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MICROBIOLOGY

Verticillium lecani (Zimm.): A potential entomopathogenic fungus

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Abstract

Development of insect resistance and risk to the environment due to indiscriminate use of conventional chemical pesticides for insect pest management favored the introduction of one of the new alternatives as biological control. Entomopathogenic fungi are one of the most versatile biological control agents for sustainable management. The most considerable fungal species are *Metarhizium spp.*, *Beauveria spp.*, *Nomuraea rileyi*, *Verticillium lecanii* and *Hirsutella spp*. Out of these fungi, *V, lecanii* are opportunistic and widely distributed ascomycete fungi that has the ability to cause mycosis in a number of insects of orders Homoptera, Coleoptera and Lepidoptera. *V. lecanii* are easy to mass produce, store and are effective over a wide range of temperatures and humidity levels. It also provides a rapid kill at optimum doses and the fungus has been recently commercialized as a microbial agent for pest management. It has the additional features to produce extracelullar enzymes, such as chitinases which helps in promoting host colonization. It also shows compatibility with commonly used agrochemicals such as insecticides or fungicides and other biocontrol agents. Because of these numerous advantages, it can be considered as a potential biocontrol agent's in integrated pest management.

Highlights

- *Verticillium lecanii* as a potential entomopathogenic fungus and its mass production
- Host specificity, mode of action , pathogenicity and epizootiology of *V. lecanii*
- Factors influencing the growth and pathogenicity of *V. lecanii*
- Compatibility of *V.lecanii* with other biocontrol agents and agrochemicals

Keywords: *Verticillium lecanii*, entomopathogenic fungi, biocontrol agents, integrated pest management

Tremendous use of conventional chemical pesticides adversely affected the environment and non-target organisms. One of the major challenges in limiting pest population below threshold level is enhanced resistance of insects and pests towards chemical pesticides. The pests mutate and develop resistance to newer molecules. Biological control of insect pests has generated interest among farmers for sustainable management. Due to the side-effects of chemical pesticides, the sustainable crop production through eco-friendly pest management is essentially required in recent scenario. Among the several micro-organisms viz., bacteria, fungi, virus, protozoan's and entomopathogenic nematodes, a few have been systematically studied for their effective beneficial characteristics. The systematic study of these beneficial micro-organisms can lead to gainful exploitation in microbial control programmes (Burges and Hussey,

1998). Entomopathogenic fungi (EPF) can be used as a component of integrated pest management (IPM) of many insect pests. They are often reported as causing high levels of epizootics in nature and are the most versatile biological control agents, and are environmentally safe. An attractive feature of these fungi is that the virulence caused by contact and the action is through penetration (Nadeau *et al.*,1996). These fungi subsume a heterogenous group of over 100 genera with approximately 750 species, notified from different insects. Many of these are proved to be highly potential in pest management. The most considerable fungal species are Metarhizium spp., Beauveria spp., Nomuraea rileyi, Verticillium lecanii and Hirsutella spp.

***Verticillium lecanii* (Zimm.)**

Verticillium lecanii are opportunistic and widely distributed ascomycete fungi of the order Hypocreales. *V. lecanii* (Zimm.) is widely called the "white holo" and it causes mycosis in a number of insects of orders Homoptera, Coleoptera and Lepidoptera. *V. lecanii* attacks a wide range of insects and is grouped into the extremely diverse aggregate species. *V. lecanii* can be maintained on SMY agar at 28°C. Colonies of *V. lecanii* on SMY agar media grow white or pale yellow, cottony or velvety, turning cream in colour with mealy appearance and gradually to purplish pink pigmentation. Mycelium are composed of septate, branching hyphae, hyaline or light coloured, conidiophores erect, septate, simple or branched. Phialides formed either singly or in whorls of 3-4. The species concept, phylogeny and taxonomic status as well as the genetics of *Verticillium spp*. is somewhat unclear. Based on nuclear ribosomal RNA and mtDNA analyses strains isolated from different hosts and various locations show very high levels of polymorphisms (Kouvelis *et al.*, 2004). The genus has recently been taxonomically reviewed using rDNA sequencing to assess diversity within the taxon (Zare *et al.*, 2000; Zare and Gams, 2001). Because of this, the species *V. lecanii* has been divided into a number of new taxonomic entities, including *L. lecanii*, *L. longisporum*, *L. attenuatum*, *L. nodulosum* and *L. muscarium*. The *Verticillium spp*. group includes a number of important species that are used for the control of pests and diseases in agriculture. It is currently used as a bioinsecticide, with a minimum of 15 products being or in the process of being – commercialized worldwide (Goettel *et al.*, 2005; Faria and Wraight, 2007).

Mass production

V. lecanii (Zimm.) are easy to mass produce, store and are effective over a wide range of temperatures and humidity levels. It also provides a rapid kill at optimum doses and the fungus has been recently commercialized as a microbial agent for pest management (Faria and Wraight, 2001). Production of adequate quantities of a good quality inoculum is an essential component of the biocontrol programme. It has been found effective against whiteflies, aphids, thrips and mealybugs in greenhouses and nurseries (Faria and Wraight, 2007). The production of *V. lecanii* can be done by the following methods based on the quantity of the product desired: 1) relatively small quantities of the inoculum for laboratory experimentation and field-testing during the development of mycopesticide and 2) development of a basic production system for large-scale production by following the labour intensive and economically viable methods for relatively small size markets. Development of simple and reliable production system follows the basic multiplication procedures of submerged liquid fermentation for the production of blastospores, which are short lived, and hydrophilic (Romback, 1989) or solid state fermentation (Rousson *et al.*, 1983) for the production of aerial conidia. However, the most viable mass production technologies include making use of a diphasic strategy in which the fungal inoculum is produced in liquid culture, which is further utilized for inoculating the solid substrate(s) for conidia production (Burges and Hussey, 1981). *V. lecani* is a facultative pathogen and can be mass-produced on various substrates. The choice of a suitable and economic medium, which supports rapid growth without loss of virulence for number of generations, is one of the basic requirements in the mass production of fungi for microbial control of

insect pests. The production can be achieved using different methodologies, which can be classified in to low input and industrial technologies. However, most production of fungal spores worldwide is carried out using simple technologies that demand low inputs (Alves and Pereira, 1989). Some attempts to mass produce *V. lecani* on low cost materials have been carried out by Mazumdar *et al.*(1995) and Babu *et al.*(2008). They evaluated solid and liquid substrates, including sugar industry by-products and observed that addition of dextrose was indispensable for accelerated growth on some of them. The type of growing medium affects conidial production of fungi (El Damir, 2006). Prasad and Pal (2014) conducted study to mass produce *V. lecani* on nine different agricultural and industrial waste substrates. They found maximum yield (18^5 X 10^6 spores/ml) in Farm Yard Manure (FYM) followed by SDB (246.25, 157.25 and 180.0 X 10^6 spores/ml) respectively whereas the lowest number of spores of *V. lecanii* was obtained in sugarcane bugasse (39.0 X 10^6 spores/ml), respectively. The economics of *V. lecanii* production was also evaluated based on the final yield. Among in vitro produced media, Crushed jowar grain + 1.0g dextrose was the best low cost substrate for *V. lecanii*. The highest cost of spore production was recorded in sugarcane bagasse followed by pressmud. Sahayraj and Namasivayam (2008) found sorghum grains as ideal substrate for mass production of *V. lecanii*. The effect of different carbon and nitrogen sources on the *V. lecanii* were studied with the avalibility of Czepeck Dox media (Mehta *et al*., 2012). It was observed that *V. lecanii* showed higher growth potentials on almost all the nutrient sources such as sodium nitrate, Potassium Nitrate and Ammonium Sulphate. In these nitrogen sources, sodium nitrate promotes highest growth whereas in carbon sources, fructose was found most ideal. Sahayaraj and Namasivayam (2008) reported maximum growth and sporulation of fungi in coconut water as liquid media. Mehta *et al.* (2012) observed maximum biomass production in yeast extract media.

Host specificity

For studying host specificity the genus *Verticillium* can be used as a fruitful model. *Verticillium spp*. specificity is complex and characterized by wide range of hosts. Presently, it is difficult to provide an detailed information regarding host range of *Verticillium spp*. because (i) their identity to the species level is still unclear in most of the published studies, (ii) their host relationships have mainly been established for the organisms of economic importance (agricultural pests and plant diseases), and (iii) all studies on host suitability and pathogenicity have been conducted under laboratory and greenhouse conditions. This species complex exhibits a very wide host range, including insects, mites, nematodes and phytopathogenic fungi (Askary *et al.*, 1998 and Goettel *et al.,* 2008). Within the Insecta, *Verticillium spp.* has been shown to affect Orthoptera, Thysanoptera, Homoptera, Coleoptera, Diptera, Lepidoptera and Hymenoptera.

Mode of action

Entomopathogenic fungi vary considerably in their modes of action, virulence, and degree of host specificity (Clarkson and Charnley, 1996). Ability of fungi to adhere and penetrate the host integument is very important for successful infection (St Leger, 1993). Fungi are challenged by the complex structure of the insect cuticle and, typically, a variety of extracellular enzymes are involved in the degradation of proteins, chitin, and lipids (Charnley, 1984; St Leger, 1993). Furthermore, once the host hemocoel has been invaded, a number of other determinants such as the fungal capacity to fend off the host defence reactions and feed on the host tissues could also affect the efficacy of the pathogen (Vey, 1984). The hyphomycete *V. lecanii* (Zimm.) is ubiquitous and has a wide host range. It has the ability to develop successfully in several hosts belonging to the Nematoda (Meyer *et al*., 1990), Arachnida (Jun *et al*., 1991), and Insecta (Hall, 1981). *V. lecanii* is also a mycoparasite of rust (Allen, 1982; Spencer and Atkey, 1981) and other phytopathogenic fungi (Raghavendra-Rao and Pavgi, 1977; Askary *et al*., 1997). Despite its remarkable host spectrum and potential as a biological control agent of arthropods (Hall and Burges, 1979), plant pathogens (Whipps, 1992; Verhaar *et al*., 1996), and plant-parasitic nematodes (Meyer and Meyer, 1996). As with other plant and arthropod pathogens, variations

in pathogenicity of *V. lecanii* are related to various growth characteristics and enzymatic activities. Jackson *et al.* (1985) reported that high germination and sporulation rates as well as production of extracellular chitinases correlated with virulence of *V. lecanii* to the aphid *Macrosiphoniella sanborni*. However, there was no relationship between virulence and extracellular lipase and protease activities; whereas amylase production was associated with avirulent isolates *V. lecanii* pathogenesis also involves production and diffusion of toxins with insecticidal properties (Claydon and Grove, 1982; Gindin *et al.*, 1994). Askary *et al.* (1999) studied general traits of establishing infections by *Verticillium sp*. 198499 on potato aphid *Macrosiphum euphorbiae*. The process of aphid colonization involves (i) spore attachment to the host cuticle through a mucilaginous matrix, (ii) spore germination and coloniza tion of the cuticle surface, (iii) cuticle penetration by germ tubes, (iv) active multiplication of blastospores and invasion of host tissues and (v) release of the fungus from aphid cadavers through the production of conidiospores. Typically, the fungi's extracellular enzymes are involved in the degradation of the host insects' proteins, lipids and chitin (St. Leger *et al.*, 1996). Pathogenesis would also involve production and diffusion of toxic metabolites with insecticidal activity, as shown for other *Verticillium spp*. (Claydon and Grove, 1982; Gidin *et al.*, 1994).

Pathogenicity

Sitch and Jackson (1997) studied the pathogenicity of two isolates of *V. lecanii* towards non-target invertebrates and six species of aphid was tested in bioassays. It was found that all target aphid species were susceptible to at least one isolate. The level of susceptibility varied between aphid species. They also studied initial spore deposition, retention and germination on two susceptible aphid species and four resistant non-target insects revealed differences in the behaviour of the pathogen between the two groups. Spore density was greater on susceptible insects both immediately post-inoculation and following 24 h incubation. There was no loss of spores from *M. persicae* over 24 h. All other species showed more than 50% spore loss in the same period. However, even after 90% spore loss, large numbers of spores remained on the bigger insects such as *Agonum dorsale*. Germination and germ-tube growth were possible on such resistant non-target insects, indicating that resistance to infection occurs after this stage. There was little difference in rate of germination on the two groups. However, differences in germ-tube growth were evident, with the production of longer and narrower germ-tubes on the resistant insects. Spore density 24 h post-inoculation may be more dependent upon spore-cuticle interactions (Fargues, 1984; Rath *et al*., 1996), being a function of initial deposition and the ability of the spores to persist on the cuticle. Numerous studies have shown that pathogens can germinate and grow on insects which are not susceptible to infection by the pathogen (Milner, 1982; Boucias and Latge, 1988). Chandler *et.al.* (1993) found no correlation between pathogenicity and germination rate of strains of *V. lecanii* on whitefly scales. It was also evident from the faster rates of germination on dead insects that the cuticle of both groups of insect may contain labile or volatile fungistatic compounds (Butt *et al.*, 1992), which may have some inhibitory effect on germination on live individuals (although increased germination may be due to release of nutrients from the cuticle as a result of freezing). Differences in germ-tube growth of *V. lecanii* have often been observed to show extensive hyphal growth on susceptible insects (Drummond *et al.*, 1987; Schreiter *et al.*, 1994). However, whilst high nutrient levels have previously been associated with extensive surface hyphal growth (Hunt *et al.*, 1984) and lack of penetration or appressorium production (St Leger *et al.*, 1991; Butt *et al.*, 1995), others have found that this is characteristic of pathogens exhibiting low levels of pathogenicity (Pekrul and Grula, 1979), or growing on hard cuticle (Charnley, 1984). Gurulingappa *et al.* (2006) studied the effects of endophytic strains of *V. lecanii* on the survival, and reproduction of *A. gossypii*. Their presence in crop plants indicates the possibility of a much greater potential for contact between insect and fungus than previously recognized. The culture filtrates of *V. lecanii* significantly increased mortality and feeding-choice experiments indicate that insects may be able to detect metabolites of the fungi. The culture filtrate of fungi significantly reduced the reproduction of the aphid. The ethyl acetate and methanolic fractions of the culture filtrate and of mycelia of *V. lecanii* also caused significant

mortality and reduced fecundity of *A. gossypii*. They concluded that *A. gossypii* is affected by contact with both conidia and fungal metabolites. This broad influence indicates that this fungus may have a role in regulating insect pest populations. Steenberg and Humber (1998) bioassayed *V. fusisporum*, *V. psalliotae*, *V. lamellicola*, and species of *Acremonium* hyphomycetes against the sweet-potato whitefly (*Bemisia tabaci*) and against the housefly (*Musca domestica*) to examine their entomopathogenicity. A test was also conducted with a coleopteran (lesser meal worm, *Alphitobius diaperinus*) to further evaluate the host range for some of the fungi. *V. lamellicola* was not found pathogenic to the two species of insects treated, while varying levels of pathogenicity were shown for the other species. In general, *V. lecanii* was the most pathogenic species. Immature whiteflies appeared to be more susceptible to fungal infection than adult houseflies, and the host range for several of the fungi also included lesser mealworm.

Potential enzymes involved in pathogenesis of *V. lecanii*

Protein, chitin and lipids form the major composition of insect cuticle, the prime barrier to infection. For degradation of chemical constituents in cuticle, extracellular enzymes are secreted by entomopathogenic fungi. Infection process is facilitated by cuticle degrading enzymes. Entomopathogenic fungi produce protease, chitinase and lipase which can degrade insect cuticle (Charnley, 2003., Fang *et al*., 2005). During fungal infections, a range of hydrolytic enzymes are secreted to help promoting host colonization. Depending on the ecological niche occupied by each fungus, a particular set of enzymes mainly composed of proteases and carbohydrases, are displayed to degrade specific tissues and scavange for nutrient sources (Yakoby *et al.*,2000) . Because these enzymes work outside the fungal cell, activity as well as mechanisms that control synthesis and secretion are under the influence of several environmental factors such as ambient pH (Maccheroni Junior *et al*., 1995). *Verticillium* are gathered mycoparasitic and entomopathogenic species that produce extracelullar enzymes, such as chitinases. Ramirez-Coutino *et al*. (2006) evaluated several strains of *V. lecanii* and *V. fungicola* as chitinase producers. The strains of *V. fungicola* USDA 4519 and those of *V. lecanii* USDA 974, USDA 2460, and ATCC 26854 showed the highest activities. Natural polymeric substrates, such as chitin, have been used to support growth of *V. lecanii* as source of nutrients as well as inducer (Matsumoto *et al*., 2004). Chitin is not degraded inside the cell due to its insolubility, size, molecular complexity and heterogeneous composition but fungi secrete chitinases with different specificity, endochitinases and exochitinases, which are able to transform or hydrolyse chitin (Shirai *et al*., 2006). *V. lecanii* has been cultivated in solid substrate fermentation (SSF) using chitin as carbon source. SSF produced a highly concentrated enzymatic extract with very active chitinases (Matsumoto *et al*., 2004). Hasan *et al*. (2013) screened and proved *V. lecanii* to be an efficient producer of protein and polysaccharide degrading enzymes (amylase, protease, and lipase), hence indicating versatility in biochemical mechanisms. Halo zones produced colony growth of *V. lecanii* on agar confirmed activity of protease, amylase and lipase enzyme by the *V. lecanii* isolate. Enzymatic Index (EI) observed were: Protease – 2.195, Amylase- 2.196, Lipase- 2.147. Spectrophotometric analysis of enzymatic activity of *V. lecanii* at five different pH – 3, 5, 7, 9, 11 revealed that highest proteolytic activity of the *V. lecanii* isolate was reported at pH 7 and 9 whereas proteolytic activity was minimum at acidic pH 3. Maximum amylolytic activity of *V. lecanii* on the 7th day of inoculation was at pH 3 i.e. in an acidic environment in contrast to neutral pH 7. Maximum lipolytic activity of *V. lecanii* was found at pH 7. Maccheroni *et al.* (2004) deduced that lipase was absent at acidic pH and secreted at neutral and alkaline pH. Gomez-Santiesteban *et al.* (2004) showed lipolytic activity of *V. Lecanii* by development of inhibition zones on solidified agar. Lopez –Llorca *et al.* (1999) estimated production of extracellular lipase activity. Lipolytic activity in *V. lecanii* was followed by proteolytic activity when enzymatic activity (EA) due to degradation of substrate was studied. Ramirez-Coutino *et al*., (2010) studied the activities of Chitinase and N-acetylhexosaminidase

activities in submerged cultures of *V. fungicola* which was increased up to 5-fold and 2.5-fold, respectively when the pH of the culture medium was raised from 5 to 8.

Factors influencing the growth and pathogenicity of *V. lecanii*

Adequate development of entomopathogenic fungi as biocontrol agents requires a selection schedule of species and strains adapted to specific pests, environmental conditions and crops. Laboratory bioassays may play an important role in such a screening process. Both biology and physiology of entomopathogenic fungi depend on environmental parameters such as temperature and relative humidity (Chandler, 1992) or nutrient availability (Andersch, 1992). This is probably the reason why entomopathogens are most successful in controlled environments such as greenhouses (Moorhouse *et al.*, 1993). Adverse environmental conditions such as low moisture, UV radiation, extreme temperatures or chemicals such as some fungicides may affect negatively the performance of mycoinsecticides (Moore and Prior, 1993). For practical use of these antagonists a sound knowledge of their behaviour in respect to environmental factors is then of paramount importance. In many instances the best practice to control an insect pest is to obtain the entomopathogens from the same host or its environment (Moore and Prior, 1993).The spore germination and colony growth of the C-3 isolate of *V. lecanii* is more rapidly between 20°C and 25°C. Both germination and growth declined steeply above 25°C and ceased above 30°C (Burges, 1981). Llorca and Carbonell (1999) characterized biologically and physiologically eight *Verticillium lecanii* strains from several origins including insect pests. Of all the temperatures tested, 25°C was the best for growth and at 40°C none of the strains could grow. At 4°C and 7°C, growth was reduced in comparison to warmer temperatures. The strains had better development at pH close to 7 ($F= 27, 64$, $P< 0.01$) than at pH 3. Self-inhibition of germination of strain 50 was found when more than 0.78conidia/cm2 was plated on corn meal agar (CMA). Germination of conidia was close to 100% for all strains except one, three days after inoculation. Among extracellular enzymatic activities studied the fungal strains showed strongest pro-teolytic activities followed by lipolytic and chitinolytic activities. Some strains showed significant differences ($P < 0.05$) in conidia production. Most of the fungicides tested (especially benomyl) inhibited radial growth of strain 50 on CMA. Pathogenicity (as median lethal time, LT 50) of *V. lecanii* strains on larvae of *Galleria mellonella* varied from 2.66 ± 0.33 to 4.27 ± 0.25 days. They concluded that in vitro tests are not sufficient to select the best biocontrol strains of entomopathogenic fungi. Temperature is the environmental factor that affects most the development of entomopathogenic fungi. Its optimum value for most of them is between 25 and 30°C (Hywel-Jones N.L., Gillespie, 1990). Soil temperature and organic matter are other important factors influencing viability of conidia in soil (Storey GK, Gardner, 1987). Because of the extreme temperatures in which insect hosts can live, it is desirable to find entomopathogenic fungi capable of growing at a wide range of temperatures (Fargues *et al.*, 1992). Conidium germination and fungal development on the insect cuticle are closely influenced by temperature (Hywel-Jones NL, Gillespie, 1990, Fargues *et al.*, 1992). Temperature optimum for growth may be different from the optimum for insect infection (Fargues *et al.*, 1992). However, the former may be used as a screening criterion for strain selection together with other parameters. Other environmental parameters such as pH have also been found to affect both growth and physiology of nematophagous and entomopathogenic fungi (Lopez *et al.*, 1993).

Epizootiology

Knowledge of the epizootiology of entomopathogenic fungus is potentially important for conservation biological control in agroecosystems. Jackson *et al.* (2012) test the potential for the transmission of *V. lecanii* conidia from the soil via rain splash or wind, coffee seedlings with populations of *Coccus viridis* were placed near *V. lecanii* -inoculated soil and then subjected to artificial rain and wind treatments. Rain

splash was shown to be a potential transmission mechanism. They have also tested the dispersal of *V. lecanii* conidia by the ant *Azteca instabilis* using field and laboratory ant-exclusion experiments. *A. instabilis* was shown to transport conidia of *V. lecanii*; however, dispersal by *A. instabilis* may not be important under field conditions. Beardsley (1952) showed that the white halo of hyphae, which protruded from underneath infected scales, appeared 9 days after inoculation in laboratory studies. This corresponds well with the 2 week lag observed in this field study. Relative humidities above 93% were prerequisites for infection and speculation of the fungus in aphids (Milner and Lutton, 1986) and white-flies (Drummond *et al*., 1987); however, the highest infection rates occurred in *Myzus persicae* (Sulzer) when free water was present (Milner and Lutton, 1986). Additionally, Kohler (1980) found that outbreaks of the fungus on green scale on coffee grown in Cuba occurred during the rainy season and control by this fungus was most effective on scales on shaded plants. Easwaramoorthy and Jayaraj (1976) also found humidity and rainfall to be positively correlated with infections by the fungus but felt that maximum and minimum temperatures were more important.

Fungal metabolites

Fungal metabolites have been shown to have potential insecticidal activity against pests (Gindin *et al*., 1994; Wang *et al*., 2007). Toxins extracted from the mycelia of *V. lecanii* were deleterious to *Bemisia tabaci* (Gennadius), *Myzus persicae* (Sulzer), *A. gossypii*, *Acyrthosiphon pisum*, (Harris) and *Frankliniella occidentalis* (Pergande) (Gindin *et al*., 1994). *V. lecanii* has also been reported to produce bassionalide (Suzuki *et al*., 1977). Fungal metabolites are a potentially important mechanism for reducing insect survival and reproduction.

Compatibility with agrochemicals, other biocontrol agents and spraying equipments

One of the most important factors to take into account while selecting antagonistic fungi is their compatibility with commonly used agrochemicals such as insecticides or fungicides (Landa *et.al*., 1994). High concentrations of triadimephon and copper oxichloride inhibits the growth as well as formation of sclerotia by *V. lecanii* (Rosato *et al*., 1981). Xu *et al.* (2012) studied the effects of *V. lecanii* on the biological characteristics and life table of the whitefly predator, *Axinoscymnus cardilobus* Pang and Ren (Coleoptera: Coccinellidae) using five different conidial concentrations under laboratory conditions. The total developmental period (from egg to adult) among the treatments did not differ between fungus treatments and control. The longest total development period for *A. cardilobus* was observed when treated with 1×10^7 spore/ml. No Significant difference was found for *V. lecanii* on the percent survival of all immature stages of *A. cardilobus*. The treatment with *V. lecanii* did not elicit any significant effect on mean generation time, intrinsic rate, the finite rate of increase and longevity of *A. cardilobus* when compared with control treatment. They found that control strategies tested are compatible to a greater extent and incorporation of these have promising prospect for control of whitefly. Nilsson and Gripwall (1999) studied the viability of the fungus *Verticillium lecanii* was examined after spraying with different apparatus. The viability of *V. lecanii* was significantly decreased as the length of the pumping period in the hydraulic high-pressure sprayer increased. With increasing pressure in the hydraulic sprayer the viability of *V. lecanii* decreased significantly. The viability of the fungus was not significantly influenced by the backpack sprayer or the coldfogger.

Conclusion

Concept of biological control is increasing largely because of greater environmental awareness, food safety concerns and the failure of conventional chemicals due to an increasing number of insecticide resistant species. The application of entomopathogenic fungi *Verticillium lecanii* is one of the alternatives in biological control. *V. lecanii* are opportunistic and widely distributed entomopathogenic fungi that cause mycosis in a number of insects of orders Homoptera, Coleoptera

and Lepidoptera. The safety of this fungus for humans, environment and non-target organism offers as one of the effective alternative in integrated pest management (IPM).

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