Egyptian Journal of Plant Protection Research Institute

Volume 2: No. (3), September 2019

Egyptian Journal of Plant Protection Research Institute *is* an official journal of the Plant Protection Research Institute, *Agriculture* Research Center. It includes original research papers on basic and applied research in all aspects of plant protection. In additions to original research papers, also published reviews and scientific notes or short communications on critical issues relevant to plant protection. Subject areas include, but are not restricted to the following fields: Field pests, vegetable, aromatic, medicinal and ornamental plant pests, orchard pests, locust and grasshopper, beekeeping sericulture, spray technology, alternatives methods of control pests, survey and classification of agriculture pests and new biocontrol technology pests

Abbreviation of the Journal: Egypt. J. of Plant Prot. Res. Inst.`

Egyptian Journal of Plant Protection Research Institute

Volume 2: No. (3), September 2019

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Egypt. J. Plant Prot. Res. Inst. (2019), 2 (3): 450 - 459

Egyptian Journal of Plant
Protection Research Institute
www.ejppri.eg.net

Molecular identification of the entomopathogen *Aspergillus candidus* and its pathogenicity to the mealybug *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae) in Egyptian sugar beet fields

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ARTICLE INFO
Article History
Received: 2 / 7 / 2019
Accepted: 26 / 9 /2019

Keywords
Molecular identification, pathogenicity, *Aspergillus*, rDNA and *Phenacocus solenopsis*.

Abstract:

Several host plants are subject to infest with *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), from which is sugar beet. The infested plants suffer from reduction in photosynthetic processes. Biological control is an essential approach to control. The fungus *Aspergillus candidus* is one of the entomopathogens attacking this mealybug. The current investigation was done at "Kafr Ascar" village, Kafr El-Sheikh Governorate, Egypt during 2016/2017 and 2017/2018 seasons. Obtained results indicated that the natural mortality of *P. solenopsis* adults due to the fungus *A. candidus* infection in the field ranged between 2-19 individuals/ 50 plants from 25 October to 10 May, 2016/2017 season and the total number of dead insects recorded throughout the season was 71 individuals/ 300 plants. According to statistical analysis, the results indicated that the temperature and humidity had a significant effect on the activity of the fungus and therefore on mortality rates.The entomopathogenic fungus was isolated and identified by GATC (Biotech Sequence Company, Germany), as *A. candidus* strain HNMF075 (Ascomycetes: Eurotiaceae) for the first time in Egypt. Suspension of this fungus was made, adjusted to 5×10^{5}spores/ ml water, for using in laboratory and field tests. In a laboratory tests, the mortalities of adults were 55.00 and 95.00 two and four days after treatment, respectively. The mean of mortality was 75.00 %. Also, in a field tests, the reductions of adults were 53.00 and 67.42% two and four days after treatment, respectively, with 60.21% average reduction based on DNA sequences using rDNA, data showed that the *Aspergillus* isolate belonged to *A. candidus* with a high similarity of 94%. It is concluded that *A. candidus* strain HNMF075 is an effective biological agent against *P. solenops is* in sugar beet fields in Egypt.

Introduction

The cotton mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) is a polyphagous insect feeding on more than 202 host plant species from 55 families (Fand and Suroshe, 2015). Large populations of mealybug cause general weakening, defoliation and death of susceptible plants. As well, the honeydew excreted by the mealybugs caused growth of sooty moulds and other secondary infections that reduce photosynthesis and impair the marketability of plant products (Ibrahim *et al.*, 2015). *P. solenopsis* has a wide geographical distribution, Central America, the Caribbean, Ecuador, Chile, Argentina and Brazil (Culik and Gullan 2005). In Egypt, the occurrence of *P. solenopsis* infestation was recorded on weed plants for the first time byAbd-Rabou *et al*. (2010). In sugar beet (*Beta vulgaris* L.) fields, Vennila *et al*. (2014) showed that pigweed *Chenopodium albam* L. and spinach *Spinacia oleracea* L. are host plants of *P. solenopsis*. Anonymous (2009) recorded that mealybug is one of the insect pests of sugar beet plants in Egypt. Also, Bazazo *et al*. (2017) identified the specimens of *P. solenopsis* at Plant Protection Research Institute, Agricutural Research Center. They estimated the numbers of *P. solenopsis* infested leaves as 15-18 symptoms/200 sugar beet plants at some fields at Kafr El-Sheikh Governorate. Mealybugs are difficult to control with insecticides, as they are able to rapidly develop resistance and exhibit cryptic behavior. Further, they are covered by waxes that protect their bodies from insecticide penetration (Franco *et al*., 2004). The low efficacy of insectcide use has been encouraged biocontrol strategies (Daane *et al*., 2004). The application of entomopathogenic fungus was a valuable method of biological control of mealybugs (Mustu *et al*., 2015). Fungal pathogens have certain advantages in pest control programms over other insect pathogens like bacteria and viruses. Mass production techniques of fungi are much simpler, easier and cheaper than those of bacteria and virus. Fungi unlike bacteria or viruses directly infect through insect cuticle and do not require ingestion for infection and also sucking insects are infected (Pucheta *et al*., 2016). Entomopathogenic fungi play an important role in the natural pest control in various crops through epizootics; more than 750 species are pathogenic against insects (EL-Husseini *et al*., 2003 and 2004 and Ramanujam *et al*., 2014). *Aspergillus candidus* Link as entomopathogen against different insect pests such as, *Thaumetopoea wilkinsoni* Tams (Lepidoptera: Notodontidae) (Kenneth and Olmert, 1975); *Coccus hesperidum* L. (Hemiptera: Coccidae) (Samsinakova and Kalalova, 1975)*; Indarbela* spp. (Lepidoptera: Metarbelidae) (Singh and Singh, 1982); *Carvalhoia arecae* Miler (Heteropetera: Miriidae) (Dhileepan *et al*., 1990). Finally, research on microbial pathogens of insects is increasing considerably in recent times to find out environmental friendly alternatives to hazardous chemical insecticides (Ramanujam *et al*., 2014).

Aspergillus spp. is commonly worldwide and includes industrially and biological control important members. It is very important to isolate and identify microorganisms to be used as biological control agents against insect pests. Moreover, morphological identification of microbial isolates could be insufficient for distinguishing the species. PCR and DNA sequences are speed, sensitive and specific way for identification and are proved extremely useful in assessing the changes in fungus such as *Aspergillus* and can also provide useful taxonomic information (Rasime, 2016). Abundant gene regions, such as rDNA regions could be used for sequencing and molecular identification

(Munusamy *et al*., 2010; Peterson, 2012 and Schoch *et al*., 2012) and there are precise analyses for rDNA for differentiation of *Aspergillus* (Sabreen *et al*., 2015). Moreover, internal transcribed spacer (ITS), as called, rDNA is commonly used for taxonomic studies, barcode gene for fungal identification and phylogenetic studies (Krijgsheld *et al*., 2013 and Rasime, 2016).

This study was carried out to molecularly identify *Aspergillus* from dead *P. solenopsis* picked up from the suger beet fields. Moreover, the virulence of *Aspergillus* against the insect pest was assessed under laboratory and field conditions.

Materials and methods

1.Recording the dead *Phenacoccus solenopsis* adults with *Aspergillus candidus*:

The dead individuals of *P. solenopsis* were recorded at the experimental field (about two feddans). Sugar beet, sultan cultivar sown on 15^{th} September, 2016 at Kafr Ascar village, Kafr El-Sheikh Governorate.They were picked up by a fine brush and preserved in small sterilized vials, as monthly samples beginning from 25^{th} October to 10^{th}April 2017, for 50 plants per inspection.

2.The effect of certain climatic factors on *Aspergillus candidus* activity:

The daily records of temperature (˚C), relative humidity (RH%) and rain fall (mm/day) during the experimental season were obtained from Meteorological Department at Sakha, Agricultural Research Station. Monthly means of these factors were calculated. The relationship between these weather factors and the number of dead mealybug adults was statistically calculated. Linear correlations were fitted using IBM SPSS Statistics 19 software to reveal the correlation between mortality rates of *P. solenopsis* adults at respective weather factors.

3. Isolation of entomopathogenic fungus:

The dead insects were sterilized using 0.5% sodium hypochlorite and 75% ethyl alcohol and then rinsed in plenty of distilled water and dried with sterile filter paper. Then, the dead insects were individually kept in petri dishes (9 cm diameter) provided with moistened filter paper and incubated at 28ºC for three days to stimulate the growth of fungus.The pathogen was introduced into a petri dishes (9 cm diameter) having water agar medium and incubated for three days under 28 ºC. Finally , a piece of agar with mycelial growth was inoculated using a sterilized needle into petri dishes having Potato Dextrose Agar (PAD) medium, according to Dourou-Kpindou *et al*. (1995) (Figure,1).

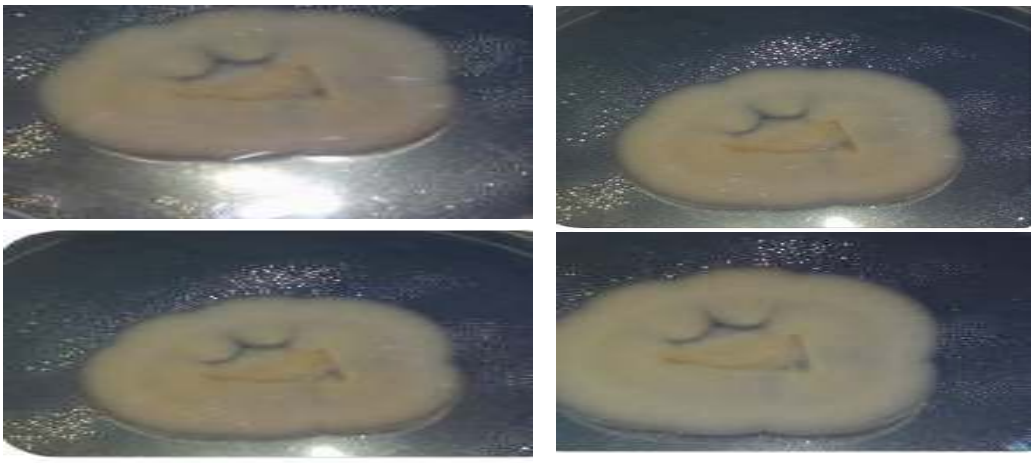

Figure (1): *Phenacoccus solenopsis* adults with fungal hyphae of *Aspergillus candidus* strain HNMF075.

4. Spore isolation for using in laboratory and field tests:

The petri dishes having sporulated fungi were washed with distilled water to exclude the fungus and formulate as a spore suspension that was adjusted to 5×10^5 spores/ml water, using the micrometer slide, to be used in tests against *P. solenopsis* adults.

5. Laboratory tests:

Forty *P. solenopsis* adults (20×2 petri dishes) for each sampling date, containing a piece of sugar beet leaf were treated with *A. candidus* spores suspension by hand sprayer (1 liter). The mortalities (%) were recorded two and four days after treatments according to Samsinakova and Kalalove (1975). The fungus was reisolated from the dead mealybug adults.

6. Field tests:

The experimental area (approximately $200 m^2$) of sugar beet was divided into two plots. Each plot was divided into four replicates. One plot was sprayed with *A. candidus* spores suspension by hand sprayer (1 liter). The other one was left untreated (as a check).The number of dead mealybug individuals were recorded two and four days after treatment according to Samsinakova and Kalalova (1975). The spraying was done on 10thJanuary 2018. The reductions were calculated using Henderson and Tilton (1955) formula:

$$\text{Reduction}(\%) = 1 - \left(\frac{\text{No in control before spray}}{\text{No in control after spray}} \times \frac{\text{No in treated after spray}}{\text{No in treated before spray}}\right) \times 100$$

7. DNA isolation, PCR and sequencing:

Total DNA was isolated using CTAB method Doyle and Doyle (1990). Concentration of obtained DNA (ng/µl) and purity were determined on Nanodrop-photometer, and then kept at 20^{o}C till use. Polymerase Chain Reaction (PCR) was done in a volume of 50µl using 60ng genomic DNA, 0.2 mMdNTP, 1.5 mM $MgCl_2$, 5 pmol of primer and 0.5U Taq polymerase. PCR conditions were as follows: 95°C for 5 min, 35 cycles of 95 °C for 1.30 min, 55°C for 45 sec, 72°C for 1 min. then 72°C for 7 min. PCR products were separated by 1.2% agarose gels electrophoresis, which run with 1X TAE buffer. The 1Kbp DNA ladder was also run on each gel as a molecular weight standard. The amplification of 5.8S ribosomal RNA gene and internal transcribed spacer was done from fungal genomic DNA by PCR. PCR purified products of the rDNA of the strains were analyzed for nucleotide sequence determination by using ABI PRISM® 3500XL DNA Sequencer (Applied Biosystems).

8. Data analysis and phylogenetic analysis:

A pairwise comparison among isolate and other sequences from GenBank database with the BlastN algorithm to determine relative phylogenetic positions was performed to produce a dendrogram using neighbor-joining (NJ) trees.

Results and Discussion

1. Recording the natural mortalities of *Phenacoccus solenopsis* adults with *Aspergillus candidus*:

Data in Table (1) showed that the number of dead adults was low on October 25th and November 16th (3 and 6 individuals/50 plants, respectively). The number jumped to 15 individuals/50 plants on December 18th. The dead numbers reached 17 and 19 individuals/50 plants on January 19th and February 20th, respectively. The numbers decreased sharply and reached to 9 and 2 individuals/50 plant on March 23rd and April 10th, respectively.Throughout the season, the total number of dead adults was 71 individuals/ 300 sugar beet plants.

2. The effect of certain weather factors on *Aspergillus candidus* activity:

A fitted linear regression model showed that the mortality rates of *P. solenopsis* adults were positively correlated with the temperature (y = 0.7

x + 16, r2 = 0.825, p < 0.001) and (y = 0.6 x + 80, r2 = 0.516, p < 0.001). These results suggested that the high temperature and relative humidity enhanced the role of the entomopathogenic fugus in killing *P. solenopsis* adults.

Table (1): Natural mortality of *Phenacoccus solenopsis* adults collected from 50 sugar beet plants /sampling date due to *Aspergillus candidus* during 2016/2017 season.

Date	No. of *Phenacoccus solenopsis* dead	Mortality%	Temperature (^{o}c)	Humidity (RH %)	Rain (mm/day)
October, 25	3	4.23	29.44	80.71	-
November,16	6	8.45	25.82	91.28	-
December,18	15	21.13	20.07	90.14	7.1
January, 19	17	24.94	18.82	91.71	8.1
Feburay, 20	19	26.76	17.01	90.00	8.7
March, 23	9	12.68	20.44	91.00	-
April, 10	2	2.82	25.08	81.00	-
Total	71	-	-	-	-

Pell *et al.*, (2001) indicated that the environmental conditions particularly, humidity and temperature play an important role in the infection and sporulation of entomopathogenic fungus. Humidity (RH %) is required for spore germination and sporulation outside the host. Most of the entomopathogenic fungi in tropical and subtropical areas require an optimum temperature of 25-30 °C for successful control of insect pests. Samsinakova and Kalalova (1975) reported that the relatively high natural mortality of scale insects depends on temperature and humidity conditions. Also, Dhileepan *et al*. (1990) showed that the possible reason for the wide spread incidence of *A. candidus* against the spindle bug, *Carvalhoia arecae* in the field during the raining season.

3. Pathogenicity of *Aspergillus candidus* to *Phenacoccus solenopsis* in laboratory tests:

Table (2) showed that the mortalities of adults were 55.00 and 95.00% two and four days after treatment, respectively. The overall mortality recorded throughout the experimental period was 75.00%. Roberts and Yendel (1971) and Pierre (1985) indicated that *Aspergillus* spp. are imperfect fungi and are most frequently associated with insect diseases. Singh and Singh (1982) reported that *A.candidus* induced about 100% mortality under laboratory conditions against *Indarela* spp. Insects. Also, Dhileepan *et al*. (1990) showed that *A. candidus* is a virulent entomopathogen to *C. arecae* killing 50% within two days of inoculation and 100% in four days after inoculation. Finally, Samsinakova and Kalalova (1975) proved that *A. candidus* caused about 85% to 100% mortality against *Coccus hesperidum* L. (Hemiptera: Coccidae) insect, two days after treatment under laboratory conditions.

Table (2): Mortality percentages of *Phenacoccus solenopsis* adult treated with *Aspergillus candidus* suspension (5×10^5) /ml water in the laboratory, 2017/2018.

Duration after treatment (day)	No.of adults before treatment	No. of dead adults after treatment	Mortality (%)
2	20	11	55.00
4	20	19	95.00
Overall	40	30	75.00

4.Effect of *Aspergillus candidus* suspension on *Phenacoccus solenopsis*in the field tests:

Table (3) indicate that the reduction of *P. solenopsis* opulations were 53.00 and 67.42% two and four days after treatment, respectively. Overall mean of reduction was 60.21%. El-Husseini (1981) proved that the entomopathogen fungi were effective microbial control agents in crops with vegetation contributes to the presence of high relative humidity in the microclimate within plants as occurring in sugar beet fields. Ramanujam *et al*. (2014) showed that entomopathogenic fungi play an important role in the natural pest control in various crops through epizootics.

Table (3): Reduction of *Phenacoccus solenopsis* due to *Aspergillus candidus* suspension (5×10^5) /ml water in the field, 2017/2018 season.

Treatment		*Aspergillus candidus*	Control
Before spray	Total	17	16
	Mean	4.25	4.00
After 2days	Total	11	22
	Mean	2.75	5.50
	Red.(%)	53.00	-
After 4days	Total	9	26
	Mean	2.25	6.50
	Red.(%)	67.42	-
Overall mean of reductions		60.21	-

5. Molecular identificaion:

The method to identify isolated fungus at the species level was developed using the rRNA genes. The partial sequence with 600bp was amplified, sequenced and compared with other sequences in GeneBank. Based on BLST, the selected isolate was closely related to various species of *Aspergillus* and has 94% identical with *A. candidus* (Figures , 2 and 3).

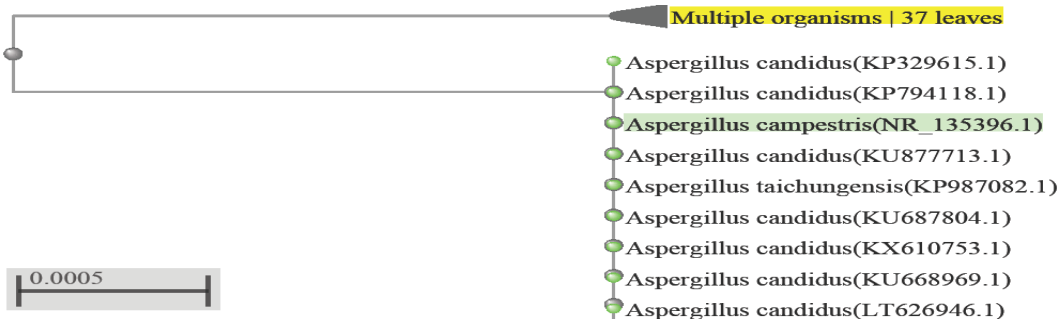

Figure (2): Phylogenetic tree of the nucleotide sequences of the PCR product of rRNA gene from BLAST.

Moreover, *Aspergillus* sp. taxonomy is complex, which makes its identification unreliable as a result of intraspecific similarities (Gontia-Mishra *et al*., 2013). Therefore, molecular characterization looks to be a confident tool in the identification isolated fungus based on the sequencing of the 18 S rRNA genes (Hunt *et al*., 2004). Sequences of rDNA regions and phylogenetic analysis have been used as targets for microbial isolates identification. It could be concluded that the use of DNA sequences gives a better picture of *Aspergillus* identification to be used as a biological control for insects.

Description	Max score	Total score	Query cover	E value	Ident	Accession
Aspergillus candidus isolate HNMF075 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complet	459	459	99%	3e-125	94%	MH725571.1
Aspergillus sp. strain Bdf-2 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal trans	459	459	99%	3e-125	94%	MH681592.1
Aspergillus tritici strain BXMA1-4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete seq	459	459	99%	3e-125	94%	MH634482.1
Aspergillus candidus IPBCC.18.1399 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence	459	459	99%	3e-125	94%	LC387830.1
Aspergillus candidus strain HDN15-152 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, comple	459	459	99%	3e-125	94%	MH430037.1
Aspergillus candidus isolate SFC102207 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene,	459	459	99%	3e-125	94%	MF186135.1
Aspergillus candidus isolate 117 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequ	459	459	99%	3e-125	94%	MH345957.1
Aspergillus sp. 4 JS-2017 genomic DNA sequence contains ITS1, 5.8S rRNA gene and ITS2, isolate FMR:15877	459	459	99%	3e-125	94%	LT798907.1
Aspergillus sp. 3 JS-2017 genomic DNA sequence contains ITS1, 5.8S rRNA gene and ITS2, isolate FMR:15736	459	459	99%	3e-125	94%	LT798906.1
Aspergillus sp. 3 JS-2017 genomic DNA sequence contains ITS1, 5.8S rRNA gene and ITS2, isolate FMR:15733	459	459	99%	3e-125	94%	LT798905.1
Aspergillus sp. 2 JS-2017 genomic DNA sequence contains ITS1, 5.8S rRNA gene and ITS2, isolate FMR:15444	459	459	99%	3e-125	94%	LT798904.1
Aspergillus sp. 1 JS-2017 genomic DNA sequence contains ITS1, 5.8S rRNA gene and ITS2, isolate FMR:15226	459	459	99%	3e-125	94%	LT798903.1
Aspergillus sp. 1 JS-2017 genomic DNA sequence contains ITS1, 5.8S rRNA gene and ITS2, isolate FMR:15224	459	459	99%	3e-125	94%	LT798902.1
Aspergillus candidus strain CHNSCLM-0393 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and inter	459	459	99%	3e-125	94%	MF681708.1
Aspergillus tritici isolate NB-DR-n internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete seq	459	459	99%	3e-125	94%	MG519717.1
Aspergillus tritici genomic DNA containing ITS1, 5.8S rRNA gene and ITS2, strain CCF 4653	459	459	99%	3e-125	94%	HG915890.2
Fungal endophyte isolate GZWMJZ-056 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal t	459	459	99%	3e-125	94%	KY038595.1
Fungal endophyte isolate GZWMJZ-055 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal t	459	459	99%	3e-125	94%	KY038594.1
Aspergillus candidus strain SW140 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete se	459	459	99%	3e-125	94%	KY260674.1
Aspergillus candidus strain SW84 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcri	459	459	99%	3e-125	94%	KY260665.1
Aspergillus tritici strain sp2-8-1 18S ribosomal RNA gene, partial sequence; and internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transc	459	459	99%	3e-125	94%	MF716581.1
Aspergillus tritici isolate ITS_4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spac	459	459	99%	3e-125	94%	MG022438.1

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Query  1    AGCGGGTGACAAAGCCCCATACGCTCGAGGACCGGACGCGGTGCCGCCGCTGCCTTTCGG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  456  AGCGGGTGACAAAGCCCCATACGCTCGAGGACCGGACGCGGTGCCGCCGCTGCCTTTCGG  397

Query  61   GCCCGTCCCCGGGGGTACCGGGGACGGGGCCCAACACACAAGCCGTGCTTGAGGGCAGCA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  396  GCCCGTCCCCGGGGGTACCGGGGACGGGGCCCAACACACAAGCCGTGCTTGAGGGCAGCA  337

Query  121  ATGACGCTCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGAC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  336  ATGACGCTCGGACAGGCATGCCCCCCGGAATACCAGGGGGCGCAATGTGCGTTCAAAGAC  277

Query  181  TCGATGATTCACTGAATTCTGCA-TTCACATTAGTTATCGCATT-CGCTGGG-TCTTCAT  237
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Sbjct  276  TCGATGATTCACTGAATTCTGCAATTCACATTAGTTATCGCATTTCGCTGGGTTCTTCAT  217

Query  238  CGATGCCG-AAC-AAGA-ATC-ATTGT-GAA-GTT--GAC-GAT-G-TA-CAA-CGACT-  283
            |||||||| ||| |||| ||| ||||| ||| |||   ||| ||| | || ||| |||||
Sbjct  216  CGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTGACTGATTGGTAACAATCGACTC  157

Query  284  AGACTG-ACTT  293
            |||||| ||||
Sbjct  156  AGACTGCACTT  146
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Figure (3): *Aspergillus candidus* isolate HNMF075 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

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Egypt. J. Plant Prot. Res. Inst. (2019), 2 (3): 460 - 464

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Effect of formulation types on the efficacy of indoxacarb against cotton leafworm *Spodoptera littoralis* (Lepidoptera: Noctuidae)

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ARTICLE INFO
Article History
Received: 4 / 7 / 2019
Accepted: 11 / 8 /2019

Keywords
Indoxacarb, formulation types, efficiency, cotton leafworm, *Spodoptera littoralis* and Lepidoptera.

Abstract:

The primary objectives of formulation technology are to optimize the biological activity of the pesticide and to give a product which is safe for use. The present study aimed to determine the more formulation type which had more the effect on the efficiency of indoxacarb against cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Three formulation types of Indoxacarb; emulsifiable concentrate (EC), suspension concentrate (SC) and water dispersible granule (WG) were prepared in four concentrations and evaluated against 3rd instar larvae of cotton leafworm *S. littoralis*. Also, the physicochemical properties of the spray solution of these formulation types were determined. The results indicated that the EC was more formulation type effect on the efficiency of indoxacarb where the mortality percentage increased with it compared with other both formulation types. Also the medium lethal concentration was less with EC compared with SC and WG. The physicochemical properties of the spray solution illustrated that the values of surface tension and pH of ECwere less than SC and WG. On the other hand, the viscosity and conductivity values were more with EC than SC and WG. In conclusion, the formulation type may be increase the efficiency of the active ingredient. In this study EC concentrate was more effective on the efficiency of indoxacarb than SC and WG.

Introduction

For an insecticide to be effectively used in the control of insects, it must first be prepared into a form suitable for a particular application method. This preparation of an insecticide is called a formulation and involves the addition of various chemical solvents or diluents to improve the effectiveness or physical properties of the insecticide. The formulation improves the properties of a chemical for handling, storage, application and may substantially influence effectiveness and safety (Burges, 1998). Any given active

ingredient can often be purchased in more than one formulation, for example, the active ingredient indoxacarb is available as an emulsifiable concentrate (EC), a suspension concentrate (SC) and a water dispersible granule (WG) thus, the same active ingredient is available in three different products. Indoxacarb is anoxadiazine pesticide developed by Du Pont Ltd., New Zealand that acts against lepidopteran larvae. Its main mode of action is via blocking of neuronal sodium channels. The use of insecticides such as indoxacarb insecticides plays an important role in controlling the Egyptian cotton leafworm in Egypt and will likely continue to be used until a more biological system with minimum environmental risks based management could be developed. The cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is a destructive prolific and highly polyphagous insect in Egypt that causes various ravages not only for cotton plants but also for other field crops and vegetables. It is considered to be a major pest of great economic importance in many countries since it attacks a multitude of host plants (Lobna *et al.,* 2013 and Heidi *et al.*, 2015). The aim of this study was to investigate the effect of three different formulation types on increasing the effectiveness of indoxacarb against cotton leafworm, *S. littoralis* under laboratory conditions.

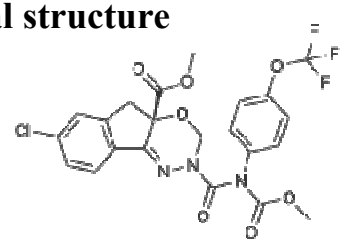
Materials and methods

1. Tested pesticide used:

Indoxacarb:

Indoxacarb is an oxadiazine pesticide developed by Du Pont Ltd., New Zealand that acts against lepidopteran larvae. Its main mode of action is via blocking of neuronal sodium channels.

Chemical structure



IUPAC name:

Methyl7-chloro-2,5-dihydro-2-[[(methoxycarbonyl)[4(trifluoromethoxy)phenyl] amino]carbonyl] indeno[1,2-e][1,3,4]oxadiazine-4a(3*H*)-carboxylate

2. Indoxacarb formulation types used:

2.1. EC: Emulsifiable concentrate

2.2. SC: Suspension concentrate = Flowable concentrate

2.3. WG: Water Dispersible Granule

3. Insect pest used:

The laboratory strain of cotton leafworm *S. littoralis* was obtained from Plant Protection Research Institute, Agricultural Research center, Giza, Egypt to bioassay test. It was reared on castor oil leaves in laboratory under constant conditions of 27 ± 2°C, photoperiod of 14 hrs light and 10 hrs dark and 65 ± 5 % RH.

4. Bioassay tests:

Three formulation types of indoxacarb (emulsifiable concentrate, suspension concentrate and water dispersible granule) as well as control, were evaluated against cotton leafworm *S. littoralis* by leaf-dip bioassay method using castor oil leaves as described by Tabashnik *et al*. (1991). Four concentrations were prepared from each formulation type of indoxacarb (15, 30, 50 and 100 ppm). Castor oil leaves were first washed with distilled water then dipped in pesticide solution of different concentrations for 30 sec. and then air-dried. Five replicates were used for each concentration. Then leaves were placed individually into plastic cups (replicate). Twenty individuals of third instar larvae of *S. littoralis* were placed in each prepared plastic cups. Each cup was tightly covered with a piece of fine cotton cloth by means of a rubber band. Larvae were allowed to feed for 48 hrs on treated leaves. Larval mortality was recorded after 48 hrs post treatment. The mortality percentages were corrected by Abbott's formula (Abbott, 1925). Results

were illustrated graphically as log/probit regression lines. Median lethal concentration (LC_{50}), slope values and 95 % fiducially limits were estimated by Finney's probit analysis method (Finney, 1971). Also, the toxicity index was calculated according to Sun's equation (1950).

5. Spray solution properties at field dilution rate:

The physicochemical properties for the spray solution of the three formulations were determined according to the following standard methods:

5.1. Surface tension: It was determined by using Du-Nouytensiometer for solutions containing 0.5 % (W/V) surfactant according to **ASTM D-1331 (2001).**

5.2. Viscosity: It was determined by using Brook field viscometer Model DVII+ Pro, where centipoise is the unit of measurement according to **ASTM D-2196 (2005).**

5.3. Electrical Conductivity: It was determined by using Cole-Parmer PH/Conductivity meter 1484-44, where µmhos is the unit of electrical conductivity measurements according to **Dobrat and Martijn (1995).**

5.4. PH: It was determined by using Cole-Parmer PH conductivity meter 1484-44 according to **Dobrat and Martijn (1995).**

Results and discussion

The efficiency of three formulation types (EC, SC, WG) of indoxacarb was evaluated on the third instar larvae of laboratory strain of cotton leafworm *S. littoralis* under laboratory condition. As shown in Table (1) the highest mortality percentage was with the treatment by EC followed by SC and the lowest mortality percentage was with the treatment by WG formulation type.

Based on the LC_{50} values of the tested formulation types, the present results indicated that all the tested formulation have larvicidal activities against 3th instar larvae of *S. littoralis*. As shown in Table (2), EC formulation of indoxacarb proved to be the most toxic formulation compared with other tested formulations, the corresponding LC_{50} value was 9.66 ppm followed by SC, where the corresponding LC_{50} value was 24 ppm then WG, LC_{50} value was 51.84 ppm. These results indicated that the EC formulation type was more effective on the efficiency of indoxacarb against *S. littoralis* compared with the SC and WG formulations.

It was reported that the physicochemical properties of the spray solution of the pesticide formulation determine strongly the efficiency of the pesticides. The increase in viscosity and electrical conductivity could result in an increase in the pesticide efficiency as stated by Tawfik and EL-Sisi (1987) and Richardson (1974). The three formulations under study showed high and relative viscosity and conductivity values. But the emulsifiable concentrate (EC) formulation showed the lowest surface tension followed by the suspension concentrate (SC) and the water dispersible granule (WG) formulation, indicating the greater larvicidal efficiency according to Osipow (1964) who stated that the decrease in surface tension of the spray solution can result in an improved wet-ability and spreading on the treated surface with a consequence increase in the pesticide activity (Table, 3). Also, the pH of spray solution (level of acidity) plays an important role in the stability and effectiveness of pesticides. The lower pH in the spray solution leads to prevent degradation of pesticide active ingredients caused by the high pH spray solution (Tawfik and EL-Sisi, 1987). In the present study, EC formulation was lowest in pH value compared with other both formulations. Another explanation for the higher efficiency of the EC formulation compared to the SC and the WG is the solvent used for the preparation of each formulation, EC

formulation contain an organic solvent while the other two formulations contains an aqueous solvent. In spite of the latter formulations were environmentally friendly than the other former one.

Table (1): Effect of three formulation types from indoxacarb, emulsifiable concentrate (EC), suspension concentrate (SC) and water dispersible granule (WG) on the mortality % of cotton leafworm *Spodoptera littoralis* under laboratory condition.

Indoxacarb conc.	15 ppm			30 ppm			50 ppm			100 ppm		
Formulation types	EC	SC	WG	EC	SC	WG	EC	SC	WG	EC	SC	WG
Mortality % corrected by Abbot's formula	62	31	13	89	58	23	96	79	45	96	93	82

Table (2): Susceptibility of the 3rd instar larvae of the cotton leafworm *Spodoptera littoralis* to three formulation types, emulsifiable concentrate (EC), suspension concentrate (SC) and water dispersible granule (WG), from indoxacarb.

Formulation type	LC_{50} ppm	Confidence limits 95%		Slope ± SE	Toxicity index %
		Lower (ppm)	Upper (ppm)		
EC	9.66	3.00	15.09	2.15 ± 0.54	100
SC	24.00	16.65	30.82	2.35 ± 0.47	40.25
WG	51.84	43.44	63.35	3.61 ± 0.58	18.63

Table (3): Physicochemical properties of the spray solution of the tested three formulations emulsifiable concentrate (EC), suspension concentrate (SC) and water dispersible granule (WG) at field dilution rate.

Physical properties and formulation	Surface tension Dyne/cm	Viscosity Cm/poise	Conductivity µ mohs	PH
EC	31.5	9.87	495	6.31
SC	42	8.87	363	7.80
WG	47.6	8.18	296	8.25

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Egypt. J. Plant Prot. Res. Inst. (2019), 2 (3): 465 - 479

Egyptian Journal of Plant Protection Research Institute

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Redescription and population dynamic of some genera from subfamily Phyllocoptinae (Prostigmata: Eriophyoidae)

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ARTICLE INFO
Article History
Received: 9/ 7 / 2019
Accepted: 5 / 9 /2019

Keywords
Taxonomy, population dynamics, Eriophyoidae, *Tegonotus mangiferae, Oxycenus maxwelli* and Egypt.

Abstract:

Four species of eriophyoid mite belonged to three genera from subfamily Phyllocoptinae Nalepa (Prostigmata: Eriophyoidae) are redescribed. In addition, the population dynamics of two species, *Oxycenus maxwelli* (Keifer) and *Tegonotus mangiferae* (Keifer) was conducted at El-Fayoum Governorate on olive and mango trees during two successive years 2017 and 2018. The samples were collected from leaves, buds, branches and grass. The obtained results showed that, four species were recorded and illustrated then arranged in taxonomical key, while the population dynamics were recorded two peaks *O.maxwelli* and *T. mangiferae*. The mentioned species were varied in their occurrence rate according to different locations and host plants. On the other hand, the population was positively correlated with the prevailing temperatures and was negative significant correlation with the relative humidity for two successive seasons.

Introduction

The subfamily Phyllocoptinae (Prostigmata) is one of the most specious taxa in Eriophyoidea. It includes more than 1100 species belonging to nearly 100 genera (Oldfield, 1996 and Chetverikov, 2006). So far sixty eight of eriophyid species have been recorded in Egyptian fauna, twenty nine species and nineteen genera of them are belonging to subfamily Phyllocoptinae varied in their hosts where sixteen species are reported on fruit trees, nine species reported on ornamental plants, two species reported on vegetable crops and two species on grasses (Hassan, 1934; Attiah, 1955; Soliman and Abou-Awad, 1978; Zaher *et al*., 1978; Zaher and Abou-Awad, 1979; Abou-Awad, 1981; Zaher, 1984; Abou-Awad and Nasr, 1983; Abou-Awad and Elsawi, 1993 and Abou-Awad *et al*., 2011. The tribe Tegonotiniwas established by Bagdasarian (1978) and

consists of about 146 species in 25 genera. Among them, 46 species belonged to genus *Tegonotus* Nalepa. They are easy to differentiate from all other Phyllocoptinae by the presence of lateral spines or lobes on the opisthosoma. The olive bud mite *Oxycenus maxwelli* (Keifer) causes enough damage in bud andleaves. Furthermore, *O. maxwelli*as the common name reveals falls into the bud mite category because they lay their eggs near buds and feed on bud tissue. On the other hand, *Tegonotus mangiferae* (Keifer) attacks the lower surface of mango leaflets causing leaves deformations (Zaher, 1984).Therefore, the present study aims to throw a light on the taxonomical changes in subfamily Phyllocoptinae according to the Egyptian agroecosystem changesduring the few recent decades and also, the role of environmental fluctuation on the populations of the eriophyoid mites *O. maxwelli* and *T. mangiferae*.

Materials and methods

Survey of certain species belonging to tribe Tegonotini Bagdasarian, family Phytoptidae Murray were conducted at four Egyptian Governorates, Qualiubiya, Giza, EL-Fayoum and El Behera Governorates during two successive years 2017 and 2018. This study was as a part of a comprehensive work on eriophyoid mites .The samples were collected during two years (2017 and 2018) from leaves, buds, branches and grass. The samples were individually bagged in tightly-closed plastic bags and transported the same day to the laboratory. Collected mites were removed using a fine hair brush under dissection stereo-microscope, then preserved in 70% ethanol. Selected mites were cleared and mounted on micro-slides by using Keifer medium according to Keifer (1975), then dried at 40°C for one week (Zhang, 2003) and finally examined under a Carl Zeiss compound microscope. The type materials are deposited as slide mounted specimens in the mite collection of the Agricultural Research Center (ARC), Plant Protection Research Institute (PPRI), Fruit Acarology Department, Dokki, Egypt.

Identification to a specific family, subfamilies and genus of subfamily Phyllocoptinae were described using the genera world key by Amrine (2003). The species was identified using the published descriptions of family Phytoptidae species. In addition, the identification specimens were compared with the collection specimens' mite which, located in Plant Protection Research Institute (ARC).

On the other hand, the population dynamics of two Eriophyoids mites, *O. maxwelli* and *T. mangiferae* were conducted at EL-Fayoum Governorate during the two successive years 2017 and 2018.

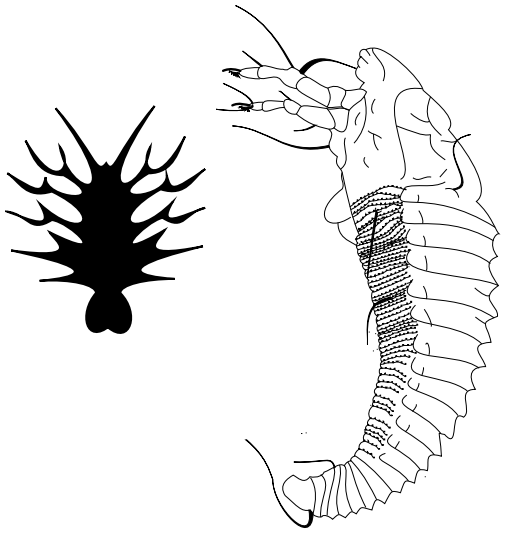
Results and discussion

1. Taxonomical studies:

Data in Table (1) shows the taxonomical differences between three tribes, Tegonotini Bagdasarian, Phyllocoptini Nalepa and Anthocoptini Amrine and Stasny belonged to subfamily Phyllocoptinae in Egypt which provided as follow:

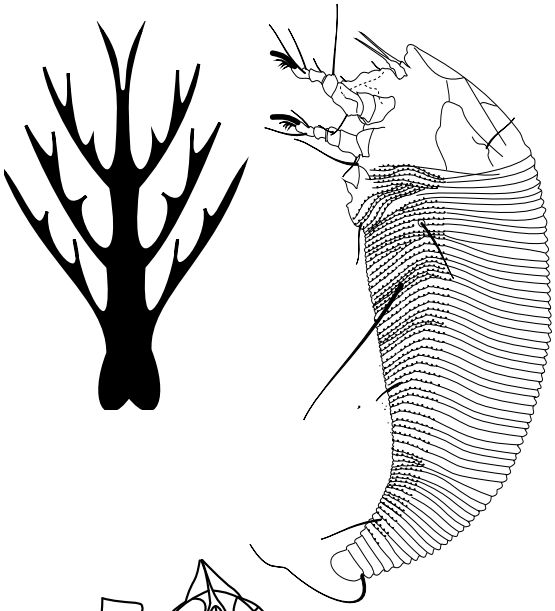
Table (1): Key (1), tribes' subfamily Phyllocoptinae Nalepa1892 in Egypt

1. Empodium entire; scapular tubercles and setae present; opisthosoma, viewed dorsally, with lateral lobes or pointed projections from some or all annuli, or with a plate behind prodorsal shield bearing lateral extensions…….**Tegonotini Bagdasarian 1978 (Key2).**

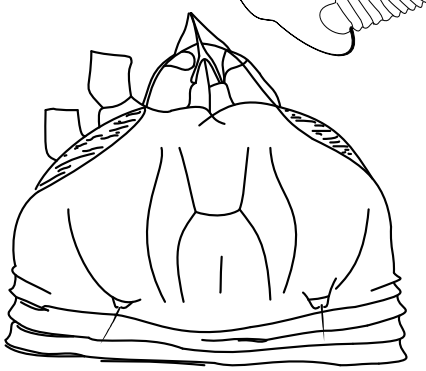


- Empodium entire; Scapular tubercles and setae present; opisthosoma viewed dorsally with annuli evenly downcurved over lateral opisthosomal margins; dorsum varying from evenly arched in cross section to flattened, ridged or furrowed……..2

2. Scapular setae usually with well-formed, often plicate, tubercles placed ahead of rear shield margin, directing setae forward, up or centrad; if tubercles and setae are near rare shield margin, thin tubercles are subscylindrical and bent forward or the alignment of their bases is longitudinal or diagonal to the body **……..……..Phyllocoptini Nalepa 1892**



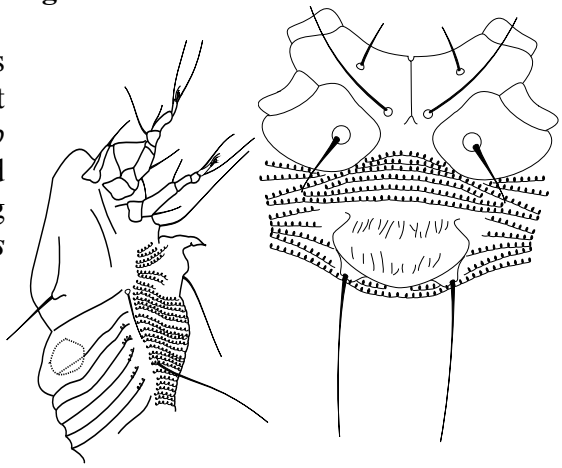
- Scapular setae with tubercles on or very near the rear shield margin, directing setae to rare, usually divergently; scapular tubercles either subcylindrical, or the alignment of their bases is transverse to the body…**…….Anthocoptini Amrine and Stasny 1994**



The presented study was concentrated on tribe Tegonotini Bagdasarian that included three genera and four species (Table, 2).

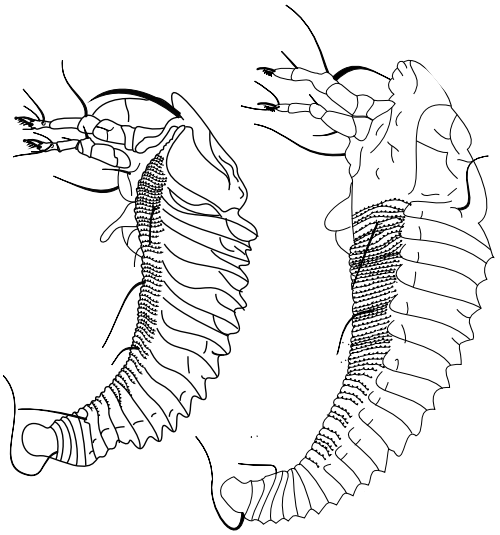
Table (2): Key (2), genera of tribe Tegonotini Bagdasarian 1978

1. Prodorsal shield and annular projections rounded, not spin-like. Prodorsal shield without posterior lobe over opisthosoma. Coxal setae *1 b* present. A deep cleft between prodorsal shield and opisthosoma; first annuals large, projecting higher than other annuli **.....*Neotegonotus* Newkirk and Keifer 1971.....Key(3)**



- Prodorsal shield and opisthosoma not separated by a deep cleft; first annulus not enlarged**…….....**2

2. Posterior opisthosoma with a dorsal depression just above setae *f*; *sc* near rare shield margin, directed posteriorly ***Oxycenus*** **Keifer 1961……...…….Key (4)**
- Posterior opisthosoma without rear depression; *sc* variable; scapular setae ahead of rear shield margin direction variable; Tibial setae present; *sc* directed up, medially or laterally. Frontal lobe not emarginated**.....*Tegonotus* Nalepa 1890.....(Key5)**



Key (3): Species of genus *Neotegonotus* Newkirk and Keifer 1971

Genus *Neotegonotus* represented by only one species (*Neotegonotus sycamori* Abou-Awad, 1984) in Egypt.

***Neotegonotus sycamori* Abou-Awad, 1984 (Figure, 1):**

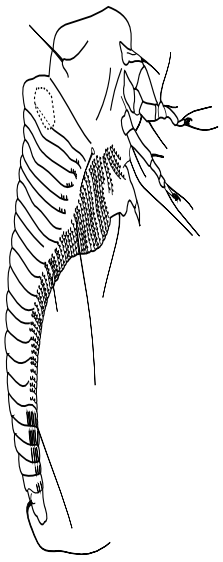
This species was recorded on leaves of *Ficus sycamorus* L. (Moraceae).

The common taxonomic characters are:

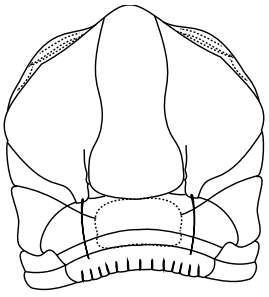
1. 4 rayed featherclaw.
2. Dorsal shield with prominent anterior lobe.
3. Shield design tending to be obscure and marked by internal line from each tubercle, extending around shield margin to form as semicircular disc.
4. Complete admedian line, meeting and forming nearly Jug shape.
5. Coverflap genitalia of female with close-set longitudinal ribs in two series ,

Synonyms: *N/A*

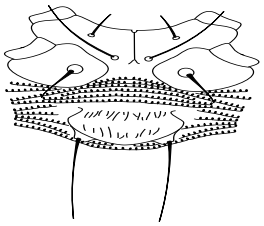
Host plant: Recorded on leaves of *F. sycamorus*.



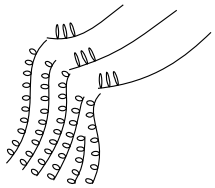
lateral of male



Anterior dorsal

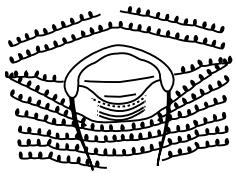


Genetal of female



Lateral

Empodium



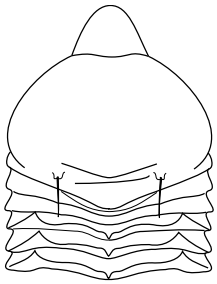
Genetal of male

Figure (1): *Neotegonotus sycamori*

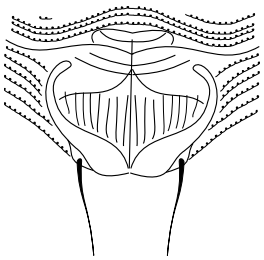
Key (4): Species of genus *Oxycenus* Keifer 1961

Genus *Oxycenus* represented by two species (*Oxycenus maxwelli* (Keifer, 1939) and *Oxycenus niloticus* Zaher and Abou-Awad, 1979) in Egypt.

1.Dorsal shield without any pattern except two transverse lines posteriorly; ventral microtubercles oval shape; coverflap genetalia with 16 longitudinal idges**.....*Oxycenus niloticus* Zaher and Abou-Awad 1979**

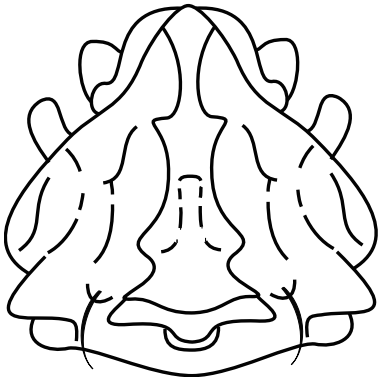


Anterior dorsal

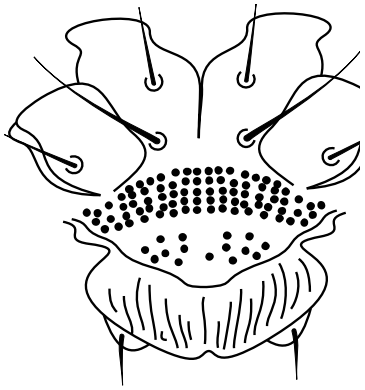


Genetal of female

- Dorsal shield with longitudinal broken lines ; ventral micro tubercles not oval; coverflap genetalia with 17 longitudinal ridges…….***Oxycenus maxwelli* (Keifer1939)**



Anterior dorsal



Genetal of female

***Oxycenus niloticus* Zaher and Abou-Awad, 1979) (Figure, 2):**

Synonyms: *N/A*

Host plant: Recorded on leaves and buds of *Olea europaea* L. (Oleaceae)

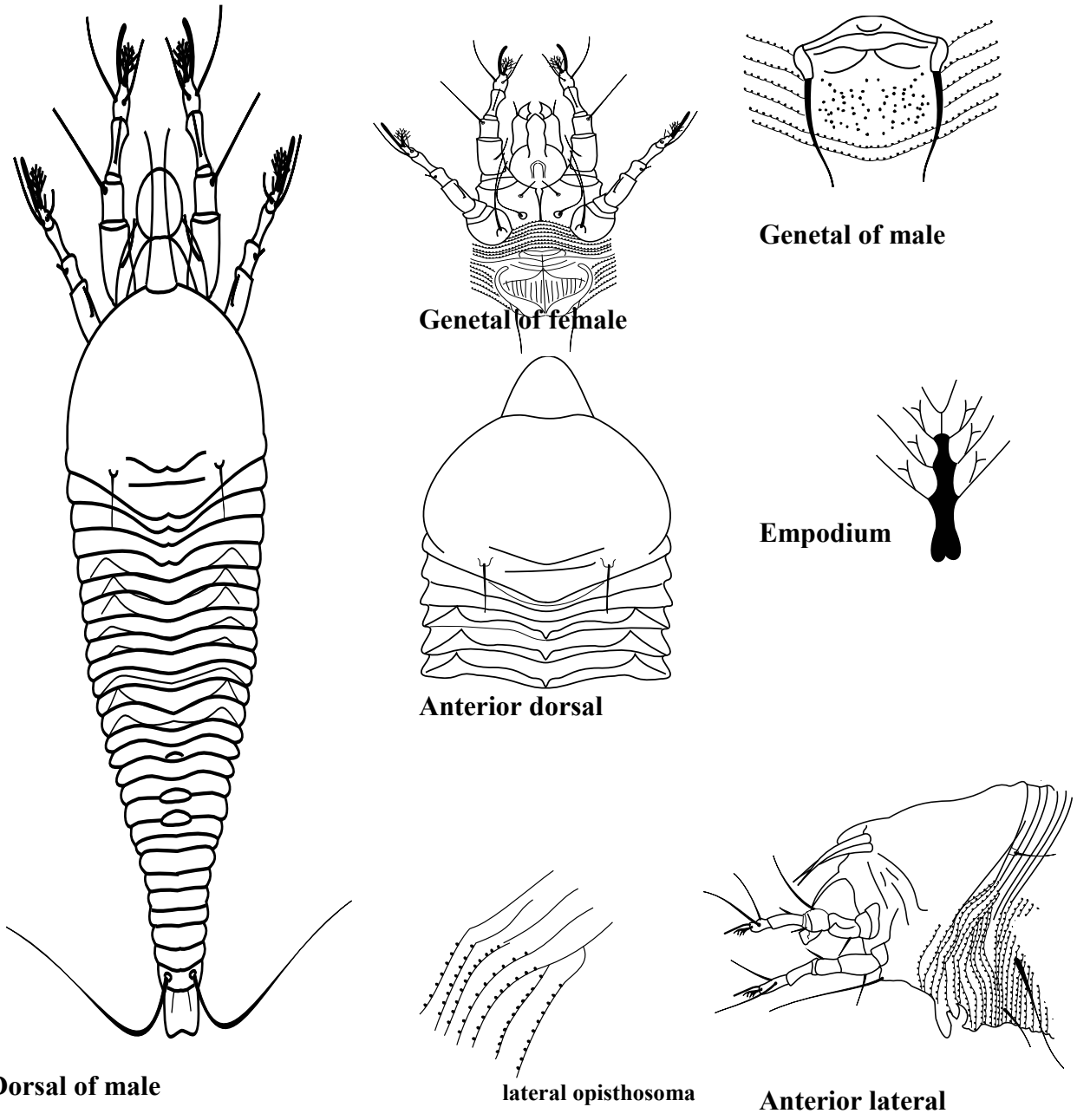


Figure (2): *Oxycenus niloticus*

***Oxycenus maxwelli* (Keifer 1939) (Figure,3):**

Synonyms: *Oxypleurites maxwelli* (Keifer 1939)

Host plant: Recorded on buds of*Olea europaea* L. (Oleaceae)

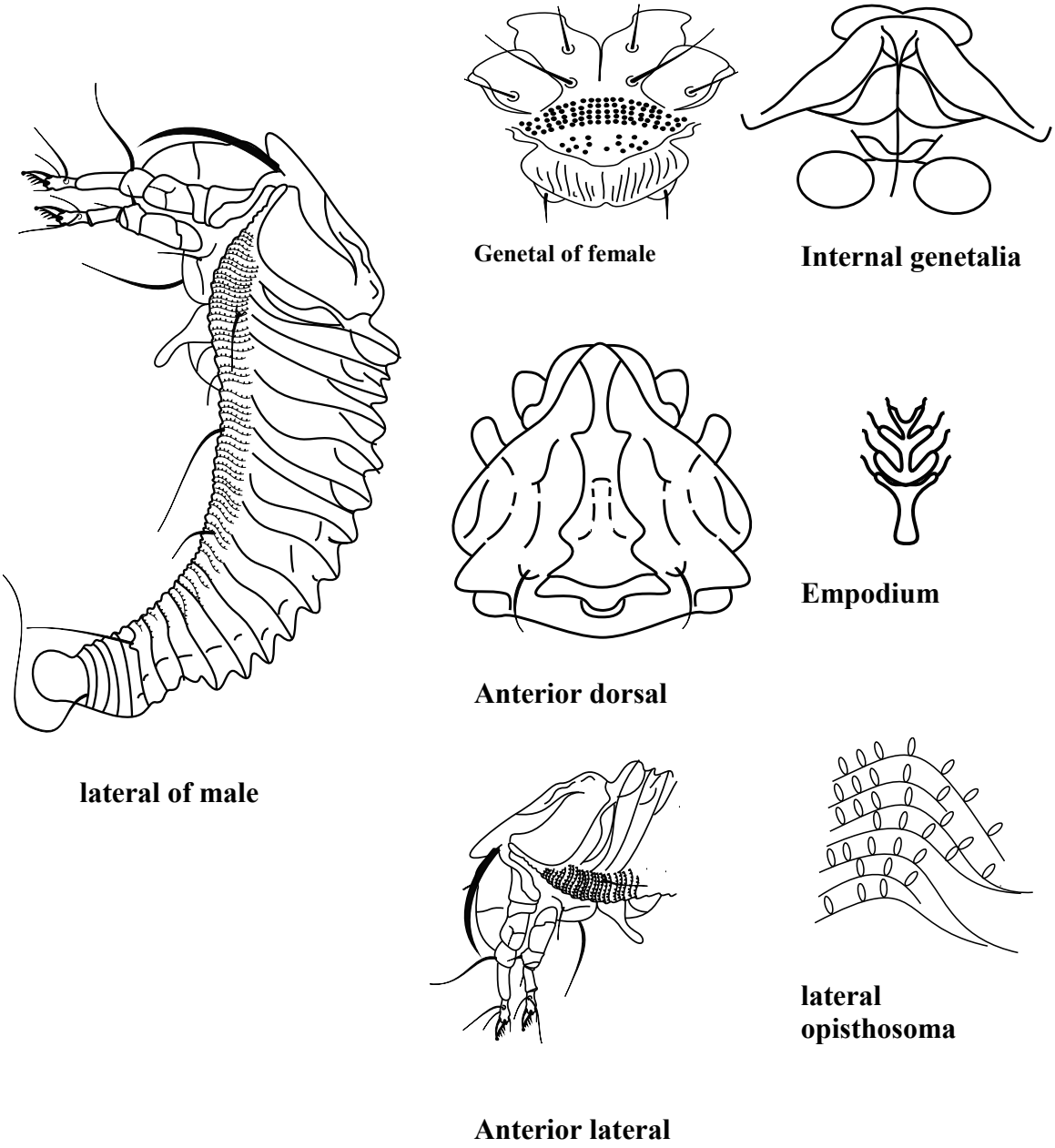


Figure (3): *Oxycenus maxwelli*

Key (5): Species of genus *Tegonotus* Nalepa 1890

Genus *Tegonotus* represented by only one species (*Tegonotus mangiferae* (Keifer, 1946)) in Egypt.

***Tegonotus mangiferae* (Keifer 1946) (Figure, 4):**

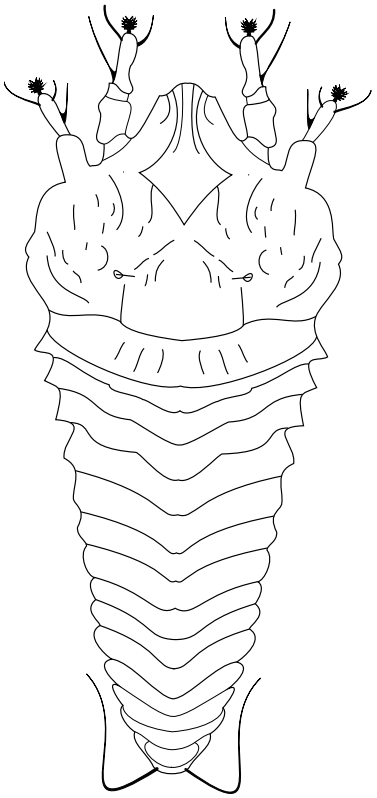
This species was recorded on leaves of *Mangifera indica* L.(Anacardiaceae)

The common taxonomic characters are:

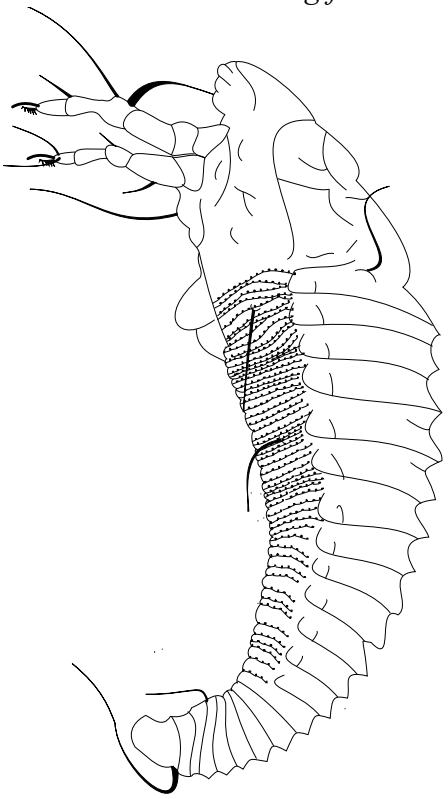
1. 6 rayed featherclaw.
2. Dorsal shield sub triangular and rough, dorsal tubercles a head of the rear margin, middorsal longitudinal ridges on thanosome.
3. Lateral tergal lobes pointed especially the anterior one.
4. Complete admedian line, meeting and forming nearly Jug shape.
5. Side of coverflape genitalia centrally converging ribs, base of coverflape with granulated lines.

Synonyms: *Oxypleurites mangiferae* Keifer 1946

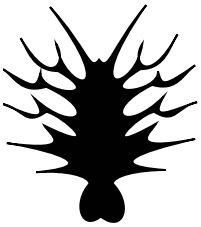
Host plant: Recorded onleaves of *Mangifera indica* L.(Anacardiaceae)



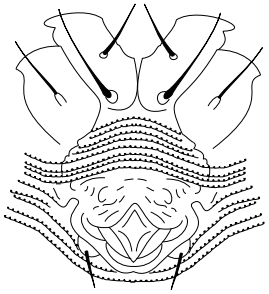
Dorsal of male



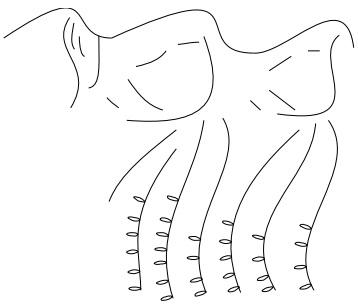
lateral of male



Empodium



Genetal of female



lateral opisthosoma

Figure (4): *Tegonotus mangiferae*

2. Ecological studies:

2.1. Population dynamics of *Oxycenus maxwelli* :

Figure (5) showed monthly average population number of *O. maxwelli* during year 2017. It showed that, the population started with low individuals number, then increased gradually by the temperature increasing from January, 2017 till reached the first peak population level at 70 individuals per 20 leaves in May, 2017when the temperature and relative humidity were recorded 32°C and 30% at the first peak, respectively.Then the population decreased gradually by the temperature increasing fromJune, 2017 till reached the minimum population levels at 35 *O. maxwelli* individuals in July, 2017, while the temperature and relative humidity were recorded 36°C and 33% , respectively. The *O. maxwelli* population returned to increase again by the temperature decreased from August, 2017 till reached 100 individuals in September, 2017. While the temperature and relative humidity were 31°C and 41%, respectively. This was the second peak level of *O. maxwelli* individuals. On the other hand, the population number of *O. maxwelli* began to decrease again from October, 2017, by the temperature decreased, till reached the 22 individuals at the end of year 2017, while the temperature and relative humidity were 20 and 49%, respectively.

Figure (6) showed monthly average population number of *O. maxwelli* during year 2018. It shows that, there was no differentiation between the data obtained for either year 2017 and 2018. The two peaks levels were recorded 68 and 160 individuals at May and September 2018. While the temperature were 32 and 33°C, respectively and the relative humidity were 29 and 40%, respectively.

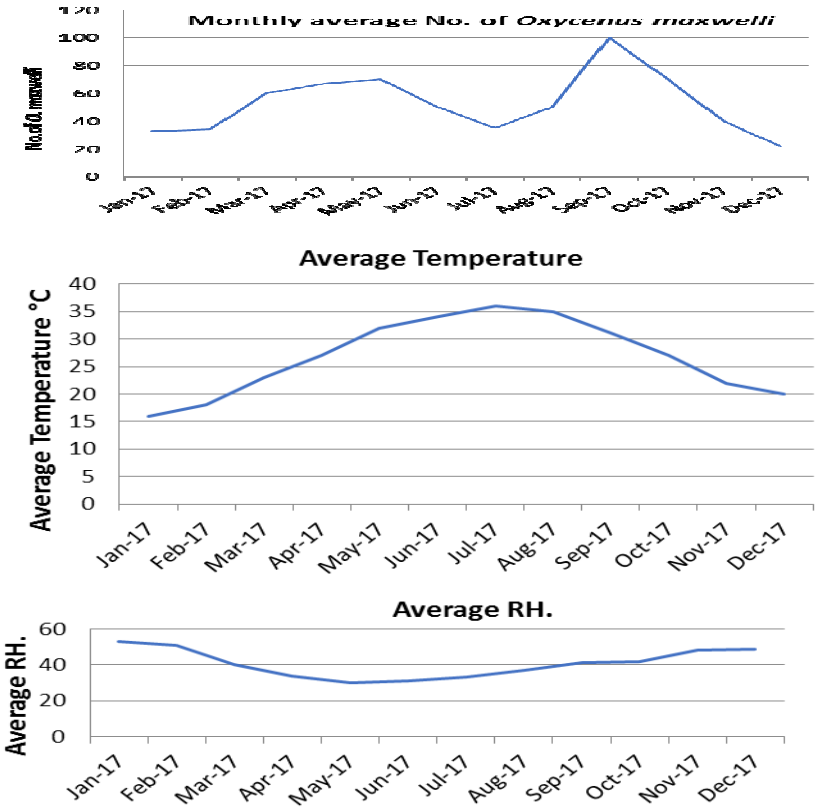


Figure (5): Monthly average numbers of *Oxycenus maxwelli* during year 2017

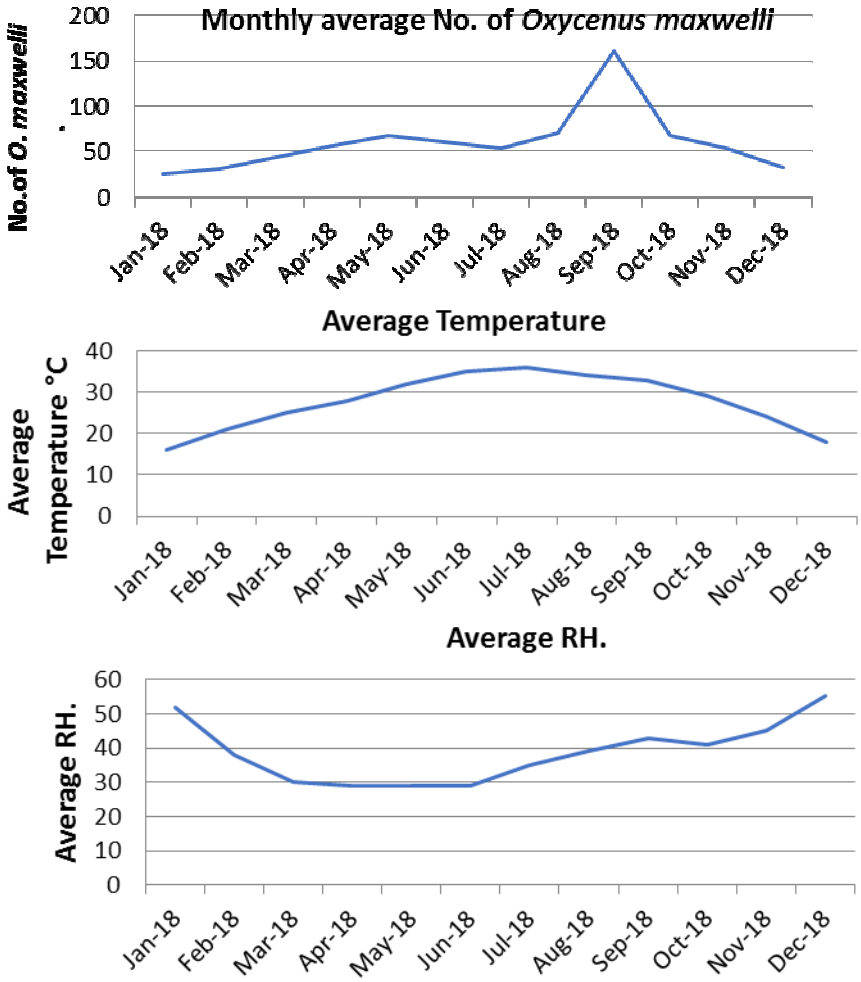


Figure (6): Monthly average numbers of *Oxycenus maxwelli* during year 2018

2.2.Population dynamics of *Tegonotus mangiferae*:

The population trend of the eriophyid mite species *T. mangiferae* was differed from the eriophyid mite *O. maxwelli* that mentioned above, whereas the Figure (7) showed that, the population was started with high numbers, 312 individual, at temperature 16°C and relative humidity 53 %. Then the population was decreased gradually from February , 2017 till reached the minimum population level at 132 individual in June, 2017 when the temperature and relative humidity were recorded 34°C and 31%, respectively. The population began to build up his number again until reached the peak levels at 724 individuals in October, 2017, while the temperature and relative humidity were 27°C and 42%, respectively. Finally, the population returned to decrease again in November (544 individuals) and December (404 individuals), 2017. While the temperature was 22°C and 20°C and relative humidity were 48%and 49%, respectively. On the other hand, Figure (8) showed monthly average population number of *T. mangiferae* during year 2018, which shows no different from obtained data in year 2017. The peak level was recorded 872 individuals at October when the temperature was 29°C and the relative humidity 41%.

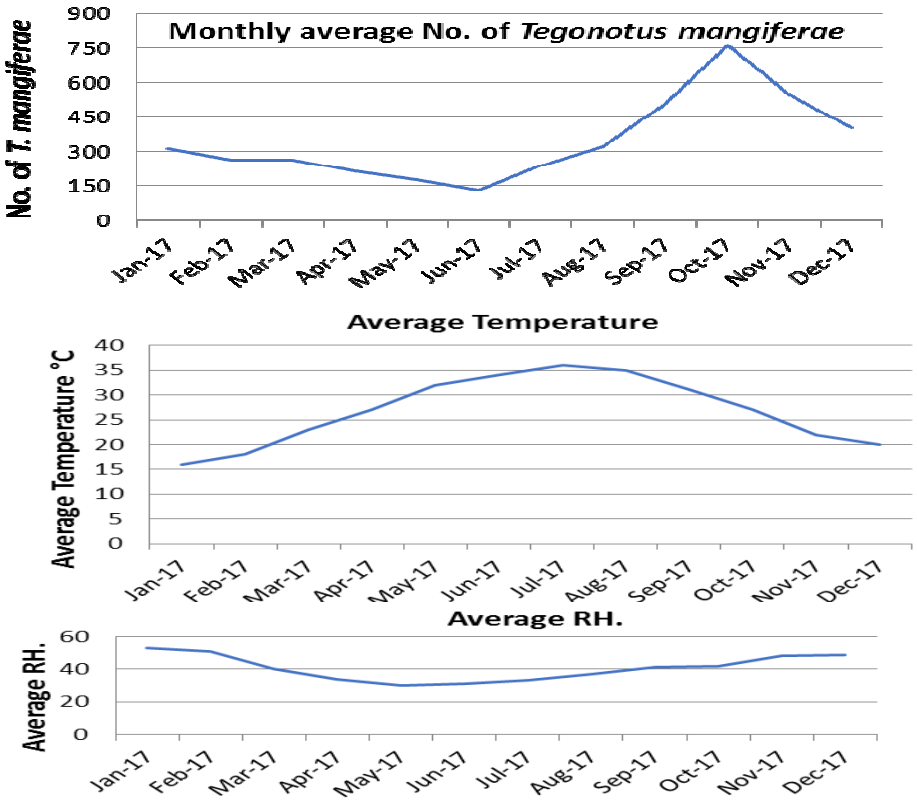


Figure (7): Monthly average numbers of *Tegonotus mangiferae* during year 2017

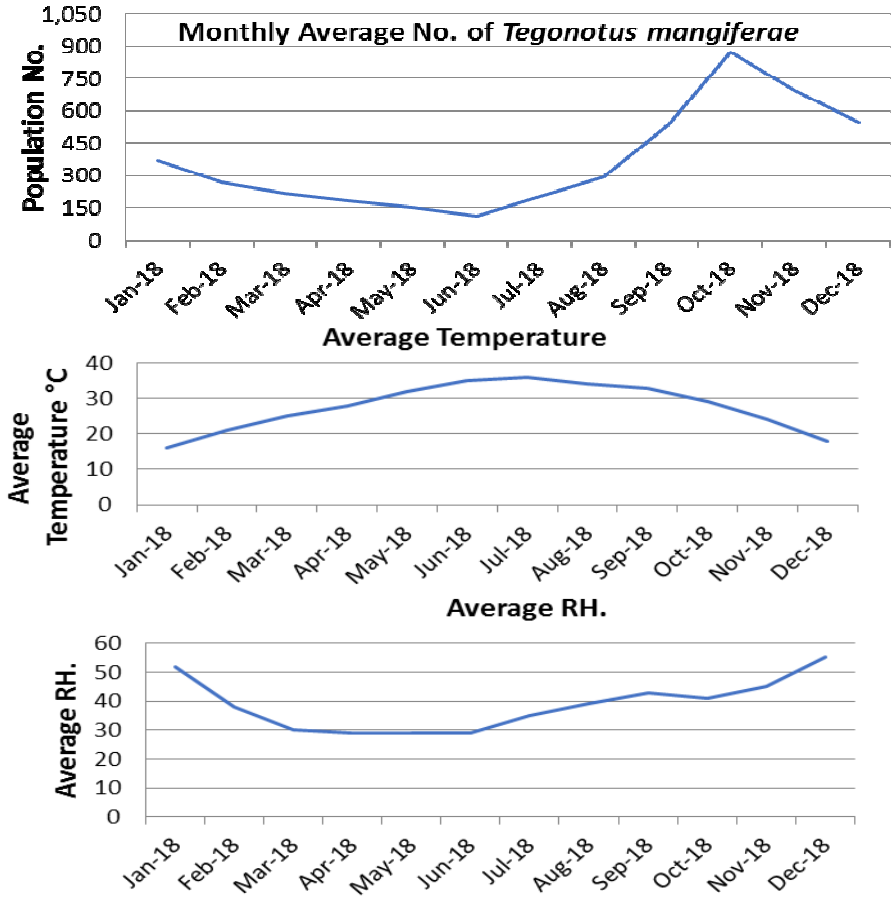


Figure (8): Monthly average numbers of *Tegonotus mangiferae* during year 2018

Although the subfamily Phyllocoptinae is one of the important group in terms of their economic damage, it included twenty nine species in Egypt while, it included 1100 species world wide. Only one collective work had been conducted by Zaher (1984). This work was included subfamily Phyllocoptinae which, contents of three tribes i.e.: *Tegonotini* Bagdasarian; *Phyllocoptini* Nalepa and *Anthocoptini* Amrin & Stasny. Our studies indicated that Tribe Phyllocoptini has three genera i.e: *Neotegonotus* Newkirk and Keifer, *Oxycenus* Keifer, Tegonotus Nalepa. Ancient studies were placed the two genera *Tegonotus* and *Oxycenus* into genus *Oxypleurites* (Keifer, 1939 and 1946). These during his reported the two species *Oxypleurites maxwelli* (Keifer, 1939) and *Oxypleurites mangiferae* Keifer, 1946. Actually, our studies placed the two species under genera *Oxycenus* Keifer (*Oxycenus niloticus* Zaher and Abou-Awad 1979 and *Oxycenus maxwelli* (Keifer) 1939 on leaves and buds of Olea *europaea* L.) and *Tegonotus* Nalepa (*Tegonotus mangiferae* (Keifer, 1946 on leaves *Mangifera indica* L.). Table (3) showed the correlation coefficient between average temperatures and relative humidity on population of mites on olive and mango trees during two successive years 2017 and 2018. The population was positively correlated with the prevailing temperatures for two successive seasons, while it was negative significant correlation with the relative humidity for two successive seasons (Table, 3). Although the data indicates that the temperature between 25 and 31°C is most convenient for increasing the population numbers of either *O. maxwelli* or *T. mangiferae* which achieved during the two months may and September, 2017, there were some months have the same temperature ranges which are suitable for increasing the eriophyid mites. It may be concluded that during certain periods, the population numbers of either *O. maxwelli* or *T. mangiferae* are affected with other ecological factors. In addition, it may become more predominant so as to over shadow other factors. These results concur with those of previous studies showing that aerial dispersal of eriophyid mites occurs throughout the season and seems to be independent of population density or host plant quality (Sabelis and Bruin 1996). Lawson *et al.* (1996) reported similar observations for the European red mite, *Panonychus ulmi* (Koch.) and speculated that a density-independent proportion of mites committed to disperse. Sabelis and Bruin (1996) made the same tentative interpretation from data on vagrant species of eriophyids. The underlying mechanisms that trigger dispersal in some proportion of females in every population remain unknown. However, dispersal-related mortality is probably high and may be partially affected by within-tree for either *O. maxwelli* or *T. mangiferae* distributions. Allen and McCoy (1979) found high eriophyid populations in the northand south-bottom quadrants of trees, where temperatures were favorable for development and the lowest mite densities in the south-top quadrant, where lethal temperatures were recorded.Most leaflets of mango trees are infested with powdery mildew of mango, *Oidium mangiferae* Berthet that gives a reason for this distribution pattern, research on the aerial dissemination of fungal spores from a plant canopy may help our understanding of the dispersal success of eriophyid. Aylor (1990) reviewed the dissemination of fungal pathogens by wind. The majority of fungal spores are often produced in the lower portions of the plant canopy because of favorable conditions there. Lower wind-speed and less turbulence in the lower canopy limit the escape of those spores except during a period around midday, when wind-speed and turbulence near the ground are usually

highest. Gradients of disease severity typically decrease rapidly with increasing distance from the source of spores. However, eriophyid mites carried upward from the canopy and transported long distances would likely suffer extremely high mortality as a function of prolonged exposure to the elements, and a greatly reduced probability of being deposited on a suitable host plant. Regardless of the mortality that occurs during dispersal which affect on the achieved the eriophyid mited beak point, these data show that some mites were carried from a grove to an adjacent grove downwind and that the percentage of mites arriving at the downwind location depended on the distance between the groves.

Table (3): Correlation coefficient between average temperatures and RH% on the population of mites on olive and mango trees during two successive years 2017 and 2018.

Correlation factors	Population correlation coefficient values of			
	O. maxwelli		*T. mangiferae*	
	2017	2018	2017	2018
Temperature	0.425	0.580	0.438	0.646
Rh	-0.443	-0.104	-0.204	-0.283

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Effect of different prey types and temperature on biological aspects of predatory mite *Protogamasellus discorus* (Acari: Gamasida: Ascidae) with special references of chemical analysis of prey

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ARTICLE INFO

Article History

Received: 7 / 7 / 2019

Accepted: 12 / 9 /2019

Keywords

Predatory mite, *Protogamasellus discorus*, biology, temperature and prey.

Abstract:

The predatory mite *Protogamasellus discorus* Manson (Acari: Gamasida: Ascidae) was isolated from soil under debris of palm trees at Giza Governorate and reared under laboratory conditions of (25 and 30 ºC) on bulb mite *Rhizoglyphus robini* Claparède (Acari: Astigmata: Acaridae), larvae of *Musca domestica* L. (Diptera: Muscidae) , free living nematode *Rhabditella masculata* and three species of fungi *Fusarium oxysporium*, *Asperagillus niger* and *Pencilium notatum*. Obtained results showed that significant effects of different prey and temperature on biological aspects, fecundity and reproduction, whereas, life cycle duration lasted (9.9 and 9.4), (12.5 and 10), (10.7 and 9.4), (15.6 and 13.1), (10.9 and 9.6) and (9.2 and 8.8) days, when the predatory mite *P. discorus* fed on the above mentioned diets at 25 and 30 ºC, respectively. Female fecundity affected by temperatures and food types, where it is generally increased as temperature increased, also it being higher with free living nematodes (61.4 and 64.5) followed by *R. robini* (59.6 and 62.0) and larvae of *M. domestica* (32.3 and 36.5) eggs at 25 and 30 ºC, respectively, while, deposited eggs are very low with fungi, the rate of reproduction was greater at 25℃ when mite fed on fungi and at 30℃ on other prey where, the highest rate obtained with nematodes while the lowest with *P. notatum*. Chemical analysis of some prey showed that free living nematodes contain the highest percent of phosphorus (2.9%), so females deposited the highest number of eggs when it fed on nematodes than others.

Introduction

Predacous mites play an important role in biological control of associated pests in different habitats, i.e. aerial and soil organisms. Mesostigmatic mites consider one of the most important groups, they are numerous and differ in

their feeding habits, some are predators of aerial pests infesting different crops, while other species live in soil and organic manure feeding on soil pests, mites, immatures, while some of them are fungivorous. Researches by several authors were mainly concerned with survey, morphology and taxonomy of vast number of species in different countries of the world. The biology of some predacous and parasitic mites attracted many investigators e.g. Hoda *et al*., 1986; Nawar and Nasr, 1988; Ibrahim *et al*., 1989; Ali, 1994; Taha, 1991 and Taha *et al.,* 2006.

The present work aims to study: 1. The effects of different prey and fungi on biological developmental stages and fecundity of the predatory mite *Protogamasellus discorus* Manson (Acari: Gamasida: Ascidae) under laboratory conditions. 2. Estimation of the rate reproduction as affected by prey types and rearing temperature. 3. Analysis of phosphorus contents in the prey. 4. Chemical analysis of dry matter, crude protein, ether extracts, nitrogen and ash, of different prey.

Materials and methods

1.Collection:

The predatory mite *P. discorus* was extracted from soil under palm trees associated with acarid mites, free living nematodes and other organisms at Giza Governorate. Samples were freshly transferred to laboratory for extraction using modified Tullgren funnels for 24 h. Mites received in petri dishes filled with water. Living specimens were examined using stereomicroscope and collected by camel hair brush for biological experiments.

2. Specimens identification:

Adult individuals were firstly cleared in Nesbitt's solution, then mounted in Hoyer's medium on glass slides and examined microscopically for identification; slides were labeled with locality, stage, sex and date of mounting.

3. Rearing procedures:

The predatory mite *P. discorus* was reared in plastic rings 2.8 cm. in diameter and 2.0 cm. in depth, they were filled up to 0.5 cm. with plaster of Paris and charcoal, drops of water were added daily to maintain suitable relative humidity. For culturing mites, several adult females were placed in plastic rings supplied with food and kept in an incubator at 25 and 30 ºC. For individual rearing, newly deposited eggs were transferred singly to prepare ring. Each newly hatched larva was supplied with a known number of prey and developed individuals replaced daily by fresh ones till reaching maturity. Mites were examined twice daily with the aid of stereomicroscope. Emerging females were copulated and kept for oviposition. Observations concerning all biological aspects were recorded during the predator life span. Each rearing experiment was started with 25 newly hatched larvae, immature stages of bulb mite *Rhizoglyphus robini* Claparède (Acari: Astigmata: Acaridae), larvae of *Musca domestica* L. (Diptera:Muscidae), free living nematode *Rhabditella masculata* and three species of fungi *Fusarium oxysporum*, *Aspergillus niger* and *Penicilium notatum* were used as different types of food at 25 and 30 ºC.

4. Sources of food:

4.1. Immature stages of the bulb mite *R. robini* were obtained from rot infested onion and rearing at laboratory on dry yeast granules.

4.2. *M. domestica*, the same technique of rearing house fly in laboratory described by Mohamed (1976) was used to obtain daily fresh larvae as a main source of food.

4.3. Free living nematodes *R. musculata* as extracted from humified materials which put in Baerman's funnel for 24 hour. The extraction was added to petri dishes contain slides of ornamental bulb on potatoes as a mixture food source for rearing nematodes, petri dishes left for one week in natural condition by using a camel brush, drops of this feeding were put in each rearing cell of mites as the main source of food.

4.4. Fungal culture:

The fungal cultural of *F.oxysporum*, *A. niger* and *P. notatum* were obtained from Plant Pathology Dept., Fac. of Agric., Cairo Univ. These fungi were cultured on agar medium and species from culture.

4.5.Mixture (*)

R. robini, larvae of *M. domestica* and *R. masculata*.

4.6. Mixture (**) *F. oxysporium, A. niger and P. notatum*.

4**.7.** Mixture (***)

R. robini, larvae of *M. domestica*, *R. masculata*, *F. oxysporium, A. niger and P. notatum*.

4.8. Chemical analysis of prey:

Experiments were carried out to shed light on the effect of chemical constituents of different prey types on the whole activity of the predatory mite, *P. discorus*. The prey contents of protein, phosphorus, ether extracts, and nitrogen free extract with amino acids were analyzed in the Central Lab. for Food and Feed, Agric. Res. Center. These contents were estimated using the method of Bhargava and O'Neil (1975).

Results and discussion

1. Biological studies:

1.1. Habitat and behaviour:

The predatory mite *P. discorus* was isolated from soil under debris of palm trees at Giza Governorate. This mite species was reared in laboratory on the bulb mite *R. robini*, larvae of *M. domestica*, free living nematode *R. musculala* and three species of fungi, *F. oxysporum*, *S. niger* and *P. notatum*. Thelytoky in *P. discorus* was observed as unmated females deposited unfertilized eggs which gave rise to only females. It was observed that females deposited their eggs in the substratum. Also, cannibalism was observed for this mite species.

1.2. Hatching:

Eggs are oval white and hatching occur through a medial longitudinal slit surrounding the eggs and dividing the shell into two parts except one slide. Hatching larvae then crawls outside leaving the egg shell.

1.3. Moulting:

Any immature stage when full grown enters a semi quiescent phase. This period lasted for about one hour at a laboratory temperature, after that, the individual bend it's for legs, then stretches its forward without leaving its place and shakes its body laterally then stops. This process is repeated several times and then the mite gets ride of the skin through a ventral longitudinal slit. Moulting process lasted an hour.

1.4. Biological developmental stages:

The incubation period lasted (1.5 and 1.4), (1.7 and 1.5), (1.8 and 1.5), (2.3 and 1.8), (1.5 and 1.6) and (1.5 and 1.4) days at 25 and 30 ºC when adult females were fed on the previous diets , respectively, as shown in Table (1). It is clear that the incubation period was shorter at 30 ºC than that at 25 ºC.

1.5. Total immature stages:

The total immature stages stayed (8.0 and 8.5), (8.5 and 10.5), (7.9 and 8.9), (11.3 and 13.3), (8.1 and 9.3) and (7.8 and 7.7) days at 30 and 25 ºC, when the predatory mite *P. discorus* fed on the above mentioned diets, respectively. Obtained data cleared that food types and temperature affected on immature stages (Table, 1). These results coincided with that obtained by Taha *et al.*, (1988) and 2006.

1.6. Life cycle:

Life cycle period was greatly affected by temperature. It was short at high than low temperature; also, this period was differed according to the types applied food. The duration of life cycle was (9.2 and 9.9) and (8.8 and 9.4) days on *P. notatum* and *R. robini* at 25 and 30 ºC, resepectively, which consider were more favourable than other diets for the predatory mite *P. discorus.*

1.7. Longevity and life span:

The obtained data as shown in Table (1) cleared that female longevity was shorter (25.8 and 17.4) days when it fed on *P. notatum* and prolonged with free living nematodes (28.5 and 22.1) days at 25 and 30 ºC, respectively, while life span was short (30.0) days on *P. notatum* and prolonged to 40.0 and 39.2 days on larvae of *M. domestica* and free living nematodes.

1.7. Female oviposition:

The period of female oviposition found to be affected by different diets and temperature, it is greatly shorter (8.5 and 8.2 days) on *A.niger* and *F. oxysporum* at 30 ºC, while, on the bulb mite *R. robini* (21.1 and 16.9) days at 25 and 30 ºC, respectively (Table, 2). These results agree with those obtained by Nawar and Nasr, 1988 and Ibrahim *et al*., 1989. They found that female of *P. primitis* oviposition period affected by different types of food.

1.8. Female fecundity:

The female fecundity of the predatory mite *P. discorus* was affected by different types of food and temperature, where it is generally increased as temperature increased, also, it is being higher with free living nematodes (61.4 and 64.5) eggs followed by *R. robini* (59.6 and 62.0) and larvae of *M. domestica* (32.3 and 36.5) eggs at 25 and 30 ºC, respectively. Obtained results showed that female fecundity was very low when fed on fungi, whereas, female deposited a total average of 5.0, 4.7 and 2.3 eggs with a daily rate of 0.55, 0.53 and 0.17 eggs when it fed on *F. oxysporum*, *A.niger* and *P. notatum* at 25ºC, respectively. From the above mentioned data, it could be concluded that the free living nematodes is the most suitable diets for mass production of the predatory mite, *P. discorus.* These results are agreed with that obtained by Abou-El-Naga *et al.,* 1987 and Taha *et al*., 1988.

1.9. Feeding capacity as influenced by prey types:

As shown in Table (3) female immature (proto and deutonymph) of *P. discorus* consumed (23.8 and 33.7) and (15.5 and 21.5) individuals of *R. robini* and larvae of *M. domestica* at 25 and 30 ºC., respectively. During the oviposition period, female was at the highest efficiency, it consumed (65.1 and 81.0) and (38.3 and 49.8) individuals of *R. robini* and larvae of *M. domestica* at 25 and 30 ºC, respectively (Table, 3).

1.10. Reproduction and feeding:

This experiment was carried out by keeping virgin females of *P. discorus* in screw cupped glass container (5 cm in diameter) supplied with diet and incubated at 25 and 30 ºC. This species reproduced parthenogntically, after one month, individuals were counted, whereas, experiments was replicated five times. The obtained results in Table (4) showed that level of reproduction was greater at 30 ºC compared to its level at 25 ºC for free living nematodes *R. robini* and *M. domestica*. On the other hand, level of reproduction was greater at 25 ºC than that obtained at 30 ºC for fungi. Reproduction was affected by types of palpitated food. Free living nematodes recorded highest rate, while, fungi recorded lower reproduction. Mixture (***) recorded the highest number (330.4 and 350.4) individuals at 25 and 30 ºC, respectively, but mixture (*) recorded (310.6 and 346.8) individuals,

while mixture (**) contained all fungi under investigation recorded the lowest number of individuals (Table, 4).

2. Chemical analysis:

Chemical analysis of prey *R. robini***,** larvae of *M. domesticae* and free living nematodes were conducted for some knowledge about (1)Phosphorus, (2) Moisture and dry matters, (3) Dry matter, (4) Crude proteins, (5) Ether extracts, (6) Nitrogen free extracts, (7) Amino acids and ash. Chemical analysis of prey was carried out to shed light on its effect on the predatory mite *P. discorus* activity.

2.1. Phosphorus contents (%) of the prey:

By using the methods of Bhargava and O'Neil (1975), phosphorus contents was estimated in dry matter samples. Results, showed that free living nematodes contained the highest percentage of phosphorus (2.9 % /g. dry matter) followed by both larvae of *M. domestica* and *R. robini* (2 %) (Table, 5).

2.2. Moisture and dry matter:

Estimation of the percentage of moisture and dry matter in different preys is very important because if the percent of moisture was relatively high in the prey, the predacous mite was very active and its feeding capacity increased. Data showed that, free living nematodes contain the highest percentages of moisture reached to 95.1 %. On the other hand, organic manure recorded low percentage of moisture (13.7 %) and high percentage of dry matter reached to 86.3 % (Table, 6).

2.3. Dry matter:

The bulb mite *R. robini* contain the highest percentages of dry matter 14.7% followed by *M. domestica* 14.0% and free living nematodes 4.8% (Table, 5).

2.4. Crude proteins: Larvae of *M. domestica* contain the highest amount of crude proteins (51.5 % / g. dry matter) followed discendingly by free nematodes 40.7 % and *R. robini* 31.8 %.

2.5. Ether extracts:

Ether extracts is necessary for predacous mite to provide it with energy which is needed for its movements and reproduction. Ether extracts are recorded in all preys, but in different percentages, free living nematodes contain the highest percent of ether extract (26.6 %) followed by R. robini (24.64 %) and larvae of *M. domestica* (12.22 %), therefore, the predacous mite *P. discorus* feeding on prey containing the highest percent of ether extracts was more active than other (Table, 6).

2.6. Ash:

The bulb mite *R.robini* contains the highest percent of ash (15.0 %), followed by free living nematode (6.7 %) and larvae of *M. domestica* (5.28 %). Statistically highly significant deviations existed between the ash in different tested preys (Table, 6).

2.7. Amino acids: As shown in Table (7) by using instrume: High performance, Amino acids analyzer, Model: Beckman, system 7300 and Data system 7000.Column: Na- A/B/D 25-Cm Column. Sample Vol: 50 Ul. From Table (7), it was shown that free living nematodes contained 15 amino acids, while larvae of *M. domestica* and immature stages of *R. robini* are contained 14 amino acids. Data cleared that larvae of *M. domestica* contained the highest percentage of amino acids, while *R. robini* contained the highest percentage of two amino acids; serine (1.38 %) and Alanine (2.52 %). Free nematodes contained the lowest percentage of 13 amino acids except Aspartic acid (1.12 %) and Glutamic acid (1.33 %) (Table, 7).

Table (1): Duration of developmental stages of the predatory mite *Protogamasellus discorus* reared on different diets at 25 and 30 ºC.

Predatory stage	Temp.	Average periods in days					
		Rhizoglyphus robini	Larva of *Musca domestica*	*Rhabditella masculata*	*Fusarium oxysporium*	*Asperagillus niger*	*Pencilium notatum*
Egg	25 ºC	1.5 ± 0.5	1.7+0.5	1.8±0.7	2.3±0.7	1.5±0.5	1.5±0.5
Larva		2.3±0.5	2.1±0.6	2.3±0.4	2.0±0.5	2.7±0.5	2.3±0.5
Protonymph		3.1±0.6	3.7±0.9	3.0±0.5	5.6±0.4	3.1±0.7	22.3±0.4
Deutonymph		3.1±0.5	4.7±1.3	3.0±0.7	5.7±1.1	3.5±0.6	3.1±0.8
Total immature		8.5±1.1	10.5±1.4	8.9±1.1	13.3±1.4	9.3±1.0	7.7±1.1
Life cycle		9.9±1.2	12.5±1.5	1.7±1.5	15.0±1.6	10.3±1.3	9.2±1.3
Longevity		28.5±2.4	27.5±4.6	28.5±3.8	22.8±3.7	24.1±2.4	25.8±2.1
Life span		38.4±2.1	40.0±5.1	39.2±3.8	38.4±3.3	35.0±2.8	30.0±2.9
Egg	30 ºC	1.4±0.5	1.5±0.5	1.5±0.5	1.8±0.6	1.6±0.5	1.4±0.5
Larva		2.2±0.6	2.1±0.7	2.0±0.7	2.2± 0.7	2.2± 0.4	2.3±0.8
Protonymph		3.5±0.5	3.1±0.7	2.3±0.4	3.8±0.8	2.9± 0.7	2.2±0.6
Deutonymph		2.4±0.7	3.3±0.7	3.6±0.5	5.3±0.8	3.1± 0.6	3.0± 0.6
Total immature		8.0±1.2	8.5±0.9	7.9±1.1	11.3±1.6	8.1± 1.0	7.8±0.7
Life cycle		9.4±1.1	10.0±1.1	9.4±1.0	13.1±1.7	9.6±1.3	8.8± 1.1
Longevity		21.6±1.3	21.4±1.8	22.1±2.8	19.2± 1.3	16.6± 1.6	17.4±2.3
Life span		31.0±1.1	31.4±2.3	31.5±3.6	32.3± 1.5	29.4± 2.9	25.2± 2.1

Table (2): Effect of different types of food on longevity and fecundity of the predatory mite *Protogamasellus discorus* at 25 and 30 ºC.

Type of food	Average duration in days						Number of eggs / female			
	Pre-oviposition		Oviposition		Post-oviposition		Total average		Daily rate	
	25 ºC	30 ºC	25 ºC	30 ºC	25 ºC	30 ºC	25 ºC	30 ºC	25 ºC	30 ºC
Rhizoglyphus robini	3.6±0.8	4.9+0.5	21.1±2.3	16.9±1.8	3.8±0.8	2.8±0.4	59.6 ±7.5	62.0±6.9	2.8±0.6	3.7±0.6
Rhabditella masculata	6.8±0.4	4.4±0.5	14.7±4.3	12.2± 1.9	6.9±0.9	4.5±0.7	61.4±5.0	64.5±3.2	4.2±0.4	5.3±0.2
Larvae of *Musca domestica*	3.8±1.2	2.6±0.5	20.1±4.0	16.1± 1.2	3.6±0.8	2.7±0.8	32.3±5.5	36.5±4.8	1.7±0.5	2.1±0.4
Fusarium oxysporium	5.4±0.9	4.6±0.5	9.0±2.1	8.2±1.1	8.4± 1.1	6.4±0.5	5.0±3.7	10.0±1.6	1.2±0.6	1.1±0.3
Asperagillus niger	6.8±1.3	5.0±0.7	8.8±2.0	8.5±0.8	8.5± 1.4	6.0±1.9	4.7±1.7	5.0±1.0	0.5±0.3	0.5±0.3
Pencilium notatum	4.4±1.4	4.4±0.5	12.9±1.4	5.2±1.1	13.5±2.0	7.8±0.8	2.3±0.8	2.8±0.8	0.2±0.1	0.5±0.04

Table (3): Food consumption of the predatory mite *Protogamasellus discorus* during life span at 25 and 30 ºC.

Predatory stage	*Rhizoglyphus robini*		Daily rate		Larvae of *Musca domestica*		Daily rate	
	25 ºC	30 ºC	25 ºC	30 ºC	25 ºC	30 ºC	25 ºC	30 ºC
Protonymph	10.5 ± 2.3	15.2 ±3.4	3.4 ± 4.5	4.3 ± 1.5	6.7 ±2.0	10.2 ±4.1	1.8±0.5	3.3±0.7
Deutonymph	13.5 ± 2.3	18.5 ± 2.9	4.2 ± 0.7	7.7 ± 1.3	8.8±2.9	11.3±3.3	1.8±0.6	4.3±1.1
Total immature	23.8 ± 1.9	33.7 ± 7.5	3.9 ± 0.7	4.2 ± 1.3	15.5±3.8	21.5±7.9	1.0±0.5	3.6±1.0
Oviposition	65.1 ± 13.7	81.0 ±13.7	3.1 ± 0.7	4.1 ± 0.7	38.3±9.2	49.8±10.5	1.9±0.4	3.6±0.7
Longevity	104.0±23.7	126.7±25.1	3.6 ± 0.8	4.8 ± 1.5	7552±12.1	75.0±28.2	1.8±0.7	3.5±0.5
Life span	138.8 ± 9.5	170.4±18.3	3.6 ± 0.8	5.0 ± 1.3	77.8±10.2	106±17.3	2.0±0.5	3.4±0.8

Table (4): Reproduction and feeding of *Protogamasellus discorus* at different temperature and types of food

Type of diets	No. of individuals after one month			
	25ºC		30 ºC	
	Average	Range	Average	Range
***Rhabditella masculata* (1)**	274.4	250-301	304.8	283-321
***Rhizoglyphus robini* (2)**	156.4	144-173	167.6	154-177
Larvae of *Musca domestica* (3)	147.2	125-201	153.4	133-190
***Fusarium oxysporium* (4)**	94.2	76-114	89.8	82-99
***Asperagillus niger* (5)**	18.2	0-31	17.8	0-28
***Pencilium notatum* (6)**	7.8	0-16	7.8	0-13
***mixture (1+2+3)**	310.6	280-335	346.8	290-375
**** mixture (4+5+6)**	121.0	111-140	136.4	110-155
***** mixture (1-6)**	330.4	290-360	350.4	312-384

Table (5); Percentage of moisture, dry matter and phosphorus in different preys

Content prey	Moisture	Dry matter %	Phosphorus %
Rhabditella masculata	95.18	4.82	2.9
Rhizoglyphus robini	85.30	14.70	2.0
Larvae of *Musca domestica*	86.0	14.0	2.0

Table (6): Chemical analysis of dry matter contents of tested preys

Prey	D.M. %	C. P. %	E. E. %	H. F. E. %	Ash
Larvae of *Musca domestica*	14 (100)	57.5	12.22	31.0	5.28
Rhizoglyphus robini	14.7 (100)	31.80	24.64	28.56	15.0
Rhabditella masculata	4.82 (100)	40.7	26.6	26.0	6.700

O.M.: Dry Matter, C.P.: Crude Protein, E.E.: Ether Extract, N.F.E.: Nitrogen Free Extract

Table (7) : Amino acids (%) in test preys

Amino acids	Larvae of *Musca domestica*	*Rhizoglyphus robini*	*Rhabditella masculata*
Aspartic acid	4.15	2.26	1.12
Therionine	1.82	1.09	0.40
Serine	1.18	1.38	0.37
Glutamic acid	3.41	2.19	1.33
Glycine	3.05	1.64	0.17
Alanine	2.30	2.52	0.63
Valine	2.02	1.43	0.51
Methionin	-	-	0.23
Isoleucine	1.58	1.02	0.40
Leucine	2.90	1.89	0.72
Tyrosine	0.98	0.76	0.15
Phenylalanin	2.99	1.53	0.52
Histidine	1.28	0.62	0.36
Lysine	2.83	1.66	0.45
Prginine	1.74	1.53	0.39

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Egypt. J. Plant Prot. Res. Inst. (2019), 2 (3): 488 - 492

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

Toxic effect of tomato leaves extract against the leaf miner *Tuta absoluta* (Lepidoptera: Gelechiidae) and the cotton leafworm *Spodoptera littoralis* (Lepidoptera:Noctuidae)

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ARTICLE INFO

Article History

Received: 11 / 7 / 2019

Accepted: 24 / 9 /2019

Keywords

Tuta absoluta, *Spodoptera littoralis*, tomato leaves, botanical extract and nontoxic pesticide.

Abstract:

Tomato is one of the most important vegetable crops in Egypt. The present study aims to evaluate the unused part of the tomato plant, leaves, as a botanical and non-toxic pesticide. The leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and the cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) are the most serious lepidopterous pests on the tomato crop. Also, these two insect pests have awide host range. *T. absoluta* larvae can cause yield losses of up to 80 - 100% by attacking all parts of tomato crops. While *S. littoralis* is a polyphagous and cosmopolitan pest and it can causes an estimated loss of 25.8 to 100% in crop production. Due to the problems of chemical pesticides to all organisms and environment, natural control replaced pesticides. Tomato extract contains many contents of phenolic and flavonoid compounds which were effective in control. Different concentrations of tomato extract were applied in control of *T. absoluta* and *S. littoralis* and caused high mortality proportion. In the present study, LC_{50} was 606.34 ppm for *T. absoluta* and 1161.76 ppm for *S. littoralis*. Although LC_{50} for *T. absoluta* is lower than it in *S. littoralis* and is affected highly with the tomato extract, but the extract of tomato leaves as unused part of plant crop and without any cost, so it considered a great botanical pesticide for controlling serious pests as *T. absoluta* and *S. littoralis*.

Introduction

Tomato (*Lycopersicon* spp.) is one of the most important edible andnutritious vegetable crops in Africa; it grows both on asmall and commercial large scale as a cash crop by thevegetable growers (FAOSTAT, 2010). One of the most important insect pests that are constraining tomato production is the tomato leaf miner *Tuta absoluta* (Meyrick) and the cotton leafworm *Spodoptera littoralis* (Boisd.). The tomato leaf miner *Tuta absoluta* (Lepidoptera: Gelechiidae), originated in South America and is a significant pest of tomato. Currently, Egyptian tomato fields were infested with *Tuta absoluta* since 2009 and it became one of the economic pests of tomato and other Solanaceous plants (NAPPO, 2012). *T. absoluta* larvae can cause yield losses of up to 80 -

100% by attacking tomato leaves, flowers, stems and especially fruits of tomato crops in both greenhouse and open field tomato (Desneux *et al*., 2010). *S. littoralis* (Lepidoptera: Noctuidae) is a polyphagous pest of many economically important crops such as cotton, groundnut, soybean tomato, sweet potato etc. (Senrung *et al*., 2014).

Chemical control tactics have been the primary method for managing infestation, but this strategy has become less effective due to development of insecticide resistant population (Siebert *et al.,* 2012). Occurring often slow acting crop protectants are usually safer to humans and the environment than conventional pesticides. Therefore, the use of botanical insecticides has been recommended ever more as a suitable alternative of plant protection with minimum negative risk (Isman *et al.*, 2007). Tomato is a good source of phenolic compounds, pigments, antioxidants and other nutrients, these compounds prevent oxidative changes in cell by reducing the level of free radicals (Norma *et al*., 2015). The aim of this study was to determine the toxicity of tomato leaves extract on *T. absoluta* and *S. littoralis.*

Materials and methods

1. Insects:

1.1. Rearing of *Tuta absoluta:*

Tomato leaves including *T. absoluta* were collected form the unsprayed farm of Agriculture College, Mansoura University (Dakahlia, Egypt). The larvae was reared for two generations before the beginning of the tests on leaves of unsprayed tomato which were provided daily, in laboratory under constant conditions of 25 ± 2°C, photoperiod of 14 h light and 10 h dark and 70±10 % RH. The adults were kept separately and mated on the third day of emergence in clean jars (4 lb.) adults were fed on 10% honey solution, fresh green leaves of unsprayed tomato were provided for egg laying (Bajonero and Parra, 2017).

1.2. Rearing of *Spodoptera littoralis*:

A laboratory strain of cotton leafworm, *S. littoralis* (Lepidoptera: Noctuidae) (maintained on above 30 generations) which was initiated from freshly collected egg-masses supplied from the division of cotton leafworm of Plant Protection Research Institute (PPRI), Dokki, Egypt. Larval stages were reared on castor leaves, which were provided daily, in laboratory under constant conditions of 27 ± 2°C, photoperiod of 14 h light and 10 h dark and 65 ± 5% RH. The adult were kept separately and mated on the third day of emergence in clean jars (4 lb.), adults were fed on 10% honey solution, fresh green leaves of tafla, *Nerium oleander* (L.) were provided for egg laying.

2. Preparation of plant sample and extraction:

Leaves of tomato plant, 961 sorts, were left to dry at room temperature for one month then the dried leaves were grinded into fine powder. Powder was soaked in a mixture of hexane, acetone and ethanol solvents of equal proportion (1:1:1) in a flask for about one week. Finally, the flask was shaked in a shaker and its contents were filtered. The solvents were evaporated under reduced pressure and the crude extract was weighted and kept in deep freezer until use.

2.1. Preparing the stock solution of the tested plant extract:

Convenient stock, concentrations of tomato extract, was prepared on basis of the tested plant weight and the volume of the distilled water (w/v) in the presence of tween 80 (0.1%) as emulsifier. The stock concentrations were kept in glass

stoppered bottles and stored under refrigeration. Such stock solutions were prepared periodically. Four diluted concentrations for the plant extract for each insect pest were used to draw the LC-P Lines. Four replicates were used for each concentration.

3. Method of application:

3.1. Spray method:

The 3rd instar larvae of the *T. absoluta* were used for application. Four concentrations were used as well as four replicates for each concentration. Ten individuals of larvae for each replicate were applied to estimate the mortality line. Different concentrations were sprayed directly on the leaves contains the larvae. The concentrations used were 250, 500, 1000 and 2000 ppm. The same number of leaf discs per treatment was dipped into distilled water water as an untreated check. The percentage of mortality was recorded after one, three, five and seven days and the data were corrected relatively to control mortality (Abbott, 1925). LC_{50} values were determined using probit analysis statistical method of (Finney, 1971).

3.2. Leaf dipping method:

The 2nd instar larvae were used to determine the toxicity action of the tomato leaves extract. Tomato leaf discs were cut and dipped into the treatments for 20 seconds, then left for air dryness, 10 larvae for each replicate were released to each leaf disc placed. Four concentrations and three replicates were used to estimate each concentration-mortality line. The concentrations used were 500, 1000, 2000 and 4000 ppm. The same number of leaf discs per treatment was dipped into distilled water water as an untreated check. Before and after treatment, larvae were maintained under laboratory conditions (constant temperature 25 ±2 °C and 70 ± 5 % RH. After 24 h of treatment.The percentage of mortality was recorded after one, three, five and seven days.The data were corrected relatively to control mortality (Abbott, 1925). LC_{50} values were determined using probit analysis statistical method of Finney (1971).

Equation: Sun, 1950 (to determine LC_{50} index**)**

Toxicity index for LC_{50} =

$$\frac{LC_{50} \text{ of the most effective compound}}{LC_{50} \text{ of the least effective compound}} \times 100$$

Results and discussion

The data in Table (1) demonstrated that, although the extract concentrations were low, the mortality rate of the larvae of *T. absoluta* was high and when the concentrations increased, the total mortality increased. Also, the mortality rate of 2nd instar larvae of S. littoralis was high especially with high concentations. The used concentrations in *T. absoluta* were 250, 500, 1000 and 2000 ppm. While, in *S. littoralis,* the used concentrations were 1000, 2000, 4000 and 8000 ppm. This means that, the cooperated concentrations between *T. absoluta* and *S. littoralis* were 1000 and 2000 ppm. The total mortality rates in concentration 1000 ppm were 66.67 and 46.66 % against *T. absoluta* and *S. littoralis*, respectively. Also, the total mortality rates in concentration 2000 ppm were 83.33 and 66.67 % against *T. absoluta* and *S. littoralis*, respectively. So, the effect of tomato leaves extract was more effective on larvae of *T. absoluta* than *S. littoralis*. The effectiveness of tomato leaves extract in controlling of pests was in agreement with Abd- Allah *et al.* (2017) which proved the effectiveness of tomato leaves extract on *Aphis gossypii*.

Table (1): Corrected mortality % of 3rd instar larvae of *Tuta absoluta* and 2nd instar larvae of *Spodoptera littoralis* treated with tomato leaves extract under laboratory conditions 27±2 ◦C and 65±5% RH.

Treatment	Conc. (ppm)	Mortality after treatments %				Total Mortality %
		One day	Three days	Five days	Seven days	
Tuta absoluta	250	3.33	3.33	10	6.67	23.33
	500	10	3.33	20	10	43.33
	1000	16.67	3.33	30	16.67	66.67
	2000	30	10	33.33	10	83.33
Spodoptera littoralis	1000	-----	3.33	20	23.33	46.66
	2000	-----	20	23.33	23.33	66.67
	4000	-----	20	26.67	26.67	73.34
	8000	6.67	26.67	30	30	93.34

Tuta absoluta* (Lepidoptera: Gelechiidae) and the cotton leafworm *Spodoptera littoralis

However, the results in Table (2) and Figure (1) demonstrated that, LC_{50} was 606.34 ppm and 1161.76 ppm for *T. absoluta* and *S. littoralis*, respectively. LC_{90} was 2888.98 and 7844.91 ppm for *T. absoluta* and *S. littoralis*, respectively. The toxicity index was 100% and 52.19 % for *T. absoluta* and *S. littoralis*, respectively. The previous results proved that, the extract of tomato leaves was very effective in controlling the most dangerous lepidopterous pests on tomato plants, but *T. absoluta* was more affected with tomato leaves extract than *S. littoralis.* Esther *et al.* (2008) proved a significant effect of tomato extract against lycopene level of tomato. Hussein *et al.* (2015) proved the more effectiveness of plant extracts against *T. absoluta* than the effectiveness of plant extracts against *S. littoralis* (Ankita and Sangeeta, 2017).

Table (2): Efficiency of tomato leaves extract against 3rd instar larvae of *Tuta absoluta* and 2nd instar larvae of *Spodoptera littoralis*

Treatment	Conc. (ppm)	Corrected mortality%	LC_{50}	LC_{90}	Slope± S.D.	Toxicity index LC_{50}	LC_{90}/LC_{50}	R	P
Tuta absoluta	250	23.33	606.34	2888.98	1.89±0.2	100	4.76	0.999	0.982
	500	43.33							
	1000	66.67							
	2000	83.33							
Spodoptera littoralis	1000	46.66	1161.76	7844.91	1.55±0.2	52.19	6.75	0.967	0.145
	2000	66.67							
	4000	73.34							
	8000	93.34							

R: Regression P: Propability

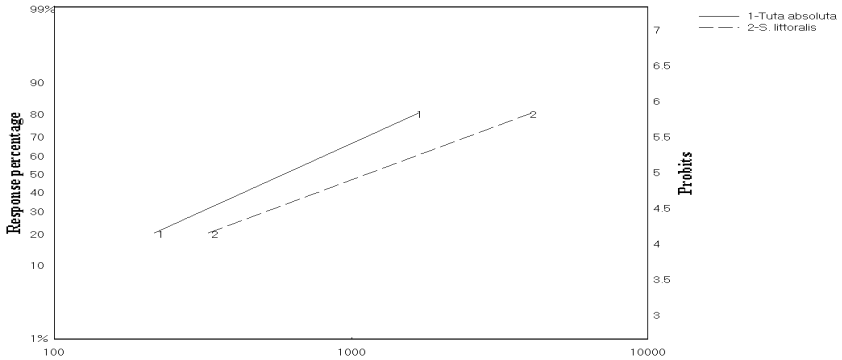

Figure (1): LC-P line for tomato leaves extract of *Tuta absoluta* and *Spodoptera littoralis.*

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Egypt. J. Plant Prot. Res. Inst. (2019), 2 (3): 493 - 497

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Toxic effect of cinnamon, castor plant oils and their combination on *Tetranychus urticae* (Acari: Tetranychidae)

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ARTICLE INFO

Article History

Received: 8 / 7 / 2019

Accepted: 3 / 9 /2019

Keywords

Tetranychus spp., cinnamon oil, castor oil, *Tetranychus urticae* and biological effect.

Abstract:

The biological effects of cinnamon oil, castor oil and their compination were studied under laboratory conditions against adult female of the two spotted spider mite, *Tetranychus urticae* Koch. (Acari: Tetranychidae)**.** Also, LC_{50} of each treatment was established and the obtained results revealed that the mixture of the two oils and cinnamon oil were more effective than the castoroil. LC_{50} was 1100.92, 2928.97 and 7856.59 ppm for the mixture of the two oils, cinnamon oiland castor oil, respectively, for *T. urticae*. The study indicated that, the mixture of cinnamon oil and castor oil was more effective than each one alone on *T. urticae*.

Introduction

Red spider mites, *Tetranychus urticae* Koch. (Acari: Tetranychidae) is a notorious pest of economically important agricultural crops as well as ornamental plants (Navajas, 1998). It has been reported to attack about 1200 species of plants (Zhang, 2003), of which more than 150 are economically important (Jeppson *et al.,* 1975 and Xie *et al.,* 2006). It causes damage to sweet corn, beans, peas, hops, grapes, deciduous fruit trees, strawberries and many other fruit, vegetables, flowers and ornamental plants (Johnson and Lyon, 1991).

Commercially available synthetic acaricides are usually expensive and may be needed to be imported for use by farmers. They also tend to have detrimental effects on the environment and can be hazardous to humans. These negative effects have resulted in an increasing interest for natural plant-based pesticides which are assumed to be safer than the synthetic pesticides (Yanar *et al*., 2011). Natural plant extracts play an increasingly prominent role as alternatives to synthetic pesticides due to the increasing concern on health hazards, environmental pollution and negative effects on non target organisms (Sharma *et al*., 2006 and Mariam *et al.,* 2015).

Cinnamon oil (*Cinnamomum verum*), the bark oil consists of cinnamaldehyde (80– 90%), eugenol, eugenol acetate, cinnamyl acetate, cinnamyl alcohol, methyl eugenol, benzaldehyde, benzyl benzoate, linalool, monoterpene, hydrocarbon, caryophyllene, safrole and others, such as pinene, phyllandrene, cymene and cineol (Health, 1978). Castor oil (*Ricinus communis*) is producing non-edible plant oil provide a better economical alternative (Deligiannis *et al*., 2009) and

using pressing and extractionmay offer vegetal oils. This can also be used as bio-oil (fuel without transesterification) which can then being completely biodegradable (Boza and Saucedo, 2011). Castor bean is a naturally occurring plant, inexpensive and an environmental-friendly resource (Jumat *et al.*, 2010).

The present work was aimed to evaluate the biological aspects of cinnamon *oil*, castor oil and their mixture against the two spotted spider mite *T.urticae*.

Materials and methods

1. Rearing mites:

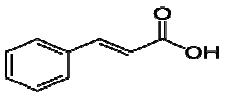
T. urticae was collected from unsprayed castor bean plants and reared at 25± 2° C and 60± 5% RH.

2. Plant oils:

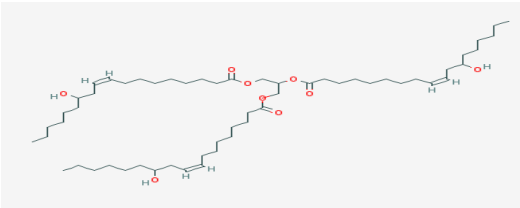
cinnamon oiland castor oil was bought from Essential Oil Extracts Center, National Research Center.

-*Cinnamon*oil contains cinnamic acid, $C_9H_8O_2$.

cinnamic acid formula (Vogt, 2010)



-Castor oil contains ricinoleic acid $C_{57}H_{104}O_9$ Osol *et al.* (1975)



-Mixture of the two plant oils made by mixing cinnamon oil and castor oil 1:1 proportion.

3. Preparing the stock solution the tested plant oils:

Convenient stock concentrations of each oils were prepared on basis of the tested plant oil weight and the volume of the distilled water (w/v) in the presence of tween 80 (0.1 %) as emulsifier. The stock concentrations were kept in glass stoppered bottles and stored under refrigeration. Such stock solutions were prepared periodically. Four diluted concentrations for each plant oil were used to draw the LC-P lines. Three replicates were used for each concentration.

4. Toxicity test:

The toxicity of cinnamon oil, castor oil and their mixture was evaluated against adult females of *T. urticae*. Thirty newly emerged adult females were transferred to the lower surface of castor leave discs (2.5 cm. diameter) placed separately on moist cotton wool in petri dishes. Each petri dish contains three replicates, ten individuals in each replicate. Each acaricide had four concentrations which were sprayed on the individuals. Mortality was recorded for 7 days after treatment. The mortality percentage was estimated and corrected according to the **Abbott's formula (1925)**. LC_{50} values were determined using probit analysis statistical method of **Finney (1971)**.

Equation: Sun, 1950 (to determine LC_{50} index**)**

Toxicity index for LC_{50}=

$$\frac{LC_{50} \text{ of the most effective compound}}{LC_{50} \text{ of the least effective compound}} \times 100$$

Results and discussion

1. Effect of the cinnamon oil, castor oil and their mixtureon adult female of two- spotted spider mite, *Tetranychus urticae* (Koch):

Data given in **Table (1)** showed thatthe mixture of cinnamon and castor oils caused high mortality proportion on the two spotted spider mite *Tetranychus urticae* then cinnamon oil, while the castor oil caused mortality less than the mixture and cinnamon oil. These findings were in agreement with Health, (1978); Abd El- Wahab (2003) and Ghada and Amal (2015) .

Table (1): Corrected mortality % of two spotted spider mite *Tetranychus urticae* treated with cinnamon oil, castor oil and their mixture under laboratory conditions 25±2 ˚C and 65±5% RH.

Treatments	Conc. (ppm)	Mortality after treatments %				Total Mortality %
		One day	Three days	Five days	Seven days	
Castor oil	5000	-----	6.67	20	6.67	33.34
	10000	-----	13.33	26.67	13.33	53.33
	15000	13.33	20	33.33	13.33	80
	20000	20	20	33.33	13.33	86.66
Cinnamon oil	1000	-----	6.67	-----	13.33	20
	5000	20	6.67	20	20	66.67
	10000	26.67	-----	40	13.33	80
	15000	13.33	26.67	40	13.33	93.33
Castor oil+ Cinnamon oil	500	-----	-----	20	13.33	33.33
	1000	26.67	-----	26.67	----	53.34
	5000	6.67	13.33	40	6.67	66.67
	10000	26.67	6.67	26.67	33.33	93.33

Data in Table (2) and Figure (1) revealed that cinnamon oil and castor oil mixture were more effective than castor oil with LC_{50}, 2928.97 ppm, 1100.92 ppm and 7856.59 ppm, respectively. However, the toxicity index was 100% for the mixture, 37.59% for cinnamon oil while was 14.01 for castor oil. The slope values indicated that the oil mixtures had the lowest value which was 1.15, followed by 1.8 and 2.6 for cinnamon oil and castor oil, respectively. Also, LC_{90}/ LC_{50} values were 3.08, 4.96 and 12.88 for castor oil, cinnamon oil and the mixture, respectively. These results were in agreement with Mwandila *et al*. (2013) and Mariam *et al.* (2015).

Table (2): Efficiency of cinnamon oil, castor oil and their mixture against two spotted spider mite *Tetranychus urticae*.

Treatment	Conc. (ppm)	Corrected mortality%	LC_{50}	LC_{90}	Slope± S.D.	Toxicity index (LC_{50})	LC_{90}/ LC_{50}
Castor oil	5000	33.34	7856.59	24230.82	2.6± 0.3	14.01	3.08
	10000	53.33					
	15000	80					
	20000	86.66					
Cinnamon oil	1000	20	2928.97	14524.98	1.8± 0.17	37.59	4.96
	5000	66.67					
	10000	80					
	15000	93.33					
Castor oil + Cinnamon oil Mixture	500	33.33	1100.92	14182.7	1.15± 0.13	100	12.88
	1000	53.34					
	5000	66.67					
	10000	93.33					

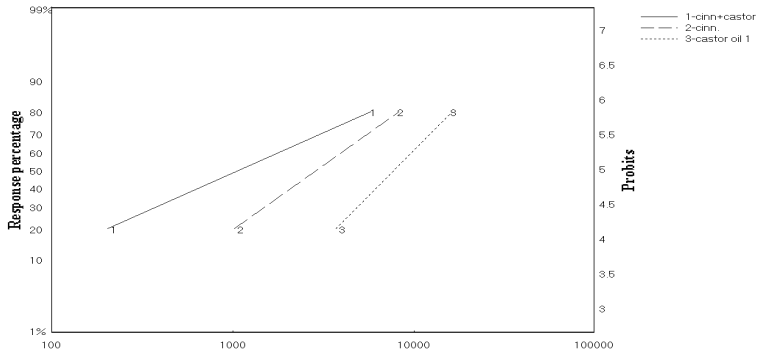


Figure (1): LC-P lines for cinnamon oil, castor oil and their mixture against adult female of two spotted spider mite *Tetranychus urticae*.

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Egypt. J. Plant Prot. Res. Inst. (2019), 2 (3): 498 - 504

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Effect of temperature on life history of predatory mite *Amblyseius californicus* (Acari: Phytoseiidae) associated with the scale *Aulacaspis tubercularis* (Hemiptera: Diaspididae) infesting mango

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ARTICLE INFO

Article History

Received: 4 / 8 / 2019

Accepted: 25 / 9 /2019

Keywords

Predatory mite, armored scale, temperatures, biology and mango.

Abstract:

Amblyseius californicus (McGregor) (Acari: Phytoseiidae) is a beneficial predatory mite endemic to the Eastern Mediterranean region. This species is considered a generalist predatorand readily consumes small soft-bodied pest species as well as pollen or plant exudates. *Amblyseius* has attracted substantial interest as a biological control agent of mites, thrips and whiteflies in greenhouse and nursery crops. The present studies were conducted to study the effect of temperatures on development and food consumption of *Amblyseius californicus* (McGregor) feed on the scale *Aulacaspis tubercularis* Newstead (Hemiptera: Diaspididae) under constant temperatures. The results showed that the developmental period increased with temperature decrease. Fecundity, longevity and lifespan were longer at 25°C and lower at 27°C. Feeding capacities were increased with increasing temperature from 22°C to 25°C and then decreased at 27°C. The highest means of total prey consumption of females were recorded during oviposition, when they devoured an average of 103.0, 189.3, 109.0 and 79.0 prey at 20, 25, 30, and 35°C, respectively. The highest values of the mean prey consumption by postoviposited females was observed at 25°C (108.42 preys) followed by 27°C (76.61 preys), respectively.

Introduction

Scale insect injures leaves and fruits affecting the commercial value of the fruits and their export potential. Colyn and Schaffer (1993) , Pena *et al.* (1998) and Joubert *et al*. (2000) mentioned that *Aulacaspis tubercularis* Newstead (Hemiptera: Diaspididae) injures the leaves and fruits of mango trees *Mangifera indica* L. (Anacardiaceae) affecting the commercial value of the fruits and their export potential. Mango trees considered as one of the most popular fruit in Egypt contains a high percent of sugar, protein, fats, salt and vitamins. It played an important role in food industrialization such as juices, which wanted with large amounts of export according to good reputation of

Egyptian varieties. Now , the Egyptian agricultural strategy is to increase the quality level of exported crops to certain European countries , for this reason many efforts has been done to increase the total cultivated areas of mango in Egypt , as a favorable fruits in many countries . Scale insects are usually considered as the most important pests which infesting mango trees in many countries of the world. Phytoseiid mites have been studied extensively with respect to their potential for the biological control of phytophagous mites in greenhouses, on strawberry and deciduous fruit (Helle and Sabelis, 1985). McMurtry and Croft (1997) listed phytoseiids now being used or with the potential of being used in control programs against agricultural and horticultural pests. One of these species being used in control programs is *Amblyseius californicus* (McGregor) (Acari: Phytoseiidae), also known as *Neoseiulus californicus*. This predatory mite originates from field of Egypt. It is used extensively in biological control programs against red spider mites (*Tetranychus urticae* Koch., Tetranychidae) on a global scale (Hart *et al*., 2002). *A. californicus* is widely used in the Mediterranean region, particularly southern France, Italy and Spain, where it is reported to occur naturally (Raworth *et al*., 1994 and Castagnoli and Simoni, 1999).

Biological events of the arthropods in relation to key environmental factors are necessary to determine the extent of their influence on the population dynamic of the predator and/or pests. Temperature has long been recognized as a primary environmental factor influencing the rate of development of arthropods (Høye and Cull, 2018).

The present investigation was oriented to study feeding capacity of *A. californicus* against *A. tubercularis* infesting mango under constant temperatures.

Materials and methods

1. Prey culture:

The scale *A. tubercularis* was found on leaves of naturally infested mango trees at Giza, Egypt. Samples were taken and transferred to laboratory and reared under laboratory conditions.

2. Predator culture:

A laboratory colony of *A. californicus* was collected from mango orchard at Giza Governorate. It was mass cultured in the laboratory on castor leaves infested with *T. urticae* as prey. The experiment was under the same conditions.

3. Experimental procedure:

The experiments were conducted at three constant temperatures (22, 25 and 27±1ºC) with relative humidity of 70±5%. Thirty gravid females of *A. californicus* were taken randomly and transferred to rearing substrates. Females were left 24 hours and their oviposited eggs were used to start biological aspects. Thereafter, when a sufficient number of eggs were laid, the adult females were removed and eggs from the same age were obtained to start the experiment. Observations were made at 6 hourly intervals to see if the eggs had hatched. After the eggs hatching to larvae, the larval individuals of larvae were transferred very carefully onto leaf disks of castor leaves (3 cm in diameter). Leaf discs were placed with the upper surface facing down on cotton layer in petridishes (6 cm in diameter). Water was added when needed to maintain the suitable moisture. The leaf margin was surrounded by a cotton strip to prevent the mites escaping. A few cotton threads were placed on the surface of leaves to serve as shelter and oviposition sites. Ten replicates were maintained for each temperature, so 40 petri dishes were maintained simultaneously. All the petri dishes were kept in incubators maintaining the desired temperature. Immature stages of *A. tubercular* is which was given as food for the

predatory mite *A. californicus*. Duration of the developmental stages, pre-oviposition, oviposition, post-oviposition periods, longevity, fecundity, lifespan and food consumption were recorded by taking observations using the stereomicroscope.

4. Statistical analysis:

Data were subjected to statistical analysis using F-test and means were compared according to Duncan's multiple range test. Developmental thresholds (t_o) becalculated according to the method of (Weinberg and Lange 1980) and the thermal units (TU) needed for the developments of each stage were calculated according to Madubunyi and Koehler (1974).

K (TU) = T (t-t_0), where,

K (TU) = Thermal units (day-degree),
(T) = Duration (in days),
(t) = Exposure temperature (ºC),
(t_0) = temperature threshold (ºC).

Results and Discussion

The present investigations were carried out to study the effect of constant temperature regimes of 22, 25 and 27ºCon the development and feeding capacity of *A. californicus* against *A. tubercularis* infesting mango under constant temperatures to estimate temperature developmental threshold (t_0ºC) and thermal units (TU) (day-degrees) required for the development of the immature stages.

1. Effect of constant temperatures on the immature stages:

The results indicated that the pest developed successfully from egg to adult emergence over the temperatures ranged from 22 to 27ºC. The duration of the immature stages under the different constant temperature regimes are given in Table (1).

1.1.Egg stage:

The incubation periods at various constant temperatures are given in Table (1). As shown, the time required for completion of embryogenesis decreased gradually as temperature increased. The incubation periods lasted from 2.15±0.07, 1.11±0.12, to 1.01±0.08 days at the constant temperatures of 22, 25 and 27ºC, respectively. There were significant differences between the incubation periods at the tested temperatures. The longest incubation period (2.15 days) was recorded at 22ºC and the shortest (1.01 days) was revealed at 27ºC. This may suggest that the constant temperature of 27ºC was the most preferable tested temperature for the development of the egg stage.

1.2.Larval stage:

The larval durations tended to be shortened with an increasing of temperature. The results in Table (1) showed that the average duration of larval stage of *A.californicus*. Means of 1.13±0.05, 1.01±0.01 and 0.90±0.03 days were recorded at temperatures of 22, 25 and 27ºC, respectively. Statistical analysis showed that significant differences between values of mean durations of larval stage at the tested temperatures.

1.3. Protonymphal stage:

The duration of the protonymphal stage is shown in Table (1). Results show that the protonymphal stage lasted 1.65±0.04, 0.55±0.02 and 0.15±0.05 days at 22, 25 and 27ºC, respectively.

1.4. Deutonymphal stage:

The duration of the deutonymphal stage is shown in Table (1). Results showed that the deutonymphal stage lasted 1.21±0.08, 1.00±0.05 and 0.85±0.06 days at 22, 25 and 27ºC, respectively with significant differences.

1.5. From egg to adult emergence:

Data in Table (1) showed that the developmental times for the immature stages of *A. californicus* were inversely related to temperature. Total developmental time (egg to adult) ranged from 1.80 days at 22°C to 1.30 days at 27°C. As shown in Table (1), the total developmental time from egg to adult emergence was correlated significantly with the corresponding temperatures. This result seems to be logic, since the

duration of any developmental stages or/and physiological process are negatively correlated with temperature within the tolerant zone of temperatures. The foregoing results concerning, the duration in relation to temperature clearly indicated that temperature of 27ºC was the most preferable temperature for development of *A. californicus*.

Development temperature relationship expressed as rate of development (100/y) is shown in Table (2). Data in Table (1) were used to calculate regression equations, which were used in estimation threshold of temperature. As shown in Table (2), it seems that the equation fit the observed rather well, as indicated by high values of coefficient of determination. Extrapolation of the regression line to the temperature axis resulted in a threshold temperature of 10.30 ºC. The calculated thermal units, using this threshold as a base temperature, were about 86.20 day degrees (Table, 2).

The observation that developmental period decreased with increasing temperature was also observed in *Amblyseius fallacis* (Garman) by Smith and Newsom (1970), *A.citrifolius* (Denmark and Muma) by Moraes and McMurtry (1982), *Amblyseius swirskii* Athias-Henriot by Yousef *et al*. (1982) and Onzo *et al*. (2012) and in *Amblyseius coccosocius* Ghai and Menon by Saha *et al*. (2001). Incubation period was a maximum at 20°C andminimum at 30°C and these findings are in agreement with the findings of Sharma and Sadana (1984).

Means for the total prey consumption by *A. californicus*on a diet of *A. tubercularis* immatures at constant temperatures are presented in Table (3). The larvae of this predator did not feed during the experiment and predation activity started just after. Data analysis revealed a significant effect of temperature on total food consumption by *Amblyseius californicus*except larval stage where there was no significant effect. The total number of prey consumed by the protonymphs increased with increasing temperature from 2.11 prey at 22°C to 4.15 prey at 25°C, and then decreased to 3.01 prey at 27◦C. Feeding capacities of deutonymphs followed a similar trend as protonymphs. It was ranged from 9.11 preys at 25°C to 6.04 preys at 25°C. The highest mean number of total preyconsumed by protonymphs and deutonymphs was 13.31 preys which were obtained at 25°C.

Adult of *A. californicus* started prey consumption after emergence at all temperatures tested. During the preoviposition period, predators devoured an average of 3.70, 13.71, and 6.20 preys at 22, 25 and 27◦C, respectively. The maximum means for total food consumption of the predator was recorded during the oviposition period; it consumes an average of 53.31, 89.30 and 67.11 preys at the same temperatures. The highest and lowest values for the mean prey consumption by postoviposited females were observed at 25°C (89.30 preys) and 22 °C (53.31 preys). During adulthood, the highest number of prey consumed was at 25°C (108.42 preys), which decreased to 76.61 and 58.11 individuals at 27 and 22°C, respectively.

The present study showed that temperature affects the feeding capacity of all life stages of *A. californicus* except the larval stage where it developed to the protonymphal stage without feeding. Non feeding larval behavior may be a mechanism to avoid sibling cannibalism. Similar findings have been reported for otherphytoseiid species by (El-Banhawy *et al*., 2000; Kouhjani *et al*., 2009 and Fatemeh *et al*., 2011).

During immature stages of *A. californicus*, food consumption increased with increasing temperature from 22 to 25°C. Therefore, it could be concluded that theoptimal temperature for predation

of this predator was about 25°C. A clear reduction was observed in the mean number of prey consumption from 25 to 27°C. The findings of Metwally *et al.,* (2005) and Fatemeh *et al*. (2011) support our results.

Although we observed the same trend in all experiments, the obtained values were different because of different prey and predator species were used in the experiments. Furthermore, several other factors, such as relative humidity, photoperiod, presence of pollen and the type of experimental arena may also affect apredator's feeding (Fernando and Hassell, 1980). The females during oviposition consumed a significantly higher number of preys, suggesting that females need extra food for egg production during this period. This new information is in agreement with other findings (Kouhjani *et al*., 2009). To the best of our knowledge, little previous study has been made concerning the predation of this species; therefore we could not compare the results with previous published studies. However, there are numerous investigations on other phytoseiid species, revealing the effect of temperature on these predators food consumption. The results from the current study would help us to gain a better insight into the efficiency and practical application techniques of a predator in biological control programs of spider mites. According to the findings, *A. californicus* could be a beneficial biocontrol agent in both greenhouses and field when temperature is above 22 °C, however, to optimize results, additional experiments should be performed. From an overall evaluation of results it appeared that 25°C was the most suitable temperature for the growth of *A. californicus*. At this temperature the total developmental period was moderated with a longer ovipositional period, higher fecundity and longer longevity.

Table (1): Development of the immature stages and preoviposition period (days) of the predator mite *Amblyseius californicus* reared on *Aulacaspis tubercularis* at constant temperatures.

Temp. (°C)	Developmental time (in days) ± SD					
	Egg	Larvae	Protonymph	Dutonymph	Total	Preoviposition
22	2.15±0.07a	1.13±0.05a	1.65±0.04a	1.21±0.08a	6.14±0.24a	1.80±0.16a
25	1.11±0.12b	1.01±0.01b	0.55±0.02b	1.00±0.05b	3.67±0.20b	1.60±0.13b
27	1.01±0.08c	0.90±0.03c	0.15±0.05c	0.85±0.06c	2.91±0.22c	1.30±0.09c
Total	4.27±0.27	3.04±0.09	2.71±0.11	3.36±0.19	12.72±0.66	4.70±0.23
Mean ± SD	1.42±0.09	1.01±0.03	0.90±0.04	1.12±0.06	4.24±0.22	1.57±0.08

Means followed by the same litters vertically are not significantly different at 0.05 level of probability.

Table (2): Regression equations, lower developmental thresholds (t_0) and thermal units (TU) of *Amblyseius californicus* reared on *Aulacaspis tubercularis* at constant temperatures.

Stage	Regression equations	R^2	Developmental thresholds (t_0)	Thermal units
Egg	Y = 0.52+0.04x	0.97	11.60	22.30
Larva	Y =1.48+0.13x	0.95	11.10	7.50
Protonymph	Y =0.71+0.06x	0.97	10.40	14.60
Deutonymph	Y =0.65+0.07x	0.98	9.90	15.20
Egg to adult	Y =0.18+0.02x	0.98	10.90	59.20
Preoviposition	Y =0.29+0.04x	0.95	8.20	27.50
Egg to adult oviposition	Y =0.12+0.01x	0.98	10.30	86.20

Table (3): Prey consumption of different stage of *Amblyseius californicus* reared on *Aulacaspis tubercularis* at constant temperatures.

Temp. (°C)	No. prey consumed / stage ± SD							
	Immature stage				Adult stage			
	Larva	Protonymph	Deutonymph	Total	Pre.	Oviposition	Post.	Total
22	0.01 ±0.01**c**	2.11 ±0.02**c**	7.30 ±0.06**c**	9.42 ±0.09**c**	3.70 ±0.02**c**	53.31 ±2.14**c**	1.10 ±0.21**c**	58.11 ±2.37**c**
25	0.05 ±0.00**a**	4.15 ±0.03a	9.11 ±0.05**a**	13.31 ±0.08**a**	13.71 ±0.01**a**	89.30 ±4.48**a**	5.41 ±0.27**a**	108.42 ±4.76**a**
27	0.02 ±0.01**b**	3.01 ±0.01**b**	6.04 ±0.11**b**	9.07 ±0.13**b**	6.20 ±0.12**b**	67.11 ±1.85**b**	3.30 ±0.21**b**	76.61 ±2.18**b**
Total	0.08 ±0.02	9.27 ±0.06	22.45 ±0.22	31.80 ±0.30	23.61 ±0.15	209.72 ±8.47	9.81 ±0.69	243.14 ±9.31
Mean ± SD	0.03 ±0.01	3.09 ±0.02	7.48 ±0.07	10.60 ±0.10	7.87 ±0.05	69.91 ±2.82	3.27 ±0.23	81.03 ±3.01

Means followed by the same litters vertically are not significantly different at 0.05 level of probability.

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Egypt. J. Plant Prot. Res. Inst. (2019), 2 (3): 505-513

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

Biological control of *Macrosiphum rosae* (Hemiptera: Aphididae) infesting rose plants by releasing the predator *Coccinella septempunctata* (Coleoptera: Coccinellidae) under glasshouse

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ARTICLE INFO

Article History

Received: 9/ 7 / 2019

Accepted: 18 / 9 /2019

Keywords

Biological control, rose, rose aphid, *Macrosiphum rosae*, *Coccinella septempunctata* and glasshouse.

Abstract:

The rose aphid *Macrosiphum rosae* L. (Hemiptera: Aphididae) is one of the most important pests on roses (*Rosa gallica*) in the world and it causes economic damage. This study was carried out to evaluate the management of rose aphid *M. rosae* by releasing different levels of the seven spotted lady beetle *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). This study was carried out at two locations (Governorates), Elorman Garden (Giza Governorate) and International Garden (Alexandria Governorate) during season 2018 under glasshouse conditions. At Giza Governorate, in the first level of release (30 eggs/plant), the reduction percentages in the population of *M. rosae* increased gradually whereas it were 25.0, 37.0, 46.9, 59.8 and 69.5% on mid-February, first-March, mid-March, first-April and mid-April, respectively. Also, in the second level of release (60 eggs/plant) the reduction percentages in the population increased gradually whereas it were 28.4, 42.2, 54.7, 65.1 and 73.8% in the same dates, respectively. Lastly, in the third level of release (90 eggs/plant) the reduction percentages in the population increased gradually whereas it were 32.2, 47.7, 58.4, 68.2 and 77.2% in the same dates, respectively. The same trend was achieved at Alexandria Governorate. Statistical analysis showed that were highly significant differences between the three releasing levels (30, 60 and 90 eggs/plant) of *C. septempunctata* predator in reduction of *M. rosae* at both the two locations compared to control.

Introduction

Rose (*Rosa gallica*) considers one of the most important cut flowers and ornamental plants in Egypt and all over the world which cultivated in the open field and under greenhouse conditions. Also, its cultivated area increased gradually during the last years, especially in the new reclaimed areas for purposes local consumption and exportation to the foreign markets. Rose named king of flowers because it found from oldest countries and it is the favorite flower for

human all over the world. Although developing live and highly technology but love human to rose still and increase. The human love to the roses due to their beautiful colors, style of flowers, smells and tolerant the inferable weather factors. Later rose became one of the important components for international income for many countries all over the world through exporting these roses to the different countries (Emam, 2009).

Rose plants infested with large scale of insects belong to many orders and families such as aphids, as an important group of insects which are belonged to order Hemiptera. *Macrosiphum rosae* L.(Hemiptera: Aphididae) commonly known as rose aphid considers one of the most important insect of rose plants and many other ornamental plants. Jaskiewicz (2006) reported that the strong infestation by the rose aphid, *M. rosae* resulted in the deformation of stems, leaves and flowers. Derek (2015) in Australia who reported that *M. rosae* is a serious pest on rose and it is reproducing, parthenogenticcally and viviparously all year round. It feeds mainly on the young leaves and developing flower-buds of roses.

The seven spotted lady beetle *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) is the commonest lady beetle known in Egypt, it is an important predator of many aphid species, eggs and small nymphs of mealybugs, jassids, eggs and larvae of cotton leafworm (Bilashini *et al.*,2017). The adults and small stages are often encountered in large numbers on the plants infested with aphids. They feed on these harmful insects and often play a great role in suppressing them under control. Both the adult and larval stages feed on insects harmful to plants, such as aphids and scale insects (Anonymous, 1997). Adults can be killing up to 100 aphids per day (Arnett *et al.,* 2015). The seven spotted lady beetle *C. septempunctata* lives in a wide variety of habitats any place where there are plants and aphids may attract these species (Fleming, 2000).The lady beetle kills its prey outright and then devours it (Waldbauer, 2007). Under field conditions, numerous coccinellids consume nectar, honeydew, pollen, fruit, vegetation and fungus. These non-prey foods are used by coccinellids to increase survival when prey is scarce, reduce mortality during diapause, fuel migration and enhance reproductive capacity. Each of these non-prey foods has unique nutritional and defensive characteristics that influence its suitability for lady beetles (Lundgren, 2015).

This study was carried out to evaluate the releasing of different levels of the seven spotted lady beetle *C. Septempunctata* to control rose aphid *M. rosae* biologically.

Materials and methods

1. Mass rearing of the seven spotted lady beetle *Coccinella septempunctata* and its prey the cowpea aphid *Aphis craccivora*:

1.1. Mass rearing of *Aphis craccivora* as a prey:

The cowpea aphid *Aphis* craccivora Koch. (Hemiptera: Aphididae) is considered the most preferable prey for mass production of *C. septempunctata*. Strong culture of this aphid should be available during the rearing time to maintain the predator rearing process. The broad bean *Vicia faba* seeds were planted in plastic trays (25 X 40 X 15 cm) or foam trays (60 X 25 X 20 cm with 109 wholes) contained peat moss. The seeds were planted at 1-2 cm deep and followed with irrigation and fertilizers as required. When the first leaflet appeared after about one week from cultivation. Bean leaves were infested with *A. craccivora* which distributed over the new foliage of cultivated trays. Culturing of broad bean plants and artificial aphid infestation was a continuous process carried out at weekly intervals.

The infested trays were followed until the population of *A. craccivora* increased and become suitable for using as prey to the lady beetle *C. septempunctata*. *A. craccivora* colonies were cultured under laboratory conditions (23±2ºC and 60±5% RH.) on broad beans (*V. faba*). Such leaves of beans were infested by different stages of aphids and kept under a glass chimney which its upper opening was covered with white muslin. The potted plants were irrigated and fertilized whenever necessary and kept in wooden cages (100 X 135 X 135 cm) with nylon gauze sides using the method described by (Mangoud, 2003 and Mahyoub *et al.*, 2013). *A. craccivora* and *C. septempunctata* instars were originally collected from an agricultural field.

1.2. Mass rearing of *Coccinella septempunctata*:

When the population of *A. craccivora* increased and reached to suitable density individuals (approximately 100 individuals/ plant) on broad bean plants these plants were inoculated with *C. septempunctata*. The stock culture of ladybird was obtained from infested plants and transferred to laboratory. Only 10 adult ♂+ 10 adult ♀ of ladybird (to prevent larval cannibalism) were transferred to rearing cages (30 cm diameter X 25 cm high) and kept in wooden cages (100 X 135 X 135 cm) with nylon gauze sides. To maintain the predator culture, a suitable number of the prey was daily offered to the predator (Mahyoub *et al.,* 2013).

1.3. Egg picking:

The method for egg laying [black polyethylene strips fixed inside a plastic cylindrical (10 cm length X 2 cm diameter) for laying eggs and put in the rearing pots. After laid egg-masses, they were removed from plastic cylinders to separate the egg-masses from the cylindrical plastic and to be ready to stick on the carton paper card for releasing. The plastic cylinder was checked twice/ day for egg-masses because of the cannibalistic habits of the adults, especially when there was a shortage of host food. In order to provide the developing larva with sufficient food throughout their developmental period, it was necessary to increase the amount of food with the advancement of their development (Mahyoub *et al.*, 2013).

2. Release of *Coccinella septempunctata*:

Releasing was conducted on rose plants grown at the two locations, Elorman Garden (Giza Governorate) and International Garden (Alexandria Governorate) during season 2018 under glasshouse conditions. Both at the two places, glasshouse divided into three replects (5 X 8m for each) for rose seedlings which were sown during November 2017. Each replect for each release level and each replect also divided into six plots three plots for that release level and the other three plots used as control. The normal release and recommended agricultural practices were applied, also no chemical control against aphid were used during the whole experimental period.

Naturally, the numbers of *C. septempunctata* stages were recorded. Therefore, three levels of *C. septempunctata* eggs; first level consists of 30 eggs (one card), second level consists of 60 eggs (two cards) and the third one consists of 90 eggs (three cards) were released to encouragement the normal predator population to reduce the aphid. *C. septempunctata* were released (one time) by the beginning of February on rose plants at both the two locations in 2018 season.

Samples were randomly taken bi-weekly at both the two locations and counting started from the beginning of February in rose plants. Ten new plants were examined from each plot (five leaves and three flowers for each plant), were made by a hand lens for counting life insects and the predator and took the

mean numbers. Both surfaces of the leaf were inspected for the presence of aphid (Mangoud, 2000).

3. Statistical analysis:

The obtained results were statistically analysed and the percent reduction of *M. rosae* after *C. septempunctata* released was calculated according to Hendrson and Tilton equation (1955) as follow**:**

% Mortality = 1 – (Ta ✕ Cb / Tb ✕ Ca) ✕ 100

Where:Ta = No. of insect in treat plot after treatment.

Cb = No. of insect in check before treat.

Tb = No. of insect in treat plot before treatment.

Ca = No. of insect in check after treat.

The data was subjected to analysis of variance (ANOVA) and the means were compared by L.S.D. test at 0.05 level, using SAS program (SAS Institute, 1988).

1. In Giza Governorate:

Three levels of *C. Septempunctata* eggs, first level (30 eggs on one card), second level (60 eggs on two cards) and the third level (90 eggs on three cards) were released (one time) on the beginning of February on rose plants during 2018 season.

I.1. First level of release (30 eggs/ plant):

Results in Table (1) and Figure (1) indicated that the number of *M. rosae* in the 1st release plot decreased gradually from 40 on the 1st February to 32, 28, 25, 20 and 16 individuals/plant, on mid-February, first-March, mid-March, first-April and mid-April, respectively as compared to control which aphid populations changed from 45 individuals/ plant,on first-February to 48, 50, 53, 56 and 59 individuals/ plant, at the same dates, respectively. The present results showed that the percent reduction of *M. rosae* in 1st release plot increased gradually to reach 25.0, 37.0, 46.9, 59.8 and 69.5% at the same dates, respectively.

Results and discussion

Table (1): Population fluctuations of *Macrosiphum rosae* in the 1st plot release at level (30 eggs) of *Coccinella septempunctata* in Giza Governorate.

Date	Release plot	Control	% Reduction
First -February	40	45	-
Mid- February	32	48	25.0
First- March	28	50	37.0
Mid- March	25	53	46.9
First- April	20	56	59.8
Mid -April	16	59	69.5
F (0.05)	**232.43**		
L.S.D	**1.78**		

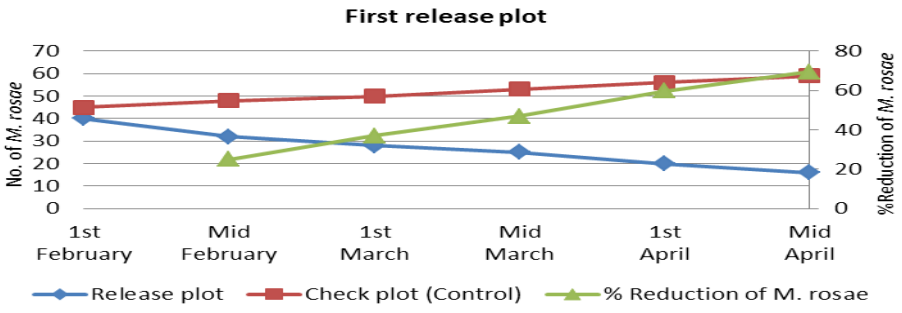

Figure (1): Population fluctuations of *Macrosiphum rosae* in the 1st plot release at level (30 eggs) of *Coccinella septempunctata* in Giza Governorate.

I.2. Second level of release (60 eggs/ plant):

Results in Table (2) and Figure (2) indicated that the number of *M. rosae* in the 2nd release plot decreased gradually from 47 on the 1st February to 37, 31, 26, 21 and 17 individuals/plant on mid-February, first-March, mid-March, first-April and mid-April, respectively as compared to control which aphid populations changed from 50 individuals/plant, on first-February to 55, 57, 61, 64 and 69 individuals/plant, at the same dates, respectively. The obtained results showed that the percent reduction of *M. rosae* in 2nd release plot increased gradually to reach 28.4, 42.2, 54.7, 65.1 and 73.8% at the same dates, respectively.

Table (2): Population fluctuations of *Macrosiphum rosae* in the 2nd plot release at level (60 eggs) of *Coccinella septempunctata* in Giza Governorate.

Date	Release plot	Chick plot (Control)	% Reduction
First- February	47	50	-
Mid -February	37	55	28.4
First- March	31	57	42.2
Mid- March	26	61	54.7
First –April	21	64	65.1
Mid –April	17	69	73.8
F (0.05)	**254.21**		
L.S.D	**1.75**		

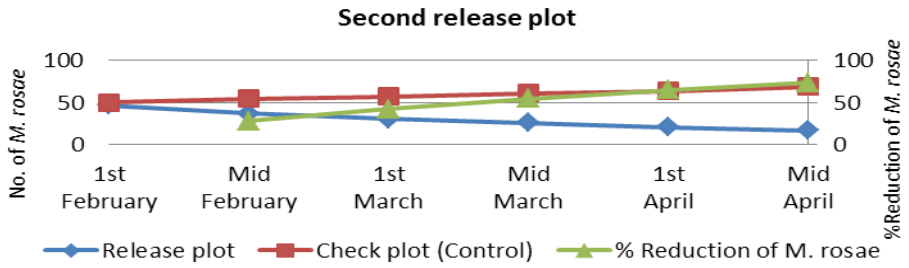

Figure (2): Population fluctuations of *Macrosiphum rosae* in the 2nd plot elease at level (60 eggs) of *Coccinella septempunctata* in Giza Governorate.

I.3. In third level of release (90 eggs/ plant):

Results in Table (3) and Figure (3) indicated that the number of *M. rosae* in the 3rd release plot decreased gradually from 49 on the 1st February to 37, 30, 25, 20 and 15 individuals/plant, on mid-February, first-March, mid-March, first-April and mid-April, respectively as compared to control which aphid populations changed from 53 individuals/ plant, on first-February to 59, 62, 65, 68 and 71 individuals/ plant, at the same dates, respectively. The results showed that the percent reduction of *M. rosae*in 3rd release plot increased gradually to reach 32.2, 47.7, 58.4, 68.2 and 77.2% at the same dates, respectively.

Table (3): Population fluctuations of *Macrosiphum rosae* in the 3rd plot release at level (90 eggs) of *Coccinella septempunctata* in Giza Governorate.

Date	Release plot	Control	% Reduction
First- February	49	53	-
Mid- February	37	59	32.2
First - March	30	62	47.7
Mid- March	25	65	58.4
First- April	20	68	68.2
Mid -April	15	71	77.2
F (0.05)	**242.56**		
L.S.D	**1.59**		

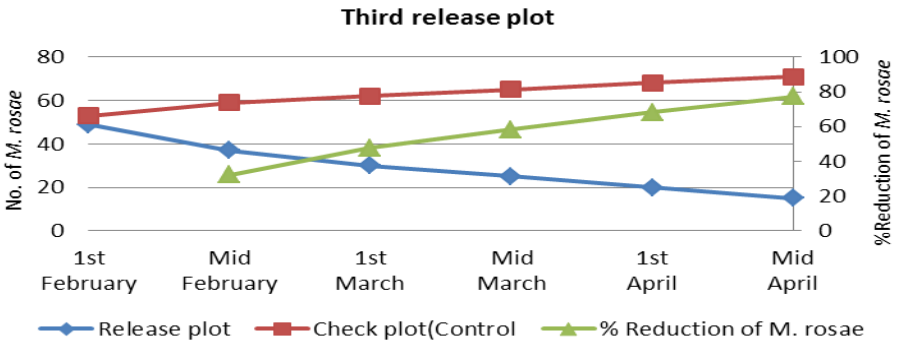

Figure (3): Population fluctuations of *Macrosiphum rosae* in the 3rd plot release at level (90 eggs) of *Coccinella septempunctata* in Giza Governorate.

2. In Alexandria Governorate:

2.1. First level of release (30 eggs/ plant):

Results in Table (4) and Figure (4) indicated that the number of *M. rosae* in the 1st release plot decreased gradually from 35 on the 1st February to 28, 25, 22, 17 and 14 individuals/plant, on mid-February, first-March, mid-March, first-April and mid-April, respectively as compared to control which aphid populations changed from 40 individuals/ plant, on first-February to 44, 46, 49, 52 and 55 individuals/plant, at the same dates, respectively. In addition, the results showed that the percent reduction of *M. rosae* in1st release plot increased gradually to reach 27.3, 37.9, 48.7, 62.7 and 70.9% at the same dates, respectively.

Table (4): Population fluctuations of *Macrosiphum rosae* in the 1st plot release at level (30 eggs) of *Coccinella septempunctata* in Alexandria Governorate.

Date	Release plot	Control	% Reduction
First -February	35	40	-
Mid- February	28	44	27.3
First - March	25	46	37.9
Mid -March	22	49	48.7
First- April	17	52	62.7
Mid April	14	55	70.9
F (0.05)	**298.21**		
L.S.D	**1.67**		

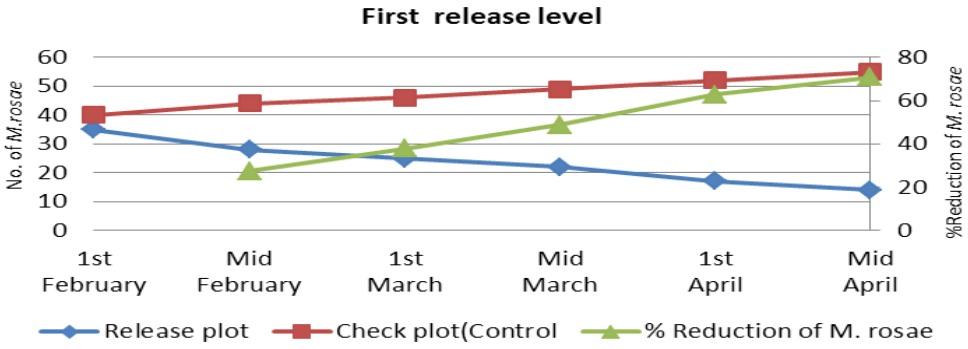

Figure (4): Population fluctuations of *Macrosiphum rosae* in the 1st plot release at level (30 eggs) of *Coccinella septempunctata* in Alexandria Governorate.

2.2. Second level of release (60 eggs/ plant):

Results in Table (5) and Figure (5) indicated that the number of *M. rosae* in the 2nd release plot decreased gradually from 43 on the 1st February to 34, 28, 23, 18 and 14 individuals/ plant, on mid-February, first-March, mid-March, first-April and mid-April, respectively as compared to control which aphid populations changed from 45 individuals/ plant, on first-February to 49, 52, 55, 58 and 62 individuals/ plant, at the same dates, respectively. In addition, the results showed that the percent reduction of *M. rosae* in2nd release plot increased gradually to reach 27.4, 43.7, 56.2, 67.5 and 76.4% at the same dates respectively.

Table (5): Population fluctuations of *Macrosiphum rosae* in the 2nd plot release at level (60 eggs) of *Coccinella septempunctata* in Alexandria Governorate.

Date	Release plot	Control	% Reduction
First- February	43	45	-
Mid -February	34	49	27.4
First- March	28	52	43.7
Mid -March	23	55	56.2
First –April	18	58	67.5
Mid –April	14	62	76.4
F (0.05)	**274.21**		
L.S.D	**1.35**		

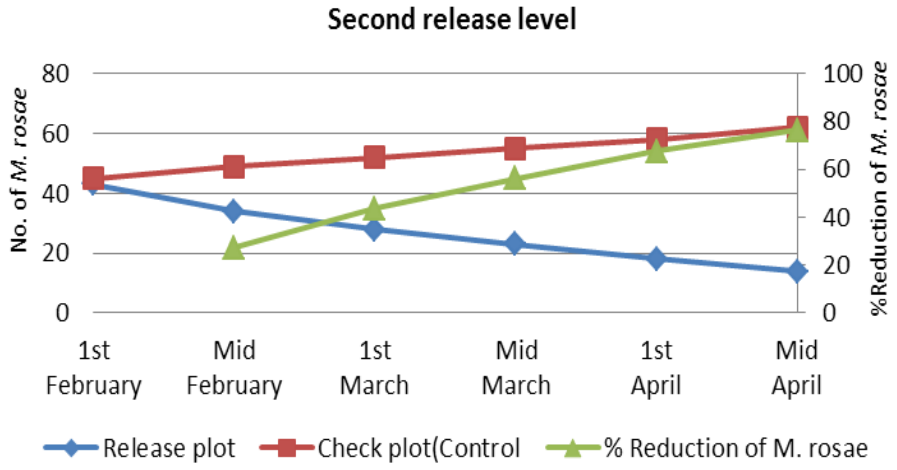

Figure (5): Population fluctuations of *Macrosiphum rosae* in the 2nd plot release at level (60 eggs) of *Coccinella septempunctata* in Alexandria Governorate.

2.3. In third level of release (90 eggs/ plant):

Results in Table (6) and Figure (6) indicated that the number of *M. rosae* in the 3rd release plot decreased gradually from 45 on the 1st February to 32, 26, 21, 16 and 12 individuals/ plant, on mid-February, first-March, mid-March, first-April and mid-April, respectively as compared to control which aphid populations changed from 48 individuals /plant, on first-February to 51, 54, 57, 60 and 63 individuals/plant, at the same dates, respectively. In addition, the results showed that the percent reduction of *M. rosae* in the 3rd release plot increased gradually to reach 33.1, 48.6, 60.7, 71.6 and 79.7% at the same dates, respectively.

Table (6): Population fluctuations of *Macrosiphum rosae* in the 3rd plot release at level (90 eggs) of *Coccinella septempunctata* in Alexandria Governorate.

Date	Release plot	Control	% Reduction
First- February	45	48	-
Mid- February	32	51	33.1
First - March	26	54	48.6
Mid- March	21	57	60.7
First- April	16	60	71.6
Mid -April	12	63	79.7
F (0.05)	**245.11**		
L.S.D	**1.96**		

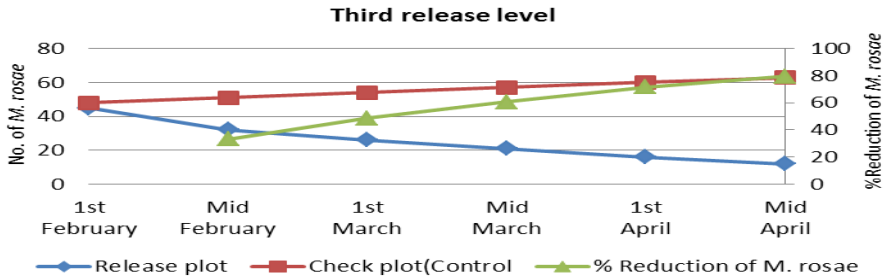

Figure (6): Population fluctuations of *Macrosiphum rosae* in the 3rd plot release at level (90 eggs) of *Coccinella septempunctata* in Alexandria Governorate.

Statistical analysis showed that were highly significant differences between the three releasing levels (30, 60 and 90 eggs/ plant) of *C. septempunctata* predator in reduction of *M. rosae* at both the two locations compared to control.

These results obtained are in agreement with those obtained by Mangoud (2009) who found that the seven spotted lady beetle *C. septempunctata* is an important predator of aphids play a good role in reducing the population density of the woolly apple aphid *Eriosoma lanigerum* (Hausmann) (Hemiptera : Aphididae) attacking apple trees. Also, these results are in agreement with those obtained by Mangoud (2003) who stated that the seven spotted lady beetle *C. septempunctata* is an important predator of aphids play a good role in reducing the population density of the green peach aphid *Myzus persicxae* (Sulzer) and the cotton aphid *Aphis gossypii* Glover (Hemiptera: Aphididae) attacking apple trees.

Also, these results are in harmony with those obtained by Hoyt and Madsen (2005). They found that the control of aphid species complex is complicated by the continue dispersal of aphids from the roots to the aerial portions of the tree and a corresponding dispersal in the opposite direction. Release *C. septempunctata* adopted here can cope very well with this behaviour. Brar and Kanwar (2005) in field experiments in India found *C. septempunctata* was an effective predator against *A. craccivora* infesting fenugreek germplasm. El-Aish *et al.* (2004) stated that the role of the predator *C. septempunctata* in biological suppressing of cereal aphids showed that the eggs last 2-3 days and the 1st, 2nd, 3rd and 4th larval instars were lasted 3, 2, 2 and 4 days, respectively, the pupal stage lasted 8 days at the room temperature. The adult predator consumed 46.13 aphids, while the larval consumed 26.9 aphids daily. Fang *et al.* (2012) found that the coccinellids *C. septempunctata* good controlling of cabbage aphid *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae) in cotton fields at yellow rever valley in China.

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Egypt. J. Plant Prot. Res. Inst. (2019), 2 (3): 514 - 525

Egyptian Journal of Plant
Protection Research Institute
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Field evaluation of insect pests infesting *Phaseolus vulgaris* and their natural enemies in Beheira Governorate

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ARTICLE INFO
Article History
Received: 22/ 7/ 2019
Accepted: 30 / 9/2019

Keywords
Survey, *Phaseolus vulgaris*, population dynamics and Egypt

Abstract:

Common bean *Phaseolus vulgaris* (L.) is considered one of the most important leguminous vegetable crops in Egypt. Field studies were conducted at El-Rahmaneia region, Beheira Governorate during 2017 and 2018 seasons on common bean (*P. vulgaris*). This study aimed to evaluate certain pests infesting *P. vulgaris* and their natural enemies. The crop is sown in mid of February and harvested in June and second planting dates of the experiment were carried out from September to November. The results showed that ten insect species belonging to eight families and five orders according to feeding behavior were noticed. The results recorded the major pests during two successive summer plantation 2017 and 2018. The highest total number recorded by *Aphis craccivora* Koch (Hemiptera: Aphididae). exhibited 1100.33 individuals/25 leaves and the lowest total number recorded by *Ophiomyia phaseoli* (Tryon) (Diptera: Agromyzidae) as 64.33 individuals/ 25 leaves during summer season 2017 and 2018, respectively.The highest total number recorded during spring seasons during both 2017 and 2018, representedby *A. craccivora* being 1125.63 individuals/ 25 leaves and the lowest number of *O. phaseoli* being 74.00 individuals/ 25 leaves and the results noticed no significant difference between the two seasons. The results indicated that 12 species of predators belonging to eleven families were recorded. While the recorded parasitoids were 12 species in five families. The present work here recorded the highest mean number of total mines (occupied and empty) caused by *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) larvae were significantly represented higher in summer plantation (March) during 2017 and 2018 represented by 21.95 and 18.31 individuals/ 25 leaves, respectively. The parasitoids were recorded parasitized the leafminers were *Opius dissitus* (Muesebeck) (Hymenoptera: Braconidae), *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) and *Halticoptera* sp. (Hymenoptera: Pteromalidae). Also the persentage of parasitism of a forementioned parasitiods was studied.

Introduction

Common bean *Phaseolus vulgaris* (L.) is an annual leguminous plant that belongs to the family Leguminaceae, common bean is the most important grain legume for direct human consumption with production more than twice that of the next most important grain legume, chickpea (Gepts *et al*., 2008). The common bean provides one of the most important sources of protein (Boudoin and Maquet, 1999 and Arulbalachandran and Mullainathan, 2009) and is rich in vitamins, 2 minerals and dietary fiber (Kelly and Scott, 1992 and Ndegwa *et al*., 2006). The immature pods of these beans are also an important food source in many locations around the world, where they are known as green beans, snap beans, french beans or string beans. They are important foods in most tropical and subtropical countries of the world and they are second only to cereals as a food source for humans and animals (Graham and Vance, 2003). Legume crops are also important for their nitrogen fixing capabilities (Piha and Munns, 1987; Keyser and Li, 1992 and Amannuel *et al*., 2000), and can be used in crop rotation systems to improve soil conditions. Nitrogen fixation by legume crops offers an alternative to nitrogen fertilizers which may present a serious environmental problem (Nason and Myrold, 1992 and Brentrup *et al*., 2001). Leguminous plant species are susceptible to many biotic stresses, including attacks by many different insect pests and diseases. Pest and disease problems are the major constraints tothe agricultural productivity of the common bean, particularly in the tropics (Graham and Vance, 2003). Worldwide, yield losses due to insect pests alone have been estimated to be from 35% to 100% annually (Singh and Schwartz, 2011). Pest problems prohibiting more extensive productionof legume crops include such diseases as brown rust, powdery mildew and insect pests such asaphids, caterpillars, leafhoppers and whiteflies. The optimum sowing date varies according to the planted cultivar; the sowing time of crop is critical factor in determining the environmental condition at planting .Sowing date can be important in determining the success of the crop and in maximizing seed yield (Dapoah *et al*., 2000). *P. vulgaris* plants are liable to be attacked by several pests. Many insects belonging to the different orders, Lepidoptera, Diptera, Hemiptera and Thysanura as well as mite pests Tetranychidae attack *P. vulgaris* (Awadalla *et al.,* 1991; Berlinger, 1986; Schuster *et al*., 1996; Cohen and Berlinger, 1986; Schuster and Everett, 1983; Parrella, 1987; Abd El-Gawwad, 2008; Parrella *et al.,* 1985 and Saleh, 2011).The two spotted spider mite, *Tetranychus urticae* Koch. (Acari: Tetranychidae) attacks the broad range of crops including soybean, cowpea and common bean and etc. (Razmjou *et al*., 2009). In Egypt, *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) causing an economically significantly loses and damaged to many bean crops (Shahein and EL-Maghraby, 1988). In contrast, flower thrips start infesting at the vegetative stage and migrate later into flower buds and flowers (Kasina *et al*., 2006). This study aimed to evaluate the population fluctuation of certain pests and their natural enemies infesting *P. vulgaris*.

Materials and methods

Field studies were conducted at El-Rahmaneia, Beheira Governorate during 2017 and 2018 seasons. Insect pests and their parasitoids as well as predators were sampled at approximality weekly. The experimental area of 1/4 feddan (1050 m^2) was divided into three replicates about (350 m^2 for each). Randomized complete block design with three replications was used each year. A plot was made up of five rows, 4 m long at spacing of 60 X 40 cm. Two seeds were

planted per hole and thinned after three weeks after seedling emergence. Manual weeding was done as at when found, no herbicide was applied. The seeds of *P. vulgaris* were sown in plots on the two sowing dates at the mid of February and the second date on mid of August 2017 and 2018, respectively.The crop is sown in the mid of February and harvested in June and second planting dates of the experiment was from September to November. Samples of 25 leaves/ replicate were collected randomly at early morning each weekly until the harvest. Numbers of insect stages, egg and movable stages of spider mite were counted and kept in paper bag and transferred to the laboratory to inspect and count the number of each investigated pest. The total numbers were recorded and the mean number was calculated. The insect pests encountered survey were collected and preserved as dry specimen and the specimens were identified.

1. Survey and population density of insects and their natural enemies:

All insect pests and predators were weekly counted on 25 *P. vulgaris* leaves randomly chosen. Standard sweeping insect net (35 cm in diameter cloth cone 75 cm long) was used for collecting flying insects or those existing on plant leaves. Twenty-five strokes every week were implemented. Direct observations were used to study the occurrence of lady bird beetles (Coleoptera: Coccinellidae), syrphid flies (Diptera: Syrphidae) and spiders (Arachnida: Araneae). The sampling procedures indicated previously for predators continued through the plant growth stages. The trapped arthropods were transported to the laboratory in polyethylene bags and spread on a white paper sheet for identification, counting, stage and status of existing on *P. vulgaris* plants.

2. Population fluctuation of the leafminers as amajor pests of *Phaseolus vulgaris* and their parasitids:

Stems, upper andlower surfaces of all the leaves of the selected plants were carefully examined for leafminers during 2017-2018 seasons. Both the immature and adults were counted. The number of leafminers was also recorded on 25 leaves. The leaves bearing the leafminers were collected and placed separately in semi-transparent plastic boxes. The organisms were reared in the laboratory at room temperature.The samples were reared of 1-2 weeks until the adult parasitoids emerged from their host *L. trifolii* or *Ophiomyia phaseoli* (Tryon) (Diptera: Agromyzidae). The emerged wasps were carefully collected and transferred into 96 % ethanol for later identification. The number of leafminers (mines and larvae) was recorded. The number of pupae from each replicates was recorded and counted. The adult emergence of leafminers or parasitoids are collected and identified as *Opius dissitus* (Muesebeck) (Hymenoptera: Braconidae), *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) and *Halticoptera* sp. (Hymenoptera: Pteromalidae).

The emerging parasitoids were counted and the percentage of parasitism was calculated as followed Par. % =NP/T x 100 Where NP. = number of parasitized larvae (pupa), T. = total number of larva or pupa.

Twenty-five double net strokes were taken weekly. The trapped parasitoids were transported to the laboratory in polyethylene bags, separated on a white paper sheet for identification and counting.

3. Specimens identification:

Specimens' identification was done at the Biological Control Laboratory at Rice Research and Training Center, Sakha Agricultural Research Station, Kafr El-Sheikh and Taxonomy Department, Plant Protection Research Institute, Dokki, Giza**.**

4. Statistical analysis:

The analysis of variance and Duncan's Multiple Range Tests (DMRT) were used (SAS, 2003).

Results and discussion

1. Survey and population density of insects:

Data presented in Table (1) showed that nine insect species belonging to eight families and five orders according to feeding behavior were identified.These results agree with those of Daiber (1994) who found that the foliage *P. vulgaris* sown in autumn is damaged by the larvae of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) and larvae of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) and the pods sown during spring and summer are attacked by larvae of *H. armigera*, *Thrips* sp. and *Megalurothrips sjostedti* Trybom (Thysanoptera: Thripidae); Ibrahim (1999) found that *P. vulgaris* plants are attacked by several insect pests and the most serious pests are *Aphis crassivora* (Koch.) (Hemiptera: Aphididae) and *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae); Gamila *et al*. (2016) recorded insects attacked *P. vulgaris* plants, *T. urticae, A. craccivora, L. trifolii, B. tabaci, Empoasca decipiens* Paoli (Hemiptera: Cicadellidae)*, Thrips tabaci* Lindeman (Thysanoptera: Thripidae)*, A. gossypii*. Magouz *et al*. (2011) evaluated that certain *P. vulgaris* varieties and in order to breeding lines of bean, *P. vulgaris* for their relative susceptibility to spider mite *Tetranychus cucurbitacearum* Sayed and whitefly *B. tabaci*. *P.vulgaris* was suitable host to development of aphid and *T. urticae*. The incidences of the four studied pests were significantly and positively correlated with maximum temperature and maximum relative humidity (Hanafy *et al*., 2014).

Table (1): Insect pests recorded from *Phaseolus vulgaris* plant during 2017and 2018 seasons.

Order	Family	Scientific name
Hemiptera	Pentatomidae	*Nezara viridula* (L.)
	Aphididae	*Aphis craccivora*(Koch.) *Aphi sgossypii* (Glover)
	Cicadellidae	*Empoasca decipiens*(Paoli)
	Aleyrodidae	*Bemisia tabaci*(Gen.)
Lepidoptera	Noctuidae	*Spodoptera litoralis* (Bosi.)
Thysanoptera	Thripidae	*Thrips tabaci*(Lin.)
Tetranychidae	Acarididae	*Tetranychus urticae*(Koch.)
Diptera	Agromizidae	*Lirimyza trifolii* (Burgess) *Ophiomyia phaseoli*(Tryon)

Data summarized in Table (2) recorded the main pests during two successive summer seasons mid of February plantation (2017 and 2018).Total number of *A.gossypii* 80.67 individuals/ 25 leaves, *B. tabaci* (immature stages) represented by 222.33 individuals/ 25 leaves, *A. craccivora* 1100.33 individuals/ 25 leaves, *T. tabaci* 112.66 individuals/ 25 leaves, *E. decipiens* 339.33 individuals/ 25 leaves, *O. phaseoli* 64.33 individuals/ 25 leaves, *T. urticae* 923.67 individuals/ 25 leaves, *L. trifolii* 427.33 individuals/ 25 leaves, *N. viridulla* 183.33 individuals/ 25 leaves, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) 126.00 individuals/ 25 leaves. Moreover, there was a highly significant difference between the number of insects collected by using sample plant and sweep net during summer planting date in two seasons. The resultsare in agreement with of El -Gindy (2002) and Hashem (1997) who mentioned that both of *A. craccivora* and *A. gossypii* has two generation on bean plants and Abd El-Gawwad (2008) indicated that the mean number of *L. trifolii* population on *P.vulgaris* plants reached its maximum on April during the two seasons, 2005 and 2006 in summer plantation and EI-

Sayed *et al.* (1991) showed that highest rate of infestation with *B. tabaci* (immature stages) on bean leaf in all plantations (early summer, summer and winter). Also, El-Khayat *et al.* (1994) estimated the relative population density of *B. tabaci* stages on leaves of summer vegetable crops at two locations in Qalubiya Governorate. Amaar *et al.* (2014) revealed that minimum and maximum temperatures had no significant negative effects on the seasonal fluctuation of *T. urticae* during 2011, but in the second season recorded significant negative effects for the tested factors, respectively. While the mean percentages of relative humidity had insignificant positive effect in both seasons and Abo-zaid (2011) who showed that the main pests infesting green bean plants during three successive seasons 2008, 2009 and 2010 during summer plantation were *T. urticae* which the most abundant pest in first season, followed by *L. trifolii, A. craccivora, B. tabaci* and *E. discipiens*.

Table (2): Total number of the main pests recorded on *Phaseolus vulgaris* plants during summer seasons during 2017 and 2018.

Pest species	Summer 2017		Summer 2018		General
	Plant samples	Sweeping net	Plant samples	Sweeping net	Total number
***Aphis gossypii* (Glover)**	36. 33±2.082aa	4.33±1.528b	33.33±3.512aa	6.67±.2.082bb	80.67
***Bemisia tabaci* (Gen.)**	125.00±4.509a	0.00±.000cc	97.33±2.517b	0.00±.000c	222.33
***Aphis craccivora* (Koch.)**	569.33±8.505a	35.33±4.509cc	469.0±123.964b	26.67±5.859c	1100.33
***Thrips tabaci* (Lin.)**	54.33±5.132a	0.00±.000bb	58.33±5.508aa	0.00±.000b	112.66
***Empoasca decipiens* (Paoli)**	68.33±1.528c	125.67±5.508a	58.0±2.000d	87.33±2.517b	339.33
***Ophiomyia phaseoli* (Tryon)**	0.00±.000cc	36.00±2.000a	0.00±.000c	28.33±3.512b	64.33
***Tetranychus urticae* (Koch.)**	591.67±52.994a	0.00±.000cc	332.0±262.092b	0.00±.000c	923.67
***Lirimyza trifolii* (Burgess)**	232.0±2.000a	0.00±.000cc	195.33±10.504b	0.00±.000c	427.33
***Nezara viridula* (L.)**	65.67±5.508aa	30.33±9.504bb	58.0±3.000a	29.33±4.041b	183.33
***Spodoptera litoralis* (Bosi.)**	46.67±3.055aa	16.33±4.041bb	40.33±10.504a	22.67±3.055b	126.00
Total	1789.33	248.00	1341.66	201.00	1579.98

Data expressed as Mean ± S. D.
Values followed by the same letter (s) with in a column are not significantly different from eachother at P=0.05.

Data shown in Table (3) recorded the main pests during two successive spring seasons of 2017 and 2018. The Total number of *A. gossypii* was 94.33 individuals/25 leaves , *B. tabaci (*immature stages) represented by 236.33 individuals/25 leaves, *A.craccivora* 1125.63 individuals/25 leaves, *T.tabaci* 113.66 individuals/25 leaves, *E. decipiens* 302.66 individuals/25 leaves, *O.phaseoli* 74.00 individuals/25 leaves, *T. urticae* 1450.00 individuals/25 leaves, *L. trifolii* 453.67 individuals/25 leaves, *N.viridulla* 221.99 individuals/25 leaves, *S.littoralis* 133.00 individuals/25 leaves. Moreover, there was a highly significant difference between the number of insects between sample plant and sweep net during spring planting date during two seasons.

These results are accordance with Mahmoud *et al.* (2011) who studied the population fluctuation of the leafhopper *E. decipiens* on some plantations such as broad bean, green bean, pea, lupine, potato and squash during winter season of 2008-2009 at El-Kanater El-Khairia farm, Kalubia Governorate. The data indicated that *E. decipiens* had two peaks during its winter activity.

Table (3): Total number of the main pests recorded on *Phaseolus vulgaris* plants during spring seasons during 2017 and 2018.

Pest species	Spring 2017		Spring 2018		General
	Plant samples	Sweeping net	Plant samples	Sweeping net	Total numbers
***Aphis gossypii* (Glover)**	44.33±8.505aa	6.33±2.517bb	38.67±3.512a	5.0±2.000b	94.33
***Bemisia tabaci* (Gen.)**	136.33±6.506a	0.00±.000cc	100.0±10.000b	0.00±.000c	236.33
***Aphis craccivora* (Koch.)**	580.0±10.000a	42.33±2.517cc	470.0±20.000b	33.3±3.512c	1125.63
***Thrips tabaci* (Lin.)**	59.33±2.517a	0.00±.000cc	54.33±4.041b	0.00c	113.66
***Empoasca decipiens* (Paoli)**	67.33±3.055cc	105.0±5.000a	46.0±6.000c	84.33±4.041b	302.66
***Ophiomyia phaseoli* (Tryon)**	0.00±.000cc	40.33±5.508a	0.00±.000c	33.67±3.51b2	74.00
***Tetranychus urticae* (Koch.)**	786.67±77.675a	0.00±.000cc	663.33±60.277b	0.00±.000c	1450.00
***Lirimyza trifolii* (Burgess)**	250.67±11.015a	0.00±.000cc	203.0±2.646b	0.00±.000c	453.67
***Nezara viridula* (L.)**	75.0±5.000a	42.33±2.517cc	65.33±5.033b	39.33±4.041c	221.99
***Spodoptera litoralis* (Bosi.)**	55.0±5.568aa	26.33±3.512b	51.67±7.638a	34. 67±4.509bb	133.00
Total	2054.66	262.65	1692.33	195.63	4225.27

Data expressed as Mean ± S. D.
Values followed by the same letter (s) with in a column are not significantly different from each other at P=0.05

2. Survey and population density of predators:

The trend of occurrence of the predatory species at El Rahmaneia region is shown in Table (4). It was obvious that (12 species) were belonging to eleven families included *Coccinella undecimpunctata* L., *Scymnus* sp. (Coleoptera: Coccinellidae); *Orius* sp. (Hemiptera: Anthocoridae); *Ischnura senegalensis* (Rambur) (Odonata: Coenagrionidae); *Chrysoperla carnea* (Stephens) (Chrysopidae: Neuroptera); *Paederus alfieri* Koch. (Coleoptera: Staphylinidae) and some spider.

Table (4): List of abundant predator species collected from *Phaseolus vulgaris* plants during 2017 and 2018 seasons.

Family	Scientific name
Coccinellidae	*Coccinella undecimpunctata* (L.)
	Scymnus spp.
Staphylinidae	*Paederus alfierii* (Koch.)
Coenagrionidae	*Ischnura senegalensis* (Rambur)
Chrysopidae	*Chrysoperla carnea* (Stephens)
Anthocoridae	*Orius* spp.
Salticidae	*Ballus* sp.
Thomisidae	*Thomisius* sp.
Philodromidae	*Thanatus* sp.
Araneidae	*Singa* sp.
Miturigidae	*Cheiracanthium* sp.
Tetragnathidae	*Tetragnatha* sp.

The trend of the population density of the predators on *P. vulgaris* plants depends mainly on the densities of aphids. The mean number of predators fluctuated during March and April and increased gradually to reach its maximum during May at summer seasons, then decreased towards the end of the season during first June, as shown in Table (5). The mean number of predators fluctuated during September and increased gradually to reach its maximum during October and November at the spring seasons, then decreased towards the end of the season during first December as illustrated in Table (5).

Table (5): Monthly means of population density of predators on *Phaseolus vulgaris* plant during 2017 and 2018 at summer and spring seasons.

Month	Summer season			Month	Spring season		
	2017	2018	Mean		2017	2018	Mean
March	0.0	2.0	1.0	Sept.	4.0	5.45	4.72
April	2.40	3.75	3.08	Oct.	28.2	34.0	31.1
May	15.25	20.0	17.63	Nov.	23.75	30.5	27.12
June	3.0	0.0	1.50	Dec.	2.2	3.45	2.83

3. Survey and population density of parasitoid species:

Data represented in Table (6) showed that the recorded parasitoids were *Diglyphus* sp. (Eulophidae: Hymenoptera) and *Opius* sp. (Braconidae: Hymenoptera). It is cleared that the parasitize larvae of leafminer, *Chromatomyia horticola* Goureau (Diptera: Agromyzidae) in appreciable population. On the basis of relative abundance of *Diglyphus* sp. is considered as major parasitoid on bean ecosystem limiting the population of bean leafminer whereas, *Opius* sp. recorded population. The parasitoids recorded are *Sphegigaster* sp., *Halticoptera* sp., *Gelis* sp., *Ophion* sp., *Brachymeria* sp., *Trissolcus* sp., *Cotesia* sp., *Opius* sp., *Chelonus* sp., *Bracon* sp. and *Hyposter* sp. These results are agreed with Gencer (2004) who reported seven parasitoids species belonging to the Eulophidae (Chalcidoidea). Of these, *Diglyphus isaea* (Walker), *Neochyrsocharis Formosa* (Westwood) and *Neochyrsocharis arvensis* Graham were found to be the most common parasitoids of leafminers. Darvas *et al*. (1999) reported *Diglyphus begini* (Ashmead) as the dominant species on *Chromatomyia fuscula* (Zetterstedt) in south eastern Norway. Mekhlif and Abdul-Rassoul (2002) reported that *D. iseae* and *Cirrospilus vittatus* Walker were found to be dominant larval parasitoids on *C. horticola*.

Table (6): Hymenopterous parasitoids species collected from *Phaseolus vulgaris* plants during 2017 and 2018 seasons.

Family	Genus and species
Pteromalidae	***Sphegigaster* sp.** ***Halticoptera* sp.**
Ichneumonidae	***Gelis* sp.** ***Ophion* sp.**
Chalcididae	***Brachymeria* sp.**
Platygastridae	***Trissolcus* sp.**
Braconidae	***Cotesia* sp.** ***Opius* sp.** ***Chelonus* sp.** ***Bracon* sp.** ***Hyposter* sp.** ***Diglyphus* sp.**

4. Population fluctuation of the leafminers as major pests of *Phaseolus vulgaris* and their parasitoids

Results shown in Table (7) showed that the highest mean number of total mines (occupied and empty) caused by *L.trifolii* larvae were significantly represented higher in summer plantation during 2017 than spring plantation during 2017 represented by 21.95 and 16.19 individuals/25 leaves, respectively.

Table (7): Population fluctuation of *Liriomyza trifolii* and *Ophiomyia phaseoli* larvae on *Phaseolus vulgaris* plants during 2017 season.

Investigation Date	2017						Investigation Date	2017					
	Mines		Total mines	Pupa	Adult			Mines		Total mines	Pupa	Adult	
	Empty	Occupied by larvae			*Liriomyz atrifolii*	*Ophiomyi aphaseoli*		Empty	Occupied by larvae			*Liriomyz atrifolii*	*Ophiomyi aphaseoli*
9 March	2.00	1.00	3.00	1.00	0.25	0.0	14 Sept.	2.00	1.25	3.25	1.0	0.0	0.0
16 March	6.25	3.00	9.25	2.25	0.50	0.25	21Sept.	4.25	2.0	6.25	1.75	0.25	0.0
23 March	17.25	7.25	24.50	4.00	2.25	1.0	28 Sept.	13.75	5.25	19.0	3.50	0.75	0.25
30 March	29.75	9.25	39.00	6.25	1.75	0.50	5 Sept.	22.50	4.75	27.25	2.75	0.50	0.0
6 April	19.75	9.50	29.25	7.75	2.25	0.75	12 Oct.	15.25	7.50	22.75	5.25	1.25	0.50
13 April	12.00	6.25	18.25	3.25	1.25	0.75	19 Oct.	9.50	4.25	13.75	2.50	0.20	0.0
20 April	32. 50	12.00	44.50	6.75	4.75	2.00	26 Oct.	25.25	8.0	33.25	4.25	2.25	1.25
27 April	38.25	18.75	57.00	8.00	3.00	0.75	2 Oct.	34.00	12.25	46.25	6.25	2.0	1.25
4 May;8	14.75	7.00	21.75	3.00	1.25	1.00	9 Nov.	12.75	3.0	15.75	2.0	0.75	0.0
11 May	6.75	4.0	10.75	2.25	1.00	0.75	16 Nov.	3.50	1.0	4.50	0.0	0.25	0.0
18 May	3.25	2.0	5.25	0.25	0.0	0.0	23 Nov.	2.25	0.0	2.25	0.0	0.0	0.0
25 May	1.0	0.0	1.0	0.0	0.0	0.0	30 Nov	0.0	0.0	0.0	0.0	0.0	0.0
Total	151.0	80.0	263.5	44.75	18.25	7.75	Total	145.0	49.25	194.25	29.25	8.20	3.25
Mean	12.58	6.67	21.95	3.73	1.52	0.65	Mean	12.08	4.10	16.19	2.44	0.68	0.27

Results summarized in Table (8) showed that the highest mean number of total mines (occupied and empty) caused by *L. trifolii* larvae were significantly represented higher in summer plantation during 2018 than spring plantation during 2018 represented by 18.31 and 16.10 individuals/ 25 leaves, respectively. These results are going in line with Devkota (2015), who determined the seasonal abundance and spatial distribution of *L. trifolii* on bean plants. He found that bean was planted four times from November 2013 to January 2015. *L. trifolii* recorded highest in activity on two weeks after cultivated three planting dates (November – December, 2013; May-June 2014 and September–October 2014) and the highest abundance of leafminer recorded during November, May and September planting and the lowest population seemed in December plantation. During the spring planted crop, numbers of parasitoids were significantly higher than in winter planting (Bouhssini *et al*., 2008). The results of Bassiony (2019) revealed that the average infestation caused by *L.trifolii* on *P. vulgaris* was 241 larvae/ 25 leaflet and recorded high infestation during the second of February.

Table (8): Population fluctuation of *Liriomyza trifolii* and *Ophiomyia phaseoli* larvae on *Phaseolus vulgaris* plants during 2018 season.

Investigation Date	2018						**Investigation Date**	2018					
	Mines		Total mines	Pupa	Adult			Mines		Total mines	Pupa	Adult	
	Empty	Occupi			*Liriomyzatri folii*	*Ophiomyi aphaseoli*		Empty	Occupied by larvae			*Liriomyzatri folii*	*Ophiomyiap haseoli*
8 Mar.	0.00	0.00	0.00	0.00	0.0	0.0	13 Sept.	1.00	0.25	1.25	0.50	0.0	0.0
15 Mar.	4.25	2.25	6.50	1.25	0.50	0.0	20 Sept.	2.75	1.50	4.25	1.00	0.25	0.25
22 Mar.	15.0	6.25	21.25	3.00	1.25	1.0	27 Sept.	11.25	6.0	17.25	3.50	1.00	0.50
29Mar.	26.75	6.25	33.00	4.25	1.50	1.00	4 Sept.	23.75	3.25	27.00	2.25	1.25	0.25
5Apr.	17.25	7.75	25.00	5.25	2.75	0.50	11 Oct.	16.75	6.75	23.50	4.75	1.75	1.50
12Apr.	14.00	8.25	22.25	4.25	2.25	0.0	18Oct.	15. 0	5.75	20.75	3.25	1.20	1.00
19Apr.	29. 50	9.00	38.50	5.75	3.25	1.00	25 Oct.	22. 50	9.25	31.75	6.75	3.25	0.50
26Apr.	30.25	16.25	46.50	6.00	2.00	0.25	1 Oct.	31.50	13.0	44.50	5.25	1.0	1.00
3May	11.75	5.50	17.25	1.00	0.25	0.25	8Nov.	9.25	5.25	14.50	3.50	0.75	0.25
10May	3.75	1.50	5.25	0.25	0.00	0.00	15 Nov.	4.00	2.0	6.00	0.25	0.0	0.0
17May	2.25	1.0	3.25	0.25	0.0	0.0	22Nov.	2.0	0.25	2.25	0.0	0.0	0.0
24May	1.0	0.0	1.0	0.0	0.0	0.0	29 Nov	0.25	0.0	0.25	0.0	0.0	0.0
Total	126.25	64.00	219.7	31.25	13.75	4.00	Total	102.50	53.25	193.25	31.00	10.45	5.25
Mean	11.48	5.33	18.31	2.60	1.15	0.33	Mean	10.25	4.44	16.10	2.58	0.87	0.44

During summer planting date (Figure, 1), the number of parasitoids (*Opius dissitus* Muesebeck, *D. isaea* and *Halticoptera* sp.) recorded 2 individuals/ 25 leaves on 9th of March 2017 and one peak of 13.25 individuals/ 25 leaves and 13.38% were recorded on 20 March its peak of 23.0 individuals/ 25 leaves and 23.23% in 27 of April (Figure, 1). During spring plantation, the number of parasitoids began with 2.25 individuals/ 25 leaves and 3.36% in14 September 2017 then it increased to reach its peak of 15.25 individuals/ 25 leaves on the second of October and 22.74% (Figure, 2). The parasitism rate of both of *O. dissitus* and *D. isaea* showed insignificant fluctuations allthrough the 12 investigation. The season started with parasitism peak for *O. dissitus* then the percentage tended to decrease till the end of the season with exception of a slight increase in the last inspection (Bassiony, 2019).

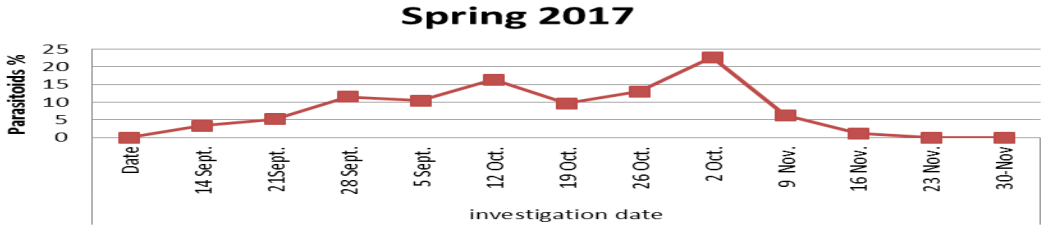

Figure (1): Parasitism percentage of *Opius dissitus*, *Diglyphus isaea* and *Halticoptera* sp. on *Phaseolus vulgaris* during summer season 2017.

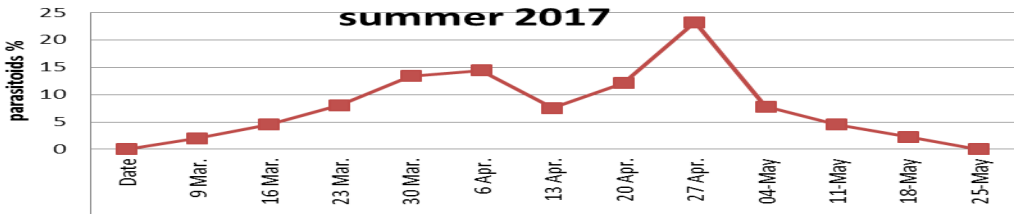

Figure (2): Parasitism percentage of *Opius dissitus*, *Diglyphus isaea* and *Halticoptera* sp. on *Phaseolus vulgaris* during spring date on 2017.

During summer planting date (Figure, 3), the number of parasitoids (*O. dissitus*, *Diglyphus isaea* and *Halticoptera* sp. recorded 3.0 individuals/ 25 leaves in 15th March 2018 and one peak of 10.5 individuals/ 25 leaves and 13.31% were recorded on 15 March its peak of 20.0 individuals/ 25 leaves and 25.97% in 26th April (Figure, 3). In spring plantation, the number of parasitoids began with 0.75 individuals/ 25 leaves and 1.09% in 13 September 2018 then it increased to reach its peak of 16.25 individuals/ 25 leaves on 31st October and 23.71% (Figure, 4). These results agreement with Bhat and Bhagat (2009) reported the occurrence of 7 hymenopteran parasitoids of agromyzid leafminer, *C. horticola* from Kashmir. The various parasitoids recorded were 5 eulophids (*Chrysocharis horticola* Mani, *D. horticola* Khan, *Pediobius indicus* Khan and *Euderus agromyzae* Gangrade) and 2 braconids (*Opius* sp. and *Dacnusa* sp.).

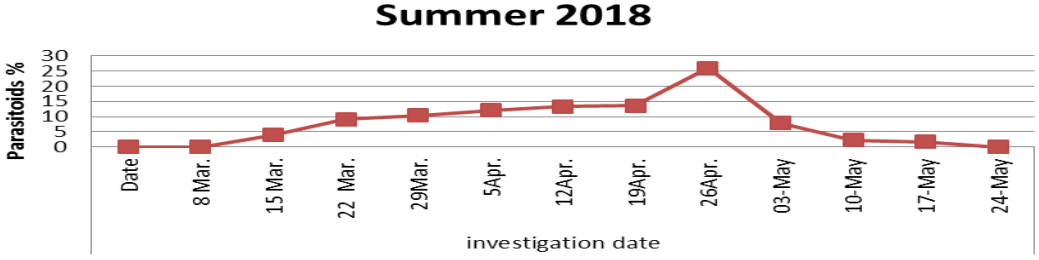

Figure (3): Parasitism percentage of *Opius dissitus*, *Diglyphus isaea* and *Halticoptera* sp. on *Phaseolus vulgaris* during summer date on 2018.

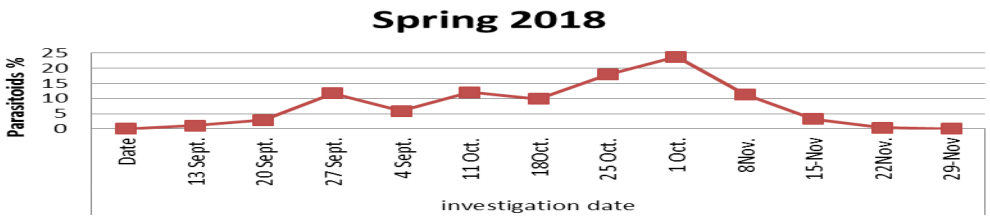

Figure (4): Parasitism percentage of *Opius dissitus*, *Diglyphus isaea* and *Halticoptera* sp. on *Phaseolus vulgaris* during spring date on 2018 season.

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Egyptian Journal of Plant Protection Research Institute

Volume 2:No. (3), September 2019

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Cite references in the text by name and year in parentheses. Some examples:

• Negotiation research spans many disciplines (Abd-Rabou, 1996).

• This result was later contradicted (Simmons and Abd-Rabou, 2009).

• This effect has been widely studied (Abd-Rabou ,1998; Simmons *et al.*, 2002; Evans and Abd-Rabou, 2005and Abd-Rabou *et al.*, 2005).

List style

Reference list entries should be alphabetized by the last names of the first author of each work.

Journal article

Abd-Rabou, S. (1998): The efficacy of indigenous parasitoids in the biological control of *Siphoninus phillyreae* (Homoptera: Aleyrodidae) on pomegranate in Egypt. Pan-Pacific Entomolgists, 74 (3): 169-173.

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