<0.05$). Starches with large granule sizes are known for their accelerated swelling rate, high viscosity and ability to withstand shear during processing (Schirmer et al., 2015). Addy et al. (2014) also reported that the size and shape of granules had a very strong influence on the functional, textual and utilization potential of starch. Generally, the swelling power increased with the increase in temperature, and this could be linked to the higher diffusion of water through the amorphous region of amylose and the dissolution of associative chains at higher temperatures. Other researchers have reported higher swelling power for other food crops. Sasaki and Matsuki (2014) reported 13-18 g/g for different varieties of wheat starches. According to Li and Yeh (2001), the swelling power of potato starch was reported as 16.26-30.30 g/g. The open structures and effect of genetics could be responsible for variation in starch contents of different crops (Abiodun and Akinoso, 2015). According to Addy et al. (2014), yam starches with low amylose content were reported to have high swelling power and the increase in randomness of granules resulted in higher swelling of the starches. A similar observation was reported by Abiodun and Akinoso (2015) for trifoliate starches. Thus, the high content of amylopectin in MRB is an indication of the suitability of their starches for incorporation in food products where swelling is desirable.

Table 2. Effect of harvesting period on swelling power of aerial yam starches

Cultivar	Harvesting period (months)	Ripeness	Swelling power (g/g)			
			Temperature (°C)			
			60	70	80	90
Tob2857	6	MUB	0.76 ± 0.05^{fg}	1.70 ± 0.03^{f}	4.39 ± 0.02^{g}	6.49 ± 0.02^{f}
		MRB	1.43 ± 0.02^{bc}	2.47 ± 0.04^{c}	5.06 ± 0.01^{d}	8.13 ± 0.05^{c}
	7	MUB	1.01 ± 0.01^{d}	2.81 ± 0.03^{b}	4.26 ± 0.11^{g}	7.14 ± 0.09^{e}
		MRB	1.50 ± 0.10^{b}	2.84 ± 0.01^{b}	5.83 ± 0.04^{b}	8.53 ± 0.04^{b}
	8	MUB	$0.87 \pm 0.05^{d-f}$	2.45 ± 0.01^{c}	4.87 ± 0.05^{ef}	7.26 ± 0.03^{e}
		MRB	1.72 ± 0.04^{a}	3.34 ± 0.02^{a}	6.15 ± 0.03^{a}	9.85 ± 0.03^{a}
Tob3059	6	MUB	0.53 ± 0.01^{f}	1.41 ± 0.02^{g}	2.65 ± 0.07^{h}	5.11 ± 0.06^{h}
		MRB	0.82 ± 0.03^{c}	1.96 ± 0.03^{e}	5.01 ± 0.05^{de}	7.15 ± 0.07^{e}
	7	MUB	0.74 ± 0.02^{fg}	1.61 ± 0.04^{f}	4.21 ± 0.04^{g}	6.26 ± 0.10^{fg}
		MRB	0.95 ± 0.03^{de}	2.21 ± 0.03^{d}	5.34 ± 0.05^{de}	7.98 ± 0.03^{d}
	8	MUB	0.68 ± 0.05^{gh}	1.94 ± 0.01^{e}	4.75 ± 0.10^{f}	6.23 ± 0.05^{g}
		MRB	1.24 ± 0.05^{bc}	2.23 ± 0.05^{d}	5.32 ± 0.02^{c}	8.26 ± 0.01^{c}

Values in the same column with different superscripts are significantly different ($p < 0.05$)

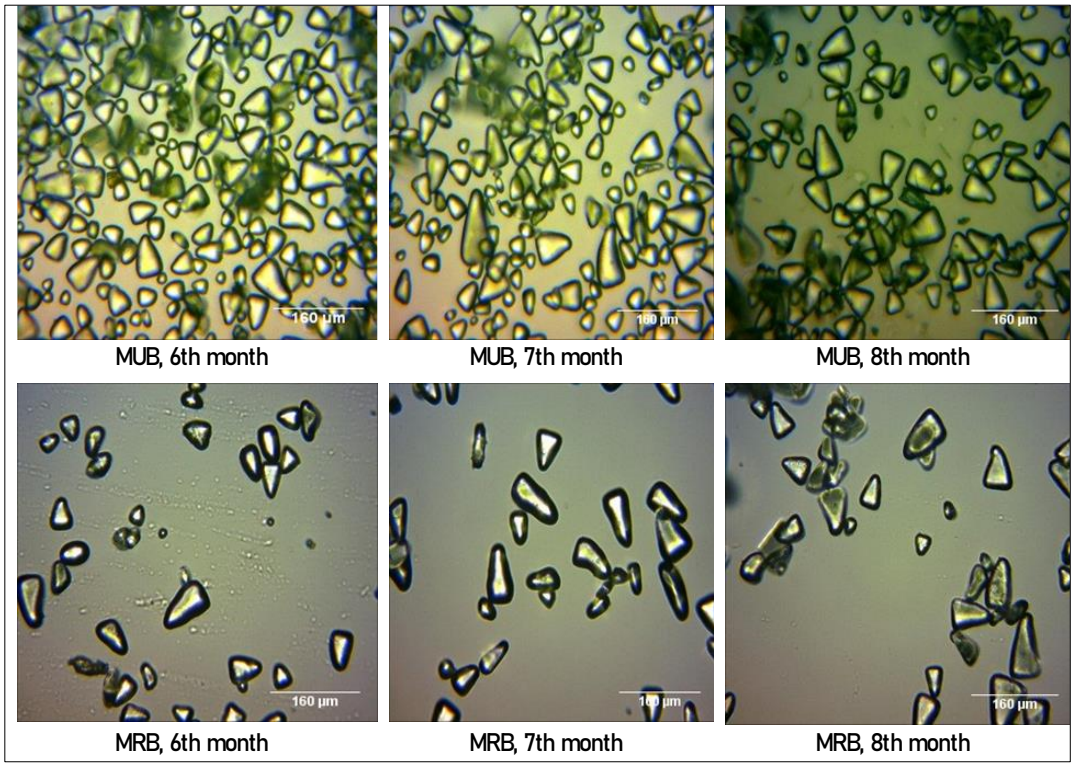

Figure 2. Starch granule morphology of Tob2857 bulbils at different harvesting periods (MUB, matured unripe bulbils; MRB, matured ripe bulbils)

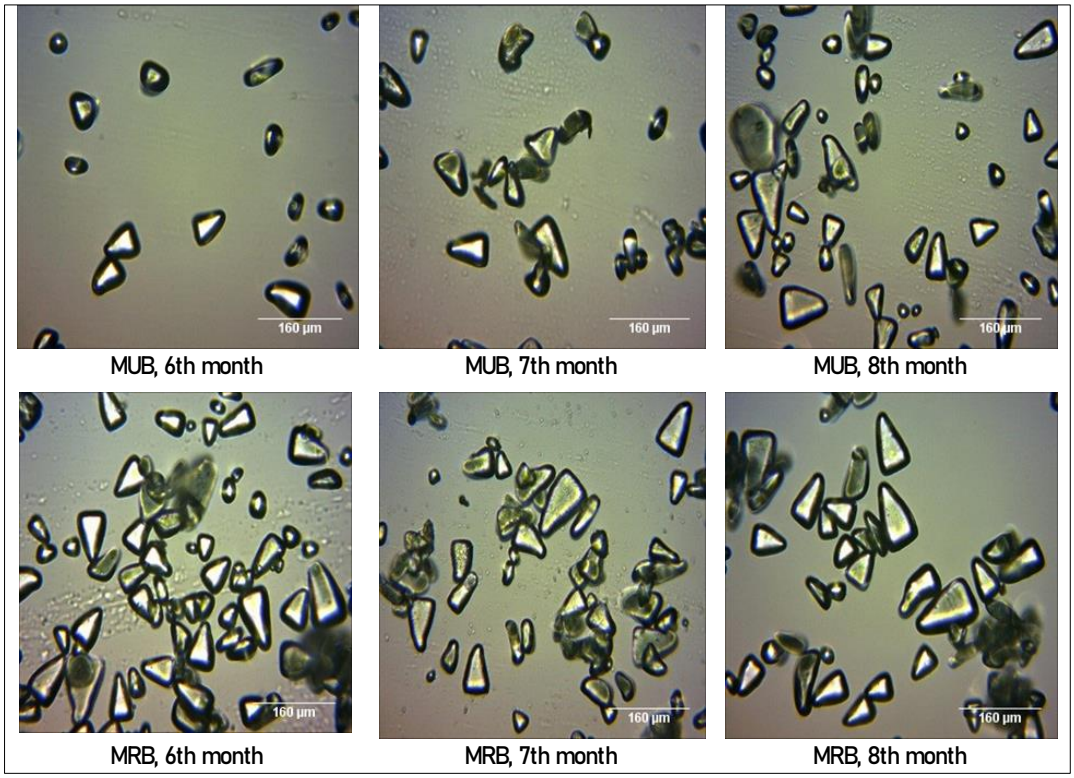

Figure 2. Starch granule morphology of Tob3059 bulbils at different harvesting periods (MUB, matured unripe bulbils; MRB, matured ripe bulbils)

3.3. Water binding, solubility and wettability

Table 3 shows the mean values for water binding capacity (WBC), solubility and wettability of AYB starches. Sample MRB of Tob3059 (8th month) had the highest WBC which differed significantly ($p<0.05$) from all the MUB samples regardless of the harvesting period and cultivars. The WBC ranged from 78.16 to 102.16% and the values increased with the harvesting period for MRB (Tob3059). A similar trend was observed for their MUB harvested between 6 and 7th month after planting. All the MRB showed significantly higher ($p<0.05$) values than MUB harvested in the same month. According to Oke et al. (2013), a lower value of WBC could be attributed to the loss of soluble components of starch during starch extraction. Apart from the leaching of soluble components, the morphology of starches (size, shape and distribution of particles in starches), salts and the presence of sulphur in the starches could contribute to increasing WBC (Abiodun and Akinoso, 2015). Kone et al. (2014) concluded that WBC is an important parameter and a pointer to good textured quality products with higher resistance to the syneresis effect of starch. The solubility of starches samples from MUB and MRB differed significantly ($p<0.05$). MUB of Tob2857 had the highest value in the 6th month and was significantly different ($p<0.05$) from MRB regardless of the harvesting period. Also, the solubility of all MRBs was lower than their respective MUB harvested in the same month. The values of solubility in this study decreased slightly with the harvesting period for MUB of both cultivars and MRB of Tob3059 (between 6 and 8th month). Although solubility had a detrimental effect on the WBC of the starches, higher values have been reported to aid the finely dispersed colloidal liquid with a homogenous structure (Akinoso et al., 2021). This observation was in agreement with the findings of Libra et al. (2011) who indicated the lower starch solubility value (0.01-1.17%) of mauve aerial yam grown in Cote d'Ivoire to maturity effect. The values (2.86-3.98%) observed for MUB of Tob2857 were within the range (2.98-6.68%) reported for water yam starches in Nigeria (Oke et al., 2013).

The wettability of MUB (Tob2857) was lowest (13.00 sec) in the 6th month, and that of the 7 and 8th months (15.00 sec) showed no significant change with the harvesting period. The increase in harvesting period (6-8th months) of MRB showed a relatively high amount of wettability with values ranging from 18.00-25.00 sec and there was a significant difference ($p<0.05$) between MUB and MRB of the same cultivars harvested at the same month except Tob3059 harvested at the sixth month. Wettability measures the ease of samples dispersing in water and the result suggests a faster rate for dissolution of MUB than MRB, which is an advantage in the production of weaning food (Akinoso et al., 2021). The results of MRB (18.00-25.00 sec) were compatible with those found by Oke et al. (2013) who reported wettability values of 17.40-25.62 sec for water yam starches in Nigeria.

Table 3. Water binding capacity, solubility and wettability of aerial yam starches

Cultivar	Harvesting period (months)	Ripeness	Water Binding Capacity (WBC) (%)	Solubility (%)	Wettability (sec)
Tob2857	6	MUB	78.16 ± 0.83^{f}	3.98 ± 0.22^{a}	13.00 ± 1.45^{f}
		MRB	84.56 ± 1.32^{de}	2.06 ± 0.21^{c}	18.00 ± 2.01^{e}
	7	MUB	80.10 ± 0.37^{f}	3.34 ± 0.18^{a}	15.00 ± 1.72^{f}
		MRB	88.21 ± 1.00^{cd}	2.50 ± 0.20^{bc}	21.00 ± 1.33^{cd}
	8	MUB	80.18 ± 0.64^{f}	2.86 ± 0.09^{b}	15.00 ± 2.00^{f}
		MRB	86.76 ± 1.23^{cd}	2.31 ± 0.30^{bc}	23.00 ± 2.41^{abc}
Tob3059	6	MUB	80.12 ± 0.88^{f}	2.15 ± 0.33^{c}	20.00 ± 2.12^{de}
		MRB	95.47 ± 1.20^{b}	1.17 ± 0.21^{d}	22.00 ± 2.20^{bcd}
	7	MUB	84.82 ± 0.58^{d}	2.02 ± 0.30^{c}	20.00 ± 2.36^{de}
		MRB	97.38 ± 0.71^{b}	1.21 ± 0.13^{d}	24.00 ± 2.62^{ab}
	8	MUB	81.72 ± 1.13^{ef}	1.96 ± 0.20^{c}	22.00 ± 1.45^{bcd}
		MRB	102.16 ± 0.87^{a}	1.16 ± 0.10^{d}	25.00 ± 1.45^{a}

Values in the same column with different superscripts are significantly different ($p < 0.05$)

3.4. Pasting properties of AYB starches

The peak viscosity of AYB starches varied significantly from 261.50 RVU (MUB of Tob3059) to 528.92 RVU (MRB of Tob3059) (Table 4). Starches of MRB had the highest value in the 8th month and MUB had the lowest in the 6th month.

Table 4. Pasting properties of aerial yam starches

Cultivar	HP (month)	Ripeness	Peak viscosity (RVU)	Hot paste (RVU)	Break down (RVU)	Cold paste (RVU)	Setback (RVU)	Time (min)	Temperature (°C)	Pasting time (min)
Tob2857	6	MUB	330.08±23.34^{c}	304.38±32.51^{cde}	25.70±25.55^{b}	412.13±32.38^{bc}	107.75±3.41^{d}	5.83±0.18^{abc}	87.30±2.30^{a}	5.83±0.18^{abc}
		MRB	521.13±37.52^{a}	451.75±11.89^{a}	69.38±28.36^{a}	568.25±60.44^{a}	116.50±2.18^{ab}	5.74±0.19^{abc}	84.78±1.56^{b}	5.74±0.19^{abc}
	7	MUB	288.75±19.34^{de}	269.00±24.72^{ef}	19.75±14.27^{b}	371.00±28.36^{cde}	102.00±1.69^{e}	5.92±0.21^{a}	88.26±1.90^{a}	5.92±0.21^{a}
		MRB	515.33±14.31^{a}	447.82±34.72^{a}	67.51±22.31^{a}	561.32±45.51^{a}	113.50±3.40^{bc}	5.64±1.20^{bc}	84.32±1.21^{b}	5.64±1.20^{bc}
	8	MUB	312.72±25.16^{cd}	289.00±37.23^{def}	23.72±26.62^{b}	401.25±28.52^{bcd}	112.25±3.19^{abc}	5.89±0.15^{ab}	87.82±2.30^{a}	5.89±0.15^{ab}
		MRB	528.92±43.65^{a}	458.38±50.21^{a}	70.54±31.35^{a}	576.79±64.75^{a}	118.41±3.25^{a}	5.61±0.16^{c}	84.64±1.40^{b}	5.61±0.16^{c}
Tob3059	6	MUB	267.42±24.51^{e}	250.47±42.35^{e}	16.95±15.28^{b}	321.89±29.96^{f}	71.42±2.14^{h}	5.87±0.11^{ab}	87.16±1.26^{a}	5.87±0.11^{ab}
		MRB	405.92±42.16^{b}	339.91±24.45^{bc}	66.01±21.66^{a}	435.41±35.16^{b}	95.50±2.62^{f}	4.68±0.20^{e}	84.26±1.42^{b}	4.68±0.20^{e}
	7	MUB	261.50±31.34^{e}	243.64±27.51^{f}	17.86±14.64^{b}	304.14±61.52^{f}	60.50±1.57^{i}	5.31±0.14^{d}	87.56±2.17^{a}	5.31±0.14^{d}
		MRB	399.67±27.44^{b}	334.75±32.32^{bcd}	64.92±25.37^{a}	433.33±33.26^{bc}	98.58±3.11^{f}	4.82±0.16^{e}	84.35±1.15^{b}	4.82±0.16^{e}
	8	MUB	293.83±17.76^{cde}	274.63±40.43^{ef}	19.20±13.60^{b}	348.97±31.52^{def}	74.34±2.52^{gh}	5.24±0.18^{d}	87.64±1.22^{a}	5.24±0.18^{d}
		MRB	425.67±40.71^{b}	359.37±31.46^{b}	66.30±27.48^{a}	461.87±37.32^{b}	102.50±2.42^{e}	5.18±0.13^{d}	84.18±1.25^{b}	5.18±0.13^{d}

Values in the same column with different superscripts are significantly different ($p < 0.05$), HP: Harvesting period

The peak viscosities of MRB were significantly higher ($p<0.05$) than the MUB regardless of the harvesting period and the cultivars. The higher peak viscosity of MRB showed the maximum swelling of the granule starches before disintegration and could be an added advantage for food product development that requires strong gel strength and elasticity. Abiodun and Akinoso (2015) also relate the ability of starch to swell freely before breakdown to peak viscosity. Kaushal et al. (2012) opined peak viscosity to be an equilibrium point between granules swelling and breakdown. It is also a valuable parameter useful during the formulation of ingredients and serves as mechanical stress resistance during mixing and kneading. The higher peak viscosity of MRB at different harvesting periods is in consistent with the findings of Akinwande et al. (2008) who observed higher peak viscosity for yam starches with an increase in the swelling power of starches granules. Genetic factors, growing conditions, phosphorus content, starch content and interactions among the components may play an important role in the behavior and texture of starch granules (Schirmer et al., 2015). According to Kaushal et al. (2012), the hot paste viscosity is the starch granules' ability to resist breakdown at high temperatures under mechanical shear stress, the values ranged from 269.00 to 458.38 RVU. The MRB of Tob3059 had the highest in the 8th month and the values reduced in the starches harvested earlier. The hot paste viscosities of both cultivars had lower values than their respective peak viscosities in the same month. In a similar trend to peak viscosity, the starch granules of MRB had significantly higher paste values (334.75-458.38 RVU) than MUB (243.64-304.38 RVU) at all the harvesting periods. The hot paste values for the MUB in this study were within the range of 84.04-356.79 RVU reported by Ezeocha and Okafor (2016). Abiodun and Akinoso (2015) reported a similar trend for trifoliate yam cultivated in Nigeria, in which white had much more hot paste than starches of yellow varieties. For breakdown viscosity, the pattern of results between MUB and MRB at different harvesting periods were similar to peak and hot paste viscosities results. The 8th month harvest of MRB starches had the highest (70.54 RVU), followed by its 6th month (69.38 RVU) while the 6th month harvest of MUB (Tob3059) had the lowest (16.95 RVU) breakdown viscosity. According to Schirmer et al. (2015), higher breakdown viscosity of starches is an indication of lower resistance to heat and shear stress during cooking, and the values obtained in this study were lower than the maximum level of 184.37 RVU reported by Oke et al. (2013) for starches of water yam cultivars cultivated in Nigeria. Thus, lower breakdown viscosity of AYB suggests better stability of the starches under hot conditions compared to starches from water yam.

Cold paste viscosity of the starches ranged from 304.14 RVU (MUB of Tob2857) to 576.79 RVU (MRB of Tob3059). Tob3059 had the lowest value in the 7th month, while Tob2857 had the highest in the 8th month, but there was no significant difference ($p<0.05$) between starches from the same peel color at different harvesting periods. Since cold paste viscosity provides appropriate information for the gelling ability of the starch samples after cooking, higher cold paste viscosity of MRB is an indication of stronger gel formation after cooking compared to MUB starches. Chung et al. (2014) pointed out that continuous aggregation of leached amylose molecules rapidly during cooling results in the formation of amylose chains, responsible for final product viscosity. In a similar study, Schirmer et al. (2015) attributed such an increase in final product viscosity to the formation of an amylose junction zone during the cooling of starches.

The setback viscosities, an estimate of the difference between the final and hot paste viscosities provide knowledge on the tendering of starch to retrogradation (re-association of starch). The setback viscosities of aerial yam starches were between 60.50 and 118.41 RVU. As seen from Table 4, as the harvested period significantly influenced the setback value of MUB, they did not have a generally remarkable effect on MRB irrespective of the cultivars. The starches of MRB of Tob2857 had the highest value at all harvesting periods. The high cohesive paste and low retrogradation tendency during cooling were mentioned in different studies regarding high setback viscosity (Arici et al., 2016; Chung et al., 2014). Since the staling of pastries (bread) is an essential problem in bakeries and other related food products, setback viscosity is, therefore, an important parameter during the incorporation of starches in such foods (Akinoso et al., 2021; Lawal et al., 2024). The food applications such as pounded yam that requires highly cohesive paste could make use of MRB with high setback values (102.00-118.41 RVU), while low setback values (60.50-102.50 RVU) of MUB are added advantage in low viscosities and paste stability food products (weaning food) at low temperature. The selection of AYB for food processing could vary depending on the final quality features of the products in terms of viscosity or texture. The result is in agreement with the observation of Arıcı et al. (2016) on the pasting properties of taro starches.

Pasting time ranged from 4.68 to 5.92 min. The MUB harvested in the 6th month had the lowest pasting time, while pasting time ranged from 4.68 to 5.92 min. The MUB of Tob2857 harvested in the 6th month had the highest pasting time while the MRB of Tob3059 had the lowest. Also, starches from MUB with higher peak time recorded low peak, hot paste, breakdown and cold paste viscosities. According to Addy et al. (2014), higher pasting and cooking time of starches were projected by elevated levels of amylose content which inhibits swelling, similar to those reported in this research for pasting time. The pasting times at different harvesting periods of aerial yam were within the range of values (4.52-6.30 min) reported for taro and trifoliate yam flours (Abiodun and Akinoso, 2015; Arici et al., 2016). For pasting temperature, the values recorded (84.18-88.26 °C) were higher than values (62.20-65.80 °C) reported for sweet potato (Chung et al., 2014) but within the range of values (78.05-86.05 °C) reported for different varieties of water yam (Oke et al., 2013). In the consideration of ideal starch for different food products, energy consumed during production may play a significant role in the cost of the product (Olatoye et al., 2014).

Table 5. Morphological parameters of aerial yam starches

Cultivar	Harvesting period (months)	Ripeness	Shapes	Sphericity	Size (µm)
Tob2857	6	MUB	Ovo triangular oblong	0.66 ± 0.16^{a}	20.87 ± 1.36^{def}
		MRB	Ovo triangular oblong	$0.55 \pm 0.15^{b-e}$	23.97 ± 1.75^{c}
	7	MUB	Ovo triangular oblong	0.59 ± 0.14^{b}	22.48 ± 1.25^{cde}
		MRB	Ovo triangular oblong	0.53 ± 0.11^{def}	24.24 ± 1.64^{c}
	8	MUB	Ovo triangular oblong	0.64 ± 0.15^{a}	23.66 ± 1.42^{c}
		MRB	Ovo triangular oblong	0.48 ± 0.17^{g}	27.39 ± 1.53^{b}
Tob3059	6	MUB	Ovo triangular oblong	0.58 ± 0.20^{b}	16.80 ± 1.92^{g}
		MRB	Ovo triangular oblong	0.57 ± 0.16^{bcd}	19.59 ± 2.07^{f}
	7	MUB	Ovo triangular oblong	0.51 ± 0.18^{efg}	20.25 ± 1.29^{ef}
		MRB	Ovo triangular oblong	$0.54 \pm 0.18^{c-f}$	24.35 ± 2.34^{c}
	8	MUB	Ovo triangular oblong	0.53 ± 0.17^{def}	23.22 ± 1.58^{cd}
		MRB	Ovo triangular oblong	0.50 ± 0.14^{fg}	32.34 ± 2.30^{a}

Values in the same column with different superscripts are significantly different ($p < 0.05$), HP: Harvesting period

4. Conclusion

The effect of the harvesting period on the functional and pasting properties of aerial yam starches was investigated to determine their suitability for different food applications in the food industry. The condition of bulbils at harvest (unripe and ripeness) played an important role in the functional and pasting properties of the starches. The MUB starches of both cultivars (Tob2857 and Tob3059) showed higher resistant starch, solubility and amylose content than their corresponding MRB starches while swelling power increased significantly ($p<0.05$) with the harvesting period and cooking temperature of both cultivars.

The pasting properties of MUB and the corresponding MRB also varied with harvesting period and cultivars. MRB of both cultivars had a high value of peak viscosity in the 8th month, providing useful information to the potential application of aerial yam starches in food processing. The granular sizes of the starches were generally high and varied with the harvesting period and conditions of bulbils at harvest.

Compliance with Ethical Standards

Conflict of interest

The author declares no conflict of interest.

Authors' contributions

All authors contributed to the emergence of the manuscript and approved the final version.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Unravelling the impact of varied organic fertilizer sources on the vegetative and reproductive traits of okra growth and development

Rupesh Kumar MEHTA [1*], Chetana ROY [2], Begam Kumari CHAUDHARY [1], Aniksha MOKTAN [1], Astha KARKI [1], Adhish Kumar ROY [1], Sujata KOIRALA [1], Hikesh GURAGAIN [1], Raju KHATRI [2]

[1] Faculty of Science and Technology, G. P. Koirala College of Agriculture and Research Centre, Purbanchal University, Gothgaun, Morang, Nepal
[2] Faculty of Agriculture, College of Natural Resources Management, Agriculture and Forestry University, Bardibas, Nepal

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* CONTACT

entersgz1907@gmail.com

A B S T R A C T

Nepal's pursuit of sustainable food production and rural livelihoods faces challenges amid evolving environmental pressures. Okra (*Abelmoschus esculentus*) cultivation, integral to Nepalese agriculture, demands innovative approaches to enhance productivity while minimizing environmental impact. This study investigates the efficacy of varied organic fertilizers on okra growth and development, aiming to identify sustainable alternatives to chemical fertilizers. The research was conducted at the G.P. Koirala College of Agriculture and Research Centre in Sundarharaicha, Morang, Nepal, from June to August 2023. A Randomized Complete Block Design (RCBD) with eight treatments replicated three times was employed, including recommended NPK dosage and various organic sources. Observations on plant height, primary branches, pods per plant, pod length, diameter, weight per pod, and yield per plant were recorded. Statistical analysis revealed significant variations among treatments. The highest yield was obtained with the recommended NPK dosage (108.84 g/plant), closely followed by biofertilizers like 100% mustard cake (103.70 g/plant) and goat manure (104.28 g/plant). The lowest yield was observed in the control group (76.99 g/plant). Notably, NPK fertilizer consistently outperformed organic alternatives in promoting okra growth and yield. However, among organic fertilizers, mustard cake and goat manure emerged as promising alternatives, showcasing comparable results to synthetic fertilizers. These findings underscore the importance of balanced nutrient management in optimizing okra productivity. Future research should explore integrated nutrient management strategies, combining organic and synthetic inputs, to enhance sustainability and resilience in Nepalese agriculture.

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1. Introduction

Nepal, renowned for its geographical diversity and agricultural heritage, faces a pivotal juncture in its quest for sustainable food production and rural livelihoods (Sharmin et al., 2023; Yadav et al., 2024a). Among the myriad crops cultivated, okra (*Abelmoschus esculentus*), locally known as "bhindi," holds a significant place, both culturally and economically (Mehata et al., 2022a; Mehata et al., 2023b). Okra, belonging to the Malvaceae family, is a warm-season, annual vegetable prized for its tender pods and nutritional value (Yadav et al., 2023; Bhandari et al., 2019). Cultivated worldwide, it thrives in tropical and subtropical climates, making it an ideal crop for Nepal's diverse agroecological zones (Kumar et al., 2017; Singh Bamboriya et al., 2022). As per the Ministry of Agriculture and Livestock Development's report in 2020, the national production of okra amounted to 11.3 tons per hectare, resulting in a total of 122,101.6 metric tons across an area spanning 10,781.4 hectares (Fasakin et al., 2019; Yadav et al., 2023). Its cultivation not only sustains local food security but also serves as a potential source of income for smallholder farmers (Bamboriya et al., 2018; Meiriani et al., 2023). However, sustaining optimal okra yields amidst evolving environmental challenges and agricultural practices remains a critical concern, particularly in the eastern regions of the country (Giri et al., 2020; Al-Joboory et al., 2021).

The cultivation of okra in Nepal traces back centuries, deeply ingrained in traditional farming practices (Abd Alla et al., 2015; Udin et al., 2022). Despite its resilience to various climatic conditions and adaptability to diverse soils, enhancing okra yields remains a persistent challenge (Singh Bamboriya et al., 2022; Mehata et al., 2023c). Although concerted efforts to enhance agricultural productivity through the adoption of modern farming techniques, including the use of chemical fertilizers (Regmi et al., 2022; Kumari Sah et al., 2024), the outcomes have been mixed, with concerns emerging regarding their long-term sustainability and environmental impacts (Yadav et al., 2024b). The widespread use of chemical fertilizers in Nepalese agriculture has led to a myriad of consequences (Sarker et al., 2018), including soil degradation, water pollution, and adverse effects on human health (Nawalkar et al., 2007; Kumar et al., 2017; Mehata et al., 2023a). Moreover, the dependency on these inputs poses economic challenges for resource-constrained farmers (Omotoso et al., 2018), exacerbating inequalities and perpetuating unsustainable agricultural practices (Muhammad et al., 2020; Riasat et al., 2022). Considering these challenges, there is a growing imperative to explore alternative approaches to enhance okra productivity sustainably (Aluko, 2020; Ter and Maga, 2023). Organic farming, grounded in principles of ecological stewardship and natural resource conservation, emerges as a viable solution (Adekiya et al., 2018; Abbas et al., 2019). By eschewing synthetic inputs in favor of organic fertilizers such as compost, manure, and biofertilizers, organic farming not only mitigates environmental harm (Aggrey et al., 2023; Ishwar et al., 2024; Majhi et al., 2024), but also enhances soil fertility and resilience over the long term (Chattoo et al., 2022; Sikdar et al., 2023).

This study aims to identify alternative biofertilizers that could mitigate these issues and offer sustainable solutions. By conducting comprehensive research into the efficacy of various organic fertilizers on okra growth and yield, this study aims to provide valuable insights for both scientific understanding and practical application in agricultural policies and practices. Ultimately, the goal is to promote the adoption of sustainable alternatives to chemical fertilizers among smallholder farmers in Nepal. Through this endeavor, we aspire to pave the way for a more resilient and equitable agricultural future, ensuring the well-being of both people and the environment.

2. Materials and methods

2.1. Experimental site

From June 2023 to August 2023, the field experiment was carried out at the G.P. Koirala College of Agriculture and Research Centre in Sundarharaicha, Morang, Nepal. The climate in Sundarharaicha, Morang, Nepal, is tropical. The region has 20.87 to 34.45 °C average annual temperature which changes along with 135.87 mm of average precipitation. Situated 151 meters above sea level, the location is 26° 40' 49.9" North latitude and 87° 21' 16.8" East longitude. Using a soil test kit box, the experimental site's soil characteristics were assessed qualitatively (Table 1).

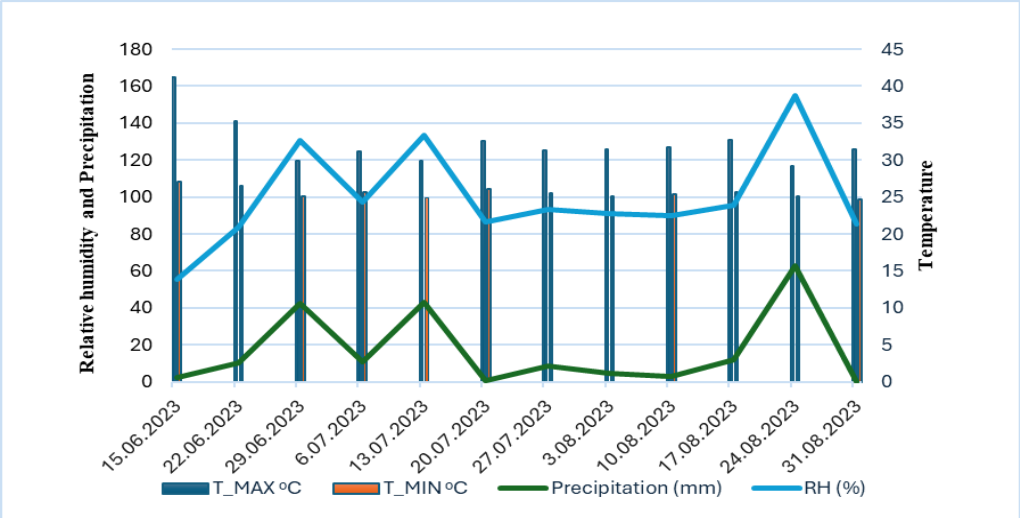

Figure 1. Meteorological data of the research site during study period

Table 1. Characteristics of soil in the research area

Serial Number	Soil characteristics	Properties
1	Organic matter	3.9%
2	pH	6.6
3	Soil texture	Sandy loam
4	Nitrogen	High
5	Phosphorous	Slightly moderate
6	Potassium	moderate

2.2. Experimental design and cultivation practices

The research was conducted following a Randomized Complete Block Design (RCBD), comprising eight distinct treatments replicated three times. In total, 24 plots were established, each occupying an area of 4.5 m² (3m*1.5m), resulting in a combined experimental area of 245 m². A gap of 1.5 m was maintained between two consecutive replications, while a spacing of 0.5 m separated adjacent treatments. Each plot accommodated 25 okra plants, with row-to-row spacing set at 0.6 m and plant-to-plant spacing at 0.3 m. Field preparation commenced seven days prior to sowing, with seeds soaked for 24 hours before planting. Specific treatments were applied to their designated plots, each receiving its calculated dosage. The recommended fertilizer application dosage, provided by the Nepal Agriculture Research Council (NARC), Tarahara, Sunsari, Nepal, stood at 200:180:60 kg NPK/ha, translating to 90:81:27 g NPK per 4.5 m². At sowing, the complete doses of potassium and phosphorus, along with half of the nitrogen dose, were uniformly distributed. The remaining nitrogen dose was split into two applications: one during the flowering stage and the other post-weeding. Irrigation was performed every seven days. The treatment details and their respective doses, symbol are outlined in table 2.

Table 2. Treatment details along with doses

Serial Number	Treatments	Symbol	Doses
1	RD of NPK	T1	200:180:60 kg NPK ha^{-1}
2	Mustard cake 100%	T2	6 tons ha^{-1}
3	Goat manure	T3	10 tons ha^{-1}
4	Mustard cake 75%	T4	4.5 tons ha^{-1}
5	Vermicompost	T5	8.5 tons ha^{-1}
6	Poultry manure	T6	16.67 tons ha^{-1}
7	FYM	T7	15 $tons^{-1}$
8	Control	T8	-

Note: RD: Recommended dose, FYM: Farmyard manure

2.3. Data observations and collection

There were altogether 25 plants in each plot. Out of twenty-five plants, five plants were selected randomly, and the data were collected on the following parameters.

2.3.1. Plant height

The stature of the plants was gauged at intervals of 30, 45, 60, and 75 days after sowing (DAS), employing a measuring tape. The vertical dimension of the plant, from its base to its apex, was assessed weekly until the plant reached a stage of economic yield.

2.3.2. Primary branches

The number of primary branches was enumerated at 30, 45, 60, and 75 DAS, capturing the branching pattern of the plants over time.

2.3.3. Number of pods per plant

The aggregate count of pods per plant (P/P) was assessed for each treatment and replica, commencing from the day of 50% flowering within each plot. Subsequently, counts were made at 30, 45, 60, and 75 DAS to monitor pod development.

2.3.4. Pod length and diameter

Upon reaching 50% maturity, the length and diameter (from midpoint of okra fruit with the help of vernier caliper) of pods were recorded. Pod length was measured manually utilizing a measuring scale, while pod diameter was determined using a vernier caliper. The average dimensions of harvested pods were computed by dividing the total length/diameter by the total number of pods per plant across all treatments and replications.

2.3.5. Pod weight

The weight of harvested pods was quantified using a digital weighing machine. The acquired data were then extrapolated to express yield in tons per hectare, based on the yield per plot.

2.4. Statistical Analysis

Initially, the data was organized in a sequential manner for both replication and treatment blocks using MS Excel 2021 (Microsoft Corporation, Washington, USA). Subsequently, ANOVA analysis was conducted utilizing statistical software (R Studio, Version 4.2.2, Boston, Massachusetts, USA). To evaluate variations in means across various treatments with a significance level of 5%, Duncan's Multiple Range Test (DMRT) was utilized.

3. Result and discussions

3.1. Growth observation parameters

3.1.1. Plant height

The application of various organic fertilizer sources resulted in notable disparities in the plant height of okra, as outlined in Table 3. Initially ranging from 50 to 65 cm, the plant height markedly surged to a maximum of 181 cm. The average height stood at 57.47 cm at 30 days after sowing (DAS), steadily escalating up to 165.82 cm at 60 DAS, before a slight decrease to 169.24 cm. Although statistically non-significant overall, there were variations among treatments at 30, 45, and 60 DAS, with highly significant differences observed at 75 DAS. The highest average plant height was attained with the recommended NPK dosage (132.26 cm), followed by Mustard cake (100%) at 128.40 cm, Poultry manure at 127.60 cm, and Mustard cake (75%) at 127.15 cm. These findings align closely with those of a study by Chattoo et al. (2022), which reported a maximum plant height of 181.01 cm, mirroring our result of 181.44 cm with the recommended NPK dosage. Consistently, studies by Mehata et al. (2022) and Yadav et al. (2023) also observed peak plant heights around 180 cm.

Table 3. Effect of different organic fertilizer combination on plant height

Treatments	Plant Height (PH)				Average PH
	30 DAS	45 DAS	60 DAS	75 DAS	
NPK	61.11^{a}	109.07ab	177.39^{a}	181.44^{a}	132.26^{a}
Mustard cake 100%	61.62^{a}	109.66^{a}	170.00^{ab}	172.34^{abc}	128.40^{ab}
Goat manure	58.15^{ab}	106.80^{ab}	159.08^{b}	166.45^{bc}	122.62^{bc}
Mustard cake 75%	58.89^{ab}	107.11^{ab}	166.57^{ab}	176.06^{ab}	127.15^{ab}
Vermicompost	57.49^{ab}	107.80^{ab}	165.26^{ab}	161.62^{cd}	123.04^{abc}
Poultry manure	57.43^{ab}	104.87^{ab}	169.00^{ab}	179.11^{a}	127.60^{ab}
FYM	55.48^{ab}	101.70^{ab}	160.25^{b}	164.94^{bc}	120.59^{bc}
Control	51.47^{b}	98.83^{b}	158.18^{b}	151.98d	115.11^{c}
Grand mean	57.75	105.73	165.71	169.24	124.59
SEM	1.05	1.17	1.79	2.21	1.35
CV (%)	9.90	4.98	4.40	3.90	3.93
F-value	NS	NS	NS	**	*

Note: *** Significant at 0.1 % level of significance. ** Significant at 1% level of significance. * Significant at 5 % level of significance. DAS: Day After Sowing. CV: Coefficient of variance. SEM: standard error mean.

NPK fertilizer likely facilitated balanced nutrient provision, fostering vigorous growth. Mustard seed cake might have contributed by gradually releasing nutrients, albeit slightly less effectively than NPK. Similarly, vermicompost, goat manure, and farmyard manure (FYM) yielded comparable plant heights ranging from 120 to 125 cm, as supported by Shampazuraini et al. (2023). This uniformity could be attributed to their rich organic content, enhancing soil fertility and structure, thereby sustaining consistent plant growth. The control group's minimal height (115.11 cm) underscores nutrient deficiency, impeding robust growth. The absence of fertilization restricts essential nutrient availability, resulting in stunted plants, corroborating previous findings by Abbas et al. (2019) and Singh et al. (2023) highlighting nutrients' pivotal role in promoting plant height.

3.1.2. Primary branches

Table 4 delineates the effects of various organic sources on primary branches, showcasing significant variations among treatments. Notably, the primary branches displayed a remarkably high significance level at 0.1%. The progression of the main primary branches, commencing at 3.76 on day 30, exhibited a consistent rise, culminating at a peak of 4.73 by day 75. The most noteworthy observation was the highest number of primary branches recorded with the recommended NPK dose, reaching 5.58, closely followed by 100% mustard cake at 4.63. This outcome echoes the findings of Yadav et al. (2023), indicating the efficacy of NPK in fostering robust branch growth due to its balanced nutrient profile. Interestingly, mustard cake at 75%, alongside goat manure, vermicompost, poultry manure, and farmyard manure, yielded comparable primary branches ranging from 4 to 4.5. This uniformity underscores the ability of these organic sources to provide sufficient nutrients, thereby promoting consistent branch development.

Table 4. Effect of different organic fertilizer combination on primary branches

Treatments	Primary branches (PB)				Average PB
	30 DAS	45 DAS	60 DAS	75 DAS	
NPK	4.04^{a}	5.71^{a}	6.54^{a}	6.03^{a}	5.58^{a}
Mustard cake 100%	3.76^{b}	4.61^{b}	5.19^{b}	4.95^{b}	4.62^{ab}
Goat manure	3.82^{ab}	4.25^{bc}	4.70^{bc}	4.59^{bc}	4.34^{b}
Mustard cake 75%	3.83^{ab}	4.25^{bc}	4.71^{bc}	4.60^{bc}	4.34^{bc}
Vermicompost	3.80^{ab}	4.21^{bc}	4.61^{bc}	4.51^{bc}	4.28^{bc}
Poultry manure	3.74^{b}	4.12^{bc}	4.60^{bc}	4.50^{bc}	4.24^{bc}
FYM	3.73^{b}	4.10^{bc}	4.62^{bc}	4.52^{bc}	4.24^{bc}
Control	3.38^{c}	3.90^{c}	4.30^{c}	4.21^{c}	3.94^{c}
Grand mean	3.76	4.39	4.90	4.73	4.44
SEM	0.04	0.12	0.15	0.12	0.10
CV (%)	3.76	6.65	7.46	6.07	5.63
F-value	**	***	***	***	***

This finding resonates with similar observations documented in studies by Al-Joboory et al. (2021) and Abbas et al. (2019), further validating the role of organic fertilizers in enhancing primary branch proliferation. Conversely, the control group exhibited the lowest average number of primary branches at 3.95. This deficiency is attributed to the absence of supplementary nutrients, corroborating findings from Yadav et al. (2023) and Mehata et al. (2022). Such results underscore the vital necessity of supplemental nutrients in stimulating primary branch growth, emphasizing the pivotal role of fertilization strategies in optimizing crop yield and quality.

3.1.3. Pod number per plant

Table 5 outlines the influence of various organic fertilizer sources on the growth and development of pods number per plant, revealing notable disparities among treatments at a significant level of 0.1% throughout the growth period. Initially, pod numbers were modest, averaging just 0.77 per plant across all treatments. However, as the days progressed, the formation of pods number per plant gradually increased, peaking at 4.73 by the 75th day after sowing. The highest pod number count per plant was observed in response to the recommended dose of NPK, reaching an impressive 4.93. This finding resonates with the results reported by Sikdar et al. (2023) and Al-Joboory et al. (2021), emphasizing the pivotal role of NPK in promoting optimal nutrient availability for robust pod development. NPK's balanced combination of essential nutrients, including nitrogen, phosphorus, and potassium, likely played a crucial role in enhancing flower and fruit formation, ultimately leading to higher pod numbers per plant compared to other treatments. Among the various biofertilizers tested, including Mustard cake (100% and 75%), goat manure, poultry manure, and vermicompost, pod numbers ranged between 3.52 and 4.08. These results suggest that these biofertilizers provided a balanced array of essential nutrients, supporting consistent pod development throughout the growth period. However, it's worth noting that their nutrient profiles might not have been as comprehensive as the recommended NPK dosage, resulting in slightly lower pod counts per plant. The findings from Yadav et al. (2023) also corroborate our observations, further strengthening the argument for the efficacy of NPK and the comparable performance of biofertilizers in pod development. Conversely, the lowest pod counts were observed in the farmyard manure (FYM) and control groups, averaging around 3.10 pods per plant. This can be attributed to nutrient deficiencies in these treatments, with FYM likely providing insufficient nutrients and the control group entirely lacking supplemental nutrients. These conclusions find support in the results reported by Mehata et al. (2022) and Bertrand et al. (2024), which also documented minimal pod numbers under the FYM and control treatments. In summary, our study underscores the importance of nutrient management in influencing pod development in okra plants, with NPK emerging as the most effective treatment and biofertilizers providing viable alternatives for sustainable agriculture practices.

Table 5. Effect of different organic fertilizer combination on pod number per plant

Treatments	Pod number per plant (PP)				Average PP
	30 DAS	45 DAS	60 DAS	75 DAS	
NPK	1.07^{a}	4.69^{a}	7.96^{a}	6.03^{a}	4.93^{a}
Mustard cake 100%	0.84^{b}	3.97^{b}	6.57^{b}	4.95^{b}	4.08^{b}
Goat manure	0.75^{bc}	3.73^{bc}	6.34^{b}	4.59^{b}	3.85^{b}
Mustard cake 75%	0.74^{bc}	3.70^{bc}	6.49^{b}	4.60^{b}	3.88^{b}
Vermicompost	0.74^{bc}	3.74^{bc}	6.40^{b}	4.51^{b}	3.84^{b}
Poultry manure	0.73^{bc}	3.61^{bc}	6.23^{b}	4.50^{b}	3.76^{b}
FYM	0.68^{c}	3.50^{c}	5.38^{c}	4.52^{b}	3.52^{c}
Control	0.63^{d}	2.96^{d}	4.60^{c}	4.21^{b}	3.10^{d}
Grand mean	0.77	3.73	6.24	4.73	3.87
SEM	0.02	0.10	0.20	0.15	0.11
CV (%)	9.25	6.08	7.14	7.06	5.52
F-value	***	***	***	***	***

3.1.4. Pod length

Application of various organic fertilizers alongside the recommended NPK dose significantly impacts okra pod length, as evidenced in Table 6. Results were highly significant at the 0.1% level, though initially insignificant during growth. The recommended NPK dose outperforms, yielding a pod length of 13.53 cm, closely followed by goat manure at 13.08 cm.

Table 6. Effect of different organic fertilizer combination on pod length (cm)

Treatments	Pod length (PL)				Average PL
	30 DAS	45 DAS	60 DAS	75 DAS	
NPK	3.54^{a}	17.04^{a}	17.57^{a}	15.98^{a}	13.53^{a}
Mustard cake 100%	3.47^{a}	15.65^{ab}	17.03^{ab}	15.08^{ab}	12.81^{ab}
Goat manure	3.52^{a}	15.99^{ab}	17.34^{a}	15.48^{ab}	13.08^{a}
Mustard cake 75%	3.52^{a}	15.63^{ab}	17.10^{ab}	15.48^{ab}	12.93^{ab}
Vermicompost	3.48^{a}	15.77^{ab}	16.95^{ab}	15.15^{ab}	12.84^{ab}
Poultry manure	3.52^{a}	15.48^{ab}	17.29^{ab}	15.54^{ab}	12.96^{ab}
FYM	3.43^{a}	14.28^{b}	15.66^{b}	14.52^{b}	11.97^{b}
Control	3.07^{b}	11.19^{c}	13.32^{c}	11.93^{c}	9.88^{c}
Grand mean	3.44	15.13	16.53	14.89	12.50
SEM	0.04	0.38	0.31	0.26	0.24
CV (%)	5.46	6.62	5.10	3.82	4.78
F-value	NS	***	***	***	***

Previous studies Regmi et al. (2022) and Sikdar et al. (2023) support these findings, attributing NPK's balance and goat manure's organic enrichment to enhanced growth. Similarly, Mehata et al. (2022) found comparable results. Mustard cake (100% and 75%), vermicompost, poultry manure, and FYM yielded slightly lower pod lengths (11-13 cm), likely due to nutrient supply imbalances or slower release rates compared to NPK and goat manure. These results align closely with prior research Mehata et al. (2022) and Yadav et al. (2023). The control group exhibited the lowest pod length (9.88 cm), likely owing to nutrient deficiency and lack of organic supplementation, consistent with earlier findings Bertrand et al. (2024).

3.1.5. Pod diameter

The utilization of various organic fertilizers alongside the recommended chemical fertilizer dosage demonstrates noteworthy impacts on the pod diameter of okra plants, as detailed in Table 7. These findings were highly significant at the 0.1% threshold throughout the growth and development of okra life cycle. Within these treatments, the recommended NPK dose showcased remarkable outcomes, yielding the widest pod diameter at approximately 5.16 cm, closely trailed by goat manure at 5.03 cm. Prior investigations Regmi et al. (2022) and Sikdar et al. (2023) corroborates these results, attributing the optimal nutrient balance from the recommended NPK dosage and the soil enrichment with organic matter from goat manure to the robust development of pods. Similarly, Mehata et al. (2022) observed analogous outcomes in their research. Mustard cake (at 100% and 75% concentrations), vermicompost, poultry manure, and FYM demonstrated comparable results, albeit slightly lower than those of NPK and goat manure, ranging from 4 to 5 cm in diameter. This disparity could stem from the supply of essential nutrients, albeit potentially imbalanced or released at slower rates, consequently resulting in slightly diminished pod diameters compared to NPK and goat manure. These findings closely mirror previous investigations by Mehata et al. (2022) and Yadav et al. (2023). The lowest pod diameter observed in the control group, measuring at 3.76 cm, likely ensued from the dearth of nutrients and inadequate organic matter in the absence of supplementary treatments. This conclusion finds substantial support in the preceding study conducted by Bertrand et al. (2024).

Table 7. Effect of different organic fertilizer combination on pod diameter (cm)

Treatments	Pod diameter (PD)				Average PD
	30 DAS	45 DAS	60 DAS	75 DAS	
NPK	1.63^{a}	5.89^{a}	6.64^{a}	6.49^{a}	5.16^{a}
Mustard cake 100%	1.59^{ab}	5.61^{a}	6.23^{ab}	6.08^{ab}	4.87^{a}
Goat manure	1.61^{ab}	5.62^{a}	6.53^{ab}	6.38^{ab}	5.03^{a}
Mustard cake 75%	1.60^{ab}	5.62^{a}	6.24^{ab}	6.05^{ab}	4.87^{a}
Vermicompost	1.54^{ab}	5.36^{ab}	6.07^{b}	5.92^{b}	4.72^{a}
Poultry manure	1.56^{ab}	5.50^{ab}	6.18^{ab}	5.98^{ab}	4.80^{a}
FYM	1.49^{bc}	4.73^{b}	5.41^{c}	5.27^{c}	4.22^{b}
Control	1.40^{c}	3.90^{c}	4.92^{c}	4.85^{c}	3.76^{c}
Grand mean	1.55	5.28	6.03	5.88	4.67
SEM	0.01	0.14	0.12	0.11	0.09
CV (%)	4.05	8.32	4.76	4.53	4.93
F-value	**	**	***	***	***

3.1.6. Fresh weight per pod

The integration of various organic fertilizers alongside the recommended chemical fertilizer dosage significantly influences the fresh weight per pod of okra plants, as indicated in Table 8. These findings were highly significant at the 0.1% threshold throughout the growth and yield period. Initially, the overall mean fresh pod weight among these treatments stood at a modest 25.69 g. However, as days progressed, there was a notable increase in pod weight, reaching a peak of approximately 28.70 g at 60 days after sowing. Among the assortment of fertilizers, synthetic fertilizer NPK emerges as the frontrunner, yielding the highest pod weight, nearly hitting the 29.93 g mark. This outcome aligns robustly with the findings of a previous investigation by Yadav et al. (2023). The superiority of the recommended NPK dosage can be attributed to its meticulously balanced blend of nitrogen, phosphorus, and potassium, which fosters optimal plant growth and development, consequently enhancing pod weight. Similarly, among several organic fertilizers, including mustard cake, goat manure, vermicompost, and poultry manure, slightly lower fresh pod weights are observed compared to NPK. Corresponding results were also documented in the earlier study by Mehata et al. (2022) and Abbas et al. (2019). Conversely, the control group displayed the lowest pod weight, measuring just 22.27 g, consistent with earlier findings from Bertrand et al. (2024). This diminished weight in the control group may be attributed to the absence of supplementary fertilization, resulting in inadequate nutrient uptake and limited support for robust pod development.

Table 8. Effect of different organic fertilizer combination on fresh weight per pod (gram)

Treatments	Fresh weight per pod (WP)				Average WP
	30 DAS	45 DAS	60 DAS	75 DAS	
NPK	28.93^{a}	29.24^{a}	32.04^{a}	29.53^{a}	29.93^{a}
Mustard cake 100%	26.89^{ab}	27.20^{ab}	29.57^{b}	26.90^{ab}	27.64^{b}
Goat manure	26.29^{b}	26.60^{b}	29.16^{bc}	26.19^{abc}	27.06^{b}
Mustard cake 75%	25.82^{b}	26.13^{b}	29.56^{b}	25.93^{abc}	26.86^{b}
Vermicompost	25.77^{b}	26.08^{b}	29.51^{b}	25.87^{abc}	26.80^{b}
Poultry manure	25.50^{b}	25.18^{b}	28.90^{bc}	28.83^{ab}	27.10^{b}
FYM	24.87^{b}	25.18^{b}	27.04^{c}	24.36^{bc}	25.36^{b}
Control	21.49^{c}	21.80^{c}	23.89^{d}	21.92^{c}	22.27^{c}
Grand mean	25.69	25.92	28.70	26.19	26.62
SEM	0.46	0.46	0.50	0.60	0.47
CV (%)	5.05	4.99	4.21	8.73	4.80
F-value	***	***	***	*	***

3.1.7. Yield per plant

The incorporation of various organic fertilizers alongside the recommended chemical fertilizer dose significantly impacts the yield per okra plant, as detailed in Table 9. These results maintained a high level of significance at the 0.1% threshold throughout the growth and yield stages of okra plants. Initially, the average yield per plant across these treatments was modest, measuring at 19.95 g. However, as the cultivation period progressed, there was a noticeable surge in yield, peaking at approximately 130.34 g by the 60th day post-sowing. Within the spectrum of fertilizers tested, synthetic NPK fertilizer emerged as the top performer, yielding the highest output per plant, nearly reaching 108.84 g. This finding strongly corroborates previous research conducted by Yadav et al. (2023). The superiority of the recommended NPK dosage can be ascribed to its meticulously balanced composition of nitrogen, phosphorus, and potassium, which fosters optimal plant growth and development, consequently elevating overall yield. Following this trend, biofertilizers such as 100% mustard cake and goat manure yielded 103.70 g and 104.28 g respectively. Similarly, among the biofertilizers tested, 75% mustard cake, vermicompost, poultry manure, and FYM exhibited comparable results, ranging from 90 to 102 g. Conversely, the control group displayed the lowest yield per plant, measuring just 76.99 g, consistent with earlier findings from Bertrand et al. (2024). This diminished yield in the control group can be attributed to the absence of supplementary fertilization, resulting in inadequate nutrient uptake and limited support for robust overall yield. In conclusion, the study underscores the significant impact of fertilizer type on okra plant yield, with synthetic NPK fertilizer demonstrating the highest efficacy, closely followed by biofertilizers like mustard cake and goat manure. These findings emphasize the importance of balanced nutrient application in maximizing crop productivity.

Table 9. Effect of different organic fertilizer combination on yield per plant (grams)

Treatments	Yield per plant (grams)				Average yield
	30 DAS	45 DAS	60 DAS	75 DAS	
NPK	29.97^{a}	131.39^{a}	142.34^{a}	131.66^{a}	108.84^{a}
Mustard cake 100%	21.95^{b}	128.80^{a}	136.56^{ab}	127.50^{a}	103.70^{ab}
Goat manure	19.11^{bc}	130.50^{a}	138.97^{ab}	128.55^{a}	104.28^{ab}
Mustard cake 75%	18.55^{bc}	126.40^{a}	134.84^{ab}	125.10^{a}	101.22^{b}
Vermicompost	18.54^{bc}	124.41^{a}	131.49^{b}	122.68^{b}	99.35^{b}
Poultry manure	18.91^{bc}	123.72^{a}	131.60^{b}	122.96^{b}	99.29^{b}
FYM	17.49^{bc}	112.20^{b}	121.44^{c}	113.28^{b}	91.10^{bc}
Control	15.15^{c}	90.66^{c}	105.48^{d}	92.69^{c}	76.99^{c}
Grand mean	19.95	121.01	130.34	120.55	97.97
SEM	0.97	2.88	2.50	2.60	2.05
CV (%)	12.84	5.04	4.08	4.19	4.29
F-value	***	***	***	***	***

4. Conclusion

Our research underscores the critical role of fertilizer selection in shaping okra yield and growth parameters. While synthetic NPK fertilizer consistently yielded the highest results, our study identified promising organic alternatives, notably 100% mustard cake and goat manure, which demonstrated comparable efficacy. These findings stress the importance of transitioning towards sustainable agricultural practices to mitigate environmental risks associated with chemical inputs. Our study contributes valuable insights to agricultural decision-making, advocating for adopting of organic fertilizers to promote soil health and environmental sustainability. Furthermore, we recommend further exploration of integrated nutrient management strategies and the long-term impacts of biofertilizers on soil health and crop resilience. By advancing our understanding of sustainable agricultural practices, our research ensures food security, supports rural livelihoods, and fosters environmental stewardship in Nepal and beyond.

Compliance with Ethical Standards

Conflict of interest

The author declares no conflict of interest.

Authors' contributions

Rupesh Kumar MEHTA: Conceptualization, funding acquisition, investigation, methodology, resources, software, supervision, writing – review & editing, validation, visualization. **Chetana ROY**: Methodology. **Begam Kumari CHAUDHARY**: Data curation, methodology, writing-original draft. **Aniksha MOKTAN**: Data curation, writing-original draft. **Astha KARKI**: Writing-original draft. **Adhish Kumar ROY**: Data curation, methodology, writing-original draft. **Sujata KOIRALA**: Data curation, writing-original draft. **Hikesh GURAGAIN**: Data curation, methodology. **Raju KHATRI**: Investigation, writing – review & editing, writing – original draft. All authors have read and agreed to the published version of the manuscript.

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Body weight, scrotal parameters and semen characteristic of Kano Brown Buck Kids fed *Pluerotus ostreatus* solid state fermented sugarcane scrapings

Emmanuel ANASO [1*], Olurotimi OLAFADEHAN [1], Ayoola John SHOYOMBO [2], Emeka Solomon FIDELIS [3]

[1] Department of Animal Science, University of Abuja, Abuja, Nigeria
[2] Department of Animal Science, Landmark University, Omu-Aran, Kwara State, Nigeria
[3] Department of Agricultural Economics, University of Abuja, Abuja, Nigeria

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*CONTACT

dranasoeub@gmail.com

ABSTRACT

The study evaluated the effect of substitution of *Pleurotus ostreatus* biodegraded sugarcane scrapings (BSS) for corn bran on the growth performance and reproductive potential of Kano Brown bucks. Twenty-one healthy buck kids (6 – 7 months of age, with an average initial BW of 9.44 ± 0.39 kg) were stratified into three treatments and fed: (1) a total mixed diet containing no BSS (0% BSS; control), (2) the control diet containing 15% BSS substituting 50% corn bran and (3) the control diet containing 30% BSS substituting 100% corn bran on dry matter basis (DM) for 12 weeks in a completely and fully randomized design. Intakes of DM, crude protein and organic matter, and sperm concentration varied in the order: 15% BSS > 0% BSS >30% BSS ($p<0.05$). Final BW, semen volume, initial fructose, scrotal length (SL) and scrotal circumference (SC) were greater in 15% BSS diet than 0 and 30% BSS diets. Semen pH and color, sperm progressive motility, viability and abnormalities, and live spermatozoa were not affected by diets. Whereas testosterone level was greater in 0 and 15% BSS diets, libido was lower in 30% BSS diet. Final BW was positively correlated with SC ($p=0.030$; $r = 0.510$) and SL ($p = 0.048$; $r = 0.472$). It was concluded that up to 30% biodegraded sugarcane scrapings can be used in a complete diet for bucks without negatively impacting final body weight and semen quality, though 15% BSS was more impactful and recommended.

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ORCID> 0009-0001-6886-8557 (EA), ORCID> 0009-0002-6949-3205 (OO), ORCID> 0000-0003-2937-7779 (AJS), ORCID> 0000-0003-4276-2735 (ESF)

1. Introduction

Ruminant production in sub-Saharan African is principally constrained by dearth and unsteady quantity and quality of the year-round feed availability which affect both the growth (productive) and sexual (reproductive) performance of the animal (Olafadehan and Adewumi, 2009). This problem of soaring cost of conventional feedstuffs used in ration formulation necessitates quest by animal nutritionists for alternative, non or less competitive, available and cheap feedstuffs, particularly lignocellulosic residues constituting environmental hazard due to improper disposal (Olafadehan et al., 2014).

Production of sugarcane, an extensively cultivated plant in the northern Nigeria, is associated with concomitant production of a variety of its coproducts among which sugarcane scraping or peel is an important abundantly available coproduct (Anaso et al., 2021; Olafadehan et al., 2021). In spite of the availability, it has a limited utilization in livestock due to its low crude protein (CP) and lignified nature (Anaso et al., 2021).

By improving the CP and dissolving the lignin barrier, solid state fermentation (SSF) with fungus of lignocelluloses has been utilized to increase the nutritional value. This has changed the lignocellulosic structure and made the carbs accessible for more effective conversion (Isroi et al., 2011). There have been reports of white rot fungus producing ligninolytic enzymes, such as laccase, manganese peroxidase, and lignin peroxidase, and effectively mineralizing lignin into carbon dioxide and water (Sanchez, 2010; Isroi et al., 2011), Furthermore, using white rot fungus in solid state fermentation of lignified roughages is an environmentally benign method of using them instead of physical or chemical treatment. Whereas most of the previous studies on the utilization of solid-state fermented lignocelluloses in the diets of ruminants focused on the productive performance, information on the reproductive potential or fertility, particularly of male animals, is rarely available. A thorough assessment of the animals' testicular metrics, especially scrotal length and circumference, as well as their semen qualities, may provide some light on how well the food treatment of the animals satisfies their needs for adequate reproduction. The objective of the current study was to assess the body weight, scrotal parameters, and reproductive performance of bucks that were fed diets that included sugarcane scrapings that had been biodegraded by *Pleurotus ostreatus*.

2. Materials and methods

2.1. Experimental location

The University of Abuja Teaching and Research Farm in Abuja, Nigeria, served as the site of the experiment. Situated at an elevation of 456 m, the location is situated between latitudes 8º 55'N and 9º 00'E and longitudes 7º 00'N and 7º 05'E. The place experiences 1100–1650 mm of precipitation and 25.8–42°C of yearly temperature.

2.2. Preparation and biodegradation of sugarcane scrapings

While fresh SS was gathered from nearby sugarcane processors, chopped into 1-2 cm lengths, and air-dried at room temperature (25–30°C), *Pleurotus ostreatus* was purchased from a respectable commercial producer in Nigeria. The SS was mixed with distilled water in a 1:1 ratio in each previously cleaned, dried, and sterilized container to bring the moisture content down to 67% prior to the *Pleurotus* inoculation for solid state fermentation. The SS were autoclaved twice, once at 121°C for 15 minutes each time, with cooling intervals in between to eradicate any living microbes. The prepared SS were cooled in an aseptic environment before being infected with P. ostreatus spores in a 25:1 ratio. The inoculation room was then maintained at 30°C and 100% relative humidity until mycelia formed. Following a 21-day period of inoculation and SSF, the mycelia growth and biodegradation of the biodegraded SS (BSS) were stopped by autoclaving the material. After being dried to a consistent weight, the BSS were bagged and kept until they were required for feeding.

2.3. Experimental animals, management and diets

Kano Brown male goats (n =21; 6 to 7 months of age; 9.44 ± 0.39 kg body weight) for the experiment were purchased from an open market.

The goats were housed in separate 1.2 m^2 cages within a 6 m x 8 m x 4 m pen. The pen and its immediate surrounds were carefully cleansed with Hypo® (sodium hypochlorite, caustic soda, and de-mineralized water) and antiseptic (Morigad) two weeks before their arrival. Prophylactic treatments were given to the animals during their two-week quarantine. These included an oral anti-stress medication called Vitalyte®, a subcutaneous injection of the live PPR vaccine (1 mL at 102.5 of the TCID50 PPR virus) at the neck region, a subcutaneous injection of an anti-parasitic medication called Avomec® at 0.5 mL/25 kg of body weight (BW), and an intramuscular injection of a long-acting oxytetracycline HCl at 1 mL/10 kg BW. Using different levels of SS 0 (control), 15 and 30% inclusion levels, three whole diets were developed (Table 1). The NRC's (2007) recommendations were followed when designing the diets that addressed the needs of growing goats. During a 12-week period (from January to March), buck kids were fed at 5% of their body weight (based on dry matter; DM) and were balanced for body weight. The goats were then randomly assigned to one of the experimental diets. As the trial went on, the amount of feed provided to the goats was adjusted to make sure some was left over. Feeding took place twice a day, at 8:00 and 16:00. They had unlimited access to clean water every day.

Table 1. Ingredient and chemical composition (% DM) of the experimental diets

	Level of BSS inclusion, %		
	0	15	30
Ingredient			
Maize	25	25	25
Corn bran	30	15	0
BSS	0	15	30
Groundnut cake	17	17	17
Cowpea husk	25	25	25
Salt	0.5	0.5	0.5
Limestone	2	2	2
Premix	0.5	0.5	0.5
Chemical composition			
Crude protein	15.7	15.8	15.9
Ether extract	5.78	5.80	5.82
Organic matter	93.8	92.0	91.6
Non-fibre carbohydrate	33.6	29.7	27.2
Neutral detergent fibre	38.7	40.6	42.6
Acid detergent fibre	21.0	23.1	25.2

BSS: Biodegraded Sugarcane Scrapings

2.4. Feed intake and body weight

Feed intake was calculated by deducting the weight of the feed supplied the day before from the weight of the leftover feed. The calculation of nutrient intake involved multiplying the animal's feed intake (measured in dry matter) by the nutrient's (measured in dry matter) content in the diets. The individual initial body weight (BW) was measured at the start of the experiment, and the individual final BW was determined right before the goats were fed at the end of study using a standard goat weighing scale (UmaTech Scales)

2.5. Testicular and semen parameters

Scrotal length (SL) was measured weekly with a flexible measuring tape as the distance along the caudal surface of the scrotum, from its point of attachment to the tip of the scrotum as described by Akpa et al. (2012). Scrotal circumference (SC) is the maximum dimension around the pendulous scrotum after pushing the testes firmly into the scrotum (Akpa et al., 2006). It was measured by using measuring tape.

Semen was collected from all the bucks from each treatment through electro-ejaculation, using an automatic electro-ejaculator (Autojact, Neovet) with 12 V and 5 A as outlined by Zemjanis (1970). The ejaculates were obtained by the automatic method with animals standing in a chute followed by a sequence of 1-35 stimuli adding up a total of 30 s to 5 min. The semen ejaculatory volume was determined immediately in the collection vial graduated in milliliter (mL). The samples were kept in water bath at 37°C, and evaluations were made in sequence according to CBRA (1998) manual.

2.6. Chemical analysis

Following AOAC (2000) protocols, diet samples were analyzed for their proximate elements. Neutral and acid detergent fiber were determined according to the method described by Van Soest et al. (1991). Semen samples was evaluated immediately for volume through direct reading of millimeter graduation of the collection vial, and the result was expressed in milliliter (mL). Semen pH was determined by dipping a litmus paper into the ejaculate and monitoring the corresponding color changes (Anaso et al., 2023). Semen appearance was determined by visualization of consistency of ejaculates and classified as: creamy marble, creamy, thick milky, milky and watered (Anaso et al., 2023). Semen initial fructose evaluation was carried out immediately after collection according to Mann (1948). Smear of each semen sample was prepared, air dried, labelled and kept for further examination. The progressive motility was determined by placing 10 µL of semen into 1 mL of Tris dilution buffer, hydroxymethylaminomethane, (3.0 g), sodium citrate (2.0 g) and fructose (1.0 g). Then a 10 µL-aliquot of the diluted semen sample was placed between preheated slide and coverslip (37 ℃) and evaluated under optical microscope (100 x magnification). The progressive motility was expressed in percentage. The concentration of the spermatozoa was determined using a hemocytometer that were crossed with microscopic grids containing 25 large squares with each containing sixteen smaller squares. The total number of smaller squares on the hemocytometer was 400. Sperm cells were counted diagonally from top left to the bottom right and from top right to the bottom left in five large squares or a total of 80 smaller squares (Rekwot et al., 1997). Prior to counting, formaldehyde was used as a dilution reagent. A drop of semen was taken from each sample using automatic pipette and diluted with formaldehyde at 1:100. The hemocytometer was mounted into the microscope and an absorbable tube as used to pipette a drop of the solution into the hemocytometer chamber. The absorbable tube was blown before pipetting to avoid air bubbles in the absorbable tube. The result obtained was recorded as the sperm cell concentration for the sample.

Sperm morphology was determined from 95 slide smears stained with Giemsa at 7.5% (Doles Laboratory), diluted in distilled water and immersed in this solution for two h. Afterwards, the slides were kept upright until they dried completely and viewed under the microscope to get the normal and abnormal sperm percentage. Normal spermatozoa and spermatozoa abnormalities were classified according to principles used for rabbits by Barth and Oko (1989). The result was expressed in percentage. The live to dead sperm ratio was estimated by the preparation of a smear of individual semen sample using eosin-nigrosine stain immediately after collection. A drop of semen was diluted and placed on a clean glass slide using automatic pipette. A drop of the eosin-nigrosine solution was placed alongside the semen on the slide. A gentle circular turning of the slide was done to allow a uniform mixture of the two samples. A one-quarter of the part of another clean slide was placed on top of the first sample and the two slides were gradually and carefully drawn apart to prepare a thin smear on the first slide. This was allowed to dry and thereafter labelled. This was done for each sample, and they were later mounted on the microscope for counting the live and dead sperm cells. The principle is that the dead sperm cells accept the stain and appear stained while the live sperm cells reject the stain and remain unstained (Hancock, 1951). Testosterone concentration in serum was measured by radioimmunoassay via assay kits (Siemens, Mexico, D.F). Libido (reaction time to does) was determined by exposing bucks to estrogenized doe. A stop-watch was used to take time for mounting without intromission and ejaculation and duly recorded as described by Angel-Garcia et al. (2015).

2.7. Data analysis

The obtained data was subjected to and met normal distribution conditions. Subsequent results for body weight, scrotal parameters and semen characteristic were subjected to analysis of variance (ANOVA) in a completely randomized design using the SPSS BASE 23 (SPSS software products, USA). Duncan multiple range test (DMRT) of same software was used to test the significant difference between the means at ($p \leq 0.05$ level of significance.

The statistical model is shown below:

$Yij = \mu + tij + eij$

Where:

Yij = the general response to the specific parameter under investigation,

μ, the general mean peculiar to each observation,

tij = the fixed effect of the dietary treatment on the observed parameters, and

eij = the random error term for each estimate

Linear relationships between BW and SL and BW and SC were estimated by simple regression analysis while multiple regression was used to estimate the contribution of both SL and SC to variation in the final BW. The statistical model for the multiple regression was:

$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \varepsilon$

Where:

Y = final BW; X_1 = SL; X_2 = SC; β_0 = the intercept; β_1 and β_2 are the slopes and ε is the error. The R^2 and P values were used to express the degree of the significances.

3. Results and discussion

3.1. Chemical composition of experimental diets

Replacement of corn bran with BSS, a poor-quality roughage, slightly alter the chemical composition of the diets, implying that SSF of SS enhanced its nutritive value to almost similar level to that of the corn bran (Table 1). Previous studies (Akinfemi, 2010; Khattab et al., 2013) reported enhanced nutritive value in form of increased CP and reduced fiber contents of *Pleurotus ostreatus* fermented lignocellulosic materials.

3.2. Feed intake and body weight

Feed intake was affected ($p<0.05$) by the diets in the order: 15% BSS > 0% BSS > 30% BSS (Table 2). There was no ($p>0.05$) difference in the initial BW among the three experimental groups. Final BW (16.1 kg > 14.6 kg > 13.6 kg) indicates that 15% BSS in treatment 2 was greater ($p<0.05$) than 0 and 30% BSS.

Table 2. Feed intake and bodyweight of Kano Brown goats fed biodegraded sugarcane scrapings (BSS) meal

Parameter	Level of BSS inclusion, %			SEM
	0 (n*=7)	15 (n=7)	30 (n=7)	
DM intake, g/d	354^{b}	374^{a}	319^{c}	3.39
Crude protein intake, g/d	55.6^{b}	59.1^{a}	50.7^{c}	0.53
Organic matter intake, g/d	332^{b}	344^{a}	291^{c}	3.16
Initial body weight, kg	9.50	9.50	9.40	0.38
Final body weight, kg	14.6^{b}	16.1^{a}	13.6^{b}	1.17

BSS. Biodegraded Sugarcane Scraping. n*: Animal numbers. ^{abc} Means in the same row without common letter are different at P<0.05

The DM intake of ruminants is a function of many factors such as chemical composition, characteristics and palatability of feeds (Olafadehan and Okoye, 2017). The higher feed intake of 15% BSS suggests superior palatability, nutritive value, digestibility and ruminal fermentation at this inclusion level (Olafadehan et al., 2014; Olafadehan and Adebayo, 2016; Kholif et al., 2021). The optimum feed intake of 15% BSS diet would have undoubtedly ensured availability of energy and various nutrients for body activities like body weight gain and physiological and reproductive functions (Olafadehan and Adewumi, 2009;2010). The insignificant difference in initial body weight of the goats indicates the effectiveness of the randomization of the animals which further justified the use of complete randomized design. The higher final BW of animals in 15% BSS diet is a direct response to the improved feed as well as the nutrient intake and hence availability, utilization, assimilation and conversion to the body weight. This result indicates a synergy between the nutritional components of both corn bran and BSS which had equal combinations in 15% BSS diet unlike in either sole corn bran-based diet (0% BSS) or sole BSS based diet (30% BSS).

However, similar final BW of 0 and 30% BSS diets plausibly implies that BSS can completely replace corn bran in a practical goat ration without compromising the final BW of the animals.

3.3. Semen quality and testicular parameters

Semen color was the same (creamy) for the diets. Semen pH, sperm viability, progressive motility, abnormalities and live spermatozoa were not ($p>0.05$) affected by diets (Table 3). Semen volume was higher ($p<0.05$) in 0 and 15% BSS than in 30% BSS. Semen concentration was affected ($p<0.05$) in the order: 15% BSS > 0% BSS > 30% BSS. The testosterone level and libido test (measured as the reaction time when exposed to female) followed the same trend and were higher ($p<0.05$) for 15 and 30% BSS diet relative to 0% BSS diet.

Table 3. Semen quality and testicular parameters of goats fed biodegraded sugarcane scrapings (BSS) meal

Parameter	Level of BSS inclusion, %			SEM
	0 (n*=7)	15 (n=7)	30 (n=7)	
Semen color	Creamy	Creamy	Creamy	
pH	6.38	6.43	6.68	0.07
Ejaculatory volume, mL	0.29^{b}	0.33^{a}	0.27^{b}	0.00
Progressive motility, %	84.0	84.3	84.0	3.64
Sperm viability, %	82.0	83.0	80.0	3.34
Sperm concentration, x 10^{6}	322^{b}	397^{a}	312^{c}	2.98
Live spermatozoa, %	80.0	85.0	80.0	3.88
Sperm abnormalities, %	12.0	10.0	15.0	5.19
Testosterone, ng/mL	3.05^{a}	3.06^{a}	2.87^{b}	0.36
Libido, seconds	13.3^{b}	12.4^{b}	14.9^{a}	0.63
Initial fructose, mg/dL	803^{b}	830^{a}	780^{b}	2.95
Scrotum length, cm	9.14^{b}	9.43^{a}	9.11^{b}	1.15
Scrotum circumference, cm	16.6^{b}	17.0^{a}	16.4^{b}	0.97

abc: Means in the same row without common letter are different at P<0.05. n*: Animal numbers

Evaluation of semen quality is essential because of the imperativeness of a good semen quality in achieving adequate fertility in farm animals. In fact, semen quality and sexual behavior are the essential standards that determine male reproductive efficiency. The parallel results for semen pH, sperm viability, progressive motility, abnormalities and live spermatozoa indicate that BSS can be included in buck diets without negatively impacting these semen traits. The bucks' comparable semen colors are consistent with earlier studies on goats and rams (Oyeyemi et al., 2011; Ososanyo et al., 2013), which noted a creamy color feature. Translucent semen generally indicates low concentration, blood stains and strange colors indicate poor quality or contamination, and creamy white semen often indicates acceptable quality. The unaffected semen's pH did not match Osinowo's (2016) reported value of 6.9. The 15% BSS had greater sperm and semen EV concentrations, indicating that the diet had a significant impact on spermatogenesis and that the goats' nutritional status was enhanced while on this diet. The improved nutritional status may be attributed to the increased supply of nutrients for spermatogenesis process arising from increased the enhanced feed and nutrient intake (particularly protein intake). Though not reported in this study, the diet might have also possibly improved nutrient digestibility (Gado et al., 2015) and ruminal microbial growth and production of microbial protein (Gado et al., 2009). Enhanced nutrient digestibility has been reported to promote nourishment of the sertoli cells and seminal fluid that nurse the germ cells (Gado et al., 2015). The increase in sperm concentration and volume, of goats on 30% BSS, therefore, signals the possibility of high fertility during service or insemination (Oyeyemi and Okediran, 2007). The unaffected sperm progressive motility was within the above the minimum motility of 50% (Oyeyemi et al., 2000) and 65% (Osinowo, 2016) required for satisfactory fertility. Osinowo (2016) asserts that strong, progressive motility is an essential measure of sperm viability and sperm level, which might be high or low. Motile cells are usually innately viable, and viability is crucial in the determination of non-motile cells that are alive or dead. The marginal effect of the diets on of the sperm viability suggests diets did not adversely impact spermatogenesis (Sumalatha, 2010) and livability of the sperm cells.

It appears BSS is a nutritionally adequate feedstuff can be included in the diets of bucks up to 30% without compromising semen characteristics and fertility because low-quality or inadequate diet has been linked to cases of low-live sperm count. (Irkham et al., 2017).

The similar sperm abnormalities across the treatment groups did not surpass the earlier reported permissible limit in rams fed pineapple waste-based diets (Ososanya et al., 2013). Generally, high levels of sperm abnormalities indicate poor quality semen. Therefore, it could be inferred that nutritional treatments did not cause any negative effects on semen quality in the current study. Testosterone, produced from the leydig cells of the testes, is vital for spermatogenesis and male characteristics (Sekoni et al., 2010) and its vascular distribution throughout the body is a major contributory factor to the libido of males (Sajjad et al., 2007). Though the testosterone was higher for 0 and 15% BSS diets than for 30% BSS diet, the levels were within the range of 2.10 – 10.8 ng/mL for White goats in Türkiye (Polat et al., 2011) and 0.1 – 10 ng/mL for Creole bucks (Delgadillo et al., 1999), suggesting that testosterone concentration was unaffected by the 30% BSS threshold level in the present investigation. Given its favorable association with other semen characteristics, testosterone has been identified as a critical component in the formation of superior quality semen, Cornwall (2009) attributed low sperm quality to low testosterone levels. Given that testosterone has been shown to increase male sexual behavior, it is possible that the higher libido (lower reaction time to doses) in the 0 and 15% BSS is related to testosterone levels (Gado et al., 2015). The increased initial fructose of 15% BSS suggests that the diet increased the amount of nutrient and energy for spermatozoa (Wilke et al., 2009; El-Gindy et al., 2020). Generally, libido (sex drive) is an important component of male fertility.

Scrotal length and circumference are important indicators when observing animals for breeding soundness. Higher SC and SL of goats fed 15% BSS diet indicate that the diet may improve the reproductive performance and breeding soundness of the bucks. Earlier studies (Azizunnesa et al., 2013) attributed increased scrotal circumference and growth rate to nutritional plane, implying that 15% BSS diet is perhaps nutritionally superior to the other diets. Both SC ($p=0.030$; $r = 0.051$) and SL ($p = 0.048$; $r = 0.472$) had a notably positive correlation with the final BW (Table 4), in consonance with previous findings (Olafadehan et al., 2015). However, multiple regression of final BW on SC and SL indicates that final BW was insignificantly ($p>0.05$) positively correlated ($r = 0.513$) with SC (X1) and SL (X2) with 26.3% ($R^2 = 0.263$) variation in final BW attributable to these testicular parameters. However, SC might have had a higher contribution ($R^2 = 0.260$) to the final BW than SL ($R^2 = 0.222$). The multiple regression equation is $Y = 4.17 + 0.099XI + 0.581X2$. Akpa et al. (2012) reported a positive and significant correlation between testicular dimensions and body measurements and implied that males with greater scrotal sizes may also have larger body morphology better suited for reproduction as seen in this experiment.

Table 4. Linear relationships between body weight (Y) and testicular parameters (X) of goats

Dependable variables	Regression equation	R	R^2	SEM	P-value
Scrotal length	Y = 10.6 + 0.449X	0.472	0.222	0.210	0.048
Scrotal circumference	Y = 2.97 + 0.708X	0.510	0.260	0.298	0.030

4. Conclusions

- The final body weight, testicular characteristics, semen quality, and fertility of bucks can all be positively impacted by substituting their diet with biodegraded sugarcane scrapings instead of maize bran.

- For enhanced and superior final body weight, testicular parameters, semen quality and fertility, 15% BSS, replacing 50% of corn bran, is recommended in the diet of bucks.

Compliance with Ethical Standards

Conflict of interest

The author declares no conflict of interest.

Authors' contributions

Emmanuel ANASO: Writing and editing. **Olurotimi OLAFADEHAN:** Statistical analysis and major editing. **Ayoola John SHOYOMBO:** Minor editing. **Emeka Solomon FIDELIS:** Minor editing.

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Data availability

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Explanation of morphological and biochemical diversity of autochthonous grapes grown in Türkiye (Kelkit Basin) using multivariate analysis

Ahmet SÜMBÜL [1*], Ercan YILDIZ [2]

[1] Sivas Cumhuriyet University, Susehri Timur Karabal Vocational School, Sivas-Türkiye
[2] Erciyes University, Faculty of Agriculture, Department of Horticulture, Kayseri-Türkiye

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*CONTACT

asumbul3188@gmail.com

ABSTRACT

Grapes are widely grown around the world thanks to their different uses and nutritional importance. The demand for grapes is changing day by day in line with consumer preferences. This situation reveals the importance of identifying and protecting autochthonous grape varieties. This study was carried out to evaluate the morphological and biochemical characteristics of a previously unexplored autochthonous grape (*Vitis vinifera*) population using multivariate analyses. Morphological and biochemical characteristics were evaluated using principal component analysis (PCA), correlation analysis and hierarchical clustering analysis based on Ward's method. In the study, bunch weight varied between 71.67 g and 554.17 g, berry weight varied between 1.54 g and 10.98 g, and the number of seeds in berries varied between 0.00 and 3.50. Among the biochemical properties, total antioxidant content varied between 10.12% and 91.75%, total phenolic content varied between 123.77 mg 100 g^{-1} and 664.58 mg 100 g^{-1}, total flavonoid content varied between 16.48 mg 100 g^{-1} and 270.92 mg 100 g^{-1} and total anthocyanin content varied between 3.35 mg 100 g^{-1} and 74.42 mg 100 g^{-1}. The coefficient of variation (CV) among the characteristics examined ranged from 5.16% to 102.58%. As a result of PCA, the first two components explained 43.43% of the variation. The autochthonous grapes examined were divided into two main groups with different sub-clusters as a result of hierarchical clustering analysis. As a result of multivariate analysis, was detected significant variation among autochthonous grapes. The variations obtained show that the germplasm examined will be a valuable genetic resource for future grape breeding.

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ORCID> 0000-0001-9510-0992 (AS), ORCID> 0000-0003-1445-2385 (EY)

1. Introduction

Türkiye has a special position in the world in terms of plant genetic resources. The Mediterranean and Near Eastern Centers which are the centers of plant diversity and origin defined by Vavilov (1951), intersect on Türkiye. Türkiye, located at the intersection of two different gene and diversity centers, is the gene center of grapes, as well as many fruit species (Sümbül et al., 2024). Türkiye, geographically located between 36º - 42° north latitude, is in the most suitable climate zone for viticulture in the world (Sabır, 2008). Türkiye is very rich in local grape genotypes resulting from natural hybridization, mutation and selection (Aradhya et al., 2003). The genetic resources of Türkiye, one of the homelands of grapevine, are gradually disappearing as a result of adverse events such as global climate change, urbanization, low number of varieties used in trade, natural disasters and various stress factors. Breeders using grapevine genetic resources can develop new varieties that are resistant to various stress factors and have high quality. In addition, grapevine genetic resources provide a valuable resource to breeders in solving problems that may be encountered. As a result, collecting, identifying, and protecting local grape resources is important for the future of grape growing (Sümbül et al., 2024).

Grapevines differ from each other in terms of bunch and berry characteristics depending on the region where they are grown. In the characterization of grapevine genotypes, ampelographic methods based on morphological and pomological characteristics were first used. Ampelographic methods are widely used in the characterization of grapevine genotypes based on their phenotypic characteristics. Morphological characteristics increase diversity in terms of desired agricultural characteristics (Iezzoni and Pritts, 1991; Khadivi-Khub and Anjam, 2014).

Grape fruit, which is rich in vitamins and minerals, is also rich in phytochemicals with antioxidant properties. Phenolic compounds are the most important phytochemical group that is a powerful defense tool in the fight against free radicals for humans and show antioxidant properties (Yang and Xiao, 2013). Phenolic compounds, which are abundant in grape fruit, have anticancer, anti-inflammatory, anti-aging and antimicrobial effects (Xia et al., 2010) as well as protective and preventive properties against cancer, cardiovascular diseases, cataracts, diabetes, Alzheimer's and eye diseases (Yahia, 2017). Consumer preferences in grapes are generally in the direction of physical properties such as bunch size, berry size, berry colour and berry shape. However, with the discovery of the health effects of grapes, consumer preferences have changed towards products that are beneficial for health (Filimon et al., 2017). Consumer demand for products with rich biochemical content such as grapes has revealed that biochemical content is also an important criterion in the characterization of genotypes. As a result, biochemical properties have been used in many studies on the characterization of grapes (Eyduran et al., 2015; Küpe et al., 2020; Özden and Deveci, 2023).

Grapes are one of the important commercial products of regions with temperate and tropical climates due to their high adaptability to different climatic and soil conditions, variety of usage areas and high nutritional value (Çelik, 2006). The global economic importance of the grapevine has led to a large proportion of clonally propagated genetic resources (Boz et al., 2011). The heterozygotic genetic structure of grapevines and the preservation of heterozygosity by clonal propagation can lead to the emergence of different types (Arroyo-Garcia et al., 2006). Thanks to this diversity in grapes, there are still many undefined genotypes in the gene centres of grapes (Magris et al., 2021). In recent years, with the understanding of the importance of genetic resources, many studies have been carried out both in Türkiye (Eyduran et al., 2015; Keskin, 2017; Küpe et al., 2020; Özden and Deveci, 2023; Güler and Karadeniz, 2023; Sümbül et al., 2023) and in the world (Khadivi-Khub et al., 2014; Vafaee et al., 2017; Abiri et al., 2020) to define the morphological and biochemical contents of local grapes.

Leaf, bunch and berry characteristics and biochemical contents of grapes are widely used in characterization studies of grapevine genotypes. This study is the first study to identify autochthonous grapes (*Vitis vinifera*) in the Kelkit Basin, Türkiye. The aim of the study is to determine the morphological and biochemical diversity of the grapes in the region by multivariate analysis.

2. Material and methods

2.1. Plant materials

The material of the study consisted of 60 autochthonous grape (*Vitis vinifera*) genotypes. The leaf characteristics of grape genotypes were determined in healthy leaves above the middle part of the shoots. Grape fruit were collected on harvest date specific to the genotypes. Collected fruit and leaves were placed in labeled plastic transport containers and transported to the laboratory in ice boxes. Pomological analyzes were carried out on the fruits transported to the laboratory, and necessary measurements were carried out on the leaves. Samples were taken from the fruits for biochemical analysis and stored at -20°C until analysis. The names of the genotypes and some descriptive characteristics are given in Table 1. Descriptive information of the genotypes was selected from the "Descriptors for Grapevine (Vitis spp.)" (Anonymous, 1997) list of descriptives fit for purpose.

Table 1. Some descriptive characteristics and local names of genotypes

Genotype	Code	OIV 204	OIV 225	OIV 241	Genotype	Code	OIV 204	OIV 225	OIV 241
Kokulu	U1	Medium	Green-Yellow	Seeded	Karadeniz	U31	Dense	Blue-Black	Seeded
Siyah üzüm 1	U2	Medium	Dark red-Violet	Seeded	Müşkü	U32	Dense	Green-Yellow	Seeded
Adıyaman	U3	Very Loose	Green-Yellow	Seeded	Beyaz üzüm 2	U33	Dense	Green-Yellow	Seeded
Mor üzüm 1	U4	Medium	Dark red-Violet	Seeded	Mor üzüm 3	U34	Very Loose	Dark red-Violet	Seeded
Beyaz üzüm 1	U5	Loose	Green-Yellow	Seeded	Ağ üzüm	U35	Dense	Green-Yellow	Seeded
Alyanak	U6	Medium	Green-Yellow	Seeded	Çavuş 1	U36	Medium	Green-Yellow	Seeded
Cemin	U7	Loose	Dark red-Violet	Seeded	Müşküle	U37	Medium	Green-Yellow	Seeded
İstanbul	U8	Loose	Green-Yellow	Seeded	Emcoğlu	U38	Very Loose	Green-Yellow	Seeded
Gazova 1	U9	Dense	Green-Yellow	Seeded	Dağ üzümü	U39	Loose	Dark red-Violet	Seeded
Mor üzüm 2	U10	Medium	Dark red-Violet	Seeded	Danagözü	U40	Medium	Green-Yellow	Seeded
Uzun üzüm	U11	Loose	Green-Yellow	Seeded	Çavuş 2	U41	Medium	Green-Yellow	Seeded
Parmak üzümü	U12	Loose	Green-Yellow	Seeded	Çekirdeksiz 1	U42	Loose	Red	Seedless
Dökülen	U13	Very Loose	Green-Yellow	Seeded	Siyah çekirdeksiz	U43	Loose	Red-Grey	Seedless
Kara üzüm 1	U14	Medium	Blue-Black	Seeded	Tokat üzümü	U44	Dense	Green-Yellow	Seeded
Gazova 2	U15	Dense	Green-Yellow	Seeded	Mor üzüm 4	U45	Loose	Red	Seeded
Siyah üzüm 2	U16	Medium	Blue-Black	Seeded	Bursa üzümü	U46	Medium	Green-Yellow	Seeded
Sarı üzüm 1	U17	Medium	Green-Yellow	Seeded	Sarı yanak	U47	Medium	Green-Yellow	Seeded
Gazova 3	U18	Loose	Green-Yellow	Seeded	Beyaz üzüm 3	U48	Loose	Green-Yellow	Seeded
Siyah gazova	U19	Very Loose	Blue-Black	Seeded	Güççük	U49	Loose	Green-Yellow	Seeded
Şirelik	U20	Loose	Green-Yellow	Seeded	Kara Salkım	U50	Very Dense	Dark red-Violet	Seeded
Pembe üzüm 1	U21	Loose	Dark red-Violet	Seeded	Keribar	U51	Dense	Green-Yellow	Seeded
Kara üzüm 2	U22	Medium	Blue-Black	Seeded	Ak üzüm	U52	Medium	Green-Yellow	Seeded
Pembe üzüm 2	U23	Loose	Dark red-Violet	Seeded	Davut üzümü	U53	Dense	Green-Yellow	Seeded
Dedem	U24	Loose	Green-Yellow	Seeded	Işıklar	U54	Very Dense	Green-Yellow	Seeded
Sık üzüm	U25	Very Dense	Green-Yellow	Seeded	İri mor	U55	Medium	Dark red-Violet	Seeded
Gevrek	U26	Very Dense	Green-Yellow	Seeded	Yeşil üzüm	U56	Dense	Green-Yellow	Seeded
Siyah Gevrek	U27	Dense	Dark red-Violet	Seeded	Geççi	U57	Very Dense	Green-Yellow	Seeded
Sarı üzüm 2	U28	Loose	Green-Yellow	Seeded	Mor üzüm 5	U58	Medium	Red	Seeded
Keçi memesi	U29	Medium	Green-Yellow	Seeded	Uzun kara	U59	Loose	Dark red-Violet	Seeded
Tatlı kara	U30	Medium	Blue-Black	Seeded	Çekirdeksiz 2	U60	Medium	Green-Yellow	Seedless

2.2. Morphological and physico-chemical characterization

Morphological characteristics consisted of mature leaf, bunch and berry characteristics. Leaf width, leaf length, main vein length and petiole length were measured with a ruler, while petiole thickness was measured with a digital caliper with a precision of 0.01 mm. The leaf area was calculated in cm^2 in the Image J package program on the leaf images photographed from an equal distance. Bunch weight, berry weight and 100 seed weight of the genotypes were determined with a precision balance with an accuracy of 0.01 g. Bunch width and bunch length were measured with a ruler, while berry width and berry length were measured with a digital caliper with a precision of 0.01 mm. The skin color of the berries was measured in terms of L*, a* and b* with a color measuring device (PCE-XXM 30, UK).

Measurements were made bidirectionally from the middle parts of the berries. Chroma (Chroma = $(a^2+b^2)^{1/2}$) and hue angle values (Hue angle = arctan(b/a)) were calculated using a* and b* values (McGuire, 1992). Fruit juice was obtained from 100 berries taken from the central parts of the bunches. From the obtained fruit juice, amount of total soluble solids (TSS) was determined in % Brix with a digital hand refractometer (PAL-1 Atago, USA), pH level was determined with a pH meter, and titratable acidity (TA) was determined in tartaric acid according to the titration method with a pH meter (Hanna pH212; USA) (Cemeroğlu, 2007). Maturity index was determined by the ratio of TSS to TA.

2.3. Biochemical characterization

Extraction: For biochemical analyses, 100 berries taken from the central part of the bunches were homogenized using a blender. 1 g of the homogenized sample was weighed and homogenized by adding 10 mL of 80% methanol. The samples were kept in the refrigerator (+4ºC) in the dark during a day. Then, the samples were shaken at 200 rpm for 30 min at room temperature and filtered with filter paper.

Total Phenolic Content: Total phenolic content was determined using Folin-Ciocalteu solution according to the method described by Slinkard and Singleton (1977). The absorbance of the resulting solution was read in a UV-Vis spectrophotometer (Shimadzu, Japan) at a wavelength of 765 nm. The values were calculated as mg gallic acid (GAE) 100 g^{-1} fresh weight (fw).

Total Flavonoid Content: Total flavonoid content was determined according to the method described by Karadeniz et al. (2005). The absorbance of the resulting solution was read in a UV-Vis spectrophotometer (Shimadzu, Japan) at a wavelength of 510 nm. The values were calculated as mg catechin (QE) 100 g^{-1} fw.

Total Anthocyanin Content: Total anthocyanin content was determined according to the pH-differential method described by Giusti et al. (1999). In this method, total monomeric anthocyanin content was determined at two different wavelengths (520 and 700 nm) and two different pH values (1.0 and 4.5). The values were calculated as mg malvidin 3-glycoside 100 g^{-1} fw.

Total Antioxidant Content: Total antioxidant content was determined according to the DPPH (1.1-diphenyl-2-picryl-hydrazyl) antioxidant activity method described by Brand-Williams et al. (1995). The absorbance values of the samples were determined in a UV-Vis spectrophotometer (Shimadzu, Japan) at a wavelength of 517 nm and the values were calculated according to the control and expressed as % inhibition.

2.4. Statistical analysis

In order to evaluate morphological and biochemical characteristics, minimum, maximum and mean values and CV showing the variation between the data were calculated. Analyzes based on morphological and biochemical characteristics were carried out in the JMP Pro 17.0 (SAS Institute Inc., Cary, NC, USA) statistical package program. PCA was used to determine the degree of influence of the examined characteristics and the relationship between the genotypes, hierarchical clustering analysis was used to group the examined characteristics and genotypes and correlation analysis based on Ward's method was used to determine the relationship between the examined characteristics.

3. Results and discussion

3.1. Morphological and Biochemical Analysis

Bunch and berry characteristics of grapevines are generally preferred in variety identification studies because they provide variety-specific information. Among the genotypes examined, bunch frequency was generally classified as loose and medium. In terms of berry skin color, more than half of the genotypes (37 genotypes) have green-yellow skin color. While three of the genotypes were seedlessness, the presence of seeds was detected in the other genotypes (57 genotypes) (Table 1). Grape genotypes examined within the scope of the study are generally in the table grape class. However, there are also grape genotypes with potential for wine grapes (U9, U15, U25, U26, U27, U31, U32, U33, U35, U44, U50, U51, U53, U54, U56, U57). In table grapes, berry skin color (Vafee et al., 2017; Somogyi et al., 2020) and seedlessness (Reisch et al., 2012) are important criteria affecting consumer preferences.

Berry skin color, berry size and bunch shape are important quality criteria for table grapes (Harindra Champa, 2015). While large-berry and loose bunches are preferred for table grapes, medium and small-berry and dense bunches are preferred for wine grapes (Melo et al., 2015).

Statistical information on the morphological and biochemical characteristics of grape genotypes are presented in Table 2. Wide variations have been observed among the characteristics examined. The coefficient of variation showing the change of the properties was determined at the lowest pH value (CV: 5.16%) and the highest a* value (CV: 102.58%). In addition, CV value of 19 of the 28 characteristics analyzed was found more than 20%. Characteristics with a CV value of more than 20% show more significant differences between genotypes and these characteristics can be used to distinguish genotypes. As a matter of fact, characteristics with high CV values have a wider selection range, while characteristics with low CV values are more stable among genotypes (Khadivi-Khub and Etemadi-Khah, 2015). In studies on morphological characteristics of grapes, CV varied between 0.00 % and 258.46 % (Khadivi-Khub et al., 2014; Vafaee et al., 2017; Akhram et al., 2019; Abiri et al., 2020; Güler and Karadeniz, 2023).

Table 2. Descriptive statistics of morphological and biochemical characteristics of genotypes

No	Traits	Abbreviation	Unit	Min.	Max.	Mean	SD	CV (%)
1	Leaf Width	LW	cm	9.15	16.45	12.33	1.67	13.54
2	Main Vein Length	MVL	cm	6.80	13.33	9.75	1.54	15.76
3	Leaf Length	LL	cm	8.87	17.38	13.15	1.93	14.70
4	Leaf Area	LA	cm^2	51.38	220.30	109.41	30.56	27.93
5	Petiole Length	PL	cm	3.72	9.90	6.05	1.37	22.71
6	Petiole Thickness	PT	mm	1.34	2.83	1.89	0.28	14.62
7	Number of Lobes	NL	Number	3.00	11.00	5.15	1.16	22.56
8	Bunch Weight	BW	g	71.67	554.17	238.23	111.09	46.63
9	Bunch Width	BWi	cm	7.18	15.83	10.69	2.04	19.08
10	Bunch Length	BL	cm	8.53	24.82	17.32	3.53	20.38
11	Berry Weight	BrW	g	1.54	10.98	3.78	1.74	45.97
12	Berry Width	BrWi	mm	12.31	24.63	16.82	2.31	13.74
13	Berry Length	BrL	mm	13.13	28.45	18.40	3.66	19.91
14	100 Seed Weight	SW	g	0.00	9.65	6.13	1.93	31.46
15	Number of Seed	NS	Number	0.00	3.50	2.30	0.76	32.97
16	L*	L	Code	23.85	50.80	37.66	10.15	26.96
17	a*	a	Code	-19.39	96.56	23.81	24.42	102.58
18	b*	b	Code	4.26	39.39	19.25	11.95	62.08
19	C*	C	Code	17.89	96.85	37.68	15.78	41.88
20	Hue*	H	Code	3.57	125.85	46.08	35.50	77.03
21	TSS	TSS	%	12.80	20.30	15.53	1.88	12.11
22	pH	Ph	Code	3.03	4.02	3.44	0.18	5.16
23	TA	TA	mg/L	0.33	1.47	0.76	0.22	28.68
24	TSS/TA	TSS/TA	Code	10.74	51.31	22.34	7.55	33.79
25	Total Antioxidant	TAnt	%	10.12	91.75	40.13	17.77	44.28
26	Total Phenolic	TP	mg GAE 100 g^{-1}	123.77	664.58	359.08	104.80	29.19
27	Total Flavonoid	TF	mg QE 100 g^{-1}	16.48	270.92	81.52	50.83	62.35
28	Total Anthocyanin	TAnth	mg malvidin 3-glycoside 100 g^{-1}	3.35	74.42	23.29	21.71	93.21

As a result of the study, leaf characteristics (leaf width, leaf length, main vein length, leaf area, petiole length and petiole thickness) showed wide variations among genotypes. Among leaf characteristics, the largest CV was determined in leaf area (27.93%), petiole length (22.71%) and number of lobes (22.56%). Leaf width varied between 9.15 cm and 16.45 cm, main vein length varied between 6.80 cm and 13.33 cm, leaf length varied between 8.87 cm and 17.38 cm, leaf area varied between 51.38 cm^2 and 220.30 cm^2, petiole length varied between 3.72 cm and 9.90 cm, and petiole thickness varied between 1.34 cm and 2.83 cm. Leaf characteristics of the genotypes in the study coincide with the research findings of Sümbül et al. (2023). Leaf lobe numbers of genotypes varied between 3 and 11. Although the number of lobes of the leaves varied according to genotypes, our study findings were found compatible with the literature (Vafaee et al., 2017; Abiri et al., 2020). Leaf characteristics, which provide objective information about genotypes, are of great importance in grapevine genotypes identification studies (Santiago et al., 2007).

Among the bunch and berry characteristics, the highest CV was found in bunch weight (46.93%) and berry weight (45.97%). These characteristics were followed by number of seeds (CV= 32.97%) and 100 seed weight (CV= 31.46%). While the bunch weights of the genotypes varied between 71.67 and 554.17 g, the average bunch weight was determined as 238.33 g. The bunch width varied between 7.18 and 15.83 cm, and the bunch length varied between 8.53 and 24.82 cm. Among the genotypes, berry weights varied between 1.54 and 10.98 g, berry widths varied between 12.31 and 24.63 mm, and berry lengths varied between 13.13 and 28.45 mm. According to the literature, the bunch and berry weights of grapes vary according to genotypes and growing regions. The bunch and berry weights obtained as a result of the study are similar to the previous studies. Bunch weights of grapes were reported to vary between 62.75 - 214.99 g (Vafee et al., 2017) and 33.65 - 890.70 g (Razi et al., 2019) in a study conducted in Iran, 71.00 - 872.00 g (El Oualkadi and Hajjaj, 2019) in a study conducted in Morocco, 77.70 - 583.55 g (Akhram et al., 2019) in a study conducted in Pakistan and 195.60 - 272.70 g (Habib et al., 2020) in a study conducted in Tunisia. In studies conducted in different regions of Türkiye, bunch weight varied between 60.57 and 876.38 g (Keskin, 2017; Serhat et al., 2017; Özden and Deveci, 2023; Sümbül et al., 2023; Güler and Karadeniz, 2023). It has been stated that the berry weight of grapes varies between 1.50 and 5.94 g by Khadivi-Khub et al. (2014), between 0.64 and 3.74 g by Vafee et al. (2017), betweeen 0.64 and 3.47 g by Razi et al. (2019) and between 2.35 and 4.97 g by Habib et al. (2020). In the studies conducted in Türkiye, berry weights of grapes were determined between 3.10 - 5.40 g by Keskin (2017), between 1.20 - 6.70 g by Serhat et al. (2017), between 1.53 - 7.44 g by Sümbül et al. (2023) and between 1.29 - 9.48 g by Güler and Karadeniz (2023).

Seed characteristics are a frequently used distinguishing feature in the identification of diversity of grape genotypes (Benito et al., 2017). In the study, number of seeds per berry and 100 seed weight showed great variation among genotypes. The number of seeds per berry varied between 0.00 and 3.50, while the 100 seed weight varied between 0.00 and 9.65 g. The number of seeds per berry in grapes was reported to vary between 0.00 and 4.00 by Khadivi-Khub et al. (2014), between 0.00 and 3.00 by Vafaee et al. (2017), between 0.00 and 4.00 by Abiri et al. (2020) and between 0.88 and 5.50 by Güler and Karadeniz (2023). The seedlessness of varieties is a desirable characteristic in table grape growing. This situation was revealed in a study on grape production projection (Sümbül and Yıldız, 2022).

The skin color values of berries showed wide variations among genotypes. Among the color values, a* value had the highest CV (CV= 102.58%), while L* value had the lowest CV (CV= 26.96%). The average L*, a*, b*, C and Hue color values were determined as 37.66, 23.81, 19.25, 37.68, 46.08, respectively. Although berry skin color in grapes is specific to genotypes, berry skin color intensity is affected by factors such as the location, sunlight utilization and training method of grapes. There may be color differences even in bunches on the same grapevine (Kılıç et al., 2011). Differences in berry skin color of the grapes are important in determining harvest dates and consumer preferences (Vafaee et al., 2017; Somogyi et al., 2020).

Among the fruit must characteristics of the genotypes (TSS, pH, TA and TSS/TA), the highest CV was found in TSS/TA (33.70%). Among the genotypes, TSS varied between 12.80% and 20.30%, pH varied between 3.03 and 4.02, TA varied between 0.33 and 1.47, and TSS/TA varied between 10.74 and 51.31. The must characteristics of grapes depend on genetics but are also affected by climate and environmental conditions. TSS in grapes was reported between 15.00 and 25.40 in Iran (Khadivi-Khub et al., 2014) and between 17.00 and 21.03 in Tunisia (Habib et al., 2020). In studies conducted in Türkiye, TSS of grapes was reported between 7.83 - 26.39 in Bolu (Güler and Karadeniz, 2023) and between 14.00 - 24.13 in Kayseri (Sümbül et al., 2023). In order to ensure unity on the maturity criterion in grapes, the International Organization of Vine and Wine (OIV) has reported that TSS will be considered ripe between 12.5 and 16 °Brix (OIV, 2008). In addition, according to the Turkish Standards Institute Table Grape Standard, it is stated that the TSS value of table grapes should be at least 13% for seeded varieties and at least 14% for seedless varieties (Polat, 2016). In this regard, the genotypes within the scope of the study are generally suitable for table consumption.

Grapes are known as a natural and rich source of antioxidants due to their phenolic compound and anothcyanin contents. Among the biochemical properties examined in the study, the highest CV was detected in the total anthocyanin content (93.21%) and the lowest CV was detected in the total phenolic content (29.19%).

In the genotypes, total antioxidants varied between 10.12% and 91.75%, total phenolics varied between 123.77 and 664.58 mg GAE 100 g^{-1}, total flavonoids varied between 16.48 and 270.92 mg QE 100 g^{-1} and total anthocyanins varied between 3.35 and 74.42 mg malvidin 3-glycoside 100 g^{-1}. In studies conducted on grapes, it has been reported that total phenolics vary between 237.12 and 4680.00 mg GAE kg^{-1} (Revilla et al., 2010; Aydın, 2015; Oktay, 2022; Özden and Deveci, 2023), total flavonoids vary between 96.26 and 1440.00 mg QE kg^{-1} (Aydın, 2015; Soltekin, 2019; Küpe et al., 2021; Oktay, 2022; Özden and Deveci, 2023) total antioxidants vary between %19.79 and %81.46 (Özden and Özden, 2014; Soltekin, 2019; Balbaba and Bağcı, 2021; Küpe et al., 2021), and total anthocyanin vary between 24.00 and 1914.00 mg kg^{-1} (Crupi et al., 2012; Gervasi et al., 2016; Özden and Deveci, 2023). It is known that genotypic effects, climatic conditions, growing conditions, soil structure and harvest and post-harvest storage conditions are effective on the biochemical content of fruits (Yahia, 2017). Recently, it has been claimed that the biochemical contents of grapes can be used in studies on the identification of grape genotypes (Laurentiu and Popa, 2018). In addition, it is thought that determining the biochemical content of grapes may be important in the introduction and consumption of new varieties (Özden and Deveci, 2023).

3.2. Principal Component Analysis (PCA)

PCA is widely used to explain the degree of influence of the studied characteristics or patterns of variation among genotypes. The first three basic components provide significant savings in time in the characterization of genotypes (Iezzoni and Pritts, 1991). Within the scope of the study, PCA of 28 characteristics was performed. In order to reveal the components explaining the largest variation as a result of PCA, components with eigenvalues greater than 1 were evaluated. As a result of the analysis, there are 7 components with eigenvalues greater than 1. These 7 components explain 83.09% of the total variation. However, the first three principal components explained 57.06% of the total variation. PCA1 explained 23.43% of the variation, PCA2 explained 20.00% of the variation, and PCA3 explained 13.63% of the variation. While our findings are similar to the study results of Khadivi-Khub et al. (2014) (PCA3 53.98%), they were found higher than the results of Vafaee et al. (2017) and Abiri et al. (2020) (PCA3 32.41%, PCA3 25.82%, respectively). According to the PCA, the contribution of each characteristic to the principal components varied. L* and b* values from berry skin color values, total antioxidant content, total flavonoid content and total anthocyanin content from biochemical properties, bunch weight from bunch properties showed the highest effect on PC1. Leaf characteristics (leaf width, leaf length, leaf area, leaf main vein length, petiole thickness and petiole length) showed the highest correlation with PC2, while bunch weight, berry width, berry length, berry weight and 100 seed weight and TSS showed the highest correlation with PC3 (Table 3; Figure 1).

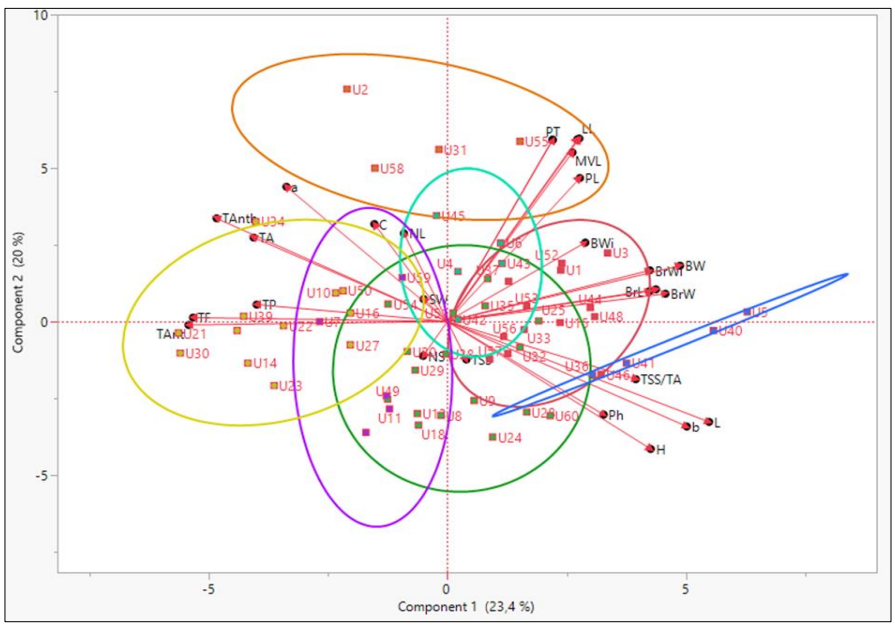

Figure 1. Biplot graph of the first two principal components in the investigated grape genotypes

Table 3. Principal component analysis and contribution rates of the investigated characteristics

Traits	PCA 1	% Cont	PCA 2	% Cont	PCA 3	% Cont	PCA 4	PCA 5	PCA 6	PCA 7
LW	0.11	1.29	**0.33**	**10.89**	-0.19	3.47	0.08	0.09	-0.12	0.10
MVL	0.14	1.82	**0.31**	**9.45**	-0.18	3.29	0.15	0.07	0.01	-0.05
LL	0.14	2.03	**0.33**	**11.07**	-0.18	3.26	0.12	0.10	0.01	-0.03
LA	0.14	1.97	**0.33**	**10.97**	-0.22	4.91	0.06	0.00	-0.02	-0.06
PL	0.14	2.05	**0.26**	**6.81**	-0.09	0.87	0.08	0.14	-0.20	-0.08
PT	0.11	1.29	**0.33**	**10.95**	-0.15	2.40	0.03	-0.05	-0.01	-0.05
NL	-0.05	0.22	0.16	2.56	0.07	0.44	0.17	0.05	0.03	0.08
BW	**0.25**	**6.32**	0.10	1.03	**0.26**	**6.71**	-0.02	-0.03	0.26	-0.21
BWi	0.15	2.22	0.14	2.06	0.23	5.18	-0.03	-0.07	0.42	-0.18
BL	0.23	5.08	0.06	0.34	0.17	2.87	0.00	0.04	0.41	0.13
BrW	0.24	5.55	0.05	0.25	**0.37**	**13.35**	0.00	0.04	-0.14	-0.06
BrWi	0.22	4.79	0.09	0.87	**0.38**	**14.55**	0.04	0.05	-0.11	0.01
BrL	0.22	4.75	0.05	0.30	**0.32**	**10.54**	-0.12	-0.05	-0.11	-0.08
SW	-0.03	0.07	0.04	0.16	**0.28**	**8.06**	0.33	0.06	-0.32	0.16
NS	-0.03	0.07	-0.06	0.39	0.11	1.30	0.35	0.19	-0.27	0.17
L	**0.28**	**8.00**	-0.18	3.34	-0.07	0.54	0.26	-0.04	0.08	0.15
a	-0.17	3.03	0.24	6.00	0.18	3.11	-0.23	0.10	0.04	0.36
b	**0.26**	**6.69**	-0.19	3.65	-0.12	1.39	0.26	-0.05	0.11	0.17
C	-0.08	0.62	0.18	3.14	0.11	1.24	-0.13	0.07	0.17	0.68
H	0.22	4.83	-0.23	5.38	-0.14	2.07	0.26	-0.08	0.07	0.05
TSS	0.02	0.04	-0.07	0.48	**-0.26**	**6.64**	-0.14	0.32	0.29	0.13
Ph	0.17	2.85	-0.17	2.87	0.01	0.00	-0.22	0.33	-0.20	0.07
TA	-0.21	4.39	0.15	2.33	0.03	0.07	0.14	-0.41	0.06	0.15
TSS/TA	0.20	4.14	-0.11	1.10	-0.06	0.36	-0.18	0.51	0.00	-0.03
TAnt	**-0.28**	**7.84**	-0.01	0.00	0.12	1.47	0.26	0.28	0.13	-0.09
TP	-0.21	4.25	0.03	0.09	0.11	1.30	0.31	0.23	0.29	-0.13
TF	**-0.27**	**7.56**	0.01	0.00	0.07	0.42	0.26	0.29	0.15	-0.13
TAnth	**-0.25**	**6.23**	0.19	3.52	0.04	0.19	-0.19	0.11	-0.09	-0.28
Eigenvalue	6.56		5.60		3.82		2.59	1.99	1.56	1.13
Variance	23.43		20.00		13.63		9.27	7.12	5.59	4.05
Cumulative Variance	23.43		43.43		57.06		66.33	73.45	79.04	83.09

* Cont: Contribution

3.3 Heatmap analysis and hierarchical clustering analysis

As a result of heatmap and hierarchical clustering analysis, genotypes were divided into two main groups (Figure 2). Each group was again divided into two subgroups within itself. In group A, U5, U36, U41 and U40 genotypes constitute the A2 subgroup, while the A1 subgroup is divided into two subgroups within itself. Group B is divided into two subgroups. These subgroups were again divided into two subgroups. Group A consists of genotypes with white skin color, while group B generally consists of genotypes with colored skin color. However, subgroups B1-2 and B2-1 consisted of genotypes with both white and colored skins. The genotypes of A1/1 subgroup in A1 group have high values of LW, MVL, LL, BWi, BL, L*, b*, Hue and TSS characteristics, while the genotypes of A1/2 subgroup have high values of L*, b*, Hue and TSS characteristics. BW, BL, BrW, BrW, BrWi, BrL, L* and SW traits of genotypes in A2 group had high values. Genotypes in B1 group showed high values for LW, LA, MVL, LL, PT, PL, BW, BWi, A*, Chroma and TAnth content characteristics, while genotypes in B2 group showed high values for TAnth, NS, TAnt and TF characteristics. Heatmap analysis can classify genotypes based on morphological characteristics. As a matter of fact, heatmap analysis has been used to group genotypes in many studies (Gündeşli et al., 2023; Yaman et al., 2023; Say et al., 2024). Identification of highly distinctive characteristics is important for the morphological characterization of genotypes. Because the correct selection of morphological characteristics that distinguish genotypes saves both time and cost. These characteristics are important for grapes where homonym and synonyms states are observed and have a wide variety richness.

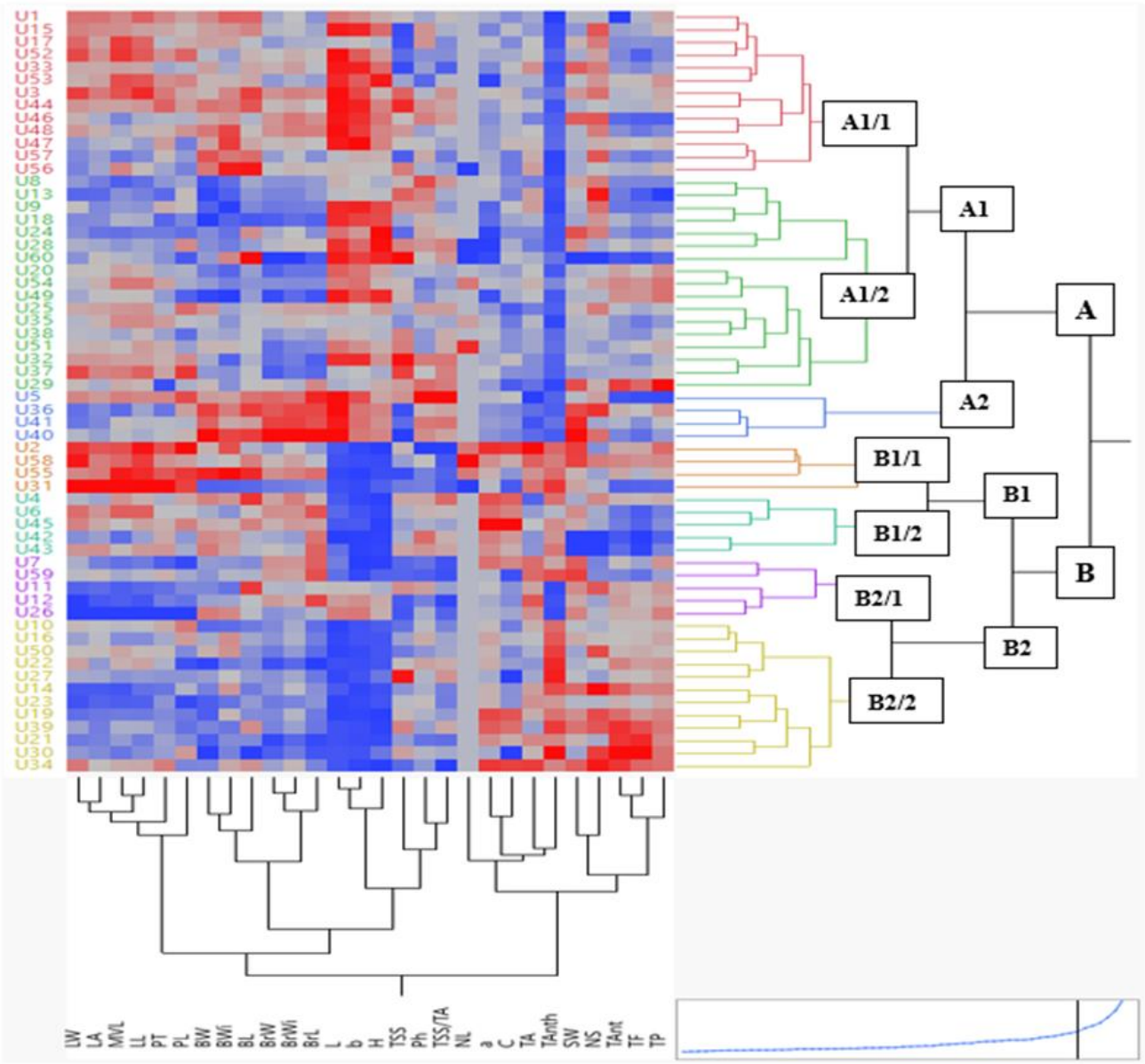

Figure 2. Heatmap analysis and hierarchical clustering of investigated grape genotypes based on morphological and biochemical characteristics

This study is the first study to describe the diversity of autochthonous grapes in the Kelkit Basin of Türkiye. The results of the study showed that there were significant differences between individuals in terms of morphological and biochemical characteristics. These differences are valuable genetic resources for the development of new grape varieties suitable for various usage purposes. While U5, U36, U40, U41 genotypes stand out with their cluster and berry characteristics, U42, U43 and U60 genotypes stand out with their seedless characteristics. These genotypes are candidates for becoming varieties. U34, U30, U21, U39, U19 genotypes stand out with their biochemical contents. The inclusion of these genotypes in breeding programs may enable the development of high-quality grape varieties. In addition, multifaceted statistical approaches were used to evaluate the diversity among autochthonous grapes in the region. Multivariate statistical approaches have divided individuals into different groups. As a result of the study, it was concluded that multivariate statistical approaches are a useful method that can be used in evaluating inter-individual variability.

Compliance with Ethical Standards

Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contributions

Ahmet SÜMBÜL: Methodology, Investigation, Conceptualization, Validation, Data curation, Formal analysis, Writing - original draft, Visualization. **Ercan YILDIZ**: Methodology, Conceptualization, Validation, Review and editing.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Impact of coconut water and grape juice blend on physico-chemical and sensory properties of wine

Nabin KHADKA [1], Manita CHAUDHARY [2], Ganga SANGROULA 1*, Aayusha REGMI [2], Sabin Bahadur KHATRI [1], Navin GAUTAM [1]

[1] Department of Food Technology, Tribhuvan University, Central Campus of Technology, Dharan, Sunsari-Nepal
[2] Department of Food Technology, Tribhuvan University, Nilgiri College, Itahari, Sunsari-Nepal

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* CONTACT

samyogsangroula511@gmail.com

A B S T R A C T

This study focused on crafting a unique wine by blending grape juice with coconut water (CW) in varying ratios (1:2, 2:2, and 2:1), supplemented with crystallized refined table sugar to maintain a 15 °Brix. Fermentation, initiated with the wine yeast S. Cerevisiae VR21, at 0.2 g/100 mL of must, occurred at room temperature. Daily monitoring of physio-chemical parameters [total soluble solids (TSS), pH, and acidity] provided insights into substrate utilization kinetics and fermentation dynamics. The chemical constituent analysis examined the impact of grape juice concentration on the CW wine's chemical properties. During the period of fermentation, the findings indicated significant changes in must constituents; pH was found to be decreased, TSS was decreased and attained steady state however, acidity continuously rose. Subsequently, a sensory analysis using GenStat software version 12.1 evaluated the formulated samples' appearance/color, aroma, taste/body, and overall acceptability. Sensory analysis revealed a preference for formulations with CW and grape juice concentration ratios of 2:2 and 1:2. Particularly, within these ratios, a formulation with a higher proportion of grape juice exhibited superior concentrations of aldehyde, total phenolic content, and antioxidant activity, suggesting enhanced overall quality. This research provides a comprehensive understanding of the chemical and sensory aspects of these distinctive coconut water wine blends.

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1. Introduction

Winemaking is a centuries-old technology that has become a highly profitable biotechnology process (Chakraborty et al., 2014). Since the beginning of civilization, the process of creating wine has been known and has developed with agricultural and human advancement (Chambers and Pretorius, 2010). A variety of raw materials have been used to make wine, either to add flavor or to add important chemical components (Gubhaju, 2006). Wine is a low-alcohol beverage produced by partially or fully fermenting fresh grapes or grape juice (He et al., 2023). Furthermore, wine includes a variety of nutrients that the human body needs, including sugar, vitamins, amino acids, mineral elements, and polyphenols. These nutrients are mixed with alcohol, organic acids, and fragrance components to provide the wine with a more distinct sensory flavor and a better nutritional value (Fia et al., 2018).

Coconut (*Cocos nucifera* L.) is a monoecious perennial palm belonging to the Arecaceae family (Niral and Jerard, 2019). Coconuts are tropical fruits originating from Southeast Asia and are extensively grown in tropical Africa and Latin America (Foale et al., 2020). More than 80% of the world's coconuts are produced in the Asian-Pacific region (Samosir et al., 2006). With their significant export value and influence on local economies and cultures, coconuts are among the most crucial sources of revenue for many of the countries in this region (Samosir et al., 2006). Coconut water (CW), or liquid endosperm, makes up around 25% of the total weight of the nut. Moreover, the drink has a somewhat sweet and acidic flavor (pH 5.6), and the clear liquid within young green nuts comprises around 5% total solids by weight (Purkayastha et al., 2012).

CW nutritional value varies depending on age, type, location, soil condition, and environmental conditions (Chourio et al., 2018). CW, derived from young green coconuts, is a natural and nutritious beverage rich in minerals, sugars, proteins, and vitamins (Sunil et al., 2020). It includes minerals, amino acids, phytohormones, and helpful bioactive components, such as vitamin C, vitamin B, potassium, calcium, magnesium, sodium, glutamic acid, lysine, arginine, alanine, cytokinin, and others (Matsui et al., 2008; Rethinam and Krishnakumar, 2022; Yong et al., 2009). Furthermore, CW is utilized for various food products like tender coconut water, snowball tender nut, vinegar, etc. for its various health benefits, which include boosting the immune system, detoxification, curing hangovers, etc. (Emojevwe, 2013).

Due to its improved flavor, natural hydration characteristics, functional health benefits, and nutritional advantages, CW consumption is now rising globally and is among the beverage categories with the greatest rate of growth (Campbell-Falck et al., 2000; DebMandal and Mandal, 2011). Once the water is withdrawn or separated from the inner cavity, it degrades rapidly. Additionally, spoilage is primarily caused by microbiological activity, physical impurities, storage conditions, and the types of packing materials applied (Ciou et al., 2011) Green coconut water is highly susceptible to oxidation by native enzymes like peroxidase and polyphenol oxidase, which causes nutritional and color losses (Matsui et al., 2008). Globally, about 14% of produced coconut is lost Shahbandeh (2023). Lindner et al. (2013) state that fermented foods are becoming more widely accepted due to their practical advantages. Thus, the fermentation of the green coconut water could be the best way of preserving it, which is rarely studied. CW only contains 5-6% soluble solids, so for the fermentation study, the addition of fresh grape juice is suggested by Satheesh and Prasad (2013). The main objective of the research work is to study the effect of grape juice and coconut water blends on the production of coconut water-based fermented beverages.

2. Materials and methods

2.1. Description of study area

The research was performed from June 2023 to July 2023 in the research lab of Nilgiri College Itahari, Sunsari, Nepal. Geographically, the experimental area is situated at Latitude 26º 40′ 0.01″ N, and Longitude 87º 16′ 59.99″ E with an average altitude of 117.63 m (385.93 ft.). The minimum and maximum temperatures range from 10 and 42 ºC respectively, and the average annual precipitation falls up to 2007 mm.

2.2. Raw materials

Coconut water processed and packaged by Tipco F & B, Ltd., Thailand, was purchased from the supermarket

in Itahari, a sub-metropolitan city. CW was claimed to be free from any type of contaminants, and there were no added sugars or preservatives. Matured, fresh, and sound-quality purple grapes were purchased from the local market in Itahari. Crystallized refined cane sugar was obtained from sugarcane juice, purchased from the local market in Itahari, and commercial wine yeast, Saccharomyces cerevisiae VR 21, was taken from the lab of Nilgiri College, Itahari, Sunsari, and applied for attenuation and fermentation, respectively.

2.3. Experimental method

All the raw materials were collected. The grapes were first sorted, the stem removed, and then washed with potable water. Then the whole grapes were crushed gently and ground in an electric grinder to extract the juice. The crushed grapes were subjected to straining on a clean muslin cloth. Gentle hand pressing was done to extract clear grape juice. Thus, the obtained juice was blended with CW in three different proportions: 1:2, 1:1, and 2:1 by volume and coded as samples A, B, and C respectively. The total soluble solid content of the must was adjusted to 15-degree brix (°Bx) with pulverized table sugar and then transferred to the amber-colored glass bottle for fermentation. The experiments were conducted in triplicate for each blend. A pure yeast culture at a rate of 0.2 g/100 mL (Boulton et al., 1998) must be inoculated, no artificial preservatives were added and allowed to ferment at the temperature of 28 ºC until a constant total soluble solid was obtained. When the constant TSS was observed the fermentation was indicated to be completed after 5 days, the wine was racked into amber-colored glass bottles and then pasteurized at 68 °C for 5 minutes. The prepared wine was then stored at a refrigerated temperature (at 4 °C) and then subjected to sensory analysis. During the fermentation, total soluble solids, pH, and acidity were analyzed daily.

2.4. Analytical methods

2.4.1. Total soluble solids and pH

The pH and Total soluble solid (TSS) was determined by the method given by FSSAI (2021).

2.4.2. Total acidity

Using phenolphthalein indicators, a 10 mL sample was pipetted out and titrated with 0.1 N NaOH. The total acidity was determined by the titrimetric method as per AOAC (2005).

Total acidity (tartaric acid) (g/L of wine) = (V X 0.00375 X 1000) / V_1.

Where,

V_1 is the volume of wine taken

V is the volume of standard NaOH used for titration in mL

Note: 1 mL of 0.05N is equivalent to 0.00375 g

2.4.3. Volatile acidity

For volatile acidity determination, 50 mL of distillate that was collected during the determination of ethyl alcohol was titrated against standard NaOH using the phenolphthalein indicator (FSSAI, 2021).

$$\text{Volatile acidity as acetic acid (g/L)} = \frac{V \times 0.003 \times 1000}{V_1}$$

Where,

V_1 is the volume of sample taken (mL)

V is the volume of standard NaOH used for titration (mL)

Note: 1 mL of 0.05N NaOH is equivalent to 0.003 g of acetic acid

2.4.4. Alcohol content

The alcohol concentration was assessed using the specific gravity technique as described in FSSAI (2021). For the identification, 200 mL of sample was taken in a distillation flask with 50 mL of distilled water. The content

was then distilled to collect 200 mL of distillate. The distillate was gently poured into a specific gravity bottle maintained at a temperature of 20 ℃. Finally, specific gravity was determined, and the alcohol content was obtained from the standard specific gravity vs. alcohol content chart.

2.4.5. Ester

The ester contents of samples were determined as per the method described by the FSSAI (2021). Briefly, 200 mL of the sample was used for distillation, and 50 mL of distillate was recovered, and neutralized with 0.1 N NaOH, an addition of 5 mL of 0.1 N NaOH, and refluxed for one hour. Then, the cooling and back titration of unspent alkali against 0.1N sulfuric acid were carried out. Blank titration was carried out simultaneously with 50 mL of distilled water. The difference in titer value in milliliters of standard sulfuric acid gave an equivalent ester. The values were expressed in grams per 100 liters of ethyl alcohol as ethyl acetate.

$$\text{Ester as ethyl acetate (g/100L abs. alcohol)} = \frac{V \times 0.0088 \times 100 \times 1000 \times 2}{V_1}$$

Where,

V is the difference of the titer value of std. H_2SO_4 used for blank and sample (mL)

V_1 is the alcohol by volume (%abv)

Note: 1 mL of 0.1 N NaOH is equivalent to 0.0088 g of ethyl alcohol

2.4.6. Aldehyde

After keeping the flask containing 50 mL of distilled liquor and 10 mL of bisulfate solution for 30 min, the addition of 25 mL of standard iodine solution was performed, then back titration against the standard thiosulphate solution using a starch indicator to the light green endpoint was performed. (FSSAI, 2012). Similarly, a blank was performed using 50 mL of pure water, Then, analogous aldehyde content was calculated by applying the difference in titer value in milliliters of sodium thiosulfate solution (FSSAI, 2012).

$$\text{Aldehyde as acetaldehyde (g/100L abs. alcohol} = \frac{V \times 0.0011 \times 100 \times 1000 \times 2}{V_1}$$

Where,

V_1 is the alcohol by volume (%abv)

V is the difference in titer of the blank and sample (mL) of sodium thiosulfate solution

Note: 1 mL of 0.05 N sodium thiosulfate corresponds to 0.0011 g acetaldehyde.

2.4.7. Higher alcohol

The higher alcohol content in the wine samples was determined by the titrimetric method as described in FSSAI (2021). For the determination of higher alcohol, a solution obtained from the determination of esters was mixed with 50 mL of distilled water. Sodium chloride was added for extraction up to four times, with progressively larger portions of carbon tetrachloride (40, 30, 20, and 10 mL each). Then, the extract was filtered after washing three times with a saturated sodium sulfate solution, and 50 mL of the oxidizing mixture was added, which was refluxed for two hours. After reflux, it was transferred to the distillation assembly using 50 mL of water. About 100 mL of distillate was titrated against NaOH using a phenolphthalein indicator. Likewise, blank was run, and higher alcohol was determined.

$$\text{Higher alcohol as amyl alcohol (g/L absolute alcohol)} = \frac{V \times 0.0088 \times 100 \times 1000 \times 2}{V_1 \times V_2}$$

Where,

V is the difference in titer

V_1 is the volume of the sample

V_2 is alcohol % (v/v)

2.4.8. Methanol

To begin with, 500 milliliters of wine were taken, and 50 milliliters of distillate were obtained. The distillate's alcohol concentration was then changed to 5% abv. via the addition of purified water. Next, three separate 50 mL volumetric flasks were filled with 2.0 mL of potassium permanganate solution. One milliliter of each of the chilled samples, standard methanol solution, and the blank solution was added to separate flasks labeled "sample," "standard," and "blank." Afterward, an ice bath was used to immerse all three flasks for thirty minutes. Additionally, each flask was filled with 1.0 mL of chromotopic acid, 15 mL of sulfuric acid, and a small amount of dry $NaHSO_3$. The flasks were then put in a hot water bath for 15 minutes. Following, the cooling of each flask, 50 mL of distilled water was added to the capacity. Using a spectrophotometer, the absorbance of each solution was then measured at 575 nm (FSSAI, 2021).

$$\text{Methanol content (g per 100 L abs. alcohol)} = \frac{A_2 \times C \times D \times 1000 \times 100 \times 100}{A_1 \times S}$$

Where,

A_2 is the absorbance of the sample solution

C is the concentration of methanol standard solution

D is the dilution factor for the sample solution

A_1 is the absorbance of the methanol standard solution

S is the ethanol content (%) of the liquor sample (v/v)

2.4.9. Tannin

For the standard curve, pipetted-out standard tannic acid solutions (0.0, 0.2, 0.4, 0.6, 0.8, and 1.0 mL) were placed in a 100 mL volumetric flask with 75 mL of distilled water. Next, 100 mL of distilled water was added to the mixture after adding 5 mL of Folin-Dennis reagent and 10 mL of sodium carbonate solutions. After thoroughly mixing, the solution was set aside for half an hour. The absorbance of each standard using a reagent blank was determined, and the mg of tannic acid vs. absorbance was plotted for the determination of tannin in wine.

For the determination of tannin in the wine samples, in a 100 mL volumetric flask with 80 mL of distilled water, 1.0 mL of sample wine was pipetted out. Afterward, 10 mL of sodium carbonate solution and 5.0 mL of Folin-Dennis reagent were added, and distilled water was used to bring the volume to 100 mL. Following thorough mixing of the prepared solution, the absorbance was measured against a reagent blank and allowed to stand for 30 min. Finally, mg of tannic acid was obtained using a standard curve, and the calculation was done to express the value in g per liter of wine (FSSAI, 2021).

2.4.10. Total phenolic contents

Total phenolics was determined by the Folin-Ciocalteu method as mentioned by Stratil et al. (2008) with a slight modification. At first, 20, 40, 60, 80, and 100 ppm of a standard gallic acid solution were prepared. The absorbances of all standard solutions were noted at the 760 nm wavelength. The absorbance vs. gallic acid concentrations were linearly plotted. Aliquots of 300 microliters were combined with 1.5 mL of diluted Folin-Ciocalteau's reagent (10 times) and 1.2 mL of 7.5% sodium carbonate in a test tube containing the appropriately diluted samples.

2.4.11. Antioxidant activity

The antioxidant activity, expressed as free radical scavenging activity (RSA), in the prepared wine, was determined by the diphenyl-p-picrylhydrazyl (DPPH) method, as mentioned by Hwang and Lee (2023). In summary, the sample (100 µL), methanol (4.4 mL), and DPPH radical methanol solution (0.5 mL, 1 mmol/L) were combined for 15 seconds and allowed to react at room temperature for 30 min. Using a spectrophotometer single-beam UV-visible spectrophotometer using a quartz cuvette, the mixture's absorbance was determined at 517 nm. Using gallic acid, a standard curve for the standard compound's concentration and the rate at which DPPH radicals are scavenged was drawn. In milligrams of gallic acid equivalents per liter of wine, the sample's DPPH radical scavenging activity was reported.

2.5. Sensory evaluation

To ensure customer acceptance, obtained beverages underwent a sensory examination. Clean wine glasses with silent surroundings were used to offer the samples. Nine points were used to rate the sensory qualities (color/appearance, scent, taste, and overall acceptability). A hedonic scale rating test with ten semi-trained panelists, including instructors and students, ranging from dislike highly (1) to like exceedingly (9) of Nilgiri College, those who were familiar with alcoholic beverages. They assessed the beverages in terms of organoleptic parameters.

2.6. Statistical evaluation

A statistical application called GenStat version 12.1 was used to evaluate all of the data collected for the research work. Using an ANOVA, the mean and Tukey HSD at the 5% level of significance were calculated. Additionally, MS Excel was used to create the general diagram and graph.

3. Result and discussion

3.1. Chemical constituents of coconut water and grape juice

The general constituents of coconut water (CW) and grape juice were determined and tabulated in Table 1. The constituents of CW were in the range mentioned by Shayanthavi et al. (2024) and the content was claimed to be free from any added sugar, preservatives, fat, and cholesterol. Researchers concluded that various factors might affect the constituents of CW. Factors noted are the age of the coconut, variety of coconut, climatic conditions, etc. (Tan et al., 2014; Adubofour et al., 2016; Halim et al., 2018; Shayanthavi et al., 2024). The values obtained were compared with those of Sousa et al. (2014) and were in the range. Climate, storage temperature and condition, and juice extraction mechanisms are the factors that might affect the constituent composition of grape juice (Umar et al., 2022).

Table 1. General constituent of coconut water (CW) and grape juice

Parameters	Coconut water	Grape juice
pH	5.1 ± 0.0058	3.29 ± 0.01
TSS (°Bx)	5.2 ± 0	13 ± 0
Acidity (%)	0.0898 ± 0.0023 (as malic acid)	0.21 ±0.003 (as tartaric acid)
Reducing sugar (% dextrose equivalent)	2.32 ± 0.54	0.1812 ± 0.015
Ash content (%)	0.365 ± 0.00058	0.898 ± 0.05

Note: values are the mean of triplicate and ± represents the standard deviation of the values.

3.2. Changes in TSS, pH, and acidity during fermentation

Changes in total soluble solids, pH, and acidity during the course of fermentation for five days are shown in Table 2. According to the statistical study, the components significantly changed during the fermentation process. All three samples with grape juice (GJ): CW proportions: 1:2, 1:1, and 2:1 by volume and coded as samples A, B, and, C respectively, show a similar pattern of changes during storage. The rate of changes might be altered due to the compositional variations in the fermenting must due to the blend. Soares et al. (2016) studied the changes during the fermentation of CW wine by adjusting TSS to 15 °Brix with table sugar and observed a similar pattern in soluble solids change.

They observed an 11% depletion of soluble solids in 30 days of fermentation. The rate of change in TSS might be different due to the fact that they use 100 ppm of SO_2 and the difference in temperature. Total sugar drops steadily, while reducing sugar increases constantly until the first 3 days but quickly decreases thereafter (Xia et al., 2011). The rapid depletion in TSS and pH was reported by Lee et al. (2013) while CW was subjected to fermentation at 28 °C for 2 days. The depletion of soluble solids indicates the corresponding production of ethanol, organic acids, and other volatiles, which ultimately increase the total titratable acidity, and reduce pH. Satheesh and Prasad (2013), reported that a CW and grape juice blend results in a higher percentage of constituents compared with coconut water wine.

Table 2. Changes in TSS, pH and acidity during the course of fermentation

Parameters	Samples	Fermentation time (days)					
		0	1	2	3	4	5
TSS (°Brix)	A	15 (0)[a]	12.033 (0.057)[a]	9.033 (0.057)[c]	6.067 (0.057)[b]	5.367 (0.057)[a]	5 (0)[a]
	B	15 (0)[a]	13.033 (0.057)[b]	8.1 (0.1)[b]	6 (0)[b]	5.4 (0.057)[a]	5 (0)[a]
	C	15 (0)[a]	12.033 (0.057)[a]	7.033 (0.057)[a]	5.033 (0.057)[a]	5.367 (0.057)[a]	5 (0)[a]
	Mean	15	12.36	8.056	5.7	5.4	5.0
	SEM (±)	-	0.047	0.06	0.038	0.04	-
	CV (%)	-	0.5	0.9	0.8	1.1	-
	LSD	-	0.11	0.14	0.094	0.11	-
	F-Test	NS	***	***	***	NS	NS
pH	A	4.56 (0)[a]	4.490 (0.026)[c]	4.023 (0.068)[a]	4.033 (0.057)[c]	3.787 (0.321)[b]	3.753 (0.080)[a]
	B	4.2 (0)[a]	4.167 (0.057)[b]	3.983 (0.028)[a]	3.783 (0.028)[b]	3.793 (0.012)[b]	3.167 (0.029)[a]
	C	4 (0)[a]	3.883 (0.029)[a]	3.683 (0.029)[a]	3.477 (0.040)[a]	3.590 (0.017)[a]	3.583 (0.029)[a]
	Mean	4.25	4.180	4.0	3.76	3.72	3.8
	SEM (±)	-	0.032	-	0.035	0.018	-
	CV (%)	-	1.0	-	1.2	0.6	-
	LSD	-	0.08	-	0.087	0.044	-
	F-Test	NS	***	NS	***	***	NS
Acidity (% as tartaric acid)	A	0.1137 (0.001)[a]	0.1203 (0.001)[a]	0.1070 (0.0001)[a]	0.2417 (0.001)[a]	0.1827 (0.0025)[a]	0.2133 (0.023)[a]
	B	0.1337 (0.0006)[b]	0.1393 (0.0006)[b]	0.1513 (0.0023)[b]	0.2420 (0.0017)[a]	0.3147 (0.0254)[b]	0.3353 (0.0002)[b]
	C	0.1337 (0.0006)[b]	0.1517 (0.0011)[c]	0.1383 (0.0006)[c]	0.3447 (0.008)[b]	0.3377 (0.0046)[b]	0.4107 (0.0023)[c]
	Mean	0.12	0.13	0.13	0.27	0.27	0.32
	SEM (±)	-	-	-	-	0.01	0.01
	CV (%)	0.6	0.7	1.0	1.7	5.4	4.2
	LSD	-	-	-	-	0.02	0.02
	F-Test	***	***	***	***	***	***

Note: Values are the mean of the triplicate and ± indicates the standard deviation of the triplicate data. Mean followed by the same alphabet in the same column signifies no significant difference between the samples, whereas mean followed by a different letter in the same column is significant and tested at the 5% level of significance. Abbreviations: Sample A: one-part GJ and two-part CW, Sample B: equal part of GJ and CW, Sample C: two-part of GJ and one part of CW, LSD: least significant difference, ***Significant at the 0.1% level of significance, NS: Not-Significant.

3.3. Physicochemical properties of samples

The physicochemical properties of the prepared samples are presented in Table 3. Statistical analysis revealed that there was a significant effect of the CW and grape juice blend at a 95% level of confidence. Ester, aldehyde, methyl alcohol, tannin, total phenolic compounds, and antioxidant activity increased significantly when the grape juice proportion was increased. Further addition of sucrose in fermentation must increase the alcohol content and flavor components (H. Liu et al., 2017; J. Liu et al., 2022).

In this experiment, all the samples were adjusted to a total soluble solid of 15 % with a varying blend of CW, grape juice, and sucrose. Grape juice contains a higher amount of coloring pigments, fermentable sugars, and phytochemicals. That might be a reason for the higher contents of the chemical and phytochemical constituents. Aeration of the must, temperature, yeast type, fermentation process, and fruit ripeness are the

primary factors that determine the chemical composition of wine (Buglass, 2011). Depending on the nature of the must, the ester content in wines ranges widely from 25 mg/L to 300 mg/L, the ester content of all three samples is within the range revealed by Ronald and Bakker (2004). The aldehyde content was least in sample A increased with an increase in grape juice content in composition samples A and B was within the range as reported by Rai (2009) however, sample C showed higher aldehyde content, which might be due to the chemical constituents of grapes (Buglass, 2011).

The tannin, and phenolic content increases in the composition of grape juice this might be due to the antioxidant activity, polyphenolic compounds, anthocyanins, and other pigments aligned with Yuyuen et al. (2015). The tannin content of all the compositions is within the range as revealed by Harbertson et al. (2008). Pectin content influences the methanol concentration to some extent. Grapes have less pectin than most other fruits. Therefore, out of all fruit-based, fermented beverages, wine has the lowest methanol level (Buglass, 2011). Red wine tends to contain more methanol (between 120 mg/L and 250 mg/L of total wine volume) because of its higher peel content (Hodson et al., 2017). Yuyuen et al. (2015) reported that an increase in the concentration of ripening berries can increase the concentration of tannins and total phenolic content in wine. The increase in tannins and phenolics leads to an increase in antioxidant activity. Phenol content is greatly influenced by a variety of fruits and climatic factors such as soil, exposure to heat, light, and water availability (Eseberri et al., 2022).

Table 3. Chemical properties of the prepared samples

Samples	A	B	C
Ethyl alcohol (ABV)	7.1 ± 0.087^{a}	7.97 ± 0.029^{b}	7.05 ± 0.087^{c}
Esters (mg/L)	192.67 ± 2.52^{a}	184.33 ± 3.79^{b}	193.18 ± 3.27^{a}
Aldehyde (g/100L)	29.19 ± 1.73^{a}	40.92 ± 1.46^{b}	91.82 ± 1.05^{c}
Methyl alcohol (mg/100L)	210.67 ± 1.23^{a}	225 ± 2^{b}	240.37 ± 1.57^{c}
Tannin (mg/L)	37.63 ± 1.24^{a}	39.49 ± 0.81^{a}	74.89 ± 1.64^{b}
Phenolics (mg/L)	2.9 ± 0.025^{a}	18.46 ± 0.68^{b}	29.32 ± 1.54^{c}
Antioxidant activity (mg gallic acid/L)	2.83 ± 0.76^{a}	6.6 ± 0.53^{b}	11.89 ± 0.93^{c}
Higher alcohol (%)	19.68 ± 0^{a}	5.05 ± 0^{b}	7.38 ± 0^{c}

Note: Values are the mean of the triplicate and ± indicate the standard deviation of the triplicate data. Same alphabet in the same row signifies no significant difference between the samples at $p<0.05$, whereas a different letter in the same row signifies a significant difference. **Abbreviations:** Sample A: one-part GJ and two-part CW, Sample B: equal part of GJ and CW, Sample C: two-part of GJ and one part of CW, ABV: Alcohol by volume.

3.4. Sensory analysis of samples

The result of the sensory evaluation of prepared wine samples is presented in Figure 1. Statistical analysis showed a significant effect of formulation on the preference of the panelists towards appearance, aroma, and overall acceptability at the 5% level of significance. According to Yuyuen et al. (2015), ripened berries contain high concentrations of anthocyanins and total phenol contents, as well as higher concentrations of sugar and total red pigments, which results in high color concentration. Thus, panelists favor sample C, which contains two parts of grape juice and only one part of coconut water.

Certain scents found in fermented beverages are esters, which are produced when the alcohol and acids in the beverage react. Esters can form later on through chemical reactions or during fermentation under the influence of yeast. Small amounts of higher alcohol contribute positively to quality, while excessive amounts may detract from quality. So, smell or aroma depends upon the aromatic compounds formed during fermentation and aging (Hazelwood et al., 2008). In the present study, panelists prefer sample C to sample A significantly. That might be due to significantly higher esters and aldehyde content in sample C than in sample A. Panelists do not find a significant effect of the blend on the taste and body of the wine. That might be because there are no significant residual sugar or alcohol levels in the final product. Research has demonstrated that several parameters, including residual sugar in the sample, alcohol concentration, fermentation day, and aromatic components, influence sample flavor (Naknaen et al., 2010).

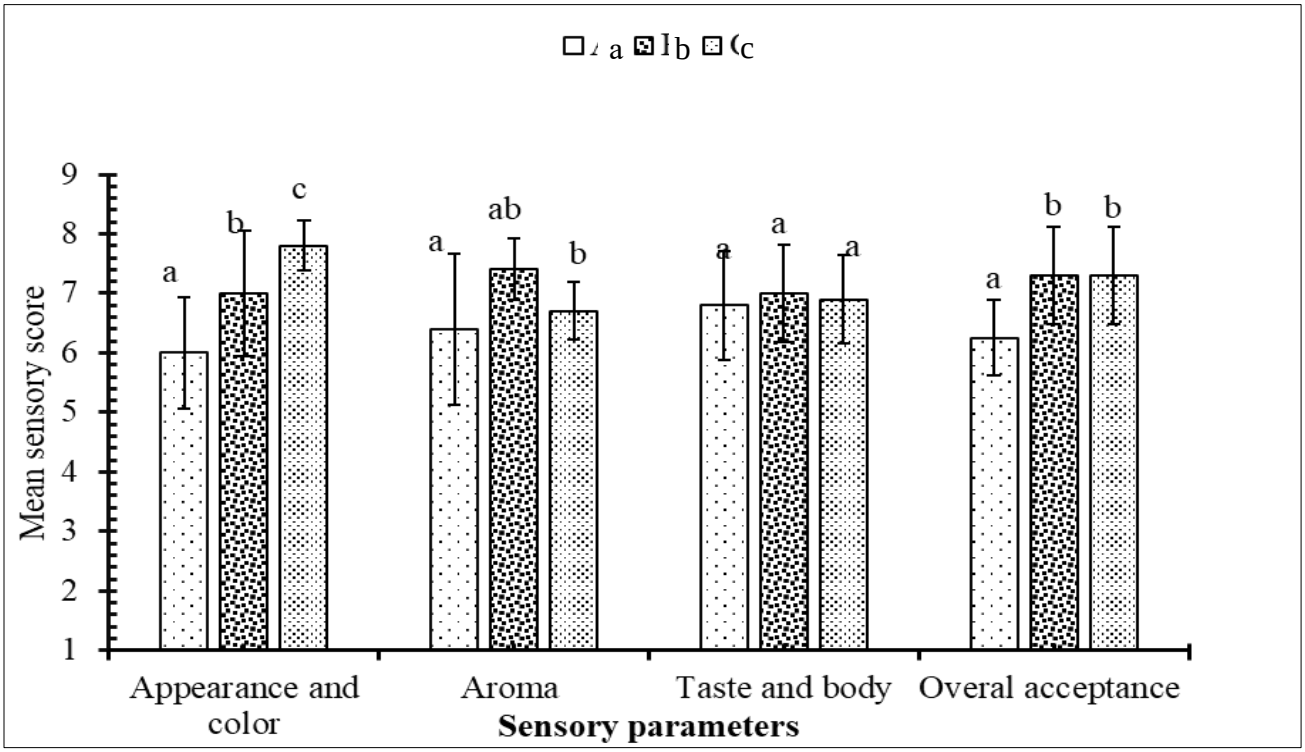

Figure 1. Mean sensory score for the sample parameter. Abbreviations: Sample A: one-part GJ and two-part CW, Sample B: equal part of GJ and CW, Sample C: two-part of GJ and one part of CW. Note: bars represent ± standard deviation of the sensory scores, and the bars with the same alphabets demonstrate that the samples do not significantly vary from one another.

3.5. Selection of best blend composition

Sensory evaluation revealed that there is no significant difference between wine containing the same proportion of grape juice and CW and wine containing double the proportion of grape juice and coconut water in terms of overall acceptability. So, according to color preference and antioxidant content, sample C was taken as the best among the three formulations. An increase in the concentration of grape juice increases the phenolic content, antioxidant activity, color, density, and color index (Poliana et al., 2010). Additionally, Yuyuen et al. (2015) suggest samples containing a higher concentration of grape juice are likely to produce wine with higher acceptance quality.

3.6. Limitations and recommendation

A microbiological analysis of fresh coconut water was not performed during the laboratory work. After the preparation of the wine, it was not adequately matured. To ensure robust conclusions, we recommended further research to be continued by performing microbiological analysis, and maturation of the wine.

4. Conclusions

This study can conclude that grape juice can be blended for the preparation of CW blend wine. The concentration of grape juice has a significant effect on the physicochemical and sensorial characteristics of the wine. An increase in grape juice concentration results in a higher concentration of phytochemical contents. Grape juice blended with CW wine was found to be highly acceptable by the panelists. Two parts of grape juice can be blended with one part of CW for the wine preparation.

Compliance with Ethical Standards

Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contributions

Nabin KHADKA: Conceptualization, Supervision, Methodology, Software, Resources, Validation, Data Curation, Writing- review & editing; **Manita CHAUDHARY**: Methodology, Formal analysis, Resources, Validation, Writing-original draft, Writing- review & editing. **Ganga SANGROULA**: Writing- reviewing & editing, Writing-original draft, Software, Validation, Supervision, Data curation. **Aayusha REGMI**: Software, Resources. **Sabin Bahadur KHATRI**: Software, Resources. **Navin GAUTAM**: Software, Resources.

Ethical approval

The participants in the sensory evaluation voluntarily took part and scored the samples with full satisfaction.

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Data availability

The data underlying this study are available on request from the corresponding author.

Consent for publication

We humbly give consent for this article to be published.

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Factors affecting usage of digital advisory tools and services by vegetable farmers in Nepal

Kiran Kumar GUPTA[1], Udit Prakash SIGDEL[1], Om Prakash SINGH[1], Pankaj Raj DHITAL[1], Ram Hari TIMILSINA[1], Asmita GUPTA[2], Raksha UPRETI[3]

[1] Department of Agricultural Extension and Rural Sociology, Faculty of Agriculture, Agriculture and Forestry University, Rampur, Chitwan-Nepal
[2] Nepal Polytechnic Institute,College of Agriculture, Engineering, Medicine and Veterinary Science, Purwanchal University, Bharatpur-Nepal
[3] Himalayan College of Agriculture Science and Tehnology, Salyanthan, Kirtipur, Kathamandu-Nepal

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*CONTACT

kkgkirangupta@gmail.com

ABSTRACT

It is evident that several factors condition the usage of Digital advisory tools and services (DATS) namely the internet, mobile phone, TV, radio, social media in the agricultural sector. The core, socio-economic factors and regional differences in technology adoption are being addressed. With the use of a multistage purposive sampling technique the study was steered to assess factors affecting the DATS use in Lumbini province. Using survey research design and administering pre-tested semi-structured interview schedule; 135 samples were randomly selected from Rupandehi (Terai), Palpa (Midhill) and Rukum East (Mountain) districts. The data were analyzed using multivariate probit regression model along with apt scaling and indexing techniques. The findings reveal that the tool's performance expectancy, the ease of use and relevance to user needs were the core factors while age, education level, experience, group membership and geographical variation were influencing factors shaping farmers' behavior for choosing different information sources. The results indicate that vegetable farmers rely on a variety of information resources which can be grouped under locality, cosmopolite, traditional and modern DATS. These sources either complement or substitute one another. This implies that no single source fully satisfies all of a farmer's information needs. If information providers get abreast of how likely it is that farmers will choose any particular source of information, they can guide efforts and shape policies to communicate via those channels in specific areas, generating higher impact. In light of the findings, the study accentuates the need for policy reforms to boost information sources that are tailored to specific regional and socio-economic profile of farmers.

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ORCID> 0009-0005-2853-1246 (KKG), ORCID> 0000-0003-3865-8820 (UPS), ORCID> 0000-0001-5564-5031 (OPS), ORCID> 0009-0008-9165-980X (PRD), ORCID> 0000-0001-7721-5120 (RHT), ORCID> 0009-0009-9277-7127 (AG), ORCID> 0009-0003-9031-9751 (RU)

1. Introduction

The use of agricultural information by farmers has become increasingly crucial for taking decisions in farm management (Opara, 2008). Additionally, farmers' need for dire information have evolved as a result of depleting natural resources, diversifying farming practices, transforming dietary habits, increasing agro-business and overt climate change impacts. With rapid technological advancements and rapidly changing agricultural systems, there is a heightened need for efficiently transferring up-to-date knowledge to growers via different media platforms (Birkhaeuser et al., 1991).

Commercially done vegetable farming occupies a noteworthy role in uplifting rural lives in the Lumbini Province. The voice telephony has reached 126.04% and the internet coverage up to 130.64% in the country (Nepal Telecommunication Authority [NTA], 2022). Digital advisory tools and services (DATS) has not only changed the farming style but also the life-style of commercial farmers (Gyawali, 2022). Agricultural information plays significant role in enabling vegetable growers to take decision on sowing, soil improvement, controlling pests and diseases and seeking the best market prices for their produces (Armstrong et al., 2012). Information on vegetables and price in the market develops confidence and income to farmers (Pudasainee and Chaulagain, 2020). With real time market information, farmers can make informed decisions on vegetable crop selection, timing of harvest, and market channels to go through. Vegetable farmers can also get forecasting on heavy rains and floods; which enables them to prepare better for reducing the possible loss (GC et al., 2022). Overall, DATS have the potential to revolutionize commercial farming by enhancing productivity, reducing risks, and promoting sustainable practices.

Vegetable growers often face challenges related to crop management, market access, and profitability. The supply chain of agri-inputs was highly disturbed during the months long COVID-19 pandemic (FAO, 2020). It either left famers devoid of guidance and supervision in their farming or delays in advisory services (Pradhan, 2020). In such crisis, farmers can remain in touch with the agricultural advisors through DATS (Bhusal, 2020). The adoption of DATS among vegetable growers in Nepal is still low and there is a need to evaluate the factors that undermine their adoption and usage (Bhusal et al., 2021).

Thapa (2018) report that farmers in Nepal are least benefitted by using digital tools in terms of access to timely information about quality seeds, market rates, agriculture loans and subsidies, livestock and irrigation. The source of information for farmers is their local social network or their own experience. Chhetri (2016) argue that famers prefer local resources to professional resources for fetching information. In regard to weather forecast information, they rely on past occurrences and farming practices which are less precise. For instance, unseasonal rains in 2021 destroyed huge amount of ready-to-harvest paddy in Nepal which could have been substantially reduced provided that farmers were pre-informed via weather forecast mechanisms (Prasain, 2021). Moreover, the bargaining power of farmers is also compromised. Traders may exploit farmers by offering lower price due to farmer's ignorance of existing market price (Svensson and Yanagizawa, 2009).

Alike other developing countries: with the development of DATS, vegetable farmers in Nepal are embracing different digital tools for fetching farming related information. In addition to the written and audio-visual media digital technologies like information and communication technologies (ICTs), sensor, robotics, analytical technologies, machine learning, and artificial technologies have emerged. The digital tools used in Nepal of concern in this research are mobile SMS, agriculture related applications, websites and agri-web portals, e-mail, digital display boards, call centers, social media, radio, TV, mobile calls.

Some studies have revealed essential features that could make digital advisory tools appealing to users for adoption. These features include simplicity, user-friendliness, availability, and reliability. The accessibility and interoperability of these tools were also recognized as core factors for their successful use (Bracken, 2022). By the same token, research conducted by Mittal and Mehar (2016), Nwokoye et al. (2019) and Nikam et al. (2021) have suggested that age, sex, education level, farm size, income, ownership of smart phones, internet access effect on the farmer's choice decision for available DATS. Eloquently, it is necessary to understand the governing core and influencing factors associated with farmers in preferring amongst available information source.

Amongst all these, this research aims to empirically analyze the factors determining the usage of DATS by vegetable farmers in Lumbini province. This research's findings will be helpful in guiding the extension community for sustainable and impactful use of DATS in the vegetable farming sector.

2. Materials and methods

2.1. Data collection

This study used a survey research design. Three districts namely Rupandehi (Terai), Palpa (Mid hill) and Rukum East (High hill) were purposefully selected considering the geographical variation. The selected districts are vegetable baskets of major cities like Butwal, Tansen, Ghorahi, Tulsipur, Nepalgunj in the region. The study was accomplished utilizing a multistage sampling technique. Right after fixing the research districts, vigorous discussion with concerned stakeholders was organized to purposefully select the respective local levels, Gaindahawa Rural Municipality, Siddarthanagar municipality and Butwal Sub-metropolitan municipality in Rupandehi, Tansen municipality, Rampur municipality and Tinau Rural Municipality in Palpa and Sisne RM, Bhume RM and Putha Uttarganga RM in Rukum East (Figure 1). Altogether 135 households were randomly sampled, 15 from each local level using the random table in excel.

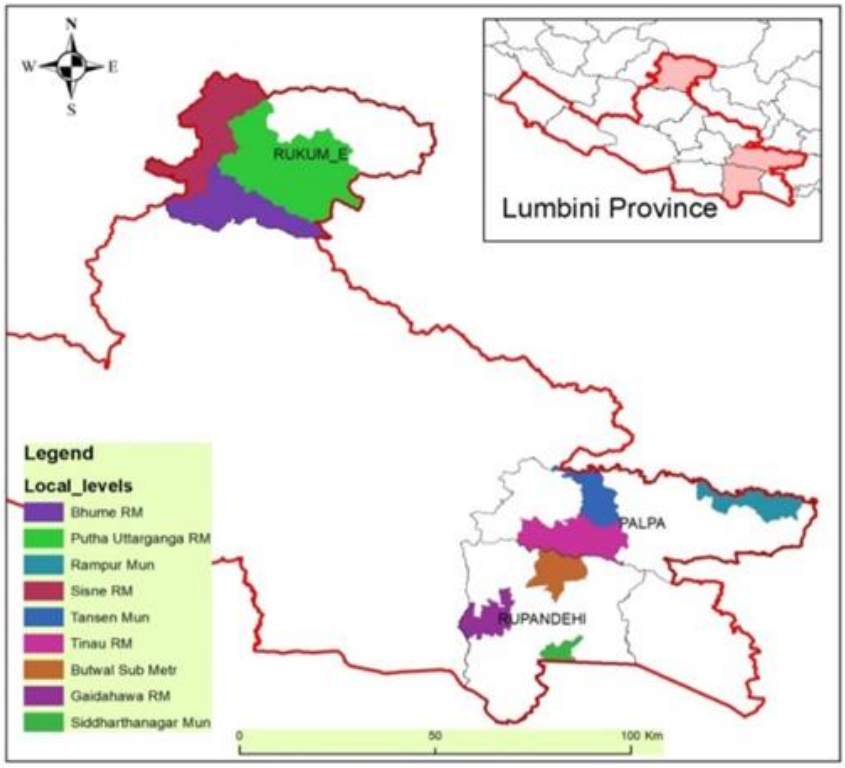

Figure 1. Map of the study site

The primary data was collected using household survey (135), Focused Group Discussion (FGD 3) and Key Informants Interview (KII 5) methods. The sampled respondents were interviewed by face-to-face method using semi-structured pre-tested interview schedule. An array of published materials like bulletins, books, journals, research articles, web surfing, progress reports and relevant publications served as secondary sources.

2.2. Operationalization of DATS

Digital advisory tools and services means software, programs, platforms, applications that can be run with computers or electronic gadgets. DATS incorporate text, audio and visual stimuli (Oikonomou and Patsala, 2020) that enable farmers to make informed decisions.

Computer, web sites, web portals, e-mail, RSS are regarded under the term internet whereas the Youtube and official Facebook pages are treated under the term social media. The term printed media encompasses newspaper, booklets, pamphlets, posters, agriculture magazines. The basis of consideration is on the review of the study of Sigdel et al. (2022). In the field survey, vegetable farmers identified 14 different sources for obtaining information on agricultural activities. For analysis, based on common characteristics, these various sources were classified into four domains (Table 1). The four categories are personal locality, cosmopolite, traditional DATS and modern DATS.

Table 1. Assembling different information sources according to common traits

S.N.	Class	Agricultural information-sources included
1	Personal Locality	Neighbors, local leaders, peer farmers
2	Cosmopolite	Extension workers, Farmer groups/ co-operatives, agrovets/ input suppliers
3	Traditional DATS	Radio, TV and printed media (newspaper, leaflet, pamphlet, folder)
4	Modern DATS	Mobile (Call and SMS), Internet, Agricultural applications (online and offline), social media and video conferencing

2.3. Explanation of variables (explanatory and dependent) used

Four different information sources' use is taken as dependent variable. Age, years of schooling, number of educated family members, years of experience in vegetable farming, association in farmer group/ co-operative, farm size and physiographic parameters represented by the district dummies are explanatory variables. Mittal and Mehar (2015) have recorded that age, level of education, and farm income are key factors influencing farmers' decisions when choosing among various information sources. The explanation of individual variables used in this research are described in Table 2.

Table 2. Explanation of the variables

Variables	Elaboration
1. Dependent variable	
Use of different sources of agriculture information	Use of one of the mentioned sources (personal locality, cosmopolite, traditional DATS and modern DATS for agriculture information (users=1; non-users=0)
2. Independent/ Explanatory variables	
Age	Respondents' age (in years) during data collection
Education (Years of schooling)	Years of formal education of the respondents
Farm size	Total land used for cultivation purpose (ha)
Membership in Farmer's group/ co-operative	Membership (=1 If respondent is a member of any farmers group/ co-operative, 0 otherwise)
Farming experience	Vegetable farming experience in years
Educated family size	Number of educated members in the respondent's family
District dummies	Representing different districts to address geographical variability
Rupandehi dummy	Terai (=1 if respondent is resident of Rupandehi, 0= otherwise)
Palpa dummy	Midhill (=1 if respondent is resident of Palpa, 0= otherwise)

Note: Rukum East is taken as benchmark

2.4. Likert scale for perception measurement

Perception of respondents regarding use of DATS for fetching agricultural information was analyzed using 5-point Likert scale. Rensis Likert (1932) developed it for measuring opinion, perception and attitudes of individuals. Perception of respondents was measured at 5 agreement levels; strongly agree (SA), agree (A), neutral (N), disagree (DA) and strongly disagree (SDA). The weightage values 1, 0.5, 0, -0.5, -1 was assigned for SA, A, N, DA and SDA responses respectively.

Scoring of scale (Tanujaya et al., 2022)

Respondent's perception regarding various DATS for agriculture information was analyzed using index value. As, the index of agreement was estimated by:

Iagg=Σ(Si fi)/N

Σ= Summation

Iagg= Index of agreement

Si= Scale weightage

Fi= Frequency of respondents occupying a given level of agreement

N= Number of respondent farmers in total

Then, specific points were assigned to each classification and index value was calculated.

$$\text{Index value of particular statement} = \frac{\begin{array}{c}\text{Frequency of SA}\times 1+\\ \text{Frequency of A}\times 0.5+\text{frequency of N}\times 0+\\ \text{Frequency of DA}\times -0.5+\text{ Frequency of SDA}\times -1\end{array}}{\text{Frequency of total respondents}}$$

Based on a comparison of mean index value of each statement calculated from index values of Rupandehi, Palpa and Rukum East, ranking was done.

2.5. Multivariate probit regression analysis

The data gathered show that farmers utilize a variety of information sources, and it is believed that these sources are used concurrently to meet similar information needs. The null hypothesis states that no significant link exists between vegetable growers' socio-economic and institutional characteristics and use of the given sources. It infers that, regardless of factors like age, education, experience, farm size, number of educated members in family, organizational involvement, or geographic location, farmers tend to rely on certain information sources. To assess their choice among four groups of information sources, either multinomial or multivariate regression models can be used. Multinomial models assume that irrelevant alternatives are independent, meaning the error components of the alternatives are unrelated (Greene, 2003). Since farmers often rely on multiple sources simultaneously, the error terms of these sources may be related. In the wake of contemporaneous correlation amongst given choices, the multivariate regression model is ideal. Previous studies; too have employed multivariate probit regression to analyze the factors distressing the usage of varied agricultural information sources (Jenkins et al., 2011; Mittal and Mehar, 2015). Mittal and Mehar (2015) used this method to investigate the factors influencing farmers in adopting "face-to-face communication, advice from other farmers, traditional media, and modern ICT". They suggest that the multivariate probit model improves estimation efficiency when multiple sources are used at the same time.

Model specification

Mathematically,

$$Y_{i1} = X'_{ij_1}\beta 1 + \varepsilon_{i1}$$

$$Y_{i2} = X'_{ij_2}\beta 2 + \varepsilon_{i2}$$

$$Y_{i3} = X'_{ij_3}\beta 3 + \varepsilon_{i3}$$

$$Y_{i4} = X'_{ij_4}\beta 4 + \varepsilon_{i4}$$

Where, i= farmer identification, Y_i1=1 for respondent using locality sources (0: if not), Y_i2=1 if respondent using cosmopolite sources (0: if not), Y_i3=1 if farmer using traditional DATS (0: if not), Y_i4=1 if farmer exploiting modern DATS (0: if not), X_i^'= vector of factors determining access to the sources, β_j= vector of unidentified parameters (j=1, 2, 3, 4) and ε = error term. By assuming, error terms are mutually exclusive, four individual binary logit or binary probit forms can be employed. But in this case, the error terms are stochastically dependent on each other. Therefore, it will be wise to run the multivariate probit equation as mentioned below.

$$Y_{ij} = X'_{ij}\beta'_j + \varepsilon_{ij}$$

Where, Y_ij (j=1, 2, 3, 4) are four varied information pathways confronted by the ith respondent (i= 1, …., 135), X_ij^' is a 1 × k vector of observed variables determining the choice decision, β_j is a 1 × k vector of unrealized parameters and ε_ij is the unseen stochastic error term. Let's assume the error terms (in j= 1, 2, 3, 4) terms follow normal distribution with mean vector = 0 and are multivariate in nature. Then, simulated maximum probability can be used to calculate the unidentified Ass parameters. This technique applies to the Geweke, Hajivassiliou and Keane (GHK) smooth recursive conditioning simulator) for evaluating the multivariate normal distribution (Cappellari and Jenkins, 2003). The widely preferred software: STATA (version 17) is administered for estimation.

Question of multicollinearity between the explanatory variables was resolved computing a condition index factor which was typically recommended by Belsley et al. (1980). The condition index value measured less than 4, which showed that there would be no severe multicollinearity problem in our data. Furthermore, we calculated pairwise correlation amongst error terms for endogeneity testing where farmers' choice of information sources was endogenously determined and tested it significant to show that if multivariate probit model was appropriate.

3. Results

3.1. Core factors affecting DATS use

Table 3 depicts the core factors that motivate the respondent farmers for using digital advisory tools and services in vegetable farming. Result depicted that the ease of use (0.75) was the major motivating core factor in Rupandehi followed by performance expectancy (0.72). Similar case was found in Rukum East, whereas in Palpa performance expectancy of tool (0.84) followed by relevance to user needs (0.78) were the major motivating core factors for the vegetable growers in the surveyed region. Overall, the performance expectancy of tools (0.78) was the major driving factor while the cost of tools (0.39) and farmer advisory compatibility (0.37) were found to be the least motivating core factors in all three districts.

Table 3. Core factors affecting the use of specific DATS by respondents

Statements	Index Value			Mean	Rank
	Rupandehi	Palpa	Rukum East		
Performance expectancy of tool	0.72	0.84	0.77	0.78	I
The ease of use	0.75	0.67	0.78	0.73	II
Peer recommendations	0.64	0.48	0.46	0.53	V
Trust in embedded technology	0.64	0.67	0.55	0.62	IV
The cost of tool	0.42	0.31	0.43	0.39	VIII
Usual habits	0.47	0.48	0.47	0.47	VI
Relevance to user needs	0.58	0.78	0.69	0.68	III
Farmer advisor compatibility	0.37	0.35	0.39	0.37	IX
Farmer skills and behavior	0.45	0.45	0.47	0.46	VII

The above index value varies from -1 to 1; positive value reverberates to agreement

3.2. Influencing factors affecting decision-choice of different information-sources

Table 4 presents regression results of the run probit model. It highlights the calculated results on the aspects influencing selection-decision of four information sources available to the farmers in Lumbini province. We justify the application of multivariate probit model by the existence of positive likelihood ratio value. The conjoined nullity of variable coefficients comprised in the calculation is rejected as the value of wald $\chi 2$ test is significant.

This study uses seven explanatory variables to analyze their effect on usage of different sources. The results obtained by the model show that age, years of schooling, number of educated family members, years of experience in vegetable farming, association in farmer group/ co-operative have negative coefficient for personal locality sources while farm size and physiographic dummy variables have positive coefficients. Among them, education level, experience years and membership in FG/co-operatives have significant effects at 5%, 5% and 1% respectively.

Farm size and age do not have any significant effect while the Rupandehi and Palpa are positively significant for personal locality sources of information. In context of cosmopolite sources age, schooling years, group membership and farm size have positive coefficient. Years of schooling and membership in groups act significantly at 5% level of significance. Likewise, the number of educated family members, years of experience in vegetable farming and physiographic dummies possess negative values. Among them, vegetable farming years is significant at 1%.

As shown by run model results, for the traditional media sources; age, number of educated family members, experience, membership in group and physiographic dummies have positive coefficients out of which only age variable is significant at 5% significance-level. Education level and farm size affect negatively but have no significant effect on use of traditional DATS for agricultural information.

In the case of modern DATS use for agriculture information; age, years of schooling, vegetable farming experience, association in FGs/ co-operative, physiographic dummies are the factors that affect positively. Years of schooling, Rupandehi dummy and number of educated family members have positive and significant effect on the use of modern DATS at 1%, 5% and 10% respectively.

Table 4. Estimated farmer characteristics regarding utility of agricultural information sources

Attributes	Personal locality	Cosmopolite	Traditional DATS	Modern DATS
Age	-0.016	0.026	0.032**	0.039
	(0.015)	(0.020)	(0.015)	(0.035)
Years of schooling	-0.085**	0.095**	-0.034	0.537***
	(0.036)	(0.045)	(0.035)	(0.146)
Number of educated family members	-0.063	-0.074	0.025	0.419*
	(0.095)	(0.117)	(0.094)	(0.231)
Year of experience in vegetable farming	-0.086**	-0.160***	0.017	0.025
	(0.041)	(0.054)	(0.042)	(0.086)
Association in Farmer Group or co-operative	-0.932***	0.817**	0.081	1.105
	(0.331)	(0.369)	(0.305)	(0.755)
Farm size	0.378	0.803	-0.220	-0.447
	(0.420)	(0.570)	(0.406)	(1.326)
District dummies				
Rupandehi	0.872**	-0.648	0.587	4.769**
	(0.361)	(0.484)	(0.377)	(1.964)
Palpa	0.623**	-0.139	0.147	0.001
	(0.308)	(0.411)	(0.309)	(0.622)
Constant	2.561***	-0.185	-0.955	-4.850**
	(0.895)	(1.147)	(0.837)	(2.444)
Log Likelihood value	-74.73	-41.74	-75.72	-16.95
Wald $\chi 2$ test (9)	28.82***	25.56***	19.12**	20.38***
LR test of errors		12.37*		
Number of observations		135		

Source: Field survey, 2022, Note: Rukum East is used as benchmark dummy. Figures in parentheses represent robust standard errors. *, **, *** represent statistical significance at 10%, 5% and 1% level of significance.

4. Discussion

4.1. Core factors affecting DATS use

The results highlighted that the performance expectancy of tools, the ease of use and relevance to user needs were the major driving factor while the cost of tools and farmer advisory compatibility were found to be the least motivating core factors in all three districts. Similar to the findings, Brackley (2022) reported that simplicity, easy to use, accessibility and perceived usefulness characteristics of DATS motivated users to adopt them.

4.2. Influencing factors affecting DATS use

Results indicate that years of schooling, experience in vegetable farming, membership in farmer groups or cooperatives, and physiographic factors significantly influence farmers' choice of personal locality sources for agricultural information. The negative coefficients for years of schooling, experience in vegetable farming, and group membership suggest that as farmers' education, experience, and group involvement increase, they tend to rely less on locality sources. Conversely, the positive and significant coefficients for the Rupandehi and Palpa dummies indicate a higher likelihood of using local sources for agricultural information compared to the reference district. Additionally, the significant-positive constant value suggests, all other factors remaining the same, the studied vegetable farmers are highly inclined towards personal locality sources.

In case of cosmopolite information sources, the coefficients for years of schooling and membership in farmer groups or cooperatives are positively significant, indicating that as education level and group membership increase, vegetable farmers highly remain confident on cosmopolite resources. However, the negatively significant coefficient for years of experience in vegetable farming suggests that more experienced farmers are less inclined to prefer cosmopolite sources for agricultural information.

Regarding 'Traditional media' information sources, the positive and significant coefficient for age implies that older farmers place greater value on information provided by traditional media. The coefficients for education level and farm size are not significant in relation to the use of traditional media.

Modern DATS, which includes mobile phone calls and SMS, internet, social media, and agriculture applications, exhibit positive-significant linkage with years of schooling and the number of educated family members. This indicates that educated farmers put huge confidence to modern DATS, as they can easily access, integrate, and use these sources according to their needs. Interestingly, the likelihood of using modern digital tools increases with the number of educated family members, as farmers may receive assistance from their spouse, children, or siblings in operating these tools. Group membership has a positive but not significant effect on DATS use for agricultural information. This is in contrary to the findings of Nwafor et al. (2020), who argue that group associated vegetable farmers are more aware of DATS. The district Rupandehi positively and significantly influences the adoption of modern DATS by farmers. The negative-significant coefficient advocates that, ceteris paribus, the respondents rarely solely rely on modern DATS. Though users appear more motivated to use modern DATS, they do so mainly as a complement to traditional sources.

In summing up, the estimated results reject the Ho that posited farmer characteristics don't affect the use of above agricultural information-sources. As level of education of respondent's augments, they incline towards modern DATS for information than that of locality sources. The findings are in congruent with Bakari et al. (2018), and Mittal and Mehar (2015), who also found education to significantly impact DATS usage. The significant-negative coefficient of years of experience suggest that experienced farmers already have extensive knowledge regarding vegetable farming. So, they regard local and cosmopolite sources of less worth. Though, "farm size is considered as proxy for farmer's economic status", it does not have any significant effect on adoption of different sources. This gist accords with Bakari et. al. (2018) where non-significant result was recorded. The result contradicts Luqman et. al. (2019) and Derso et. al. (2014). They arbitrated; large farmers have more resources, market surplus, interconnection with stakeholders unlike most of the small farmers whose production is subsistence type. Members of farmer's group/ co-operative are preferring cosmopolite sources to locality sources as they have higher exposure to first-hand information delivered from agrovets, group executives, extension advisors. The physiographic dummy variable captures the variation due to physical infrastructure, demography, and geographic constraints. Empirically, these findings remain in line with the results presented by Mittal and Mehar (2015).

5. Conclusion

This study focused to evaluate the factors affecting the DATS usage in the farm information uptake by vegetable growers. There are a wide range of DATS coming downstream that are promising to significantly improve the way farmers can complete their work. The study also identified key factors that influence farmers' decision in choosing them.

The results revealed that the performance expectancy of tool, the ease of use and relevance to user needs were the core driving factor. Similarly, socio-economic-institutional traits of farmers such as age, education level, experience, size of farm and group membership, are significantly linked with the application of various agricultural information sources. These findings can be utilized to develop programs tailored to farmers' specific profiles. In simpler terms, information providers can use these insights to better communicate with their target growers either solely or in companion with other sources. The insights highlight the complementary nature of different information sources, and the vital role education can play in connecting users to new exciting avenues. Overall, while farmers manifest belief on numerous sources, they heavily depend on locality and cosmopolite sources yet. Additionally, we acknowledge that the existing policy should be directed towards enhancing farmer's access to, expertise in and high quality of information consistent to farmer's conditions for motivating them to use DATS.

6. Limitations and future research direction

Acknowledging the limitations of the current study, there exists a considerable scope for conducting additional research on the utilization of DATS by farmers.

- The present study solely concentrated on vegetable farmers of Rupandehi, Palpa and Rukum East district of Lumbini province; however, replication of the research should be carried in other crops and locations in the future.
- Future research could investigate the factors determining use of specific DATS by extension personnel.

Compliance with Ethical Standards

Conflict of interest

No competing interest attested.

Authors' contributions

Kiran Kumar GUPTA: Conceptualization, project administering, Data curing and analysis, Writing original draft . **Udit Prakash SIGDEL**: Formal analysis, review and editing. **Om Prakash SINGH**: Review and editing. **Pankaj Raj DHITAL**: Draft preparation. **Ram Hari TIMILSINA**: Draft preparation. **Asmita GUPTA**: Draft preparation. **Raksha UPRETI**: Draft preparation

Ethical approval

The participants in the sensory evaluation voluntarily took part and scored the samples with full satisfaction.

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Data availability

The data underlying this study are available on request from the corresponding author.

Consent for publication

Not applicable.

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Determination of distribution and population change of Drosophilidae (Diptera) species in cherry and peach orchards in Tokat province (Türkiye)

Hüseyin Bilal TAŞLIOĞLU[1], Turgut ATAY[2]*

[1] Middle Black Sea Transitional Zone Agricultural Research Institute, Tokat-Türkiye
[2] Tokat Gaziosmanpaşa University, Faculty of Agriculture, Department of Plant Protection, Tokat-Türkiye

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*CONTACT

turgut.atay@gop.edu.tr

ABSTRACT

In this study, the presence and prevalence of Drosophilidae species were studied in cherry and peach plantations of Tokat province in 2021-2022. In addition, the population of the species belonging to the family was monitored for two years in one peach and cherry plantations in central district of Tokat. According to the results, a total of 10 species were recorded. These species are *Drosophila hydei* Sturtevant, 1921, *D. immigrans* Sturtevant, 1921, *D. melanogaster* Meigen, 1830, *D. phalerata* Meigen, 1830, *D. simulans* Sturtevant, 1919, *D. subobscura* Collin, 1936, *D. suzukii* (Matsumura, 1931), *Gitona distigma* Meigen, 1830, *Scaptomyza pallida* (Zetterstedt, 1847), *D. transversa* Fallen, 1823. Of these, 10 species were determined in the central district of Tokat, 9 species in Erbaa, 8 species in Turhal and 6 species in Pazar. *D. subobscura* Collin, 1936 was the most common species in cherry and peach fields in central district of Tokat in 2021 and 2022. In Erbaa, *D. subobscura* was the most common species in cherry fields in 2021 and *D. hydeii* Sturtevant, 1921 and *S. pallida* (Zetterstedt, 1847) took the first place in terms of prevalence in peach fields. In 2022, the most common species in cherry and peach fields was *D. subobscura* in Erbaa. While *D. hydei* was the most common species in cherry and peach plantations in Turhal in 2021, *D. immigrans* Sturtevant, 1921 in cherry fields, and *D. subobscura* in peach fields were common in 2022. In the cherry fields in Pazar, the common species was *D. immigrans* in 2021 and *D. subobscura* in 2022. *D. suzukii* (Matsumura, 1931), one of the important species in the family, was recorded in cherry and peach plantations in central district of Tokat and Erbaa, and in peach plantations in Turhal. *D. suzukii* was seen for the first time on 25 Aug. (2 specimens) in the cherry fields of Tokat Centre in 2021, while it could not be detected in the peach fields. In 2022, the first adult detection in cherry areas was made on 29 Aug. (7 specimes) and in peach areas on 08 Aug. (1 specimen). This study is the first detailed study on Drosophilidae family in Tokat (Türkiye) province.

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ORCID> 0000-0002-8109-6301 (HBT), ORCID> 0000-0002-9074-0816 (TA)

1. Introduction

Drosophilidae is a family of Diptera with a rich species diversity with 4700 species belonging to 77 genera worldwide (Bächli, 2023). Only 36 species are known in our country (Koçak and Kemal, 2013). Most drosophilid species are saprophagous and it is known that they consume decomposing plant materials (Schmitz et al., 2007). Some species of the family have caused significant quality and quantity losses in fruit areas, especially in recent years (Živković et al., 2019). In Türkiye, there are suitable habitats for the species of Drosophilidae family due to the fact that there are many different climatic conditions, geographical conditions are very variable regionally and host plant diversity is high. Some of the species of Drosophilidae family may pose a threat to agricultural production because they feed on cultivated plants, have a rapid spread and reproduction potential, easily adapt to climatic conditions, have a high number of generations and are polyphagous. Although many species of the family feed on rotten fruits on the ground, species such as *Drosophila suzukii* (Matsumura, 1931) and *Zaprionus indianus* Gupta, 1970 attack ripe and healthy fruits (Lee et al., 2011; Walsh et al., 2011; Özbek-Çatal et al., 2019). Especially in recent years, the prevalence of *D. suzukii* and *Z. indianus* species, which are invasive pests of the family, throughout the country and causing significant damages (Orhan et al., 2016; Özbek-Çatal et al., 2019) has increased the interest in the family in our country (Kaçar and Koca, 2017; Efil, 2018; Kasap and Özdamar, 2019; Zengin, 2020; Özbek-Çatal et al., 2021).

Drosophila suzukii, which is an invasive quarantine pest and has spread rapidly both in the world and in our country in recent years, is one of the important factors preventing fruit production. This species is a polyphagous pest that damages many fruits, especially of fruits (Bieńkowski and Orlova-Bienkowskaja, 2020; Çatal et al., 2021). *D. suzukii*, whose native land is Asia, was first detected in strawberry fields in Italy in Europe, then invaded western countries and America, infected many parts of these countries in a short time and threatened fruit production (Lee at al., 2011). In our country, it was first detected in strawberry orchards in Erzurum province in 2014, and its presence was observed in grape, nectarine, apple, pear, plum and cherry areas in Çanakkale, Adana, Karaman and Uşak provinces (Orhan et al., 2016; Efil, 2018; Öğür et al., 2018; Kasap and Özdamar, 2019; Zengin and Karaca, 2019). *Zaprionus indianus*, another invasive species belonging to the family, originates from tropical Africa and is known to be widespread throughout Central and South America in recent years. It has also been detected in some European countries (Soto et al., 2006; Yassin et al., 2008; Kremmer et al., 2017). It was determined for the first time in Türkiye in Adana, Hatay, Mersin and Osmaniye in 2019 (Özbek-Çatal et al., 2019). It is known that *Z. indianus* is a primary pest in about 80 fruit species such as figs, apples and strawberries and causes serious damage to fruits (Yassin and David, 2010). In our country, it has been determined that it damages figs, persimmon, blackberry, cherry, peach and plum (Özbek-Çatal et al., 2019). The presence of Z. tuberculatus, another species belonging to the genus *Zaprionus*, in our country was revealed in 2012 (Patlar et al., 2012) and is known as a secondary pest.

Studies on the family Drosophilidae in Türkiye are quite limited. Especially after the detection of the invasive species *D. suzukii*, *Z. indianus* and *Z. tuberculatus* in our country, studies on the detection and prevalence of these species and other species belonging to the family in our country have increased (Kaçar and Koca, 2017; Efil, 2018; Özbek-Çatal et al., 2019; Kasap and Özdamar, 2019). In the province of Tokat, which is an important fruit producer and in the transitional zone in terms of climate, no study has been carried out to determine the species belonging to the Drosophilidae family. In this study, it was aimed to determine the presence, distribution, first adult times and population fluctuations of the species belonging to the Drosophilidae family in the cherry and peach fields of Tokat province.

2. Material and methods

2.1. Determination of the distribution of species of the Drosophilidae

The surveys were carried out for two years in 4 districts (cherry and peach orchards in Centre, Erbaa and Turhal districts and only cherry orchard in Pazar district) where fruit is intensively cultivated in Tokat province. In the surveyed orchards, 2% of the districts with 50-100 da production, 1% of the districts with 101-1000 da production and 0.1% of the districts with 1001-10000 da production were sampled (Bora and Karaca, 1970) (Table 1, 2).

Table 1. Pest survey area in fruit orchards (Anonymous, 2020)

Districts	Total Area (decare)		Estimated Investigated Area (decare)	
	Peach	Cherry	Peach	Cherry
Erbaa	250	150	2.5	1.5
Pazar	-	350	-	3.5
Turhal	460	345	4.5	3.5
Centre	7.750	4.550	7.7	4.5

Table 2. Information on the gardens where traps were hung

Districts/Villages	Orchard	Coordinates	Altitude
Tokat/Centre/Kömeç	Peach	40.35876°N, 36,45025°E	621m
Tokat/Centre/Kemalpaşa	Cherry	40.36301°N, 36,50897°E	611m
Tokat/Turhal/Çarıksız	Peach	40.32504°N, 36,26169°E	608m
Tokat/Turhal/Çarıksız	Cherry	40.33115°N, 36,26230°E	651m
Tokat/Erbaa/Karayaka	Cherry	40.72877°N, 36.60200°E	272m
Tokat/Erbaa/Salkımören	Peach	40.72527°N, 36.61157°E	275m
Tokat/Pazar/Seyitali	Cherry	40,26889°N, 36,28472°E	629m

Particular attention was paid to the selection of untended and unsprayed orchards for the surveys. In order to determine the distribution of the species, traps were hung in the selected orchards one month before harvest. 100 mL of apple cider vinegar was placed in 500 mL plastic bottles with about 10 holes with a diameter of 3 mm. The traps prepared in this way were hung on the outer parts of the trees with 3 traps per garden (Figure 1). The traps were hung in the southeast direction of the trees at a height of 1.5 m from the ground (Grassi et al., 2011; Öğür et al., 2018; Zengin, 2020). For monitoring purposes, apple cider vinegar traps were checked weekly until harvest.

Figure 1. Vinegar traps hung in cherry and peach orchards

2.2. Determination of the first adult emergence time and population monitoring

In the centre of Tokat, studies were also carried out to determine the first adult emergence and population monitoring. For this purpose, population monitoring studies were carried out for two years in peach orchards with 100 trees in Kömeç and cherry orchards with 100 trees in Kemalpaşa, villages of Tokat-Centre. The traps prepared as mentioned above were hung in 4 pieces in each orchard at least 1 month before the mole fall period, when the fruits start to sweeten depending on the phenology of the fruit variety. The traps were checked weekly until one month after the fruit was harvested. The obtained data were correlated with the climatic data (daily average temperature and daily average relative humidity) obtained from Tokat Meterology Directorate. Temperature and humidity values are given as weekly average temperature and weekly average relative humidity. Graphs were created for the first 5 species with high population density. Specimens caught in the traps were preserved in 70% alcohol.

Identifications of the determined species were made according to Markow and O'Grady (2006), Miller et al. (2017) and Yuzuki and Tidon (2020). The identification of the species that could not be identified and the confirmation of the identifications were carried out by Dr. Burcu ÖZBEK ÇATAL (Çukurova University Pozantı Vocational School-Pozantı/Adana) and Assoc. Prof. Dr. Asime Filiz ÇALIŞKAN KEÇE (Çukurova University, Faculty of Agriculture, Department of Plant Protection-Adana).

3. Results

3.1. Determination of the distribution of species of the Drosophilidae

According to the results obtained, a total of 10 species were identified in Tokat province (Table 3). When the determined species are analyzed according to districts, years and fruit types 8 species were found in the cherry orchard in Tokat Centre in both 2021 and 2022, 8 species in 2021 and 9 species in 2022 in the peach orchard. In Turhal district, 4 species were found in 2021 and 6 species in 2022 in the cherry orchard, 5 species in 2021 and 7 species in 2022 in the peach orchard. In Erbaa district, 5 species were found in the cherry orchard in both 2021 and 2022, and 6 species were found in the peach orchard in both 2021 and 2022. In Pazar district, 3 species were found in 2021 and 6 species were found in 2022 in the cherry orchard (Table 3).

Table 3. Drosophilidae species detected in cherry and peach orchards by districts in 2021-2022

Species	Year	Cherry				Peach		
		Centre	Turhal	Erbaa	Pazar	Central	Turhal	Erbaa
Drosophila hydei Sturtevant, 1921	2021	+	+	+	+	+	+	+
	2022	+	+	+	+	+	+	+
D. immigrans Sturtevant, 1921	2021	+	+	+	+	+	+	+
	2022	+	+	+	+	+	+	+
D. melanogaster Meigen, 1830	2021	+	-	-	-	+	-	+
	2022	+	+	-	-	+	+	+
D. phalerata Meigen, 1830	2021	+	-	-	-	+	-	-
	2022	+	+	+	+	+	-	-
D. simulans Sturtevant, 1919	2021	+	-	-	-	+	-	-
	2022	+	-	+	-	+	+	+
D. subobscura Collin, 1936	2021	+	+	+	+	+	+	+
	2022	-	+	+	+	+	+	+
D. suzukii (Matsumura, 1931)	2021	+	-	+	-	+	+	-
	2022	+	-	-	-	+	+	+
Gitona distigma Meigen, 1830	2021	-	+	+	-	-	+	+
	2022	+	+	-	-	+	+	-
Scaptomyza pallida (Zetterstedt, 1847)	2021	+	-	-	-	+	-	+
	2022	-	-	-	+	-	-	-
D. transversa Fallen, 1823	2021	-	-	-	-	-	-	-
	2022	+	-	-	+	+	-	-

Turhal district in 2021, *D. hydei* (76 adults in cherry and 49 adults in peach) was the most frequently detected species in both cherry and peach orchards, followed by *D. immigrans* (13 adults in cherry and 12 adults in peach). In the 3rd place was *D. subobscura* (5 adults in cherry, 5 in peach). It was observed that *D. hydei* had a high population in June in both cherry and peach orchards in 2021 (Table 4). In 2022, *D. immigrans* (327 adults) had the highest population in cherry fields, followed by *D. subobscura* (137 adults) and *D. hydeii* (15 adults). In the cherry orchard, *D. immigrans* and *D. subobscura* were caught in traps in very high numbers in late May and early June. In peach orchards, the highest number of specimens was obtained from *D. subobscura* (49 adults), followed by *D. hydeii* (27 adults) and *D. immigrans* (21 adults). The highest number of *D. subobscura* and *D. immigrans* were caught in the traps at the end of May, while *D. hydei* was caught in the traps in mid-July and early August (Table 5).

Table 4. Drosophilidae species caught in traps in cherry and peach orchards in Turhal district in 2021

Species	Orchard	24 May.	31 May.	07 Jun.	14 Jun.	21 Jun.	28 Jun.	05 Jul.	12 Jul.	19 Jul.	26 Jul.	02 Aug.	09 Aug.
Drosophila hydeii	Cherry	1	0	32	10	8	25	-	-	-	-	-	-
D.hydeii	Peach	0	2	10	13	7	0	1	0	0	11	5	0
D.immigrans	Cherry	4	0	6	2	0	1	-	-	-	-	-	-
D. immigrans	Peach	1	0	2	3	1	0	0	0	0	0	1	4
D.subobscura	Cherry	2	0	0	2	1	0	-	-	-	-	-	-
D. subobscura	Peach	0	0	0	1	0	1	0	0	0	2	1	0
D. suzukii	Cherry	0	0	0	0	0	0	-	-	-	-	-	-
D.suzukii	Peach	0	0	0	0	0	0	0	3	0	0	0	1
Gitona distigma	Cherry	1	0	0	0	0	0	-	-	-	-	-	-
G.distigma	Peach	0	0	0	0	1	0	0	0	0	0	1	2

Table 5. Drosophilidae species caught in traps in cherry and peach orchards in Turhal district in 2022

Species	Orchard	23 May.	30 May.	06 Jun.	13 Jun.	20 Jun.	27 Jun.	04 Jun.	11 Jun.	18 Jun.	25 Jun.	01 Aug.	08 Aug.	15Aug.	22Aug.	29Aug.
Drosophila hydeii	Cherry	0	2	4	3	2	1	3	-	-	-	-	-	-	-	-
D. hydeii	Peach	1	2	0	3	5	0	0	0	5	0	3	5	2	1	0
D. immigrans	Cherry	250	26	51	0	0	0	0	-	-	-	-	-	-	-	-
D. immigrans	Peach	5	1	3	0	1	0	0	0	3	2	3	1	0	2	0
D. melonogaster	Cherry	0	0	0	0	0	0	0	-	-	-	-	-	-	-	-
D. melonogaster	Peach	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
D. phalerata	Cherry	2	0	0	0	0	0	0	-	-	-	-	-	-	-	-
D. phalerata	Peach	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D. simulans	Cherry	0	0	0	0	0	0	0	-	-	-	-	-	-	-	-
D. simulans	Peach	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1
D. subobscura	Cherry	71	14	33	3	5	9	2	-	-	-	-	-	-	-	-
D. subobscura	Peach	18	5	1	1	1	0	0	0	3	0	4	0	9	7	0
D. suzukii	Cherry	0	0	0	0	0	0	0	-	-	-	-	-	-	-	-
D. suzukii	Peach	0	0	0	0	0	0	0	0	0	0	1	0	0	1	3
Gitona distigma	Cherry	0	0	0	0	2	1	1	-	-	-	-	-	-	-	-
G. distigma	Peach	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0

In Erbaa district, *D. subobscura* (11 adults) was the most frequently detected species in the traps in the cherry orchard in 2021, followed by *D. hydei* (9 adults). In 3rd place was *D. immigrans* (5 adults). *D. subobscura* was most abundant in the traps in mid-May, while *D. hydei* and *D. immigrans* were seen in early June. In the peach orchard, *D. hydei* and *Scamptomyza pallida* (9 adults) shared the first place, followed by *D. immigrans* (5 adults). *D. hydei* and *D. immigrans* were detected in high numbers in the traps in mid-May and *S. pallida* in early June (Table 6). In 2022, the population density in the cherry orchard was determined as *D. subobscura* (63 adults), *D. immigrans* (10 adults) and *D. hydei* (5 adults), respectively. When we look at the months of the year, it is understood that *D. subobscura* was most abundant in early June, *D. immigrans* in early May and *D. hydei* in mid-June. In the peach orchard, the order of density was *D. subobscura* (36 adults), *D. suzukii* (16 adults) and *D. immigrans* (3 adults) and the months of occurrence were determined as early June for *D. subobscura*, August for *D. suzukii* and late August for *D. immigrans* (Table 7).

Table 6. Drosophilidae species caught in traps in cherry and peach orchards in Erbaa district in 2021

Species	Orchard	17 May.	24 May.	31 May.	07 Jun.	14 Jun.	21 Jun.	28 Jun.	05 Jul.	12 Jul.	19 Jul.	26 Jul.	02Aug.	09 Aug.
Drosophila subobscura	Cherry	5	1	1	1	2	1	0	-	-	-	-	-	-
D. subobscura	Peach	1	0	0	1	0	1	0	0	0	0	0	0	0
Gitona distigma	Cherry	1	0	0	0	0	0	1	-	-	-	-	-	-
G. distigma	Peach	1	0	0	0	0	0	0	0	0	0	0	0	0
D. immigrans	Cherry	1	0	0	2	0	1	1	-	-	-	-	-	-
D. immigrans	Peach	4	0	1	0	0	0	0	0	0	0	0	0	0
D. hydeii	Cherry	0	0	2	4	2	0	1	-	-	-	-	-	-
D. hydeii	Peach	3	2	2	1	0	0	0	0	0	0	0	1	0
Scamptomyza pallida	Cherry	0	0	0	0	0	0	0	-	-	-	-	-	-
S. pallida	Peach	0	0	0	5	0	4	0	0	0	0	0	0	0
D. suzukii	Cherry	0	0	0	0	0	1	0	-	-	-	-	-	-
D. suzukii	Peach	0	0	0	0	0	0	0	0	0	0	0	0	0
D. melonogaster	Cherry	0	0	0	0	0	0	0	-	-	-	-	-	-
D. melonogaster	Peach	0	0	0	0	0	0	0	0	0	0	0	1	0

Table 7. Drosophilidae species caught in traps in cherry and peach orchards in Erbaa district in 2022

Species	Orchard	23 May.	30 May.	06 Jun.	13 Jun.	20 Jun.	27 Jun.	04 Jul.	11 Jul.	18 Jul.	25 Jul.	01Aug.	08Aug.	15 Aug.	22 Aug.	29 Aug.
D.subobscura	Cherry	9	0	19	7	23	5	0	-	-	-	-	-	-	-	-
D.subobscura	Peach	8	3	10	0	0	0	0	3	5	0	1	0	1	1	4
D.immigrans	Cherry	4	0	3	1	2	0	0	-	-	-	-	-	-	-	-
D.immigrans	Peach	0	0	0	0	0	0	0	0	0	0	0	1	0	2	0
D.hydeii	Cherry	0	0	1	2	1	0	1	-	-	-	-	-	-	-	-
D.hydeii	Peach	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
D.simulans	Cherry	0	0	1	0	0	0	0	-	-	-	-	-	-	-	-
D.simulans	Peach	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
D.phalerata	Cherry	0	0	1	0	0	0	0	-	-	-	-	-	-	-	-
D.phalerata	Peach	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D.suzukii	Cherry	0	0	0	0	0	0	0	-	-	-	-	-	-	-	-
D.suzukii	Peach	0	0	0	0	0	0	0	0	2	2	-	2	4	2	4
D.melonogaster	Cherry	0	0	0	0	0	0	0	-	-	-	-	-	-	-	-
D.melonogaster	Peach	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

In Pazar district, the highest population density was observed in *D. immigrans* (49 adults) in the cherry orchard where traps were hung in 2021. This was followed by *D. hydei* (15 adults) and *D. subobscura* (8 adults). When it was analysed in terms of the time of peak densities, it was found that D. immigrans and *D. hydei* were detected during June and *D. subobscura* was detected at the end of June (Table 8). In 2022, *D. subobscura* (290 adults), *D. hydei* (29 adults) and *D. immigrans* (23 adults) constituted the first three ranks according to population density, and when their densities according to months were examined, it was determined that *D. subobscura* and *D. immigrans* were seen intensively in the traps in May-June and *D. hydei* in late May-early June (Table 9).

Table 8. Drosophilidae species caught in traps in cherry orchard in Pazar district in 2021

Species	Orchard	17 May.	24 May.	31 May.	07 Jun.	14 Jun.	21 Jun.	28 Jun.	05 Jul.
Drosophila immigrans	Cherry	8	0	0	17	3	12	9	0
D. subobscura	Cherry	1	0	1	0	0	0	6	0
D. hydeii	Cherry	0	0	1	1	4	4	5	0

Table 9. Drosophilidae species caught in traps in cherry orchard in Pazar district in 2022

Species	Orchard	23 May.	30 May.	06 Jun.	13 Jun.	20 Jun.	27 Jun.	04 Jul.
Drosophila immigrans	Cherry	6	5	3	5	1	3	0
D. subobscura	Cherry	65	32	32	63	66	28	4
D. hydeii	Cherry	3	9	11	4	1	0	1
D. phalerata	Cherry	0	3	3	0	3	4	0
D. transversa	Cherry	0	0	1	0	1	1	0
Scamptomyza pallida	Cherry	0	0	0	0	1	0	0

3.2. Determination of the first adult emergence time and population monitoring

The first adult of *Drosophila subobscura* in Tokat-Centre-Kemalpaşa village cherry orchard in 2021 was seen in the traps on 21.04.2021 (14.7°C, 61.4% RH), the number of insects caught reached the highest number with 120 individuals on 19.05.2021 (18.8°C, 60.8% RH) and the maximum number of insects caught by the end of June was 2 on a weekly basis (Figure 2a). In 2022, the first adult was detected in the traps on 09.05.2022 (14.6°C, 56.9% RH), reached the highest number with 263 individuals on 16.05.2022 (15.3°C, 58.3% RH) and the number of insects caught by the end of July did not exceed 5 (Figure 2b). The first adult emergence of *D. subobscura* in Tokat-Centre-Kömeç village peach orchard in 2021 was on 28.04.2021 (18.6°C, 54.3% RH), the number of insects caught in traps reached the highest level with 31 individuals on 19.05.2021 (18.8°C, 60.8% RH) and the number of adults caught in the following counting intervals did not exceed 2 (Figure 2c). In 2022, the first adult emergence was detected on 25.04.222 (18.3°C, 53% RH), reached the highest number with 68 individuals on 16.05.2022 (15.3°C, 58.3% RH) and the number of adults captured from the end of May did not exceed 6 (Figure 2d).

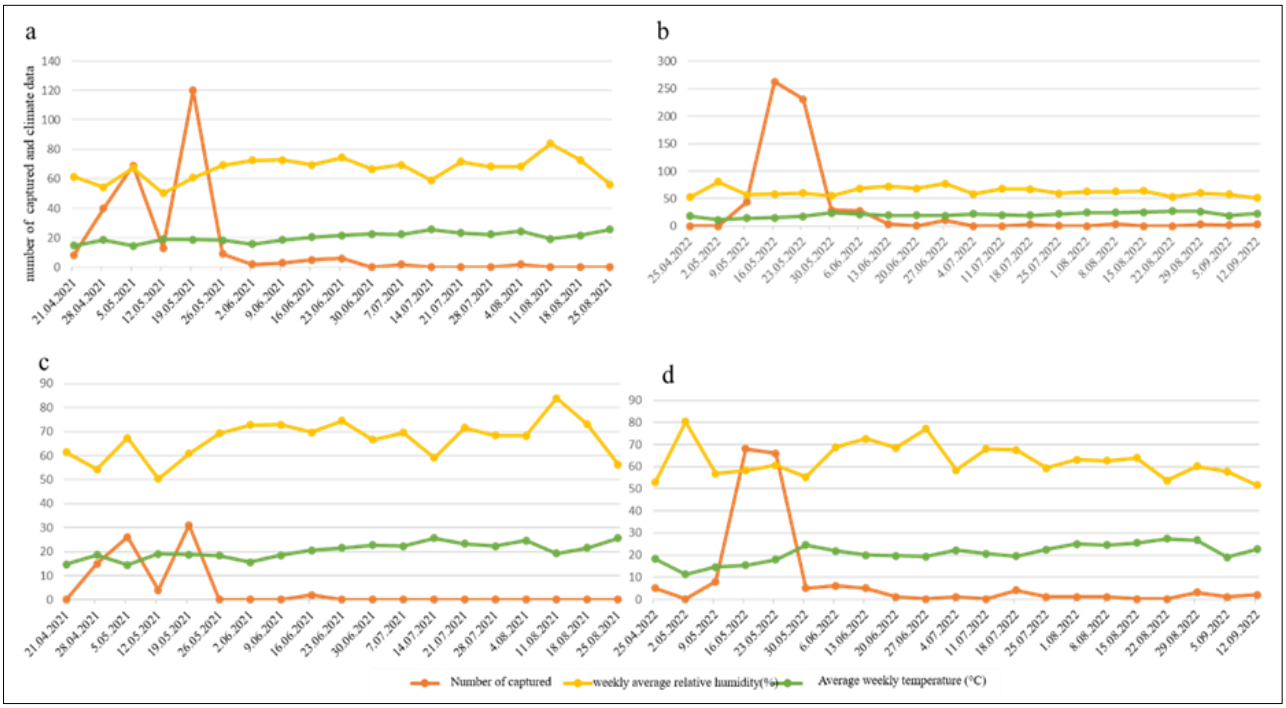

Figure 2. Population density of *Drosophila subobscura* in cherry and peach orchards in Tokat-Centre in 2021-2022 (a-2021 cherry, b-2022 cherry, c-2021 peach, d-2022 peach)

Drosophila immigrans appeared in the cherry orchard of Tokat-Centre-Kemalpaşa village in 2021 on 28.04.2021 (18.6°C, 54.3% RH) and in 2022 on 09.05.2022 (14.6°C, 56.9% RH) and the number of adults caught in traps during the vegetation did not exceed 4 individuals in both years (Figure 3a, b). The first adult of *D. immigrans* in the peach orchard of Tokat-Centre-Kömeç village in 2021 was detected on 28.04.2021 (18.6°C, 54.3% RH) and in 2022 on 13.06.2022 (20°C, 72.7% RH), and the number of adults caught in traps during the vegetation did not exceed 5 individuals in both years (Figure 3d, c).

The first adult of *Drosophila hydei* in Tokat-Centre-Kemalpaşa village cherry orchard in 2021 was detected on 28.04.2021 (18.6°C, 54.3% RH) and the highest population density was recorded on 19.05.2021 (18.8°C, 60.8% RH) with 16 individuals (Figure 4a). In 2022, the first adult was seen on 16.05.2022 (15.3°C, 58.3% RH) and the number of adults caught in other counts made in traps during the season in both years did not exceed 4 (Figure 4b). In the peach orchard of Tokat-Centre-Kömeç village, *D. hydei* was first seen in traps on 19.05.2021 (18.8°C, 60.8% RH) in 2021 and on 16.05.2022 (15.3°C, 58.3% RH) in 2022, and the number of adults caught in traps did not exceed 2 in both years during the vegetation (Figure 4c, d).

Figure 3. Population density of *Drosophila immigrans* in cherry and peach orchards in Tokat-Centre in 2021-2022 (a-2021 cherry, b-2022 cherry, c-2021 peach, d-2022 peach)

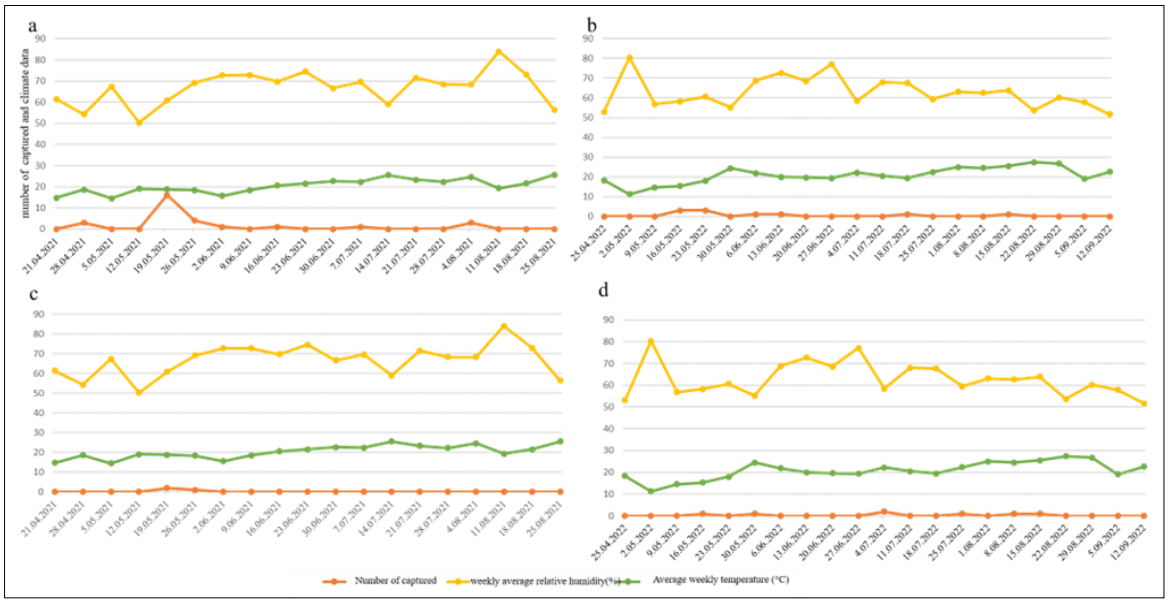

Figure 4. Population density of *Drosophila hydei* in cherry and peach orchards in Tokat-Centre in 2021-2022 (a-2021 cherry, b-2022 cherry, c-2021 peach, d-2022 peach)

Drosophila melanogaster was first detected in the cherry orchard of Tokat-Centre-Kemalpaşa village in 2021 on 28.04.2021 (18.6°C, 54.3% RH) and the highest number of adults in the traps was found on 19.05.2021 (18.81°C, 60.8% RH) with 9 individuals. Afterwards, the number of individuals caught was quite low and reached 7 at most (Figure 5a). In 2022, the first detection was made on 27.06.2022 (19.4°C, 77.1% RH), and the number of individuals caught did not exceed 3 (Figure 5b). *D. melanogaster* was first caught in traps on 05.05.2021 (14.5°C, 67.3% RH) in the peach orchard of Tokat-Centre-Kömeç village in 2021, and the number of adults caught weekly did not exceed 4 (Figure 5c). In 2022, the first detection was made on 15.08.2022 (25.5°C, 63.4% RH) and the highest population density was determined on 05.09.2022 (19°C, 57.8% RH) with 10 individuals. In the other 2 counting intervals, the number of insects in the traps did not exceed 7 (Figure 5d).

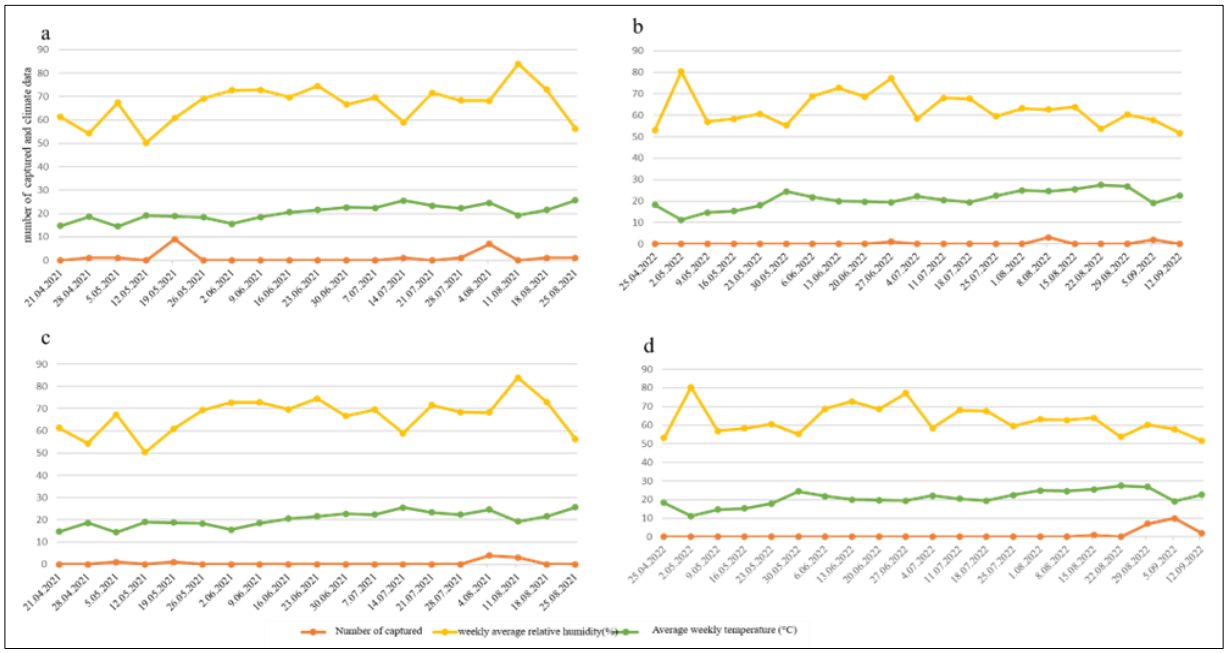

Figure 5. Population density of *Drosophila melonigaster* in cherry and peach orchards in Tokat-Centre in 2021-2022 (a-2021 cherry, b-2022 cherry, c-2021 peach, d-2022 peach)

Drosophila suzukii was detected only on 25.08.2021 (25.6°C, 56.3% RH) in the cherry orchard of Tokat-Centre-Kemalpaşa village in 2021 (Figure 6a). In 2022, it was first detected on 29.08.2022 (26.8°C, 60.2% RH) and the highest population density was determined on 05.09.2022 (19.04°C, 57.8% RH) with 16 individuals. In the next single counting interval, 1 individual was detected (Figure 6b). *D. suzukii* was not detected in the peach orchard of Tokat-Centre-Kömeç village in 2021. In 2022, the first adult was detected on 08.08.2022 (24.5°C, 62.6% RH) and the highest population density was observed on 12.09.2022 (22.6°C, 51.7% RH) with 6 individuals (Figure 6c).

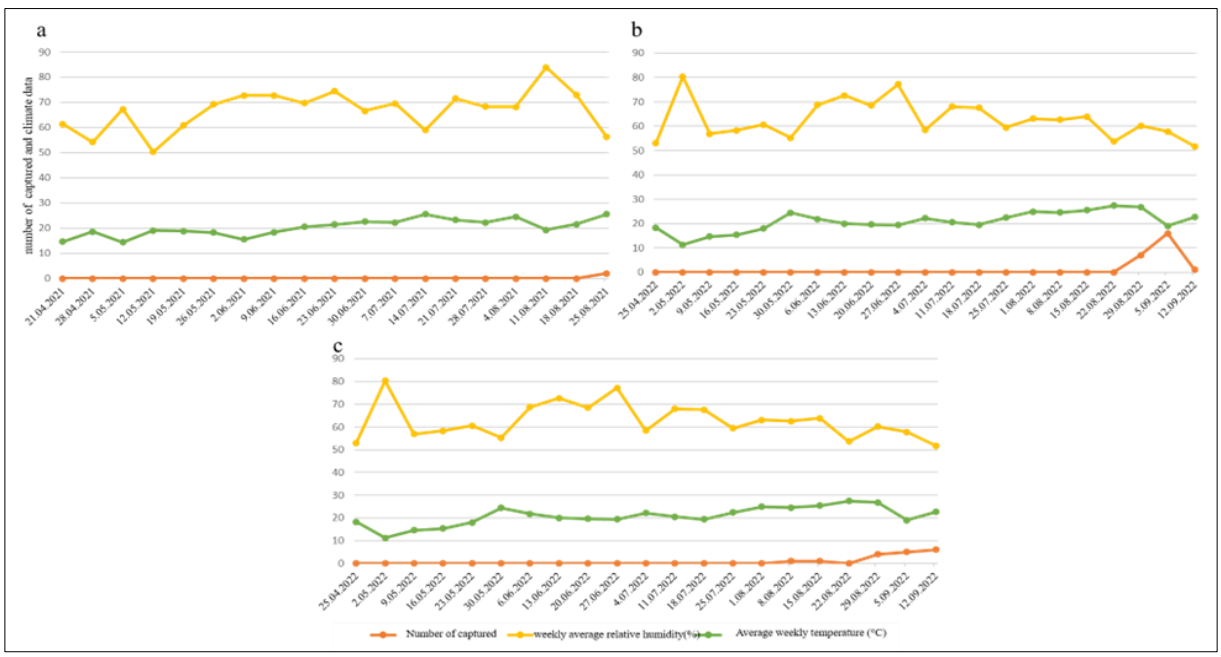

Figure 6. Population density of *Drosophila suzukii* in cherry and peach orchards in Tokat-Centre in 2021-2022 (a-2021 cherry, b-2022 cherry, c-2022 peach)

Drosophila simulans was not detected in the cherry orchard of Tokat-Centre-Kemalpaşa village in 2021. In 2022, the first adult detection was made on 23.05.2022 and the highest population was obtained on the same date (18°C, 60.6% RH) with 8 individuals. In other counting intervals, the number of adults in the traps did not exceed one. *D. simulans* was caught in the traps for the first time on 19.05.2021 (18.8°C, 60.8% RH) in 2021 and on 16.05.2022 (15.3°C, 58.3% RH) in 2022 in the peach orchard of Tokat-Centre-Kömeç village and the number of adults detected at other counting intervals did not exceed 1 in both years.

Drosophila phalerata was detected for the first time on 05.05.2021 (14.5°C, 67.3% RH) in the cherry orchard of Tokat-Centre-Kemalpaşa village in 2021, and at most 1 individual was seen at other counting intervals. In 2022, the first adult was detected on 16.05.2022 (15.3°C, 58.3% RH) and reached the highest number with 11 individuals caught on 23.05.2022 (18°C, 60.6% RH). No adults were detected at other counting intervals. In both 2021 and 2022, no adult of *D. phalerata* was detected in the peach orchard of Tokat-Centre-Kömeç village.

Drosophila transversa was not detected in the cherry orchard of Tokat-Centre-Kemalpaşa village and peach orchard of Tokat-Centre-Kömeç village in 2021. In 2022, it was detected for the first time in Tokat-Centre-Kemalpaşa village cherry orchard on 16.05.2022 (15.3°C, 58.3% RH) and in Tokat-Centre-Kömeç village peach orchard on 23.05.2022 (18°C, 60.6% RH). The number of detections in the traps did not exceed 1 at other time intervals.

Gitona distigma was not detected in the cherry orchard of Tokat-Centre-Kemalpaşa village and in the peach orchard of Tokat-Centre-Kömeç village in 2021. In 2022, only one was detected in the cherry orchard of Tokat-Centre-Kemalpaşa village on 16.05.2022 (15.3°C, 58.3% RH) and in the peach orchard of Tokat-Centre-Kömeç village on 25.07.2022 (22.4°C, 59.4% RH).

Scamptomyza pallida was detected as 1 specimen on 19.05.2021 (18.8°C, 60.8% RH) in the cherry orchard of Tokat-Centre-Kemalpaşa village in 2021 and was not seen in 2022. In both 2021 and 2022, it was not found in the traps in the peach orchard in Tokat-Centre-Kömeç village.

4. Discussion

In this study, the presence and prevalence of the species belonging to the family Drosophilidae in the cherry and peach fields of Tokat province were tried to be determined. In addition, population monitoring of the species belonging to the family was carried out for two years in one peach and cherry orchard determined in Tokat-Centre. As a result of the study, 10 species belonging to the family were identified in Tokat province. Considering the number of species obtained on the basis of districts, 10 species were identified in Tokat centre, 9 species in Erbaa, 8 species in Turhal and 6 species in Pazar.

It was observed that *D. subobscura*, *D. immigrans*, *D. hydei* and *D. melonogaster* were prominent in terms of prevalence and density, respectively. Özbek-Çatal et al. (2021), in their study conducted in the orchards of the Eastern Mediterranean Region, determined a total of 11 species belonging to the family and reported that *D. immigrans*, *D. melanogaster* and *D. subobscura* were common in the region. Similarly, Zengin (2020), detected a total of 13 species belonging to the family in the fruit fields of Uşak province and stated that *D. subobscura* was the most common species. In addition, Başpınar et al. (2022), detected totally 11 species of Drosophilidae in the orchards of Aydın province and reported that *D. subobscura* was the most abundant species, followed by *D. immigrans* and *D. melanogaster*.

Drosophila suzukii, one of the important species in the family, was found in cherry and peach fields in Tokat-Centre and Erbaa districts, and in peach fields in Turhal district. It was observed that the population of *D. suzukii* increased from the end of August to mid-September 2022 in the cherry and peach areas of Tokat Centre and from mid to late August 2022 in the peach areas of Erbaa district. Arıdıcı-Kara and Ulusoy (2020), detected D. suzukii in cherry and peach orchards in the Eastern Mediterranean Region and stated that the species caused significant damage especially in cherries. Öğür et al. (2018), reported that the pest was detected in cherry orchards in Karaman province. Kasap and Özdamar (2019), reported that D. suzukii was observed in Çanakkale vineyards in September-February every year and its population peaked in December. Again, Zengin and Karaca (2019), in their study conducted in Uşak province in 2017-2018, reported that D. suzukii density started to increase from the end of September in both years and started to decrease from the end of November.

Tokat province is an important agricultural city located in the transition zone of the Central Black Sea Region and has a variable climate due to its location in the transition zone. This variability has caused the variability of product pattern and varieties in the province and has brought Tokat to an important position in fruit production. The fact that the climatic conditions of the province are suitable for agricultural production has a positive effect on the diversity and intensity of the species that cause damage in these products. As a matter of fact, the number of species obtained as a result of this study carried out in Tokat-Centre and 3 districts with two different fruit species is not a small number compared to the studies covering larger areas and more fruit species in the literature. The study revealed that Drosophilidae family species are widespread in Tokat province. It is important to carry out similar studies in other cultivated plants which are intensively cultivated in the province, and which may be possible hosts of the species belonging to the family.

Compliance with Ethical Standards

Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contributions

Hüseyin Bilal TAŞLIOĞLU: Methodology, Investigation, Field studies, Writing - original draft. **Turgut ATAY**: Methodology, Investigation, Writing - original draft.

Ethical approval

Not applicable.

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The impact of several insecticides against the legume pod borer, *Maruca vitrata* Fab. (Lepidoptera: Crambidae) on cowpea

Vivek LAHUTIYA[1], Dipesh Kumar MEHATA[1]*, Akshita SINGH[2], Bishnu YADAV[1]

[1] Faculty of Science and Technology, G.P. Koirala College of Agriculture and Research Centre, Purbanchal University, Gothgaun, Morang-Nepal
[2] Department of Entomology and Agricultural Zoology, Institute of Agricultural Science, Banaras Hindu University, India

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***CONTACT**

mehatadipesh643@gmail.com

A B S T R A C T

Cowpea (*Vigna unguiculata*), a widely cultivated grain legume in Nepal and a member of the Fabaceae family, faces significant production challenges due to the legume pod borer (*Maruca vitrata*). This pest is a major threat, limiting both the yield and productivity of cowpea crops. The research was laid out in Randomised Completely Block Design with seven treatments and three replications. The treatments comprise chlorantraniripole 18.5% SC, emamectin benzoate 5% SG, spinotoram 11.7% SC, dimethoate 30% EC, azadiractin 0.07 % EC and BT+ *Saccharopolyspora spinosa* and control. Chlorantraniliprole demonstrated the highest effectiveness, reducing larval populations to just 0.16 after the fourth application, while achieving the maximum fruit yield of 13 t/ha. Emamectin and spinetoram also performed well, both decreasing larval counts to below 1.0 and producing comparable yields of 12.90 t/ha and 12.89 t/ha, respectively. In contrast, biological treatments, such as *Bacillus thuringiensis* var. Kurstaki, exhibited moderate success in pest control, resulting in a lower yield of 10.19 t/ha. Azadirachtin and the untreated control plots experienced the highest infestation rates, leading to significantly lower yields of 8.04 t/ha and 4.70 t/ha, respectively. Chlorantraniliprole also proved superior in reducing fruit damage, limiting it to just 1.55%, compared to the high damage rate of 42.04% observed in the untreated control. These findings highlight the strong efficacy of chemical insecticides, especially chlorantraniliprole, in controlling *Maruca vitrata* infestations and enhancing cowpea productivity. Future studies should focus on integrating biological agents with chemical treatments to minimize environmental impacts and prevent resistance, while maintaining high yields and effective pest control.

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ORCID> 0000-0003-1287-4920 (VL), ORCID> 0000-0002-2942-9199 (DKM), ORCID> 0009-0006-4322-1526 (AS), ORCID> 0000-0003-2249-3056 (BY)

1. Introduction

Pulse crops are prominent source of proteins, carbohydrates, and fats along with vitamins and minerals like phosphorous, calcium, iron, etc. (Kumar, 2005). They are cultivated in most of the tropical or sub-tropical regions of the world (Singh et al., 2020). Among several pulses crop cowpea is also one of the widely grown grain legume crop species in Nepal (Pant et al., 2021; Aryal et al., 2021). The crop flourishes well in areas where the temperature ranges from 27-35 °C, comfortably adapted to a wide range of soil types and various cropping systems but not so in the case of alkaline soil (Kumar, 2005). The botanical name of cowpea is *Vigna unguiculate* pronouncedly also known as black pea or southern pea. It belongs to the family Leguminiaceae and sub-family Fabaceae (Pant et al., 2021). Cowpea contains about 90% of dry matter (DM), 17-18% of crude protein (CP) & 13-15% of an ash (Owade et al., 2020). It can be used in multiple ways such as food, feed, forages, fodder, green manuring, and as a vegetable too (Pant et al., 2021). It enhances soil fertility due to its N2 fixing ability as well as being helpful in preventing soil erosion (Doyle et al., 2013). Emphasizing the uses of cowpea, Nepal has managed to produce around 9186 Mt of cowpea utilizing an area of 6752 hectares (Ha) (Aryal et al., 2021).

However, its production and productivity is confined by some factors like nutritional deficiency, climatic extremities, diseases outbreak, and majorly insect pests of several genera. Some major pests of cowpea include cowpea pod borers, aphids, sucking bugs, and leaf hoppers (Pant et al., 2021). Originating from the Ind-Malaysian region, legume pod borer (LPB), *Maruca vitrata* (Lepidoptera, Crambidae) is one of the most severely damaging pests of cowpea crops (Yule and Srinivasan, 2014), (Yadav and Singh, 2014). It is widely distributed in tropical and sub-tropical regions of the world but the majority of the LPB population is found in Asian and African regions and is polyphagous (Aryal et al., 2021). It feeds on more than 39 host plants belonging to 26 different genera and 6 families extensively on plants from the papilionaceae family. Eggs of LPB are greenish white and are laid singly or in batches of 2-6 on the underside of the leaves, terminal shoots, and floral buds, which hatch in 2-3 days (Ba et al., 2019), (Ashigar and Umar, 2016). They are oval and translucent and measures around 0.65 * 0.45 mm (Ba et al., 2019). Depending on the climatic condition and host plant the five instars larval stage lasts up to 8-10 days (Ashigar and Umar, 2016). The body is tube shaped and measures about 11-12 mm long and 2.1-2.4 mm wide with a slender head and a pair of dark brown spots on each segment (Ba et al., 2019). The pupae are initially red brown but later change their color from red brown to dark brown when fully developed measuring about 13 mm in length (Ba et al., 2019; Ashigar and Umar, 2016). Female pupae of LPB do not have any rings but male pupae bear a small distinct ring on the last abdominal segment (Ba et al., 2019). An adult LPB has a wingspan of 13-25 mm with a dark brown body color (Ba et al., 2019). The forewings are brown with white spots and black edges whereas the hindwings are translucent (Ashigar and Umar, 2016). Mostly the reproductive parts of at least 73 of the host plant species get infected by the pests of these species leading to 20-80% of crop loss (Ekesi, 1999; Srinivasan et al., 2021; Sharma and Franzmann, 2000). The infection is initiated by the larvae in leaves where it feeds inside the rolled and webbed leaves (Sharma and Franzmann, 2000).

Further, the infestation proceeds to floral buds, flowers, and pods. The larvae feed on the structures by webbing them which prevents these larvae from adverse factors and natural enemies (Sharma and Franzmann, 2000). Most of the farmers heavily rely on chemical insecticides for the control and management of this pest. However, cultural practices and the employment of biological control agents are being focused on the recent days (Ekesi, 1999; Yule and Srinivasan, 2013). The newly emerged larvae can only be killed via chemical insecticides before they bore into the flowers. Therefore, this leads to frequent spraying of insecticides by the farmers for the control and management of LPB resulting in chemical residue in food stuffs (Aryal et al., 2021; Yule and Srinivasan, 2013). The chemical residue hampers soil health, water condition and, also human health (Pant et al., 2021; Aryal et al., 2021). The application of botanical and biological agents like: Bacillus thuringiensis, parasitoid wasps (which is the classical biological control agent used for the control of LPB), and azadirachtin which is found to have a significant impact in reducing LPB population (Yule and Srinivasan, 2013; Yule and Srinivasan, 2014; Dannon et al., 2012).

The aim of this study is to evaluate the effectiveness of various Insecticides against the legume pod borer (*Maruca vitrata*) in cowpea, assess the damage caused, and identify optimal control methods.

The findings will guide farmers in selecting effective management strategies that enhance yield and quality while ensuring consumer safety by minimizing health risks associated with pesticide use.

2. Materials and methods

2.1. Description of the study area

Between March 2023 and May 2023, the study was conducted in the research field of the Prime Minister Agriculture Modernization Project (PMAMP), Katahari Municipality, Morang, Nepal. The location is at an altitude of 73.74 meters above sea level, with geographic coordinates of latitude 26° 28' 10.4118" N and longitude 87° 21' 3.1431" E. Maximum and Minimum temperatures in the region are 33.9°C and 10.5°C, respectively, with an average annual rainfall of 1891.8 mm. Figure 1 represents the experimental site of the study.

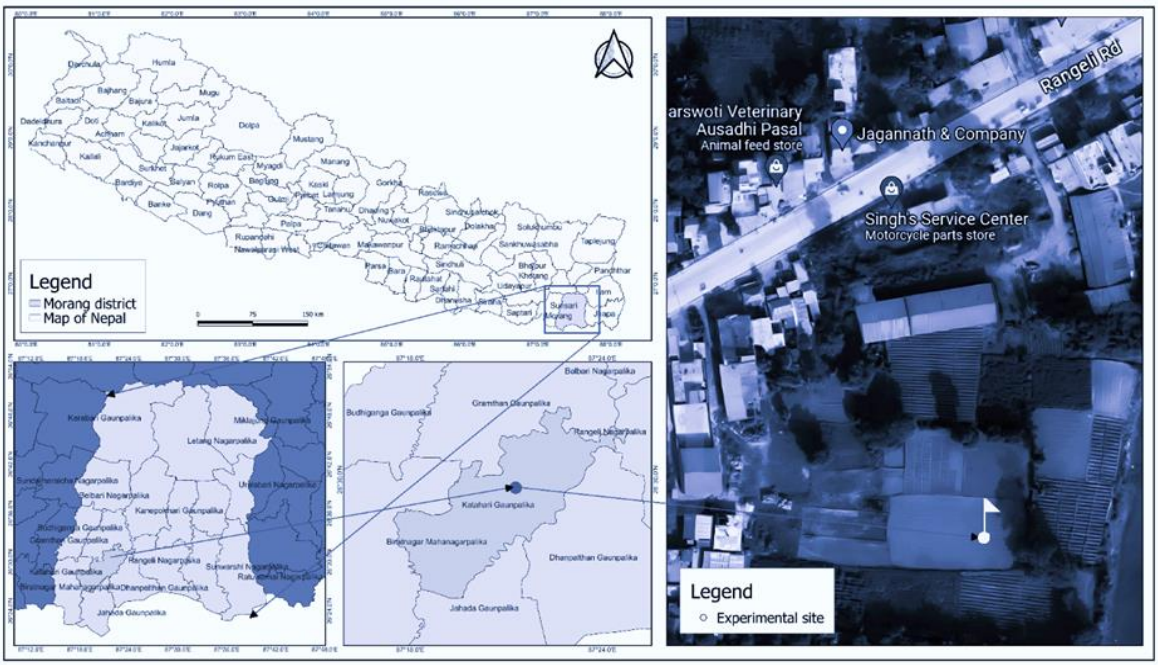

Figure 1. Map illustrating the experimental site of the study

2.2. Trial design

To determine the efficacy of insecticides against LPO (*Maruca vitrata*) in cowpea (*Vigna ungliculata*), the entire research was laid out in Randomized Completely Block Design (RCBD). There were seven treatments and three replications. The treatments were randomly assigned to the experimental plots to get uniform distribution. The experimental units were arranged in a spatial manner, with 1.0 m distance in between the replications and 0.8 m between the treatments. The overall area of the experimental area was 265.1 m^2 which was divided into 21 plots, each with an area of 4.5 m^2. The plants were planted by maintaining a plant-to-plant distance of 30 cm (P-P) and row-to-row distance of 60 cm (R-R).

2.3. Planting material

To determine efficacy of various Insecticides against *Maruca vitrata*, the crop or cowpea variety selected was Anna green F1. The seeds of this variety were obtained from the National Agriculture Research Council (NARC), Tashara, Morang, Nepal. The seed packets had some specifications labelled on them such as the minimum physical purity of the seeds as 98%, which ensures the absence of impurities in the seeds, the genetic purity of the seeds as 96%and the germination percentage was labelled as 97%, ensuring high rate of successful germination of the seeds.

2.4. Cultural practices

Before the start of the experiment, the land was brought to a cultivable condition via various steps. The land was measured and made free of weeds; The land was ploughed with rotavator followed by harrowing to make a land even. The depth of ploughing was adjusted to about 15-20 cm. The seeds of cowpea for the experiment were directly sown in the plots on March 15, 2023. A dibbler was used to make a hole of 2.0 cm for the placement of seeds. Two seeds per hole were placed so as to ensure successful germination in the case of seeds. After two weeks, gap filling and thinning were done to ensure one healthy plant per hill with total of 30 plants per plot. At the time of field preparation, the land was incorporated with well decomposed and rotten Farmyard manure at the rate of 15 tons per hectare. The fertilizers used as sources of nutrients were urea, diammonium phosphate (DAP), and muriate of potash (MOP). At the rate of 40:60:40 Kg NPK/ha. The full dose of potassium (K) and phosphorous (P) along with half dose of nitrogen (N) was applied at the time of field preparation. The remaining half dose of nitrogen was applied in further two split doses i.e., one after gap filling and another during flowering stage to ensure proper nutrient supply at different growth stages for the plants. The irrigation schedule involves watering the plants twice a day, one during the morning time and another during evening depending on the field moisture condition. For the management and control of weeds, an application of pre-emergence herbicide pendimethalin 50% EC was used. In addition to this, two hand weeding was also performed at 25 and 40 DAS respectively. A fungicide named SAAF (carbendain 12 % + mancozeb 63 % WP) at a rate of 2 gm/L of water was sprayed to avoid of any fungal infestation. Staking was done with the help of bamboo and ropes to support the plants and facilitates easy monitoring, spraying and harvesting. Harvesting was done manually by picking the matured pods from the plants in every 3 days. All the cultural practices such as irrigation, weeding, fungicide application, staking and harvesting followed the guidelines of NARC, Tarrahara, Morang.

2.5. Treatments details

The Insecticides used in the experiment were brought from Koshi Agro Traders, Biratnagar. Insecticides selected for this experiment were of different categories including chemical, botanical, and biological agents based on their proven efficacy against LPB. The total of 7 treatments were applied in this experiment, out of which 6 were Insecticides and remaining was water spray or control group. The selection of these treatments was based on recommendation from local agricultural extension services and previous research, also commonly used insecticides of the region were included. To facilitate easy identification and references, each pesticide treatment was assigned a unique code or name. LPB infestation reached a critical level (Economic Threshold Level or ETL) on the 38th day after sowing (DAS). At this point, one live larva was found on average for every six plants. To control the pest, four rounds of insecticide spraying were conducted. Each spraying was done a week apart, starting on the 38th DAS. The insecticides were sprayed using a motorized knapsack sprayer, which was set to a pressure of 40 psi and had a tank capacity of 16 liters. To reduce environmental impact, the spraying was always done in the late evening.

Table 1. Different insecticides used in the experiment including brand name, application rates, and treatment numbers

S.N.	Treatments	Brand Name	Dose/L	Treatment No.
1.	Chlorantraniliprole18.5% SC	Coragen	0.2 ml/l of water	T1
2.	Emamectin Benzoate 5% SG	Indogulf	1.5 ml/l of water	T2
3.	Spinotoram 11.7% SC	Largo	1 ml/l of water	T3
4.	Dimethoate 30% EC	Rogorasa	2 ml//l of water	T4
5.	Azadirachtin 0.03 % EC	Multiplex	2 ml//l of water	T5
6.	*Bacillus thuringiensis var. Kurstaki (BK) + Saccharopolyspora spinosa 15 % SC*	Multiplex	2 ml//l of water	T6
7.	Water spray	N/A	N/A	T7

2.6. Data observation and collection

For data collection and observation, the healthy plants were tagged randomly keeping border plants neglected. The data was collected on number of pods, weight of normal pods, number of damaged pods and weight of damaged pods, number of larvae in damaged pods, No. of Larvae in 12 flowers before treatments and after 7 days of successive treatments.

2.7. Statistical analysis

For further investigation, the gathered data were imported into MS Excel (2019). Using SPSS, the Kolmogorov-Smirnov and Shapiro-Wilkinson tests of normality were performed to see whether the data were normal (Buragohain et al., 2021). A square root transformation (SQRT) was used to normalize the data when the data did not adhere to the assumptions of normality (Devkota et al., 2016). R-Studio statistical software was then used to do an Analysis of Variance (ANOVA). Using the methods given by Gomez and Gomez (1984), this research sought to identify significant differences between the treatments. At a significance threshold of p 0.05, post-hoc tests like Tukey's HSD were used to detect treatment effects.

3. Results

3.1. Impact of insecticides on pod borer larval count in cowpea flowers before and one week post-each spray

3.1.1. Impact of *Maruca vitrata* infestations on cowpea before treatment spray

Before the spray treatments, *Maruca vitrata* infestations on cowpea plants did not show statistically significant differences across the treatments as presented on table 2. The infestation levels varied slightly, with Spinetoram having the lowest mean infestation at 14.91 ± 3.92, and both emamectin and dimethoate showing the highest mean infestation at 16.02 ± 4.06 and 16.02 ± 3.94, respectively. Chlorantraniliprole, *Bacillus thuringiensis* var. *Kurstaki (BK)* + *Saccharopolyspora spinosa* 15 % SC, azadirachtin, and water spray had infestation levels between these extremes, all averaging around 15.41 to 15.63. The overall mean infestation level across all treatments was 15.50, with a standard error of ±1.12, and the coefficient of variation (CV%) was 5.46%, indicating low variability among the treatments.

3.1.2. Effect of insecticides on larvae after multiple consecutive sprays:

The effect of insecticides on larval counts in cowpea flowers after consecutive sprays shows a clear trend of decreasing larval populations with each application. Chlorantraniliprole consistently had the most significant reduction, dropping from 2.49 ± 1.728 after the first spray to just 0.16 ± 0.813 after the fourth spray. Similarly, emamectin and spinetoram also showed notable reductions, with larval counts decreasing from around 3.5 to below 1 by the final spray. In contrast, treatments like *Bacillus thuringiensis* var. *Kurstaki (BK)* + *Saccharopolyspora spinosa* 15 % SC and azadiractin showed moderate effectiveness, with larval counts declining but remaining relatively higher than other insecticides. *Bacillus thuringiensis* var. Kurstaki (BK) + *Saccharopolyspora spinosa* 15 % SC reduced larvae from 7.94 ± 2.903 to 2.27 ± 1.663, while azadiractin reduced from 9.47 ± 3.157 to 3.24 ± 1.934. Dimethoate was less effective compared to the top treatments but still showed a consistent decline over time. Water spray, serving as the control, had the highest larval counts throughout, starting at 20.10 ± 4.532 and only reducing to 6.19 ± 2.580 after four sprays. The grand mean larval counts for each spray dropped steadily from 7.47 to 2.07.

Table 2. Impact of insecticides on pod borer larval count in cowpea flowers before and one week post-each spray

Treatment	Before spray	First spray	Second spray	Third spray	Fourth spray
Chlorantraniliprole	15.63 ± 4.016^{a}	2.49 ± 1.728^{d}	1.41 ± 1.380^{c}	0.80 ± 1.132^{d}	0.16 ± 0.813^{c}
Emamectin	16.02 ± 3.935^{a}	3.44 ± 1.985^{cd}	2.05 ± 1.584^{c}	1.24 ± 1.306^{cd}	0.69 ± 1.088^{c}
Spinetoram	14.91 ± 3.924^{a}	3.58 ± 2.019^{cd}	2.13 ± 1.606^{c}	0.99 ± 1.186^{cd}	0.83 ± 1.142^{c}
Dimethoate	16.02 ± 4.063^{a}	5.30 ± 2.409^{c}	3.22 ± 1.925^{c}	2.11 ± 1.594^{c}	1.13 ± 1.225^{c}
Bt K + S. spinosa	15.50 ± 3.992^{a}	7.94 ± 2.903^{b}	5.11 ± 2.363^{b}	3.32 ± 1.955^{b}	2.27 ± 1.663^{b}
Azadiractin	15.02 ± 3.949^{a}	9.47 ± 3.157^{b}	6.30 ± 2.607^{b}	3.83 ± 2.080^{b}	3.24 ± 1.934^{b}
Water spray	15.41 ± 3.986^{a}	20.10 ± 4.532^{a}	16.44 ± 4.106^{a}	12.19 ± 3.560^{a}	6.19 ± 2.580^{a}
Grand mean	15.50	7.47	5.23	3.50	2.07
SEM (±)	1.12	0.70	0.56	0.34	0.35
CV%	5.46	5.76	6.53	10.99	14.14
F value	NS	***	***	***	***

Data was transformed by (√(x+0.5)) before statistical analysis, and the parentheses show the transformed value. Mean sharing same letter within column are non-significant. Means followed by different letter within each column are significantly different, DMRT ($p \leq 0.05$). SEM: Standard error of mean. CV: Coefficient of variation. *** Significant at 0.1% level of significance. NS: Non-significant

The F values for all sprays were highly significant at 0.1% level of significance, indicating that insecticide treatments had a strong effect on larval reduction. Overall, chlorantraniliprole emerged as the most effective treatment, with the others also reducing larval counts, albeit to varying degrees.

3.2. Effect of Insecticides on total number of fruits, total damaged number of pods and total weight of damaged pods per eight plants in cowpea against legumes pod borer

3.2.1. Total fruit number per eight plants

There was a significant difference in the total fruit number per eight plants across the treatments, with a grand mean of 251.38 fruit which is shown in Table 3. Chlorantraniliprole produced the highest fruit number (310.33), closely matched by spinetoram (308.00) and emamectin (307.66). These three treatments dramatically increased fruit production, significantly outperforming dimethoate (286.00), which fell into the same statistical group as the top treatments but still trailed behind them. Biological treatments, such as *Bacillus thuringiensis* var. Kurstaki (BK) + *Saccharopolyspora spinosa* 15 % SC (243.33) and azadiractin (192.00), showed considerably lower fruit numbers, suggesting weaker pest control. At the bottom end, the water spray control (112.33) yielded the fewest fruit, illustrating the impact of heavy pest pressure without protection. In summary, chlorantraniliprole was the most effective in maximizing fruit production, with spinetoram and emamectin providing almost equal results.

3.2.2. Total fruit weight per eight plants (g)

Table 3, illustrates the results of total fruit weight per eight plants displayed significant differences, with an average weight of 4184.78 g. The heaviest fruit production was achieved with chlorantraniliprole (5128.90 g), with emamectin (5125.86 g) and spinetoram (5115.50 g) close behind, demonstrating their effectiveness in enhancing fruit size and overall yield. In contrast, dimethoate (4776.51 g) was less effective but still provided considerable improvement over biological treatments like *Bacillus thuringiensis* var. Kurstaki (BK) + *Saccharopolyspora spinosa* 15 % SC (4018.59 g) and azadiractin (3217.66 g), which lagged significantly. The untreated water spray group yielded the lowest fruit weight (1910.42 g), underscoring the importance of pest control in maintaining fruit quality and quantity. Among the treatments, chlorantraniliprole slightly outperformed the others, but all three top chemical treatments were highly successful in increasing fruit weight.

3.2.3. Total damaged fruit per eight plants

The number of damaged fruits per eight plants varied widely among the treatments presented in Table 3, with a grand mean of 21.90 damaged fruit. Chlorantraniliprole led with the fewest damaged fruit (4.66), clearly surpassing all other treatments in its ability to control pest damage. Both emamectin (9.66) and spinetoram (10.00) were also highly effective but allowed slightly more damage compared to chlorantraniliprole. Dimethoate (19.33) performed moderately, showing its lower effectiveness in damage control. Biological treatments fared worse, with *Bacillus thuringiensis* var. Kurstaki (BK) + *Saccharopolyspora spinosa* 15 % SC (26.33) and azadiractin (37.33) allowing significantly more damaged fruits. The water spray treatment had the highest number of damaged fruit (46.00), highlighting the severe damage caused in the absence of any pest control. Clearly, chlorantraniliprole was the most potent in minimizing fruit damage, followed by emamectin and spinetoram.

3.2.4. Total damaged fruit weight per eight plants (g)

Table 3, shows the total weight of damaged fruit also showed significant differences across treatments, with a grand mean of 262.98 g. Chlorantraniliprole resulted in the lightest damaged fruit weight (58.62 g), indicating its effectiveness in not only reducing the number of damaged fruits but also limiting the extent of damage. emamectin (116.10 g) and spinetoram (118.77 g) also performed well, though they allowed more damage than chlorantraniliprole. Dimethoate (232.92 g) provided only moderate protection, allowing substantially higher damage. Biological treatments were far less effective, with *Bacillus thuringiensis* var. Kurstaki (BK) + *Saccharopolyspora spinosa* 15 % SC (322.54 g) and azadiractin (444.80 g) recording significantly heavier damage.

The untreated water spray treatment had the highest damaged fruit weight (547.11 g), underscoring the necessity of effective pest control. Overall, chlorantraniliprole provided the strongest reduction in damaged fruit weight, proving its superiority over other treatments.

3.2.5. Percentage of fruit damage

The percentage of fruit damage varied significantly presented in Table 3, with a grand mean of 12.40%. Chlorantraniliprole provided exceptional protection, reducing fruit damage to just 1.55%, the lowest among all treatments. Emamectin (3.12%) and spinetoram (3.22%) were also highly effective, though their damage percentages were approximately double that of chlorantraniliprole. Dimethoate allowed a higher damage percentage (6.75%), while biological treatments performed poorly, with *Bacillus thuringiensis* var. Kurstaki (BK) + *Saccharopolyspora spinosa* 15 % SC (10.78%) and azadiractin (19.38%) showing much higher levels of fruit damage. Unsurprisingly, the untreated water spray group had the highest damage percentage (42.04%), indicating severe crop loss without intervention. Among all treatments, chlorantraniliprole was clearly the most efficient at minimizing fruit damage, followed by emamectin and spinetoram.

3.2.6. Yield (t/ha)

There were significant differences in yield among treatments at 0.1% level of significnace, with a grand mean of 10.53 t/ha as given in table 3. Chlorantraniliprole produced the highest yield (13.00 t/ha), slightly ahead of emamectin (12.90 t/ha) and spinetoram (12.89 t/ha), demonstrating its superior pest control and productivity benefits. Dimethoate (11.98 t/ha) provided reasonable yield improvement but remained less effective than the top three treatments. The biological treatments performed poorly, with *Bacillus thuringiensis* var. Kurstaki (BK) + *Saccharopolyspora spinosa* 15 % SC (10.19 t/ha) and azadiractin (8.04 t/ha) delivering significantly lower yields. The lowest yield was recorded in the untreated water spray group (4.70 t/ha), confirming the devastating impact of uncontrolled pest pressure on overall crop production. Chlorantraniliprole was the most successful treatment in maximizing yield, closely followed by emamectin and spinetoram, all of which substantially outperformed biological treatments and the control.

Table 3. Efficacy of Insecticides on total fruit number, damage, and yield performance in cowpea infested by pod borer

Treatment	Total fruit number/eight plants	Total fruit weight/eight plants (g)	Total damaged fruit/eight plants	Total damaged fruit weight/eight plants (g)	Percentage of fruit damage	Yield (t/ha)
Chlorantraniliprole	310.33^{a}	5128.90^{a}	4.66^{f}	58.62^{d}	1.55^{f}	13.00^{a}
Emamectin	307.66^{a}	5125.86^{a}	9.66^{e}	116.10^{c}	3.12^{e}	12.90^{b}
Spinetoram	308.00^{a}	5115.50^{a}	10.00^{e}	118.77^{c}	3.22^{e}	12.89^{b}
Dimethoate	286.00^{ab}	4776.51^{ab}	19.33^{d}	232.92^{b}	6.75^{d}	11.98^{c}
Btk + S. spinosa	243.33^{b}	4018.59^{bc}	26.33^{c}	322.54^{b}	10.78^{c}	10.19^{c}
Azadiractin	192.00^{c}	3217.66^{c}	37.33^{b}	444.80^{a}	19.38^{b}	8.04^{d}
Water spray	112.33^{d}	1910.42^{d}	46.00^{a}	547.11^{a}	42.04^{a}	4.70^{e}
Grand mean	251.38	4184.78	21.90	262.98	12.40	10.53
SEM (±)	15.43	278.7	1.22	15.88	2.32	0.64
CV%	10.63	11.53	9.66	10.46	32.49	10.48
F value	***	***	***	***	***	***

Means followed by different letter within each column are significantly different, DMRT ($p \leq 0.05$). SEM: Standard error of mean. CV: Coefficient of variation, *** Significant at 0.1% level of significance. NS: Non-significant

4. Discussion

The experiments revealed that chemical insecticides were highly effective in controlling *Maruca vitrata* larvae in cowpea, with chlorantraniliprole 18.5% SC being the most effective. This aligns with findings by Aktar et al. (2020), who also reported Chlorantraniliprole's superior performance compared to other chemicals. However, the percentage reduction over control plots was lower in Katahari, Morang, possibly due to climatic factors or *Maruca vitrata* tolerance.

Emamectin benzoate and spinetoram followed in efficacy, consistent with research by Anusha et al. (2014), though their reduction rates in Katahari were lower, likely due to higher infestations or host susceptibility. Dimethoate 30% EC was the least effective, possibly due to resistance, as it's an older insecticide. Biocontrol with *Bacillus thuringiensis* (B.T.) was less effective than chemical insecticides, as its slow toxin release likely delayed suppression. Azadirachtin-treated plots had higher larval counts, which may be due to the decomposition of active ingredients or slower larval suppression, as reported by Aryal et al. (2021). In reducing damaged pods, chlorantraniliprole was again most effective, like Aryal et al. (2021). Emamectin benzoate and spinetoram showed statistically similar results in pod protection, possibly due to their being of the same generation of insecticides, with findings comparable to Ashigar and Umar (2016). Dimethoate was less effective due to its older formulation, and B.T. had an intermediate effect, likely due to slower action, like findings by Ba et al. (2019). Azadirachtin was the least effective, comparable to results by Anusha et al. (2014), while control plots experienced the highest infestation levels. In the experiments conducted in Katahari, Morang, chlorantraniliprole 18.5% EC resulted in the highest yield per hectare. This yield surpassed the findings of Aryal et al. (2021), likely due to the higher genetic potential of the cowpea variety used, favorable soil, climatic conditions, and superior management practices. Emamectin benzoate and spinetoram produced statistically similar yields, with emamectin benzoate yielding more than that reported by Priyadarshini et al. (2013), again attributed to better conditions in Katahari. Dimethoate-treated plots recorded lower yields compared to other chemical insecticides, though still higher than Priyadarshini et al. (2013), possibly due to similar factors. The biocontrol agent *Bacillus thuringiensis* (B.T.) resulted in moderate yields, aligning with the findings of Yule and Srinivasan (2013), although yields in Katahari were higher than those reported by Swathi et al. (2019) due to favorable conditions. Azadirachtin was the least effective, leading to the lowest yield among the treatments, consistent with the findings of Srinivasan et al. (2021). Control plots, as expected, produced the lowest yields due to the lack of pest management interventions.

5. Conclusion

LPB is one of the significant pests that reduces cowpea crop production yield and productivity. Insecticides have a far greater overall effectiveness and yield than bio-control agents and botanical pesticides. However, as advised in IPM tools, these pesticides should only be used as the final option for pest management and should be applied at the prescribed dose or less if possible. Particularly for sustainable cowpea agriculture, safer options such as the use of bio-control agents (*B. thrungiensis*) or botanical pesticides (azadiractin) should be promoted because they are safe to the environment and human health.

Compliance with Ethical Standards

Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contributions

Vivek LAHUTIYA: Conceptualization, funding acquisition, investigation, Data curation,methodology. **Dipesh Kumar MEHATA:** Conceptualization, funding acquisition, investigation,methodology, resources, software, supervision,writing–review & editing, validation, visualization. **Akshita SINGH:** Data curation,methodology, Revision. **Bishnu YADAV:** Data curation,methodology. All authors have read and agreed to the published version of the manuscript.

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Not applicable

Consent for publication

Not applicable.

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