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Pharmacological Studies of Syrian Rue (*Peganum harmala* L., *Zygophyllaceae*)

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Abstract: Syrian rue (*Peganum harmala* L., *Zygophyllaceae*) has been used in traditional medicine of Central Asia, the Middle East, and Caucasus areas (Azerbaijan) for centuries, mainly as ritual and psychedelic plant. At full growth, this erect, dichotomously branched shrub is about 1 m in height with a dense foliage consisting of narrow, linear, pinnate leaves with acute spreading lobes, and small solitary, axillary, white flowers and globe capsules enclosing numerous angular seeds. All parts of the plant (including roots) contain alkaloids. The seeds contain β-carbolineses (harmine, harmalol and harman) with the active hallucinogen being the alkaloid harmine. The seeds contain a red pigment used for coloring wool and carpets and for use as a spice and, in traditional medicine, as valuable aphrodisiac.

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1. Introduction

Syrian rue (*Peganum harmala* L., Zygophyllaceae) is a native plant of the Middle East that has become widely distributed in dry places throughout Central Asia and the Caucasus (Azerbaijan) (as a wild plant and as a weed in grain plantations). For many centuries, Syrian rue was used in traditional medicines of the Middle East, Central Asia, Azerbaijan, and India as ritual, psychedelic plant, for coloring wool in carpets, and as a spice [1, 2, 3, 4, 5, 6]. Traditional uses of the plant include treatment of stomach pain, external wounds and rashes.

The plant, which is hallucinogenic and toxic, is used for antispasmodic and painkilling effects, particularly in treatment of Parkinson's disease, eye afflictions, rheumatism, nervous disorders, and impotence. Smoke from burning pods with seeds is a traditional intoxicant, relaxant, and sexual stimulant in countries of Central Asia. The smoke from burning seed pods

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which commonly named "Esphand" is also believed to have antiseptic properties and used for treatment of palsy and lumbago [7]. Seeds in the form of powder are given as anthelmintic against tapeworms [5]. A decoction of the seeds is considered useful in the treatment of fevers and malaria [8]. Among the many benefits claimed for *P. harmala*, are use as a vasorelaxant [9], as a treatment for hypertension and diabetes [3], as a, analgesic and insecticide, and of behavior modifier of pests [10].

The chemical composition of *P. harmala* has been investigated since the 1970s [2, 11]. Macro- and microelements content which can have a direct impact on pharmacological activities of medicinal plants [12], are also a key to understand many diseases [3]. Among the important phytochemicals of *P. harmala* β- carboline alkaloids constitute the most important constitutions of this plant. This class of alkaloids is well known for several pharmacological activity include neuropsychological effects, that as an major investigated mechanism these compounds can facilities dopaminergic effects and intract with D_1 and D_2 dopamine receptors in brain also observed some cross interaction between these chemicals and benzodiazepine receptors [13, 14, 15]. *P. harmala* main alkaloids (harmine and harmaline in seeds and roots) can be played role in antinociceptive and antidepressant effects of this plant [16, 17, 18].

Medicinal plants used in herbal medicine for treatment of depression accumulated K and Mg most among other macro-elements and Se, Sr, and Ba among microelements. Mg and K are known as supplements used to reduce anxiety, depression, and effects of stress on central nervous system and cardiovascular system [3, 12, 19]. This study investigated both the macro and microelements in aboveground parts of *P. harmala*.

2. Material and Methods

The study was performed in Spring 1994 in Biological Center of National Academy of Sciences (Baku, Azerbaijan) by Dr.Mamedov with assistance of graduate students [20]. Plant material was collected from Zagulba (Apsheron peninsula, near Baku). Voucher specimen was identified by Dr.Mamedov and kept in Herbarium of Komarov Botanical Institute of National Academy of Sciences.

Analysis of macro- and microelements of *Peganum harmala* showed that aboveground parts of the plant accumulated K and Ca most among other macro-elements and Zn, Sr, Mo, Se, and Ba among microelements (Table 1.)

Treatment of Hypertension and Diabetes: A. Tahroui and colleagues did an ethnopharmacological survey in south-eastern Morocco which showed *P. harmala* along with five other plants are used in folk medicine for the treatment of hypertension and diabetes. Their survey shows that the alcoholic extract of *P. harmala* is taken orally and this treatment is considered effective in the region [21].

Vasorelaxant: H. Berrougui and colleagues show the vasorelaxant effects of harmine and harmaline [9]. These two alkaloids abundantly present in *P. harmala,* and are readily extractable via alcoholic extraction. Both alkaloids are not effective as a contact poison but active in vapor form [5].

Analgesic Effects: L. Farouk and colleagues [22] injected experimental mice with formaline, which causes acute pain, then they treated the mice with alkaloid extract of *P. harmala*. Animals treated with alkaloid extract of *P.harmala* showed ~35 to 69% pain inhibition.

Insecticidal Effects: In 2008 R. Jbilou and colleagues have studied the effects several plant extracts (including *P. harmala* extract) on α-amylase activity and off-spring production of the red flour beetle, *Tribolium castaneum* (Herbst). Their study shows that treatment with *P. harmala* methanolic extract causes a 58±5.7 percent larval mortality. This is quite significant

in the experimental group; however, the authors do not clarify whether they had controlled for the possible effects of methanol or not (10). A paste of the seeds made with mustard oil is used to kill head lice [3].

Anti-cancer properties: F. Jahaniani and colleagues report xanthomicrol present in *P. harmala* to be cytotoxic but also a possible anti-cancer agent [23].The authors have tested extracts of *P. harmala* on different human cell lines and the extract shows obvious cytotoxic properties. F. Jahaniani also claim in vivo tumor suppressing effects for *P. harmala* alcoholic extract administration in mice.

Table 1. Macro- and micro-element content of *Peganum harmala* L. (above ground parts).

Chemical element	Concentration	References
Ash	10.55	Mamedov et al., 1994
macro-elements (mg/g)		
Ca	16.55	Mamedov et al., 1994
K	32.95	Mamedov et al., 1994
Mg	5.9	Mamedov et al., 1994
Fe	0.15	Mamedov et al., 1994
micro-elements (μg/g)		
Mn	13	Mamedov et al., 1994
Cu	37	Mamedov et al., 1994
Zn	78.5	Mamedov et al., 1994
Mo	64.8	Mamedov et al., 1994
Cr	0.45	Mamedov et al., 1994
Al	10.2	Mamedov et al., 1994
Ba	69.76	Mamedov et al., 1994
Se	0.19	Mamedov et al., 1994
Ni	2.06	Mamedov et al., 1994
Sr	190	Mamedov et al., 1994
Pb	0.98	Mamedov et al., 1994
B	59	Mamedov et al., 1994

Depression and sleep-loss treatment: According to traditional herbal medicine of Azerbaijan Syrian rue is useful for treatment of insomnia and depression [2]. In Spring 1994 Dr.Mamedov studied possibility of use *Peganum harmala* for treatment of mild to moderate depression, anxiety and insomnia in Baku (Azerbaijan). Plant material for the study collected from Zagulba (Apsheron peninsula, near Baku), Voucher specimen was identified by Dr.Mamedov and kept in Herbarium of Komarov Botanical Institute National Academy of Sciences. Result of this study was reported in May 1994 at the seminar in National Institute of Post-graduate Training for Medical Doctors in Baku. The group of ten young veterans of war in Karabakh (Azerbaijan) suffering from medically recognized mild to moderate depression, anxiety and sleeping disorder was selected for the study. The men aged from 19 to 25 years old and were outpatients of City Psychiatric Clinic. Patients were required to be free of all psychotropic medications for at least 4 weeks before study entry. *P.harmala* was administered in traditional way. The whole herb were used, 50g of dried pods used each time. Every day bedrooms were heavily fumigated with burning dried pods of *P.harmala* before sleep time. As a result of studies 8 men (19-25) reported to their primary physicians overall improvement and

better sleep after six weeks (80 percent), one man (23 years old) asked for one more week and reported improvement after 7 weeks (10 percent), and one man (25 years old) reported no improvement after 6 weeks and discontinued use of *P.harmala* (10 percent) (Table 2.). There were no side effects reported after using plant. Studies have shown that Syrian rue is safe and might be used as an alternative to conventional drugs for treatment of mild to moderate depression, anxiety and insomnia. *P.harmala* chemical compounds offered various effects on central nervous system (CNS). Among of these compounds, alkaloids have unique position in CNS neurotransmitters receptors regulation changes. Harmine, harmaline and tetrahydroharmine that known as harmala alkaloids, belong to β-carbolines. These alkaloids are found mostly in the seeds and the roots (2–7% by dry weight). β-carbolines chemical compounds could change CNS neurotransmitters and caused some behavioral changes such as hallucinogenic effects (23, 24, 25). Psychoactivity of these alkaloids due to direct activation of the 5-HT2A or 5- HT2C receptors (26). Harmala alkaloids also interact with benzodiazepine receptors that caused a mild sedative effect [27]. Competitive and reversible inhibition of monoamine oxidase type-A (MAO-A) enzymes have been determined for haramine and harmaline, similar serotonin uptake inhibition also detected for tetrahydroharmine [28].

Table 2. Using of *Peganum harmala* L. for treatment of mild to moderate anxiety and depression

Number of people	Age	Days	Result	Percent
8	19-25	42	Improvement	80
1	23	49	Improvement	10
1	25	42	No improvement	10
10				100

Note: Study participants were required to not take any psychotropic medication for at least four weeks prior to the study.

3. Results and Discussion

We used whole plant for our trial. Meanwhile, we didn't determine which biologically active compounds were responsible for observed pharmacological actions. Syrian rue has a big potential as medicinal plant. Although, plant contains toxic alkaloids and was never used internally, its use as psychedelic and ritual plant is widely spread in Middle East and Central Asia for centuries. Future studies are needed to identify biologically active compounds responsible for healing abilities of Syrian rue.

4. Conclusion

Syrian rue (*Peganum harmala* L., Zygophyllaceae) used for centuries in traditional medicine. Our study shows that *Peganum harmala* has a potential for treatment of anxiety and depression. More clinical studies are needed in order to confirm activity of *Peganum harmala* for treatment of anxiety and depression.

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The Effects of Increasing Mycorrhiza Applications on Some Biological Properties of Baby Carrot (*Daucus carota* L.) Plant

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Short Communication

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The Effects of Increasing Mycorrhiza Applications on Some Biological Properties of Baby Carrot (*Daucus carota* L.) Plant

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Abstract: The study was done to determine the effect of increasing mycorrhiza application on some biological properties of baby carrot plant. According to the pot experiment results, important increases in some biological properties of baby carrot plant were determined with increasing mycorrhiza applications. The tuber diameter were determined as 9.79 cm, 11.09 cm, 12.58 cm, 13.60 cm, 13.80 cm and 14.25 cm; height of leaf 12.98 cm, 15.11 cm, 15.00 cm, 16.07 cm, 17.79 cm and 16.81 cm; number of leaf 7.11 cm, 7.44 cm, 6.99 cm, 7.89 cm, 7.66 cm and 8.11 cm at I. dose: 0 ml /pot, II. dose: 120 ml / pot, III. dose: 150 ml / pot, IV. dose: 180 ml /pot, V. dose: 210 ml /pot and VI. dose: 240 ml/ pot, respectively. These root diameter and height of leaf increases were determined significant at the level of 5 %, statistically. The effect of mycorrhiza application on number of leaf was not found statistically significant.

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1. Introduction

Carrot (*Daucus carota* L.) is considered a basic vegetable in many countries. Various cultivars are grown in the world's mild areas [1]. It is known that growth conditions and management practices affect the concentrations of these compounds in the storage roots of carrots which are important for both human nutrition and taste [2-4]. Carrot (*Daucus carota* L.) is a good source of natural antioxidants, especially carotenoids and phenolic compounds [5]. The demand for organic vegetables is increasing day by day in domestic and international market. Carrot (*Daucus carota* L.) is highly nutritious and preferred as salad vegetable in common household.

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Mycorrhiza use has increased rapidly to accelerate plant growth and improve soil [6, 7]. Researches has shown that mycorrhiza accelerates plant growth, increases the plant's fight against stress, increases the plant's need for sugar -which is important for this carrot plant- and increases the plant's photosynthesis rate [6-10].

For organic carrot is nourishing very fast, farmers are gradually adopting organic carrot cultivation using vermicompost as organic source of nutrients [11]. This is the case in mycorrhiza. According to the some earlier research results, some biological properties of different plants increased with mycorrhiza applications [12, 13]. Root and shoot height of plants, yield and uptake of nutrient elements from the soil increased with increasing mycorrhiza application to the soils [14].

One other earlier research was done determine the influence of mycorrhiza inoculation on plant growth, yield and fruit quality of tomato plants grown in greenhouse conditions and to detect the influence of different doses of Symbion VAM (mainly *Glomus fasciculatum*) as mycorrhiza source. According to results root infection rate increased with Symbion VAM inoculation and in parallel with this increase plant growth and total and marketable yield increased. Besides, fruit diameter, red color (a), vitamin C and pH of fruit juice increased [15].

This research was done to determine the effect of increasing mycorrhiza application on some biological properties of baby carrot (*Daucus carota* L.) plant.

2. Material and Methods

This pot experiment was carried out greenhouse conditions in December 2016 to March 2017, Tekirdag (40°98'N, 27°48'E) Turkey. Pot experiment was done Namik Kemal University, Agricultural Faculty, Department of Soil Science and Plant Nutrition.

Soil samples filled in pots 4 kg soil /pot. Research was designed as three replications according to randomized block experimental design. The variety of baby carrot (Zengarden Firm) was used for the research (Figure 1). Baby carrots seeds were sown in pots.

After germination, three plants were left in each pot. Some chemical and physical properties of soil sample as, pH: 6.5, EC $x10^6$: 700, organic matter: 3.9 %, lime: 5.2 %, exchangeable potassium (K_2O: 128 kg da^{-1}), available phosphorus (P_2O_5: 9.25 kg da^{-1}) and texture: Clay (C).

Six mycorrhiza doses (I. dose: 0 ml /pot, II. dose: 120 ml / pot, III. dose: 150 ml / pot, IV. dose: 180 ml /pot, V. dose: 210 ml /pot and VI. dose: 240 ml/ pot) were applied 6 days after sowing to the sample of the plants. Plants were harvested 60 days after sowing.

Some biological properties of baby carrot plants (*Daucus carota* L.) -root diameter, height of leaf, number of leaf- were determined. Then experiment analysis results were evaluated SPSS 21 statistically program. ANOVA variance analysis was done and Duncan multiple comparison tests were done on this research results. The climatic conditions necessary for growing of the plants have been provided in the laboratory (Table 1). In addition no pesticides were used during the growing period.

Figure 1. Area of research and post harvest baby carrot plants (original).

Table 1. Average some climate data in laboratory during the experiment period.

Month	Average temperature (^{0}C)	Average humidity (%)
December	7.4	90
January	7.3	92
February	7.6	91
March	9.6	89

3. Results and Discussion

The effect of mycorrhiza applications on some biological properties of baby carrot (*Daucus carota* L.) plant is given in Table 2. According to the Table 2, number of leaf of baby carrot was obtained 7.11 and 8.11 for I. dose and VI. dose, respectively. Height of leaf of baby carrot was obtained 12.98 and 16.81 for I. dose and VI. dose, respectively. Root diameter of leaf of baby carrot was obtained 9.79 and 14.25 for I. dose and VI. dose, respectively.

Consequently, height of leaf and root diameter of baby carrot (*Daucus carota* L.) increased with increasing mycorrhiza application to the pots. These increases were found statistically significant at the level of 5 %, except number of leaf. Similar results were obtained earlier researchers [15].

Another research was done the effects of increasing vermicompost and mycorrhiza application on pepper plant growth and mineral nutrition. Three mycorrhiza doses (0, 1 and 2 g pot^{-1}) and four vermicompost doses (0, 2.5, 5 and 10 g pot^{-1}) was applied to the plants. According to results, vermicompost and mycorrhiza applications positive affected on fresh weight, dry weight and some nutrient element contents of pepper plant [16]. This result is similar our research results.

Eissenstat et al., [17] would have reported mycorrhiza colonization can enhance root of plants. Many variables involved in building roots might affect root longevity, including root diameter. Mycorrhizal associations have evolved to improve the fitness of both plant and fungal symbionts [18]. The beneficial effects of mycorrhizal fungi on plant performance are essential for sustainable management of agricultural [19].

Table 2. The effect of increasing mycorrhiza application on some biological properties of baby carrot plant, *, **, ***

Mycorrhiza doses	Number of leaf	Height of leaf	Root diameter
I. dose	7.11±5.0 ns	12.98±2.3b	9.79±5.4b
II. dose	7.44±6.9 ns	15.11±23.6ab	11.09±29.1ab
III. dose	6.99±5.7 ns	15.00±16.3ab	12.58±11.1ab
IV. dose	7.89±6.9 ns	16.07±5.0a	13.60±18.8a
V. dose	7.66±3.3 ns	17.79±15.8a	13.80±19.1a
VI. dose	8.11±6.9 ns	16.81±20.1a	14.25±18.1a

*: values are average of three replications,
**: each parameter evaluated individually,
***: $p<0.05$, ns: none significant

4. Conclusion

According to this pot experiment results, increasing doses of mycorrhiza application was increased some biological properties on baby carrot (*Daucus carota* L.) plant. This result is important quality of baby carrot plant. Also, according to the earlier researchers, mycorrhiza applications were positive effects of some soil physical, chemical and biological properties. On the other hand, mycorrhiza and another different organic fertilizers and organic materials should be applied to the agricultural soils for quality plant production and sustainable soil fertility.

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The Accumulation of Phenolic Compounds in Genetically Selected *Amaranthus hybridus* is Influenced by Endophytic Natural Growth Regulator

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Abstract: Amaranth (*Amaranthus spp*. L.) (*Amaranthaceae*), an endemic plant in Central and South America, grows worldwide, being cultivated in many temperate and tropical countries. Although several species of amaranth are frequently considered weeds, the plant is recognized as a food, constructive medicine, a source of protein and minerals livestock feed. The plant is widely cultivated, promoted, and increasingly consumed as a leafy vegetable and traditional medicine in Africa. Despite progressive genetic improvement and modern plant growing technologies, unfavorable climatic and ecological factors reduce the yield, and quality of the active plant botanicals. The role of bio-transformed endophytic microbial plant growth regulator formulation (BESF) on yield and accumulation of phenolic compounds in amaranth leaves is poorly understood. The current study assessed the effects of pre-sowing seed treatments with 0.0 %, 0.2 % and 0.4 % concentrations of BESF solution on germination, leaf yield, flavor and phenolic content in genetically selected *Amaranthus hybridus* var. *cruentus*. Data collected were subjected to analysis of variance (ANOVA). Significant treatment means were separated using Tukey test at $p \leq 0.05$. BESF significantly increased fresh marketable leaf yield by over 360 kg/ha (29 %) compared to the control. The total flavonoid content in the leaves was raised by 34 % and 47 % with 0.2 % and 0.4 % BESF solution treatments respectively, compared to control. Maximum concentration levels of rutin, apigenin, apigetrin, and quercetin was obtained with 0.4% BESF solution treatment. An analysis of the collected data suggest that BESF was effective in overall improvement in leaf yield, chemical content, and flavor of *A. hybridus* var. *cruentus*, allowing us to recommend BESF application to raise *A. hybridus* var. *cruentus* leaves for nutrition and pharmacological applications.

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1. Introduction

Amaranth (*Amaranthus spp*. L) (*Amaranthaceae*), an endemic plant of South and central America that now grows worldwide, is being cultivated in many temperate and tropical countries as a source of food, high quality forage and silage crop, and medicinal and ornamental applications [1- 4]. Although several species of amaranth are frequently considered weeds, the plant grows rapidly in hot weather conditions, accumulates concentrations of desirable

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bioactive constituents and is recognized as an effective food and medical source with macro- and micro- nutrients and other healthful bioactive compounds (secondary metabolites). In Africa, the plant is widely cultivated for leaf, under various agro-climatic and soil conditions during hot seasons, especially when common vegetables are scarce or difficult to locate [5, 6].

Thus, amaranth is promoted and increasingly consumed as leafy vegetable, and traditional medicine, where all parts of the plant are used as medicine to heal a number of human and animal diseases in most African communities [1, 2, 3, 5]. Freshly harvested amaranth leaf can be quickly boiled and consumed as a vegetable, mixed with locally grown spices along with fermented staple breads, such as wassa or kocho processed from enset (*Enset ventricosum*), tef (*Ergrostis tef*), sorghum, wheat, barley, legumes or root crops in most parts of Ethiopia [7]. With the population explosion from 25 million in 1977 to 100 million in 2016, there is an increasing challenge to satisfy the growing demand for fresh vegetables. This food need becomes overwhelmingly difficult and very limited in subsistent farming practices in highlands during long period of dry and hot seasons of the year.

The unfavorable climatic and ecological factors continue to reduce the growth, yield, and quality of active botanicals such as *A. hybridus* var *cruentus* despite the use of the latest achievements of genetic improvement, and modern plant growing technologies. Nigist [8] demonstrated the positive effects of urea and compost on growth and grain yield of *A. hybridus* var. *cruentus* in Ziway, Ethiopia. Onyango *et al*. [9] reported that the application of manure and mineral fertilizers improved seed germination, leaf yield, and mineral content of *A. hypochondriacus* in Kenya. It is important, however, to note that amaranth grown on land where chemical fertilizers are used or on nitrogen-rich soils, they accumulate nitrates and oxalate in the leaves, where nitrates are suspected to be implicated in stomach cancers, blue babies syndrome, and some other health problems [10-12], including being fatal to cattle in large quantities. It is desirable, therefore, to raise vegetable amaranth organically by using alternative method, such as use of BESF rather than chemical fertilizers.

Currently, recommended agronomic practices to improve the *A. hybridus* var *cruentus* leaf yield, nutritional quality, flavor, and its biochemical profile under organic cultivation do not exist. Furthermore, there has been no study to determine any induced response of *A. hybridus* var *cruentus* to pre-sowing seed treatments with BESF in respect to germination, leaf yield, flavor, and the biochemical contents in the leaves. BESF is a bio-transformed form of endophytic preparation from symbiotic endophytes (symbionts) of known medicinal plants origins, such as Echinacea. Though "symbiotic endophytes" may well be all microorganisms inhabiting plant organs while having a mutual symbiotic continuum ranging from mutualism to parasitism [13, 14] with possibility to transform to pathogenic [15, 16].

The main objective of our current study was, therefor, to evaluate the induced response of a genetically selected *A. hybridus* var. *cruentus* to pre-sowing seed treatment with BESF as a natural growth regulator, where we measured seed germination, morphology, leaf yield and flavor, and conducted lab tests for bioactive compounds. It is assumed that BESF will serve as an alternative to chemical fertilizers to raise vegetable amaranth that is, nutritious, healthy, and tasty.

2. Material and Methods

Amaranth (*A. hybridus* var. *cruentus*) seeds for the growth of plants in this study were purchased from a local farmer in Southern Ethiopia (ca 2100 m.a.s.l.), and subjected to a seed germination test to determine the seed viability [17]. Subsequently, the seeds were subjected to one of the following pre-sowing treatments for 12 h: Control (0.0 % pure water); 0.2 % and 0.4 % BESF plant growth regulator solution. The treated seeds were directly sown in organically farmed field of Gudar in southern Ethiopia during the rainy season (early July – mid September 2013). The leaves were harvested at maturity just before flowering and then weighed, boiled,

and subjected to a flavor/taste test with a panel of 12 local people who were familiar with the plant.

2.1. Chemical analyses

Air dried leaves were weighed, ground, and extracted with 70 % ethanol (1 part leaf to 5 parts ethanol) for 24 h using continuous Soxhlet extractor apparatus. The ethanol extract was subsequently filtered and concentrated with a Büchi 461 rotary-evaporator (BUCHI Corporation, New Castle, Delaware, USA) at ca 40 °C.

Total phenolic contents (TPC) of the leaf extracts were measured using gallic acid, according to the method described by Singleton and Rossi [18], using Folin–Ciocalteau reagent with Na_2CO_3 (20%). The absorbance of the solutions was measured at 650 nm against the reagent used as a blank. A standard calibration plot was generated at 650 nm using known concentrations of gallic acid. The concentrations of phenols in the test samples were calculated from the calibration plot and expressed as g of gallic acid equivalents (GAE) per 100 grams tissue dry-weight basis (g GAE/100 g dwt). Total flavonoid content in the extracts were measured according to the methods described by Zhishen *et al*. [19] and Mabry *et al*. [20], with the flavonoid contents expressed as quercetin equivalents (QE), and recorded as mg quercetin per g of dried extract.

2.2. Statistical analysis

Growth, yield, and biochemical tests were subjected to analysis of variance (ANOVA) using SPSS software version 12.0 (SPSS Inc. Chicago) with differences defined as $p \leq 0.05$. Regression analysis was used for assessment of a dose-response relationship. A probability value of $p \leq 0.05$ was considered significant.

3. Results

3.1. Agronomic traits

Pre-sowing seed treatment of *A. hybridus* with symbiotic growth regulator BESF solution positively affected the seed germination, seed emergence, plant height, leaf color, and marketable fresh leaf yield both at 0.2 % and 0.4% BESF solution compared to the control (Table 1). Pre-sowing seed treatment with BESF significantly ($p \leq 0.05$) influenced fresh edible leaf yield by over 360 kg/ha compared to the control (Table 1). Furthermore, the acceptance or likeness of taste and aroma (flavor) of steamed leaf of 0.4 % treatment received the highest (88 %) point by taste testing panel of 12 people compared to the control (Table 1). Increased plant height and leaf color, and numbers (data not shown) for 0.4% concentration of BESF treatment were significantly higher compared to the control (Table 1).

Table 1. Agronomic traits of Amaranthus hybridus var creuntus (L.) Thill as affected by different levels of pre-sowing seed treatment with symbiont BESF plant growth solution.

	Concentration levels of BESF solution		
Traits	0.0 % (control)	0.20%	0.40%
Seed germination (%)	89a	92a	97b
Seed emergence (%)	91a	94a	96b
Plant height at harvest (cm)	54a	56ab	60b
Fresh marketable leaf yield (kg/ha)	516a	764b	878c
Leaf flavor (taste), steamed (%)	74a	81b	88c
Leaf color at harvest	green	green	dark green

Note: Mean values followed by the same letter are not significantly different at $p \leq 0.05$.

Table 2. Biochemical traits of *Amaranthus hybridus* var. *cruentus* (L.) Thill. as affected by pre-sowing seed treatment with different concentration of BESF solution.

	Concentration levels of BESF solution		
Chemical name	0.0 % (control)	0.20%	0.40%
Total polyphenols (PPs)	4.75a	5.87b	6.33c
Simple PPs & HBA[1]	0.60a	0.56a	0.58a
Hydroxycinnamic acid	0.22a	0.28a	0.21a
Condensed PPs	0.87a	0.93ab	1.04b
Total flavonoids	1.53a	2.05b	2.25b
Quercetin	0.32a	0.42b	0.44b
Rutin	0.42a	0.69b	0.72b
Apigenin	0.45a	0.53ab	0.61b
Apigenin-7-O-glucoside	0.34a	0.41b	0.48b

Note: Mean values followed by the same letter are not significantly different at $p \leq 0.05$.

[1]Hydroxybenzoic acid

3.2. Biochemical traits

At eight weeks after sowing, the total concentration of polyphenols (PPs) in the leaves was 4.75 mg% in control, 5.8 mg% in 0.2%, and 6.33 mg% in 0.4% concentration of BESF solution growth regulator solution treatments, which were 24% and 33%, respectively, higher than the control ($p \leq 0.05$) (Table 2). Similarly, the condensed PPs content in the leaves rose from 0.87 mg% (control) to 1.04 mg% (0.4 % BESF solution concentration), which was about 20 % higher than the control ($p \leq 0.05$) (Table 2). The content of simple PPs, hydroxybenzoic acid (HBA), and hydroxycinnamic acid in the samples remained relatively stable under all treatments. In addition, the leaves from seed treatment at 0.2% and 0.4% concentration of BESF growth regulator solution, respectively, accumulated 34% and 47% more total flavonoids compared to leaves harvested from the control group ($p \leq 0.05$) (Table 2).

As shown in Table 2, other medically important plant constituents in the amaranth leaves were significantly increased in which seeds were pre-treated with BESF. On the average, the maximum concentration of rutin, apigenin, and apigenin-7-O-gluoside (apigetrin) and quercetin (Fig. 1) was obtained at 0.4% BESF solution, which enabled an increased concentration by 71%, 36%, 41% and 38%, respectively compared to the control ($p \leq 0.05$) (Table 2). It is important to note that BESF seed pretreatment showed the highest increase in rutin content. Rutin is among the flavonoids that has been reported to have many beneficial health effects, including that of a protective effect on memory dysfunction [21]. Our results on the response of leaf vegetable *A. hybridus*, var. *cruentus* is similar to the findings of Gins *et al*. [22] that applied pre-sowing seed treatment and foliar application of *Amaranthus tricolor*, L. with symbiotic microbial growth regulator. However, it is important and interesting to note, that, unlike our method that used Echinacea plants as source of the symbiotic microbial growth regulator, Gins *et al*. [22] applied different symbiotic growth regulators that were isolated from amaranth plants grown under totally contrasting ecological conditions.

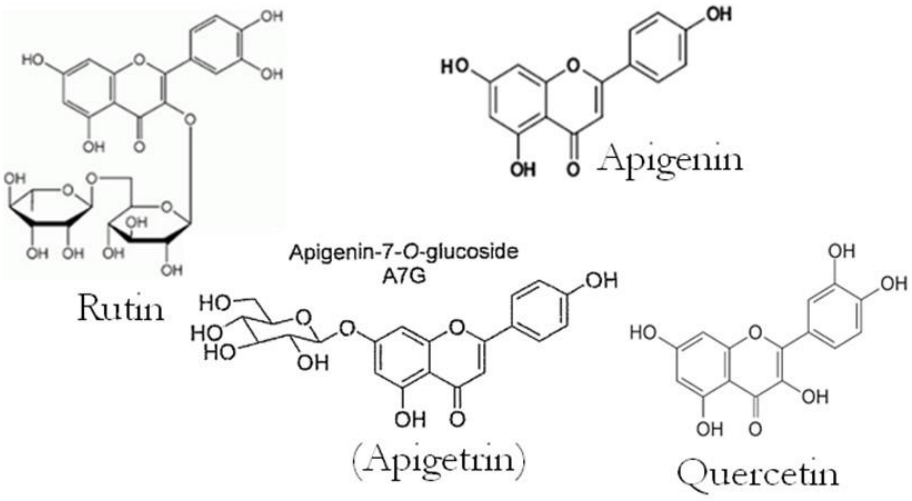

Figure 1. Biologically active constituents in vegetable *Amaranthus hybridus* var. *cruentus* (L.) Thill. leaves.

4. Discussion

Due to increasing global population with health consciousness, there is a growing demand for easily accessible, organically raised, leafy, healthful and nutritious vegetables in Africa, and elsewhere. *A. hybridus* var *cruentus* is one of those desirable popular alternatives growing widely under various agro-climatic conditions having less problems with pests and diseases. This is good news for subsistence farmers in Ethiopia, where there is limitations in rainfall, chemical fertilizers, pesticides, etc.

On the other hand, there is an increasing tendency to raise large fields of amaranth using chemical fertilizers to increase its yield [8, 9]. Amaranth grown under chemical fertilizers, urea, or nitrogen-rich soils tends to accumulate nitrates and oxalate in the leaves, where nitrate is suspected of causing stomach cancers, blue baby syndrome and some other health problems, including fatality to cattle in large quantities [10, 11, 12]. Fungal endophytes are known to occur in almost every plant species grown from the tropics to Antarctica and Arctic regions, having great potential in raising (cultivating) high quality plant products, including *in vitro* development and harvesting of pharmaceutical and nutraceutical products [23]. Endophytic symbionts can serve as elicitors of certain bioactive compounds, improve yield, and help protect plants from stressful conditions, such diseases, draught, etc. serving as alternative choice to pesticides or fungicides [24].

It has been shown that strains of symbiotic endophytes can be isolated from the host plant(s), where they can be cultivated under appropriate bioreactor to enable the endophytic bio-elicitors that enhance the root biomass and the concentration of pharmaceutically important compound, such as ginsenoside in ginseng adventitious roots [25]. Xiaolin *et al*. [25] reported the effects of endophytic bacterial elicitors on biomass and ginsenoside production in adventitious roots cultures of *Panax ginseng* C.A. Meyer (*Araliaceae*). Endophyte LB 5-3, as an elicitor, increased biomass and the content of total ginsenoside to 2.026 mg g^{-1} which was four times more than that in unchallenged roots (25). We did not determine the exact identity of the endophytic strain that we obtained from Echinacea. However, there is good evidence, from our experiment on *A. hybridus* var *cruentus*, that, symbionts can positively affect growth, yield and the content of healthful bioactive components in plants grown under unirrigated subsistence farming fields in southern Ethiopia highland. Hence endophytic symbiont (BESF) can be successfully used by subsistence farmers to produce high quality *A. hybridus* var *cruentus* as vegetable, with less or no nitrate, but higher content of bio-active secondary

metabolites with flavorful taste. It is, however, important that locally identified and processed symbionts suitable for specific ecology must be developed and be available at an affordable price. For durable self-sufficiency, farmers need to be continuously trained or educated to enable them to responsibly practice sustainable, environmentally friendly agriculture and food production. Therefore, our program is focused to improve the challenges facing the rural agriculture, environment, and the human health by developing sustainable practices of agriculture and food production. In our present experiment, we have obtained promising results using a microbial symbiont BESF that increased leaf yield the bioactive chemical components with nutritional health and pharmacological applications. The symbiont BESF can be applied for organically raising valuable alternative vegetables, such as *A. hybridus* var *cruentus*.

5. Summary and Conclusion

Due to rapidly growing population and demand for fresh food, there is an increasing tendency to raise amaranth using major chemical fertilizers, such as nitrogen and phosphorus to increase the yield. Amaranth grown under chemical fertilizers or nitrogen-rich soils is known to concentrate nitrates and oxalate in the leaves. Nitrates are suspected to have caused health problems, such as stomach cancers, "blue baby syndrome" (methemoglobinemia) and fatal to cattle. In addition to being suspected of being contributors to the loss of soil microbial biodiversity and soil degradation, the cost of chemical fertilizers are steadily increasing as a consequence of the increasing energy prices. Hence, search for sustainable, environmentally friendly symbiotic microbial plant growth regulators is consistently increasing.

The main objective of our study was to evaluate the influence of pre-sowing seed treatment with bio-transformed fungal endophyte (symbiont) BESF on genetically selected *A. hybridus* var. *cruentus* raised in organically farmed field. We measured major traits, such as seed germination, morphology, leaf yield and flavor, and conducted biochemical tests in the lab for major bioactive compounds (secondary plant metabolites) of nutritional health value. Our research demonstrated that pre-sowing seed treatment of genetically selected *A. hybridus* var *cruentus* with BESF significantly improved agronomic traits, while enhancing the accumulation of bioactive constituents, such as apigenin, apigetrin, rutin, and quercetin compared to the unchallenged control. The blanched leaf flavor (palatability) as fresh vegetable was improved compared to the untreated plot. Our results show that BESF can be used successfully to grow vegetable *A. hybridus* var *cruentus* by subsistence farmers where they will be able to produce high quality plant material with no or less nitrates, to feed their families, domestic animals, and be able to market excess yield at premium prices in local markets. This study has shade some light on the promising prospects of using the symbiotic microbial growth elicitor BESF in one location, one growing season with one *A. hybridus* var *cruentus*. Therefore, we recommend further tests to elucidate the induced response of the selected variety to the application of microbial symbiont BESF under various environmental (growing) conditions, and cultivation practices in the country.

Conflict of Interst

None declared.

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Biodiversity of Fungi in Strawberry Fields in Anamur, TURKEY

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Abstract: Strawberry is a delicious and aromatic fruit, which can be consumed as fresh and also is suitable for industry. However, strawberry is exposed to many fungal diseases that end with the loss of the product up to % 15 before harvest. The aim of this study is to determine the fungi that present in the field whether or not pathogenic. Samples were collected from different strawberry fields in Anamur in April 2016. Morphological identification was made according to the shape and color of the colonies, mycelium and spore structures. For molecular identification, ITS gene region was used. According to morphological and molecular methods, seven different fungal genera were found on strawberries.

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1. Introduction

Biodiversity is the establishment of environment administrations to which human prosperity is personally connected [1]. It is one of the essential parts of nature and it guarantees the survival of earth definitely.

Fruits are the comestible part of a mature ovary of flowering plants, which are normally eaten raw [2]. Strawberry is one of these fruits. However, fruits are easily spoilt and usually have active metabolism during the storage stage [3]. The importance of fruit in human nutrition cannot be overestimated as it provides essential growth factors such as vitamins and minerals necessary for proper body metabolism [4]. The high concentration of various sugars, minerals, vitamins, amino acids, and low pH also enhances the successful growth and survival of various parasitic and saprophytic forms of fungi [5]. Annual reports have shown that % 20 of fruits and vegetables produced are lost to spoilage [6].

Soil biodiversity impacts a gigantic scope of biological system forms that add to the maintainability of life on earth [7]. Biological activity is an essential factor in the physical and substance development of soils [8].

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There are 110.000 defined fungi species were present in the World but it is estimated that 1.5 million fungi species exists [9]. The ITS region, one of the polymorphic DNA sequences among fungal species, is now considered to be a good candidate for accurate detection and can be largely separated from all other species by this application. It is important to determine the diversity of fungi, which cause diseases on strawberries and their ecological and genetic effects. Abdullah et al. (2016) studied fungal biodiversity of post-harvest rot of some fruits in Yemen. They found 16 fungal genera and 39 species [10]. Jensen et al. (2013) studied characterization of microbial communities on strawberries and found *Penicilium* spp were abundant [11].

In this study, fungi that cause disease in strawberry will be detected by morphological and molecular methods.

2. Material and Methods

Sample Collection: Samples were collected aseptically from the strawberry fields from Anamur in April 2016. Thirty rotten strawberry fruits were collected and kept in the portable refrigerator until brought to the laboratory.

Isolation of Fungal Species: One gram of strawberry fruits were weighted and homogenized in 9 ml of 0.85% Physiological Saline Water (PSW). 100 µL of these homogenised samples were inoculated on Rose Bengal Agar (RBA) and Potato Dextrose Agar (PDA). Samples than incubated at 27 °C for 5 days. After incubation, different fungi were selected and isolated from the mixed colony under the same incubation conditions.

Morphological Identification: Morphological identification of the fungi was made according to Samson [12]. Mycelium and spore structures smeared on a slide, dyed with lactophenol cotton blue and visualized under the microscope. Colonial shapes were determined and used in morphological identification.

Molecular Identification: Fungi samples were put in 1.5 ml Eppendorf tubes using a sterile toothpick. Then samples have reduced the powder using liquid nitrogen. DNA isolation of the samples was realized with 2X CTAB isolation protocol according to Doyle and Doyle [13]. Concentration and purity of the samples were measured with a Nanodrop Spectrophotometer (Thermo). ITS gene region was used to identify the species. Two universal ITS primers were used (ITS1:5'TCCGTAGGTGAACCTGCGG'3, ITS4: 5'TCCTCCGCTTATTGATATGC'3) [14]. PCR reactions were realized at initial denaturation 94 ºC 5 min, denaturation 94 ºC 30 sec, annealing 60 ºC 30 sec, extension 72 ºC 60 sec with 35 cycles and a final extension at 72 ºC 10 min. Reagents concentrations were 10X Taq Buffer, 0.5M dNTP mix, 10 pM from each primer, 7.5 mM MgCl2 and 1U Taq polymerase (GenMark) with the final volume of 25 µl. Agarose gel electrophoresis of the PCR products were observed with 1.4 % agarose concentration on 90 V 40 min. 100 bp DNA ladder was used for size comparison of the products. After PCR products were sent to DNA sequencing (Macrogen, Holland).

Data Analysis: Sequence results were aligned with the ones in GenBank using BLASTn software to find out the species of the samples. MEGA 7.0 was used to infer phylogenetic tree using maximum parsimony method.

3. Results

Morphological Identification: According to morphological methods seven different species were found (Table 1). Colony shape, mycelium and spore structures were investigated. Seven different species were spotted according to Samson [10].

Table 1. Morphological identification of the species.

No	Name
1	*Botrytis cinera*
2	*Mucor sp.*
3	*Fusarium sp.*
4	*Alternaria alternata*
5	*Aspergillus niger*
6	*Mucor circinelloides*
7	*Pestalotiopsis sp.*

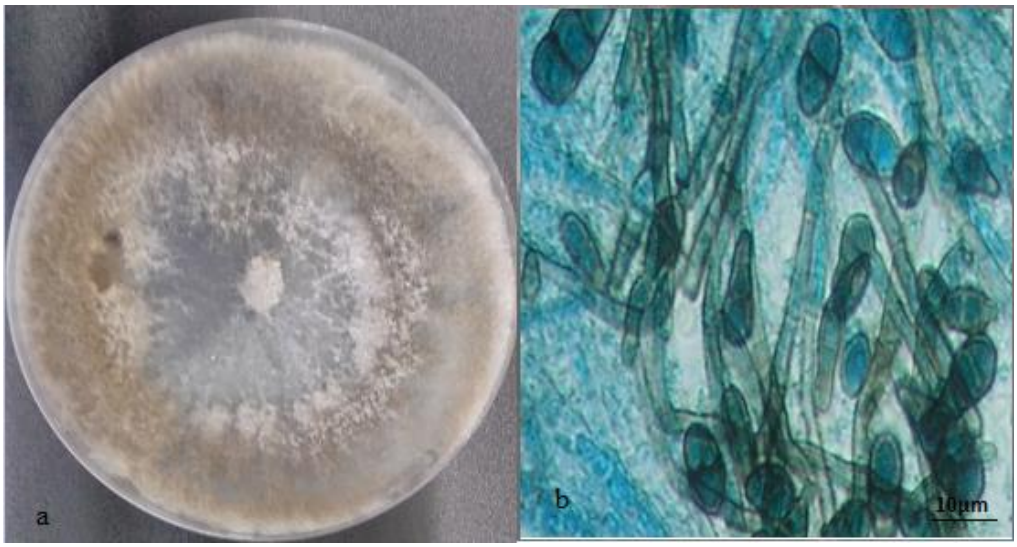

Figure 1. Morphological identification structures (*Botrytis cinera*). A) Colony image B) Mycelium image

Molecular Identification: ITS rDNA gene region was used to identify fungal species. After amplification PCR products were sent to sequencing to Macrogen (Holland). Molecular identification was made by comparing sequences with GenBank using BLASTn. Seven fungal species were found in accordance with morphological results (Table 2).

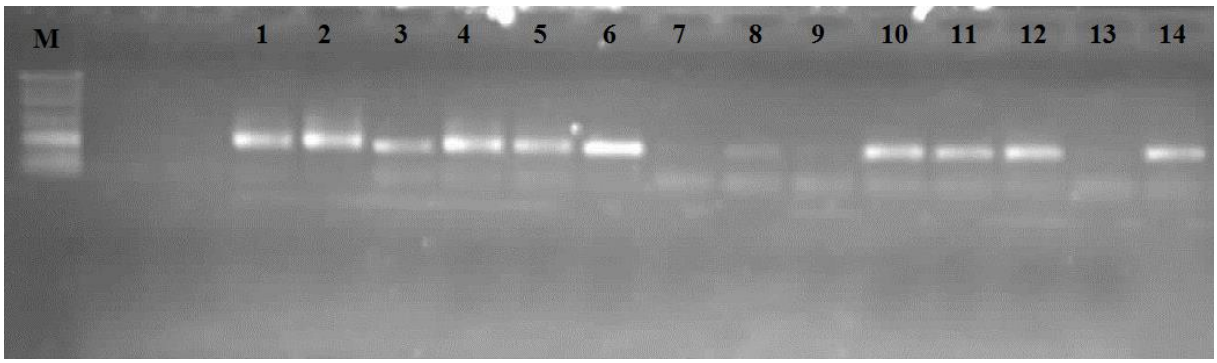

Figure 2. ITS PCR results of samples. (M: 100bp marker (GenMark), 1-14: Samples)

Table 2. Molecular Identification of species.

No	Name	Number of Isolates	Accession No
1	*Botrytis cinera*	10	KP151607.1
2	*Pestalotiopsis clavispora*	1	JF327826.1
3	*Mucor circinelloides*	4	KJ584557.1
4	*Mucor racemosus*	6	JN205991.1
5	*Alternaria alternata*	2	KP661568.1
6	*Fusarium oxysporum*	3	GQ121286.1
7	*Mucor fragilis*	4	JF327830.1

MEGA 7.0 was used to infer a phylogenetic tree. Maximum parsimony method was used to construct a tree (Figure 3). MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm with search level 1 in which the initial trees were obtained by the random addition of sequences (10 replicates).All positions with less than 95% site coverage were eliminated.

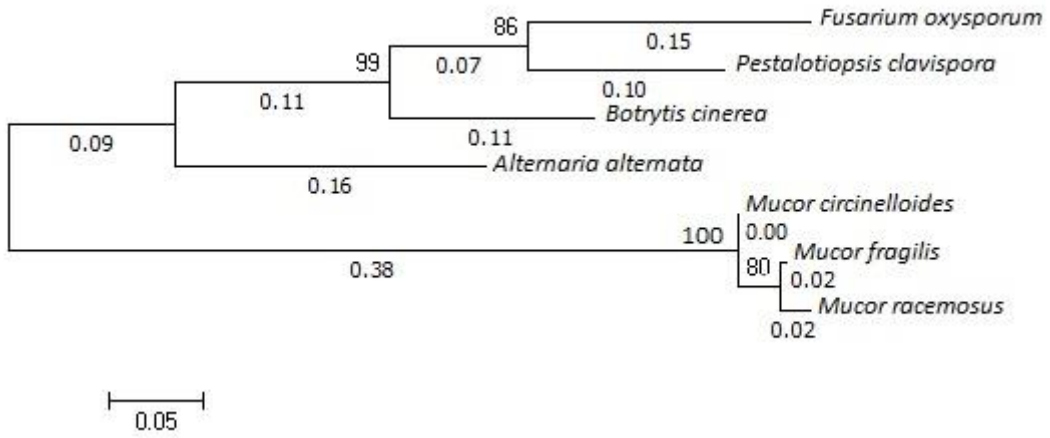

Figure 3. The evolutionary history was inferred using the Maximum Parsimony method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA7.

4. Discussion

Because only spoiled fruits were used in this study all species found were associated with diseases. If other plant parts and soil were used more species can be found both pathogenic and non-pathogenic. Literature shows that procedures, such as gathering and transporting, natural products may experience physical damage that builds post-reap misfortune and the likelihood of contagious pollution [15, 16].

Kasiamdari et al. (2002), isolated *Rhizoctonia solani* CFM1 isolate from soil-grown cabbage, designed two primer sequences from the ITS gene region by Nested-PCR method and indicated that molecular methods would provide more advantages than microscopic methods [17]. Staats et al. (2004) used the DNA sequence of 3 nuclear protein-coding genes (RPB2, G3PDH and HSP60) to classify *Botrytis* spp. And compared them to conventional classifications. Phylogenetic analyses indicated that *Botrytis* spp. Separated from Sclerotiniaceae species, of the species had only 4 species, while line 2 contained 18 species

[18]. Khairnar et al. (2011) studied the soil-borne fungal biodiversity of some fruit crops in India and found 21 fungal species and suggested that all fungal species can be controlled with 500 ppm Moximate [19]. Abdelfattah et al. (2015) researched fungal biodiversity of olive and found 195 different Operational Taxonomic Units (OTUs). They found Ascomycota was the most abundant phyla that can be found in olives [20]. Mailafia et al. (2017) researched fungi associated with fruit species and identified six different fungi and one yeast species [6].

Pestalotiopsis clavispora causes crown rot and leaf spot on strawberries [21, 22]. Botrytis cinera is the cause of gray mold disease [23]. *Alternaria alternata* is the cause of leaf spot disease over 380 plant species [24]. *Mucor circinelloides* is both a plant and human pathogen [25]. *Mucor racemosus* is a plant pathogen that can cause allergic reactions in humans [26]. *Mucor fragilis* is reported as a growth promotor in plants [27]. *Fusarium oxysporum* is the cause of fusarium wilt disease [28].

5. Conclusion

This study was conducted in order to find fungal biodiversity on strawberries. As a result of this study seven fungal species were identified both by morphological and molecular methods. Spoiled fruits were used in study therefore all fungi identified were pathogenic. Although fungicides were used in the field fungal diseases, such as gray mold, can still be seen frequently. Further studies must be conducted to prevent these diseases.

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The Effect of Increasing Mycorrhiza Applications on Nutrition of Pak Choi (*Brassica rapa* L. *subsp. chinensis* L.) Plant

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The Effect of Increasing Mycorrhiza Applications on Nutrition of Pak Choi (*Brassica rapa* L. *subsp. chinensis* L.) Plant

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Abstract: The study was done to determine the effect of increasing mycorrhiza application on some macro and micro nutrient element contents of pak choi (*Brassica rapa* L. *subsp. chinensis* L.) plant. According to the experiment results, important increases in some macro and micro nutrient element contents of pak choi plant were determined with increasing mycorrhiza applications. The contents were determined as P (0.38 %, 0.42 %, 0.45 %, 0. 49 % and 0.51 %), K (4.01 %, 4.30 %, 4.41 %, 4.56 % and 4.70 %), Ca (1.83 %, 2.01 %, 2.06 %, 2.20 % and 2.36 %), Mg (0.14 %, 0.15 %, 0.15 %, 0.16 % and 0.16 %), Fe (309 mgkg^{-1}, 417 mgkg^{-1}, 678 mgkg^{-1}, 1009 mgkg^{-1}and 1696 mgkg^{-1}), Cu (5.49 mgkg^{-1}, 6.10 mgkg^{-1}, 6.53 mgkg^{-1}, 7.05 mgkg^{-1}and 7.63 mgkg^{-1}), Mn (45.90 mgkg^{-1}, 52.23 mgkg^{-1}, 60.20 mgkg^{-1}, 70.40 mgkg^{-1}and 80.00 mgkg^{-1}) and Zn (32.23 mgkg^{-1}, 35.40 mgkg^{-1}, 37.00 mgkg^{-1}, 40.70 mgkg^{-1}and 46.86 mgkg^{-1}) at I. dose, (control): 0 ml plant^{-1}, II. dose: 15 ml plant^{-1}, III. dose: 20 ml plant^{-1}, IV. dose: 30 ml plant^{-1} and V. dose: 40 ml plant^{-1}, respectively. These P, K, Ca and Mg contents increases were determined significant at the level of $P<0.05$, statistically. The highest nutrient element contents of pak choi plant were obtained V. dose: 40 ml plant^{-1}applications for P, K, Ca, Mg, Fe, Cu, Mn and Zn nutrient elements.

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Pak choi (*Brassica rapa* L. *subsp. var. chinensis* L.),

Exotic vegetable,

1. Introduction

As a highly rated leafy variety of vegetables and a marvelous food alternative, brassicas is grown for its enlarged, edible, terminal buds; and is preferably eaten almost everywhere in the world as well [1]. This green vegetable was made known around the world by the efforts of the travelers and immigrants [2-4].

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Pak choi name was given syn. *Brassica chinensis* L. (1759), *Brassica campestris* L. subsp. *chinensis* (L.) [5], *Brassica rapa* L. subsp. *chinensis* (L.) [6] in China. This vegetable which has been known since the 5th A. D. and grown widely in China and Taiwan is classified within the group which is identified as Chinese vegetables [7, 8]. Its leaves are edible and it has 50-60 days of vegetation approximately [9, 10].

A greenhouse experiment was made for the effect of increasing mycorrhiza application on the some nutrient element contents of maize plant (*Zea mays* L.) [11]. According to the research results, N, K, Fe and Cu contents of maize plant increased with increasing mycorrhiza application.

The effect of salt (0 and 100 mg Na Cl kg^{-1}) and increasing zinc applications (0, 25, 50 mg Zn kg^{-1}) application on phosphorus and zinc uptake of maize (*Zea mays* L.) plant with mycorrhiza and non-mycorrhiza conditions. At the end of the experiment it was determined that mycorrhiza inoculated applications provided a significant increase in fresh weight, dry weight, phosphorus and zinc contents compared to the non-mycorrhiza applications [12].

The research was done to determine the effect of increasing mycorrhiza application on some macro and micro nutrient element contents of pak choi plant.

2. Material and Methods

Using high tunnel cold greenhouse covered by polyetilen (PE) with UV additive which belongs to Namik Kemal University, Vocational College of Technical Sciences, Plant and Animal Production Department, the experiments were carried out autumn in Tekirdag city (40°98' N, 27°48' E) Turkey in 2016.

Research was designed as three replications according to randomized block experimental design. The white mini variety of Pak Choi (Zengarden Firm) was used for the research (Figure 1). Seeds were sown in multi-celled trays filled with peat (Klasmann-Deilmann, Potground H, Germany) in October. Some properties of the used peat are: 160-260 mgl^{-1} N, 180-280 mgl^{-1} P_2O_5, 200-150 mgl^{-1} K_2O, 80-150 mgl^{-1} Mg, pH: 6, 70 % organic matter and 35 % C. When the seedlings became 2 to 3 true leaves (21th days for pak choi after seed sowing) they were planted to pre-prepared places in high tunnel cold greenhouse with 10x10 cm intervals and 10 plants in each parcel.

Then five mycorrhiza doses (I. dose, (control): 0 mlplant^{-1}, II. dose: 15 mlplant^{-1}, III. dose: 20 mlplant^{-1}, IV. dose: 30 mlplant^{-1}and V. dose: 40 mlplant^{-1}were applied before a month from harvesting time and plants were harvested 54 days after seed sowing. Some macro and micro nutrient elements (P, K, Ca, Mg, Fe, Cu, Mn and Zn) contents of plants were determined via ICP-OES instrument [13]. Then experiment analysis results were evaluated SPSS 21 statistically program. ANOVA variance analysis was done and Duncan multiple comparison tests were done on this research results.

Some chemical properties of the soil samples which used in this experiment it can be seen in Table 1. The some climate data measured inside the tunnel during the growing of the plants it can be seen in Table 2. Since there were no diseases and pests, no pesticides was used during the growing period.

Figure 1. A general view from pak choi experiment and post-harvested plant (Original).

According to the Table 1, pH value alkaline, salt less, low organic matter content, little lime, medium available phosphorus, exchangeable K, Ca and Mg sufficient, also, micro element (Mn, Cu, Fe and Zn) contents are sufficient of experiment area soil

Table 1. Some chemical properties of soil samples

Soil properties	Results
pH	8.01
Salinity (%)	0.07
CaCO3 (%)	2.74
Organic matter (%)	1.35
Ca (%)	0.54
P ($mgkg^{-1}$)	36.40
K ($mgkg^{-1}$)	253.80
Mg ($mgkg^{-1}$)	473.10
Mn ($mgkg^{-1}$)	5.68
Cu ($mgkg^{-1}$)	0.81
Fe ($mgkg^{-1}$)	7.43
Zn ($mgkg^{-1}$)	0.97

Table 2. Average some climate data in unheated greenhouse during the experiment period.

Month	Average temperature (^{0}C)	Maximum temperature (^{0}C)	Minimum temperature (^{0}C)	Average humidity (%)
October	16.50	19.03	14.01	87
November	12.30	15.01	9.60	89
December	7.40	10.50	4.30	90

3. Results and Discussion

The effect of increasing mycorrhiza application on some macro nutrient element content of pak choi plant is given in Table 3. According to the Table 3, phosphor content of pak choi plant was obtained 0.38 % and 0.51 % for I. dose and V. dose, respectively. Potassium content of pak choi plant was obtained 4.01 % and 4.70 % for I. dose and V. dose, respectively.

Calcium content of pak choi plant was obtained 1.83 % and 2.36 % for I. dose and V. dose, respectively. Magnesium content of pak choi plant was obtained 0.14 % and 0.16 % for I. dose and V. dose, respectively.

P, K, Ca and Mg contents of pak choi plant increased with increasing mycorrhiza application (Table 3). These increases were found statistically significant at the level of $p<0.05$. These results were found to be some earlier researchers [11, 14].

Table 3. The effect of increasing mycorrhiza application on some macro nutrient element contents of pak choi plant, (%)

Mycorrhiza doses	P	K	Ca	Mg
I.dose (Control)	0.38±0.31**c**	4.01±0.18**d**	1.83±0.77**d**	0.14±0.07**a**
II. dose	0.42±0.13**b**	4.30±0.30**c**	2.01±0.29**c**	0.15±0.21**ab**
III. dose	0.45±0.08**b**	4.41±0.51**bc**	2.06±0.11**c**	0.15±0.13**ab**
IV. dose	0.49±0.09**a**	4.56±0.73**ab**	2.20±0.99**b**	0.16±0.15**a**
V. dose	0.51±0.06**a**	4.70±0.59**a**	2.36±0.62**a**	0.16±0.80**a**

*: values are average of three replications,
**: each parameter evaluated individually,
***: $p<0.05$

The effect of increasing mycorrhiza application on some micro nutrient element content of pak choi plant is given in Table 4. According to the Table 4, iron content of pak choi plant was obtained 309 mgkg^{-1} and 1696 mgkg^{-1}for I. dose and V. dose, respectively. Cupper content of pak choi plant was obtained 5.49 mgkg^{-1}and 7.63 mgkg^{-1}for I. dose and V. dose, respectively.

Zinc content of pak choi plant was obtained 32.23 mgkg^{-1}and 46.86 mgkg^{-1}for I. dose and V. dose, respectively. Manganese content of pak choi plant was obtained 45.90 mgkg^{-1}and 80.00 mgkg^{-1}for I. dose and V. dose, respectively.

Fe, Cu, Zn and Mn contents of pak choi plant increased with increasing mycorrhiza application according to the Table 4. These increases were found statistically significant at the level of $p<0.05$. These results were found to be some earlier researchers [15-17].

Table 4. The effect of increasing mycorrhiza application on some micro nutrient element contents of pak choi plant, $mgkg^{-1}$

Mycorrhiza doses	Fe	Cu	Zn	Mn
I.dose (Control)	309±4.3**d**	5.49±0.02**d**	32.23±0.10**d**	45.90±0.4**d**
II. dose	417±3.9**d**	6.10±0.02**c**	35.40±0.10**c**	52.23±0.2**d**
III. dose	678±5.5**c**	6.53±0.01**c**	37.00±0.02**c**	60.20±0.4**c**
IV. dose	1009±19.3**b**	7.05±0.08**b**	40.70±0.10**b**	70.40±0.1**b**
V. dose	1696±4.9**a**	7.63±0.04**a**	46.86±0.30**a**	80.00±0.5**a**

*: values are average of three replications,
**: each parameter evaluated individually,
***: $p<0.05$

Mycorrhiza could also enhance crop quality not only by enrichment in macronutrients (like P) [18, 19] and in micronutrients [20-22]. Mycorrhiza yield mineral element contents are decreasing, compromising the nutritional value of food. The use of beneficial bacteria results in an increase in the concentration or availability of mineral elements in the production. Micro nutrient is regarded as a promising strategy to overcome malnutrition in terms of nutrition [20, 23]. Beneficial bacteria use, macro and micronutrient element affects the soil intake positively [24, 25]. According to Gianinazzi and Gollotte, 2010 [26] increasing mineral nutrient and water uptake by plants. And promote plant growth while reducing fertilizer requirement.

4. Conclusion

According to this greenhouse experiment results, increasing doses of mycorrhiza application was increased some macro (N, P, K, Ca and Mg) and micro (Fe, Cu, Zn and Mn) element contents of pak choi plant. These increases were found to be statistically significant at the level of $p<0.05$. This result is very important nutrition and quality of pak choi plant. Because, excess chemical fertilizers application to the agricultural soils was destroyed soil fertility and quality. Therefore, mycorrhiza, another different microbial fertilizers, other organic fertilizers and organic materials should be applied to the agricultural soils for quality plant production and sustainable soil fertility. Also, according to the earlier research result, mycorrhiza applications were improved of some physical, chemical and biological properties of the agricultural soils.

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In Vitro Evaluation of Antimicrobial and Antibiofilm Potentials of Essential Oil of *Tanacetum argenteum* (Lam.) Willd. subsp. *canum* (K.Koch) Grierson var. *canum*

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Abstract: This study was performed to determine the antimicrobial and antibiofilm activities of essential oil of *Tanacetum argenteum (Lam.)* Willd. subsp. *canum (K.Koch)* Grierson var. *canum*. The plant was collected from Amasya. The antimicrobial activity was determined by disc diffusion and microdilution broth methods. Essential oil was active against indicator organisms. The maximal inhibition zone diameter were as follows: *E. cloacae* ATCC 28355 (14mm), *P. fluorescens* ATCC 55241 (16mm), *P. aeruginosa* ATCC 27853 (19mm), *S. sonnei* RSKK 8177 (18mm), *E. coli* ATCC 25922 (21mm), *E. coli* O157:H7 (13mm), *Y. enterocolitica* RSKK 1501 (11mm), *C. jejuni* ATCC 33291 (12mm), *K. pneumoniae* ATCC 27736 (13mm), *S. enteritidis* RSKK 171 (12mm), *S. aureus* ATCC 33862 (26mm) and *S. aureus* ATCC 25923 (29mm), *M. luteus* NRLL-B-4375 (20mm), *E. faecalis* ATCC 19433 (19mm), *B. cereus* NRRL-B-3711 (30mm), *B. cereus* RSKK (32mm), *C. albicans* ATCC 10231 (19mm) and *C. tropicalis* (17mm). The minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) were ranged from 62.5-900 µg/ml and 125-1000 µg/ml, respectively. Plant essential oil was the most active against *B. cereus* NRRL-B 3711 and *B. subtilis* RSKK 867. Also, the essential oil has shown a strong anti-candidal activity against two *Candida* species. For determining the antibiofilm effect, various dilutions of oil were studied on *M. luteus, E. cloacae, P. fluorescens, Y. enterocolitica and S. enteritidis* by using microplate biofilm assay. According to results, antibiofilm effect was found as weak. Maximum antibiofilm effect was determined on *E. cloacae* ATCC 28355 with 32.92% biofilm inhibition rate.

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1. Introduction

The incidence of infectious agents such as human, animal and plant pathogens has increased dramatically over the past few decades. The most common of these pathogens are developed multi-drug resistance and there is a reduction in the number of effective drugs.

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Because of this, the new and effective natural compounds against pathogens are searched by scientists. Aromatic plants and their essential oils are commonly used for the treatment or prevention of disease in traditional medicine as antimicrobial agents. Moreover, their pharmacological effects such as antimicrobial, antioxidant and anticholinesterase are scientifically proven [1, 2]. The chemical analysis of *Tanacetum* species has been well documented [3-11]. Also, the essential oils and sesquiterpenes isolated from some *Tanacetum* species have various biological properties, such as antifeedant, insecticidal, antimicrobial, larvicidal, cytotoxic, anticoagulant and antifibrinolytic, etc. [12-19]. Plant oils in traditional medicine are used to treatment of infectious diseases. Particularly, searching of new drugs against to resistant microorganisms and/or biofilm forming microorganisms has received much attention. Because, treatment of chronic infections related with biofilm are very difficult as biofilms are exceptionally resistant to antibiotics and host immune response. Therefore, natural products have also been screened for their antimicrobial or antibiofilm activity.

T. argenteum (Lam.) Willd. subsp. *Canum* (K.Koch) Grierson var. *canum* used in our study has distributed in south Anatolian regions of Turkey [20]. Its local name is Bodur Pireotu. In the literature, there are many works about the composition essential oil and sesquiterpenoids of *T. argenteum* subsp. *canum* [4, 21]. But, we have not found any published report on the antimicrobial and antibiofilm effects of essential oil of this subspecies. Therefore, present study will be the first scientific research to provide data that the antimicrobial and antibiofilm activity of this essential oil. Also we expect that results obtained from this study will become a good reference for detailed screening of other biological and pharmacological properties.

2. Material and Methods

Plant materials: *T. argenteum* (Lam.) Willd. subsp. *canum* (K.Koch) Grierson var. *canum* plant was collected during the flowering period and natural populations in A5 Amasya (between Direkli village and Yassıçal town, rocky areas and steppes, at 1300 m, 20.06.2010, Cansaran 5402) which is a city in the Black Sea Region of Turkey. The identification of plant was confirmed by Assoc. Prof. Dr. Arzu Cansaran according to the description given by Davis [20] and Cansaran et al. [22].

Test microorganisms: *Enterobacter cloacae* ATCC 28355, *Pseudomonas fluorescens* ATCC 55241, *Pseudomonas aeruginosa* ATCC 27853, *Shigella sonnei* RSKK 8177, *Escherichia coli* ATCC 25922, *Escherichia coli* O157:H7, *Yersinia enterocolitica* RSKK 1501, *Campylobacter jejuni* ATCC 33291, *Klebsiella pneumoniae* ATCC 27736, *Salmonella enteritidis* RSKK 171, *Staphylococcus aureus* ATCC 33862, *Staphylococcus aureus* ATCC 25923, *Micrococcus luteus* NRLL-B-4375, *Enterococcus faecalis* ATCC 19433, *Bacillus cereus* NRRL-B-3711, *Bacillus cereus* RSKK 867, *Candida albicans* ATCC 10231 and *Candida tropicalis* were used as test organisms in this study. Microorganisms tested in present study were obtained from Bacteriology Laboratory, Department of Biology, Pamukkale University (Denizli) and were regenerated twice before use in the activity studies. Tryptic Soy Broth (Merck), Tryptic Soy Agar (Merck), Sabouraud Dextrose Broth (Oxoid) and Sabouraud Dextrose Agar (Oxoid) were used for bacteria and yeasts, respectively. The culture was aerobically incubated and adjusted by comparing with 0.5 McFarland Standard Dilutions in all manipulations.

Extraction of the essential oil: Air-dried aerial parts of plants were subjected to hydro-distillation for 3-5 h using a Clevenger-type apparatus to obtain essential oil in a yield of 1.905% (v/w) for *T. argenteum* subsp. *canum* var. *canum* based on the dry weight of the samples. The essential oil was stored in a sealed dark vial at 4 °C.

Determination of inhibitory effect by the disc diffusion method: The antimicrobial activity of essential oil was assigned by disc diffusion method (23). 100 µl suspensions of

microorganisms were spread on the solid medium plates. Empty sterilized disks of 6 mm (Schleicher and Schuell, No. 2668, Germany) were each impregnated with 50 µl of essential oils. The discs injected with essential oils were placed on the inoculated agar. Petri dishes were sealed with sterile laboratory parafilm to avoid eventual evaporation of the essential oils. The dishes were left for 2 h at 4 °C to allow the diffusion of oil, and then were incubated at 37°C for 24 h (at 30°C for *M. luteus* NRRL-B-4375, at 42°C for *C. jejuni* ATCC 33291 and at 28°C for yeasts). The diameters of the inhibition zones formed on the medium were evaluated in millimetres. All the tests were performed in duplicate. The inhibition zones were compared with those of Meropenem and Nystatin as antibiotic and antifungal.

Analysis of the minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC): MIC value of essential oils was determined by the broth microdilution method in 96-well microplates [24]. 95 µl of TSB, 100 µl of the essential oil and 5 µl of the culture were added into each wells. Our controls (with no cells and no essential oils) were also included on each microplate. The MIC was defined as the lowest concentration of the essential oils where no growth was visually observed. The growth of the microorganism was indicated by turbidity. After determination of the MIC, the microplates were mixed and 25 µl from each well (MIC and higher concentrations) was plated on solid medium. After 24 hours for bacteria and 48 h for yeasts, the lowest concentration of essential oil which no growth of colonies (98%) was considered as MLC. Standard powder of Meropenem and Nystatin were dissolved in distilled water and DMSO, respectively.

Antibiofilm activity: Crystal violet method was applied using 96-well polystren plate for antibiofilm activity [25]. The culture suspensions was adjusted at 0.5 McFarland standard tube and 100 µl was dispensed into each well of 96-well plates in the presence of 100 µl essential oil (1/2 MIC, ¼ MIC and 1/8 MIC) or 100 µl TSB (control). The plates were incubated for 48 h at 37 °C and 30 °C. Following incubation, crystal violet staining assay was performed. Measure the optical density (OD) of each of these samples at a wavelength of 550 nm. Each experiment was performed in duplicate. And the biofilm inhibition percentage was calculated by using the following formula:

$$[(OD_{\text{growth control}} - OD_{\text{sample}}) / OD_{\text{growth control}}] \text{ x}100$$

3. Results and Discussion

Undoubtedly, infectious disease agents cause major problems in the worldwide and antimicrobial resistance is a critical public health issue. Therefore, the scientific community has been focused on various fields to combat pathogens. Biological and pharmacological properties of aromatic plants and their seconder metabolites are scientifically screened to control the colonization and growing of pathogens in environment. It is the known fact that essential oils obtained from aromatic plants are very rich in complex compounds and the biological and pharmacological properties of some of them are confirmed scientifically. Moreover, it was reported their potential applications as natural and safe food preservatives or an antibacterial agent for both pharmaceutical and pesticide industries [26, 27]. According previous reports, camphor, borneol and 1,8-cineole (eucalyptol) were common compounds of the essential oils of *Tanacetum* species and antimicrobial potentials of these compounds were well determined [28-31]. The antimicrobial and cytotoxic activities of caryophyllene oxide and thujone, the main constituents of the oil of *T. argenteum* subsp. *canum* var. *canum* [21], were also verified by some researchers [32, 33].

In the present research, the antimicrobial activity of essential of *T. argenteum* subsp. *canum* var. *canum* collected from Amasya (between Direkli village and Yassıçal town) was examined against some pathogens on the basis of disc-diffusion and microdilution assay. The activity results, quantitatively estimated by the presence or absence of inhibition zones and also

MIC and MLC values, were given in Table 1 and 2. According to the initial antimicrobial screening test, it was shown that the activity of essential oil was good against all the used organisms. But, *E. coli* 0157:H7, *Y. enterocolitica* RSKK 1501, *Campylobacter jejuni* ATCC 33291, *Klebsiella pneumoniae* ATCC 27736 and *Salmonella enteritidis* RSKK 171 were more resistant than other bacteria. The inhibition zones of disc, MIC and MLC values for the microorganisms were in the range of 11-32 mm, 62.5-900 µg/ml and 125-1000 µg/ml, respectively. The essential oil was more active against gram-positive bacteria than gram negative bacteria.

Table 1. Growth inhibition zones of pathogens in millimetres (±standard deviation) of *Tanacetum argenteum* subsp. *canum* var. *canum*

Tested bacteria	Inhibition zone diameter (mm; 50 µl)	Antibiotics	
		Meropenem (10 µg)	Nystatin (100 U)
Enterobacter cloacae ATCC 28355	14±0	22±0	NT
Pseudomonas fluorescens ATCC 55241	16±0	24±0	NT
Pseudomonas aeruginosa ATCC 27853	19±0	23±0	NT
Shigella sonnei RSKK 8177	18±0	22±0	NT
Escherichia coli ATCC 25922	21±2	27±0	NT
E. coli O157H7	13±0	25±0	NT
Yersinia enterocolitica RSKK 1501	11±1	25±0	NT
Campylobacter jejuni ATCC 33291	12±0	18±0	NT
Klebsiella pneumoniae ATCC 27736	13±0	19±0	NT
Salmonella enteritidis RSKK 171	12±2	24±0	NT
Staphylococcus aureus ATCC 33862	26±0	12±0	NT
Staphylococcus aureus ATCC 25923	29±0	10±0	NT
Micrococcus luteus NRLL, B-4375	20±2	5±0	NT
Enterococcus faecalis ATCC 19433	19±0	9±0	NT
Bacillus cereus NRRL,B-3711	30±2	10±0	NT
Bacillus cereus RSKK 867	32±0	8±0	NT
Candida albicans ATCC 10231	19±0	NT	18±0
Candida tropicalis (clinic isolate)	17±0	NT	16±0

NT: Not tested; ATCC: American Type Culture Collection; NRRL: a culture collection of the Agricultural Research Service (ARS); RSKK: Refik Saydam National Type Culture Collection

As known, spore forming bacteria are critical issue in food industry. For example, *Bacillus cereus* is an important bacterium for the safety of prepared foods. Because, spores of *B. cereus* are found widely in environment such as soil, water, dust, etc. and they produce toxins in foods [34]. In our study, *Bacillus cereus* NRRL-B-3711 (30 mm) and *Bacillus cereus* RSKK 867 (32 mm) were the most sensitive bacteria among all tested organisms. The MIC and MLC values of essential oil against these bacteria were 62.5 µg/ml and 125 µg/ml, respectively. On the other hand, it was noted that the bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*, known to be multi-resistant to antibiotics and concern to public health, were moderate sensitive to the essential oil. For example, *Escherichia coli* O157:H7 is an enterohemorrhagic foodborne pathogen and causes human disease [35]. *Campylobacter jejuni* is the most important source of gastrointestinal illness. Moreover, its infection happens more frequently than do infections caused by *Salmonella* and *Shigella* species, or *Escherichia coli* O157:H7 [36]. It is well known that such infections are fatal in children and elderly. Therefore,

the use of natural antimicrobial agents to combat pathogens is important issue. Because, there are no toxic effects of the plant extracts. MIC values of the essential oil against *E. coli* 0157:H7 and *C. jejuni* ATCC 33291 were 850 µg/ml and 875 µg/ml, respectively. MIC and MLC values of essential oil against *P. aeruginosa* were also determined as 150 µg/ml and 300 µg/ml. Also, *S. aureus* was another pathogen species for public health in our study and it was found that the essential oil has strong antibacterial activity against *Staphylococcus aureus* ATCC 33862 (26 mm) and *S. aureus* ATCC 25923 (29 mm). Essential oil was active against *Candida tropicalis* and *C. albicans*. It was clear from Table 1 and 2 that essential oil of *T. argenteum* subsp. *canum* was not only active against bacteria but it was also effective against yeast species tested in present study. It is considered from this result that this oil is a broad-spectrum.

Table 2. Values of MIC and MLC of *Tanacetum argenteum* subsp. *canum* var. *canum* essential oil

Tested bacteria	MIC (µg/ml)	MLC (µg/ml)	Antibiotics	
			Meropenem (µg/ml)	Nystatin (µg/ml)
Enterobacter cloacae ATCC 28355	350	875≥	62.5	NT
Pseudomonas fluorescens ATCC 55241	350	875≥	40.0	NT
Pseudomonas aeruginosa ATCC 27853	150	300≥	40.0	NT
Shigella sonnei RSKK 8177	250	750≥	40.0	NT
Escherichia coli ATCC 25922	150	325≥	62.5	NT
E. coli O157:H7	850	1000>	62.5	NT
Yersinia enterocolitica RSKK 1501	850	1000>	62.5	NT
Campylobacter jejuni ATCC 33291	875	1000>	40.0	NT
Klebsiella pneumoniae ATCC 27736	850	1000>	40.0	NT
Salmonella enteritidis RSKK 171	900	1000>	62.5	NT
Staphylococcus aureus ATCC 33862	175	300≥	125.0	NT
Staphylococcus aureus ATCC 25923	175	300≥	125.0	NT
Micrococcus luteus NRLL-B-4375	150	300≥	175.0	NT
Enterococcus faecalis ATCC 19433	150	300≥	125.0	NT
Bacillus cereus NRRL-B-3711	62.5	125≥	125.0	NT
Bacillus cereus RSKK 867	62.5	125≥	125.0	NT
Candida albicans ATCC 10231	200	450≥	NT	100
Candida tropicalis (clinic isolate)	225	425≥	NT	125

MLC: minimal lethal concentration; MIC: minimal inhibition concentration; NT: Not tested

Table 3. Antibiofilm effect of *Tanacetum* essential oil (%)

Bacteria	% Inhibition		
	½ MIC	¼ MIC	1/8 MIC
Enterobacter cloacae TCC 28355	32.92	29.30	18.40
Micrococcus luteus NRRL-B 4375	24.10	18.65	12.00
Pseudomonas fluorescens ATCC 55241	15.62	8.16	-
Yersinia enterocolitica RSKK 1501	12.54	8.12	6.02
Salmonella enteritidis RSKK 171	10.26	-	-

-: no inhibition;

Particularly, searching of new drugs against to resistant microorganisms and/or biofilm forming microorganisms has received much attention. Because, treatment of chronic infections related with biofilm are very difficult as biofilms are exceptionally resistant to antibiotics and host immune response [37]. Therefore, natural products have also been screened for their antibiofilm activity. In this study, *T. argenteum* subsp. *canum* oil was also studied for its antibiofilm activity. The antibiofilm effect of the oil was tested at the ratio of 1/2, 1/4 and 1/8 MICs. It was found that, the oil exhibited moderate antibiofilm activity on *E. cloacae* ATCC 28355 (32.92, 29.30 and 18.40% for ½ MIC, ¼ MIC and 1/8 MIC, respectively) and less antibiofilm activity on *S. enteritidis* RSKK 171 (maximum 10.26%), *P. fluorescens* ATCC 55241 (maximum 15.62%) and *Y. enterocolitica* RSKK 1501 (maximum 12.54%). Moreover, any antibiofilm activity could not be determined on other bacteria and candida species (Table 3).

4. Conclusion

In conclusion, essential oil of *T. argenteum* subsp. *canum* var. *canum* showed antimicrobial and antibiofilm activity various pathogens. In the literature, there are many works about the biologic and pharmacologic activity and content of essential oils *Tanacetum* species. But, there was no published report about the antimicrobial or antibiofilm effect of *T. argenteum* subsp. *canum* var. *canum*. Since, to the best of our knowledge, no or limited data is available about antimicrobial and antibiofilm activity of this essential oil, our aim was to investigate its effect on some pathogen bacteria and yeasts. The results of the present study have confirmed our hypothesis that this essential oil has the antimicrobial and antibiofilm effect on viability of tested pathogens. Last of all, present study will be the first scientific research to provide data that the antimicrobial and antibiofilm activity of this essential oil possesses on some pathogen organisms.

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Biodiversity of Bacteria Isolated from Home-Made Wine and Vinegar

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Abstract: Wine is an alcoholic beverage made grapes fermented without the addition of sugars, acids, enzymes, water. It has been consumed by human beings in religious ceremonies since ancient times. Vinegar is sour juice that is used as a sweetener in meals, in salads, or as a preservative such as brine. It has a great variety of industrial, medical, and domestic uses are still commonly practiced today. The aim of this study was to determine the bacterial biodiversity of home-made wine and vinegar using classic and molecular methods. Morphological, cultural and biochemical identifications were made according to the Bergey's Manual of Systematic Bacteriology. For molecular identification 16S rDNA-PCR method was used. PCR results of these samples were send to the sequencing. BLASTn software was used to match our sequences with the ones in GenBank. In this study, bacteria colonies were isolated from home-made wine and vinegar. According to molecular results acetic acid and lactic acid bacteria were found.

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1. Introduction

Biodiversity is the foundation of ecosystem to which well-being of all living things is dependent variety of the living beings that exist known as biodiversity. It is one of the basic components of nature and it ensures the survival of earth by all means. Biodiversity relies upon the climatic conditions and areal parts of the district [1].

Vinegar is expended worldwide as a sustenance sauce and additive. It is the oldest preservative of vegetables, meat and fish [2]. Fermentation of the wine and vinegar is a spontaneous microbiological process [3]. There are different techniques to produce. The customary advances depend at first glance microbiota that is immobilized on various help materials, (for example, beech-wood shavings) or it is drifting on the surface of ethanol containing substrates [4]. Winemaking is older than the recorded history and the development of this technology begins nearly 7000 years ago. Different organisms found on the surface of grape skins and the indigenous microbiota related with winery surfaces take an interest in these regular wine maturation [5].

The conversion rate of liquid-state fermentation, the speed of acid production, and the flavor of vinegar are all dependent on the quality of the acetic acid bacterial strains, the selection

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and culture of excellent acetic acid bacteria have attracted much attention from scholars [6]. The aim of this study was to determine the bacterial biodiversity of home-made wine and vinegar using molecular methods.

2. Material and Methods

2.1. Sample collection and bacterial isolation

Home-made wine and vinegar samples were collected aseptically from the villages of Aydın. Bacterial growth was realized on HS (Hestrin-Schramm) Agar at 30ºC for 72 h. After incubation, each different colony were isolated and stocked in skim milk [7].

2.2. Identification of microorganisms

Morphological, cultural and biochemical identifications were made according to the Bergey's Manual of Systematic Bacteriology [8]. For molecular identification, DNA isolation of the samples were made according to De Boer and Ward (1995) [9]. After isolations DNA concentration and purity was measured with nanodrop spectrometer (Thermo Scientific). Their purity were between the values of 1.73 and 2.20. For PCR 16S universal rDNA primers were used (27F: 5'- AGA GTT TGA TCM TGG CTC AG-3', 1492R: 5'- CGG TTA CCT TGT TAC GAC TT-3'). 16S rRNA PCR reactions were carried out at initial denaturation 95ºC 5 min, denaturation 94ºC 40 sec, annealing 50ºC 40 sec, extension 72ºC 40 sec with 35 cycles and a final extension at 72ºC 10 mins. Reagents concentrations were 10X Taq Buffer, 0.5M dNTP mix, 10 pM from each primer, 7.5 mM $MgCl_2$ and 1U Taq polymerase with the final volume of 25 µl. PCR products were sent to the sequencing (GATC BioTech, Germany) after electrophoresis at 1.4% agarose gel at 90 V 40 min.

3. Results and Discussion

3.1. Morphological and Biochemical Identification

Morphological and biochemical tests were done according to Bergey's Manual of Systematic Bacteriology [8]. Results were shown in Table 1.

Table 1. Classical identification of bacteria from wine and vinegar samples.

Sample Number	Gram Staining	Cell Shape	G	L	S	M	NR	SH	H_2S	C	GH
1	_	Rod	_	_	_	_	+	_	+	+	+
2	_	Rod	_	_	_	_	+	_	+	+	+
3	_	Rod	_	_	_	_	+	_	+	+	+
4	_	Rod	_	_	_	_	+	_	+	+	+
5	_	Rod	_	_	_	_	+	_	+	+	+
6	_	Rod	_	_	_	_	+	_	+	+	+
7	_	Rod	_	_	_	_	+	_	+	+	+
8	_	Rod	_	_	_	_	+	_	+	+	+
9	_	Rod	_	_	_	_	+	_	+	+	+
10	_	Rod	_	_	_	_	+	_	+	+	+
11	_	Rod	_	_	_	_	+	_	+	+	+
12	_	Rod	_	_	_	_	+	_	+	+	+
13	_	Rod	_	_	_	_	+	_	+	+	+
14	+	Rod	+	+	+	_	+	_	+	_	+
15	+	Coc	_	+	+	+	+	_	+	_	+
16	+	Rod	+	+	+	_	+	_	+	_	+

G: Glycerol, L: Lactose, S: Sucrose, M: Mannitol C: Citrate, NR: Nitrate Reduction, SH: Starch Hydrolyse, GH: Gelatine Hydrolyse

3.2. Molecular identification

PCR results of these samples (Figure 1) were send to the sequencing (GATC BioTech, Germany). Molecular identification was made by comparing sequence results with Genebank using BLASTn software. Our analysis showed that there 26 different strains and 16 different species (Table 2). MEGA 6 software was used for evolutionary analysis. Maximum likelihood method was used to infer evolutionary history. Maximum likelihood tree was shown in Figure 2.

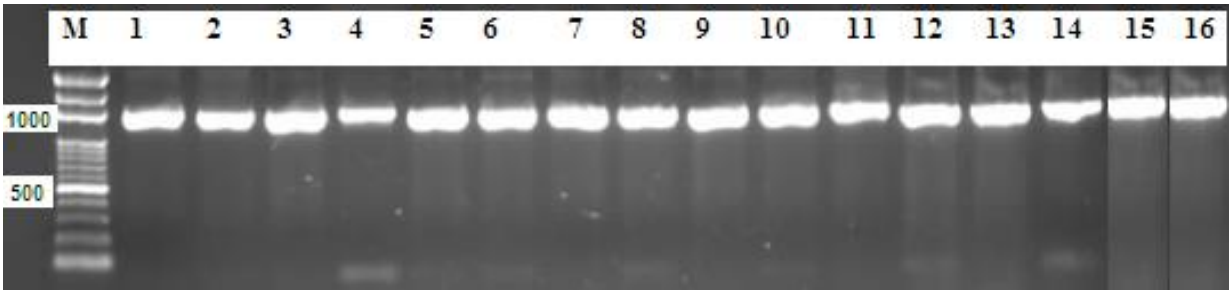

Figure 1. 16S rDNA PCR results of the samples (M: Marker (100bp), 1-16: Samples

Table 2. Molecular identification of the species from wine and vinegar samples.

No	Name	Number of Strains	Accession No
1	*Komagataeibacter saccharivorans* strain MI5SAII	1	KY287776.1
2	*Gluconacetobacter europaeus*	1	FN429075.1
3	*Komagataeibacter saccharivorans* strain JCM 25121	6	NR_113398.1
4	*Gluconacetobacter saccharivorans*	2	AB759966.1
5	*Gluconacetobacter xylinus* strain 1-5	4	KF030727.1
6	*Gluconobacter oxydans* strain A292	1	DQ523497.1
7	*Gluconacetobacter europaeus*	2	FN429075.1
8	*Acetobacteraceae bacterium* J2	1	GU213109.1
9	*Komagataeibacter xylinus* strain ATCC 53524	1	KX216689.1
10	*Acetobacter pasteurianus* strain NH6	1	KR150441.1
11	*Gluconacetobacter hansenii*	1	KF155166.1
12	*Acetobacter ghanensis* strain CIFT MFB 15295 HSA15	1	KP240986.1
13	*Acetobacter pasteurianus* strain L1	1	MF179549.1
14	*Leuconostoc mesenteroides* strain 10-7	1	KJ477420.1
15	*Lactococcus lactis strain* RCB462	1	KT260674.1
16	*Lactobacillus brevis strain* JNB23	1	JQ741972.1

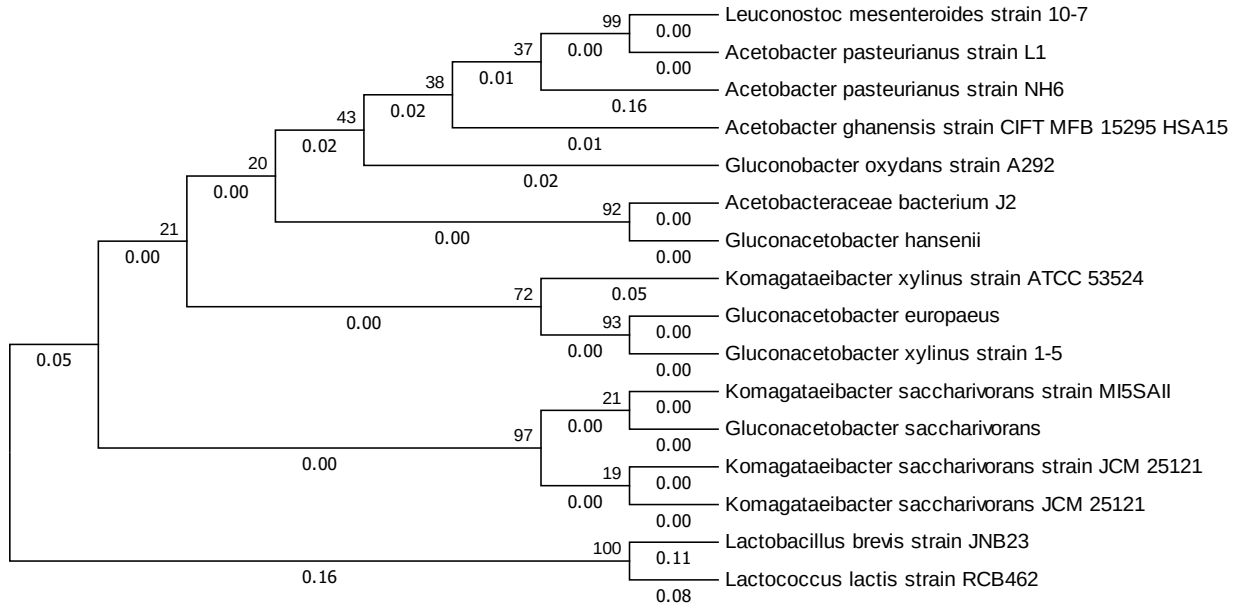

Figure 2. Molecular Phylogenetic analysis by Maximum Likelihood method

The evolutionary history was inferred by using the Maximum Likelihood method based on the Jukes-Cantor model. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log-likelihood value. Evolutionary analyses were conducted in MEGA6.

4. Discussion

It can be seen that different strains of same species grouped together except one *A. pasterianus* strain. This can be caused because of a polymorphism earlier in the branch. *Gluconobacter* and *Acetobacter* genuses grouped separetly while each of their species grouped together as expected. Ruiz et al (2010) studied bacterial biodiversity of Tempranillo wines and found *Oenococcus oeni* was dominant species. Besides *Oenococcus oeni; Gluconobacter oxydans*, *Asaia siamensis*, *Serratia* sp., and *Enterobacter* sp. was also observed [10]. Torija et al. (2010) studied to develop a Real-Time PCR assay for acetic acid bacteria in wine and vinegar using Taqman minor grove binder probes [11]. Sharafi et al. (2010) studied characterization and optimisation indigenous acetic acid bacteria and isolated thirty-seven acetic acid bacteria from *Acetobacter* and *Gluconobacter* members and observed *Acetobacter pasteurianus* was dominant species [12].

Wu et al. (2010) studied diversity of Acetobacter pasteurianus strains from cereal vinegars and isolated 21 strains with 16S-PCR method [13]. Kommanee et al. (2012) studied the restriction analysis of 16S-23S rRNA gene internal transcribed spacer regions (ITS) using TaqI, AluI, HpaII, and AvaII revealed that forty-seven bacterial isolates found in fruits and flowers collected in Thailand belong to the genus Acetobacter [14]. Wu et al. (2012) studied biodiversity of yeast and lactic acid bacteria of traditional Chinese vinegar and found 47 yeast isolates, 28 lactic acid bacteria isolates and 58 acetic acid bacteria isolates [15]. Trček et al. (1997) and Hidalgo et al. (2012) suggested in a vinegar with high percentage of acetic acid (>6%) the dominant species are *Komagataeibacter europaeus*, *Komagataeibacter oboediens* and/or *Komagataeibacter intermedius*, whereas in vinegar with low percentage of acetic acid (<6%), the dominant species are *Acetobacter aceti*, *Acetobacter pasteurianus* and/or *Acetobacter pomorum* [16, 17]. Petri et al. (2013) studied wine related lactic acid bacteria with

multiplex PCR method and found 13 different species [18]. Wang et al. (2015) investigated Acetobacter bacteria in Zhenjiang vinegar and found six significant acetic acid bacteria strains which two of them was dominant, *Acetobacter aceti* and *A. pasteurianus* [19]. Štornik et al. (2016) studied cultivable acetic acid bacteria from apple cider vinegar and observed 96 bacteria from organic and 72 bacteria from conventional apple cider vinegar using 16S and 23S rRNA restriction analysis [20]. Treck et al. (2016) collected unfiltered vinegar samples and microbial analyses carried out by Illumina MiSeq sequencing of 16S rRNA gene variable regions. They showed that in all wine vinegar samples *Komagataeibacter oboediens* (formerly *Gluconacetobacter oboediens*) was a predominating species and the acetic acid and lactic acid bacteria were two major groups of bacteria in apple cider vinegar [21]. It is possible to find both acetic acid and lactic acid bacteria in fermentative products. Lactic acid bacteria include the genera of *Leuconostoc* sp., *Lactococcus* sp., *Lactobacillus* sp., *Enterococcus* sp., *Pediococcus* sp., *Bifidobacterium* sp., *Aerococcus* sp. [22]. The acetic acid bacteria and lactic acid bacteria can easily be mixed in terms of their morphological and biochemical properties. Therefore, the diversity of these bacteria can be demonstrated more safely using molecular techniques including inter-delta/PCR, PCR-RFLP, ERIC/PCR analysis, as well as 16S rRNA and 26S rRNA partial gene sequencing [23].

5. Conclusion

In this study, bacteria were isolated from home-made wine and vinegar. We determined *Komagataeibacter saccharivorans*, *Gluconacetobacter europaeus*, *Gluconacetobacter saccharivorans*, *Gluconacetobacter xylinus*, *Gluconobacter oxydans*, *Acetobacteraceae bacterium*, *Acetobacter pasteurianus*, *Komagataeibacter xylinus*, *Gluconacetobacter hansenii* and *Acetobacter ghanensis* as acetic acid bacteria. In additional *Leuconostoc mesenteroides*, *Lactococcus lactis* and *Lactobacillus brevis* as lactic acid bacteria were identified. The acetic acid bacteria and lactic acid bacteria have important roles in food and beverage production, in the bioproduction of industrial chemicals, to improve the preservation, nutritional value, and sensorial characteristics of a variety of fermented foods and products derived from animal and vegetable origins.

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The Effects of Medicinal Plants on Cancer Cell Lines and Efficacy of Experimental Animal Model

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Abstract: The use of medicinal plants as an alternative treatment is a historical process and has been known for a long time so that classical treatment can be more effective in wound and cancer treatment. The purpose of this study was to investigate the effects of plant extracts used for therapeutic purposes in cancer cell lines in vitro wound model and in vivo experimental animal model in order to obtain this information. The plants, olive oil (Oleocanthal), mistletoe (*Viscum album*), Common Centaury (*Centaurium erythraea*), *Momordica charantia, Inula viscosa, Citrus aurantium*, Thyme oil (Thymus vulgaris) and algae (*Jania longifurca*), were used. MCF-7, MB-MDA-231, 67NR and 4T1 for breast, NB2a for neuron, L929 for fibroblast and normal somatic mesenchymal stem cell for comparison were selected for in vitro wound models. As an in vivo breast cancer model, female Balb/c mice were injected with 4T1 cells and skin wound healing in rats was investigated. The effects of medicinal plants were evaluated using MTT assay for viability and proliferation, immunocytochemistry staining NOS for oxidative stress and TGFbeta1 for wound healing. It was found that plant extracts reduced antioxidative damage and inhibited apoptosis. It was observed that oxidative stress and apoptosis were increased in cancer cells, but less effective in invasive cell lines. In vivo experiments showed that wound healing was accelerated and that these rates were achieved with antioxidative and antiapoptotic effects. It has been concluded that medicinal plants are beneficial for treatment of difficult diseases in which patient quality of life is very effective and they should be used as scientific-based medical applications.

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1. Introduction

Nowadays, breast cancer has become an increasingly important health problem and it is the second cause of cancer related deaths after lung cancer among women [1]. Early diagnosis and subsequent treatment are difficult and can result in death. Tumor cells with uncontrolled proliferation accumulate to form masses, furthermore tumors can squeeze normal tissues,

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infiltrate into them, or destroy them. If the cancer cells are separated from the tumor, they can go to other parts of the body through the blood or lymphatic circulation. In places where they grow, they form tumor colonies and continue to grow. The spread of cancer to other parts of the body in this way is called metastasis [2, 3].

Cancers are classified according to their appearance under the organ and the microscope. Different types of cancer grow at different rates, show different spreading patterns and respond to different treatments. For this reason, in the treatment of cancer patients, different types of treatment are applied according to the existing cancer type. Chemotherapy as well as alternative treatment options are also used in the treatment of cancer [4]. Especially in recent years plant extracts have been started to be used under the name of phytotherapy. In addition, there are plants that are widey used by the population and stil subject to experimental and clinical trials such as olive oil (oleocanthal), mistletoe (*Viscum album*), *Centaurium erythraea*, *Momordica charantia, Inula viscosa, Citrus aurantium*, thyme oil (*Thymus vulgaris*) and algae (*Jania longifurca*) [5-9]. Because Oleocanthal is a phenylethanoid, a type of natural phenolic compound, has antiinflammatory and anticancer properties and several studies have demosrated that it can promote apoptosis in tumor cells [10, 11]. On the other hand, it has been found that it can be used as an antioxidant. *V. album, C. erythraea, M. charantia, I. viscosa, C. aurantium*, Thyme oil (*T. vulgaris*) *J. longifurca* are very rare in the literature [12-14].

Additionally, the plants extracts are also used for wound healing. Wound healing is one of the unsolved problems of medicine nowadays and it needs to be improved especially in cases of poor recovery and salvation from diabetes. Herbal therapies are a popular product of our time and have been started to be applied to humans in current medical treatment.

Nitric oxide (NO) is catalyzed by the enzyme nitric oxide synthase (NOS) and has an important role in the cellular metabolism. There are three different NOS in mammals that synthesize NO: endothelial NOS, inducible NOS and neuronal NOS. The increase of NO levels into the cells, caused oxidative stres and cell death, also wound healing is delayed. Because of its short half-life, to detect NO is difficult, so levels of NOS are investigated [15, 16].

Transforming growth factor (TGFbeta) beta super family is involved in cell proliferation and differentiation. TGFbeta isoforms (TGFbeta1, 2, 3, 4 and 5) are critical for vascular diseases and wound healing process. Especially, TGFbeta 1 and 2 are released in response to the tissue injury and regulate cell proliferation, differentiation, and migration during wound healing [17].

The aims of our study were to detect the cytotoxic effects of oleocanthal, *V. album*, *C. erythraea*, *M. charantia*, *I. viscosa*, *C. aurantium*, Thyme oil (*T. vulgaris*), *J. longifurca* on MCF-7, MB-MDA-231, 67NR and 4T1 breast carcinoma cell lines, NB2a neuroblastoma cell line, L929 fibroblast cell line and to determine the effects of *V. album* extract on in vivo-breast cancer model and skin wound healing.

2. Material and Methods

67NR and 4T1 breast cancer cells were seeded in DMEM-F12, NB2a neuroblastoma and L929 fibroblast cell line were cultured in DMEM medium, MCF-7 and MB-MDA-231 breast cancer cells were maintained in RPMI-1640 medium at 37^0C and 5% CO2 incubator. Adipose tissue derived mesenchymal stem cells (MSC) were isolated from rat fat tissue and seeded in alpha-MEM medium. Also MSCs were differentiated into neurons (N-MSC) by administration of serum-free medium plus 20 µl combinations of epidermal growth factor (EGF) and fibroblast growth factor, neuronal differentiation was performed using a Mouse Rat Neural Stem cell Functional İdentification Kit (SC013; RD Systems, Minneapolis, MN). For MTT ((3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) (M6494, Invitrogen) cytotoxicity analysis, all cells were seeded in 96-well plates (45.000 cells/well in 250 µL medium) for 24 hours and then plant extracts at different dilutions (0, 0.1, 1, 10, 100 µgr/ml) were applied for

24 hours. Subsequently, the medium containing extracts was removed and 100 µL of fresh medium and 10 µL MTT (5 mg/ml in distiled water) were added into each well and plate was incubated at 37^0C for 4 hours. Later, medium containing MTT was decanted and the cells were exposed to the dimethyl sulphoxide (DMSO, A3672, AppliChem, Darmstadt, Germany) (100 µL DMSO/well) for 10 min. The absorbance was measured at a wavelenght of 570 nm using an UV visible spectrophotometer (ELx800UV, BioTek) and IC_{50} dose for each plant extract was calculated using GraphPad InStat 3.0 statistical software [18].

The effects of all extracts were investigated at IC50 dose in vitro wound model. For this purpose, all cell lines were seeded in 24 well plates (15.000 cells/well in 500 µL medium) and a wound model was created by a pipette tip in (+) plus at the confluent phase [19]. The effect of extracts on wound healing at IC_{50} dose was investigated by immunocytochemical analysis with eNOS, iNOS and TGFbeta1.

The effects of thyme oil (*T. vulgaris*) in the experimental skin wound healing model and the effect of *V. album* in the experimental breast cancer model were evaluated. The experimental skin wound healing was created by aseptic conditions in the Wistar albino rats where the back region was shaved to remove the skin 1.5x1.5 cm^2 and rat were divided in three groups, control (no application, sham (topical application of salin buffer) and thyme oil (topical application) [20]. The wound closure process was morphologically assessed by topical application of thyme oil daily. For experimental breast cancer, $1x10^5$ 4T1 breast cancer cells were injected orthotopically into the right breast in 50 µL of medium to Balb / c female rats [21]. From the tenth day, intratumoral injection of 50 µL of the extract of *V. album* (15 mg / kg) was injected every day for 15 days while tumor formation was initiated. During the experiment, the tumor size was measured using a ruler every two days by formula: Tumor size = (length x $width^2$)/2 [22]. At the end of the experiment, the data about the size of tumor tissue were evaluated by one-way ANOVA analysis using GraphPad InStat 3.0 statistical software.

3. Results

All plant extracts were found to have toxic effects on cancer cells except for mesenchymal stem cells. Following MTT analysis, the % cell survival and IC_{50} doses of all extracts were shown in Figure 1 and 2.

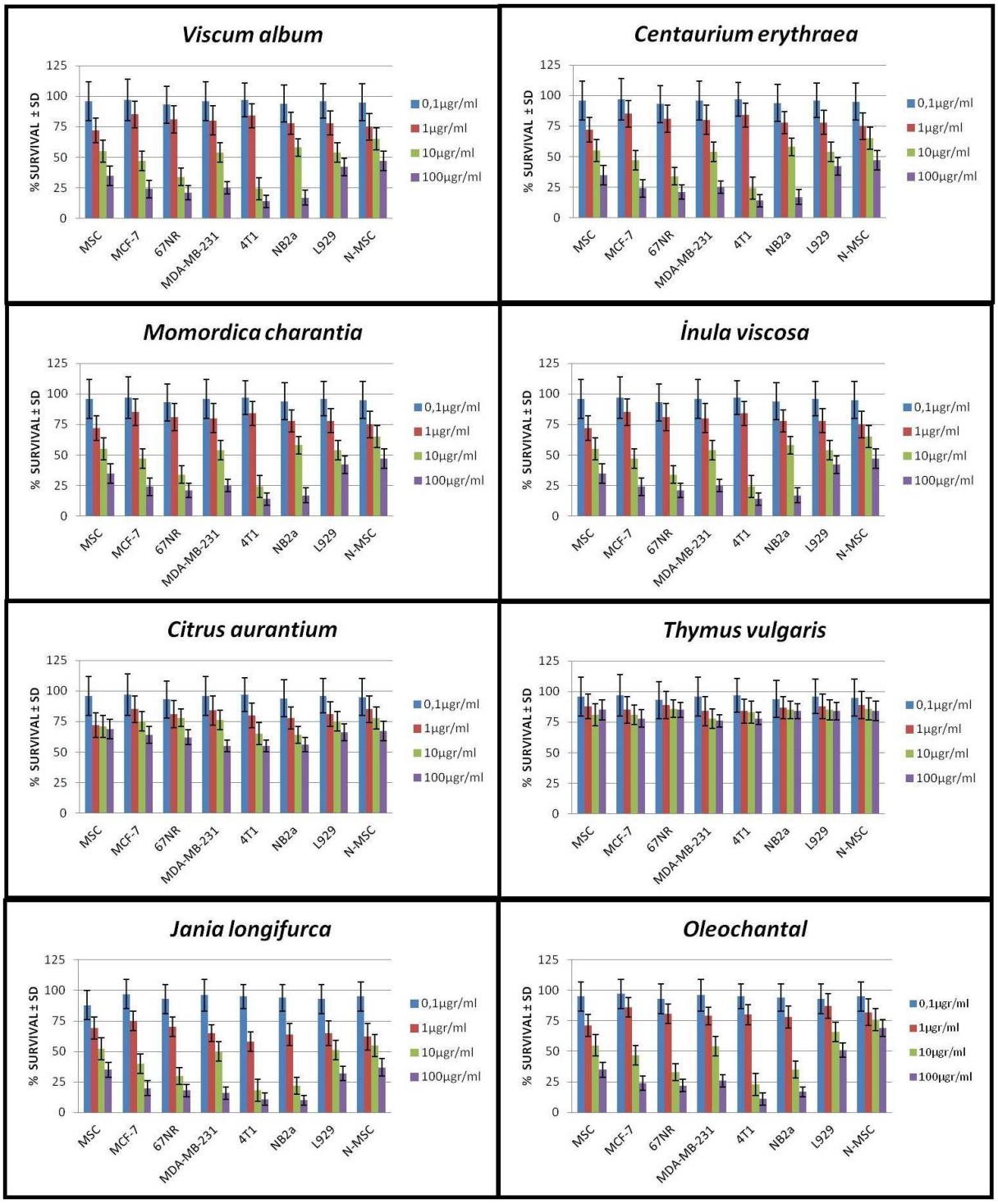

Figure 1. The % survival of all cells for each exracts.

IC_{50} Doses	MSC	MCF-7	67NR	MDA-MB-231	4T1	NB2a	L929
Oleochanthal	8.75 µgr/ml	7.25 µgr/ml	6.00 µgr/ml	8.00 µgr/ml	5.25 µgr/ml	6.00 µgr/ml	9.00 µgr/ml
V. album	8.50 µgr/ml	7.65 µgr/ml	6.25 µgr/ml	8.25 µgr/ml	6.00 µgr/ml	8.25 µgr/ml	8.00 µgr/ml
C. erythraea	8.75 µgr/ml	7.50 µgr/ml	6.00 µgr/ml	8.00 µgr/ml	5.75 µgr/ml	7.55 µgr/ml	8.25 µgr/ml
M. charantia	8.50 µgr/ml	7.25 µgr/ml	5.75 µgr/ml	7.50 µgr/ml	5.25 µgr/ml	7.50 µgr/ml	8.25 µgr/ml
I. viscosa	8.55 µgr/ml	7.15 µgr/ml	5.65 µgr/ml	6.65 µgr/ml	5.00 µgr/ml	7.25 µgr/ml	8.50 µgr/ml
C. aurantium	11.75 µgr/ml	10.55 µgr/ml	10.25 µgr/ml	10.00 µgr/ml	9.65 µgr/ml	9.25 µgr/ml	10.50 µgr/ml
T. vulgaris	12.25 µgr/ml	11. 25 µgr/ml	10.75 µgr/ml	10.25 µgr/ml	10.25 µgr/ml	10.50 µgr/ml	11.00 µgr/ml

Figure 2. IC_{50} doses of all extracts for each cells.

When the effects of extracts in the in vitro wound model were examined by inverted microscopy, it was found that all of the wound healing delayed the wound closure but the rate of wound healing was different in each cells. When NOS and TGFbeta1 staining were examined, there was an increase of NOS and TGFbeta1 stainings in cancer cells compared to mesenchymal stem cells (Figure 3).

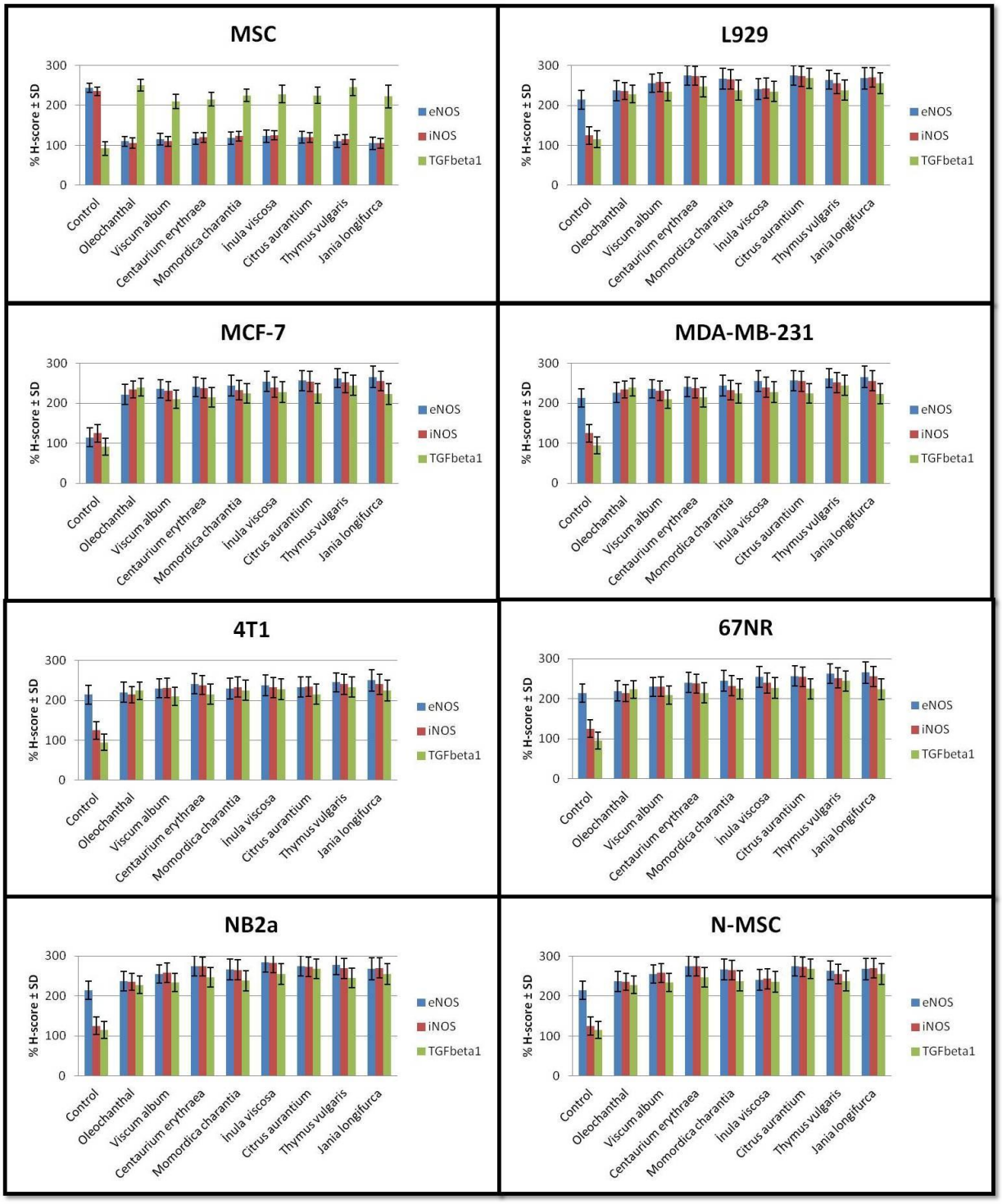

Figure 3. Immunocytochemical staining of eNOS, iNOS and TGFbeta1 in all cells.

In this study, to assess the effects of thyme oil in the experimental wound model, wounds were measured with a ruler on the 1st, 7th and 9th days. Analyses of the measurements revealed that healing in the wound area was achieved in all groups, and healing in the control groups was slower than in the application groups. The most effective and significant ($p < 0.05$) healing was found in the Thyme oil applied animals (Figure 4).

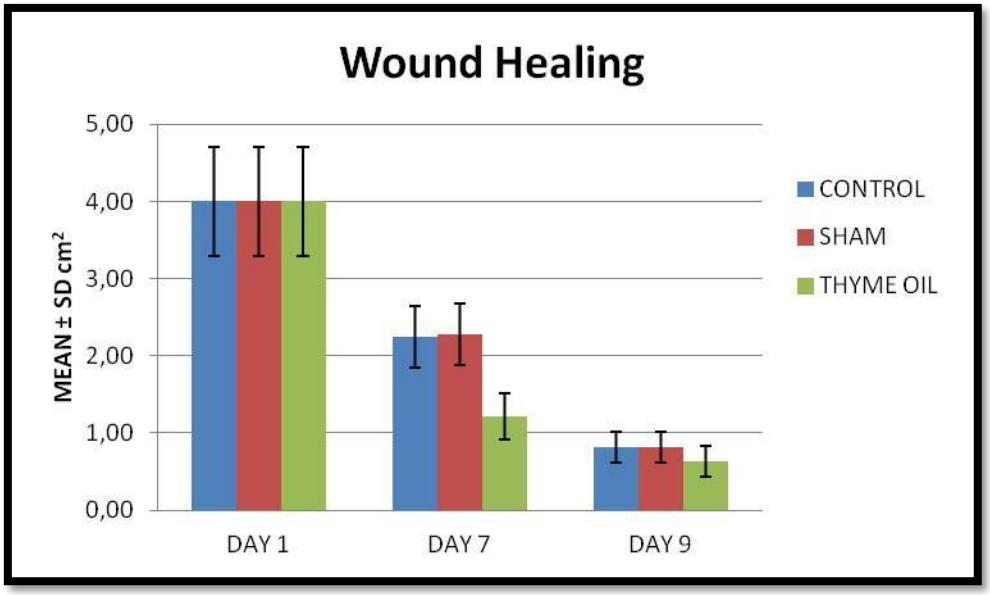

Figure 4. Wound healing with tyme oil in rat skin.

In the breast cancer model, increased oxidative stres markers, eNOS and iNOS, were observed in adenocarcinomas induced by 4T1 cells and the antiproliferative effected tumor growth rate slowed down. Only the neurotoxic effect of *J. longifurca* extract was shown and this was demonstrated in an experimental animal model in which it had serious clinical consequences (Figure 5).

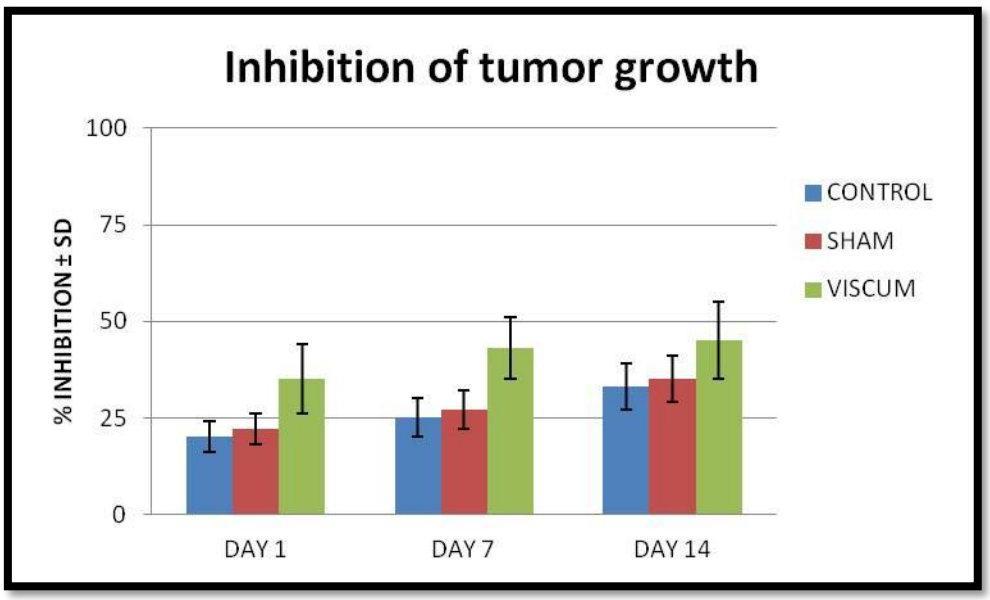

Figure 5. Inhibition of tumor growth after the application of *Viscum album* extract.

4. Discussion

In our study, the effects of oleocanthal, *Viscum album*, *Centaurium erythraea*, *Momordica charantia*, *Inula viscosa*, *Citrus aurantium*, Thyme oil (*Thymus vulgaris*) *Jania longifurca* on MCF-7, MB-MDA-231, 67NR and 4T1 breast carcinoma cell lines, NB2a neuroblastoma cell line, L929 fibroblast cell line were investigated. In addition, in vivo

experiments, anticancerogenic effects of *V. album* extract in breast cancer model and skin wound healing model were examined. All medical plants were found useful for both cancer cells and the cells in wound. They may induce oxidative stress and cell death for cancer cells in culture and they have antioxidant and antiapoptotic effects in wound healing.

Oleocanthal contains phenolic compounds and has anti-inflammatory properties. It has been reported that oleocanthal has cytotoxic effects on human melanoma cells. The cytotoxic effect was supported with the inhibition of c-Met activity, ERK1/2 and AKT phosphorylation and downregulation of Bcl-2 expression [23]. The pentacyclic triterpenes form *V. album* exhibit immunomodulatory, antitumor, and wound healing activity. Especially, compounds of lectins and viscotoxins of *V. album* has anticancer effects via apoptosis [24]. *C. erythraea* is used in traditional medicine because of antidiabetic, digestive, antipyretic and antiflatulent effects. *C. erythrea* extract was a protective effect on diabetic rats by reducing oxidative stres and pancreatic β-cells' damage [12]. *M. charantia* is widely used in Southeast Asia and Indo-China. It has been stated that it has anticancer, antidepressant, antidiabetic, anti-inflammatory, antimicrobial, antiobesity, antioxidant, and antiulcer properties via phytochemical components include alkaloids, charantin, flavonoids, glycosides, phenolics, tannins, and terpenoids. It triggers AMP-activated protein kinase (AMPK) and CaMKK (Ca(2+)/calmodulin-dependent protein kinase) and mTOR/p70S6K and/or the AKT/ERK/FOXM1 (Forkhead Box M1) signaling was suppressed in tumor cells. Also anticancer properties occured by activation of apoptosis signaling of caspases and mitochondria [25]. These observations are similar to our results.

The genus Inula grows in the regions of Europe and Asia. This genus is used as herbal products expectorants, antitussives, diaphoretics, antiemetics, and bactericides. The extract of *I. viscosa* L. were studied and it has been shown that it inhibited the cell growth in HeLa and SiHa cells in a dose-dependent manner. The anticancer and antimicrobial properties were occured in the presence of methylated quercetins isolated from *I. Viscosa* [26]. Another anticancer agent is genus Citrus which contain flavonoids [27]. *C. aurantifolia* from genus Citrus is widely used because of its antibacterial, anticancer, antidiabetic, antifungal, anti-hypertensive, anti-inflammation, anti-lipidemia, and antioxidant properties. The anticancer effect of *C. aurantifolia* is occurs by inhibiting metastasis and angiogenesis signal pathways [28]. Nar isolated from *T. vulgaris* was shown to trigger apoptotic cell death via cell cycle arrest at S- and G2/M-phases in human colorectal and breast cancer cells. Also it caused the down-regulation of apoptosis and cell-cycle regulatory genes such as Cdk4, Cdk6, Cdk7, Bcl2, x-IAP and c-IAP-2. *T. vulgaris* inhibited the migration and invasion of HCT116 cells [29, 30]. In our experiments, the oil of *T. vulgaris* has antiproliferative effect on cancer cells by oxidative stres.

The research about the *J. longifurca* has shown that extracts of *J. longifurca* were toxic in MCF-7 cells and caused apoptosis and inhibition of proliferation [31]. In our study, we determined the toxic effect of *J. longifurca* in breast cancer cell lines, and also in neuroblastoma cells.

The use of medicinal herbs both in preventive medicine and in cases where treatment is difficult is an application that must be done as a medical procedure based on scientific data. Investigation of these effects using current techniques is very important to prevent harmful effects of herbal products after unconscious use.

5. Conclusion

In this study, the mechanisms by which the beneficial effects of the herbal plants used among the people are revealed and influenced. The neurotoxic algae extract proved to be very important in terms of showing the meaning of these scientific studies. The frequent use of algae, based on the beneficial effects of the vast majority of them, emphasized that this harmful effect

should be kept in mind. It was pointed out that herbal treatment practices may be meaningful in breast cancer treatment and should be studied in animal models, indicating that cancer patients may be an important alternative treatment product for the quality of life. It has been concluded that medicinal herbs are beneficial for treatment difficult diseases, where patient quality of life is highly affected, and they should be used as medicinal therapies.

Conflict of Interest

None declared.

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