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Original paper

Entomopathogenic Fungi on Fabae bean Aphid, Aphis craccivora (Koch) (Hemiptera: Aphididae)

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Abstract

The Aphids, *Aphis craccivora* (Koch) is a serious pest in agricultural fields in Egypt. Entomopathogenic fungi are biological control agents of insects. The study was carried out on the aphid by using of Bio Catch, *Verticillium lecanii*, Bio Power, *Beauveria bassiana* and Bio Magic, *Metarhizium anisopliae* on *A. craccivora*. Laboratory experiments were done to measure the pathogenicity of three commercial compounds from entomo-pathogenic fungi, Bio Catch, *V. lecanii*, Bio Power, *B. bassiana*, and Bio Magic, *M. anisopliae* against adults and nymphs of *A. craccivora*. Three concentrations were used from all compounds, 10^7, 10^8 and 10^9 spores/ml. at 22 ± 2°C and 75 ± 5 R.H. The concentration (10^9 spores/ml.) gave 100% mortality with *V. lecanii* and *B. bassiana* then *M. anisopliae*. The Lowest LC_{50} value of 2.1 x 10^6 spores/ml. was recorded by *V. lecanii*, which showed higher virulence compared to other entomopathogenic fungi. The LC_{50} values of *V. lecanii*, *B. bassiana* and *M. anisopliae* were 2.1 x 10^6, 4.3 x 10^6 and 6.4 x 107 spores ml., respectively. At the highest concentration of 10^9 spores/ml., the Median LT_{50} values for *V. lecanii*, *B. bassiana* and *M. anisopliae* were 4.2, 5.2 and 7.0 days, respectively.

Keywords *Aphis craccivora*, Entomopathogenic Fungi, control.

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Introduction

The faba bean, *Vicia faba*, is one of the most important leguminous crops as a source of plant protein in Egypt. This crop is highly susceptible to infestation with *Aphis craccivora* (Koch) (EL-DEFRAWI, 1998). There are over 700 species of entomopathogenic fungi reported (RABINDRA and RAMANUJAM, 2007), however, some species have been effectively utilized widely as microbial control agents such as *Beauveria bassiana*, that attack a wide range of pests. The fungus *B. bassiana* (Hyphocreales, Ascomycota) is a facultative saprophytic fungus infects and kills insects and other arthropods. This fungus inhabits a variety of plant species as endophytes (BARELLI et al, 2016). Entomopathogenic fungi infect the insect through penetration of the cuticle. Germ tubes grow through the layers of the cuticle using the enzymatic action and finally enter the haemocoel (ANDERSON et al, 1995). Aphids are dangerous insect pests all over the world. It is important to appreciate the effect of entomopathogenic fungi on the aphid pest at different developmental stages (WANG and KNUDSEN, 1993; SHIMAA et al, 2020). Aphids can be attacked by entomopathogens of Zygomycetes but the entomophthoralean fungi are the major fungal pathogens of aphids (HUMBER, 1991). *Verticillium lecanii* is one of the most important Hyphomycetes parasites of aphids. It can attack a broad spectrum of insects both in tropical and temperate regions (HALL, 1984; HUMBER, 1991 and SABRY et al, 2011). The fungus can rarely affect aphids under field conditions (FENG et al, 1990, HUMBER, 1991, HATTING et al, 1999, ABDEL-RAHEEM and LAMYA AHMED EL-KERIDIS, 2017 and ABDEL-RAHEEM, 2011, 2019, ABDEL-RAHEEM et al, 2009, 2010, 2016a & b 2019, 2020a & b). *A. craccivora* is one of the most common and well-known insect pests throughout the world (MINKS and HARREWIJN 1987; BLACKMAN and EASTOP 2006; SADEGHI et al. 2009; ISMAIL and ABDEL-RAHEEM; 2010, ISMAIL et al, 2016; ZAKI and ABDEL-RAHEEM, 2010). Aphids are important piercing-sucking insects during feeding cause significant loss of a plant's phloem sap, which is essential for plant growth (DIXON, 1998). Indirectly, cowpea aphid also disturbs the photosynthesis process by the presence of fungus on the leaves that is supported by the aphids' honeydew secretion (KLINGLER et al, 2001, SMITH and BOYKO, 2007). Plant damage increases because of the aphids' role as vectors for numerous plant viruses (ALDRYHIM and KHALIL, 1993; SMITH and BOYKO, 2007), such as faba bean necrotic yellow virus, broad bean yellow mosaic virus, and bean leaf roll virus (WEIGAND and BISHARA, 1991).

Many of entomopathogenic fungi are a great potential for the management of sucking pests (RABINDRA and RAMANUJAM, 2007; ABLA and ABDEL-RAHEEM 2020; SABBOUR et al, 2020; MOHAMED ABDEL-RAHEEM, 2020).

The present investigation aimed to clarify the efficiency of Entomopathogenic Fungi to control one of the most serious pests of agriculture crop and plant viral vector, *A. craccivora.*

Materials and Methods

Plant cultures

Faba bean *Vicia faba* seeds (10 cm diameter, 15 cm high) containing were kept in Lab. Fourteen days after cultivation rearing of aphids.

Rearing of aphids

Aphids were reared for the bioassay in the laboratory by using the method of Yeo et al. (2003). Initially 20 adult apterous aphids were inoculated on fresh cowpea seedlings in the trifoliate stage. The inoculated aphids reproduced parthenogenetically, and the newly formed one day old 1st nymphs were reared on the same plant. After 24 h, the inoculated adult aphids were removed from the seedlings and were used for the bioassay studies.

Commercial Indians Compounds

This Compounds were Provided from Company of Saiefe Gaarah at Cairo, Egypt, Bio Catch, *V. lecanii*, Bio Power, *B. bassiana* and Bio Magic, *M. anisopliae.*

Preparing of the concentrations

Three concentrations were used (C_1) 10^7, (C_2) 10^8 and (C_3) 10^9 spores/ml. and add 0.5% Tween 80. The spores were counted in the suspension using a haemocytometer (Swastik Scientific Company, India) blood cell counting chambers (Hirscmann 0.1 mm x 0.0025 mm^2). A haemocytometer is essentially a microscope slide bearing a small well of known depth. The base of which is marked with squares of known dimensions. During use the well is covered with a special coverslip (usually 0.4 mm thick).

Bioassay

Three concentrations (10^9, 10^8, 10^7 spores/ml.) were prepared for *V. lecanii*, *B. bassiana* and *M. anisopliae.* Each concentration was replicated three times.

Totally 30 aphids were used for each treatment. Mortality of aphids was recorded separately at 24 h interval up to seven days. Dead aphids were collected daily, and placed in Petri-dish containing a moist filter paper and kept in humid chamber. The dead aphids which produced mycelial growth were considered for the mortality count. Neonate aphids were counted and removed daily. Mortality data was corrected with that in control by using the Abbott's formula (ABBOTT, 1925). The data was then analyzed by probit analysis (FINNEY, 1971) and the Median Lethal Concentration (LC_{50}) and the Median Lethal Time (LT_{50}) values were computed by using statistical computer programme, Statistical Package of Social Sciences (SPSS).

Statistical analysis

The per cent corrected cumulative mortality of each fungus was subjected to ANOVA test and the means were separated by Duncan's Multiple Range Test (DMRT).

Results and Discussion

The % mortality of nymphs of *A. craccivora*

Table 1 showed that percent mortality of nymphs was 15.2% after 3rd days, at the concentration (10^9 spores/ml.) of *V. lecanii.* After 3rd days of infection with *B. bassiana and M. anisopliae* recorded of percent mortality ranging between 7.0 to 10.5 and 5.5 to 9.0%.

All the entomopathogenic fungi in concentration (10^9 spores/ml.) produced high mortality ranging from 75.0 to 100 percent, after 8th days of infection.

Table 1. Percent Mortality of nymphs of *A. craccivora* at different time using Entomopathogenic Fungi compounds at 22 ± 2°C and 75 ± 5 R.H.

Entomopathogenic Fungi compounds	Concentrations (Spores/ml.)	Corrected mortality %						
		Day						
		2nd	3rd	4th	5th	6th	7th	8th
Bio Catch, *V. lecanii*	C_3	0.0	15.2	33.6	46.5	80.0	95.2	100
	C_2	0.0	11.5	27.0	42.8	77.0	85.0	90.0
	C_1	0.0	8.7	22.5	35.2	63.0	75.0	80.0
Bio Power, *B. bassiana*	C_3	0.0	10.5	22.5	41.5	76.0	85.0	90.0
	C_2	0.0	8.5	18.0	35.0	65.0	80.0	85.0
	C_1	0.0	7.0	15.0	22.9	50.0	75.0	79.9
Bio Magic, *M. anisopliae*	C_3	0.0	9.0	20.8	28.9	70.2	80.0	85.0
	C_2	0.0	7.0	16.5	21.2	53.0	70.0	80.0
	C_1	0.0	5.5	12.0	17.0	45.0	55.0	75.0

(C_1) 10^7, (C_2) 10^8 and (C_3) 10^9 spores/ml.

The percent mortality of adults of *A. craccivora*

Table 2 indicates that percent mortality of adults was 15.0% after 3rd days from infection, at a concentration of (10^9 spores/ml.) of *V. lecanii.* After 3rd days of infection by *B. bassiana and M. anisopliae* the percent mortality ranging between 12.0 to 9.0 at 10^9 & 10^8 spores/ml. and 11.0 to 7.5% at 10^9 & 10^8 spores/ml. All the entomopathogenic fungi in concentration (10^9 spores/ml.) produced high mortality ranging from 65.0 to 100%, after 8th days of infection. Yokomi and Gottwald, 1988; Saleh, Saleh, et al., 2016; Abdel-Raheem, et al., 2010; 2020 and Abdel-Raheem, 2011, also reported the percent mortality of three aphid species *Myzus persicae, Aphis gossypii* and *Aphis citricola* at 10^6-10^7 spores/ml. after four days. Smitha, 2007 and Ekesi, et al., 2000 mentioned the similar result with 91 and 93% mortality of *A. craccivora* at 7th day of infection.

Table 2. Percent Mortality of adults of *A. craccivora* at different time using Entomopathogenic Fungi compounds at 22 ± 2°C and 75 ± 5 R.H

Entomopathogenic Fungi compounds	Concentrations (Spores/ml.)	Corrected mortality %						
		day						
		2nd	3rd	4th	5th	6th	7th	8th
Bio Catch, *V. lecanii*	C_3	0.0	15.0	30.0	50.0	80.0	88.0	100
	C_2	0.0	12.0	25.0	45.0	76.0	82.0	95.0
	C_1	0.0	10.0	20.0	37.0	73.0	80.0	90.0
Bio Power, *B. bassiana*	C_3	0.0	12.0	22.0	40.0	79.0	85.0	90.0
	C_2	0.0	9.0	19.0	34.0	65.0	77.0	85.0
	C_1	0.0	7.0	14.0	25.0	45.0	68.0	79.0
Bio Magic, *M. anisopliae*	C_3	0.0	11.0	20.0	30.0	65.0	80.0	85.0
	C_2	0.0	7.5	13.5	25.8	48.0	75.0	78.0
	C_1	0.0	6.0	10.9	18.0	47.0	55.0	65.0

(C_1) 10^7, (C_2) 10^8 and (C_3) 10^9 spores/ml.

The Cumulative mortality of *A. craccivora*

After 8th days from infection the corrected cumulative percent mortality was analyzed by ANOVA and the results are presented in Table 3. *V. lecanii* was closely followed by *B. bassiana* causing 90.0% mortality, which was as effective as *V. lecanii*.

Table 3. Dose mortality of entomopathogenic fungi against *A. craccivora*

Entomopathogenic Fungi compounds	LC_{50} (Spores/ml.)	95% Fiduciallimits (spores ml-1)	Relative toxicity
Bio Catch, *V. lecanii*	2.1×10^6	1.1×10^6 - 4.0×10^6	1.1
Bio Power, *B. bassiana*	4.3×10^6	1.1×10^6 - 3.3×10^7	1.7
Bio Magic, *M. anisopliae*	6.4×10^7	3.2×10^7 - 1.3×10^8	1.8

The median Lethal Concentration (LC_{50})

Table 3 shows the LC_{50} values and the relative toxicity of the three entomopathogenic fungi. Low LC_{50} value of 10^7 spores/ml. for *V. lecanii* against *A. craccivora* was 2.1×10^6 spores/ml. Low LC_{50} value of 1.2×10^4 spores ml-1 for *V. lecanii* against *Brevicoryne brassicae* and 2.7×10^4 spores ml-1 against *A. gossypii* was reported by Derakshan et al. (2007) and Karindah et al. (1996). Liu et al. 1999 mentioned that for *B. bassiana* was 1.2×10^4 spores ml-1 and Chandler, 1997 mentioned that for *M. anisopliae* was 2.45×10^6 spores ml-1.

Also, Saranya et al. 2010, mentioned that concentration (10^8 spores ml-1) 100% mortality was obtained with *V. lecanii* and *H. thompsonii* followed by *B. bassiana*, *M. anisopliae* and *C. oxysporum*. Mortality declined with the decrease in concentrations. The lowest LC_{50} value of 2.5×10^4 spores ml-1 was recorded by *V. lecanii* and *H. thompsonii* isolates.

The median Lethal Time (LT_{50})

Table 4 obtained the LT_{50} values at different concentrations. LT_{50} values decreased with increase in concentrations.

LT_{50} value At 10^9 spores/ml. from *V. lecanii*, *B. bassiana* and *M. anisopliae* were 4.2, 5.2 & 7.0 days respectively.

Nirmala et al. (2006) mentioned the similar results for *B. bassiana* with LT_{50} value of 3.17 days. Hesketh et al. 2008 and Saranya et al. 2010 also agree with them. They mentioned that LT_{50} value was 3.31 days with *V. lecanii* against *Aphis fabae*.

Table 4. Time mortality response of fungal isolates against *A. craccivora*

Entomopathogenic Fungi	Median Lethal Time (Days)		
	C_3	C_2	C_1
Bio Catch, *V. lecanii*	4.2	5.5	6.0
Bio Power, *B. bassiana*	5.2	6.5	6.9
Bio Magic, *M. anisopliae*	7.0	7.6	8.5

(C_1) 10^7, (C_2) 10^8 and (C_3) 10^9 spores/ml.

Conclusion

Entomopathogenic fungi, Bio Catch, *V. lecanii*; Bio Power, *B. bassiana* and Bio Magic, *M. anisopliae* against *A. craccivora* as biological control agent are promising in the future. The authors advise farmers to use *V. lecanii* against *A. craccivora* in IPM program. Bio Catch was the highest pathogenicity against the nymphs of *A. craccivora* then Bio Power and Bio Magic.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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