Efficacy of different entomopathogenic fungi against cowpea aphid, *Aphis craccivora* (Koch)

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ABSTRACT

Laboratory bioassay studies were carried with six different concentrations of *Beauveria bassiana* (Bals.) Vuill., *Metarhizium anisopliae* (Metsch.) Sorokin., *Verticillium lecanii* (Zimm.) Viegas., *Hirsutella thompsonii* (Fisher) and *Cladosporium oxysporum* (Berk. and Curt.) against the adults of *Aphis craccivora.* In the high concentration (10^8 spores ml^{-1}) 100% mortality was obtained with *V. lecanii* and *H. thompsonii* followed by *B. bassiana, M. anisopliae* and *C. oxysporum*. Mortality declined with the decrease in concentrations. The lowest LC_{50} value of $2.5x10^4$ spores ml^{-1} was recorded by *V. lecanii* and *H. thompsonii* isolates, which showed higher virulence compared to other isolates. The LC_{50} values of *B. bassiana, M. anisopliae* and *C. oxysporum* were $4.5x10^4$, $8.9x10^5$ and $7.4x10^5$ spores ml^{-1} respectively. At the highest concentration of 10^8 spores ml^{-1}, the Median LT_{50} values for *B. bassiana, H. thompsonii, V. lecanii, C. oxysporum* and *M. anisopliae* were 3.63, 3.64, 3.90, 5.24 and 5.54 days, respectively. The LT_{50} values were found to be inversely proportional to the spore concentrations. Among the five entomopathogenic fungi, *V. lecanii, H. thompsonii* and *B. bassiana* were found to be the promising virulent isolates. By testing their field efficacy, they can be used as potential biocontrol agent for the management of cowpea aphid.

Key words: *Aphis craccivora*, Crop Pest, Entomopathogenic Fungi, median lethal concentration

INTRODUCTION

Cowpea aphid, *Aphis craccivora* (Koch) is a threat to cowpea growers in all over the country. Both nymphs and adults suck plant sap and cause serious damage right from the seedling to pod bearing stage. Due to heavy infestation, young seedlings succumb to death, whereas the older plants show symptoms such as stunting, crinkling and curling of leaves, delayed flowering, shriveling of pods and finally resulting in yield reduction. Considering the adverse effect of insecticides, pest management through biological control is encouraged using predators, parasites and pathogens. Among the different microbial agents, entomopathogenic fungi (EPF) are gaining importance in pest control. More than 750 species of fungi are pathogenic to insects and many of them offer a great potential for the management of sucking pests (Rabindra and Ramanujam, 2007). A study was conducted to evaluate the bioefficacy of *Beauveria bassiana* (Bals.) Vuill., *Metarhizium anisopliae* (Metsch.) Sorokin., *Verticillium lecanii* (Zimm.) Viegas., *Hirsutella thompsonii* (Fisher) and *Cladosporium oxysporum* (Berk. and Curt.) against the adults of *A. craccivora.*

MATERIALS AND METHODS

Rearing of aphids

Uniform aged aphids were reared for the bioassay in the laboratory by using the method of Yeo *et al.* (2003). Initially 20 adult apterous aphids were inoculated on fresh cowpea seedlings in the trifoliate stage. The inoculated aphids reproduced parthenogenetically, and the newly formed one day old first instar nymphs were reared on the same plant. After 24 h, the inoculated adult aphids were removed from the seedlings and were used for the bioassay studies.

Fungal isolates

Pure cultures of the entomopathogenic fungi *B. bassiana, M. anisopliae, V. lecanii* and *H. thompsonii* obtained from the National Bureau of Agriculturally Important Insects (NBAII), Bangalore and one local isolate *C. oxysporum* were reisolated after proving the Koch postulates. These fungi were then subcultured in Sarbaourd's Maltose Agar enriched with one per cent yeast extract (SMA+Y) media and incubated at room temperature for 10 days and stored in refrigerator. All the fungal isolates were subcultured once in three weeks. To maintain the virulence, after six subculturing all the fungal isolates were subjected to pathogenicity test and again reisolated for further studies.

Preparation of spore concentrations of the fungal isolates

All the five fungal isolates were cultured in 100ml SMA+Y liquid medium in 250ml conical flask and incubated at room temperature for 10 days. After sporulation, ground with ordinary mixer and made into liquid spore suspension.

This was filtered through double layered muslin cloth to remove the mycelial mat. The suspension was shaken thoroughly with a drop of Teepol solution for uniform dispersion of the spores in the solution. The spore count was made by using a haemocytometer. All the cultures were adjusted to 1x 10^8 spores ml^{-1} from which the lower concentrations were prepared by serial dilution technique for bioassay studies.

Bioassay

Cowpea seedlings were raised in small paper cups of size 7.5x7.5cm in the laboratory. Fifteen days old seedlings were used for the bioassay studies. Six different spore concentrations ($1x10^8$, $1x10^7$, $1x10^6$, $1x10^5$, $1x10^4$, $1x10^3$ spores ml^{-1}) were prepared for *B. bassiana, M. anisopliae, V. lecanii, H. thompsonii* and the local isolate *C. oxysporum.* Each concentration was replicated three times. One day old adult apterous aphids were inoculated on the cowpea seedlings using a camel hairbrush @ 10 aphids per seedling. Totally 30 aphids were used for each treatment. After inoculation of aphids, the respective concentrations of all the fungal spore suspensions were sprayed on the seedlings using an atomizer. Aphids sprayed with 0.05 per cent Teepol solution served as control. After spraying, seedlings were kept under belljar to avoid the escape of aphid population and to maintain the humidity. The mouth of the cup was closed with white paper, for the easy collection of dead aphids

Mortality of aphids was recorded separately at 24 h interval up to seven days. Dead aphids were collected daily, and placed in Petridish containing a moist filter paper and kept in humid chamber. The dead aphids which produced mycelial growth were considered for the mortality count. Neonate aphids were counted and removed daily from the seedlings. Mortality data was corrected with that in control by using the Abbott's formula (Abbott, 1925). The data was then analysed by probit analysis (Finney, 1971) and the Median Lethal Concentration (LC_{50}) and the Median Lethal Time (LT_{50}) values were computed by using statistical computer programme, Statistical Package of Social Sciences (SPSS).

Statistical analysis

The per cent corrected cumulative mortality of each fungus was subjected to ANOVA test and the means were separated by Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Mortality of adults of *A. craccivora*

Mortality of aphids was monitored at 24 h interval upto seven days. The data of corrected per cent mortality at different time intervals presented in Table 1 indicates that the mortality increased with increase in time interval. Mortality of aphids was 3.33 per cent observed with in 24 h, at the highest concentration (10^8 spores ml^{-1}) of *B. bassiana, V. lecanii* and *H. thompsonii*. After 48 h of exposure, among the five fungal isolates *B. bassiana, H. thompsonii* and *C. oxysporum* recorded of per cent mortality ranging between 3.33 to 13.33 at 10^8 and 10^7spores ml^{-1} and 3.33 to 6.66 per cent at 10^6spores ml^{-1}. All the concentrations of *B. bassiana* and *C. oxysporum* recorded mortality of aphids after 72 h only; mortality in different concentrations ranged between 6.66 to 43.33 and 6.66 to 20.00 per cent in *B. bassiana* and *C. oxysporum* respectively. However, mortality was recorded in other fungal isolates namely *M. anisopliae, V. lecanii* and *H. thompsonii* at the highest concentration (10^8 spores ml^{-1}) only; they produced 6.66 to 35.71 per cent mortality.

At 120 h (5 days) after treatment the highest per cent mortality was obtained in the highest spore concentration of 10^8 spores ml^{-1} in *V. lecanii* (88.46%) followed by *H. thompsonii* (80.70%) and *B. bassiana* (73.33%). *Cladosporium oxysporum* and *M. anisopliae* resulted in lower mortality of 46.43 and 42.86 per cent respectively. At 10^7 spores ml^{-1}, *B. bassiana* and *V. lecanii* obtained more than 50 per cent mortality 66.66 and 76.96 per cent respectively. *Hirsutella thompsonii, HhhhhM. anisopliae* and *C. oxysporum* recorded the least mortality ranging between 3.33 to 10.34 per cent at the least concentration 10^3 spores ml^{-1}. At 144 h (6 days) and 168 h (7 days) after treatment there was marked increase in the mortality of aphids. Among the five isolates, *V. lecanii* at 10^8 spores ml^{-1} showed cent per cent mortality on the 6^{th} day followed by *H. thompsonii* (96.50%).

All the fungal isolates in the highest spore concentration (10^8 spores ml^{-1}) produced high mortality ranging from 77.50 to 100 per cent, after seven days of treatment. Among the five isolates *V. lecanii* and *H. thompsonii* gave cent per cent mortality. Yokomi and Gottwald (1988) also reported cent per cent mortality of three aphid species *Myzus persicae, Aphis gossypii* and *Aphis citricola* at 10^6-10^7 spores ml^{-1} after four days. Increased mortality of *H. thompsonii* at 10^8 spores ml^{-1} has also been reported by Smitha (2007); she obtained 90 per cent mortality of banana mealy bug, *Geococcus* sp. after three days of inoculation.

At the highest concentration of 10^8 spores ml^{-1}, *B. bassiana* and *M. anisopliae* also gave appreciable reduction in population showing 96.66 and 80.76 per cent respectively. Ekesi *et al.* (2000) also got similar result with 91 and 93 per cent mortality of *A. craccivora* at 7^{th} day of treatment.

Table 1. Effect of fungal isolates on the mortality of adults of *A. craccivora* at different time interval

Fungal Isolate	Dose(spores/ml)	Corrected mortality (%)						
		Hours						
		24	48	72	96	120	144	168
B. bassiana	10^8	3.33	10.00	26.66	43.33	73.33	86.66	96.66
	10^7	0.00	6.66	43.33	60.00	66.66	76.66	83.33
	10^6	0.00	6.66	33.33	40.00	53.33	66.67	70.00
	10^5	0.00	0.00	20.00	33.33	40.00	43.33	50.00
	10^4	0.00	0.00	6.66	20.00	33.33	36.66	40.00
	10^3	0.00	0.00	6.66	10.00	23.33	26.66	26.66
M. anisopliae.	10^8	0.00	0.00	6.66	24.14	42.86	51.85	80.76
	10^7	0.00	0.00	6.66	13.79	17.86	33.33	61.53
	10^6	0.00	0.00	3.33	3.33	13.79	29.62	50.00
	10^5	0.00	0.00	3.33	6.89	10.34	29.62	38.47
	10^4	0.00	0.00	0.00	10.34	17.24	18.51	23.07
	10^3	0.00	0.00	0.00	3.33	6.89	14.14	19.23
V. lecanii	10^8	3.33	3.33	17.86	53.84	88.46	100.00	100.00
	10^7	0.00	0.00	32.14	50.00	76.96	92.30	100.00
	10^6	0.00	0.00	17.86	42.30	50.00	76.92	84.00
	10^5	0.00	0.00	21.43	32.14	46.15	57.69	60.00
	10^4	0.00	0.00	7.14	15.39	23.07	34.61	44.00
	10^3	0.00	0.00	0.00	11.54	23.07	26.92	28.00
H. thompsonii	10^8	3.33	13.33	35.71	60.71	80.70	96.50	100.00
	10^7	0.00	13.33	28.57	39.29	46.18	76.91	96.00
	10^6	0.00	3.33	7.14	21.43	26.94	61.53	80.00
	10^5	0.00	0.00	10.72	26.94	28.57	57.69	60.00
	10^4	0.00	0.00	7.14	14.29	23.07	42.30	44.00
	10^3	0.00	0.00	0.00	3.33	3.33	7.69	20.00
C. oxysporum	10^8	0.00	6.66	20.00	24.14	46.43	64.28	77.50
	10^7	0.00	3.33	16.66	13.79	37.93	57.13	60.71
	10^6	0.00	3.33	13.33	13.79	31.03	39.29	49.99
	10^5	0.00	3.33	10.00	10.34	27.58	35.71	39.29
	10^4	0.00	0.00	6.66	13.79	24.14	25.00	28.57
	10^3	0.00	0.00	6.66	3.45	10.34	14.29	17.86

Loureiro and Moino (2006) recorded 100 % mortality of *Myzus persicae* with *B. bassiana* and *M. anisopliae* applied at 10^6 and 10^7 spores ml^{-1}, respectively. The local isolate, *C. oxysporum* recorded 77.57 per cent mortality with 10^8 spores ml^{-1}. Ramegowda *et al.* (2007) had reported 93.33 per cent mortality in *C. lanigera*, ten days after treatment. A progressive reduction in the mortality of aphids was observed with decreasing concentrations. In the lower concentration, 10^3 spores ml^{-1} the mortality of aphids ranged between 17.86 to 28.00 per cent. At in adequate spore concentration the time taken for multiplication may be prolonged resulting in a reduced control of the aphids.

Cumulative mortality of *A. craccivora*

The corrected cumulative mortality per cent at seven days after treatment was analysed by ANOVA and the results are presented in Table 2. The cumulative per cent mortality of *A. craccivora* with the fungal isolates was found to be statistically on a par at 10^8 concentration. At the highest concentration of 10^8 spores ml^{-1}, *V. lecanii* and *H. thompsonii* produced cent per cent mortality and was found to be equal to other fungal isolates. It was closely followed by *B. bassiana* causing 96.66 per cent mortality which was as effective as *V. lecanii* and *H. thompsonii*. In the next lower concentration also, *V. lecanii* produced cent per cent cumulative mortality of aphids. *Hirsutella thompsonii* and *B. bassiana* were found to be on a par with *V. lecanii* producing 96.00 and 83.33 per cent mortality respectively.

Median Lethal Concentration (LC_{50})

The data presented in Table 3 shows the LC_{50} values and the relative toxicity of the five fungal isolates. Among the

Table 2. Dose mortality response of fungal isolates against *A. craccivora*

Fungal isolate	Heterogenety (χ2)	Regression equation	LC_{50}(spores ml^{-1})	95% Fiduciallimits (spores ml^{-1})	Relative toxicity
B. bassiana	4.724	Y = 2.0507+0.44×	4.5×10^4	1.1×10^4 - 3.4×10^5	1.8
M. anisopliae	4.303	Y = 2.7301+0.62×	8.9×10^5	4.1×10^5- 1.8×10^7	35.6
V. lecanii	4.303	Y = -2.7308+4.3×	2.5×10^4	1.5×10^4- 4.0×10^4	1.0
H. thompsonii	3.681	Y = -2.025+0.34×	2.5×10^4	1.5×10^4- 4.0×10^4	1.0
C. oxysporum	1.277	Y = -1.891+0.32×	7.4×10^5	2.6×10^5- 1.6×10^6	29.6

five fungal isolates, *V. lecanii* and *H. thompsonii* caused 50 per cent mortality at the lowest concentration of 2.5 $x10^4$ spores ml^{-1}. This was followed by *B. bassiana* ($4.5x10^5$ spores ml^{-1}), *C. oxysporum* ($7.4x10^5$ spores ml^{-1}) and *M. anisopliae* ($8.9x10^5$ spores ml^{-1}). It is understood that higher the LC_{50} values, higher will be the relative toxicity . Low LC_{50} value of $1.2x10^4$ spores ml^{-1} for *V. lecanii* against *Brevicoryne brassicae* and $2.7x10^4$ spores ml^{-1} against *Aphis gossypii* was reported by Derakshan *et al.* (2007) and Karindah *et al.* (1996) respectively is in conformity with the present finding. LC_{50} value obtained in the present study was lower than that reported by Smitha (2007) for *Hirsutella* sp ($5.2x10^4$ spores ml^{-1}), but higher than reported by Liu *et al.* (1999) for *B. bassiana* ($1.2x10^4$ spores ml^{-1}) and Chandler (1997) for *M. anisopliae* ($2.45x10^6$ spores ml^{-1}). The difference in the LC_{-50} values might be due to the difference in the virulence of fungal isolates and the host species. Among the five fungal isolates, *V. lecanii* and *H. thompsonii* were the most virulent isolates with the lowest LC_{50} and relative toxicity. This was followed by *B. bassiana* with a relative toxicity value of 1.8. *Metarhizium anisopliae* and *C. oxysporum* recorded higher LC_{50} and relative toxicity values. Both these isolates less virulent compared to other isolates.

Median Lethal Time (LT_{50})

Variation in LT_{50} values at different concentrations was evident (Table 4). LT_{50} values decreased with increase in concentrations. At 10^8 spores ml^{-1}, low LT_{50} value was recorded by *B. bassiana*, *H. thompsonii* and *V. lecanii* as 3.63, 3.64 and 3.90 days respectively. Nirmala *et al.* (2006) also attained similar results for *B. bassiana* with LT_{50} value of 3.17 days. The LT_{50} value of 3.31 days has been obtained for *V. lecanii* against *Aphis fabae* by Hesketh *et al.* (2008) also agree with the present finding. *Metarhizium anisopliae* and *C. oxysporum* recorded higher LT_{50} values of 5.54 and 5.24 respectively at 10^8spores ml^{-1}. Under laboratory conditions, *V. lecanii* and *H. thompsonii* were found to be more virulent recording cent per cent mortality within seven days after treatment. Other fungal isolates also showed promising result. The lowest LC_{50} and LT_{50} values of *V. lecanii, H. thompsonii* and *B.bassiana* indicate its higher virulence against *A.craccivora*. they can be used as potential biocontrol agent after field experiments for the management of cowpea aphid.

Table 3. Time mortality response of fungal isolates against *A. craccivora*

Fungal isolate	Median Lethal Time (Days)					
	10^8	10^7	10^6	10^5	10^4	10^3
B.bassiana	3.63	4.51	5.83	5.98	6.88	8.11
M.anisopliae	5.54	6.61	7.01	7.43	8.65	8.91
V.lecanii	3.90	3.96	4.86	5.65	7.25	7.87
H.thompsonii	3.64	4.52	5.84	5.98	6.89	8.12
C.oxysporum	5.24	5.96	6.75	7.34	8.19	10.17

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