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Influence of Site of Oxyfluorfen Application on Growth, Pigments, Photosynthesis and Yield Attributes of *Glycine max* Plants

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Abstract: The diphenyl ether herbicide, oxyfluorfen effect was compared between foliar treatment and soil drench application on *Glycine max* plant. Spray of oxyfluorfen led to significant decrease in shoot length, number of leaves, total leaf area as well as fresh and dry weight of leaves and shoot after 45 days from sowing and a non significant decrease was manifested after 90 days from sowing. Soil drench with oxyfluorfen induced non-significant effect on the above mentioned parameters. Root length, number of nodules and root fresh and dry weight were non significantly affected by oxyfluorfen spraying and decreased significantly by soil drench. In the majority of cases, pigments content of soybean leaves as well as its photosynthetic activity decreased significantly by spraying oxyfluorfen; and non-significantly affected by soil drench. On the other hand, foliar application of oxyfluorfen decreased growth promoting substances (IAA, GA₃ and cytokinin) with simultaneous increase in IAA oxidase and ABA content of soybean plant after 45 days from sowing, whereas at the same stage, soil drench with oxyfluorfen affects these growth regulators non significantly. After 90 days from sowing both the site of application of oxyfluorfen appeared with insignificant effect on growth regulators content of soybean plant.

Key words: Growth, oxyfluorfen, soybean, yield

Introduction

Recently, the importance of using herbicides in agriculture has been greatly realized in the world as means of obtaining an increase in grain yield and improve its quality, particularly in view of labour shortage and technology development (Saad El-Din *et al.*, 1996).

A common feature of plants exposed to oxyfluorfen is the retardation in their growth and metabolism as well as its yield and yield attributes (Castro & Moraes, 1980; Heatherly *et al.*, 1992; Vora & Mehta, 1999; Nakamura *et al.*, 2000; Lagana *et al.*, 2000; Cheol Soo *et al.*, 2000). In this respect Halwanker *et al.* (1987) reported that oxyfluorfen decreased plant height of soybean but increased seed yield per plant, seed index and biological yield per plant. However, El-Quesni (1993) indicated that total carbohydrates, crude protein were increased by application of oxyfluorfen to soybean plant.

The response of plants to the mode of herbicides application were particularly relevant in the reviews by Currier & Dubing (1959), Oliver *et al.* (1968), Robertson & Kirkwood (1970), Bukovac (1976), Evart (1977), Guang-Guo & Williams (2000). In this connection, Crowley *et al.* (1978) stated that when diphenyl ether herbicides placed in the root zone it was more toxic to all parts of the plant than when applied to the shoot zone.

The present investigation was undertaken to elucidate the effect of site of oxyfluorfen application on the growth, pigments, photosynthesis and growth regulators content of *Glycine max* plant as well as its effect on yield attributes and chemical composition of the yielded seeds.

Materials and Methods

Plant material and growth conditions: Seeds of *Glycine max* (L var. cutler 71) were obtained from the Agricultural Research Center, Ministry of Agriculture, Giza. The diphenyl ether, oxyfluorfen (goal), 2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-trifluoromethyl benzene (23.6% E.C.), produced by Rohm and Haas Company was applied; either as soil drench or as foliar spraying at the recommended dose (according to announcement of Ministry of Agriculture of Egypt).

A homogenous lot of *Glycine max* seeds were surface sterilized with 0.001M HgCl₂ solution for three minutes and then washed thoroughly with distilled water. The sterilized seeds were sown in earthenware pots (35 cm in diameter) filled with 5 kg soil (sand: clay 2/1 v/v). The pots were kept in green house and the plants were subjected to natural day/night conditions (minimum/maximum temperature and relative humidity were 29.2/33.2°C and 35/45% respectively at mid-day during the experimental period) and irrigated with tap water. After two weeks the pots were divided into three sets; the plants of the 2nd

and 3rd set treated with oxyfluorfen solution as foliar spray and soil drench respectively while the plants of the first set remained without treatment and considered as control.

The soil of the three sets was supplemented with a phosphorus fertilizer in the form of triple-superphosphate. Samples from the treated and untreated soybean plants were taken after 45 days from sowing and finished after 90 days from sowing.

The samples of each were ten replicates for growth measurements include shoot length, number of leaves, total leaf area, fresh and dry weight of leaves and shoot, root length, number of nodules and fresh & dry weight of root as well as yield determinants (number of pods/plant, number of seeds/pod, total number of seeds/plant and fresh & dry weight of seeds). On the other hand, the samples were triplicated for pigments, photosynthetic activity. In addition, carbohydrates and protein analysis of the yielded seeds were detected. Other samples were collected and deep frozen in methanol, for at least 24 hours, before the determination of growth regulators. Data were obtained and the mean values were computed for the previous parameters.

Estimation of leaf area: The leaf area was estimated using the following equation: area = 0.7(length x breadth), as described by Quarrie and Jones (1979).

Estimation of growth regulating substances: The extraction and separation of plant growth substances followed the procedures described by Shindy and Smith (1975). For bioassay of auxins, the straight growth test of *Hordeum* coleoptiles sections as described by Foda and Radwan (1962) was used. For measurement of gibberellin substances, the lettuce hypocotyl bioassay adopted by Frankland and Wareing (1960) was followed. The technique used to assay the activity of cytokinin was that described by Esashi and Leopold (1969); cotyledons of *Xanthium brasiliicum* seeds were used as the test specimen. ABA was bioassayed by the straight-growth test of *Triticum* coleoptile segments as recommended by Wright (1969). To assay the IAA-oxidase, the plant material was extracted following the method of Kar and Mishra (1976). The activity of this enzyme was assayed following the method of Gorden and Weber (1951) as described.

Estimation of photosynthetic pigments: Pigments (chlorophyll "a", chlorophyll "b" and carotenoids) were measured by the spectrophotometric method as recommended by Metzener *et al.* (1965).

Estimation of photosynthetic activity (¹⁴C-light fixation): As described by Shaddad (1979) and modified by Aldesuquy (2000) a definite fresh mass of 2nd leaf discs was introduced into the fixation apparatus (Fig. 1). An aqueous solution of ¹⁴C-sodium

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carbonate of known activity (3.7 MBQ cm^{-3}) was pipetted into the apparatus followed by H_2SO_4 (10%). The evolved $^{14}\text{CO}_2$ passed over and radioactivity of the green leaf discs was measured using Packard Scintillation Counter model 526.

Estimation of carbohydrates: The method of extraction was essentially that described by Younis *et al.* (1969). The polysaccharides were determined in the dry residue after alcohol extraction according to Naguib (1967).

Estimation of protein content: The method adopted for estimation of protein was essentially those described by Bradford (1976). The results were first subjected to the analysis of variance (ANOVA). When ANOVA showed a significant ($P < 0.05$) effect, the least significant differences were used to compare treatments (Snedecor and Cochran, 1967).

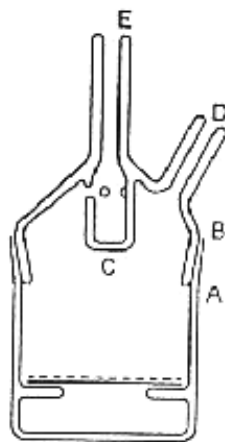


Fig. 1: $^{14}\text{CO}_2$ -fixation apparatus. A: main container, B: lid; C: inner container (1 cm^3 capacity); D, E: side arms.

Results and Discussion

Measurements of shoot growth parameters (shoot length, number of leaves, total leaf area and fresh & dry weights of leaves and shoot) revealed that spraying of soybean plants with oxyfluorfen decreased these parameters; markedly ($P < 0.05$) after 45 days from sowing and non significantly after 90 days from sowing. However, soil drench with oxyfluorfen affect these shoot parameters non-significantly during the two stages of plant growth (Table 1). Regarding root measurements (root length, number of nodules and fresh & dry weight of root), treatments with oxyfluorfen decreased these parameters, either non-significantly by spraying or significantly ($P < 0.05$) by soil drench, throughout growth period (Table 2). On the other hand, percent of water content of both shoot and root of soybean plant non-significantly affected by oxyfluorfen treatment (Tables 1 & 2).

In this connection, the inhibition of the growth of roots by diphenyl ether herbicides has been reported in maize (Hoppe, 1977). Also Ebner *et al.* (1968) found that soybean roots in the culture media, containing diphenyl ether herbicides, were reduced in growth and so was the whole plant in time. Moreover, Vanstone and Stobbe (1979) found that oxyfluorfen at 100 g/ha led to leaf injury symptoms of *Vicia faba* plant. Kocher and Lozch (1975) reported that diphenyl ether herbicides markedly inhibited root growth and meristematic activity of shoot of the sued plant. Similarly, Halwanker *et al.* (1987) and El-Quesni (1993) reported that treatment with oxyfluorfen decreased plant height of soybean. Also Saad El-Din *et al.* (1996) stated that plant growth characters of soybean (plant height, no of leaves, no of branches, dry wt of stem and leaves), which measured at 60 and 75 days after sowing, were affected significantly by application of oxyfluorfen at 0.5 L/fed . Vanstone (1978) and Vora & Mehta (1999) stated that oxyfluorfen inhibits seed germination, growth

and meristematic activity of the treated plants.

It is clear from the above results that application of oxyfluorfen to roots was more harmful than that of shoot application (Tables 1 & 2), these results are supported by Crowley *et al.* (1978), who observed that root-applied diphenyl ether herbicides enhanced adventitious root development in oat shoots, whereas when applied to the shoots, this growth response was inhibited. Chlorophyll a, chlorophyll b, chlorophyll a/b ratio, total chlorophylls and consequently total pigments of soybean plant decreased ($P < 0.05$) by foliar application of oxyfluorfen, during the two stages of plant growth. However, the application of this herbicide through soil decreased these pigments ($P < 0.05$) at the first stage (45 days from sowing) and non-significantly after 90 days from sowing. On the other hand, throughout plant growth and development, oxyfluorfen treatments affect carotenoids content non significantly (Table 3). All over growth period of *Glycine max* plants, treatment with oxyfluorfen decrease the photosynthetic activity; markedly ($P < 0.05$) by foliar application and non significantly by soil drench, meanwhile the two treatments of oxyfluorfen appeared without significant effect on soluble/insoluble ratio of photosynthetic activity (Table 4).

The results are in good conformity with those obtained by Kocher and Lotzch (1975) who stated that the diphenyl ether herbicides reduce the chlorophyll content in leaves of the used plant, but they reported that photosynthesis and respiration of intact plants were not directly inhibited by these herbicides. Moreland *et al.* (1970) investigated the effect of diphenyl ether herbicides on chloroplast and mitochondria reaction and concluded that these compounds acted primarily as inhibitors of chloroplast noncyclic electron transport and coupled photophosphorylation. They also suggested that interference with ATP generation could be one of the mechanism by which the phototoxicity of these herbicides is expressed. However Pereira *et al.* (1971) suggested that the effect of the diphenyl ether herbicides on photosynthesis and respiration might be secondary in nature. Moreover Vanstone (1978) found that oxyfluorfen affected photosynthesis only after the leaf tissue began to wilt. Recently, (Lee *et al.*, 2000), stated that use of oxyfluorfen interfere with the biosynthetic pathway of chlorophylls of rice plants.

It is clear from the results presented in Tables 3 & 4 that spraying effect of oxyfluorfen is more significant in pigments as well as photosynthetic activity of soybean plant than that by soil drench, this may be due to the fact that the diphenyl ether herbicides absorbed by both leaves and roots of plants but very little long distant transport occurred (Matsunaka, 1976).

In conclusion, the response of leaf area to oxyfluorfen treatments is in harmony with the change in pigments content and photosynthetic activity of soy bean plants (Tables 1,3&4), thus Vanstone and Stobbe (1979) investigated that the diphenyl ether herbicides induce chlorosis and necrosis when applied to leaves which has been attributed to loss of membrane integrity. They also found that certain of these compounds inhibit seed germination, growth and meristematic activity.

On the day 45 from sowing, foliar application of oxyfluorfen caused a decrease and increase ($P < 0.05$) in content of IAA & GA_3 and IAA oxidase respectively while cytokinin and ABA content was affected by this treatment non significantly (Table 5). However, at the same stage of plant development, soil drench with oxyfluorfen led to non-significant effect in IAA, GA_3 , ABA and IAA oxidase and decreased cytokinin ($P < 0.05$), in relation to the control values. On day 90 from sowing both oxyfluorfen treatments appeared without significant effect on these growth regulators and IAA oxidase content of soybean plant (Table 5). These results are in accord with the results reported by Shimabukuro *et al.* (1978) who stated that diphenyl ether herbicides inhibit IAA of oat and wheat coleoptile segments. They also suggested that these compounds function as strong auxin antagonists. Moreover, Pereira (1971) and Vanstone (1978) suggested that diphenyl ether herbicides action appeared to in part involve altered endogenous growth regulators response, the same was detected by Nakamura *et al.* (2000) working on mungbean leaves.

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Table 1: Effect of oxyfluorfen treatments on growth parameters of *Glycine max* shoot

Parameters	Days of sowing																
	Shoot length (cm)		No. of Leaves /plant		Leaf area (cm ²)		Fresh wt. of shoot (g/plant)		Dry wt. of shoot (g/plant)		Fresh wt. of leaves (g/plant)		Dry wt. of leaves (g/plant)		% of water content		
	45	90	45	90	45	90	45	90	45	90	90	45	90	45	90	45	90
Treatments	45	90	45	90	45	90	45	90	45	90	90	45	90	45	90	45	90
No	22.15	29.53	7.11	19.52	18.31	90.15	21.75	46.81	2.32	8.05	10.181	22.13	1.151	3.58	89.75	82.8	
Spraying	19.05*	28.45	6.06*	18.5	16.11*	86.11	18.97*	45.5	1.72*	8.17	8.71*	21.67	0.892*	3.12	90.93	82.04	
Solid drench	21.13	29.12	6.92	19.09	17.9	89.29	20.71	46.12	2.05	7.98	9.15	21.95	0.967	3.42	89.6	82.71	
L.S.D at 5% level	0.97	1.52	0.83	1.23	0.97	4.21	1.21	1.56	0.27	0.62	1.21	2.45	0.21	0.511	2.65	2.87	

* significant at P< 0.05

Table 2: Effect of oxyfluorfen treatments on growth parameters of *Glycine max* root

Parameters	Days of sowing									
	Root length (cm)		No. of bacterial nodules/plant		Fresh wt. of root g/plant		Dry wt. of root g/plant		% of water content	
	45	90	45	90	45	90	45	90	45	90
Treatments	45	90	45	90	45	90	45	90	45	90
No	12.36	20.12	6.98	20.11	1.13	2.45	0.131	0.301	88.41	87.71
Spraying	12.15	18.17	6.95	20.15	1.15	2.4	0.128	0.298	88.87	87.58
Soil drench	9.81*	16.35*	6.66*	18.19*	0.89*	2.21	0.116*	0.271*	86.97	87.74
L.S.D. at 5% levels	1.33	1.93	0.25	1.16	0.11	0.2	0.01	0.02	2.11	2.26

* Significant at P< 0.05

Table 3: Effect of oxyfluorfen treatments on pigments (mg/g dry wt) of *Glycine max* leaves.

Parameters	Days of sowing											
	Chlorophyll "a"		Chlorophyll "b"		Total Chlorophylls		Chl. a/b		Carotenoids		Total pigments	
	45	90	45	90	45	90	45	90	45	90	45	90
Treatments	45	90	45	90	45	90	45	90	45	90	45	90
No	1.03	0.715	0.618	0.426	1.648	1.141	1.667	1.678	0.381	0.405	2.029	1.546
Spraying	0.715*	0.608*	0.509*	0.315*	1.227*	0.923*	1.405*	1.93*	0.362	0.391	1.586*	1.314*
Soil drench	0.83*	0.683	0.521*	0.389	1.351*	1.072	1.593*	1.756	0.375	0.412	1.726*	1.484
L.S.D. at 5% levels	0.18	0.091	0.082	0.051	0.212	0.071	0.06	0.13	0.025	0.018	0.27	0.102

Table 4: Effect of oxyfluorfen treatments on photosynthetic activity of *Glycine max* leaves (Cpm x 10⁻³/ g fresh wt.)

Parameters	Days of sowing							
	Soluble		Insoluble		Total photosynthates		Soluble/insoluble	
	45	90	45	90	45	90	45	90
Treatments	45	90	45	90	45	90	45	90
No	45.18	62.21	17.28	25.65	62.46	87.86	2.615	2.425
Spraying	35.67*	57.13*	14.21*	20.01*	49.88*	77.14*	2.51	2.855
Soil drench	41.91	60.33	17.15	25.11	59.06	85.44	2.44	2.403
L.S.D. at 5% levels	8.13	4.04	2.81	3.65	7.123	8.15	0.191	0.44

Table 5: Effect of oxyfluorfen treatments on growth regulators content (µg/g F.wt) and on IAA oxidase (activity/g F.wt) of *Glycine max* plant

Parameters	Days of sowing									
	IAA		GA ₃		Cytokinin		ABA		IAA oxidase	
	45	90	45	90	45	90	45	90	45	90
Treatments	45	90	45	90	45	90	45	90	45	90
No	6.13	7.65	2.19	3.15	6.82	7.63	2.65	2.95	405.61	511.8
Spraying	4.89*	7.08	1.95*	2.71	6.64	7.42	2.6	2.63	482.14*	591.6
Soil drench	5.73	7.21	2.03	3.2	6.35*	7.28	2.68	2.87	417.16	505.11
L.S.D. at 5% levels	1.11	0.65	0.18	0.55	0.27	0.65	0.16	0.4	20.43	83

Table 6: Effect of oxyfluorfen treatments on yield components and physiological aspects of developing seeds of *Glycine max* plants

Parameters	Days of sowing							
	No. of pods /plant	No. of seeds /pod	Total no. of seeds/plant	Fresh wt. of seeds (g/pod)	Dry wt. of seeds (g/pod)	Total carb. (mg/100g d.wt)	Total protein (mg/100g d.wt)	Relative grain yield
Treatments	45	90	45	90	45	90	45	90
No	8.911	5.81	51.773	3.56	0.362	2168	3418	100
Spraying	8.816	5.621	49.555	3.41	0.356	2077	3316	95.72
Soil drench	8.712	5.431	47.315	3.33*	0.347	1915	3276	91.39*
L.S.D. at 5% levels	0.21	0.39	3.66	0.13	0.022	366	481	5.81

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In the majority of cases, soil drench with oxyfluorfen has no significant effect on growth regulators and IAA oxidase content (Table 5) this is supported that oxyfluorfen was readily absorbed by roots of faba bean (*Vicia faba*) and green foxtail (*Setaria viridis*) from a nutrient solution, however, translocation to shoot was limited; faba bean (4.6%) and green foxtail (2.2%).

The changes in growth regulators content of soy bean plants treated with oxyfluorfen are in good support with the changes in growth rate of shoot and root maintained for the same plant (Tables 1,2&5). In accord with these results Shimabukuro *et al.* (1978) investigated that the diphenyl ether herbicides inhibited IAA-stimulated elongation of oat and wheat coleoptile segments by 51% and 13% respectively. They found also that increasing the IAA concentration partially counteracted the inhibitory effect of these herbicides.

Except for the significant decrease in fresh weight of seeds and relative grain yield of soybean plant, treated with oxyfluorfen by soil drench, a non significant response was maintained in number of pods & seeds, fresh & dry weight of seeds and relative grain yield as well as total carbohydrates and protein of the yielded seeds of soybean plants treated with oxyfluorfen by both spraying and soil drench (Table 6). In this connection, Saad El-Din *et al.* (1996) found that oxyfluorfen as pre-emergence herbicide at 0.5 and 0.75 L/ha

caused significant increment in yield components and chemical composition of the yielded seeds. Also, Vora and Mehta (1999) stated that significant positive correlation was reported for bulb yield and yield attributes of garlic. On the other hand Satao *et al.* (1999) and Ghosheh & Shannag (2000) recorded the decrease in the yield of cotton and onion respectively in response to oxyfluorfen application.

It is authenticated that yield is a function of many factors among which the shoot growth and pigment content of the developing leaf area are the most important ones (Aldesuquy, 2000). The examination of the results in Tables 1,3,4&6 clear that the changes in growth rate and pigments content as well as photosynthetic activity of soy bean plants, treated with oxyfluorfen, were accompanied by non-significant responses in yield and yield attributes. These responses may be presumably being due to the fact that the applications of oxyfluorfen cause damage to shoot and root but the plant recovered and yield was not reduced (Ebner *et al.*, 1968). It is emerged from the above mentioned results that either spray or soil drench with oxyfluorfen alter the growth, pigments, photosynthesis and growth regulators contents of soybean plants as well as its yield and yield attributes and these effects seemed to depend on: (1) the site of oxyfluorfen uptake, and (2) the age of the plant used. The treatment of oxyfluorfen by foliar application is more safety to soybean plants.

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