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Relative susceptibility of brinjal spotted beetle, *Henosepilachna vigintioctopunctata* (Fabricius) to certain isolates of *Beauveria bassiana* (Bals.) Vuill

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ABSTRACT: Pathogenicity of *Beauveria bassiana* (Bals.) Vuill isolates to the brinjal spotted beetle, *Henosepilachna vigintioctopunctata* (Fabricius) has been recorded for the first time. Virulence of the isolates was assessed at different developmental stages of the beetle from laboratory bioassay experiments. Pathogenic strains showing rapid germination and profuse sporulation were found to be more virulent to the pest. LT_{50} values of the second instar larvae ranged from 1.33 to 4.8 days in the different isolates. Larval stages appeared to be more susceptible than prepupal and adult stages. Field performance of the pathogen tested by applying the mass multiplied inoculum to the infested brinjal plants revealed persistence of the biocontrol agent in the soil for 30 days.

KEY WORDS: *Beauveria bassiana*, field application, *Henosepilachna vigintioctopunctata*, persistence, virulence

The brinjal spotted beetle, *Henosepilachna vigintioctopunctata* (Fabricius) is one of the serious pests infesting brinjal, *Solanum melongena* (L.) and a wide range of cucurbitaceous plants throughout the cropping season. Both adults and grubs skeletonise the leaves and reduce yield. Entomopathogenic fungi are promising candidates for biological control of a number of pests (Roberts and Wraight, 1986). *Beauveria bassiana* (Bals.) Vuill is one of the extensively used mycopathogens for the control of many important Coleopteran pests namely blister beetle, *Lytta nutali* (Miranpuri and Khachatourians, 1994) and *Coleomegilla maculata* (Coccinellid) (Todorova *et al.*, 1996). *Beauveria bassiana* isolates were identified as pathogenic to *H. vigintioctopunctata* in our preliminary bioassays and hence further investigations were planned with the objective of

understanding virulence of the isolates and obtain pest specific strains for subsequent use in biocontrol programme.

MATERIALS AND METHODS

Four isolates of *Beauveria bassiana* namely 4-1(NRRL 13050 - an USDA-ARS collection), 4-2 (from Bangalore), 4-3 and 4-5 (two isolates from Guntur) were used for assessing virulence against the brinjal spotted beetle. Sabaraud's dextrose agar plates were inoculated with 10µl of $1x10^8$ spores/ml spore suspension and growth was recorded from 3rd day onwards by taking diameter of the perfectly round colonies. Spore harvest was made on 10th day in Tween 80 (0.02%) solution and spores were estimated using Neubaeur haemocytometer. Five replicates were maintained for each isolate for growth and sporulation.

Laboratory bioassay

Second instar grubs of the brinjal spotted beetle, collected from the heavily infested brinjal plants were transferred on to the fresh brinjal leaves collected in the form of twigs. The spore suspension ($3x10^{10}$ spores /ml) in Tween 80 (0.02%) was sprayed with a hand atomiser using 15grubs for each treatment with four replicates. Controls were maintained by spraying the grubs with Tween 80 (0.02%) solution and treatment schedules were repeated with grubs at prepupal stage.

Field application

The fungus was mass multiplied on sorghum grains, bran and husk. By 10th day the fungus showed profuse growth and sporulation. The fungus spores were mixed with fresh bran and jaggery and dusted on heavily infested brinjal plants grown in the field plots keeping the spore concentration of the inoculum at $7x10^{12}$ spores /g during September, 1996 at Visakhapatnam. An untreated control plot was maintained for comparison. Mortality rates were noted by counting the number of dead grubs on 10 plants from each of the control and treated lines at 3 days interval. Persistence of the fungus in the soil was studied by collecting soil samples of the treated field from four randomly selected areas and plated by serial dilution method.

RESULTS AND DISCUSSION

Quantitative data on rate of germination, growth and sporulation of *Beauveria bassaina* isolates presented in Table 1 reveal differences among the four isolates. Isolate 4-5 showed germination, profuse growth pattern, better spore output and least LT_{50} value compared to the other strains indicating positive correlation between growth rate, sporulation and virulence. Isolates 4-1 and 4-2 showing extensive hyphal growth and puffy colonies, were the poor sporulators. Pekrul and Grula (1979) reported extensive surface growth associated with less virulent isolates and highly virulent isolates showing germ tubes oriented towards the cuticle facilitating effective penetration in *Beauveria bassiana* isolates investigated by them.

Data on laboratory bioassay experiments presented in Table 2 and 3

Table 1. Germination, growth and sporulation of *Beauveria bassiana* isolates

Isolate number	TG* $_{50}$	Growth rate of the colonies (Mean diam in cm)				
		3rd day	4th day	5th day	7th day	8th day
4-1	18 h	0.88 ± 0.14	1.06 ± 0.08	1.28 ± 0.08	1.68 ± 0.04	$6x10^7$
4-2	20 h	0.74 ± 0.13	0.94 ± 0.08	1.04 ± 0.05	1.54 ± 0.05	$2.7x10^7$
4-3	16 h	0.92 ± 0.13	1.12 ± 0.04	1.16 ± 0.05	1.78 ± 0.04	$6.5x10^7$
4-5	12 h	0.92 ± 0.05	1.14 ± 0.08	1.22 ± 0.04	1.82 ± 0.07	$7x10^7$

* Time taken for 50 per cent germination of spores

revealed that at second instar grubs, least LT_{50} value (1.33 days) was observed with isolate 4-5 followed by isolate 4-3 (1.69days) during the month of September (Temperature 31.32 ± 1.39° C and relative humidity 80.46 ± 8.2%). Isolates 4-5 and 4-3 appear to be more virulent as was evident from LT_{50} and higher regression values at 2nd instar grub and prepupal stages. Within 24h after death, profuse mycelial growth all over the body leading to sporulation was observed in the 4-5 isolate. The relative virulence of the four isolates with respect to mortality rates at second instar larval and prepupal stages are in agreement. However, LT_{50} values at the early larval stages were significantly lower for all the isolates compared to the corresponding values at prepupal stage. The differential response suggests that application of the pathogen be better targeted against young larval stage rather than older stage.

The pest load in brinjal field treated with mass multiplied inoculum showed

Table 2. Probit analysis of mortality rates of *B. bassiana* isolates to 2nd instar grubs of *H. vigintioctopunctata*

Isolate number	Percent mortality on 5th day	LT_{50}	Fiducial limits	Regression equation
4-1	53	3.94	1.318 - 11.784	Y = 4.441 + 0.749x
4-2	50	4.86	0.867 - 27.334	Y = 4.550 + 0.649x
4-3	70	1.69	0.308 - 9.347	Y = 4.797 + 0.938x
4-5	82	1.33	0.313 - 5.732	Y = 4.790 + 1.516x

* Time taken for 50 per cent mortality in days

reduction to 50 per cent in plot 1 and to 35 per cent in plot 2 by 6th day. Control plot on the other hand showed a minimal mortality value of 10 per cent. Drastic reduction in the pest load to 80 and 75 per cent was observed in the plot one and two, respectively by 10th day after application. Some of the dead grubs observed on the plants showed mycosis on the body.

The soil samples collected from the field showed *Beauveria bassiana* colonies, indicating persistence of the biocontrol agent in the soil till 30 days after application. On the other hand, soil samples from the control field did not yield any *Beauveria bassiana* colonies. Persistence of the viable fungal spores in the soil samples till 30th day after application indicated positive signal towards this proposition. Roberts and Campbell (1977), Ignoffo *et al.* (1978), and Ling and Donaldson (1981) reported conidia of *Beauveria bassiana* surviving in or on the surface of the soil for longer period.

In the present study, though 4-5 and 4-3 isolates were identified to be virulent against the *H. vigintioctopunctata,* profuse mycosis on the dead larvae could be obtained only in 4-5 isolate. Mycosis facilitates continuos supply of inoculum for further infection which is necessary for optimum performance of the pathogen at field level. In addition to the speed of kill, ability to proliferate and sporulate on the cadavers is a desirable feature for developing pest specific strains of the biocontrol agent.

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Table 3. Probit analysis of mortality response of *B. bassiana isolates* to prepupae o *H. vigintioctopunctata*

Isolate number	Percent mortality on 5th day	LT_{50}	Fiducial limits	Regression equation
4-1	58	3.69	1.401 - 9.728	Y = 4.0073 +1.745x
4-2	45	5.67	3.135 - 10.267	Y = 3.592 + 1.861x
4-3	50	4.64	3.578 - 6.032	Y = 2.724 + 3.405x
4-5	65	2.75	2.831 - 21.439	Y = 4.325 +1.533x

* Time taken for 50 per cent mortality in days

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