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Metarhizium based mycoinsecticides for forest pest management

O. K. Remadevi, T. O. Sasidharan*, M. Balachander and N. Sapna Bai*

ABSTRACT

Explorative surveys were conducted to collect native isolates of the entomopathogenic fungus, *Metarhizium anisopliae* from forest pests and soil to study their potential for developing mycoinsecticides for management of selected forest pests. Nine promising isolates identified from among a collection of 25 isolates were examined for their pathogenicity against the forest pests, *Hyblaea puera, Paliga machoeralis, Spilarctia obliqua, Hypsipyla robusta* and *Odontotermes* spp. Besides this, the comparative conidia germination of the isolates under selected cultural conditions was also examined to identify the candidate isolates for pesticide development. The LC_{50} of the isolates ranged from 0.01 $x10^5$ to 759.21 $x10^5$ for the different pests. Isolate Ma2 was the most virulent against *H. puera, P. machoeralis* and *Odontotermes* sp. with significantly low LC_{50} values, viz., 0.65, 0.11 and 0.01$x10^5$ conidia, respectively for the three pests. Ma7 showed lowest LC_{50} of 1.20 and 1.77$x10^5$ spores against *S. obliqua and H. robusta*. Ma13 also showed high virulence against *Odontotermes* sp. The isolate Ma2 showed significantly higher germination under all the conditions evaluated. Radial growth and biomass, which are critical factors for selecting a strain for mass production, was also highest in Ma2 (64.5mm and 5.1gram/lit) followed by Ma7 (42.5mm and 4.6gram/lit). These observations indicate at least two of these isolates hold good potential for formulating a biopesticide for forest pest management.

Key Words: Forest pests, mycoinsecticides, *Metarhizium anisopliae*, pest management

INTRODUCTION

Teak is one of the most valuable timber species in tropical forests of India. Defoliation in teak is caused by two major insects pests, *Hyblaea puera* Cramer (Lepidoptera: Hyblaeidae) and *Paliga machoeralis* Walker (Lepidoptera:Pyralidae). Repeated stripping in the same season by these defoliators adversely affects the tree growth, resulting in qualitative and quantitative loss of timber (Priya and Bhat, 1997). The shoot borer, *Hypsipyla robusta* (Moore) (Lepidoptera: Pyralidae) is a serious pest of meliaceous forest trees in India, such as *Swietenia macrophylla* King, *S. mahogany* Jacq. and *Toona ciliata* M. Roem. Although *Swietenia* sp are grown in plantations in many states, the establishment is difficult because of shoot borer attack during the sapling stage (Newton *et al*., 1993). The Bihar hairy caterpillar, *Spilarctia obliqua* (Walker) (Lepidoptera: Arctiidae) is a polyphagous pest having a very wide range of host plants. It has been recorded feeding on as many as 33 host plants including many agricultural crops and garden plants and also on numerous species of forest shrubs and trees (Yadav *et al*., 2001). Many termite species also cause severe economic damage to timber and timber products, living plants and even plastics (Rath, 2000). In India, estimated losses caused by termites are reported to range between 10 and 50% (Remadevi *et al*., 2008). The arboreal termites, especially *Odontotermes* spp. are common in plantations of many timber yielding species in India and their management is possible only for shorter duration with the application of insecticides like Chlorpyriphos (Remadevi *et al*., 1998).

Chemical methods are largely unsuitable for vast areas of plantation due to the potential hazards and damage they inflict on the environment. Continuous use of pesticides chemicals has resulted in adverse impact on humans, animals and the environment due to toxic effects in food chain, development of resistance in target pests and destruction of natural enemies (Joshi *et al*., 2000, Paray and Rajabalee, 1997). Due to these ill effects of pesticides, alternative ecofriendly strategies are being increasingly appreciated and practiced for pest management (Padmaja *et al*., 2005). Therefore, more safe agents with appropriate application methods need to be identified to meet the current challenges. Biopesticides have been proved as one of the feasible alternatives to chemicals in agriculture in recent times and therefore merit for a large scale try in forestry too, especially in large plantations, obviously with carefully designed application methods.

The present study was aimed at identifying promising native isolates of *Metarhizium anisopliae* effective against selected forest pest insects. Development and application of formulations of the identified isolates could supplement existing control methods and reduce dependence on synthetic chemicals.

MATERIALS AND METHODS

Survey, collection and isolation of fungal strains

Regular surveys were conducted in nurseries and plantations in the states of Karnataka, Andhra Pradesh, Kerala and Tamil Nadu. Diseased insect specimens and soil samples were collected. The samples were brought to the laboratory for observation and isolation of any fungal pathogen using Potato Dextrose Agar medium (HiMedia) fortified with 1% yeast extract at 26 ± 1° C in darkness. The isolated *Metarhizium* colonies were further purified through repeated sub-culturing and preserved in cold. Isolates Ma1, Ma2 and Ma3 were collected from mummified lepidopteran larvae. Ma4 and Ma7 were recovered from the teak skeletoniser pest, *Eutectona machoeralis* (Lepidoptera). Ma5 was isolated from teak tree orchard soil. Ma13 was obtained from a coleopteran host while Ma19 and Ma20 were isolated from unknown host insects.

Pathogenicity of *Metarhizium* isolates

Of the twenty five isolates, based on an initial screening with a single concentration (10^7 conidia ml^{-1}), nine virulent isolates were selected for multiple concentration assay (10^4, 10^5, 10^6 and 10^7 conidia ml^{-1}) against *H. puera*, *P. machoeralis*, *S. obliqua*, *H. robusta* and *Odontotermes* sp at 26±1°C. Third instar larvae of the four lepidopterans were dipped separately in the spore suspensions and transferred to petri plates containing artificial diet/host plant leaves. For *Odontotermes* sp, 1ml of each fungal suspension was poured into a sterile petri plate and allowed to dry partially. The field collected workers of the termite species were allowed to walk on the dried fungal suspension in the Petri plate for 1min. The contaminated workers were transferred aseptically to sterile Petri plates containing small blocks of rubber wood and sealed in a sterile plastic bag containing moist cloth and incubated at 26±1°C in dark. Mortality was observed at 24 h intervals up to eight days. Mycelia growth and conidia formation were confirmed by further incubation of the cadavers at 26±1°C and 90±1% RH.

Germination response of the fungal isolates

Based on the results of bioassay, three isolates which had the lowest LC_{50} against the tested pests were chosen to study their germination response in PDAY medium with different cultural conditions (temperature, RH and darkness). Germination was assessed after 14hrs post inoculation. Percentage of germination was determined by counting 100 spores for each isolate at 400x magnification. The radial growth was estimated by inoculating 5mm fungus culture disc in to PDAY plates and biomass by inoculating 10ml of spore suspension ($3x10^6$ conidia ml^{-1}) in PDAY broth. The daily growth was measured by measuring the colony diameter up to 7 days and biomass production was determined at the end of 7days by dry weight method.

Data analysis

One way ANOVA was performed to analyse the germination response of the three isolates. Probit analysis was used to determine LC_{50} of the nine selected promising isolates (Finney, 1971).

RESULTS AND DISCUSSION

Among the nine isolates tested, Ma2 showed lowest LC_{50} value against *H. puera*, *P. machoeralis* and *Odontotermes* sp. followed by Ma19 for *H. puera*, Ma7 for *P. machoeralis* and Ma13 for *Odontotermes* sp. Ma7 showed lowest LC_{50} value for *S. obliqua* and *H. robusta* (Table 1). Several

Table 1. LC_{50} of selected pests exposed to *Metarhizium anisopliae* isolates

Isolates	*H. puera*	*P. machoeralis*	*S.obliqua*	*H. robusta*	*Odontotermes* sp.
Ma1	$1.98x10^5$	$00.61 x10^5$	$39.93 x10^5$	$42.95 x10^5$	$0.08 x10^5$
Ma2	$0.65 x10^{5*}$	$00.11 x10^5$	$02.11 x10^5$	$49.86 x10^5$	$0.01 x10^{5*}$
Ma3	$22.09 x10^5$	$04.72 x10^5$	$15.23 x10^5$	$26.99 x10^5$	$0.03 x10^{5*}$
Ma4	$46.62 x10^5$	$77.64 x10^5$	$54.73 x10^5$	$21.81 x10^5$	$0.27 x10^5$
Ma5	$201.92 x10^5$	$60.12 x10^5$	$157.60 x10^5$	-nil-	$0.04 x10^5$
Ma7	$1.67 x10^5$	$0.15 x10^{5*}$	$01.20 x10^{5*}$	$1.77 x10^5$	$1.31 x10^5$
Ma13	$123.14 x10^5$	$32.33 x10^5$	$759.21 x10^5$	$39.70 x10^5$	$0.02 x10^{5*}$
Ma19	$0.88 x10^5$	$46.34 x10^5$	$78.62 x10^5$	$61.07 x10^5$	$0.44 x10^5$
Ma20	$7.84 x10^5$	$13.86 x10^5$	$24.87 x10^5$	$126.27 x10^5$	$1.30 x10^5$

isolates of *Metarhizium anisopliae* tested in this study demonstrated high levels of virulence against the pests under laboratory conditions. Three isolates, Ma2, Ma7 and Ma13 demonstrated high virulence for all the five pests studied. Pandit and Samanta (1995) studied the effect of *B. bassiana* and *M. anisopliae* on *Spilarctia obliqua* resulting in mortality of 75.91%. *Odontotermes obesus* collected from an agro forestry location showed 43.67% mortality when treated with muscardine fungi (Khan *et al*., 1993). Conidia from *M. anisopliae var. dcjhyium* were highly virulent to *O. formosanus,* causing approximately 100% mortality when treated with a concentration of 3 x 10^8 conidia/ml.

The germination response was significantly higher ($P \leq 0.01$) for Ma2 under all the cultural conditions studied. Ma7 ranked second in germination response (Table 2). Radial growth and biomass were significantly higher in the case of Ma2 which showed a growth of 64.5 mm and biomass of 5.1g per litre of broth after 7 days of inoculation ($P \leq 0.01$). Ma7 recorded 42.5 mm radial growth and 4.6g biomass whereas Ma13 showed 32.2 mm growth and 3.2g biomass.

Table 2. Germination response of three promising isolates to different cultural conditions

Isolates	Conidia germination(%)				
	PDAY medium at pH 6.0				
	26±0.2 ºC	28±0.2 ºC	28±0.2 ºC +RH 95±5%	28±0.2 ºC +RH 95±5% +Darkness	Storage at 4 ºC
Ma2	99.6	95.6	98.3	97.3	97.0
Ma7	86.6	79.3	94.0	81.6	83.0
Ma13	92.0	78.0	93.3	77.6	92.0
CD($P \leq 0.05$)	1.02687	1.79097	0.79844	1.72722	0.99652
CD($P \leq 0.05$)	1.35308	2.35892	1.05164	2.28535	1.31397

Radial growth and biomass which are also critical factors for selection of the most efficient isolate, were higher for the three virulent isolates. The germination of the isolate Ma2 under the tested conditions was more promising than the other two isolates. Germination of *B. bassiana* and *M. anisopliae* was reported to be declining at the upper temperature limit of 35ºC (Walstad *et al.,* 1970). Studies that elucidate the factors affecting host-pathogen interactions will be of great value in the selection of hyper virulent strains for the future. Other characteristics besides different mass production protocols must also be evaluated before contemplating on the development of a biopesticide using these isolates. Fungal epizootics were reported in *H. puera* by Sandhu *et al.* (1993) which indicates prospects of augmentative pest control using *M. anisopliae* in forestry. The present study indicates the prospects of developing a mycoinsecticide from the selected isolates for managing the above studied forest pests.

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O. K. Remadevi, T. O. Sasidharan*, M. Balachander, and N. Sapna Bai*

Institute of Wood Science and Technology, Bangalore 560 003, Karnataka, India. Fax: 91-080-23340529, Phone: 91-080-22190153, Email: okremadevi@icfre.org, E-mail: okremadevi@gmail.com

*Ashoka Trust for Research in Ecology and the Environment, Royal Enclave, Srirampura, Jakkur P.O., Bangalore 560064, Karnataka, India.

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