Pakistan J. Zool., vol. 52(1), pp 131-137, 2020. **DOI**: https://dx.doi.org/10.17582/journal.pjz/2020.52.1.1.131.137

Potential of *Lecanicillium lecanii* (Zimm.) as a Microbial Control Agent for Green Peach Aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae)

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ABSTRACT

The utilization of entomopathogenic fungi as potential microbial control against different insect pests is important due to their eco-friendly nature. The virulence potential of 3 different isolates of *Lecanicillium lecanii* formerly recognized as *Verticillium lecanii* (V-2, V-3 and V-5) with different bioassay materials (conidia, filtrate and the binary combination of conidia + filtrate) were evaluated against green peach aphid (*Myzus persicae*) under control condition. Conidial bioassay of fungal isolates were evaluated with three different concentrations (1×10^6, 1×10^7 and 1×10^8 conidia/ml), filtrate bioassay was estimated by applying 7th day fungal filtrate and binary combination (1ml conidia +1ml filtrate) of V2×V2, V3×V3 and V5×V5 were tested against *M. persicae*. Mortality data were recorded after 2, 4, 6 and 8 days post-treatment. In conidial bioassay, V-3 showed maximum aphid mortality (95%) and V-5 showed the lowest mortality (70%) at the highest concentration (1×10^8 conidia/ml) at 8th-day post-treatment whereas, in filtrate bioassay, 98% mortality was achieved in V-3, and recorded mortality up-to 74% in V-5. Similarly, in the binary combination of fungal conidia 1×10^8 conidia per ml and its filtrate, combination (V-3×V-3) revealed the highest *M. persicae* mortality 91%, while combinations, V-2×V-2 and V-5×V-5 resulted in lowest *M. persicae* mortality 79% and 65%, respectively. The results revealed that in all types of bioassays, V-3 isolate was the most virulent and its filtrate application was found to be the most effective against *M. persicae*.

Article Information
Received 18 December 2018
Revised 11 March 2019
Accepted 20 April 2019
Available online 10 October 2019

Authors' Contribution
AH and DQ Conceived and designed the experiment. AH and TN Conducted the experiment. AH and TN analyzed the data. AH and AB drafted the manuscript.

Key words
Entomopathogenic fungi, *Lecanicillium lecanii*, Virulence, *Myzus persicae*, Bioassay.

INTRODUCTION

The green peach aphids, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) is an important pest of many economical agricultural crops. Aphids destroy crops by direct feeding as well as by transmitting various plant viruses (Diaz *et al*., 2009). *M. persicae* is a cosmopolitan and polyphagous pest which infests particularly cruciferous vegetables in China (Ye *et al*., 2005). Microbial pesticides especially entomopathogenic fungal pesticides have key importance in integrated pest management (IPM) because these organisms cause diseases in insects and suppress their growth and rate of multiplication (Fan *et al*., 2007; Thomas and Read, 2007).

For the control of different aphid species, various entomopathogenic fungi such as *Isaria fumosorosea, Lecanicillium* spp., *Metarhizium anisopliae* and *Beauveria bassiana* have been commercially used (Mohammed *et al*., 2018). *L. lecanii* formerly recognized as *Verticillium lecanii* (Gams and Zare, 2001) is a unique entomopathogenic fungus (EPF), isolated from many diseased insect pests i.e. aphids, whiteflies, scales (De Faria and Wraight, 2007) also found to cause infection in plant parasitic nematodes. *V. lecanii* as bio-insecticide has been used to control black bean aphid *Aphis fabae* (Hemiptera: Aphididae) under control conditions (Saruhan, 2018). The entomopathogenic fungi have been suggested as a major role in the development of new bioactive agents for the control of plant pathogen and insect pests (Isaka *et al*., 2005; Lozano-Tovar *et al*., 2013; Shin *et al*., 2017).

Petch (1925) first time exploited *V. lecanii* for the control of green scale, *Coccus viridis* (Alavo, 2015). Molecular analysis of internal transcribed spacer (ITS) sequences and morphological characteristics of genus *Lecanicillium* showed that *V. lecanii* complex contains 5 clades. These clades are *L. lecanii*, *L. attenuatum, L. muscarium, L. nodulosum* and *L. longisporum*. *L. lecanii* are specifically more effective against soft scale insects, *L. muscarium* against whitefly and thrips and *L. longisporum* against aphids, whitefly and thrips (Gams and Zare, 2001; De Faria and Wraight, 2007; Goettel *et al*., 2008). *L. longisporum* and *L. lecanii* have special traits like hosts

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0030-9923/2020/0131-0001 $ 9.00/0

specificity, long shelf life, a specific mode of action and non-toxicity to the environment (Panahi and Loni, 2013). Conidia of this fungus are produced asexually and become the basis of infection in insect pests of crops. Infection through conidia starts when they are attached to the host cuticle, germinate following the activation of enzymatic reaction and invaded in the body of the insect by germ tube, appressoria and penetration pegs (Samson *et al*., 1988).

Abiotic parameters like temperature, humidity and sunlight are very specific for the conidial based application (Burges 1998; Ownley *et al*., 2004). These factors alter the germination of conidia and the slow germination of conidia effects the total market-percentage of conidial based fungal pesticides (St Leger *et al*., 2009). To combat this problem, the use of fungal liquid broth is the alternate method to enhance the efficacy of a fungus (Kim *et al*., 2010). The EPF secreted bioactive compounds in liquid broth culture that has antifeedant activity and can be purified and used as insecticides like abamectin and spinosad (Copping and Menn, 2000; Godfrey *et al*., 2005; Quesada-Moraga *et al*., 2006). Refined culture filtrates of entomopathogenic fungi, *L. lecanii* and *B. bassiana* decreased the reproductive rate of aphids (Kim *et al*., 2010; Khan *et al*., 2012) and prevent feeding by the larva of *Spodoptera littoralis* and *Bemisia tabaci* (Quesada-Moraga *et al*., 2006; Wang *et al*., 2007). Increased in fungus concentration decreased the number of adult parasitoid and also negatively effects its developmental stages (Fazeli-Dinan *et al*., 2016). Filtrate culture contains many enzymes like chitinases, lipases and proteases and these enzyme helps in the infection process by degrading the cuticle of insects. The concentration of enzyme can be enhanced by the use of different additives in the culture media like colloidal chitin for chitinase production (Kim *et al*., 2010).

In the present study, three isolates of entomopathogenic fungus, *L. lecanii* (V-2, V-3 and V-5) with different application materials (conidia, filtrate and binary combination of conidia + filtrate) were used for mortality of green peach aphid, *M. persicae* to determine the most virulent isolate of *L. lecanii*, most appropriate application material and combined effectiveness of fungal conidia with fungal filtrate.

MATERIALS AND METHODS

Aphid stock

M. persicae were obtained from the insectary of the Beijing Academy of Agriculture and Forestry Sciences (BAAFS) Beijing, China. The *M. persicae* populations were maintained in incubator on potted broad bean, *Vicia faba* plants in cages at temperature 27±2°C, 55-60% R.H., photoperiod (light: dark) of 16:8 h and plants were replaced every week in State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China.

Fungal isolates

Three isolates of *Lecanicillium lecanii*, namely V-2, V-3 and V-5 were cultured on potato dextrose agar (Agar 20g/L, Dextrose 20g/L, and Potatoes 200g/L) plates and incubated at 27±2°C in dark for 20 days.

Conidial suspension

The conidia were harvested from twenty days of fungal culture plates using distilled water along with 0.02% Tween 80®. The conidial suspensions were collected and filtered through cheesecloth. Conidial concentrations were estimated 1×10^6, 1×10^7 and 1×10^8 conidia per ml by using hemocytometer. Conidial viability was tested before doing bioassay according to the methods described by Hywel-Jones and Gillespie (1990).

Fungal filtrate

Potato dextrose broth (PDB) (Potatoes 200g/L, Dextrose 20g/L) was used for the production of fungal filtrate. First primary culture was prepared by adding 6 ml conidia, harvested from 20 days PDA plates and added to 100 ml of PDB. Primary culture was incubated in dark for 4 days at 160 rpm at 26°C temperature. The secondary culture was prepared by adding 10ml of primary culture into 500ml PDB and incubated in dark on a rotatory shaker for 7 days at 160 rpm and 26°C temperature. For the collection of filtrate secondary culture was centrifuged at 12000 rpm at 4°C for 30 min. The supernatant after centrifugation was filtered through 0.45 µm-pore-size filter (Millipore Corp) to get the filtrate.

Bioassays

To check the efficacy of conidia, filtrate and binary combinations, bioassays were performed under the controlled laboratory conditions. For the bioassay study of binary combination (filtrate + conidia), the uppermost concentration 1×10^8 conidia/ml of each isolate was mixed with the 1ml filtrate of the same isolate. The combinations were V2×V2, V3×V3 and V5×V5. All treatments were performed on 90-mm Petri dishes containing a thin layer of 1.5% agar (for maintaining moisture for leaf), and a 60mm detached leaf disk of Chinese cabbage was placed in the plate. Ten apterous adult *M. persicae* were released on a leaf of each plate and a 2ml sample of each treatment was sprayed on the *M. persicae* by using a small sprinkler. All the treatments were placed in a chamber at 25±2°C temperature and 70.0±5.0% R.H. for 8 days using

10 replications, for each bioassay one control was used. Mortality data were recorded after 2, 4, 6 and 8 days post-treatment.

Data analysis

The virulence activity of different isolates of *L. lecanii* as conidia, filtrate and binary combination (filtrate + conidia) were analyzed by Statistix software (version 8.1) (Tallahassee, FL). Factorial analysis of variances (ANOVA) was used to test the data and the means were separated, using the LSD test. Data were expressed as Means ± SE, statistical significance was set at the conventional $\alpha < 0.05$ level.

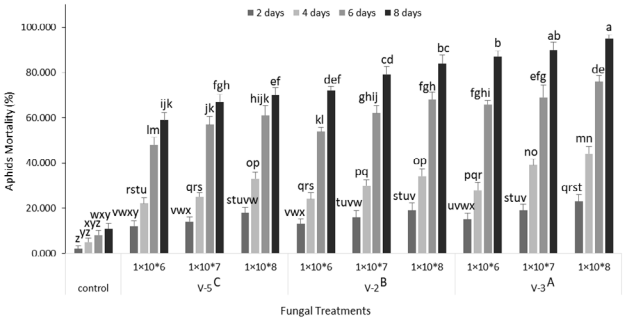

Fig. 1. Percentage mortality of green peach aphid, *Myzus persicae* by conidial application of three different concentrations (1×10^6, 1×10^7 and 1×10^8 conidia/ml) at different time intervals (2nd, 4th, 6th and 8th day) by *Lecanicillium lecanii* isolates (V-2, V-3 and V-5). One control was set for all the three isolates. Error bars indicate ± SE and columns headed by different letters represent significant difference ($P < 0.05$) according to LSD test.

RESULTS

Conidial virulence

In conidial bioassay studies, *L. lecanii* isolates exhibited different levels of *M. persicae* mortality with different conidial concentrations after the time interval of 2, 4, 6 and 8 days post-treatment. In all isolates, the concentration of conidia was directly proportional to the percentage mortality of *M. persicae*. The highest percentage mortality of *M. persicae* was observed with V-3 isolate in uppermost concentration (1×10^8 conidia/ml) on 8th-day post treatment and it was 95% followed by 84% with V-2 and 70% with V-5 isolate (Fig. 1). According to statistical analysis of all isolates of *L. lecanii* there was a substantial influence of different treatment ($F=679.16$; $P < 0.001$*) time ($F=775.30$; $P < 0.001$*) and their interaction ($F=59.41$; $P < 0.001$*) on the mortality of *M. persicae* (Table I). Percentage mortality of *M. persicae* in control treatment was considerably lower (11%) after 8th-day post-treatment, compared to all other concentrations of *L. lecanii*.

Filtrate virulence

In all isolates, filtrate treatments indicated comparatively high virulence than the conidial treatments. Significant influence of different treatments ($F=817.17$; $P < 0.001$*) time ($F=293.69$; $P < 0.001$*) and their interaction ($F=32.98$; $P < 0.001$*) on the mortality of *M. persicae* was observed (Table II). Maximum mortality of *M. persicae* was observed in V-3 isolate (98%) on the 8th day of treatment. The V-2 demonstrated 87% mortality, while V-5 represents 74% mortality. The overall mortality percentage of all isolates of the fungus were higher for filtrate as compared to conidial treatment (Fig. 2).

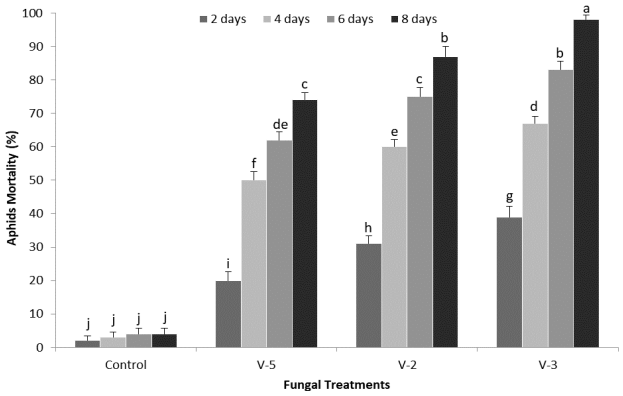

Fig. 2. Percentage mortality of green peach aphid, *Myzus persicae* by filtrate application of *Lecanicillium lecanii* isolates (V-2, V-3, and V-5) after time intervals (2nd, 4th, 6th and 8th day). Error bars indicate ± SE and columns headed by different letters represent significant difference ($P < 0.05$) according to LSD test.

Binary combination (conidia + filtrate) virulence

The application of binary combination (conidia + filtrate) gave significant control of *M. persicae*. Substantial influence of different treatments ($F=48.62$; $P < 0.001$*) time ($F=702.07$; $P < 0.001$*) and their interaction ($F=7.44$; $P < 0.001$*) was observed on the mortality of *M. persicae* (Table III). After 8 days post-treatment V3×V3 combination gave 91% mortality, whereas V2×V2 represent 79% and V5×V5 provided 65% mortality. The result indicates that among these isolates V3×V3 showed the highest percent mortality of *M. persicae* (Fig. 3).

DISCUSSION

The virulence potential of the entomopathogenic fungus, *V. lecanii* isolates for the control of aphids varied among different aphid species and even for same species

(Ferrari *et al*., 2001; Blanford *et al*., 2003). Our study exhibited that not only different isolates have different virulence but the different materials of application (conidia, filtrate and binary combination) of the same isolate also differ the virulence potential. Results indicated that from all the isolates used in this study, the isolate (V-3) is the most

Table I.- Three-way factorial analysis of variance for conidial bioassay of *Lecanicillium lecanii* isolates V-2, V-3 and V-5 against green peach aphid, *Myzus persicae*.

Source	DF	SS	MS	F-value	P-value
Replication	9	1258	139.8		
Fungal	2	8584	4291.9	56.73	< 0.001*
Time	3	175964	58654.7	775.30	< 0.001*
Treatments	3	154144	51381.4	679.16	< 0.001*
Fungal x Time	6	2720	453.3	5.99	< 0.001*
Fungal x Treatment	6	2870	478.3	6.32	< 0.001*
Time x Treatment	9	40451	4494.5	59.41	< 0.001*
Fungal x Time x Treatment	18	1320	73.4	0.97	0.4942
Error	423	32002	75.7		
Total	479	419312			
CV/GM		23.59/36.875			

*$P < 0.001$ (highly significant) and $P < 0.05$ (significant); three-way factorial ANOVA at $\alpha = 0.05$

Table II.- Two-way factorial analysis of variance for filtrate bioassay of *Lecanicillium lecanii* isolates V-2, V-3 and V-5 against green peach aphid, *Myzus persicae*.

Source	DF	SS	MS	F-value	P-value
Replication	9	1535	170.6		
Treatments	3	109773	36590.8	817.17	< 0.001*
Time	3	39452	13150.8	293.69	< 0.001*
Time x Treatments	9	13293	1476.9	32.98	< 0.001*
Error	135	6045	44.8		
Total	159	170098			
CV/GM		14.05 /47.625			

*$P < 0.001$ (highly significant) and $P < 0.05$ (significant); two-way factorial ANOVA at $\alpha = 0.05$.

Table III.- Two-way factorial Analysis of variance for binary bioassays of *Lecanicillium lecanii* isolates V-2, V-3 and V-5 against green peach aphid, *Myzus persicae*.

Source	DF	SS	MS	F-value	P-value
Treatments	2	6124	3062.0	48.62	< 0.001*
Time	4	176860	44214.9	702.07	< 0.001*
Time x Treatments	8	3747	468.3	7.44	< 0.001*
Error	225	14170	63.0		
Total	239	204056			
CV/GM		18.23 /43.533			

*P < 0.001 (highly significant) and $P < 0.05$ (significant); two-way factorial ANOVA at $\alpha = 0.05$

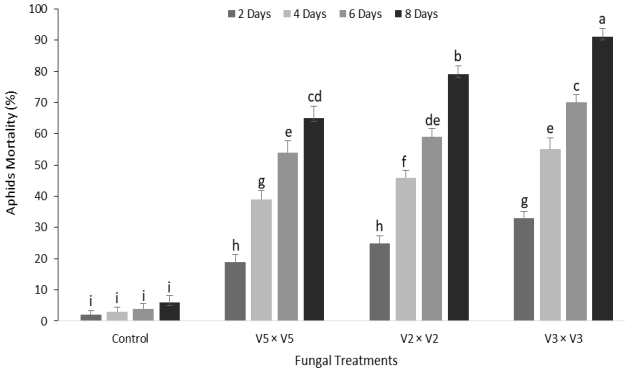

Fig. 3. Percentage mortality of green peach aphid, *Myzus persicae* by binary combination of *Lecanicillium lecanii* isolates V2×V2, V3×V3 and V5×V5 after time intervals (2nd, 4th, 6th and 8th day). Error bars indicate SE ± and columns headed by different letters represent significant difference ($P < 0.05$) according to LSD test.

virulent and its filtrate application found most efficient in controlling *M. persicae*. Filtrate application represented higher mortality (98%) on 8th-day post-treatment. In conidial and combined applications (conidia + filtrate), *M. persicae* mortality was recorded 95% and 91% respectively at the highest conidial concentration. The concentration of conidia was directly proportional to the mortality, so the highest concentration 1×10^8 conidia/ml represented the highest mortality in conidial bioassay experiment. The combined (conidia + filtrate) bioassay indicated lowest aphid mortality, that may be the incompatible effects of conidia with the filtrate. Halimona and Jankevica (2011) demonstrated similar results according to our study for the conidial treatment. They use different concentrations of the entomopathogenic fungus, *B. bassiana* against *Metopeurum fuscoviride* and *A. fabae* and reported that the highest concentration (1×10^8 spores per ml) exhibited the highest percent mortality on seventh 7th-day post-treatment. Liu *et al*. (2002) and Wright *et al*. (2005) also demonstrated that the mortality of aphids increased with the increase of conidial concentration and exposure time. Similarly, Ansari *et al*. (2004) described that the mortality percentage affected by the concentration of conidia, temperature and exposure time. In studies conducted by Akmal *et al*. (2013), the mortality percentage was directly proportional to the concentration of conidia, by using different concentration of *B. bassiana* (1×10^6, 1×10^7 and 1×10^8 conidia/ml) *Rhopalosiphum padi*, *Schizaphis graminu*, *Lipaphis erysimi* and *Brevicoryne brassicae* and found that, all concentrations were effective for the control of aphids but the highest concentration, (1×10^8 conidia/ml), gave the highest percentage mortality.

According to filtrate treatment, our results are similar to Kim *et al*. (2010) which revealed that degradation of insect body was observed higher in filtrate treated aphids and the reduction of aphid population was more in high concentration and high dose filtrate treatment. The maximum efficacy was due to the production of metabolites in the culture filtrate that helps to degrade the cuticle and also deform the hemocoel. As compared to the filtrate, conidia need more time to germinate and release required enzymes for the degradation of the insect body. Bateman and Alves (2000) and Altre and Vandenberg (2001) demonstrated that the use of filtrate application for the control of insect was the best method and also effective for those insects which had short life cycle because these insects have more chances to shed the conidia from their body by molting since germination of conidia needs a specific time. According to their finding, our results were similar to that of filtrate had more ability to control the insect population because they contained toxic enzyme for the degradation of the insect body. Through filtrate optimization, the production of enzymes could be enhanced easily as compared to fungal genetic manipulation. Filtrate production, storage and transportation were more suitable than conidia and it meets the commercialization requirements. Khan *et al*. (2012) demonstrated the virulence potential of six entomopathogenic isolates of two fungi, *V. lecanii* and *B. bassiana* both as conidial and filtrate bioassay. The results of their studies showed that filtrate application gave the highest control of aphid as compared to the conidial application. Their results also demonstrated that the concentration of conidia is directly proportional to the percent mortality of insect and the maximum concentration of conidia gave the maximum mortality.

According to combined application our results are similar to those of Yun *et al*. (2017) who studied the dual behavior of two fungi, *M. anisopliae* (SD3) and *B. bassiana* (SD15) against *B. cinerea* and *M. persicae*, and observed that the use of cultural filtrates with blastospores gave the highest mortality of *M. persicae*. Moreover, the mortality percentage was close to the combination of cultural filtrates with blastospores but the combination of cultural filtrates with aerial conidia gave the lowest mortality as compared with the application of filtrate. So, it is obvious that the filtrate application of the fungus has maximum virulence potential.

CONCLUSION

Entomopathogenic fungi are among the alternative tools beside the chemicals for controlling *M. persicae*. Results of this study indicated that different isolates of *L.*

lecanii have different virulence potential. The different application materials of the same isolate and its dosage also affect its pathogenicity under controlled conditions. Filtrate application is the most suitable material for the control of *M. persicae*. Binary combination of fungal conidia with its fungal filtrate has some incompatible effect and cannot be used effectively for the control of *M. persicae*. So, further studies are required to evaluate the efficacy and compatibility of tested isolates of *L. lecanii* against *M. persicae* under field conditions.

ACKNOWLEDGMENT

This study was supported by the National Key Research and Development Program of China (2017YFD0200900). The authors would like to thank the Graduate School of the Chinese Academy of Agricultural Sciences (GSCAAS) for providing a Ph.D. fellowship for this study.

Conflict of interest statement

The authors declare no competing interests.

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