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

**ND Wayal, SK Mehendale, GM Golvankar, VS Desai and KV Naik**

**Abstract**
The present study was undertaken to find out the bioefficacy of culture filtrates *M. anisopliae* from different liquid media against *A. crassivora* under laboratory condition during the year 2015-2017 in the Agricultural Entomology Department, College of Agriculture, Dapoli (Maharashtra). In this regards, green muscardine fungus, *M. anisopliae* was tested against aphid, *A. craccivora*. In this experiment, total nine liquid broth treatments were evaluated *viz*., Coconut water, Carrot broth, Potato dextrose broth, Coon's medium, Corn flour medium, Czeapeks medium, Molasses, Gram flour medium and control (sterilized distilled water), respectively. The highest mean mortality of *A. craccivora* 86.67 per cent was recorded in treatment $T_6$- Czeapeks medium which, was at par with $T_1$- coconut water (83.33%). No mortality was revealed in control treatment.

**Keywords:** bioefficacy, *M. anisopliae*, *A. crassivora*, culture filtrates liquid 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. Entomopathogenic fungi are natural common enemies of arthropods particularly, insects and they can be used in the management of pest population in agro ecosystem as well as some domestic pest in urban area (Lacey and Kaya, 2007) [7].

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 Mechnikoff in 1879 infecting the larvae of wheat cockchafer. In India, Nirula (1957) [10] first reported the said fungus inhabiting the breeding site of *Oryctes rhinoceros* L. Steinhaus (1949) [13] found it to have a wide distribution as that of the white muscardine fungus, *B. bassiana. M. anisopliae* is an important candidate among the entomopathogenic fungi, for use in bio-intensive pest management strategies.

Many entomopathogenic fungi produce metabolic compounds that may be toxic to insects (Vey *et al*. 2001) [14]. Production of metabolites and their control efficacy against mosquitoes differed among fungal isolates and culture media or media composition (Mohanty *et al*. 2008) [9]. Culture filtrates of entomopathogenic fungi such as *Lecanicillium lecanii* and *B. bassiana* reduce aphid survival rates (Kim *et al.* 2010 and Khan *et al*. 2012) [6, 5] and deter feeding by whitefly and larva of *Spodoptera littoralis* (Kim *et al.* 2010 and Gurulingappa *et al*. 2011) [6, 3]. These culture filtrates may include various enzymes, such as protease, chitinase, and lipase, which are important in the process of infection by conidia. These enzymes can be induced by additives such as colloidal chitin in culture media (Kim *et al.* 2010) [6]. Filtrate from cultures in broth amended with chitin or colloidal chitin showed higher toxicity to insects due to induced chitinase (Mohanty *et al*. 2008, Mohanty *et al*. 2009 and Binod *et al*. 2007) [9, 8, 1]. However, insecticidal activity of culture extracts of an isolate of entomopathogenic fungus (*Metarhizium anisopliae*) was not influenced by media composition (Quesad – Moraga *et al*. 2006) [12]. Production of toxin, particularly destruxin, differs according to fungal isolate,

culture composition, and pH (Wang *et al*. 2004)[15]. Consequently, culture extracts or filtrates may contain secondary metabolites or compounds having different insecticidal activity. The type and concentration of a compound may vary according to fungal isolate, composition of culture media, and culture condition. Because both spores and compounds are killing factors, whole fungal cultures that include spores may have higher efficacy and consistency. Therefore, use of culture filtrates or fungal culture including both spores and secondary metabolites may increase the speed of killing compared to conidia or spores only. In this study, we conducted bioassays with different cultural filtrates of *M anisopliae* that produces compounds or metabolites that are toxic to the cowpea aphid (*Aphis craccivora* Koch) and to find culture media that can maximize such an effect.

## Materials and Methods

The present investigation was carried out in 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 ± $2^0$C temperature, uniform light (1600 Lux) provided by fluorescent tubes (7200 K) over a light and dark cycle of 16/8 hours.

### 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 $26^0$C ± $2^0$C in an incubator till sporulation and the master culture was maintained in refrigerator. Mass multiplication of *M. anisopliae* was done by using different liquid media (culture media) mention in the below Table 1. Mass multiplication process is discussed below.

### Preparation of liquid media

**Table 1:** Composition of liquid medium (For 100 ml)

| Treatment No. | Broth/liquid Medium | Quantity for 100 ml |
|---|---|---|
| $T_1$ | **Coconut water** | 100 ml |
| $T_2$ | **Carrot broth** | |
| | Carrot | 25 g |
| | Dextrose | 20 g |
| | Water | 100 ml |
| $T_3$ | **Potato dextrose broth** | |
| | Potato | 25 g |
| | Dextrose | 20 g |
| | Water | 100 ml |
| $T_4$ | **Coon's (broth) medium** | |
| | Sucrose | 0.72 g |
| | Dextrose | 0.36 g |
| | Magnesium sulphate | 0.12 g |
| | Potassium nitrate | 0.20 g |
| | Distilled water | 100 ml |
| $T_5$ | **Starch (Corn flour) + water** | |
| | Corn meal | 2 g |
| | Peptone | 2 g |
| | Dextrose | 2 g |
| | Distilled water | 100 ml |
| $T_6$ | **Czeapek's Dox medium** | |
| | Sucrose | 3 g |
| | Sodium nitrate | 0.2g |
| | Potassium diphosphate | 0.1 g |
| | Magnesium sulphate | 0.05 g |

| | | |
|---|---|---|
| | Ferrous sulphate | 0.05 g |
| | Distilled water | 100 ml |
| $T_7$ | **Molasses + yeast + water** | |
| | Molasses – 10 ml | 1 ml |
| | Yeast – 3 g | 0.3 g |
| | Distilled water – 1000 ml | 100 ml |
| $T_8$ | **Gram flour + water** | |
| | Gram flour | 2 g |
| | Peptone | 2 g |
| | Dextrose | 2 g |
| | Distilled water | 100 ml |
| $T_9$ | **Control (Water)** | 100 |

**Coconut water**
The tender coconut water (100 ml) was drawn out in 250 ml conical flask aseptically. The flask was plugged with non-absorbent cotton and sterilized in autoclave at $121^0$C at 15 psi for 1 h and the same was used as medium.

**Potato dextrose broth**
Peeled potato (25 g) was mixed in 100 ml water and boiled to have extract. Extract of potato and dextrose (20 g) were mixed and filled in 100 ml conical flask. Volume adjusted to 100 ml by adding required distil water, flask were then autoclaved as usual.

**Carrot broth medium**
Peeled carrot (25 g) was mixed in 100 ml water and boiled to have extract. Extract of carrot and dextrose were mixed and filled in 100 ml conical flask. Volume adjusted to 100 ml by adding required distil water, flask were then autoclaved as usual.

**Molasses + yeast + water, Gram flour + Water, Czeapek's broth medium and Coon's medium**
Known quantity as mentioned earlier (in Table 1) was mixed in distilled water (100 ml), poured in conical flask (100 ml), plugged with non-absorbent cotton and sterilized in autoclave at $121^0$C for 1 h. After sterilization, flasks were kept for cooling.

**Inoculation of the fungus**
With the help of cork (size 5 mm) one bit of PDA containing *Metarhizium* was inoculated in each flask in aseptic condition. After inoculation, the flasks were incubated at room temperature $28^0$C. After 3 days of inoculation mycelial growth was developed on broth media.

**Mass rearing of *A. craccivora***
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 *A. craccivora* was maintained. These nymphs were further used for testing the efficacy of *M. anisopliae* against aphid.

**Bio-efficacy of *M. anisopliae* culture filtrates from different liquid media against aphid, *A. craccivora***
**Experiment details:**
Statistical design: - CRD (Complete randomized design)
No. of repetition: - 3
No. of treatments: - 9 (Liquid media)
No. of aphid per treatment: - 10

**Culture medium (liquid)**
The treatment details are given in below Table No. 2

**Table 2:** Details of various liquid media for sporulation of *M. anisopliae*

| Treatment No. | Broth/liquid Medium | Volume (ml) |
|---|---|---|
| $T_1$ | Coconut water | 100 |
| $T_2$ | Carrot broth | 100 |
| $T_3$ | Potato dextrose broth | 100 |
| $T_4$ | Coon's (broth) medium | 100 |
| $T_5$ | Starch (Corn flour) + water | 100 |
| $T_6$ | Czeapek's Dox medium | 100 |
| $T_7$ | Molasses + yeast + water | 100 |
| $T_8$ | Gram flour + Water | 100 |
| $T_9$ | Control (Water) | 100 |

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**
The culture filtrate of each liquid medium was taken into a saloon sprayer. This suspension was filled in saloon sprayer 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 each treatment. After spraying, mortality was observed 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**
**Bio-efficacy of culture filtrates of *M. anisopliae* against *A. craccivora***
Efforts were made in the present investigation to utilize culture filtrate of green muscardin fungus *M. anisopliae* against aphid. The results are summarized below.
The data on per cent mortality of aphids are presented in Table 3. Results of the experiment indicated that all the treatments were significantly superior over control. The highest mean mortality of 86.67 per cent was recorded in treatment $T_6$- Czeapeks medium which, was at par with $T_1$- Coconut water (83.33%) as also $T_1$ was at par with $T_4$- Coon's medium (73.33%). $T_4$ further was at par with $T_5$- Corn flour medium (70%) and $T_3$-Potato dextrose broth (63.33%). In the remaining treatments, $T_8$- Gram flour medium, $T_2$- Carrot broth and $T_7$- Molasses the mean mortality recorded was 60.00, 60.00 and 56.67 per cent, respectively and all were at par.
Padmanban *et al.* (1997) [11] tested the pathogenicity of *M. anisopliae and B. bassiana* on white grubs. They sprayed the spore suspension of *M. anisopliae* prepared from Potato

Dextrose Broth (PDB) medium at the rate 104,105 and 106 spores/ml. They recorded no mortality due to application of *B. bassiana* and *M. anisopliae* with the spore suspension prepared from PDB.

Mohanty *et al.* (2008) [9] studied efficacy of culture filtrates of five strains of *M. anisopliae* isolated from insects were evaluated against *Anopheles stephensi* Liston, and *Culex quinquefasciatus* Say. The culture filtrates released from the strains of *M. anisopliae* in the Yeast phosphate soluble starch (YpSs) broth and chitin broths were filtered and used for the bioassays after a growth of 7 days. Among the culture filtrates of five strains, *M. anisopliae* 892 was found to be more effective against both the mosquitoes. The $LC_{50}$ values of culture filtrates of *M. anisopliae* 892 in chitin broth was lower than the $LC_{50}$ of culture filtrates in (YpSs) against first and fourth instars of both the mosquitoes.

Jeong *et al*. (2013) [4] conducted bioassays with 47 fungal culture filtrates in order to evaluate the potential of secondary metabolites produced by entomopathogenic fungi for use in aphid control. Among 47 culture filtrates cultured potato dextrose broth, filtrate of *B. bassiana* Bb08 showed the highest mortality (78%) against green peach aphid three days after treatments. These results indicate that the fungal culture fluid or culture filtrate of *B. bassiana* Bb08 cultured in Adamek's medium has potential for development as a mycopesticide for aphid control.

Boruah and Dutta (2014)[2] used two bio formulation of *M. anisopliae* (1 X $10^6$ spores/ml) *viz.,* suspension concentrate and liquid formulation mixed with glycerol (10%) + sunflower oil (0.5%) and glycerol @ 10 per cent + sunflower oil @ 1 per cent, respectively. Liquid formulation with glycerol 10.0 % + sunflower oil 0.5% was found highly pathogenic to aphid and killed 80 per cent of the test population at 30 days after spraying. *M. anisopliae* was observed over the surface of the aphid body covering head, thorax, abdomen and all other appendages. Bio formulation of *M. anisopliae* amended with adjuvants and oils were highly effective against cowpea aphid and found to be superior to the formulation without adjuvants and oils.

In general, results indicated that the lowest mean mortality of 56.67 per cent was recorded in the treatment $T_7$- Molasses while the treatment T6- Czeapeks medium with 86 per cent mortality was the best treatment. In control (sterilized distilled water) no aphid mortality was observed.

In present study, 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 3:** Bioefficacy of culture filtrates of *M. anisopliae* from different liquid media against *A. craccivora*

| Treatment No. | Treatment | Mortality of aphids (%) | | | Mean per cent mortality |
|---|---|---|---|---|---|
| | | RI | RII | RIII | |
| 1 | Coconut water | 90 (71.57) | 80 (63.43) | 80 (63.43) | 83.33 (66.14) |
| 2 | Carrot broth | 60 (50.77) | 50 (45.00) | 70 (56.78) | 60.00 (50.85) |
| 3 | Potato dextrose broth | 70 (56.78) | 80 (63.43) | 70 (56.78) | 63.33 (52.78) |
| 4 | Coon's medium | 70 (56.79) | 80 (63.43) | 70 (56.79) | 73.33 (59.00) |
| 5 | Corn flour medium | 70 (56.79) | 80 (63.43) | 60 (50.76) | 70 (57.00) |
| 6 | Czeapeks medium | 90 (71.57) | 90 (71.56) | 80 (63.43) | 86.67 (68.86) |
| 7 | Molasses | 60 (50.77) | 60 (50.77) | 50 (45.00) | 56.67 (48.85) |
| 8 | Gram flour medium | 60 (50.77) | 50 (45.00) | 70 (56.78) | 60.00 (50.85) |
| 9 | Control | 0 (0.29) | 0 (0.29) | 0 (0.29) | 0 (0.29) |
| S.E. ± 2.66 | | | | | |
| C.D. at 5% 7.19 | | | | | |

*Figures in the parentheses are arc sine values

## Conclusion

During the present investigation, it can be concluded that the highest mean mortality of *A. craccivora* (86.67%) was recorded in treatment $T_6$- Czeapeks medium which, was at par with $T_1$- coconut water (83.33%). No mortality was revealed in control treatment of liquid medium experiments.

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