Phytomyza orobanchia Kalt.as a biological control agent of Orobanche crenata Forsk, in Alexansria, Egypt

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Abstract

Background

The parasitic weed, *Orobanche crenata* Forsk (Orobanchaceae) is a noxious root parasite which seriously damage the production of many crops, particularly in the Leguminosae such as faba bean *Vicia faba*. Significant yield losses in faba bean fields can be attributed to its parasitism.

Results

The occurrence of *Phtomyza orobanchia* Kalt fly in faba bean fields naturally infested by the parasitic weed *Orobanche crenata* Forsk, was studied in two locations namely Abies and Nubaria farms during three successive faba bean seasons, 2020–2023 during, February, March and April (cool to warm temperature). The fly population was generally low in the 3 seasons in February, but increase to its maximum in late March in the 1st season and April in the 2nd and 3rd seasons. In Nubaria farm most larvae and puparia of *Ph.orobanchia* fly were found in fruits capsules with significantly lower proportions in plant base and stem parts. The fly preferred to attack the middle and upper fruits than lower ones. In Abies farm most of *Ph. orobanchia* flies were significantly found in plant base followed by plant capsules and the lowest proportion was in the plant stem. The Broomrapes are very prolific, producing thousands of tiny ripe Seeds/ plant. Thirty flowering Broomrape shoots produce 1,320 seed capsules. In one capsule, 4721.49 ripe seeds had been counted and an average of 207,247minute seeds can be produced per plant.

Conclusions

Infestation with *Phytomyza* fly is efficient in reducing *Orobanche* seed yield so has a great effect in the reduction of *Orobanche* seed bank. The work suggested that *Ph. orobanchia* could be a promising biological control agent of *Orobanche* spp. in Egypt, especially if resistance faba bean cultivar was grown and repeated field mass release techniques could be adopted.

Highlights

• The parasitic weed, was found parasitizing faba bean crop in Alexandria.

• Weekly variations of population dynamics of on spikes showed that, the fly population was generally low in the in February, but increase to its maximum in late March or April feeding only on weed.

• Most larvae and puparia of fly were found in fruits capsules or plant base of the weed.

• Infestation with is efficient in reducing seed yield and has a great effect in the reduction of seed bank.

Background

The parasitic weed species, *Orobanche crenata* Forsk (Orobanchaceae) is noxious root parasites which seriously constrain the production of many crops, including Leguminosae and Solanaceae families in Egypt (Parker & Riches 1993; Hegazi et al. 2024a). Various control methods have been tested for broomrape control, but none has proved entirely satisfactory (Saghir 1979; Saghir et al. 1980; Linke & Saxena 1991). The production of a large number of seeds (30,000-200,000 per plant) (Schroeder 1994), which remain viable in the soil for several years, and the intimate physiological interaction with their host plants limits the application of conventional weed control measures for *Orobanche* control.

Several records show that biological control of *Orobanche* has been successfully achieved in the field. The fly *Phytomyza orobanchia* Kalt (Diptera: Agromyzidae), occurs widely in Egypt, but it was too often killed by routine use of insecticides (Rezene Fessehaie & Parker 1992; Hegazi et al. 2024 b).

It has been shown that this insect provided excellent control of *Orobanche* spp. in the former USSR (Klyueva & Pamukchi 1983; Horvath & Wittmann 1988), Turkey (Giray & Nemli 1983), Syria (Linke et al. 1990; Linke 1992) and in India (Manjunath & Nagurkatti 1977).

Broomrape fly, *Ph. orobanchia* is oligophagous feeding only on *Orobanche* species. It feeds mainly on the immature seeds destroying between 11% and 90% of seeds (Sauerborn 1991). Also, it causes reduction in the length of shoots, number of capsules/ shoot. One larva can destroy all seeds in the small capsule of broompare, but the big capsule needs more than one larva for complete reduction of seeds. The natural capacity of *Ph. orobanchia* to reduce the *Orobanche* population is mainly limited by low temperatures, cultural practices (soil preparation, crop rotation, irrigation and the use of insecticides against crop pests) and natural enemies (micro- organisms, parasitoids and predators). The aim of the present study was to determine the efficiency of *Ph. orobanchia* as a biocontrol agent of *O. crenata* in faba bean (*Vicia faba* L.) fields in Alexandria, Egypt.

Methods

The occurrence of *Ph.orobanchia* fly in faba bean fields naturally infested by the parasitic weed *O.crenata*, was studied in two locations namely Abies and Nubaria farms during three successive faba bean seasons, 2020–2023 (February-April) .In both locations faba bean cultivar, Giza 843 was cultivated. The cultivar is known for its resistance to *O. crenata* infestation. The recommended agricultural practices for faba bean were applied. Notably, no fertilizers or chemical treatments were administered throughout the duration of the seasons. In each visit, thirty random samples per location and 3 reps. /Expt. were gently uprooted for investigation. Identification of *Ph. orobanchia* fly was carried out following Spencer (1973). All data of the present work was from broomrape plants at full flowering to early ripening stage. All *O.crenata* shoots. were collected randomly per Expt., with at least two meters between samples by walking diagonally across the fields and along the peripheries (Abbasher 1994).

Natural incidence and infestation rates with agromyzid fly, Ph. orobanchia

Variations of population dynamics of *Ph. orobanchia* during the three successive faba bean seasons (2020–2023) cultivated in Nubaria area 45 k west Alexandria were studied. Samples of *O.crenata* shoots at full flowering to early ripening stage were successively weekly collected from faba bean farms in the area and dissected. The number of flies in different parts of the spike (= capsules, stem and tubercle) was recorded weekly during February, March and April (cool to warm temperature). Density of *Ph. orobanchia* population on the parasitic weed was determined by counting larval and pupal stages following the dissection of the capsules and stems.

Distribution pattern of Ph.orobanchia fly in O.crenata spikes

In April of the first season, new samples of broomrapes were examined in faba bean (*Vicia faba* L.) farms of two locations namely Abies and Nubaria where the fruit capsules, stems and plant base of parasitic weed were dissected and number of *Ph.orobanchia* flies in each was recoded.

In Nubaria farm another set of parasitic weed of well-developed and almost of equal lengths were collected to study the effect fruit capsule level on infestation rate by *Ph.orobanchia* flies. All fruit capsules were detached from upper, middle and lower levels of the parasitic plant and checked for fly infestation, where percentage of insect infestation in fruits of each level was recorded.

Orobanche seed yield and its reduction due to Ph. orobanchia parasitism

Sets of well-developed Orobanchia spikes were collected from Nubaria faba bean farm in 2023 season to study the *Orobanche* seed yield and its reduction due to *Ph. orobanchia* parasitism. All capsules of the plant samples were separated and counted. Capsules were then air-dried. The fruit capsules were dissected and classified into healthy fruits containing no flies and other sets of fruits containing 1,2 and 3 or more fly pupae. /Capsule. The weight of seeds produced from healthy and *Phytomyza* infested capsules by one, two and three or more pupae/capsule were determined and used to calculate the *Orobanche* seed yield and its reduction due to *Phytomyza* fly. The ripe seeds in the fruits were then weighed on a microbalance of high accuracy (± 0.01 mg; Sartorius AG, Goettingen, Germany). Then weight and number of ripe seeds / healthy and infested fruits was recorded.

Climate in the farms

The daytime temperatures in February range from 17°C to 22°C (63°F to 72°F), while nighttime temperatures can drop to 8°C to 12°C (46°F to 54°F). Rainfall is generally minimal, though occasional temperatures typically range from 18°C to 25°C (64°F to 77°F), while nighttime temperatures can showers can occur, particularly in Alexandria. The temperature in March gradually warm up. Daytime vary from 10°C to 15°C (50°F to 59°F). remains relatively low, though occasional showers are possible, especially in coastal areas.In April, temperatures start to rise, with daytime highs ranging from 20°C to 30°C (68°F to 86°F). Nighttime temperatures are cooler, typically between 12°C and 18°C (54°F to 64°F). April is generally dry, with minimal rainfall across most sites.

Statistical analysis

Where appropriate, data were subjected to one-way analysis of variance (ANOVA) to determine differences between means. Some data were subjected statistically using T-test ($p > 0.05$). Data are presented as means of number ± SE.

Results

Occurrence of Ph. Orobanchia in faba bean fields

The soil of faba bean farms was heavily infested with *O. crenata*. Some of the infested faba bean plants by *O. crenata* in locations near sites treated with insecticides were *Ph. orobanchia* free. Weekly variations of population dynamics of *Ph. orobanchia* on *O. crenata* spikes during three successive faba bean seasons (2020–2023) cultivated in Nubaria area 45 k west Alexandria are tabulated in Table 1 and illustrated in Fig. 1. The data in Table (1) indicated that the mean number of *Ph. orobanchia* on *O. crenata* spikes in the 1st season (2020–2021) was low during the first week of February 6 (16.29 ± 1.17/ spike) reaching its significant peak ($F = 51.5$, df = 7,16, $P < 0.05$) on March 21 (42.16 ± 1.95/spike). On the 2nd and 3rd seasons the fly populations was generally lower than in the 1st season. The mean number of fly population was low in February 6 (6.56 ± o.96/spike) increased significantly ($F = 51.5$, df = 7,16, $P < 0.05$) in April 3 (17.7 ± 1.55/ spike), in the second season. In the 3rd season, the population was also low in February 15 (10.17 ± 0.12/spike), significantly increased ($F = 28.73$, df = 7,16, $P < 0.05$) in April 7 (26.78 ± 1.00/spike). Weekly occurrence of *Ph. orobanchia* flies on *O. crenata* spikes shows in Fig. 1.The fly population was generally low in the 3 seasons in February, but increase to its maximum in late March in the 1st season and April 3 in the 2nd and April 7 in the 3rd seasons. Up to 42.2,17.7 and 26.8 larvae and pupae have been counted in one shoot of these dates, respectively. So, the population differed from year to year.

Table 1
Ph. orobanchia fly population dynamics during three faba been seasons cultivated in Nubaria 45 Km far from Alexandria

Sampling date	Season		
	2020–2021	2021–2022	2022–2023
	Total insect no./ Plant		
6-Feb	$16.29^{e} \pm 1.17$	$6.56^{d} \pm 0.96$	$13.7^{c} \pm 0.23$
15-Feb	$16.29^{e} \pm 1.17$	$6.56^{d} \pm 0.96$	$10.17^{d} \pm 0.00$
22-Feb	$21.08^{d} \pm 1.53$	$8.75^{d} \pm 1.65$	$13.52^{c} \pm 0.13$
9-Mar	$28.25^{c} \pm 2.46$	$11.33^{c} \pm 0.6$	$14.81^{c} \pm 0.55$
16-Mar	$35.21^{b} \pm 1.27$	$13.73^{b} \pm 0.7$	$15.64^{c} \pm 0.60$
21-Mar	$42.16^{a} \pm 1.95$	$16.12^{a} \pm 0.8$	$18.15^{b} \pm 0.93$
3-Apr	$19.68^{d} \pm 0.92$	$17.7^{a} \pm 1.55$	$17.26^{c} \pm 4.19$
7-Apr	$19.68^{d} \pm 0.48$	$8.61^{d} \pm 0.42$	$26.78^{a} \pm 1.00$

When the letter per each column is the same, the data for separately season are not significantly different ($P < 0.05$)

Distribution pattern of Ph. orobanchia fly in O.crenata spikes

The eggs of *Ph. orobanchia* fly are deposited into fruit capsules or under the epidermis of the shoots. The larvae feed on the immature seeds and subepidermal tissue, sometimes penetrating into the shoots where they feed. Pupation takes place in the capsules but some in the stem or deeper in the hidden part of the stem base near the ground. In the 1st season (2020–2021), the distribution pattern of *Ph. orobanchia* fly in different parts of *O.crenata* spikes was studied at the same time (April) in the two locations of faba bean farms, namely Abies and Nubaria farms (Fig. 2). The results revealed that differences between the investigated fields were significant. The distance between the 2 farms was 45 km, however in Nubaria farm most larvae and puparia of *Ph. orobanchia* fly were found in fruits capsules with significantly lower proportions in plant base and stem parts ($F = 6703.96$, df = 2,6, $P < 0.05$). While in Abies farm most of *Ph. orobanchia* flies were significantly found in plant base followed by fruit capsules and the lowest proportion was in the plant stem ($F = 998.9$, df = 2,6, $P < 0.05$).

In Nubaria farm, in 2020–2021 season, the fruit capsules of *O.crenata* spikes samples were divided into 3 levels, upper, middle and lower ones to study the influence of capsule level on fly infestation rate. Fruits of each part of plant spike were examined for the presence of *Ph.orobanchia* flies (Fig. 3).Infestation

rates by the fly among these groups of fruits were significant (F = 18.41, df = 2,6, P < 0. 05). It seems that the fly prefers to attack the middle and upper fruits than lower ones.

C. Effect of parasitism by Ph. orobancia on Orobanche seed yield.

As shown in Table 2, two sizes of *Orobanche* fruit capsules were observed, small weighed 31.3 ± 2.97 mg and large ones weighed 94.9 ± 6.4 mg. The difference was significant ((t = 31.3, P < 0. 05). The ratio between the 2 sizes was almost 1:1. The average number of ripe seeds found/capsule was 2484.2 ± 341.8 and 6958.7 ± 493 of tiny seeds in small and large fruit capsules, respectively, The larvae of *Ph..orobanchia* mine the fruit capsules and feed on the immature seeds. Shrinkage and rottening of the fruit capsules suggest symptoms of fly infestation. Dissecting these fruits will show complete destruction of some or all seeds and accumulation of larval faeces. One seed capsule is generally sufficient for six larvae up to pupation. However, *Ph. orobanchia* fly infestation reduced *O. crenata* seeds by destroying 59.6, 91.36 and 100% of the seed capsules (Table 3) when the capsule attacked by 1,2, or 3 of *Ph. orobanchia* larvae, respectively. The weight of ripe seeds (size 0.3 × 0.2 mm) in the healthy capsule and those infested by 1,2 and 3 fly larvae was 24.85 ± 3., 8.85 ± 2.6,2.15 ± 0.9 and 0.0 mg, respectively. The difference was significant (F = 1347.1, df = 3, 36, P < 0. 05). In Nubara, The flowering Broomrape shoot (n = 30 × 3 reps.) infesting faba bean cultivar (Giza 843) produce 1,320 seed capsules. In one healthy capsule 4721.49 ripe seeds had been counted and an average of 207, 247minute seeds per plant.

Table 2
Weight (mg) and seed number in healthy air-dried capsule of *O. crenata* capsules

Capsule		Capsule Weight (mg)	Ripe Seeds/ Capsule	
			Weight (mg)	Number
Small	Range	19.7–54.1	7.1–26.3	1347.5–4991.3
	Mean	31.3 ± 2.97	13.9 ± 1.8	2484.2 ± 341.8
Large	Range	61.7–130.1	23.6–50.7	4478.9–9622.1
	Mean	94.9 ± 6.4	36.6 ± 2.6	6958.7 ± 493
Mean		63.1 ± 8.1	24.85 ± 3.1	4721.49 ± 590.48

Table 3
Effect Infestation level by *Ph. Orobanchia* fly larvae on reducing seed production in *O. crenata* capsules

Insect no./ Capsule		Capsule weight (mg)	Ripe Seeds/ Capsules		Seed reduction ٪
			weight (mg)	No.	
None	**Range**	19.7–130.1	7.1–50.7	1347.5–9622.1	0.0
	Average (± SE)	63.1 ± 8.1	24.85 ± 3.1a	4721.49 ± 590.5	
1	**Range**	7.9–54.4	0.0–35.6	0.0–6756.3	59.604
	Average (± SE)	29.93 ± 4.6	8.85 ± 2.6b	1907.3 ± 669.08	
2	**Range**	11.1–44.3	0.0–6.6	0.0–1252.57	91.36
	Average (± SE)	22.5 ± 3.5	2.15 ± 0.9 c	408.03 ± 171.6	
3 or more	**Range**	7.8–44.1	0.0–0.0	0.0–0.0	100
	Average (± SE)	28.1 ± 3.7	0.0 ± 0.0d	0.0	

For the weight of ripe seeds, when the letter per column is the same, the data for are not significantly different ($P < 0.05$)

Discussion

The occurrence of *Ph. orobanchia* in Egypt seems to be limited. The absence of *Ph. orobanchia* in some fields might be associated with the application of high rate of insecticides. This agrees with the observations of Parker & Riches (1993) and Schroeder (1994) who reported that the use of insecticides against crop pests can largely eliminate *Ph. orobanchia*. The fly population was generally low in the 3 seasons of the study in February, but increase to its maximum in late March in the 1st season and April in the 2nd and 3rd seasons. The eggs of *Ph.. orobanchia* fly are deposited into *Orobanche* shoots and significant number of fly larvae pupate in the fruit capsules. Up to 19–64 larvae and puparia have been counted in one sprout (n = 40 sprouts) in Yugoslavia (Mihajlović 1986). In Nubaria, Egypt, the number of larvae and puparia /plant ranged 6–42 fly. In Bulgaria, 100 flowering Broomrape sprouts produced 2,302 seed capsules, (Klein and Kroschel ,2002). In the present study, the flowering Broomrape shoots (n = 30 × 3 reps.) infesting faba bean cultivar (Giza 843) produce 1,320 seed capsules. In one capsule ,4721.49 ripe seeds had been counted and an average of 207,247minute seeds can be produced per plant. So the Broomrapes are very prolific, producing thousands of minute Seeds /plant that can be dispersed by field activities and wind. The fly effectively destroyed *O. crenata* capsules. The fly has been found to be efficient in reducing seed capsules of *Orbanche* pps. by 96% in Yugoslavia. The fly has shown effective

destruction of *O. ramosa* and *O. cernua* seed capsules by 81.4% and 71.7%,respectively under natural conditions at Malima (Ethiopia), (Rezene & Parker ,1992;Elzein et al. 1999).In Hungary and former USSR reduction in seed production per capsule of *O. cumana* on sunflower of 60–85% caused by *Ph. orobanchia*. Sauerborn, 1991 reported that one fly larva can destroy all seeds in the small capsule of broompare, but the big capsule needs more than one larva for complete reduction of seeds. In the present work, three of *Ph. orobanchia* larvae/capsule were able to destroy all immature seeds. Similar results have been reported in USSR (Okazova ,1973; Girling et al. (1979). Hence, the reduction of Orobanche seed production by *Phytomyza* fly prevents supplementary infestation and dissemination. Infestation with *Phytomyza* is efficient in reducing *Orobanche* seed yield and may have a great effect in the reduction of Orobanche seed bank. In this study, 65.5% of the larvae and pupae were found in stems of *O. crenata*. Significant number of *Ph. orobanchia* pupae remained in dried seed capsules and in stem base of *O. crenata* in Nubaria and Abies farms, could be subjected to predatory arthropods including soil ants (Hegazi et al. 2024b).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

The manuscript has not been published in completely or in part elsewhere.

Author Contribution

E. M. H. H. conceived the idea, wrote the first draft of the manuscript and general supervision of the research group, and was a major contributor in writing the manuscript. W. E. K., S. M. A., S. F. A. S., M. A. A., M. A. H., A. M. S., M. A. E. and M. M. A. F. collected the data and participated in its design and coordination and aided to draft the manuscript.

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Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

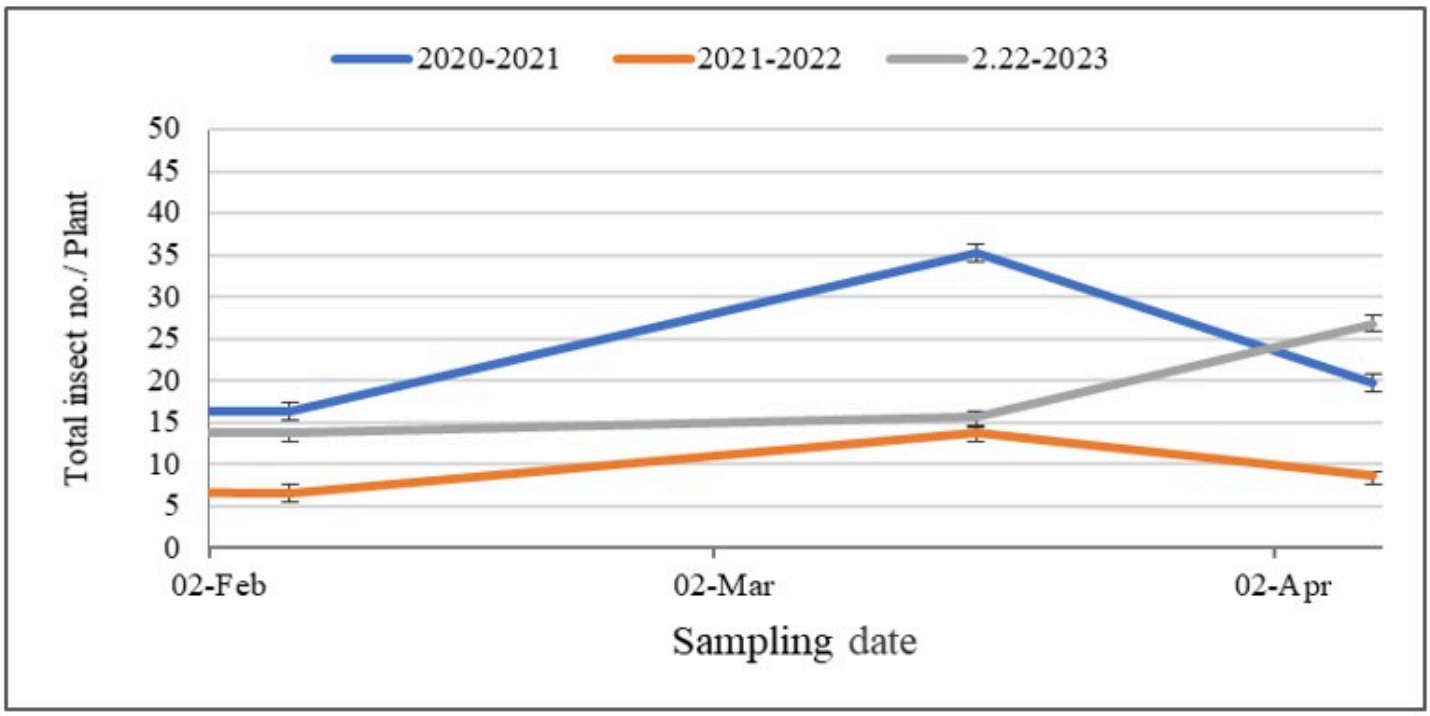

Figure 1

Ph. orobanchia fly population dynamics during three faba been seasons cultivated in Nubaria ,45 Km far from Alexandria

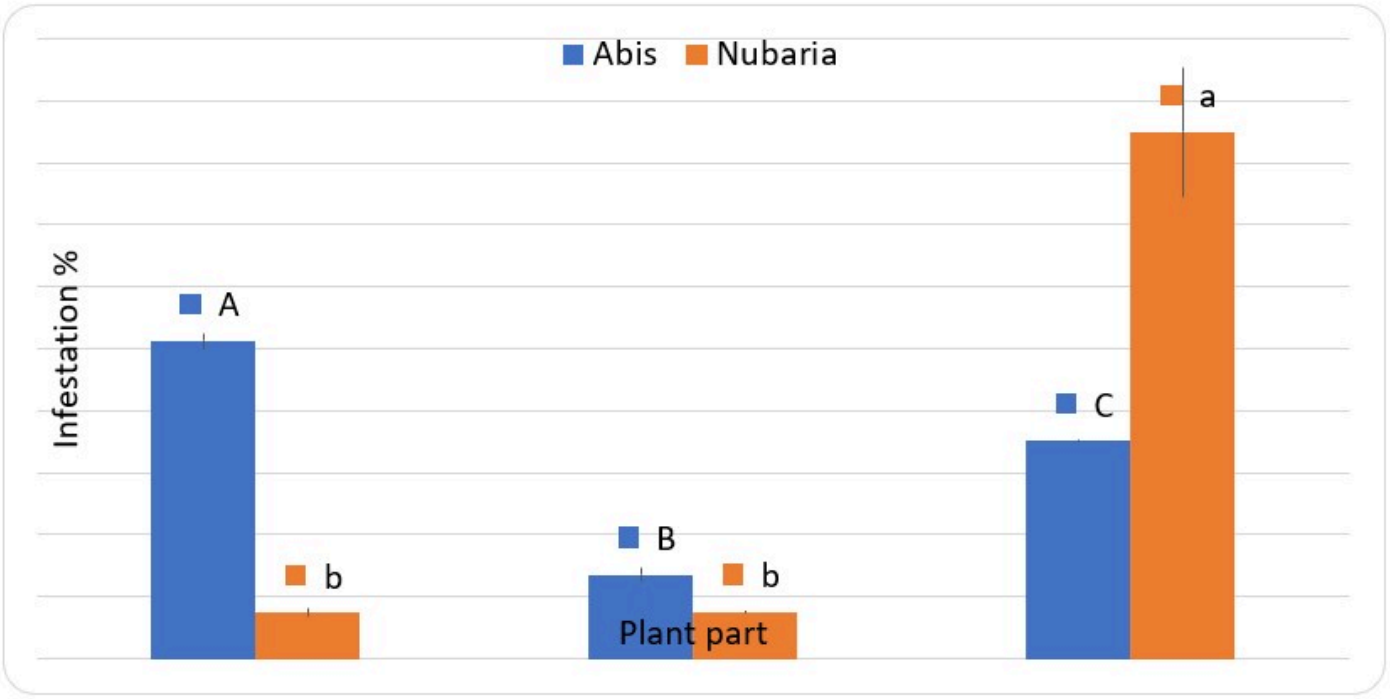

Figure 2

Distribution pattern *of Ph. orobanchia* on different parts of infested *O. crenata*, in 2020- 2021 season (Abies, Alexandria, and Nubaria farms). For each farm, bars with the same letter are not significantly different (P0.05)

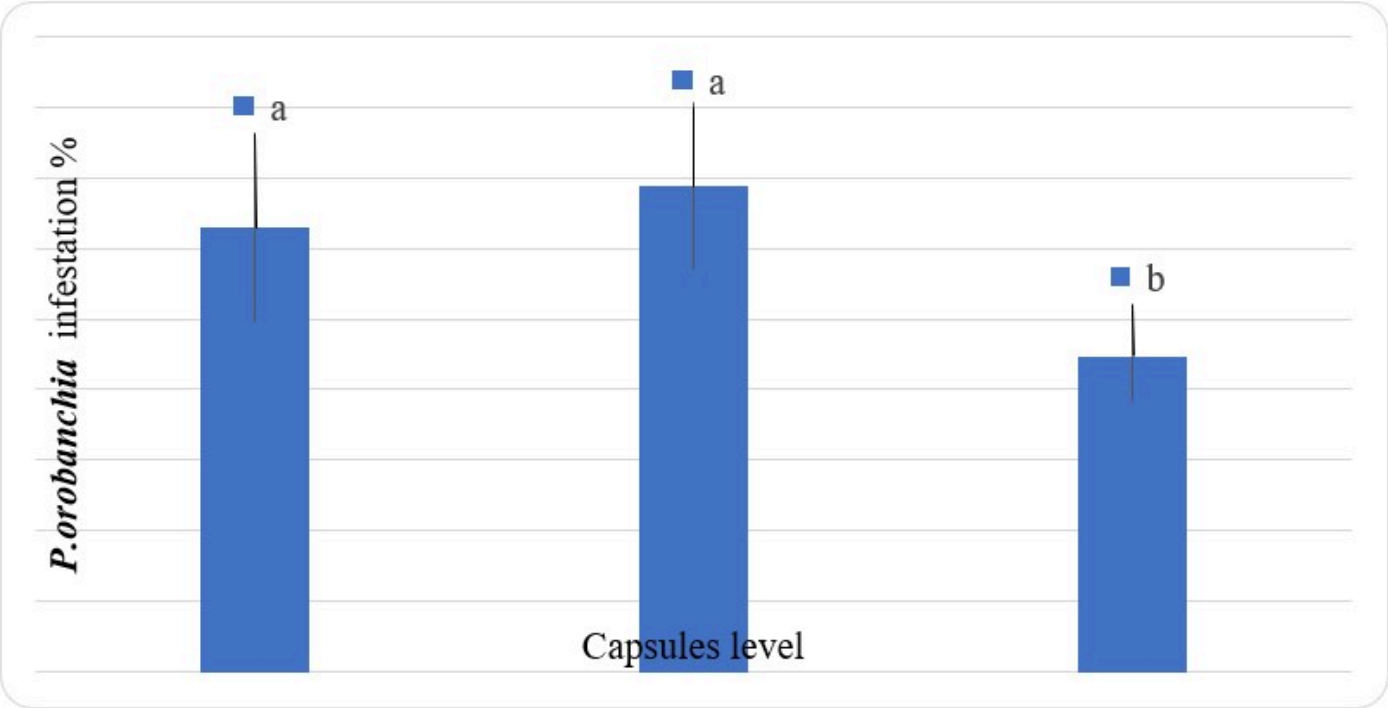

Figure 3

Influence of capsule level on infestation rate by *Ph. orobanchia* (2020-2021 season) in Nubaria farm. Bars with the same letter are not significantly different (P0.05)

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