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Effect of entomofungal pathogens on sugarcane woolly aphid, (*Ceratovacuna lanigera* Zehntner) and its predators

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ABSTRACT: **Four isolates in each of *B. bassiana, M. anisopliae* and *V. lecanii* were tested for their pathogenicity to the sugarcane woolly aphid (SWA), *Ceratovacuna lanigera* Zehntner in oil emulsion formulations under field conditions at Arabhavi, Karnataka. Mycosis was observed with six isolates *viz.*, *B. bassiana*, Bb4 (10%), Bb5a (19.8%), Bb6 (8.3%) and *M. anisopliae*, Ma2 (4.7%), Ma3 (16.2%) and Ma4 (42.3%). Pathogenicity was confirmed by re-isolation of respective fungal isolates from the mycosed aphids. None of the *V.lecanii* isolates showed mycosis on *C.lanigera*. In the laboratory bioassay studies, *M. anisopliae* (Ma4) and *B.bassiana* (Bb5a) were found pathogenic to the predator of sugarcane woolly aphid, *Dipha aphidivora* causing 29.3 and 10.4 per cent mycosis. *M. anisopliae* (Ma 4) isolate was also found pathogenic to another predator of SWA, *Micromus* sp. causing 29.14 per cent mycosis.**

KEY WORDS: *Beauveria bassiana, Ceratovacuna lanigera, Dipha aphidivora, Metarhizium anisopliae*, oil in water emulsion, *Verticillium lecanii*

INTRODUCTION

The sugarcane woolly aphid, *Ceratovacuna lanigera* Zehntner has become a serious pest in India after its unprecedented level of infestation in sugarcane in Kolhapur and Pune Districts of Maharashtra during 2002 (Rabindra *et al.*, 2002). Later, the pest has spread to Karnataka, Tamil Nadu, Andhra Pradesh and caused severe loss to the crop. Adults and nymphs cause appreciable damage to sugarcane crop by sucking plant sap from the lower surface. Severe infestation results in stunted growth, drying up of leaves and significant loss in crop yield. Insecticides are the primary weapons in insect management, used by the farmers in endemic area of SWA (West Maharashtra and North Karnataka) in order to bring down the population under control. Considering the adverse effects of insecticides, pest management through biological control is being encouraged using parasites, predators and pathogens. Among the pathogens, fungal pathogens *viz.*, *Beauveria bassiana* (Bals.) Vuill., *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Verticillium lecanii* (Zimm.) are frequently used in insect management due to their wide host range and easy and cheap mass production technology.

The white halo fungus, *V. lecanii* was tested for its efficacy under field condition against woolly aphid and 6.7 to 36.7 per cent mortality of woolly aphid was reported (Anon., 2003) in Maharashtra. In the present study, preliminary screening of four isolates of these pathogens as oil emulsion formulations was attempted based on their efficacy against other sucking pests under field conditions. As the pathogens used for screening are

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neither specific nor native pathogens, laboratory testing of these formulations was carried out with the promising predators of SWA *viz.*, *Dipha aphidivora* and *Micromus igorotus* to assess their safety. The efficacy of pathogens against SWA and their safety to predators are discussed in this paper.

MATERIALS AND METHODS

1. Pathogenicity of different fungal isolates to *C. lanigera* in oil emulsion formulations

Four isolates each of *B. bassiana*, *M. anisopliae* and *V. lecanii* collected from different insect hosts (Table 1) and maintained on Potato Dextrose Agar medium (PDA) slants in a refrigerator at 8-10°C at the Project Directorate of Biological control, Bangalore, were used in the studies.

a. Preparation of dry conidial powder of fungal isolates

The twelve isolates of fungi were cultured on broken raw rice (150g / bag) in polypropylene bag (20 × 28cm) and incubated at 25 ± 0.5°C, 90% ± 1 per cent relative humidity for 15 days. After incubation, the sporulated rice of each of the isolate was dried in sterile aluminium trays under a laminar flow chamber (30-32°C) until the moisture content of the medium reached 10.0 per cent. Moisture content of the medium was ascertained using moisture meter. After drying, the sporulated rice was vigorously agitated and then passed through a fine sieve (105 microns) to collect spore dust (conidial powder). The moisture content of the spore dusts of all isolates was maintained below 4-5 per cent using a desiccator.

b. Preparation of oil in water emulsion formulation of fungal isolates

For the preparation of oil in water emulsion formulation, one gram of spore dust of each isolate was transferred to the vials containing five ml of sterilized mixture of sunflower oil and Tween -80 (9:1 ratio) and mixed thoroughly by rigorous shaking (Vimala Devi *et al.*, 2002). The spore concentration (cfu /ml) of each formulated isolates was estimated by serial dilution technique. Before spraying, prepared formulations of twelve isolates were individually mixed with 95 ml of water and shaken vigorously to get oil in water emulsion.

c. Pathogenicity test with oil emulsion formulations

The pathogenicity of fungal formulations was tested *in situ* on SWA infested four months old ratoon crop (Co 8011) at Arabhavi, Belgaum dist., Karnataka. Pretreatment counting of aphids from five infested leaves from each treatment plot was taken at random. Then, the

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

Sl. No.	Fungal isolates	Host insect	Place of collection
1	*B.bassiana* - Bb 3	*Neochetina eichhorniae* Warner	Bangalore
2	" - Bb 4	*Spodoptera litura* F.	Bangalore
3	" - Bb 5a	*Hyphothenemus hampei* (Ferrari)	Madikeri
4	" - Bb 6	Indet. tree hopper	Bangalore
5	*M. anisopliae* -Ma 2	*Amsacta albistriga* (Wlk.)	Davangere
6	" - Ma 3	*Oryctes rhinoceros* L.	Kasargod
7	" - Ma 4	*Plocaederus ferrugineus* L.	Puttur
8	" - Ma 5	*Holotrichia serrata* F.	Coimbatore
9	*V. lecanii* - Vl 1	*Spodoptera litura* (F.)	Bangalore
10	" - Vl 2a	*Lepidosaphes beckii* (Newman)	Madikeri
11	" - Vl 3a	*Coccus viridis* (Gr.)	Madikeri
12	" - Vl 5	*Maconellicoccus hirsutus* (Gr.)	Pune

oil emulsion formulation of each isolate was sprayed on the infested leaves using a garden sprayer of one-liter capacity. For control water containing Tween 80 (0.5 %) was sprayed. The dead aphids from the treated leaves from 3rd day to 5th day of treatment were collected at random and transferred to sterile wet papers in petri plates to confirm the mortality of aphids due to fungus by checking external fungal growth on dead aphids using microscope. The per cent mycosis for each treatment was calculated. Dead samples without mycosis were considered as mortality due to other causes such as asphyxiation. Isolation of the respective fungal organisms from the mycosed aphids was carried out on potato dextrose agar plates to confirm their pathogenicity.

2. Susceptibility of *D. aphidivora* to *M. anisopliae* and *B. bassiana*

Laboratory bioassays were carried out to test the infectivity of *B. bassiana* (Bb5a) and *M. anisopliae* (Ma 4) to *D. aphidivora*. Sugarcane leaves infested by woolly aphid were collected from the affected fields and the leaves were cut into 20 cm long pieces. For each treatment, thirty leaf pieces with predators ranging from 41-59/ treatment (3-12mm size) were used. The leaf pieces were sprayed with the spore suspension of *B. bassiana* (Bb5a) and *M. anisopliae* (Ma 4) at the concentration of 1×10^8 spores/ml, prepared in Tween 80 (0.5%). The treated leaves were placed in plastic containers (10 leaf pieces for each plastic container) and kept under 25 ± 0.5°C and 90 ± 1 per cent relative humidity in an incubator for six days. Spray with Tween 80 (0.5%) and unsprayed check were also maintained. Mortality of *D. aphidivora* was recorded on 6th day of the treatment and the dead larvae were transferred to sterilized petri plates containing wet filter paper. Mycosis of the predator was assessed by microscopic observation and the per cent mycosis was calculated. Isolation of the respective fungus from the mycosed predator was carried out on the potato dextrose agar plates to confirm the pathogenicity.

RESULTS AND DISCUSSION

1. Pathogenicity of different fungal isolates to *C. lanigera* in oil emulsion formulations

The results of pathogenicity tests of oil emulsion formulations of different isolates of *B. bassiana*, *M. anisopliae* and *V. lecanii* to *C. lanigera* carried out *in situ* in sugarcane plants at Arabhavi, Karnataka are presented in Table 2. Among the twelve isolates tested, mycosis was observed with six isolates *viz.*, *B. bassiana*, Bb4 (10.0%), Bb5a (19.8%), Bb6 (8.3%), and *M. anisopliae*, Ma2 (4.7%), Ma3 (16.2%) and Ma4 (42.3%). The respective fungal isolates were reisolated from the mycosed aphids indicating their pathogenicity to *C. lanigera*. None of the isolates of *V. lecanii* caused mortality of woolly aphid due to mycosis. The reasons might be low concentration of spore suspension, low level of spore production in rice medium, poor compatibility with oil and inability to withstand the climatic conditions. However, *V. lecanii* derived from some other host recorded 6.7 to 36.7 per cent woolly aphid mortality in a field study conducted in Maharashtra

Table 2. Pathogenicity of oil emulsion formulation of entomofungal pathogens to *C. lanigera*

Sl. No	Fungal isolate	Concentration of suspension (spores/ml)	Per cent mycosis
1	*B.bassiana* - Bb 3	8.5×10^6	0.0
2	" - Bb 4	1.35×10^7	10.0
3	" - Bb 5a	2.25×10^8	19.8
4	" - Bb 6	5.30×10^7	8.3
	Mean		9.6
5	*M. anisopliae* -Ma 2	9.00×10^7	4.7
6	" - Ma 3	7.5×10^7	16.2
7	" - Ma 4	1.65×10^8	42.3
8	" - Ma 5	1.50×10^8	0.0
	Mean		15.8
9	Control (Tween 80)	0.5%	0.0

(Anon., 2003).

The present study infers that an isolate of *M. anisopliae* (Ma 4) was found superior compared to isolates of *B. bassiana* and *V. lecanii*. The difference in the efficacy of isolates in terms of mortality of aphids might be due to inherent capacity such as high CFU (colony forming units) production, fast growth and sporulation. Being the hemipteran derived pathogen Bb6 caused only 8.3 per cent mortality among the isolates of *B.bassiana*. In contrast, the homopteran derived isolate of *B. bassiana* caused 72–86 per cent mortality of *M.persicae* when sprayed twice @10^9 spores/ml (Miranpuri and Khachatourians, 1993).

2. Susceptibility of the predators, *D. aphidivora* and *Micromus* sp. to *B. bassiana* and *M. anisopliae*

In the laboratory bioassay studies, it was observed that *M. anisopliae* (Ma 4) and *B.bassiana* (Bb 5a) were found pathogenic to *D.aphidivora* causing mycosis and the fungi could be reisolated from the mycosed predator. *M. anisopliae* (Ma 4) caused the highest mycosis of *D.aphidivora* (29.3%) followed by *B.bassiana* (Bb 5a) showing 10.4 per cent mycosis. With regard to *Micromus* sp., *M. anisopliae* (Ma 4) was pathogenic and caused 29.14 per cent mycosis in the laboratory bioassays.

In the preliminary screening study with oil emulsion formulations of different isolates against sugarcane woolly aphid under field condition, the fungal pathogens could cause mortality ranging from 4.7 to 42.3 per cent due to mycosis alone. In the safety tests under laboratory condition with the effective isolates of fungal pathogens against the native predators of woolly aphid such as *D.aphidivora* and *Micromus* sp, it is concluded that Ma 4 and Bb 5a are equally unsafe causing appreciable mortality. Hence, the utilization of these fungal isolates as a management tool for woolly aphid has to be analysed thoroughly prior to large scale application by giving main consideration to safety to non-target beneficial organisms in the sugarcane ecosystem.

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