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Eco friendly and Economic Farm Level Production Method for *Metarhizium anisopliae*

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ABSTRACT

Entomo pathogenic fungi play a crucial role in any IPM programme. Among these fungi, the green muscardine fungus *Metarhizium anisopliae* (Metschnikoff) Sorokin is an important one due to its effectiveness against a wide range of insect pests including soil inhabiting ones and grubs of rhinoceros beetle. Adoption of *Metarhizium* for managing rhinoceros beetle grubs as manure pit application is not gaining popularity due to the non availability of formulation. On farm production method involving solid state fermentation system has the lacuna of availing pure culture, maintaining the culture and its high cost. Moreover, there are chances of occurrences of health problems by inhalation of conidia produced as dry powder form in the on farm production methods practiced nowadays .In this backdrop, present study has been conducted, wherein 2 economic media *viz*. coconut water and rice gruel water with and without addition of sugar and two types of inoculation methods *i.e.* inoculating with pure culture of fungus and with talc based formulation were evaluated. The results revealed that coconut water inoculated with talc based formulation was superior in supporting growth of the fungus and produced more number of colony forming units, which was at par with that of potato dextrose broth inoculated with pure culture of fungus. Hence, the farmers can exploit the possibility of utilizing talc based formulation of *Metarhizium* purchased from a reputed and reliable source for on farm multiplication using coconut water as it is easily available and economical.

Key Words:,Coconut water, Metarhizium anisopliae, On farm production method, Talc based formulation

INTRODUCTION

Adoption and success of integrated pest management depend on the availability of eco friendly pest management options. *Metarhizium anisopliae*, commonly called as green muscardine fungus is a broad spectrum entomo pathogenic fungus, which is highly effective to various groups of insect pests. In different agro-ecosystems, entomo pathogenic fungi are employed as bio control agents for reducing pest population and consequently their damages (Inglis *et al,* 2001). In the present era of good agricultural practices; safe food production methods demand hugely onbio agents for pest management. Ability of *Metarhizium* to suppress immature stages of rhinoceros beetle is a boon to farmers in Kerala where coconut is a major crop with rhinoceros beetle being its important pest.

Management of rhinoceros beetle in immature stage is highly advantageous to the farmers since it not only reduces the damage by rhinoceros beetle, but reduces the chances of red palm weevil, diseases like bud rot and leaf rot. Recommended practice of crown cleaning and filling with sand and botanicals/ insecticides are not widely practiced among farmers due to high cost and non-availability of skilled labour. Application of *Metarhizium* as talc based / liquid formulation in manure pits is highly effective in managing immature stages of rhinoceros beetle (Varma, 2013). *Metarhizium* multiplied in coconut water medium effectively controlled rhinoceros beetle grubs without harming earthworms in vermicomposting tanks (Gopal *et al,* 2006). Non availability of formulation leads to its poor adoption. If there is a suitable on farm production method,

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adoption of this bio agent for pest management would automatically increase. Limitation to the present system of on farm production method adopting solid state fermentation are the high cost of mother culture, difficulty in availing the pure culture and its maintenance, which requires laboratory skills. Under these circumstances, a laboratory study was conducted to evaluate different economic liquid media and methods of inoculation for on farm production of *Metarhizium*.

MATERIALS AND METHODS

The study was undertaken in the bio control laboratory of KrishiVigyan Kendra Kollam located at 8.99^0N and 76.82^0E, during the year 2017-18.The experiment was laid out in completely randomized design with 10 treatments and 3 replications. Two liquid media *viz.* coconut water and rice gruel water were utilized with and without addition of 2g sugar along with potato dextrose broth under two inoculation methods(T_1- Coconut water inoculated with talc based formulation (tbf) of fungus, T_2- Coconut water inoculated with pure culture of fungus, T_3-Coconut water +sugar inoculated with tbf of fungus, T_4- Coconut water +sugar inoculated with pure culture of fungus,T_5-Rice gruel water inoculated with tbf of fungus of fungus,T_6-Rice gruel water inoculated with pure culture of fungus,T_7- Rice gruel water +sugar inoculated with tbf of fungus,T_8- Rice gruel water +sugar inoculated with pure culture of fungus,T_9-Potato dextrose broth(PDB) inoculated with tbf of fungus,T_{10}-PDB inoculated with pure culture of fungus).Two inoculation methods evaluated were-inoculating the sterilized medium with mother culture of the fungus and inoculating with 1g of talc based formulation having minimum cfu of $1x10^8$.

***Preparation of pure culture of fungus*:** Mother culture of *Metarhizium anisopliae* from Kerala Agricultural University obtained in potato dextrose agar (PDA) slants were sub cultured in PDA slants and petri plates in PDA medium under sterile condition in a laminar air flow cabinet using inoculation loop and kept under room temperature for completion of mycelial growth, which were used for mass production.

***Preparation of talc based formulation(tbf)*:** This was achieved by growing fungus as a mat over potato dextrose broth under liquid state fermentation system and mixing it with sterile talc at optimum moisture and colony forming units(cfu) levels.

Potato dextrose broth(PDB):For 1litre of broth, 200g thoroughly washed, sliced potatoes were boiled in a litre of water for about 30minutes. After the potatoes were being cooked well, broth was separated by straining. Twenty grams of dextrose was added to this hot broth and dissolved. The potato dextrose broth (PDB) thus prepared would be transferred to sterile conical flasks and sterilized using pressure cooker.

***Preparation of media and sterilization*:** Two easily available and economic liquid media i.e. rice gruel water (*kanjivellam* in Malayalam) and coconut water was evaluated in the study. 100ml each of coconut water and rice gruel water was collected and filtered using muslin cloth, which was taken in 250ml conical flasks and sterilized using a pressure cooker. To another set of these two media 2g sugar was added before sterilization.

Pressure cooker was kept on high flame in a LPG stove till 3whistles were blown, and then the flame was lowered to minimum for 20m before putting off. After cooling under normal room temperature, the media were taken out for inoculation.

***Inoculation*:** Two methods of inoculation were done on each set of media. One set of media was inoculated using normal procedure i.e. inoculation using pure culture maintained in a petriplate with a sterile inoculation loop under laminar air flow cabinet. Talc based formulation of 1g *Metarhizium* was added to another set of media using a sterile spatula. After inoculation the flasks were incubated at room temperature (26 ± 2^0C) till the surface of media was fully covered with mycelium. Observations on speed of mycelial mat coverage (no. of days taken for complete spread of

Table1. Effect of different media and methods of inoculation on different parameters.

Sr. No	Speed of growth (days)	Biomass produced (g)	Population of *Metarhizium* at different dilutions (cfu/g)		
			dilution (10^{-10})	dilution (10^{-11})	dilution (10^{-12})
T_1	3.67	4.35	180.33	143.00	136.33
T_2	3.67	3.30	75.67	66.00	69.33
T_3	3.67	3.32	93.67	77.33	50.00
T_4	3.67	1.87	77.33	47.33	20.33
T_5	13.67	0.62	68.33	43.33	33.00
T_6	7.33	1.60	29.67	24.00	20.00
T_7	14.00	0.73	84.00	46.33	44.33
T_8	7.33	1.53	148.00	131.00	97.00
T_9	3.67	9.39	61.67	53.33	36.67
T_{10}	3.67	4.76	68.67	55.67	41.33
CD	1.319	1.148	NS	NS	NS
CV	12.04	21.411	77.406	84.863	103.607

mycelial mat over the medium), biomass produced (grams/100mlmedium) were taken.

After the complete spread of mycelium, media along with mycelium produced were mixed thoroughly with 500g sterile talc separately under aseptic condition. One gram of this talc based formulation was taken and subjected to serial dilution. The dilutions of 10^{-10}, $10^{-11}$and 10^{-12}were plated in PDA medium then incubated under room temperature. Numbers of colony forming units were counted and recorded 3days after of inoculation.

RESULTS AND DISCUSSION

Growth of mycelium

Growth of mycelium was fast and the entire surface was covered within 3.67d in the treatments with coconut water under both inoculation methods which were statistically similar to the treatments with PDB *i.e.* inoculation methods under these media produced no difference in growth of mycelium. At the same time rice gruel water inoculated with tbf of *Metarhizium* took 13.67d for the complete growth of mycelium. Rice gruel water+ sugar inoculated in the same manner also took 14d for complete growth whereas rice gruel water and rice gruel water+sugar inoculated with pure culture took 7.33d for the complete surface coverage by mycelium. The results indicate the suitability of coconut water over rice gruel water in supporting the growth of the fungus. Growth of fungus in coconut water under both inoculation methods was as fast as the standard procedure of inoculating PDB with pure culture. The interesting result found was that the addition of sugar to coconut water did not make any difference in speed of growth of fungus (Table 1).

Bio mass production

Production of mycelia bio mass after the complete spread was the highest in potato dextrose broth inoculated with tbf of the fungus (9.393g) which was followed by the standard procedure *i.e.* PDB inoculated with pure culture of the fungus(4.763g) that was on par with the treatment coconut water with tbf of fungus which was followed by the treatment T_3 (coconut water+sugar inoculated with tbf of fungus), T_2 (coconut water inoculated with pure culture),T_4 (coconut water+sugar inoculated with pure culture of fungus),T_6 (rice gruel water inoculated with pure culture of fungus), $T_{8(}$rice gruel water+sugar inoculated with pure culture of fungus),T_7 (Rice gruel water +sugar inoculated

with tbf of fungus), T_5(Rice gruel water inoculated with tbf of fungus). Coconut water proved its ability in biomass production of *Metarhizium*. Inoculation with talc based formulation in PDB is found superior to all other treatments.

Population of fungus

From the data (Table 1), it was clearly visible that at all the 3 dilutions plated, treatments did not differ significantly from the standard procedure(T10). Hence it can be ascertained that all the media in both inoculation methods were effective as that of standard procedure. Present study was in confirmation with the findings of Dunda (2017). Anith *et al* (2016) also established the suitability of coconut water in supporting growth of *Pseudomonas fluoresens*. Coconut water is highly efficient in supporting multiplication of micro organisms.This might be due to the nutrient rich composition of amino acids, vitamins and minerals in coconut water, thus it can be utilized as an efficient and cheap media for the multiplication of microorganisms (Survase *et al,* 2007; Unagul *et al,* 2007; Anith *et al,* 2014).

CONCLUSION

Considering all the three parameters tested, coconut water was found equally effective or superior to PDB. While, there is no significant variation observed among the methods of inoculation. Hence farmers can utilize talc based formulation of *Metarhizium* purchased from a reputed and reliable source for the on farm multiplication using coconut water which is always wasted in all the households and abundantly available as a byproduct of copra industry.

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