



Ecological, biological and physiological studies on some cereal aphid species infesting wheat plants and their parasitoids at Assiut

A THESIS

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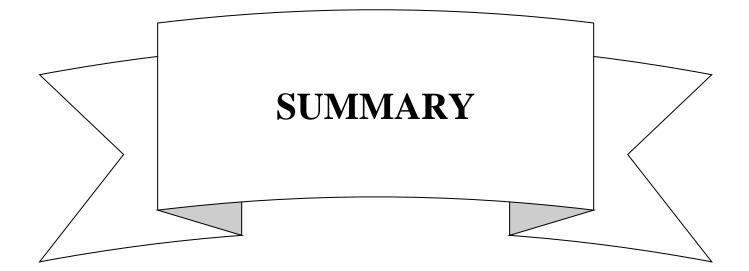
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Summary

The present investigations were undertaken at the Experimental Farm of Assiut University, Agronomy Department, throughout 2013-2014 and 2014-2015 wheat growing seasons in addition to some laboratory experiments.

The objectives of this work were:

- 1- Studying the population of cereal aphids.
- 2- Studying factors (biotic and abiotic) affecting cereal aphid populations.
- 3- Studying the mechanisms underlying aphid-parasitoid dynamics in the field.
- 4- Studying the effect of some agricultural practices such as wheat cultivars and nitrogen fertilization on the aphid populations.
- 5- Determine yield losses due to aphid infestations.
- 6- Studying some biological aspects of the main cereal aphid species *Rhopalosiphum padi* L. and *Schizaphis graminum* (Rondani) as well as their common primary parasitoid *Diaeretiella rapae* (M'Intosh) under different constant temperatures.
- 7- Studying biochemical and physiological changes in wheat leaves in relation to nitrogen fertilization and how these changes affect physiological parameters of both *R. padi* and *S. graminum*.
- 8- Studying the impact of parasitization by hymenopterous parasitoid *D. rapae* on chemical composition and some antioxidant enzymes of its host *R. padi* under laboratory condition.
- 9- Scanning electron microscope studies for *R. padi* and *S. graminum* and their dominant parasitoid *D. rapae*.

1-Ecological studies

1.1. Aphid species infesting wheat plants and their natural enemies1.1.1. Aphids

Four cereal aphid species belonging to Order Homoptera Family Aphididae were found infesting wheat plants in Assiut. These species were; the corn leaf aphid, *Rhopalosiphum maidis* (Fitch); the oat birdcherry aphid, *Rhopalosiphum padi* (Linnaeus); the greenbug, *Schizaphis graminum* (Rondani) and the English grain aphid, *Sitobion avenae* (Fabricius).

1.1.2. Natural enemies

1.1.2.1. Predators

Chrysoperlla carnea Steph, (Chrysopidae: Neuroptera); *Coccinella undecimpunctata* L. (Coccinellidae: Coleoptera); *Nabis* spp. (Nabidae: Hemiptera); *Orius* spp. (Anthocoridae: Hemiptera); *Paederus alfierii* (Koch) (Staphilinidae: Coleoptera); *Scymnus* spp. (Coccinellidae: Coleoptera); *Syrphus corollae* Fab. (Syrphidae: Diptera) and some unidentified species of true spiders found in association with cereal aphids during the study periods.

1.1.2.2. Parasitoids

The following primary parasitoid species emerged from mummies of cereal aphid belonging to Fam. Aphidiidae from wheat fields: *Diaeretiella rapae* (M'Intosh), *Paraon necans* (Mackauer) and *Aphidius gr. colemani* Viereck and three hyperparasitoid species belonging to two different families, *Alloxysta* sp; *Pachyneuron* sp, and *Phaenoglyphis* sp.

1.2. Population studies of aphid species infesting wheat plants

The population of cereal aphids on wheat plants began to appear on wheat plants on 1st week of January with few numbers when the plants were in the tillering stage. Afterwards, the population of aphids increased gradually to reach a peak of abundance during the end of February. Then the number of aphids began to decline until the end of March, when the aphid disappeared from the plants.

The multiple regression analysis show that the eight selected variables (plant age, predators, parasitoids, effective temperatures (day-gegrees), max. and min. temperature, max and min. relative humidity) were together responsible for about 82 % of the increase in cereal aphid populations during the wheat growing seasons.

The efficiencies of the most effective variable, plant age, parasitoid as biotic factors and max. temperature as abiotic factors were 29.88,15.72 and 8.28% respectively.

1.3. Incidence of parasitoids in relation to cereal aphids

Field and laboratory studies were carried out to determine the relative abundance of the hymenopterous parasitoids in relation to cereal aphid populations during 2013-2014 and 2014-2015.

1.3.1. Mummification rate

The mummified aphids appeared on wheat plants during the period extended from the second week of January up to the end of March. The percentage of parasitism was relatively low, generally <3% until the end of February. Maximum parasitism (31.22 and 6.35%) was recorded during the end of March in both growing seasons.

1.3.2. Parasitization rate

Regardless of aphid species the parasitization rate was 50.87% during 2014 and 42.50 during 2015 with an overall average of 46.68.

Generally, clearly revealed that the parasitism frequencies in the field for any given date were always low as compared with those reared from the live aphids in the laboratory. At the peak population, the rates of parasitism in the field were 2.91 and 2.79 % as compared with 43.00 and 35.00% (parasitization rate). Data show that rates of parasitism of reared aphids were 14.78 and 12.55% times higher than the rates of parasitism in

Summary

the field during the peak of aphid populations during 2014 and 2015 seasons, respectively.

1.4. Agricultural practices

1.4.1. Effect of some agricultural practices on the aphid infestation

1.4.1.1. Effect of wheat cultivars

Data showed that the two tested wheat cultivars have been infested approximately with the same number of aphids. The average numbers of aphids / tiller were 201.30 and 205.07 and 556.05 and 422.63 on Sakha 93 and Sids 1 during 2014 and 2015, respectively.

1.4.1.2. Effect of nitrogen fertilization

Significant differences were found between the numbers of aphids and different levels of N- fertilization. Unfertilized plants harboured the lowest number of aphids (61.23 and 137.37 aphids/tiller during 2014 and 2015, respectively). Whereas those receiving 1.5 fertilization times the recommended rate of fertilization (120 N- units/ feddan) harbour the highest number of aphids (403.27 and 889.97 aphids / tiller during 2014 and 2015, respectively).

- **1.4.2.** Effect of agricultural practices in relation to aphid infestation on the grain yield of wheat
- **1.4.2.1** Grain yield in relation to wheat cultivars and aphid infestation

The average grain yield in the check plants were 242.53 and 252.10 gm/ 0.25 m^2 and the average were 192.89 and 203.27 in the plants which exposed to the natural aphid infestation on Sakha 93 and Sids 1, respectively. The reduction of the grain yield due to aphid infestation was 20.47 and 19.37% in Sakha 93 and Sids 1, respectively.

1.4.2.2 Grain yield in relation to nitrogen fertilization and aphid infestation

In the check plants (free from aphid infestation) the average grain yield gm / 0.25 m² in the plots which did not receive fertilizer and those received 40, 80 and 120 N-units / feddan were, respectively, 160.63, 228.80, 280.61 and 319.20 gm / 0.25 m². Meanwhile in the plants which were exposed to the natural aphid infestation, the averages grain yield / 0.25 m² were 136.66, 186.26, 219.53 and 249.86 gm / 0.25 .The reduction due to aphid infestation were 14.92%, 18.59%, 21.77% and 21.72% in the plots without adding fertilizers and those supplied with 40, 80, 120 N-units, respectively.

Regardless of wheat cultivars and nitrogen fertilization, the results showed that the reduction in the grain yield due to aphid infestation ranged from 20.41% during 2014 and 19.48% during 2015 with an average of 19.03%.

2- Biological studies

The effect of constant temperatures of 18°, 22° and 26 °C on the development and survival *R. padi* and *S. graminum* as well as their common parasitoid *D. rapae* were studied.

2.1. Aphids

2.1.1. Nymphal stage:

The time needed for the development of nymphal instars decreased significantly with the increase in temperatures. The highest percentage of survival (86.67 and 78.33%; 85 and 83.33% for *R. padi* and *S. graminum*, respectively) was observed at 22° and 26° C whereas, the lowest (66.67 and 75% for *R. padi* and *S. graminum*, respectively) was noticed at 18°C. The highest indices of efficiency for development (IE) (14.18 and 14.89 for *R. padi* and 10.58 and 12.18 for *S. graminum*) were obtained at 22° and 26° C whereas, the lowest (4.64 and 5.16 for *R. padi* and *S. graminum*) were obtained at 22° and 26° C whereas, the lowest (4.64 and 5.16 for *R. padi* and *S. graminum*) were obtained at 22° and 26° C whereas, the lowest (4.64 and 5.16 for *R. padi* and *S. graminum*).

Summary

graminum, respectively) were obtained at 18 °C .Developmental threshold of the nymphal stage was calculated to be 12.66° and 10.37° C for *R. padi* and *S. graminum*, respectively) .The thermal units required for development of the nymphal stage of both aphid species were 67.95 and 103.72 day-degrees. About 63 generation of *R.padi* and 50 of *S. graminum* could be develop in one year under Assiut conditions, out of which about 6 generations could develop on wheat growing season.

2.1.2. Adult stage (aptera)

Adult longevity decreased with the increase of temperatures for both *R. padi* and *S. graminum*. The number of progeny per aptera reached 15.18 \pm 3.22, 35.76 \pm 14.94 and 25.32 \pm 12.49 nymphs / female for *R. padi* and 15.19 \pm 5.98, 54.95 \pm 19.05 and 44.16 \pm 15.09 nymphs / female for *S. graminum* at 18°, 22° and 26°C, respectively .

Mean generation time (GT) for *R. padi* lasted for about 20 and 11 and 10 days at 18°, 22 and 26°C, respectively. For *S. graminum*, the duration of one generation lasted for about 25, 13 and 13 days at 18°, 22° and 26°C, respectively.

The population doubling time (DT) was 6.66, 2.24 and 2.48 days for *R. padi* and 7.17, 2.49 and 2.56 days for *S. graminum* at 18°, 22° and 26°C, respectively.

Net reproductive rate (R_0) increased by 8.35, 28.02 and 16.88 times within a single generation for *R. padi* and by 10.96, 40.33 and 31.64 times for *S. graminum*, at 18°, 22° and 26°C, respectively.

The values of r_m were 0.1041, 0.3092, 0.2792 for *R. padi* and were 0.0966, 0.2775 and 0.2706 for *S. graminum* at 18, 22 and 26°C, respectively

According to the values of the finite rate of increase (λ) the population of *R. padi* and *S. graminum* could be multiplied about 1.1097,

1.3624 and 1.3221; 1.1014, 1.3198 and 1.3107 times per aptera per day, respectively at 18, 22 and 26°C, respectively.

Generally, it could be concluded that the temperatures 22° and 26° C seems to be the most suitable condition for survival and reproduction of *R. padi* and *S. graminum* in the laboratory.

2.2 .Hymenopterous parasitoid (D. rapae)

The data indicate that the durations for the whole life cycle (from oviposition to adult emergence) decreased as the temperature increased. The durations of the whole life cycle lasted 21.61 ± 0.59 , 12.31 ± 0.64 and 10.01 ± 0.69 days for *D. rapae* reared on *R. padi* and 20.69 ± 0.64 , 11.88 ± 0.85 and 10.19 ± 0.62 days for parasitoid reared on *S. graminum* at 18°, 22° and 26° C, respectively.

The calculated developmental thresholds (t_0) of *D. rapae* were 10.68° and 9.69° C when reared on *R. padi* and *S. graminum*, respectively. By using these values as a base temperature, averages of 150.29and 161.45day-degrees were required, for the development of *D. rapae* on *R. padi* and *S. graminum*, respectively. About 50 generation of *D. rapae* could be develop in one year under Assiut conditions, out of which about 6 generations could develop on cereal aphids during the wheat growing season. From the present work, appears that developmental thresholds for *D. rapae* within the thresholds recorded for *R. padi* and *S. graminum*, suggesting that parasitoids plays a distinguish role in natural regulation of cereal aphid populations and biological control of cereal aphids in Upper Egypt (Assiut).

3- Physiological studies

3.1. Effect of different nitrogen fertilization levels on biochemical changes in leaves of two different wheat cultivars (Sakha 93 and Sids 1)

The present study aimed to estimate the effect of different nitrogen fertilization levels (0, 40, 80 and 120 N-Units/ feddan) on biochemical composition of leaves of two different wheat cultivars (Sakha 93 and Sids 1). The total nitrogen, total protein, soluble protein, soluble carbohydrate and total free amino acids in wheat leaves of both wheat cultivars increased by increasing nitrogen fertilization levels. While, the content of total phenolic compounds increased when the plants were under stress (insufficient nitrogen) at control or low nitrogen level (40 N units).

3.2. Effect of different nitrogen fertilization levels on nutrient components and some antioxidant enzymes of *R. padi* and *S. graminum*

This study aimed to estimate the biochemical changes in nutrient components and some antioxidant enzymes of *R. padi* and *S. graminum* fed on Sakha 93 and Sids 1 wheat cultivars supplied with different nitrogen fertilization levels (Free (control), 40, 80 and 120 N- units). Data showed significant increase in total protein, carbohydrate and lipid contents of *R. padi* and *S. graminum* collected from both wheat cultivars at different nitrogen fertilization levels. On contrast to the data of biochemical parameters, the antioxidants activities in aphids decreased as the doses of nitrogen fertilization increased for both wheat cultivars.

3.3. Effect of parasitization by *D.rapae* on some physiological parameters of its host, *R. padi*

This experiment was conducted to evaluate the effect of parasitization by *D. rapae* on total proteins, carbohydrates, and lipids content of *R. padi* after 5 days of parasitization. Our results revealed that

the total proteins, carbohydrates and lipids of *R. padi* parasitized either as 2^{nd} instar or as 4^{th} instar decreased significantly after 5 days of parasitization when compared to unparasitized aphids. Also there was no significant difference in the concentration of total proteins, carbohydrates and lipids in the aphids parasitized as 2^{nd} instar and dissected either as 4^{th} nymphal instar (parasitized- 4^{th}) or as adult (parasitized-ad).

The activities of the antioxidant enzymes (SOD and CAT) of *R*. *padi* increased significantly in the aphids parasitized either as 2^{nd} or 4^{th} nymphal instar compared to nonparasitized aphids. Also our results clearly indicated significant increase in GSH level in parasitized aphids compared to unparasitized.

4- Morphological studies

4.1. The morphological and Ultra-structural studies on *R. padi* and *S. graminum*

The electron microscopy showed that the head and their compound eyes are found to be similar in structure in both alate and apterous forms of *R. padi* and *S. graminum* aphids. The compound eyes are naked of sensilla in both aphid species.

The antennae of both aphid species are composed of scape, pedicel and flagellum. Both alate and apterous forms of *R. padi* and *S. graminum* has four styloconic sensilla scattered in the apical part of the antenna while the trichoid sensilla I and basiconic sensilla II are found scattered along the surface of all segments of the antenna

Although proximal primary rhinaria and distal primary rhinaria are found on 5^{th} and 6^{th} antennal segments of both alate and apterous forms of *R. padi* and *S. graminum*, the secondary rhinaria are found only in the alate forms of both aphid species. Secondary rhinaria differ in position according to aphid species. They are scattered on the third and fourth antennal segments of *R. padi* while, in *S. graminum* they found only on third antennal segments.

The caudas are cylindrical in shape in both alate and apterous forms of both aphid species. They bear trichoid sensilla (type I and II) and basiconic sensilla I. The surface of abdomen of both *R. padi* and *S. graminum* bears a number of seta from trichoid sensilla that are similar in structure but differ in size. Also, the abdomen bears basiconic sensilla I which arrange in rows and differ in their density in both alate and apterea forms.

4.2. The morphological and Ultra-structural studies of *D. rapae* parasitoid

This study aimed to describe the diversity, morphology and ultrastructure of the sensilla distributed in different parts of both male and female of *D. rapae* by using scanning electron microscopy.

The head bears two large compound eyes with oval shaped, composed of aggregations of large numbers of visual elements called ommatidia. In females, the trichoid sensilla (Type I, II and III) and basiconic sensilla II are scattered among the ommatidia of the compound eyes while in males, only the trichoid sensilla type I and II are recorded

Antennae of males and females are clavate and are very similar in their general structure to those of other hymenopterous parasitoid species, which composed of 3 basic segments (scape with basal radicula, pedicel and flagellum). There are sexual dimorphism in the number of antennal segments in both male and female. In female, the flagellum is composed of 12 sub-segments while in males it is composed of 14 sub-segments. All the antennal segments are covered by four different types of sensilla; trichod sensillae type I (ts I), elongated Placoid sensillae (Pl), Basiconic sensilla II (Bs II) and Chaetica sensillae (Ch). Scanning electron microscopy of *D. rapae* ovipositor showed that the ovipositor is cylindrical and needlelike in shape with teeth at its tip. The tip of the two external valves protecting the ovipositor shows the presence of numerous basiconic sensilla II and covered with some sensilla trichode (I and II). Digitus (dig) is glabrous and provided by few number of cheatica sensilla.

From the present study concluded that:

- 1. Cereal aphids are considered the most important pests infesting wheat plants. The development of aphid populations continues through January until April.
- 2. Aphids performance under field conditions might be affected by several biotic and abiotic factors. Plant age, parasitoids and max. temperature were the most important factors affecting aphid populations.
- 3. The hymenopterous parasitoids are considered to be potentially important regulators of cereal aphids in wheat ecosystem. They appear to be synchronized with the start of aphid populations and they may be able to affect the growth of the aphid population. Thus, it is more convenient to do not use chemical control at this time. In case of the need to use aphicides, selective insecticide is recommended as much as possible.
- 4. Heavy applications of nitrogenous fertilizer may not affect insect directly, but they bring about changes in host-plant morphology, biochemistry, and physiology, which can improve nutritional conditions of plants and become more susceptible to herbivores. Thus, the optimum rate of nitogen fertilization is about 80 units/feddan.

- 5. Under laboratory conditions, the range of 22–26°C constant temperatures could be considered the most favorable regime for development and reproduction of both aphid species.
- 6. Developmental thresholds for *D. rapae* was within the thresholds recorded for *R. padi* and *S. graminum*, suggesting that parasitoids plays a distinguish role in natural regulation of cereal aphid populations and biological control of cereal aphids in Upper Egypt (Assiut).
- 7. The parasitization by *D. rapae* alter their host's metabolism for their own benefit to ensure successful nourishment and maturation Consequently, further studies are required on the host/parasitoid interaction between aphids and their parasitoid *D. rapae* to provide additional information about their specific metabolic interdependencies.
- 8. Morphological characters data revealed that there were differences between alate and apterous forms of both aphid species and males and females of parasitoid *D. rapae*.

Finally, I hope that these informations may be useful to establish monitoring and sampling plans for these important pests as a first step in developing IPM programs.