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Research Note

Effect of entomofungal pathogens on mortality of three aphid species

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ABSTRCT: **The pathogenicity of twelve fungal isolates belonging to *Beauveria bassiana* (Bals.) Vuill., *Metarhizium anisopliae* (Metschinikoff) Sorokin and *Verticillium lecanii* (Zimmerman) against *Aphis craccivora* Koch, *Aphis gossypii* Glover and *Rhopalosiphum maidis* Fitch was studied using detached leaf bioassay technique. All twelve isolates of the three fungi were found to be pathogenic to *A. craccivora* and *A. gossypii* at a concentration of 1x 10^7 spores/ml. All isolates except Bb3 and Bb4 of *B. bassiana* were pathogenic to *R. maidis*. The mortality ranged from 2 to 74 per cent in *A. craccivora*, 14 to 80.8 per cent in *A. gossypii* and 6 to 50 per cent in *R. maidis*. Bb5a isolate of *B. bassiana* caused highest per cent mortality in *A. gossypii* (80.8%) and *R. maidis* (50%) indicating its broad spectrum action. Vl1 isolate of *V. lecanii* recorded maximum mortality (80.8%) of *A. craccivora*. *R. maidis* was relatively less susceptible to the three fungi than *A. craccivora* and *A. gossypii*. The LC_{50} of Bb5a for three days old nymphs of *A. gossypii* was 6.57x10^5 spores /ml. The LT_{50} of Bb5a for three days old nymphs of *A. gossypii* was highest (9.67 days) for the lowest dose of 10^6spores/ml, which decreased with increasing concentration. The highest dose 10^9spores/ml recorded the lowest LT_{50} of 1.76 days.**

KEY WORDS: *Aphis craccivora, Aphis gossypii, Beauveria bassiana, Metarhizium anisopliae, Rhopalosiphum maidis, Verticillium lecanii*

Aphids are well known sucking pests on a wide array of economically important crops and forest trees, all over the world. In India, 800 species of aphids have been described (Ghosh and Basu, 1995). *Aphis craccivora* Koch and *Aphis gossypii* Glover is polyphagous attacking several crop plants like, pulses, oilseed crops and cotton. *Rhopalosiphum maidis* Fitch attacks sorghum, maize and other cereals. These sucking pests cause severe losses in several agricultural crops. The chemical sprays are not cost effective and eliminate the beneficial parasitoids and predators from these cropping systems. Biological control approaches are very much required for the management of sucking pests in order to maintain sustainable production in these crops. For sucking insects, entomopathogenic fungi are the most appropriate microbial bioagents as they infect the insect cuticle directly through contact and do not require to be ingested for infection to set in. Several fungal species like *Beauveria bassiana* and *Metarhizium anisopliae* (Liu *et al.*, 1999; Ekesi *et al.*, 2000), *Fusarium pallidoroseum* (Sunitha and Mathai, 1999) and *Paecilomyces fumosoroseus* (Chen and Feng, 1999) have been reported pathogenic to aphids. In India, *F. pallidoroseum* was found

Table 1. List of entomopathogenic fungal isolates used in the study

Sl.no.	Fungal isolate	Host insect	Place of collection
1.	*Beauveria bassiana* -Bb3	*Neochetina eichhorniae* Warner	Bangalore
2.	*B. bassiana* -Bb4	*Spodoptera litura* (Fabricius)	Bangalore
3.	*B. bassiana* -Bb5a	*Hypothenemus hampei* (Ferrari)	Madikeri
4.	*B. bassiana* -Bb6	Tree hopper	Bangalore
5.	*Metarhizium anisopliae* -Ma2	*Amsacta albistriga* (Walker)	Davangere
6.	*M. anisopliae* -Ma3	*Oryctes rhinoceros* (Linnaeus)	Kasargod
7.	*M. anisopliae* -Ma4	*Plocaederus ferrugineus* (Linnaeus)	Puttur
8.	*M. anisopliae* - Ma5	*Holotrichia serrata* (Fabricius)	Coimbatore
9.	*Verticillium lecanii* -Vl 1	*S. litura*	Bangalore
10.	*V. lecanii* -Vl 2a	*Lepidosaphes beckii* (Newman)	Madikeri
11.	*V. lecanii* -Vl 3a	*Coccus viridis* (Green)	Madikeri
12.	*V. lecanii* -Vl 5	*Meconellicoccus hirsutus* (Green)	Pune

effective against *A. craccivora* in Kerala. However, the pathogenicity of other entomopathogenic fungi on these sucking pests were not carried out. Hence, in the present study, the pathogenicity of four isolates each of *Beauveria bassiana* (Bals.) Vuill., *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Verticillium lecanii* (Zimm.) on the mortality of three aphid species *viz. A. craccivora*, *A. gossypii*, *R. maidis* were assessed under laboratory conditions to identify potential candidates for field evaluation.

Four isolates each of the fungal pathogens, *B. bassiana*, *M. anisopliae* and *V. lecanii* collected from different insect hosts (Table 1) and maintained at Project Directorate of Biological Control, Bangalore were used in the laboratory bioassay studies against three aphid species *viz. A. craccivora*, *A. gossypii* and *R. maidis*. These isolates were grown on potato dextrose agar medium (PDA) slants and stored in a refrigerator at 5°C until further use.

Field collected *A. craccivora*, *A. gossypii* and *R. maidis* were multiplied on 1-2 months old healthy plants of cowpea, cotton and maize, respectively in the net house and used for bioassay studies.

The spore suspension of different isolates were prepared using 10-day old respective PDA cultures in Petri-plates. The spores of each isolate were harvested by flooding the plate with sterile distilled water containing 0.02 per cent Tween 80 and scraping the surface with sterile spatula. Then the suspension was passed through a double-layered muslin cloth and the filtrate was diluted with known quantity of 0.02 per cent Tween-80 emulsion to get spore concentration of $1X10^7$ spores/ml. The required spore concentration was adjusted with the help of a Neubauer's improved haemocytometer.

The pathogenicity of different isolates was determined by detached leaf bioassay technique (Yokomi and Gottwald, 1988) with slight modification. One or two heavily infested host leaves (100-150 insects/ leaf) with all stages of aphid were dipped gently in spore suspension for 2-3 seconds and was drained and shade dried completely. The petioles of leaves were inserted immediately into a glass vial containing water to maintain the turgidity of leaves and placed inside a bigger container (8 X 15cm), covered with muslin cloth. This whole set up was then kept inside a

growth chamber for 24 hours at 25 ± 0.5°C, 90 ± 2 per cent relative humidity.

For further maintenance of the inoculated aphids, clean and fresh leaves were rinsed initially with distilled water and then dipped in sodium hypochlorite (0.25%) solution for two minutes followed by two rinses in distilled water. After complete drying under aseptic condition, they were placed individually in Petri-plates containing sterilized agar medium (1%). To avoid bacterial contamination, streptomycin sulphate @ 100ppm was added to the medium before pouring it into sterile plates. After 24 hours of fungal inoculation, 25 live aphids/ replication were transferred to leaves placed over the agar medium. The Petri-plates were then sealed with parafilm to avoid escape of aphids as well as to enhance the settlement of aphids on the leaf and maintained in an incubator at 25±0.5°C. The newly hatched nymphs were removed from the plates aseptically in Laminar flow system to avoid confusion in counting.

Observations on mortality of aphids were recorded on 10th day after treatment. The mortality of aphids due to fungal pathogen was easily identified by mummification and dense white mycelial growth on the aphids treated with all isolates. In the case of *M. anisopliae*, the infected aphids appeared to be green mass on sporulation. The dead insects were left on the leaf surface itself for confirmation of death due to pathogen. On 10th day, all dead insects including those with mycelial growth were placed over a wet filter paper inside a Petri-dish. Death of aphids due to fungus was confirmed by microscopic observation of spores with lactophenol. Each treatment was replicated twice with an untreated control where leaves with aphids were dipped gently in Tween 80 emulsion (0.02%). The per cent mortality of aphids was calculated and after correcting the data for control mortality (Abbott, 1925), the data were subjected to analysis of variance.

The dose and time required to kill 50 per cent of the population (LC_{50} and LT_{50}) of *A. gossypii* due to Bb5a was calculated by probit analysis (Finney, 1971), using modified detached leaf bioassay technique. Six concentrations of spore suspensions *viz.* 10^4·10^5, 10^6, 10^7, 10^8, 10^9 spores/ml of Bb5a were prepared from 15 days old broth culture (PDA) as described earlier. To obtain the uniform stage aphid culture, only adult aphids were released on cotton leaves for 24 hours and fresh nymphs were collected. The nymphs were reared on cotton for 3 days and utilized in this study. As per the procedure detailed earlier, leaves with nymphs were treated and observations on mortality were recorded at every 24 hours interval up to 10 days after treatment. The dead aphids on each observation were removed and their death due to fungus was confirmed further sporulation on incubation and microscopic observation. The LC_{50} and LT_{50} of Bb5a were analyzed by probit analysis (Finney, 1971).

Laboratory bioassays revealed that at a concentration of 1 X 10^7 spores/ml, all the fungal isolates except Bb3 and Bb4 of *B. bassiana* produced mortality of *A. craccivora*, *A. gossypii* and *R. maidis* (Table 2).

Among the twelve isolates tested, *B. bassiana* isolates showed mortality ranging from 16.7 to 60.45 per cent, *M. anisopliae* isolates causing 20- 60 per cent mortality and *V. lecanii* showing 2 – 74 per cent mortality of *A. craccivora* (Table 2). Vl 1 isolate of *V. lecanii* showed maximum mortality of 74 per cent followed by Bb5a of *B. bassiana* (60.45%) and Ma 4 of *M. anisopliae* (60%). Ekesi *et al.* (2000) found that *B. bassiana* CPD11 isolate and *M. anisopliae* CPD4 and 5 isolates were highly pathogenic to *A. craccivora* causing a mortality range of 58– 91per cent, 64 – 93 per cent and 66-100 per cent, respectively at 7 days post treatment. Zaki (1998) also reported 100 per cent mortality of *A. craccivora* infesting cucumber with *B. bassiana* at a dose of 1mg/ ml. The present study indicated that Vl 1 isolate of *V. lecanii* was found highly pathogenic to *A. craccivora* causing 74.0 per cent mortality (Table 2).

B. bassiana isolates showed mortality of *A. gossypii* to the extant of 15 per cent (Bb6), 49 per cent (Bb4), 50.8 per cent (Bb3) and 80.8 per cent (Bb5a)(Table 2). The mortality due to *M. anisopliae* isolates varied from 20.0 per cent (Ma2) to 38

Table 2. Mortality of three aphid species caused by different isolates of *B. bassiana*, *M. anisopliae* and *V. lecanii* (Lab. Bioassay)

Sl.no.	Fungal isolate	Per cent mortality		
		A. craccivora	*A. gossypii*	*R. maidis*
1.	*Beauveria bassiana* - Bb3	16.7	50.8	0.0
2.	*B. bassiana* - Bb4	43.4	49.0	0.0
3.	*B. bassiana* - Bb5a	60.5	80.8	50.0
4.	*B. bassiana* - Bb6	26.8	15.0	6.00
5.	*Metarhizium anisopliae* - Ma2	40.0	38.0	34.0
6.	*M. anisopliae* - Ma3	54.0	36.0	18.0
7.	*M. anisopliae* - Ma4	60.0	36.0	26.0
8.	*M. anisopliae* - Ma5	20.0	20.0	18.0
9.	*Verticillium lecanii* -Vl 1	74.0	68.0	16.0
10.	*V. lecanii* -Vl 2a	18.0	18.0	12.0
11.	*V. lecanii* -Vl 3a	18.0	42.0	18.0
12.	*V. lecanii* -Vl 5	2.0	14.0	8.0
	SEM ±	4.5	3.1	4.3
	CD (P=0.05)	13.9	9.6	13.3

per cent (Ma2) and with *V. lecanii* isolates from 14 per cent (Vl 5) to 68 per cent (Vl 1). This study indicated that isolate Bb5a was found highly pathogenic to *A. gossypii* causing 80.8 per cent mortality (Table 2).

It was observed that all isolates of *M. anisopliae* and *V. lecanii* were found pathogenic to *R. maidis*, whereas, two isolates of *B. bassiana* (Bb3 and Bb4) did not cause any mortality and the isolates of *M. anisopliae* and *V. lecanii* showed less percentage of mortlatity (18-34% and 8-18%, respectively). Yokomi and Gottwald (1988) observed rapid mortality of *M. persicae* and *A. gossypii* due to *V. lecanii* treatment at concentrations of 10^6-10^7 conidia ml^{-1}. However, in the present study, *V. lecanii* isolates were found less virulent on *A. gossypii* (Table 2).

The LC_{50} of Bb5a isolate on *A. gossypii* was 6.57×10^5 spores/ml (Table 3). This result is in conformity with that of Ekesi *et al.* (2000), who reported the LC_{50} of *B. bassiana* (CPD11) and *M. anisopliae* (CPD 4 and 5) for adults of *A. craccivora* as 6.8×10^5, 3.1×10^5 and 2.7×10^5 conidia ml^{-1}, respectively. The mortality of aphids began 24 hours after treatment. Liu *et al.* (1999) reported the LC_{50} values for six aphid-derived *B. bassiana* isolates against *M. persicae* to range from 1.2×10^4 – 1.55×10^6 conidia/ml. In the present study, the LT_{50} values decreased with increasing concentration of Bb5a (Table 3) and at the highest concentration of 1.0×10^9 conidia/ml, fifty per cent of the aphid population was killed in 1.76 days (Table 3). Variations in the LT_{50} of 6 aphid-derived isolates of *B. bassiana* were reported in *M. persicae* (Liu *et al.*, 1999).

Table 3. Dose and time mortality response of *A. gossypii* to *B. bassiana* (Bb 5a)

Dose mortality response					
LC_{50}(spores/ml)	χ^2 Value	Regression equation	Fiducial limit (spores/ml)	Slope ± SE	
$6.57x10^5$	2.013 (NS)	Y= - 3.01839+ 0.51885x	$1.10x10^6$- $3.77x10^5$	0.52 ± 0.039	
Time mortality response					
Concentration (spores/ml)	LT_{50} (days)	χ^2 Value	Regression equation	Fiducial limit (days)	Slope ± SE
10^6	9.67	9.96 (NS)	Y= -1.20 + 2.38x	11.33 - 8.55	1.80 ± 0.168
10^7	4.31	17.65 (S)	Y= -1.46533 + 2.31088x	4.91- 3.71	2.31 ± 0.157
10^8	3.17	28.63 (S)	Y= -1.20 + 2.38x	3.79 - 2.50	2.38 ± 0.153
10^9	1.76	84.55 (S)	Y= -0.73 + 2.98x	2.46 - 0.94	2.98 ± 0.183

NS – Not significant; S - Significant

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