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A N I N T E R N A T I O N A L J O U R N A L

Measuring of the Genotoxic and Potential Antioxidant Effects of Essential Oil Obtained from *Satureja Hortensis* Against to *Phaseolus Vulgaris*

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ABSTRACT

It is known that, there are many ways for increasing the yield at the crops specially cereal products. Genetic manipulations and physiological interventions are the primary studies that aim to obtain products at high quality and amounts. It is known that, the usage of synthetic chemicals for physiological inverventions negatively affects organism. But using natural plant products instead of these chemicals is a subject that draws attention of today scientific environment. In this study, this situation was taken into consideration and the essential oil obtained from Satureja hortensis (SEO) plant were applied to bean seeds. The genotoxic and physiological effects of SEO at four different dosages were detected against to Phaseolus vulgaris seedlings. According to obtained data, essential oil that were applied at different doses decreased the genomic stability of the bean seeds up to 30.77% in accordance with the increased dosage. The essential oil applied in the same way lead to stress on enzyme activities of seedlings. Among the antioxidant enzymes, while, significant changeswere observed at Superoxide dismutase and Peroxidase enzymes according to the control, no significant change was seen at Ascorbate peroxidase level.

Keywords: Antioxidant Enzymes, Biyopesticide, Essential Oil, GTS

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INTRODUCTION

Cereal products and legumes have an important place in the food sector. These products are used as an important nutritional source in every country and especially in the developing countries. Therefore, agricultural techniques in increasing the quality and yield ratio of the cereal products carry a big importance.Along with the developing technology, it is know that there are various ways of increasing the yield at the crop products. Genetic manipulations and physiological interventions are the primary studies that aim to obtain products at high quality and amounts. But it is a reality that the products are developed by adding genetic codes and making interventions to the chromosome numbers for the producing desired product, make the community anxious. On the other side, it is known that synthetic chemical improvers generally are used as devoted to increase the harvest amount. The pesticides that eliminate other organisms except for the plant desired to be produced are the exogenic substances that blocks many cellular metabolic pathway and therefore physiologically affects the organism. It was observed in various studies that these chemicals protects the plant from harmful pests and at the same time gives harms to the plant itself. Previous studies states that annually 2,5 million ton pesticide is used in the cereal production in the world and annual damage amounts that these pesticide lead is 100 milliard dollars [(1)]. Today it is known that harmful effects of the synthetic chemicals do not remain limited to the metabolism and in the same way it affects that genome which is a universal effect. In the previous studies, it was stated that synthetic chemicals expands to the environment especially at the usage stage [(2)]. For example; detection of mercury (Hg) and dicloro difenil tricloroethan (DDT) residuals even at the poles where human activity is limited put forth dimensions of these chemicals expansions [(3)]. While most of afore-mentioned chemicals are abolished from the market due to their harmful effects, day by day new chemical substances are synthesized. While these synthesized chemicals disturb the ecological balance, on the other side they reach to toxic dosages at high organisms by increasing at the every stages of the food chain.

Knowing that synthetic chemicals have harmful effect directs the human towards natural plant components. Because plant components are products of a natural struggle and their crack up after fulfilling their function is easier than synthetic products. If these specifications of the natural plant components are taken into consideration, the problems seen at production of important food crops such as cereals and legumes may be precluded.

Some of the plant components that may be used at the natural plant struggle are the essential oil (EO) that obtained from aromatic plants. The essential oil (natural oils, etheric oils) and aromatic extracts; it is widely used in fragrance and taste industries, at composition of perfume, food additives, cleaning products, cosmetic and drugs, source of aroma chemicals or starting substance for synthesis of the aroma nature identical or semi-synthetic beneficial aroma chemical[(4)].

Lamiceae family is one of the plant family which volatiles content is available at higher amounts. Important results were obtained from the studies that were done with this family. For example; it was observed that essential oil obtained from the plant of *Thuja orientalis* decreased the germination of most of wild seeds depending on the increased dosage [(5)]. It was observed that *Origanum dubium L.* essential oil lead to important decreases at germination ratios and root lengths of the seeds belonging to *Rumexcrispus L., Amaranthus retroflexus L., Sinapis arvensis L. Physalisangulata L.* species[(6)].

Although they are natural, the plant components may lead to problems, acute disorders or toxic effects at the livings [7-9]. It is recorded that they may even lead to fatal effects [10]. Also allelopathic interactions of aforementioned plant components were examined and found to prevent the development of weeds [11]. In a similar manner, it was detected that root and body developments of the weeds such as *Lomatium rigidum* and *Phalaris brachystachys* at which extracts obtained from *Satureja hortensis* species were negatively affected [12]. In this case, determination of applicable dosage has importance.

Satureja hortensis is belonging to Lamiaceae plant family is the one of the plants that have the relevant volatiles at high amount. As a matter of course, it has some specifications that limit usage of essential oil at the bio-control. For example; these may be deemed as disadvantages that they are volatile, insoluable in water and not present at the plants at high amounts [13]. But these are non-negligibly important that they are fast metabolized, natural and very effective at low concentrations. Therefore, it is required that possible toxic and physiologic effects of essential oil will be determined before their usage as bio-control agents. By starting from these information, toxic effect of essential oil obtained from *Satureja hortensis* againist *Phaselous vulgaris* seedlings were determined by Randomly Amplified Polymorphic DNA (RAPD) and physiological effect by measuring antioxidant enzyme levels.

MATERIALS AND METHODS

Satureja hortensis L. plants were collected from Bingol city of Turkey at 1100-1200 latitude during the flowering stage in July 2013. The identification of plant materials was confirmed by a plant taxonomist, Prof. Dr. Yusuf KAYA from Ataturk University, Erzurum, Turkey.

Commercial seeds of *Phaseolus vulgaris* were used for EO treatment. Equally sized seeds were chosen and sterilized according to Bozari et. al., [14]. After the surface sterilization with NaOCl, seeds were washed with double-distilled water and dried with the sterile filter paper. Fifteen seeds were germinated in 15cm diameter Petri dishes on two layers of sterile Whatman No. 1 filter paper. The seeds were exposed to four different (0.1, 0.2, 0.4, 0.8µl/ml) concentrations of EO. Tween 20 was added to the distilled water to dissolve the EO. Only double distilled water with Tween 20 was used for the control group. Three replicates were made for each concentration [14].

Genomic DNA was extracted by using the before reported procedure [15]. 15 RAPD primers were selected and eight of them gave polymorphic bands and performed for the study. The primer sequences were (5'→3') TGCTCTGCCC (**OPB-6**), GTCCACACGG (**OPB-8**), TGGGGGACTC (**OPB-9**), CTGCTGGGAC (**OPB-10**), GTAGACCCGT (**OPB-11**), CAGCACCACA (**OPA-16**), CTGGACGTGA (**OPW-19**) and AAGGCTCACC (**OPY-24**). RAPD technique was performed as Aksakal et. al,.[16]. Genomic template stability (GTS, %) calculated with the following formula $GTS=\left(1-\frac{a}{n}\right)*100$ where "a" is the total polymorphic bands counted in each treated sample and "n" is the total bands in the control. Polymorphic bands were existed from missed bands and/or appearance of new bands against to control.

The root and stem samples of the germinated seeds were used to evaluate the antioxidant enzymes. Superoxide dismutase (SOD) was evaluated by using the technique performed by Agarval and Pandey [17]; peroxidase (POX) by Ye et.al., [18] and ascorbate peroxidase (APX) by Nakano and Asada [19].

Homogenized leaf tissues (0.5 g) were ground in a mortar with liquid nitrogen and extracted in 500 µL of 10 mmol L^{-1} potassium phosphate buffer (pH 7.0) containing 4% (w/v) polyvinyl pyrrolidone and 1 mmol L^{-1} ethylenediaminetetraacetic acid (EDTA) followed by centrifugation at 12,000 × g for 15 min at 4°C. The supernatant was used as an enzymes source (SOD, POX and APX) [20].
The statistical analyses were carried out using the package software SPSS 22.0 for windows.

RESULTS AND DISCUSSION

The plants come across so many types of biotic and abiotic stress factors at the natural environment. These exogen related substances may lead to so many physiological problems at the livings as well as genetic changes. The pesticides that used at the plant control are one of these substances. Pesticides are the mixtures that are formed from the substance(s) and used to prevent or decrease the harmful organisims and keep these organisims under control during production of agricultural products.
It has been documented that synthetically produced chemicals lead oxidative stress change the genetic structure of the plants, thus damage the genomic stability in plants [21-23]. The increase in amount of reactive oxygen species (ROS) due to stress factors, may lead to lose of cell membranes functions such as mitochondria, chloroplast and paroxysm and therefore necrosis [24, 25]. ROS can induce several damages in plants by degradation of proteins, inactivation of enzymes, DNA alterations, and interfere in various pathways of metabolic importance. Plants improve different protection systems as antioxidant enzymes to scavenge these ROS [26]. Plants have a protective system that is consisted of membrane bounded antioxidants, water soluble antioxidant such as glutathione and ascorbate and enzymatic antioxidants such as Superoxide dismutase (SOD), Ascorbate peroxidases (APX), catalase (CAT) and peroxidase (POX) inorder to struggle with destructive effects of reactive oxygen species cause [20, 27, 28]. In case of an increase in the amount of ROS, they are systematically transformed into harmless forms by enzymatic antioxidant system at the apoplastic region where cellular damages starts [24, 29-31].
It is known that superoxide radicals are very reactive and they may also lead oxidation of many biochemical components in the cell.. It was stated that lipidperoxidation of this radicals lead to membrane damage, cellular toxicity and single chain break at DNA [32-34]. Superoxide radicals may produce hydroxyl radical which are more toxic molecule by reacting with the hydrogen peroxide [35, 36]. While, SOD catalyzing transformation of relevant radical to hydrogen peroxide and molecular oxygen, APX enzymes detoxify peroxides by using ascorbate as a substrate and POX break down hydrogen peroxide [37, 38].
According to the results obtained from RAPD in this study, polymorphism ratio of bean seedlings were generally high. Number of the polymorphic bands at all dosages were determined as total 4 units as 2 new formed and 2 descending band. Polymorphic bands at high ratios were observed at OPY-24, OPW- 19 and OPA-16 primers among applied primers. The GTS values were 58.97%, 56.42%, 30.77% and 38.46% for 1, 2, 4 and 8 µl/ml, respectively (Table 1).It seems the essential oil have changed the genomic stability of the seedlings in a dose dependent manner.

Table 1. The number of bands in control and molecular size of disappearance (-), and/or appearance (+) of DNA bands (base pair) for all primers in the EO treated bean seedlings.

Primer	Control		0.1µl/ml	0.2µl/ml	0.4µl/ml	0.8µl/ml
OPB-6	2	+	*ND	*ND	*ND	*ND
		-	*ND	*ND	*ND	*ND
OPB-8	5	+	253	253	253	253
		-	551, 410, 131	551, 410	551, 410	551, 410,131
OPB-9	3	+	*ND	*ND	325	*ND
		-	*ND	275, 244	275,	275,
OPB-10	2	+	*ND	*ND	*ND	*ND
		-	*ND	*ND	254	*ND
OPB-11	4	+	*ND	*ND	*ND	*ND
		-	261, 233, 95	*ND	233	261,233,95
OPA-16	3	+	502, 298, 265, 110, 95	505, 502, 298,265	502, 298, 265, 110	502, 298, 265, 110
		-	*ND	176, 124	*ND	*ND
OPW- 19	12	+	501	501	501	501
		-	590, 478	590, 478	585, 520, 494, 478, 251, 133, 102, 75	585,520, 494, 478, 301, 251, 102, 75
OPY-24	8	+	*ND	*ND	*ND	175, 150, 75
		-	512	594, 555, 512	594, 555, 512, 490, 301, 207, 110	*ND
Total Band	39		16	17	27	24
Polymorphism			41.03	43.58	69.23	61.53
GTS Value			58.97	56.42	30.77	38.46

*ND:Not Detected

It was also observed that RAPD band densities significantly changed (Figure 1).

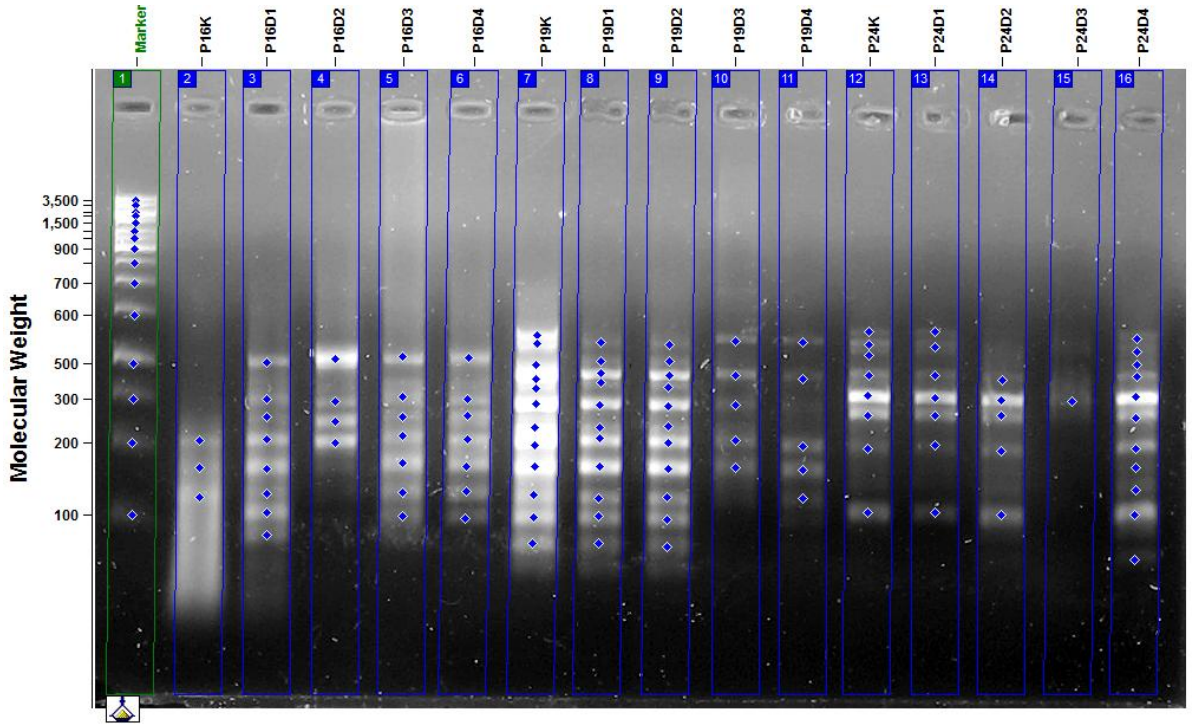

Figure 1. RAPD profiles of Essential oils treated and non-treated (control) Phaseolus vulgaris seedlings with

different primers (P: Phaseolus, 16: Primer 16, 19: Primer 19, 24: Primer 24, K: Control, D1: first dose (0.1µl/ml), D2: second dose (0.2µl/ml), D3: third dose (0.4µl/ml), D4: fourth dose (0.8µl/ml)).

On the other hand, as seen from Table 2, it was detected that applicatoin of EO significantly increased the superoxide dismutase enzyme amount in the samples obtained from germinated parts of bean seeds at the first concentration (1 µl/ml), but this increase gradually decreased towards control at increased concentrations. It was detected that the ascorbate peroxidase enzyme increased at first two concentrations but they were available only at lower levels at 4 and 8 µl/ml concentrations compared to the control. But it was detected that the difference between control and application concentrations were not significant. On the other hand, it was detected that the peroxidase enzyme level was independently different than the applied concentrations. The POX level increased at 1 µl/ml concentration compared to the control and at 2 µl/ml concentration a significant decrease was observed compared to the control. At 4 and 8 µl/ml concentrations, it was detected that the level of POX were again increased and these values were important according to the control.

Table 2. The values of cellular SOD, APX and POX enzyme activity of bean seeds that treated with essential oil for seven day.

Doses	**Superoxide dismutase (SOD) S.E***	**Ascorbate peroxidase (APX) S.E**	**Peroxidase (POX) S.E**
Control	0,503±0,07[a]	0,060±0,004 [a]	0,662±0,001 [b]
1µl/ml	0,711±0,05[b]	0,064±0,007 [a]	0,831±0,007[d]
2µl/ml	0,682±0,03[a,b]	0,069±0,004 [a]	0,539±0,007 [a]
4µl/ml	0,643±0,03 [a,b]	0,052±0,003 [a]	0,688±0,01 [c]
8µl/ml	0,637±0,06 [a,b]	0,053±0,001 [a]	0,672±0,002 [b,c]

Means within each column followed by the same letter are not significantly different at the $p < 0.05$ level as determined by Duncan's multiple range test.
*S.E.:Standard Error

According to the data obtained from this study, it was observed that, the essential oil applied to bean seeds affected SOD enzyme level. Although there was a significant change at the first concentration compared to the control, the difference in the level of SOD at higher concentrations was not significant. In this case, increased concentration of the essential oil changed the genetic strauctureof the plants and reduced the synthesis of enzyme. This case may be explained with the increase of polymorphism ratio (Table 1). The other scenario is that; SOD that provides the peroxidation of lipids may also oxidise the lipid derivative essential oilat an increased level, therefore, limits the activity of essential oil. But this case is inadequate for solely explaining decrease of genomic stability at increased concentrations.

On the other side, POX is an enzyme available at the fruits and vegetables. It protects the cell from oxidative stress that hydrogen peroxide causes. This enzyme catalyzes degeneration of many aromatic components such as phenols and hydroquinols by using H_2O_2 [(39-41)]. It is known that peroxidase activity increases at the plants under the stress [(34)]. Peroxidase regulates level of harmful oxygen radicals produced under unfavorable environmental factors and protects the plants in this way [(24, 42)]. According to the data gained from this study, it was observed that peroxidase amounts changed independently from the concentration. Germinated seeds get stressed even at the lowest concentration of the applied essential oil and triggered the antioxidant defense system at a level that may be deemed to be significant compared to the control. Although, the highest peroxidase

level was observed at the lowest EO concentration, the mentioned increase in peroxidase levels were deacreased at 2, 4 and 8 µl/ml EO concentrations, but still it showes that EO gave important damages at higher concentrations. Such that, it may be thought that natural plant components triggered DNA damage or physiologically blocks enzyme production ways.

APX Ascorbate peroxidase (APX) is a scavenging enzyme against H_2O_2 and hydroxyl radicals behave in a similar way as in chloroplast and other cell components [43]. It was observed in this study that this enzyme providing protection of the organelles such as chloroplast did not show a significant decrease or increase compared to the control. This case was attributed to the inmature chloroplast organelles that available at the germinated seeds.

CONCLUSION

The problems that pesticides may cause in terms of environment, health and economy are very well known in the developed countries. Therefore, agricultural products are consuming continuously inspected in terms of environment and human in all developed countries especially in EU [44]. But, nowadays for the obtaining of the maximum yield at unit area in a short time, application of pesticide has been still a widely used control way to preventing the food deficiency for the increased human population. On the other side, various ways are tried to be applied for reliable food production. One of these ways is known as bio-control. Natural plant components that are easy to be decompose, has no toxicity or low toxicity are amoung the products that will be used at the control of agricultural processes. A similar natural substance was used in our study. Even if usage of natural components have lower genotoxicity than chemical pesticides [21, 23] more studies should be performed in order to confirm the reliability of these natural components.

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