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Evaluation of Bioassay of Entomopathogenic Fungi, *Beauveria Bassiana* (Bals.) Vuill and *Metarhizium Anisopliae* (Metchnikoff) Sorokin on Red Pumpkin Beetle, *Aulacophora Foveicollis* Lucas

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***Abstract* – Bioassays with entomopathogenic fungi, *B. bassiana* and *M. anisopliae* on Red pumpkin beetle, *A. foveicollis* 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 *A. foveicollis* was lower for *B. bassiana* when compared to *M. anisopliae.* The LC $_{50}$ of *B. bassiana* estimated were 5.27×10^8 and 2.15×10^7spores ml^{-1} against the adults and grubs of *A. foveicollis* respectively and a spore concentration of 11.42×10^{10} and 4.91×10^7spores ml^{-1} of *M. anisopliae* was required to cause 50 per cent mortality of the adults and grubs of *A. foveicollis* respectively at the highest test dose evaluated.**

Keywords* – *A. Foveicollis,* Bioassay, *B. Bassiana*, *M. Anisopliae.

I. Introduction

Red pumpkin beetle, *Aulacophora foveicollis* Lucas is a major polyphagous pest of Cucurbits grown on the tropical and sub-tropical countries of the world. The beetle is known to cause a substantial yield loss of about 30-100 % in field level (Dhillon *et al.*, 2005) [1]. The larval as well as the adult stages of the beetle cause severe damage to almost all cucurbits by feeding on the leaves and stem. The adult beetles also feed on the flower buds and flowers. The beetles are mainly controlled by chemical measures, which are not ecofriendly [2].

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) [3] [4] [5]. 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 Red pumpkin beetle, *A. foveicollis*.

II. Materials and Methods

The isolates of the fungi, *B. bassiana* isolate, PDBC Bb 5 and *M. anisopliae* isolate, PDBC Ma 4 used for the studies were obtained from National Bureau of Agriculturally Important Insect Pests (NBAII). 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 all the test insects were captured initially from the field. The insects thus collected were kept in rearing jars along with their respective 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 of *A.foveicollis* were reared on roots and stems of pumpkin/cucurbits kept in soil in plastic trays of size 30 cm diameter and 20 cm height. The decayed plant parts were removed periodically to keep the trays clean. The grubs preferred fresh and juicy stems and they moved from decayed stems to fresh stems in the rearing trays. On maturity, the grubs pupated in soil and these were collected and kept in jars for adult emergence. Further, the adults were transferred to jars containing fresh stems for egg laying. Leaves were also provided as food for the adults. The stems with oviposition punctures were then transferred to trays for larval rearing.

Bioassay of the two fungi *viz., B. bassiana* and *M. anisopliae* was carried out against the adults and grubs of the red pumpkin beetle to determine the LC_{50}, LC_{90} and LT_{50} values. The doses for bioassay against the adults and grubs were fixed based on preliminary studies. 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). The 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) [6] [7] [8].

III. Results and Discussions

Mortality of the adult *A. foveicollis* inoculated with different spore concentrations of *B. bassiana* was noticed from the fifth day after inoculation and the mortality rate increased with increase in the spore concentration (Table 1). The mortality percentage ranged from 12.96 to 50, 18.52 to 74.07 and 25.93 to 100 per cent at 10, 15 and 18 DAT, respectively in the spore concentration ranging from 2.9×10^4 to 2.9×10^8 spores ml^{-1}. The minimum time (9.494 days) required for 50 per cent kill of the test insect was obtained at a spore concentration of 2.9×10^8 spores ml^{-1}. At the lowest dose of 2.9×10^4 spores ml^{-1}, 23.52 days was required to achieve 50 per cent kill of the beetle. The LC $_{50}$ values obtained by the probit analysis of dose-mortality responses of the insect on the fifth, tenth, fifteenth and eighteenth DAT were 5.27×10^8, 2.75×10^8, 0.73×10^8 and 0.05×10^8 spores ml^{-1}, respectively. The corresponding LC $_{90}$ values were 10.22×10^8, 7.61×10^8, 4.62×10^8 and 0.31×10^8 spores ml^{-1} at 5, 10, 15 and 18 DAT respectively.

The grubs of *A. foveicollis* treated with *B. bassiana* was also found dead on the fifth day and the mortality ranged from 11.11 per cent to 46.67 per cent at spore concentration of 2×10^3 to 2×10^7 spores ml^{-1}. As the spore concentration increased from 2×10^3 to 2×10^7 spores ml^{-1}, the mortality increased from 24.45 per cent to 64.45 per cent and 44.45 per cent to 100 per cent at 7 and 14 DAT, respectively (Table 2). At the highest concentration of 2×10^7 spores ml^{-1}, the time taken for 50 per cent kill was 5.382 days and the time increased as the concentration of spores decreased and at a concentration of 2×10^3 spores ml^{-1} 14.238 days were taken to bring about 50 per cent kill. The LC $_{50}$ values corresponding to the fifth, seventh and fourteenth DAT were 2.15×10^7, 1.09×10^7 and 0.009×10^7 spores ml^{-1}, respectively. Based on the cumulative percentage mortality, the LC $_{90}$ values obtained were 5.46×10^7, 4.02×10^7 and 0.25×10^7 spores ml^{-1} at 5, 7 and 14 DAT, respectively.

The adult beetles of *A. foveicollis* treated with *M. anisopliae* were seen dead on the seventh day, mortality ranging from 3.71 to 18.52 per cent was also recorded. The rate of mortality at 10, 15 and 22 DAT ranged between 7.41 to 40.74, 29.63 to 75.93 and 44.44 to 98.15 per cent, respectively in the spore concentrations ranging from 5.2×10^6 to 5.2×10^{10} spores ml^{-1} (Table 3). The time required for the mortality of 50 per cent population of the *A. foveicollis* adults was recorded as 11.374 days at the highest spore concentration of 5.2×10^{10} spores ml^{-1}, whereas the lowest spore concentration of 5.2×10^6 spores ml^{-1} recorded the maximum time of 21.973 days. Analysis of log dose-mortality responses between the fungi and the test insect showed that the spore concentration required to bring 50 per cent kill of the beetles was 11.42×10^{10}, 6.73×10^{10}, 0.99×10^{10} and 0.02×10^{10} spores ml^{-1} on 7, 10, 15 and 22 days after inoculation, respectively. The LC $_{90}$ values for the corresponding days were 20.62×10^{10}, 16.39×10^{10}, 8.14×10^{10} and 2.22×10^{10} spores ml^{-1}, respectively.

The *A. foveicollis* grubs infected with *M. anisopliae* were found dead on the seventh day after inoculation and the mortality ranged from 2.22 to 17.78 per cent in the different test doses of the fungi. Mortality ranging from 13.33 to 35.55, 22.22 to 51.11 and 51.11 to 97.78 per cent were observed at 9, 12 and 16 DAT respectively (Table 4). To obtain 50 per cent kill of the grubs the minimum time required was 10.708 days at the highest spore concentration of 2.3×10^7 spores ml^{-1} whereas in the lowest concentration of 2.3×10^3 spores ml^{-1} the maximum time span of 15.688 days was required for achieving mortality of half the population of the test grubs. The spore concentration for causing mortality of 50 per cent of the grubs was obtained as 4.91×10^7, 3.79×10^7, 2.08×10^7 and 0.13×10^7 spores ml^{-1} at 7, 9, 12 and 16 DAT, respectively. The corresponding LC $_{90}$ values recorded were 8.66×10^7, 9.19×10^7, 7.59×10^7 and 1.07×10^7 spores ml^{-1} respectively.

Table 1. Cumulative per cent mortality, LT $_{50}$ and probit analysis of dose-mortality responses of adults of A. foveicollis 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	18	
2.9×10^8	25.93	50.00	74.07	100.00	9.494
2.9×10^7	18.52	40.74	66.67	87.04	11.374
2.9×10^6	14.82	25.93	53.71	64.82	14.659
2.9×10^5	3.71	18.52	29.63	40.74	19.891
2.9×10^4	0	12.96	18.52	25.93	23.520
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	5.27	3.78 - 6.75	10.22	8.52 - 10.97	13.808	Y = 3.325 + 1.363 x
10	2.75	2.27 - 4.21	7.61	5.94 - 9.39	10.491	Y = 3.826 + 1.724 x
15	0.73	0.34 - 1.89	4.62	3.28 - 6.04	28.334	Y = 4.553 + 2.241 x
18	0.05	0.02 - 0.09	0.31	0.22 - 0.36	12.410	Y = 5.689 + 2.224 x

Table 2. Cumulative per cent mortality, LT $_{50}$ and probit analysis of dose-mortality responses of grubs of A. foveicollis 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	7	14	
2×10^7	46.67	64.45	100.00	5.382
2×10^6	31.11	44.45	84.45	7.927
2×10^5	24.45	35.55	66.67	10.432
2×10^4	17.78	26.67	53.33	12.859
2×10^3	11.11	24.45	44.45	14.238
Control	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores ml^{-1} $\times 10^7$)	Fiducial limit for LC_{50} (spores ml^{-1} $\times 10^7$)	LC_{90} (spores ml^{-1} $\times 10^7$)	Fiducial limit for LC_{90} (spores ml^{-1} $\times 10^7$)	χ^2	Regression equation
5	2.15	1.46 - 4.32	5.46	3.66 - 11.74	4.871	Y = 3.511 + 1.109 x
7	1.09	0.69 - 1.91	4.02	2.79 - 7.54	3.726	Y = 3.969 + 2.100 x
14	0.009	0.0063 - 0.015	0.25	0.17 - 0.48	3.127	Y = 4.725 + 2.077 x

Table 3. Cumulative per cent mortality, LT $_{50}$ and probit analysis of dose-mortality responses of adults of A. foveicollis 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)
	7	10	15	22	
5.2×10^{10}	18.52	40.74	75.93	98.15	11.374
5.2×10^9	12.96	31.48	62.96	92.59	13.179
5.2×10^8	7.41	20.37	50.00	72.22	16.511
5.2×10^7	3.71	18.52	35.18	59.26	19.263
5.2×10^6	0	7.41	29.63	44.44	21.973
Control	0	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} $\times 10^{10}$)	χ^2	Regression equation
7	11.42	9.61 - 13.24	20.62	17.46 - 22.01	7.692	Y = 2.933 + 1.591 x
10	6.73	4.82 - 8.64	16.39	13.08 - 18.45	8.583	Y = 3.388 + 2.893 x
15	0.99	0.28 - 1.69	8.14	6.77 - 8.82	12.092	Y = 4.396 + 2.001 x
22	0.02	0.009 - 0.07	2.22	1.62 - 2.52	23.994	Y = 3.817 + 1.092 x

Table 4. Cumulative per cent mortality, LT $_{50}$ and probit analysis of dose-mortality responses of grubs of A. foveicollis 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)
	7	9	12	16	
2.3×10^7	17.78	35.55	51.11	97.78	10.708
2.3×10^6	11.11	24.45	42.22	88.89	11.957
2.3×10^5	6.67	22.22	35.55	71.11	13.411
2.3×10^4	2.22	15.55	28.89	62.22	14.480
2.3×10^3	0	13.33	22.22	51.11	15.688
Control	0	0	0	0	0

Probit analysis

Days after treatment	LC_{50} (spores $ml^{-1} \times 10^7$)	Fiducial limit for LC_{50} (spores $ml^{-1} \times 10^7$)	LC_{90} (spores $ml^{-1} \times 10^7$)	Fiducial limit for LC_{90} (spores $ml^{-1} \times 10^7$)	χ^2	Regression equation
7	4.91	3.79 - 6.04	8.66	6.88 - 9.71	6.026	Y = 2.835 + 1.685 x
9	3.79	2.26 - 17.41	9.19	5.31 - 45.54	2.015	Y = 2.423 + 2.111 x
12	2.08	1.19 - 8.12	7.59	4.43 - 34.39	3.838	Y = 2.476 + 1.483 x
16	0.13	0.06 - 0.53	1.07	0.79 - 1.23	11.671	Y = 4.151 + 2.418 x

Bioassay of *B. bassiana* against the adult beetles of *A. foveicollis* showed that a period of 9.494 days was required to bring fifty per cent mortality at the highest spore concentration of 2.9×10^8 spores ml^{-1} tested. From the probit analysis, it was seen that to achieve 50 per cent mortality in a shorter period of five days a higher concentration of 5.27×10^8 spores ml^{-1} was essential and for the corresponding day the LC_{90} value was 10.22×10^8 spores ml^{-1}. With respect to the grubs, the LC_{50} value on the fifth day was 2.15×10^7 spores ml^{-1} and that was a lower concentration when compared to the concentration required for the adult *A. foveicollis*. When the adults of *A. foveicollis* was treated with *M. anisopliae* the time required for the mortality of 50 per cent population of the beetles was recorded as 11.374 days at the highest spore concentration of 5.2×10^{10} spores ml^{-1} and in the case of grubs a total of 10.708 days at the highest spore concentration of 2.3×10^7 spores ml^{-1} was recorded. The results of the probit analysis clearly indicates that there is a sharp increase in mortality of the treated insects 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 *A. foveicollis* was more when compared to *M. anisopliae* as indicated by the mortality rates of the test insect and the test dose of the fungal spore suspension used for the bioassays. As indicated by the mortality rates, both entomopathogenic fungi are potential pathogens of red pumpkin beetle and ecofriendly and disseminate naturally, hence biological control of *A. foveicollis* using *B. bassiana* and *M. anisopliae* is effective over chemical control.

IV. References

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