Suitability of different media and pathogencity of *Metarhizium anisopliae* (Metschnikoff) Sorokin against *Aphis craccivora* (Koch)

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MAY, 2017

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A thesis submitted to the

DR. BALASAHEB SAWANT KONKAN KRISHI VIDYAPEETH, DAPOLI
(Agricultural University)
Dist. Ratnagiri (Maharashtra State)

In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE (AGRICULTURE)

In

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CERTIFICATE

This is to certify that the thesis entitled, **"Suitability of different media and pathogencity of *Metarhizium anisopliae* (Metschnikoff) Sorokin against *Aphis craccivora* (Koch)"** submitted to the Faculty of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri, Maharashtra State, in the partial fulfillment of the requirements for the degree of ***MASTER OF SCIENCE (Agriculture)*** in **AGRICULTURAL ENTOMOLOGY**, embodies the results of a piece of bona-fide research carried out by **Miss. Wayal Nirmala Dunda (Reg. No. 2401)** under my guidance and supervision and that no part of this thesis has been submitted for any other degree or diploma or published in other form. All the assistance and help received during the course of investigation and the sources of literature have been duly acknowledged by her.

Place: Dapoli
Date: May 2017

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"If you accept the expectations of others, especially negative ones,
Then you never will change the outcome."

"Coming together is beginning, carrying together is progress and keeping together is success", this phrase comes to be true, while completing the post graduation.

Success is not possible lonely and any creativity is possible only after the involvement of many minds and hands to beautiful it .Emotions cannot be expressed adequately in words it is nearly the formalities .Traditional formal words of acknowledgment will not project the picture of volcano of feeling while expressing a deep sense of gratitude to my known and unknown hands which push and put me on right path and enlightened me with their experience, knowledge and wisdom and shall ever remain grateful to them.

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Place:

Date: (Wayal Nirmala Dunda)

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THESIS ABSTRACT

The present studies were undertaken at Quarantine laboratory of "Plant Pathology Department, and Agricultural Entomology Department, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist: Ratnagiri (M.S.) during 2015-17. The present research was carried out to standardize the method for mass culturing the green muscardin fungus, *M. anisopliae* under konkan conditions and to test the efficacy of *M. anisopliae* against *Aphis craccivora* (Koch) under laboratory conditions.

Studies on the mass production of green muscardin fungus, *M. anisopliae* on solid media indicated that treatment T_8-Banana psuedostem was the most suitable solid medium for multiplication of the fungus under Konkan condition. Mean spore production of T_8-Banana psuedostem after 20 days of inoculation was 951.3 x 10^8spores per gram which was at par with T_2-Tea waste (930.67 x 10^8) and was second best media. Whereas; Sugarcane baggase

produced lowest number of spores (41.33 x 10^8) at 20 days of inoculation of the fungus. Studies on the mass multiplication of *M. anisopliae* on liquid / broth medium showed that the T-6 Czeapeks broth medium was the most suitable medium for mass multiplication of *M. anisopliae* (average mat weight of dry fungus 1554 mg), after 20 days of inoculation which was at par with T_5-corn flour medium (1545.67 mg)., which also supported good growth of the fungus.

Results of the bio-efficacy of *M. anisopliae* 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%). However, in liquid/ broth medium 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%). No mortality was revealed in control treatment of both solid and liquid medium experiments.

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.

CHAPTER I

INTRODUCTION

The use of pesticides has gradually become a part of our modern agricultural practices and its consumption has increased remarkably in the past, causing serious health and environmental problems in developing countries including India. Chemical control is generally practiced by farmers for higher gains, but its injudicious use has created many problems. Sole reliance on chemical control leads to problem of pesticide resistance, resurgence of pests, pesticide residues, destruction of beneficial fauna and environmental pollution.

Natural enemies like parasitoids and predators are the main sources of reduction in populations of noxious insect pests, Pfadt (1980). Indiscriminate use of insecticides has resulted in killing of natural enemies and large scale hazardous effects on ecosystem. Adoptions of Integrated Pest Management (IPM) strategies ensure safety of environment. In view of reducing the unnecessary use of insecticide, promoting biological control through applying biopesticides can significantly prevent pest population and conserve the biodiversity.

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. *Bacillus thuringiensis* (bacteria) is very effective in controlling many lepidopterous larvae like cabbage worm, sugarcane stem borer etc. Nuclear polyhedrous viruses (NPV) have the effective control over *Spodoptera liturra* (Fabricius) and *Helicoverpa armigera* (Hubner). Entomopatogenic fungi are employed as biocontrol agents reducing pest population and consequently their damages in different agro-ecosystem, Inglis *et al.* (2001). 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).

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. In India, Nirula (1957) 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. Steinhaus (1949) 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.

M. anisopliae can be used for management of cow pea aphid Boruah and Dutta (2014). Singh *et al.* (2011) studied two strains of *M. anisopliae* and of *B. bassiana* against tea termite. Flores *et al.* (2012) indicated that the fungi *B. bassiana, M. anisopliae* and *Isaria fumorosa* had a considerable potential to control all stages of white fly (*B. tabacci*) in green house cultivated bean. Ekesi e*t al.* (1999) demonstrated combine effect of intercropping cowpea with maize and application of *M. anisopliae* on Thrips.

The key factor which decides the success and adaptability of a bio-agent is its easy availability in time and space at affordable cost. *M. anisopliae* being a facultative fungal pathogen, which can grow on organic material and readily, sporulate on semi synthetic media like PDA or carrot malt agar. Natural media, which are invariably rich in Carbon and nitrogen were, proved to support the growth and sporulation of the fungus. The most convenient and durable development stage of hypomycetes fungi is the dusty spores (conida) which are easy for application and storage and also a natural distributive stage. For mass production, many workers found the best various nutrient materials among them Jenkins *et al.* (1998) and Sharma *et al.* (1999) founded Rice, Feng *et al.* (1994) revealed rice bran and also Humphreys *et al.* (1989) and Burges (1998) reported rice bran, barley or corn extract to be a good medium for mass multiplication. While, Feng (1994) revealed coconut water to be better than PDB.

Industrial production systems for some entomopathogenic fungi use a biphasic method in which the fungal inoculant-mycelia or hyphae is produced in liquid culture and this is transferred to solid substrates in order to increase conidal production, Guillon (1997). However, cost of all the microbiological media is rising at a fast pace. To tackle this problem, some new microbiological media should be designed which, are efficient as well as cost effective. This may be achieved by using agricultural wastes as raw material for microbial media. Rachappa *et al.* (2005) studied that broken rice proved to be the most ideal substrate for mass production with higher conidal productivity and yield followed by sorghum.

In Konkan region, 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. Coconut water is also available in abundant quantity. 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, *Aphis craccivora* (Koch), the present research work entitled suitability of different media and pathogencity studies of *Metarhizium anisopliae* (Metschnikoff) Sorokin *against Aphis craccivora* (Koch) was undertaken with the following objectives.

1. To study the performance of different solid media for sporulation of *M. anisopliae* under laboratory condition.
2. To study the performance of different liquid media for sporulation of *M. anisopliae* under laboratory condition.
3. Bioefficacy of *M. anisopliae* from different solid media against *A. crassivora* under laboratory condition.
4. Bioefficacy of culture filtrates *M. anisopliae* from different solid media against *A. crassivora* under laboratory condition.

CHAPTER II
REVIEW OF LITERATURE

The most important species of fungus *Metarhizium anisopliae* (Metsch.) Sorokin 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 pest. This green muscardine fungus was discovered by Mechnikoff in 1879 infecting the larvae of wheat cockchafer. In India, Nirula (1957) 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. The review of the work done on effect of suitability of different and pathogencity studies of *Metarhizium anisopliae* (Metschnikoff) Sorokin *against Aphis craccivora* (Koch) are presented in this chapter under following sub headings;

2.1 To study the performance of different solid media for sporulation of *M. anisopliae* under laboratory condition.

2.2 To study the performance of different liquid media for sporulation of *M. anisopliae* under laboratory condition.

2.3 Bioefficacy of *M. anisopliae* from different solid media against *A. crassivora* under laboratory condition.

2.4 Bioefficacy of culture filtrates *M. anisopliae* from different solid media against *A. crassivora* under laboratory condition.

1.1 To study the performance of different solid media for sporulation of *M. anisopliae* under laboratory condition.

Mohan and Pillai (1982) used cassava chips mixed with rice bran supplemented with urea or waste fish meal extract as a nitrogen source for mass production of *M. anisopliae*. The yield of *M. anisopliae* spores obtained at 0.5 per cent nitrogen supplement level using urea and waste fish meal extract was 370 and 450 spores per µg of substrate, respectively.

Padmanban *et al.* (1997) multiplied the fungi *M. anisopliae* and *Beauveria bassiana* (Balsamo) Vuillemin on media like PDA, MA, CMA, OMA, and coconut water and sorghum grain. They obtained maximum sporulation of *M. anisopliae* on sorghum grain (4.3 x 10^8 spores / ml) but *B. bassiana* did not sporulate in any of the above tested media.

Bhide (2001) used grains of Rice, Wheat, Sorghum, Bajra, Cowpea and Green gram for sporulation of *M. anisopliae*. Maximum spore production was recorded on Sorghum grain (2.575 x 10^8 spores per/g) followed by Bajra grains which also supported good growth of the fungus.

Kumar *et al.* (2005) studied variability in growth and sporulation of five isolates of *Arthrobotrys dactiloides* (Drechsler) on five agar, six bran and five grain media. They used some general media like bran of pea, wheat, rice, gram, lentil, pigeon pea and grains like wheat, maize, sorghum, barley and gram for growth and sporulation of *A. dactyloides*. In grain media maximum number of spores was recorded in gram grain agar followed by barley and maize grain agar media.

Rachappa *et al.* (2005) used various grain media like Sorghum, Maize, Rice, Barley, Pearl millet, Little millet, Italian millet, Finger millet, Bengal gram, Green gram, Red gram and Black gram. The most common dietary ingredients with yeast (1per cent) were evaluated for the fungus *M. anisopliae* in order to select the best substrate for mass production of the pathogen. Chickpea supported spore yield as much as maize, wheat and rice (8.66 g of conidia/100 g) and was the best substrate to support the growth and development of the fungus for higher yield of spores.

Pandey *et al.* (2010) tested eight grain based media, for *B. bassiana* among them; chickpea was the best culture medium giving highest weight of biomass (0.80 g) and viability (87.00%) whereas, the highest conidial count (6.9×10^7 conidia ml^{-1}) was observed in case of cowpea. For the growth and sporulation of *M. anisopliae* isolate, green gram was most suited grain medium with highest biomass (0.87 g).

Senthamizhlselvan *et al.* (2010) used two solid media: Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA) for the growth of *B. bassiana*. In general, highest mycelial growth was observed in *B. bassiana* isolates and *Verticillium psalliotae* Treschow BPH isolate and lowest mycelial growth was noticed in *V. psalliotae* isolate on PDA and also similar on SDA. Highest spore count was noticed in *B. bassiana* isolate BbMtKKL 2107 (8.90×10^8 spores / ml).

Banu (2012) studied sporulation of fungus *Lecanicillium lecanii* (Zimmerman) on solid state fermentation. Five grains *viz.*, rice, wheat, sorghum, pearl millet and finger millet along with PDA were used. Among various grains tested, maximum spore production of 9.84×10^8 spores g^{-1} was recorded in rice grain followed by wheat grains (9.12×10^8 spores g^{-1}), sorghum (8.92×10^8 spores g^{-1}) and pearl millet (8.11×10^8 spores g^{-1}). The lowest cost ratio over Sabouraud's dextrose Agar with yeast extract [SDAY] was recorded in sorghum.

Bhadauria *et al.* (2012) isolated *B. bassiana* strain from diseased caterpillar of *Spodoptera litura* (Fabricius) and mango mealy bug from field and was inoculated on Sabouraud's dextrose Agar with yeast extract (SDYA) media. Fifteen whole grains *viz.* Wheat, Maize, Paddy, Sorghum, Rice, Groundnut, *Jhangora*, Chick pea, Lentil, Pea, Black gram, *Rajma*, Soybean, Green gram, Cow pea and Pearl millet were used for estimating the sporulation of *B. bassiana*. Highest conidal count (9.06×10^{7} conidia ml^{-1}) was observed on cowpea media followed by soybean.

Yadav *et al.* (2013) used different solid substrates such as grains, vegetable wastes, Maize, Bran, Cotton seed, Rice husk and Wheat for production of fungi like *B. bassiana* and *M. anisopliae*. Result showed that rice was a best solid substrate for spore production and their viability but fungus also grew equally well on maize or other grains.

Media like Czapek's Dox agar (CZA), Potato dextrose agar(PDA), Sabouraud dextrose agar with Yeast extract (SDAY) and Sabouraud maltose agar (SMA) were used for mass multiplication of four species of entomopathogenic fungi *viz. L. lecanii*, *M. anisopliae,Fusarium pallidoroseum* (Cooke) Sacc and *B. bassiana* by Banu and Rajalakshmi (2014). Maximum sporulation of *M. anisopliae* was recorded in SDAY medium. Maximum growth and sporulation of *B. bassiana* was recorded in CZA and PDA media, respectively.

Ingle (2014) isolated pure culture of *Nomuraea rileyi* (Farlow) Samson from naturally infected cadaver of *S. litura*. The dry Sorghum, Wheat, Maize, Pearl millet, Barley, Pigeon pea and Rice grains media as well as Sabouraud's maltose agar + yeast extract (1%) (SMAY), Potato dextrose agar (PDA), Carrot agar medium (CAM), Malt extract agar medium (ME), Sabouraud's dextrose agar + yeast extract (SDA) were also used for study. Significantly higher growth of *N. rileyi* was noticed on SMAY medium i.e. 40.50 mm. Among the substrates tested, sorghum (4.73 x 10^9spore/ml) and maize (4.60 x 10^9 spore/ml) were found to support the production of maximum spore load of *N. rileyi.*

Sidana and Farooq (2014) studied comparison with commercial media for all the eight fungal strains of *Aspergillus Niger* Van Tighem, *Candida albicans*, *Saccharomyces cerevisiae*, and *Fusarium* sp., and four unidentified species F1, F2, F3, and F5, were also grown on potato dextrose agar (PDA), Sabouraud dextrose agar (SDA), cornmeal agar, Sugarcane Bagasse Medium (SBM), Sugar alone, and Commercial Media. Maximum fungal growth was obtained in the case of potato dextrose agar and minimum growth was in sugar agar medium (SAM). Maximum vegetative growth was seen in case of PDA and maximum spores were seen in SBM.

Rajput and Shahzad (2015) studied effect of carbon and nitrogen sources on growth and sporulation of *Trichderma polysporum* (Link) Rifai on organic substrates. Five selected substrates *viz.,* Rice grains, Sorghum grains, Millet grains, Wheat straw and Rice husk (less suitable substrates) were used for multiplication of *T. polysporum.* Highest population *T. polysporum* was observed on sorghum grains (53.2x10^8cfu g^{-1}) followed by millet grains (47.433x10^8 cfu g^{-1}). Rice grains, wheat grains, wheat straw and rice husk performed moderately and produced 29.30x10^8, 23.3x10^8, 20.33x10^8 and 12.60x10^8 cfu $g^{-1}$of substrate, respectively. Increasing concentrations of nitrogen sources *viz.*, DAP, NPK and ammonium nitrate showed positive correlation with the growth of these fungus while Urea showed toxic effect and suppressed the growth of the *T. polysporum.*

Latifian *et al.* (2014) studied the most appropriate medium for the production of *M. anisopliae* in solid phases to produce clamydospores and conidiophores. For solid phase, plant material, including sugarcane, corn, barley, rice, millet and sorghum were used. Among the solid media, *M. anisopliae* recorded the maximum spore production (2.8×10^6 spores/ml) on Rice followed by Sorghum seeds (2.45×10^6).

Eight whole grains *viz.* polished rice, Brown rice, Wheat, Ragi, Sorghum, Bajra, Barley and Maize were used by Karengala *et al.* (2016) for checking the fastest growth of *B. bassiana*, *M. anisopliae* and *L. lecanii.* Among the different grains, they reported that brown rice gave the highest growth rate with highest weight fungal biomass (94 g) While, the *M. anisopliae* showed highest growth and fungal biomass (83 g) and *B. bassiana* recorded more growth and fungal biomass (87 g) in maize.

1.2 To study the performance of different liquid media for sporulation of *M. anisopliae* under laboratory condition.

Bastoz *et al.* (1987) reported that bean broth was the best out of the four liquid culture media tested for producing spores of the entomopathogenic fungus, *M. anisopliae*. The other media tested were rice broth, potato broth and water.

Danger *et al.* (1991) tested coconut water, a cheaper and easily available agricultural waste from copra making industry for mass production of *M. anisopliae*. They observed spore load of *M. anisopliae* (46.66×10^6) on aseptically drawn out coconut water and was superior and the cheapest medium for mass production of the *M. anisopliae*.

Padmanban *et al.* (1997) multiplied the fungi *M. anisopliae* and *B. bassiana* on media like coconut water, potato dextrose broth (PDB). They obtained maximum sporulation of *M. anisopliae* in potato dextrose broth

(1.2 x 10^6spores/ml) but *B. bassiana* did not sporulate in any of the above tested media.

Sharma *et al.* (1999) selected molasses yeast broth as a synthetic medium for mass production of the fungus *M. anisopliae*. The production of *M. anisopliae* conidia was 8x10^8conidia/ml in selected media.

Bhide (2001) selected carrot broth, potato dextrose broth, coconut water, with addition of various ingredients like sucrose and yeast for the multiplication of the *M. anisopliae*. The maximum spore production (307x10^6 spores) was recorded in carrot broth medium.

Two isolates (*Mf189* and *Mf324*) of *Metarhizium flavoviride* Gams and Rozsypal were grown by Fargues *et al.* (2001) on different seven media like Ademek, Catroux, Goral, Jackson, Jenkins–Prior, Kondryatiev, Paris etc. *Mf324* produced submerged propagules in all media, except in Paris medium, in which blastospore production was erratic and occurred. Highest concentrations of *M. flavoviride* propagules were harvested after 72 h incubation from Adamek, followed by Catroux, Jackson, and Jenkins – Prior media. The maximal yield, observed after 72 h culture of*Mf189* in these media, was ca. 300 times greater than the initial concentration.

Rachappa *et al.* (2005) used coconut water, rice gruel and molasses (4%) supplemented with (1%) yeast compared with synthetic liquid media (saborauds maltose broth and potato dextrose broth) for their suitability for growth and spore production of *M. anisopliae.* Both semi-synthetic broth media (PDB and SMYB) were superior over natural liquid media (coconut water, rice gruel and molasses).Spore yield of potato dextrose broth was 12.3 x 10^8.

Mascarin *et al.* (2010) worked on production of fungi *B. bassiana* and *Isaria fumorosa* Wizein biphasic fermentation using agro-industrial products and residues. The three main ingredients used at different combination were rice broth, molasses and dry yeast extract. Besides, complete medium (CM) and potato-dextrose (PD) that used as standards, other natural and alternative culture media included sugarcane molasses, rice broth + yeast, molasses + yeast + rice broth, which resulted in the highest viable propagules concentration. Their findings also supported the use of liquid culture fermentation as a cost-effective process to rapidly produce high yields of stable and infective blastospores of either *B. bassiana* or *I. fumorosa*.

Pandey *et al.* (2010) tested ten media *viz.* Potato Dextrose Broth (PDB), Sabouraud's Dextrose Broth (SDB), Czeapeks Dox Broth (CDB), Malt Extract Broth (MEB) and chitin containing media with or without yeast extract against *B. bassiana* and *M. anisopliae* were prepared. Among synthetic media, Sabouraud dextrose medium with yeast extract was significantly superior over all other media for *M. anisopliae* and *B. bassiana* and supported the maximum biomass (1.13 g, 1.00 g), conidial count (5.10×10^7, 4.80×10^7conidia ml^{-1}) for *B. bassiana* and *M. anisopliae,* respectively. In all media, addition of yeast increased the growth of fungi. SDYB medium was the best with regards to biomass production, conidial count (4.80×10^7 conidia ml^{-1}) of *M. anisopliae* and *B. bassiana.*

Senthamizhlselvan *et al.* (2010) tested broth (PDB and SDB) to attain maximum growth and sporulation of *B. bassiana* (BbMdKKL 2106, BbMtKKL 2107), *Fusarium pallidoroseum* (Cooke sacc) (FmNvKKL 2124, FpCmKKL 1526, FpEvKKL 2119), *Verticillium psalliotae* (Treschow) (VpNlKKL2121 and VpPmKKL 2120). With reference to SDB media tested, highest yield was noticed in FpCmKKL 1526 (2.10g) and it was on par with BbMtKKL 2107 (1.81g), BbMdKKL 2106. Among different isolates of *B. bassiana* and *F. pallidoroseum,* leaf folder isolate recorded highest mycelial weight while lowest weight was noticed in all other isolates with respect to PDB.

The study of Agarwal *et al.* (2012) was to emphasize in pest management of crop, optimization of media for growth of *M. anisopliae* and bioassay of its different formulations for their efficacy as marketable and easily applicable biopesticides. Its different formulations *viz.* Bentonite oil-based liquid formulation (BOBLF), oil based liquid formulation (OBLF). Carrier based powder formulation (CBPF) and there spore count/ml was 1.7x 10^8, 1.2 x 10^8 and 0.9x10^6, respectively. The fungus *M. anisopliae* showed fast growth on a medium maize powder i. e. modified Czeapek's dox media. As also evident from the study, Bentonite was an effective carrier of the *M. anisopliae* in terms of being economical, maintaining the biological activity and increasing the ease of application.

Bhadauria *et al.* (2012) used liquid media such as a potato dextrose broth and saboraud's dextrose broth for sporulation of *B. bassiana*. Liquid media, SDB produced significantly higher spore production of test fungus. Biomass production of *B. bassiana* was 0.69 g and 0.63 g on SDB and PDB, respectively.

Kotwal *et al.* (2012) studied the effect of different parameters on growth and sporulation of *M. anisopliae* and revealed that dry mycelia weight amongst nine media tested Emerson Yeast Phosphate Soluble starch medium (0.450 g), Potato dextrose agar (0.303 g), Cotton cake extract medium (0.343 g), Cotton leaf extract medium (0.226 g), Jowar meal extract medium (0.260 g), Luria agar medium (0.245 g), Cotton seed extract medium (0.273 g), Jaggery yeast extract medium (0.385 g). Amongst them Saboraud's agar + yeast medium (0.566 g) was found best for growth and sporulation of *M. anisopliae*.

Yadav *et al.* (2013) evaluated liquid media such as coconut water at variable moisture content and yeast extract concentration for mass production of two entomopathogenic fungi: *B. bassiana* and *M. anisopliae.* The mass production of fungal spores using diphasic liquid-solid fermentation i.e. LUBILOSA system which was particularly well adapted to the production of *B. bassiana* and *M. anisopliae.* They also revealed that a cheap and effective liquid medium for the mass production of fungi could be prepared using sugar (sucrose) as the carbon source and waste brewer's yeast as the nitrogen source.

Ingle (2014) isolated pure culture of *N. rileyi* from naturally infected cadaver of *S. litura.* Broth media like Barner's medium and Czapek's medium were used for sporulation of fungi *N. rileyi*. Among the ten different broth media evaluated, maximum dry mycelial weight of *N. rileyi* isolates was noted in Sabouraud's maltose + yeast extract broth (SMYB) and observed as a best supporting medium that yielded 0.680 g dry mycelial weight. SDB yielded second highest fungal biomass (0.609 g) followed by BCY broth (0.590 g). YPSSB, Carrot broth, MEB, PDB, Czapek's and Barner's broth were also supported the growth.

Latifian *et al*. (2014) studied the most appropriate medium for the production of *M. anisopliae.* In liquid phase's plant foods, including potatoes, wheat flour, rice flour, and corn flour and sugarcane molasses are used to produce clamydospores and conidiospores of *M. anisopliae*. Among the liquid media, sugar cane molasses produced significantly higher spore (7.6×10^{10} spores/ml). The lowest spore production was reported on potato culture media (7.4×10^2 spores/ml).Hence, Sugarcane molasses extract was elected to produce clamydospores and conidia of *M. anisopliae* due to maximum produce and economic performance.

Patil *et al.* (2014) conducted research for evaluation of nine media of various nutrient sources on the basis of per cent surface coverage and biomass. Sabouraud's dextrose (SD) broth + 1 per cent Yeast extract, Sabouraud's maltose (SM) broth + 1 per cent Yeast extract, Potato peptone (PP) broth, Yeast extract glucose (YEG) broth, Potato dextrose (PD) broth, Potato maltose (PM) broth, Malt extract (ME) broth, Potato glucose (PG) broth and Potato dextrose (PD) broth + 1 per cent yeast extract were prepared. Sabouraud's dextrose broth with yeast extract proved to be the superior which, gave significantly highest cfu ($12.33\text{x}10^8$/ml) and biomass (7.20g). The lowest (48.33%) medium surface coverage and least biomass (1.57g) and cfu (4.33×10^8/ ml) were registered in medium with malt extract

1.3 Bioefficacy of *M. anisopliae* from different solid media against *A. crassivora* under laboratory condition.

Very few references pertaining to bio-efficacy solid media of *M. anisopliae* were available hence references related to other insects and entomopathogenic fungi are reviewed herewith.

Ferron *et al.* (1975) studied the susceptibility of *Oryctes rhinoceros* (L.) adults to *M. anisopliae*, *B. bassiana, and Benthonella tenella* Jefrreys by spraying titrated suspension of spores on the insect integument. They reported that the beetles were susceptible only to strains of *M. anisopliae* of the major type and recorded 100 per cent mortality at a concentration of $1\text{x}\ 10^7$conidia/ml.

Choudhari (1976) observed the colonies of *Coccidohystrixs sinsolita* (Green) on brinjal which were infected by fungus, *M. anisopliae*. In control tests, he dusted brinjal plants severally infested by *C. insolita* in field with spores obtained from cultures of the fungus grown on molasses-peptone agar. After 12-15 days, he observed heavy mealy bug mortality and the plants regained vigour after about a month. Further he concluded that *M. anisopliae* could be used for eradicating mealy bug infestations on brinjal.

Latch (1976) Selected strains of *M. anisopliae* were grown on oat grain and tested in field experiments against *Oryctes rhinoceros* L. It was found that *M. anisopliae* survived in breeding site materials for at least 24 months, with survival not greatly affected by the type of material or by seasonal factors. Naturally occurring breeding sites were examined 3 months after they had been surface treated with oat grain inoculum of *M. anisopliae*. In most sites all the larvae had been killed by the fungus while some contained both diseased and apparently healthy larvae.

Latch and Fallon (1976) used selected strains of *M. anisopliae* against *Oryctes rhinoceros* (L.) on coconut. The fungus was found to survive in breeding site materials for at least 24 months, while without survival not greatly affected by the type of material or by seasonal factors. Naturally occurring breeding sites were examined 3 months after they had been surface treated with oat grain inoculum of *M. anisopliae*. In most sites, all the larvae killed by the fungus while some contained both diseased and apparently healthy larvae.

Babu *et al.* (1983) studied the pathogencity of *M. anisopliae* var. major to the scarabaeid, *Oryctes rhinoceros* L. When the fungus was applied at $34.4 x 10^4$ spores/g farm yard manure, it caused 100 per cent mortality of *O. rhinoceros* larvae.

Darwis (1990) reported that the minimum effective concentration of *M. anisopliae* to control the larvae of *O. rhinoceros* in the laboratory at room temperature was 10^6conidia/kg sawdust. This concentration killed 90 per cent of the larvae.

Chabchoul and Taborsky (1991) studied the effect of treating potatoes with 3 different doses of *M. anisopliae* (1,2 and 4 g at a concentration of 2.8x107 conidia/g against Colorado potato beetle. They observed the greatest effect on fourth instar larvae in the soil.

Padmanban *et al.* (1997) tested the pathogencity of *M. anisopliae and B. bassiana* on white grubs. They multiplied the fungi on various media like PDA, MA, CMA, OMA and sorghum grain. They sprayed the spore suspension of *M. anisopliae* prepared from sorghum grain which gave 16.2 to 100 per cent mortality after 48 hours of the treatment. No mortality was observed due to application of *B. bassiana*.

Theunis and Aloali (1998) tested the strains of *M. anisopliae* against *Papuana uninodis* L., a pest of okra. The most effective strain against *P. uninodis* larvae and adults was Ma TB 101. For the adults, starin Ma F1 384 of *M. anisopliae* from *Popiliia japonica* L. was almost equally effective. They found that the strain Ma TB 101 was signifiacantly more effective against both adults and larvae than all other isolates.

Ambethgar *et al.* (1999) reported the infection of cashew root and stem borer, *Plocaederus ferruginous* L. by *M. anisopliae* in Tamil Nadu. They also reported the mortality rate of around 19-26 per cent under natural conditions. They isolated the fungus on PDA medium and confirmed the pathogenicity and also recorded cent per cent mortality of the larvae 10 days after inoculation.

Bhide (2001) stated that there was a mortality of 50 per cent adults of *O. rhinoceros* due to application of a spore load of $3x10^8$per ml of the suspension of *M. anisopliae*. In the first instar, the body of the grub became brittle and braked into small pieces. The results revealed that highest mean mortality 42.5 was recorded by application of a spore load of $50x10^7$ per kg cow dung, all the treatments were significantly superior over the control.

Sahayaraj and Borgio (2010) isolated *M. anisopliae* on Potato Dextrose Agar (PDA). Four spore concentrations such as 1.7×10^4, 2.6×10^5, 1.9×10^6 and 1.6×10^7 spores/ml were prepared from the stock and applied on *Pericallia ricini* Fabricius, *Helicoverpa armigera* (Hubner), *Mylabris Pustulata* (Thunb.) and *A. craccivora* and *S. litura*. Lowest (1.84×10^4) and highest (2.38×10^5) LC_{50} values were recorded on *A. craccivora* and *M. pustulata,* respectively. Among all the tested pests, the mycelial growth was observed only on *A. craccivora*.

Antonio *et al.* (2012) studied *B. bassiana, M. anisopliae* and *I. fumorosa* as biological control agents for whitefly, *B. tabacci*. Mortality of eggs, first, second and third instar nymphs, pupae and adults were monitored on leaves located at three different heights in the canopy. The *M. anisopliae* was significantly more effective against eggs, first, second and third instar nymphs and pupae. Their results indicated that mixed applications of *M. anisopliae* and *B. bassiana* could maximize the likelihood for control of all stages of *B. tabacci*.

Banu (2012) studied solid state fermentation, five grains *viz.*, Rice, Wheat, Sorghum, Pearl millet and Finger millet along with PDA were used for multiplication of *L. lecanii.* Virulence of spores produced in solid substrates (Spores @ 1x10^7 ml^{-1}) were sprayed onto the *Paracoccus marginatus* Williams & Granara de Willinkand 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.

Hussein *et al.* (2012) studied pathogenicity of *B. bassiana* and *M. anisopliae* against greater wax moth *Galleria mellonella* L. In this study, 90 isolates of *B. bassiana* and 15 isolates of *M. anisopliae* were screened for proteases and lipases production. In the bioassay, the selected isolates evidenced high virulence against the 4th instar of *G. mellonella* larvae. The isolates BbaAUMC3076, BbaAUMC3263 and ManAUMC3085 realized 100 per cent mortality at concentrations of 5.5 x 10^6 conidia ml^{-1}, 5.86 x 10^5 conidia ml^{-1}, and 4.8 x 10^6 conidia ml^{-1}, respectively.

Sajap *et al.* (2012) used *I. fumorosa* and *M. anisopliae* for controlling Tiger moth, *Atteva sciodoxa*, a pest of *Eurycoma longifolia* (Simaroubaceae). The larvae were separately sprayed with concentrations of 1 x 10^2 to 1 x 10^5 conidia ml^{-1} of each fungal isolate. Both fungi were pathogenic to third instar larvae of *A. sciodoxa.* However, *M. anisopliae* was more virulent than *I. fumorosa*. The median effective concentrations for *M. anisopliae* and *I. fumorosa* were 4.23 x 10^3 and 8.24 x 10^4 conidia ml^{-1}, respectively. *M. anisopliae* killed 48 to 88 per cent larvae while *I. fumorosa,* 26 to 62 per cent larvae for the highest and lowest concentrations, respectively. This study indicated the potential of *M. anisopliae* for controlling *A. sciodoxa.*

Pokhrel *et al.* (2014) conducted experiment to evaluate the level of effects by different concentrations of indigenous entomopathogenic fungus, *M. anisopliae* spores by dipping the non-target organism, the fourth instar silkworm larvae, *Bombyx mori* L. The mortality of silkworm larvae due to fungus started only after five days of the treatment and finally caused 70, 44.44, 28.88, 26.66, 23.33 and 3.33 percent death of silkworm larvae with 10^7, 10^6, 10^5, 10^4, 10^3 and 10^2spores per ml of fungal conidial concentrations, respectively. The laboratory result indicated that even a small number of conidia of *M. anisopliae* caused mortality and was found hazardous to silkworms.

Hemalatha *et al.* (2015) collected *Scirtothrips dorsalis* Hood and *Thrips tabacci* (Lindeman) from the field, mass reared and maintained on the 60 days old chilli and tomato plants. Five different *Metarhizium* fungal spore concentrations (1 x 10^8 to 1 x 10^4spores ml^{-1}) were prepared and each concentration sprayed on thrips using atomizer. Thrips sprayed with 0.05 per cent Tween 80 solution served as control. *M. flavoviride* var. *minus* had higher virulence against both chilli and tomato thrips than *M. anisopliae* species.

Visalakshi *et al.* (2015) conducted research on sugarcane for white grub management using two entomopathogenic fungi, *B. bassiana* and *M. anisopliae* at sugarcane field. Reduction in white grub damage over control was high in FYM enriched with *M. anisopliae* @ 5x10^{13} spores ha^{-1} (89.5%) followed by FYM enriched with *B. bassiana* @ 5x10^{13}spores ha^{-1} (81.96%). Both the entomopathogenic fungi were found effective in reducing the white grub population and plant damage.

1.4 Bioefficacy of culture filtrates *M. anisopliae* from different solid media against *A. crassivora* under laboratory condition.

Very few references pertaining to bio-efficacy of *M. anisopliae* from different liquid/broth media were available hence, references related to other insects and entomopathogenic fungi irrespective of liquid media are reviewed herewith.

Sivapragasam and Tey (1994) tested the three isolates of the entomopathogenic fungi, *M. anisopliae* against the larvae of *O. rhinoceros* scarabaeid. They followed the dipping of third instar larvae in conidal suspension of 10 billion spores/ml, which led to the development of brown lesions on the body of the larvae.

Fernando *et al*. (1995) inoculated the larvae and adults of *O. rhinoceros* L. with fungus *M. anisopliae*. All the isolates caused 100 per cent mortality of larvae and adults when treated with suspension of 10^7conidia/ml. The local isolates caused a slow death rate in larvae in adults compared to other isolates.

Padmanban *et al.* (1997) tested the pathogenicity of *M. anisopliae and B. bassiana* on white grubs. They sprayed the spore suspension of *M. anisopliae* prepared from PDB media at the rate 104,105 and 106spores/ml on white grubs. They recorded no mortality due to application of *B. bassiana* and *M. anisopliae* with the spore suspension prepared from PDB.

Cherry *et al.* (1999) used biocontrol agent *viz.*, fungi (*B. bassiana and M. anisopliae*), bacteria (*Bacillus thuringiensis and Serratia marcesens*), and viruses (*granuloviruses* and *cytoplasmic polyhedroviruses*) to control maize stem borer. Amongst *B. bassiana* isolates possessing the capacity to grow systemically in the maize plant are considered one of the more interesting candidates for development as microbial control agents despite limited control in preliminary trials.

Ekesi *et al.* (1999) conducted field experiments to assess the combined effects of intercropping cowpea with maize and the application of *M. anisopliae* and synthetic insecticide Karate (Lambda cyhalothrin) on the density of *Megalurothrips sjostedti* Trybom of cowpea. Thrips density and damage were significantly lower in the intercrop treated with fungus than in the control plots. Results suggest that *M. anisopliae* is a promising candidate for the management of *M. sjostedti*, especially within a cowpea/maize intercropping systems.

Sharma *et al.* (1999) selected molasses yeast broth as a synthetic medium for mass production of the fungus *M. anisopliae*. They observed high virulence of *M. anisopliae* ($8x10^8$) against *Maladera insanabilis* L. and *Holotrichia consanguinea* L. with $LT_{50}$of 7.95 and 16.20 respectively.

No mortality was recorded in the grubs of Rhinoceros beetle by the application of remainants of carrot broth medium. There were no any symptoms of fungal infection on the grubs. This indicated that the remainants of carrot broth medium after removal of fungal mat does not contain any fungal spore in it. (Bhide, 2001).

Ansari *et al.* (2008) reported combined use of the entomopathogenic nematodes (EPNs), *Heterorhabditis bacteriophora* Poinar, *Steinernema feltiae* Bovien, and *Steinernema krausse I.* (Steiner) and EPF *M. anisopliae* were evaluated for control of third-instar black vine weevil, *Otiorhynchus sulcatus* Fabricius. The combined application of EPNs with *M. anisopliae* resulted 100 per cent larval mortality.

Mohanty *et al.* (2008) 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.

Akbarian *et al*. (2012) studied the pathogenicity of six isolates of *B. bassiana* and two isolates of *M. anisopliae* investigated. Five different concentrations from each isolate, 1 x 10^7, 5 x 10^7, 1 x 10^8, 5 x 10^8 and 1 x 10^9 conidia ml^{-1}, were applied in bioassays on 2nd and 4th instars larvae. Based on these results susceptibility of second instar larvae to all *B. bassiana* and *M. anisopliae* isolates was significantly higher than fourth larval instar of Colorado potato beetle.

Balachander *et al.* (2012) compared the virulence of six native and two standard ARSEF isolates of *M. anisopliae* against this Mahogany shoot borer pest *Hypsipyla robusta* (Moore). The mycotoxic effect of ARSEF 2596 and ARSEF 3603 showed LD_{50} value of 3.7 per cent and 5.6%. However, IWSTMa7 was highly lethal with significant lowest LD_{50} value of 2.6 per cent.

Banerjee *et al.* (2012) evaluated four different fungal pathogens *viz., B. bassiana, F. solani, V. lecanii, P. fumosoroseus* against cowpea aphid. Throughout the study, it was observed that per cent reduction of aphids due to *V. lacanii*-3 increased with time. Among different concentration of *V. lecanii* -3 @ 1x10^{10}conidia/ml showed highest per cent reduction of 79.32.

Jeong *et al*. (2013) 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 Beauveria 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.

Kapoor *et al.* (2013) exposed larvae and adult females of *Aedes aegypti* (L.) to fungal suspensions of three virulent strains of *M. anisopliae* viz., M34412, M34311 and M81123.Values of LC_{50} varied from 5.92 x 10^3 conidia/ml for M34412, 3.49 x 10^4 conidia/ml for M34311 and 5.12 x 10^5 conidia/ml for M81123. Based on virulence and stability, the most promising strain, *M. anisopliae* M34412 was used for lethal exposure time determination. An exposure time of only 4 h was necessary to cause 50 per cent mortality.

Boruah and Dutta (2014) 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 per cent 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.

Kumar *et al.* (2015) conducted field trial to check the efficacy of *B. bassiana* and *M. anisopliae* against *Kharif* Grass Hopper (*Hieroglyphus banian*), Yellow Stem Borer (*Scripophaga incertulas*) and Rice Hispa

Beetle (*Dicladispa armigera*). Used *B. bassiana* @ 5 kg/ha, *M. anisopliae* @ 5 kg/ha, and chloropyriphos @ 3.5 l/ha were applied. The overall mean population after spraying of different treatments on the polled data it is found that *B. bassiana* @ 5 kg/ha followed by T_{5}- *M. anisopliae* @5 kg/ha. Biocontrol agents like *B. bassiana* and *M. anisopliae* are being used as an alternate of chemical and it is cheap and ecofriendly.

Mane *et al.* (2015) tested pathogenicity of entomopathogenic fungi, *M. anisopliae*, *Beauveria brongniartii* (Sacc.) and *B. bassiana* against white grub, *Leucopholis lepidophora* (Blanchard). Among overall concentrations, $2x10^{8}$ conidia ml^{-1} concentration was the most promising for highest grub mortality in each entomopathogenic fungi. The results showed that *M. anisopliae* was found to be more pathogenic than *B. brongniartii* and *B. bassiana.*

CHAPTER III

MATERIALS AND METHODS

The present investigation entitled "suitability of different and pathogencity studies of *Metarhizium anisopliae* (Metschnikoff) Sorokin against *Aphis craccivora* (Koch)" 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 present research was carried out to standardize the method for mass culturing the green muscardin fungus, *M. anisopliae* under Konkan conditions and to test the efficacy of *M. anisopliae* against *Aphis craccivora* (Koch) under laboratory conditions. The material used and the methods adopted during the investigation are discussed in the following chapter.

3.1. MATERIALS

3.1.1. Chemicals

The details of the various laboratory chemicals used in the present investigation for media preparation are given below:

3.1.1.1 Chemicals for media preparation:

i. Sucrose as a energy source
ii. Agar-agar as a solidifying agent

3.1.1.2 Chemicals for surface sterilization

i. Mercuric chloride ($HgCl_2$)
ii. Ethyl alcohol (70 %)

3.1.2. Glass wears

i. Conical flasks of capacity 250, 500 ml
ii. Beakers of capacity 500 ml
iii. Petri plates of size 100 x 20 mm
iv. Pipettes of capacity 10 ml
v. Micropipettes of capacity 100-1000 µ
vi. Measuring cylinders of capacity 10 and 1000 ml

3.1.3. Laboratory Equipments

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

3.1.4. Others

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

3.1.5. 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.

3.1.6. Culture medium (solid)

The treatments details given in Table No.1

Table 1: Composition of solid medium.

Treatment No.	Media	Weight (g)
T1	Sorghum grain (Standard medium)	50
T2	Tea waste (without sugar and milk)	50
T3	*Nagli* husk	50
T4	Soybean chunks	50
T5	Sugarcane baggase	50
T6	Wheat grain	50
T7	Tea waste (with sugar and milk)	50
T8	Banana psuedostem	50

Table 2: Composition of liquid medium (For 100 ml)

Treatment No.	Broth/liquid Medium	Quantity for 100 ml
T1	**Coconut water**	100 ml
T2	**Carrot broth** Carrot Dextrose Water	 25 g 2.0 g 100 ml
T3	**Potato dextrose broth**	

	Potato Dextrose Water	25 g 2.0 g 100 ml
T4	**Coon's (broth) medium** Sucrose Dextrose Magnesium sulphate Potassium nitrate Distilled water	 0.72 g 0.36 g 0.12 g 0.20 g 100 ml
T5	**Starch (Corn flour) + water** Corn meal Peptone Dextrose Distilled water	 2 g 2 g 2 g 100 ml
T6	**Czeapek's Dox medium** Sucrose Sodium nitrate Potassium diphosphate Magnesium sulphate Ferrous sulphate Distilled water	 3 g 0.2g 0.1 g 0.05 g 0.05 g 100 ml
T7	**Molasses + yeast + water** Molasses – 10 ml Yeast – 3 g Distilled water – 1000 ml	 1 ml 0.3 g 100 ml
T8	**Gram flour + water** Gram flour Peptone Dextrose Distilled water	 2 g 2 g 2 g 100 ml
T9	**Control (Water)**	100

3.2 METHODOLOGY

3.2.1 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 $26^0C \pm 2^0C$ in an incubator till sporulation and the master culture was maintained in refrigerator. Mass multiplication of *M. anisopliae* using different solid and liquid media is discussed below.

3.2.2. Experiment details:-

Statistical Design: - CRD (Complete randomized design)

No. of Repetition: - 3

No. of Treatments: - 8

Table 3: Details of various treatments given for standardization of solid media for sporulation of *M. anisopliae*.

Treatment No.	Solid media	Weight (g)
T1	Sorghum grain (Standard media)	50
T2	Tea waste (without sugar and milk)	50
T3	*Nagli* husk	50
T4	Soybean chunks	50
T5	Sugarcane baggase	50
T6	Wheat grain	50
T7	Tea waste (with sugar and milk)	50
T8	Banana psuedostem	50

3.2.3 Mass multiplication 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^{0}C, 15 psi for 1 h. After sterilization, bags were kept for cooling. With the help of cork (size 5 mm), abit of PDA containing *Metarhizium* was removed and inoculated in each bag in aseptic condition. After inoculation, the bags were incubated at room temperature (28^{0}C). 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.

3.2.4. Method of spore count:

After 20 days of inoculation, the spore mass developed on grains and also other media was taken at 1 g from each polypropylene bag and crushed properly in small amount of sterilized water and filtered with the muslin cloth. Further, the volume was adjusted to 10 ml in each of the test tube and all test tubes were well shaken. Then eight test tubes, each containing 9 ml sterilized distilled water were arranged serially. With the help of a micropipette (1000 μl), 1 ml spore suspension was taken from the test tube containing 10 ml stock suspension and was added in the 1st test tube containing 9 ml sterilized distilled water. In this way, the total volume in the first test tube became 10 ml and it was the first dilution of spore suspension. This way 1 ml quantity of spore suspension from first test tube was transferred to another test tube containing 9 ml sterilized distilled water to make volume of second test tube 10 ml and this was the second dilution of spore suspension. After repeating the same procedure of dilution next dilutions were made up to 8th test tube. In this way, eight dilutions *viz.* 1:10, 1:100, 1:1000, 1: 10000, 1:100000, 1:1000000, 1:10000000, 1:100000000 were available in 1 to 8 test tubes, respectively. From 8th test-tube, 0.5 ml representative suspension was taken in amicropipette aseptically and transferred at the center of sterilized Petri plate. Three such Petri plates were prepared. The sterilized PDA medium was melted, cooled to 40^{0}C and one pinch (500 mg) crystals of antibiotic, streptomycin was added aseptically to prevent the bacterial growth. The plates were shaken gently to mix the medium and spore suspension. The plates were incubated at room temperature and fungal growth was monitored after 24 h. Colonies from each plate were counted by digital colony counter within 24-36 h from inoculation.

3.2.5 Mass multiplication on broth liquid/media

For the multiplication of *M. anisopliae*, different broth/liquid media were used as mentioned below.

Experiment details:

Statistical design: - CRD (Complete randomized design)

No. of repetition: - 3

No. of treatments: - 9

Table 4: Details of various liquid media for sporulation of *M. anisopliae*.

Treat. No.	Broth/liquid Medium	Volume (ml)
T1	Coconut water	100
T2	Carrot broth	100
T3	Potato dextrose broth	100
T4	Coon's (broth) medium	100
T5	Starch (Corn flour) + water	100
T6	Czeapek's Dox medium	100
T7	Molasses + yeast + water	100
T8	Gram flour + Water	100
T9	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. Only 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. Only 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 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. 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.

3.2.6 Method of determining mat weight

After 3 - 4 days, the mycelial mat of *Metarhizium* was monitored on surface of the liquid media. After sufficient development of the mat (20 days after inoculation), the media was filtered through funnel fitted with filter paper (What man # 2). Each filter paper disc was initially weighed. Mat was collected along with filter paper and remaining suspension was stored in the conical flask. The mat was dried in hot air oven at 100^{0}C for 5 min and then the weight of the mass taken by deducting filter paper weight.

3.2.7 Mass rearing of *Aphis craccivora* (Koch)

The nymphs of aphid were collected in large numbers from their breeding places from the cow pea plants. The field collected nymphs were reared on cow pea 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 (Plate XI).

3.2.8 Testing the 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: - 8 (Solid media)

No. of aphid per treatment: - 10

Three cow pea seedlings in small plastic cup with 10 aphid nymphs per seedling were maintained to take treatments (Plate X).

3.2.9 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.

3.2.10 Testing the Bio-efficacy of culture filtrate *M. anisopliae* from different liquid media against Aphids.

Experiment details:

Statistical design: - CRD (Complete randomized design)

No. of repetition: - 3

No. of treatments: - 9 (Liquid media)

No. of aphid per treatment: - 10

3.2.11 Method of application of fungal suspension of liquid medium.

The culture filtrate of each liquid medium was taken into a saloon sprayer. Remaining procedure was similar as mentioned earlier under 3.2.8. (Plate XII).

3.2.12 Method of recording observation:-

The aphids were observed daily for the symptoms of fungal infection and mortality in both the media tests. After spraying, mortality was observed within 24, 48, 72, and 96 h and dead aphids were counted to determine percent mortality. The data before analysis of variance was subjected to arcsine transformation and presented.

CHAPTER IV
RESULTS AND DISCUSSION

Studies on the mass multiplication of *Metarhizium anisopliae* (Metschnikoff) Sorokin using different solid and liquid media and tested its pathogencity against Aphid, *Aphis craccivora* (Koch) were conducted in the Quarantine laboratory of Department of Plant Pathology and Department of Entomology, College of agriculture, Dapoli during 2016-17. The results of various experiments are presented and discussed in this chapter.

4.1 Standardization of method for mass multiplication of *M. anisopliae.*

The commercial utilization of *M. anisopliae* as a bio-pesticide necessitates its mass production. Several workers have employed various substrates for its mass multiplication at different localities in the world, but under Konkan conditions the mass multiplication of *M. anisopliae* on different agricultural residue media has not been tried yet. So it was felt necessary to standardize method for mass multiplication of *M. anisopliae,* for this purpose different solid and liquid/broth media were tested and the results of various media are given below.

4.1.1 Mass multiplication of *M. anisopliae* on different solid media.

During the present investigation the efforts were made to obtain maximum sporulation of *M. anisopliae* within a shortest period and also a cheaper medium which will suffice the said purpose. For the purpose of which, the grains like, Sorghum, Wheat and also some agricultural residues like Banana pseudostem, Sugarcane baggase, *Nagli* husk, Tea waste and Soybean chunks, were tested as a solid media. The results of a statistically designed laboratory experiment are summarized in Table 5 and depicted in Fig. 1(Plate I,II,III,IV).

The result indicated that there were significant differences among various treatments. The higher conidia production after 20 days of inoculation was recorded in the treatment T_8- banana pseudo stem with 951.33 x 10^8spores per g which, was at par with T_2-Tea waste (930.67 x 10^8) which also supported good growth of the fungus. Among other treatments, treatment T3-*Nagli*husk produced 528.67 x 10^8spores followed by T_1- Sorghum grain (454.67 x 10^8) which at par with T_7-Hotel tea waste (397.33 x 10^8) while other treatments *viz.,* T_6- Wheat grain and T_4-Soybean chunks produced 327.33 x 10^8, 172 x 10^8spores, respectively. The minimum spore production was recorded in sugarcane baggase (41.33 x 10^8).The results in general indicated that amongst various solid media tested, the banana pseudo stem was found to be the most suitable medium for mass multiplication of *M. anisopliae* followed by Tea powder waste. Among these two media banana pseudo stem contains rich cellulose. Similarly, tea powder waste contains dried tea leaves which, also represents cellulose material. This might have favored mycelial growth and more number of spores. Banu (2012) obtained maximum sporulation of fungus *Lecanicillium lecanii* (Zimmerman) on solid state fermentation with rice grains (9.84 x 10^8 spores g^{-1}) followed by wheat grains (9.12 x 10^8 spore's g^{-1}). Sharma *et al.* (1997) and Bhide (2001) obtained the maximum sporulation of *M. anisopliae* on sorghum grains. Thus the results of present investigation are in line with above.

Table 5: Mass multiplication of *M. anisopliae* on solid medium.

Treat. No.	Treatment (Solid media)	Conidia production ($x 10^8$) per g			Mean conidia production ($x 10^8$) per g
		RI	RII	RIII	
1	Sorghum grain	474 (21.772)*	446 (21.119)	444 (21.071)	454.67 (21.32)
2	Tea waste (without milk and sugar)	974 (31.209)	938 (30.627)	880 (29.665)	930.67 (30.5)
3	*Nagli* husk	524 (22.891)	544 (23.324)	518 (22.760)	528.67 (22.99)
4	Soybean chunks	180 (13.416)	160 (12.649)	176 (13.266)	172.00 (13.11)
5	Sugarcane baggase	62 (7.874)	24 (4.899)	38 (6.164)	41.33 (6.312)
6	Wheat grain	332 (18.221)	358 (18.921)	292 (17.088)	327.33 (18.08)
7	Hotel tea waste (with sugar and milk)	452 (21.260)	338 (18.385)	402 (20.050)	397.33 (19.9)
8	Banana pseudo stem	942 (30.692)	952 (30.854)	960 (30.984)	951.33 (30.84)
S.E. ±					0.51
C.D. at 5%					1.52

*Figures in the parentheses are square root transformed values

4.2.1 Mass multiplication of *M. anisopliae* on liquid/broth medium.

In order to obtain maximum growth of the *M. anisopliae* within shortest period, the efforts were made to multiply it on various liquid or broth media. The results of a statistically designed laboratory experiment are presented in Table 6 and depicted in Fig. 2. (Plate VII,VIII,IX). The maximum mycelium mat weight after 20 days of inoculation was recorded in T_6- Czeapek's broth medium (1554 mg) which, was at par with T_5- corn flour medium (1545 mg). Further, next best treatment T_8- Gram flour medium (1196 mg) was at par with T_2- Carrot broth (1121 mg) and T_1- Coconut water (1064 mg). Also T_2- Carrot broth was at par withT_1-Coconut water andT_7- Molasses (985 mg). Further, Molasses was at par withT_3- potato dextrose broth (938mg). In control treatment T_9- Sterilized distilled water no fungal mat was recorded. However, 558 mg weight of the bit of agar which was initially inoculated in the sterile distilled was recorded. Among the liquid media tested, T_4- Coon's medium was found to be the weakest medium which produced lowest number of spores (661mg) at 20 days of inoculation.

Table 6: Mass multiplication of *M. anisopliae* on liquid/broth medium.

Treat. No.	Treatment	Mycelial (Mat) Production			Mean weight of dry fungal mat (mg)
		RI	RII	RIII	
1	Coconut water	1014 (31.84)*	1046 (32.34)	1132 (33.65)	1064 (32.61)
2	Carrot broth	1167 (34.16)	1110 (33.32)	1086 (32.95)	1121 (33.48)
3	Potato dextrose broth	998 (31.59)	894 (29.9)	922 (30.36)	938 (30.62)
4	Coon's medium	697 (26.4)	618 (24.86)	670 (25.88)	661.66 (25.71)
5	Corn flour medium	1503 (38.77)	1326 (36.41)	1806 (42.5)	1545.67 (39.23)
6	Czeapeks medium	1510 (38.86)	1536 (39.19)	1616 (40.2)	1554 (39.42)
7	Molasses	1066 (32.65)	971 (31.16)	918 (30.3)	985 (31.37)
8	Gram flour medium	1087 (32.96)	1209 (34.77)	1292 (35.94)	1196 (34.56)
9	Control- (Sterile distilled water)	566 (23.79)	563 (23.73)	545 (23.35)	558 (23.62)
S.E. ±					0.78
C.D. at 5%					2.31

*Figure in the parentheses are square root values

The results in general revealed that the Czeapeks medium was found to be the best suitable liquid medium for mass multiplication and was found at par with corn flour medium. Further, two flour media and coconut water also emerged as promising media for mass production of *M. anisopliae*. Danger *et al*, (1991) reported that the aseptically drawn out coconut water was the superior and cheapest medium for mass production of *M. anisopliae*. During present investigation also coconut water gave better fungal development. Further, Pandey *et al.* (2010) revealed that Sabouraud's Dextrose Yeast Broth medium was the best with regards to biomass production, conidial count (4.80×10^7 conidia ml^{-1}) of *M. anisopliae* and *B. bassiana.* Thus the present findings are in accordance with that of above findings.

4.3.1. 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 7, (PLATE X) and presented in Fig.3. 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).

Table 7: Bioefficacy of *M. anisopliae* from different solid media against *A. craccivora*

Treat. No.	Treatment	Mortality of aphids (%)			Mean per cent mortality
		RI	RII	RIII	
1	Sorghum grain	90 (71.57)*	80 (63.43)	90 (71.57)	86.67 (68.86)
2	Tea waste (without sugar and milk)	90 (71.57)	80 (63.43)	80 (63.43)	83.33 (66.14)
3	*Nagli* husk	70 (56.79)	60 (50.76)	60 (50.76)	63.33 (52.78)
4	Soybean chunks	70 (56.79)	80 (63.43)	70 (56.79)	73.33 (59.00)
5	Sugarcane baggase	50 (45.00)	60 (50.76)	70 (56.78)	60 (50.85)
6	Wheat grain	70 (56.78)	70 (56.78)	60 (50.76)	66.67 (54.78)
7	Hotel tea waste (with sugar and milk)	60 (50.76)	50 (45.00)	60 (50.76)	56.67 (48.85)
8	Banana pseudo stem	80 (63.43)	70 (56.78)	80 (63.43)	76.67 (61.22)
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

Among reamaining 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* against is available for comparison hence other reference are included here with

Sahayaraj *et al.* (2010) 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) 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 &Granara de Willinkand 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) 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 cow pea 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 (plate XIII). This fungal growth became olive green in colour after three days and covered entire body (plate XII).

4.4.1. 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 (Plate XII). The results are summarized below.

Table 8: Bioefficacy of culture filtrates of *M. anisopliae* from different liquid media against *A. craccivora*

Treat. 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 sin values

The data on per cent mortality of aphids are presented in Table 8 and depicted in Fig. 4. Results of the experiment indicated that all the treatments were significantly superior over control. The highest mean mortality of 86.67per 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 48.85 per cent, respectively and all were at par.

Padmanban *et al.* (1997) 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) 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) 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) 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.

The infected aphids became inactive and sluggish, green spots were observed on the body after about 2 days of infection which was the first external sign of infection (plate XIII). Later green mycelial growth of the fungus was observed on the body of aphids.

CAHPTER V

SUMMARY AND CONCLUSIONS

The green muscardine fungus *viz*., *Metarhizium anisopliae* (Metsch.) Sorokin (Deuteromycotina: Moniliales) is already reported to be a very useful fungus for the management of *Aphis craccivora*. Although, the techniques for its mass multiplication and effectiveness has not been tried in Konkan region of Maharashtra. Therefore, efforts were made during the present investigation to standardize the method for its mass multiplication and to find out the effectiveness of this fungus from different solid and liquid media against *Aphis craccivora* (Koch) under laboratory conditions. The results of various experiments conducted in the Quarantine laboratoryof Department of Plant Pathology and Department of Agril. Entomology, College of Agriculture, Dapoli during 2016-17 are summarised in the following chapter.

Studies on the mass production of green muscardin fungus, *M. anisopliae* on solid media indicated that treatment T_8-Banana psuedostem was the most suitable solid medium for multiplication of the fungus under Konkan condition. Mean spore production of T_8-Banana psuedostem after 20 days of inoculation was (951.3×10^8) spores per gram which was at par with T_2-Tea waste (930.67×10^8) and was second best media. Whereas; Sugarcane baggase produced lowest number of spores (41.33×10^8) at 20 days of inoculation of the fungus. Studies on the mass multiplication of *M. anisopliae* on liquid / broth medium showed that the T-6 Czeapeks broth medium was the most suitable medium for mass multiplication of *M. anisopliae* (average mat weight of dry fungus 1554 mg), after 20 days of inoculation which was at par with T_5-corn flour medium (1545.67 mg). Further, Treatment T_8-Gram flour medium (1196 mg) was at par with T_2-Carrot broth (1121 mg) and T_1-Coconut water (1064 mg), which also supported good growth of the fungus.

Results of the bio-efficacy of *M. anisopliae* against *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%). However, in liquid/ broth medium 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%). No mortality was revealed in control treatment of both solid and liquid medium experiments.

As far as the symptoms of infection by *M. anisopliae* on aphids were concerned, the infected aphids were found at the surface of soil and plant. Subsequent symptoms observed were inactiveness, development of coppery fungal growthon the body which, further developed to olive green muscardin fungus matall over the body parts.

From the results of present studies, it could be concluded that the Banana psuedostem, Tea waste and Czeapeks broth medium were found to be the best for mass multiplication of *M. anisopliae* under laboratory conditions from solid and liquid media, respectively. The *A. craccivora* could be effectively infected and killed by spores of *M. anisopliae.*

LITERATURE CITED

Agarwal, R., Choudhari, A., Tripathi, N., Patil, S., Agnihotri, S. and Bharti, D. (2012). Biopesticidal formulation of *Metarhizium anisopliae* (Metsch.) Sorokin effective against larvae of *Helicoverpa armigera. Int. J. Agric. Food Sci.,* **2**(2):32-36.

Akbarian, J., Ghosta, Y., Shayesteh, N. and Safavi, S. (2012). Pathogenicity of some isolates of *Beauveria bassiana* (Bals.) Vuill. And *M. anisopliae* on 2nd and 4th larval instars of Colorado potato beetle, *Leptinotarsadecemlineata* (Say) (Col.:Chrysomelidae), under laboratory conditions. *African J. Microbio. Res.,* **6**(34):6407-6413

Ambethgar, V., Lakshman, V., Dinakaran, D. and Selvarajan, M. (1999). Mycosis of cashew stem and root borer, *Plocaederus ferruginous* L. by *M. anisopliae* from Tamilnadu (India). *J. Ent. Res.,* **23**(1):81-83.

Ansari, M. A., Shah, F. A. and Butt, T. M. (2008). Combined use of entomopathogenic nematodes and *M. anisopliae* as a new approach for blackvine weevil, *Otiorhynchus sulcatus*, control. *Entom. Exp. Applicata.*, **10**.1111:1570-7458

Antonio, F., Micaela, P. D., Rodriguez, N. S., Mayra, M. and Miguel, A. R., (2012). Mycoinsecticide effects of *B. bassiana*, *M. anisopliae*, and *I. fumosorosea* on the whitefly *B. tabaci* (Homoptera: Aleyrodidae) in different strata of bean. *African J. Microbio. Res.*, **6**(45):7246-7252.

Babu, S. P.C., Balsubramanian, M. I. and Jayraj, S. (1983). Studies on the pathogenicity of *M. anisopliae* var. major Tolloch in *Oryctes rhinoceros* (L.). *Res. Pub. Tamil nadu Agril. Uni. Coimbatore,* India. No. **1** pp.32.

Balachander, M., Remadevi, O. K., Sasidharan, T. O., and Bai, S. N. (2012). Virulence and mycotoxic effects of *M. anisopliae* on Mahogany shoot borer, *Hypsipyla Robusta* (Lepidoptera: Pyralidae). *J. For. Res.,* **23**(4):651-659.

Banerjee, S. C., Khan, K. H. and Prasanna, P.M. (2012). Efficacy of different fungal pathogen against cow pea aphid, *Aphis craccivora* Koch under lab and field condition. *Int. J. Plant Prot.*, **5**(1):68-71.

Banu, J. G. (2012). Effect of solid substrates on growth and sporulation of *Lecanicillium lecanii* and pathogenic activity to Mealy bug. *Ann. Plant Prote. Sci.*, **21**(1):176-223.

Banu, J. G. and Rajalakshmi, S. (2014). Standardization of Media for Mass Multiplication of Entomopathogenic Fungi, *Indian J. Plant Prot.,* **42**(1):91-93.

Bastoz Cruze, B. P., Abreu, O. C., Oliveira, D. A. and Chiba (1987).Growth of *M. anisopliae* in natural liquid culture media. *ENT. Newsletter.*, 18:3.

Bhadauria, B. P., Puri, S. and Singh, P. K. (2012). Mass production of entomopathogenic fungi using agricultural products. *The Bioscan,* **7**(2):229-232.

Bhide, S. G. (2001). *The use of M. anisopliae for the control of Rhinoceros beetle infesting coconut palms*. M.sc. (Agri.) thesis (Unpublished), Submitted to Dr. Balasaheb Sawant Konkan Krushi Vidhyapeeth Dapoli, Maharashtra.

Boruah, S. and Dutta, P., (2014). Preliminary evaluation of bioformulations of *M. anisopliae* against cowpea aphid, *Aphis craccivora. Insect Envi.,* **20**(2).

Burges, H. D. (1998). Formulation of mycoinsecticides. In: Formulation of Microbial Biopesticides. *Kluwer Academic publi.,* Dordrecht, pp. 131 – 185

Chabchoul, H. and Taborsky, V. (1991). Use of *M. anisopliae* against Colarado Beetles, *Leptinotarsa decemlineata* (Say*). Agric. Tropica-et-Sbtropica.*, **24**:31-38.

Choudhari, S. (1976). *M. anisopliae* on brinjal mealy bug and its use in the biological control of the pest. *Current sci.,* **45**(1):34

Cherry, A. J., Lomer, C. J., Djegui, D. and Schulthess, F. (1999). Pathogen incidence and their potential as microbial control agents in IPM of Maize stem borers in West Africa. *BioControl.*, **44**(3):301-327.

Danger, T. K., Geetha, S. P., Jaypal and Pillai G. B. (1991). Mass production of the Entomopathogen *M. anisopliae* in coconut water wasted from copra making industry. *J. Plant. Crops.*, **19**(1)**:**54-59.

Darwis, M. (1990). Minimum effective concentration of *M. anisopliae* to control *Oryctes rhinoceros* larvae. *Balaipenelitian Kelapa.*, **15**(4):133-136.

Ekesi, S., Maniania, N., Nyarko, K. and Onu, I., (1999). Effect of intercropping cowpea with maize on the performance of *M.*

anisopliae against *Megalurothrips Sjostedti* (thysanoptera:Thripidae) and Predators. *Envi. Ento.,* **28**(6):1154-1161.

Fargues, J., Smits, N., Vidal, C., Vey, A., Vega, F., Mercadier, G. and Quimby,P. (2001). Effect of liquid culture media on morphology, growth, propagules production, and pathogenic activity of the Hyphomycete, *M. flavoviride. Mycopathologia.,* **154**: 127-138.

Feng, M. G., Poprawski, T. J. and Khachataurians, G. G. (1994). Production, formulation and application of the entomopathogenic fungus, *B. bassiana* for insect control: current status. *Biocontrol Sci. and Tech.,* **4**: 3-34.

Fernando, L .C. P., Kangaratnam, P. and Narangoda, N.K. (1995). Some studies on the use of *M. anisopliae* for the control of *Oryctes rhinoceros* in Shri Lanka. *Cocos.,* **10**:46-52.

Ferron, P., Robert, P. H. and Deotte, A. (1975). Susceptibility of *Oryctes rhinoceros* (L.). Adults to *M. anisopliae. J. Invertibr. Pathol.*, **25**(3):313-319.

Flores A., Micaela, P., Rodriguez, N., Mayra, M., Miguel, A. and Ramos, L. (2012). Mycoinsecticide effects of *B. bassiana*, *M. anisopliae*, and *I. fumosorosea* on the whitefly *B. tabaci* (Homoptera: Aleyrodidae) in different strata of bean. *African J. Microbio. Res.*, **6**(4):7246-7252

Guillon, M. (1997) Production of biopesticides: scale up and quality assurance. In: BCPC Symposium Proceedings No. 68. Microbial insecticides: novelty or necessity. Farnham, UK; *The British Crop Prot. Council.*, pp. 151-162

Hemalatha, S., Ramaraju, K. and Jeyarani, S. (2015). First record of *M. flavoviride* Gams & Rozsypal var. minus on Brown plant hopper from Tamil Nadu, India with infectivity on chilli and tomato thrips. *J. Ento. Res.,* **39**(1):15-20.

Humphreys, A. M., Matewele, P., Trinci, A. P. J., and Gillespie, A. T. (1989). Effect of water activity on morphology, growth and blastospore production. *Mycological Res.*, **92**: 257- 264.

Hussein, K. H., Rahman, M. A., Ahmed, Y., Abdel, M. S. and Jin, H. J. (2012). Pathogenicity of *B. Bassiana* and *M. anisopliae* against *Galleria Mellonella. Phytoparasitica.,* **40**(2):117-126.

Ingle, Y. V. (2014). Effect of different growing media on mass production of *N. rileyi. Int. J. Env. Sci.*, **4**(5):976-4402.

Inglis, G. D., Goettel, T. M. and Strasser, B. (2002). Use of hyphomycetous fungi for managing insect pest. Fungi as biocontrol agentes. Wallingford: *CAB International.*, P.23-69.

Jenkins, N., Gabriel H., Langewald J., Cherry, A. and Chris, J. (1998). Development of mass production technology for aerial conidia for use as mycopesticides. *Biocontrol News and Info.*, **19**(1):21N – 31N

Jeong, J., Kim, Jeong, G., Han, J. and lee, S. (2013). Biological control of aphid using fungal culture and culture filtrates of *B. bassiana. Mycobiology.,* **41**(4):221-224.

Kapoor M., Pawar, P.V., Joseph, M., Sen, A. and Deshpande, M. V. (2013). Evaluation of biocontrol potential of *M. anisopliae* strains against larvae and adults of *Aedes aegypti. J. Biological Control.*, **27**(3):194-203.

Karengala, S., Chintala, S. and Lenin A. E. (2016). Selection of a suitable medium for the mass production of some selected entomopathogenic fungus. *Int. J. Recent Sci. Res.,* **7**(7):12370-12372.

Kotwal, S., Parate, R. L., Mane, S. S., and Deshmukh, V. V. (2012).Influence of nutrition and different physical parameters on growth and sporulation of *M. anisopliae*. *Int. J. Sci, Env. and Tech.*, **1**(5):479-484.

Kumar, A., Singh, A. P. and Singh, D. V. (2015). Field evaluation of *B. bassiana* and *M. anisopliae* against major in sect pest on paddy (*oryza sativa l.*).*Soc. Recent Deve. Agric., Prog. Agric.,* **15**(1):62-65.

Kumar, D., Singh, K. P., and Jaiswal, K. P. (2005). Screening of different media and substrates for cultural variability and mass culture of *Arthrobotrys dactyloides* Drechsler. *Mycobiology.*, **33**(4): 215-222.

Latch, G.C. and Falloon, R. E. (1976). Studies on the use of *M. anisopliae* to control *Oryctes rhinoceros*. *BioControl.*, **21**(1):39-48.

Latch, G. C. M. (1976). Studies on the susceptibility of *Oryctes rhinoceros* to some Entomogenous fungi. *Entomophaga*, **21**(1):31-38.

Latifian, M., Rad, B. and Amani, M. (2014). Mass production of entomopathogenic fungi *M. anisopliae* by using agricultural products based on liquid-solid diphasic method for date palm pest control. *Int. J. Farm. and Allied Sci.,* **3**(4):368-372.

Mane, P. B. and Mohite, P.B. (2015).Pathogenicity of entomopathogenic fungi against White grub, *Leucopholis lepidophora* (Blanchard) infesting Sugarcane under pot culture experiment. *Int. J. Plant Prot.*, **8**(1):41-44.

Mascarin, G. M., Jackson, M. A., Kobori, N. N., Behle, R.W. and Júnior, I.D. (2010). Liquid culture fermentation for rapid production of desiccation tolerant blastospores of *B. bassiana* and *I. fumosorosea* strains. *J. Invertebrate Patho.*, **xxx–xxx**,YJIPA 6616.

Mohan, K. S. and Pillai, G. B. (1982). A method for Laboratory-Scale cultivation of *M. anisopliae. Folia Microbiol.*, **27**:281-238.

Mohanty, S. S., Kamaraju, R., Mittal, P. K. and Dash, A. P. (2008). Efficacy of culture filtrates of *M. anisopliae* against larvae of *Anopheles stephensi* and *Culex quinquefasciatus*. *J. Indian Micro. B. tec.*, **35**(10):1199-1202

Nirula, K. K. (1957). Observation on the green muscardine fungus in population of *Oryctes rhinoceros*. *J. Econ. Entomol.*, **50**:767-770.

Padmanban, B., Sukamaran, A. S., Ramanujam, B. and Raman, R. (1997). Testing of pathogenicity of *M. anisopliae* and *B. bassiana* on white grubs. *Annual Report*, CPCRI, Kasaragod (kerala), pp.92-93.

Pandey, R. and Kanaujia, K. R. (2010). Suitability of different synthetic and grain based liquid media for the mass culture of entomopathogenic fungi, *B. bassiana* and *M. anisopliae. J. Entomol. Res.*, **34**(4): 305-309.

Patil, S. D., Kadam, J. R., Chandele, A. G., Wagh, S. S. and Jadhav, R. S. (2014). Growth, development and viability of *M. anisopliae* on media with various nutrient sources. *Int. J. Plant Prot.,* **7**(2):420-423.

Pfadt, R. E. (1980). Fundamentals of applied entomology. *Macmillan Company, New York. pp.*, **99**-104, 24-126.

Pokhrel, M. R., Thapa, R. B., Mandal, C. K., Keller,S. F. and Y. D. G C. (2014). Evaluation of effects of indigenous entomopathogenic fungus, *M. anisopliae* on target organism, the *Bombyx mori* Lin., *J. Biopest.,* 7**(Supp.)**:75-82.

Rachappa, V., Lingppa, S., Patil, R. K. and Hugar, P. S. (2005).Suitability of media and containers for mass production of *M. anisopliae. Karnataka J. Agric. Sci.,* **18**(3):680-684.

Rajput, A. Q. and Shahzad, S. (2015). Growth and sporulation of *Trichoderma polysporum* on organic substrates by addition of carbon and nitrogen sources. *Pak. J. Bot.,* **47**(3): 979-986.

Sahayaraj, K. and Borgio, J. (2010). Virulence of entomopathogenic fungus *M. anisopliae* on seven insect pests. *Indian J. Agric. Res.,* **44**(3):195-200.

Sajap, A. S., Rozihawati, Z., Omar, D. and Lau, W. H. (2012). *Isaria fumosorosea* and *M. anisopliae* for controlling *Atteva sciodoxa* (Lepidoptera: Yponomeutidae), a pest of *Eurycoma longifolia. J. Tropical For. Sci.,* **26**(1):84-91.

Senthamizhlselvan, P., Alice, J., Sujeetha, R.P. and Jeyalakshmi, C. (2010). Growth, sporulation and biomass production of native entomopathogenic fungal isolates on a suitable medium. *J. Biopesticides.*, **3**(2):466-469.

Sharma, s., Gupta R. B. L. and Yadava, C. P. S. (1999). Mass multiplication and formulation of entomopathogenic fungi and their efficacy against White grub. *J. Mycol. Pl. Pathol.,* **29**(3)**:**299-305.

Sidana, A. and Farooq, U. (2014). Sugarcane Bagasse: A Potential Medium for Fungal Cultures. *Chinese J. Bio.*, **10**.1155:840-505.

Singh, D., Singh B. and Dutta B., (2011). Potential of *M. anisopliae* and *B. bassiana* in the Control of Tea Termite *Microtermes obesi* Holmgren in Vitro and under Field Conditions. *J. Pest Sci.,* **84**(1):69-75

Sivpragasam, A. and Tey, C. C. (1994). Succeptibility of *Oryctes rhinoceros* (L.) to three isolates of *M. anisopliae*. *MAPPS Newsletter.*, **18**(2):13-14.

Steinhaus, E. A. (1949). Principples of Insect Pathology. *McGraw-Hill Book*, New York.

Theunis, W. and Aloali, I. L. (1998). Selection of highly virulent fungal isolate, *M. anisopliae* Ma TB 101, for control of taro beetle, *Papuana uninodis*(Colioptera:Scarabaeidae). *Biocontrol Sci. and Techno.,* **8**(2):187-195.

Visalakshi, M .and Bhavani, B. (2015). Field efficacy of *B. bassiana* and *M. anisopliae* against white grub, *Holotrichia consanguinea* blanch damaging sugarcane in Andhra Pradesh. *J. Res. ANGRAU*, **2**(3&4)13-17.

Yadav, S., Tandan, N. and Kumar, K. (2013). Mass production of entomopathogens *B. bassiana* and *M. anisopliae* using rice as a substrate by diphasic liquid-solid fermentation technique, *I. J. A. B. R,* **3**(3):331-335.

APPENDIX-I

ABBREVIATIONS USED

%	:	Per cent
@	:	At the rate
=	:	Equal to
^0C	:	Degree Celsius
C.D at 5%	:	Critical difference at 5 per cent
et al.	:	And others
etc.	:	et cectra
FYM	:	Farm yard manure
Fig.	:	Figure
g	:	gram(s)
h	:	Hour
ha^{-1}	:	Per Hectare
i.e.	:	That is
m^2	:	Square meter
µl	:	Micro liter
Min.	:	Minimum

Max.	:	Maximum
mg	:	Milligram(s)
ppm	:	Parts per million
psi	:	Pound per square inch
S.Em. ±	:	Standard error
Var.	:	Variety
Viz.,	:	Namely

INTRODUCTION

APPENDICES

LITERATURE CITED

LITERATURE CITED

MATERIALS AND METHODS

REVIEW OF LITERATURE

REVIEW OF LITERATURE

RESULTS AND DISCUSSION

SUMMARY AND CONCLUSION

कृषि किटशास्त्र विभाग

कृषिमहाविद्यालय, दापोली

प्रबंध का शीर्षक	:	"विभिन्नमीडियाकीउपयुक्तताऔरमेटारायजियमअनिसोप्लीय (मेचिंकोफ़) सोरोकिनकेरोगजनकताएफिस क्रसिवोरा (कोच) केखिलाफ"
छात्र का नाम	:	निर्मला दूंदा वायाल
पंजीकृत क्रमांक	:	२४०१
संशोधन मार्गदर्शक	:	आचार्य एस. के. मेहेंदले
पदवी	:	एम.एस्सी. (कृषि)
मुख्य विषय	:	कृषि किटशास्त्र
पूर्णता का वर्ष	:	२०१७

सारांश

वर्तमानअध्ययन "प्लांटपैथोलॉजीविभागकीसंगरोधप्रयोगशालाऔरकृषिकीटविज्ञानविभाग, डॉ।बालासाहेबसावंतकोकणकृषिकृषिविद्यापति, दापोली, जिलारत्नागिरी (एमएस) 2015-17 केदौरानकिएगए।कोंकानस्थितियोंकेतहतहरीमास्कैक्रिनकवक, एम।एनिसोप्लीयाकेजनसंचारकेलिएविधिकोमानकीकृतकरनेकेलिएवर्तमानप्रयोगकियागयाथाऔरप्रयोगशालास्थितियोंकेतहतअपिक्रेसीवोवोरा (कोच) केखिलाफएम।अनिसोप्लीआकीप्रभावकारीताकापरीक्षणकरनेकेलिएकियागयाथा।

हरेरंगकीमास्कैर्डिनकवककेबड़ेपैमानेपरउत्पादनपरअध्ययन, ठोसमीडियापरएम।एनिसोप्लीआनेसंकेतदियाकिउपचारटी 8-केलेपीयूडीओस्टेमकोंकणहालतकेतहतकवककेगुणनकेलिएसबसेउपयुक्तठोसमाध्यमथा। 20 दिनकीटीकाकरणकेबादटी 8-केनपीयूडीओस्टेमकामतलबबीताहुआउत्पादन 951.3 x 108 ग्रामप्रतिग्रामथाजोकिटी 2-टीकचरा (9 30.67 x 108) केबराबरथाऔरदूसरासर्वश्रेष्ठमीडियाथा।जहाँतक; गन्नाबैगनेकवककेटीकाकरणके 20 दिनोंमेंसबसेकमसंख्यामेंबीजों (41.33 x 108) काउत्पादनकिया।तरल / शोरबामाध्यमपरएम।अनिसोप्लीयकेद्रव्यमानगुणोंपरअध्ययनसेपताचलाहैकिटी -6 Czeapeksशोरबामध्यमएम।एनिसोपलिआ (द्रव्यकवकनका 1554 मिलीग्रामकाऔसतचटाईवजन) 20 दिनोंकेबाद, केद्रव्यगुणनकेलिएसबसेउपयुक्तमाध्यमथा।टीओसी-मकईकाआटामध्यम (1545.67 मिलीग्राम) केबराबरथा, जोकिटीका, जोकवककेअच्छेविकासकाभीसमर्थनकरताहै।

एफीसक्रेसीवॉरोकेअप्सराकेखिलाफएम।अनिसोप्लीआकेजैव-प्रभावकारिताकेपरिणामबतातेहैंकिसभीउपचारनियंत्रणकेमुकाबलेबेहतरथे।एफिडकीउच्चतममृत्युदर (86.67%) उपचारटी 1- ज्वारअनाजमेंदर्जकीगईथी, जोटी 2-चायकचरे (83.33%) केबराबरथी।हालांकि, तरल / शोरबामाध्यममें 86.67 प्रतिशतकीउच्चतममृत्युदरटी -6 केइलाजकेमामलेमेंदर्जकीगई, जोकिटी 1- नारियलपानी (83.33%) केबराबरथा।ठोसऔरतरलमाध्यमिकप्रयोगोंदोनोंकेनियंत्रणकेउपचारमेंकोईमृत्युनहींहुई।

जहाँतकएनिसोप्लीयनेएनम्फ्सपरसंक्रमणकेलक्षणोंकासवालउठायाथा, संक्रमितनिम्फमिट्टीऔरपौधेकीसतहपरपाएगएथे।बादकेलक्षणोंकोदेखागयानिष्क्रियता, शरीरपरतांबेकेकवकविकासकाविकासहोताहै, जोशरीरकेसभीभागोंमेंजैतूनहरेरंगकीमास्किंगिनकवककीचटाईकेलिएविकसितहोताहै।

कृषि किटकशास्त्र विभाग

कृषि महाविद्यालय, दापोली

प्रबंधाचे नाव	:	"विविधमीडियाचीउपयुक्तताआणिमेटारिझियमअनिसोप्ली (मेट्चिकोनॉफ्फ) सोरोकीनच्यारोगकारकतेसअपीसक्रोसिवोरा(कोच) विरुद्ध"
विद्यार्थ्याचे नाव	:	निर्मला दूंदा वायाळ
नोंदणी क्रमांक	:	२४०१
संशोधन मार्गदर्शक	:	आचार्य एस. के. मेहेंदळे
पदवी	:	एम.एस्सी. (कृषि)
मुख्य विषय	:	कृषि किटकशास्त्र
सदरीकरणाचे वर्ष	:	२०१७

सारांश

सध्याचेअभ्यास "वनस्पतीरोगविज्ञानविभागआणिकृषीकीटकशास्त्रविभाग, डॉ. बाळासाहेबसावंतकोकणकृषीविद्यापीठ, 2015-17 दरम्यानरत्नागिरी (एम.एस.), दापोली, च्याअलगदप्रयोगशाळेतहातीघेण्यातआले. सध्याच्यासंशोधनासाठीहिरव्यामॉस्किर्फिनफंगसचेजनकल्याण, कोनकनपरिसीमाअंतर्गतएम. अनिसोप्लीय, आणिप्रयोगशाळेच्यापरिस्थितीनुसारअप्ससक्रेसीव्होव्होरा (कोच) विरुद्धएम. अनिसोप्लीयाचीकार्यक्षमतातपासण्यासाठीसध्याचेसंशोधनकेलेगेले.

ग्रीनमॉस्कर्किनफंगसचेजननउत्पादनावरअभ्यास, घनमिडियावरएम. अनिसोप्लीयनेसूचितकेलेकीउपचारटी -8- केणpsuedostemकोंकणस्थितीतबुरशीचेगुणधर्मअसणारेसर्वातयोग्यठोसमाध्यमहोते. 20 दिवसाच्याटीकाझाल्यानंतरटी-के-केळ्याचेसापाचेउत्पादनसुमारे 951.3 x 108 चौरसप्रतिग्रामहोतेजेटी 2-टीकचरा (9 30.67 x 108) च्याबरोबरीनेहोतेआणिदुसरेसर्वोत्तममाध्यमहोते. तर; ऊस-

गॅसनेबुरशीच्यारोगप्रतिबंधकआवरणाच्या 20 दिवसातकमीतकमीअवयव (41.33 x 108) निर्माणकेले. द्रव / मटनाचारस्साच्यामाध्यमातुनएम. एनिसोप्लियाच्याद्रव्याचागुणोत्तरांवरीलअभ्यासांवरूनअसेदिसूनआलेकीटी -6 सेझेपिक्सचेमटनाचारस्सामध्यमएम. एनिसोपिलिया (सूतबुरशीचे 1554 एमजीचेसरासरीचटईवजन) 20 दिवसांनंतरबहुतेकगुणोत्तरांसाठीयोग्यमाध्यमहोते. टीओ-कॉर्नफ्लोमिडियम (1545.67 एमजी) च्याबरोबरीनेअसलेल्याटीकामुळे, तसेचबुरशीच्यावाढीलाहीपाठिंबाहोता.

एपीसक्रोसिवोराच्याअप्सराविरुद्धएम. अनिसोप्लीयच्याजैव-कार्यक्षमतेचेपरिणामउघडकीसआलेकीसर्वउपचारहेनियंत्रणाबाहेरश्रेष्ठआहेत. उपचारांतटीए- ज्वारीचेधान्य (83.33%) सारख्याअंदाजेदरानेअफीड (86.67%) उच्चतममृत्युदरनोंदवलागेला. तथापि, द्रव / मटनाचारस्सामाध्यमामध्येउपचारसरासरी T6 - Czeapeksमाध्यम 86.67 टक्केउच्चतममृत्युदरनोंदवलागेला, तोटी 1 - नारळपाणी (83.33%) च्याबरोबरीहोता. दोन्हीठोसआणिद्रवमध्यमप्रयोगांवरनियंत्रणउपचारांवरमतभेदनाही.

एम. एनिसोपलीनंबोफाच्यासंसर्गाचीलक्षणेलक्षातघेता, संक्रमितलिम्फिसमातीआणिवनस्पतीच्यापृष्ठभागावरआढळूनआले. त्यानंतरच्यालक्षणांचीलक्षणेनिष्क्रियता, शरीरावरतांबेफुफ्फुसांच्याविकासाचेविकासहोते, ज्यामुळेशरीराच्यासर्वभागावरऑलिव्हहरीमॉस्सेक्रिनफंगसचीचटणीपुढेवाढली.

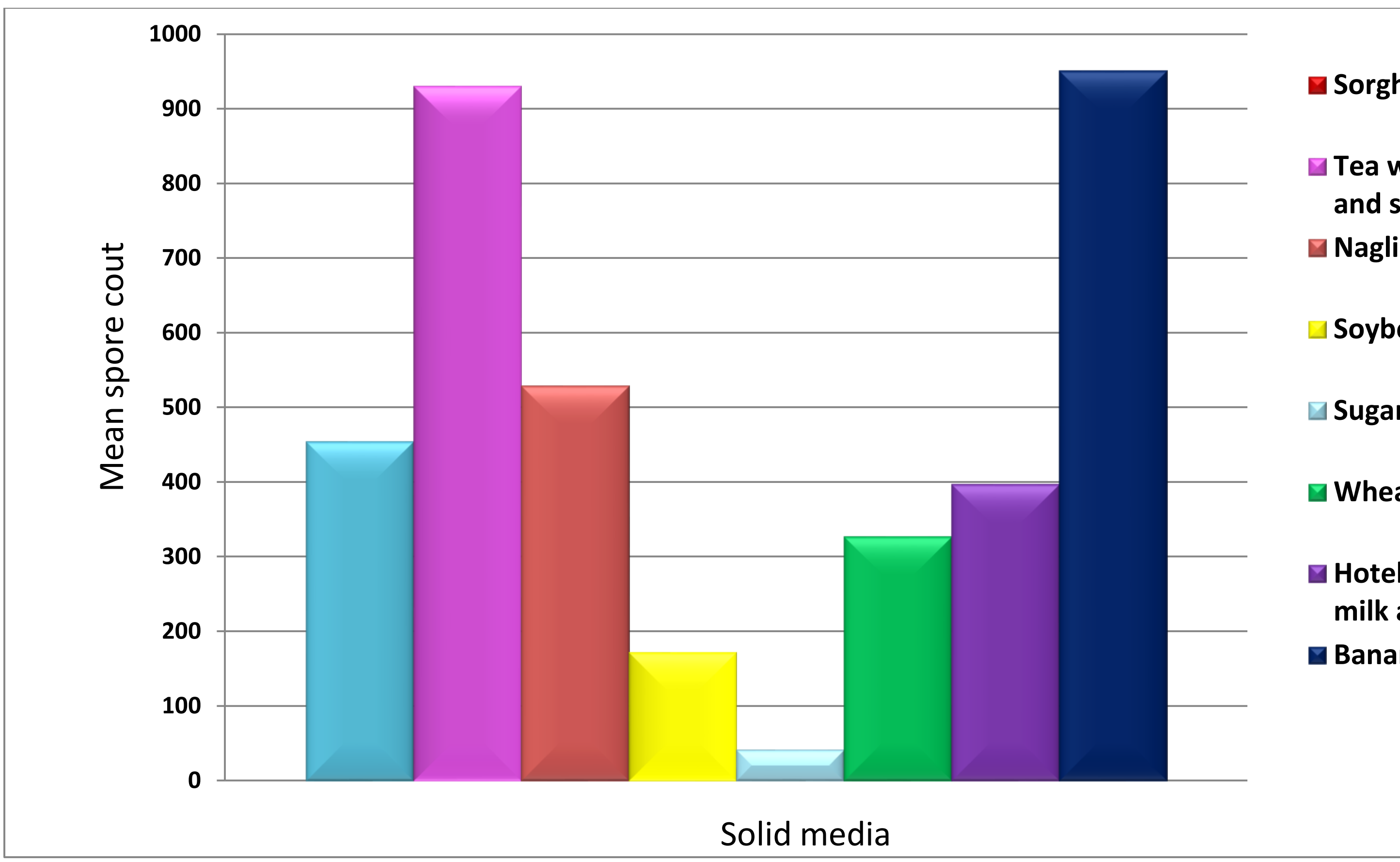

Fig. 1: Mass multiplication of *M. anisopliae* on solid media.

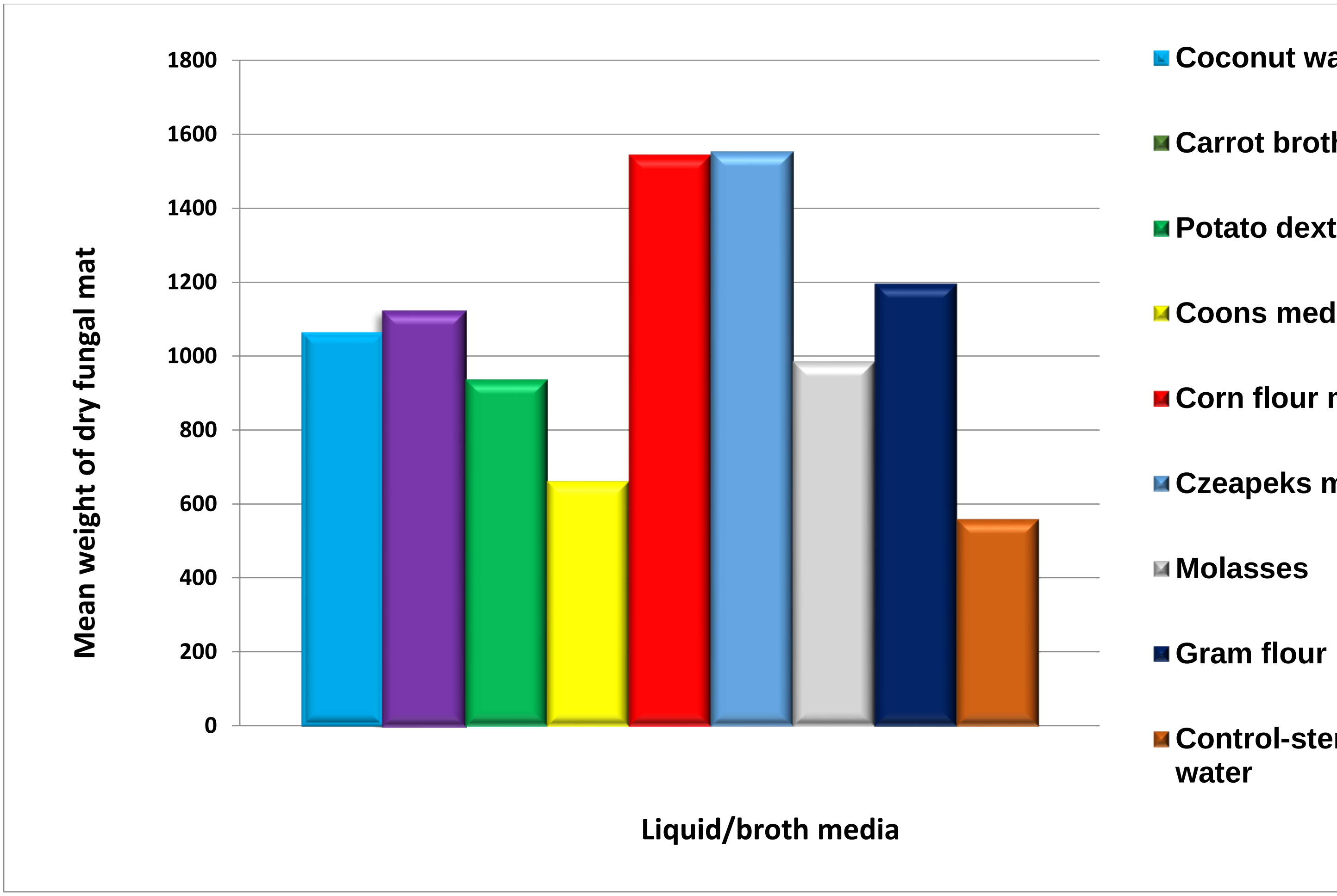

Fig.2 Mass multiplication of *M. anisopliae* on liquid/broth medium.

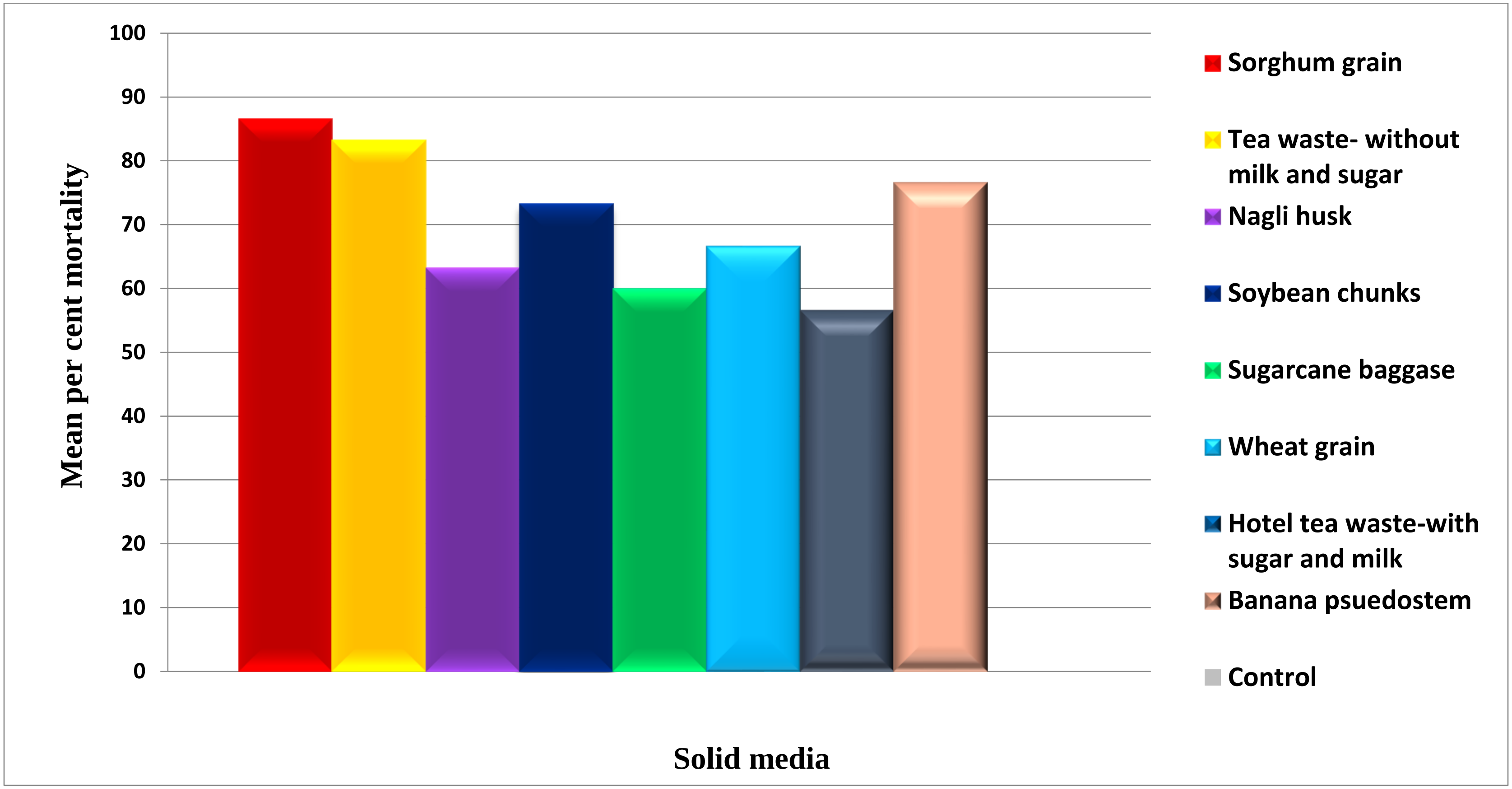

Fig. 3: Testing the bio-efficacy of *M. anisopliae*from solid media against *A. craccivora*

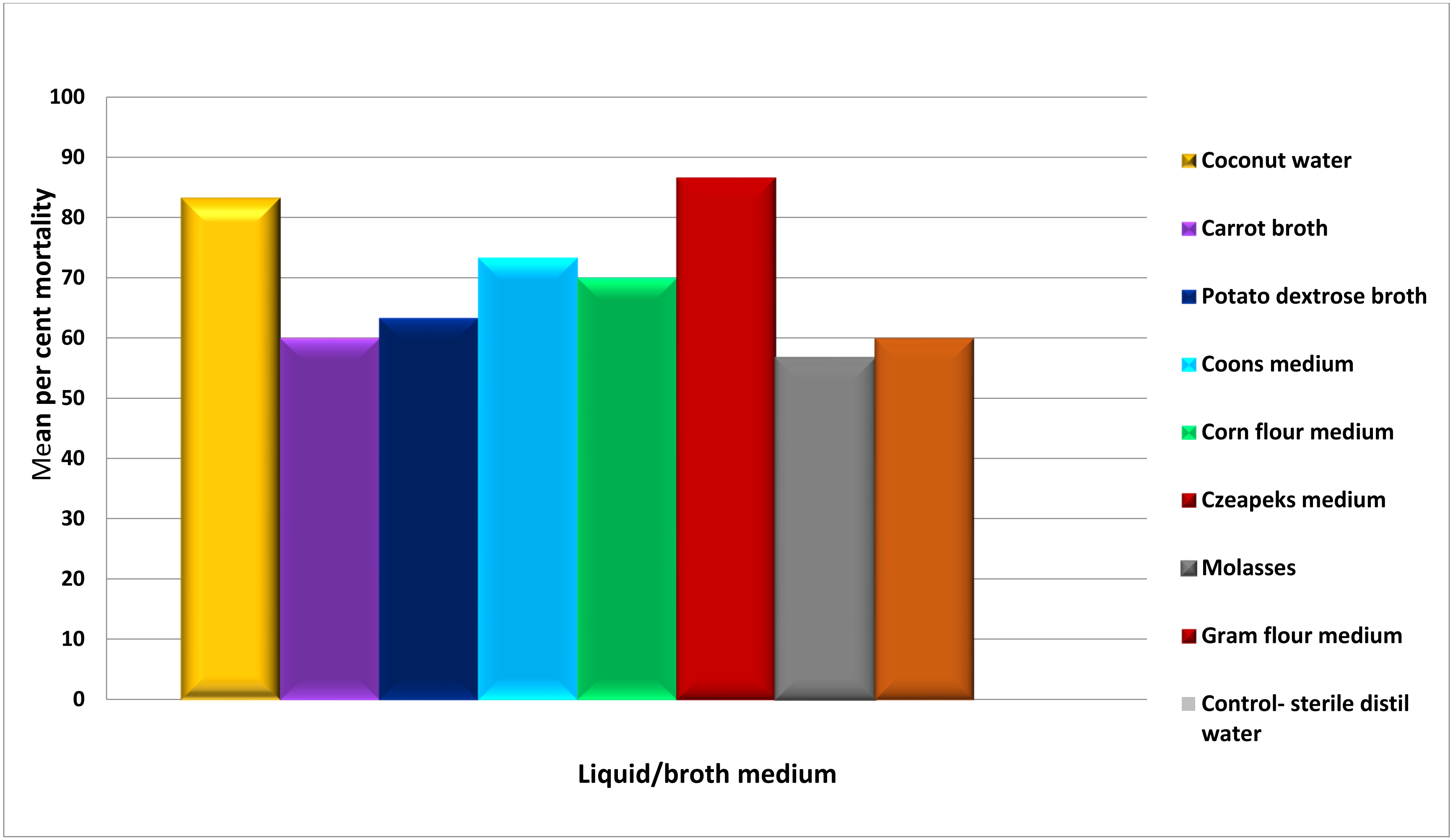

Fig.4: Bio-efficacy of culture filtrates of *M. anisopliae*from different liquid media against *A. craccivora*

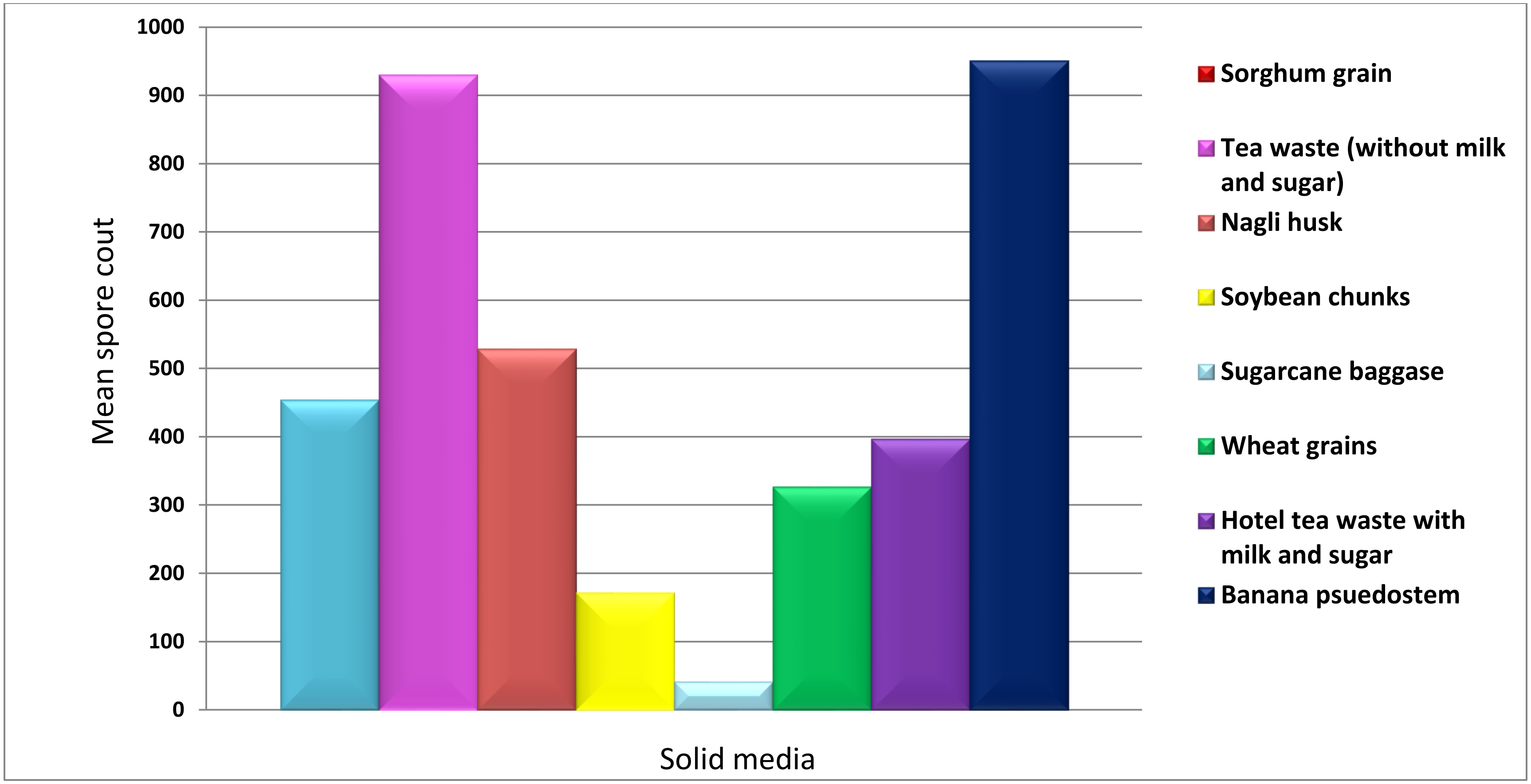

Fig. 1: Mass multiplication of *M. anisopliae* on solid media.

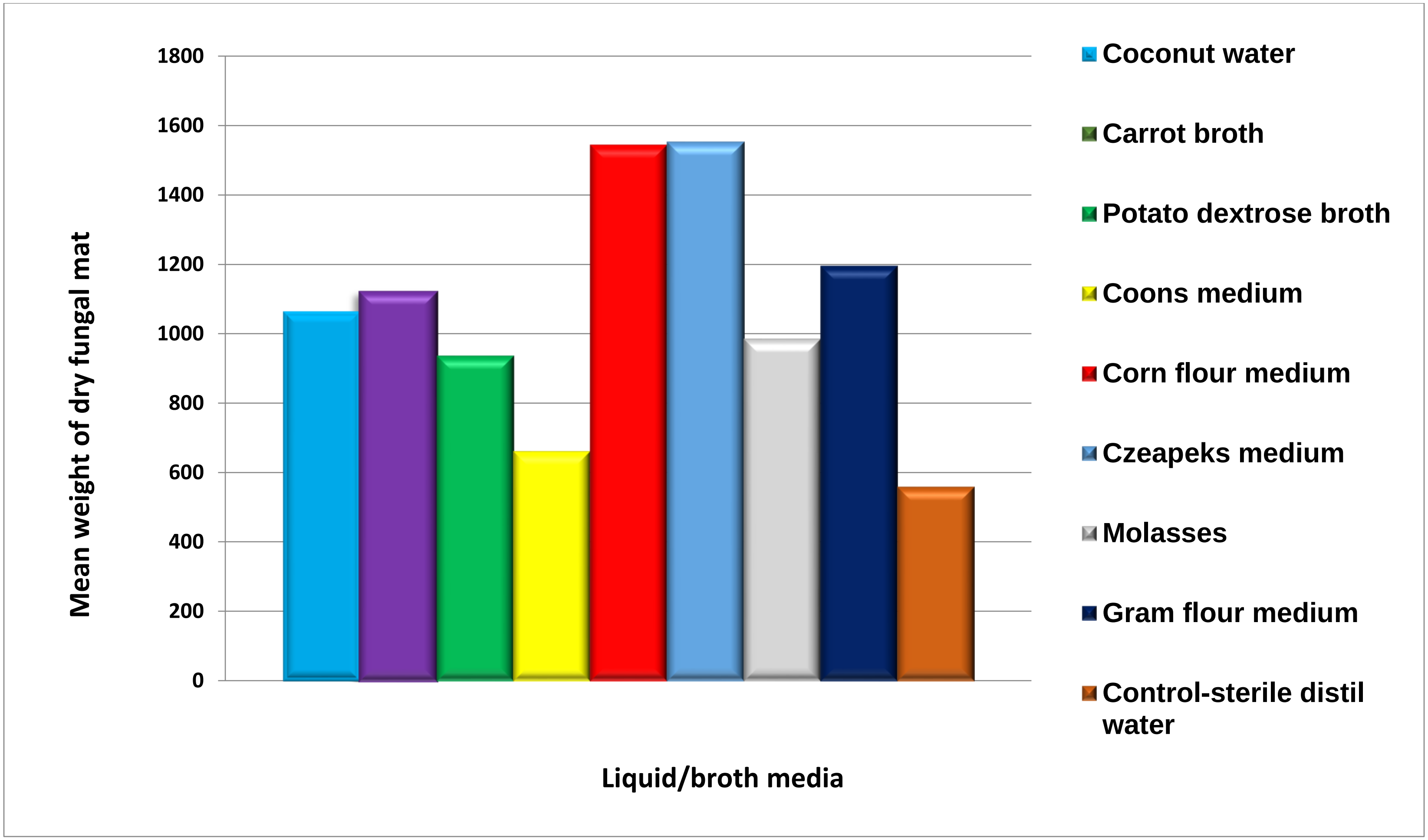

Fig.2 Mass multiplication of *M. anisopliae* on liquid/broth medium.

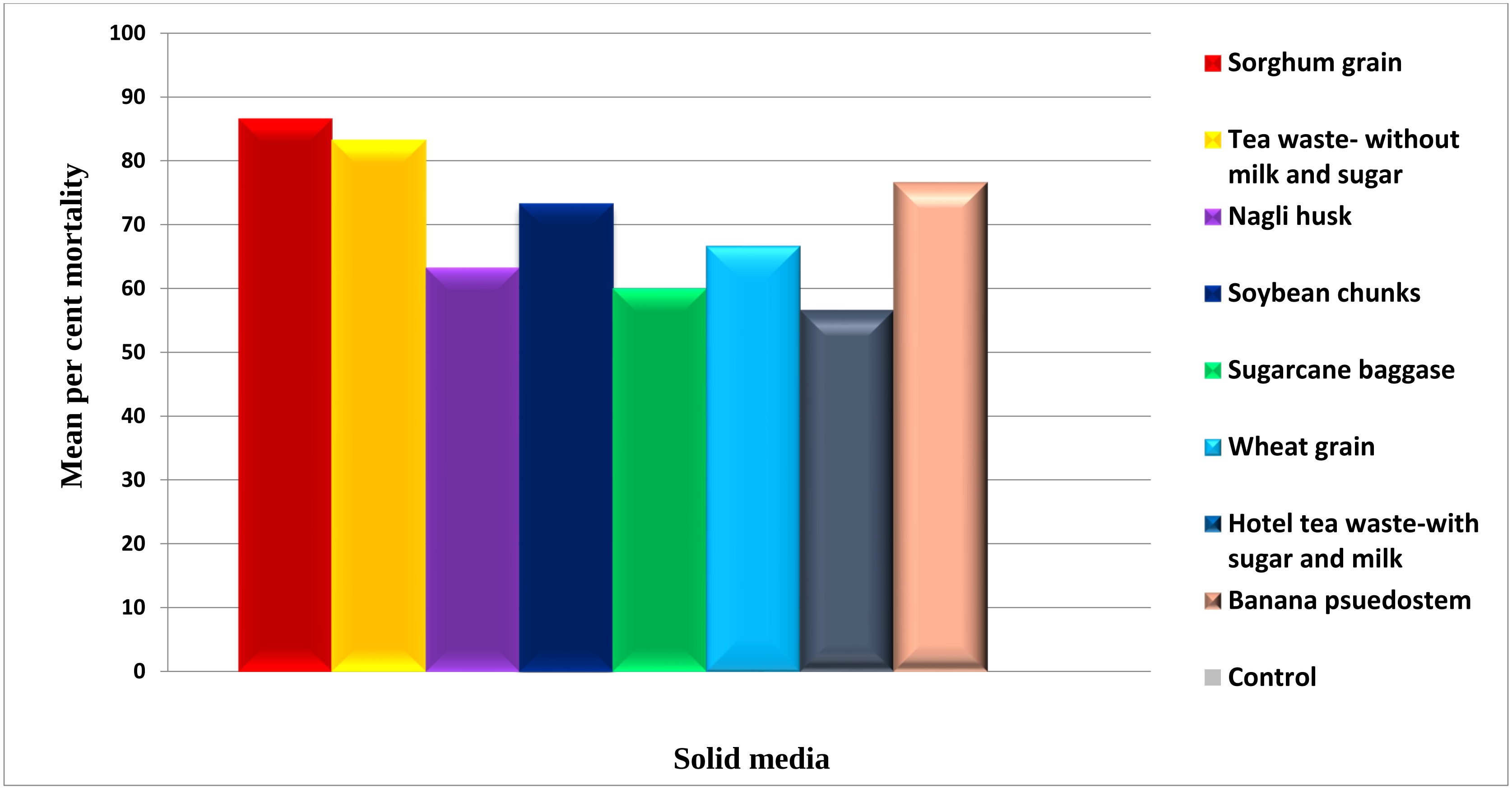

Fig. 3: Testing the bio-efficacy of *M. anisopliae*from solid media against *A. craccivora*

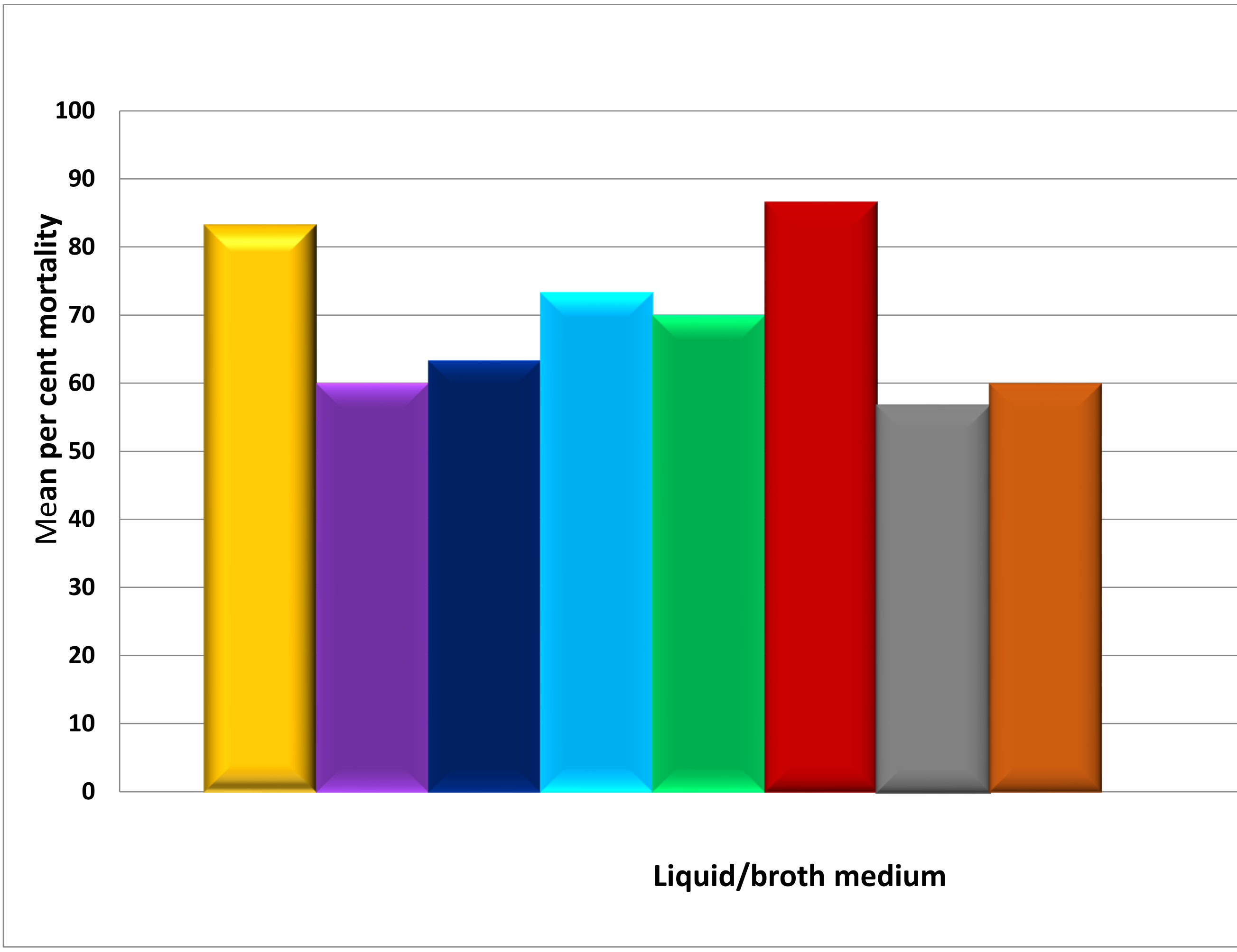

Fig.4: Bio-efficacy of culture filtrates of *M. anisopliae*from different liquid media against *A. craccivora*

PLATE I

Polybag method for mass culturing the *Metarhizium anisopliae*

PLATE II

Polybag method for mass culturing the *Metarhizium anisopliae*

PLATE III

Polybag method for mass culturing the *Metarhizium anisopliae*

PLATE IV

Polybag method for mass culturing the *Metarhizium anisopliae*

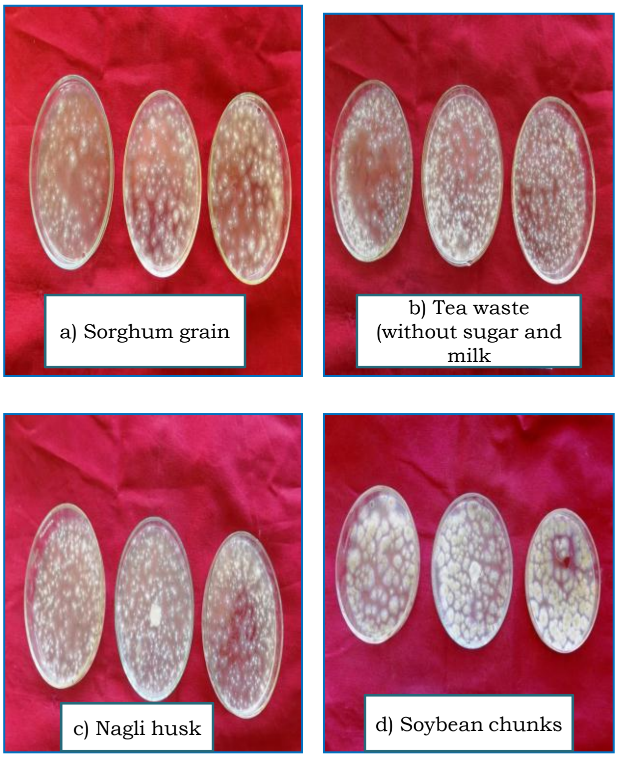

PLATE V

Fungal spore of *Metarhizium anisopliae* on

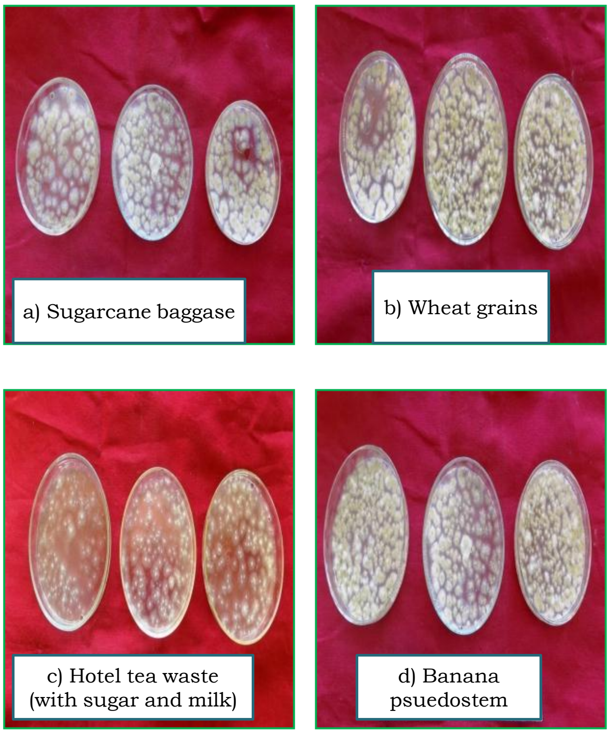

PLATE VI

Fungal spore of *Metarhizium anisopliae*

PLATE VII

Mycelial mat of *M. anisopliae* developed on broth/ liquid media

PLATE VIII

Mycelial mat of *M. anisopliae* developed on broth/ liquid media

PLATE IX

Mycelial mat of *M. anisopliae* developed on broth/ liquid media

PLATE X: Bio-efficacy of *M. anisopliae* from solid media against *A. craccivora*

PLATE XI: Aphid colony developed on twigs of cowpea

PLATE XII: Bio-efficacy of culture filtrates of *M. anisopliae* from different liquid/broth medium against *A. craccivora*

PLATE XIII:
Coppery growth of *Metarhizium anisopliae* on Aphid body

PLATE XIV
Infected Aphid by *Metarhizium anisopliae*

PLATE XV
Fungal spores of *Metarhizium anisopliae* grown on Aphid body

Treat. No.	Treatment (Solid media)
1	Sorghum grain
2	Tea waste (without milk and sugar)
3	*Nagli* husk
4	Soybean chunks
5	Sugarcane baggase
6	Wheat grain
7	Hotel tea waste (with sugar and milk)
8	Banana pseudo stem

Treat. No.	Treatment
1	Coconut water
2	Carrot broth
3	Potato dextrose broth
4	Coon's medium
5	Corn flour medium
6	Czeapeks medium
7	Molasses
8	Gram flour medium
9	Control- (Sterile distilled water)

Treat. No.	Treatment
1	Sorghum grain
2	Tea waste (without sugar and milk)
3	*Nagli* husk
4	Soybean chunks
5	Sugarcane baggase
6	Wheat grain
7	Hotel tea waste (with sugar and milk)
8	Banana pseudo stem
9	Control (Sterile distilled water)

Treat. No.	**Treatment**
1	Coconut water
2	Carrot broth
3	Potato dextrose broth
4	Coon's medium
5	Corn flour medium
6	Czeapeks medium
7	Molasses
8	Gram flour medium
9	Control (Sterile distilled water)