

EFFICACY OF GLYPHOSATE HERBICIDE AGAINST DODDER AND ITS IMPACT ON SOME BIOCHEMICAL PARAMETERS ON HOST PLANT *Nerium oleander* L. AND A SURVEY OF HOST RANGE OF SOME ORNAMENTALS AT FAYOUM.

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ABSTRACT

Post – attachment applications of glyphosate at low rates (200 and 400 ppm) controlled (86-90%) of attached dodder, *Cuscuta campestris* Yunker on *Nerium oleander* L. after two months of the treatment without any phytotoxicity. Also, glyphosate at the same rates reduced seed germination, length and fresh weight of dodder. Moreover, both glyphosate at the rates (200 and 400 ppm) increased leaf area, leaf fresh weight and percentage of leaves dry weight of *N. oleander* as compared to the check. The obtained results also indicated that both rates of the herbicide markedly increased the leaf contents from total carbohydrate, total reducing and non reducing sugars, chlorophylls, carotenoid, xanthophylls, prolin, total indoles, ascorbic acid and antioxidant enzymes activity in the leaves. On the contrary, all dodder control treatments decreased free phenols in the leaves as compared to the check. On the other hand, this parasite was found attacking eleven ornamental plants belonging to different families at Fayoum Governorate. Some of these hosts were new recording as susceptible hosts by *C. campestris* in Egypt. These new hosts included five ornamentals: *Nerium oleander* L., *Adhatoda vasica* L., *Ficus nitida* L., *Ipomoea horsfalliae* L. and *Cestrum diurnum* L.

Key words: *antioxidant enzymes, chemical constituents, dodder control, glyphosate, Nerium oleander, ornamentals.*

1. INTRODUCTION

The genus *Cuscuta* contains numerous species, all of which are known in English by the common name "dodder". All species of *Cuscuta* are obligate stem and leaf parasites. The field dodder (*C. campestris* Yunker.) a parasitic weed is one of several dodder species present in the flora of Egypt (Parker and Wilson, 1986). This species is a parasite not only of many crops and vegetables but also of numerous ornamental species. Also, the field dodder is the most widespread agricultural problem of all *Cuscuta* species, one plant is capable of producing several thousand seeds (Gimesi, 1966, 1987). The dodder seedlings have twined about a host plant, haustoria from the parasite soon penetrate the stem and leaf of the host. Vascular cells of the parasite contact those cells of the host and the materials from the phloem and xylem of the host are diverted into the parasite which grows rapidly and becomes large, while the host plant is severely damaged or killed (Dawson, 1987; Christopher *et al.*, 2009). *Cuscuta*

plants can be killed by cutting or removed the host plants or by spraying the host and parasite with a selective herbicide. Among the herbicides which were screened for their activity on field dodder, glyphosate. Lethal quantities of glyphosate reach the dodder by direct contact and by translocation from the host to the parasite (Dawson and Saghir, 1983). Glyphosate is a standard herbicide because it controlled *Cuscuta* selectively at the very low rates 75 to 150 g/ha to alfalfa foliage (Dawson, 1990). Fenugreek plants (Ali and El-Yazal, 2003), clover (Soliman and Abd El-Hamid, 2009), at 50 ppm to citrus trees (Abu-Irmaileh and Fuilk, 1989) and at 300 ppm to some ornamental plants (Hock *et al.*, 2008). The objective of this research was to determine the proper rates of glyphosate herbicide that would control dodder without causing phytotoxicity to *N.oleander* shrubs as well as the effect of herbicide on some biochemical constituents and antioxidants enzymes for the host in addition to recognise the host range of field dodder at Fayoum Governorate.

2. MATERIALS AND METHODS

2.1. Field experiments

In April 2009, the heavy infestations of field dodder (*C. campestris*) on evergreen shrubs of *N. oleander* at Fayoum (Keman fars region) were sprayed with Herbazed (glyphosate 48% WSC) at several levels 50, 100, 200, 400 and 800 ppm. Only one treatment per shrub was applied to the *N. oleander* because of the limited number of infested shrubs. Each branch represented one replicate (4 branches / shrub), the treated shrubs were marked by an adhesive tape, treatments were replicated four times. The data were recorded after 2, 4 and 8 weeks to evaluate the level of dodder control. Dodder control was measured by counting the attached dodder that wrapped around the host and produced visible haustoria according to (Dawson, 1972) as follows:

$$\text{Control percentage} = 100 - \frac{(\text{No. attached dodder in treatment})}{(\text{No. attached dodder in check})} \times 100$$

2.2. Laboratory experiments

Dodder seed germination was tested in the laboratory, seed lots were collected from each glyphosate treated dodder and untreated check. Mature seeds were selected from each lot for germination and growth. The seeds were dipped for 25 minutes in concentrated sulfuric acid, washed with tap water, air dried before use (Graph *et al.*, 1987). Twenty five seeds per dish were placed on filter paper moistened with 3 ml/dish of distilled water. Dishes were covered and incubated at $25 \pm 2^\circ\text{C}$. Germination percentages were counted after 10 days of incubation. The shoots were excised at the seed level and their length were calculated. Shoot fresh weight was also recorded (Giannopolitis, 1979).

2.3. Data recorded

2.3.1. Growth character

For each treatment, host leaf samples were collected in the morning after 70 days post spraying. Samples of each treatment were taken and the average of the following characters were recorded: fresh and dry weight of leaf (g) and total leaf area (cm^2) was estimated by using an area meter, model Li 3000 from LICORE, USA.

2.3.2. Chemical analysis

For each treatment, host leaf samples were collected in the morning after 70 days from spraying. Each sample was analyzed twice for: photosynthetic pigments (chlorophyll a, b and carotenoids) as mg/g fresh weight was extracted from fresh leaves by acetone (80%) then, their concentrations were determined according to Welburn and Lichtenthaler (1984). Total carbohydrates as mg/g dry weight was extracted

by sulphuric acid (0.1 N) then determined colorimetrically by using phenol-sulphuric acid reagent according to the method described by (Michel *et al.*, 1956), total and reducing sugars as mg/g dry weight were determined in ethanolic extract using phosphomolybdic acid reagent as described by (A.O.A.C., 1995), total indoles as mg/g dry weight were determined in ethanolic extract using 4-dimethyl- amino- benzaldehyde reagent as described by (Larson *et al.*, 1962), free proline concentration as mg/g dry weight was extracted by sulfosalicylic acid then determined colorimetrically using acid ninhydrin and toluene reagent as described by (Bates *et al.*, 1973), ascorbic acid as mg/100 g fresh weight was extracted by metaphosphoric acid then, determined using 2,6- dichlorophenol indophenol as described by (A.O.A.C., 1995). Total and free phenols as mg/g dry weight were determined in ethanolic extract using Folin-Denis reagent as described by (A.O.A.C., 1995). Peroxidase activity was determined by the method described by (Maehly and Chance, 1954), catalase activity was determined by the method described by (Beers and Sizer, 1952). Polyphenol oxidase was determined according to (Taneja and Sachar, 1974) and ascorbic acid oxidase was determined according to (Dawson and Magee, 1955).

2.4. The host range of *C. campestris*

Field survey was carried out to study the host range of *C. campestris* at Fayoum Governorate. The susceptible hosts of ornamental plants were collected from various areas to determine its spread. Specimens were brought to the laboratory for proper identification, and parasitism was qualified by the presence of sucker attachments.

2.5. Statistical analysis

All data were statistically analyzed according to Snedecor and Cochran (1980). Means were compared using the least significant difference (L.S.D.) test at 0.05 significance level.

3. RESULTS AND DISCUSSION

3.1. Dodder control

The data in Table (1) show that glyphosate at 200 and 400 ppm controlled 86 and 90% of dodder within two months of application without any injury to the shrubs of *N. oleander*. Glyphosate at the rate of 400 ppm exhibited a higher control for dodder than the rate of 200 ppm. However, this difference is not significant even at the 0.01 level. The efficiency of both glyphosate rates was more or less the same. Although glyphosate at the higher rate (800 ppm) caused 95% control but phytotoxic effect on the

Table (1): Control percentage of dodder measured periodically after the application of glyphosate on *N.oleander*.

Treatment	Conc. (ppm)	Control percentage			
		Weeks after application			Mean
		2	4	8	
Check	-	(-)	(-)	(-)	(-)
Glyphosate	50	58.4	38.0	(-)	32.1
	100	75.0	59.0	29.8	54.6
	200	94.1	90.2	73.7	86.0
	400	100	90.8	79.3	90.0
	800	100	100	85.0	95.0
L.S.D. 0.05	-	19.04	15.62	10.73	-

host plant was observed, while at the lower rate (50 ppm) had little or no significant effect on dodder (32.1% control). Therefore, the injury of the host was due to dodder infection and not to the side effect of the herbicide activity. Thus, glyphosate rates at 200 or 400 ppm are recommended for use in *N. oleander* without inducing any phytotoxicity.

The data in Table (2) reveal that glyphosate caused significant reduction in seed germination of dodder to 69.0, 60.5 and 47.3% at 200, 400 and 800 ppm, respectively, while it was 92% in the check treatment. Also, the length of dodder seedling was reduced to 8.0, 7.3 and 5.8 cm and the fresh weight to 0.095, 0.092 and 0.084 mg at the same rates, respectively, whereas the length and fresh weight were 10.5 cm and 0.146 mg in the control. This decrease was not significant at the lower rates (50 and 100 ppm) of glyphosate. These results indicate that the low rates (200 or 400 ppm) of glyphosate could control field dodder selectively in *N. oleander* after the parasite is attached to the host plant. These results are in line with those of Zahran *et al.* (1995) who found that the best treatments for the dodder control on duranta shrubs were glyphosate + herbex (500 + 500 ppm) or glyphosate + sceptor (500 + 500 ppm). Both treatments killed 100% of *C.campestris*. Also, Abu-Irmaileh (1987) showed that glyphosate gave excellent control of dodder (*C.monogyna*) on citrus at rates as low as 50 ppm within four months without harming citrus trees. Seed germination and growth of dodder were affected significantly by glyphosate treatments at 100 ppm and higher. Bewick *et al.* (1988) mentioned that glyphosate controlled dodder selectively in cranberry and carrot after the parasite is attached to the host plant. They added that large amount of dodder tissue (threads and seeds) present on the host plant may have it protected from the harmful effects of glyphosate application. Giannopolitis (1979) reported that

ethofumesate is a potent inhibitor of dodder seed germination and stem elongation. Metolachlor and propyzamide are also active inhibitors but at higher concentrations than ethofumesate.

3.2. Growth characters

Data in Table (3) show that all treatments of glyphosate improved the growth of *N.oleander* compared with the infected untreated plants and reduced the adverse effect of dodder on leaf area. The best results were obtained by applying glyphosate at 200 and 400 ppm. The increases gained were 21.55 and 25.53% more than the check, respectively. Also, the increments in leaf fresh weights were 11.16 and 14.42% and leaf dry weights were 47.57 and 58.10% at 200 and 400 ppm more than the check, respectively.

3.3. Chemical constituents

3.3.1. Pigment concentrations in the leaves

The data presented in Table (4) indicate that treating plants with glyphosate at 200 and 400 ppm increased the plastid pigments significantly compared to the check. The increases were 14.27 and 23.15% for chlorophyll a; 43.89 and 53.05% for chlorophyll b; 68.75 and 87.50% for total carotenoids and 81.08 and 91.89% for xanthophyll over the check, respectively.

3.3.2. Carbohydrate concentrations in the leaves

The data in Table (5) reveal that concentrations of total carbohydrates in the leaves were significantly decreased due to dodder infection. On the contrary, total carbohydrate concentrations were increased due to glyphosate treatments. The maximum increases obtained by glyphosate at 200 and 400 ppm were 57.26 and 60.99% for total carbohydrate. While, these increases were 46.04 and 51.45% for total sugars and 17.86 and 31.46% for reducing sugars. Regarding the concentration of non reducing sugars in leaves, the higher increase was obtained by glyphosate at 200 and 400 ppm. This increase reached 78.07 and 74.17% over the check, respectively.

Table (2): Effect of glyphosate applications on seed germination, length and fresh weight of dodder seedlings.

Treatment	Conc. (ppm)	Seed germination (%)	Length (cm)	Fresh weight (mg)
Check Glyphosate	-	92.0	10.5	0.146
	50	85.3	11.3	0.153
	100	82.7	10.0	0.128
	200	69.0	8.0	0.095
	400	60.5	7.3	0.092
	800	47.3	5.8	0.084
L.S.D. 0.05	-	9.96	1.64	0.032

Table (3): Effect of glyphosate herbicide on the growth of *N.oleander* leaves.

Treatment	Conc. (ppm)	Total leaf area (cm)	Leaf fresh weight (g)	Dry weight of leaves (%)
Check Glyphosate	-	25.66	2.15	21.65
	50	28.15	2.24	22.44
	100	29.96	2.30	29.23
	200	31.19	2.39	31.95
	400	32.21	2.46	34.23
	800	27.17	2.25	24.08
L.S.D. 0.05	-	1.66	0.09	3.15

Table (4): Effect of glyphosate herbicide on chlorophyll a,b, carotenoids and xanthophyll of *N.oleander* leaves.

Treatment	Conc. (ppm)	Chlorophyll (A) mg/g	Chlorophyll (B) mg/g	Carotenoides mg/g	Xanthophyll mg/g
Check Glyphosate	-	1317	262	112	37
	50	1477	349	122	42
	100	1489	356	148	52
	200	1505	377	189	67
	400	1622	401	210	71
	800	1357	219	119	40
L.S.D. 0.05	-	118	30.6	24.1	5.70

Table (5): Effect of glyphosate herbicide on total carbohydrate, total, reducing and non reducing sugars of *N.oleander* leaves.

Treatment	Conc. (ppm)	Total carbohydrate mg/g	Total sugars mg/g	Reducing sugars mg/g	Non reducing sugars mg/g
Check Glyphosate	-	241	25.15	13.38	11.77
	50	304	31.73	14.32	17.41
	100	337	32.24	14.74	17.50
	200	379	36.73	15.77	20.96
	400	388	38.09	17.59	20.50
	800	235	25.85	10.53	15.32
L.S.D. 0.05	-	12.2	2.07	1.93	2.16

3.3.3. Free prolin, total indoles, ascorbic acid, total and free phenoles

Data in Table (6) show that free prolin, total indoles, ascorbic acid and total phenol in the leaves were decreased in the plants of *N.oleander* infected with dodder. In contrast, applying glyphosate at 200 and 400 ppm increased these constituents. The increases gained were 23.52 and 31.20% in free prolin; 75.15 and 77.89% in total indoles; 44.33 and 47.98% in ascorbic acid and 66.79 and 71.40% in total phenol over the check, respectively. On the other hand, free phenol concentrations were decreased by 10.78 and 12.63% at 200 and 400 ppm under the check, respectively.

The positive effect of glyphosate on growth characters of *N.oleander* infected plants may be due to the role of the herbicide in controlling dodder selectively after the parasite is attached to the host plant (Dawson, 1990) which improve early plant growth, the rate of essential nutrients absorption, e.g., (N.P.K.) and the increase in plant growth, hormones such as indole acetic acid. In this respect, Dawson and Saghir (1983) and El-Saht *et al.* (1994) generally reported that herbicides effect on plant growth was found to be associated with marked changes in the metabolism of the treated plants. Also, they added that lethal quantities of herbicide reach the dodder by translocation from the host to the parasite.

Table (6): Effect of glyphosate herbicide on some chemical constituents of *N.oleander* leaves.

Treatment	Conc. (ppm)	Free prolin mg/g	Total indoles mg/g	Ascorbic acid mg/100g	Free phenoles mg/g	Total phenoles mg/g
Check Glyphosate	-	6.12	10.95	10.15	2.69	5.21
	50	6.65	15.19	12.65	2.66	6.14
	100	6.73	18.17	13.10	2.42	8.25
	200	7.56	19.18	14.65	2.40	8.69
	400	8.03	19.48	15.02	2.35	8.93
	800	7.15	11.13	12.10	2.65	6.55
L.S.D. 0.05	-	0.53	1.83	1.26	0.08	0.44

3.3.4. The enzymatic activity

Data in Table (7) indicate that the plants of *N.oleander* sprayed with glyphosate increased the leaf content of antioxidant enzymes such as peroxidase, catalase, polyphenol oxidase and ascorbic acid oxidase than unsprayed control. Glyphosate at 200 and 400 ppm induced the higher increase of enzymatic activity. The relative increase percentages were 21.15 and 26.18% for peroxidase; 39.15 and 43.12% for catalase; 29.11 and 32.06% for polyphenol oxidase and 19.03 and 23.02% for ascorbic acid oxidase over the check, respectively.

Moreover, the infection of plants with dodder reduces the different metabolic processes which are responsible for normal plant growth. The adverse effect on the synthesis of chlorophyll a, b, carotenoids, xanthophylls, sugars, prolin and plant auxin concentration which occurred as a result of dodder infection was reduced by treating the plants with glyphosate used in this investigation. The positive effect of glyphosate on the activities of catalase, peroxidase, polyphenol oxidase and ascorbic acid oxidase may be due to rather sensitive effect of glyphosate on these enzymes. In this connection, Yin *et al.* (2008) found that

Table (7): Effect of glyphosate herbicide on antioxidant enzymes of *N.oleander* leaves.

Treatment	Conc. (ppm)	Peroxidase activity	Catalase activity	Polyphenol oxidase activity	Ascorbic acid oxidase activity
Check Glyphosate	-	100.00	100.00	100.00	100.00
	50	111.21	131.01	120.15	109.22
	100	113.33	136.21	126.02	111.25
	200	121.15	139.15	129.11	119.03
	400	126.18	143.12	132.06	123.02
	800	113.26	133.44	128.00	111.64
L.S.D. 0.05	-	7.31	5.82	5.54	9.02

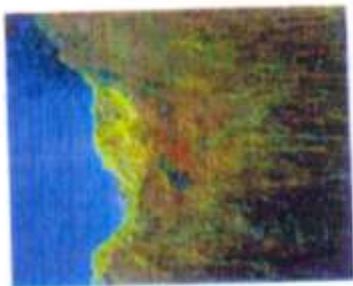


Fig. (2): Yellow threads extended of *Cuscuta* .

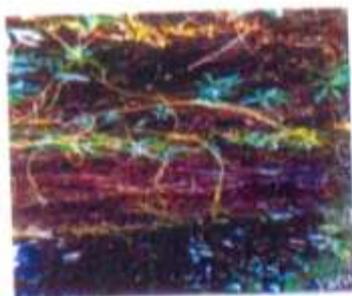


Fig. (1): The habitus of *Cuscuta* on stem and leaves

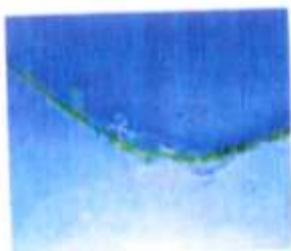


Fig.(6): *Cuscuta* on *Nerium oleander* .



Fig. (5): large amounts of *Cuscuta* seeds on the host.

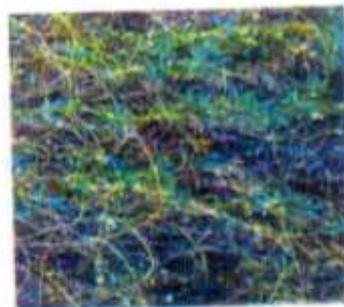


Fig.(4): *Cuscuta* on *Ipomoea horifoliae*.



Fig.(7): *Cuscuta* on *Ficus nitida*

isoproturon-induced oxidative stress resulted in a substantial change in activities of the majority of antioxidant enzymes including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX). Activities of the antioxidant enzymes showed a general increase at low isoproturon concentrations and a decrease at high isoproturon concentrations. Also, similar results were obtained by El-Saht (2001) using metribuzin herbicide on maize plants and by Moldes *et al.* (2008) using glyphosate herbicide on soybean plants.

Generally it is clear that glyphosate enhanced all the biochemical parameters in the leaf of host plant *N. oleander* and there are no adverse effect on the host plant at the levels of application with glyphosate at 200 and 400 ppm.

3.4. Survey study on *C. campestris*

The survey study was aiming to identify the susceptible hosts of ornamental plants attacked by *C. campestris* in Fayoum Governorate. *C. campestris* was found parasitizing eleven ornamentals belonging to different plant families. Some of these hosts were previously recorded by Abu-Irmaileh (1987) and Hock *et al.* (2008) such as *Canna indica* L., *Ocinum basilicum* L., *Ixora acuminata* L., *Hibiscus mutabilis* L., *Majorana hortensis* Jacq. and *Celosia argentea* Dc. Al-Menoufi *et al.* (1985) recorded the occurrence of this parasite on some of the previous hosts in addition to weed and crop plants in the delta region. Also, Ali (1996) reported the presence of this species on *Dodonia vescosa* L., *Duranta plumieri* Jacq., *Luffa aegyptiaca* L., *Tropaeolum majus* L. and *Sesbania aegyptiaca* L. in Middle Egypt. Some others were new recorded of *C. campestris* in Egypt included five ornamentals: *Nerium oleander* L., *Ficus nitida* L., *Adhatoda vasica* L., *Ipomoea horsfalliae* L. and *Cestrum diurnum* L. as shown in Table (8) and Figs. (1 to 7).

Table (8): Susceptible hosts of ornamentals by *C. campestris*.

Ornamental plants	
A. Old recording	B. New recording
1. <i>Canna indica</i> L.	1. <i>Adhatoda vasica</i> L.
2. <i>Celosia argentea</i> Dc.	2. <i>Cestrum diurnum</i> L.
3. <i>Hibiscus mutabilis</i> L.	3. <i>Ficus nitida</i> L.
4. <i>Ixora acuminata</i> L.	4. <i>Ipomoea horsfalliae</i> L.
5. <i>Majorana hortensis</i> Jacq.	5. <i>Nerium oleander</i> L.
6. <i>Ocinum basilicum</i> L.	

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تأثير مبيد الحشائش الجليفوسيت على مكافحة الحامول وكذلك المكونات الكيميائية والانزيمات المضادة للاكسدة
لنباتات الدفلة مع حصر المدى العوائلى لنباتات الزينة فى الفيوم

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ملخص

أدت معاملة الحامول المتطفل على الدفلة بمبيد الجليفوسيت بتركيز 200 جزء فى المليون الى مكافحة الحامول بنسبة 86% بعد شهرين من المعاملة فى حين أدت المعاملة بتركيز 400 جزء فى المليون الى مكافحة بنسبة 90% ، كما أدى الرش على بذور الحامول المتواجدة بكمية كبيرة على العائل بنفس المعدلات السابقة (200 و 400 جزء فى المليون) الى نقص معنوى فى نسبة انبات البذور وكذلك تقليل طول ووزن الحامول مقارنة بالكنترول وقد أدت هذه المعدلات من تطبيق الجليفوسيت الى زيادة خصائص نبات الدفلة من حيث متوسط مساحة الورقة ومتوسط الوزن الطازج للورقة ونسبة المادة الجافة فى الاوراق. تبين النتائج ايضا ان كلا المعدلين قد أدى الى احداث زيادة ملحوظة فى محتوى اوراق النبات من الكربوهيدرات الكلية والسكريات الكلية والمختزلة وغير المختزلة والكلوروفيلات والكاروتينويدات والزانثوفيل والبرولين والاندولات الكلية وكذلك زيادة نشاط الانزيمات المضادة للاكسدة وهى الكتاليز والبيرواكسيد يز والبولى فينول اكسيديز وحامض الاسكوربيك اكسيديز وعلى العكس من ذلك فقد أدت المعاملة بمبيد الحشائش الى احداث نقص فى محتوى الاوراق من الفينولات الحرة.

من ناحية اخرى وجد الحامول الحقلى متطفلاً على احدى عشر نوعاً من نباتات الزينة فى محافظة الفيوم منها خمسة انواع تسجيل جديد للاصابة بهذا الطفيل وهى الدفلة، الادهاثودا، الفيكس، زهرة الساعة ومسك الليل وجميعها حديثة التطفل بهذا النوع وتؤدى العدوى اذا استمر التطفل بدون مكافحة الى اصفرار الاوراق وذبول ثم موت النبات فى النهاية.

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