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Original Research Article

Phytotoxic Effect of Quizalofop-P-Ethyl on Soybean (*Glycine max* L.)

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ABSTRACT

In this study, phytotoxic effects of phenoxy herbicide Quizalofop-P-Ethyl (QPE, ethyl (R)-2-[4-[(6-chloro-2-quinoxalinyl)oxy]phenoxy] propionate) in *Glycine max* L. was investigated. The effective concentration (EC50) value was determined as approximately 0.4 M. Morphological and anatomical experiments were carried out using QPE concentrations of 0.4 M (EC50) and 0.8 M (EC50x2) on 5th and 10th days, by a control for each combination. QPE concentrations were applied with spraying method in 2-3 leaf stage. The phytotoxic effects were determined by morphological and anatomical experiments. Root and seedling growth, chlorophyll and carotenoid contents and seedling and leaf anatomy were identified. QPE exposure significantly reduced the amount of carotenoid and chlorophyll b pigments except of chlorophyll a in all treatment. Parallel to the increase in concentrations of QPE, there was a reduction in root and seedling length and also the lengths of the anatomical parts of seedlings were changed when compared with the control group. It is vital to confirm that the usage of QPE should be subject to control since it might have a toxic effect on farmers who applied the herbicide and humans who consumed the plant.

Key Words: Quizalofop-P-Ethyl, *G. max*, Morphology, Anatomy, Chlorophyll, Herbicide

Quizalofop-P-Ethyl'in Soya Fasulyesi (*Glycine max* L.) Üzerindeki Fitotoksik Etkileri

ÖZET

Bu çalışmada bir fenoksi herbisit olan Quizalofop-P-Ethyl'in (QPE, ethyl (R)-2-[4-[(6-chloro-2-quinoxalinyl)oxy]phenoxy] propionate) *Glycine max* L. üzerindeki fitotoksik etkileri araştırıldı. Etkili konsantrasyon (EC50) değeri yaklaşık 0.4 M olarak belirlendi. Morfolojik ve anatomik deneyler her kombinasyon için bir kontrol ile, 0.4 M (EC50) ve 0.8 M (EC50x2) QPE konsantrasyonları kullanılarak 5. ve 10. günlerde gerçekleştirildi. QPE konsantrasyonları 2-3 yapraklı dönemde spreyleme yöntemiyle uygulandı. Fitotoksik etkiler morfolojik ve anatomik deneyler ile belirlendi. Kök ve fide gelişimi, klorofil ve karotenoid içeriği ve kök ve fide anatomisi tanımlandı. QPE muamelesi klorofil a hariç, karotenoid ve klorofil b pigment miktarını önemli ölçüde azalttı. QPE konsantrasyonundaki artışa paralel olarak, kök ve fide boyunda azalma ve ayrıca kontrol grubuyla karşılaştırıldığında fide anatomik kısımlarının boyunda değişiklikler olmuştur. Herbisiti uygulayan çiftçiler ve bitkiyi tüketen insanlar üzerinde toksik etkiye sahip olabileceğinden, QPE kullanımının kontrole tabi olması gerektiğini belirtmek önemlidir.

Anahtar Kelimeler: Quizalofop-P-Ethyl, *G. max*, Morfoloji, Anatomi, Klorofil, Herbisit

INTRODUCTION

Pesticides which are used in the modern agricultural practices for disease control have some dangerous effects (Pandy et al. 1994). Different pesticides and plant growth regulators are being used extensively in modern agriculture; though the use of these chemicals has become a necessity, their frequent and indiscriminate use has many undesirable consequences in culture plants (Aksoy et al. 2007). Pesticides from a broad range of classes are widely used in various combinations and at different stages of cultivation and during postharvest storage to protect crops against a range of pests and fungi, and/or to provide quality preservation. Pesticides can be carried to the final products, such as infant foods, even following food processing (Wang and Cheung 2006).

The herbicides, one of the pesticides, are used in agriculture in controlling weeds. QPE is a phenoxy herbicide compound. It is absorbed rapidly through leaf surfaces and quickly hydrolyzes to fluazifop acid. The acid is transported primarily in the phloem and accumulates in the meristems where it disrupts the synthesis of lipids in susceptible species (Urano 1982, Erlingson 1988). The indiscriminate use of herbicides in agriculture, as well as the increase of pollution in ecosystems due to industrial development, justifies the evaluation of the toxicity of these chemicals (Marcano et al. 2004). Currently, the literature is unavailable on the phytotoxic effects of QPE herbicide in plant systems. The purpose of this study was to investigate the effects of QPE

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herbicide on root and seedling growth, the upper and lower surface of leaf stoma indexes, seedling and leaf anatomy and chlorophyll and carotenoid contents were identified in *G. max* plant. The commercial form of the pesticide was tested because this is the form that is utilized in agriculture and introduced into the environment. *Glycine max* was chosen as an experimental plant material as it is a crop of global importance and is one of the most frequently cultivated crops worldwide. As biomarkers give information about the development and growth of the plants, the results may suggest a tolerance value of soybean according to its growth.

MATERIALS AND METHODS

Chemical material

Quizalofop-p-Ethyl, [Ethyl (2R)-2-[4-(6-chloroquinoxalin-2yloxy) phenoxy] propionate] (The End EC) is a post-emergence phenoxy herbicide widely used in soybean fields for weed control. The commercial form of the pesticide was tested. It is a 5% emulsifiable solution produced by Agrogeneral Company and applied for control weeds in soybean fields at rate of 50g (recommended dose) active ingredient per decare. The structural formula of QPE was shown in Figure 1.

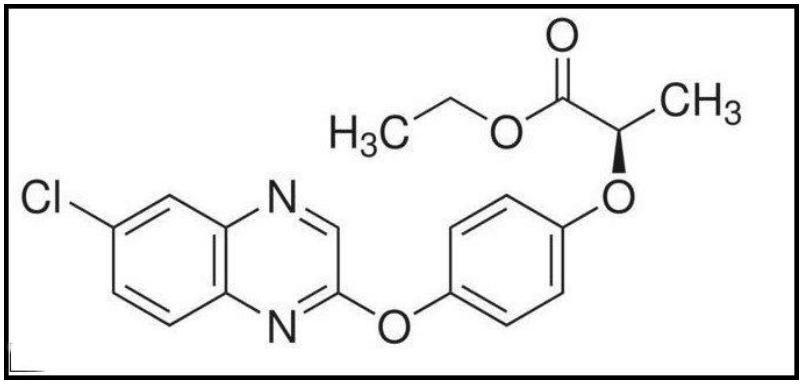

Figure 1. The structural formula of QPE

Determination of EC50

Surface sterilized *G. max* seeds were allowed to produce roots in distilled water for 24 h, where after the fifty homogeneous seeds transferred to the distilled water and ten different concentrations of QPE (0.0125, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 ve 6.4 M) for 72 h. For preperation the QPE solution, a stock solution of QPE was prepared in distilled water and the test concentrations were obtained by diluting the stock solution. The root lengths in the control and QPE treated goups were measured and the relative reduction in root length was calculated (T/C%) after treatment for 72 h. Three replicates were made for each concentration.

Procedure for treatment

Healthy and proximate equal-sized soybean seeds were selected. The seeds were sterilized with 2.5% sodium hypochlorite solution for 10 min and washed in distilled water. 50 seeds of *G. max* (2n=12) was planted in each Petri dish. The seeds in each treatment group were placed on filter paper in Petri dishes at 25°C under fluorescent lights in a 16-h-light/8-h-dark cycle. The seeds were treated with EC50 and EC50x2 concentrations and control groups were germinated in distilled water. At the end of 5th,7th and 10th days, the root and shoot lengths of the germinated seeds were measured with a milimetric ruler. The root length was determined by radicula formation bases of *G. max* seeds nonexposed and exposed to QPE. After seedling formation, for the phytotoxicity analysis, the seedlings were grown in soil media: 95% peat and 5% humus (pH=6-7) in pots placed at 25°C under fluorescent lights in a 16-h-light/8-h-dark cycle.

Anatomical experiments

The materials for the anatomical studies were fixed and preserved in 70% alcohol. All the sections were taken by hand and the observations were performed on the superficial sections of leaves. Superficial sections were stained with aniline blue and then well stained sections were mounted with glycerine-gelatine in order to obtain permenant slides (Makbul et al. 2010) and photographed with an Olympus BX51 microscope and DP71 camera. All of the measurements and observations were performed 10 times on different slides.

Chlorophyll and carotenoid contents

The content of Chl a, Chl b and total carotenoids (xanthophylls and carotenes) in the leaves of the plants were determined by a UV-mini 1240 Shimadzu scanning and recording spectrophotometer. Chlorophyll was extracted from leaf tissue samples at 6-8 leaf stage (approximately 10 mg each) with 80% acetone, the absorbance of the extracts was measured at 470, 646 and 663 nm wavelengths. Chlorophyll and carotenoid concentrations were calculated from the spectrophotometric data using the formulae of Lichtenthaler and Wellburn (1983).

Chlorophyll a (μgml^{-1}) = 12.21 (A_{663}) - 2.81 (A_{646})
Chlorophyll b (μgml^{-1}) = 20.13 (A_{646}) - 5.03 (A_{663})
Carotenoids (μgml^{-1}) = [1000A_{470} - 3.27(chl a) – 104(chl b)] /227

Statistical Analysis

The statistical analysis of data were carried out using SPSS for Windows version 16.0 statistical software (SPSS Inc, Chicago, USA). Statistically significant differences between the groups were compared using one-way analysis of variance (ANOVA) and Duncan's test. The data are displayed as means ± standard deviation (SD), and p-values less than 0.05 are considered ''statistically significant.''

RESULTS

EC50 indicate the effective concentration for 50% growth inhibition. EC50 values was determined ~0,4 M for QPE. So, all experimental procedures were carried out using QPE concentrations of 0,4 M (EC50) and 0,8 M (2xEC50). After the treatment with different concentrations of QPE, we observed that seed germination decreased while the concentrations increased for each concentration of QPE in 5th day (Fig. 2).

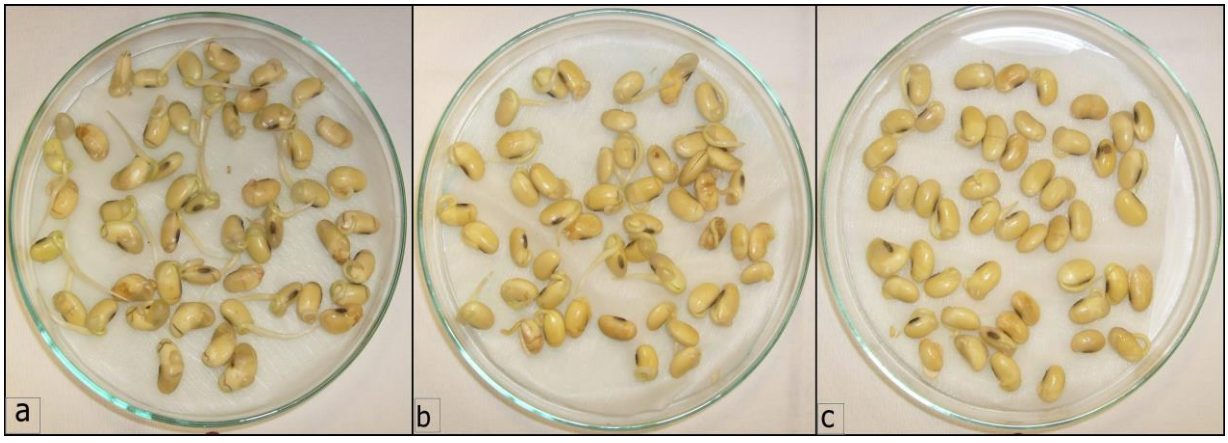

Figure 2. The germinated *G.max* seeds treated with QPE concentrations after 5 days, a) control, b) 0.4 M, c) 0.8 M QPE

The amount of leaf pigments of *G. max* were significantly affected by QPE treatment, which represented in Table 1. From the table, with increased QPE concentration, the distinct reduction amount of chlorophyll b and carotenoid content of leaves could be detected in 10th day. Under QPE stress, the amount of chlorophyll b content and carotenoids of *G. max* was more affected than the amount of chlorophyll a content. The amount of carotenoid and chlorophyll b content of *G. max* decreased approximately by 44% and 61%

respectively at the highest QPE concentration, while for chlorophyll a, the decrease was around 3% at the end of 10th day. The amount of carotenoid contents of *G. max* decreased with QPE treatment but the rate of reduction of amount of carotenoids was slower than amount of Chl b content. In this study the carotenoid contents decreased with QPE treatment but the rate of reduction was slower than the amount of Chl contents.

Table 1. Chlorophyll (Chl) and carotenoid concentration mean and standart deviation (±SD).

	5th day			10th day		
Conc.	Chl-a	Chl- b	Carotenoid	Chl-a	Chl- b	Carotenoid
Control	0,85 ± 0,02[a]	1,11 ± 0,18[a]	12,22 ± 0,30[a]	0,89 ± 0,76[a]	0.79 ± 0,53[a]	11,22 ± 0,03[a]
0.4 M	0,84 ± 0,05[a]	0,73 ± 0,04[b]	10,46 ± 0,45[b]	0,82 ± 0,09[a]	0,43 ± 0,12[a]	7,98 ± 0,85[b]
0.8 M	0,83 ± 0,02[a]	0,65 ± 0,12[c]	9,23 ± 0,07[c]	0,81 ± 0,03[a]	0,40 ± 0,05[a]	6,78 ± 0,43[c]

Data are shown as mean±confidence interval of three replicates. Different letter superscripts in the same columns indicate significant differences between treatments (P<0.05).

The effects of QPE on root and seedling growth of *G. max* was evaluated by taking into consideration the average of the root and seedling length after 10 days exposure. *G. max* showed a significant decrease in root and seedling length at a concentration of 0.8 M (Fig. 3 and 4.). *G. max* showed a significant decrease in seedling growth at concentration of 0.8 M after 5 days (Fig. 5) and 10 days exposure (Fig. 6). All anatomical parts length of soybean seedlings decreased significantly in all treatment of QPE after 10 days exposure but the rate of reduction in 0.8 M QPE concentraion was more than 0.4 M QPE concentration compared to the control group (Table 2).

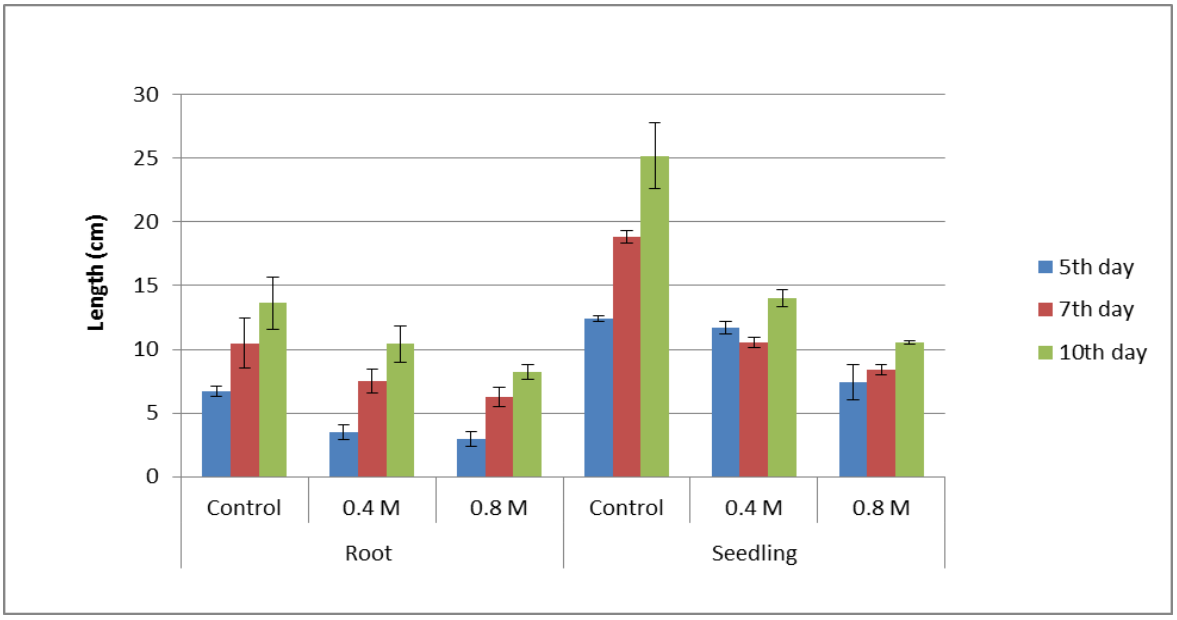

Figure 3. The average length of *G. max* root and seedling after 10 days exposure

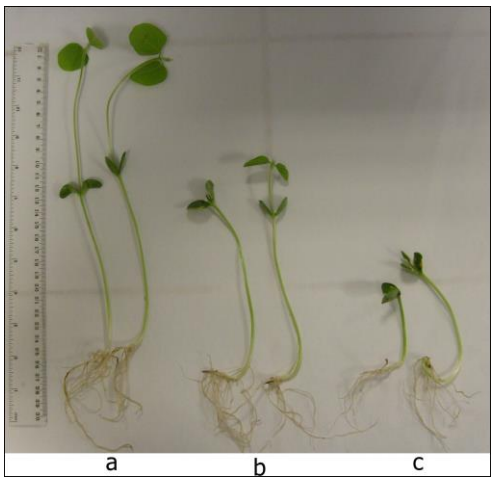

Figure 4. *G. max* root and seedling growth after 10 days exposure, a) control, b) 0.4 M, c) 0.8 M QPE

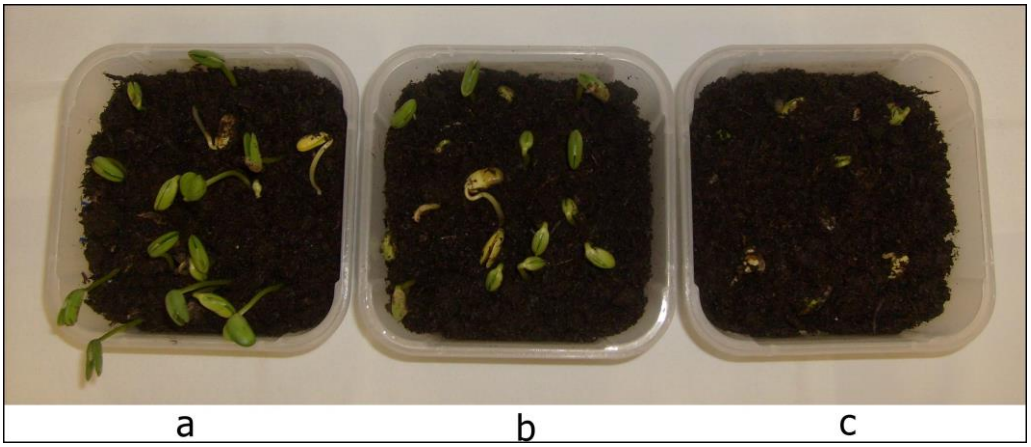

Figure 5. *G. max* seedlings after 5 days exposure, a) control, b) 0.4 M, c) 0.8 M QPE

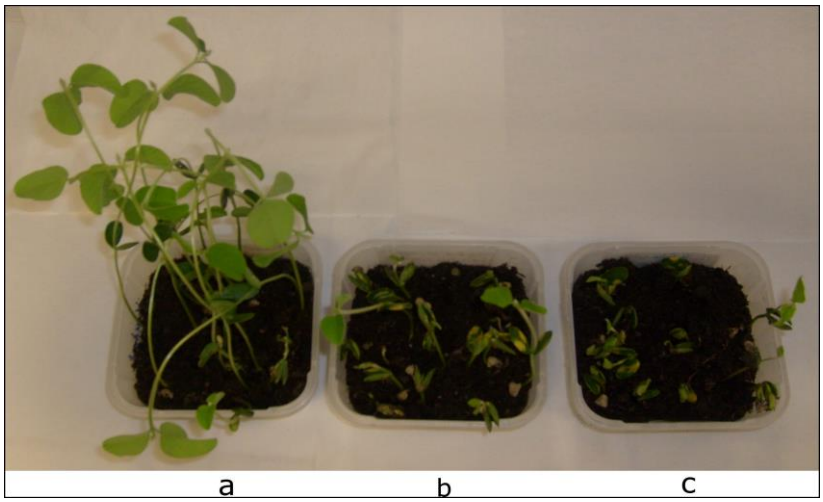

Figure 6. *G. max* seedlings after 10 days exposure, a) control, b) 0.4 M, c) 0.8 M QPE

Table 2. Anatomical parts length mean and standart deviation of *G.max* (±SD).

Anatomical parts of the seedling (μm)	Control	0.4 M	0.8 M
Cuticula	2.01 ± 0.83^{a}	1.98 ± 0.03^{b}	$1.75\ 0.05^{bc}$
Epidermis	32.02 ± 0.02^{a}	$31,45 \pm 0.93^{ab}$	$26,38 \pm 0.05^{c}$
Cortex parenchyma	$451,26 \pm 0.21^{a}$	$318,2 \pm 1.56^{b}$	$262,1 \pm 0.47^{c}$
Sclerenchyma	$96,23 \pm 0.43^{a}$	$46,08 \pm 0.76^{b}$	$42,78 \pm 0.04^{bc}$
Phloem	$137,7 \pm 0.71^{a}$	$94,85 \pm 0.48^{b}$	$92,13 \pm 0.84^{b}$
Xylem	$127,81 \pm 0.04^{a}$	$101,78 \pm 0.12^{b}$	$98,03 \pm 0.63^{c}$
Trachea (width)	$38,27 \pm 0.19^{a}$	$32,49 \pm 0.69^{ab}$	$29,43 \pm 0.81^{c}$
Trachea (length)	$34,95 \pm 0.84^{a}$	$34,65 \pm 0.32^{a}$	$32,52 \pm 0.72^{a}$

Data are shown as mean±confidence interval of three replicates. Different letter superscripts in the same columns indicate significant differences between treatments ($P<0.05$).

DISCUSSION

Toxic effects of environmental pollutants may be evaluated by analyzing macroscopic (root growth decrease) as well as cytological parameters (types and frequencies of chromosome aberrations) (Smakakincl et al. 1996). The toxicity of QPE herbicide was found because of it showed an inhibition in root growth of *G. max*. The fact that the root growth decrease over 45% strongly indicates the presence of toxic substances (Fiskesjö 1985) having sublethal effects on plants (Hidalgo et al. 1989). Disturbance of seed germination and mitosis could produce severe consequences for root growth and development. This was clearly demonstrated for the roots of *Pisum sativum* and *Zea mays* with different concentrations of the sulfonylurea herbicides chlorsulfuron and metsulfuron methyl which caused severe injuries of the root growth (Fayez et al. 1994). Ruiz-Santaella et al. (2003) found that the herbicide cyhalofop-butyl from the family of phenoxy acetic acid decreases seed germination by 50% in *Echinochloa muricata*. Sasaki et al. (1968) reported that the use of herbicide 2,4-dicloro-phenoxy acetic acid (2,4-D) in *Pinus* decreased the seed germination percentage and similar results were found by Chopra and Singh (1978) in *Guizotia* which was treated with the same herbicide 2,4-D. Inhibition of germination may result from the interference of lead with important enzymes. Early seedling growth was also inhibited by lead in soya bean, rice, maize, barley, tomato, eggplant and certain legumes (Nagajyoti et al. 2010). Lead also inhibited root and stem elongation and leaf expansion in *Allium* species (Gruenhage and Jager 1985), barley and *Raphanus sativus* (Juwarkar and Shende 1986). We observed reduction in carotenoid content our study and this reduction showed that QPE not only induce the degradation of pigments but also reduced the biosynthesis mechanism. As most of the enzymes responsible in carotenoid synthesizing are membrane-bound, so the reduction of carotenoid biosynthesis might be due to the inhibition of these enzymes by interacted QPE. The inhibition of membrane proteins and lipids by various herbicides has already been reported (Stelzer and Gordon 1988, Michelangeli et al. 1990). The QPE treatment in seeds and leafs of *G. max* with different concentrations resulted in the reduction in germination of seeds, root and seedling length, amount of the leaf pigment content of soybean leaves and decrease in the length of all anatomical parts of soybean seedlings as statistically significant. It is vital to confirm that the usage of QPE should be subject to control since they may have a toxic effect on farmers and on humans consumed the plant. It also point to the importance of phytotoxicity testing of the applied chemicals like pesticides before use.

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REFERENCES

Aksoy Ö, Dane F, Sanal FE, and Aktaç T (2007). The effects of fusilade (Fluazifop-p-butyl) on germination, mitotic frequency and a-amylase activity of lentil (*Lens culinaris* Medik.) seeds. Acta Physiol. Plant 29: 115-120.

Chopra S, and Singh RP (1978). Effect of gamma rays and 2,4-d on germination, growth and morphogenetic responses in *Guizotia abyssinica*. Phytomorphology 92: 82-87.

Erlingson M (1988). Fusilade: a strategy for long-term control of couch (*Elymus repens*) weeds. Weed Control 1: 158-165.

Fayez GA, Gerken I, and Kristen U (1994). Ultrastructural responses of root caps to the herbicides chlorsulfuron and metsulfuron methyl. Plant Soil 161: 1-8.

Fiskesjö G (1985). The allium test as a standard in environmental monitoring. Hereditas 102: 99-112.

Gruenhage L, and Jager IIJ (1985). Effect of heavy metals on growth and heavy metals content of *Allium porrum* and *Pisum sativum.* Angew. Bot. 59: 11-28.

Hidalgo A, Gonzalez-reyes JA, Navas P, and Garcia-herdug G (1989). Abnormal mitosis and growth inhibiton in *Allium cepa* roots induced by propham and chlorpropham. Cytobios 57: 7-14.

Juwarkar AS, and Shende GB (1986). Interaction of Cd-Pb effect on growth yield and content of cd, pb in barley. Ind. J. Environ. Heal. 28: 235-243.

Lichtenthalker HK, and Wellburn AR (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem. Soc. Trans. 11: 591-592.

Makbul S, Saruhan N, Güler N, Durmuş N, and Güven S (2011). Changes in anatomical and physiological parameters of soybean under drought stress. Turk. J. Bot. 35: 369-377.

Marcano L, Carruyo I, Del Campo A, and Montiel X (2004). Cytotoxicity and mode of action of maleic hydrazide in root tips of *Allium cepa* L.. Environmental Research 94: 221-226.

Michelangeli F, Robso MJ, East JM, and Lee AG (1990). The conformation of pyrethroids bound to lipid bilayers. Biochim. Biophys. Acta 1028: 49-57.

Nagajyoti PC, Lee KD, and Sreekanth TVM (2010). Heavy metals, occurrence and toxicity for plants: a review. Environ. Chem. Lett. 8: 199-216.

Pandy RK, Shukla R, and Datta S (1994). Chromotoxic effects of one fungicide (Dithane M-45) and two insecticides (Aldrex-30 and Metacid-50). Cytologia 59**:** 419-422.

Ruiz-Santaella JP, Bakkali Y, Fischer AJ, and De Prado R (2003). Is it possible to detect *Echinochloa spp*. tolerance to accase inhibiting herbicides using a simple quick tolerance test?. Commun. Agric. Appl. Biol. Sci. 68: 331-334.

Sasaki S, Kozlowski TT, and Torrie JH (1968). Effect of pretreatment of pine seeds with herbicides on seed germination and growth of young seedlings. Can. J. Bot. 46: 255-262.

Smakakincl V, Stegnar P, Lovka M, and Toman MJ (1996). The evaluation of waste, surface and ground water quality using the allium test procedure. Mutation Research 368: 171-179.

Stelzer KJ, and Gordon MA (1988). Interactions of pyrethroids with gramicidin containing liposomal membranes. Biochim. Biophys. Acta 938: 114-120.

Urano K (1982). Onecide, a new herbicide fluazifop-butyl. Japan Pesticide Information. 41: 28-31.

Wang J, and Cheung W (2006). Determination of pesticides in soy-based infant formula using liquid chromatography with electrospray ionization tandem mass spectrometry, J. AOAC Int. 89: 214-224.