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Bioassay of entomopathogenic fungi, *Beauveria bassiana* (Bals.)Vuill and *Metarhizium anisopliae* (Metchnikoff)Sorokin.,against Sweet potato tortoise beetle, *Metriona circumdata* H.

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Abstract

Bioassays with entomopathogenic fungi, B. bassiana and M. anisopliae on sweet potato tortoise beetle, M. circum data were conducted to assess the LC_{50}, LC_{90} and LT_{50} values and fiducial limits and regression parameters. The results of the study indicated that the mortality of the insects was dependent on the concentration of the spore suspension of the fungus. The cumulative per cent mortality varied with dose and time for both adult beetles and grubs for both the fungi. The dose and time taken for mortality of the adults and grubs of M. circumdata was lower for B. bassiana when compared to M. anisopliae. The LC_{50} of B. bassiana estimated were 7.26×10^9 and 4.96×10^8 spores ml^{-1} against the adults and grubs of M. circumdata respectively and a spore concentration of 6.13×10^{10} and 4.64×10^9 spores ml^{-1} of M. anisopliae was required to cause 50 per cent mortality of the adults and grubs of M. circumdata respectively at the highest test dose evaluated.

Key words: Bioassay, B. bassiana, M. anisopliae, sweet potato, tortoise beetle

I. INTRODUCTION

Sweet potato tortoise beetle, *Metriona circumdata* H. (Family: Cassididae) is one of the major canopy inhabiting defoliator of sweet potato found throughout the crop period. Both adults and grubs damage entire foliage by extensive feeding of the green matter, which seriously affects the tuber production and the size of the tubers. The pest infests many weeds, ornamentals and few food crops coming under Family Convolvulaceae.

B. bassiana and *M. anisopliae* are promising entomopathogenic fungi extensively used for biological control of a wide range of economically important insect pests (Coates *et al.,* 2002; McGuire *et al.,* 2005) and differences exists in host specificity, virulence and time taken for pathogenesis among both the fungi towards various pests of crops (Ferron *et al.,* 1991) [1] [2] [3]. A perusal of literature on the pathogenicity of *B. bassiana* and *M. anisopliae* to the pest in the present study showed that no work has been undertaken on the assessment of the pathogenicity of both the fungi to the sweet potato tortoise beetle, *M. circumdata*.

II. MATERIALS AND METHODS

B. bassiana isolate, PDBC Bb 5 and *M. anisopliae* isolate, PDBC Ma 4 used for the studies were obtained from The National Bureau of Agriculturally Important Insect Pests (NBAII), Bengaluru. The

fungal isolates were subcultured and maintained on Potato Dextrose Agar (PDA) media at 27 ± 5 ° C. Mass production of the fungi for laboratory experiments was done in Potato Dextrose Broth (PDB).

The adults as well as the immature stages of *M. circumdata* were captured initially from the sweet potato field. The insects thus collected were kept in rearing jars along with host plants for fifteen days in order to screen the diseased insects. The healthy disease free insects were further reared in the laboratory to obtain the stock culture for the experiments. The grubs and adults of *M. circumdata* were reared on fresh sweet potato twigs with young leaves which were kept in polypet jars. The cut ends of the sweet potato vines were rolled with moist cotton balls for keeping them fresh and green. The eggs were laid singly on the ventral sides of the leaves and the grubs that emerged from these eggs were further transferred to fresh trays along with food. Those pupated on the plant parts were collected and kept separately for adult emergence.

For bioassay, the spore count of the suspension was estimated through haemocytometer counts and five serial dilutions of the corresponding fungal spore suspensions were prepared from 14 day old stock culture of the fungus grown in potato dextrose broth (PDB). Third instar grubs and newly emerged adults from the culture stock of the insects were used for the study. The fungal spore suspension was uniformly sprayed on to the adults and grubs using an atomizer and were then released into rearing jars with fresh food. Three replications were maintained for each adults and grubs. The insects treated with distilled water alone served as control for the experiment. The mortality of the adults / grubs was recorded every day. The log dose probit mortality data was statistically analysed after necessary correction using Abbott's formula (Abbott, 1925) and the LC_{50}, LC_{90} and LT_{50} values and fiducial limits and other regression parameters were worked out using SPSS Statistics Version 21 (IBM CORP., 2012) as explained by Fang *et al.* (2005)[4] [5] [6].

III. RESULTS AND DISCUSSION

The results of the bioassay showed that on the fifth day after inoculation with *B. bassiana*, the mortality of the adult *M. circumdata* was noticed and it ranged from 2.22 to 17.78 per cent. The mortality percentage ranged from 11.11 to 40.00 per cent, 20.00 to 84.45 per cent and 35.55 to 100 per cent on 10, 15 and 20 DAT in the different doses ranging from 2.52×10^5 to 2.52×10^9 spores ml^{-1}, respectively (Table 1). Death of 50 per cent adults of *M. circumdata* was seen at 10.23 days at the higher spore concentration of 2.52×10^9 spores ml^{-1} whereas 23.533 days was required to bring 50 per cent kill at the lowest concentration of 2.52×10^5 spores ml^{-1}. The probit analysis of log dose- mortality responses between the fungus and the insect indicated the LC_{50} values as 7.26×10^9, 3.73×10^9, 0.07×10^9 and 0.0056×10^9 spores ml^{-1} at 5, 10, 15 and 20 DAT, respectively. A spore concentration of 14.04×10^9, 10.76×10^9, 2.91×10^9 and 0.16×10^9 spores ml^{-1} was necessary to cause 90 per cent kill of the test insects at the 5, 10, 15 and 20 days after inoculation respectively.

Table 1. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of adults of M. circumdata treated with different spore concentrations of B. bassiana

Concentration (spores ml^{-1})	**Cumulative per cent mortality at different intervals after treatment (Days)**				**LT_{50} (Days)**
	5	10	15	20	
2.52×10^9	17.78	40.00	84.45	100.00	10.230
2.52×10^8	15.55	37.78	75.55	95.55	11.157

2.52×10^7	11.11	31.11	68.89	84.45	12.775
2.52×10^6	6.67	22.22	37.78	64.45	17.073
2.52×10^5	2.22	11.11	20.00	35.55	23.533
Control	0	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores $ml^{-1} \times 10^9$)	Fiducial limit for LC_{50} (spores $ml^{-1} \times 10^9$)	LC_{90} (spores $ml^{-1} \times 10^9$)	Fiducial limit for LC_{90} (spores $ml^{-1} \times 10^9$)	χ^2	Regression equation
5	7.26	5.22 - 10.83	14.04	11.71 - 17.21	4.983	Y = 2.818 + 1.373 x
10	3.73	2.34 - 7.43	10.76	6.61 - 12.76	8.694	Y = 3.090 + 1.680 x
15	0.07	0.01 - 0.35	2.91	1.57 - 5.23	33.196	Y = 4.448 + 2.735 x
20	0.006	0.009 - 0.009	0.16	0.09 - 0.32	18.241	Y = 4.298 + 1.889 x

The percentage of mortality ranging from 7.41 to 20.37 was noticed on the fifth day after inoculation for the grubs of *M. circumdata* and the rate increased as days progressed. The mortality in the ranges of 14.82 to 40.74 per cent, 27.78 to 68.52 per cent and 51.85 to 98.15 per cent was recorded at 10, 15 and 20 DAT respectively (Table 2). The LT_{50} was computed for the various doses and the shortest duration was 10.892 days and the highest was 20.052 days, corresponding to the highest and lowest doses of 1.3×10^8 spores ml^{-1} and 1.3×10^4 spores ml^{-1}, respectively. The spore concentration required to cause 50 per cent mortality of the test insects was 4.96×10^8, 1.85×10^8, 0.26×10^8 and 0.005×10^8 spores ml^{-1} at 5, 10, 15 and 20 DAT, respectively, and the corresponding LC_{90} values were 10.76×10^8, 5.13×10^8, 2.88×10^8 and 0.58×10^8 spores ml^{-1}, respectively.

Table 2. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of grubs of M. circumdata treated with different spore concentrations of B. bassiana

Concentration (spores ml^{-1})	**Cumulative per cent mortality at different intervals after treatment (Days)**				**LT_{50} (Days)**
	5	10	15	20	
1.3×10^8	20.37	40.74	68.52	98.15	10.892
1.3×10^7	18.52	31.48	55.56	87.04	12.893
1.3×10^6	16.67	25.93	44.44	72.22	14.515
1.3×10^5	12.96	24.07	31.48	66.67	16.175
1.3×10^4	7.41	14.82	27.78	51.85	20.052
Control	0	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^8$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^8$)	LC_{90} (spores ml^{-1} $\times 10^8$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^8$)	χ^2	Regression equation
5	4.96	3.23 - 8.77	10.76	9.11 - 13.47	3.015	Y = 2.271 + 1.099 x
10	1.85	1.12 - 7.19	5.13	3.01 - 21.76	3.621	Y = 2.528 + 1.096 x
15	0.26	0.09 - 1.05	2.88	1.31 - 4.47	10.218	Y = 3.137 + 1.456 x
20	0.005	0.001 - 0.05	0.58	0.16 - 1.11	11.65	Y = 3.807 + 1.451 x

M. circumdata adult beetles treated with *M. anisopliae* was found dead from the tenth day after inoculation and the rate of mortality was between 8.89 to 35.55 per cent. The mortality further increased to 28.89 to 71.11 per cent and 48.89 to 97.78 per cent at 15 and 20 DAT, respectively in the spore concentrations ranging from 3.97×10^6 to 3.97×10^{10} spores ml^{-1} (Table 3). The fungus killed 50 per cent of *M. circumdata* in 11.361 days at the highest spore concentration of 3.97×10^{10} spores ml^{-1}, where as a period of 19.608 days was needed at the lowest concentration. The spore concentrations of 6.13×10^{10}, 0.0913×10^{10} and 0.008×10^{10} spores ml^{-1} was necessary to kill 50 per cent population of the test insects at 10, 15 and 20 DAT, respectively. For the corresponding days, the dosage required for killing 90 per cent of the *M. circumdata* was computed as 14.03×10^{10}, 8.29×10^{10} and 1.79×10^{10} spores ml^{-1} respectively.

Table 3. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of adults of M. circumdata treated with different spore concentrations of M. anisopliae

Concentration (spores ml^{-1})	**Cumulative per cent mortality at different intervals after treatment (Days)**			**LT_{50} (Days)**
	10	15	20	
3.97×10^{10}	35.55	71.11	97.78	11.361
3.97×10^{9}	24.44	68.89	88.89	12.968
3.97×10^{8}	17.78	55.55	77.78	14.970
3.97×10^{7}	15.55	48.89	66.67	16.390
3.97×10^{6}	8.89	28.89	48.89	19.608
Control	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^{10}$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^{10}$)	LC_{90} (spores ml^{-1} $\times 10^{10}$)	Fiducial limit for LC_{90} (spores ml^{-1}	χ^2	Regression equation

				$\times 10^{10}$)		
10	6.13	3.88 - 17.64	14.03	8.67 - 43.22	3.241	Y = 2.831 + 1.994 x
15	0.0913	0.30 - 0.25	8.29	4.41 - 13.23	13.489	Y = 3.757 + 1.096 x
20	0.008	0.001 - 0.013	1.79	0.81 - 2.86	14.980	Y = 4.760 + 2.485 x

The percentage mortality in the grubs of *M. circumdata* treated with *M. anisopliae* initiated on the fifth day after inoculation. After a lapse of 20 days, the mortality percentage reached 98.15 per cent at the highest dose of 2×10^9 spores ml^{-1} whereas at the lower doses of 2×10^5 spores ml^{-1} the mortality percentage was only 44.44 (Table 4). At the highest spore concentration of 2×10^9 spores ml^{-1} the time taken for killing 50 per cent population of test insects was 10.885 days and the lowest concentration recorded 20.347 days. The lethal concentration to kill 50 per cent *M. circumdata* grubs were 4.64×10^9, 2.39×10^9, 0.74×10^9 and 0.001×10^9 spores ml^{-1} at 5, 10, 15 and 20 days after treatment, respectively. The corresponding LC_{90} were 8.92×10^9, 5.88×10^9, 4.12×10^9 and 0.89×10^9 spores ml^{-1} respectively.

Table 4. Cumulative per cent mortality, LT_{50} and probit analysis of dose-mortality responses of grubs of M. circumdata treated with different spore concentrations of M. anisopliae

Concentration (spores ml^{-1})	**Cumulative per cent mortality at different intervals after treatment (Days)**				**LT_{50} (Days)**
	5	10	15	20	
2×10^9	20.37	42.59	66.67	98.15	10.885
2×10^8	18.52	35.18	59.26	87.04	12.462
2×10^7	12.96	25.93	42.59	79.63	14.881
2×10^6	3.71	14.82	35.19	62.97	17.645
2×10^5	0	3.71	24.07	44.44	20.347
Control	0	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^9$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^9$)	LC_{90} (spores ml^{-1} $\times 10^9$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^9$)	χ^2	Regression equation
5	4.64	2.89 - 7.58	8.92	8.11 - 10.92	13.493	Y = 2.553 + 1.388 x
10	2.39	2.05 - 4.69	5.88	5.21 - 7.98	17.007	Y = 3.625 + 1.881 x
15	0.74	0.41 - 2.52	4.12	2.96 - 5.71	12.370	Y = 3.750 + 2.137 x
20	0.001	0.0009 - 0.026	0.89	0.26 - 1.95	21.625	Y = 4.578 + 2.422 x

Bioassay of *B. bassiana* against adult *M. circumdata* showed that a period of 10. 23 days was required to bring fifty per cent mortality at the highest spore concentration of 2.52×10^9 spores ml^{-1} tested. It was seen from the probit analysis of log dose - mortality responses that a concentration of 7.26×10^9 spores ml^{-1} was required to bring fifty per cent mortality in five days, whereas to achieve LC_{90}, a concentration of 14.04×10^9 spores ml^{-1} was required. Also a much lower concentration was enough to bring mortality in grubs, the LC_{50} was only 4.96×10^8. The LC_{90} values on the fifth day for adults and grubs were 14.04×10^9 spores ml^{-1} and 10.76×10^8 spores ml^{-1} respectively. A sharp increase in mortality of the treated insects was observed as the treatment dose was increased and the corresponding time taken was lowered. At lower doses onset of mortality was further delayed for both the fungi. The virulence of *B. bassiana* towards the adults and grubs of *M. circumdata* was more when compared to *M. anisopliae* as indicated by the mortality rates of the test insect.

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