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Biology of *Acanthiophilus helianthi* Rossi (Diptera: Tephritidae): A Safflower Pest of Southern Iran

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ABSTRACT: The safflower fly is one of the most important pests of safflower in Iran. Losses caused by larval feeding leads to disrupted plant activities, reduction in flower buds, and, ultimately, to decreased quality and quantity of crop. This study was conducted on *Acanthiophilus helianthi* Rossi (Diptera: Tephritidae) at the Gachsaran Agricultural Research Station in Iran from November 2008 to July 2009. Sampling was performed weekly using a two-stage cluster sampling method. For the life cycle studies, the infected flower heads were collected from an experimental field plot and were developed in cages (160×160×100 cm) from egg to adult under laboratory conditions(27±2℃; relative humidity, 60%; and 16/8 L:D).The results showed that the first adults emerged gradually in mid April 2009. Female *A.helianthi* had a pre-oviposition period of 5.8 ± 1.0 days and the average fecundity was 27 ± 3.2 eggs. The Eggs were laid in the bracts of flower heads singly or in clusters of 3-18. The Incubation period was 3.8 ± 0.6 days under field conditions and 3.4 ± 0.6 days under cage conditions. The egg dimensions were 1.19 × 0.19 mm. Three larval instars occurred, and the larval phase was 7-10 days. The mean body dimensions (L × W) of larvae were 4.79 × 1.71mm. Pupa were coarctate and become sluggish before last molt. The pupal period was 7.5 days (range,6-9 days). The mean pupal dimensions of male and female cocoons were 3.95 × 1.42 and 4.55 × 2.27 mm, respectively. The flies spent their entire lifespan from egg to adult inside the flower heads of safflower plants. Males emerged earlier than females, but the longevity of the adult females (12 ± 3.0) was significantly greater than that of males (8 ± 1.0). Analysis of aggregated male and female sampling data showed that the sex ratio was 1:1.28.

Key words: *Acanthiophilus helianthi*, Safflower fly, Biology, Safflower, Iran

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is an important oilseed crop and an essential component of cropping systems in the dry regions and marginal areas of the world (Sabzalian et al. 2008). Like other crops, safflower suffers from various diseases and insects (Weiss 2000). The most serious safflower pest in Asia and Europe is the safflower fly *Acanthiophilus helianthi* Rossi (Tephritidae), and sometimes known as the shoot fly or capsule fly (Talpur et al. 1995; Zandigiacomo and Iob 1991). In Asia, the safflower fly devastates most production areas in Iraq (Al-Ali et al. 1977), Pakistan (Talpur et al. 1995), and India (Vaishampayan and Kapoor 1970; Verma et al. 1974). In Iran, seed-yield loss due to the safflower fly is estimated to be 30-70% for different safflower cultivars (Sabzalian et al. 2010).

The safflower fly is a polyphagous insect belonging to the Tephritidae family (Ashri 1971). Adult flies lay eggs on the inner side of involucral bracts of safflower green heads (Narayanan 1961; Ashri and Knowles 1960). Heavy infestations of safflower fly occurs during the reproductive phase of the plant, and the fly prefers to lay its eggs inside developing heads throughout the flowering stage (Talpur et al. 1995). Larvae hatch from eggs, penetrate the head bracts, and feed on receptacle tissue or the whole seed (Faure et al. 2004; Jkhmola and Yadav 1980; Narayanan 1961; Ricci and Ciriciofolo 1983). Larval feeding on seeds causes significant losses in seed weight, yield, and seed marketability (Ashri 1971).

A large number of plants including weeds are alternate hosts of *A. helianthi*, and some are used by this pest for both feeding and reproduction. *Chenopodium virgata, Polygonum aviculare, Salsola kali, Acroptilon repens, Carthamus oxyacantha, Cuscuta campestris* and *Convolvolus arvensis* serve as alternative host plants for *A. helianthi* (Hegazi and Moursi 1983; Selim 1977; Singh et al. 1982).

The increasing impact of *A. helianthi* has elicited concern among entomologists who are looking for pest management options. The biology and behavior of *A. helianthi* has been described by some entomologists in various parts of Iraq (Al-Ali et al. 1977), Pakistan (Rahoo et al. 1997), India (Verma et al. 1974), and Egypt

(Hegazi and Moursi 1983). However, little information is available on the biology of this pest in the dry zone of Iran (Bagheri 2007), and no information is available for Gachsaran, Iran. Therefore, the main objectives of this study were to elucidate the biological, behavioral, and morphological aspects of *A. helianthi* in the Gachsaran zone of Iran. Results presented here may be helpful for future planning of large-scale safflower cultivation in similar environmental conditions of the tropics, especially for pest management purposes.

MATERIALS AND METHODS

Experimental plot

Studies were conducted on a 50×50 m safflower plot located within the premises of the Agricultural Research Station in Gachsaran (8° 35´ N latitude and 89° 51´ longitudes) in southern Iran during November 2008 to July 2009. The seed of the safflower variety “Sina” obtained from the oilseeds division of the research institute were planted within the experimental plot, following standard agricultural practices. Approximately 2500 plants were in the experimental plot. Plants were fertilized with NPK fertilizer once every 3 months, and watering was performed when necessary. No insecticides, herbicides, or fungicides were used.

Experimental insects

A. helianthi began to attack the plants 5 to 6 months after planting (during the formation of flower heads). Field and laboratory experiments were started after *A. helianthi* became available in the experimental plot.

Experimental protocol

Rearing studies

A.helianthi was reared under controlled conditions in cages at the Gachsaran Agricultural Research Station, to determine the oviposition, fecundity, longevity and for developmental experiments. Adult *A.helianthi* were collected from the experimental plot, reared outdoors in wooden framed cages (160 × 160 × 100 cm), and covered with organdy cloth. An opening of 100 ×70 cm was made in the organdy cloth cover on one side of each cage for the safflower plants and the insects.

Safflower plants of approximately 130 cm in height and grown in polythene soil containers (80 cm diameter and 60 cm high) were placed separately inside each cage. The egg clusters collected from the safflower plot together with parts of the receptacles on which they were found were stapled on-to flower heads of potted plants without disturbing the eggs. Egg clusters were examined daily until the eggs hatched.

Life stage studies

Upon hatching, the first instar larvae from each egg cluster were transferred to a potted plant placed inside another cage. These larvae were left undisturbed to feed, molt, and metamorphose into pupa and adults. Adults were carefully observed and sexed using morphological features. The pre-oviposition, oviposition, and post-oviposition periods were studied under laboratory conditions. Adult male and female insects were collected from the rearing cages within 24-h. of the last molt. Batches of the three males and one female each were placed separately in 20 rearing jars (90 cm internal diameter × 70 cm high). A 20-30 cm long piece of safflower flower head was placed inside each jar, which provided nourishment and surfaces on which the insects could rest and oviposit. A 5-cm thick layer of plaster of paris was laid at the bottom of each jar to provide sufficient moisture to prevent the safflower flower head from wilting. The mouth of each jar was covered with muslin cloth to allow aeration. Insects in the rearing jars were monitored daily until they died. Pre-oviposition, oviposition and post-oviposition periods were recorded.

Twenty pairs of newly emerged adults collected from the rearing cages were placed inside a new rearing cage containing a potted safflower plant. The insects were allowed to mate and oviposit. The number of egg clusters produced each day by the 20 females was recorded. In a separate experiment, newly emerged adults males (n = 20) and females (n = 20) were collected from the rearing cage and placed separately, with a 20-30 cm long piece of safflower flower head, in the rearing jars described above. Insects in the rearing jars were monitored daily to determine adult longevity until all of the insects died. The incubation period and egg viability were studied both in the laboratory and field. Newly laid egg clusters were randomly selected from the rearing cages (n = 20) and the field (n = 20) and observed daily until hatching. The number of eggs in each cluster was recorded. The number of unhatched eggs in each cluster was recorded after the incubation period. The incubation period and egg viability inside the cage and under field conditions were compared using a *t* – test. Larvae were examined daily and the number of instars was determined by molts and by measuring head capsule size.

Morphological studies

Fifty infected flower heads were collected from the experimental plot and brought into the laboratory. In the laboratory, each flower head was opened, and the eggs were, transferred individually to a glass slide. The length and width of 100 randomly selected eggs were measured under a light microscope fitted with a micrometer eyepiece. Then, the length and width of first, second, and third instar larvae were measured in the same manner; the pupae and adults were measured using a pair of dividers and a millimeter scale. The morphological features of the eggs, larvae, pupae, and adults were examined under a stereomicroscope (×25).

Field studies

Sex ratio, mating, and oviposition behavior of *A. helianthi* were studied under field conditions. To determine the sex ratio, adult *A. helianthi* captured in a sweeping net were sexed and counted once per week. We could distinguish adult males from females using morphological differences in the abdominal tips. The sex ratio of adults was determined using the chi-square test.

Preliminary observations of mating and egg laying behavior were conducted in the field. Focal animal sampling (Martin and Bateson 1986) was chosen (observing one individual until the end of the desired behavior) and duration of the behavior was recorded (n = 50).

Measurement of physical environmental factors

Daily maximum and minimum temperature within the experimental plot were measured using four maximum and minimum thermometers. Relative humidity (RH) was measured using a thermo-hydrograph.

RESULTS AND DISCUSSION

A. helianthi was established throughout the study period, as insecticides, herbicides, or fungicides were not used. Subsequently, *A. helianthi* found in the experimental plot were identified by comparing their morphological characters with voucher specimens from the Insect Taxonomy Research Institute of Iran. The adult *A. helianthi*, found in Gachsaran was a grey or slightly green colored, medium sized fly. Sexes differed in size,; the female has an average length of 5.2 ± 0.7 mm, whereas the male was slightly smaller with an average length of 4.7 ± 0.5 mm. The female also had a characteristic spear like ovipositor at abdominal tip. Adults were relatively inactive during the early morning, evening, and night, and typically remained on the lower surface of leaves. During the day (8.0 h to 18.0 h) adults became more active and were found on both the upper and lower surfaces of flower head bracts. Newly emerged adult females were ready to mate 2 days after emergence from the pupa. Males and females began to copulate about 1 day after exit from the pupae, and mating occurred usually during the day. Males and females mated multiple times, usually with different partners, and each mating episode lasted 1-2 h. Females typically mated multiple times during a three days period before starting to oviposit. Mating continued throughout the oviposition period.

Table1. Duration of various *A. helianthi* life parameters

	Duration(in days)		
Life history parameter	minimum	maximum	Average (±SE)
Pre-oviposition period	4	8	5.8±1.0
Oviposition period	8	14	11.0±1.2
Post-oviposition period	3	7	6.0±2.0
Male longevity	6	11	8.0±1.0
Female longevity	9	15	12.0±3.0
Incubation period (in field)	2	7	3.8±0.6
Incubation period (in lab)	2	6	3.4±0.6
First instar larvae	2	3	2.5±0.1
Second instar larvae	2	3	2.6±0.1
Third instar larvae	3	4	3.6±0.2
Pupae period	6	9	7.5±0.0

A female produced 2- 4 egg clusters during her lifespan with an average of 2.8 ± 1.0. The number of eggs in a cluster obtained from the rearing cage ranged from 4-18 with a mean of 10 ± 2.0, whereas egg clusters obtained from the experimental plot ranged from 5-20 eggs with mean of 11 ± 2.1. The difference in means between eggs in a cluster laid in rearing cages and in the experimental plot was not statistically significant. The total number of eggs laid by a female during her lifetime ranged from 10-37 with a mean of 27 ± 3.2.

Rahoo et al. (1997) stated that the mean number of eggs in an *A. helianthi* egg cluster in Pakistan (in the field) was 10. The mean number of eggs in a cluster at Gachsaran (in the field) was 11.0 ± 2.1. Despite the differences in climatic conditions between Pakistan and Iran the mean number of eggs in a cluster in both

places was approximately the same; indicating that the number of eggs in a cluster is an inherent trait unaffected by climatic differences.

Under laboratory conditions, the incubation period ranged from 2-6 days with a mean of 3.4 ± 0.6 days, whereas under field conditions it ranged from days with a mean of 3.8 ± 0.6 days (Table 1). The difference between the incubation period under laboratory and field conditions was not significantly different. Egg viability recorded from egg clusters collected from the rearing cages was 81.85%, whereas that of egg clusters collected from the experimental plot was 83.28%. No significant difference between the viability of eggs laid in rearing cages and in the experimental plot was observed.

Longevity of the adult females was significantly greater (*t*-test; $p<0.01$) than that of the males; females lived for 9-15 days with a mean of 12 ± 3.0 days, whereas the longevity range of the males was 6-11 days with a mean of 8 ± 1.0 days (Table 1). Egg viability appears to be affected by ambient RH, especially when it fluctuates drastically (Bagheri 2007). These authors reported that egg viability was 56% at 30% (RH) and increased gradually with increasing RH, reaching a maximum of 85% at 87% RH. Meteorological data recorded during the present study showed that the RH at Gachsaran fluctuated between 75 and 83% with a mean of 78 ± 2.3%, and egg viability remained high throughout the study period. The mean egg viability (83.28%) was similar to the maximum percent viability (85%) recorded by Bagheri (2007).

Table2. Comparison of biological parameters records of A. helianthi between the present study and results of other studies.

	Bagheri, 2006	Jakhmola,1980	Keyhanian, 2007	Ricci and Ciriciofolo, 1983	Zandigiacomo,1983	Present study
Number of generations/year		-	2	-	2	3
Sex ratio	1.1:0.9	-	-	-	1:1	1:1
Longevity of females (days)	-	-	-	-	-	12±3.0
Number of pupae in each flower head	-	-	1-13	-	5.4	1-11
Yield losses (%)	25-70	96-99	10-33	14-79	59	39-78
Duration of larval stage	15	-	11-12	-	-	7-10

A .helianthi has three larval instars. Larvae are elongate and sub_cylindrical with a milky-white colored integument. The main difference between instars is body size and length of the cephalopharyngeal skeleton. The cephalopharyngeal skeleton of first instar larvae ranges in size from 0.5 to 0.10 mm and is shaped differently than that of second and third instars. The mean duration of the first instar larvae was 2.5 ± 0.1 days (Table 1). The shape of the cephalopharyngeal skeleton of second instar larvae was similar to that of the third instar. The cephalopharyngeal skeleton of second instar ranged from 0.20 to 0.35 mm. The mean duration of the second larval period was 2.6 ± 0.1 days (Table 1). The cephalopharyngeal skeleton of third instar larvae ranged from 0.40 to 0.65 mm in length. The mean duration of the third instar larvae was 3.6 ± 0.2 days (Table 1). Larvae at this stage are pale yellow in color and much more active than less developed instars.

Males emerged earlier than females, so that in the first and second week of sampling, the number of males in the sweeping net was 10 times greater than that of females, but over time, the male: female ratio gradually became closer, so that the final sex ratio was 1:1.28.

During the study period, the daily minimum temperature fluctuated from 24.2 to 27.5 ℃ and the daily maximum temperature ranged from 37 to 39.5 ℃. The daily temperature and RH inside the rearing cages were only marginally higher than those in the experimental plot. However, fecundity, mean oviposition period, and percentage of viable eggs were not significantly different inside the cages compared to the field conditions. Therefore, (using cages) is recommended for biological studies of *A. helianthi*. Studies of *A. helianthi* have shown that the mean duration of pre-oviposition, oviposition and post-oviposition are 5.8 ± 1.0, 11 ± 1.2, and 6 ± 2.0 days, respectively. In the present study, egg incubation time was relatively longer than the value reported by Rahoo, et al. (1997), (incubation period of 2-4 days, mean of 2.9 days), which might be attributed to different host varieties,. In our study *A. helianthi* adults survived on a water and- honey diet for 3-17 days, (mean, 10 ± 1.0 days,), which is considerably longer that on a water and sugar diet, which is approximately 2-12 days, with an average of 7.5 ±1.0 days (Bagheri 2007). Female longevity (12 ± 3.0 days) was longer than males (8 ± 1.0 days), which was consistent with other studies (Bagheri 2007 and Rahoo et al. 1997), (Table 2). We report a different sex ratio (1:-1.28) for *A. helianthi* than that (1:1) reported by Keyhanian (2007), (Table 2). Genetic heterogeneity of the local *A. helianthi* populations and inherent demographic stochasticity of *A .helianthi* individuals, as well as the use of safflower as a host may account for the minor inconsistencies between our results and those of other studies. *A. helianthi* is normally active for 4 months from April to July, in the Gachsaran region where it has three generations per year. Although the number of generations per year was quite close to that reported for Iraq (Al-Ali et al. 1977), a study conducted in Italy reported two generations per year (Zandigiacomo and Iob, 1991), (Table 2). *A. helianthi* larvae feed mainly on safflowers, but can also feed

on some species of Compositae (Hegazi and Moursi 1983). Further research is required to clarify the host effect on the biology and feeding behavior of *A. helianthi*.

CONCLUSION

The results obtained from field studies are often preferred and more reliable, as they account for the effect of numerous unknown influencing factors that could otherwise be controlled in a laboratory study. However, for the same reason, lower accuracy of results from field observations is common. Alternatively, laboratory studies often produce consistent and reproducible results, but they do not provide a comprehensive image of the inherent natural randomness. Neither lab nor field studies are perfect alone, and hence this study benefited from a combination of both laboratory trials and field observations.

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