https://doi.org/10.51483/IJAGST.1.4.2021.24-32

ISSN: 2710-3366

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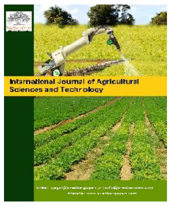

Research Paper **Open Access**

Ameliorative Impact of Glyphosate Herbicide Treatments on Dodder (*Cuscuta campestris*) Control in *Nerium oleander* L. Shrubs

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Article Info

Volume 1, Issue 4, November 2021
Received : 25 June 2021
Accepted : 14 October 2021
Published : 05 November 2021
doi: 10.51483/IJAGST.1.4.2021.24-32

Abstract

This study was conducted to evaluate the impact of Glyphosate herbicide on dodder control on *Nerium oleander* L. shrubs. Glyphosate herbicide was applied at several rates (50, 100, 200, 400 and 800 ppm) to control the parasitic weed *Cuscuta campestris* on *Nerium oleander* L. shrubs. Results revealed that applying glyphosate at very low rates (200 and 400 ppm) controlled (86-90%) of attached dodder, *Cuscuta campestris* Yunker on *Nerium oleander* L. after two months of treatment without any phytotoxicity and also improved growth parameters (leaf area, leaf fresh weight and percentage of leaves dry weight of *N. oleander* as compared to the check). Data also showed that dodder control by the use of glyphosate at the same rates reduced seed germination, length and fresh weight of dodder. The obtained results also indicate that glyphosate herbicide at the two rates were markedly increased chemical constituents of leaves (total carbohydrate, total, reducing and non-reducing sugars, chlorophylls, carotenoid, xanthophylls, prolin, total indoles, ascorbic acid and antioxidant enzymes activity). On the contrary, all dodder control treatments decreased free phenols in leaves as compared to the check.

Keywords: *Nerium oleander L.*, Antioxidant enzymes, Chemical constituents, Dodder control, Glyphosate

1. Introduction

Nerium oleander L. is normally existing in Mediterranean regions of Africa and Europe, it is an evergreen shrub or small tree. Leaves are linear, leathery and dark green to grey green, with dissimilar light yellowish veins. Florae are in groups at the landfill of branches, they are white to pink to deep red, with five dispersal petals. The fruit is a narrow pod and encompasses numerous silky-haired seeds. The juice is dense, sticky and clear (Hamad, 2021).

Invasive holoparasitic plants of the genus Cuscuta (dodder) threaten African ecosystems due to their rapid spread and attack on various host plant species. Most Cuscuta species cannot photosynthesize and hence rely on host plants for nourishment. After attachment through a peg-like organ called a haustorium, the parasites deprive hosts of water and nutrients, which negatively affects host growth and development. Despite their rapid spread in Africa, dodders have

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attracted limited research attention, although data on their taxonomy, host range, and epidemiology are critical for their management. Here, we combine taxonomy and phylogenetics to reveal the presence of field dodder (*Cuscuta campestris*) and Cuscuta kilimanjari (both either naturalized or endemic to East Africa) (Masanga *et al.*, 2021).

The use of various herbicides such as Glyphosate for dodder control has been studied by many investigators among them, Ali and El-Yazal, (2003) on Fenugreek plants, Soliman and Abd El-Hamid (2009) on clover, Hock *et al.* (2008) on some ornamental plants, Goldwasser *et al*. (2012) on tomato, Ziveh *et al*. (2013)on sugar beet, Zaroug *et al*. (2014) on onion, Sariæ-Krsmanoviæ *et al*. (2015) on alfalfa, Meighani *et al*. (2017), Hoseyni *et al*. (2018) on sugar beet and El-Yazal and Ali (2019) on Fenugreek plants.

The present work aimed 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.

2. Materials and Methods

2.1. Field Experiments

Herbazed (glyphosate 48% WSC) at several levels 50, 100, 200, 400 and 800 ppm were sprayed in April 2009 to the heavy infestations of field dodder (*C. campestris*) on evergreen shrubs of *N.oleander* at Fayoum (Keman fars region) with only one treatment per shrub was applied to the *N.oleander* because of the limited number of infested shrubs.., Each branch was represent 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 dodder control. Dodder control was measured by counting the attached dodder that wrapped around the host and produced seen haustoria in accordance to (Dawson, 1972) as follows:

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

2.2 Laboratory Experiments

Dodder seed germination changed into examined within side the laboratory, seed lots were collected from each glyphosate treated dodder and untreated check. Mature seeds have been decided on from every lot for germination and growth. The seeds have been dipped for 25 min in concentrated sulfuric acid, washed with tap water, air dried earlier than 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°C. Germination percentages were counted after 10 days of incubation. The shoots were excised at the seed level and their length was calculated. Shoot fresh weight was also recorded (Giannopolitis, 1979).

3. Data Recorded

3.1. Growth Character

For every treatment, host leaf samples had been collected within side the morning after (70 days post spraying), samples of every treatment had been taken and the average of the following characters had been 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.

3.2. Chemical Analysis

For every treatment, host leaf samples had been collected within side the morning after (70 days from spraying), every sample was analyzed twice for: photosynthetic pigments (chlorophyll a, b and caroteniods) as mg/g fresh weight was extracted from fresh leaves with the aid of using acetone (80%) then, their concentrations had been decided in line with Welburn and Lichtenthaler (1984), total carbohydrates as mg/g dry weight was extracted with the aid of using sulphuric acid (0.1 N) then decided colorimetrically with the aid of using the usage of phenol-sulphuric acid reagent according to the method described by Herbert *et al.* (1971), total and reducing sugars as mg/g dry weight had been decided in ethanolic extract the usage of phosphomolybdic acid reagent as defined with the aid of using (A.O.A.C., 1995), total indoles as mg/g dry weight had been decided in ethanolic extract the usage of 4-dimethyl-amino-benzaldehyde reagent as defined with the aid of using (Larson *et al.*, 1962), free proline awareness as mg/g dry weight was extracted with the aid of using sulfosalicylic acid then decided colorimetrically the usage of acid ninhydrin and toluene reagent as defined with the aid of using Bates *et al*. (1973) with slight modification as described by Ennajeh *et al*. (2006), ascorbic acid as mg/100 g fresh weight was extracted by metaphosphoric acid then, decided the usage of 2,6- dichlorophenol indophenol as defined with the aid of using (A.O.A.C., 1995), total and free phenols as mg/g dry weight had been decided in ethanolic extract the usage of Folin-Denis reagent as defined with the aid of using (A.O.A.C., 1995), peroxidase activity was decided with the aid of using the technique defined with the aid of using (Maehly and Chance, 1954), catalase activity was decided with the aid of using the technique defined with the aid of using (Beers and Sizer, 1952), polyphenol oxidase was decided in line with (Taneja and Sachar, 1974) and ascorbic acid oxidase was decided in line with (Dawson and Magee, 1955).

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 have been added to the laboratory for proper identification, and parasitism changed into certified with the aid of using the presence of sucker attachments.

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.

6. Results and Discussion

6.1. Dodder Control

The data in Table 1 display 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. Since the efficiency of both glyphosate rates is more or less the same. Although glyphosate at the higher rate (800 ppm) caused 95% control but phytotoxic effect on the 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 herbicide activity. Thus, glyphosate rates at 200 or 400 ppm are recommended for use in *N. oleander* without inducing any phytotoxicity.

Table 1: Control Percentage of Dodder Measured Periodically After 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	-

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, 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 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 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.

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	-	92.0	10.5	0.146
Glyphosate	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

6.2. Growth Characters

Data in Table 3 display that every one remedies of glyphosate improved the growth of *N. oleander* plant 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 leaves dry weights were 47.57 and 58.10% at 200 and 400 ppm more than the check treatment, respectively.

Table 3: Effect of Glyphosate Herbicide on Growth of *N. oleander* Leaves

Treatment	Conc. (ppm)	Total Leaf Area (cm)	Leaf Fresh Weight (g)	Dry Weight of Leaves (%)
Check	-	25.66	2.15	21.65
Glyphosate	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

6.3. Chemical Constituents

6.3.1. Pigments Concentration in Leaves

The data provided in Table 4 suggest that treating plants with glyphosate at 200 and 400 ppm increased the plastid pigments appreciably in comparison to the check. The will increase had been 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.

6.3.2. Carbohydrates Concentration in Leaves

The data in Table 5 display that concentrations of total carbohydrates in leaves had been appreciably reduced because of dodder infection. On the contrary, total carbohydrates concentrations were increased due to glyphosate treatments. The most will increase acquired via way of means of 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 concentration of non reducing sugars in leaves, the better growth becomes acquired via way of means glyphosate at 200 and 400 ppm. This increase reached 78.07 and 74.17% over the check, respectively.

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	-	1317	262	112	37
Glyphosate	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	-	241	25.15	13.38	11.77
Glyphosate	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

6.3.3. Free Prolin, Total Indoles, Ascorbic Acid, Total and Free Phenoles

Data in Table 6 display that free prolin, total indoles, ascorbic acid and total phenol in leaves have been reduced below the plants of *N. oleander* infected with dodder. In contrast, applying glyphosate at 200 and 400 ppm increased these

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	-	6.12	10.95	10.15	2.69	5.21
Glyphosate	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

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 opposite, free phenoles awareness has been reduced with the aid of using 10.78 and 12.63% at 200 and 400 ppm under the check, respectively.

6.3.4. The Enzymatic Activity

Data in Table 7 suggest that the plants of *N. oleander* sprayed with glyphosate increased the leaves 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.

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	-	100.00	100.00	100.00	100.00
Glyphosate	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

Generally it is clear that glyphosate enhanced all the biochemicals 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.

7. Discussion

It is clear from the present data 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*. 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 + scepter (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.

The positive effect of the herbicide on growth characters of *N. oleander* infected plants may be due to the role of herbicides on control dodder selectively after the parasite is attached to the host plant Dawson (1990), which improve early plant growth and caused improvement in the rate of essential nutrients absorption, e.g. (N.P.K.).Also to the increase in plant growth hormones such as indole acetic acids. In this respect, Dawson (1990) 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 glyphosate reach the dodder by translocation from the host to the parasite. In this concern, Sarić-Krsmanović *et al*. (2015) conducted a study to investigate to see how glyphosate, propyzamide, imazethapyr, and diquat herbicides are able to curb field dodder in alfalfa of Pot and field trials. The highest effectiveness of 95% and 97.5%, was reported by two glyphosate application rates (288 and 360 g a.i. ha) respectively. Also, chemical control plays an important role in weed control in sugar beet production, until become established; they are very susceptible to competition from weeds. That is one reason why many growers like to use pre plant or pre-emergence herbicides. Early poste-mergence herbicide applications also help reduce competition from weeds while the sugar beets are small (Morishita, 2003). Several studies have been conducted to evaluate the effects of herbicides on weeds. Longden (1989) reported that Weed did not affect the concentration of sugar (sucrose), potassium, sodium, á amino nitrogen, or invert sugar in the crop beets. Root and sugar yields were progressively reduced by increasing densities of weed beet.

It is clear from the present data also that infection of plants with dodder reduce the different metabolic processes which are responsible for normal plant growth. The adverse effect on the syntheses of chlorophyll a, b, carotenoids, anthocyanin, sugars, total free amino acids, proline, N, crude protein and plant auxin concentration which occurred as a result of dodder infection was reduced by treating the plants with the herbicide used in this investigation. In this connection, El-Saht *et al*.(1994) found that chlorophyll a, b and carotenoids concentration of both castor bean and maize plants treated with low concentrations of metribuzin herbicide were unaffected whereas at high concentration of herbicide significantly decreased the chlorophyll pigment concentration. Also El-Yazal and Abd El-Samie (2002) reported that the increase in anthocyanin may be attributed to the increase in the concentration of sugars in plants. Also El- Saht (2001) reported that anthocyanin is utilized as a precursor with cytoprotective function in the secondary metabolism, this would led to a defense mechanisms in castor bean and maize seedling and plants treated with low concentration of metribuzin herbicides. Thus, the increased level of anthocyanin indicates an index for a good mechanism of plant resistance towards the changes in the environmental conditions. The reduction of total carbohydrates concentration in leaves of plant under treatment with glyphosate herbicides at high concentration might be due to the inhibiting effect on Hill reaction (El-Saht and El-Maghraby, 1998). The increase in total and reducing sugars concentration in the present study agreed with those obtained by Hasaneen *et al.* (1994) they observed that contents of natural products with cytoprotective functions (reducing sugars and sucrose) as well as activities of $\propto$ and B-amylase of castor bean plants supplemented with herbicide were increased significantly whereas at high concentrations of these herbicide an opposite response was apparent. Moreover the increase in total free amino acids and free prolin may be due to the interruption of protein synthesis and proteolysis which occurs in plants under herbicide treatment stress. Some results were also obtained by El-Saht and El-Maghraby (1998) they concluded that most herbicides are known to induce changes in the levels of natural products with cytoprotective function . The present results are in general agreement with those obtained by El-Saht (2001).

It is clear that at low concentration of the used herbicide, the reduction in the concentration of total indoles was reduced while at high concentration it increased as compared to the control plants.

This reduction in IAA at high concentrations of both herbicides may be attributed to increasing activity of IAA oxidase under herbicide stress conditions. The increase in levels of IAA oxidase may have some influence on the amounts of the transported IAA, which could influence the final levels that occur in a tissue at any one time. Such increasing effect of total phenols may be attributed to that phenolic compounds constitute a part of cellular solvents and provide a reducing environment that could be adaptive mechanism for scavenging oxygen free radicals during stress, El-Shewy *et al.* (2001).

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 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.

These results are generally in full agreement with those obtained by us and many investigators, Seif El-Yazal and Abd El-Samie (2002) on Faba bean; Ali and Seif El-Yazal (2011) on *Nerium oleander* L; Goldwasser *et al*. (2012) on tomato; Ziveh *et al*. (2013) on sugar beet; Zaroug *et al*. (2014) on onion; Sarić-Krsmanović *et al*. (2015) on alfalfa ; Meighani *et al*. (2017) and Hoseyni *et al*. (2018) on sugar beet, Seif El-Yazal and Ali (2019) on fenugreek plants.

8. Conclusion

Applying glyphosate at very low rates (200 and 400 ppm) controlled (86-90%) of attached dodder, *Cuscuta campestris* Yunker on *Nerium oleander* L. after two months of treatment without any phytotoxicity and also improved growth parameters. Dodder control by the use of glyphosate at the same rates reduced seed germination, length and fresh weight of dodder. Glyphosate herbicide at the two rates were markedly increased chemical constituents of leaves (total carbohydrate, total, reducing and non-reducing sugars, chlorophylls, carotenoid, xanthophylls, prolin, total indoles, ascorbic acid and antioxidant enzymes activity). On the contrary, all dodder control treatments decreased free phenols in leaves as compared to the check.

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Cite this article as: Mohamed Ahmed Seif-El-Yazal and Ibrahim Hamed Hussein Ali (2021). Ameliorative Impact of Glyphosate Herbicide Treatments on Dodder (*Cuscuta campestris*) Control in Nerium oleander L. Shrubs. *International Journal of Agricultural Sciences and Technology*. 1(4), 24-32. doi: 10.51483/IJAGST.1.4.2021.24-32.