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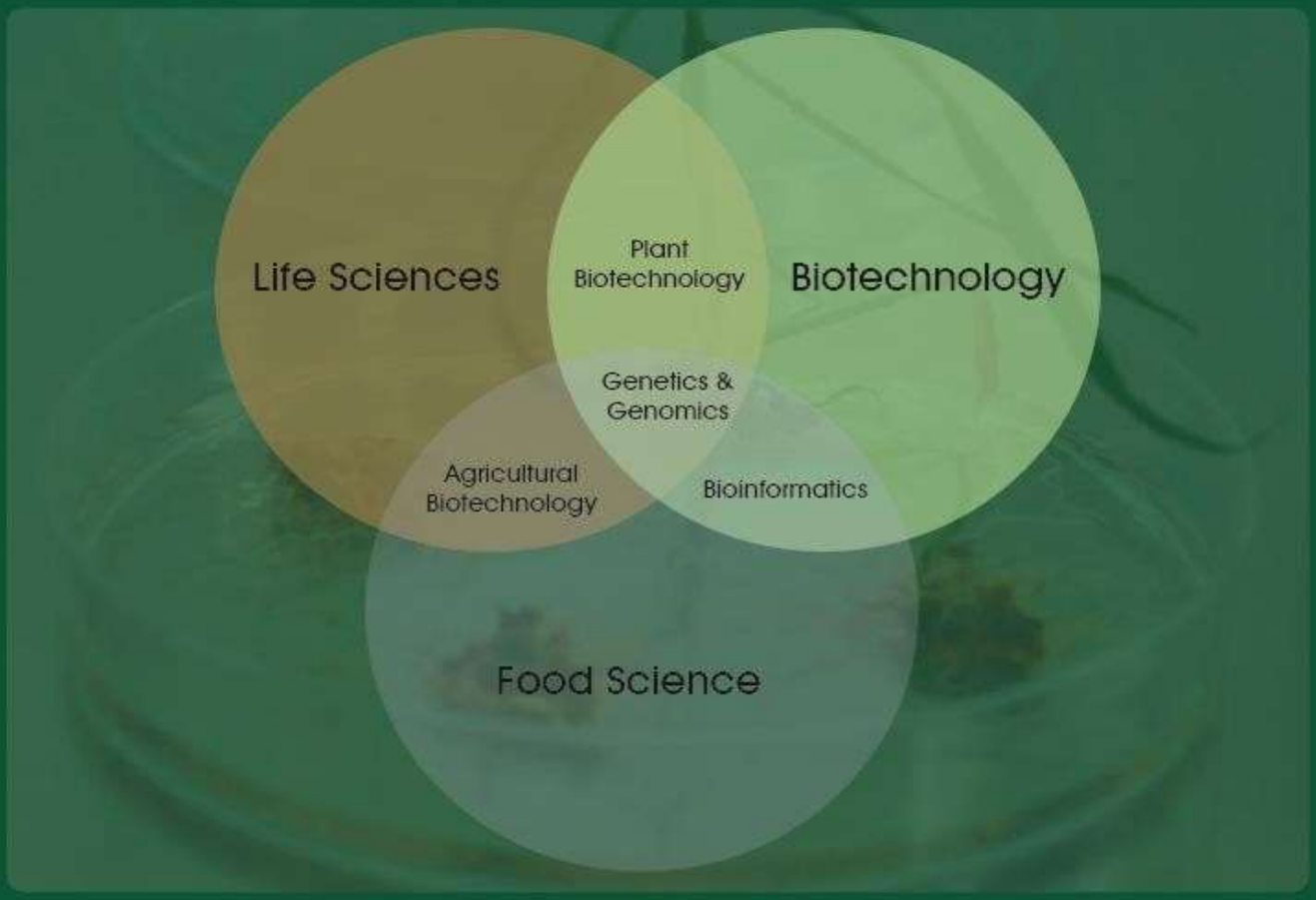

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Editörden;

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"International Journal of Life Sciences and Biotechnology" olarak dergimizin altıncı sayısını yayın hayatına sunmaktan mutluluk ve onur duyuyoruz. "International Journal of Life Sciences and Biotechnology" dergisi araştırma- geliştirme ve uygulama ilkeleri baz alınarak yayınlanan uluslararası hakemli açık erişimli akademik bir elektronik dergidir.

"International Journal of Life Sciences and Biotechnology" dergisi Yaşam Bilimleri, Biyoloji, Biyoteknoloji, Biyomühendislik, Ziraat Bilimleri, Gıda Biyoteknolojisi ve Genetik alanlarındaki ilgili araştırmacılara, kurum ve kuruluşlara teorik ve pratik uygulamalarda katkı sağlamayı, tarafsızlık ve bilim etiği ilkelerine bağlı kalarak çözüm temelli, yenilikçi ve katma değeri olan çalışmalara odaklanan, güncel ve geleceği tartışan çalışmaların yayınlanmasını hedeflemektedir.

Bu düşüncelerle altıncı sayısını yayınladığımız "International Journal of Life Sciences and Biotechnology" dergisini, makaleleri ile onurlandıran akademisyenlere, Fikir / Görüş / Öneri / Katkı ve Eleştirileri ile değerlendirme süreçlerine katkılarından dolayı hakem ve yayın kurullarında yer alan kıymetli bilim insanlarına yürekten teşekkür ediyoruz. Bir sonraki sayıda görüşmek ümidiyle…

15.04. 2020
Editör
Dr. Öğrt. Üyesi Yilmaz KAYA

From The Editor;

Dear Readers and Authors,

As “International Journal of Life Sciences and Biotechnology”, we are pleased and honored to present the fifth issue of the journal. "International Journal of Life Sciences and Biotechnology" is an international double peer-reviewed open access academic journal published on the basis of research-development and code of practice.

The aims of this journal are to contribute in theoretical and practical applications in relevant researchers of Life Sciences, Biology, Biotechnology, Bioengineering, Agricultural Sciences, Food Biotechnology and Genetics institutions and organizations in Turkey, and to publish solution based papers depending on the principle of impartiality and scientific ethics principles, focusing on innovative and added value work, discussing the current and future.

With these thoughts, We are especially thankful to academicians honoring with the articles, valuable scientists involved in editorial boards and reviewers for their contributions to the evaluation processes with through their opinions/ideas/contributions/criticisms in sixthy issue of "International Journal of Life Sciences and Biotechnology". Hope to see you in the next issue…

15.04.2020
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İçindekiler/ Contents

Research article

Ahmed, M.U., R.O. Arise, and I.Y. Sudi, Evaluation of the Antidiarrhoeal Activity of Aqueous Root and Stem Bark Extracts of Annona senegalensis. International Journal of Life Sciences and Biotechnology, 2020. 3(1): p. 1-17. DOI: 10.38001/ijlsb.656112

Evaluation of the Antidiarrhoeal Activity of Aqueous Root and Stem Bark Extracts of *Annona senegalensis*

Maryam Usman Ahmed[1]*, Rotimi Olusanya Arise[2], Ismaila Yada Sudi[1]

ABSTRACT

Diarrhoea is a leading cause of deaths amongst children below five years. *Annona senegalensis* plant is used traditionally to treat diarrhoea. This study aims at evaluating the antidiarrhoeal potency of the aqueous root and stem bark extracts of *Annona senegalensis*. Antimicrobial, antioxidant and antidiarrhoeal activity of aqueous extracts of the root bark (ARB), stem bark (ASB) and mixture of stem and root barks (AM) of *Annona senegalensis* were assessed. Stool inhibition, enteropooling, inhibition of gastro-intestinal motility and electrolyte secretion potential of the extracts were assessed in castor oil - induced diarrhoeic rats following administration of 100, 200 and 400 mg/kg body weight ASB, ARB and AM. The aqueous stem bark (ASB) had the greatest total antioxidant capacity, H_2O_2 scavenging activity and the ferric reducing assay power. The zone of inhibition of AM against *Escherichia coli*, *Bacillus aureus* and *Shigella dysentriae* was greater than 6 mm. All the extracts inhibited stooling by a percentage ranging from 17 – 24.7 but was significantly lower ($p < 0.05$) than the standard drug (30.0%). Inhibition of gastrointestinal tract transit was significantly increased by all the extracts but low dose (100 mg/kg b. wt. of ARB and ASB) significantly increased it the most. The weight and volume of intestinal fluid was significantly decreased by 100 mg/kg b. wt. ARB. The mixture (AM) exhibited synergistic antimicrobial effect. The aqueous stem bark exhibited good antioxidant property, antisecretory and pro-absorptive property while the root exhibited good antienteropooling activity. Isolation of bioactive compounds in the extracts should be carried out.

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Annona senegalensis, antidiarrhoeal, enteropooling, gastrointestinal transit, intestinal fluid

Introduction

Diarrhoea is an increase in the frequency, volume and water content of stool [1]. It is a leading cause of death amongst children below five years old, accounting for 9% of all deaths among children [2]. Globally, an estimate of 1.7 billion cases of diarrhoea diseases is recorded annually [3]. Diarrhoea can be classified as either infectious or non-infectious [4]. Causative agents in infectious diarrhoea include bacteria such as *Escherichia coli*,

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Shigella species, *Bacillus aureus*; virus e.g *rotavirus* and parasites e.g *Trichinella spiralis* [5, 6]. Diarrhoea caused by enteric infections is a major factor in morbidity and mortality [7]. It is widespread in developing countries due to lack of proper hygiene, good portable water and proper or inadequate sanitation facilities. Non-infectious diarrhoea may be as a result of reaction to some drugs, food allergy, toxins and acute inflammation [8], abnormality in gastrointestinal tract function and neurohumoral mechanisms [9]. It may also occur as a result of other diseases such as irritable bowel syndrome (IBS), ulcerative colitis, intestinal inflammation [1] and HIV/AIDS [10]. Infection, inflammation and intestinal irritation leads to excessive generation of reactive oxygen species which results in oxidative stress. Oxidative stress has been reported to be one of the causes of diarrhoea [11].

Plant extract provides opportunity for new drug discoveries [12]. Plants with antidiarrhoeal activity act by either inhibiting secretion or reducing gastrointestinal tract motility [13]. *Annona senegalensis* popularly known as African custard apple (English) is known as "gwandar daaji" in Hausa, uburu-ocha in Igbo and abo in Yoruba languages. It is referred to as 'arere' in Ilorin. It is found widely distributed in Central and West Africa. The plant has several medicinal uses. The Hausas in the northern region of Nigeria use the boiled root bark for the treatment of intestinal disorders while the Senegalese chew the stem for stomach ache [14]. Local livestock farmers in Nigeria use the plant as an antihelmintic [15], [16]. The leaves and stem barks are used for the treatment of cancer of the skin and leukaemia [17]. The plant's smooth muscle relaxant [18], antibacterial [19], anti-inflammatory [20], antimalarial [21], anticonvulsant, sedative and central nervous depressant [22] properties have been reported. Its use as a medicinal plant may be attributed to the presence of phytochemicals such as tannins [23], flavonoids [24], saponins [25], alkaloids [26], glycosides, steroids [27], volatile oil [28] and anthocyanins [29]. The plant also contains minerals such as calcium, zinc, potassium, iron, magnesium, lead, copper, manganese, as well as ascorbic acid and amino acids [30]. Nineteen monoterpenes and sesquiterpenes have been isolated in essential oils extracted from the fruits and leaves of *A. senegalensis* from Nigeria [31]. The stem and root barks are used traditionally to treat gastrointestinal troubles and diarrhoea [32]. The methanolic stem bark extract of *A.*

senegalensis has been reported to attenuate spontaneous contractions in isolated rabbit ileum and inhibit gastrointestinal transit time in normal (diarrhoea not induced) mice [33]. The antisecretory and pro-absorptive properties of the stem bark extracts of this plant have not been studied in diarrhoeal animals. In addition, the traditional medicine practitioners in Adamawa State, Nigeria use a mixture of the root and stem of *Annona senegalensis* as a remedy for diarrhoea and this synergistic effect has not been scientifically proven. Synergistic interaction between different plant and different plant parts may increase efficiency, reduce undesirable effects and increase the bioavailability or stability of the free agents [34]. This study therefore, investigated the antidiarrhoeal activity of the stem and root bark extract in castor oil-induced diarrhoeal rats. The synergistic effect of the combined plant part on antimicrobial, antioxidant and *in vivo* antidiarrhoeal activity was also investigated.

Material and Methods

Collection and preparation of plant

Fresh stem bark and root of *Annona senegalensis* were collected in July, 2018 and authenticated at the Dept. of Plant Biology, University of Ilorin, Kwara State with a voucher number UILH/001/449. It was separately washed and air dried under shade (room temperature) and then pulverized separately using mortar and pestle into powder. The powdered samples were stored in airtight containers and kept at room temperature until required.

Extraction procedure

The powdered samples (100 g each) were soaked separately in 1 L of distilled water for 48 h at 35°C with vigorous shaking at 3 h intervals. The mixture of root and stem bark was made in ratio 1:1. The crude extracts were filtered using Whatman No. 1 filter paper. Each of the filtrates was evaporated to dryness at 40^{o}C under reduced pressure using rotary evaporator and water bath. The dried substance was stored in airtight bottles until required. The crude extracts were kept in a desiccator.

Percentage yield

Percentage yield was calculated as: weight of extract/ weight of dried powdered sample × 100

Experimental animals

Adult albino rats weighing between 130 – 150 g were purchased from the Breeding unit of Department of Biochemistry, University of Ilorin, Kwara State, Nigeria. They were housed in well ventilated cages, with free access to drinking water and given standard laboratory diet (Bendel feeds, Ilorin). The rats were handled according to the guidelines for the protection and handling of laboratory animals by the International Council for Laboratory Animal Science (ICLAS) and approved by the ethical committee of the Department of Biochemistry, University of Ilorin. Animals were allowed to acclimatize with the laboratory environment for one week before the experiment commenced.

Phytochemical screening

Qualitative phytochemical screening was carried out as described by Sofowora [35], Trease and Evans [36] and Harbone [37]

Acute toxicity test

The method described by Lorke [38] was used to the determine the LD_{50}.

In vitro antioxidant property

The method described by Benzie and Strain [39], Lingnert *et al.* [40], Ruch *et al.* [41], Kikuzaki and Nakatani [42], Ottolangi [43] was used to determine the ferric reducing power, DPPH IC_{50}, H_2O_2 scavenging activity, ferric thiocynate assay and thiobarbituric acid assay respectively.

Determination of antimicrobial activity of the plant extracts

The antimicrobial activity was done using the method of Olila *et al.* [44]. Zones of inhibition greater or equal to 6 mm diameter were regarded as having antimicrobial activity.

Stool inhibition

A total of 55 albino rats were randomly divided into eleven groups (I - XI) of five animals each. All rats were fasted for 12 hours and thereafter, received castor oil at a dose of 1 ml/animal orally (p.o.) using orogastric cannula to induce diarrhoea. Thirty minutes after castor oil administration, rats of group I (control) received 1.0 mL of 0.9% NaCl in distilled

water (normal saline), group II received 3 mg/kg loperamide (standard drug), groups III - V received 100, 200 and 400 mg/kg. body weight of aqueous root bark extract, groups VI - VIII received 100, 200 and 400 mg/kg b.wt. of aqueous stem bark extract, while group IX - XI received 100, 200 and 400 mg/kg b.wt of a mixture of root and stem bark aqueous extract p.o. respectively. The animals were placed separately in metabolic cages over white clean whatman filter paper, which was changed every hour. The severity of diarrhoea was assessed each hour for 4 hours. The total number of diarrhoea faeces of the control group was considered 100%.

% inhibition = (Control - Test) × 100/Control.

Measurement of gastrointestinal transit time using charcoal

Fifty-five (55) adult rats were fasted for 12 hours and randomly divided into eleven groups of five animals each. Castor oil (1 mL) was administered orally to the animals. One hour later, rats of group I (control) received 1.0 mL of 0.9% NaCl in distilled water (normal saline), group II received 3 mg/kg b. wt. atropine sulphate. Rats of groups III - V received 100, 200 and 400 mg/kg. body weight of aqueous root bark extract and groups VI - VIII received 100, 200 and 400 mg/kg b.wt. of aqueous stem bark extract. Group IX - XI received 100, 200 and 400 mg/kg b. wt. of a mixture of root and stem bark aqueous extract p.o. respectively. After 30 min of the administration, 1 mL of charcoal meal, (10% suspension in 5% gum acacia) was orally administered to rats in each group. The rats were sacrificed by ether (20% v/v) anesthesia and the small intestine was carefully separated from mesentery to avoid being stretched. For each animal, gastrointestinal transit was calculated as percentage distance travelled by charcoal meal to the total length of intestine. The inhibitory effect of the extracts on gastrointestinal transit was calculated relative to respective group.

Castor oil-induced enteropooling and electrolyte secretion

Fifty-five (55) adult rats selected without sex discrimination were fasted for 12 hours and divided into eleven groups of five animals each. Castor oil (1 mL) was administered orally to these rats. One hour later, group I (control) received 1.0 ml/100 g of 0.9% NaCl in distilled water (normal saline), group II received standard drug, loperamide (3 mg/kg p.o.). Rats of groups III - V received 100, 200 and 400 mg/kg. body weight of aqueous root bark

extract and groups VI - VIII received 100, 200 and 400 mg/kg b. wt. of aqueous stem bark extract. Group IX - XI received 100, 200 and 400 mg/kg b. wt of a mixture of root and stem bark aqueous extract p.o. respectively. After 2 hours of treatment, the rats were sacrificed by ether anesthesia. The edges of the intestine from pylorus to ceacum were tied with thread and the intestine removed and weighed. Intestinal fluid was milked into a graduated tube and the volume of the intestinal fluid was taken. The intestine was reweighed and differences between full and empty intestines calculated. The Na^+ and K^+ concentrations in the supernatant, after centrifugation of the intraluminal fluid was measured by flame photometry

Statistical analysis

The computation of the mean and statistical analysis was done using SPSS software version 17.0. Data is expressed as the mean ± SD for group of five animals. It was statistically analyzed with one-way analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT). For all the tests, results with p values < 0.05 was considered significant.

Results

The percentage yield of the aqueous stem extract was 38.6% while that of the root was 29.2% and the mixture was 35.3%.

Intraperitoneal administration of up to 5000 mg/kg of aqueous extract of the stem, root and mixture of stem and root of *Annona senegalensis* to rats caused no death in the two stages of the test. There were no observable changes in physical behavior of the animals. 100, 200 and 400 mg/kg b. wt. was thus chosen for this study.

Qualitative phytochemical screening shows presence of tannin, flavonoids, steroids, alkaloids, saponin and glycosides in both the root and stem of *A. senegalensis* (Table 1). Reducing sugar was present in the root but absent in the stem. Anthraquinones is absent in both the stem and the root. Quantitative phytochemical analysis shows that glycoside concentration was significantly higher in the stem bark when compared to the ARB and AM.

Table 1 Quantitative phytochemical screening of the root bark, stem bark and mixture of stem and root bark of *Annona senegalensis* (mg/100mg of extract)

	ARB	ASB	AM
Saponin	0.80 ± 0.02^{a}	0.66 ± 0.34^{a}	0.75 ± 0.03^{a}
Phenol	0.60 ± 0.12^{a}	0.54 ± 0.15^{a}	0.58 ± 0.27^{a}
Flavonoids	0.39 ± 0.22^{a}	0.37 ± 0.33^{a}	0.34 ± 0.02^{a}
Alkaloids	0.62 ± 0.11^{a}	0.83 ± 0.17^{a}	0.71 ± 0.01^{a}
Glycosides	0.03 ± 0.23^{a}	0.11 ± 0.07^{b}	0.06 ± 0.22^{c}
Tannins	0.58 ± 0.22^{a}	0.53 ± 0.19^{a}	0.30 ± 0.13^{a}

Values are mean ± S.D. of six determination. Values with different superscript along the row are significantly different ($p < 0.05$) from each other

Table 2 presents the zone of inhibition of the aqueous root and stem bark extracts of *A. senegalensis.* The zone of inhibition for all the extracts against all the bacteria was lower than the control. The mixture of stem and root bark extracts (AM) showed significant antimicrobial activity (zone of inhibition > 6mm) against *B. cereus* (10 ± 0.01), *Proteus vulgaris* (9 ± 0.30) and *E .coli* (12 ± 0.32). The root extract (ARB) also showed significant antimicrobial activity (zone of inhibition > 6mm) against *E. coli* (8 ± 0.37). The zone of inhibition for the aqueous stem bark extract (ASB) against all the micro-organisms tested for was ≤ 6 mm.

Table 2 Antimicrobial activity of aqueous root and stem bark extract of *Annona senegalensis* expressed as zone diameter inhibition (mm)

	Bacillus aureus	*Proteus vulgaris*	*Shigella dysentriae*	*E. coli*	*A. niger*	*Mucor*
ASB	5 ± 0.08	3 ± 0.30	3 ± 0.01	6 ± 0.56	0 ± 0.50	3 ± 0.60
ARB	7 ± 0.65	5 ± 0.10	7 ± 0.10	8 ± 0.37	3 ± 0.45	6 ± 0.47
AM	10 ± 0.01	9 ± 0.30	7 ± 0.10	12± 0.32	3 ± 0.12	7 ± 0.90
Control	28 ± 1.0	23 ± 0.45	28 ± 0.01	30± 0.34	14± 0.35	18 ± 0.23

Values are mean ± SD, n=6, $p < 0.05$

Table 3 shows the total antioxidant capacity of aqueous root and stem bark extracts of *A. senegalensis.* The aqueous stem bark had the highest total antioxidant capacity. The *in vitro*

antioxidant activity of the root and stem barks of *A. senegalensis* is shown in Table 4. The IC_{50} of the aqueous stem bark extract (ASB) was not significantly different ($p > 0.05$) from that of ascorbic acid but ARB and AM was significantly greater ($p < 0.05$). The H_2O_2 scavenging activity and the ferric reducing assay power of ASB and AM was significantly greater ($p < 0.05$) than ascorbic acid. The ferric thiocyanate scavenging activity and thiobarbituric acid (TBA) activity of all the extracts were significantly greater ($p < 0.05$) than that of ascorbic acids.

Table 3 Total antioxidant capacity of *Annona senegalensis* root, stem bark and its mixture

	Total antioxidant activity (mg equivalent of ascorbic acid/g of extract)
ASB	564.02 ± 0.18^{c}
ARB	418.17 ± 0.07^{a}
AM	515.58 ± 0.23^{b}

Values are mean ± S.D, n = 6. Values with different superscript down the column are significantly different from the others ($p < 0.05$)

Table 4 *In vitro* antioxidant activity of *Annona senegalensis*

	Ascorbic acid	**ARB**	**ASB**	**AM**
DPPH (IC_{50}) mg/ml	54.10 ± 0.02^{a}	91.80 ± 0.23^{c}	53.45 ± 0.13^{a}	74.30 ± 0.22^{b}
H_2O_2 Scavenging (% activity)	20.12 ± 0.15^{b}	14.23 ± 0.17^{a}	18.82 ± 0.22^{b}	16.35 ± 0.13^{b}
Ferric thiocynate (% activity)	63.01 ± 0.45^{a}	86.26 ± 0.32^{b}	85.14 ± 0.21^{b}	85.71 ± 0.11^{b}
Thiobarbituric acid % activity	72.85 ± 0.12^{a}	94.14 ± 0.13^{b}	95.54 ± 0.13^{b}	93.45 ± 0.22^{b}
Ferric reducing activity power (%)	66.29 ± 0.35^{a}	71.10 ± 0.05^{a}	79.13 ± 0.02^{b}	75.92 ± 0.23^{b}

Values are mean ± S.D, (n = 6). Values with different superscript along each row are significantly different from the others ($p < 0.05$)

Fig. 1 shows the reduction in number of stool for the first four hours. In the first three hours, there was gradual decline in the number of stool. By the 4^{th} hour, there was observable reduction in stool when compared to the control which was still high. Table 5 shows the stool inhibition and gastrointestinal tract transit inhibition by aqueous root and stem bark extracts of *A. senegalensis* in castor oil induced diarrhoeal rats. All of the

extracts inhibited stool (% ranging from 24.7 – 17) but was significantly lower ($p < 0.05$) than stool inhibition of the standard drug; loperamide.

Table 5 Stool inhibition and gastrointestinal transit inhibition in castor oil- induced diarrhoeal rats administered aqueous root and stem bark extracts of *Annona senegalensis*

Group	Mean no. defecation	% stool inhibition	%Gastrointestinal transit inhibition
I (control)	11.08 ± 0.20^{g}	-	15.60 ± 0.09^{a}
II (Loperamide)	7.70 ± 0.52^{a}	30	69.90 ± 0.05^{f}
III (100 mg/kg b.wt ARB)	9.30 ± 0.06^{f}	17	49.80 ± 0.10^{d}
IV (200 mg/kg b.wt. ARB)	9.06 ± 0.01^{e}	17.9	25.40 ± 0.10^{b}
V (400 mg/kg b. wt. ARB)	8.81 ± 0.02^{d}	20.29	37.41 ± 0.31^{c}
VI (100 mg/kg b. wt. ASB)	9.09 ± 0.01^{e}	17.7	47.12 ± 0.12^{d}
VII (200 mg/kg b.wt. ASB)	8.31 ± 0.00^{b}	24.7	25.73 ± 0.20^{b}
VIII (400 mg/kg b. wt. ASB)	8.98 ± 0.01^{e}	18.7	38.02 ± 0.21^{c}
IX (100 mg/kg b. wt. AM)	8.65 ± 0.02^{c}	21.7	22.08 ± 0.20^{b}
X (200 mg/kg b.wt AM)	9.09 ± 0.01^{e}	17.6	22.85 ± 0.18^{b}
XI 9400 mg/kg b. wt. AM)	8.71 ± 0.02^{c}	21.9	43.27 ± 0.33^{d}

Values are mean ± S.D of five determinations. Values with different superscript down the column are significantly different from the others ($P > 0.05$)

All extracts also reduced the number of defecation when compared to the normal control. Administration of 200 mg/kg b. wt. aqueous stem bark (ASB) was more efficient in reducing the number of stool after loperamide (the standard drug). All of the extracts significantly increased the % gastrointestinal transit inhibition when compared to the negative control but were all significantly lower than the standard drug, loperamide. Amongst the extracts, aqueous root bark extract (ARB) and aqueous stem bark extract (ASB) both at 100 mg/kg b.wt. and 400 mg/kg had the highest % gastrointestinal transit inhibition. There was a significant decrease ($p < 0.05$) in percentage inhibition of gastrointestinal transit of diarrhoeal rats administered higher doses (200 mg/kg and 400 mg/kg b. wt.) of ASB and ARB.

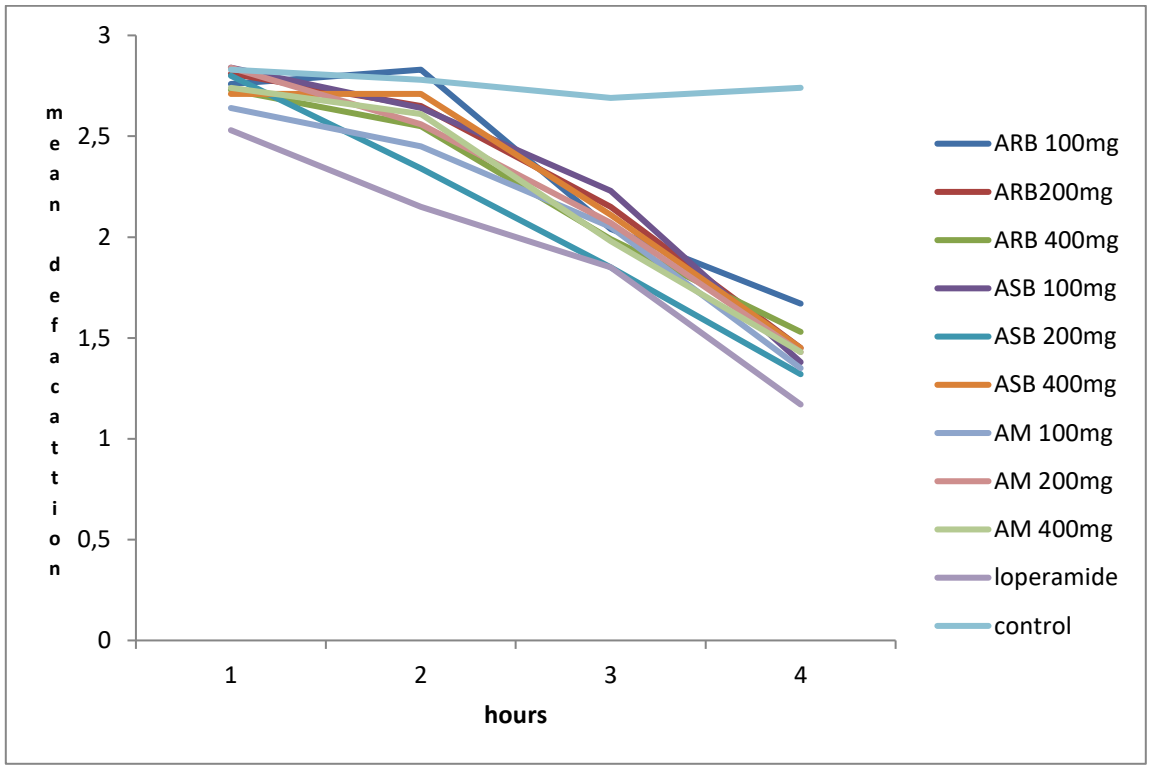

Fig 1 Hourly stool inhibition in castor oil- induced diarrhoeal rats administered aqueous root and stem bark extracts of *Annona senegalensis*

Table 6 shows the result of enteropooling and electrolyte concentration of the intestinal fluid. All of the extracts significantly decreased ($p < 0.05$) the weight and volume of the intestinal fluid of castor oil- induced diarrhoeal treated rats when compared to the negative control. The weight of the intestinal fluids of rats administered moderate doses (100 and 200 mg/kg b. wt.) of ARB was not significantly different from the standard drug loperamide. The weight of intestinal fluid of rats administered 100 and 200 mg/kg b. wt of ARB significantly decreased ($p < 0.05$) when compared with the negative control and the standard drug, loperamide while 400 mg b. wt. Aqueous mixture (AM) of root and stem bark was not significantly different ($p > 0.05$) from that of the standard drug, loperamide. Aqueous root bark extract (ARB) at 100 mg/ kg b. wt. and 200 and 400 mg/kg b. wt. of ASB significantly decreased the concentration of both Na^+ and K^+ when compared to the standard drug.

Table 6 Effect of administration of aqueous root and stem bark extracts on enteropooling and intestinal fluid electrolyte concentration

Group	Weight of intestinal fluid (mg)	Vol. of intestinal fluid (ml)	K^+ (mol/L)	Na^+ (mol/L)
I (control)	3.27 ± 0.33^{d}	3.00 ± 0.5^{c}	10.36 ± 0.49^{d}	309.00 ± 0.74^{g}
II (Loperamide)	1.63 ± 0.09^{a}	2.03 ± 0.3^{b}	9.37 ± 0.27^{c}	180.23 ± 0.02^{c}
III (100 mg/kg b.wt ARB)	2.28 ± 0.30^{a}	1.43 ± 0.25^{a}	5.46 ± 0.20^{b}	148.68 ± 0.56^{b}
IV (200 mg/kg b.wt. ARB)	2.01 ± 0.50^{a}	1.43 ± 0.22^{a}	10.49 ± 0.19^{d}	288.16 ± 0.23^{b}
V (400 mg/kg b. wt. ARB)	3.94 ± 0.67^{d}	2.63 ± 0.49^{c}	9.26 ± 0.42^{c}	319.70 ± 0.89^{g}
VI (100 mg/kg b. wt. ASB)	2.43 ± 0.43^{b}	2.26 ± 0.15^{b}	11.02 ± 0.19^{e}	303.95 ± 0.08^{f}
VII (200 mg/kg b.wt. ASB)	2.45 ± 0.05^{b}	2.23 ± 0.8^{b}	5.97 ± 0.47^{b}	130.00 ± 0.96^{a}
VIII (400 mg/kg b. wt. ASB)	3.89 ± 0.14^{d}	2.60 ± 0.6^{c}	4.60 ± 0.27^{a}	112.58 ± 0.92^{a}
IX (100 mg/kg b. wt. AM)	3.04 ± 0.29^{c}	1.50 ± 0.25^{a}	10.75 ± 0.60^{d}	274.95 ± 0.02^{e}
X (200 mg/kg b.wt AM)	3.1 ± 0.60^{c}	1.93 ± 0.33^{a}	11.75 ± 0.30^{e}	298.68 ± 0.03^{f}
XI (400 mg/kg b. wt. AM)	2.18 ± 0.27^{a}	2.03 ± 0.30^{b}	10.23 ± 0.32^{d}	265.77 ± 0.16^{d}

Values are mean ± S.D of five determinations. Values with different superscript down the column are significantly different from the others ($p < 0.05$)

Discussion

Any substance that is not toxic at 5,000 mg/kg b. wt. is considered safe [45]. The aqueous root and stem bark extracts of *Annona senegalensis* is therefore safe at doses ≤ 5000 mg/kg b.wt.

Antidiarrhoeal activity of medicinal plants is due to the presence of tannins, alkaloids, saponins, sterols, triterpenes and reducing sugar [46]. The presence of these phytochemicals in *A. senegalensis* root and stem barks may be responsible for its antidiarrhoeal activity. Reducing sugar present in ARB but absent in ASB may be responsible for the difference in antidiarrhoeal activity of the two extracts. A zone of inhibition greater than 6 mm indicates antimicrobial activity, thus AM exhibited antimicrobial activity against *E. coli, B. cereus* and *Shigella dysentriae.* The antimicrobial activity exhibited by AM when compared to with the individual plants may be due to synergistic effect. The synergistic effect exhibited by the mixture of root and stem barks (AM) of this plant may be due to formation of complex compounds which are more effective in the inhibition of particular specie of micro-organisms by either inhibiting cell wall synthesis or by causing cell death or lyses

[47]. Also, synergism of different plant parts may lead to slower development of bacterial resistance [12], [48].

The significantly greater total antioxidant capacity, H_2O_2 scavenging activity and ferric reducing assay power exhibited by ASB indicates its good antioxidant property. Free radicals induce lipid peroxidation which destroys cell membrane and leads to the inability of membranes to maintain ionic gradients which will particularly affect the efflux of sodium and potassium, thus leading to diarrhoea [49]. Plants with antioxidant property will therefore be of advantage in the treatment of diarrhoea, thus, the antioxidant potential of ASB may contribute to the antidiarrhoeal activity of ASB. The good antioxidant property can be attributed to the presence of phenolic compounds in the extract [50]]. Flavonoids and their derivatives are the most active polyphenolic antioxidant [51].

The gradual reduction in the number of stool by the extracts indicates antidiarrhoeal potential of the extracts. The significant reduction in mean number of stool demonstrates the efficacy of these extracts as an effective antidiarrhoeal agent. The significant reduction by aqueous stem bark (ASB) extract of *A. senegalensis* indicates antisecretory activity of the extract. The antisecretory property may be due to its ability to inhibit prostaglandin synthesis which has been reported to inhibit diarrhoea [52]. Flavonoids and glycosides, present in this plant extract, have been reported to inhibit release of autacoids and prostaglandins [53]. Glycoside was significantly low in the root extract when compared to the other extracts. This might be a reason for the decreased antisecretory activity of the root extract.

The inhibitory effect of ARB and ASB extracts on gastrointestinal motility indicates antimotility activity of the extracts which may be due to the presence of antimotility agents such as tannins [54] and flavonoids [55]. Tannic acid form complexes with proteins in the lumen which then lines the intestinal wall, thus reducing peristaltic movement and secretion [54], [56], [57]. It could also bring similar function by reducing intracellular Ca^{2+} inward current or by activation of the Ca^{2+} pumping system which induces muscle relaxation [58]. Also, castor oil-induced gastro-intestinal hypermotility has been suggested to be indirectly mediated by the cholinergic system since it is inhibited by atropine and loperamide, known anticholinergic agent [59]. Anticholinergic agents are known to inhibit gastro-intestinal (GI)

hypermotility [60]. Therefore, ARB and ASB may contain anticholinergic compound responsible for their antimotility activity.

Desensitization occurs when there is persistent signal at the receptors [61]. This might be a reason for the decline in the inhibitory effect of the root and stem extract at high dose of 400 mg/kg b.wt. Previous study also reported decrease in inhibitory efficiency of the stem bark on gastrointestinal transit at higher dose (≥ 100 mg/kg) [33].

A decrease in the motility of gastrointestinal tract increases the stay of the substance in the intestine and allows for better reabsorption of water and nutrients [62]. This can be a reason for the antienteropooling activity exhibited by 100 mg/kg b.wt ARB. The significant reduction in intestinal fluid Na^+ concentration of castor oil- induced diarrhoeal rats administered 200 and 400 mg/kg b. wt. ASB indicates the pro - absorptive property of the extract. This suggests that the plant contains phytochemicals that are able to stimulate Na^+ absorption by stimulating any of the apical ion transporters. Reabsorption of Na^+ via any of the channels play an important role in re-absorption of water [63]. This may contribute to the antidiarrhoeal potential of the extract.

Conclusion and Recommendation

Synergistic interaction between aqueous root and stem bark extract of *Annona senegalensis* improved antimicrobial activity against most diarrhoea causing micro-organism studied. The mixture did not show any observable improvement in the *in vivo* diarrhoea models studied. The findings from this study indicates that both the stem and root barks exhibit antidiarrhoeal activity. Both extracts exhibited good antimotility activity. The aqueous stem bark in addition exhibited good antioxidant, antisecretory and proabsorptive properties while the aqueous root bark exhibited good antienteropooling activity. The extracts (ASB and ARB) is more efficient at lower doses (100 – 200 mg/kg b. wt.). Further studies on the isolation of the bioactive compound and its synergistic interactions should be carried out.

Abbreviations

ASB: *Annona senegalensis* stem bark, ARB: *Annona senegalensis* root bark. AM: *Annona senegalensis* mixture of root and stem bark extract. b.wt. body weight, DPPH: 2,2-Diphenyl-1- Picrylhydrazyl, IC_{50}: concentration required to attain 50% radical scavenging activity. H_2O_2: Hydrogen peroxide. LD_{50}: Lethal dose.

Ethics Approval
Adults albino rats used in this research were handled according to the guidelines for the protection and handling of Laboratory animals by the International Council for Laboratory Animal Science (ICLAS) and approved by the ethical committee of the Department of Biochemistry, University of Ilorin, Nigeria.

Availability of Data and Material
All data generated or analysed during this study are included in this published article.

Competing interest.
The authors declare that they have no competing interest.

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Authors' contribution
ARO designed the study; AMU performed the laboratory experiments and drafted part of the manuscripts. SIY participated in the drafting of the manuscripts. All authors read and approved the final manuscripts.

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Research article

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Effect of Heat Treatment on Aflatoxin Contents of Tomatoes Samples in Ilorin, Kwara State, Nigeria

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ABSTRACT

Fungi contamination of agricultural products had been one of the major concerns to human due to their possibility of causing spoilage and the potential of producing potent toxins that could constitute health problems. Therefore, this study evaluates the effect of heat treatment on aflatoxin contents of tomatoes samples in some selected market in Ilorin, Kwara State, Nigeria. The spoilt and fresh tomatoes samples were randomly purchased from nine vendors for analysis. Fungi were isolated on Sabroud dextrose agar and identified using colonial and morphological characteristics. The levels of aflatoxin in the samples were determined using ELISA techniques and effects of heat treatments on aflatoxin levels of the samples were determined before and after 5, 15, 25 and 35 minutes of heat treatments at 100 ^{o}C. The results revealed the presence of fungi contaminants in all the analysed samples irrespective of their quality status. Furthermore, the aflatoxins contaminants were not detected in all the evaluated fresh samples; whereas the presence of aflatoxin in the spoilt samples was observed. The average aflatoxin contents of spoilt samples were 7.83, 8.17 and 8.62 µg/kg respectively, with no significant differences among the values. The effect heat treatments on the aflatoxin contaminated samples were observed to be time dependent. About 7.5 % non-significant reductions ($p > 0.05$) in aflatoxin contents of the samples were observed after 5 min of heat treatment. While, significant reduction of about 45.0 %, 53.1 % and 71.5 % were observed after 15, 25 and 35 minutes of heat treatments respectively. This is an indication that proper cooking at 100 ^{o}C, over a period of time could significantly reduce aflatoxin level in some food products that are usually subjected to cooking prior to consumption

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Introduction

Fungi groups had been implicated in most of the post-harvest loss of some major agricultural products. However, the major concerns about fungi presence in agricultural produce did not limit to their possibility of causing spoilage but their capability to release the potent toxins into the products [1]. The toxins of fungi origin are generally termed mycotoxins and they are reported to be the secondary metabolites of fungi metabolism [2]. The groups of reported mycotoxins producing fungi include,

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Alternaria, *Fusarium*, *Pencillium*, *Mucor* and *Aspergillus* genera. They can flourish at relatively mild acidic pH range that characterized the vegetables and fruit products.

Aflatoxins are among the most reported group of mycotoxins with high prevalence rate in most of the food products. They had been reported to be a potential mutagene and carcinogene compounds. Also, they could have some cyototoxic, teratogenic effects on human as well as causing liver serosis and immune impairment among others [3].

Tomato (*Lycopersicum esculentum*) is an herbaceous plant of family solanaceae [4]. The richness of tomato in terms of its nutritional composition has made it a good source of nutrient not only for human consumptions but also for microorganisms. It has been reported to be a good source of vitamins and some other phytonutrients such as proteins, carbohydrates as well as minerals such as potassium [5]. The use of spoilt tomatoes for soup ingredients is one of the common practices especially among the lower and middle classes in Nigeria without considering its potential health implications. These could be attributed to socio-economic factors, believe and cultural factors among others. In recent year, Nigeria had been ranked among the poorest countries with highest poverty rate [6].

The consumption of aflatoxins containing spoilt tomatoes could endanger the people's life. It has been recently reported that the rising incidence of cancer among human could probably have direct linked with consumption of spoilt tomatoes [7, 8]. In Nigeria, the preparation of soup usually involves thorough cooking processes; therefore, this study was to evaluate the effect of heat treatments on the aflatoxin levels of spoilt tomatoes used in making soup.

Material and Methods

Collection of Samples

The spoilt and fresh tomatoes fruits samples sold in some selected major market in Iloin, Kwara State Nigeria, were randomly purchased from nine vendors for aflatoxin assay. These markets were Ipata market Ilorin East Local Government Area, Oloje Market, Ilorin West local Government Area and Kunlende market, Ilorin South Local Government Area. The tomatoes samples were transported aseptically in sterilized sampling bottles to the laboratory for further processing and analyses.

Isolation and Identification of Fungi Group Present in Tomatoes Samples

The isolation and identification of fungi present in tomatoes' samples were carried out by adopting the method used by Suleiman [9] with little modifications. About 1 g from homogenized samples was aseptically serially diluted up to 10 fold dilution. About 0.1 ml from diluted samples was inoculated by streaking methods onto plate of Sabroud dextrose agar (SDA) and incubated at room temperature for the period of 4 to 7 days. Each of the distinct fungus colony was sub-cultured onto the SDA plates and incubated at room temperature for 4 to 7 days.

For identification the colonies were observed from the upper and lower part of the plates and colonial characteristics were noted. The isolates were further subjected to microscopic examination using lactophenol cotton blue stain under the low (x 10) and high (x 40) power objective lens respectively. The morphology features like spore types and presence or absence of septa in the mycelia were observed.

Effect of Heat Treatment on Aflatoxin Level of Tomatoes Samples

The aflatoxin levels of tomatoes samples were determined prior to heat treatment and after 5, 15, 25 and 35 minutes of heat treatments at 100 ^{o}C in water bath under standard conditions.

Extraction and Quantification of Aflatoxin in Tomatoes Samples

The levels of aflatoxin in tomatoes were determined using standard ELISA techniques as described by Zhang *et al*. [10] with little modification. The tomatoes fruits were initially homogenized in blender prior to analysis.

About 5 g homogenized samples were extracted with methanol water solutions (4:1) after shaking on orbital shaker for about 30 mins at 120 rpm at room temperature. The subsequent mixtures were centrifuged and 100 µL of the supernatant was buffered to pH 7.2. About 50 µL of the buffered solution was then mixed with equal volume of conjugated aflatoxin-peroxidase in microtitre plate. These procedures were replicated three times and the sealed plates were incubated at 25 ^{o}C for 30 mins in cold incubator.

The plates were further rinsed with phosphate buffer (pH 7.2) for 2 minutes before 50 µL of tetramethylbenzidine chromogen with 50 µL of urea peroxide solution were added and incubated for another 30 min at 25 ^{o}C in cold incubator. About 100 µL of 0.25 mol/dm^3 of sulphuric acid was added to terminate the reaction and the plates were

read with spectrophotometer at 450 nm wavelength. The amounts of aflatoxins were evaluated from the previously prepared standard curve.

Statistical Analysis

The average value of the triplicate analysis for each sample was evaluated and presented in table. The data were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) version 21. The analysis of variance (ANOVA) was used to compared the differences among the values and further subjected to Duncan multiple range test (DMRT) to compare the mean values for significant difference. Differences were considered statistically significant at $P \leq 0.05$ [11].

Results and Discussions

The results of aflatoxin contents and fungi contaminants presents in fresh and spoilt tomatoes samples were summarized in Table 1 below.

In fresh tomatoes samples, the aflatoxins level were below the limit of detection (LOD) value of 0.005 µg/kg of the analytical instrument for all the samples analyzed for sampling point A, B, and C (Table 1). While, the average value of aflatoxin contents in spoilt tomatoes were 8.17 ± 0.57 µg/kg, 7.83 ± 0.97 µg/kg and 8.62 ± 0.69 µg/kg for samples obtained from sampling area D, E and F respectively (Table 1). However, the differences among the values obtained for the aflatoxin from these samples were not statistically significant ($p > 0.05$).

Macroscopic and microscopic examination of the fungi isolates showed the presence of the following fungi as follows:

In the fresh tomatoes samples, *Aspergillus niger, Fusarium* sp, and *Pencillium* sp. were the three fungal species isolated from sample A and B respectively; while, the *Aspergillus flavus, Mucor* sp, alongside the *Fusarium* sp, were the three fungal species observed in sample C (Table 1).

Likewise, in the spoilt tomatoes samples, the following fungi groups were isolated:

Aspergillus niger, Aspergillus parasiticus, Yeast, Fusarium sp, and *Pencillium* sp. were the five fungal species in sample D; Also in sample E, five fungal species were observed and they were *Aspergillus niger, Aspergillus flavus, Pencillium* sp., *Fusarium* sp, and Yeast; while the four fungi isolates obtained from sample F were *Aspergillus niger, Aspergillus flavus, Yeast, Fusarium* sp. (Table 1).

The effect of heat treatments on the level of aflatoxin contents of spoilt tomatoes were presented in Table 2.

The non-significant reduction ($p > 0.05$) in the average values of aflatoxin from 8.62 ± 0.69 µg/kg to 7.97 ± 0.41 µg/kg representing about 7.54 % percentage reduction were observed after 5 minutes of heat treatment. At 15, 25 and 35 minutes after heat treatment, significant reduction ($p < 0.05$) in aflatoxin level were observed with the values of 4.74 ± 0.93 µg/kg, 4.04 ± 0.43 µg/kg and 2.46 ± 0.62 µg/kg represent about 45.01 %, 53.13 % and 71.46 % percentage reduction respectively (Table 2).

Table 1 Aflatoxin contents and fungi contaminants of fresh and spoilt tomatoes samples

Sample type	Sampling Point	Average aflatoxin level (µg / kg)	Fungi isolates
Fresh	**A**	< LOD	*Aspergillus niger, Fusarium* sp, *Pencillium* sp.
	B	< LOD	*Aspergillus niger, Fusarium* sp, *Pencillium* sp.,
	C	< LOD	*Aspergillus flavus, Fusarium* sp, *Mucor* sp.
Spoilt	**D**	8.17 ± 0.57[a]	*Aspergillus niger, Aspergillus parasiticus, Yeast, Fusarium* sp, *Pencillium* sp.
	E	7.83 ± 0.97[a]	*Aspergillus niger, Aspergillus flavus, Pencillium* sp., *Fusarium* sp, and Yeast
	F	8.62 ± 0.69[a]	*Aspergillus niger, Aspergillus flavus, Yeast, Fusarium* sp,

Results are presented as means ± standard error of triplicate analysis; Means value with the same superscript shows no significance difference; LOD = Limit of detection = 0.005 µg / kg; significant level = ($p \leq 0.05$)

Table 2 Effect of heat treatments on aflatoxin level of spoilt tomatoes

Treatment Period (minutes)	Aflatoxin level (µg / kg)	Heat treatment efficiency (%)
0	8.62 ± 0.69^{a}	-
5	7.97 ± 0.41^{a}	7.54
15	4.74 ± 0.93^{b}	45.01
25	4.04 ± 0.43^{b}	53.13
35	2.46 ± 0.62^{c}	71.46

Results are presented as means ± standard error of triplicate analysis; Means value with the same superscript shows no significance difference; LOD = Limit of detection = 0.005 µg / kg; significant level = ($p \leq 0.05$)

The presence of fungi contaminants were observed in all the tomatoes fruits samples purchased from the selected market area in Ilorin irrespective of the quality status of tomatoes sampled. The contamination of these products by fungi might occur majorly from its poor handling or due to vendors' attitude in Nigeria open market that barely subject their agricultural products to post-harvest decontamination treatments. Kaczarmarek *et al*. [12] has previously reported that the fungi are among the leading contaminants and potential spoilage agents of fruit products due to their preference for acidic condition that majorly characterized such products as tomatoes.

The most predominant fungal genera isolated from all the samples were *Aspergillus* and *Fusarium* spp, while *Penicillum, Mucor,* and Yeast were only present in some of the sample analysed. The predominant presence of *Aspergillus* spp. might have accounted for the incidence of aflatoxin in the tomatoes samples. Several authors have also reported the prevalence of aflatoxins producing *Aspergillus* spp. in fruit samples [13,14,15].

The presence of these fungi contaminants in tomatoes fruit are of major economic and public health concerns, not only because of the roles they play in food spoilage but also as a potential source of fungal toxins. Adeyeye [16] stressed that fungi contaminations

of various agricultural products could significantly reduce the value of such products and thereby lead to economic losses.

Furthermore, the aflatoxins contaminants were not detected in all the evaluated fresh tomatoes samples using the analytical instrument with 0.005 μg/kg limit of detection (LOD) value; whereas all the spoilt tomatoes samples were observed to be contaminated with aflatoxin. The average aflatoxin contents in spoilt tomatoes samples were 7.83 μg/kg, 8.17 μg/kg and 8.62 μg/kg respectively, and the differences among these values were not statistically significant ($p > 0.05$). Comparatively, these values were below the maximum allowable limits (10 μg/kg) for human consumption as the value adopted by National Agency for Food and Drug Administration in Nigeria from European Commission reported by Williams *et al*. [17].

Although, there were concerns about the consumption of aflatoxin contaminated food products to potentially contribute to high incidence rate of cancer among the people [7, 8]. However, proper cooking of pepper and tomatoes products for sauces making has been a common practice among Nigeria population most especially in the rural community. Therefore, the effect of heat treatment on aflatoxin level of spoilt tomatoes was further evaluated.

The effect heat treatments on the aflatoxin contaminated spoilt tomatoes at 100 ^{o}C under the standard conditions were observed to be time dependent. At 5 minutes period after boiling, about 7.5 % non-significant reductions ($p > 0.05$) in aflatoxin contents of the samples were observed. However, further heat treatment for about 15 min, 25 min and 35 min significantly ($p < 0.05$) reduced the aflatoxin levels to about 45.0 %, 53.1 % and 71.5 % respectively. This is an indication that proper cooking at 100 ^{o}C, over a period of time can significantly reduce aflatoxin level in some food products that are usually subjected to cooking prior to consumption. In line with this finding, Diedhiou *et al*. [18] reported a significant reduction of about 82 % in aflatoxin level of aflatoxin contaminated peanut after heat treatment over a specific period of time.

The significant reduction in aflatoxin level of spoilt tomatoes at 100 ^{o}C after 15, 25 and 35 minutes of heat treatment, under normal atmospheric pressure, has shown that proper boiling might be an effective strategy of reducing the aflatoxin level of food products.

Conclusion

In spite of the effectiveness of heat treatment in the reduction of aflatoxin level of the aflatoxin contaminated spoilt tomatoes in this study, the most effective strategy to efficiently prevent aflatoxicosis in human is through consumption of fresh quality fruit products. Pre- and post-harvest treatment processes could also reduce the level of fungal infestation, spoilage and aflatoxin contamination of agriculture products.

Abbreviations

ELISA: Enzyme-linked immuno-sorbent assay; ^{o}C : degree Celcius; µg/kg: microgram per kilogram; p: probability level; < : less than; > greater than; LOD: limit of detection

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Availability of data and material

Please contact the corresponding author for any data request.

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Research article

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Performances of Popcorn Hybrids in Three Geographical Regions of Turkey Based on Yield and Quality Traits

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ABSTRACT

Evaluation of popcorn experimental hybrids in different geographical regions gives valuable clues to breeders in selection of best hybrids for the target environments. In the present study, 49 popcorn crosses (44 test hybrids and 5 commercial checks) were tested in three different ecological condition in Turkey. A 7×7 lattice design using three replications were used. The field experiments of this research was carried out in Samsun (Northern Turkey), Çankırı (Middle Anatolia) and Antalya (South Anatolia) ecological conditions in 2016. Yield and quality parameters such as popping volume, unpopped kernels ratio and kernel size were determined. According to the results, 10 hybrids have a high performance over than 5 commercial checks. Yield and quality results showed that TBCM2015-41, 56, 62, 76 and 80 candidate hybrids were promising popcorn hybrids for the tested environments.

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Introduction

Zea mays belongs to the *Gramineae* family (Wheatgrass) and is divided into seven sub groups. Among these groups popcorn has a special significance thanks to rich nutrient content, high vitamins and minerals [1]. Popcorn also is a prudent choice for those wanting to reduce feelings of hunger while managing energy intake and ultimately, body weight [2]. A major trait that distinguishes popping maize from other types of maize is a formation of large flake after kernel popping as a response to the heat treatment [3]. Popcorn, flint corn and dent corn are different each other by grain appearances. The most important observation in terms of seed differences is the shape and size of the seeds. Popcorn is smaller and has a thicker layer of endosperm than other two types. High quality hybrid popcorn must have high and stable yield and high level of popping volume [4].

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The popping volume (PV) is the most important parameter of quality and principal trait distinguishing popping maize from other types of maize [3]. Major quality characteristics of popcorn is high PV, a good appearance, low unpopped kernel rate and kernel size. Popcorn varieties traded in the world, consist of many different species in terms of seed color, size and seed shape. No industry standards have been developed for kernel size determinations; but a commonly used measure is based on number of kernel in 10 grams and defines kernel size as follow: 52-67= large, 68-75=medium and 76-105= small, >105=very small [5].

Turkey's popcorn cultivation has increased significantly in recent years. However, production is not enough for the demand of the country. Turkey imports popcorn as a result of insufficient production [6]. Popcorn agriculture is made with farmer contract ten thousand ha in Turkey. The consumption of popcorn is dramatically increased after 1980's depend on rise of shopping centers and cinema in Turkey [7]. Hybrids with high yield and quality are needed for the country.

The objective of this research was to determine new popcorn hybrids with high yield and quality which can meet the demands not only producers but also consumers.

Materials and Methods

In the present study, 44 experimental hybrids plus 5 commercial checks total 49 popcorn crosses were tested in three regions of Turkey. The research was carried out in Antalya Bati Akdeniz Agricultural Research Institute (BATEM) (36053' N, 30042' Mediterranean of Turkey), Samsun Black Sea Agricultural Research Institute (KTAE) (41016' N, 36020' Northern Turkey) and Çankırı Karatekin University (40032'N, 33035' Middle Anatolia) ecological conditions in 2016. The meteorological conditions of locations during growing seasons are shown in Table 1. Hybrid seeds obtained from three locations were also used for quality analysis.

Tablo 1 The meteorological conditions of locations during growing season of 2016

	Antalya		Samsun		Çankırı	
	Temperature (°C)	Precipitation (mm)	Temperature (°C)	Precipitation (mm)	Temperature (°C)	Precipitation (mm)
April	18.2	11.0	13.7	44.4	12.2	52.5
May	20.3	15.0	16.3	191.8	16.5	57.0

June	26.1	18.0	21.4	98.5	22.0	50.2
July	29.1	0.0	23.5	50.9	24.3	22.5
August	26.6	0.0	24.7	59.0	23.2	20.2
September	24.6	24.0	19.5	108.2	20.2	18.5
October	21.3	5.0	14.6	39.6	12.5	31.7
November	14.7	78.0	10.6	94.3	7.8	35.8
Avaerage/ Sum	22.6	151.0	18.04	686.7	17.34	288.4

The field experiments were conducted in a 7×7 lattice design with three replications. Plots consisted of two rows. Row length was 5.0 m. 0.18 m and 0.70 m were used as on row and inter row distances, respectively. All cultural practice used in the experiments were as follow; Fertilizing was made as pure 8 kg phosphorus and 20 kg N/ha totally per decare according to soil analysis. All phosphorus and 8 kg/ha of nitrogen were given at the time of sowing as bottom fertilizer, the rest of the nitrogenous fertilizer was applied in four equal parts using drip irrigation system. Planting of popcorn seeds were done in 10th of April in Antalya location, in 2nd of May in Samsun location, in 18th of May in Çankırı location. Crops were harvested on September 20-22 in Antalya location, on October 20-22 in Samsun location, on November 1-2 in Çankırı location. The harvesting process was done manually on different dates because of plants reached to harvesting status at different dates. Experiment plots were harvested, measured and adjusted to 15% kernel moisture content to obtain grain yield (GY). Below formula was used for determination of GY. GY= Plot weight x [(100- grain moisture % /85) x ((Grain / ear ratio)/100)] Popping volume (PV), unpopped kernels ratio (UKR), kernel size (KS) traits were examined in Karamanoglu Mehmetbey University Food Engineering Department Laboratory in 2017. PV (cm^3g-1) was determined according to methods described [8]. A hot air popping machine was used for PV. The moisture content of samples was 10±0.5% before popping. Popped samples were poured into a 2000 mL plastic graduated cylinder, and volume recorded [6]. The method described by [9] was used for UKR (%). For UKR, before and after popping, the number of kernels for each sample was counted according to following formula. Percentage of unpopped kernel ratio= (Number of total unpopped kernels / Original number of kernels)×100. KS (number/10g) was measured according to [5]. All data obtained within this study was analyzed according to ANOVA procedures.

The multiple comparison of the group averages were made according to LSD multiple range tests.

Results

The difference between genotypes, locations and genotype by environment interactions for GY, PV, UKR, KS traits were found statistically significant at P <0.01 (Table 2). Due to significant environmental interaction, the locations were evaluated separately (Supplementary Table 3).

Table 2 Analysis of variance (ANOVA) for investigated traits

Source	DF	GY	PV	UKR	KS
Blok(Rep)	18	0.7315	0.0863	0.2046	0.4108
Replication	2	0.0061	<.0001	0.2450	0.5602
Location	2	<.0001	<.0001	<.0001	<.0001
Genotype	48	<.0001	<.0001	<.0001	<.0001
Genotype x location	96	<.0001	<.0001	<.0001	<.0001
LSD		66.81	0.29	1.86	2.20
CV (%)		11.79	1.23	10.63	2.74

The mean performances of the 49 popcorn crosses were given in supplementary Table 3. The average GY of genotypes was found to be 4.18 t ha-1, and it was varied between 1.91 t ha-1 (TBCM2015-86) and 6.75 t ha-1 (TBCM2015-56) in Antalya Location. Mean experiment yield in Çankırı location was 6.0 t ha-1. Yields varied between 3.85 t ha-1 (TBCM2015-61) and 7.39 t ha-1 (TBCM2015-80) in Çankırı site. Yields in Samsun location were changed between 4.52 t ha-1 (TBCM2015-99) and experiment average was 7.13 t ha-1 (TBCM2015-80) for this location (Supplementary Table 3).

The mean of PV of genotypes was found as 24.73 cm^3g-1 and the PV ranged from 19.5 (TBCM2015-89) to 30.5 (TBCM2015-76) cm^3g-1 in Antalya site. Experiment average for PV in Çankırı was found 23.39 cm^3g-1, and it was varied between 12.0 (TBCM2015-86) and 31.5 (TBCM2015-68) cm^3g-1. Experiment average was 19.3 cm^3g-1 in Samsun location and PV varied in that location from11.3 (TBCM2015-72) to 35.2 (TBCM2015-93) cm^3g-1 (Supplementary Table 3).

The average UKR of the genotypes was found 6.4%, and it varied from 2.2 (TBCM2015-76) to 12.3 (TBCM2015-52) % in Antalya Location. An experiment average was found

to be 23.5% for Çankırı location. UKR was changed between 5.2 (TBCM2015-70) and 44.8 (TBCM2015-65) % in this location. It was found that experiment average was 20.3% UKR in Samsun. Values were changed between 3.3 (Baharcin) to 52.3 (TBCM2015-49) % in this location (Supplementary Table 3).

The KS ranged from 72.3 (TBCM2015-71) to 116.3 (TBCM2015-86) number/10g and the average of KS was found 84.8 number/10g in Antalya Location. Values in this location ranged from 48.0 (TBCM2015-100) to 131.7 (TBCM2015-65) number/10g. In Çankırı location experiment average was 72.0 number/10g and values were changed between 56.7 (TBCM2015-71) and 93.6 (TBCM2015-47) number/10g. It was found that experiment average 73.2 number/10g in Samsun (Supplementary Table 3).

Discussion

According to the mean GY values, Çankırı location was the best, while Antalya was the worst. Location ecological conditions may affect the GY. Especially, unfavorable high temperature during flowering period seemed to be one of the reason for low yield in Antalya location when compared to Çankırı. While mean of checks for GY was 5.20 t ha-1 (the highest of them is 5.46 ton ha-1, Nermincin), 17 genotypes (TBCM2015-80, 56, 52, 68, 46, 87, 53, 81, 62, 98, 93, 92, 96, 73, 101, 70, 60) gave higher GY than 5.46 t ha-1 respectively.

GY is one of the most important trait for both producers and consumers. The GY of popcorn usually depends on genotype and growth conditions [10]. Different genotypes had different grain yield in different environments [6], [11]. GY of Antcin-98 and Nermincin hybrids reported to be 3.75 and 4.47 t ha-1 respectively in a study [12]. It was reported that GY of popcorn hybrids varied from 2.72 to 4.64 t ha-1 in Turkey [13]. [14] Researcher investigated 30 single cross popcorn hybrids and reported that the mean of genotypes were 4.38 t ha-1 for GY, yield ranged from 3.55 to 5.40 t ha-1. A research team investigated grain component of eight popcorn varieties and reported GY of popcorns ranged from 3.73 to 5.38 t ha-1 [15]. It was reported GY of popcorns ranged from 4.78 to 7.38 t ha-1 [16]. Our results are generally similar those studies were given. According to the average of PV of genotypes, the highest value was obtained from Antalya location, while lowest result obtained from Samsun. The studies showed that GY and PV is negatively correlated [1, 17-20]. Yield was found to be the lowest in the Antalya location, but the high PV at the same location confirms the negative relationship. Again, non-

popped kernels reduce the volume of the PV. The high nonpopped kernel rate (6.4%) in the Antalya location might be effective in finding the high PV volume. While mean of checks for PV is 24.57 cm^3g-1 (the highest of them was 26.90 cm^3g-1 (Baharcin), 3 genotypes (TBCM2015-41, 76, 93) had higher PV values than 26.90 cm^3g-1 respectively. In a study carried out with 35 inbred popcorn lines, mean of the genotypes for PV determined as 19.49 cm^3g-1 [21]. The researchers reported that there was an important positive correlation between PV and kernel size, and negative correlation between PV and UKR. It was reported that PV changed from 19.79 cm^3g-1 to 22.92 cm^3g-1 in another study [22]. Our results seem to be higher than other reports. Hybrids potential for these traits probably increased the values in our study. It was reported that the means of PV was 42.00 cm^3g-1 and changed from 38.20 cm^3g-1 to 46.50 cm^3g-1 [14]. In another study PV changed from 28.00 cm^3g-1 to 40.17 cm^3g-1 [16]. Our results in terms of these traits were lower than mentioned studies. It is thought that both environment and genotypes affected the different results.

UKR is one of the most important quality parameter and significantly affects the PV. Popcorn consumers prefer high PV, delicious, low gumminess, and low unpopped kernel. Also crop breeders have been striving to develop genotypes with superior grain yield, quality and other desirable characteristics over a wide range of different environmental conditions. As a result of this research, it was determined that genotypes TBCM2015-76,78,53,75 and, 70 are promising as low 12% according to UKR.

KS is another important quality trait and affects indirectly PV UKR [23]. Home consumers generally prefer small kernel types. Vendors usually prefer larger kernel, while medium kernels preferred by both home users and vendors [5].

Four of the hybrids studied in this research, (TBCM2015-100, 71, 46 and Antcin98) were in the small class (52-67 number/10g), 16 of them (TBCM2015-48, Elacin, 99, 83, 82, 98, 44, 60, SH9201, 53, 92, 87, 64, 96, 45 and 103) were in the medium class (68-75 number/10g) and 29 of them (TBCM2015-80, Baharcin, 70, 84, 49, 77, 56, 52, 68, 81, 97, 62, 43, 72, 61, Nermincin, 55, 101, 75, 73, 76, 78, 93, 47, 86, 65 and 67) were in the large class (78-105 number/10g). It was suggested that larger-sized grains produced greater flake size than small-medium sized grain [24].

In this research genotype x environment interaction (GEI) was statistically significant at $P < 0.01$. Likewise [25] reported that there was a significant GEI in popcorn hybrids

evaluated in different locations of Turkey. In maize breeding, the effects of Genotype x Environment interaction on the stability and adaptability are very important because of the fact that every cultivar has a connatural capacity to respond to the changes of environment [26]. But especially in popcorn, the correlations between the stability and adaptability statistics are not yet well understood, and selection of genotype based on the average crop yield is ineffective [27]. There are many methods to determine stability analysis. In the regression methods, it is assumed that performance of genotypes will may increase in good environmental. Hence, it is important to know not only average performance of genotypes but also magnitude of the genotype x environment interaction in the selection. [25].

The researchers proposed a model to test the stability of varieties under various environments [28]. They defined a stable variety as having unit regression over the environments (b= 1.00) and minimum deviation from the regression (S2di= 0).

Therefore, a variety with a high mean yield over the environments, unit regression coefficient (b=1) and deviation from regression as small as possible (S2d= 0), will be a better choice as a stable variety. The average results of both grain yield and PV and stability analyze of popcorn genotypes investigated three locations were given in Table 4, the diagrammatic presentation of GY and PV were given figure 1, 2 respectively.

Table 4 The stability parameters for both GY and PV in popcorn genotypes in three different environments

	Grain Yield (GY)						Popping Volume (PV)					
	X	b	a	VK	r2	S^2d	X	b	a	VK	r2	S^2d
2015-41	514.83	1.7	-391	0.06	0.94	3957.5	29.93	-0.83	48.65	0.01	1	0.05
2015-43	447.96	1.51	-356.6	0	1	3.41	23.23	1.67	-14.43	0.14	0.75	15.13
2015-44	525.85	0.49	263.02	0.08	0.47	5563.4	19.3	1.5	-14.49	0.14	0.71	14.27
2015-45	519.94	1.05	-41.71	0.11	0.65	12289	21.73	2.27	-29.27	0.23	0.68	38.88
2015-46	599.79	0.73	211.9	0.04	0.86	1707.7	21.93	0.88	2.05	0.14	0.45	14.98
2015-47	412.09	1.52	-400.7	0.02	0.99	513.51	19.77	0.3	13.01	0.11	0.14	8.9
2015-48	518.44	0.46	272.57	0.11	0.27	11675	24.43	2.55	-32.81	0.06	0.98	2.58
2015-49	531.38	0.44	295.54	0.02	0.94	243.62	22.53	3.1	-47.26	0.23	0.79	41.56
2015-52	613.88	0.34	434.7	0.07	0.33	4615.7	22.33	2.55	-34.94	0.17	0.82	21.78
2015-53	590.37	0.58	281.75	0.11	0.38	11299	25.43	0.41	16.23	0.14	0.15	14.6
2015-55	503.49	1.17	-121.4	0.01	0.99	206.43	21.93	1.08	-2.31	0.29	0.23	62.33

2015-56	638.88	-0.31	801.68	0.01	0.97	51.68	20.57	2.26	-30.24	0.01	1	0.07
2015-60	547.73	0.49	285.9	0	1	3.53	20.2	2.24	-30.2	0.12	0.88	10.74
2015-61	466.53	0.72	82.73	0.2	0.22	37265	20.6	2.03	-24.99	0.1	0.9	6.92
2015-62	580.67	-0.07	617.18	0.05	0.03	2692.6	20.6	3.01	-47.05	0.05	0.99	2.06
2015-64	486.1	1.36	-240.9	0.05	0.93	2847.2	20	2.07	-26.53	0.03	0.99	0.86
2015-65	540.76	1.58	-303.5	0.01	1	152.63	20.13	0.95	-1.13	0.05	0.9	1.59
2015-67	453.95	0.91	-29.82	0.08	0.73	6174.6	21.07	0.92	0.43	0.01	0.99	0.15
2015-68	613.16	0.49	349.59	0.01	0.99	74.36	23.9	2.29	-27.51	0.26	0.62	51.54
2015-70	552.12	0.28	404.44	0.01	0.96	61.44	23.87	0.52	12.08	0	1	0.01
2015-71	529.03	0.84	81.37	0.05	0.84	2757.2	23.03	0.53	11.02	0.12	0.29	11.14
2015-72	531.57	1.29	-156	0.04	0.96	1487.2	19.03	2.44	-35.88	0.01	1	0.04
2015-73	563.66	1.5	-235.6	0.02	0.99	315.13	23.3	2.48	-32.49	0.13	0.89	12.32
2015-75	481.1	1.16	-137.1	0	1	3.57	22.37	1.99	-22.46	0.1	0.9	6.99
2015-76	545.04	1.35	-173.4	0.07	0.9	4200.9	27.43	-0.22	32.37	0.22	0.02	36.47
2015-77	530.86	0.57	227.49	0.04	0.83	1313.7	25.47	0.21	20.85	0.18	0.03	25.42
2015-78	514.75	1.75	-416.3	0	1	13.01	22.07	1.74	-17.04	0.05	0.96	2.18
2015-80	692.31	0.59	378.1	0.01	0.98	124.28	20.63	1.94	-22.99	0.17	0.73	22.38
2015-81	587.72	1.36	-140.5	0.05	0.94	2491	23.7	-1.5	57.53	0.03	0.99	0.52
2015-82	481.49	1.29	-208.4	0.05	0.92	2794.5	20.6	2.2	-28.83	0.09	0.93	5.92
2015-83	543.84	0.07	508.85	0.09	0.01	7614.3	21.77	2.15	-26.55	0.02	1	0.24
2015-84	528.51	0.35	341.75	0.09	0.22	8666	22.63	1.22	-4.8	0.04	0.96	0.94
2015-86	423.83	2.05	-671.5	0.07	0.94	5072.7	16.03	1.21	-11.13	0.24	0.35	42.78
2015-87	593.15	0.47	342.8	0.12	0.25	13574	23.17	2.04	-22.66	0.05	0.98	1.58
2015-92	570.12	-0.06	600.5	0.03	0.06	1128.3	18.83	0.48	8.04	0.31	0.05	74.53
2015-93	572.1	0.87	106.68	0.13	0.48	16472	26.97	-2.55	84.2	0.04	0.99	1.2
2015-95	519.75	1.09	-63.01	0.14	0.57	18445	19.1	2.1	-28.02	0.06	0.97	2.35
2015-96	568.9	1.04	12.81	0.08	0.78	6164.5	20.53	-1.78	60.49	0.31	0.4	73.54
2015-97	530.23	0.98	9.67	0.05	0.9	2046.5	22.33	-0.37	30.56	0.28	0.03	60.57
2015-98	574.53	1.29	-113.6	0.1	0.79	9108.6	23.37	0.06	21.93	0.09	0.01	5.72
2015-99	459.45	1.66	-425.4	0.12	0.79	15049	22.27	-0.86	41.6	0.05	0.85	2.01
2015-100	440.36	1.69	-461.3	0.01	1	43.49	25.77	0.6	12.37	0.2	0.15	31.41
2015-101	561.46	1.79	-394.9	0.08	0.9	6869.6	19.7	1.15	-6.07	0.04	0.96	0.94
2015-103	540.76	0.77	129.24	0.01	0.99	105.55	25.43	-0.75	42.28	0.19	0.25	26.74
Antcin-98	542.66	1.71	-371	0	1	1.34	23.6	1.38	-7.4	0.16	0.61	19.37
Baharcin	530.66	1.78	-419.1	0.06	0.95	3463.4	26.9	-1.79	67.15	0.24	0.55	41.95

Elacin	542.99	1.63	-324.2	0	1	17.53	26.1	-1.65	63.24	0.13	0.77	12.79
Nermincin	545.68	1.07	-24.39	0.03	0.97	720.31	21.2	2.24	-29.24	0.23	0.66	40.15
Sh9201	437.87	1.61	-419.1	0.06	0.94	3274.4	25.07	0.55	12.66	0.14	0.26	14
Confidence interval	¯x=±18.0						¯x=±0.17					

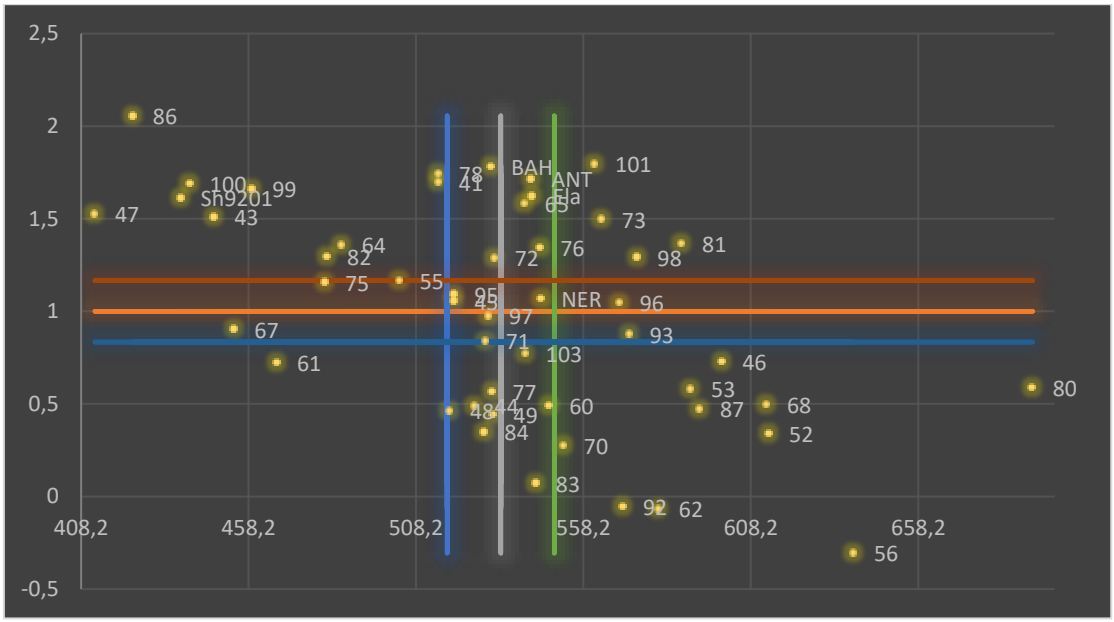

Fig 1 Scattered diagram for GY of popcorn genotypes in three locations

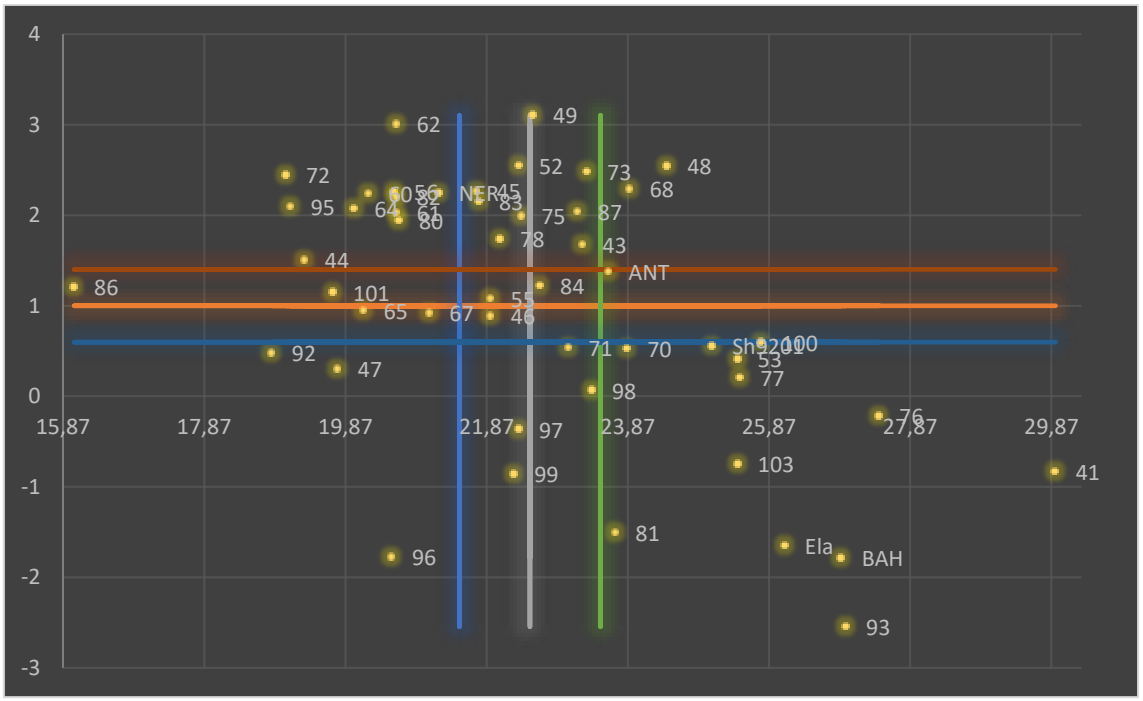

Fig 2 Scattered diagram for PV of popcorn genotypes in three locations

The GY of experimental varied between 4.12 and 6.92 t ha-1. The highest GY of experimental mean was found in TBCM2015-80, TBCM2015-56 and TBCM2015-52 respectively. While the highest of checks for GY is 5.46 t ha-1 (Nermincin), the 30 genotypes gave higher GY than Nermincin (Supplementary Table 3). TBCM2015-43,60,75,78 and 100 genotypes are thought as the most stabile genotypes due to b and R2 values of them are closed 1 and 0. TBCM2015-52, 56 and 52 genotypes giving the highest GY in research have shown good adapt in good environmental condition about GY. According to researchers [28] , TTM2015-56, 87 and 95 genotypes are thought as the most stabile genotypes due to b and R2 values of them are closed 1 and 0 respectively.

Conclusion

As a result of the research, TBCM2015-41, 56, 62, 76, 80 and 93 popcorn hybrids selected for their high yield and quality characteristics. These popcorn hybrids are promising and could be used to meet both producer and consumer demands in the future.

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Supplementary Table 3 Mean grain yield (t ha -1), popping volume (cm³ g-1), unpopped kernel ratio (%) and kernel size (number/10g) obtained from popcorn hybrids across locations

	Genotype	Grain Yield (GY)			Popping volume (PV)			Unpopped kernel (UKR)			Kernel Size (KS)		
		Antalya	Çankırı	Samsun	Antalya	Çankırı	Samsun	Antalya	Çankırı	Samsun	Antalya	Çankırı	Samsun
1	2015-41	3.14	5.85	6.46	28.2	29	32.6	8.4	11.3	36.9	92.3	71.7	67.7
2	2015-43	2.73	5.47	5.24	24.7	27.8	17.2	6.1	23.8	30.0	95.7	65.7	74.3
3	2015-44	4.64	5.08	6.05	24.9	17.7	15.3	5.4	26.1	30.9	76.3	63.3	73.3
4	2015-45	3.90	5.15	6.54	30.5	18.9	15.8	4.1	40.7	33.2	87.7	72.0	64.3
5	2015-46	5.18	6.76	6.05	26.2	19.7	19.9	7.3	28.6	17.1	73.0	56.3	69.3
6	2015-47	2.34	4.98	5.05	22.2	17.7	19.4	6.1	39.4	9.7	91.0	72.0	96.3
7	2015-48	4.72	6.21	4.62	29.2	28	16.1	9.5	14.7	12.0	75.0	64.3	65.3
8	2015-49	4.79	5.50	5.65	25.7	30.4	11.5	11.8	17.4	52.3	83.7	79.3	65.0
9	2015-52	5.79	6.82	5.80	25.3	28.3	13.4	12.3	11.6	18.7	74.7	79.7	77.3
10	2015-53	5.30	7.00	5.41	24.1	28.8	23.4	5.4	11.4	16.2	79.0	60.7	77.3
11	2015-55	3.67	5.71	5.72	19.7	29.1	17.0	7.5	18.8	16.2	81.7	82.0	79.7
12	2015-56	6.75	6.24	6.18	25.8	22.4	13.5	4.3	22.1	18.5	84.3	70.0	76.7
13	2015-60	4.91	5.79	5.74	23.3	24.8	12.5	3.2	15.2	19.9	82.3	73.7	57.3
14	2015-61	3.70	3.85	6.45	23.6	24.5	13.7	6.2	24.4	34.8	89.0	87.0	60.7
15	2015-62	5.92	6.11	5.39	28.2	22.2	11.4	9.2	25.5	16.9	90.7	68.0	75.7
16	2015-64	3.25	5.40	5.93	24.1	22.6	13.3	10.4	14.5	33.2	78.7	71.3	69.7
17	2015-65	3.58	6.54	6.10	23	20	17.4	6.8	44.8	16.4	85.0	131.7	68.0
18	2015-67	3.44	4.61	5.57	22.9	22.2	18.1	4.0	40.5	12.9	95.3	125.0	94.7
19	2015-68	5.55	6.40	6.44	24.8	31.6	15.3	6.0	37.1	18.2	84.7	70.0	79.0
20	2015-70	5.20	5.65	5.72	25	24.4	22.2	11.4	5.2	18.6	83.0	81.0	62.7

21	2015-71	4.28	5.49	6.10	26.2	20.9	22.0	10.5	30.5	29.8	72.3	69.7	56.7
22	2015-72	3.85	6.43	5.67	24.4	21.4	11.3	7.0	20.0	43.7	90.3	81.3	64.3
23	2015-73	3.89	6.51	6.51	26.8	28.3	14.8	4.4	18.0	16.2	88.0	75.0	89.7
24	2015-75	3.47	5.59	5.37	28.4	22.1	16.6	4.8	21.6	8.8	88.7	72.3	90.7
25	2015-76	3.85	5.91	6.60	30.5	22.5	29.3	2.2	17.7	9.2	92.0	74.0	87.7
26	2015-77	4.63	5.44	5.86	28.9	21.7	25.8	9.6	25.6	9.7	78.7	71.3	79.3
27	2015-78	3.13	6.33	5.99	25.1	24.8	16.3	6.5	18.2	7.5	89.0	75.0	91.0
28	2015-80	6.25	7.39	7.13	22.2	26.1	13.6	6.2	28.2	21.2	86.3	56.7	82.3
29	2015-81	4.26	6.45	6.92	19.9	22.9	28.3	7.7	17.3	16.3	85.3	78.0	70.7
30	2015-82	3.35	6.03	5.07	24.1	24.5	13.2	3.0	10.2	30.7	85.0	66.3	59.0
31	2015-83	5.30	4.90	6.12	26.3	24.1	14.9	3.6	21.1	34.2	82.7	65.3	61.7
32	2015-84	4.82	4.89	6.15	24.8	24.5	18.6	8.8	16.8	21.3	92.7	66.3	68.7
33	2015-86	1.90	6.08	4.73	22.6	12	13.5	7.4	43.8	28.8	116.3	70.7	75.3
34	2015-87	5.46	7.02	5.31	27	26	16.5	2.3	24.7	12.1	77.7	60.0	80.3
35	2015-92	5.74	5.44	5.92	25	12.5	19.0	7.3	24.1	12.3	79.7	61.7	76.0
36	2015-93	4.79	7.16	5.21	21.9	23.8	35.2	2.3	25.2	9.0	91.7	74.0	89.7
37	2015-95	3.84	5.01	6.74	22.9	22.2	12.2	3.0	21.8	14.7	81.7	64.0	82.3
38	2015-96	4.53	6.91	5.63	21.6	12.2	27.8	6.9	36.7	7.9	84.0	61.7	77.3
39	2015-97	4.20	6.25	5.45	26.1	15.9	25	2.2	30.8	6.6	85.0	65.7	83.3
40	2015-98	4.31	7.24	5.68	22.1	25.3	22.7	3.0	23.2	27.6	83.7	64.7	63.0

41	2015-99	2.75	6.52	4.51	19.5	22.6	24.7	6.5	17.4	18.8	80.7	67.7	60.3
42	2015-100	2.45	5.57	5.20	23.8	30.7	22.8	7.6	20.4	20.9	80.3	48.0	57.3
43	2015-101	3.59	7.36	5.90	21.7	21.5	15.9	5.1	9.5	25.8	91.7	90.7	69.0
44	2015-103	4.51	5.85	5.87	26.8	20.7	28.8	6.4	17.2	30.4	85.3	80.3	59.3
45	Antcin-98	3.44	6.57	6.27	24.1	28.3	18.4	6.7	21.5	38.2	80.7	60.0	59.7
46	Baharcin	3.20	6.09	6.63	26.7	20.2	33.8	8.7	37.7	3.3	73.3	67.7	84.7
47	Elacin	3.55	6.53	6.21	24.5	21.8	32	6.6	28.3	4.7	78.7	55.7	74.0
48	Nerminci	4.20	5.98	6.19	22.5	28.2	12.9	5.1	37.4	7.0	88.7	70.7	79.3
49	Sh9201	2.55	5.83	4.76	24.1	28.5	22.6	5.4	13.2	14.8	83.7	68.7	61.3
Standards Mean		3.39	6.20	6.01	24	25	24	6	27.6	13.6	81.0	64.5	71.8
Genotypes Mean		4.26	5.97	5.81	25	23	19	6	23.0	21.0	85.3	72.8	73.4
Experiment Mean		4.18c	6.00a	5.83b	25a	23b	19c	6	23.5	20.3	84.8	72.0	73.2
CV (%)		13.2	11.7	10.8	1.06	1.07	1.67	13.08	8.58	10.46	2.54	2.93	2.74
LSD		1.02	1.29	1.62	0.48	0.46	3.89	1.55	3.75	3.68	3.95	3.93	3.68
F		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Research article

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Biodegradation of 3-chloropropionic acid and production of propionic acid by novel isolated fungus *Trichoderma* sp. MF1

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ABSTRACT

Biologically synthesized chemicals have drawn a growing interest recently. The main objectives of the study are to isolate 3-chloropropionic acid (3CP) degrading fungus and to examine its ability to produce Propionic Acid (PA) as a by-product. 3CP is widely used in many pesticide and herbicide synthesis due to biocidal properties but it is toxic and recalcitrant to be removed from the biosphere. Bioremediation approaches through dehalogenation have promised removal of these xenobiotics. Fungi are selected due to its broad enzymatic capacities which is not limited like bacteria. In this research, several fungal isolates have been purified, among the isolates, strain designated as MF1 has shown greater potential in 3CP degradation. Using morphological and molecular approaches, MF1 was identified as *Trichoderma* sp. Amplification of ITS genome region (Accession No. MT126695) revealed that the MF1 isolate had 99% identity to *Trichoderma asperellum* strain AF14. Strain MF1 growth rate is 1.42cm/day on solid medium and it was able to produce biomass up to 0.855g/L in liquid minimal medium supplemented with 10mM 3CP. Whereas the growth in control medium containing 1% glucose has resulted in biomass of 1.814g/L. 90.32% of 10mM 3CP were successfully de-chlorinated within 20 days. Confirmed by HPLC, PA was the major product of dehalogenation with highest concentration of 2.72mM at day 10. Presented data can be used for the designing of by-product extraction. Dehalogenation of 3CP by *Trichoderma asperellum* MF1 have not only been successfully removed xenobiotic pollutant but also have open for new strategy on synthesis of industrial required chemicals.

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Introduction

Propionic acid (PA) is widely used as an additive in animal feed and an intermediate in biodegradable polymers which can potentially replace the petroleum-based polymers. Commercial production of PA is chemical synthesis from petroleum feedstock [1]. Growing demand of this acid draws attention towards a more economical energy efficient technology. 3-chloropropionic acid (3CP) has revealed synthesis of significant by-products of PA.

Various xenobiotic have been used extensively in agriculture for the sustained production. These compounds are highly toxic, recalcitrant and raised public concern as

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they are harmful threat to environment. Microorganisms have been identified with ability of utilizing these halogenated compounds by dehalogenation mechanism [2, 3]. However, biodegradation β-halogenated compounds like 3CP which are widely used in intermediates for pharmaceutical and pesticide productions was limited in bacterial remediation [4-6]. 3CP has been classified as chlorinated aliphatic acids and also known as β-chlorine substituted haloalkanoates. This compound has been identified as a chemical intermediate in many herbicide and chemical fertilizers [7].

Therefore, fungal remediation was proposed in this research as a new approach in bioremediation of 3CP. Our main objective was to isolate 3CP degrading fungi from pesticide exposed soil samples. Microbial dehalogenation usually produces an intermediate which subsequently converted to another form or directly utilized as carbon source. The intermediates serves as carbon source to the organism, if it is not toxic. In present study, we have proven that fungal biodegradation of 3CP have resulted in synthesis of industrial useful co-product PA.

PA is widely used in many industries as additive in animal feed and intermediate in biodegradable polymers. PA is also used in the manufacture of herbicides, chemical intermediates, artificial fruit flavours, pharmaceuticals, cellulose acetate propionate, and preservatives for food, animal feed, and grain. This important industrial substrate is expensively synthesized by chemical reactions. However, our newly isolate *Trichoderma asperellum* MF1 has been proven to synthesize PA as co-metabolic product in dehalogenation of 3CP.

Material and Methods

Sample collection and isolation

Pesticide exposed soil were collected in sterile polythene bags from local oil palm plantation area. Samples were stored at 4°C in a refrigerator for further use. Samples were prepared as described by Parvizpour, Hamid [8].One gram of soil sample was serially diluted and spread on the potato dextrose agar plates supplemented with 10mM of 3CP. All plates were incubated at 30°C for 5 to 14 days. Colonies were purified by transferring the 1 cm^3 of mycelium to a new agar plates.

Growth medium

Minimal media (100 ml) containing 3CP as the only source of carbon and energy was used to incubate the fungul culture. Herein, chloride-free minimal media was prepared in distilled water as 10x concentration basal salts containing $NaH_2PO_4.2H_2O$ (10.0 g/L), $K_2HPO_4.3H_2O$ (42.5 g/L), $(NH_4)_2SO_4$ (25.0 g/L). Trace metals at 10x concentrations: $C_6H_9NO_6$ (1.0 g/L), $MgSO_4$ (2.0 g/L), $FeSO_4.7H_2O$ (120.0 mg/L), $MnSO_4.4H_2O$ (30.0 mg/L), $ZnSO_4.H_2O$ (30 mg/L) and $CoCl_2.6H_2O$ (10 mg/L) were also added [9]. Basal salts (10 ml) and trace metals (10 ml) were added to distilled water (80 ml) and sterilized (121°C for 15 min at 15/psi). 3CP was filter sterilized through a 0.2 μm nylon-membrane and added aseptically to the media. Delta 320 pH meter (AES, Combourg, France) was used to monitor pH of the media and was adjusted to pH 5.5 ±0.2 using sodium hydroxide and/or hydrochloric acid. Fungal isolates were incubated at 30°C in a rotary incubator with agitation at 200 rpm. 1% of Glucose replaced 3CP as carbon source in control sample.

Morphological and molecular characterization

Selected isolate was grown in minimal medium with 10mM of 3CP to characterize their morphology. Purified isolate was identified based on fruiting bodies and spores viewed under light microscope after staining with lactophenol blue. The genomic DNA of the fungal isolate was prepared by modified methods of [10]. Internal Transcribed Space (ITS) regions of rRNA genes were amplified using upstream primer of ITS1 – 5' TCCGTAGGTGAACCTGCGG 3' and downstream primer of ITS4 – g' TCCTCCGCTTATTGATATGC 3'. PCR conditions are detailed in (Table 1). A phylogenetic tree was constructed using MEGA 6.0 software through Neighbour-Joining bootstrap method. The ITS partial sequence of top blast results (Accession numbers are shown in the figure 3) from NCBI and other different species from same genus were selected to investigate the evolutionary relationship.

Table 1 ITS rRNA PCR amplification conditions of *Trichoderma asperellum* MF1.

Steps	Cycles	Temperature (ºC)	Time (minute)
Initial denaturation	1	95	5
Denaturation	34	95	1
Annealing		55.5	2
Extension		72	2
Final extension	1	72	10
Hold	1	4	∞

Biodegradation of 3CP

Isolate grown in 100 mL of minimal medium adjusted to pH 5.5; supplemented with 10mM 3CP, at 30°C and 200rpm. The depletion of 3CP was detected by chromatography analysis (HPLC) using C18 column (4.6 x 150mm, 5µM) under wavelength of 210 nm. Samples were separated using an isocratic elution with a mobile phase containing potassium sulphate (20 mM) to acetonitrile at ratio of 60:40, in deionised water. The retention time of 3CP was 1.254 min at flow rate of 1.0 ml/min flow rate and 30°C column temperature. PA also confirmed through similar chromatography method detected at 1.190 min.

Growth Analysis

The growth was measured by determination of biomass produced, recovered by filtration using Whatman No. 1 filter paper (No. 1). Dehalogenation was supported with detection of free halide release by modified method of Bergmann and Sanik [11].

Results

Morphological and molecular characterization

In this study, MF1 isolate have shown a high growth rate (1.42 cm/day) on solidified minimal medium with 3CP (Fig. 1 a and b). Morphological characteristics of the strain MF1 showed that the isolate are among the genus *Trichoderma.* Specie of *Trichoderma* are mainly distinguished from other isolates through their conidiophores (Fig. 1 c).

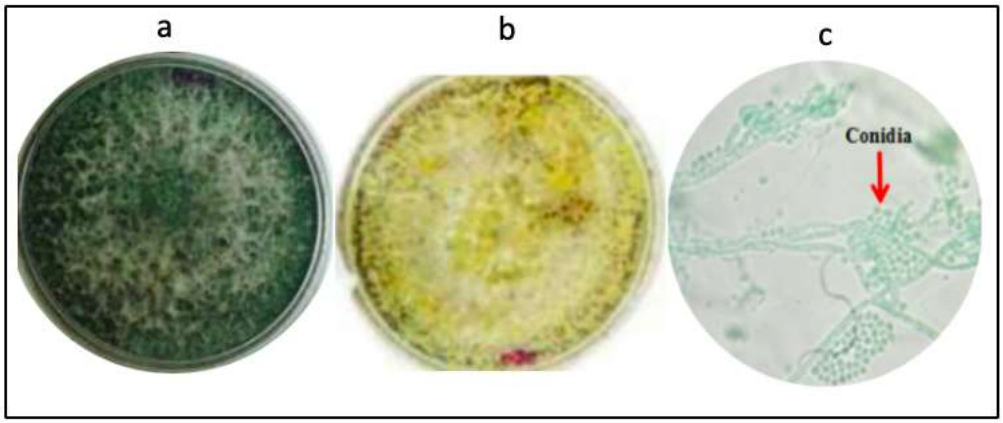

Fig 1 Morphological analysis of MF1. (a) Grown on PDA agar, (b) on solidified minimal medium supplemented with 10mM 3CP. All plates incubated at ambient temperature for 14 days, (c) shows the conidiophores of *Trichoderma* species MF1.

Based on staining result, MF1 classified as a *Trichoderma* species. Further confirmation was done through genetic identification by the amplification of the ITS gene (Fig. 2).

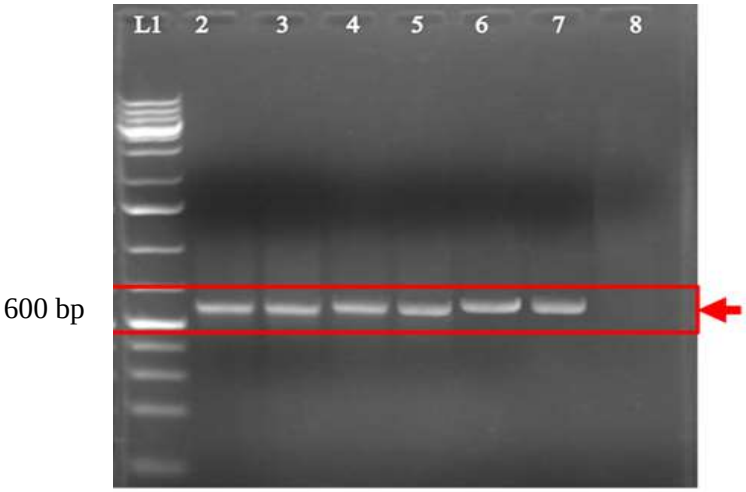

Fig 2 The amplified ITS region for *Trichoderma* strains. Lane 1: DNA ladder; Lane 2: strain MF1; Lane 3: strain MF2; Lane 4: strain MF3; Lane 5: strain MF4; Lane 6: strain MF5 and Lane 7: strain MF6. Lane 8: negative control.

Approximately 600 base pairs of the amplified ITS region was obtained by aligning the forward and reverse sequences. Phylogenetic relatives to the corresponding isolates were identified by using BLASTn online analysis tool. The isolate showed 99% similarity to *T. asperellum* strain AF14 (JX677934.1), the phylogenetic tree is shown in Fig. 3.

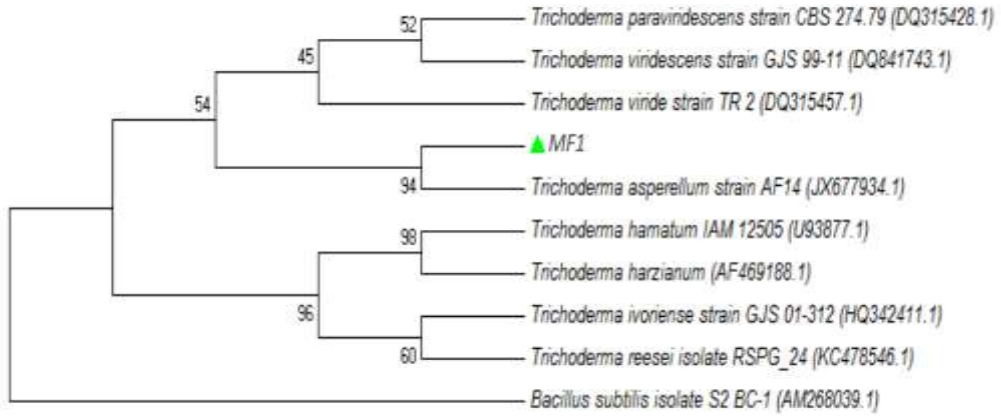

Fig 3 Phylogenetic tree showing the evolutionary relationship of isolate MF1 (Accession No. MT126695) with representative members from the genus of *Trichoderma*. The sequences were obtained from GenBank database. The tree was constructed by the neighbour-joining method using MEGA 6.0 software

Biodegradation of 3CP

Under optimized conditions, the harvested *Trichoderma asperellum* MF1 spores were subjected to growth in 10mM 3CP liquid minimal medium. MF1 produced biomass up to 0.855g/L. Whereas, in control cultures supplemented with 1% glucose as carbon source, MF1 produced biomass of about 1.814g/L (Fig. 4). This indicates the limited growth under treatment with 3CP. Increasing free chloride ions detected together with biomass have proven that there was dehalogenation of 3CP (Fig. 5).

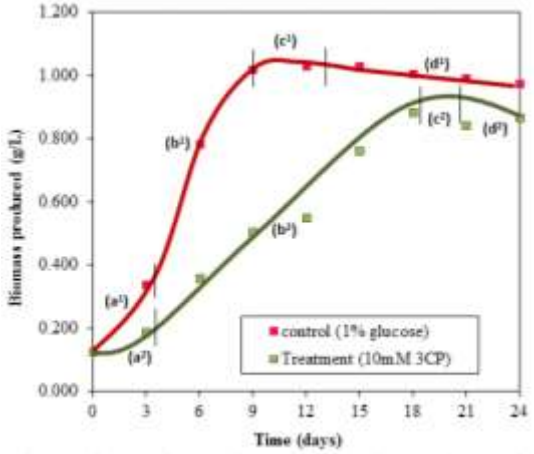

Fig 4 Growth of *Trichoderma asperellum* MF1 in presence and absence of 3CP. **$a^{1\&2}$:** lag phase; **$b^{1\&2}$:** log (exponential) phase; **$c^{1\&2}$:** stationary phase; **$d^{1\&2}$:** death phase. Growth determined through dry cell weight of biomass collected on the corresponding time.

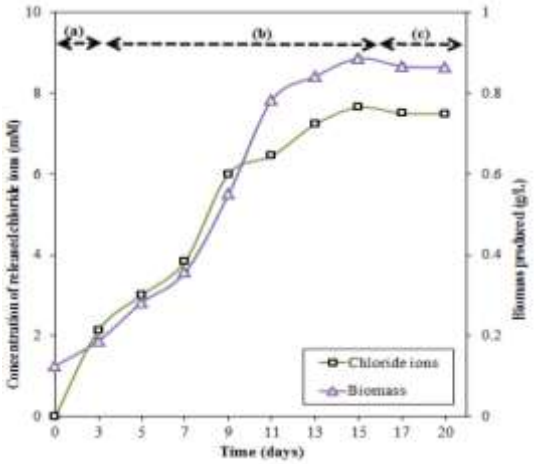

Fig 5 Dechlorination performance by *Trichoderma* strain MF1. Triplicate experiments conducted with liquid medium supplemented with 3CP at final concentration of 10mM.

Production of PA

Samples were further analysed by HPLC methods. The liberation of chloride ion almost corresponds with the disappearance of 3CP from the cultivation medium. Maximum chloride has been released through dehalogenation was 3.41mM/mL in 20 days of

incubation. About 90.32% of 3CP was disappeared from the cultivation medium. It is expected to be successfully oxidised by fungal enzymes in 20 days of treatment period. HPLC analysis also detects synthesis PA. Concentration of PA detected by HPLC reached 2.72mM at day 10 and increased to 3.77mM at day 20 of incubation (Fig. 6). Increasing concentration indicates this product was accumulated in the medium and not utilized by the fungal cell.

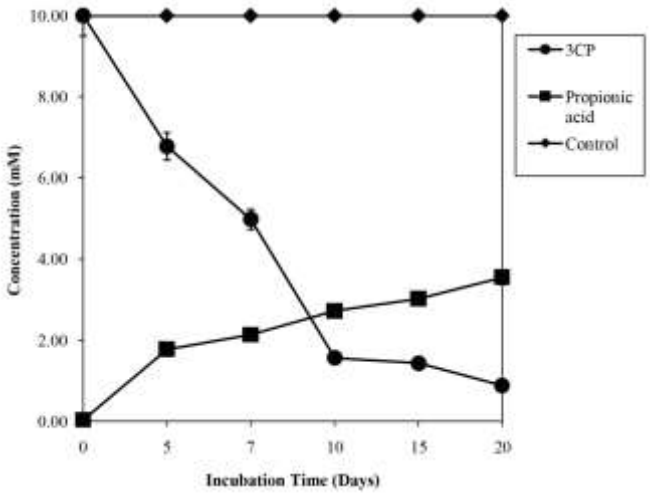

Fig 6 Disappearance of 3CP in culture medium and accumulation of PA in the culture medium.

Discussion

Bacterial remediation of β-chloro substituted organic acids such 3CP is restricted to certain groups only. This is due to the compound toxicity and recalcitrance, created by the position of halogen [7]. In current investigation, PCR amplification of ITS gene had proven that 3CP tolerant and degrading fungal isolates shared more than 99% to *Trichoderma* and *Aspergillus* species. These widely occurring organisms are of considerable environmental and biotechnological importance due to their broad metabolic diversity and array of unique enzymatic capabilities [12].

Trichoderma species are the most common fungi that were successfully isolated. Morphologically, spores formed through asexual reproduction are exposed and not enclosed within membrane such as in *Aspergillus* and *Penicillium*. Different from *Aspergillus*, the conidia of isolates form globular clusters and branched like in *Penicillium*. However, the absence of phialides, the branching tips was the key for

microscopic identification of *Trichoderma*. They also have branched conidiophore [13, 14].

Trichoderma species are the most ubiquitous saprophytic component, sustain for longer time even in increased pollution conditions [8, 15]. They are capable of synthesising various kind of enzymes which play a significant role in biotic and abiotic stress tolerance, hyphae growth, and plant growth enhancer as well as in pollutant degradation potential. Hamzah, Zarin [15] reported the degradation of up to 40% of total petroleum hydrocarbon (TPH), 100% of pristane and 74% of phytane compounds by *Trichoderma* species in 9 days of incubation at optimal physical and nutrient parameters.

There are four basic criteria which must be fulfilled in order for a given halogenated compound to be utilized by an organism as sole source of carbon and energy. Firstly, the organism must be adaptive to the concentration toxicity of supplemented halogenated compound. Secondly, the organism must either possess or synthesize dehalogenase in response to the halogenated compound which is capable of removing the substituent halogen(s) from the compound. Thirdly, the dehalogenation product should be non-toxic and easily converted to an intermediate of the organism's central metabolic pathway. Finally, the intermediate of halogenated compound should be able to enter cell either passively or by active transport in order to reach the site of central metabolic activity[16].

3CP degradation potential was previously described in *Trichoderma* sp. Parvizpour, Hamid [8] reported the dehalogenation of 3CP and the synthesis of 3- hydroxypropionic acid (3HP) as the by-product. 3HP is non-toxic carbon source which can be easily absorbed and utilized [4]. Fungal dehalogenation is different where most saprophytic fungi are equipped with lignin modifying enzymes which is non-substrate specific and able to breakdown various pollutants [17, 18].

In this study, we have proven that the accumulation of 3CP in fungal mycelium has resulted in synthesis of a reductive dehalogenase enzyme. The enzyme replaced the chloride ion with a hydrogen atom through reductive dehalogenation which is different from bacteria where it involving substitution of hydroxyl ion through hydrolytic dehalogenation [4].

Accumulation PA also proven with the biomass produced. One of the applications of PA in food industry is to delay the growth of mould and bacteria [19]. Thus, it has inhibitory

effect on *Trichoderma asperellum* MF1 and this explains the difference in maximum biomass produced in control and treatment cultures.

Conclusion

In conclusion, it is shown that biodegradation of environmental pollutants (dehalogenation of 3CP) by *Trichoderma asperellum* MF1 is producing an industrial important PA as the co-metabolites. Thus, breaking down of xenobiotic and producing non-toxic and industrially important intermediates.

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Araştırma makalesi / Research article

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Salvia officinalis L. Etanol Ekstresinin Antikolinerjik ve Antioksidan Aktivitesi ve LC-MS/MS Analizi

Mesut Işık[1]*

ÖZET

Tıbbi bitkiler, yeni ilaç gelişiminde kullanılabilecek önemli fenolik ve flavonoid bileşikleri içerdiklerinden dolayı birçok biyolojik aktiviteye sahiptir. Bu yüzden bitkilerin fenolik içeriğinin biyoaktivitelere katkısının bilinmesi önem arzetmektedir. Bu çalışmada, *Salvia officinalis* L. etanol (SOLE) ekstresinin fenolik içeriği LC-MS/MS ile belirlendi. Bu ektrenin *in vitro* olarak, antikolinerjik ve antioksidan (metal indirgeme, radikal ve lipit peroksidasyon giderme) aktiviteleri materyal metotta belirtilen yöntemlerle belirlendi. The SOLE ektresi AChE enzimi üzerine (IC_{50}: 0.136 mg / ml) inhibisyon etkisi göstermiştir. Dahası, bu bitki ektresi % 17 oranında DPPH radikal giderme aktivitesi gösterirken, % 22 oranında ABTS radikal giderme aktivitesi göstermiştir. The SOLE (10 µg/mL) ektresi lineloik asit oksidasyonunu % 24 inhibe etmiştir. Bu çıktılar oksidatif strese karşı bu bitkinin antioksidan savunma sistemlerine katkıda bulunduğunu göstermektedir. Sonuç olarak, asetilkolinesteraz inhibitör etkisi ve antioksidan aktiviteleri nedeniyle önemli biyoaktiviteye sahip olan bu tıbbi bitki ateroskleroz, Alzheimer gibi birçok hastalıkların tedavisinde kullanılabilir.

MAKALE GEÇMİŞİ
Geliş
10 Şubat 2020
Kabul
22 Mart 2020

ANAHTAR KELİMELER
Adaçayı
fitokimyasal analiz
antioksidanlar
asetilkolinesteraz
lipit peroksidasyonu

Anticholinergic, Antioxidant Activity and LC-MS/MS Analysis of Ethanol Extract from *Salvia officinalis* L.

ABSTRACT

Medicinal plants have many biological activities because they contain important phenolic and flavonoid compounds that can be used in new drug development. Therefore, it is important to know the contribution of phenolic content of plants to bioactivities. In this study, the phenolic content of *Salvia officinalis* L. ethanol (SOLE) extract was determined with LC-MS/MS. The anticholinergic and antioxidant (metal reduction, radical and lipid peroxidation removal) activities of the extract *in vitro* were determined by methods specified in the material method. The SOLE extract showed an inhibition effect on AChE enzyme (IC_{50}: 0.136 mg / ml). Moreover, the plant extract showed 17 % DPPH radical removal activity, while 22 % ABTS showed radical removal activity. The SOLE extract (10 µg / mL) inhibited lineloic acid oxidation by 24 %. The results showed that this plant contributes to antioxidant defense systems against oxidative stress. As a result, the medicinal plant, which has significant bioactivity due to its acetylcholinesterase inhibitory effect and antioxidant activities, can be used in the treatment of many diseases such as atherosclerosis, Alzheimer's.

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Giriş

Tıbbi bitkilerin birçoğu, farmakolojik olarak biyoaktif maddeler içermektedir. Son yıllarda, bitkilerde bulunan doğal biyoaktif bileşiklerin insan sağlığına olan faydalarından dolayı geleneksel olarak kullanılan tıbbi bitkilere ilgi artmıştır [1]. Buna ek olarak, bitki içeriğinde bulunan fenolik bileşikler, lipit peroksidasyonunu azalttıkları için gıdaların raf ömrünün uzatılmasında rol oynamaktadır [2, 3]. Gıda endüstrisinde kullanımı ile olası toksisiteleri ve kanserojen etkileri nedeniyle kısıtlanan sentetik antioksidanların yerine doğal antioksidan kaynaklı bitki kullanımına ilgi her geçen gün artmaktadır [4]. Bu nedenle, biyoaktif içeriğe sahip tıbbi bitkilerin araştırılması önem arzetmektedir.

Lamiaceae familyası dünya çapında yaklaşık 230 cins ve 7100 türden oluşmaktadır. Bu familyadan birçok türün tıp, gıda ve kozmetik gibi birçok alanda kullanıldığı ve uçucu yağ içeriği nedeniyle yüksek önem taşıdığı düşünülmektedir. Salvia, Menthe ve Siderites Lamiaceae familyasına ait bazı büyük cinsler arasındadır [5]. Salvia (adaçayı), geleneksel tıp ve gıda alanında kullanım amaçlı yetiştirilmektedir [6]. Adaçayı içeriğinde bulunan bileşikler antioksidan, antimikrobiyal, sitotoksik, antimutagenik, antikanser antienflamatuar, antifungal ve lipit peroksidasyonunun önlenmesi/azaltılması gibi etkilere sahip olduğu için çeşitli hastalıkların tedavisinde kullanılmaktadır [7, 8]. *S. officinalis*'in tedavi amaçlı kullanılma nedeninin içeriğindeki polifenoller olabileceği bildirilmiştir [9]. Bu yüzden aromatik bitkilerin hangi fitokimyasal içeriklerinin ne tür biyoaktivitelere sahip olabileceği araştırma konusu olmuştur [10].

Nörodejeneratif bir hastalık olan Alzheimer hastalarında 4 aylık bir süre boyunca sabit bir dozda (60 damla/gün) *Salvia officinalis* L. (SOL) ektresinin etkisine bakılmıştır. Bu çalışma sonucunda hafif ve orta derecede Alzheimer hastalığı tedavisinde SOL ektresinin etkili olabileceği bildirilmiştir [11].

Bu çalışmada, Akdeniz bölgesinde yetişen SOL etanolik ekstresinin radikal giderme ve metal indirgeme aktivitesinin yanı sıra asetilkolinesteraz aktivitesi üzerine etkisi araştırıldı. Ayrıca bu ektrenin fenolik içerik analizi LC-MS/MS yöntemi ile belirlendi. Fenolik içerik enzimatik inhibitör etkiler (asetilkolinesteraz) ve antioksidan aktivite ile ilişkilendirildi.

Materyal ve Metot

Ektraksiyonun hazırlanması

Gölgede kurutulduktan sonra değirmen içinde toz haline getirilen 1 g *S. officinalis* L. (SOL) 20 mL etanol içerisine eklendi, oda koşullarında 6 saat boyunca manyetik karıştırıcı ile karıştırıldı. Elde edilen ekstreler, Whatman No.1 kâğıdı ile süzüldü. Etanolik ekstreler falkon tüplerine konuldu ve kullanılana kadar –20 °C'de saklandı.

LC-MS/MS yöntemi ile *S. officinalis* L. etanol ektresinin içerik analizi

25 fenolik bileşiğin LC-MS/MS analizleri, bir tandem MS cihazına bağlanmış bir Nexera modeli Shimadzu UHPLC kullanılarak gerçekleştirildi. Kromatografik işlemlerin yürütülmesinde LC-30AD ikili pompalar, DGU-20A3R degazör, CTO-10ASVP kolon fırını ve SIL-30AC otomatik örnekleyici bulunur. Kromatografik ayırma işlemi, bir C18 intersil ODS-4 (3.0mm x 100mm, 2µm) analitik kolon üzerinde gerçekleştirildi. Kolon sıcaklığı 40 °C'ye sabitlenmiştir. Elüsyon gradyanı mobil faz A (Su, % 0,1 Formik asit) ve mobil faz B (Metanol, % 0,1 Formik asit)'den oluşturuldu. Çözücü akış hızı 0.3 mL / dakikada tutuldu ve enjeksiyon hacmi 2 µL olarak ayarlandı.

Antioksidan aktivite yöntemleri

FRAP demir iyonlarını (Fe^{3+}) indirgeme analizi

SOL ekstresinin indirgeme gücü Oyaizu yönteminin modifiye edilmiş şekliyle gerçekleştirildi [12, 13]. Farklı konsantrasyonlardaki SOL ekstresine (10, 20, 40 ug / mL) 2.5 mL fosfat tamponu (0.2 M, pH 6.6) ve 2.5 mL %1'lik potasyum ferrisiyanit [$K_3Fe(CN)_6$] ile karıştırıldı. Karışımlar 50°C'de 20 dakika inkübe edildi. İnkübasyondan sonra her karışıma trikloroasetik asit (2.5 mL, %10) ve $FeCl_3$ (0.25 mL, %0.1) ilave edildikten sonra santrifüj edildi (10 dakika boyunca 3.000 rpm'de). 700 nm'de karışımın absorbans değerleri kaydedildi.

Cu^{++} iyonu indirgeme kapasitesi (CUPRAC)

Cu(II)-Nc'nin Cu(I)-Nc şelatına indirgenmesine dayanan Cuprac metodu uygulandı [14]. Test tüpüne, 1 mL neocuprin'e (2,9-dimethyl-1,10-phenanthroline), 1 mL $CuCl_2$ (0.01 M) çözeltisi ve 1 mL amonyum asetat (NH_4Ac) tampon çözeltisi ilave edildi ve vortekslendi. Daha sonra farklı konsantrasyonlarda (10, 20, 40 µL) ekstreler ilave edildi ve toplam hacim su ile 4 mL'ye tamamlandı. 30 dakika oda sıcaklığında inkübasyondan sonra, absorbans değişimi 450 nm'de kaydedildi. Karışım içerisinde gerçekleşen reaksiyonla artan absorbans Cu^{++} iyonu indirgeme kapasitesinin arttığını göstermektedir.

DPPH$^{\bullet}$ giderme aktivitesi

SOL ekstresi ve standart antioksidanların DPPH$^{\bullet}$ serbest radikal giderme aktivitesi Blois metodu ile gerçekleştirildi [15]. Etanol içerisinde 0.1 mM DPPH$^{\bullet}$ çözeltisi hazırlandı ve bu çözeltinin 1 mL'si ile stok çözeltilerden (10, 20, 40 µL) alınan örnek karıştırıldı ve etanol ile 3 mL'ye tamamlandı. Bu çözeltiler vortekslendi ve karanlıkta 30 dakika inkübe edildi. Absorbans değişimi 517 nm'de spektrofotometre ile ölçüldü. Reaksiyon karışımının düşük absorbans vermesi, daha yüksek radikal giderme aktivitesinin göstergesidir.

ABTS$^{\bullet+}$ giderme aktivitesi

Bu metot, renkli ABTS$^{\bullet+}$ katyon radikalinin ektre ile muamele edilmesi sonucunda renk değiştirmesi esasına dayanır [16]. ABTS$^{\bullet+}$ katyon radikali, ABTS (2 mmol L^{-1}) çözeltisi ile 2.45 mmol L^{-1} potasyum persülfat ($K_2S_20_8$) çözeltisinin karıştırılarak karanlıkta ve oda sıcaklığında 14 saat inkübasyonu ile hazırlandı. ABTS$^{\bullet+}$ katyon radikalini kullanmadan önce, radikal çözeltisi 734 nm'de 0.750 ± 0.025'lik bir absorbans elde edene kadar sodyum fosfat tamponu (0.1 mol L^{-1}, pH 7.4) ile seyreltildi. Daha sonra ekstresi hazırlanan stok çözeltilerinden 10, 20, 40 µL alınarak üzerine hacmi 3mL oluncaya kadar fosfat tamponu eklendi. Üzerlerine hazırlanan 1 mL ABTS$^{\bullet+}$ çözeltisi eklenerek vortekslendi. 734 nm'de radikal giderme aktivitesi ölçüldü.

Linoleik asit peroksidasyonu giderme aktivitesi

Linoleik asit peroksidasyon giderme aktivite tayini ferrik tiyosiyanat metoduna göre belirlendi [17]. Bu metodun esası, linoleik asit oksidasyonu sonucu oluşan hidroperoksitin spektrofotometrik olarak 500 nm'de ölçülmesine dayanır. Yüksek absorbans, peroksidasiyon sonucu oluşan peroksit miktarının fazlalığını gösterir. Oluşan hidroperoksit ise Fe^{2+}'yi Fe^{3+}'e yükseltger. Daha sonra Fe^{3+}, ilave edilen tiyosiyanat ile kompleks oluşturarak 500 nm'de maksimum absorbans verir. İstenilen miktarlara karşılık gelen konsantrasyonlarda stok çözeltilerden (1mg/ml) vezin kaplarına otomatik pipetlerle pipetlendi ve hacim tampon çözeltiyle 2,5 mL'ye tamamlandı. Daha sonra her bir vezin kabına 2,5 mL linoleik asit emülsiyonu ilave edildi. Kontrol olarak 2,5 mL tampon çözelti ve 2,5 mL linoleik asit emülsiyonu kullanıldı. İnkübasyon 37 $^{\circ}$C'de gerçekleştirildi. Her altı saatte bir vezin kaplarından 100'er µL alındı ve 4,7 mL etanol bulunan deney tüplerine konuldu. 100 µL Fe^{2+} çözeltisi daha sonra da 100 µL SCN^{-} çözeltisi ilave edildi.

Kör 4,8 mL etanol bulunan deney tüpüne 100 µL Fe^{2+} ve 100 µL SCN^- çözeltilerinin ilavesiyle hazırlandı. Numunelerin 500 nm'deki absorbansları köre karşı okundu.

Antikolinerjik aktivitesinin belirlenmesi

Çalışmada, SOL etanol ekstresinin asetilkolinesteraz (AChE) enzimi üzerindeki inhibe edici etkisi Ellman spektrofotometrik metodu ile test edilmiştir [18]. Kısaca, 50 µl 5,5′-dithio-bis(2-nitro-benzoic)acid (DTNB), 100 µl Tris–HCl tampon (1 M, pH 8.0) ve 50 µl AChE ($5.32x10^{-3}$ U) içeren reaksiyon çözeltisi 15 dakika boyunca 30 °C'de karıştırılarak inkübe edildi. Son olarak reaksiyon, substrat olarak kullanılan 50 µl acetylthiocholine iodide (AChI) ilave edilerek başlatıldı. Substratın enzimatik hidrolizi, 412 nm'de spektrofotometrik olarak kaydedildi ve sonuç IC_{50} değeri olarak verildi [19, 20].

Sonuçlar ve Tartışma

Birçok çalışma, polifenol ve flavonoidlerin antioksidan, antimikrobiyal, antitümör gibi biyolojik aktivitelerdeki rolünü ortaya koymuştur. Bu biyoaktiviteler, aslında bileşiklerde bulunan fonksiyonel gruplara bağlıdır. Yapılan biyo-güdümlü araştırmalar, yan etkisi olan sentetik bileşiklerden ziyade onlara alternatif doğal fenolik içerikli gıdalara yöneltilmiştir [10]. Yapılan bir çalışmada fenolik ve flavonoidlerin antioksidan aktivitesinin genellikle bileşiklerin kimyasal yapılarına ve hidroksil gruplarının dağılımına bağlı olabileceği bildirilmiştir [21]. Diğer bir çalışmada, süperoksit radikalinin üretiminde önemli rol oynayan ksantin oksidazın flavonoidler tarafından inhibe edilebileceği gösterilmiştir [22, 23]. Flavonoidlerin protein kinaz, aldoz redüktaz, miyeloperoksidaz, NADPH oksidaz, fosfolipaz, lipooksijenaz gibi enzimlerin aktivitesini etkilediği de bilinmektedir. Fenol ve flavonoidlerin enzimler üzerine etkisi ve antioksidan özellikleri bunların birçok farmakolojik özelliklerini açıklamaktadır [10]. Fenolik hidroksil gruplarının sayısı serbest radikal giderme aktivitesinde önemli rol oynamaktadır [24, 25]. Kuersetin gibi aromatik orto pozisyonda hidroksillenmiş bileşiklerin reaktif oksijen türlerine (ROS) karşı anti-oksidan aktiviteye sahip olduğu gösterilmiştir [26]. ROS oluşumunda önemli rol oynayan H_2O_2, orto-kinol gibi fenolik bileşikler tarafından indirgenerek orto-kinon ve suya dönüştürülür [27]. Dolayısıyla, fenolik bileşikler hücresel biyomolekülleri oksidatif hasara karşı koruyabilir ve bu nedenle oksidatif strese bağlı dejeneratif hastalık riskini azaltabilir [28]. Adaçayı içeriğindeki rosmarinik asitin lipid peroksidasyonunun önlenmesinde etkili olduğu bildirilmiştir. Adaçayı ayrıca toplam

antioksidan aktiviteye katkıda bulunabilecek flavonoid ve diğer fenolikleri de içermektedir [4, 6].

LC-MS/MS yöntemi ile analiz edilen SOL'un fenolik bileşik kompozisyonu standart olarak tanıtılan fenolik bileşiklerle değerlendirildi. Tablo 1'de görüldüğü gibi vanilik asit, silymarin, kafeik asit, resveratrol ve luteolin miktarı diğerlerine (fumarik asit, gallik acid, bütein…) nispeten yüksek çıktığı tespit edilmiştir.

Tablo 1 Adaçayının (*Salvia officinalis* L.) fitokimyasal fenolik bileşik içeriğinin LC-MS/MS yöntemi ile kantitatif olarak belirlenmesi

Standart bileşikler	ESI IYON MOD	MRM	LOD/LOQ (µg L^{-1})	RT	R^2	Regresyon denklemi	Konsantrasyon (µg L^{-1})
Kuersetin	neg	301,1>151	22.5/25.7	6,091	0,999	Y=(13,7831)X+(-146,951)	ND
Asetohidroksamik asit	pos	76,10>43,10	2.8/8.2	1,986	0,999	Y = (150,982)X + (23,1833)	ND
Kateşin hidrat	neg	291,10>139,00	8.2/11.4	4,958	0,999	Y = (79,2933)X + (-2406,22)	ND
Vanilik asit	pos	168,80>93,00	125.5/142.2	6,026	0,998	Y = (48,0522)X + (-876,904)	10818,231
Resveratrol	pos	229,10>135,00	9.0/13.6	5,713	0,998	Y = (46,4361)X + (-1314,61)	589,313
Fumarik asit	neg	115,20>71,00	25.2/31.3	3,674	0,999	Y = (20,2986)X + (-762,592)	482,415
Gallik asit	neg	169,20>125,00	0.90/1.6	4,134	0,999	Y = (65,3835)X + (-2699,84)	427,359
Kafeik asit	neg	179,20>135,00	6.3/10.7	5,283	0,996	Y = (124,785)X + (-487,132)	702,146
Phloridzin dihidrat	neg	435,00>273,10	61.0/207.0	5,646	0,999	Y = (33,4069)X + (-1396,90)	ND
Oleuropein	neg	539,10>377,20	0.05/1.0	5,643	0,999	Y = (25,9240)X + (-558,916)	ND
Hidroksisinnamik asit	neg	163,20>119,00	8.7/16.1	5,738	0,995	Y = (13,1516)X + (717,421)	ND
Ellagik asit	neg	300,90>145,10	0.101/0.333	5,895	1,000	Y = (5,25903)X + (-1167,31)	ND
Mirisetin	neg	317,10>150,90	55.4/59.6	5,858	0,999	Y = (37,0934)X + (2684,23)	ND
Protokatekuik asit	neg	181,20>108,00	30.3/35.4	5,875	0,994	Y = (526,954)X + (23026,1)	ND
Silymarin	neg	481,00>301,00	0.5/1.2	5,978	0,995	Y = (31,9969)X + (-1823,79)	3146,396
2-hidroksi-1,4 naftakinon	neg	173,20>144,90	0.5/1.5	6,058	0,997	Y = (203,469)X + (29033,1)	ND
Bütein	neg	271,10>135,00	22.7/28.6	6,084	0,999	Y = (49,3543)X + (367,917)	26,438
Naringenin	neg	271,10>150,90	5.4/6.4	6,104	0,996	Y = (317,241)X + (33733,3)	
Luteolin	neg	285,20>132,90	0.5/2.5	6,190	0,998	Y = (34,6668)X + (3721,79)	6397,142
Kaempferol	neg	285,10>116,90	206.6/214.3	6,288	0,999	Y = (2,63905)X + (-206,494)	ND
Kurkumin	neg	367,00>149,00	0.1/0.75	6,516	0,997	Y = (227,706)X + (-10111,1)	ND
Thymoquinone	neg	164,20>149,00	1.5/4.5	6,632	0,999	Y = (60,4553)X + (2285,92)	ND
Alizarin	neg	239,20>210,90	65.2/77.5	6,800	0,998	Y = (3,97487)X + (1614,23)	ND
Hidroksibenzoik asit	neg	137,20>93,00	30.5/40.25	6,130	0,999	Y = (735,804)X + (-498,102)	ND
Salisilik asit	neg	137,20>93,00	4.2/7.6	6,104	0,999	Y = (746,369)X + (6072,41)	ND

Çoklu reaksiyon izleme (MRM), Alıkonma zamanı (RT), R^2: determinasyon katsayısı, LOD/LOQ (µg/L): Belirleme sınırı/Tayin sınırı, N.D: not detected

Yapılan bir çalışmada, HPLC ile belirlenen kafeik asit, karnosol ve toplam tanımlanmış fenolikler, uygulanan üç antioksidan test ile anlamlı ($p < 0.05$) korelasyon göstermiştir. Luteolin ve ABTS testi arasında pozitif anlamlı korelasyon saptandı ($r = 0.50$). Ferulik asit ($r = 0.60$), karnosik asit ($r = 0.83$), metil karnosat ($r = 0.62$) ve hispidulin ($r = 0.49$) FRAP testi ile anlamlı korelasyon göstermiştir. Korelasyonlardaki bu farklılıkların, çok çeşitli fenolik bileşiklerin yapısındaki farklılıklardan kaynaklanabileceği öne sürülmektedir [29]. Araştırmacılara göre, fenolikler ile antioksidan aktivite arasındaki önemli korelasyonlar, bitki ekstrelerinde bulunan fenolik bileşiklerin toplam antioksidan kapasitesine katkı sağlayabileceği hipotezini destekleyebilir. Çeşitli çalışmalar, bitki ekstrelerinde bulunan rosmarinik asidin toplam antioksidan kapasitesine önemli katkı sağlayabileceğini göstermiştir [7, 30].

Bu çalışmada, SOL ekstresinin metal indirgeme gücü FRAP ve CUPRAC yöntemi ile araştırılmıştır. Tablo 2'de görüldüğü gibi, Demir ve bakır indirgeme aktivitesinin (FRAP and CUPRAC) troloksa yakın çıkması antioksidan kapasitesinin yüksek olduğunu göstermektedir. Bu SOL ektresi 0.2 mg/ml konsantrasyonda yaklaşık % 22 ABTS radikal giderme aktivitesi gösterirken, % 17 DPPH radikal giderme aktivitesi göstermiştir. Tablo 2'de gösterildiği gibi, bu bitki ektresi orta derece radikalleri giderme kapasitesine sahiptir. Bu çalışmada, SOL'un içeriğinde bulunan vanilik asit, silymarin, kafeik asit, resveratrol ve luteolin gibi fenolik bileşiklerin radikal giderme ve metal indirgeme kapasitesine önemli katkı sağlayabileceği söylenebilir.

Asetilkolinesteraz (AChE) enziminin aşırı aktivitesi ile kolinerjik sistemde nörotransmitter asetilkolin hidrolizi artar. Bu durum Alzheimer hastalığı gelişimine sebep olmaktadır. Alzheimer hastalığının semptomatik tedavisinde kullanılan AChE inhibitörlerinin hücreleri oksidatif hasardan koruduğu bilinmektedir. Bu hastalık için kullanılan bazı AChE inhibitörlerinin kardiyovasküler hastalarda anjiyogenezin artmasında rol oynadığı da vurgulanmıştır [31-33].

Tablo 2 de gösterildiği gibi SOLE ektresi AChE üzerine inhibisyon etkisi göstermiştir (IC_{50}: 0,136 mg/ml, R^2: 0,965). Yapılan birçok çalışmada flavonoid ve fenolik bileşiklerin anti-asetilkolinesteraz aktiviteye sahip olduğu bildirilmiştir. Örneğin, yapılan bir çalışmada, ellagic asidin tirozinaz ve asetilkolinesteraz üzerine güçlü inhibitor etkisinin olduğu bildirilmiştir [34]. Fenolik bileşiklerin nörokoruyucu etkilerinden dolayı Alzheimer hastalığının tedavisinde önemli bir rol oynayabilirler [35, 36]. Günümüzde

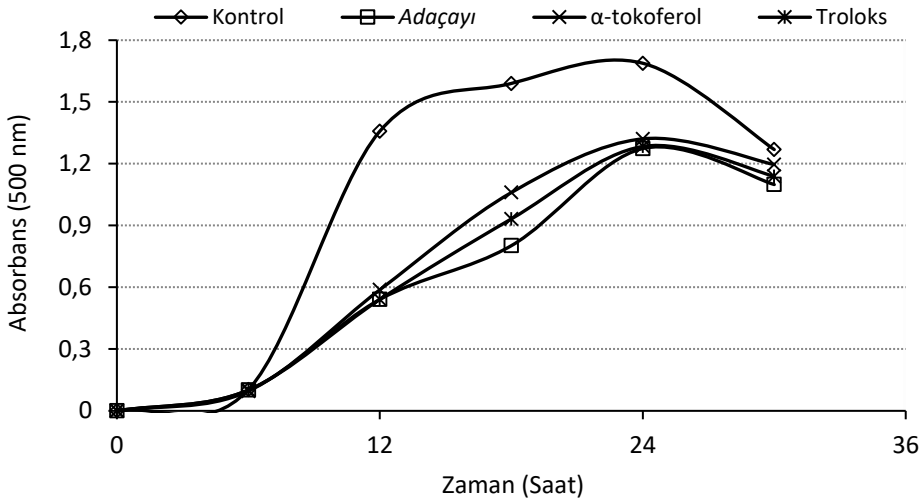
yaygın kullanılan donepezilin, ellagic asitten yaklaşık 10000 kat daha güçlü AChE inhibitör etkisine sahip olduğu bilinmiştir [37]. Bu çalışmada, LS-MS/MS içerik analizine bakıldığında ellagic asit tespit edilmemiştir. SOL'un içeriğinde bulunan vanilik asit, silymarin, kafeik asit, resveratrol, luteolin ve diğer fenolik asitlerin AChE üzerine inhibisyon etkisinin olabileceği sonucuna varılabilir.

Tablo 2 *Salvia officinalis* L. ektresinin radikal giderme, metal indirgeme aktivitesi ve AChE üzerine inhibisyon etkisi

Antioksidanlar	DPPH[a] (0,2 mg/ml)	ABTS[a] (0,2 mg/ml)	FRAP Testi[b] (0,2 mg/mL)	CUPRAC Testi[b] (0,2 mg / mL)	AChE inhibisyonu	
					IC_{50} (mg/ml)	R^2
S. officinalis L.	17,601±1,674	22,153±1,945	0,183±0,015	0,438±0,029	0,136±0,014	0,965
BHA	71,819±5,462	83,668±7,674	0.454±0,032	-		
BHT	46,329±3,647	48,353±3,648	0.624±0,041	-		
Troloks	81,188±7,423	80,063±7,064	0.252±0,018	0,516±0,037		

Standart antioksidanlar (BHA, bütillenmiş hidroksianisol; BHT, bütillenmiş hidroksitoluen, troloks)
[a]Değerler yüzde radikal giderme aktivitesi olarak ifade edildi
[b]Değerler absorbans olarak ifade edildi. Yüksek absorbans, yüksek metal indirgeme kapasitesini gösterir

Ferrik tiyosiyanat metodu ile lineloik asit peroksidasyon giderme kapasitesi en çok kullanılan antioksidan parameterlerden biridir. Ferrik tiyosiyonat metodu, lipit peroksidasyonu boyunca meydana gelen peroksitin miktarını ölçer. Bu analizde, hava oksijeni tarafından oksitlenen ve emülsiyonda linoleik asitin peroksidasyonu sonucu oluşan hidroperoksitler dolaylı olarak ölçülür [17, 33]. Çalışmamızda, SOL ekstresinin (10 µg/mL) zamanla lipit peroksidasyonunu inhibe ettiği gösterilmiştir (Şekil 1). SOL, troloks ve a-tokoferolün lineloik asit oksidasyonu üzerine inhibisyon etkisi 24. saatte sırasıyla yaklaşık %24, %24 ve %22 olarak bulunmuştur. Sonuçlarımız, bitki içeriğinde bulunan fenolik bileşiklerin lipit peroksidasyonunu önleyebileceğini göstermektedir. Yapılan bir çalışmada, BHT (10-400 µg/mL aralığında) doza bağımlı olarak lineloik asit oksidasyonunu % 34-49 aralığında inhibe ederken, *Ocimum basilicum* etanolik ektresi ise aynı dozda % 12-29 aralığında inhibe etmiştir [38]. Yukarıdaki çalışmada 10 µg/mL'de *Ocimum basilicum* etanolik ektresi lineloik asit oksidasyonunu % 12 inhibe ederken, çalışmamızda ise SOLE ektresi % 24 inhibe etmiştir.



Şekil 1 Standart antioksidanlar (α-tokoferol ve troloks) ve *Salvia officinalis* L. etanol ekstresinin (10 µg mL^{-1}) lineloik asit peroksidasyonu giderme aktivitesi

Sonuç olarak, SOL'un birçok fenolik bileşik içerdiği ve bu içeriğinden dolayı önemli düzeyde antikolinerjik ve antioksidan özellik (metal indirgeme, radikal ve lipit peroksidasyon giderme) sergilediği gösterilmiştir. Bu araştırma, doğal olarak zengin antioksidan kaynağa sahip bu bitkinin oksidatif strese bağlı gelişen birçok hastalığın önlenmesin/tedavisinde kullanılabileceğini gösterebilir.

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Research article

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Study of Chemical Composition and Antimicrobial Properties of Rosemary (*Rosmarinus Officinalis*) Essential Oil on *Staphylococcus Aureus* and *Escherichia Coli in vitro*

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ABSTRACT

Nowadays, with increasing bacterial resistance, finding new antimicrobial agents to replace herbal remedies with less side effects than conventional drugs has provided. The aim of this study was to investigate the chemical composition and antimicrobial properties of Rosemary (Rosmarinus officinalis) essential oil on Staphylococcus aureus and Escherichia coli *in vitro*. In this experimental study, rosemary was prepared from Tabriz city and the essential oil was extracted after drying by water distillation using Clevenger apparatus. The essential oil components were identified by Gas chromatography mass spectrometry (GC-MS). Essential oil of 10, 5, 2.5, 1.25, 0.625 and 0.312% of essential oil was used to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Based on the results of GC-MS analysis, 19 compounds were identified, of which 1.8 Cineole and α-pinen had the highest essential oil composition. MIC and MBC of essential oils were obtained on S. aureus (0.625 and 1.25%) and E. coli (1.25 and 2.5%), respectively. The results of this study showed that rosemary essential oil has remarkable antibacterial activity and can be used as a suitable replacement for synthetic antibiotics.

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Introduction

The use of herbal compounds for the treatment of diseases is an old practice in different parts of the world, especially in developed countries. In the past century, there has been a growing focus on medicinal plants with antimicrobial properties that can resolve common problems with the use of antibiotics for reasons such as the increased use of chemical drugs and the increasing resistance of bacteria to these substances [1, 2]. However, most plant species with medicinal properties remain unexplored and there is still much time left to discover new and valuable plant sources [3, 4]. Medicinal plants

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are one of the important sources of antimicrobial agents in different countries [1]. About 60% to 90% of the population in developing countries use herbal remedies [5]. As such, plants can be considered as a source of potentially useful chemicals that are only partially exploited. These potentially useful chemicals can be used not only as medicines but also as unique starting points for the production of pharmaceutical analogues as well as an interesting tool to better understand biological phenomena [6, 7]. Rosemary, scientifically named Rosmarinus officinalis, belongs to the Lamiacea family and is known as a spice and medicinal plant in many countries. It has antibacterial, antioxidant and antifungal effects and is native to the Mediterranean and Asian regions [8, 9]. Rosemary contains oleoresin and tannins, pinene, camphor, bornyl acetate, etc. Rosemary also contains large amounts of salicylates [10, 11]. In traditional medicine, this herb is used for anti-asthmatic, digestive, sedative, relieving, headache, circulatory disorders, visual acuity, anti-rheumatic and irritant effects [12, 13]. Numerous pharmacological effects including antioxidant effect, growth factor stimulation, antimicrobial and antiviral activity and inhibition of hepatotoxicity have been reported for this plant [14-17].The aim of this study was to investigate the antimicrobial properties of Rosemary essential oil on S. aureus and E. coli in laboratory conditions.

Materials and Methods

Extraction and essential oil making

In this *in vitro* study, plant samples were collected from natural areas of Tabriz's greenhouses. After identification and approval of the scientific name by Herbarium Natural Resources of Agriculture Jihad, the samples were dried in a large, convenient space and prepared for grinding. Essential oil extraction was performed using Clevenger apparatus. The essential oil was dehydrated with sodium sulfate and passed through a 0.45 μm microfilter and stored in a dark glass container at 4 ° C until identification and determination of the chemical constituents.

Analysis of essential oil constituents using GC-MS

The constituents of essential oils were identified using Retention indices and mass spectra of the compounds and compared with standard mass spectra and valid references. To do this, the prepared sample of the essential oil was first injected into the gas chromatography apparatus and the most suitable temperature programming of the column was obtained

for complete separation of the essential oil compounds. The essential oil was then injected into a gas chromatograph coupled to a mass spectrometer and the mass spectra of the compounds were obtained.

Bacterial strains

Standard strains were used to evaluate the antibacterial effects of rosemary essential oil. These strains, including S. aureus ATCC 25923 and E. coli (ATCC 25922) were obtained from the University of Tehran Microbial Collection. The strains were then identified using culture media and biochemical tests.

Determination of MIC and MBC

One hundred µl of Mueller Hinton Broth was poured into the wells and then 100 µl of essential oil was added to the first well of each row. After mixing the contents of the first well, 100 µl of it was removed and added to the next well. Discard 100 µl of the final well. A control row was assumed for each experiment row (corresponding to one essential oil). The dilution steps of the essential oils were carried out in the same test sequence. Then 100 µl of the bacterial suspension (1.5 106 cfu / ml) was added to each well, but not to the control well. Microdilution method was used to perform this test. Finally, they were incubated at 37 ° C for 24 hours. The presence of turbidity (compared to the control row) indicates bacterial growth and the transparency indicates that the bacteria are not growing. Then, all wells in which bacterial growth was observed were sampled and cultured on MBC plate. Plates were then incubated for 24 hours at 37 ° C. Plates in which bacterial not growth was visible were considered MBC. Each experiment was repeated 5 times to reduce the error of the experiment.

Results

According to the chemical analysis of plant essential oils by GC / MASS, 19 compounds were identified in rosemary that comprised 96.57% of the essential oil. The essential oils were 1.8 Cineole (21.8%), α-pinen (18.7%), Camphor (14.6%), Linalool (13.4%) and Camphene (7.2%), respectively. The results of MIC and MBC of rosemary essential oil by microdilution broth on S. aureus and E. coli are presented in Table 2. The results showed that rosemary essential oil has antimicrobial effects. In general, based on the results of this study, it can be said that Gram-positive bacteria are more susceptible to essential oils than Gram-negative bacteria.

Table 1 Chemical compounds of essential oils of Rosemary

No	Compounds	Percentage of compounds (%)
1.	1,8 Cineole	21.8
2.	α-pinen	18.7
3.	Camphor	14.6
4.	Linalool	13.4
5.	Camphene	7.2
6.	Borneol	5.2
7.	limonene	4.9
8.	Verbenone	3.5
9.	Myrtenole	2.4
10.	Myrcene	2.05
11.	Verbenone	1.02
12.	α-Thujene	0.7
13.	B-Pinen	0.41
14.	Trephine	0.32
15.	3-Carene	0.14
16.	P-Cymenene	0.1
17.	Camphonelal	0.08
18.	Iso-Pinocamphone	0.05
Total Identified Constituents 96.57		

Table 3 MIC and MBC of Rosemary essential oil on E. coli and S. aureus (in percent)

Extract concentration / Bacteria	MIC	MBC
Staphylococcus aureus	0.625	1.25
Escherichia coli	1.25	2.5

Discussion

Rosemary essential oils are a combination of esters, aldehydes, alcohols, ketones and terpenes, which are classified into two groups of major and minor constituents [18]. The main constituents make up about 85% of the essential oils, the quantity and quality of the essential oils vary with the climate, soil composition and age of the plant [19]. In this study, 19 compounds of rosemary essential oil were identified which comprised 96.57%

of the essential oil. The essential oils were 1.8 Cineole (21.8%), α-pinen (18.7%), Camphor (14.6%), Linalool (13.4%) and Camphene (7.2%), respectively. Malakootian and Hatami identified 20 compounds in 2013 with rosemary essential oil analysis, of which 82.99% included 1.8 Cineole, Borneo, -pinen α, Camphor, Linalool, Camphene, limonene and Verbenone [20]. In 2010, Zaouali et al., isolated 25 compounds from the rosemary essential oil analysis. This is inconsistent with the present study because the time and stage of plant picking, plant age, soil type, climate, and the essential oil extraction method are effective on the effective constituents of the plant [21].Okoh et al., in South Africa introduce Verbenone as the main rosemary compound [22]. Linares et al [23], santoyo et al [24] and Angioni et al [25]obtained similar results to the present study. Malakootian and Hatami, by examining non-growth halo at 32 µg of essential oil, showed that non-growth halo was higher than non-growth halo gentamicin, penicillin, streptomycin and erythromycin. MIC and MBC in this study were 3,000 and 3,200, respectively. The time required for the complete destruction of Escherichia coli was 25 minutes [20]. Nascimento et al., Studying the antimicrobial effects of rosemary essential oil on different bacteria, showed that the rate of growth inhibition of this essential oil on S. aureus was 18 mm [26]. In a 2007 study by Fu et al., The antimicrobial effects of rosemary essential oil showed that the growth rate of this essential oil on S. aureus was 18 mm [27]. Other studies have reported the effects of rosemary essential oil on gram-positive S. aureus and B. cereus bacteria [28, 29]. In a study of rosemary essential oils in 2018, ahmady-asbchin and Mostafapour showed that the plant has antibacterial activity against E. coli, S. aureus, S. epidermidis, E. faecalis and P. mirabilis, this property varies depending on the essential oil and the bacterial sex. The highest effect was on P. mirabilis and the least on E faecalis [30]. Soltan Dallal et al. Examined the antimicrobial effects of rosemary essential oil by disk diffusion and dilution methods on methicillin-resistant S. aureus, the diameter of the non-growth halo was 20 mm and MIC and MBC were 40.1 and 81.2 mg/ml, respectively [31]. Comparing these results, it can be said that the effect of rosemary essential oil was more than the extract. Mashreghi M, Momtazi studies of the antimicrobial effects of the alcoholic extract of rosemary on E. coli O157 showed that the extract had little effect in the early stages of bacterial growth and its effects on bacterial growth and proliferation [32]. Antimicrobial effects of rosemary essential oils on bacteria and fungi indicate effective antimicrobial activity of these essential oils. In one study, it

was shown that the MIC of rosemary essential oil on Propionibacterium acne was 0.56 mg / ml (14) [24].

Conclusion

Although the present study provides information on the chemical constituents and antibacterial effects of rosemary essential oil, due to time and financial constraints, the effects of all chemicals of this essential oil on different types of microbes have not been possible, Therefore, Researchers need to take a holistic view of this topic. In general, the results of *in vitro* studies showed that the essential oil of this medicinal plant contains various compounds that can have antibacterial activity against E. coli and S. aureus. However, clinical trials on patients after consuming rosemary essential oil are recommended to confirm this data so that it can eventually be made available to patients in the category of medicinal plants.

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Research article

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Photosynthetic-Related Properties of Oil Palm Leaves Treated with Different Amount of Fertilizer

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ABSTRACT

Oil palm trees need suitable amount of fertilizer for improvement of growth and productivity. Different oil palm planting materials would require different amount of nitrogen-potassium (NK) fertilizer. Nitrogen promotes growth of plant leaves while potassium helps overall function of oil palm including formation of sugar and starch. This study focused on the determination of photosynthetic-related properties including the amount of soluble sugar, starch and chlorophyll from six planting materials of oil palm indicated as A,B,C,D,E and F. Palms were supplemented with different levels of fertilizer; T1 for control, T2 and T3. In this study, we observed that the application of the fertilizer at T2 and T3 levels for planting materials A, B, and C resulted in the increased amount of total soluble sugar compared to control, T1. The total starch content and the total chlorophyll content fluctuated between treatments and types of planting materials, hence no clear trend was observed. These results could be used to estimate the behaviour or biochemical responses of oil palm from different planting materials towards different levels of NK fertilizer.

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Introduction

Oil palm is one of the main commodity in Malaysia with over 19.5 million tonnes of crude palm oil produced from a planted area of 5.8 million hectares in 2018. The management of this huge industry requires holistic integration in the aspect of socio-economic and environmental sustainability [1]. To cater the needs of environmental protection and at the same time achieving high productivity in oil palm growth and yield production, effective fertilizer management is crucial. Suitable type and amount of fertilizer will help to boost the growth of plants besides preventing plant toxicity and pollution problems [2]. Nutrient deficiency in oil palm can be seen through the leaf color and other plant growth morphology. Nitrogen is one of the main macronutrients, and naturally limiting element for plant growth. Nitrogen fertilizer is important for growth of

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oil palm, promotes green color and helps to avoid leaf yellowing. As the main component in amino acids synthesis, application of fertilizer containing nitrogen will help plant development, hence increase crop yields [3, 4].

Nitrogen can improve the yield of oil palm by 49% in the presence of high potassium rate, similar as 25% yield of oil palm can be gained in response to potassium when high nitrogen rate was applied [5]. The fertilizer also enhances photosynthesis and photo-protection of plant, which help in high yield production. However, excessive amount of nitrogen, especially nitrate, gives rise to nitrogen pollution of soils and underground waters [6]. Nitrogen over-fertilization will also cause certain nutrients deficiencies, which lead to a reduction in plant growth, crop quality, and potentially lead to nitrogen loss due to soil erosion, leaching below the root zone rather than being absorbed effectively by the plant [6]. Besides nitrogen, potassium is another element that is important for oil palm functions, including the formation of sugar and starch. Hence, a recommended fertilizer level for optimum growth of oil palm could be referred in operating the plantation [7].

For planters, quality of seeds is also important to ensure good yield production. Hence, choosing the right planting material from the begining is crucial. Different progenies might have different requirements for nutrients, dissimilar efficiencies in nutrient uptake and utilization of resources [8]. Therefore, selection of high yielding planting materials with sustainable and affordable fertilization is preferred. To date, the optimum amount of NK fertilizer required vary greatly among planting materials.

Therefore, this study mainly focused on the effect of different amount of fertilizer on oil palm photosynthetic-related properties of the leaves including soluble sugar, starch, and chlorophyll content

Methodology

Plant Samples

Leaves samples from four-year old oil palm planting materials, designated as A, B, C, D, E and F, were collected from an oil palm plantation in Johor, Malaysia. These plants were supplied with different level of NK fertilizer indicated with T1 as control, T2 as the first level amount of NK and T3 as the second level of fertilizer (doubled amount of NK in T2).

Determination of soluble sugar content

Mixture of 0.5 g leaf samples and distilled water was made in 15 ml conical tube and the volume was made up to 10 ml with distilled water. The mixture was vortexed and incubated for 10 minutes. Anthrone, 0.2 g was dissolved in 100 ml of 95% sulphuric acid. Standard curve was obtained by using sucrose as the standard solution for sucrose quantification. The centrifugation of ground dry sample and distilled water was carried out for 10 minutes at 3400 rpm. The supernatant was gained by performing a filtration. The mixture of 4 ml sample and 8 ml anthrone reagent was incubated for 5 minute in a water bath at $100^{O}C\cdot$ An absorbance reading was obtained by a spectrophotometer (UV160U) at wavelength of 620 nm. The results were reported in mg sucrose per gram dry sample using sucrose standard curve plotted before [9].

Determination of starch content

The homogenization of 0.5 g dry leaf samples was carried out in hot 80% ethanol. After 5 minute of sample centrifugation at 5000 rpm, the residue was obtained. Addition of 6.5 ml of 52% perchloric acid and 5 ml of distilled water into residue was performed. Centrifugation of solution and supernatant separation were carried out. The supernatant was filtered using a filter paper. The filtration was continued till 100 ml of supernatant was obtained. 1 ml of mixture for a test tube was obtained from addition of 100 µl (0.1 ml) of supernatant to distilled water. Then 4 ml of anthrone reagent were added into the test tube. The mixture was heated for 8 minutes in $100^{o}C$ water bath then cooled at room temperature. Absorbance reading was obtained from spectrophotometer (UV160U) at wavelength 630 nm. The results were reported in mg glucose per gram dry sample using starch standard curve plotted before [9].

Determination of chlorophyll content

Leaf samples of 0.5 g were ground in 10 ml of 80% (v/v) acetone using a mortar and pestle. Addition of 0.5 mg calcium carbonate was carried out to avoid the emergence of pheophytin and the filtration of the extract was performed using a filter paper. The samples were washed continuously till they turn colorless. Extract combination, 20 ml was obtained, and the absorbance for chlorophyll (A and B) was recorded at 663 nm and 645 nm respectively [10]. The obtained chlorophyll (A and B) was calculated with formulas as below:

Total chlorophyll: 20.2(A645) + 8.02(A663) (1)

Chlorophyll A: 12.7(A663) - 2.69(A645) (2)

Chlorophyll B: 22.9(A645) - 4.68(A663). (3)

Statistical analysis

The experiments were conducted in triplicates and the data obtained were analyzed using Microsoft Excel and IBM SPSS Statistics 20.0 software.

Results and Discussion

Total soluble sugar

Total soluble sugar content in the leaves of planting materials A-F were determined (Figure 1). The results showed that, except for planting materials B, D and E, there were no significant difference in soluble sugar content detected in other planting materials when supplied with different levels of fertilizer.

In planting material B, soluble sugar produced was 148 % and 150 % higher than T1 for T2 and T3, respectively. Planting material D had 28 % less soluble sugar in T2 compared to T1. On the other hand, planting material E showed fluctuated level of total soluble sugar where the amount was significantly decreased 47% compared to control and then increased 50 % from T1 to T3.

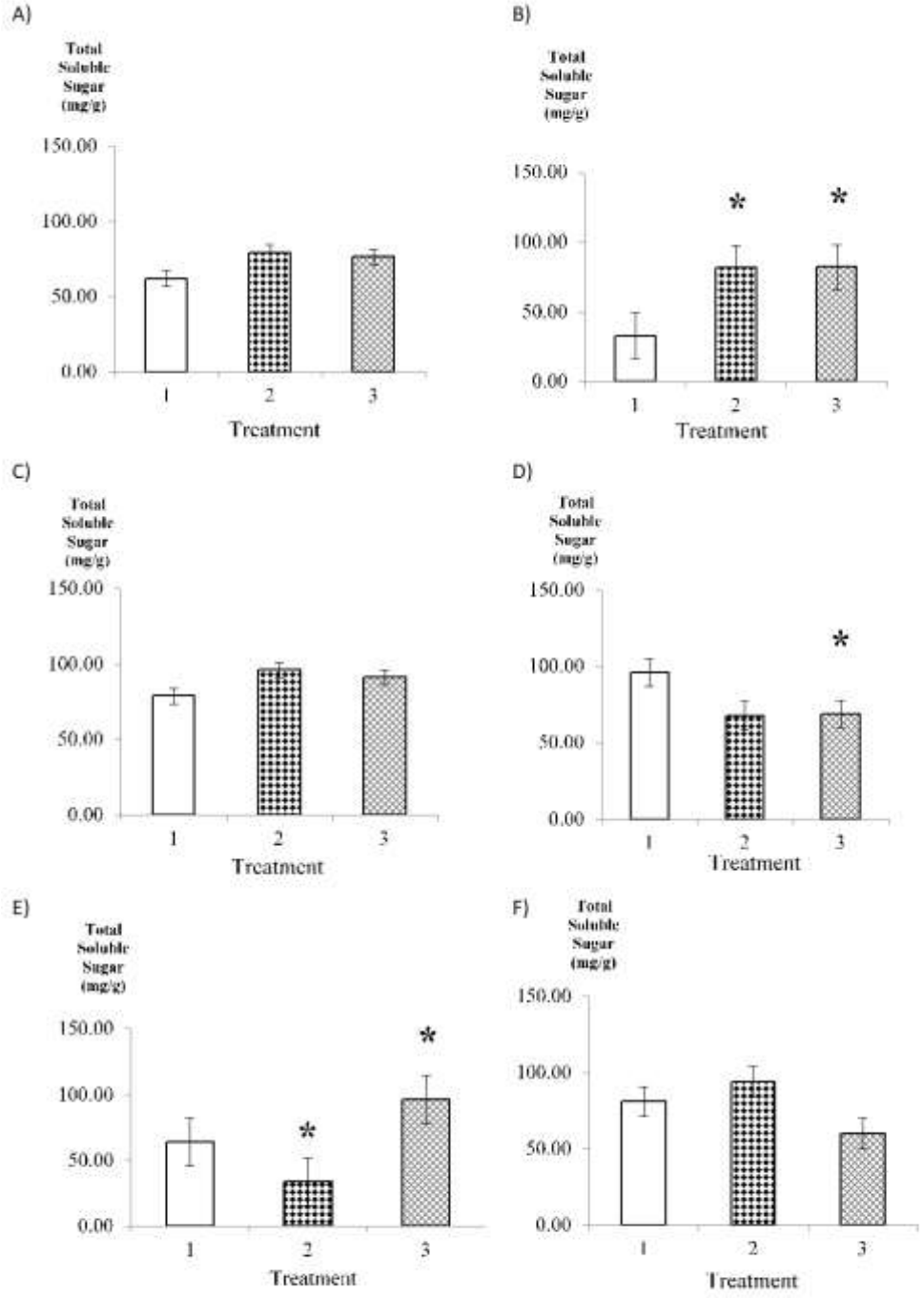

Fig 1 Total soluble sugar content in the leaves of oil palm. (A-F): Planting material A-F treated with different amount of fertilizer. Data are mean ± standard error, n=3. (* indicates significant difference in total soluble sugar content compared to control using t-test; $p<0.05$)

Soluble sugars supply carbohydrates from source to organs. They have important functions in plants, especially as metabolic resources, cell structural constituents and

signalling compounds related to plant development. Among the processes are plant flowering, photosynthesis, and other activities which ultimately influence growth and yield production [11,12,13]. Photosynthesis will be enhanced by lower level of sugar. Whereas at high level of sugar, plant growth and carbohydrate storage will be promoted [13]. Several genes coding for photosynthetic enzymes were influenced by the levels of soluble sugars in plant cells [14]. Generally, nutrient deficiencies will decrease sugar concentrations, however this is also depends on plant species and other environmental factors. In our study, different planting materials react dynamically towards diferent levels of fertilizer as shown in their soluble sugar production.

Total starch content

We analyzed the starch content in the leaves of oil palm (Figure 2). As shown in the results, only planting material B had 34 %, significantly lower starch content in the leaves of oil palm at T3 compared to T1. Starch content decreased with higher NK fertilizer for this planting material, although the decrease at T2 was not significant. Other planting materials (A,C,D,E and F) supplemented with different levels of NK fertilizer did not show any significant differences.

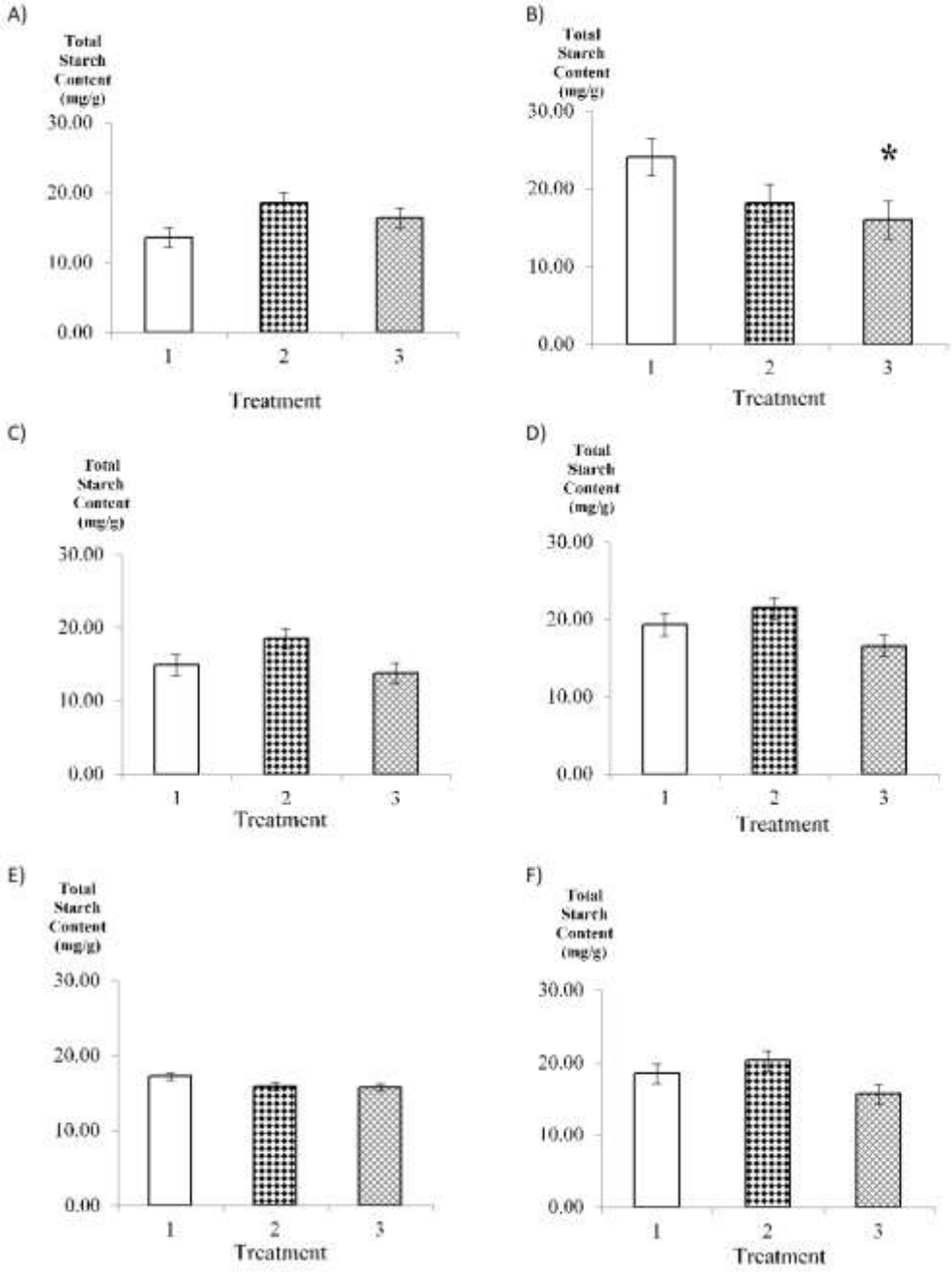

Fig 2 Total starch content in the leaves of oil palm. (A-F): Planting material A-F treated with different amount of fertilizer. Data are mean ± standard error, n=3. (* indicates significant difference in total starch content compared to control using t-test; $p<0.05$)

Plants store carbohydrate mainly in the form of starch. Hydrolysis of starch will lead to an increase in soluble sugar content [15]. When carbohydrate is mobilized, for instance during pruning, the photosynthetic capacity of oil palm reduces [16]. Inhibition of leaf

photosynthesis also observed when starch accumulated [17]. We did not found a clear trend on starch content in the leaves of different oil palm planting materials when treated with different levels of fertilizer. A study conducted previously on potato starch and sugar content showed that fertilizer supplied only gave significant effects to starch content at 1% probability level, but insignificant effect to soluble sugar content [18]. The level of starch and soluble sugar content also influenced by the type of fertilizer applied. Song et al., 2016 reported that organic fertilizer application caused starch accumulation and increased of soluble sugar content in tobacco plants [19].

Total chlorophyll content in oil palm leaves

Chlorophyll content in plant leaves is another photosynthetic-related parameter that have been determined in this study (Figure 3). The results showed that chlorophyll content in planting material B and D showed significant responses when supplied with slightly higher level of fertilizer. The chlorophyll content dropped significantly at T2 and slightly increased at T3, which were insignificant for both planting materials. The total chlorophyll content for T2 was 43% lower than T1 in planting material B, while the same treatment gave 50 % lower level of chlorophyll content in planting material D when compared to control.

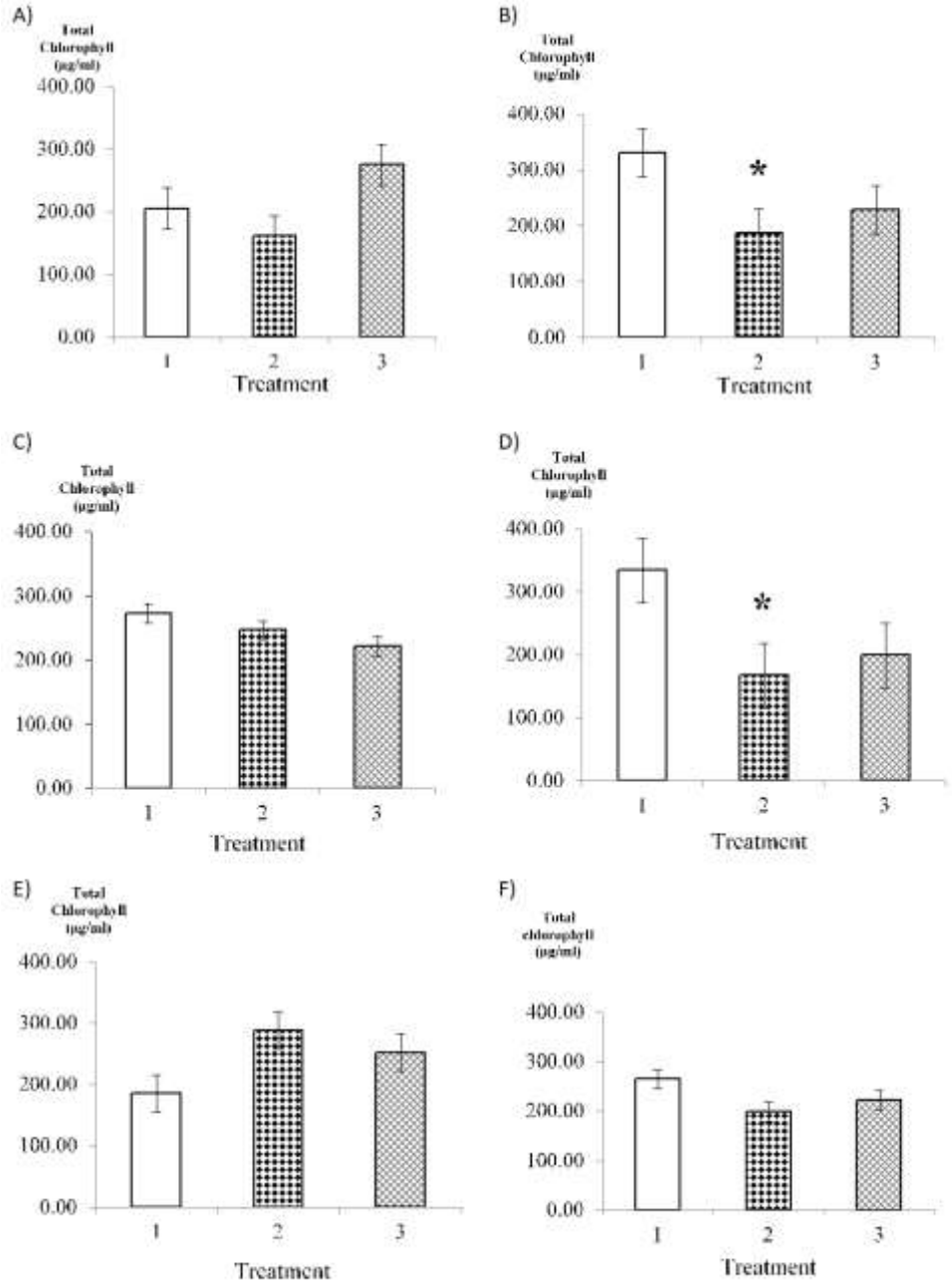

Fig 3 Total chlorophyll content in the leaves of oil palm. (A-F): Planting material A-F treated with different amount of fertilizer. Data are mean ± standard error, n=3. (* indicates significant difference in chlorophyll content compared to control using t-test; $p<0.05$)

Chlorophyll content in plants normally influenced by nutrients and light availability besides plant chemical metabolites. Chlorophylls are crucial to plants as they are required for photosynthesis. A study conducted previously on oil palm showed that nitrogen fertilizer did not increase leaf chlorophyll content although the stomatal density increased [20].

Conclusion

Photosynthetic-related properties of oil palm leaves from different planting materials supplemented with different amount of fertilizer were analyzed. Differed responses of each planting material were observed by which no clear trend was recorded in the total soluble sugar, starch content and chlorophyll content in the oil palm leaves. This preliminary findings suggest that further work is required to understand the behaviour of various oil palm planting materials towards chemical fertilizer application.

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Derleme makale /Review article

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Bitki gelişimini Teşvik Eden Rizobakteriler Tarafından Üretilen Metabolitler ve Bitki Gelişimine Etkileri

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ÖZET

Kuraklık, tuzluluk, ağır metaller, sıcaklık gibi abiyotik faktörler bitki gelişimini olumsuz etkilemekte ve bitkisel verimin azalmasına neden olmaktadır. Bu stres faktörleri bitki morfolojisi ve fizyolojisi üzerinde olumsuz etkilere sahiptir. Bitkiler bu stres faktörlerinden korunmak için, bazı savunma mekanizmalarını kullanırlar. Bitki metabolizması üzerinde önemli etkiye sahip oldukları bilinen küçük moleküller olan metabolitler, bitkilerin strese karşı uyarılmasında da önemli bir rol oynamaktadır. Son yıllarda yapılan çalışmalarda rizobakteriler tarafından üretilen oksin, sitokinin, gibberellin, etilen ve absisik asit gibi metabolitlerin strese karşı konakçı toleransı yaratmada önemli olduğu gösterilmiştir. Ayrıca, rizobakteriler tarafından üretilen bu metabolitler; bitki gelişimini teşvik etmiş, mineral fosfatın ve diğer besin maddelerinin çözünmesinde, strese karşı direncin arttırılmasında, toprak agregatlarının stabilizasyonunda ve toprağın organik madde içeriğinin iyileştirilmesinde yardımcı olmuştur. Bu derlemede, bitkilerin stres toleransını indükleyen rizobakteriler tarafından üretilen metabolitler ile ilgili yapılan çalışmalar özetlenmiştir.

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ANAHTAR KELİMELER
Bitki gelişimini teşvik,
stres faktörleri,
abiyotik faktörler,
rizosfer,
metabolit

Metabolites by Plant Growth Promoting Rhizobacteria and Their Effects on Plant Grown

ABSTRACT

Drought, salinity, heavy metals, abiotic factors such as temperature adversely affect plant growth and cause a decrease in plant yield. These stress factors have negative effects on plant morphology and physiology. To avoid these stress factors; plants use some defense mechanisms. Metabolites, which are small molecules known to have significant effects on plant metabolism, also play an important role in stimulating plants against stress. Recent studies have shown that metabolites such as auxin, cytokine, gibberellin, ethylene and absicic acid produced by rhizobacteria are important in creating host tolerance to stress. In addition, these metabolites produced by rizobacteria; It promotes plant growth, helps dissolve mineral phosphate and other nutrients, increases resistance to stress, stabilizes soil aggregates and improves soil organic matter content. In this review, the studies on metabolites produced by rhizobacteria inducing stress tolerance of plants are summarized.

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Giriş

Dünyada tarım arazisinin yaklaşık% 25'inin kuraklıktan, yaklaşık% 5-7'sinin de tuzdan etkilendiği düşünülmektedir [1]. Abiyotik streslerin, su alımını azaltarak, bitkinin fizyolojik ve biyokimyasal süreçlerini olumsuz olarak etkilediği, bitki büyümesini engellediği rapor edilmiştir [1,2]. Kadmiyum, kurşun ve cıva gibi ağır metaller toksik olup, çoğunlukla topraklarda düşük konsantrasyonlarda bulunurlar. Bununla birlikte, toprak-bitki sistemindeki hareketlilikleri nedeniyle, bitkiler tarafından kolayca alınırlar ve sürgünlere iletilirler [1]. Bitkide metal konsantrasyonlarındaki artışın, büyümenin gecikmesine, nekroz, besin alımının eksikliğine, düşük enzim aktivitesine ve dolayısıyla fitotoksisiteye neden olduğu bildirilmiştir [3].

Fitohormonlar, bitki metabolizması üzerinde belirgin bir etkiye sahip olan ve bitkinin belli organlarında sentezlenen, büyüme düzenleyicileridir [4] ve abiyotik streslerin azaltılmasında önemli rol oynarlar [5]. Bununla birlikte, abiyotik streslerin; bitki gelişimini etkileyen oksinler, gibberellinler, apsisik asit (ABA), jasmonik asit ve salisilik asit (SA) gibi endojen fitohormon seviyelerini değiştirdiği [6,7], kuraklık ve tuz stresinin ise bitki dokusundaki fitohormon konsantrasyonlarını engellediği bildirilmiştir [7].

Tarımsal ürünlerin iyileştirilmesi ile ilgili araştırmalarda büyük ilerleme kaydedilmiş, bu ilerlemede mikrobiyal teknoloji ve genetik mühendisliğinin önemli katkıları olmuştur. Rizosfer mikroorganizmaları ile bitki stres toleransını arttırmaya yönelik birkaç strateji önerilmiştir [8]. Bitki ile ilişkili mikroorganizmaların bitki dokusunda endofitik veya simbiyotik olarak veya kök yüzeyini kolonize ederek çeşitli metabolik aktif maddeler üretip birbirleriyle işbirliği yaptığı da açıklanmıştır [9,10]. Bazı rizobakteriler tarafından gibberellinler [7], sitokininler [11], ABA [12], oksin üretimleri gibi bitki büyüme düzenleyicilerinin biyosentezi ile bitki büyümesi ve besin kazanımının uyarılması ilişkilendirilmiştir [13].

Topraklar; mantar, bakteri ve bitkiler de dahil olmak üzere çeşitli organizmaların kaynağıdır [14]. Rizosfer, bitki kök sızıntılarının zengin besin bileşenlerini içermesinden dolayı (toprak ve diğer habitatlarla karşılaştırıldığında) mikroorganizmaların yoğun bir şekilde kolonize olduğu [1], kök tarafından salınan

çeşitli besinleri kullanan mikroorganizmaları [12] çeken, amino asitler, şekerler, yağ asitleri ve diğer organik bileşikleri içeren ve besin açısından zengin bir ortamdır.

Mikroorganizmalar; fitohormonlar (oksinler, sitokininler, gibberellinler ve ABA) dahil olmak üzere biyolojik olarak aktif bileşikleri, antifungal bileşikleri ve enzimleri sentezler. Bu mikrobiyal metabolitlerin bitki büyümesinde, beslenmesinde ve gelişmesinde hayati rol oynadığı yapılan araştırmalarda açıklanmıştırr [15,16]. Sayılan özelliklerdeki mikroorganizmalar, bitki gelişimini uyarabilmiş, çeşitli abiyotik ve biyotik stres faktörlerine direnç sağlayabilmiş, bitkilerin besin kazanımını iyileştirerek çeşitli toprak kaynaklı patojenlerden koruyabilmiştir [17]. Bu derlemede, rizosferdeki bakteriler tarafından sentezlenen metabolitler ve bitki gelişimi üzerine etkileri ile ilgili son yıllarda yapılan çalışmalar özetlenmiştir.

Rizosfer Bakterilerinin Rizosferde Metabolit Üretimi

Kök çevresi ve kök sızıntılarından etkilenen bölge rizosfer olarak adlandırılmaktadır. Köklerden salgılanan karbonhidratlar, organik asitler, vitaminler, nükleotidler, flavonoidler, enzimler, hormon ve uçucu bileşikler kök çevresindeki mikrobiyal aktiviteyi uyarmaktadır. Rizosfer bölgede bakteri yoğunluğunun diğer mikroorganizmalar içinde en yoğun olduğu açıklanmıştır [14]. Rizobakterilerin başlıca yararlı aktiviteleri arasında; topraktaki minerallerin bitkinin alabileceği forma dönüştürülmesi, azot fiksasyonu, patojenlerin bastırılması ve bitki büyümesini teşvik eden hormonların üretimi bulunmaktadır [9,14]. Mikroorganizmalar, bitkilerde besinsel ve hormonal dengeyi düzenleyerek, sistemik toleransı indüklemiş, bitkilerin strese karşı tepkilerini azaltmışlardır. Mikroorganizmaların bitki büyümesini ve stres toleransını iyileştirme mekanizmalarından biri, rizosfer veya kök dokusunda metabolit/fitohormon sentezleme yetenekleridir [18]. Mikrobiyal fitohormonlar, bitki dokusundaki endojen büyüme düzenleyicilerinin metabolizmasını etkilemiş; kuraklık, tuzluluk, aşırı sıcaklık ve ağır metal toksisitesine maruz kalan kök morfolojisinin iyileştirilmesinde anahtar rol oynamıştır [1,15].

Acinetobacter, Pantoea, Pseudomonas, Rhizobium ve *Sinorhizobium, Bacillus, Enterobacter, Brevibacillus Cellulosimicrobium, Mycobacterium, Ochrobactrum* ve *Paenibacillus*'in çeşitli izolatları [16], orkide rizosferinden izole edilen IAA üreten *Mycobacterium* türleri [19] ve buğday rizosferinden izole edilen *Azotobacter,*

Azospirillum, Cellulomonas ve *Mycoplana* [6] gibi farklı cins ve türlere ait mikroorganizmalar çeşitli fitohormonları üretmişlerdir.

Piccoli ve ark. [20] tarafından yapılan bir araştırmada ABA üreten endofitik diazotrofik bakteri *Arthrobacter koreensis* izole edilmiş ve tanımlanmıştır. IAA, GA3 ve jasmonik asit, halofit çalıların (*Prosopis strombulifera*) köklerinden izole edilen *Klebsiella* sp. ve *Enterobacter sp.*'in endofitik suşları, IAA sentezleyebilmiştir [21]. Mishra ve ark. [22], ekstrem ortamlarda IAA üretebilen *Ochrobactrum* spp. ve *Pseudomonas* spp. olarak tanımlanan bakterileri izole etmiştir. *Halomonas desiderata, Bacillus megaterium, B.cereus, B.subtilis, Escherichia coli* ve *Pseudomonas fluorescens* G20-18'in sitokinin sentezlediği bildirilmiştir [23,24]. *Bacillus, Klebsiella, Leifsonia* ve *Enterobacter* cinsine ait izolatlar, kabak rizosferinden izole edilmiştir. Bu bakteri izolatları Cd ile kirlenmiş toprakta IAA üretmiş ve mısır büyümesini arttırdığı Ahmad ve ark. [25] tarafından rapor edilmiştir.

Naz ve ark. [26] tarafından yapılan bir araştırmada ise; bitkilerin kök gelişimini uyaran sitokinin üreten; *Arthrobacter, Bacillus, Azospirillum* ve *Pseudomonas* gibi türler izole edilmiştir. ABA, çeşitli bitkilerin kök bölgesinden izole edilen mikroorganizmalarda saptanmıştır. Karadeniz ve ark. [23], *Proteus mirabilis, Klebsiella pneumoniae, B.megaterium* ve *B.cereus*'u ABA üreten bakteri olarak rapor etmişlerdir. *B.pumilus, B.licheniformis, Acetobacter* sp., *Bacillus* sp., *Azospirillium* sp.; gibberellin üreticileri olarak saptanmıştır [27,28].

Salomon ve ark. [29] *Vitis vinifera*'nın rizosferinde ABA üreten *B. licheniformis* Rt4M10 izolatı ve *P.florescens* Rt6M10 izolatını izole etmiş ve tanımlamışlardır. Ayçiçeği köklerinden izole edilen *Achromobacter xylosoxidans* SF2 besi ortamında ABA üretmiştir [30]. Tuzlu toprakta yetişen bitkilerle ilişkili IAA üreten bakteriler arasında Rizobia'nın oksin, sitokinin ve apsik asitleri sentezlediği, bitki büyümesini, gelişimini ve ürün verimini arttırdığı saptanmıştır [31]. Aktinobakterilerin; IAA, CK, GB benzeri metabolitleri ürettiği de tespit edilmiştir [32,33]. Ruanpanun ve ark. [34], yüksek miktarda IAA üreten *Streptomyces* sp.'yi izole etmişlerdir. Shutsrirung ve ark. [32] tarafından yapılan çalışmada ise endofitik aktinomisetlerden *Streptomycetes, Nocardia, Nocardiopsis, Spirillospora, Microbispora* ve *Micromonospora*'nın IAA ürettiğini tespit edilmiştir. Endofitik aktinobakteri *Streptomyces coelicolor* DE07 ve *S.geysiriensis* DE27'nin fitohormon sentezi su stres koşullarından etkilenmemiştir [35].

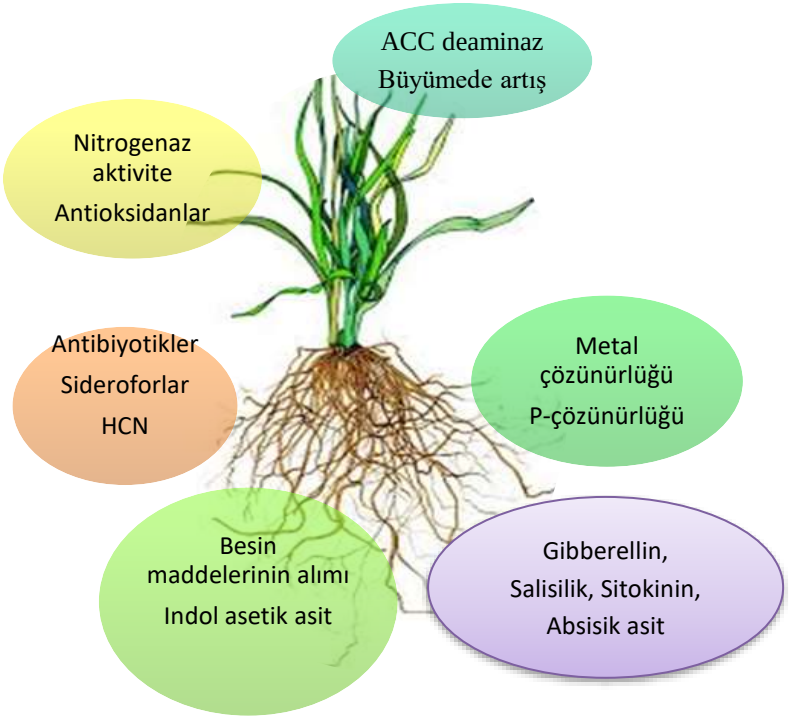
Ozmotik stres koşullarında *A.brasilense*, tarafından üretilen IAA üretimi, strese duyarlı *A.brasilense* Sp7 izolatı ile karşılaştırıldığında daha yüksek bulunmuştur [36].

Yüksek tuz konsantrasyonları içeren ortamlarda rizosfer mikroorganizmaları çeşitli fitohormonları sentezleyebilmiştir [6]. Başka bir çalışmada ise, %4 NaCl içeren ortamda; *Pseudomonas putida, P.extremorientalis, P.chlororaphis* ve *P.aurantiaca* IAA üretebilmiştir [6]. *Pseudomona*s sp. ve *Bacillus* sp.'nin çeşitli izolatları yüksek tuz koşulları altında (200–400 mM NaCl) IAA üretmiş ve tuz stresinde mum çiçeğinin bitki biyokütlesini arttırmışlardır [37].

Bacillus ve *Pseudomonas* izolatları, 2.2 µg/ml'nin üzerinde IAA sentezlemiş, *A.xylosoxidans* ve *B.halotolerans* tarafından üretilen GA3 sırasıyla 36.5 ve 75.5 µg/ml olarak belirlenmiştir [12]. Kültür ortamında *L.fusiformis* Ps14, *B.subtilis* Ps8 ve *P.putida* Ps30 izolatları sırasıyla 0.3, 1.8 ve 4.2 µg/ml ABA üretmiştir. Rizosfer bakterilerinden *B.licheniformis* MML2501 (18 µg/ml) ve *Pseudomonas* sp. PRGBO6 (6.8 µg/ml)'nin SA ürettiği bildirilmiştir [38,39].

Bitki Stres Toleransında Üretilen Mikrobiyal Metabolitler

Mikroorganizmalar düşük miktarlarda fitohormonları (metabolitleri) sentezleyerek tuzluluk, sıcaklık, kuraklık ve metal toksisitesi gibi çeşitli stres koşullarında bitki gelişimini ve bitkinin stres toleransını arttırmışlardır [40-42]. Fitohormon üreten mikroorganizmaların bitkilerde abiyotik stresin hafifletilmesi üzerindeki yararlı etkisi yapılan çalışmalarda da bildirilmiştir (Şekil 1) [1,43].



Şekil 1 Rizosfer bakterilerinin bitki gelişiminde etkili olan mekanizmaları

Bazı fitohormon üreten bakteri izolatları ve bunların abiyotik stresi azaltma yetenekleri Tablo 1'de verilmiştir. Birçok çalışmada abiyotik stres koşullarında bitki gelişiminin uyarılmasında IAA üretimi ve bitkilerle ortak yaşayan bakterilerin olumlu etkileri rapor edilmiştir.

Tablo 1 Stres koşullarında bazı rizosfer bakterileri tarafından üretilen fitohormonlar

Rizobakteri	**Üretilen fitohormon***	**Bitki**	**Stres koşulları**	**Kaynak**
Bacillus licheniformis	IAA	Buğday	Tuz stresi	Singh ve Jha [44]
Serratia sp.	IAA	Nohut	Besin stresi	Zaheer ve ark. [45]
Bacillus cereus	IAA	Buğday	Tuz stresi	Egamberdieva ve ark. [46]
Pseudomonas sp.	IAA	Mısır	Tuz ve sıcaklık stresi	Mishra ve ark. [22]
Achromobacter xylosoxidans, *Bacillus pumilus*	SA	Ayçiçeği	Kuraklık stresi	Forchetti ve ark. [47]
Pseudomonas putida	IAA	Soya fasulyesi	Tuz stresi	Egamberdieva ve ark. [46]
Bacillus	ABA	Çeltik	Tuz stresi	Shahzad ve ark. [48]

amyloliquefaciens				
Azospirillum lipoferum	GB	Buğday	Kuraklık stres	Marulanda ve ark. [49]
Micrococcus luteus	CK	Mısır	Kuraklık stresi	Raza ve Faisal [50]
Bacillus subtilis	CK	Mazı	Kuraklık stresi	Liu ve ark. [42]
Azospirillum lipoferum	GB	Buğday	Kuraklık stresi	Creus ve ark. [51]
B.amyloliquefaciens	ABA	Çeltik	Tuz stresi	Shahzad ve ark. [48]
Serratia marcescens	SA	Mısır	Tuz stresi	Lavania ve Nautiyal [52]
Bacillus aryabhattai	IAA, GA, ABA	Soya fasulyesi	Sıcaklık stresi	Park ve ark. [53]
Burkholderia sp., Bacillus sp., *Leifsonia* sp.	IAA	Domates mısır	Cd stresi	Ahmad ve ark. [25]; Durado ve ark. [54]
Bacillus sp.	SA	Mısır	Cr stresi	Islam ve ark., 2016
Kluyvera ascorbata SUD 165	siderofor, IAA	Kanola, Domates	ağır metal (Ni, Zn, Pb)	Burd ve ark. [56]

*sitokinin (CK), gibberellin (GB), indol-3-asetik asit (IAA), salisilik asit (SA), absisik asit (ABA)

Hordeum secalinum'dan izole edilmiş *Curtobacterium flaccumfaciens* E108 ve *Ensifer garamanticus* E110 izolatları arpanın tuz stresine karşı direncini ve verimini arttırmıştır [57].

Rizosfer bakterisi olan *B.licheniformis* HSW-16, tuz stresinden kaynaklı hasarı hafifletmiş ve tuzlu toprak koşulları altında IAA üretimi ile buğdayın büyümesini uyarmıştır [44]. Benzer incelemeler Upadhyay ve ark. [58] tarafından belirlenmiş, araştırıcılar tuza toleranslı *B.subtilis* ve *Arthrobacter* sp.'nin buğday biyokütlesi ve toplam çözülebilir şeker içeriğini arttırdığını, bitki dokusunda sodyum konsantrasyonunu azalttığını saptamışlardır. Tuza toleranslı bir yabancı ot (*Psoralea corylifolia* L.) köklerinden izole edilen tuza toleranslı ve IAA üreten *Enterobacter* sp. NIASMVII uygulamasının buğdayın (*Triticum aestivum* L.) tohum çimlenmesini arttırdığı rapor edilmiştir [28]. Başka bir çalışmada, *Pseudomonas* spp. ekstrem koşullardan izole edilmiş, IAA'i tuz stresi (500 mM NaCl) ve yüksek sıcaklık (40 ^{o}C) altında sentezleyerek mısır gelişimini uyarmış, kök gelişimini ve mısırın verimini arttırmıştır [22]. Bianco ve Defez [59]'e göre bitkilerin IAA tarafından abiyotik stresin olumsuz etkilerinden korunması, gelişmiş hücresel savunma sistemleri ile ilişkilendirilmiştir. Kültür ortamında IAA'i sentezleyen tuza toleranslı türler olan

Serratia plymuthica RR-2-5-10, *Stenotrophomonas rhizophila* e-p10, *P.fluorescens* SPB2145, *P.extremorientalis* TSAU20, *P.fluorescens* PCL1751 sera koşullarında hıyar ile aşılanmış; hıyarın biyokütlesi ve veriminde sırasıyla %9 ve %24 oranında artışa neden olmuşlardır [40].

Marulanda ve ark. [49], kuraklık sırasında *P.putida* ve *B.megaterium* ile yonca tohumlarının muamelesi ile yonca (*Trifolium repens* L.) biyokütlesinde artış olduğunu belirlemişler, bu değişiklikler ile artan IAA arasında bir ilişki bulmuşlardır. IAA üreten bakterilerin ayrıca, besin açısından zayıf toprak koşulları altında bitki büyümesini ve gelişimini arttırdığı tespit edilmiştir. Nohut nodüllerinden izole edilen *Serratia* sp.'nin IAA ürettiği saptanmış, bu da besince eksik topraklarda nohut tane veriminin artmasına katkıda bulunmuştur [45].

Mikrobiyal fitohormonlar, metal-bitki etkileşimlerinde, bitkilerin fitoekstraksiyonunu iyileştirmede önemli bir rol oynamıştır. Bakır kontamineli topraklarda IAA üretimi ile *A.xylosoxidans* Ax10, *Brassica juncea'nin* kök gelişimini artırmıştır [60]. Zaidi ve ark. [61] tarafından benzer sonuçlar gözlemlenmiştir, IAA sentezleyen *B.subtilis* ile aşılı hardal bitkisinin (*B.juncea* L.) kök gelişimini uyardığı ve Ni birikiminin azaldığı incelenmiştir. *B.megaterium* MCR-8 izolatı, 68.5 mg/25 ml konsantrasyonundaki oksin üretimiyle *Vinca rosea*'da Ni stresini hafifletmiş, kök ve yeşil aksam ağırlığını arttırmıştır. *B.megaterium* MCR-8 ile muamele edilen bitkilerde süperoksit dismutaz (SOD), katalaz (CAT), peroksidaz (POD) ve askorbat peroksidaz (APX) gibi savunma ile ilgili enzimler, toplam fenoller, flavonoidlerin birikimleri muamelesiz bitkilerle karşılaştırıldığında artmıştır [62]. Yapılan başka bir çalışmada ise; Cd stresinin mısır tohumlarının çimlenmesini ve fide büyümesini inhibe ettiğini, oysa Cd' a toleranslı ve IAA üreten bakterilerden *Leifsonia* sp. ve *Bacillus* sp.'in Cd bulaşık topraktaki mısırın filiz ve kök gelişimini kontrollere göre önemli oranda arttırdığı açıklanmıştır. Cd'a toleranslı *Burkholderia* sp. SCMS54 ise, IAA üreterek Cd stresinde domatesin stres toleransını ve bitki gelişimini artırmıştır [25].

Islam ve ark. [55], Cr toksisitesinin mısır büyümesini önemli ölçüde engellediğini, fotosentetik pigment ve karbonhidrat metabolizması gibi fizyolojik süreçlerini olumsuz yönde etkilediğini ve prolin, H_2O_2 ve MDA seviyelerini arttırdığını bildirmiştir. Bu koşullarda, Cr dirençli *P.mirabilis*'in T2Cr ve CrP450 nolu izolatları SA ile kombine

edildiğinde, Cr'nin toksik etkisini hafifletmiş, mısırda antioksidant aktiviteleri arttırarak oksidatif stresi azaltmış, kök ve yeşil aksam gelişimini arttırmıştır [63].

IAA üreten *B. subtilis* NUU4 ve *Mesorhizobium ciceri* IC53'nin birlikte nohut (*Cicer arietinum* L.) ile aşılanması; sadece *Mesorhizobium ciceri* IC53 ile aşılanan veya aşılanmamış kontrol bitkilerle karşılaştırıldığında, tuz stresi altında nohutun kök, biyokütle ve nodül oluşumunu arttırmıştır [64]. Birçok çalışmada, sitokinin üreten bakteri izolatlarının kök gelişimi üzerindeki olumlu etkileri de bildirilmiştir. Örneğin, mısırın sitokinin üreten bakteri *Micrococcus luteus* chp37 ile aşılanması, kurak koşullarda fotosentetik pigmentler dâhil olmak üzere bitkinin fizyolojik özelliklerini olumlu yönde değiştirmiştir [50]. Rizosfer bakterilerinden *Arthrobacter, Bacillus, Azospirillum* ve *Pseudomonas*'in sitokinin üreten izolatları, soya fasulyesinin kök ağırlığını ve ayrıca tuz stresi altında da bitki dokusundaki prolin içeriğini arttırmıştır [26]. Sitokinin üreten *B. subtilis* ile aşılı *Platycladus orientalis* (oryantal mazı) kök ağırlığını % 13.9 oranında arttığı, aşısız kontrollere göre yapraklarda sitokinin konsantrasyonunda % 47.52 oranında arttığı bildirilmiştir [9]. *B.licheniformis* ve *P.fluorescens* tarafından ABA üretiminin su stres koşullarında asmanın gelişimini stimüle ettiği Salomon ve ark. [29] tarafından açıklanmıştır.

Normal ve tuzlu koşullar altında *B.amyloliquefaciens*'in RWL1 izolatı ABA (0.32-0.14 ng/ml) üretmiştir [48]. Tuz stresinde; bakteriyel aşılama, çeltik bitkisinin kök ve yeşil aksam gelişimini, bitki dokusundaki SA konsantrasyonunu önemli ölçüde arttırmıştır. Park ve ark. [53]; *Bacillus aryabhattai* SRB02 izolatını soya fasulyesi rizosferinden izole etmişler ve soya fasulyesi ile aşıladıklarında ise soya fasulyesinde nodül oluşumunun önemli ölçüde arttığını belirlemişlerdir. Aynı araştırıcılar, kültürde izolatın 2 ng/ ml ABA ürettiğini ve soya fasulyesinin kuraklık stres toleransını kontrol bitkilerine göre önemli oranda arttırdığını tespit etmişlerdir.

Diğer fitohormonların etkilerine benzer şekilde, SA üreten endofitik bakteriler *A.xylosoxidans* ve *B.pumilus*'ın kurak koşullarda ayçiçeği fidelerinin biyokütlesini arttırmıştır [30]. Tuza toleranslı olan ve SA üreten *Serratia marcescens* NBRI1213'ün mısır kök ve sürgün gelişimini, topraktan besin kazanımını teşvik ettiği; tuzlu koşullarda bitki stres toleransını arttırdığı Lavania ve Nautiyal [52] tarafından bildirilmiştir. Bazı bakteriler, bitki dokusunda hormonal denge ve fizyolojik süreçlerin düzenlenmesinde etkili olan çeşitli metabolitleri (fitohormon) üretmiştir. *Sphingomonas*

sp. LK11 ve *S.marcescens* TP1, kültür ortamında sırasıyla 12.31 ve 10.5 µM/ml IAA üretmiş; soya fasulyesinin kök ve sürgün gelişimini arttırmış, kontrol bitkilerle karşılaştırıldığında jasmonik asit içeriği azalmış, ABA ve gibbrellindeki artışla soya fasulyesi kök gelişimini stimüle etmiştir [10]. *B.aryabhattai* SRB02 ile muamele edilen bitkilerin dokularında daha yüksek konsantrasyonda; IAA, JA, GA12, GA4 ve GA7 belirlenmiştir. ABA üreticisi olan *Azospirillum lipoferum* ve *A.brasilense* sp. ile aşılanan mısır için de benzer gözlemler rapor edilmiştir. Bu çalışmalar, bitkilerin stres toleransını indükleyen bitki ile ilişkili mikroorganizmalar tarafından bitki dokusunda fitohormon modulasyonunun bulunduğunu göstermektedir.

Sonuç

Mikrobiyal kökenli fitohormon uygulamalarının; değişen veya ekstrem çevre koşulları altında potansiyel uygulamalarının sağlanması, bitkilerin abiyotik ve biyotik stres toleransının arttırılması için önemli bir araç olduğu kanıtlanmıştır. Bitki büyümesinin uyarılması, abiyotik streslere tolerans ve patojenlere direnç gibi mikroorganizmaların aracılık ettiği bitki üzerindeki faydalı etkiler, mikroorganizmaların bitki dokularında oksin, gibberellinler, SA, ABA ve sitokinin üretebilme yeteneğine dayanmaktadır. Bu nedenle, bitki ile ilişkili mikroorganizmalar, bitki dokusundaki hormon seviyelerini ve metabolizmayı, özellikle kuraklık, tuzluluk, besin eksikliği veya ağır metal kirliliği gibi dış baskıların zarar verici etkilerini önleyebilecek potansiyele sahiptir. Stres koşullarında, mikroorganizmalar tarafından bitki dokularında metabolit dengesinin düzenlenmesi, bitkisel üretimde sürdürülebilir yaklaşımların geliştirilmesinde önemli olabilir. Bunların bitkiye özgü özellikler olup olmadığını belirlenmesi ve mikrobiyal metabolitler ile bitkilerin stresli ortamlardaki tepkilerinin anlaşılmasında farklı bitki türleri üzerinde yapılacak daha fazla araştırmaya ihtiyaç vardır. Fitohormonların antagonistik veya sinerjik etkileşimlerini ortaya çıkarmak için, genetik mekanizmaları kullanmak, mikrobiyal metabolit uygulanmasından sonra spesifik genlerin ekspresyonuna yol açan reseptörlerin tanımlanması ile ilgili çalışmalar yapılmalıdır. Bitki üzerinde metabolit üreten mikroorganizmaların performansına ilişkin çalışmalar yapılmalı ve bu mikrobiyal aşıların, yerli mikrofloraya karşı besin ve niş için rekabetçi olmasına dikkat edilmelidir. Konukçu-mikroorganizma-stres etkileşimlerinin ve bunların proteomik, genomik, metagenomik gibi omik tabanlı yaklaşımlarını içeren mekanizmalarının araştırılması gerekmektedir

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Review article

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The Important of Flax (*Linum usitatissimum* L.) In Terms of Health

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ABSTRACT
The flax is one of the oldest known cultivated plants of the World that benefited from seeds and fibers. It is an important nutrient because of the rich α-linolenic acid (ALA, omega-3 fatty acid), lignans, short chain polyunsaturated fatty acids, soluble and insoluble fibers, phytoestrogenic lignans (secoisolariciresinol diglycoside-SDG), waxy and mucilage compounds, protein and antioxidant compounds. In addition, the seed contains components effective in reducing cardiovascular disorders, diabetes, digestive system, urinary tract disorders, osteoporosis, cancer, arthritis, autoimmune and neurological diseases. Seed protein content varies between 20-30%, of which approximately 80% consists of globulins and 20% glutelines. The amino acid level is so rich and gluten-free. The unconscious consumption of seed may cause toxicity due to trypsin, myo-inositol phosphate inhibitors, cadmium and cyanogenic glycosides in the seed. The seed should be consumed as milled flour after absolute heat treatment. Side effects may be seen in pregnant and young men due to hormonal effects of lignan. It may be oxidized shortly after grinding due to the fatty acids present in the seed. Flax fibers (Linen) have been used in house and fabric textiles, sailing and tent making since 7040 BC. The fibers are gold-yellow in color, the qualitative and moisture absorption capacity is very low, easily creasing. For this reason, the linen keeps the skin cool and does not sweat. Fiber is also an important source for healthy insulation materials, biocomposites, structural application materials for automotive, contributing both to the environment and to human health. In this paper, the effects of flax on human health and methods of traditional uses in Turkey were discussed with references.

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Introduction

Flax (*Linum usitatissimum* convar. *usitatissimum*) is the only economic important genus of Linaceae family, annual, used both of fiber and seed. Flax, one of the oldest plants in the world means “very useful” [1]. The cultivation for flax fiber is need climate condition of relatively high humidity, warm and rainy in winters, cool in summers while for oil / seed production was suitable of drier and warmer climates [2]. Its homeland is region from the west of the Mediterranean to India. Flax, which is accepted as a medicinal plant for the European Pharmacopoeia, grows today in a wide range of areas

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including Tropical and moderate climates [3]. Its use by humans is based on the beginning of agriculture.

In recent years, dyed flax fibers found in excavations at the Dzudzuana cave in Georgia have shown that fibers over 30,000 years of age dating back to the Upper Palaeolithic period, prehistoric hunter collectors use flax fiber to produce or develop everyday objects [4, 5]. In the historical process, it has been stated that flax is grown and woven both by Ottoman historians, traveler historians such as Evliya Çelebi, Cuinet, Strabon and archives of the Republican Period. Excavations in different parts of Anatolia have indicated that prehistoric village inhabitants cultivated barley, wheat and flax on the banks of the Tigris River. The historical findings in Anatolia have shown that flax wovening was very old, back to 7040 BC. [6,7]. Flax is one of the most mentioned plants in the 800 recipes made of 77 herbal, animal and mineral drugs in papyrus at 1550 BC.[8]. It has been included in herbal prescriptions for the treatment of various diseases [9]. In this reviewed paper, the effects of flax on human health and methods of traditional uses in Turkey (Anatolia) were discussed with references.

Physical and chemical properties of flax fiber (Linen)

Flax fibers are the most used fiber source after cotton in the World. The linen obtained from the stems contain 75% cellulose, 5% hemicellulose, 4% lignin [10, 11], 3% fat and waxy substances, 0.5% ash and 12.5% water [12,13,14]. The degree of polymerization of flax fibers is 18000 and has a longer polymer chain than cotton. Each linen chain consists of 18000 cellulose units, which are 18000 micrometers long and 8.8 nanometers thick [2]. The length of a single fiber cell is 6-65 mm and the diameter is 0.02 mm [15,16]. The length of a fiber varies between 90-125cm. The tensile strength of the fibers is 6.5-8 gr / denier, elongation at break 1.8% (dry) and 2.2% (wet), specific gravity 1.54. The flax fibers are resistant to distortion up to 120^{o}C [12] .Wear resistance is moderate. Stability and thermal conductivity are good [17]. The fibers are brownish, ivory, gray, light, yellowish (4,12,15,18). Flax fiber is easily damaged by high concentrations of acids. However, it is not affected by low density acids if washed immediately. It has excellent resistance to alkalis and does not break down by strong alkalis. Fibers are not affected much by bleaches such as cold chlorine and hypochlorite, boiling water, sun and detergent [12,13,19] Flax fiber cannot be spun due to its fiber structure. Therefore, it can be used by mixing with fibers such as cotton, silk and wool

[20,21]. Compared to hemp fibers, it is relatively thin and soft and has low elasticity. It ranks first among natural bast fibers in terms of durability. The best sweat absorbing after cotton [22,16]. The linen fabrics keep the body dry and cool because of these properties. Whereas polyester worn over the skin has been shown to result in skin with higher relative humidity than fabrics made from cotton and linen [23]. Linen, which is a natural bast fibre, has unparalleled characteristics such as a feel of freshness and a magnificent brilliance. It is very hygienic and imparts an air of satisfaction and style to the wearer [24]. 100%-linen and linen-blended fabrics are more permeable to air than cotton fabrics, which confirms the suitability of linen fabric for winter wear. Linen and linen-blended fabrics have higher thermal insulation values than cotton fabrics. Linen fabric has a higher moisture vapour transmission rate [25,16].

Many healthy products such as shirts, jackets, dresses, blouses, trousers, home textile products, bed linen, ornaments, bedding, towels are made of flax fibers [18]. In addition to, it is used in the production of environmentally friendly materials such as surgical thread, sewing thread, wall coverings, wallpaper, high-quality papers (money, cigarettes), lightweight fabrics for aeronautics, automotive interior coating materials [26], noise-resistant materials in construction and automotive industry, insulation material, biocomposites, bioplastics, pulp [21,27].

Chemical structure of flaxseed

The chemical content of the flaxseed varies according to the environmental conditions in which the plant grows and the characteristics of the genotypes. The flaxseeds consist of about 35-45% lipids [28,18], 30% dietary fibre, between 20 to 30 % protein [29,30], 10% musilaj and glycoside linamarin 5-6% gum [31]. Major lignan present in flaxseed is secoisolariciresinol diglycoside (SDG) [32]. The total protein content composed of mainly 80% globulins (linin and conlinin) and 20% glutelin [30,29]. Flax protein is relatively rich in arginine, aspartic acid and glutamic acid. However lysine, methionine and cysteine are the limiting amino acids and gluten-free [33]. Flaxseed oil is composed of 73% polyunsaturated fatty acids, 18% monounsaturated fatty acids and 9% saturated fatty acids. It is also the richest known plant source of the omega 3 (n-3) fatty acid, α-linolenic acid (ALA), which comprises 55% of the total fatty acids [34,35,36]. Flaxseed oil is mainly found as triacylglycerols (98%) with lower contents of phospholipids (0.9 %) and free fatty acids (0.1%) [37]. In addition to, cellulose,

hemicellulose and lignin are insoluble fiber constituents abundantly found in flaxseed while mucilage gums form is the soluble fiber fraction [38]. Flaxseed is an equally good source of minerals, particularly, phosphorus, magnesium, calcium, iron, zinc and very little amount of sodium [39]. However, flaxseed contains toxic components such as trypsin, myo-inositol phosphate inhibitors, cadmium, cyanogenic glycosides [40].

Flaxseed and Health

The flax is a valuable plant from which we benefit from both seed and fiber. Flaxseed is an important source of edible oil [41] as well as traditional treatment (medicinal purposes). In traditional treatment throughout history, the flaxseeds have been used to reduce inflammation, calming, colds, coughing and fever [42], to treat of lung diseases, severe colds and cough, as muscle relaxant by American Indian [1] to heal abdominal pain in ancient Egypt and Rome [43]. Flaxseed consumption in the diet prevents serious diseases like coronary diseases, cancer, diabetes, obesity, gastrointestinal, renal and bone disorders [39]. Effects of flaxseed on gastrointestinal problems, constipation, glucose tolerance, hypocholesterolemic effect and fermentation have been described comprehensively in many articles [44,45, 46].

Flaxseed is the good source of functional food or functional components [47,37,39] due to such components as lignans (secoiso-laricrecinol diglucoside-SDG), polysaccharides [35], α-linolenic acid [48] and soluble flaxseed gum (FG; Flax musilage) [30] which are positively effective in preventing many diseases. Ibrügger et al. [49] have reported that flaxseed fiber may be applicable to control appetite, thus the ingestion of the flaxseed fiber, both as a powder and tablets, have caused to increase satiety and decrease energy intake at a subsequent meal. Flaxseed is one of the richest sources of lignan [50] and there are many studies showing that it is a source of quality phytoestrogens [51,52,53]. The major lignan in flaxseed is secoisolariciresinol diglucoside (SDG), phenolic compounds are coumaric acid and ferulic acid. The lignans of flaxseed have possess a high activity especially as antioxidant and antibacterial [54]. Also, researchers have reported that flaxseed might be have health-promoting effects due to these properties [55,56]. Lignans have been shown as a supplementary food source that can serve as an alternative to traditional estrogen therapies in the prevention and treatment of hormone-based cancers [57]. In vivo studies have been observed that the addition of flaxseed to 10% in mice feeding diets [53] and 5% in mammals reduces tumor growth rate and

metastasis [57]. The high content of SDG (secoiso-lariciresinol Diglucoside) lignin in flaxseed has got possibly a breast tumor-reducing effect [58]. However, the researchers have determined of flaxseed lignan SDG appears to be a promising naturally-occurring therapeutic agent for insulin resistance and type-2 diabetes. It has shown significant-beneficial effects on glucose tolerance and insulin sensitivity [59,60].

The flaxseed gum (FG) diet by affecting the abundance of some specific bacteria in the stomach of obese rats, via suppressing appetite has created the effect of anti-obesity. Thus, A proper dose flaxseed gum (FG) consumption could be reduced the body weight, body fat and total triacylglycerols [61]. Alzueta et al. [62] was indicated that flaxseed gum (FG) is a potential prebiotics due to could selectively stimulate the growth of Lactobacilli in vivo.

The researchers have shown that the use of degreased and ground flaxseed has statistically significant decrease in total cholesterol and LDL cholesterol levels [63,64]. So, addition of flaxseeds to diets can be reduced the risk of cardiovascular disease by improving fat profile in hyperlipidemic patients [39,65].

Flaxseed is one of the richest of sources of phytoestrogens to reduce menopause complaints of menopausal women [66]. It has reasoned significant reductions in menopausal symptoms of women, to consume phytoestrogen-rich soy products and diet products containing flaxseed in 145 women suffering from menopause during the 12-weeks [67]. The researchers have determined that may be ability to protect from breast, colon and ovarian cancer by preventing the formation of tumor in vivo studies conducted on rats of flaxseed eat [68]. The flaxseed oil has proven to be a good cytotoxic agent on the oral cancer cell lines by experiments. It can be used as a biomedicine against cancer which can reduce the adverse effects of cancer treatment and to be a life saving drug for oral cancer suffering people which can help many in the future [69].

ALA, which is abundant in linseed oil, is a fatty acid that cannot be synthesized in the body and must be taken from the outside. It is an 18-carbon n-3 (Omega-3) fatty acid that is essential for humans [70]. Omega-3 fatty acids exhibit anti-inflammatory [71,72,73], anti-thrombotic, vasodilatory, anti-atherogenic properties and can modulate lipid metabolism in a beneficial way [74]. Natalucci-Hall and Starr [75] have suggested the homeopathic usage of omega-3 which is effective food supplement for reducing

Attention Deficit Disorder without Hyperactivity symptoms in patients. ALA-rich flaxseed oil has health benefits in obesity due to its ability to improve adipocyte function [76]. The flaxseed oil diet has also proved to be better at reducing hepatic nitric oxide concentrations in diabetic rats. These diets have also significantly lowered hepatic expression of inflammatory biomarkers. The omega-3 diets have proved to be beneficial in protecting against injury and potential complications in liver tissue of diabetic rats [70,71]. In addition to Tomaz et al. [77] found that adding 20% flaxseed to daily diet may be important to prevent cardiovascular diseases according to their experiment with healthy rats. However, Rajaram [78] has stated that although there has been an increase in studies that ALA provides protection against cardiovascular diseases for the last 10 years, there are still deficiencies, and it would be beneficial not to make specific recommendations for prevention of public cardiovascular diseases until strong evidence is revealed in the future. The flaxseed have potentials [32]. Nowadays, flaxseed oil is often used as nutritional supplements [79]. Expecially for vegans, flaxseed oil or flaxseeds are one of the major sources of omega-3 fatty acids [70,39]. Therefore it may be prudent to include foods containing ALA in the diet [78].

Traditional Uses of Flaxseed In the Turkey

Flaxseed is known by local names such as, sârek, seyrek, zârek [80] bızıktan seed, siyalek, zergenek [81] in Turkey and has been consumed in many different ways since ancient times. Flaxseed has used for especially for pain relief, wound healing, bronchitis, cough treatment [82,11] and burn treatment [83], as ointment and mush [84]. The water obtained by boiling flaxseed (infusion method) has a curative effect on stomach and intestinal disorders, gastritis treatment, asthma and bronchitis when drunk on an empty stomach [85,11]. It has strong laxative effect [11] if ground flaxseed is consumed with spinach [86]. Especially it is very effective against chronic constipation because of the mucilage substances in the seed. In order to overcome this problem, it is reported to be beneficial to consume morning and evening, ground flaxseeds, 2 tablespoons, on an empty stomach and withplenty of warm water. It is recommended that the consume be continued for several weeks in order to regulate the defecation [87]. The yellow colored flax oil obtained from flaxseed by the cold-press method has used to against eczema, hemorrhoids and shortness of breath [88]. When one tablespoon of ground flax seed (about 30-40 g) is mixed in 1 bowl of yogurt and eaten, It is mentioned

that this mixture balances blood pressure, sugar, is good for bone resorption, stomach, cough, lost weight among the local public [81]. The same mixture is also frequently consumed by breast cancer patients in recent years [86].

An equal amount of flaxseed and hemp seeds are lightly roasted. Then a powder mixture is obtained by grinds together with the seizure sugar. Consuming three meals /a tablespoon per day from this powder mixture is found very effective for eliminating breast swelling of the puerperal women in the post-natal and to increase breast milk [80].

It has stated that the mixture prepared by grinding roasted flaxseed, hazelnut and seizure sugar together, consuming 1 tablespoon on an empty stomach in the morning, is beneficial for people with weak and stomach discomfort. In addition to, in the morning, consumption of 1 tablespoon from roasted-ground flaxseed and honey mixture has mentioned to show good effect for lung diseases. In interviews with consumers, they have said that when roasted-ground flaxseed is mixed with tahini (ground sesame) and grape molasses, it keeps the body warm and delays hunger and energizes the body. Also, it has a lowering effect on cholesterol, if flaxseed is boiled and drunk [86].

Nowadays, still in traditional treatment of flaxseed mush has known to use to ripen swollen wounds. Fistly, ground flaxseed is cooked with milk, then is applied to the swollen swelling which does not flow after being mush as warmish. This process is repeated several times in day [80, 86] Also, It has reported that applying hot flaxseed mush on the chest is a cough remover [11, 89].

The Oleum lini (flaxsed oil) has reddish brown and special scented which is obtained by the pressing after roasting of flax seeds. Oleum lini and lime water mixture has very effected to use as externally for wound and burn treatment [11] In addition, this oil has used in veterinary medicine especially as a laxative and to lubricate buffalo skins [84]. It can be used by friction in all skinless skin diseases, including calluses by human [89].

The Anti-nutrients and Toxicity in Flaxseed

Whole flaxseeds and cold press flaxseed contain anti-nutrients which may pose adverse health effects as cyanogenic glycosides (250–550 mg/100 g) [90,91]. Hydrogen cyanide released from flaxseed is minimal and lower than the toxic or lethal dose while the phytic acid content of flaxseed meal is 2.3–3.3% which result in decreased absorption of nutrients. The release of hydrogen cyanide from recommended daily intake of flaxseed

(1–2 tablespoons/day) is approximately 5–10 mg. This value is very low considering that the toxic dose is 50-60mg [39]. Also, human beings can detoxify cyanide levels below 30–100 mg/day [92]. Althought, no reports on cyanide poisoning after consumption of linseed were found in the literature [40,89] the more research is needed on this subject.

Roasting is generally done to remove cyanogenic glycosides [93,94,95]. The seed should be consumed as milled flour after absolute heat treatment (approximately min. 35^{o}C) for safe consumption. Side effects may be seen in pregnant and young men due to hormonal effects of lignan. It may be oxidized shortly after grinding due to the fatty acids present in the seed. It should be stored in the refrigerator after grinding fresh [86]. Flaxseed meal obtained after cold press is toxic because it contains cyanogenetic glycosides and should not be given directly to animals [84,96]. It should not be taken just before bedtime [89].

In adults, high-quality studies have indicated that daily ground flaxseed intake may range from 38 g [97] to 40 g [98,99] to lead a healthy life under the specified conditions. Althought, It is need to determined of the minimum consumption amount of flaxseed to explore its therapeutic potential for all population groups including pregnant and lactating women and to learn possible problems posed by its overdose via in vivo research (100).

Conclusion

The present review shown that flaxseed, fiber and its oil have different uses and many health benefits of since ancient times with reference to evidence based literatures, it has still consumed in traditional. Today, many people suffer from many diseases such as cardiovascular diseases, diabetes, hypertension, osteoporosis, cancer, arthritis, autoimmune and neurological diseases, obesity. According to literature, flaxseed has seen as a product that can be take into place in the diet of people to reduce to negative effect of diseases. However, there is a need to increase the researches on the mentioned effects especially in traditional usage and to support them with scientific data. In the reviewed literature, even if no toxicity cases have been recorded to date for flaxseed, warnings about its use due to cyanogenic glycosides and anti-nutritions should be taken into consideration until their effects are proven by scientific data.

Competing Interests
The authors declare that they have no competing interests.

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Atrazine ve Glyphosate'ın Toprak Karbon Mineralizasyonuna Olan Etkileri

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ÖZET

Tarımsal ekosistemlerde yabancı otların kontrolünde en çok tercih edilen yöntemlerden birisi herbisit kullanımıdır. Mikrobiyal aktive toprakta herbisitlerin davranışlarını belirleyen en önemli faktörlerdendir. Karbon mineralizasyonu toprak kalitesinin biyolojik indikatörlerinden olup topraktaki tüm canlıların hayati faaliyetlerini sürdürebilme kapasitesini yansıtmaktadır. Bu derlemede tarım alanlarında yabancı ot kontrolünde en fazla kullanılan bileşikler olan atrazine ve glyphosate'ın toprakta karbon mineralizasyonuna olan etkileri özetlenmiştir.

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ANAHTAR KELİMELER
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Organik madde mineralizasyonu

Effects of Atrazine and Glyphosate on Soil Carbon Mineralization

ABSTRACT

One of the most preffered methods for weed control is application of herbicide in agricultural ecosystems Microbial activity is one of the most important factors that determines behavior and decomposition of herbisicides in soil. Carbon mineralization is one of the biological indicators of soil quality while it reflects the sustainability of vital functions of all living beings in soil. In this review, effects of atrazine and glyphosate that are most used in agricultural areas for weed control on soil carbon mineralization were summarized.

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Giriş

Bitkisel üretimin korunmasında ve ürüne zarar veren organizmaların kontrolünde kullanılan kimyasallara pestisit denilmekte olup bu maddeler yeterli besin üretiminin sağlanması ile insan ve hayvan sağlığının korunmasına yardımcı olmaktadır. Pestisit kalıntıları havanın, suyun, toprağın ve gıdanın önemli bir kontaminasyon kaynağı olabilmekte ve bu da ekosistemlerdeki pek çok bitki ve hayvan toplulukları için bir tehdit haline gelmektedir [1-5]. Herbisitler, pestisitlerin önemli bir grubu olmanın yanında sürdürülebilir tarımda kullanılan araçlardan birisidir. Bu maddeler, bitkisel ve

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dolayısıyla hayvan üretimin artışına destek sağlarken tarımsal üretimde insan emeğinin azaltılmasına yardımcı olmaktadır [6].

Herbisitler tarımda en çok kullanılan pestisit sınıfı olup bütün herbisitler içinde glyphosate dünyada en fazla kullanılan kimyasal olup 2017 itibariyle 1.35 milyon metre tona ulaşacağı bildirilmiştir [7]. Glyphosate gibi pestisitlerin toprak mikrobiyal kommunitelerine olan etkilerinin araştırılması, mikroorganizmaların biyojeokimyasal süreçlerini sürdürmesinde kritik bir role sahip olması, patojenlerin kontrolü ve ekosistemlerin insanlığa hizmet sağlaması konularında oldukça önemli bir faktör olduğu belirtilmiştir [7].

Günümüzde 200'den fazla etken madde herbisit olarak kullanılmakta ve bunların birçoğunun toksisite potansiyeli değerlendirilmiş olup JMPR (Joint Meeting on Pesticide Residues) tarafından kabul edilebilir günlük alım dozları belirlenmiştir [8]. Genel olarak yeni geliştirilen herbisitlerin memelilere olan toksik etkilerinin düşük olduğu bildirilmiştir [9]. Ancak herbisitlere maruz bırakılan bir veya birkaç tür hayvanda gelişim ve üremenin etkilendiğini gösteren deneysel ve anekdot niteliğinde kanıtların sayısı arttığı ifade edilmiştir. Bazı herbisitlerin insanlarda doğuştan kusura neden olduğu bulunmuştur. Örneğin, 2.4-D (2.4-Dichlorophenoxyacetic acid) ile dioxin, oryzalin ve picloram gibi herbisitlerle kombine olarak uygulanması insanlarda üreme problemlerine neden olduğu bildirilmiştir [9]. Dioxin içeren 2.4.5-T'nin (2.4.5-Trichlorophenoxyacetic acid) bazı formülasyonlarına maruz kalan işçilerde üç nadir görülen kansere (Hodgkin hastalığı, yumuşak doku kanseri ve Hodgkin olmayan lenfoma) görüldüğü bildirilmiştir [10]. Glyphosate'ın toksik olmayan düşük konsantrasyonlarının in vitro insan plasental hücrelerinde aromataz enziminin bozulmasına yola açtığı ve östrojen sentezinden sorumlu olan aromataz enzim aktivitesini azalttığı bildirilmiştir [11]. 2015 yılında IARC (International Agency for Research on Cancer) pek çok pestisidin insanlar kansere neden olma risklerini yeniden değerlendirdi ve glyphosate'ın insanda olası karsinojenik etkiye yol açan bir madde olarak 2A kategorisine aldığını bildirmiştir [12].

Atrazine C3 bitkilerinde fotosentezde görev yapan spesifik enzimleri hedefleyen ve yaygın bir biçimde kullanılan bir herbisittir [13]. Bu herbisit dünya çapında mısır ve şeker kamışı tarlalarında geniş yapraklı yabancı otların kontrolünde kullanılmakta olup tarımsal alanların yakınlarındaki su kaynaklarında en fazla tespit edilen kimyasallardan

birisi olduğu bildirilmiştir [14, 15]. Atrazine'in sudaki varlığı düşük konsantrasyonlarda bile pek çok sucul canlısına toksik olduğu ve kurbağalarda hermafroditizme yol açtığı bulunmuştur [16-18]. Ayrıca tatlı su alglerinin büyümesini inhibe ettiği ve toprak solucanlarında DNA aberasyonlarına yol açtığı ifade edilmiştir [19, 20]. Atrazine'in insanlarda endokrinal bozulmalara, düşük yapma ve düşük doğum ağırlığına sebep olduğu belirtilmiştir [21-23]. Atrazine, gine domuzunda derinin hassaslaşmasına neden olurken 30 ile 60 gün süresince uygulanan 30 mg/kg atrazine dozu koyunlarda ölümcül olduğu ifade edilmiştir [9]. Bu toksik etkiler Avrupa Birliği'nin bu herbisitin kullanımını tamamen yasaklanması gerektiği konusundaki iddiasını desteklemektedir [24]

Pestisitlerin tarımsal ekosistemlerdeki davranışlarının ayrışma sürecine bağlı olduğu bilinmektedir. Ayrışma, pestisit moleküllerinin birbirlerinden ayrıldığı kimyasal bir işlem olup bu işlem ışıkla parçalanma, mikrobiyolojik ayrışma, kimyasal ayrışma ve bitki detoksifikasyonu kapsamaktadır. Ayrıca pestisit parçalanması, pestisidin kalıcılığını, zararlı organizma kontrolündeki etkinliği ile toprak ve su kaynaklarının kontaminasyon riski oluşup oluşmamasını belirlemektedir [25-29]. Bu derlemede Atrazine ve Glyphosate'ın toprakta karbon mineralizasyonuna etkileri ve bazı önemli herbisitlerin bu biyolojik aktiviteye olan etkileri incelenmiştir

Toprakta Karbon Mineralizasyonu

Mikroorganizmalar, toprakta besin döngülerinin sürdürülmesini sağlamaktadır ve bu önemli aktivite olmasaydı, pek çok gerekli element bitkilere yarayışlı hale gelmeyeceği bilinmektedir [30]. Bunun tam tersi düşünüldüğünde yeşil bitkiler yoluyla karbon (C) ve enerji girişi olmasaydı, toprak sadece kayaların parçalanmasıyla oluşan mineral partiküllerden meydana gelecekti. Bitki ve hayvan kalıntıları ile mikrobiyal aktivite, toprağa organik katkılar sağlamanın yanında mineral toprak parçacıklarının agregatlaşmasına ve dolayısıyla daha verimli hale gelmesine yardımcı olmaktadır [31]. Toprakta mikroorganizmalar, mikrofauna ve makrofaunanın oluşturdukları kompleks bir besin ağının konağı olarak görev yapmaktadır [32, 33].

Bitki ve hayvan kalıntılarının ayrışmasında büyük bir rolü olan mikroorganizmalar aynı zamanda toprakta organik karbonun (TOK) da önemli bir rezervuarıdır. Bununla beraber, toprak karbonca zengin organik maddenin büyük bir kısmını tutmaktadır. Bu kısım belli bir dereceye kadar ayrışmakta ve zamanla yoğunlaşarak dayanıklı bir karbon

deposu olan humusa ve mikrobiyal ayrışmaya fiziksel olarak dayanıklı diğer moleküllere toprak matriksinde dönüşebilmektedir. Tarımsal sistemlerde toprak işlemesi organik karbonu toprak mikroorganizmalara daha yarayışlı hale getirirken bozulmamış topraklarda TOK birikme eğilimindedir [34].

Topraktaki mikrobiyal metabolik aktivite genelde katabolik solunumun ölçülmesiyle belirlenmekte olup solunumun küresel karbon döngüsünde temel bir süreç olduğu ve toprakta enerjinin bölünmesinde hayati bir role sahip olduğu bilinmektedir. Aerobik solunum süreçlerinde O_2 tüketilir, CO_2 salınır ve bu şekilde ekosistemden atmosfere doğru bir karbon kaybı meydana gelmektedir. Toprakta organik madde, hazır yarayışlı ölü örtü ve bitkisel salgılar kimyasal olarak değişime uğrar ve mikrobiyota solunum süreçleri yoluyla bu maddelerdeki bitki besin elementleri mineralize olurlar. Küresel iklim değişiminin toprakta karbon depolanması, bağlanması ve salınımı ile yakından ilişkili olduğundan toprakta karbondioksit oluşumu son yıllarda oldukça önem kazandığı bilinmektedir [35, 36]

Çeşitli uygulamaların ve farklı madde girişlerinin toprak mikrobiyal aktivitesine olan etkilerinin tespit edilmesinde toprakta CO_2 salınım yöntemi yaklaşık 100 yıldır kullanılmaktadır [37-40]. Laboratuvarda kurutulan ve elenen bir toprak tekrar nemlendirilip inkübe edildiğinde ölçülen 3 günlük karbon mineralizasyonu, arazi nemli toprakta ölçülen 24 günlük karbon mineralizasyonuyla önemli derecede örtüştüğü bildirilmiştir [41]. Ayrıca, kurutulan (40°C ve 60°C) ve tekrar nemlendirilen topraklardaki kısa dönemli karbon mineralizasyonunun (1-3 günlük), uzun dönemli (100 günlük) CO_2 oluşumu ve toprak mikrobiyal biyokütle karbonu ile önemli bir biçimde korelasyon gösterdiği ifade edilmiştir [42, 43]. Toprakta inkübasyon süresince mineralize olan karbonunun toprak organik karbonuna olan oranına " Karbon mineralizasyon oranı" denilmektedir [39, 40, 44]

Atrazine İle İlgili Yapılan Çalışmalar

Atrazine, günümüzde halen pek çok sayıda geniş yapraklı yabancı otun kontrolünde yaygın bir biçimde kullanılan bir herbisit olup yeraltı ve yer yüzey sularında sıklıkla tepsit edilmektedir. Atrazine'in toprakta ayrışmasını sağlayan ana süreç mikrobiyal ayrışmadır. ^{14}C-atrazin'in arazi koşullarına uyumlu bir toprakta, bu koşullara adapte olmayan bir toprağa göre daha hızlı ayrıştığı bulunmuştur [45]. Aynı çalışmada araştırıcılar, her iki toprağa glukoz ilave edip laboratuvar koşullarında inkübe

ettiklerinde atrazin mineralizasyonunu etkilenmediğini ancak yüksek miktarda azot ilavesinde (2.5 g N/kg toprak) atrazine mineralizasyonunu durdurduğunu bildirmişlerdir [45]. Toprağa atrazine'in uygulama dozunun 2 (94 mg/ kg toprak), 4 (188 mg/ kg toprak) ve 6 (282 mg/kg toprak) katı uyguladığında karbon mineralizasyonunun arttığı bildirilmiş olup uygulama dozunun 2 kat artması halinde toprağa giren atrazin karbonunun %67'sinin, 4 kat dozda %78'den fazlasının ve 6 kat dozda %28'inin mineralize olduğu tespit edilmiştir [46]. Ayrıca araştırmacılar, atrazine'in toprak karbon mineralizasyonuna duyarlı olduğunu ifade etmişlerdir [46]. Atrazine'nin 0,2 ile 1000 mg/kg arasında 10 farklı dozu yarı kurak bir bölgeden alınmış toprağa karıştırıldığında mikrobiyal solunumun arttığını ve sadece toprak mikrobiyal popülasyonunun çok küçük bir kısmının atrazine'i tamamen CO_2 ve H_2O'ya dönüştürdüğü bildirilmiştir [47]. Buna ek olarak atrazine'in kısmi ayrışmasıyla meydana gelen organik ürünlerin daha fazla çeşitteki toprak mikroorganizmalarınca kullanılabileceğini bildirmişlerdir [47]. Glyphosate (2.16 mg etken madde/g toprak) ve atrazine'in (2.25 mg etken madde/g toprak) tavsiye edilen dozlarını ve bunun 2 katını beraber tropik bir toprağa karıştırıldığında atrazine varlığının geçici olarak toprakta mikrobiyal biyokütleyi azalttığı ve karbon mineralizasyonunu arttırdığı belirlenmiştir. Aynı çalışmada karbon mineralizasyonundaki artışın herbisit toksisitesine dirençli organizmaların ölü biyokütleyi mineralize etmiş olabileceğini ifade edilmiştir [48].

Glyphosate İle İlgili Yapılan Çalışmalar

Glyphosate, ekim öncesi ya da çıkış sonrasında yabancı otların kontrolünde kullanılan, yapraklara uygulanan ve seçici olmayan bir herbisittir. Bu herbisit bitkinin yaşayabilmesi için gerekli olan aromatik aminoasitlerin sentezini engellemekte olup toprakta kil minerallerince adsorbe olabilmektedir [49]. Atrazine'in ve Glyphosate'ın tavsiye edilen tarla dozlarının 2 (94 mg/kg toprak), 4 (188 mg/ kg toprak) ve 6 (282 mg/kg toprak) katı beraber toprağa uygulandığında karbon mineralizasyonun kontrole göre sırasıyla %244, %46 ve %76 arttığını bulmuşlardır [46]. Aynı çalışmada araştırmacılar, bu herbisitlerin karbon mineralizasyonun 4 ve 6 kat dozlarındaki azalışın atrazine'nin mineralizasyonunda meydana gelen klor iyonunun mikrobiyal toksisiteye yol açabileceğini bildirmişlerdir. Glyphosate'ın tavsiye edilen dozu (47 µg/g toprak) ile bunun 2 (94 µg/g toprak), 3 (140 µg/g toprak) ve 5 (234 µg/g toprak) katı topraklara karıştırıldığında kümülatif karbon mineralizasyonunun doz artışına bağlı arttığı

bulunmuş olup yüksek dozlarda bile toprak mikroorganizmalarının glyphosate'ı doğrudan ve sürekli olarak parçalayabildikleri bildirilmiştir [49]. Bu çalışmaya ek olarak glyphosate'ın tavsiye edilen tarla uygulama dozu (6 l/ha) ve bunun 2 katı (12 l/ha) zeytin topraklarına karıştırıldığında karbon mineralizasyonunu arttırıcı özelliğine sahip olduğu ve toprak mikroorganizmalarının glyphosate'ı karbon kaynağı olarak kullanabildikleri ifade edilmiştir [50]. Başka bir çalışmada, glyphosate'ın düşük dozları (1.5 ve 15 mg etken madde/kg toprak) topraklara karıştırıldığında karbon mineralizasyonunda kontrol ile uygulamalar arasında istatistiksel bir fark olmadığı bulunmuştur. Ancak aynı çalışmada herbisitin yüksek dozu (1500 mg etken madde/kg toprak, GLY) ve bununla beraber diamonyum fosfat gübresi (250 mg/kg toprak, GLY+G) toprağa karıştırıldığında ise her iki uygulamada topraktan CO_2 çıkışının arttığını ancak GLY+G uygulamasının GLY uygulamasına göre C girişini %16 azalttığı belirlenmiştir [51]. Osmaniye'de bir turunçgil bahçesinde glyphosate'ın püskürtme öncesi ve sonrası alınan toprakları ile kontrol amacıyla alınan Osmaniye Korkut Üniversitesi Kampüs'ü toprağına herbistin tavsiye edilen tarla dozunun kendisi, 2 ve 4 katı karıştırıldığında turunçgil toprağında bütün dozların karbon mineralizasyonunu önemli bir biçimde arttırırken kampüs toprağındaki artışın önemsiz olduğu ifade edilmiştir [52].

Sonuç ve Öneriler

Karbon mineralizasyonu toprakta mikrobiyal aktivitenin belirlenmesinde kullanılan eski ama güvenilir bir yöntemdir. Karbon mineralizasyonundaki değişimler, pestisit toksisitesinin belirlenmesinde önemli bir kriter olarak değerlendirilmektedir.

Laboratuvar koşulları altında yapılan çalışmalarla atrazine ve glyphosate'ın tavsiye edilen tarla dozları topraklara karıştırıldıklarında mikrobiyal aktiviteyi olumsuz etkilemediği ancak karbon mineralleşmesinin bu herbisitlere duyarlı olduğu söylenebilir. Buna ek olarak, aynı herbisitlerin tavsiye edilen tarla dozlarının iki ve dört katları uygulandığında toprak karbon mineralizasyonunu teşvik ettiği ve hatta toprak mikroorganizmalarının bu herbisitleri yaşamsal faaliyetlerini sürdürebilmek amacıyla enerji kaynağı olarak kullanabileceği yapılan çalışmalarda ortaya çıkarılmıştır. Buna karşın, arazide pek çok faktörün herbisitlerin toksisite potansiyelini maskeleme veya azaltma ihtimali olduğundan dolayı, laboratuvar ile arazi uygulamaları arasında fark çıkabilir. Bu yüzden bu herbisitlerin toprak karbon mineralizasyonuna olan etkileri

konusunda genel bir sonuca varılması için arazi koşullarında denenmesi gerekmektedir. Glyphosate'ın ve atrazine'in tarım arazilerinde uygulama sıklıkları fazla olduğundan dolayı toprağın fonksiyonunda ve bitkisel üretimde meydana gelebilecek potansiyel negatif etkilerinden kaçınmak için bu kimyasalların kritik sınırlarının tanımlanması amacıyla daha fazla araştırma yapılmasına ihtiyaç duyulmaktadır.

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