International Journal of Plant Protection | **Volume 5 | Issue 1 | April, 2012 | 68-71**

IJPP

Research Article

Efficacy of different entomopathogenic fungi against cowpea aphid, *Aphis craccivora* Koch under laboratory and field condition

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ARITCLE INFO

Article Chronicle :
Received : *05.11.2011*
Revised : *12.12.2011*
Accepted : *08.02.2012*

Key words :
Cowpea aphid, *Aphis craccivora*, Entomopathogenic fungi, *Verticilium lecanii*

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ABSTRACT

Six isolates of entomopathogenic fungi *viz., Beauveria bassiana, Fusarium solani, Verticillium lecanii*-1, *Verticillium lecanii*-2, *Verticillium lecanii*-3 (VL-3) and *Paecilomyces fumosoroseus* each at 1 x 10^8 conidia/ml were bioassayed against nymphs and adults by dipping method. The virulent fungal entomopathogen, *Verticilium lecanii*-3 was evaluated under field condition at three different concentrations *viz*., 1x10^7, 1x10^8, 1x10^9 spores/ml. It was found that VL-3 showed higher per cent mortality of 73.99 and 57.73 of adult and nymphs of *Aphis craccivora* , respectively. Under field condition VL-3 @1x10^9 spores/ml showed higher per cent mortality of aphids (71.62) compared to other two lower concentrations. This study indicates the scope of using *V. lecani* for the management of cowpea aphid under field condition.

How to view point the article **:** Suresh, B.C., Khader Khan, H. and Prasanna, P.M. (2012). Efficacy of different entomopathogenic fungi against cowpea aphid, *Aphis craccivora* Koch under laboratory and field condition. *Internat. J. Plant Protec*., **5**(1) : 68-71.

INTRODUCTION

Cowpea aphid, *Aphis craccivora* Koch is an important pest of wide range of leguminous crops such as cowpea, groundnut, pigoenpea, chickpea, peas, mungbean and urdbean. Both nymphs and adults infest all the stages of crop growthby sucking cell sap from tender shoots, flower stalks and pods resulting in stunting of plant growth and can cause yield loss of 20-40 per cent (Singh and Allen, 1980) due to transmission of virus diseases such as rosette, mottles, stunt and stripe (Porter *et al*., 1984). It is a difficult pest to control with insecticides because of its polyphagous nature with very short life cycle and high reproduction rates. Fungi have been considered the principal group of aphid pathogens, the most prevalent and widely encountered species belonging to the order Entomopthorales (Zygomycetes). In particular environments (green house/tropical regions) Deutermycetous species also significantly reduce aphid numbers. *V. lecanii* is a well documented, extremely wide spread entomopathogen and spectacular epizootics are observed on its most common hosts *viz*., aphids and scales in tropical and sub tropical region (Hall, 1980). In this context efforts have been made to evaluate efficacy of fungal entomopathogens on cowpea aphids under laboratory and field conditionss.

MATERIALS AND METHODS

Four different fungal pathogens were evaluated against cowpea aphid *viz*., *Beauveria bassiana* (Balsano) Vuillmin, *Fusarium solani* (Marts) Sacc., *Verticillium lacanii* (Zimn.) Viegas and *Paecilomyces fumosoroseus* (wize) Brown and smith. These were obtained from different sources (Table A).

Isolation and purification of entomopathogenic fungi from the infected aphids

The entomopathogenic fungi were isolated from field collected dead insect specimens adopting the procedure of Lomer and Lomer (1995). The specimens were surface sterilized with 0.1 per cent sodium hypochlorite solution and rinsed with sterile distilled water to remove the traces of sodium hypochlorite in order to prevent toxicity to the fungus. Surface sterilized specimens were planted on 20 ml water agar Petriplates and incubated at 25 ± 1°C under 95 per cent RH. The fungi were sub-cultured and purified by hyphal tip method (Tuite, 1969).

Table A : Details of different fungal pathogens used for evaluation against *Aphis craccivora*

Sr. No.	Fungal pathogen	Isolates	Source	Natural hosts
1.	*Beauveria bassiana*	Bb	Survey	Aphid
2.	*Fusarium solani*	Fs	Survey	Aphid
3.	*Verticillium lecanii*	VL-1	BCRL	Aphid
		VL-2	BCRL	Whitefly
		VL-3	BCRL	Mealy bug
4.	*Paecilomyces fumosoroseus*	Pfr	BCRL	Whitefly

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Rearing of *Aphis craccivora:*

The stock culture of *Aphis craccivora* was maintained on caged cowpea plants raised in plastic pots. Initially about 20-25 adults aphids were inoculated on 6-7 days old cowpea seedlings to get uniform aged aphids for bioassay studies. After 24 hours of transfer, the adult aphids were removed from the seedlings (Blackman, 1988).

Laboratory bioassay:

The test was conducted on fresh adults and third instar nymphs. Uniform aged adults and nymphs were collected, fungal suspension of 6 fungal isolates at a uniform concentration of $1x10^8$ conidia/ml and a control (Water + Tween-80 0.05%) were treated by dipping method. For each treatment four replications were maintained. 24 aphids were used for each replication. The treated aphids were transferred into the excised seedling cages, by closing the lids they were incubated in lab condition with average temperature of 26°C and RH80 per cent. Observation was taken on 3rd and 6th days after treatment for nymphs and adult's aphids.

Field trials:

The cowpea crop was raised in 80 sq. m. of land. The experiment was laid out in randomized block design with five treatments and four replications. The treatments as water suspensions were at concentrations $1x10^7$, $1x10^8$ and $1x10^9$ spores/ml. A spray of water and chlorpyriphos @ 2 ml/lit served as control and standard check, respectively.

Spray suspension was prepared on the day of spraying. *V. lecanii*-3 which was mass produced on bran medium was homogenized with known quantity of distilled water in Tween-80 0.05 per cent to prepare spray suspension. Then the contents were filtered through 300-mesh sieve and spore count of the suspension was done using haemocytometer and different concentrations were prepared by using cereal dilution technique.

In all the treatments, spraying was done with a hand atomizer with spray fluid volume of 250 lit/ha. Two sprays were given at an interval of 9 days and spraying was carried out during evening hours. Population density of aphids was assessed following the method of Banks (1954).

Ten plants of each treatment were removed from and aphids on each plant counted and mean number of aphids for each treatment was calculated. Pre-treatment and post-treatment populations were calculated by multiplying the mean number of aphids for each of the classes by the appropriate number of stems of each treatments and the products, when added, given an estimate of the aphid population of the plants examined in the plot. Post treatment count were taken on 3, 6 and 9th days after each spray. The per cent reduction in aphid population was calculated using the formula given by Henderson and Tilton (1955) :

$$\textbf{Per cent reduction} = [1- (T_a/T_b \times C_b/C_a)] \times 100$$

where, T_b = No. of aphids before treatment

T_a = No. of aphids after treatment

C_b = No. of aphids in the control plot before treatment

C_a = No. of aphids in the control plot after treatment

The data analysis was done using ANOVA for RCBD and interpreted at the probability level of 5 per cent. The means were compared using Duncan multiple range test (DMRT).

RESULTS AND DISCUSSION

The results obtained from the present investigation are presented below :

Laboratory bioassay:

Out of six fungus isolates tested, *V. lecanii*-3 was found to be significantly superior at 3 and 6 days after treatment with per cent mortality of 26 and 73.99, respectively. There was significant per cent mortality in aphid population in treatment *V. lecanii*-3 as compared to control (4.00%), after 6 days of treatment at the spore load of $1x10^8$ conidia/ml. The other fungal isolates, *B. bassiana* (65.24%), *F. solani* (61.86%), *V. lecanii*-1 (58.62%), *V. lecanii*-2 (58.28%) and *P. fumosoroseus* (50.67%) were significantly inferior to *V. lecanii*-3 (Table 1). The present results confirm the reports of Miranpuri and Khachatourian (1995) where in various isolates of *V. lecanii* showed significantly higher pathogenicity against the aphid, *Sitobion avenae* compared to *B. bassiana*. Similarly, the present investigation also in close agreement with Askary *et*

al. (1998) where in out of three strains of *V. lecanii* (DAOM 198499, DAOM 216596 and Vertilac) tested against *M. euphorbiae*, Vertilac and DAOM 198499 strains were more virulent to aphid compared to DAOM 216596 strain.

Similarly, there was significant reduction in the nymphs population in all the tested entomofungal pathogens as compared to check in the laboratory. Highest per cent reduction was achieved in *V. lecanii*-3 (57.73) followed by *Beauveria bassiana* and *F. solani* with per cent reduction of 55.56 and 54.72, respectively at constant spore load of $1x10^8$ conidia/ml after sixth days of treatment (Table 1).

In the present study, per cent reduction of nymphs of *A. craccivora* was considerably low compared to adults under laboratory conditions. This might be due to shedding of conidia with exuviae during nymphal ecdysis. Present findings are in conformity with Vestergard *et al.* (1995) where they reported similar results of *Metarrhizium anisopliae* against nymphs and adults of flower thrips, *Frankliniella occidentalis*.

Field trials with *V. lecanii:*

The field studies with *V. lecanii* on cowpea aphid was encouraging. After third day of first spray there was significant difference among the treatments with respect to per cent reduction of *A. craccivora*. Standard check chlorpyriphos showed significantly greater per cent reduction (98.83) compared to all other treatments. However, *V. lecanii*-3 at different concentration showed less per cent reduction compared to standard check but it was significantly superior over control. Throughout the study, it was observed that per cent reduction of aphids due to *V. lecanii*-3 increased with time (Table 2). This might be due to requirements of long incubation period for germination, penetration and establishment inside the host body. Among different

Table 1 : Efficacy of entomofungal pathogens against cowpea aphid, *Aphis craccivora*

Tr. No	Fungal pathogens @ $1x10^8$ conidia/ml	Per cent mortality			
		Nymphs		Adults	
		3 DAT	6 DAT	3 DAT	6 DAT
1.	*Beauveria bassiana*	33.25 (35.20)^{ab}	55.56 (48.19)^{b}	18.01 (25.13)^{b}	65.24 (53.88)^{b}
2.	*Fusarium solani*	32.23 (34.25)^{bc}	54.72 (47.72)^{b}	14.01 (22.01)^{cd}	61.86 (51.86)^{c}
3.	*Verticillium lecanii*-1	29.33 (32.78)^{c}	48.00 (43.85)^{c}	15.91 (23.43)^{bc}	58.62 (49.97)^{d}
4.	*Verticillium lecanii*-2	25.00 (29.99)^{d}	44.66 (41.93)^{d}	12.51 (20.73)^{d}	58.28 (49.76)^{d}
5.	*Verticillium lecanii*-3	35.21 (36.39)^{a}	57.73 (49.44)^{a}	26.00 (30.65)^{a}	73.99 (59.43)^{a}
6.	*Paecilomyces fumosoroseus*	19.17 (25.94)^{e}	34.22 (35.77)^{e}	10.36 (18.25)^{e}	50.67 (45.38)^{e}
7.	Water (control)	2.90 (8.02)^{f}	4.18 (11.79)^{f}	1.33 (3.85)^{f}	4.00 (11.54)^{f}
	F test	*	*	*	*
	S.E. $\pm$	0.579	0.243	0.638	0.591
	C.D. (P=0.05)	1.742	0.736	1.967	1.792

Average of four replications
- Figures in the parenthesis are Arc sign transformed values
- Days After Treatment(DAT)

Means followed by same letter in a column are not significant by DMRT

Table 2 : Efficacy of *Verticillium lecanii*-3 against *Aphis craccivora* on cowpea under field condition

Sr. No.	Treatments	Dosage (Conidia/ml)	Per cent reduction					
			First spray			Second spray		
			3DAS	6DAS	9DAS	3DAS	6DAS	9DAS
1.	*Verticillium lecanii*-3	$1x10^8$	25.31 (30.20)^{c}	40.64 (39.42)^{c}	51.56 (45.47)^{c}	61.81 (51.63)^{c}	68.55 (55.89)^{d}	76.12 (60.12)^{d}
2.	*Verticillium lecanii* -3	$1x10^9$	30.38 (33.45)^{b}	41.22 (39.94)^{bc}	54.10 (47.35)^{bc}	64.58 (53.68)^{b}	72.22 (58.15)^{c}	77.05 (61.41)^{c}
3.	*Verticillium lecanii* -3	$1x10^{10}$	31.34 (34.04)^{b}	43.76 (41.57)^{b}	56.82 (48.97)^{b}	63.80 (53.01)^{bc}	74.74 (59.85)^{b}	79.32 (62.98)^{b}
4.	Chlorpyriphos 20EC	2ml/lit	98.83 (83.79)^{a}	96.87 (79.81)^{a}	91.77 (73.33)^{a}	98.31 (82.53)^{a}	96.65 (79.45)^{a}	91.64 (73.19)^{a}
5.	Control	Water	0.00 (0.00)^{d}	0.00 (0.00)^{d}	0.00 (0.00)^{d}	0.00 (0.00)^{d}	0.00 (0.00)^{e}	0.00 (0.00)^{e}
		F test	*	*	*	*	*	*
		S.E. $\pm$	1.038	0.6756	1.109	0.6175	0.5362	0.4102
		C.D. (P=0.05)	3.20	1.97	3.29	1.85	1.65	1.21

Average of four replications
- Figures in the parenthesis are Arc sign transformed values
- Means followed by same letter in a column are not significant by DMRT
- Days After Spray(DAS)

concentration of *V. lecanii*-3@ $1x10^{10}$ conidia/ml showed highest per cent reduction of 79.32.

The results corroborate with the findings of Easwaramoorthy and Jayaraj (1978) on the effectiveness of white halo fungus, *Cephalosporium lecanii* @ 16×10^6 conidia/ml against the coffee green bug, *Coccus virids* with 73.1 per cent mortality. Similarly, Sunitha *et al.* (1999) reported that *F. pallidoroseum* at the concentration of $7x10^6$ conidia/ml found to be as effective as quinalphos 0.05 per cent against *Aphis craccivora* under field condition. Wayne *et al.* (1984) found that a single aqueous spray of Vertilac (Commercial formulation of *V. lecanii*) consistently and effectively controlled *Myzus persicae* on chrysanthemum in green house conditions.

In future, requirements for effective fungal pathogen for control of aphid pest is likely to increase due to concern about environmental safety measures. In this context, the present study paved biocontrol based on *V. lecanii* that could be used as a potential agent for managing aphids.

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