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Compatibility of neem based oil nimbicidine with entomopathogenic fungi

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The entomopathogenic fungi are one of the insect pathogen with significant host range and host specificity. Growth and conidial survival of entomopathogenic fungi can be affected by environmental factors or by biopesticides and chemical products used to protect crop plants. In this study compatibility of *Beauveria bassiana* and *Verticellium lecanii* with neem based commercial formulation (Nimbecidine) and effect of this pesticide on growth, colony formation and biomass production were studied. The formulations of pesticides were tested in the concentration of 0.2%, 0.4% and 0.6% for Nimbecidine. From this study, interesting observation was noted. Nimbecidine is highly compatible with *B. bassiana* *V. lecanii* at these concentrations.

Bio pesticides /Toxicology

Agriculture is the back bone of Indian economy, and agricultural development is central to all strategies for planned development. The use of plant product chemicals has greatly increased in our country with the advent of high yielding varieties of chemical fertilizers a quantum jump in pest attack and disease infestation were observed leading to chemicalization in plant protection sector. These chemical pesticides are toxic chemicals designed to kill agricultural pests but can also cause problems with human health if exposed to in large amounts. In recent years as growing concern has been expressed about the hazards of excessive use of agro-chemicals, there has been increasing interest in alternative technologies. An emergent contradiction in the contemporary development of biological control is that of the prevalence of the substitution of periodic releases of natural enemies for chemical insecticides and the dominance of biotechnologically developed transgenic crops. The relative abundance of entomopathogenic fungi was estimated for 10 sites in each of indigenous forest, pasture, and cropland habitats by baiting soil samples with Galleria larvae. There are, however, many other fungi that infect and kill mosquitoes at the larval and/or adult stage. In some cases, compatible products may be associated with entomopathogenic fungi, increasing control efficiency (Moino and Alves, 1998 and Quintela and McCoy, 1998). In general, biofertilizers or insecticides used in organic agriculture are not very standardized due to their home made nature, being prepared by farmers themselves or by small companies. Thus, the variability of these products composition might be quite large.

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However, with the recent increase in organic food production, and in the use of products in systems where the entomopathogenic fungi

B. bassiana and *M. anisopliae* are also used, a study on the compatibility among them is needed (Hirose *et al.*, 2001). The present study deals with the compatibility of three biofertilizers and neem oil with *B. bassiana* and *M. anisopliae.*

Materials and Methods

The present work was carried out at Research and Development Center T. Stanes and Co Ltd., which is recognized by Department of scientific and Industrial Research (DSIR), Coimbatore. Potato Carrot Agar (PCA) medium was used for culturing the fungi *Verticillium lecanii* and for culturing *Beauveria bassiana* SDA (Saboraud's Dextrose Agar) medium was utilized. The fungi multiplied in a complete medium and maintained it for 5 days at 27±2°c in BOD incubator. It has been originally isolated by T. Stanes and Co Ltd and Institute of Microbial technology (IMTECH) Chandigarh, India (source- T. Stanes and Co Ltd). The botanical insecticide namely Nimbecidine, was added to the above said entomopathogenic fungi at different concentration to test their influence on mycelial growth and sporulation.

The Petri plates containing the medium were then inoculated with 0.3cm disc of actively growing fungal cultures. For each treatment, two replicas were made. Control plates were maintained on the medium without Nimbicidine. The Petri plates were incubated at 27±2°C and the optimum period of growth was noted. The radial growth of colony was measured in mm on two directions at right angle to each other and the average of two was taken. The percentage inhibitions of fungus growth in various treatments were assessed. The radial mycelial growth of entomopathogenic fungi and percentage of inhibition in the growth of them were studied by poison food technique (PFT). Measured quantity of SDA broth and PDA were inoculated with the culture of entomopathogenic fungi (*Beauveria bassiana and Verticellium lecanii)* respectively form the conical flasks which were already incubated on the shaker. The inoculated conical flasks were incubated for 5-6 days with proper aeration. Then the fungal cultures were exposed to serial dilution to observe the colony formation in the medium treated with different concentration of Nimbecidine.

Results and Discussion

In the present study, significant reduction of radial mycelial growth was recorded against *V. lecanii* and *B. bassiana* with Nimbecidine (Table-1). It was very interesting to note that, after 5 days of incubation there was no growth was recorded in *V. lecanii* irrespective of Nimbecidine treatments (0.2%, 0.4% and 0.6%) whereas on 8the day there was a growth in all the concentrations. This growth was continued even after 11th day. The maximum grown was recorded in 0.2% nimbecidine (21.5mm),then 0.4% (11.0mm) and 0.6% (11.3%) the lowest growth in 0.6% of Nimbecidine might be due to the presence of organic acids, propanic, butyric, malic, succinic and tartaric) in the product (Hirose *et al.* 2001).

Table-1: Radial mycelial growth of *Beauveria bassiana* on nimbecidine treated medium.

% of Nimbecidine concentration	Radial mycelial growth in (mms)											
	5th day				8th day				11th day			
	R 1	R 2	Mean	% of inhibition	R 1	R 2	Mean	% of inhibition	R 1	R 2	Mean	% of inhibition
0	7	6.5	6.7	0	14	12	13	0	20	21	20.5	0
0.2	7	6.5	6.7	0	12	10	11	15.38	13.5	12	14.7	28.01
0.4	6	5.5	5.5	17.91	13	12	11.5	22.38	16	15	15.5	32.25
0.6	4	4	4	40.2	9	8	8.5	34.61	11	11	11	46.34

The antibacterial agent, bacterimycin caused the highest reduction levels for *V. lecanii* with no radial growth and spore germination. The reductions were entirely different from Nimbecidine treatment. Nimbecidine was low to moderately toxic to *V. lecanii* whereas in bacterimycin it was not compatible and also was observed to be highly toxic in action towards the growth of this fungus. Among two different agrochemicals tested (viz.

Nimbecidine and Bacterimycin) both were observed to inhibit to the growth of *M. anisopilae and P. lilacinus.* The inhibition caused by neem oil agreed with the results obtained by Aguda *et al.,* (1986) and Gonzalez *et al.,* (1996), which verified the negative effect caused by neem on *M. anisopliae* germination and conidiogenesis. This was confirmed through the observation made on the radial growth and sporulation in the present study. The observation on the bio-mass weight of the mycelium was also observed to be greatly reduced when the tow biocontrol agents were treated with the Nimbecidine. Bajan *et al.,* (1998) also observed a reduction in the vegetative growth of *B. bassiana* colonies caused by a commercial formulation of neem.

Table -2: Radial mycelial growth of *Verticillium lecanii* on nimbecidine treated medium.

Nimbecidine concentration in (%)	Radial mycelial growth in mms											
	5th day				8th day				11th day			
	R 1	R 2	Mean	% of inhibition	R1	R2	Mean	% of inhibition	R1	R2	Mean	% of inhibition
0	18	17	17.5	0	35	29	32	0	40	38	39	44.87
0.2	0	0	0	100	13	14	13.5	57.81	23	20	21.5	71.79
0.4	0	0	0	100	11	7	9	71.87	12	10	11	70.51
0.6	0	0	0	100	8	7	7.5	76.56	12	11	11.5	0.00

Treatment with least concentration of Nimbecidine showed higher spore count in the culture inoculated medium. *B.bassiana* exhibited higher spore count in 0.2% concentration of Nimbecidine. In case of 0.4% chemical treatment the spore count as 120 CFU/ml, 63CFU/ml was observed. Lower spore count was observed in 0.6% of the Nimbecidine treatment as 36CFU/ml. These results were confirmed by measurement of radial growth. From these results we can conclude that Nimbecidine treatment slightly reduce the growth of *B. bassiana* at 3 different concentrations. Nimbecidine treatment with *Verticellium lecanii* at 0.2 concentrations showed less spore count when compared to the culture grown on the medium without Nimbecidine. 0.4% of chemical pesticide treatment showed spore count as 262CFU/ml, 62CFU/ml. So the result concluded that least concentration showed more spore count than higher concentration. So the additions of Nimbecidine with the culture medium slightly alter the growth of the *Verticellium lecanii.* In addition, under field conditions, vegetative growth inhibition may not be a good indication of fungicidal effects such as spore viability (Loria *et al.,* 1983). It is very necessary to understand that this study suggests that for sustainable farming there should be an integration of the chemicals with biological in disease management, insect management and nutrient supply systems. Along with this we need to stress the conservation of nature, taking note on toxic residues and pollution caused by it. The deposition of chemical residues may lead to a lethal dosage finally, causing various types of cancers in man, especially colon and para colon cancer. This may also be a result of mutation due to the chemicals, affecting the different macromolecules in our body. Though this might not be a visible in the present generation, there are chances of its demonstration in the coming generation. Hence the introduction of integrated Pest Management is necessary in order to reduce the level of chemicals and include more biological, though its action is comparatively slow. In the present study undertaken various concentrations of two different chemicals used. This is to study if the lower concentrations are compatible with the biological. Hence were can reduce the application of chemicals and use more biological.

At some concentrations the agrochemicals were observed to be compatible with these entomopathogens. This study should be extended to the field level in order to confirm the laboratory findings, since various environmental factors like soil, climate, sunlight, humidity etc. affect the survival of the

plant and also of the biological agents. This study has thrown light in demonstrating the compatibility of entomopathogenic fungi with some of the well-known and commonly used botanical pesticide neem oil (Nimbicidine). It is therefore evident that these agrochemicals can be used along with entomopathogenic fungi for the control of insect, pests and for controlling the bacterial diseases at a same time. The various laboratory studies suggest that field evaluations under different biotic and abiotic factors must be done to confirm the compatibility of entomopathogenic fungi with commonly used agrochemicals.

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