Investigation of Efficiency of *Pytomyza orobanchia* Kaltenbach (Diptera: Agromyzidae) on *Orobanche aegyptiaca* Pers. and *O. ramosa* Linnaeus (Orobanchaceae) in Tomato Fields at Diyarbakır and Mardin Provinces

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This study was carried out to investigate efficiency of *Phytomyza orobanchia* Kaltenbach, 1864 (Diptera: Agromyzidae) on *Orobanche aegyptiaca* and *O. ramosa* parasitizing tomato crops under natural conditions, in Southeast Anatolia Region (Diyarbakır and Mardin provinces) in 2008. Trials were practiced using a randomized parcel design with five release ratios (0, 4, 6 and 8 pupae/cage and open field control) and with six replicate each. Highly significant differences were detected among treatments in both locations. The differences among treatments were ordered from the most effective ones to the least as; 8, 6 pupae/cage, open field control, 4 pupae/cage, and control lattices that without releasing pupae. The efficiency of *P. orobanchia* increased significantly by increasing the number of released *P. orobanchia* pupae, while there was no effect in control cages. Compared to pupae released cages open fields showed almost the same activity levels of *P. orobanchia* as that four pupae or sometime slightly higher than four pupae. Obtained data may provide valuable information for improving effective biological control program in the frame of integrated pest management of *Orobanche aegyptiaca* and *O. ramosa*.

Key words: Tomato, *Orobanche aegyptiaca, O. ramosa*, *Phytomyza orobanchia*, efficiency, biological control.

Diyarbakır ve Mardin İllerinde Domates alanlarında Canavarotu Türleri *Orobanche aegyptiaca* Pers. ve *O. ramosa* Linnaeus (Orobanchaceae) üzerinde *Pytomyza orobanchia* Kaltenbach (Diptera: Agromyzidae)'nın Etkinliğinin Araştırılması

Bu çalışma, *Orobanche aegyptiaca* ve *O. ramosa* canavar otu türleri üzerinde beslenen *Phytomyza orobanchia* Kaltenbach, 1864 (Diptera: Agromyzidae)'nın bu türler üzerinde etkinliğini araştırmak amacıyla Mardin ve Diyarbakır illerinde domates yetiştirilen alanlarda 2008 yılında yürütülmüştür. Denemeler tesadüf parselleri deneme desenine göre 5 karakter (0, 4, 6 ve 8 pupa salınmış kafesler ve açık alan kontrolleri) ve 6 tekerrür olarak kurulmuştur. Her iki lokasyonda da karakterler arasında çok önemli fark görülmüştür. Karakterler arasındaki fark etkinlik durumuna göre en yüksekten en düşüğe doğru; 8 pupa salınmış kafesler, 6 pupa salınmış kafesler, açık alan kontrolleri, 4 pupa salınmış kafesler ve pupa salımı yapılmamış kafesler olarak sıralanmıştır. Pupa salımı arttıkça *P. orobanchia* etkinliği önemli ölçüde artarken, pupa salınmamış kafesler de etkinlik hiç görülmemiştir. Açık alanda *P. orobanchia* etkinliği ile kafes altındaki etkinlik karşılaştırıldığında açık alan etkinliğinin 4 pupa salımı yapılmış kafeslerden biraz daha etkin olduğu saptanmıştır. Elde edilen veriler, *O. aegyptiaca* ve *O. ramosa*'nın kontrol altına alınması için entegre mücadele çerçevesinde biyolojik mücadele programının geliştirilmesinde kıymetli bilgiler sağlayabilir.

Anahtar Kelimeler: Domates, *Orobanche aegyptiaca, O. ramosa*, *Phytomyza orobanchia*, etkinlik, biyolojik Mücadele.

Introduction

Tomato is one of the most cultivated productions in Turkey where it is the fourth country in the world after China, India and USA with 11 820 000 tons production (Anonymous, 2013). Tomato could be grown in so many regions of the country including Southeast Anatolia region. In Southeast Anatolia Region, tomato has been suffered from parasitic weeds and some other plant protection problems. The parasitic weeds seriously affect many crops causing significant yield losses. The most common parasitic weed species that cause problem in Turkey are; *Orobanche* spp., and *Cuscuta* spp. Percentage of damage causing by

these two parasitic weeds ranged between 30 -70 % (Aksoy et al. 2004). *Orobanche* species are notorious parasitic weeds, feeding on plant through roots of many wild and cultivated plants including tomato, lentil, faba bean, green bean and many other crops in Turkey, Egypt and Mediterranean Countries (Salem and Abd El-Sala 2013). In Southeast Anatolia region, there is a complex of *O*. *ramosa* and *O*. *aegyptiaca* species in tomato growing fields (Bayram and Çıkman 2009). There is no practical use of chemical control possibility in tomato fields against broomrape; it could be controlled partly by taking cultural measures (flooding) and mechanical control (hand-picking) methods. Recently, studies against parasitic plants generally have been focused on possibilities for using biological control and breeding of resistant cultivars. Fortunately, bio-control agent, *P. orobanchia* was found associated with broomrapes in tomato growing fields of the region. In Southeast Anatolia region, *P. orobanchia* has four generations annually, the 1st generation emerges from overwintered pupae, 2nd generation lives in broomrape of lentil fields, while 3rd and 4th generations live in broomrapes that parasitize summer growing plants, especially tomato cultivars, and then it goes to diapause as pupae in winter in broomrape remains (Bayram and Çıkman, 2014). In tomato fields at Diyarbakır province, the infestation and density of *P. orobanchia* on broomrape species were ranged between 5-95% (Bayram and Çıkman, 2009). Biological control of broomrape by using the dipteran *P. orobanchia* has been shown as promising in Eastern Europe, the former USSR and Mediterranean basin and it was introduced as the most effective bio-agent (Al-Eryan et al., 2004). The presented study aimed to evaluate efficiency of *P. orobanchia* under natural conditions in tomato growing fields at Mardin and Diyarbakır provinces.

Materials and Methods

Stock Culture of *Phytomyza orobanchia* Pupae

Stock culture of *P. orobanchia* pupae was collected from *Orobanche* stems and capsules of lentil fields where vegetation begins more early than tomato. The pupae inside broomrapes and those collected from stems and capsules were placed into plastic boxes and preserved under climatic room conditions. Studies concerning the broomrape fly efficiency were carried out in tomato cultivated fields under natural conditions at two provinces (Diyarbakır and Mardin) in Turkey in 2008.

Working Sites

Trials were carried out at Mardin province, in Kızıltepe district on July 3rd 2008 and at Diyarbakır province in Ergani district on June 16th 2008, when new broomrape emerged in tomato fields. Mardin and Diyarbakır trials lasted till July 30th and 29th 2008 harvesting time, respectively. Samples of broomrapes were collected at the beginning and the end of the trials, for estimating natural efficiency of *P. orobanchia* in two districts. As broomrape species of tomato fields in the southeast Anatolia Region was determined as a complex of *O. ramosa* and *O. aegyptiaca* (Bayram and Çıkman 2009) including İn Çukurova Region (Aksoy et al. 2004), when trials were established broomrape species was regarded as the same and according to the size of lattices newly emerged 1-2 broomrapes were covered properly and *P. orobanchia* pupae were released into lattices.

Methods

Trials were designed as a randomized parcel design, consisted of five treatments (without *P. orobanchia* pupae, 4, 6 and 8 pupae / lattices (cages) released and open field control) with six replications for each treatment. Number of releasing pupae/ treatment was the same as per lattices, regardless to the number of broomrape. However, as the size of lattices was small (40x40x50cm), broomrapes were given the opportunity to grow properly inside lattices, so the place of lattices embed on generally was preferred as one or two broomrapes maximally. Newly emerged fleshy broomrapes were covered with lattices (40x40x50 cm) covered with ventilated cloth properly and *P. orobanchia* pupae were released inside these lattices according to trial design.

After removal of cages broomrape spikes of each cage were collected and separately placed into transparent polyethylene bags. Tag information of cages was written on them, and brought to the laboratory. In order to evaluate efficiency of *P. orobanchia*, capsules and stems (spikes), the tubercles of broomrapes were examined. Also, capsules of each broomrape plant and number of pupae inside it were counted to estimate the ratio of the number of pupae per broomrape plant. The

number of pupae in stems, total number of pupae for each broomrape plant and percentage of pupae in capsules were calculated. As some data had zero, square root transformation was applied to give more reliable results for the analysis of the classifications, for determining the variation coefficients and the level of significance. Accordingly, data of tomato fields at both provinces combined and then combined analysis of the both locations were constituted.

Statistical Analysis

Data of the effect of releasing different numbers of *P. orobanchia* pupae on broomrape were analyzed using JMP (statistic software) program (Anonymous, 2007). A one-way analysis of variance (ANOVA) was conducted for statistical comparison among means of developed *P. orobanchia*. Classification of differences between releases ratios were determined by Least Significant Differences (LSD) at probability level of $P \leq 0.05$. Statistical comparison of an average of female fecundity of *P. orobanchia* was calculated.

Results

Determination of Efficiency of *Phytomyza orobanchia* on *Orobanche aegyptiaca* and *O. ramosa*

For evaluation of *P.orobanchia* efficiency, total number of pupae and percentage of pupae in capsules were used as the most important parameters. Efficiency of *P.orobanchia* on controlling broomrape in tomato fields at Mardin and Diyarbakır provinces was given in table (1). In terms of all examined parameters; (№ of pupae in capsules, № of pupae in stems, № of total pupae and the percentage of pupae in capsules); 8 pupae released cages manifested the highest efficiency, while lattices without pupae were found the least. The 8 pupae released lattices showed nearly 70% effectiveness, followed by 6 pupae (50%), open fields (23%) and 4 pupae (17%), while without release showed no activity at Mardin location. Correspondent percentages were 22, 4, 16 and 9% respectively, at Diyarbakır location. The highest effectiveness of *P. orobanchia* was found at Mardin location as 70%, while at Diyarbakır location only 22%.

According to data of all parameters and combined variance analysis at both locations, the percentages of effectiveness of different releasing rates were given in table (2). Compared to replications; there wasn't any difference among replications. Compared to locations; pupae in capsules was found significant (at 5%), pupae in stems, total pupae and the ratio of pupae in capsules were found highly significant (at 1%). Compared to the characters; there was no any difference between the characters in terms of pupa in stems, while pupae in capsules, total pupae and the ratio of pupae in capsules were found highly significant (at 1%). Compared to interaction among releases and locations; there wasn't any difference in terms of pupae in stems and tubercles and the number of total pupae, while pupae in capsules was found significant (at 5%), and the ratio of pupae in capsules was found highly significant (at 1%) table (2).

There was significantly difference between two locations (Mardin and Diyarbakır) in terms of all parameters (Table 3). At Mardin province, the efficiency of *P. orobanchia* was found higher than Diyarbakır province.

Table 1. Average of *Phytomyza orobanchia* efficiency on broomrape in tomato lattices that received releasing of pupae in open fields of Mardin and Diyarbakır provinces.

Provinces	Trials	Number of Capsules	Number of Pupae in Capsules	Number of Pupae in Stems	Number of Total Pupae	Percentage of Pupae in Capsules
Mardin	8 Pupae	89.40	61.00	8.67	69.67	68.23
	6 Pupae	90.24	45.33	4.50	49.83	50.23
	4 Pupae	72.44	12.17	5.17	17.33	16.80
	Without Pupae	119.39	1.17	1.33	2.50	0.98
	Open Field	59.00	13.67	2.17	17.50	23.17
Diyarbakır	8 Pupae	122.68	26.50	0.17	25.67	21.60
	6 Pupae	37.69	1.50	0	1.50	3.98
	4 Pupae	282.91	24.67	0	24.67	8.72
	Without Pupae	83.64	0	0	0.00	0
	Open Field	143.92	23.33	2.67	26.00	16.21

Table 2. Combined variance analysis of both locations

Variance Sources	S.D.	Means of Square			
		Number of Pupae in Capsules	Number of Pupae in Stems	Number of Total Pupae	Percentage of Pupae in Capsules
Locations	1	23.28*	15.78**	39.66**	79.36**
Replications	10	3.67	0.51	3.89	1.16
Characters	4	46.66**	1.89	47.23**	54.79**
Characters x Locations	4	17.69*	2.50	19.09	15.55**
Error	40	6.40	1.12	7.61	3.53
General	59				
CV (%)		74.70	97.54	77.23	53.15

** p<0.01 highly significant, * p<0.05 significant

Insignificant difference between the numbers of pupae in stems was recorded for all characters, while the difference of the number of pupae in capsules, the number of total pupae and the percentage of pupae in capsules were recorded as significant. Compared to the characters; in terms of the number of pupae in capsules, the number of total pupae and the percentage of pupae in capsules 8 pupae released were ordered as “a”, 6 pupae, open field and 4 pupae released shared the same group as “b”, while control lattices that without pupae were categorized in another group as “c” (Table 4). According to averages of both locations in terms of all examined parameters, treatments were ordered from the most effective to the least as; 8 and 6 pupae released lattices, open field control, 4 pupae released lattices and lattices that without pupae, respectively.

Although the activity of *P. orobanchia* was changed in terms of all parameters such as the number of pupae in stems, the number of pupae in capsules, total number of pupae and the percentage of pupae in capsules it was also changed in each location (Mardin and Diyarbakır provinces), but it could be said that the activity of *P. orobanchia* in open field was lower than that of 6 pupae released lattices and it was slightly higher than four pupae released lattices.

The activity of *P. orobanchia* in open tomato fields come across 4 pupae released level or a little bit higher than 4 pupae. There was a strong relationship between the released numbers of pupae in cages and obtained number of pupae afterwards, also, there was a strong relationship between broomrape and host plant as well. The difference of *P. orobanchia* efficiency in both locations among treatments was determined as highly significant. The activity of *P. orobanchia* in Diyarbakır location has been observed lower than Mardin location.

Table 3. Classification of both locations

Provinces	No. of Pupae in Capsules	No. of Pupae in Stems	No. of Total Pupae	Percentage of Pupae in Capsules
Mardin	26. 67 a	4.37 a	31.37 a	31.88 a
Diyarbakır	15.20 b	0.57 b	15.57 b	10.05 b
LSD $_{0.05}$	1.10	0.41	1.13	0.62

Means within a row sharing the same letter are not significantly different (P ≤ 0.05, LSD multiple range test).

Table 4. Classification of the characters

Characters	No. of Pupae in Capsules	No. of Pupae in Stems	No. of Total Pupae	Percentage of Pupae in Capsules
8 Pupae	43.75 a	4.42	47.67 a	44.87 a
6 Pupae	23.42 b	2.25	25.67 ab	27.10 b
4 Pupae	18.42 b	2.58	21.00 b	12.71 b
Open Field	18.50 b	2.42	21.75 b	19.64 b
Without Pupae	0.58 c	0.67	1.25 c	0.49 c
LSD $_{0.05}$	2.09	-	2.28	1.70

Means within a row sharing the same letter are not significantly different (P ≤ 0.05, LSD multiple range test).

Discussion

According to the analysis of both tomato growing fields; there is strong relationship between released the number of pupae in lattices and obtained the number of pupae from released pupae, and also there is a strong relationship between broomrape and host plant as well. Considering the difference between treatments from the most effective ones to the least ones trials were ordered as 8 pupae, 6 pupae, open field control, 4 pupae, and control lattices that without pupae. The efficiency of *P. orobanchia* was observed in all lattices that pupae released. The least activity of *P. orobanchia* observed in control lattices that without pupae. The broomrape samples collected from the same tomato fields both at the beginning and at the end of trials for comparing field condition to pupae released lattices. The activity level of *P. orobanchia* in open field samples was equivalent or a little bit more than four pupae released lattices. Even effectiveness of *P. orobanchia* in tomato fields is the same as four pupae or more, the broomrapes of tomato fields of the Southeast Anatolia Region has a complex of *O*. *ramosa* and *O*. *aegyptiaca* (Bayram and Çıkman 2009), they have ability to produce so many stems, capsules and seeds. Only one tubercle of broomrape could produce 30-50 stems and just 5-6 broomrape tubercles could have over 200 stems/m², it means highly extreme density. In such a situation, *P*. *orobanchia* may not alone enough to control broomrape.

If the amount of broomrape in the field is higher, the activity of *P. orobanchia* on per broomrape will be lower, but if the amount of broomrape lower or broomrape has less capsules and stems, the activity of *P. orobanchia* on per broomrape will be higher. It is completely depend on distribution of host and parasitoid. It could be said that there is a negative coloration between host and parasitoid. If infestation level of broomrape is minimal or moderate; it is enough to control broomrape by *P. orobanchia*. However, considering broomrape seeds live in the soil for many years and could produce millions of seeds, releasing of *P. orobanchia* individuals should be made periodically for many years and in further infested fields, additional control methods should be used.

P. orobanchia life cycle depends on fresh broomrape parts, so which part of broomrape has more suitable water and food, females laying their eggs inside it. At the beginning of season, *P. orobanchia* prefers newly emerged broomrape stems, when capsules occur and stems getting harder, it prefers capsules, when broomrape getting older and dryer then newly emerged *P. orobanchia* larva moves from the stem towards tubercle to pupate inside and remain until the next year generation. This cycle always continue to year by year, or from one host plant to another (Bayram and Çıkman, 2016).

Except for adult stage of *P. orobanchia*, it is impossible to reproduce *P. orobanchia* without broomrape by manual ways or different methods. Moreover, the preservation temperature did not affect fecundity of *P. orobanchia* and subsequently had no more effect on its beneficial role for biological control (Abu-Shall, Amany, 2012). However, the efficiency of *P. orobanchia* under natural conditions is limited at low temperatures, cultural practices and natural enemies. The efficiency of *P. orobanchia* could be increased only by positive interventions, so in order to strengthen the natural population and efficiency of *P. orobanchia*, at the beginning of broomrape emergence, inundative releases of adults should be considered (Klein and Kroschel 2002). Additional releasing of 500-1000 *P. orobanchia* adult individuals per hectare caused a decrease of 96% of broomrape seeds. However, due to 10-15 years longevity of broomrape seeds, further infestations will occur in the following cropping seasons. Therefore, in order to minimize broomrape infestation, releasing of *P. orobanchia* should be repeated for at least 3-4 years (Kroschel and Klein 1999). At the beginning of spring, additional release of *P. orobanchia* adults will increase the activity of all next generations. Considering each female individual lays 18-200 eggs (Tawfik et al. 1976) and *P. orobanchia* could complete 4 generations in a year (Bayram and Çıkman, 2014; Jafarzadeh and Pourmirza, 1999).

If the subterranean parts of broomrape (in which *P. orobanchia* pupae live inside) could be collected from tomato fields and protected properly in the autumn and winter period and emerged adult could be released in the subsequent spring, this effort would give an additional support to control broomrape in lentil fields and in summer growing plants such as tomato, sunflower and tobacco (Bayram and Çıkman, 2016). Thus, by collecting broomrape from tomato fields the damage of broomrape and subsequently the broomrape seed bank in the soil would be decreased, and by

releasing newly emerged adults, effectiveness of *P. orobanchia* would be increased in four generations. Therefore, broomrapes should be collected away from the fields before they spill their seeds in the soil and should be protected into a "Phytomyzarium" or in a suitable enclosed place in the winter. The benefit of this effort may not be seen directly in the same year as biological control. However, considering the long-term and comprehensive control method there is no doubt that the quality, quantity and amount of tomato production will be improved and density of broomrape will be decreased year by year (Bayram and Çıkman, 2016).

According to a survey study carried out by Aksoy et al., (2004), in Çukurova region, *P. orobanchia* was only found in lentil fields with a very low population, while couldn't find in bean and tomato fields. On the contrary, in Southeastern Anatolia region, especially in Mardin and Diyarbakır provinces, both lentil and tomato fields showed very high density of *P. orobanchia* that caused over 95% infestation to broomrape (Bayram and Çıkman 2014). The existence and efficiency of *P. orobanchia* in both locations are a source of sight for any subsequent biological control studies. Therefore, protection of *P. orobanchia* population in Southeastern Anatolia region is necessary (Bayram and Çıkman 2016).

P. orobanchia is a hopeful agent for controlling broomrape and for integrating with other control methods. Future studies should focus on protecting the last generation of *P. orobanchia* in broomrape remains during autumn and winter then releasing adults at the beginning of next season in the subsequent spring. The awareness of farmers should be raised about the beneficial role of this biological control agent and about the seriousness of the heavy treatments by pesticides.

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