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Research Article

Effect of common insecticides on the growth of Entomopathogenicfungi, *Zoophthora radicans* (Brefeld) Batko

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Abstract

Zoophthora radicans was isolated from rice leaf folder cadaver and grown in the Sabouraud dextrose agar yeast extract medium and different insecticide emulsions were mixed separately and tested for growth of fungus. After seven days of inoculation, 80-90 per cent reduction in mycelial growth of *Zoophthora radicans* were reported with triazophos,imidacloprid, chlorpyriphos and cypermethrin and 50-79 per cent reduction in mycelial growth were reported with monocrotophos, quinalphos, spinosad and econeem. After 14 days of inoculation, except chlorpyriphos percent reduction of mycelial growth of triazophos, imidacloprid, cypermethrin decreases and they were in the range of 50-79 per cent. In general chlorpyriphos is most toxic to the fungal growth and 81.64 per cent reduction in mycelial growth was reported when compared to 57.46 per cent reduction in econeem.

Keywords: *Zoophthora radicans*, rice leaf folder, mycelial growth, econeem .

Introduction

By 2020, the world would require 500 million tonnes of milled rice. To achieve this, the rice production has to be enhanced to the tune of 5 tonnes per hectare on an average. One of the constraints for the farmers to obtain this yield is losses caused by insects. When prophylactic insecticides are constantly used, the ecosystem gets disturbed causing pest outbreaks, besides inducing rapid development of insecticide resistance. In the recent past 10 years, insecticide resistance against some active ingredients has been recorded in many places. Insecticide resistance can be a major threat to the sustainability of rice production. (Robert Zeigler, 2011).

The synthetic chemical pesticides have been the widely used approach to reduce the estimated 45% gross crop loss due to pests and diseases that amounted to 290 billion rupees per annum. More and more quantities of synthetic pesticides are being used for agricultural intensification to feed on ever growing population. As the most of insecticides are banned, it becomes inevitable to find alternate, viable and effective biopesticides.

In India, biopesticides represent only 3.00% of the overall pesticide market and is expected to exhibit an annual growth rate of about 2.3% in the coming years. Consumption of biopesticides has increased from 219 metric tonns in 1996-97 to 683 metric tonns in 2010-11 and about 85% of the biopesticides used are neem -based products. Consumption of chemical pesticides has significantly, been reduced from 56,114 metric tonns to 43,584 metric tonns during the same period (Thakore, 2006).

In India, development and use of mycoinsecticides against various pest problems have received greater attention. *Z. radicans* has been isolated from natural infections of various insect pests such as leafhopper and plant hopper (Ben Ze’ev and Kenneth, 1981), larvae of *Helicoverpa armigera, H. punctifera* (Glare and Milner, 1991), larvae of *Plutella xylostella* (Pell *et al.,* 1993) and many other pests. So the present study was conducted to know the compatibility of *Z.radicans* with few selected commercial insecticides.

Materials and Methods

Zoophthora radicans was isolated from rice leaf folder cadaver and grown in the Sabouraud dextrose agar yeast extract medium. The effects of selected insecticides like econeem, chlorpyriphos, cypermethrin, imidacloprid, monocrotophos, quinalphos, spinosad and triazophos on the radial growth of *Z. radicans* were evaluated. The insecticides dose was calculated on the basis of field application rates *viz.*500 lit of spray fluid /ha. The SMA medium of twenty ml was sterilized in each individual boiling tube and the insecticide emulsions of required concentrations were mixed into the melted sterile SMA aseptically, thoroughly mixed, poured into sterile petri dishes and allowed to solidify under laminar flow cabinet. An agar disc along with mycelium mat of *Z. radicans* was taken from the periphery of 10 days old colony by 10 mm diameter cork borer and transferred to the centre of SMA plate. The growth medium (SMA) without insecticide and inoculated with mycelia disc served as untreated check. The plates were sealed with paraffin film and incubated at room temperature for 14 days to allow the maximum growth. Each treatment was replicated thrice. The diameter of growing culture in excess of the plugs in each dish was measured on 7th day after inoculation (DAI) (when radial growth in the control plate fully covered by medium) and also on 14th day after inoculation. The data was expressed as percentage growth inhibition of *Z. radicans* by insecticide treated PDA (Hokkanen and Kotiluoto, 1992).

X=Y-Z/ Y×100 where X, Y, Z stand for percentage of growth inhibition, radial growth of fungus in untreated check and radial growth of fungus in poisoned medium respectively. The pesticides were further classified in evaluation categories of 1-4 scoring index. 1=Harmless, 2=Slightlyharmless, 3= Moderately harmful, 4=Harmful in toxicity tests *in vitro* according to Hassan's classification scheme (Hassan and Charnley, 1989). All the treatments were replicated four times.

Results and Discussion

The influences of different insecticides on the mycelial growth of *Z. radicans* were detailed. All the treatments to the fungal growth. Insecticides like imidacloprid, cypermethrin and triazophos were less supporting showed significant differences relating to control. After seven days of inoculation among the randomly selected insecticides, only chlorpyriphos was greatly inhibiting growth of fungus (9.41 mm) and colony diameter recorded on econeem, quinalphos, monocrotophos and spinosad were 30.33 mm, 21.33 mm, 17.48 mm, 14.67 mm respectively and they were slightly toxic the growth and colony diameter recorded were 13.67 mm, 11.35 mm and 10.33 mm respectively. After fourteen days of inoculation, chlorpyriphos was recorded 12.67 mm and rated as moderately harmful.

However, in further studies, seven insecticides like spinosad, econeem, quinalphos, imidacloprid, triazophos, monocrotophos and cypermethrin recorded mycelial growth of 20 mm, 32.33 mm, 24.50 mm, 16.00 mm, 27.67 mm, 22.34 mm and 28.22 mm and were rated as slightly toxic. In brief, among the selected insecticides, except a few (imidacloprid, triazophos, chlorpyriphos and cypermethrin), all other insecticides can be safely used along with the mycopathogen. While comparing toxicity of insecticides against *Z. radicans* on 7 days and 14 days after inoculation, toxicity of insecticides was reduced on 14 days after inoculation. On 7 DAI, imidacloprid and triazophos were moderately toxic, during 14 DAI the toxicity reduced to slightly toxic. Although the different insecticides tested in the present investigations inhibited the growth of *Z. radicans* the poisoned medium *in vitro*, the combined use of the fungus and insecticides cannot be completely ruled out.

Among the selected insecticides, except a few (imidacloprid, chlorpyriphos, triazophos and cypermethrin), all other insecticides could be carefully used along with the mycopathogen. Chlorpyriphos was moderately toxic (80-90%) on 7and also on 14 days after inoculation based on the Hassan's grade. Li and Holdam (1994) observed extremely detrimental effect of chlorpyriphos, tempephos and malathion to mycelial growth and sporulation of *M. anisopliae*. Chlorpyriphos had been reported to strongly inhibit the growth and sporulation of *B. bassiana* (Barbosa

Table 1. Effect of common insecticides on the growth of *Z. radicans*

S. No.	Insecticides	7 days after inoculation			14 days after inoculation		
		Mean diameter (mm)⋆	Per cent reduction⋆⋆	Grade	Mean diameter (mm)⋆	Per cent reduction⋆⋆	Grade
1.	Triazophos	10.33 [f] (3.22)	85.02 [a] (66.70)	3	27.67 [c] (5.30)	63.60 [d] (52.18)	2
2.	Chlorpyriphos	9.41 [f] (3.06)	86.37 [a] (66.90)	3	12.67 [g] (6.30)	81.64 [a] (64.15)	3
3.	Imidacloprid	13.67 [e] (3.70)	80.19 [b] (64.15)	3	16.00 [f] (4.00)	78.95 [a] (62.87)	2
4.	Monocrotophos	17.48 [d] (4.20)	74.67 [c] (59.30)	2	22.34 [d] (4.70)	67.63 [c] (55.08)	2
5.	Quinalphos	21.33 [c] (4.60)	69.08 [d] (53.10)	2	24.50 [d] (4.90)	67.76 [c] (55.08)	2
6.	Spinosad	14.67 [e] (3.80)	78.74 [b] (60.45)	2	20.00 [e] (4.50)	73.68 [b] (59.31)	2
7.	Econeem	30.33 [b] (5.50)	56.04[e] (47.73)	2	32.33 [b] (5.70)	57.46[e] (49.48)	2
8.	Cypermethrin	11.35 [f] (3.40)	81.24 [b] (64.15)	3	28.22 (5.30)[d]	59.11[e] (36.15)	2
9.	Untreated check	69.00 [a] (8.30)	--	--	76.17 [a] (8.70)	--	--

Each value is mean of four replications. Figures in parenthesis are square root***** /arc sin** transformed values. In a column means followed common letter are not significantly different at five percent level (DMRT)
Grade1 = Harmless (<50% reduction in beneficial capacity), 2= Slightly harmful (50-79%), 3= Moderately harmful (80-90%), 4= Harmful (>90%) in toxicity tests *in vitro* according to Hassan's classification scheme (Hassan, 1989).

and Moreira, 1982) in a dose-dependent manner even at concentrations lower than recommended rates of field use.

While comparing toxicity of insecticides against *Z. radicans*on 7 and 14 days after inoculation, toxicity of insecticides was reduced on 14days after inoculation. On 7 DAI, imidacloprid and triazophos were moderately toxic (80-90%), during 14 DAI its toxicity reduced to slightly toxic (50-79%).The growth of fungus on imidacloprid and triazophos observed in this study is in corroboration with the findings of Kaakeh *et al.* (1997) and Gardner and Kinard (1998). They found that this insecticide slightly toxic to conidial germination but also to mycelial growth. With regard to neem based pesticides, Gupta *et al.* (1999) reported that econeem was slightly harmful to *Beauveria bassiana* and can be combined with fungus to tackle the pest problem. In the current study also, mycelial growth of *Z. radicans* on econeem treated culture was more when compared to chemical insecticides and it shows positive signal in utilizing the neem based pesticides with entomopathogenic fungi for the control of insects.

Some authors reported, emulsible neem oil inhibits mycelia growth and the production and germination of spores of *B. bassiana* (Bajan *et al*., 1998). Other authors do not report fungi toxic effects caused by emulsible oil or by neem seed extract in concentrations above 2.5% (Rodriguez-Lagunes *et al*., 1997). Therefore, to solve this contradiction, further research is needed to investigate the effect of neem on enzymatic activity of *B. bassiana* that would help to make sure the compatibility between these two factors.

Hassan and Charnely, (1989) also observed inconsistent interactions between chemical insecticides and entomopathogenic fungi.

Ghini and Kimati, (2000) emphasized that organophosphate (OP) compounds interfere directly on membrane permeability of the cells and synthesis of enzymes, consequently affecting the metabolic process of the organism. As a result, it inhibits the enzyme that converts phosphatydilethanalamine in to chitin. Probably, the same mechanism of inhibition was responsible for drastic reduction of *P. fumosoroseus* sporulation and vegetative growth. Chemical insecticides cause varied levels of inhibitions of vegetative growth and spore germination of entomopathogen, depending on concentration of active ingredient and chemical nature.

Although the different insecticides tested in the present investigations inhibited the growth of *Z. radicans* in the poisoned medium *in vitro*, the combined use of the fungus and insecticides could not be completely ruled out.

Certain insecticides have been combined at sub-lethal doses with entomopathogenic fungi for obtaining better control of the pest species. For instance, increased mortality due to mycosis of *Beauveria* by addition of reduced doses of insecticides had been established in *Melolontha melolontha* L. (Ferron, 1971). Similarly, Benz (1971) established positive results on the combined use of reduced doses of insecticides with *B.bassiana* for the control of coleopterous insects.

Considering this, it is worth exploring the effects of these insecticides at sub-lethal doses on the fungus as two-in one mix strategy. While doing so, adequate precaution should also be taken because these compounds at sub lethal doses may end up with complications like resurgence of sucking insects. The laboratory results on artificial media may not be reproducible in field as there will be degradation of toxicants. Thus, when the product is determined in the laboratory, no doubt that its selectivity under field condition will stand.

On the other hand, the high toxicity under *in vitro* of a given formulation may suggest similar toxicity under field conditions. However for field studies the inhibition of conidial germination should be the key factor to be considered. It needs field confirmation. The mycopathogen, to be considered as an integral part of plant protection has to meet the requirement of compatibility with many other measures recommended for management of pests. Interval between insecticide spray and the mycopathogen inoculation decides the effectiveness and strategy development for the conjunctive or supplementary use of mycopathogens. New techniques through biotechnology or genetic engineering may be helpful for strain improvement of fungal pathogens for tolerance to agrochemicals.

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