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THE INSECT PATHOGENIC FUNGUS *Verticillium lecanii (Zimm.) Viegas* AND ITS USE FOR PESTS CONTROL: A REVIEW

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ABSTRACT

Chemical insecticides play an important role in the control of plant damage and plant diseases. However, extensive use of these products has led to the disruption of ecosystems because of several reasons such as death of non-target species, accumulation of pesticide residues in the environment and food, and buildup of pesticide resistance in the target species. Biological control is one of the alternatives to chemical pesticides and it can be described as the limitation of the abundance of living organisms and their products by other living organisms. Predators, parasitoids, fungi and other beneficial organisms can be used for the biocontrol of insect pests. The fungus *Verticillium lecanii* is one of the members of *Deuteromycetes* and it can be used for crop protection. This paper is a review of the international literature related to *V. lecanii* for the bio-control of insects of agricultural importance.

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1 Introduction

Chemical pest control occupies an important place among the plant protection methods. Before World War II, chemical pest control was mainly restricted to the application of nicotine and some arsenical products (Schepers, 1989). Sprayed on the crops, they kill the pests that come into contact with these compounds, but they have neither systemic nor residual effects. Since the war, chemical control of insects has made rapid progress, starting with the development of DDT formulations and other chlorinated hydrocarbons such as lindane, which showed a residual effect but had no systemic properties (Schepers, 1989).

The persistence of residues of the chlorinated hydrocarbons causes accumulation in the food chain and since this phenomenon has become known, unrestrained application of those products no longer seems justified and is already prohibited in many countries. The development of systemic insecticides starting with the formulation of organophosphorous compounds, opened new perspectives in pests control (Schepers, 1989). Later, the carbamate insecticides added more possibilities, as did the development of synthetic pyrethroids. All these products played an invaluable role in the control of plant damage and plant diseases (Schepers, 1989). However, extensive use of chemical insecticides has led to the disruption of ecosystems because of the death of non-target species, the accumulation of pesticide residues in the environment and food, and the build up of pesticide resistance in the target species (FAO, 1989; Devonshire, 1989).

Biological control can be described as the limitation of the abundance of living organisms and their products by other living organisms. The term encompasses both the biotic component of natural control, which is, the naturally occurring regulation of the numbers of a species by the action of its natural enemies, and also, applied or manipulative biological control, which is the use by man of biological means to control a plant or animal (or its products) that has become a pest (Carver, 1989).

Predators, parasitoids, fungi and other beneficial organisms can be used for the bio-control of insect pests. The fungus *V. lecanii* is one of several *Deuteromycetes* species that can be used for crop protection. This work is a review of the international literature related to *V. lecanii* for the bio-control of insects of agricultural importance.

2 Biology and Ecology of *Verticillium lecanii*

V. lecanii lacks a sexual phase (perfect stage) and reproduces by means of non-motile, asexual spores called conidia. Germination of these conidia produces hyphae and after subsequent growth, these hyphae produce conidiophores which finally produce conidia (Alexopoulos & Mims, 1979). *V. lecanii* is able to grow on both living and dead materials (Schuler, 1991). It is non-fastidious and can grow on all conventional mycological media so far tested, e.g. Czapeck-Dox, Malt extract, Sabouraud and Potato dextrose agars, including a media containing chitin as the sole source of carbon and nitrogen. It has capability to produce conidia on solid media; in contrast, *V. lecanii* assumes a semi-yeast morphology in liquid media (Hall, 1981a).

V. lecanii is one of the most common and important entomophagous *Hyphomycetes* fungi occurred on coccids, aphids, thrips, *Diptera*, *Homoptera*, *Hymenoptera*, *Lepidoptera* and mites and in all the climatic regions. Other important substrates for *V. lecanii* are rusts and other fungi. It is a consequence of this habit that the species is frequently isolated from soil; it has also been isolated from leaf litter of oak, ash and birch, tea leaves, barley seed, baker´s yeast, beet seed and bursting corn kernels (Domsch et al., 1980; Sewify & Mabrouk, 1990; Andreeva & Chternchis, 1995). During greenhouse experiment, *V. lecanii* could control the cucumber powdery mildew (*Sphaerotheca fuliginea*), keeping the mildew severity with partially resistant cucumber variety, below 15% of infected leaf area or under economic threshold (Verhaar et al., 1993; Verhaar et al., 1996). Transmission electron microscopy observations have provided evidence that *V. lecanii* has also potential to colonize mycelial structures of *Sphearotheca fuliginea* and reduced the pathogenicity of this phytopathogenic fungus under laboratory conditions (Askary et al., 1997; Askary et al., 1998). Isolates of *V. lecanii* readily isolated from coffee rust lesions under laboratory conditions and at high relative air humidity, showed hyperparasitic and antibiotic properties. Other isolates of *V. lecanii* of insect origin were also able to parasitise *Hemileia vastatrix* (Eskes et al., 1991).

The parasitism *of V. lecanii* has been well demonstrated against many other phytopathogenic fungi like *Puccinia graminis var. tritici* (Hänssler et al., 1981), *Puccinia striiformis* (Mendgen, 1981), *Uromyces appendiculatus* (Grabski & Mendgen, 1985; Grabski & Mendgen, 1986), *Phaeoisariopsis personata* and *Puccinia arachidis* (Ghewande, 1989; Ghewande, 1990; Zambettakis et al., 1985), *Uncinula necator* (Heintz & Blaich, 1990), *Puccinia horiana* (Srivastava et al., 1985; Whipps, 1993); *Puccinia coronata* (Leinhos & Buchenauer, 1992) and *Puccinia allii* (Uma & Taylor, 1987).

This fungus also occurs on several species of Nematodes (Schuler, 1991). The parasitism of *V. lecanii* on cysts or eggs of several nematode species has been well reported (Hänssler, 1990; Uziel and Sikora, 1992; Meyer et al., 1990). The cyst wall of *Heterodera schachtii* was penetrated by *V. lecani* mycelium only after 60 hours of inoculation. Inside the cyst cavity, the fungus passed through the eggshells and colonized the larvae. *V. lecanii* secreted specific enzymes into the culture medium which enable him to degrade constituents of the cyst as well as the eggshell (Hänssler, 1990). Wild or mutant strains of *V. lecanii* showed efficacy against nematodes under greenhouse conditions (Meyer & Meyer, 1995; Meyer &

Meyer, 1996; Meyer, 1994; Reddy et al., 1996; Meyer & Huettel, 1996; Meyer et al., 1997). Like all the other entomopathogenic fungi, *V. lecanii* infects its invertebrate hosts through the external cuticle. Three phases have been recognised in the development of insect mycosis: adhesion and germination of the fungal spores on the host cuticle, penetration of the insect integument by a germ tube, and development of the fungus inside the insect body, generally resulting in death of the infected host. Under good humidity conditions, the dead host is covered by the fungal spores and hyphae (Quinlan, 1988; Zimmermann, 1984).

Humidity and temperature are the most important climatic factors which influence the growth of *V. lecanii* (Schuler, 1991). Virtually, all fungi require high humidity for spore germination, growth and sporulation. *V. lecanii* conidia require high humidity to germinate and possibly do so in water film only (Hall, 1981a). The optimal temperature requirement for spore germination and colony growth or infection may vary from isolate to isolate, between 15°C and 25°C (Hall, 1981a; Ekbom, 1979; Easwaramoorthy & Jayaraj, 1977; Barson, 1976; Hirte et al., 1989; Sermann et al., 1991; Hsiao et al., 1992). At 5°C, spores of *V. lecanii* can germinate and grow slowly and above 30°C its germination and growth may cease.

3 Insecticidal potential of *V. lecanii*

The fungus appears to have been first observed in Ceylon (Sri Lanka) about 1861, on diseased *Lecanium coffeae*. It was subsequentely found by Zimmermann on *Lecanium viride* on coffee, in Java (Indonesia) and was briefly described by him under the name *Cephalosporium lecanii*, in a short paper in 1898. Zimmermann stated that each dead scale was surrounded by a white fungus. He cultivated the fungus on nutrient agar and directed attention to the possibility that this fungus can be utilised for controlling the scale-insect (Petch, 1925).

Guegnen published an exhaustive work in 1905 on fungus-parasites of man and animals, and reported a new conidial form, *Acrostalagmus coccidicola,* found by him on coccid in a greenhouse in Paris (Parkin, 1906). Guegnen cultivated this fungus on several media and described it. He attempted to infect an undetermined coccid by applying the fungal spores to the insect with a brush, but without success. According to the description, *Acrostalagmus coccidicola* does not differ morphologically from *Cephalosporium lecanii* (Petch, 1925). In 1905, Dop published a paper on a new fungus parasite of *Aspidiotus* from Martinique; he stated that the advent of the fungus has practically saved the cocoanut palm cultivation in this Island; the fungus was referred to the genus *Hyalopus*; thus, it is probably near akin to *Cephalosporium* (Parkin, 1906).

Parkin (1906) reported fungus similar to *Cephalosporium lecanii* from Ceylon on *lecanium viride, L. hemisphaericum* and *L. nigrum* and stated that the ease whereby the fungi can be artificially cultivated is a point in their favour for their possible use for controlling the scale-insects. Successful infection of the green-bug (*Lecanium viride*) on the Java coffee by an artificial culture of *Cephalosporium* was then cited by him. At that time, conditions for successful inoculation were therefore somewhat obscure. In 1909, information with regard to the distribution and effectiveness of insect-pathogenic fungus in the West Indian Islands has been collected by various researchers (South, 1910). The fungi which were commonly reported by these researchers are: the white-headed fungus (*Ophionectria coccicola E. and E.*), the black fungus (*Myriangium duriaci Mont*.), the shield scale fungus (probably *Cephalosporium lecanii Zimm*) (South, 1910).

In some districts of these islands where the general conditions are favourable to their growth, the parasites of certain species of insects exist naturally in large numbers and were responsible for the comparative rarity of these species in those districts (South, 1910). Work was carried out in order to introduce the parasite into places in which the conditions are favourable to its growth, but in which it has not previously been known to occur and to produce it where possible by artificial means.

Methods of introducing these fungi include spraying the spores and portions of the mycelium of the fungi onto trees which it is intended to infect, tying infected material into trees which it is desired to infect and finally, planting among the trees to be infected, small trees whose foliage is well infected with various parasitic scale fungi, so that the leaves of the small trees come into contact with those of the larger ones. With regard to the artificial formation of conditions suitable to these fungi in localities where they are naturally unfavourable, two methods were suggested: spraying trees with clean water, once or twice a week and allowing the trees attacked by scale insects to become covered with a fairly thick growth of Bengal beans (*Mucuna pruriens*); the beans were supposed to create damp conditions.

The results with *Cephalosporium lecanii* and another fungus were said to be encouraging (South, 1910). Petch (1925) collected specimens of insect pathogenic fungi from several localities and cultivated them on nutritive media for further study and reported that most of the insect pathogenic fungi showed similarity with *Cephalosporium lecanii*.

V. lecanii has been employed in Brazil for controlling the green scale of coffee by Viegas who gave him in 1939, its current name (Schuler, 1991). Gams (1971), after cultural and morphological examination of type specimens, regarded the identifying characteristics of previously determined *Cephalosporium* as well as other associated species insufficient to justify their separation as distinct species; he, therefore, proposed that all these species should be amalgamated as synonyms of *Verticillium lecanii*. This fungus has never been implicated, in temperate climates in epizootics, although it is frequently isolated from individual insects (Barson, 1976; Hall, 1981a).

Indoors, in the somewhat tropical environment of greenhouses, in North Europe and USA, it frequently decimates populations of scales and aphids, e. g. 100% mortality of several target insects was reported in limited trials (Hall, 1981a).

The potential of the fungus has been realised in glasshouse systems in Europe thanks to the extensive research in U. K., in years 70. For that, laboratory methods for obtaining uniform batches of conidiospores and stock insects have been developed together with a bio-assay technique, using isolated apterous insects on leaf discs, in order to quantify accurately the pathogenicity of *V. lecanii* conidiospores against aphids. A single spore isolate of *V. lecanii* obtained from a diseased aphid *Macrosiphoniella sanborni* infesting chrysanthemums was therefore cultured on Sabouraud dextrose agar and spore concentration was determined using improved hemacytometer. The bio-assay technique consists in treating artificially reared aphids with the fungal spore on filter paper in Buchner funnel, by pouring gently a known amount of the appropriate spore suspension on them. After treatment, aphids were singly placed on leaf discs in high humidity assay cells which were kept in perplex cages at high humidity for the duration of the experiment (Hall, 1976). This assay technique provides a good measurement of the pathogenicity of batches of conidiospores of *V. lecanii*.

Once the efficacy of the pathogen proved under laboratory, it has been tested on aphid-infested Chrysantemum plants in greenhouse. These plants were sprayed with conidial suspension with pneumatic hand sprayer (Hall, 1979; Hall & Burges, 1979). All these laboratory and greenhouse experiments led to the development of two commercial products “Vertalec” and "Mycotal" based on strains specifically selected for use against aphids and whiteflies (Gardner et al., 1984; Ramakers, 1989). *V. lecanii* has been tested experimentally against a range of pests, in a number of countries, with varying results which were said in general to be encouraging (Kitazawa et al., 1984; Helyer & Wardlow, 1987; Saito, 1988; Gour & Dabi, 1988; Gopalokrishnan, 1989; Ravensberg et al., 1990a; Ravensberg et al., 1990 b; Byrne, 1991; Van der Schaaf et al., 1991; Meade & Byrne, 1991; Masuda & Kikuchi, 1992; Pinna, 1992; Chandler et al., 1993; Zukauskiene & Sirvinskas, 1993; Helyer, 1993; Ravensberg et al. 1994; Miranpuri and Khachatourians, 1994; Fournier, 2000; Gindin et al., 2000; Wang et al., 2004; Nirmala et al., 2006; Liande et al., 2007; Jeong et al., 2007; Van et al., 2007; Goettel et al., 2008; Jeong et al., 2008; Chavan et al., 2008; Shinya et al., 2008).

The effectiveness of this bio-control agent has been proved also in soil against the soilborne stages of the western flower thrips (*Frankliniella occidentalis*) (Hirte et al., 1994; Sermann et al., 1994; Sermann et al. 1996; Beyer et al., 1997a; Beyer et al., 1997b). Nevertheless, despite the fact that greenhouses offer an attractive environment for the exploitation of the fungus, the effectiveness of *V. lecanii* still depends on high humidity and selection of strains infecting the host rapidly or investigation of methods able to favour the fungus action is essential.

The effects of pesticides on spores germination, mycelial growth and sporulation as well as on infection have been investigated quantitatively and qualitatively with strains of *V. lecanii*. Some chemicals were shown to be incompatible while others proved relatively harmless to the fungus (Hall, 1981b; Khalil et al., 1985; Saito & Yabuta, 1996). Synergistic inhibitory action of innocuous chemicals on conidiospores germination of the pathogen has been reported. On the basis of all these studies, it is concluded that a careful selection of pesticides and fungicides would permit the combined use of *V. lecanii* and chemicals in integrated control programmes (Hall, 1983).

Similarly, the combined use of *V. lecanii* with other biological control agents such as the whitefly parasites *Encarsia formosa*, *Amblyseius spp*. and other pests antagonists has been investigated (Kanagaratnam et al., 1979; Bennison et al., 1990; Van der Schaaf et al., 1991; Buxton & Wardlow, 1992). Results showed that integration of *V. lecanii* with these bio-control agents is also possible.

The virulence of two strains of *V. lecanii* originally isolated respectively from the aphid *Myzus persicae* and whitefly (*Trialeurodes vaporariorum*) was bioassayed against 3 different aphids’ species. These strains (V24 and V18) were capable of infecting all three aphids species, but their virulence determined by LC_{50} and LT_{50} varied. The strain (V24) isolated from *M. persicae* showed the highest virulence against the homologous aphid species, but the whitefly derived strain (V18) showed also the highest virulence against one of the aphid species.

The variability of the bioassay results and the impossibility of defining clearly traits associated with the virulence of a fungus strain toward a specific insect species have been discussed and spores improvement using adjuvants was suggested (Alavo et al, 2002a). However, greenhouse trials aimed at controlling *M. persicae* in chinese cabbage using *V. lecanii* blastospore suspension either pure or with adjuvants such as soyflour and rape oil were discouraging; the product failed to control the aphid populations (Alavo et al, 2002b). Some other adjuvants were said to improve germination and infection rate of *V. lecanii*; nevertheless, effective pest control using *V. lecanii* conidia suspension combined with such additives was not demonstrated (Jin et al., 2006; Zhangyan et al. 2006). Fluorescent microscopy investigations revealed almost 100% spore loss from the cuticle of aphid larvae before infection. Spore loss was attributed to the rapid moulting of aphids larvae and the unreliable control of aphids using *V. lecanii* was discussed (Alavo et al, 2002b; Alavo, 2000).

Nowadays, a formulation of *V. lecanii* is commercialized under the name of ‘Mycotal®’ only for use against Whitefly larvae. This product efficacy is said to be improved if applied together

with adjuvant based on emulsifiable vegetable oil (Koppert, 2015).

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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