EVALUATION OF SOME ENTOMOPATHOGENIC FUNGI AND THE PREDATOR *COCCINELLA SEPTEMPUNCTATA* (COLEOPTERA: COCCINELLIDAE) AGAINST CEREAL APHIDS IN EGYPT

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INTRODUCTION

Cereal aphids have been found infesting wheat plants in Egypt (Abdel-Rahman, 2000). *Rhopalosiphum padi* (L.), and *Rhopalosiphum maidis* (Fitch) are the more serious pests on cereal, causing damage to wheat and many other crops (Abdel-Rahman, 1997; Abdel-Rahman, 2000; El-Heneidy *et. al.*, 2006 and Abdel-Rahman, *et al.*, 2006).

Coccinella septempunctata is considered the most important predator controlling the cereal aphids. Hafez (2003) found that *C. septempunctata* is an important predator in reducing the aphids infestations. Hanaa *et. al.* (2004), El-Heneidy *et. al.* (1999); Helenius, (1991) and Mcconnell and Kring, (1990) found that *C. septempunctata* had decreased the infestation with *R, padi* to 53%. Yakoty and Bolling (1999) reported that neem extracts and the parasite *Diaeretiella rapae* (Mantch) decreased Russian wheat aphids under laboratory conditions. Yakoty *et. al.* (1997) found that *Chrysoperla carnea* (Steph.) decreased the infestation of aphids. The entomopathogenic fungi are considered among the biological control agents against insect pests including aphids (Sewify, 1998; and Abdel-Rahman, 2004). In Egypt, few studies revealed the effect of entomopathogenic fungi on the population dynamics of some pests (Abdel-Rahman, 2001 and 2004); Abdel-Rahman and Abdel-Mallek, 2001and Abdel-Rahman, *et. al.*, 2006).

The present work aims to evaluate the effect of entomopathogenic fungi after release of the predator *C. septempunctata* on aphids in wheat fields.

MATERIAL AND METHODS

The aphids *R. padi* and *R. maidis* were reared under laboratory conditions according to Abdel-Rahman (2000) .

Adults of the predator *Coccinella septempunctata* were collected from the fields. They were transferred to the laboratory and reared under laboratory conditions, 22 ± 2 °C and 65±5% RH, feeding on aphids *R. padi* and *R. maidis* (Hanaa *et al.*, 2004).

Cultivation of the fungi

Beauveria bassiana (BR3), *Verticillium lecanii* and *Metarhizium anisopilae* (RM3) were kindly obtained from Prof. Dr. Alain Vey, Mycology unites; National De La Research Scientifique, Univ. Montpellier. *Paecilomyces fumosoroseus (P.f.)* (Apopka strain 97) and *Nomuraea rileyi (N.r.)* (Farlow) (Samson) were obtained from Florida University and reproduced in N.R.C Microbiology Dept. The fungi were primarily purified using the mono-spore technique. They were propagated in Petri-dishes (10cm) on potato dextrose agar medium (PDA) enriched with 1% peptone, 4% glucose, and 0.2% yeast and incubated at 26 C° Seven-days old cultures with well developed spores were harvested by washing with 10 cc sterilized water +0.03% Tween-80 and used as stock suspensions with known spore count and kept in refrigerator at 4 C°, from which the fungi were sub-cultured or produced for laboratory evaluation tests (infectivity and bioassay tests) or adjusted as conidiospores concentrations of $1X10^6$ /ml and mixed with 1% sunflower oil for field application using the spraying technique. Large amount of conidiospores was produced by culturing on liquid medium (Rombach *et al.*,, 1988) in conical flasks or in 1L cell culture glass bottles or on rice grains in 0.5 kg marmalade jars.

Susceptibility of aphids *R. padi* and *R. maidis* to fungi under laboratory conditions

The fungi *B.b.; M.a.; V.l., N.r., P.f.* at the concentrations 107; 10^6; 10^5; 10^4 10^3 and 10^2 spores/ml were tested against *R. padi* and *R. maidis* . Bioassay techniques were employed according to Abdel-Rahman (2001). The percentage of mortality determined and corrected according Abbott, (1925) and LC_{50} calculated according Finney (1964). The experiments were replicated 4 times.

Efficacy of predation of *Coccinella septempunctata* on aphids adults and nymphs

Predator was put in plastic cups 11X12 cm. Each predator supplied by 10 individuals of each species of aphids. The number of consumed adults of *R. padi* and *R. maidis* were calculated each 24 h and the predator was supplied every day by newly 10 individuals of *R. padi* and *R. maidis* during the life cycle till 21 days . The experiment was replicated 4 times. The same methods were applied with the aphid nymphal stages.

Field trials

The field trials were carried out during two wheat successive seasons (2005-2006) at Eben-Malek farm at El –Nobaryia region (N. R. C), to study the effectiveness of the tested fungi on *R. padi* and *R. maidis* in the wheat fields. Wheat plant (Variety Giza 164) were cultivated at mid-November during two wheat successive seasons in area of half feddan and the formulations were then applied as single treatment in randomize plots. Regular agricultural practices were normally performed and no chemical control was used during the study period, weeds were removed by hand. Five plots were sprayed by water as control.

The predator *C. septempunctata* (15 predators/m^2) was released three times with intervals of 15 days. Weekly field samples of green pods were collected randomly from each plot until harvest, the percentage of infestation were calculated.

After releasing the predator, *B. bassiana (B.b.), M. anisopilae (M.a.),* and *P. fumosoroseus (P.f.)* were applied at the sunset as a single treatment in a randomize plot, by the rate of $1X10^6$ spores/ml by five liter sprayer. Each treatment was replicated four times. Four plots were treated by water as control. Samples of the titter were collected weekly and transferred to laboratory for investigations. The percentages of mortality were calculated and the infested of, *R. padi* and *R. maidis* were estimated.

Yield assessment

Data presented yield weight figures in kgs for treated and untreated plots. The yield weight averages were determined at wheat harvest seasons (2005 and 2006).Yield loss was calculated according to the following equations:

$$\text{Yield loss} = \frac{\text{potential yield - actual yield}}{\text{Potential yield}} \times 100$$

Potential yield was the yield of *B. bassiana* treated wheat (which gave the best results among the tested pathogens, taking as a base for comparing other products)

RESULTS AND DISCUSSIONS

Under laboratory conditions, data shows that LC_{50} s of *R. maidis* ranged between 42.5 and 67.5 $X10^5$ spores/ml after treatment with the different fungi (Table1). When the aphid *R. padi* was treated by the microbial control agents the LC_{50} s recorded 37.7, 46.7, 58.9, 58.8 and 36.7 $X10^4$ spores/ml , after treated with different

concentrations of *B.b., M.a., V.l., N.r., P.f.*, respectively (Table 2). Abdel-Rahman *et. al.* (2001 and 2004); Abdel-Rahman and Abdel-Mallek (2001) controlled cereal aphids under laboratory and field conditions by entomopathogenic fungi. Abdel-Rahman, *et. al.* (2006) controlled the cereal aphides by the fungus *B. bassiana*, they found that the infestation was reduced after fungi applications under laboratory and field conditions. Sabbour and Sahab (2005 and 2007) suggested that the fungi reduce the infestations with cabbage and tomato pests under laboratory and field conditions. Data in Table 3, show that the consumed number of *R. maidis by* the predator adult stages significantly increased during the first and second week to 78.6, 91.7 individuals but during the third week the aphids numbers were significantly decreased to 66.9 individuals. One day after placing nymphal stage of both *R. maidis* and *R. padi* the percentage mean of consumed numbers were 65.9 and 68.7, respectively. After 5-7 days the percentage mean number recorded the highest consumed number (96.9 and 98.1) by the predator *C. septempunctata* (Table 3 and 4). The same results were obtained by Hanaa *et al.*,(2004), Formusoh and Wild (1993), Mcconnell and Kring (1990), Ferran *et al.*,(1989), Alhag *et al.*, (1996) and Subhash, (1996). They suggested that during the third nymphal stage of cereal aphids, predation efficacy of *Coccinella* spp. was very high. Data in (table 5) show that after predator release the results showed that the percentage mean number of infestations with *R. maidis* and *R. padi*, decreased significantly to 61 and 67% as compared to 100 and 99% in the control (Table 5). In the plots treated with fungi only, the percentage mean of infestation ranged between 16-18% with *R. maidis* and 10-18% with *R. padi.* After fungi and predator applications, the percentage mean numbers of infestations were highly significant where they decreased to 7% for *R. maidis* and 9% of *R. padi*, followed by *B.b* and predation application, respectively (Table5). Mesbah *et al.*, (2004) reported that the treatments of sugar beet by the fungi *B. bassiana* after releasing the egg parasitoid *Trichogramma evanescens* reduced the infestation of some lepidopterous insect pests. Sabbour (1992) found that the fungus *B. bassiana* could control the potato tuber moth and not affecting *T. evanescens* and *Microbracon instabilis.* The same results were obtained by Adel- Rahman *et al.*, (2006). The yield assessments after treatments with the fungi and the predator *C. septempunctata* showed that during season 2005 the highest yield of the wheat recoded, 1243 kg/feddan in the plots treated with *B. bassiana* as compared to 1115 kg/feddan in the control plots. The weight of the crop in all tested treatments ranged between 1201 and 1243 kg/feddan. This shows significant reduction of the yield ranged between 0 and 17 % in the different plots treated with the fungi during season 2005 (Table 6). During season 2006, the yield weight was significantly increased after treatments with different fungi tested as compared to 1102

kg/feddan in the control plots (Table 6). Accordingly, the percent of reduction in the yield ranged between 0-13 % as compared to 60% in the control. The same results were obtained by Mansour (1999) who found that the yield increased after treatments with the fungus *B. bassiana*. Sarhan (2004) found that the infestation with the potato tuber moth was by the parasitoid and predatorrelease. Hassan (1993) suggested that the combinations of parasitoids, predator and applied pathogens provided good control for certain maize pests. Hassan *et. al.* (1987); Takada *et. al.* (2001) and El-Mandrawey *et. al.* (2004) suggested that the released of *T. evanescens* followed by applications of *B. thuringiensis* gave a good control of *Ostrinia nubilalis* in the field. Abo- Sheaesha and Agamy, (2004) reported that *B. thuringiensis* (Agerin) or egg parasitoid *T. evanescens* are suitable candidates for controlling citrus flower moth *Prays citri* in lime orchards within IPM system. Mesbah *et. al.* (2004) reported that *B. thuringiensis, B. bassiana* and *T. evanescens* were mainly effective as biocides and reduced the infestations of the sugar beat insect pests in Kafer El-Sheikh.

TABLE (I)

Effect of tested fungi on *R. maidis* under laboratory conditions.

Fungi	LC_{50}	Slope	Variance	95%confidence limits
B.b.	$42.5 X10^5$	0.6	1.4	2.26-6.68
M.a.	$54.7 X10^5$	21	1.7	3.27-8.99
V.l.	$66.8 X10^5$	1.2	2.1	4.38-9.93
N.r.	$67.5 X10^5$	1.36	2.3	4.45-8.91
P.f.	$44.7 X10^5$	15	1.8	2.23-6.78

TABLE (II)

Effect of tested fungi on *R .padi* under laboratory conditions.

Fungi	LC_{50}	Slope	Variance	95%confidence limits
B.b.	$37.7 X10^4$	1.5	1.6	2.68-7.78
M.a.	$46.7 X10^4$	1.2	1.4	3.35-6.78
V.l.	$58.9 X10^4$	1.4	1.6	4.46-9.81
N.r.	$58.8 X10^4$	1.6	0.8	4.03-8.85
P.f.	$36.7 X10^4$	0.7	0.6	3.01-8.73

TABLE (III)

Efficacy of predation of *C. septempunctata* on adult and nymphal stages of *R. maidis* under laboratory conditions.

Adult age (days)	% means number of consumed aphids	Nymph age (days)	% means number of consumed aphid
1-7	78.6 ±10.1	One day old	65.9 ±9.3
6-14	91.7±11.3	2-4	77.8 ±10.2
15-21	66.9 ±12.1	5-7	96.9±9.8
		8-11	88.7±11.1
F value=	27.3	F value=	25.2
Lsd=	11.6	Lsd=	10.1

TABLE (IV)

Efficacy of predation of *C. septempunctata* on adult and nymphal stages of *R. .padi* under laboratory conditions.

Adult age(days)	% means number of consumed aphids	Nymph age (days)	% means number of consumed aphid
1-7 6-14 15-21	90.6±8.9 95.3±10.3 60.1±10.2	One day old 2-4 5-7 8-11	68.7±11.4 75.6±12.3 98.1±9.9 84.1±11.5
F value= Lsd=	26.4 9.15	F value= Lsd=	24.8 10.4

TABLE (V)

Bio-effect of the tested fungi on the infestation % number of cereal aphids in the field.

Fungi tested	%(mean number of infestation) After fungi application only		after fungi and predation	
	R. maidis	*R. padi*	*R. maidis*	*R. padi*
B.b.	16	18	7	9
M.a.	16	11	10	10
P.f.	18	10	8	8
Predation only	61	67		
Control (untreated & non predation)	100	99		
F value= LSD5%=	22.6 11.9			

TABLE (VI)

Assessment of damage in the relation to treatments with fungi and predation with *C. septempunctata.*

Treatments	Season 2005		Season 2006	
	Wt of wheat kg/ feddan	%of yield loss kg/feddan	Wt of wheat kg/fed	%of yield loss kg/feddan
B.b.	1243 ± 77.8	0	1257 ± 65.9	-
M.a.	1201 ±56.9	17	1222 ± 77.9	13
P.f.	1243 ± 78.4	-	1256 ± 66.8	-
control	1115 ± 59.7	51	1102 ± 71.3	60
F value= LSD5%=	47.66 119.5		34.5 123.4	

SUMMARY

The present investigations were carried out during two successive wheat growing seasons 2005 and 2006. The entomopathogenic fungi *Beauveria bassiana, Metarhizium anisopliae, Verticillium lecanii, Nomuraea rileyi, Paecilomyces*

fumosoroseus and the predator *Coccinella septempunctata* were evaluated under laboratory and field conditions against aphids *Rhopalosiphum padi* and *Rhopalosiphum maidis*. Data shows that the fungus *B. bassiana* significantly resulted the best results followed *P. fumosoroseus, M. anisopliae, V. lecanii and N. riley* against *R. padi* and *R. maidis*. LC_{50} s for *R. maidis* and *R. padi* treated with the entomopathogenic fungi ranged between 42.5 and 67.5 $X10^5$ spores/ml and for *R. padi* between 63.7 and 58.9 $X10^4$ spores/ml, respectively. Under field conditions, the treatments with the different fungi after releasing the predator decreased the infestations with the cereal aphids. Also, the percentage of yield loss was significantly decreased to 17% after predator releasing and fungi treatments, as compared to 51% in the untreated plots during season 2005. During season 2006 the percentage of yield loss in the treated plots was 13% as compared to 60% in the control.

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