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Bioefficacy of *Metarhizium anisopliae* (Metschn.) Sorokin from different solid media against *Aphis craccivora* (Koach) under laboratory condition

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Abstract
The present investigation were undertaken to study the bioefficacy of *M. anisopliae* conidia from different solid media against *A. crassivora* under laboratory condition during the year 2015-2017 in Agricultural Entomology Department, College of Agriculture, Dapoli (Maharashtra). The efforts were made in the present study to utilize an entomopathogenic fungus for aphid management. In this regards, green muscardine fungus, *M. anisopliae* was tested against *A. craccivora*. In this experiment, total eight solid media were evaluated *viz*., Sorghum grain, Tea waste (without sugar and milk), *Nagli* husk, Soybean chunks, Sugarcane baggase, Wheat grain, Hotel tea waste (with sugar and milk), Banana pseudo stem. Results of the bio-efficacy of *M. anisopliae* conidia from these solid media against the nymph of *A. craccivora* revealed that all the treatments were significantly superior over the control. The highest mean mortality of aphid (86.67%) was recorded in treatment T_1- Sorghum grain which, was at par with T_2-Tea waste (83.33%). As far as the symptoms of infection by *M. anisopliae* on nymphs were concerned, the infected nymphs were found at the surface of soil and plant. Subsequent symptoms observed were inactiveness, development of coppery fungal growth on the body which, further developed to olive green muscardin fungus mat all over the body parts.

Keywords: bioefficacy, *M. anisopliae*, *A. crassivora*, solid media, mortality etc.

Introduction
Microbial control has been considered as an important tool in IPM to conventional chemical control. The microorganisms like bacteria, virus, fungi, protozoa, rickettsia and nematodes have the capacity to affect the pest. Entomopatogenic fungi are employed as biocontrol agents reducing pest population and consequently their damages in different agro-ecosystem, Inglis *et al.* (2001) [3]. The use of fungi in the control of agriculturally harmful pests depends on different factors including the ability to produce high concentration of stable propagules at a reasonable cost, Joronski (1986) [4].

The most important species of fungus, *M. anisopliae* and *Beauveria bassiana* (Balsamo) Vuillemin are insect pathogenic fungi which have to meet several host challenges like producing enough new infectious spores in each operation for maintaining viable population. The green muscardin fungus *M. anisopliae* (Deuteromycotina: Moniliales) is already reported to be very useful fungus for the management of many insect pests. This fungus was discovered by Mechnik off in 1879 infecting the larvae of wheat cockchafer. In India, Nirula (1957) [6] first reported the said fungus inhabiting the breeding site of *Oryctes rhinoceros* L. After great exploratory surveys and pathogencity studies, many workers have suggested that the fungus could be effectively used in microbial control of some other pest. Soil is the main reservoir for many entomopathogenic fungi, but only a few strains obtained from soil have been used against insect pest.

Aphids are one of the most destructive pests in crop production such as pepper, cucumber, and eggplant (Milner, 1997 [5]. Many entomopathogenic fungi produce metabolic compounds that may be toxic to insects (Vey *et al*. 2001) [11]. Boruah and Dutta (2014) [2] reported that the *M. anisopliae* can be used for management of cowpea aphid. Pandey (2013) [7] stated that the *M. anisopliae* and *B. basiana* were most effective as compared to chlorpyriphos against cutworm. Singh *et al.* (2011) [10] studied two strains of *M. anisopliae* and of *B. bassiana* against tea termite.

In *Konkan* region of Maharashtra state, agricultural residue or waste material like *Nagli* husk, rice husk, banana pseudo-stem and hotel waste tea powder, sugarcane baggase etc.

are found in large amount. These raw waste materials are available in market at cheaper cost. With a view to generate more information on different aspects of the efficacy of different media on sporulation of the fungus, *Metarhizium* and its effectiveness as an biological control agent of the pest aphid, *A. craccivora* the present research work entitled 'Bioefficacy of *M. anisopliae* from different solid media against *A. crassivora* under laboratory condition was under taken.

Materials and Methods

The present investigation was carried out in Quarantine laboratory of "Plant Pathology Department and Agricultural Entomology Department, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist: Ratnagiri (M.S.) during the academic year 2015-2017. The details of the various laboratory chemicals used in the present investigation for media preparation are given below:

Chemicals for media preparation

1. Sucrose as a energy source
2. Agar-agar as a solidifying agent

Chemicals for surface sterilization

1. Mercuric chloride ($HgCl_2$)
2. Ethyl alcohol (70 %)

Glass wears

1. Conical flasks of capacity 250, 500 ml
2. Beakers of capacity 500 ml
3. Petri plates of size 100 x 20 mm
4. Pipettes of capacity 10 ml
5. Micropipettes of capacity 100-1000 µ
6. Measuring cylinders of capacity 10 and 1000 ml

Laboratory Equipments

- Refrigerator
- Hot air oven
- Electronic Digital balance
- Autoclave
- Laminar air flow bench
- Incubator

Others

Trays, caps, Polypropylene bags, Aluminium foil, Non-absorbent cotton, Spirit lamp or Gas burner, Forceps, Bacterial needle, and Cork were used for maintaining the aseptic culture.

Experimental Conditions

All *In vitro* studies were carried out aseptically in laminar air flow chamber. The Experiments were conducted under well-defined conditions of culture room maintained at 25 ± 2^0C temperature, uniform light (1600 Lux) provided by fluorescent tubes (7200 K) over a light and dark cycle of 16/8 hours.

Culture medium (solid)

The treatments details given in below Table No.1.

Table 1: Composition of solid medium

Treatment No.	Media	Weight (g)
T_1	Sorghum grain (Standard medium)	50
T_2	Tea waste (without sugar and milk)	50
T_3	*Nagli* husk	50
T_4	Soybean chunks	50
T_5	Sugarcane baggase	50
T_6	Wheat grain	50
T_7	Tea waste (with sugar and milk)	50
T_8	Banana psuedostem	50
T_9	Control (Sterile distilled water)	50 ml

Standardization of media for mass multiplication of *M. anisopliae*

A master culture of the test fungus, *M. anisopliae* was obtained from Biocontrol laboratory, Department of Agricultural Entomology college of Agriculture, Dapoli and used for mass multiplication. From this, inoculated test tubes were maintained at 26^0C ± 2^0C in an incubator till sporulation and the master culture was maintained in refrigerator. Mass multiplication of *M. anisopliae* by using different solid media (culture media) mention in the above Table 1 is given below.

Mass multiplication of *M. anisopliae* on solid media

For the multiplication of *M. anisopliae*, different solid media were used as mentioned above. In this experiment, dry grains of Wheat, Sorghum, Soya chunks were purchased from local market. Agricultural residues like Banana pseudo stem, *Nagli* husk, and Sugarcane baggase as well as hotel Tea waste were collected from college campus. The grains (150 g) were taken and boiled in tap water for 25 minutes to hold the maximum moisture for better growth and sporulation of *M. anisopliae*. The same were cooled and kept as 50 g boiled grains per three different plastic polypropylene bags having capacity 500 gm. Banana psuedostem, *Nagli* husk, Sugarcane baggase and Tea waste were soaked in water for 3 h to hold maximum moisture for growth and sporulation of *M. anisopliae*. The excess water was drained out from the media. The material was placed in polypropylene bag, closed by putting non-absorbent cotton plug and then sterilized in autoclave at 121^0C, 15 psi for 1 h. After sterilization, bags were kept for cooling. With the help of cork (size 5 mm), a bit of PDA containing *Metarhizium* was removed and inoculated in each bag in aseptic condition. After inoculation, the bags were incubated at room temperature (28^0C). After 3 days of inoculation, mycelial growth developed on grains and organic residues was mixed thoroughly in the bag to enhance faster growth of the fungus.

Mass rearing of *Aphis craccivora* (Koch)

The nymphs of aphid were collected in large numbers from their breeding places from the cowpea plants. The field collected nymphs were reared on cowpea seedlings grown in small plastic cups. Thus, the culture of *Aphis craccivora* was maintained. The nymphs were further used for testing the efficacy of *M. anisopliae* against aphid.

Bio-efficacy of *M. anisopliae* from different solid media against cowpea Aphid

Experiment details

Statistical design: - CRD (Complete randomized design)

No. of repetition: - 3

No. of treatments: - 9 (Solid media)

No. of aphid per treatment: - 10

Three cowpea seedlings in small plastic cup with 10 aphid nymphs per seedling were maintained to take treatments.

Method of preparation and application of fungal suspension of solid medium

Five g of each solid medium along with fungal spores was thoroughly agitated in 100 ml of sterilized distil water in conical flask. The suspension simply filtered with muslin cloth to separate out the debris of substrate, and collected in same conical flask again. This suspension was filled in 1 saloon spray and was calibrated 5 times by spraying the material once which, was measured as 0.5 ml. Thus uniform application of spray material was achieved.

Method of recording observation

The aphids were observed daily for the symptoms of fungal infection and mortality in solid media tests. After spraying, mortality was observed within and dead aphids were counted to determine per cent mortality. The data before analysis of variance was subjected to arcsine transformation and presented.

Results and Discussion

Bioefficacy of *M. anisopliae* from different solid media against *A. craccivora*

The efforts were made in the present investigation to utilize an entomopathogenic fungus for aphid management. In this regards green muscardine fungus, *M. anisopliae* was tested against *A. craccivora*. The data on per cent mortality of aphids are presented in Table 2. The results of the experiment indicated that all the treatments were significantly superior over the control. The highest mean mortality of aphid 86.67 per cent was recorded in treatment T_1- Sorghum grain which was at par with T_2-Tea waste (83.33) further, T_2 was at par with T_8- Banana pseudo stem (76.67) as alsoT_8 was at par with T_4- Soybean chunks (73.33) while T_4- was further at par with T_6- Wheat grain (66.67).

Among reaming treatments T_3- *Nagli* husk, T_5- Sugarcane baggase, T_7- Hotel tea waste revealed mean mortality of 63.33, 60.00 and 56.67 per cent, respectively and all were at par with each other and also with T_6. No mortality was revealed in control. No such work with *M. anisopliae* is available for comparison hence other reference are included here with

Sahayaraj *et al.* (2010) [8] isolated *M. anisopliae* on Potato Dextrose Agar (PDA). Four spore concentrations such as 1.7 x 10^4, 2.6 x 10^5, 1.9 x 10^6 and 1.6 x 10^7 spores/ml were prepared from the stock and applied on *Pericallia ricini* Fabricius, *Helicoverpa armigera* (Hubner), *A. craccivora* and *S. litura*. Lowest LC_{50} value (1.84 x 10^4) was recorded on *A. craccivora*. Among all the tested pests, the mycelial growth was observed only on *A. craccivora*.

Banu (2012) [1] studied solid state fermentation, with five grains *viz*., Rice, Wheat, Sorghum, Pearl millet and Finger millet along with PDA for multiplication of *L. lecanii*. Virulence of spores produced in solid substrates (Spores @ 1 x 10^7 ml^{-1}) were sprayed onto the *Paracoccus marginatus* Williams and Granara de Willink and the insect mortality was recorded. Among different grains tested, spores multiplied on rice and sorghum recorded maximum mortality of 96 per cent at 9 days after inoculation.

Sajap *et al.* (2012) [9] used *M. anisopliae* for controlling Tiger moth, *Atteva sciodoxa*. The fungus was pathogenic to third instar larvae of *A. sciodoxa. M. anisopliae* killed 48 to 88 per cent larvae.

In present investigation, aphids were found with fungal growth on leaves and stem of cowpea seedling. They became inactive and sluggish. Coppery growth was initially observed on the body after two days of infection which was the first external sign of the infection. This fungal growth became olive green in colour after three days and covered entire body.

Table 2: Bioefficacy of *M. anisopliae* from different solid media against *A. craccivora*

Treat. No.	Treatment	Mortality of aphids (%)			Mean per cent mortality
		RI	RII	RIII	
T_1	Sorghum grain	90 (71.57)*	80 (63.43)	90 (71.57)	86.67 (68.86)
T_2	Tea waste (without sugar and milk)	90 (71.57)	80 (63.43)	80 (63.43)	83.33 (66.14)
T_3	*Nagli* husk	70 (56.79)	60 (50.76)	60 (50.76)	63.33 (52.78)
T_4	Soybean chunks	70 (56.79)	80 (63.43)	70 (56.79)	73.33 (59.00)
T_5	Sugarcane baggase	50 (45.00)	60 (50.76)	70 (56.78)	60 (50.85)
T_6	Wheat grain	70 (56.78)	70 (56.78)	60 (50.76)	66.67 (54.78)
T_7	Hotel tea waste (with sugar and milk)	60 (50.76)	50 (45.00)	60 (50.76)	56.67 (48.85)
T_8	Banana pseudo stem	80 (63.43)	70 (56.78)	80 (63.43)	76.67 (61.22)
T_9	Control (Sterile distilled water)	0 (0.29)	0 (0.29)	0 (0.29)	0 (0.29)
S.E. ± 2.31					
C.D. at 5% 6.83					

*Figures in the parentheses are arc sine values

Conclusion

The results of the bio-efficacy of *M. anisopliae* against the nymphs of *A. craccivora* revealed that all the solid media treatments were significantly superior over the control. The highest mean mortality of aphid (86.67%) was recorded in treatment T_1- Sorghum grain which, was at par with T_2-Tea waste (83.33%). No mortality was revealed in control treatment of solid medium experiments.

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