JBiopest 8(1):23-27(2015)

Studies on factors influencing the viability of entomopathogenic fungi *Metarhizium anisopliae* in soil adapting culture dependent method

S. Karthick Raja Namasivayam, Aarthi, R. and Anbazhahan, P.

ABSTRACT

Environmental concerns and health risks associated with the use of synthetic chemical pesticides have stimulated efforts to develop biological control agents for integrated pest management. There are a number of microbial agents in which fungi are of special relevance. The present work is carried out to determine the effect of pH, moisture content, temperature and synthetic pesticides on the colony count of *Metarhizium anisopliae* in soil under *in vitro* condition adopting culture dependent methods. Maximum count of fungal colonies was recorded in. pH 7.5, temperature 30^0C, moisture content 80% and all the tested concentration of synthetic pesticides al lower concentration. Compatibility study clearly revealed the possible utilization of the fungal organism in the biointensive integrated pest management programme.

.

MS History: 02.01.2015 (Received)-21.05.2015 (Revised)-28.05.2015 (Accepted)

Key words: *Metarhizhium anisopliae*, soil, abiotic factors, compatibility, colony count, viability.

Citation: S. Karthick Raja Namasivayam, R. Aarthi and P. Anbazhahan. 2015. Studies on factors influencing the viability of entomopathogenic fungi *Metarhizium anisopliae* in soil adapting culture dependent method. *Journal of Biopesticides*, 8(1):23-27.

INTRODUCTION

Synthetic pesticides have been the mainstay of insect pest control for over 50 years. However, insecticide resistance, pest resurgence and concern over the environmental impact of agricultural inputs give urgency to the search for alternative, biologically based forms of pest control (Lal, 2001).

There is growing interest in the exploitation of naturally occurring micro organisms for the control of crop pests, weeds, and diseases. Biological control agents (BCA's) may offer more environmentally friendly alternatives to chemical pesticides.. The development of pest control measures using microorganisms especially entomopathogens has received increasing attention in recent years (Brar *et al.,* 2004, Enkerli *et al.,* 2004). Entomopathogens such as bacteria, virus, fungi, protozoa and nematode, which play a major role in the natural regulation of pest population and if properly utilized can be useful augmentative bio-control agents, their relative specificity to target pest groups, safety to non-target beneficial organisms including host plants, animals and their ability to cause epizootics make them alternative candidates in sustainable pest management Sharma, 2004). Fungi that cause disease in insects are known as entomopathogenic fungi including at least fourteen species of entomophthoraceous fungi attack aphids. (Stalker and Campbell, 1983).

The soil habitat is considered as excellent habitat for insect pathogenic fungi and other microorganisms since it is protected from UV radiation and buffered against extreme biotic and abiotic influences (Sergio *et al.,* 2011). Insect pathogenic fungi in the genera Beauveria, Conidiobolus, Metarhizium and Paecilomyces are all commonly found in the soil (Nicolai *et al*., 2007). Many other fungal species have also been reported on diseased soil-inhabiting insects in various regions of the world and fungal epizootics in soil insect populations are also well documented (Bing and Xing, 2008). *Metarhizium anisopliae* Sorokin Sensu lato (Ascomycetres: Hypocreales) has been studied extensively for the biological control of a wide range of insect pests, fungi belonging to the genera *Metarhizium* have been found to be effective against several species of

insects, including Lepidoptera. *Metarhizium anisopliae* is a widely distributed soil-inhabiting fungus.The spore of *M. anisopliae* can be formulated as dust and sprayable formulation. It is used to control termites, mosquitoes, leaf hopper, beetles etc. *Metarhizium anisopliae*, formerly known as *Entomophthora anisopliae* is a fungal organism that grows naturally in soils throughout the world and causes disease in various insect pests; it thus belongs to the entomopathogenic fungi. It is known to infect over 200 insect species, including termites (Padmaja, 2005; Karthick Raja Namasivayam and Vinoth Kumar, 2009). Due to the increasing awareness about the use of *M. anisopliae* as the biocontrol agent, it is important to determine the effect of various external factors on the viability and other integrated pest components (Goettel and Hajek, 2003). In the present study, the various factors influencing the viability of *M. anisopliae* inoculated in soil has been carried out.

MATERIALS AND METHODS

Soil Sample

M. anisopliae was isolated from the soil sample collected from groundnut field, Chengalpet,Kanchipuram district,Tamil Nadu. A 2 kg soil was collected from 5 points of the sampling site and randomly mixed to obtain homogenous sample, the homogenized soil samples were kept in sterile polythene bags and brought to the laboratory.

Physicochemical of soil analysis

Before microbial analysis, soil sample was processed by the method of Asensio *et al.* (2003). Soil aggregates were broken by hands, trays with soil were kept open until moisture was at equilibrium. Soil texture pH electrical conductivity organic matter nitrate, phosphorous, potassium, calcium, magnesium sulphur, sodium, zinc, iron, copper were determined for all soil collected. These measurements were determined in State Forest Research Institute, Kolappakkam, Chennai, Tamil Nadu.

Isolation of *M.anisopliae*

Soil dilution method was adopted for the isolation of the fungal strain. One gram of homogenized sample was suspended in 99 mL of sterilized distilled water mixed well and serially diluted. 1 mL of aliquots was transferred to sterile petriplates, 20 mL of sterile molten CTC (chloramphenicol, Thiabendazole, cycloheximide media) + PDA Agar media consisting of potato dextrose agar supplemented with 0.5 gm/L Chloramphenicol 0.01 gm, Thiabendazole 0.25 gm, Cyclohexamide was added, allowed to solidify. The seeded plates were incubated at 25°Cin an incubator for 3-7 days. Fungal colonies were isolated after the incubation period, respective fungal colonies were purified and the pure culture was stored on CTC media agar slant. Identification of fungal culture was determined by morphological characteristics and microscopic examination of the spores by lactophenol cotton blue staining (Humber, 1997).

Inoculum preparation

Fungal inoculam was prepared from 7 days old Sabouraud maltose yeast extract agar (SMYA) slant culture by scrapping off with a sterilized glass rod. A homogenous conidial suspension was prepared in sterile distilled water by adding a few drops of the wetting agent Tween 80 (0.01%). The conidial concentration of the suspension was determined using an improved Neubauer haemocytometer (Germany) and used as the source of inoculums.

Soil Assay

Soil sample was collected from University campus in a sterile polythene bags and brought to the laboratory for further study. Effect of factors like moisture content, pH, temperature and pesticides on the viability of fungal spores inoculated in the tested soil was studied. A 25g of finely sieved soil was taken in 100mL thermo stable plastic containers, autoclaved at 15 psi pressure for 20 minutes. The moisture content was adjusted to 10%, 20% 40%, 60%, 80% by the method of Asensio *et al*,2003). One ml (10^8spores/mL) of fungal suspension was inoculated into each of the plastic containers. The samples were incubated at room temperature (37°C) for 15 days. After the incubation period, 1 gram of the sample was transferred to 100 mL of sterile distilled water and serially diluted. From each dilution 0.1mL was spread on potato dextrose agar plates. Following incubation at room temperature the number of colonies in each plate was counted.

pH

Effect of pH on the viability was carried out by addition of phosphate buffer at varying pH as listed in table 1.2.5 mL of Phosphate buffer with different pH thus prepared was added separately to the another set of soil inoculated with the fungal spores as described earlier.

Temperature

Soil samples inoculated with fungal spores was incubated at 30, 40, 50, 60, 70 and 80°C for 15 days. After the incubation period, the viability test was carried out as described earlier.

Chemical pesticides

The entomopathogenic fungi are considered as important factor of insect population reduction. So, there is the necessity of entomopathogens conservation. However for their conservation, it is important to know the pathogens compatibility with other agricultural practices to avoid losses of control efficiency. The present study deals with the compatibility of three pesticides of varying concentrations with *M. anisopliae.* The three pesticides included Agent plus (lambda cyhalothrin), Ekalux (quinolphos) and Hostathion (triazophos). Pesticides were prepared with different concentrations as 0.02, 0.04 and 0.06 % using sterile distilled water and mixed in the soil inoculated with the fungal spores. Viability study was carried out as described earlier.

RESULT AND DISCUSSION

Soil colour (light brown), Texture (silly loan), Lime status (Purified) were recorded along with EC (ds/m) (0.17), pH (7.6), nitrogen (51.7), phosphorus (11.0) and Potassium (105.0) level was also observed. Based on the observation of colony morphology as well as the size and shape of conidia, the isolated fungus was identified as *M. anisopliae*. Colonies of this fungus grow rapidly on CTC media showing dark green aerial mycelia, Conidia are some variant of ovoid to elongate. Soil physico-chemical parameters highly influenced the natural occurrence of the fungi. *M. anisopliae* isolated from the respective soil sample reveals high nutrient contents.This may favour the viability of the fungal spore and thus improved the natural occurrence of the fungi. In the present study, factors influencing the viability of fungal organism have been carried out under soil assay. Among the different moisture content tested, maximum count was recorded at 80%.Effect of moisture content on the viability and infectivity of entomopathogenic fungi has been reported earlier (Gillespie *et al.*, 1986; Thiago *et al.*, 2008). Effect of pH on the fungal viability was also studied (Table 2). Similar work was carried out by Hallsworth *et al.* (1996) who gave consistent result that *M. anisopliae* can grow over a pH range of 2.5-10.5. Among the different temperature tested, maximum fungal colony count was recorded at 30^0C 78.1×10^8 CFU/g followed by 40^0 (65.0×10^8 CFU /mL). Fungal count was not recorded at 60, 70 and 80^0C (Table 1).Temperature, in particular, is known to have a detrimental effect on biological parameters of *M. anisopliae* (Morley, 1996). This finding was supported by Kershaw *et al.* (1999) who stated that besides virulence and host specificity, temperature is an important factor in selecting an isolate for development as a mycoinsecticide. Most isolates grow well between 15^0C and 30^0C, although some develop at temperatures as low as 5-10^0C and others grow even at 35 - 40^0C.

Table 1. Effect of moisture (%), pH, temperature (^{0}C) content on colony count of *M. anisopliae.*

Parameters	Units	Colony Forming Units (CFU/g)
Moisture content	10	37×10^8
	20	54.7×10^8
	40	73.6×10^8
	60	75.3×10^8
	80	**15.0×10^9 [a]**
pH	5.5	$4.2\times\mathbf{10^8}$
	6.0	$12.0\times\mathbf{10^8}$
	6.5	$31.4\times\mathbf{10^8}$
	7.0	**77.2 [a]$\times10^8$**
	7.5	$11.8\times\mathbf{10^8}$
	8.0	$3.5\times\mathbf{10^8}$
	8.5	$3.4\times\mathbf{10^8}$
Temperature	**30**	**78.1 [a]$\times10^8$**
	40	$65.0\times\mathbf{10^8}$
	50	$50.6\times\mathbf{10^8}$
	60	-
	70	-
	80	-

Compatibility of *M. anisopliae* with the synthetic chemical pesticides was studied. In this study the entomopathogenic fungi *M. anisopliae* showed compatibility with all the three tested synthetic pesticides- lambda cyhalothrin, quinolphos and triazophos at lower concentrations (Table 2). When the concentration was increased the fungi was moderately compatible indicating that at an increasing concentration the fungal colony count was decreased. This finding was in accordance with the work done by Dinalva *et al.* (2005), who evaluated the action of pesticides in soil and his findings indicated that the action of pesticides on the fungus present in the soil was highly discrete, suggesting that these agents can be used in combination in agro-ecosystems without compromising fungal activity. Several studies on the

effect of pesticides for entomopathogenic fungi have been reported, but few researchers have performed fungus pathogenicity tests under the

Table 2. Effect of chemical pesticides (in mL) on *M. anisopliae* colony [CFU/g ($\times 10^8$ spores/mL)]

Pesticides	CFU/g ($\times 10^8$ spores/mL)			
	control	**0.02mL**	**0.04mL**	**0.06mL**
Agent plus-Lambda Cyhalothrin	123.5	48.0	33.7	28.5 [a]
Ekalux-Quinakphos	118.6	42.2	36.9	31.3 [a]
Hostathion-Triazophos	109.4	39.6	32.4	25.0 [a]

In column, the mean followed by the alphabet is statistically significant (P<0.05) by DMRT

impact of pesticides in the soil. Li *et al.* (1994) obtained results for the pathogenicity of *M. anisopliae* after application of pesticides, did not affect larva survival and no differences were found among pesticide treatments, or between these and the control. Compatibility of plant based pesticides with entomopathogenic fungi has been reported (Sahayaraj *et al.*, 2011).Further study under field trial will helpful to determine the factors which influence the viability of fungal organism

ACKNOWLEDGEMENT

We acknowledge State Forest Research Institute, Kolappakkam, Chennai, Tamil Nadu for the soil analysis.

REFERENCES

Enkerli, J. and Windsurf Nd Keffr, S. 2004. Long term field persistence of *Beauveria brongniarti* strains applied as biocontrol agents' agents European cockchafer larvae in Switterland. *Biological Control*, **29**:115-123.

Asensio, A., Carbonell, T., Jimenez, L. and Liorca, L. 2003. Entomopathogenic fungi in soils from Alicants province. *Spain Journal of Agricultural Research*, **1**: 37-45.

Bing, D.S. and Xing, Z. L. 2008. Occurrence and diversity of insect-associated fungi in natural soils in China. *Applied Soil Ecology*, **39**:100–108.

Brar, K., Dhiruga, K. and Kaul, N. 2004. The influence of sowing and harvesting date of the yield and yield attributes of four groundnut genotype planted during summer. *Indian Journal of Environment and Ecophysiology*,**2**(1): 185-188.

Clark, M. 1997. Microorganisms standard isolation techniques, *Academic Press*, London, 271-275. **PP.**

Dinalva A Mochi, Antonio C Monteiro and Jose C Barbosa 2005. Action of Pesticides to *Metarhizium anisopliae* in soil. *Neotropical Entomology*, **34**(6): 961-971.

Dinalva A Mochi, Antonio C Monteiro, Sergio A De Bortoli, Hayda O S Doria and Jose C Barbosa. 2006. Pathogenicity of *Metarhizium anisopliae* for *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) in soil with different pesticides. *Neotropical Entomology*, **35**(3): 121-127.

Goettel, M. S. and Hajek, A. E. 2000. Evaluation of non target effects of pathogens used for management of arthropods. *Evaluating indirect ecological effects of biological control*, 81-97 **PP**.

Hallsworth, J. E. and Morgan, N. 1996. Culture age, temperature and pH affect the polyol and trehalose contents of fungal propagules. *Applied Environmental Microbiology*, **62:** 2435-2442.

Karthick Raja Namasivayam, S. and Vinoth Kumar, P. 2009. Influence of media on the pathogenicity of *Metarhizium anisopliae* (M.) Sorokin against *Chilo partellus* (Swinhoe). *Journal of Biopesticides*, **2**(1): 92-93.

Kershaw, M. J., Moorhouse, E. R., Bateman, R., Reynolds, S. E. and Charnley, A. K. 1999. The role of destruxins in the pathogenicity of *Metarhizium anisopliae* for 3 species of insect. *Journal of Invertebrate pathology*, **74**: 213-223.

Lal, R. 2001. Thematic evolution of ISTRO: transition in scientific issues and research focus from 1955 to 2000. *Soil and Tillage Research*, **61**(3): 3-12.

Li, D. P. and Holdom, D. G. 1994. Effects of pesticides on growth and sporulation of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes). *Journal of Invertebrate Pathology*, **63**: 209-211.

Morley, D. 1996. Screening of *Metarhizium* and *Beaveria* spp. Conidia with exposure to simulated sunlight and a range of temperatures. *Mycology resistance*, **100:** 31-38.

Nicolai V. Meyling and Jørgen Eilenberg 2007. Ecology of the entomopathogenic fungi

Beauveria bassiana and Metarhizium anisopliae in temperate agroecosystems: Potential for conservation biological control. *Biological Control,* **43:**145–155.

Padmaja, V. 2005. Role of entomopathogenic fungi in insect pest management. In, Ignachimuthu, S.J and Jayaraj, S. (eds.) Green pesticides for integrated management. Narosa publishing house, New Delhi 324.

Sahayaraj, K., Karthick Raja Namasivayam, S. and Martin Rathi, J. 2011. Compatibility of entomopathogenic fungi with extracts and commercial botanicals. *African Journal of Biotechnology,* **10**(6): 933-936.

Sharma, 2004. Biocontrol management of pest in organic forming *Agrobios Newsletter*,2: 12-15.

Sergio R. Sánchez-Peña, Jorge San-Juan Lara and Raúl Medin. 2011. Occurrence of entomopathogenic Fungi from Agricultural and Natural Ecosystems in Saltillo, México, and their Virulence Towards Thrips and Whiteflies. *Journal of Insect Sciences*, **11**;1-14.

Stalker, H. and Campbell, W. 1983. Resistance of wild species of peanut in insect campus. *Peanut Sciences*, **10:** 30-33.

S. Karthick Raja Namasivayam*, R. Aarthi and P. Anbazhahan

Department of Biotechnology, Sathyabama University, Chennai, Tamil Nadu, India

*Communication author

Tel: 91-44-24501644, Fax: (44-24512344);

Email: biologiask@gmail.com