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Efficacy of *Phytomyza orobanchia* Kalt. in Reduction of *Orobanche crenata* Forsk. Seed Yield Under Semi-field Conditions

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ABSTRACT

The parasitic weed *Orobanche crenata* Forsk.. seriously affects the faba bean crop causing significant yield losses. Biological control using the dipteran *Phytomyza orobanchia* Kalt. (Agromyzidae) has been promising in Eastern Europe, the former USSR and Mediterranean basin. Under semi-field conditions, *P. orobanchia* pupae were released on pot containing faba bean plants infected with *Orobanche.* The applied release ratios were 1, 3, 6, 12, 24 and 48 *Phytomyza* pupae per *Orobanche* spike during the seasons of 1999, 2000 and 2001. Results revealed that the investigated release ratios had a significant reduction in *Orobanche* dry weight and seed yield through the three consecutive seasons. For determining the efficacy of *P. orobanchia* as a biocontrol agent of *O. crenata,* the changes in the *Orobanche* seed bank in the soil was estimated. Results showed that *Phytomyza* efficacy, significantly, increased with increasing the number of released *Phytomyza.* However, it significantly decreased by succession of the three release seasons, where the mean efficacy percentages were 61.85, 55.14 and 47.58% during the seasons 1999, 2000 and 2001, respectively. Results of this study indicate that although of the effectiveness of *Phytomyza* releases in reducing *Orobanche* seed production can reach 91.73%, the already infested soil in addition to new accumulated seeds will cause further infestations over the successive seasons. This finding should be undertaken in the future release programs of *P. orobanchia.*

Key Words: Biological control, *Orobanche crenata, Phytomyza orobanchia.*

INTRODUCTION

The parasitic weed *Orobanche crenata* Forsk. seriously affects the faba bean crop causing significant yield losses. Biological control is a particularly attractive means of suppressing parasitic weeds in crops because, owing to their intimate relationship with the host plant, it is difficult to apply chemical herbicides in such a way that the crop is not do adversely affected. Furthermore, parasitic weeds produce enormous numbers of minute, long-lived seeds, which makes eradication extremely difficult (CAB International 1987).

The insect natural enemies of *Orobanche* spp. have been surveyed in different countries. The dipteran *Phytomyza orobanchia* Kalt. (Agromyzidae) is the only one of these insects that has received further attention. This fly is widespread in Europe (except Scandinavia), the Mediterranean Basin and eastwards through Saudi Arabia to Uzbekistan (Spencer 1973). Females of *P. orobanchia* usually insert their eggs singly either in the petals, the stigmata or the stylets or rarely in the antheridia or calyces of the flower. After hatching, the larvae bore in the tissues towards the ovules to feed on unripe seeds (Tawfik *et al.* 1976).

The efficacy of *P. orobanchia* under natural conditions has been reported by several authors. Investigations in Syria, Egypt and Turkey revealed infestation of seed capsules of 33, 89 and 94%, respectively (Linke *et al.* 1990, Tawfik *et al.* 1976, Nemil and Giray 1983). In Morocco, the fly is able to infest 77% of the *orobanche* spp. shoots and 36% of the seed capsules (Klein, 1995). However, the natural infestation of *Orobanche* spp. by *P. orobanchia* is not sufficient to reduce the *Orobanche* population to the point that no economic damage occurs. The extensive cropping of the host plants of *Orobanche* spp. favors the development of the parasitoid and disturbs the natural equilibrium between *Orobanche* and *P. orobanchia,* which exists in the natural vegetation in non-cropping situations. In highly infested fields with *Phytomyza,* the low remainder of *Orobanche* seeds yield is enough to maintain the *Orobanche* seed bank in soil. The aim of the present study is to evaluate the efficacy of *P. orobanchia* release rates in reducing *O. crenata* seed yield under semi-field conditions for three consecutive seasons.

MATERIALS AND METHODS

Faba bean seeds, Giza 461 were sowed in pots (20 centimeter diameter) containing sterilized soil that artificially infested with the *Orobanche* seeds at rate of 0.01 g per 1 kg soil. During the time of *Orobanche* emergence, *Orobanche* capsules infested with *Phytomyza* pupae were introduced in glass vials. The latter were hung up at a high of 10 to 15 centimeters above the soil surface on wooden stakes during the bud stage of *Orobanche*. The tested ratios of *Phytomyza* pupae: *Orobanche* spike were 0:1, 1:1, 3:1, 6:1, 12:1, 24:1 and 48:1. The 0:1 ratio was used as a control. Pot contents were covered with metal-screen cloth cage. Droplets of sugar solution 50% were put on the metal-screen cloth cages for feeding the released *Phytomyza* adults. During ripening stage of *Orobanche*, capsules from each release ratio were separated and examined. At the end of season 1999, number of *Phytomyza* pupae per spike and dry weight of *Orobanche* plants per pot were determined. The ripened seeds of both intact (control) and infested spikes were weighed for estimating the percentage of *Orobanche* seed reduction due to *Phytomyza* (ORP %) as follows:

$$ORP\% = \frac{\text{Seed weight of control} - \text{Seed weight of infested spikes}}{\text{Seed weight of control}} \times 100$$

After estimation of seed weights, the yielded seeds of each release ratio were returned back to the soil in their pots and left until the next season. The same abovementioned method of release was applied for another two consecutive seasons on the same infested soil in pots. Number of pupae per spike, dry weight of *Orobanche* plants per pot and ORP% were estimated at the end of two investigated seasons, *i.e.* 2000 and 2001.

To estimate the change in the *Orobanche* seed bank in soil due to impact of *Phytomyza*, 10 randomized samples of soil (1gm of each) were picked up from each pot. Samples were put in beaker containing water to separate the seeds from soil. The floating seeds were examined and collected using stereomicroscope. Mean weight of seeds per Kg soil was calculated. For determination of the efficacy percent of *P. orobanchia* in reducing *Orobanche* seed bank (E), the formula of Klyueva and Pamukchi (1982) was applied.

$$\%E = \frac{((Sro - Sr1) - (Sco - Sc1)) \times Sco}{Sro \times Sc1} \times 100$$

Sro = *Orobanche* seeds per Kg soil before release of *P. orobanchia* .
Sr1 = *Orobanche* seeds per Kg soil after release of *P. orobanchia* .
Sco = *Orobanche* seeds per Kg soil in the control (0.0 release) before release of *P. orobanchia* .
Sc1 = *Orobanche* seeds per Kg soil in the control (0.0 release) after release of *P. orobanchia* .
Results were statistically analyzed using the ANOVA test.

RESULTS AND DISCUSSION

Under semi-field conditions, the number of grown faba bean plants was two plants per pot and number of emerged *Orobanche* spikes was 4.07 $\pm$ 0.6 spikes per pot. *P. orobanchia* pupae were released at different ratios of *Phytomyza* per *Orobanche* spike on faba bean plants infected with *Orobanche* weeds under Metal-Screen cages. The applied release ratios were 1, 3, 6, 12, 24, and 48 of *Phytomyza* pupae per *Orobanche* spike (*P/O*) for three consecutive seasons of 1999, 2000 and 2001. The mean numbers of formed pupae inside fruits and stems per spike after release of the abovementioned release ratios were 3.59, 6.89, 8.43, 9.23, 9.83 and 9.91 pupae per spike, respectively and were 6.74, 7.99 and 9.21 in seasons 1999, 2000 and 2001, respectively (Table, 1). The statistical analysis showed a significant difference in the number of *Phytomyza* pupae per *Orobanche* spike between the ratio of 1 *P/O* and other release ratios. On the other hand, no significant difference was observed between the three investigated seasons.

Table (1): Mean number of *Phytomyza* pupae per *Orobanche* spike after different release ratios of *P. orobanchia during* three consecutive seasons under semi-field conditions.

Release ratio (*Phytomyza* pupa / spike)	No. of *p. orobanchia* pupae			Mean
	1999	2000	2001	
1	5.06	1.95	3.75	3.59b
3	6.01	6.06	8.62	6.89a
6	8.16	8.06	9.06	8.43a
12	6.29	10.16	11.25	9.23a
24	7.06	11.25	11.19	9.83a
48	7.87	10.47	11.37	9.91a
Mean	6.74	7.99	9.21	7.98

Means are average of 4 replicates / treatment. L.S.D.$_{0.05}$ (Release ratios) = 3.24
Differences due to seasons and interaction are not significant at p=0.05

As shown in Table (2) the dry weight of *Orobanche* spikes per pot was significantly decreased with increasing the released numbers of *Phytomyza* pupae through the three consecutive seasons. Mean reduction percentages of dry weights of *Orobanche* per pot were 34.9, 47.02, 46.43, 49.41, 53.54 and 58.25% at release ratios of 1, 3, 6, 12, 24 and 48 *P/O*, respectively. At the same time, no significant difference was observed between the season 2000 (51.79%) and season 2001 (56.40%).

Table (2): Dry weight of *Orobanche* spikes (gram per pot) at different release ratios of *Phytomyza orobanchia* during three consecutive seasons under semi-field conditions.

Release ratio (*Phytomyza* pupa / spike)	Weight of *Orobanche* spikes			Mean	% Reduction
	1999	2000	2001		
0	3.115	4.126	4.538	3.926a	
1	3.227	2.267	2.175	2.556b	34.90
3	2.828	1.806	1.606	2.080bc	47.02
6	2.403	2.351	1.555	2.103bc	46.43
12	2.618	1.933	1.409	1.986bc	49.41
24	2.268	1.856	1.360	1.828bc	53.44
48	1.877	1.724	1.317	1.639c	58.25
Mean	2.537	1.989	1.57		
% Reduction	18.55	51.79	56.40		

Means are average of 4 replicates / treatment.
L.S.D.$_{0.05}$ (Release ratios) = 0.82
Differences due to seasons and interaction are not significant at p=0.05

Table (3) shows weights of remaining mature seeds per pot resulted from different release ratios during the three consecutive seasons. Mean percentages of seed reduction through the three investigated seasons were 66.58, 88.34, 83.68, 90.16, 81.35 and 89.9% at ratios 1, 3, 6, 12, 24 and 48 of *P/O* respectively. Weight of remained seeds per pot was significantly reduced by applying the tested release ratios. However, no significant difference was observed between the tested seasons in the weight of remained seeds per pot.

Table (3): Weight of remained seeds (gram per pot) from infested *Orobanche* spikes with *Phytomyza orobanchia* at different release ratios during three consecutive seasons under semi-field conditions.

Release ratio (*Phytomyza* pupa / spike)	Weight of *Orobanche* seeds			Mean	% Reduction
	1999	2000	2001		
0	0.253	0.374	0.532	0.386a	
1	0.138	0.078	0.172	0.129bc	66.58
3	0.066	0.055	0.013	0.045bc	88.34
6	0.080	0.092	0.016	0.063bc	83.68
12	0.037	0.076	0.010	0.038c	90.16
24	0.085	0.110	0.031	0.075bc	81.35
48	0.066	0.031	0.021	0.039c	89.90
Mean	0.079	0.074	0.044	0.125	
% Reduction	68.77	80.21	91.73		

Means are average of 4 replicates / treatment.
L.S.D.$_{0.05}$ (Release ratios) = 0.097
L.S.D.$_{0.05}$ (Interaction) = 0.168
Differences due to seasons are not significant at p=0.05

During examination the infested spikes, it was noticed that the most pupae were found in the stem. At the same time, seeds in most *Orobanche* capsules were completely consumed. However, limited numbers of capsules were partially injured and the pupation takes place inside. These observations are in agreement with Tawfik *et al.* (1976). They found that when one larva attacks an ovule and develops to one third of its size, can destroy all the enclosed seeds, during the whole larval period. On the other hand, the damage caused by one larva attacking a mature fruit does not exceed one third of its total amount of seeds. Also, Tawfik *et al.* (1976) and Al-Eryan (1996) confirmed that the presence of three or more larvae within a mature fruit results in complete destruction of all seeds. On the other hand, reduction occurred in *Orobanche* dry weight may be due to the damage resulted from larvae mining in the plant tissues of *Orobanche* stem.

In general, release of 3 – 12 pupae *Phytomyza* per spike was enough to cause 88.34 – 90.16% *Orobanche* seed reduction. Similar results were obtained by Al-Eryan and Zaitoun (2001). They reported that release by 3 pupae of *P. orobanchia* per *O. crenata* spike in addition to natural infestation was enough to get about 84.26% *Orobanche* seed yield reduction under field conditions. Irrespective of release ratio, obtained data are in agreement with those of Natalenko (1969) in Russia; Kurbanov (1970) in Uzbekistan, Sklyarov and Borshchanskaya (1973) in Ukraine; and Klein *et al.* (1999) in Morocco, the above authors reported more than 80% reduction in *Orobanche* spp. seed yield due to *Phytomyza* release. In Egypt, Kolaib (1991) reported 64% reduction in number of seeds per infested *O. crenata* capsule. Also, Shalaby *et al.* (2000) recorded 71.4 and 100% capsule infestation of *O. ramosa* when they released *P. orobanchia* as flies and infested spikes, respectively under semi-field conditions. On the other hand, the release ratio was greatly different according to the investigated region. It was 0.005, 0.06 and 2.6-3 pupae/spike in USSR (Bakina *et al.*, 1980), Morocco (Klein *et al.*, 1999) and Egypt (Kolaib, 1991 and Al-Eryan and Zaitoun 2001), respectively. However, these differences may be due to the fecundity of *P. orobanchia* females according to the geographical distribution. Since it was 180-200 (Kott, 1969), 83 (Boumezzourh, 1996) and 33 eggs (Tawfik *et al.* 1976) per female in the aforementioned countries, respectively.

Efficacy percentages of *P. orobanchia* in reducing *Orobanche* seed bank fallen in soil through three consecutive seasons were calculated and summarized in Table (4). They were 46.13, 58.87, 59.46, 46.15, 57.09 and 61.45% at release ratios of 1, 3, 6, 12, 24 and 48 *P/O* at respect. On the other hand, efficacy percent was significantly reduced from 61.85% during season 1999 to 55.14% during season 2000 then to 47.58% at season 2001. This finding concurs with the opinions of Kroschel and Klein (1999). The above authors reported that although the effectiveness of additional releases of *P. orobanchia* to reduce the *O. crenata* seed production can reach 90%, these releases decrease the additional infestation of the soil with *Orobanche* seeds. But, in fields already infested, the longevity and germination ability of *Orobanche* seeds for many years will cause a further infestation in the successive seasons. Consequently, they suggested that releases of *P. orobanchia* have to be performed repeatedly over several years. In Egypt, Shalaby *et al.* (2002) added, it seems necessary for controlling this weed to use other methods such as mechanical control, using

Table (4) Efficacy percentages of *Phytomyza orobanchia* in reducing *O. crenata* seed bank at different release ratios during three consecutive seasons..

Release ratio (*Phytomyza* pupa/spike)	% *Phytomyza* efficacy			Mean
	1999	2000	2001	
1	47.11	51.62	39.66	46.13b
3	73.51	55.74	47.35	58.87a
6	70.49	59.08	48.80	59.46a
12	41.10	46.59	49.86	46.15b
24	65.66	52.61	53.02	57.09ab
48	72.31	65.23	46.81	61.45a
Mean	61.85a	55.14ab	47.58b	54.86

Means are average of 4 replicates / treatment.

L.S. $D._{0.05}$ (Seasons) = 8.6

L.S.$D._{0.05}$ (Release ratios) = 12.18

Differences due to interaction are not significant at p = 0.05

resistant varieties, sowing date and chemical control in integration to biological control by early release of *P. orobanchia* in order to reach successful control of *Orobanche* spp. However, Puzzilli (1983) reported that release of *P. orobanchia* repeatedly for 3-4 years resulted in 92-100% reduction in *Orobanche cumana* infestation in sunflower fields. In this concern, the statistical models which have been constructed by Al-Eryan and Zaitoun (1998) and Al-Eryan and Zaitoun (2001) could be undertaken and developed during release programs. These models for predicting *Orobanche* seed yield and its reduction due to *P. orobanchia* and rot fungi (Al-Eryan and Zaitoun, 2001) and for forecasting the number of *Phytomyza* flies required to be released per feddan to get a maximum reduction in *Orobanche* seed yield (Al-Eryan and Zaitoun, 1998).

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فاعلية ذبابة الهالوك فى خفض محصول بذور الهالوك تحت ظروف شبه حقلية

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تسبب حشائش الهالوك .*Orobanche crenata* Forsk خسائر جسيمة فى محصول الفول· وتعتبر المكافحة البيولوجية باستخدام ذبابة الهالوك .*Phytomyza orobanchia* Kalt طريقة واعدة فى شرق أوروبا والاتحاد السوفيتىالسابق وحوض البحر المتوسط· أطلقت ذبابة الهالوك تحت ظروف شبه حقلية على نباتات فول مصابة بالهالوك ومنماة داخل أصص· كانت نسب الإطلاق ١، ٣، ٦، ١٢، ٢٤، ٤٨ ذبابة/نورة هالوك خلال مواسم ١٩٩٩، ٢٠٠٠ و٢٠٠١· أظهرت النتائج انخفاضاً معنوياً فى الوزن الجاف ومحصول بذرة الهالوك خلال المواسم الثلاث· وقد كان إطلاق ٣حشرات/ النورة كافيا لإحداث ٧٣،٩١% خفض فى بذور الهالوك خلال موسم ١٩٩٩ أرتفع إلى ٩٧،٥٦% خلال موسم ٢٠٠١ فى نفس التربة المصابة· لتقويم كفاءة ذبابة الهالوك كعنصر مكافحة بيولوجية للهالوك تم تقدير كمية التغير فى مخزون التربة من بذور الهالوك· أظهرت النتائج زيادة كفاءة الذبابة فى إختزال مخزون البذور بالتربة بزيادة عدد الحشرات المطلقة· ورغم ذلك انخفضت كفاءة الذبابة فى إختزال هذا المخزون بتعاقب مواسم الدراسة حيث كان متوسط نسبة الكفاءة ٦١،٨٢، ٥٥،١٤ و ٤٧،٥٨% خلال مواسم ١٩٩٩، ٢٠٠٠ و ٢٠٠١، على التوالى· تشير نتائج هذه الدراسة إلى انه بالرغم من أن كفاءة الحشرات المطلقة فى خفض إنتاج بذور الهالوك تصل إلى ٩١،٧٣%، إلا أن تراكم بذور جديدة فى التربة المصابة أصلا ببذور سابقة يؤدى إلى إصابات جديدة فى التربة المصابة خلال المواسم المتعاقبة· ويرى الباحثون وجوب أخذ هذه النتيجة فى الاعتبار عند تخطيط برامج إطلاق ذبابة الهالوك·