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Using of Entomopathogenic Fungi against Fabae bean Aphid, *Aphis craccivora* (Koch)

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Abstract : Laboratory bioassay studies were carried with three different concentrations of *Beauveria bassiana* (Bals.) Vuill., *Metarhizium anisopliae* (Metsch.) Sorokin. and *Verticillium lecanii* (Zimm.) against *Aphis craccivora*. In the high concentration (2 x10^5 spores/ ml.) 100% mortality was obtained with *V. lecanii* and *B. bassiana* followed by *M. anisopliae*. Mortality declined with the decrease in concentrations.
Lowest LC_{50} value of 2.2 x 10^3spores/ ml. was recorded by *V. lecanii*, which showed higher virulence compared to other isolates. The LC_{50} values of *V. lecanii*, *B. bassiana* and *M. anisopliae* were 2.2 x 10^3, 4.2 x 10^3 and 6.2 x 10^4spores ml., respectively. At the highest concentration of 2 x10^5 spores/ ml., the Median LT_{50} values for *V. lecanii*, *B. bassiana* and *M. anisopliae* were 4.8, 5.80 and 7.0 days, respectively. The LT_{50} values were found to be inversely proportional to the spore concentrations.
Key words : Entomopathogenic Fungi, *A. craccivora*, control.

Introduction

Aphids can be attacked by entomopathogens of Zygomycetes and Hyphomycetes, but the entomophthoralean fungi of the class Zygomycetes are the major fungal pathogens of aphids [1].Entomopathogenic Hyphomycetes include hundreds of species, but just a few of them are specific to aphids.

Verticillium lecanii is one of the most important Hyphomycetes parasites of aphids. It can however attack a broad spectrum of insects both in tropical and temperate regions, but it is distributed mainly in tropical regions [1, 2]. The fungus can rarely affect aphids under field conditions [1, 3-7].

Crop damage by aphids is largely due to virus transmission but direct feeding damage can also cause significant yield losses in outbreak years. There is increasing economic and environmental pressure to develop alternative control strategies to replace chemical control for use in integrated pest management strategies. These include the exploitation of beneficial organisms such as entomopathogenic fungi.

A. craccivora is one of the most common and well-known insect pests throughout the world [8-11].

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Aphids are feeding cause significant loss of a plant's phloem sap, which is essential for plant growth [12]. Indirectly, cowpea aphid also disturbs the photosynthesis process by the presence of fungus on the leaves that is supported by the aphids' honeydew secretion [13, 14].

Plant damage increases because of the aphids' role as vectors for numerous plant viruses [14, 15], such as faba bean necrotic yellow virus, broad bean yellow mosaic virus, and bean leaf roll virus [16].

More than 750 species of fungi are pathogenic to insects and many of them offer a great potential for the management of sucking pests [17].

The goal of this study to evaluated the efficiency of Entomopathogenic Fungi towards Aphis *craccivora*.

Materials and Methods

Plant cultures

Faba bean *Vicia faba* seeds (15cm diameter, 20 cm high) containing were kept in Laboratory 15 days after cultivation rearing of aphids.

Rearing of aphids

Aphids were reared for the bioassay in the laboratory by using the method of [18]. 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.

Fungal isolates

The entomopathogenic fungi *V. lecanii, B. bassiana* and *M. anisopliae* were re isolated after proving the Koch postulates.

Preparation of spore concentrations of the fungal isolates

All the three fungal isolates were cultured in 100ml SMA+Y liquid medium in 250ml conical flask and incubated at room temperature for 10 days. After sporulation, ground with ordinary mixer and made into liquid spore suspension.

This was filtered through double layered muslin cloth to remove the mycelial mat. The suspension was shaken thoroughly with a drop of Tween 80 solution for uniform dispersion of the spores in the solution. The spore count was made by using a haemocytometer. All the cultures were adjusted to $2x10^5$ spores/ ml. from which the lower concentrations were prepared by serial dilution technique for bioassay studies.

Bioassay

Cowpea seedlings were raised in small plastic cups of size 5x7cm in the laboratory. Fifteen days old seedlings were used for the bioassay studies. Three concentrations, ((C_1) 2 x 10^5, (C_2) 2 x 10^4, (C_3) 2 x 10^3 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. After inoculation of aphids, the respective concentrations of all the fungal spore suspensions were sprayed on the seedlings using an atomizer. Aphids sprayed with 0.05 per cent Tween 80 solution served as control.

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 from the seedlings. Mortality data was corrected with that in control by using the Abbott's formula [19]. The data was then analyzed by probit analysis [20] 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

Mortality of adults of *A. craccivora*

Mortality of aphids was monitored at 24 h interval up to 8th days. The data of corrected per cent mortality at different time intervals presented in Fig.1 indicates that the mortality increased with increase in time interval.

Mortality of aphids was 10.3 per cent observed within 3rd days, at the highest concentration (2 x10^5 spores/ ml.) of *V. lecanii*. After 3rd days of exposure, among the two fungal isolates *B. bassiana and M. anisopliae* recorded of per cent mortality ranging between 4.6 to 7.6 at 2 x 10^5 & 2 x 10^4spores/ ml. and 3.3 to 6.7 per cent at 2 x 10^5 & 2 x 10^4spores/ ml.

All the concentrations of *V. lecanii, B. bassiana and M. anisopliae* recorded mortality of aphids after 3rd days only.

At 5th days after treatment the highest per cent mortality was obtained in the highest spore concentration of 2 x 10^5 spores/ ml. in *V. lecanii* (43.7%) followed by *B. bassiana* (34.9%) and *M. anisopliae*(21.7%).

At 6th days and 7th days after treatment there was marked increase in the mortality of aphids.

All the fungal isolates in the highest spore concentration (2 x 10^5 spores/ ml.) produced high mortality ranging from 87.4 to 100 per cent, after 8th days of treatment. [21, 5, 6], also, reported cent per cent mortality of three aphid species *Myzus persicae, Aphis gossypii* and *Aphis citricola* at 10^6 -10^7 spores/ ml. after four days. Increased mortality of *H. thompsonii* at 10^8 spores /ml. has also been reported by [14]. At the highest concentration of 10^7 spores /ml., *B. bassiana* and *M. anisopliae* also gave appreciable reduction in population showing 96.66 and 80.76 per cent respectively. [22] also got similar result with 91 and 93 per cent mortality of *A. craccivora* at 7th day of treatment.

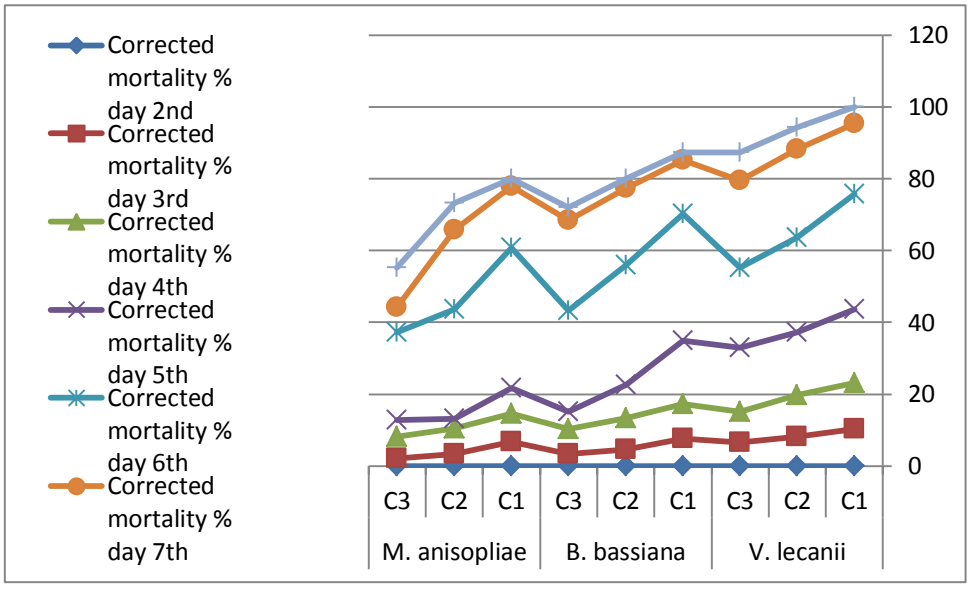

Fig. 1. % Mortality of adults of *A. craccivora* at different time and different concentration (C_1) 2 x 10^5, (C_2) 2 x 10^4, (C_3) 2 x 10^3 spores/ ml.

Mortality of nymphs of *A. craccivora*

Mortality of nymphs was monitored at 24 h interval up to 8th days. The data of corrected per cent mortality at different time intervals presented in Fig. 2 indicates that the mortality increased with increase in time interval.

Mortality of nymphs was 12.6 % observed within 3rd days, at the highest concentration ($2x10^5$ spores/ ml.) of *V. lecanii*. After 3rd days of exposure, among the two fungal isolates *B. bassiana and M. anisopliae* recorded of per cent mortality ranging between 4.9 to 8.9 at 2 $x10^5$ spores/ ml. and 4.2 to 7.9 % at 2 x 10^5 spores/ ml.

At 5th days after treatment the highest per cent mortality was obtained in the highest spore concentration of $2x10^5$ spores/ ml. in *V. lecanii* (44.9%) followed by *B. bassiana* (37.5 %) and *M. anisopliae*(25.8%).

At 6th days and 7th days after treatment there was marked increase in the mortality of nymphs.

All the fungal isolates in the highest spore concentration ($2x10^5$ spores/ ml.) produced high mortality ranging from 75.5 to 100 per cent, after 8th days of treatment.

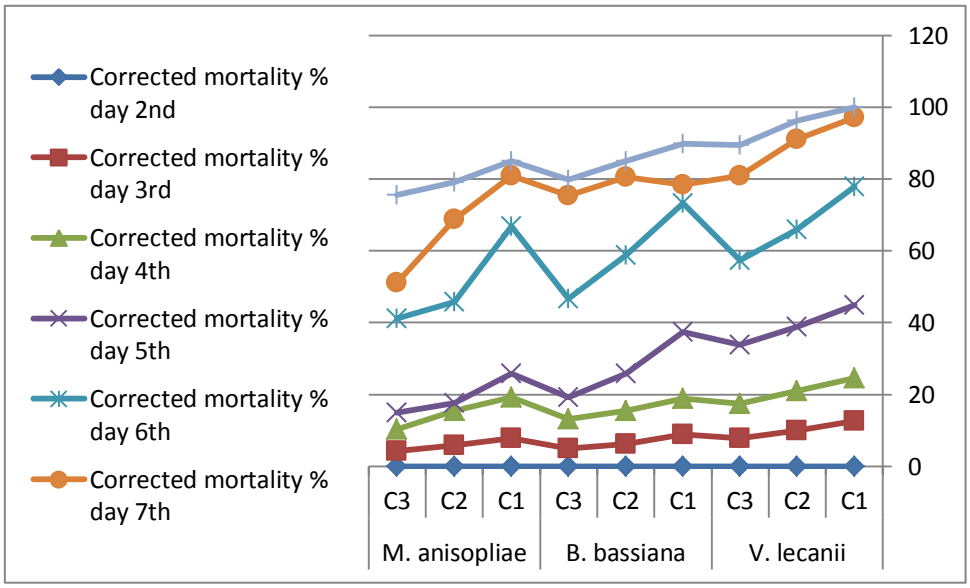

Fig. 2. % Mortality of nymphs of *A. craccivora* at different time and different concentration (C_1) 2 x 10^5, (C_2) 2 x 10^4, (C_3) 2 x 10^3 spores/ ml.

Cumulative mortality of *A. craccivora*

The corrected cumulative mortality per cent at 8th days after treatment was analyzed by ANOVA and the results are presented in Table 1. The cumulative per cent mortality of *A. craccivora* with the fungal isolates was found to be statistically on a par at $2x10^5$ concentration. At the highest concentration of $2x10^6$ spores/ ml., *V. lecanii* was closely followed by *B. bassiana* causing 89.9 per cent mortality which was as effective as *V. lecanii*.

Median Lethal Concentration (LC_{50})

The data presented in Table 1 shows the LC_{50} values and the relative toxicity of the three fungal isolates. Among the three fungal isolates, *V. lecanii* and *B. bassiana* caused 87.4 &72.0 % mortality at the lowest concentration of $2x10^3$ spores/ ml. This was followed by *M. anisopliae* 55.3% mortality at the lowest concentration of $2x10^3$ spores/ ml.

Low LC_{50} value of $2x10^3$ spores/ ml. for *V. lecanii* against *A. craccivora* was 2.2 x 10^3 spores/ ml.

The higher LC_{50} values, higher will be the relative toxicity. Low LC_{50} value of $1.2x10^4$ spores ml-1 for *V. lecanii* against *Brevicoryne brassica* and $2.7x10^4$ spores ml-1 against *Aphis gossypii* was reported by [23, 24], respectively is in conformity with the present finding. LC_{50} value obtained in the present study was lower than that reported by [14] for *Hirsutella* sp ($5.2x10^4$ spores ml-1), but higher than reported by [25] for *B. bassiana* ($1.2x10^4$ spores ml-1) and [26] for *M. anisopliae* ($2.45x10^6$ spores ml-1). The difference in the LC_{50} values might be due to the difference in the virulence of fungal isolates and the host species. Among the three fungal isolates, *V. lecanii* and *B. bassiana* were the most virulent isolates with the lowest LC_{50} and relative toxicity. This was followed by *M. anisopliae* with a relative toxicity value of 18.7.

Also, [27] 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 $2.5x10^4$ spores ml-1 was recorded by *V. lecanii* and *H. thompsonii* isolates.

Table 1. Dose mortality response of fungal isolates against *A. craccivora*

Entomopathogenic Fungi	LC_{50} (spores/ ml.)	95% Fiduciallimits (spores ml-1)	Relative toxicity
V. lecanii	2.2×10^3	1.2×10^3- 4.0×10^3	1.0
B. bassiana	4.2×10^3	1.0×10^3-3.2×10^4	1.6
M. anisopliae	6.2×10^4	3.1×10^4-1.1×10^5	18.7

Median Lethal Time (LT_{50})

Variation in LT_{50} values at different concentrations was evident (Table 2). LT_{50} values decreased with increase in concentrations. At $2x10^5$ spores /ml., low LT_{50} value was recorded by *V. lecanii*, *B. bassiana* and *M. anisopliae* as4.8, 5.80 and 7.0 days respectively. [28], also attained similar results for *B. bassiana* with LT_{50} value of 3.17 days. The LT_{50} value of 3.31 days has been obtained for *V. lecanii* against *Aphis fabae* by [26, 29, 30] also, agree with the present finding *B. bassiana* and *M. anisopliae* recorded higher LT_{50} values of 5.80 and 7.0 respectively at $2x10^5$spores/ ml. Under laboratory conditions, *V. lecanii* was found to be more virulent recording cent per cent mortality within 8th days after treatment. Other fungal isolates also showed promising result. The lowest LC_{50} and LT_{50} values of *V. lecanii* and *B. bassiana* indicate its higher virulence against *A. craccivora*. They can be used as potential biocontrol agent after field experiments for the management of cowpea aphid.

Table 2. Time mortality response of fungal isolates against *A. craccivora*

Entomopathogenic Fungi	Median Lethal Time (Days)		
	$2x10^5$	$2x10^4$	$2x10^3$
V. lecanii	4.8	5.73	6.99
B. bassiana	5.80	6.10	6.99
M. anisopliae	7.0	7.69	8.70

Conclusion

The results obtained that using of entomopathogenic fungi, *V. lecanii*; *B. bassiana* and *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 [31-39].

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