Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

J E Z S Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(2): 686-691
© 2017 JEZS
Received: 20-01-2017
Accepted: 21-02-2017

Reshma Gokak
M. Sc. Student, Department of Entomology, Kittur Rani Channamma College of Horticulture, Arabhavi - 591 218 Karnataka, India

SH Ramanagouda
Assistant Professor Department of Entomology, Kittur Rani Channamma College of Horticulture, Arabhavi - 591 218 Karnataka, India

J Jayappa
Assistant Professor Department of Entomology College of Horticulture Bengaluru - 560 065 Karnataka, India.

Correspondence
Reshma Gokak
M. Sc. Student, Department of Entomology, Kittur Rani Channamma College of Horticulture, Arabhavi - 591 218 Karnataka, India.

Collection, isolation and bioassay studies of indigenous isolates of *Lecanicillium lecanii* (Zimm.) Zare and Games against *Myzus persicae* (Sulzer)

Reshma Gokak, SH Ramanagouda and J Jayappa

Abstract
The evaluation of indigenous local isolates of *Lecanicillium lecanii* was determined against *Myzus persicae* by detached leaf smeared method. A total of three isolates of *L. lecanii* from the 30 samples were obtained based on their pathogenicity test against *M. persicae*. Among the three isolates R7BGBD (100%) and R8GAR (96.67%) showed highest mortality whereas the least mortality was found in the isolate R4HAT (56.67%) at eight days after treatment at the concentration of 2 x 10^9 cfu/1000 ml. The isolate R7BGBD was the most virulent at six and eight days after treatment, the LC_{50} values being 0.54 X 10^9 and 0.15 X 10^9 cfu/1000 ml, respectively. The order of virulence based on LC_{50} values was R7BGBD> R8GAR> R4HAT. These isolates performed better under laboratory conditions and need to be evaluated under field conditions for their promotion against sucking insect pests of various Horticulture and Agricultural crops.

Keywords: *Lecanicillium lecanii, Myzus persicae*, local isolates

1. Introduction

Management of insect pest in agriculture is important to safeguard the crop in order to increase the yield and productivity. In India, on an average 33 per cent of crop loss is mainly due to insect pest and has been estimated to be 200 billion annually (Vimala Devi *et al.*, 2012)[19]. More than 60 per cent of pesticides are used in agriculture sector for crop protection among which insecticides share larger percentage compared to fungicides, bactericides and herbicides (Wahab, 2009)[20]. Since last 25 years, chemical pesticides have become less attractive for numerous reasons including increased cost, the development of pesticide resistance, resurgence, environment and human health hazards and deleterious effects upon non-target organisms (Reddy *et al.,* 2013)[13]. Hence, the need for sustainable and eco-friendly pest management practices is strongly felt and has led to search for eco-friendly pest management strategies like use of biological control method as a best component for integrated pest management (IPM) in order to minimize the indiscriminate and injudicious use of chemical pesticides. Among which microbial pesticides are considered to be a promising alternative or best substitute to chemical pesticides of which fungus is a first organism to cause diseases in insects and are most important in insect pest management (Thomas and Read, 2007; Fan *et al.,* 2007)[18,9].

Entomopathogenic fungi are the most versatile biological control agents and are host specific with a low risk of attacking non-target organisms or beneficial insects (Roberts and Humber, 1981)[15]. Due to their biodegradable nature, they do not cause any problem of residue and contaminate aquatic ecosystem. Fungal Biocontrol agents are promising because of their virulence is caused by penetration of fungal hyphae through body wall (Nadeau *et al*., 1996)[12]. Presently there are approximately 750 species of entomopathogenic fungi from about 90 genera that have been documented to be pathogenic to insect pest (Hajek and Leger, 1994)[10]. However, a few of these species *viz., Beauveria, Metarhizium, Lecanicillium, Hirsutella, Nomuraea, Isaria,* etc. are commercially using that cause lethal infection to insects of various orders. Among these species *Lecanicillium lecanii* has been commercialized as an insect pathogen to sucking insects. However, they exhibit high level of variation among the isolates with respect to pathogenicity, virulence and viability and environmental conditions. Therefore, the identification of local isolates gained most important.

2. Materials and Methods:

2.1 Collection and isolation of *L. lecanii*

A roving survey was conducted at different villages of Belagavi districts of Karnataka state during the period of August 2015 to February 2016 covering major area of vegetable crops. During the survey, soil samples from vegetable fields and naturally infested insect specimens were collected and brought to the laboratory of Entomology KRC College of Horticulture Arabhavi and isolated the *L. lecanii*. A total of 30 places included six taluks of Belagavi district and in each taluk five villages and one field for each village were selected for soil sampling and collection of insect samples.

2.2 Isolation of *L. lecanii* through Serial dilution (soil samples)

About 0.2 g of soil sample was placed in a 1.5 ml micro centrifuge tube with 1.3 ml of 0.02% Tween-80 solution and vortexes for 15 minutes. The resulting suspension was serially diluted (10^{-9}) and plated on selective medium. After incubation for 6 days at 25ºC, the putative entomopathogenic fungi were selected by morphological characteristics (aspects of the colonies, such as color, diameter and mycelia texture) (Shin *et al.,* 2010)[17]

2.3 Isolation of *L. lecanii* from surface sterilized insect samples

The symptomatic insect specimens for mycoses were brought to the laboratory from various vegetable growing fields. Upon death or mycosis the specimens were surface sterilized using one per cent sodium hypochlorite, followed by 70 per cent alcohol and three repeated changes of sterile distilled water. They were then inoculated on selective media and kept under incubation for one week under room temperature. On development of fungal mycelia they were transferred to fresh media for further growth. The pure cultures thus developed were stored under refrigeration for further study (Reji Rani *et al*., 2015)[14].

2.4 Rearing of *M. persicae*

Cow pea plants were grown in earthen pots by sowing cow pea seeds. All the potted plants were kept in net house conditions. The wild populations of *M. persicae* were collected from the field of Kittur Rani Channamma College of Horticulture Arabhavi. When plants were 15-20 days old, the field collected *M. persiace* were released on them. The culture of *M. persicae* was maintained until all the experiments were completed. The apterous adults were taken from the culture, as and when required. All the experiments were conducted in the Biocontrol Laboratory Department of Entomology KRC College of Horticulture, Arabhavi University of Horticultural Sciences Bagalkot Karnataka state.

2.5 Bioassay study

To determine the bio-efficacy and their median lethal concentration value for isolated *L. lecanii*, a bioassay test was conducted by modifying previously reported bioassay procedures (Asi *et al.,* 2009; Diaz *et al.,* 2009)[4,7]. A preliminary experiment was run in order to decide the final concentrations for the bioassay.

Serial dilutions were prepared in 1000 ml distilled water for each formulation ($0.0625X10^9$, $0.125X10^9$, $0.25x10^9$, $0.5x10^9$, $1x10^9$ and $2x10^9$ spores/1000 ml D.W). The efficacy of *L. lecanii* against *M. persicae* was determined by using the detached leaf method. A 2.0 ml from each concentration were smeared on the detached fresh cow pea leaf with cotton wrapped to its petiole and later it was shade dried and placed on petridish containing a thin layer of water agar which is a non nutritive just to maintain moisture. A batch of 10 laboratory reared *M. persicae* nymphs were released to each Petri dish and were covered with the double layered muslin cloth. The petridishes were maintained at room temperature 27.0 ± 1.0 ºC and the relative humidity of 70.0 ± 5.0% RH. Another group of 10 *M. persiace* was released on the leaf smeared with double distilled water and maintained under the above mentioned conditions, served as control. Each treatment was replicated thrice with a 10 aphids/replication. Mortality was counted after two, four, six and eight days of treatment (DAT). Moribund aphids were counted as dead.

2.6 Statistical analysis

The per cent mortality in control was corrected by Abbott's formula (Abbott, 1925)[1]. The LC_{50} values were determined using probit analysis (Finney, 1971)[9] based computer programme STPR718 at the Computer center, KRC College of Horticulture, Arabhavi.

3. Results and Discussion

Thirty soil samples and 30 insect specimens were collected from six taluks (five villages in each taluk) of Belagavi district (Karnataka, India). A total of seven isolates (23.33 per cent) of *L. lecanii* from 30 soil samples based on their morphological and colony characteristics and one isolate (3.33 per cent) from 30 insect specimens were obtained. The occurrence of *L. lecanii* is more in Belagavi district of Karnataka state (India) compared the UK soils of both cultivated and uncultivated habitat recorded only about 0.8 per cent of *L. lecanii* as per report of Chandler *et al*. (1997)[6]. Similarly, Asensio *et al.* (2003)[2] also reported the occurrence of 4.8 per cent *L. lecanii* in South East Spain. Further, to confirm the entomopathogenic fungi, *L. lecanii* a pathogenicity test against *M. persicae* was undertaken in the laboratory conditions. Out of eight isolates screened for their pathogenicity test based on morphology and colony structure, only three isolates (from soil samples coded as R4HAT, R8GAR and from insect sample R7BGBD) yielded positive results causing death followed by development of mycelia growth on cadaver (Table 1; Plate 1). Later they were got identified with help of Agharakar Research Institute, Pune, Maharashtra, India. All the three isolates (R7BGBD, R8GAR and R4HAT) obtained from the pathogenicity test were screened for their virulence against *M. persicae* under laboratory conditions.

3.1 Mortality response of *M. persicae*

The mortality of *M. persicae* significantly increased at two, four, six and eight days after treatment in all the three isolates of *L. lecanii* (R7BGBD, R8GAR & R4HAT). The per cent mortality was found at highest concentration of 2 X 10^9 cfu/1000 ml after eight days of post treatment compared to other concentrations. While the lowest per cent mortality of *M. persicae* was found at 0.0625 X 10^9 cfu/1000 ml at two, four, six and eight days after treatment. No significant difference was observed with respect to mortality between the concentrations of 0.25 X 10^9 and 0.125 X 10^9 cfu/1000 ml at different intervals of time in all the local isolates of *L. lecanii*. Similarly, no appreciable mortality of *M. persicae* was noticed in the lower concentration (0.0625 X 10^9 cfu/1000 ml) of *L. lecanii* and it was *on par* with the control treatment at two and four days after treatment in all the isolates of *L.*

lecanii. Mortality of *M. persicae* in control treatment was noticed after six and eight days after treatment (DAT). However, it was significantly lowest compared to all other concentrations of *L. lecanii*. Further, the growth of *L. lecanii* on dead *M. persicae* was not noticed in the control treatment (Table 2; Fig. 1 and 2). The earlier reports by Yokomi and Gottwald (1988) [21] that they studied virulence of three *L. lecanii* isolates (VL4, VL6, and VL10) against *M. persicae*, *Aphis gossypii* and *A. citricola*. The virulence of VL10 and VL6 was high in *M. persicae* and *A. gossypii,* whereas VL4 virulence was low in all the three species. The concentrations of 10^6–10^7 conidia per ml showed 100 per cent mortality after four days post treatment. Another study was conducted by Li Guoxia Du Jiawei (2000)[11] on effect of four isolates of *V. lecanii* against *M. persicae* showed cent per cent mortality by three isolates after six, seven and eight days of treatment (5 x 10^6 conidia/ ml). Ashouri *et al.* (2004)[3] studied the effect of *L. lecanii* against *M. persicae* at six different concentrations (10^4, 10^5, 10^6, 10^7 and 10^8 conidia/ml) and 100 per cent mortality was noticed at 10^7 and 10^8 conidia/ml at 12th day of treatment. The present study reports that the 100 per cent mortality of test insect, *M. persicae* was recorded within 8 days of post treatment. The isolate (R7BGBD) was more virulent than the isolates of Ashouri *et al.* (2004)[3] collections. However, it was less virulent than the collections of Li Guoxia Du Jiawei (2000) [11].

3.2 Concentration – Mortality response (LC_{50})

The isolate, R7BGBD was the most virulent strain of *L. lecanii* at six and eight days after treatment. The LC_{50} values being 0.54 X 10^9 and 0.15 X 10^9 cfu/1000 ml after six and eight days after treatment, respectively. The isolate R4HAT was the least virulent strain at both six and eight days after treatment (LC_{50} values: 1.91 X 10^9 and 1.80 X 10^9 cfu/1000 ml at six and eight DAT, respectively). The order of virulence of these three isolates of *L. lecanii* at LC_{50} was: R7BGBD> R8GAR> R4HAT (Table 3 and 4; Fig. 3). A bioassay study of *V. lecanii* was conducted against *Aphis craccivora* with six different concentration *viz.,* 10^8, 10^7, 10^6, 10^5, 10^4 and 10^3 by Saranya *et al.* (2010)[16]. The results revealed that, the LC_{50} value of 2.5 x 10^4 spores per ml was recorded by *V. lecanii.* Similarly, Bouhous and Larous (2012)[5] conducted a study to evaluate the efficacy of *V. lecanii* against whitefly. The LC_{50} values for larval and egg stage were 0.5 x 10^3 and 0.59 x 10^7 spores per ml, respectively.

Table 1: Pathogenicity test of indigenous isolates of *L. lecanii* against *M. persicae* under laboratory

SL. NO.	Isolates	Infectivity	Time taken to kill test insect	Time take for mycelia growth over cadavers
1.	R1GKCB	-Ve	-	-
2.	R2HBBB	-Ve	-	-
3.	R3HBBB	-Ve	-	-
4.	R4HAT	+Ve	2 days	7 days
5.	R5CHC	-Ve	-	-
6.	R6BBC	-Ve	-	-
7.	R7BGBD	+Ve	1 day	6 days
8.	R8GAR	+Ve	1 day	6 days

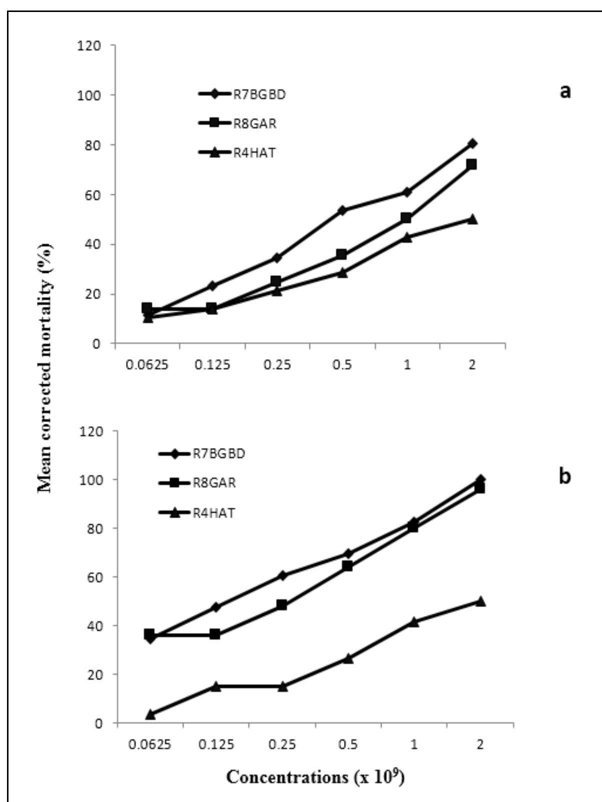


Fig 1: Concentration - corrected mortality response of *M. persicae* to R8GAR, R7BGBD and R4HATat (a) six and (b) eight days after treatment

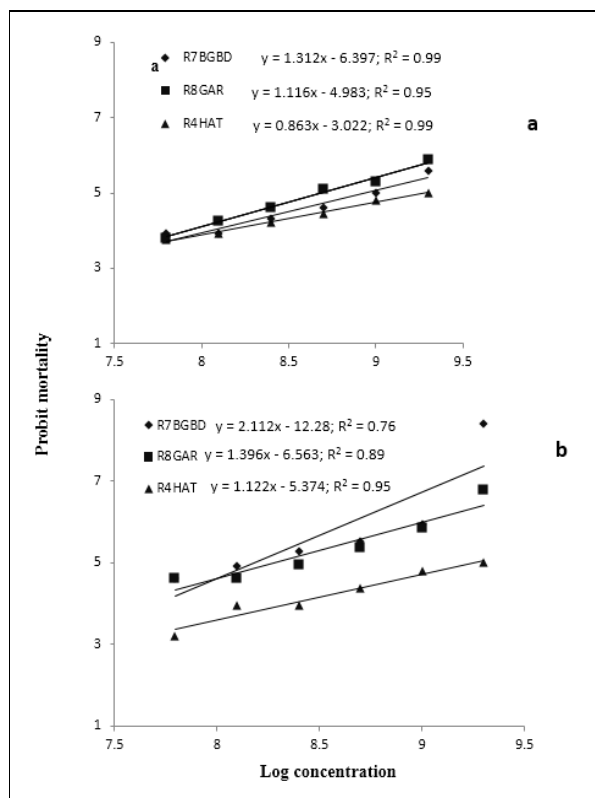


Fig 2: Log Concentration - Probit mortality response of *M. persicae* to R8GAR, R7BGBD and R4HAT at (a) 6 and (b) 8 days after treatment

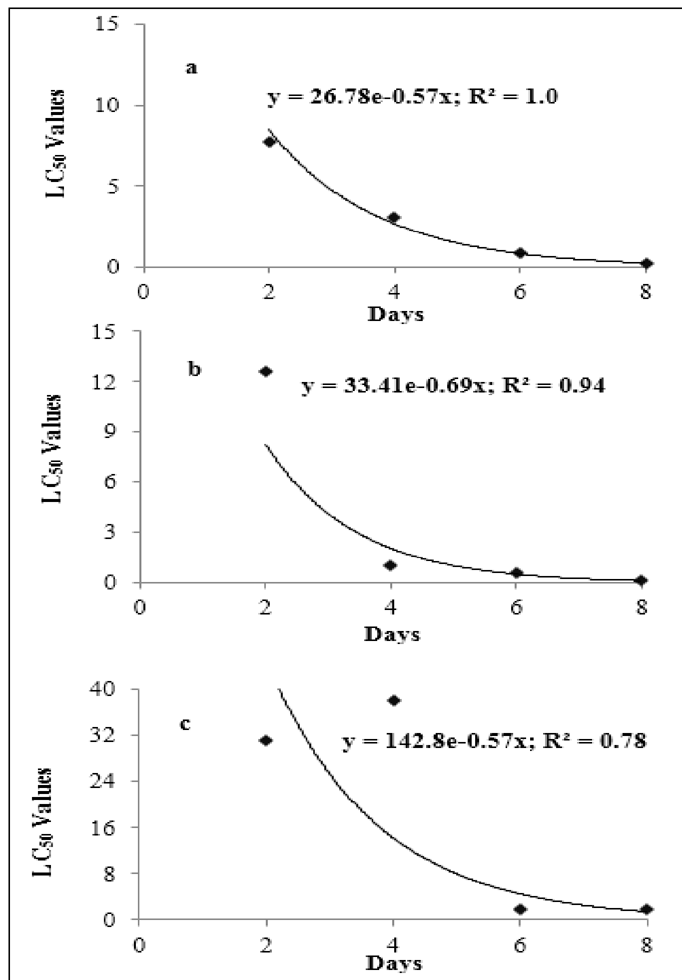
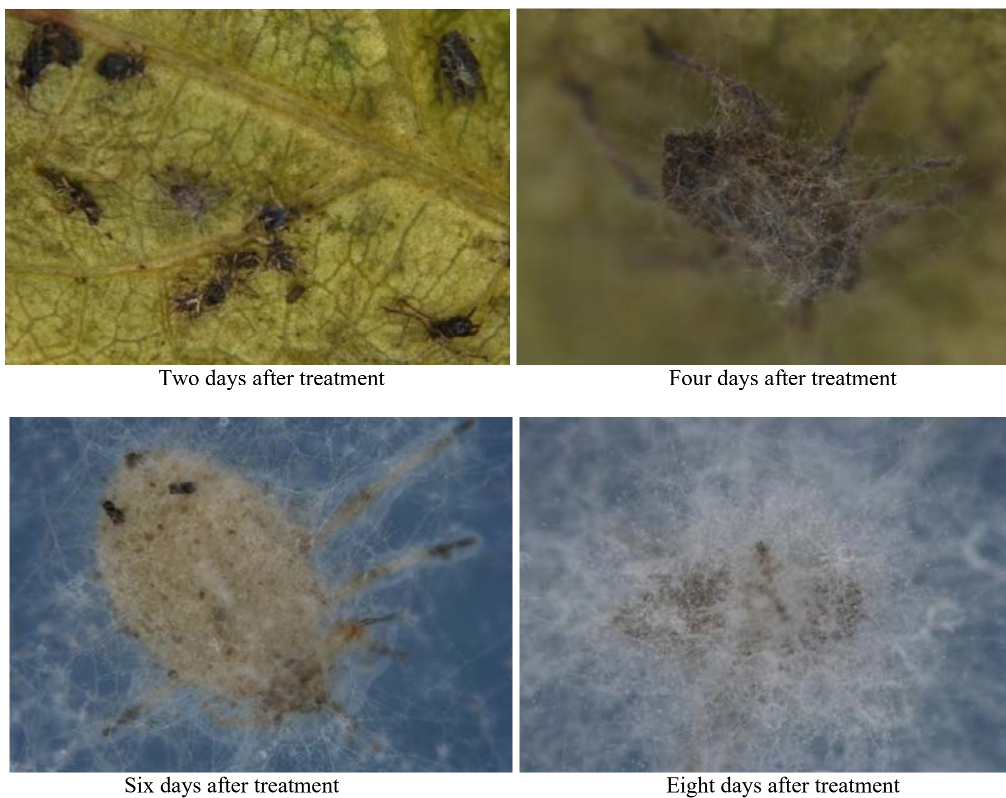


Fig 3: Trends in LC_{50} values for (a) R8GAR, (b) R7BGBD and (c) R4HAT against *M. persicae* across different days after treatments



Two days after treatment

Four days after treatment

Six days after treatment

Eight days after treatment

Plate 1: Development of *L. lecanii* on *M. persicae* over a period of time

Table 2: Bio-efficacy of local isolates of *L. lecanii* against *M. persicae*

Treatments	Concentrations (Spores/1000 ml)	Mortality (%)											
		2 DAT			4 DAT			6 DAT			8 DAT		
		R7BGBD	R8GAR	R4HAT	R7BGBD	R8GAR	R4HAT	R7BGBD	R8GAR	R4HAT	R7BGBD	R8GAR	R4HAT
T1	2 X 10^9	16.67 (23.86)a	16.67 (23.86)a	10.00 (18.43)a	53.33 (46.92)a	40.00 (39.15)a	30.00 (33.00)a	83.33 (66.14)a	73.33 (59.00)a	53.33 (46.92)a	100.00 (90.00)a	96.67 (83.86)a	56.67 (48.85)a
T2	1 X 10^9	10.00 (18.43)a	10.00 (18.43)ab	10.00 (18.43)a	36.67 (37.22)ab	26.67 (31.00)ab	16.67 (23.86)b	66.67 (54.78)b	53.33 (46.92)b	46.67 (43.08)ab	86.67 (68.86)b	83.33 (66.14)b	50.00 (45.00)a
T3	0.5 X 10^9	10.00 (18.43)a	6.67 (8.86)bc	6.67 (12.29)ab	26.67 (31.00)bc	26.67 (31.00)ab	16.67 (23.86)b	60.00 (50.77)b	40.00 (39.23)bc	33.33 (35.22)bc	76.67 (61.22)c	70.00 (56.79)c	36.67 (37.22)ab
T4	0.25 X 10^9	3.33 (6.14)b	0.00 (0.00)c	3.33 (6.14)bc	13.33 (21.14)cd	10.00 (18.43)bc	10.00 (18.43)b	43.33 (41.15)c	30.00 (33.21)cd	26.67 (31.00)cd	70.00 (56.79)c	56.67 (48.85)cd	26.67 (31.00)bc
T5	0.125 X 10^9	0.00 (0.00)b	0.00 (0.00)c	0.00 (0.00)c	6.67 (12.29)de	6.67 (12.29)c	10.00 (18.43)b	33.33 (35.22)c	20.00 (26.57)d	20.00 (26.57)cd	60.00 (50.77)d	46.67 (43.08)d	26.67 (31.00)bc
T6	0.0625 X 10^9	0.00 (0.00)b	0.00 (0.00)c	0.00 (0.00)c	0.00 (0.00)ef	6.67 (12.29)c	10.00 (18.43)b	23.33 (28.78)d	20.00 (26.57)d	16.67 (23.86)d	50.00 (45.00)e	46.67 (43.08)d	13.33 (21.14)cd
T7	Distilled water	0.00 (0.00)b	0.00 (0.00)c	0.00 (0.00)c	3.33 (6.14)f	6.67 (12.29)c	0.00 (0.00)c	13.33 (21.14)e	6.67 (12.29)e	6.67 (12.29)e	23.33 (28.78)f	16.67 (23.86)e	13.33 (17.71)d
$F_{6,12}$		*	*	*	*	*	*	*	*	*	*	*	*
S. Em±		2.69	3.417	3.144	3.144	2.547	1.95	1.95	2.473	2.59	2.59	2.092	2.98
CD		8.28	10.53	9.69	9.69	7.850	5.99	5.99	7.622	7.97	7.97	6.44	9.18
CV		27.87	46.00	39.396	39.396	11.283	9.90	9.90	7.03	8.19	8.19	3.96	8.90

Figures in parenthesis are angular transformed values/ Arc sin transformed value
* F-test is significant at 5 per cent probability, ** F-test significant at 1 per cent probability
The values in the column following same alphabet letters are not significantly different from each other
DAT - Days after treatment

Table 3: Concentration – mortality response of local isolates of *L. lecanii* against *M. persicae* at six days after treatment

Isolates	Regression equation (Y=a+bx)	LC_{50} (cfu/1000 ml)	Fiducial limits		LC_{99} (cfu/1000 ml)	Fiducial limits		X^2
			Lower	Upper		Lower	Upper	
R8GAR	Y = - 4.98+1.116x	0.87 x10^9	0.54 X 10^9	1.40X10^9	98.63 x 10^9	13.29X10^9	731.97X10^9	1.57;p>0.05
R7BGBD	Y = - 6.39+1.31x	0.54 x10^9	0.38X10^9	0.76X10^9	24.53 x 10^9	6.75X10^9	89.11X10^9	0.602;p>0.05
R4HAT	Y = - 3.022+0.863x	1.91 x10^9	0.81X10^9	4.57X10^9	9708.76 x 10^9	1.49X10^9	6315634.0X10^9	0.20;p>0.05

X^2 – Chi square

Table 4: Concentration – mortality response of local isolates of *L. lecanii* against *M. persicae* at eight days after treatment

Isolates	Regression equation (Y = a+bx)	LC_{50} (cfu/1000 ml)	Fiducial limits		LC_{99} (cfu/1000 ml)	Fiducial limits		X^2
			Lower	Upper		Lower	Upper	
R8GAR	Y = - 6.563+1.396x	0.25 x10^9	0.17 X 10^9	0.35 X 10^9	13.64X10^9	3.69X10^9	50.39X10^9	4.56; p>0.05
R7BGBD	Y = - 12.28+2.112x	0.15 x10^9	0.02 X 10^9	0.39 X 10^9	215.64x10^9	0.65X10^9	71996.64X10^9	8.91; p>0.05
R4HAT	Y = - 3.937+0.960x	1.80 x10^9	0.89 X 10^9	3.64 X 10^9	282.26X10^9	19.06X10^9	4178.61X10^9	1.28; p>0.05

4. Conclusion
Generally the entomopathogenic fungi exhibit high level of variation among the isolates with respect to pathogenicity, virulence and viability. Therefore, the identification of regional or local isolates is of utmost important. The results of the present findings also showed that all the three isolates were able to cause disease and brought the mortality at different intervals of time. However, these isolates of *L. lecanii* performed better under laboratory conditions and need to be evaluated under field conditions for their promotion against sucking insect pests of various Horticulture and Agricultural crops.

5. Acknowledgement
The authors are grateful to the Department of Science & Technology (DST), Science for Equity, Empowerment and Development Division (SEED Division), New Delhi for financial assistance under young scientist and technologist scheme to conduct this study.

6. References
1. Abbott WS. A method for computing the effectiveness of an insecticide. Journal of Economic Entomology. 1925; 18:265-267.
2. Asensio L, Carbonell T, Lopez-Jimenez JA, Lopez-Lorca LV. Entomopathogenic fungi in soils alicante province. Spanish Journal of Agricultural Research. 2003; 1(3):37-45.
3. Ashouri A, Arzanian N, Askary H, Rasoulian GR. Pathogenicity of the fungus, *Verticillium lecanii*, to the green peach aphid, *Myzus persicae* (Hom.: Aphididae). Communication Agriculture and Applied Biological Science. 2004; 69(3):205-209.
4. Asi MR, Bashir MH, Mirza JH, Afzal M, Imran S. *In vitro* efficacy of entomopathogenic fungi against cabbage aphid, *Brevicoryne Brassicae* L. Pakistan Journal of Entomology. 2009; 31(1):43-47
5. Bouhous M, Larous L. Efficiency of the entomopathogenic fungus *Verticillium lecanii* in the biological control of *Trialeurodes vaporariorum,* (*Homoptera*: *Aleyrodidae*), a greenhouse culture pest. African Journal of Microbiology Research. 2012; 6(10):2435-2442.
6. Chandler D, Hay D, Reid AP. Sampling and occurrence of entomopathogenic fungi and nematodes in UK soils. Applied Soil Ecology. 1997; 5:133-141.
7. Diaz BM, Oggerin M, Lastra, CCL, Rubio V, Fereres A. Characterization and virulence of *Lecanicillium lecanii* against different aphid species. Biocontrol, 2009. DOI: 10.1007/s1052600992189, http://dx.doi.org/10.1007/s1052600992189.
8. Fan Y, Fang W, Guo S, Pei X, Zhang Y, Xiao Y *et al.* Increased insect virulance in *Beauveria bassiana* strains over expressing an engineered chitinase. Applied and Environmental Microbiology. 2007; 73:295-302.
9. Finney JC. Probit analysis. Cambridge University Press, London, 1971, 333.
10. Hajek AE, St. Leger R. Interaction between fungal pathogens and insect hosts. Annual Review of Entomology. 1994; 39:293-322.
11. Li Guoxia, Du Jiawei. Pathogenicity and reproduction effect of four isolates of *Verticillium lecanii* on the aphid, *Myzus persicae*. www.cnki.com.cn, 2000.
12. Nadeau MP, Dunphy GB, Boisvert J. Developement of *Erynia conica* (Zygomycetes: Entomopathorales) on the cuticle of the adult black flies *Simulium rostratum* and *Simulium decorum* (Diptera : Simuliidae). Journal of Invertebrate Pathology. 1996; 68:50-58.
13. Reddy KRK, Praveen Kumar D, Reddy KRN. Entomopathogenic fungi: a potential bioinsecticide. Kavaka. 2013; 41:23-32.
14. Reji Rani OP, Shifa BS, Soni KB, Sudharma K. Isolation and screeing of indigenous entomopathogenic fungi aganist sucking pests of vegetables. International Journal of Applied and Pure Science and Agriculture. 2015; 1:9-17.
15. Roberts DW, Humber RA. Entomogenous fungi. In: Cole GT, Kendrick B, (Eds.), Biology of Conidial Fungi. Academic Press, New York, 1981, 201-236.
16. Saranya S, Ushakumari R, Sosamma J, Babu MP. Efficacy of different entomopathogenic fungi against cowpea aphid, *Aphis craccivora* (Koch). *Journal of Biopesticides*. 2010; 3(1):138-142.
17. Shin T, Choi J, Bae S, Koo H, Woo S. Study on selective media for isolation of entomopathogenic fungi. International Journal of Industrial Entomology. 2010; 20(1):7-12.
18. Thomas MB, Read AF. Can fungal biopesticides control malaria? Nature Review Microbiology. 2007; 5:377-383.
19. Vimala Devi PS, Ranga rao GV, Gopalakrishnan S, Shivakumar G. Environmental impact of microbial pesticides. ICRISAT, http://studiumpress.in/indetail.asp?id=197; 2012
20. Wahab S. Biotechnological approaches in the management of plant pests, diseases and weeds for Sustainable Agriculture. Journal of Biopesticides. 2009; 2(2):115-134.
21. Yokomi R, Gottwald TR. Virulence of *Verticillium lecanii* isolates in aphids determined by detached-leaf bioassay. Journal of Invertebrate Pathology. 1988; 51(3):250-258.