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Influence of some pesticides on entomopathogenic fungus *Lecanicillium* (= *Verticillium*) *lecanii* (Zimm.) ZARE & GAMS

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ABSTRACT

An *in vitro* study was conducted to determine the interaction effect of ten pesticides tested at field recommended dose on conidial germination, vegetative growth and sporulation of *Lecanicillium lecanii*(ZIMM.) ZARE & GAMS. Compatibility of *L. lecanii* to different pesticides was found to be varied. Conidial germination was 99.3 and 85.7% in Pongamia oil and acephate, whereas, it was totally inhibited by the presence of chlorothalonil, iprodion + carbendazim, carbendazim and thiophanate methyl indicating that these pesticides were highly toxic. Dinocap recorded as moderately toxic while endosulfan, abamectin and ethion were least toxic based to the germination of conidia. So also Iprodion + carbendazim did not and carbendazim allow *L. lecanii* to put forth mycelium growth in their presence. Thiophanate methyl, Pongamia oil, acephate, endosulfan, ethion and chlorothalonil were observed to be innocuous pesticides registering growth of mycelium upto 2.33, 2.23, 2.23, 2.03, 2.03 and 2.00 cm dia., respectively, from 0.6 cm dia. held in the center of Petri plate on 14th day after treatment. As far as sporulation is concerned, Pongamia oil alone recorded the maximum yield of 47.2x10^6 conidia/ml followed by 18x10^6 conidia/ml, in chlorothalonil as against 20x10^6 conidia/ml in control, which means that the pongamia oil exhibited synergistic effect on *L. lecanii,* yielding more conidial spores. Thus, based on *in vitro* interaction study, pongamia oil alone was found to be safe to the entamopathogenic fungus *L. lecanii* in nature and iprodion + carbendazim and carbendazim were found to be highly toxic.

Key words: Botanicals, pesticides, *Lecanicillium lecanii*

INTRODUCTION

The entomopathogenic fungus, *Lecanicillium* (=*Verticillium*) *lecanii* (Zimm.) Zare & Gams naturally infects a wide range of sucking pests such as thrips, whiteflies, aphids, etc. The effect of entomopathogenic fungi depends not only on the strain and favourable environmental conditions, but also on their interaction with other factors such as sprays of pesticides, micronutrients, hormones, etc. used by man in his attempt to increase productivity. It was demonstrated that *Verticillium lecanii* Zimm. caused over 90% mortality in whiteflies (Schaaf *et al*, 1990) and coffee green scale, *Coccus viridis* (Green) (Reddy *et al*, 1997) and produced excellent control of thrips in greenhouse grown crops (Gillespie, 1986; Meyer *et al*, 2002). Sprays of synthetic insecticides, botanical insecticides, fungicides, etc. used for the control of other pests and diseases in the same ecosystems may have better chances to interact with *L. lecanii* present in nature and possibly bring down its efficacy on the target pest. Therefore, a study on the compatibility of *L. lecanii* with some pesticides that are under use in the same environment was conducted under *in vitro* conditions to investigate the effect of interaction in terms of conidial germination, growth and conidial production.

MATERIAL AND METHODS

The commonly used pesticides in horticultural crops, viz., endosulfan, acephate, abamectin, ethion, pongamia oil (*Pongamia glabra* Vent. Jard. Malm.), chlorothalonil, iprodion + carbendazim (a combination of two fungicides, marketed as Quintol), carbendazim, thiophanate methyl and dinocap were tested to determine the influence of pesticides on *L. lecanii.* The fungus, *L. lecanii*, originally isolated from thrips, *Thrips palmi* Karvy, was used for the study (Ganga Visalakshy *et al*, 2004). Conidial germination, mycelial growth and conidial production in *L. lecanii* was determined by calibrating i) per cent germination of conidial spores ii) rate of growth of mycelium and iii) yield of conidial spores after the vegetative phase. Initially Potato Deextore Agar (PDA) was autoclaved at 1 atmospheric pressure for 20 min. and

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Table 1. Effect of various pesticides on germination of conidial spores of *Lecanicillium lecanii*

Pesticide	Dose/l	Mean no. of germinated conidia *	Per cent germination
Endosulfan	2.00ml	118.7[d]	39.6
Acephate	0.75g	256.7[b]	85.7
Abamectin	0.60ml	169.4[c]	56.5
Ethion	1.00ml	126.3[d]	42.1
Pongamia oil	2.00ml	297.7[a]	99.3
Chlorothalonil	2.00ml	0.0	0.0
Iprodion + Carbendazim	2.00g	0.0	0.0
Carbendazim	2.00g	0.0	0.0
Thiophanate methyl	1.00g	0.0	0.0
Dinocap	1.00ml	94.2[e]	31.4
Control		299.7[a]	99.9
CV (%)		2.32	-
CD *(P=0.05)*		0.73	-

* Mean of five replications

Means in the same column with same alphabet are not significantly different

pesticides were added at recommended doses (Table 1) at approx 45°C under aseptic conditions and mixed thoroughly by shaking the conical flask. The medium was then poured into sterilized Petri plates (90mm dia.) under aseptic conditions for solidification (Antonio Batista Filho *et al*, 2001). Un-amended PDA medium served as the control.

Preparation of conidial suspension

The fungus, *L. lecanii*, was cultured on PDA for two weeks in a BOD incubator at 27°C, 65% RH and a photoperiod of L16:D8. Conidia harvested from this using sterile distilled water were held in sterilized vials. The conidial load was adjusted to $3x10^3$ conidia/ml by serial dilution with sterile distilled water using sterile micro-tip pipette, with the help of a haemocytometer.

Inoculation of conidia

The surface of the pesticide amended and unamended medium in petri plate was uniformly smeared with 0.1ml of freshly prepared conidial suspension (ca. 300 conidia). Two days after the smear, total number of germinated conidia in each Petri plate was counted under a stereomicroscope. Based on conidial germination, the pesticides were grouped under four categories viz. (i) no germination: highly toxic, (ii) 1-35% germination: moderately toxic, (iii) 36-70% germination: least toxic and (iv) 71 – 100% germination: safe. Each pesticide was considered as a treatment and each treatment was replicated five times. A total of eleven treatments was used in Completely Randomized Design (CRD). Square root transformation was used to analyze difference in the germination of spores.

Rate of growth of mycelium

The medium with the isolate of *L. lecanii* cultured on PDA (stock culture) for two weeks was cut into 6mm dia. discs (with spores) using a sterile cork borer. Cut blocks were inverted and transferred to the center of pesticide + PDA amended petri plates using a sterilized inoculum loop and placed gently on the surface of the media. A disc of *L. lecanii* at the center of the unamended medium Petri plate was served as the control.

All the treated Petri plates were incubated at 27±1°C and 65 % RH in a BOD incubator with a photoperiod of L16:D8. Conidial spores that spilled from the stock culture on the surface while transferring onto the treated medium were ignored for study (like germination, mycelial growth, etc). Further, the rate of growth and the total growth of *L. lecanii* (in diameter) from the transferred block at 1st and 2nd week from inoculation of *L. lecanii* was determined. The rate of growth was calculated by the distance the fungus to which had actually grown (radial distance – initial radial distance) and dividing it by the number of days after inoculation. Per cent inhibition of mycelial growth over the control was calculated using the formula given by Vincent (1927). Based on per cent inhibition of mycelial growth, pesticides were grouped as non-toxic to very highly toxic, with six levels of categorization. A rating with 0% mycelial inhibition denotes that the pesticide in question can be used either alone or can be combined with an entomopathogenic fungus. A rating with 1-20% inhibition denotes that there is less interaction and therefore less compatibility and can be considered as least toxic. A rating with 21-40% inhibition denotes that the interaction resulted in low toxic effect on mycelial growth of *L. lecanii*. A rating with 41-60% and 61-80% inhibition denotes that the interaction resulted in moderately toxic and highly toxic effect, respectively, on mycelial growth of *L. lecanii*. A rating with 81-100% inhibition denotes that the interaction resulted in very highly toxic effect on mycelial growth of *L. lecanii*, where there is no chance of revival of the fungus. The resultant data were subjected to analysis of variance (ANOVA) using SAS (1996) package.

Yield of conidia

The conidial yield obtained from mycelia grown in the above study on rate of growth of mycelium, was recorded using improved Neubauer double ruled haemocytometer and phase contrast microscope on 14th day after inoculation. Results were expressed a number of conidia per milliliter to determine the overall

effect. Compatibility was finally decided based on mean diameter of the colony and number of conidia produced after a fortnight of incubation (Loureiro E de *et al*, 2002).

RESULTS AND DISCUSSION

Per cent germination of conidia

Results on the effect of different pesticides on germination of conidia of *L. lecanii* under *in vitro* conditions are presented in Table 1. The fungicides, viz., chlorothalonil, iprodion + carbendazim, carbendazim and thiophanate methyl were found to be highly toxic and they completely inhibited conidial germination. Majchrowicz and Poprawski (1993), similarly, reported with different pesticide , zineb + copper oxychloride and metalaxyl, that these completely inhibited germination of *Metarhizium anisopliae* (Metsch) Sorokin and *Verticillium lecanii* (Zimm.) Viegas. Dinocap was found to be moderately toxic as there was 31.4% germination. Endosulfan, ethion and abamectin allowed conidia to germinate to a large extent and, therefore, they appeared to be the least toxic. Conidial germination was not much affected in acephate and pongamia oil and these two pesticides alone were found to be safe to *L. lecanii* as there 85.7 and 99.3% germination, respectively. Germinated conidia continued to produce radial growth of mycelium in all the above treatments. Thus, acephate and pongamia oil alone were found to be safes and compatible with *L. lecanii* as far as germination of conidia is considered.

Rate of growth of mycelium

Data in Table 2 show that the pesticides produced varied levels of inhibitory effect on fungal mycelial growth. During the first week, mycelia of *L. lecanii* grew to a maximum colony dia of 1.73 cm. in pongamia oil amended medium, which is however, significantly less than the control ie., 2.03 cm dia. Abamectin, acephate, ethion, dinocap and thiophanate methyl recorded 1.40 - 1.53 cm diameter of mycelial growth, which is on par and significantly affected the fungus resulting in less rate of mycelial growth of 0.06 - 0.07 cm per day than in the control at 0.1 cm per day. The interaction of endosulfan and chlorothalonil with the fungus resulted in very less rate of mycelial growth of 0.06 and 0.04 cm, respectively. However, carbendazim and iprodion + carbendazim were found to be the most toxic chemicals, recording no increase in mycelial growth.

However, in the second week, although the maximum mycelial growth in terms of cumulative growth of colony diameter of 2.33 cm was observed in thiophanate methyl, it was significantly less than 2.73 cm recorded in the control (Table 2). This was followed by 2.23 cm mycelial radial growth in acephate and pongamia oil, 2.03 cm in ethion and endosulfan and 2.00 cm in chlorothalonil; but all were on par with each other. Toxicity of abamectin appeared to continue even in the second week, registering a colony diameter of 1.90 cm. Also, during the second week, interaction of dinocap with the fungus was so severe that there was very less mycelial growth, measuring colony diameter of 1.53 cm and was found to be significantly different from other treatments. The rate of growth of 0.03cm per day indicated that the fungus could not overcome the toxic effect although it registered growth. Iprodion + carbendazim and carbendazim remained at very high level of toxicity and did not allow the mycelium to grow further (Table 2). Machowicz *et al* (1981) also reported that carbendazim limited the growth of *V. lecanii more* than thiophanate - methyl.

The above data show that there was significant interaction among pesticides with the entomopathogenic fungus, which resulted in inhibition of *L. lecanii* growth. Among fungicides, iprodion + carbendazim and carbendazim interaction produced significantly lethal effect

Table 2. Effect of different pesticides on growth of *Lecanicillium lecanii*

Pesticide	Dose/l	Rate of growth 'cm' per day		Total mycelial growth of colony (diameter in 'cm')#	
		7th day	14th day	At 7th day	At 14th day (Cumulative growth)
Endosulfan	2.00ml	0.05	0.05	1.33^{cd}	2.03^{bc}
Acephate	0.75g	0.06	0.06	1.43^{c}	2.23^{bc}
Abamectin	0.60ml	0.07	0.05	1.53^{bc}	1.90^{c}
Ethion	1.00ml	0.06	0.05	1.43^{c}	2.03^{bc}
Pongamia oil	2.00ml	0.08	0.06	1.73^{b}	2.23^{bc}
Chlorothalonil	2.00ml	0.04	0.05	1.13^{d}	2.00^{bc}
Iprodion + Carbendazim	2.00g	0.00	0.00	0.60^{e*}	0.60^{e*}
Carbendazim	2.00g	0.00	0.00	0.60^{e*}	0.60^{e*}
Thiophanate methyl	1.00g	0.06	0.06	1.40^{cd}	2.33^{b}
Dinocap	1.00ml	0.06	0.03	1.43^{c}	1.53^{d}
Control	0.10	0.08	2.03^{a}	2.73^{a}	
CV (%)			12.79	11.22	
CD *(P=0.05)*	-	-	0.28	0.33	
CD *(P=0.01)*	-	-	0.38	0.45	

* initial disc diameter plated at inoculation

\# Mean of five replications

Means in the same column with same alphabet are not significantly different

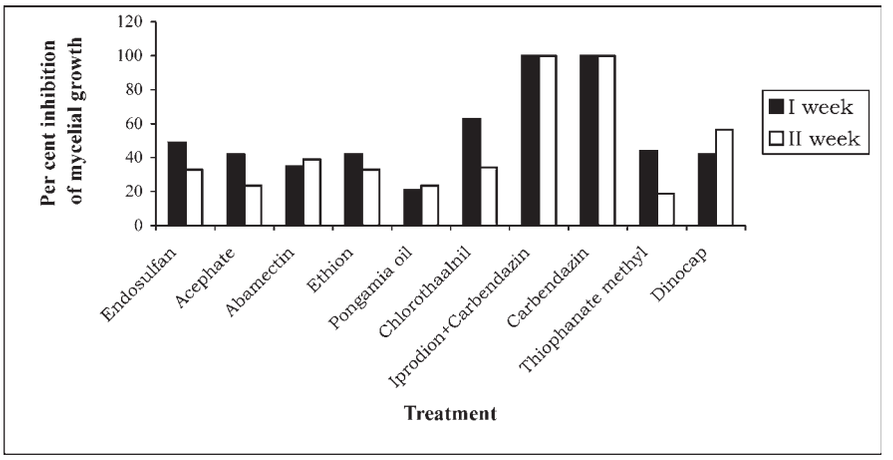

Fig 1. Per cent inhibition (over control) of mycelial growth of *L. lecanii* due to pesticides

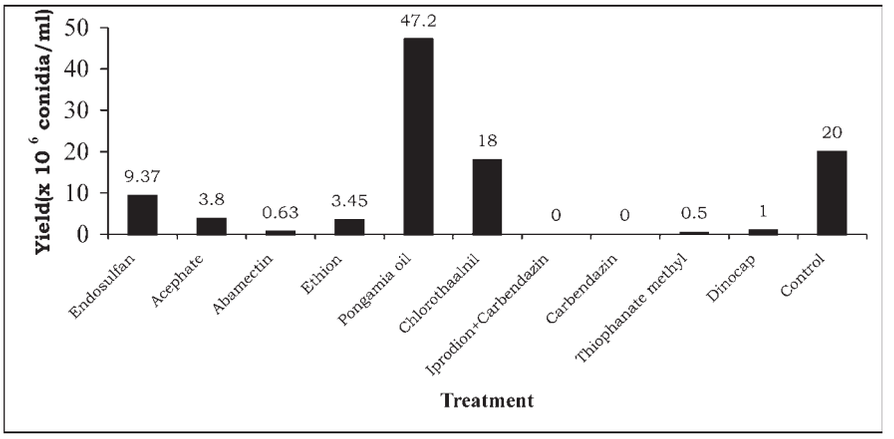

Fig 2. Conidial spore yield *L. lecanii* due to interaction with pesticides on 14th DAI.

on *L. lecanii, so much so* that even after two weeks of association, 100% inhibition of mycelial growth was observed (Fig 1). Similar observations were made by Loureiro E de *et al* (2002) with iprodione used alone. Therefore, these fungicides are considered to be the most toxic and incompatible. Further, as there was no production of spores due to interaction (Fig 2), the pathogen had of no chance revival. During the first week, chlorothalonil, although highly toxic, registering 62.93% inhibition of mycelial growth (Fig 1), became low toxic to the fungus during the second week, indicating that the fungus was able to overcome the toxic effect due to degradation of the fungicide. Degradation of the fungicide was observed in terms of increased mycelial growth, from 1.3 to 2.0 cm. Insecticides such as acephate and endosulfan, the acaricide ethion and fungicides such as dinocap and thiophanate methyl were moderately toxic, inhibiting 41.95 to 48.95% of mycelial growth during the first week. All these pesticides, however, became low toxic during the second week except dinocap, which remained moderately toxic, registering cumulative inhibition of 56.33%. Abamectin and Pongamia oil were low toxic to *L. lecanii* during the first and second weeks of interaction. The above chemicals, with exception of dinocap, thus had low toxicity and did not overly affect mycelial growth in *L. lecanii*. As there was an increase in mycelial growth in the second week due to degradation of pesticides, it may be surmised that there was sporulation after two weeks.

Yield of conidia

Significant difference was observed in conidial yield (Fig 2). Pongamia oil recorded the maximum yield of $47.2x10^6$ conidia/ml, followed by $18x10^6$ conidia/ml in chlorothalonil as against $20x10^6$ conidia/ml in the control, which means that Pongamia oil exhibited a synergistic effect on *L. lecanii* leading to higher yield of conidial spores. *L. lecanii* yielded $9.37x10^6$ and $3.45x10^6$ conidia/ml in endosulfan and ethion. Least conidial production was observed in dinocap and abamectin with 1.0 and $0.63x10^6$ conidia/ml, respectively, followed by $0.50x10^6$ conidia/ml in thiophanate-methyl.

Conidial germination was found to the totally inhibited in the presence of chlorothalonil, iprodion + carbendazim, carbendazim and thiophanate-methyl and,

therefore, these chemicals are highly toxic to *L. lecanii*. As there was, however, low rate of mycelial growth (from the inverted cut block) in chlorothalonil and thiophanate-methyl, these two fungicides can be recommended for need-based application. Thrips, one of the major pests that affect yield and quality of table grapes, is susceptible to *L. lecanii*. Therefore, these sprays in the field, affect the vegetative phase of the fungusless and inoculum of the fungus remains in the ecosystem either for enzootic or epizootic appearance when other conditions are favourable. The above observation is, however, dissimilar from that of Khalil *et al* (1985) who reported that thiophanate-methyl had little effect on conidial germination at both the recommended and sub-lethal concentrations.

Of the pesticides investigated for their compatibility, based on interaction effects on *L. lecanii* during the vegetative phase, only pongamia oil and chlorothalonil can be used along with *L. lecanii*, either alone or in combination with *V. lecanii* for control of pests and diseases. Endosulfan and ethion can be used sparingly because of their low toxicity on mycelium of *L. lecanii*. Also, the fungus yielded at least 50% of conidia as that in control. Batista Filho *et al* (2001), similarly, observed low toxicity of endosulfan to several entomopathogenic fungi including *L. lecanii*. Other treatments such as abamectin, thiophanate-methyl and dinocap resulted in very low production of conidia of *L. lecanii,and hence were* considered to the incompatible. Based on interaction of pesticides with germination of *L. lecanii* conidia, vegetative growth of mycelia and final conidial spore production (which ultimately determines vailability of the inoculum in subsequent generations), it can be considered that the fungus can be best combined with pongamia oil, and followed by chlorothalonil. The fungicides iprodion + carbendazim and carbendazim produced very severe toxic effect on the entomopathogenic fungus and are hence considered incompatible.

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