International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(5): 1637-1640

Received: 11-07-2019
Accepted: 15-08-2019

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Pathogenicity of *Metarhizium anisopliae* against *Spodoptera litura* and its compatibility with insecticides

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Abstract
The laboratory experiment was carried out at Plant Pathology Department, College of Agriculture, Nagpur during 2018-19 to assess the pathogenicity of *Metarhizium anisopliae* against *Spodoptera litura* and its compatibility with insectides. The isolates of *Metarhizium anisopliae* were collected from the field and tested against *Spodoptera litura* larvae for its pathogenicity in laboratory condition by leaf dip method. The laboratory study revealed that, the spore suspension of $1x10^9$ spore ml^{-1} recorded highest mortality of 91.66 per cent whereas, the spore suspension of $1x10^5$ spore ml^{-1} recorded minimum mortality of 33.33 per cent. The mortality rate was decreased with decrease in the spore suspension concentration and maximum per cent mortality was recorded as the increase in concentration of the inoculum. Compatibility of *Metarhizium anisopliae* was tested with insecticides *viz.*, imadicloprid, spinosad, chloropyriphos and indoxacarb by poisoned food technique and it was observed that, *Metarhizium anisopliae* slightly tolerate to the toxic effect of spinosad and imidacloprid at the recommended dose of insecticides whereas, chloropyriphos and indoxacarb produced more toxic effect at normal concentration of insecticidal dose.

Keywords: Metarhizium, anisopliae, spodoptera, litura, compatibilify, pathogenicity

Introduction
Use of biological inputs in plant protection started gaining importance as an alternative to chemical pest management practices. There mode of action and specificity make it difficult for the pest to develop resistance easily and are thus more sustainable than their chemical counterparts. Among the biological inputs, entomopathogenic fungi occupy an important place due to their vast host range. They are polyphyletic fungal group of nearly 750 species (Khachatourians and Sohail, 2008) [7]. Their ability to infect and kill insect pests is exploited in agriculture for the control of different insect pests of crops thus reducing the dependence on the hazardous chemical pesticides. Entomopathogenic fungus like *Beauveria bassiana*, *Metarhizium anisopliae*, *Nomuraea rileyi*, *Lecanicillium lecanii* and *Paecillomyces* have been widely researched among entomopahogenic fungi for their bioefficacy and based on that many commercial products have been developed (de Faria and Wraight, 2007) [5]. *Metarhizium anisopliae* is characterized as green muscardine fungus due to green colour of the sporulating colonies. The colony of *M. anisopliae* appeared white when young, but as conidia matured, the colour turned to dark green. The infective unit is conidia or blastospores which germinate and forms short germ tube bearing appresoria with infective peg attach to cuticle. The infective peg penetrates in layer of integument by enzymatic dissolution of chitin and protein. It reaches the haemocoel and internal organs and insect is filled with fungus. The death of insects occurs due to obliteration of tissues also production of toxins (destruxin A,B,C,D,E) and proteolytic enzymes secreted by the fungus. Infected insects show symptoms like loss of appetite, decreased irritability, general or partial paralysis, loss of mobility, discolouration and mummification. The lepidopterous pest causes serious havoc to most of the crops. The leaf eating caterpillar, *Spodoptera litura* Fabricius (Noctuidae; Lepidoptera) is one of the polyphagous and cosmopolitan pests. The defoliator caused serious economic damage to number of field crops and polyhouse crops. *Helicoverpa armigera* (Hubner) is another serious pest in many part of the world and also in India. It has been recorded on more than 200 hosts in India (Pawar, 1998) [12] and annual crop losses due to *H. armigera* in India has been

estimated at around Rs. 2000 crores.The number of micro-organism like fungi, bacteria, viruses, protozoa and nematodes are known to parasitise and kill insects, which have been recently included in management of pest of different crops. Under natural condition, fungi are frequent and often important natural mortality factor in insect population. All groups of insects may be affected and over 700 species of fungi have been recorded as pathogenic. Liu *et al*., (2004) [9] reported that destruxins are insecticidal metabolites of *M. anisopliae*. The structure of destruxins is classified as being a cyclic hexadepsipeptide. More than 35 different destruxins have been characterized with a wide range of insecticidal activities.

Material and Methods

The laboratory experiment was carried out at Laboratory of Plant Pathology Department, College of Agriculture, Nagpur during 2018-19 to assess the pathogenicity of *Metarhizium anisopliae* against *Spodoptera litura* and its compatibility with insectides. The isolates of *Metarhizium anisopliae* were collected from the field and tested against *Spodoptera litura*.

Pathogenicity of ten isolates of *M. anisopliae* was conducted against the *S. litura* larvae. Isolated cultures were grown on SDAY medium. The fungus when grow in medium normally produces metabolites. These metabolites especially that are excreted in the culture could be toxic to the larvae. Fifteen days old culture was taken to evaluate the effectiveness of isolates. The conidia were harvested by adding 5 ml of sterilized 0.05% Tween 80 and scraping out. The suspensions were vortexed for 3 min and then filtered through sterilized cheese cloth and make required volume of spore suspension. The experiment on pathogenicity studies was undertaken on second instar larvae of *Spodoptera litura* by leaf dip method suggested by Ma'Jun (2000) [10]. The treated larvae were incubated at 25 ± 1 ^{0}C and 90 ± 2 per cent humidity. The comparable mortality was recorded and presented in terms of per cent cumulative mortality. The isolate Ma2 is used for further studies.

The different concentrations of spore suspensions were adjusted by simple water dilution method and spore count was taken with the help of haemocytometer. The treated larvae were incubated at 25±1^{0}C and 90±2 per cent humidity. The comparable mortality was recorded after 24 hours interval upto ten days and are presented on per cent cumulative mortality after three, five, seven and ten days after treatment.

Compatibility of four insecticides *viz.,* spinosad, imidachloprid, chloropyriphos and indoxacard on variety of field crops were tested against *M. anisopliae* by adopting poison food technique (Nene and Thaplial, 1979) [11]. The insecticides were tested at recommended dose concentration. For each treatment 100 ml Sabouraud's dextrose agar + yeast extract medium was taken in 250 ml conical flask and autoclaved at 121 ^{0}C for 20 minutes. The specified concentration of insecticide was added at lukewarm temperature and mixed thoroughly by shaking the flask. The poisoned medium was poured in to Petriplates and allowed to solidify. Test fungus culture was cut in to 5 mm disc from the periphery of 10 day old pure culture with sterilized cork borer and transferred to the centre of each plates containing poisoned medium. Controls were maintained by placing fungal disc in medium without insecticides. All the plates were incubated at 27 ± 2 ^{0}C. The whole procedure was carried out under aseptic conditions. The diameter of fungal colony was measured ten days after inoculation and per cent inhibition over the control was calculated by the following equation,

$$I = \frac{C - T}{C} \times 100$$

Where,
I = Inhibition percentage
c = Colony diameter in control (mm)
T = Colony diameter in treatment (mm)

Result and Discussion

Pathogenicity of *M. anasopliae* against *S. litura*

Pathogenesis of disease development in *S. litura* due to *M. anisopliae* was observed. During first 24 hours the larvae showed no changes, feeding normally and responding to external stimuli. The incubation period ranging from 2 to 8 days. Larvae were sluggish and ceased to feed on third to fourth day after treatment then, the body become slightly bent, tough and mummified. Initial growth of fungus was noticed on the seventh days and on eight day whole body covered with white mycelia then, it turned into green colour on the insect body as earlier reported by Kotwal, (2006) [8]. The mortality shown by various isolates of *M. anisopliae* varies in the range of 46.66 to 90.00 per cent. Maximum mortality was recorded by the isolate Ma2 exhibiting 90 per cent followed by Ma9, Ma5 and Ma4 recording 86.66, 83.33 and 80.00 per cent mortality respectively. The lowest mortality was noticed in the isolate Ma3 (46.66%). The pathogenicity of *M. anisopliae* against *Spodoptera lituralis* is also reported by Amer *et al.* (2008) [2].

Effect of spore suspension

The result of the effectiveness of spore suspension increased with increasing concentration and time elapsed after treatment as earlier observed by Amer *et al*. (2008) [2]. The data indicates that highest mortality was noticed in spore concentration 1 x 10^9 spores ml^{-1}, recording 20% mortality in third day and it rapidly increased to 91.66 per cent on tenth day. The mortality increased as incubation period increased and decreased as dilution decreased. The lowest mortality 33.33 per cent at 1 x 10^5 spores ml^{-1} after ten days of treatment. Same results were reported by Han *et al*. (2014) [6] as like, mortality of insects caused by fungus increased with conidial concentration.

Compatibility of *Metarhizium anisopliae* with different insecticides

Four chemical insecticides were tested against *M. anisopliae* by poisoned food technique. Significant differences were observed among all the treatments of four insecticide tested, Spinosad showed the least inhibition 24.28 per cent at normal concentration dose. Imidacloprid were found safe to the fungus as they inhibit 35.71 per cent growth of fungus. Chloropyriphos showed most toxic effect on fungal growth as it inhibits to 74.28 per cent growth (Akbar *et al.* 2012) [1]. Indoxacarb also does not compatible with *M. anisopliae* fungus (Amrutha and Banu 2012) [3], they inhibits upto 71.42 per cent growth. The use of fungi in integrated pest management (IPM) cannot be ignored. A lot of examples exist where application of different selective chemical insecticides and fungi when used in combination provide satisfactory control against many agricultural insect pests (Quintela and McCoy, 1998; Dayakar *et al*., 2000) [13, 4].

Table 1: Pathogenicity of different isolates of *Metarhizium anisopliae* on 2nd instar larvae of *Spodoptera litura* at 10 days after treatment

Sr. No.	Isolate (1×10^9 spore ml^{-1})	Per cent mortality
1	Ma1	70.00
2	Ma2	90.00
3	Ma3	46.66
4	Ma4	80.00
5	Ma5	83.33
6	Ma6	73.33
7	Ma7	56.66
8	Ma8	63.33
9	Ma9	86.66
10	Ma10	66.66

Table 2: Efficacy of *Metarhizium anisopliae* at different concentration of spore on 2nd instar larvae of *Spodoptera litura*

Conc. (spore ml^{-1})	Per cent cumulative mortality (DAT)			
	3 Days**	5 Days***	7 Days***	10 Days***
1 x 10^9	20.00 (4.47)	66.66 (54.73)	80.00 (63.43)	91.66 (73.21)
1 x 10^8	13.33 (3.65)	36.66 (37.26)	56.66 (48.82)	76.66 (61.11)
1 x 10^7	6.66 (2.58)	23.33 (28.88)	46.66 (43.08)	70.00 (56.78)
1 x 10^6	3.33 (1.82)	13.33 (21.41)	23.33 (28.88)	36.66 (37.26)
1 x 10^5	0.00 (0.70)	10.00 (18.43)	20.00 (26.56)	33.33 (35.26)
Control	0.00 (00)	0.00 (00)	0.00 (00)	3.33 (10.51)
SE(m)±	3.33	2.72	2.35	3.26
CD(P=0.01)	13.66	11.15	9.66	13.09

** Figure in parenthesis are square root transformed value
***Figure in parenthesis are arcsin transformed value

Table 3: Compatibility of *Metarhizium anisopliae* with different insecticides

Sr. No.	Treatment	Conc. (%)	Radial mycelial growth in mm	Per cent growth inhibition
1	Imidacloprid 200SL	0.0045	22.50	35.71
2	Spinosad 45SC	0.018	26.50	24.28
3	Chlorpyriphos 20EC	0.05	9.00	74.28
4	Indoxacarb 14.5SC	0.0145	10.00	71.42
5	Control		35.00	-
	SE(m)±		1.69	
	CD (P=0.01)		7.14	

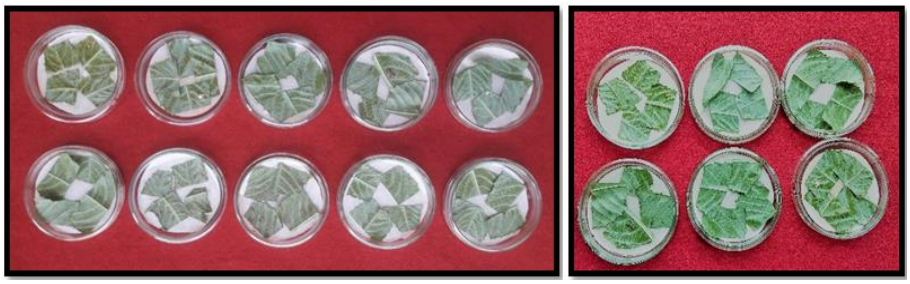
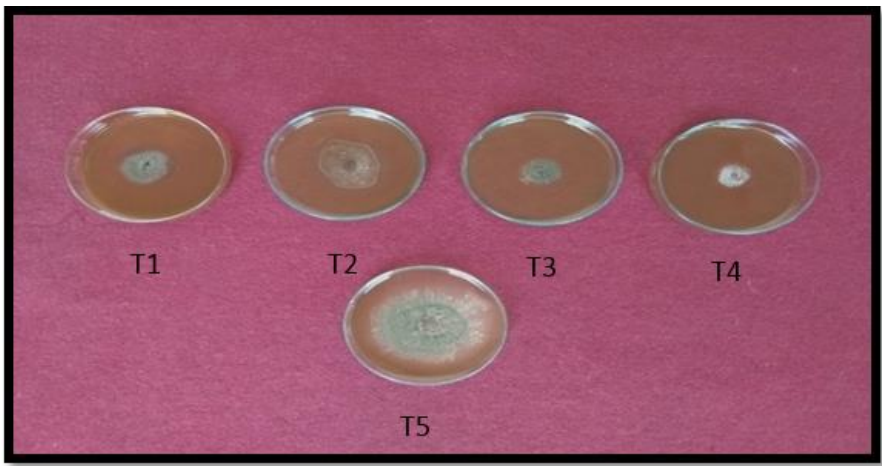


Plate 1: Pathogenicity study and efficacy of spores of *Metarhizium anisopliae* on 2nd instar larvae of *Spodoptera litura* by leaf dip method



T1- Imidachloprid T2- Spinosad T3- Chloropyriphos T4- Indoxacarb T5- Control

Plate 2: Compatibility of *Metarhizium anisopliae* with different insecticides

Plate 3: Effect of *Metarhizium anisopliae* on growth and development of *Spodoptera litura* larvae

Conclusions

Pathogenesis of disease development in *S. litura* due to *M. anisopliae* was observed and maximum mortality was recorded by the isolate Ma2. Among the various spore concentrations $1x10^9$ spore ml^{-1} recorded highest mortality while, $1x10^5$ spore ml^{-1} recorded lowest mortality. The rate of mortality was reduced with reduction in spore concentration. *Metarhizium anisopliae* showed slightly tolerate to the toxic effect of spinosad and imidacloprid whereas, chloropyriphos and indoxacarb produced more toxic effect.

Acknowledgement

Authors are thankful to Associate Dean, College of Agriculture, Nagpur and Head of the Section, Plant Pathology Department, College of Agriculture, Nagpur for providing necessary facilities for conducting the research experiment.

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