Evaluation of entomopathogenic fungus *Verticillium lecanii* (Zimmermann) Vieges and the predator *Chrysoperla carnea* (Stephens) against cowpea aphid, *Aphis craccivora* (Koch) on faba bean in EGYPT

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ABSTRACT

Three tested treatments of the entomopathogenic fungus *Verticillium lecanii* (Zimmermann) Vieges, the predator *Chrysoperla carnea* (Stephens) and *V. lecanii* followed by release of *C. carnea* in faba bean field for controlling cowpea aphid, *Aphis craccivora* (Koch) compared with control were conducted. The first treatment was *V. lecanii* repeated 4 times at 15-days intervals at the rate of 10⁸ spores/ml. The second was *C. carnea* at the same intervals at the rate of 12,000 predator [larvae (L2)]/feddan/releases. The third was *V. lecanii* applied 2 times at 15-days intervals at the same rate followed by release of *C. carnea* 2 times at 15-days intervals at the same rate. Treatments were conducted during 2006-2007 and 2007-2008 at a location in Ismailia Governorate. These treatments indicated the potential use of these treatments to control *A. craccivora* on faba bean. Reduction in *A. craccivora* population and subsequent yields were significant between treatments. Highest reduction and yield gain was observed when fungus *V. lecanii* was applied.

Keywords: Entomopathogenic, Verticillium lecanii, Chrysoperla carnea, Aphis craccivora

INTRODUCTION

Faba bean is one of the most economic winter crops in Egypt. Extensive efforts are yearly adopted to achieve both high quality and quantity of yield of this legume crop. This crop locally consumed possessing high nutrients value of proteins and carbohydrates (Bahy El-Din, 2006).

Aphids (Homoptera: Aphididae) are serious insect pests attacking nearly all plants including field crops. They feed by sucking plant sap causing leaf curl, wilting, stunting in shoot growth, reducing final yield as well as a general decline in plant vigor. Some aphids are also vectors of plant diseases. The cowpea aphid, *Aphis craccivora* (Koch) is a good example of these aphids causing serious problems to legumes (Abdel-Rahman *et al.*, 2007).

The fungus *Verticillium lecanii* (Zimmermann) Vieges has been considered as an effective biocontrol agent, against aphid pests in general. However, the fungal activity, as a biocide, is a function of its virulence under field conditions. The virulence of such a pathogen is probably associated with certain strains or isolates of the fungus, as well as its infection mechanism. The environmental conditions, particularly humidity, can also influence the fungal activity under field conditions. High humidity is essential for infection to initiate and may even be required for the development of infection on the target host (Sewify, 1989).

The green lacewings, *Chrysoperla carnea* (Stephens) is voracious predators of a wide variety of soft-bodied arthropods including aphids, scales, mealybug, caterpillars, leafhoppers, psyllids, white flies, thrips, insect eggs, spiders, mites and others (Canard and Principi, 1984). Biological control by the use of *C. carnea* has

gained importance in pest management because of its ability to control a host range soft bodied plant pests, having higher searching ability, vast geographical distribution, ease of mass production, wide adaptability in field than other predators and its tolerance to the wide ranges of ecological factors (Saminathan *et al.*, 1999 and Tauber *et al.*, 2000).

The present study amid at the releasing of *C. carnea* as a predator and the fungus *V. lecanii* for controlling *A. craccivora* during growing seasons of 2006/2007 and 2007/2008 at a location in Ismailia Governorate.

MATERIAL AND METHODS

Rearing of insects: The angoumois grain moth, *Sitotroga cerealella* (Olivier) was cultured in the laboratory as prey for rearing *C. carnea* according to the method described by Abd El-Gawad and Abd Al-Aziz (2005).

The predator *C. carnea* was cultured in the laboratory according to the method described by (Ali, 1998).

Preparation of fungus inoculums: Entomopathogenic fungus *V. lecanii* (originally isolated by Abd El-Gawad, 2000) was used. Conidiospores suspension was sprayed on *A. craccivora* and incubated at 25 °C and 65 ± 5 % R.H. Following the host death and sporulation, conidiospores were harvested from cadavers and transferred to Petri dishes containing potato–dextrose agar media (PDA). Isolated fungus was grown using autoclaved PDA media enriched with 1% peptone, 4% glucose, 2% yeast and 5 gm streptomycin (Rombach *et al.*, 1988).

The cultures were incubated at 25 °C for 10 days. Cultures with fully developed conidiospores were washed by sterilized distilled water mixed with 0.05 % Tween – 80 to obtain the stock spore suspension. Spores suspended in sterilized water were counted with a haemocytometer.

Production of conidiospores for field application: Fungal conidia (*V. lecanii*) was produced on a barley substrate composed of 50 gm of barley, 35 ml distilled water and 2 ml pressed sun flower oil. The barley mixed with water and oil was autoclaved in Erlenmeyer flasks (300 ml) for 20 min at 121 °C. Immediately after the lumps of grain were destroyed by shaking the flasks vigorously, the flasks were cooled to room temperature and inoculated with 1 ml of conidia suspension with 10^6 spores/ml, then incubated for 2-3 weeks in the dark at 25 ± 1 °C. The conidia were harvested by suspending them in 50 ml of 0.05% Tween 80. The suspension was filtrated through a double layer of muslin and the desired concentration for field application was obtained by the addition of sterile distilled water. Total spores were counted before application in the field using a haemocytometer.

Field studies: An area of about half feddan (*i.e.*2100 m²) cultivated with faba bean was chosen at Ismailia Governorate. This area was divided separately into 3 treatments and control (C.). Faba bean was sown on November, 30^{th} and 29^{th} during 2006 and 2007 seasons, respectively. All plots received the normally recommended agricultural practices. Experimental treatments started on January, 4^{th} and 5^{th} during 2006/2007 and 2007/2008, respectively. The entomopathogenic fungus *V. lecanii* (F) was sprayed 4 times at 15-days intervals at a rate of 10^8 spores/ml. *C. carnea* (P) was released 4 times at 15-days intervals by a rate of 12,000 predator [larvae (L2)]/feddan/releases. *V. lecanii* was applied 2 times at the same intervals and rate followed by *C. carnea* release 2 times at the same intervals and rate (FP) for controlling the cowpea aphid, *A. craccivora*. Sampling started on January, 11^{th} and 12^{th} and continued weekly until April, 26^{th} and 26^{th} during 2007 and 2008 seasons,

respectively. The efficacy of different treatments was measured as the number of survived individuals for the cowpea aphid, *A. craccivora* on 10 plants.

Statistical Analysis:

The formula of Henderson and Tilton (1955) was used to calculate the reduction rate among populations of the targeted the cowpea aphid, *A. craccivora* in the field after application with the three tested treatments. Obtained data were subjected to analysis of variance (ANOVA) and the means were compared by L.S.D. test at 0.05 level, using SAS (Anonymous, 1988).

Yield Assessments: Yield was evaluated at the end of the experiment for each treatment. Data are presented as estimated weight Kg/feddan.

RESULTS AND DISCUSSION

Field studies

1- Application of V. *lecanü* and releasing of C. *carnea* as a predator against A. *craccivora* in faba bean field:

Results concerning the effect of tested treatments on *A. craccivora* and their percentage reduction are presented herein.

Obtained results revealed that the numbers of *A. craccivora* adults and nymphs on faba bean plants were obviously reduced in the various treatments compared with the control. The overall means of *A. craccivora* were (69.19, 82, 102.06 & 158.88) and (81.88, 95.19, 116.13 & 176.13) on faba bean plants, respectively during 2006-2008 for the three treatments and control, respectively (Figs.1 and 2).



Fig.1: Mean numbers of *A. craccivor* as result of treating faba bean plants with the fungus *V. lecanii* (F), *V. lecanii* followed by *C. Carnea* (FP) and releasing of *C. Carnea* as a predator (P) compared with control (C) in Ismailia Governorate during 2006-2007 seasons.



Fig. 2: Mean numbers of *A. craccivor* as result of treating faba bean plants with the fungus *V. lecanii* (F), *V. lecanii* followed by *C. Carnea* (FP) and releasing of *C. Carnea* as a predator (P) compared with control (C) in Ismailia Governorate during 2007-2008 seasons.

Statistical analysis of the cowpea aphid, *A. craccivora* population for year 2006-2008 showed significant differences between the three tested treatments compared with the control. The obtained values for 2006-2007 for *A. craccivora* were F=28.87, P=0.0001 and L.S.D. = 21.00. The relative values during 2007-2008 for *A. craccivora* were F=31.15, P=0.0001 and L.S.D. = 21.26.

Average reduction of *A. craccivora* is illustrated at Fig. 3. The obtained reduction was 64.89, 57.22 and 47.52 % due to F, FP and P, respectively during 2006-2007. The relative results for the three treatments had the same trend (*i.e.* 62.44, 55.43 and 45.60 %, during 2007-2008, respectively).

Statistical analysis for 2006-2007 and 2007-2008 reduction results showed significant differences between the three tested treatments. Obtained results were F= 22.03, P = 0.0001 and L.S.D. = 5.36 for 2006-2007 and F= 20.07, P = 0.0001 and L.S.D. = 5.45 for 2007-2008.



Fig.3: Average reduction of A. craccivor as result of treating faba bean plants with the fungus V. lecanii
(F), V. lecanii followed by C. Carnea (FP) and releasing of C. Carnea as a predator (P) in Ismailia Governorate during 2006-2007 and 2007-2008 seasons.

2- The yield in the experimental field:

Results of obtained yields are illustrated in Fig. 4. Results revealed that yield was affected by the tested treatments. Applying of fungus *V. lecanii* (F) gave the highest yield followed by *V. lecanii* followed by releasing of *C. carnea* (FP) and releasing of *C. carnea* (P) only as the least rank during both growing seasons. Increase of yield was in relation to *A. craccivora* population reduction.

In conclusion obtained results revealed that applying fungus V. *lecanii* or releasing of C. *carnea* are potential factors for A. *craccivora* on faba bean under field conditions.



Fig. 4: Yield of faba bean plants (Kg/feddan) in three treatments and a control (C.) in Ismailia Governorate during 2006-2007 and 2007-2008 seasons.

Obtained results are in agreement with published results in this manner (*i.e.* Canard and Principi, 1984, Sewify, 1989, Nada, 1999, Mansour, 1999, Saminathan *et al.*, 1999 Tauber *et al.*, 2000, El-Defrawi *et al.*, 2000 and El-Khawas *et al.*, 2004). In general, using of such natural enemies in IPM programmers can be useful with other safe alternative control methods that decrease the application of harmful pesticides.

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ARABIC SUMMARY

تقييم الفطر Verticillium lecanii والمفترس Chrysoperla carnea لمكافحة من الفول على الفول البلدى في مصر

هانى أحمد سيد عبد الجواد - عاطف محمود محمد سيد معهد بحوث وقاية النباتات- مركز البحوث الزراعية

تم تقييم الفطر Verticillium lecanii والمفترس Chrysoperla carnea لمكافحة من الفول على 10⁸ V. lecanii لمكافحة من الفول على الفول البلدى بمحافظة الاسماعيلية عام ٢٠٠٦- ٢٠٠٨ واستخدمت المعدلات الأتية: الفطر (٤مـرات إطلاق) عمر spores/ml عمر رات رش) والمفترس ٢٠٠٩ C. carnea عمر ثاني/ للفدان (٤مـرات إطلاق) والفطر (٢٩٢٢ مرة رش) : المفترس ٢٠٠٩ مرات (٢٠٠٢ مرات إلى للفدان (٢مرة رش) : المفترس ٢٠٠٩ معامل. (٢مرة إطلاق) لمكافحة من الفول عمر ثاني/ للفدان (٢مرة رش) : المعدلات المعدلات الأتية معر ثاني/ للفدان (٢مرة رش) : المفتر محامل معامل.

ُ أُظهرت النتائج فاعلية هذه المعاملات الثلاثة في خفض تعداد المن وكذلك زيادة المحصول بصورة طردية مقابلة للانخفاض في تعداد المن خلال موسم النمو