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Controlling *Orobanche crenata* in Faba bean using the Herbicides Glyphosate and Imazapic with some additives

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Abstract: This investigation was carried out in the greenhouse of the National Research Center, Dokki, Cairo, Egypt, for two successive winter seasons 2011/2012 and 2012/2013. In this investigation, two herbicides, glyphosate and imazapic were tested to evaluate their efficiency in controlling broomrape in faba bean cv. Giza 2. The herbicide glyphosate was applied alone at 75 (the recommended dose), 56.5 and 37.5 ml/ fed. The herbicide imazapic was applied alone at 200 (the recommended dose), 150 and 100 ml / fed. The two herbicides were applied foliarly with the addition of 2% urea or ammonium sulfate at low doses. The two herbicides with or without urea or ammonium sulfate significantly inhibited the growth of broomrape (*Orobanche crenata*) parasitizing faba bean plants without visible injury effect on the plants. The inhibition of *O. crenata* growth was accompanied by significant increase in faba bean growth and yield. The growth and yield of faba bean varied according to the treatment applied. Maximum yield was obtained with the addition of ammonium sulfate to glyphosate at 37.5 ml / fed. or imazapic at 100ml / fed. compared with the infected control. The increase in yield approaches to that of healthy control in 2011/2012 and 2012/2013. The increase in growth was associated by increase in pigment contents in faba bean leaves. Carbohydrates and protein contents in the yielded seeds also resulted. The results suggested controlling *O. crenata* parasitizing faba bean with obtaining high yield especially with glyphosate at 37.5 ml / fed. or imazapic at 100ml / fed. combined with 2% ammonium sulfate.

Keywrods : Faba bean, Glyphosate, Imazapic, *Orobanche crenata*, Urea, Ammonium sulfate.

Introduction

Parasitic weeds of the family Orobanchaceae (*Orobanche*) are considered to be among the most serious agricultural pests of economic importance in many parts of the world[1]. The Egyptian broomrape (*Orobanche aegyptiaca* Pers.) is an obligate holoparasitic weed that causes severe damage to many important vegetable and field crops. It is considered one of the major biotic limiting factors to the production of legumes such as faba bean [2].

O.crenata and *Orobanche aegyptiaca* Pers occur in legume fields threatening production of these crops in an area of nearly 4.4 million hectares[3]. They are holoparasites, devoid of chlorophyll and totally dependant on the host for organic carbon, water and nitrogen[4]. *O. crenata* (crenate broomrape) has been known to threaten

legumes crops since antiquity [5]. It is an important pest in faba bean (*Vicia faba*) [6, 7]. In faba bean losses from 5 to 95% have been reported [6, 8] depending on the infestation level and the planting date.

Several attempts and intensive research were made in different countries to screen for potential herbicides against *Orobanche*. In general, the most limiting factor in the use of the promising herbicides is their degree of selectivity among the crops at the required rate for parasite control, especially of the foliarly applied systemic herbicides. Glyphosate is the first promising herbicide developed for *Orobanche crenata* control in faba bean [9], and is still the most important herbicide used. It has a good use in broad bean. Any substance that is translocated in the phloem but not metabolized will become concentrated in the parasite, which accumulates them in its own tissues. Herbicides applied to the host at low rates can therefore reach lethal concentrations in the parasite. This is the basis of selective, broad acre chemical control using low rates of glyphosate[10]. Glyphosate applied at 80 g a.i. ha^{-1} twice gave 100% control of emerged broomrape shoots and completely prevent parasite development in faba bean[11]. Glyphosate is best translocated from broad bean to broomrape at the tubercle and bud stage of the parasite.[12]. Applying glyphosate three times (7, 10 and 13 weeks) at rate of 75 cm^3 /fed after sowing controlled broomrape by 98.8 and 99% of broomrape and increased seed yield by 149.5 and 141.5%s compared with the untreated control [13]. Similar results were obtained [14,15, 16].

In addition to glyphosate, other herbicides, e.g. Herbicides belonging to the amino acid inhibiting imidazolinone group have been reported as efficient in *Orobanche* control in legumes and sunflower [17, 18, 19, 20, 21, 22]. Imazaquin applied at 10 g a.i. ha^{-1} twice gave 100% control of emerged broomrape shoots and completely prevent parasite development in faba bean [11]. It was found that soaking broad bean seeds for 5 min in 0·01–0·1% herbicide solutions or coating at 20–40 g ha^{-1} with imazethapyr did not affect seed germination and crop growth, and resulted in 60–80% broomrape control[18]. Furthermore, broad bean seeds treated with imazethapyr followed by an additional late post-emergence application of imazapyr at 5 g ha^{-1} resulted in excellent broomrape control (>95%). Imazapic used as pre- or post emergence at rate of 3, 4.5, 10 and 12 g/ha efficiently controlled orobanche in faba bean and ensured a satisfactory yield [23].

In this study attempts to control *Orobanche crenata* in the economic crop broad bean by the herbicides glyphosate and imazapic.

Materials and Methods

This investigation was carried out in the greenhouse of the National Research Center, Dokki, Cairo, Egypt, for two successive winter seasons 2011/2012 and /2012/2013. faba bean seeds cv. Giza 2 were obtained from the Agricultural Research Center, Ministry of agriculture, Giza, Egypt. The pots which had a 30 cm diameter (17 cm height) were infested with consistent seed weight of *Orobanche crenata* seeds at 5 cm depth from soil surface. Faba bean seeds were sown (5 seeds/pot) in 15 and 26 November at 2 cm depth under average maximum and minimum temperature 20.5±1 and 19.5±1°C. Two weeks later, thinning of faba bean was done so that two homogenous seedlings were left per pot. Ammonium nitrate and super phosphate (2: 1 w/w) were added for each pot after 15 days from sowing. The experiment consisted of 12 treatments including two control treatments. The control treatments were, infected and healthy controls. In each treatment 6 pots were used. The pots were arranged in a completely randomized design. The herbicide glyphosate was sprayed alone at the rates of 37.5, 56.3 and 75ml/fed. Addition of urea or ammonium sulfate at 2% for each was added to the reduced dose (37.5ml / fed.). The herbicide imazapic was sprayed at three rates, 100ml, 150 and 200/fed. then applied at rate of 100ml/fed. with 2% urea or ammonium sulfate. Spraying of the herbicides alone or with additives was carried out two times, the first spray was applied 45 days from sowing while the second spray was applied 21days after the first spray. Samples of *Orobanche crenata* as well as different growth criteria of the host (faba bean plants) were taken from three pots after 70 days from sowing, yield and its components were recorded from the other three pots at harvest.

Data recorded

The recorded characteristics included plant height (cm), number of leaves/ plant, and fresh and dry weight/plants (g). The fresh materials were washed carefully several times with tap water, then with distilled water and oven dried at 60°C for determination of dry weight (g/plant). The dried materials were ground to fine powder and stored in paper bags until used in further steps (extraction and testing). Faba bean yield were recorded for each treatment at the end of the season.

Biochemical analyses:

Determination of some chemical contents:

Determination of photosynthetic pigments:

Chlorophyll a, b and carotenoids (mg/g) were extracted from fresh leaves [24].

Percentage of total carbohydrates contents:

Total carbohydrates were extracted from dry finely ground yielded seeds [25] and estimated colourimetrically by the phenol-sulphoric acid method [26].

Percentage of protein contents:

Total protein contents were extracted from dry finely ground yielded seeds [27].

Statistical Analysis:

The data were statistically analyzed [28].

Results

Growth and yield

Table 1 show that the herbicides, glyphosate or imazapic at any concentration used when they were sprayed twice significantly decreased the number, fresh weight, dry weight and length of Orobanche / host. This effect also was obtained with the herbicide when combined with 2% urea or ammonium sulfate (Ams.). The effect was after 70 days from sowing and consistent at harvest in comparison to the infected control. The reduction in number and dry weight of *O. crenata* was maximum with the herbicide glyphosate at 75ml/fed. In addition, the combined application of the two herbicides with urea or Ams. were also effective. These results were coincided in the two successive seasons, 2011/2012and 2012/2013.

Table 1. Effect of the herbicides glyphosate or imazapic and their combination with urea or ammonium sulfate on the developing of *Orobanche crenata*.

Treatments (ml / fed)	70 Days after sowing				At harvest			
	No of Orobanche/host plant	Fresh biomass/ host plant	Dry biomass/ host plant	Length/ host plant	No of Orobanche /host plant	Fresh biomass/ host plant	Dry biomass/ host plant	Length/ host plant
2011/2012								
Glyphosate 37.5	2.11	8.15	1.47	6.11	7.44	44.01	10.80	37.53
Glyphosate 56.3	1.80	7.02	1.23	4.70	6.36	57.81	8.22	25.2
Glyphosate 75	1.32	5.15	0.89	4.11	4.35	23.49	5.58	22.59
Glyphosate 37.5 + 2% urea	1.83	7.11	1.27	5.50	6.60	34.77	8.34	26.25
Glyphosate 37.5 + 2% Ams.	1.75	6.80	1.19	4.50	6.24	31.02	7.35	24.93
Imazapic 100	2.21	8.68	1.58	6.32	9.27	82.17	11.52	41.16
Imazapic 150	1.95	7.66	1.38	6.00	7.50	40.23	9.66	30.63
Imazapic 200	1.56	6.08	1.05	4.23	5.37	27.72	6.57	23.31
Imazapic 100+ 2% urea	2.00	7.84	1.41	5.85	7.80	68.88	10.08	35.70
Imazapic 100+ Ams.	1.90	7.41	1.32	5.71	7.14	38.88	9.33	27.33
Healthy control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Infected control	8.24	32.00	5.85	6.66	35.13	168.00	41.19	57.78
LSD at 5%	0.15	0.25	0.19	0.44	1.12	2.11	1.28	2.28
2012/2013								
Glyphosate 37.5	2.67	8.08	1.40	4.69	8.67	33.02	8.80	46.11
Glyphosate 56.3	2.17	9.94	1.72	6.01	10.56	35.68	9.76	54.11
Glyphosate 75	1.70	6.40	1.10	4.15	6.75	30.11	7.52	42.34
Glyphosate 37.5 + 2% urea	2.22	8.26	1.46	4.99	9.06	33.63	8.96	94.14
Glyphosate 37.5 + 2% Ams.	2.11	7.77	1.38	4.55	7.84	32.35	8.06	44.90
Imazapic 100	2.83	10.85	1.94	6.14	10.85	35.23	9.86	54.82
Imazapic 150	2.45	9.35	1.68	5.50	9.95	34.30	9.47	51.23
Imazapic 200	1.91	7.25	1.27	4.30	7.58	30.82	7.71	43.23
Imazapic 100+ 2% urea	2.49	9.41	1.71	5.73	10.40	32.03	9.66	50.4
Imazapic 100+ Ams.	2.30	8.69	1.50	5.29	9.50	34.08	9.31	49.28
Healthy control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Infected control	10.11	37.22	6.59	7.11	48.67	195.74	56.83	73.6
LSD at 5%	0.14	0.18	0.14	0.25	0.33	0.42	0.45	0.245

The growth of faba bean represented by plant height, number of leaves /plant, number of branches /plant, fresh and dry weight/plant increased with the treatments of the herbicides glyphosate or imazapic as compared to the infected control (Table 2). The addition of 2% urea or Ams. to the reduced dose of either glyphosate or imazapic increased the growth of faba bean to high extent exceeded that caused by two herbicides when used alone. Maximum increase was recorded in dry weight with the addition of 2% Ams. to glyphosate at rate of 37.5 ml / fed. or imazapic at rate of 100 ml / fed. (approaching to that of healthy control) in the two seasons (Table 2).

Table 2. Effect of the herbicides glyphosate or imazapic and their combination with urea or ammonium sulfate on the growth of faba bean after 70 days from sowing.

Treatments (ml / fed)	Plant height (cm)	No. of leaves/plant	No. of branches/plant	Fresh weight(g)/plant	Dry weight(g)/plant
2011/2012					
Glyphosate 37.5	65.11	29.87	2.00	91.22	13.88
Glyphosate 56.3	63.51	29.14	1.88	90.25	13.27
Glyphosate 75	59.00	31.55	1.77	88.83	12.36
Glyphosate 37.5 + 2% urea	68.66	30.16	1.66	93.00	14.16
Glyphosate 37.5 + 2% Ams.	70.65	31.74	1.88	94.58	15.09
Imazapic 100	60.16	26.73	1.44	67.83	9.06
Imazapic 150	66.83	28.16	1.77	75.83	10.43
Imazapic 200	61.83	32.16	1.88	85.00	12.91
Imazapic 100+ 2% urea	70.33	32.88	2.00	94.83	18.20
Imazapic 100+ Ams.	71.42	34.73	2.00	98.57	19.02
Healthy control	71.20	29.33	2.10	99.83	19.33
Infected control	54.86	20.00	1.11	66.00	8.46
LSD at 5%	3.38	3.03	0.36	3.30	1.36
2012/2013					
Glyphosate 37.5	61.33	23.33	1.66	81..83	10.41
Glyphosate 56.3	62.94	27.14	1.88	90.54	13.28
Glyphosate 75	60.83	25.33	2.00	89.16	11.83
Glyphosate 37.5 + 2% urea	63.66	23.50	2.00	85.20	12.41
Glyphosate 37.5 + 2% Ams.	67.83	28.83	2.00	90.93	13.92
Imazapic 100	59.50	22.00	1.66	81.16	10.33
Imazapic 150	65.14	24.47	1.77	85.40	12.30
Imazapic 200	61.83	25.16	1.83	88.16	11.81
Imazapic 100+ 2% urea	65.83	25.33	1.83	89.73	12.16
Imazapic 100+ Ams.	66.50	26.33	2.16	98.43	14.92
Healthy control	64.16	29.16	2.50	102.00	19.83
Infected control	54.66	22.00	1.50	80.50	9.08
LSD at 5%	2.51	2.79	0.35	6.49	0.86

Spraying faba bean with the herbicides glyphosate, imazapic or their combination with 2% urea or Ams. induced significant increases in the number of pods/plant as well as number of seeds /pod, these increases reached maximum values by addition of 2% urea or Ams. to the reduced rate of both herbicides (Table 3). Similarly, weights of seeds /plant (seed yield /plant) as well as weight of 100 seeds revealed more pronounced values on the addition of 2% urea or Ams. to the herbicides. Addition of Ams. to imazapic at 100ml / fed. in the two seasons (2011/2012and 2012/2013) increased significantly yield/ plant to exceed that of healthy control although non-significant (Table 3).

Table 3. Effect of the herbicides glyphosate or imazapic and their combination with urea or ammonium sulfate on the yield and yield components of faba bean.

Treatments (ml / fed)	No. of pods/plant	No. of seeds/pod	Wt. of seeds/pod	Wt. of seeds/plant	Wt. of 100-seeds
2011/2012					
Glyphosate 37.5	8.87	2.66	2.78	23.84	80.09
Glyphosate 56.3	9.25	2.66	2.83	24.20	81.71
Glyphosate 75	11.06	2.66	2.94	25.69	86.00
Glyphosate 37.5 + 2% urea	14.00	2.66	3.00	29.2	90.00
Glyphosate 37.5 + 2% Ams.	14.43	3.10	3.10	32.65	92.83
Imazapic 100	9.00	2.66	1.80	17.2	66.00
Imazapic 150	10.66	2.66	2.43	23.7	75.33
Imazapic 200	12.33	2.66	2.53	27.5	80.66
Imazapic 100+ 2% urea	13.88	3.00	3.06	32.2	91.16
Imazapic 100+ Ams.	14.12	3.00	3.22	33.58	92.41
Healthy control	15.66	3.00	3.33	33.50	96.00
Infected control	7.66	2.66	1.53	10.73	50.80
LSD at 5%	1.15	Ns	0.23	1.25	3.09
2012/2013					
Glyphosate 37.5	12.33	1.55	1.96	22.16	72.00
Glyphosate 56.3	11.00	1.33	2.13	25.20	62.83
Glyphosate 75	12.66	1.33	2.13	25.47	64.66
Glyphosate 37.5 + 2% urea	12.00	2.66	2.30	29.30	75.00
Glyphosate 37.5 + 2% Ams.	13.66	2.55	2.65	31.99	84.76
Imazapic 100	10.66	1.33	1.93	19.57	52.23
Imazapic 150	11.00	1.66	2.00	22.6	56.10
Imazapic 200	14.00	2.00	2.10	28.64	60.26
Imazapic 100+ 2% urea	13.33	2.66	2.16	26.08	62.66
Imazapic 100+ Ams.	15.00	2.66	2.46	31.99	92.00
Healthy control	16.33	2.66	3.10	33.61	93.64
Infected control	7.33	1.33	1.53	10.21	48.00
LSD at 5%	1.13	NS	0.20	1.77	2.11

Some chemical constituents:

Photosynthetic pigments:

The results in Table 4 reveal significant increases in the synthesis of chlorophyll a, chlorophyll b and carotenoids in faba bean leaves due to combined treatments of glyphosate or imazapic with 2% urea or Ams. in comparison to the infected control. The combined treatments with glyphosate or imazapic induced maximum contents.

Table 4. Effect of the herbicides glyphosate or imazapic and their combination with urea or ammonium sulfate on the contents of some chemical constituents in faba bean.

Treatments (ml / fed)	Pigment contents (mg/g fresh weight)				% of carbohydrate	% of protein
	Chl. a	Chl. b	Car.	Total pig.		
2011/2012						
Glyphosate 37.5	0.682	0.414	0.219	1.315	41.56	24.53
Glyphosate 56.3	0.762	0.439	0.214	1.415	40.93	26.61
Glyphosate 75	0.805	0.448	0.203	1.456	45.87	29.68
Glyphosate 37.5 + 2% urea	0.787	0.462	0.234	1.483	54.39	35.50
Glyphosate 37.5 + 2% Ams.	0.894	0.479	0.265	1.638	58.12	36.86
Imazapic 100	0.649	0.349	0.179	1.177	40.79	23.29
Imazapic 150	0.663	0.401	0.221	1.285	44.53	23.88
Imazapic 200	0.721	0.449	0.253	1.423	45.21	24.89
Imazapic 100+ 2% urea	0.838	0.437	0.288	1.563	54.36	28.50
Imazapic 100+ Ams.	0.853	0.462	0.278	1.593	54.86	35.92
Healthy control	0.934	0.484	0.295	1.713	60.27	37.98
Infected control	0.615	0.316	0.169	1.1	38.68	21.50
LSD at 5%	0.074	0.012	0.009	0.071	1.98	1.17
2012/2013						
Glyphosate 37.5	0.787	0.451	0.238	1.476	41.88	25.33
Glyphosate 56.3	0.710	0.439	0.192	1.341	44.14	26.39
Glyphosate 75	0.725	0.448	0.209	1.382	47.32	28.20
Glyphosate 37.5 + 2% urea	0.820	0.418	0.177	1.415	57.35	34.71
Glyphosate 37.5 + 2% Ams.	0.934	0.449	0.301	1.684	58.02	36.52
Imazapic 100	0.682	0.356	0.184	1.222	40.20	22.70
Imazapic 150	0.690	0.342	0.193	1.225	46.70	22.37
Imazapic 200	0.720	0.411	0.228	1.359	47.54	23.51
Imazapic 100+ 2% urea	0.871	0.458	0.259	1.588	54.77	25.21
Imazapic 100+ Ams.	0.934	0.493	0.283	1.710	58.89	34.62
Healthy control	1.042	0.499	0.285	1.826	59.26	37.75
Infected control	0.681	0.316	0.168	1.165	39.12	20.86
LSD at 5%	0.051	0.035	0.012	0.076	2.89	1.15

Percentage of carbohydrate and protein in the yielded faba bean seeds

As shown in Table (4) all tested weed- control treatments on *O. crenata* significantly improved the percentages of carbohydrates and protein of faba bean seeds. Carbohydrate and protein contents in the yielded seeds get maximum values with the combined treatments of Ams. with glyphosate or imazapic.

Discussion

Many management strategies have been tried against broomrapes, but few of them have proved reliable and these are only economical in high value agriculture. Examples, Herbicides that stop the emergence of broomrape, glyphosate and imazapic are two examples.

In the present study application of the two herbicides glyphosate or imazapic at two sprays controlled the parasite without causing any damage to the host (faba bean). Great significant reduction in number and dry weight of O. *crenata* was achieved with both herbicides. These results were achieved with single application of the herbicides or on addition of 2% urea or Ams. Previous results have been reported for evaluating the efficacy of glyphosate [10, 11]. The efficiency of glyphosate against broomrape was attributed to its best translocation from

broad bean to broomrape at the tubercle and bud stage of the parasite[12]. Confirming this conclusion Broomrape was controlled by 98.8 and 99% by application of glyphosate at 75 cm ^{3} /fed [13, 14, 29]. In addition, *Orobanche* in faba bean was efficiently controlled by imazapic [16, 23]. .

The treatments of glyphosate with, without urea or Ams. inhibited significantly the development of *O. crenata* infestation and increase faba bean growth and subsequently yield (Tables 1,2 & 3) compared with the untreated control. Similar results were obtained [13, 14, 15, 16]. The present results also reveal increase in faba bean growth and yield by applying imazapic with, without urea or Ams. (Tables 1,2 & 3), these results were similar to previous work that imazapic used as pre- or post emergence at rate of 3, 4.5, 10 and 12 g/ha efficiently controlled *Orobanche* in faba bean and ensured a satisfactory yield[23]. Our results were coincided and confirmed [16]. The increase in plant growth is accompanied by different metabolic processes that may explain increases in carbohydrate and protein contents in the yielded seeds[29].

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