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CHITINASE ENZYME ACTIVITY AND PATHOGENICITY OF *LECANICILLIUM LECANII* AGAINST MUSTARD APHID *LIPAPHIS ERYSIMI* (KALT.)

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ABSTRACT

***Lecanicillium lecanii* (Zimmerman) Zare and Gams is an entomopathogenic fungi used as an alternative biocontrol agent against crop pests. *Lipaphis erysimi* (Kalt.) is an important pest of Brassica family. The present study evaluates the efficacy of three *Lecanicillium lecanii* isolates against the nymphs of *Lipaphis erysimi* (Kalt.). Bioassay revealed nymphal mortality as 66.68, 62.23 and 42.50% of *L. lecanii* NIPHM, *L. lecanii* MTCC 956 and *L. lecanii* MTCC 2056, respectively at maximum concentration (@12 g/ l). Extracellular enzyme activity of fungal isolates when evaluated revealed variation in the level of enzyme secretion. The maximum chitinase and n-acetyl-β-D-hexosaminidase activity (0.48 and 0.41 U/ ml) were observed in *L. lecanii* NIPHM on 6th day of incubation.**

Key words: *Lecanicillium lecanii*, *Lipaphis erysimi*, nymphs, mortality, chitinase, bioassay, biocontrol, Brassica family, mortality, N-acetyl-β-D-hexosaminidase

Lipaphis erysimi (Kalt.) (Hemiptera: Aphididae) commonly known as mustard aphid is one of the important pests causing yield loss in cultivated crops belonging to *Brassica* spp. (Patel, 2017: Sahoo, 2012). Various synthetic insecticides are applied during crop season for its management but high resistance to most of these insecticides and their associated environmental problems have jeopardized their continuous use (Reddy et al., 2013). Management of insect pests using entomopathogenic fungi (EPF) is regarded as an important alternative method for organic cultivation of vegetables (Gurulingappa et al., 2011; Saruhan et al., 2015; Ibrahim et al., 2020). *Lecanicillium lecanii,* is natural enemy of wide range insect pest and has been commercialized as microbial control agent for aphids (Nithya and Rani, 2019; Asi et al., 2009). The insects infected by this fungus develop white mycelial growth over insect body so this fungus is called white halo fungus. (Shinde et al*.,* 2010). The competence of this fungus lies in the fact that it can brawl against insect pest by invading insect crusts through its active penetration and control insects regardless of its feeding habit (Abdelaziz et al., 2018). *L. lecanii* commences infection via conidia attachment to insect's epicuticle by hydrophobic and electrostatic interactions followed by development of an infected structure. The germinating conidia break insect's cuticle via mechanical and enzymatic degradation and subsequently fungi grows in insect haemolymph by utilizing its nutrients and in turn produces toxins and ultimately kills insect (Anderson et al., 2011; Ritika et al., 2019; Singh and Joshi, 2020). Hydrolyzing enzymes such as chitinase, lipase and protease are major determinant of EPF virulence against target pest. Chitin hydrolysis is carried by complex enzyme system which include endochitinase; exochitinase; chitobias and β-n-acetylhexosaminidases (Hexnase) that are responsible for hydrolyzing chitin protein (macromolecule substrate) to simpler micro-nutrients required for fungal growth (Shirai and Toress-Pacheco, 2006; Chavan and Deshpande, 2013). Therefore, the present study aims to identify most virulent *L. lecanii* isolate for management of *L. erysimi* under laboratory conditions and production of chitinase and n-acetyl-β-D-hexosaminidase, which will suggest selecting the most widespread strain for biological control of aphid.

MATERIALS AND METHODS

Adults and nymphs of *L. erysimi* were collected from mustard variety (RLM 619) (*Brassica rapa*). The aphids were cultured on mustard plant in the laboratory for several generations under ambient conditions before the commencement of experiment. The fungal isolates viz *L. lecanii* MTCC 956 and *L. lecanii* MTCC 2056 procured from IMTECH, Chandigarh, India and one isolate *L. lecanii* NIPHM procured from National Institute of Plant Health Management, Hyderabad, India were used in the present study. These three *L. lecanii*

isolates were grown in Potato dextrose agar (PDA) for 21 days at 25± 2ºC temperature and 60% RH. The suspension was prepared by scrapping the harvested conidia in 10 ml distill water containing 0.1% tween-80. This conidia suspension was quantified to 10^8 conidia/ml and inoculated into sterilized rice grains and incubated at 25±2ºC for 21 days. The fully sporulated rice grains were mixed and grinded with talc powder in ratio of 1:2 and dried overnight under aseptic conditions. This talc based formulation of *L. lecanii* was evaluated at different concentrations.

Three *L. lecanii* isolates along with untreated control were evaluated against *L. erysimi.* The mustard leaves were treated with different concentrations of *L. lecanii* talc formulation (8g, 10g and 12g / l) by leaf dip method (Nazir et al., 2019). Wet filter paper disc was placed at the bottom of each petri plate before placing treated leaves. There were ten treatments with three replications each and 20 nymphs/ replication. Sterilized and wet cotton was placed around midrib to protect the leaf from drying and Petri plates were incubated at room temperature (25± 2˚C). Mortality was recorded up to 10 days. Sterilized water without fungal inoculum was used as control. Cuticle degrading enzymes viz., chitinase and hexnase activities of *L. lecanii* isolates were evaluated as described by Nahar et al. (2004) and Rocha-Pino et al. (2011), respectively. Fungal isolates @ 10^8 conidia ml^{-1} were inoculated with minimal media amended with colloidal chitin at 25± 2^0C at 150 rpm in orbital shaker for a week. The enzyme activity was determined from the broth removed at successive days which was centrifuged at 8000 rpm for 25 min for extraction of supernatant. Chitinase activity was measured using colloidal chitin as a substrate. The enzyme activity was determined by measuring absorbance at 520 nm. One unit of chitinase is recorded as the amount of enzyme released 1 mol of n-acetylglucosamine/ min. Hexnase activity was measured using p-nitrophenyl-β-N-acetylglucosamine (2.9mM) as a substrate (Tronsmo and Harman, 1993). Enzyme activity was determined at 400 nm. One unit of hexnase is measured as the amount of enzyme that released 1 mol of p-nitrophenol/ min. One-way ANOVA was used to compare the results by using CPCS1 software. Extracellular enzymatic activities mean were compared by Tukey's post hoc test in SPSS16.00 statistical software. Means with $p < 0.05$ were considered to be significantly different.

RESULTS AND DISCUSSION

The present study was undertaken to investigate effect of different concentrations of *L. lecanii* isolates against nymphs of *L. erysimi*. The lethal effect of fungi due to mycosis, were assessed as cumulative per cent mortality compared to control. The mortality was enhanced with increase in talc formulation concentration from 8 to 12 g/ l (Fig. 1 a, b, c). The results showed that mortality rate of *L. erysimi* increased significantly after 7 days of incubation and mortality depends upon exposure time and concentration. Bioassay revealed that, *L. lecanii* NIPHM recorded maximum mortality (66.68%) which was at par with *L. lecanii* 956 (62.23%) at highest concentration (12g/ l) on 10th day of treatment. However, minimum mortality (33.60%) was recorded in *L. lecanii* 2056 at lowest concentration @ 8g/ l on 10th day of incubation. The variation in mortality among various isolates of *L. lecanii* could be related to ability of fungus to counter the insect immune response (Valero-Jimenez et al., 2014). This variation in mortality could be due to various enzymes and proteins that have been characterized and are essential for virulence. Proteins of Egh16/ Egh16H family have been identified as important virulent factor contributed to penetration processes of fungus (Keppanana et al., 2019). It was reported that gas1 and gas2, are novel genes that have pivot role in appressorium formation and pathogenicity (Cao et al., 2012; Xie et al., 2015; Keppanana et al., 2019). Moreover, this fungus also produces a wide variety of cyclodepsipeptide toxic metabolites to control insect population. These finding were in concordance with Parmar et al. (2008) who reported 77.16%

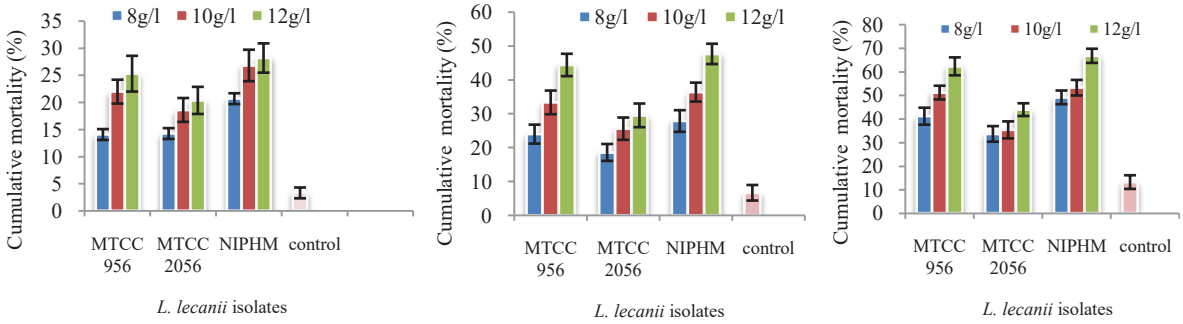

Fig. 1. Cumulative mortality (Mean± SE) of nymphs of *L. erysimi* treated with *L. lecanii* (a) 5DAT, (b) 7DAT, (c) 10 DAT

Table 1. Enzyme activity of *L. lecanii* isolates

Treatments	Chitinase activity on different days of incubation (µg of n-acetylglucosamine produced min/ ml of broth) (Mean± S.D)*							N- acetyl-β-D-Hexosaminidase activity on different days after inoculation (µg of p-nitrophenol produced min/ ml of broth) (Mean± S.D)*						
	1st	2nd	3rd	4th	5th	6th	Mean	1st	2nd	3rd	4th	5th	6th	Mean
L. lecanii MTCC 956	0.10± 0.02^{Fb}	0.29± 0.01^{Eb}	0.33± 0.02^{Db}	0.40± 0.02^{Cb}	0.50± 0.01^{Bb}	0.62± 0.03^{Ab}	0.37^{b}	0.05± 0.00^{Fb}	0.13± 0.01^{Eb}	0.25± 0.01^{Db}	0.34± 0.00^{Cb}	0.49± 0.01^{Bb}	0.52± 0.04^{Ab}	0.30^{b}
L. lecanii MTCC2056	0.05± 0.01^{Fc}	0.09± 0.01^{Ec}	0.13± 0.01^{Dc}	0.28± 0.01^{Cc}	0.34± 0.01^{Bc}	0.47± 0.02^{Ac}	0.23^{c}	0.011± 0.01^{Fc}	0.05± 0.00^{Ec}	0.08± 0.00^{Dc}	0.13± 0.01^{Cc}	0.23± 0.00^{Bc}	0.42± 0.01^{Ac}	0.15^{c}
L. lecanii NIPHM	0.16± 0.01^{Fa}	0.35± 0.02^{Ea}	0.49± 0.01^{Da}	0.55± 0.02^{Ca}	0.60± 0.02^{Ba}	0.75± 0.05^{Aa}	0.48^{a}	0.13± 0.01^{Fa}	0.29± 0.01^{Ea}	0.43± 0.03^{Da}	0.46± 0.01^{Ca}	0.55± 0.01^{Ba}	0.59± 0.00^{Aa}	0.41^{a}
Mean	0.10^{F}	0.24^{E}	0.32^{D}	0.43^{C}	0.51^{B}	0.61^{A}		0.06^{F}	0.16^{E}	0.25^{D}	0.31^{C}	0.42^{B}	0.51^{A}	

*Mean± standard deviation of six replicates; Mean value followed by same letter (a, b, c, d) in horizontal column not significantly different Tukey's post hoc test (p = 0.05); Mean value followed by same letter (A, B, C) in vertical column not significantly different Tukey's post hoc test (p= 0.05)

cumulative mortality against mustard aphid when treated with *L. lecanii*. Also, Ujjan and Shahzad (2012) used different entomopathogenic fungi strains against mustard aphid *L. erysimi* and reported 100% mortality with *L. lecanii* (PDRL), *Beauveria bassiana* (PDRL 118) and *Paecilomyces lilacinus* (PDRL812).

In the present work *L. lecanii* isolates were evaluated for chitinase and β-n-acetyl-hexosaminidase activities, two major groups of enzymes known to be synergistic and consecutive. Chitinase help the fungus in rupturing insect cuticle whereas hexnase facilitate complete degradation of chitin to n-acetylglucosamine requisite for digestion of chitinous material which in turn provided nutrition to fungus (Katta et al., 2013). A significant difference in enzyme activity was recorded when *L. lecanii* was incubated for six days. The results showed that enzyme activity increase from second day but maximum activity was observed on sixth day of incubation (Table 1). Maximum chitinase activity (0.48 Uml^{-1}) and maximum β-n-acetyl-hexosaminidase activity (0.41 Uml^{-1}) was observed in *L. lecanii* NIPHM followed by MTCC 956 and *L. lecanii* MTCC 2056. The present finding is consistent with that of Nguyen et al. (2015), who reported chitinase activity (0.528 Uml^{-1}) in *L. lecanii* culture at 6th day of incubation. Similarly, maximum extracellular enzyme activity of entomopathogenic fungi at 6th and 8th day of incubation are known (Dhawan and Joshi 2017; Mayorga-Reyes et al., 2012; Khan et al., 2012). Suresh et al. (2011) reported maximum hexnase enzyme activity after 5 days of incubation. The present study revealed that *L. lecanii* NIPHM, as pathogenic strain against nymphs of *L. erysimi*, revealed maximum enzyme activity. However, the isolate with minimum enzyme activity was found least effective against *L. erysimi*. Pelizza et al. (2012) reported *B. bassiana* isolates with highest levels of enzyme activity was more virulent against *Tropidacris collaris*. Cuticle degrading enzyme viz., trypsin, subtilisin and chitinase activity was found correlated with virulence (Pelizza et al., 2011; Svedese et al., 2013). Difference in level of enzyme secretion by three *L. lecanii* isolates can be correlated with genetic architecture of the fungi. Many factors directly or indirectly affect enzyme production and virulence. Moreover, other extracellular enzyme viz., protease, lipase, subtilisin may also have role in pathogenicity. So, more research is needed for their field evaluation and studying the complex mechanism of entomopathogenic fungal virulence.

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