STUDIES ON MASS PRODUCTION OF ENTOMOPATHOGENIC FUNGI *Metarhizium anisopliae* (METSCHNIKOFF) USING DIFFERENT MEDIA AND POTENTIAL AGAINST CHICKPEA POD BORER, *Helicoverpa armigera* (HUBNER)

THESIS

SUBMITTED TO THE

SARDAR VALLABHBHAI PATEL UNIVERSITY OF AGRICULTURE AND TECHNOLOGY, MEERUT- 250 110 (U.P.), INDIA

By

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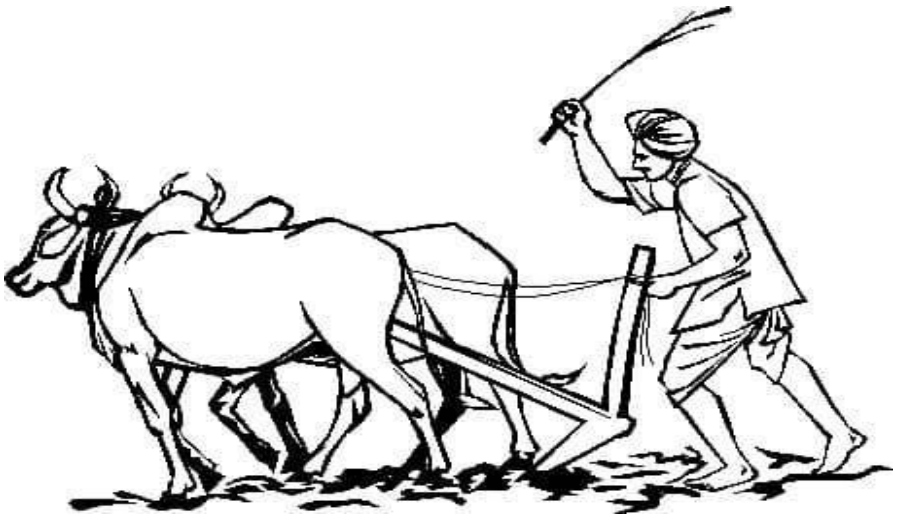

Dedicated

To

INDIAN FARMERS

RAVI SHNAKER…

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*I set my unfeigned and meek thanks to **"GOD"** who favored and invigorated me with the fortitude and capability to aptly complete my research work.*

The tenure of human in this world is supported by many others. Acknowledgement for a few might be just a trifle thing written on a piece of paper. Nevertheless, in the true essence, it gives us an opportunity to remember and express our feelings to those, whom we love, revere and share our secrets. Here I get a great chance to express my token of thanks to people who in a way helped and supported me to complete this record.

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CERTIFICATE

This is to certify that the thesis entitled "**Studies on mass production of entomopathogenic fungi *Metarhizium anisopliae* (Metschnikoff) using different media and potential against chickpea pod borer, *Helicoverpa armigera* (Hubner)"** submitted in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** with major in **Entomology** and minor in **Plant Pathology** of the College of Post-Graduate Studies, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut is a record of bonafide research carried out by **Mr. Ravi Shanker, Id. No. 4911**, under my supervision and no part of the thesis has been submitted for the award of any other degree or diploma.

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(Rajendra Singh)
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SARDAR VALLABHBHAI PATEL UNIVERSITY OF AGRICULTUREAND TECHNOLOGY, MEERUT – 250110 (U.P.), INDIA

CERTIFICATE

We, the undersigned, members of the Advisory Committee of **Mr. Ravi Shanker, Id. No. 4911**, a candidate for the degree of **Doctor of Philosophy** with major in **Entomology** and minor in **Plant Pathology** agree that the thesis entitled "**Studies on mass production of entomopathogenic fungi *Metarhizium anisopliae* (Metschnikoff) using different media and potential against chickpea pod borer, *Helicoverpa armigera* (Hubner)"** may be submitted by **Mr. Ravi Shanker** in partial fulfillment of the requirements for the degree.

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LIST OF ABBREVIATIONS

%	:	Per cent
^{0}C	:	Degree Celsius
@	:	At the rate of
a.i.	:	Active ingredient
Agri.	:	Agriculture
Agril.	:	Agricultural
Ann.	:	Annals
Annu.	:	Annual
ANOVA	:	Analysis of variance
App.	:	Applied
AS	:	Aqua Suspension
C.D.	:	Critical Difference
CFU	:	Colony Forming Unit
cm	:	Centimeter
CMC	:	Corboxy Methyl Cellulose
Con.	:	Concentration
d. f.	:	Degree of freedom
DBA	:	Days Before Application
DAA	:	Days After Application
DAT	:	Days After Transplanting
DAS	:	Day After Inoculation
DW	:	Distilled Water

SW	:	Standard Week
e.g.	:	For example
et. al.,	:	And others
etc.	:	And the rest
Fig.	:	Figure
gm.	:	Gram
Hrs	:	Hour
ha	:	Hectare
i.e.	:	That is
J.	:	Journal
kg	:	Kilogram
q	:	Quintal
Ltr.	:	Litre
LC	:	Lethal Concentration
LT	:	Lethal Time
Ma	:	*Metarhizium anisopliae*
MAS	:	Month After Storage
No.	:	Number
NS	:	Non-significant
R	:	Replication
S	:	Significant
SE	:	Standard Error
SD	:	Standard Deviation
Sp.	:	Species
Spp.	:	More than one species
viz.	:	Videlicet (That is to say)

wt.	:	Weight
ppm	:	parts per million
FYM	:	Farm Yard Manure
N	:	Nitrogen
P	:	Phosphorus
K	:	Potash
SL	:	Soluble liquid
SC	:	Suspension concentrate
EC	:	Emulsifiable Concentrate
SG	:	Soluble Granule
RBD	:	Randomized Block Design
Rs.	:	Rupees
TW	:	Tween 80
TX	:	Triton X 100

CHAPTER-1 INTRODUCTION

Chickpea, *Cicer arietinum* (L.) is an important *Rabi* season pulse crop grown and consumed worldwide, especially in the Afro-Asian countries. It is also one of the major pulse crops cultivated and consumed in India and which is also known as Bengal gram. In India, chickpea account for about 45% of the total pulse production. Similar to the case of other pulses, India is the major chickpea producing country and it contributes for more than 75% of total world's chickpea production **(Maurya and Kumar 2018).** Chickpea plays an integral part of the cropping system in the farmer's field all over the country because it fits well in the crop rotation and mixed cropping system. It is multi purpose crop and has the ability to grow under the conditions of low fertility and under varying conditions of soil and climate **(Fikre and Rubiales 2014).**

In India chickpea has been grown for decades. Chickpea fits really well in crop rotation and mixed cropping due to which it has become an integral part of our cropping systems all over the world because of its intrinsic value of higher protein content, nitrogen fixing ability and many diversified uses viz., green vegetables, germinated grain as breakfast, sweets and other relishing dishes and its indispensability as an alternate crop for crop diversification. Chickpea seed contains 18.22 percent protein, 16 percent total carbohydrates, 47 percent starch, 5 percent fat, 6 percent crud fiber, 6 percent-soluble sugar and 3 percent ash. **(Jukantil *et al.*, 2012).**

Chickpea is native to India, Afghanistan and Ethiopia. It is out of the most important pulse crop in the world, cultivated in an area of 13.884 million hectares with a production of 13.652 million tones. In India, chickpea is grown in an area of 9.85 million

hectares with production of 11.99 million tones. In India, Rajasthan is the largest chickpea growing state with an area of 2.46 million hectares with production of 2.66 million tones, followed by Maharashtra and Madhya Pradesh. Uttar Pradesh is the 4th largest producer with an area of 0.62 million hectares with a production of 0.85 million tones. **(Anonymous, 2021)**.

There are many pests infesting chickpea throughout the world in India it's about 57 species causing economic damage **(Lal OP, 1996).** Among them, gram pod borer, *Helicoverpa armigera* (Hubner) and cut worm, *Agrotis ipsilon* (Hufnage) are recognized as the major pests **(Ranga and Shanower 1999).**

Helicoverpa armigera (Hubner) is among the most harmful agricultural pests. It is geographically widespread (Europ, Asia, Africa and Oceania) and is a highly polyphagous moth whose host species include various economically important crops such as cotton, corn chickpea, tomato, sorghum, sunflower **(Akbulut *et. al.,* 2003).** Females lay their eggs on the fruits and flowers of these crops after hatching the larvae start to feed, causing significant agricultural damage. *Helicoverpa armigera* has been attributed as one of the seriouus pest status not only because of its ability to attack various hosts from various families but also for its resistance to insecticides **(Cunningham *et al.,* 1998)**.

Under natural condition, fungi are frequent and often important natural mortality factor in all groups of insect population. Over 700 species of fungi have been recorded as pathogens while most species are obligate pathogens, often quite specific and rarely found **(Ramarethinam *et al.,* 2005)**. The entomopathogenic fungi are found to be

promising in the control of insect-pests, **(Lingappa *et al.*, 2005)**. The most important entomopathogenic fungi are *Metarhizium anisopliae*, *Beauveria bassiana*, *Nomuraea rileyi*, *Verticillium lecanii* and *Hirsutella spp*.

Entomopathogenic fungi occur naturally in orchard soil, vegetable fields, and infected insects. However, several entomopathogenic fungi only occur as infections in living hosts for a relatively short time during their life cycle. In the remaing life cycle, these species presumably lurk as dormant conidia in the soil in the vicinity of the dead host cadaver. Limited saprobic growth is sometimes possible from resources contained in the host cadaver. The dead host cadavers will mostly fall to the ground and thus a reservoir of fungal material is present in the soil environment. Further, dispersal from cadavers as a focal point presumably occurs due to weather (rain and wind), soil manipulation and insect activity **(Meylinget *et. al.*, 2006)**. Conidia produced on the surface of dead host cadavers are relatively long lived.

The entomopathogenic fungus, *M. anisopliae* has been widely used as a mycopseticide to control many insects **(Feeron, 1981)**. The general mode of infection by *Metarhizium* spp. comprises six stages in the following order i.e. adhesion, germination, appressorium formation, penetration, colonization of hemolymph, and extrusion and sporulation, which can also be found in other entomopathogenic fungi. However, *Metarhizium* spp. has genes specific to each species in their mode of infection, such as the Mad1 kinase genes for the germination stage in *M. anisopliae* **(Nguyen *et al.*, 2013)**.

The two phases of culture (liquid and solid) are the most commonly used technique to mass produce *Metarhizium*, liquid fermentation is used to produce

blatospores and mycelium forms **(Perira and Roberts, 1991, Kurger *et al.*, 2014).** The solid phase is carried out in a solid substrate, which has a large surface area for aeration and physically supports the fungus conidia, and it is also used as a source of nutrients **(Jenkins *et al.*, 1998).**

Control of *H. armigera* is heavily dependent on the use of chemical pesticides. However, resistance to some commercially available insecticides has been detected in *H. armigera*. The increasing emergence of resistance problems means there is an urgent need for developing management strategies, which are less dependent on chemical insecticides and less conducive to the development of resistance problem. Therefore, use of microbial insecticides based on *Metarhizium anisopliae* (Green muscardine) plays an important role in the successful management of this pest.

Hence, keeping the aforesaid in mind to design a perfect field and laboratory experiment, this experiment was conducted under the following objectives:

1. To study the isolation, characterization and selection of most virulent strain of *Metarhizium anisopliae*.
2. To study the economic mass production and viability of *Metarhizium anisopliae* on different substrates.
3. To study the bioassay of *Metarhizium anisopliae* against *Helicoverpa armigera*.
4. To study the bioefficacy of *Metarhizium anisopliae* against *Helicoverpa armigera* on chickpea crop.

CHAPTER-2 REVIEW OF LITERATURE

This investigation under taken on mass production of entomopathogenic fungi *Metarhizium anisopliae* (Metschnikoff) using different media and potential against the chickpea pod borer, *Helicoverpa armigera* (Hubner). An overview of the literature on *M. anisopliae* about different aspects of the investigation is presented below.

2.1 To study the isolation, characterization and selection of most virulent strain of *Metarhizium anisopliae*

Ali *et al.*, (2010) conducted a survey study to isolate entomopathogenic fungi from the hazelnut grown in the region of Turkey and characterized the isolated strains in detail. In 2006 and 2007, 301 soil samples were collected randomly and analyzed for the presence of entomopathogenic fungi using the Galleria bait method. Entomopathogenic fungi were found to occur in 20.59% of the soil samples studied. Based on morphology, ITS sequence and partial sequencing of the 18S (SSU rDNA) and EF1-a genes, the isolates were identified as *Metarhizium anisopliae*. All the isolates tested were pathogenic to *M. melolontha*. *M. anisopliae var. anisopliae* KTU-27 and *Evlachovaea* sp. KTU-36 produced the highest insecticidal activity (86.6%) with 15 days of inoculation.

Franco *et al.*, (2011) collected 142 soil samples from different Mexican states i.e. Campeche (13), Michoacan (15), Nuevo Leon (25), San Luis Potosi (25), Sinaloa (17), Sonora (17), Tabasco (5), Tamaulipas (20), and Yucatán (5). Larvae of the greater wax moth, *Galleria mellonella* L. (L.) these soil samples were used as bait to detect, trap, and multiply entomopathogenic fungi *in vivo*. Twenty-three percent of the soil samples processed was positive for the presence of entomopathogenic fungi according to the macroscopic and microscopic characteristics: *Beauveria bassiana* (Bals.-Criv.) Vuill. was

detected in 12% (17 isolates), *Metarhizium anisopliae* (Metch.) Sorokin in 1% (2 isolates), and *Isaria fumosorosea* (Wize) in 10% (14 isolates). PCR generated fragments of approximately 600 base pairs (bp). The size differences between *B. bassiana* and *M. anisopliae* genera were indistinguishable on agarose gel.

Liu *et al.*, (2012) isolated 16 different isolates of *Metarhizium anisopliae* (Metsch.) from sugarcane pests in South China. All isolates were identified on the basis of the macromorphological, micromorphological and molecular characteristics. The colony morphology, mycelia and conidial yield were observed with three-agar culture media: potato dextrose agar medium (PDA), potato dextrose with 1% (w/v) peptone agar medium (PPDA), and it was found that oatmeal agar medium (OMA) and PPDA were the better culture media for vegetative growth and conidial yield (10^9 conidia/ml) than PDA (10^8 conidia/ml) and OMA (10^8 conidia/ml). To confirm whether these isolates were pathogenic to *C. venosatus*, their virulence to the sugarcane stem borer was tested in the laboratory. Both the HS (10 isolates) and LY (6 isolates) strains were pathogenic to *C. venosatus*. Several highly virulent strains were screened *in vitro* (the mortalities of the eight isolates HS1, HS6, HS7, LY2, LY3, LY6, HS3, and HS9 were from 96 to 100%), and tests for controlling the sugarcane stem borer were preliminarily performed *in vivo*. The results show that significant ($p=0.01$) reductions in the adult population were caused by the strains. Therefore, *M. anisopliae* isolated from the cadavers of *C. venosatus* Walker is a potential biocontrol agent against this pest in South China.

Bernhardt *et al.*, (2014) isolated the entomopathogenic fungi *Metarhizium* Spp. from 32 bulked soil samples of a single agroecosystem in Denmark using *Tenebrio molitor* as a bait insect. To assess the *Metarhizium* community in soil from the

agricultural field and surrounding hedgerow, 123 isolates were identified by sequence analysis of 50 end of elongation factor 1-a and their genotypic diversity was characterized by multilocus simple sequence repeat (SSR) typing. *Metarhizium brunneum* was the most frequent (78.8%) followed by *M. robertsii* (14.6%), whereas *M. majus* and *M. flavoviride* were infrequent (3.3% each) revealing co-occurrence of at least four *Metarhizium* species in the soil of the same agroecosystem. Based on SSR fragment length analysis, five genotypes of *M. brunneum* and six genotypes of *M. robertsii* were identified among the isolates. A single genotype within *M. brunneum* predominated (72.3% of all genotypes) while the remaining genotypes of *M. brunneum* and *M. robertsii* were found at low frequencies throughout the investigated area, indicating a diverse *Metarhizium* community. These results may indicate potentially favorable adaptation of the predominant *M. brunneum* genotype to the agricultural soil environment.

Pattemore *et al.*, (2014) observed that the whole genome analysis of *M. anisopliae* indicates significant macrosynteny with *M. robertsii* but with some large genomic inversions. In comparison to *M. acridum*, the genome of *M. anisopliae* shares lower sequence homology. While all alignments overall are co-linear, the genome of *M. acridum* is not contiguous enough to conclusively observe macrosynteny. Mating type gene analysis revealed both MAT1-1 and MAT1-2 genes in *M. anisopliae*, suggesting putative homothallism, despite having no known teleomorph, in contrast with the putatively heterothallic *M. acridum* isolate CQMa 102 (MAT1-2) and *M. robertsii* isolate ARSEF23 (altered MAT1-1). Repetitive DNA and RIP analysis revealed *M. acridum* to have twice the repetitive content of the other two species and *M. anisopliae* to be five

times more RIP affected than *M. robertsii*. We also present an initial bioinformatic survey of candidate pathogenicity genes in *M. anisopliae*.

Sun *et al.*, (2016) collected one strain of *Metarhizium* sp. ZJ-1, isolated from Chinese soils he evaluated it for the growth characteristics, and screened it for its virulence to *R. ferrugineus* larvae under laboratory conditions. An approximately 685-bp fragment was amplified by ITS (ITS1-5.8S-ITS2) PCR from strain ZJ-1, further phylogenetic analysis revealed a 93% similarity to *Metarhizium anisopliae.* Inoculation of 1×10^8 conidia/mL caused 100% mortality of *R. ferrugineus*, LT_{50} levels of ZJ-1 were 1.66 days (1×10^8 conidia/mL), indicating that the conidia of strain ZJ-1 was highly virulent. These results suggest that *M. anisopliae* ZJ-1 has potential as effective and persistent biological control agents for *R. ferrugineus*.

Abboud *et al.*, (2017) evaluated the entomopathogenicity of indigenous *Beauveria bassiana* and *Metarhizium anisopliae* obtained in the Gaza strip against larvae and adults of *R. ferrugineus* to identify indigenous strains potentially suitable for Red Palm Weevil biological control. *M. anisopliae* was isolated from larvae and adult dead RPW from different positions of the Gaza strip. Morphological analysis of the isolated fungi and molecular identification were performed using the PCR technique. There was a difference in the fragment size of ITS1, ITS2, and (ITS1 + 5.8S + ITS2) between the two fungal species. The length of the ITS1 region in *B. bassiana* was larger than that of the *M. anisopliae* (230 bp and 215 bp, respectively). Whereas, the ITS2 fragment in *M. anisopliae* was greater than that of the *B. bassiana* (375 bp compared to 360 bp). The mortality percentage of larvae reaches 90% after 6 days after spraying @ 3.6×10^8spores/ml of *M. anisopliae*.

Gurlek *et al.*, (2018) determined the diversity and distribution of *Beauveria* and *Metarhizium* spp. in walnut fields in Kırşehir, Turkey and evaluated their pathogenicity against *Cydia pomonella* (L.) (Lepidoptera: Tortricidae). To perform this, 90 soil samples were collected from walnut fields to isolate *Beauveria* and *Metarhizium* spp. using selective media. The isolated 40 fungi were characterized based on their morphological and molecular characteristics including Bloc and β-tubulin gene sequences. Also, eight selected fungi were tested against *C. pomonella* larvae under laboratory conditions. The fungal isolates were identified as *Beauveria pseudobassiana* (15), *B. bassiana* (12), *Metarhizium robertsii* (11), and *M. brunneum* (13). *M. brunneum* ELA-38 caused 83% mortality after 2 weeks of application with 1×10^8 conidia/ml. The *Beauveria* and *Metarhizium* spp. were found to be the common components of the soils samples collected from walnut fields and some of the fungi obtained from this work as entomopathogenic fungi.

Keppanan *et al.*, (2018) obtained different isolates of *M. anisopliae* from agricultural soil in Thekkady, India. The taxonomic identity of most virulent isolate of *M. anisopliae* TK29 was confirmed on the basis of its morphology and 18S rDNA gene sequence homology. Phylogenetic analysis confirmed that the isolated strains were related to the same species. To confirm the identity of the *M. anisopliae* isolates TK29, a partial sequence (500bp fragment) of the ITS2-ITS3 gene region was sequenced for identify and comparison with representative sequences from an earlier study.

Zhang *et al.*, (2018) obtained different isolates of *M. anisopliae* from field collected *Eupolyphaga sinensis* cadavers. The status of this fungus as a new and genetically distinct species was supported by ITS sequence comparisons. The new strain

was compared with other *M. anisopliae* isolates and was found to be highly infectious and virulent against *B. germanica*. The morphological characteristics of the fungus were broadly ellipsoid, overall range: 4.7–5.4 × 2.1–2.6 μm. Mycelium branch, with separated, colorless and smooth, wide 2.8–3.2 μm. The length of the sequencing product fragment was 531 bp. The sequence shared 95% similarity with those of the *M. anisopliae* strain (Ma-58), and a constructed phylogenetic tree demonstrated the close phylogenetic relationship between EB0732 and *M. anisopliae*.

Kilic *et al.*, (2019) isolated Ten *Metarhizium anisopliae* isolate from soil in Erzincan province through the insect bait technique. Molecular identification of the isolates was performed using ITS rDNA analysis. Based on classical and molecular methods, all fungal isolates were identified as *M. anisopliaes* and deposited into the GenBank database with MH104853 - MH104862 accession numbers. All isolates of *M. anisopliae* were pathogenic to *G. mellonella* and *T. molitor* with a mortality rate of 63.3±3.3% - 83.3±3.3% and 30±5.8% - 66.7±3.3%, respectively 12 days after application.

Vishwanath *et al.*, (2021) collected six strains of *Metarhizium anisopliae* isolates from different vegetable fields in the Nashik region of Maharashtra state of India. The identification was done on the basis of morphological characteristics. The fungus *Metarhizium anisopliae* was grown on Potato Dextrose Broth (PDB) and Sabouraud's Dextrose Broth (SDB) in the terms of development of conidia. It produces dark herbage green and olivaceous colonies with white mycelial margins and colonies were highly branched with closely packed cylindrical conidiophores. These conidiophores become colored with the development of the spores. Conidial chains were round and columnar

phialides in a dense parallel arrangement and conidia were cylindrical to oval seen when observed under a microscope.

2.2 To study the economic mass production and viability of *Metarhizium anisopliae* on different substrates

2.2.1 Mass production of *M. anisopliae*

Kulat *et al.*, (2002) reported that the Sabouraud's dextrose agar yeast (SDAY) medium was superior, resulting in the highest radial growth (4.07 cm) of *M. anisopliae* followed by Emerson YPSS medium (4.01 cm), at 10 days after inoculation (DAI). The highest spore count (9.43 × 106 spores/ml) of the fungal suspension was observed in Barner's medium, followed by Emerson YPSS medium (8.29 × 106 spores/ml) and SDAY medium (7.16 × 106 spores/ml) at 10 DAI.

Moslim *et al.*, (2005) recorded that the fungus was easily grown on barley, wheat, rice and broken corn. The fungus was then mass produced on corn as this media has produced the highest yield and cheaper compared to other substrates. High viability of spores was achieved by maintaining the spore moisture content between 40 and 60%. The age of culture also influences the yield and quality of spores. Matured cultures at age between 30 – 40 days produced yield about 10.5g spores in a bag with 250g maize and about 19.5g in a bag with 500g corn.

Soundarapandian *et al.*, (2007) reported that the spore count, radial growth, sporulation and biomass were maximum when *M. anisopliae* cultured both ozapeck dox agar and potato dextrose agar media. Four types of solid media such as rice, corn, wheat and kodomillet, were used for the mass production of *M. anisopliae* and the highest

sporulation was observed in wheat (8300×10^4). Hence, wheat is recommended as the best solid medium for the mass production of *M. anisopliae.*

Jagdeesh *et al.*, (2008) studied the mass multiplication of *M. anisopliae* on different grain media such as broken rice grains, broken corn, broken jowar-broken wheat and broken rage grains. Recorded that broken rice was followed by broken jowar seed as the most productive media for conidial production of the fungus with a yield of 3.45×10^8 and 3.2×10^8 spores per ml, respectively. The next best was broken corn 2.2×10^8 spores per ml, broken wheat 1.94×10^8 spores per ml. The broken rice yielded the highest spore, broken jowar recorded the lowest production cost 8.6 Ps/1×10^8 spores, followed by broken corn 5.6 Ps/1×10^8.

Pratap *et al.*, (2012) screened liquid media such as Potato Dextrose Broth (PDB) and Sabouraud's Dextrose Broth (SDB). Pea amended media produced maximum biomass of the test fungus, while SDB produced significantly higher spore production of the fungi. They observed the highest conidial count (9.06×10^7 conidia/ml) on cowpea media followed by soybean.

Mehta *et al.*, (2012) observed that the growth of entomopathogenic fungi like *verticillium lecanii* and *Metarhizium anisopliae* were cultured in different media to produce the highest biomass of the fungus. They used different media like vegetables, cereals, pulses, rice-washed water, boiled rice water, saw dust, fruit etc. for the production of biomass of fungi grains media, organic and non-synthetic media have been used. Among the grain media, pulse medium produced significantly higher 20.70 grams per 250 ml followed by rice 16.22 grams per 250 ml and the list biomass production of *Metarhizium anisopliae* ws observed in wheat grain was 1.15gram per 250 ml.

Bhoosreddy (2014) observed that the cultivation of *M. anisopliae* on various grain substrates showed that the sporulation of the fungus differs significantly among different substrates. The highest sporulation was recorded with broken rice (7×10^4 spores per cm^3) and corn (6.6×10^4 spores per cm^3), followed by broken jowar (5.4×10^4 spores per cm^3). The lowest sporulation was recorded with broken wheat (4.6×10^4 spores per cm^3). The results indicate that high sporulation productivity and greater spore count could be attained by use of low-cost substrates as an economical means in mass scale production of this fungus.

Latifian *et al.*, (2014) reported that the most appropriate medium for the production of clamidospores and conidiosopre from *Metarhizium anisopliae* use liquid and solid phases. The liquid phase of plant foods, includes potatoes, wheat flour, rice flour, corn flour and sugar cane molasses, and for the solid phase of plant materials, including sugar cane, corn, barley, rice, millet and sorghum were evaluated. The performance characteristics of the liquid phase compared with spore concentration and germination per cent of clamidospores. The performance and characteristics of the solid phase compared by wet weight, dry weight, conidia concentration and germination. Results showed that between different plant extracts used as liquid and solid media to produce clamidospores and conidia of *M. anisopliae* were significantly different at and 1% probability. In the end, sugar cane molasses extract and rice was elected to produce clamidspor and conidia of *M. anisopliae, respectively*, *due* to maximum production and economic performance**.**

Prasad *et al.*, (2014) recorded that a maximum yield (171.75×10^6 spores/ml) of *Metarhizium anisopliae* was obtained in Farm Yard Manure (FYM) followed by SBD

(157.25 x 10^6 spores/ml). The lowest number of spores of *M. anisopliae* was obtained in sugarcane bagasse (34.25 x 10^6 spores/ml). The economics of *M. anisopliae* were evaluated based on the final yield. Among in *vitro-produced* media, the production spores, FYM was the best low-cost substrate (Rs. 0.440) for 1 Ã— 10^6 spore production, followed by SDB (Rs. 0.68). The highest cost of spore production was recorded in sugarcane bagasse (Rs. 2.18), followed by press mud.

Anitha *et al.*, (2015) observed that **t**he production of conidiospores in jack fruit seed medium was high. The 16 days old culture was fully covered with the conidiospores. The conidiospore production recorded after 16 days of incubation was 21.2 x 10^4 spores per ml and the viable count was 41.4 x 10^7 cfu per ml. The dust formulation of *M. anisopliae* containing the adjuvants like charcoal, kaolin, wheat flour and ash was prepared to contain 3, 6, or 9 percent dry fungal powder preparations of different concentrations. The culture media and adjuvants used for the preparation are ideal, since the cost of materials is very low and the entomopathogenecity high.

Ibrahim *et al.*, (2015) reported an isolate of the fungal entomopathogen *M. anisopliae* (Metschn.) Sorokin (LIM1) was grown on cooked rice, wheat, vegetable peels and burgul in roasting bags to produce and harvest spore powder. The cultures were dried and the total yield of harvested conidia was determined. After harvesting, spores were submitted to quality control to assess concentration, germination, purity, moisture content.

Roshandel *et al.*, (2015) studied the effect of natural grain-based media on the sporulation and germination of conidia and blastospores of *Metarhizium anisopliae*. Experiments were conducted with factorial arrangement in a completely randomized

design with nine treatments and three replications. The obtained results showed that there was a significant difference in the yield of conidia among different treatments, two weeks after inoculation, ($F= 4.66$, $df= 7, 16$, $p<0.01$). The highest average yields were obtained as 3.1×10^8 and 3×10^8 conidia/g in rice flour and Sabouraud's Dextrose Agar with 1% yeast extract, respectively. The lowest level of conidia yield was 4×10^6 conidia/g which was obtained in the wheat bran medium. There was a significant difference between blastospore yields four days after inoculation of the media, ($F=2.57$, $df= 8, 18$, $p<0.05$). The highest and lowest yield of average yield of blastospores were 6.1×10^8 and 4.5×10^8 blastospores/ml and were obtained in the wheat bran + rice bran + yeast extract and rice bran + yeast extract media, respectively. The highest yield of blastospores was 6.6×10^8, 6.1×10^8 and 5.7×10^8 blastospores/ml which were obtained in the rice bran+wheat bran+yeast extract+whey powder, rice bran+wheat bran+yeast extract, and wheat bran+rice bran+magnesium sulfate media, respectively. The lowest average yield of the blastospores was obtained in the media containing grape juice.

Karengala *et al.*, (2016) recorded those various grains such as polished rice, brown rice, wheat, corn, rag, sorghum, barley and baja were evaluated for mass production of the entomopathogenic fungi *Metarhizium anisopilae*. Among the various grains, wheat showed good growth and sporulation of *M. anisopilae*.

Sujatha *et al.*, (2016) recorded that various grains such as polished rice, brown rice, wheat, corn, rag, sorghum, barley and baja were evaluated for mass production of three entomopathogenic fungi such as *Beauveria bassiana*, *Metarhizium anisopilae* and *Lecanicillium lacanii*. Among the various grains, corn grains supported maximum growth and sporulation for *B. bassiana*. Similarly, wheat supported good growth and sporulation

of *M. anisopilae* and for *L. lecanii* observed maximum growth and sporulation in brown rice.

Agale *et al.*, (2018) reported that to develop a proficient method for the deployment of *Metarhizium anisopliae* as a biocontrol agent, various grains and liquid media such as Potato Dextrose Broth and Sabouraud Dextrose Broth were screened. Significantly conidial count (67.6×10^3 spores/ml) was observed on green gram followed by sorghum in 10^3 dilutions, the assessment fungus, whereas SDB produced significantly higher spore production of the fungi. The highest conidial count (63.7×10^3 spores per ml) was observed on SDB media followed by PDB in 10^3.

Tekam *et al.*, (2018) observed that among the different substrates evaluated highest conidial count (12.68×10^7 spores per ml) was observed in cowpea media followed by pea (11.84×10^7 spores per ml). It was also clear that *M. anisopliae* can grow on various cheap and easily available grains, hence, they can be used for the mass multiplication of the fungus and produced in bulk and can be made available at the doorstep of the farmers.

Raypuriya *et al.*, (2019) reported that many natural and liquid substrates (*i.e.* rice, corn, sorghum, green-gram, chickpea, wheat, potato dextrose broth, sabouraud's dextrose broth and rice starch) were used for mass multiplication of *M. anisopliae*, and they founded that the highest spore load of *M. anisopliae* (9.78×10^{10} cfu per ml) was harvested from rice followed by SDB (8.11×10^{10} cfu per ml) and chickpea (8.00×10^{10} cfu per ml) respectively. Whereas, the minimum number of spores (2.78×10^{10} cfu per ml) was obtained from wheat. The Significantly lowest production cost was obtained in

rice (Rs. 0.26 for 1 x 10^{10} spores per ml). Therefore, rice was found to be the most appropriate and economical substrate for the mass multiplication of *M. anisopliae* following SDB and Chickpea.

2.2.2 Economic of mass production of *Metarhizium anisopliae* on different substrates

Moslim *et al.*, (2005) reported that among all treatments viz., barley, wheat, rice, and broken corn. The mass production of *Metarhizium* on corn has produced the highest yield of 10.5g spores/250g corn bag and about 19.5g/ 500g corn bag. The corn was cheaper compared to other substrates.

Prasad *et al.*, (2014) studied the economics of *M. anisopliae* evaluated based on the final yield. Among in *vitro-produced* media, the production spores, FYM was the best low-cost substrate (Rs. 0.440) for 1 Ã— 10^{6} spore production, followed by SDB Rs. 0.68. The highest cost of spore production was recorded in sugarcane bagasse (Rs. 2.18), followed by pressmud.

Anitha *et al.*, (2015) reported that the mycelia growth and production of conidia increased with increase in incubation time up to 16 days (21.2 x 10^{4} spores per ml) and the entire medium was almost completely covered with hyphae. This indicated that jack fruit seed powder was comparatively cheaper than the other synthetic media available on the market. This cheap media and low-tech mass production method was used to supply the fungal pesticides at an affordable cost.

Tekam *et al.*, (2018) studied the experiments on mass production of *M. anisopliae* undertaken on fifteen substrates for determining a suitable medium for growth and sporulation and reported the cost of production of 1 x 10^{7} spores for the substrates. The

costs of production on different substrates varied significantly from each other. Significantly production cost was recorded for pea (Rs. 0.83), this was followed by SDB (Rs. 1.39), PDB (Rs. 1.40), soybean (Rs. 1.48), but the latter two were at par with each other. The next substrate was gingerly cake (Rs. 2.01) that was significantly superior to neem cake (Rs. 2.16).

Raypuriya *et al.*, (2019) reported that many natural and liquid substrates (*i.e.* rice, corn, sorghum, green-gram, chickpea, wheat, potato dextrose broth, sabouraud's dextrose broth and rice starch) were used for mass multiplication of *M. anisopliae*, *which* found that the minimum number of spores (2.78×10^{10} cfu ml^{-1}) was obtained from wheat. The Significantly lowest production cost was obtained in rice (Rs. 0.26 for 1×10^{10} spores' ml^{-1}). Therefore, rice was found to be the most appropriate and economical substrate for the mass multiplication of *M. anisopliae* following SDB and Chickpea.

2.2.3 Viability of *Metarhizium anisopliae* on different substrates

Magalhaes *et al.*, (2004) studied that *Metarhizium anisopliae* var. *acridum* Driver & Milner (*Metarhizium flavoviride* Gams & Rozsypal), isolate CG423, is being developed as a mycoinsecticide against grasshoppers in Brazil. Conidia were harvested and stored in a drying chamber during a study period of 260 d, in a room at ~25°C. The water content (dry weight) of fresh and dried conidia was 70 and 4%, respectively. Conidia from fresh and dry preparations produced similar in vitro germination patterns and were infective with the American grasshopper *Schistocerca americana* Drury. The drying process greatly enhanced the survival of CG423 conidia, as witnessed by the nearly 100% germination rate after more than a 100-d desiccation period. Prolonged 135 and 176 day storage in the drying chamber at room temperature decreased viability to

72.5% and 28.5% respectively. In contrast, fresh conidia sealed in plastic bags and stored at ambient conditions for 52 d was inactive (91.7%, $p < 0.05$). However, after 48h on SDAY plates, the germination rate of dry conidia (88.3%) was comparable to the germination of conidia rehydrated in water, water + Tween-20, or in high humidity.

Mallikarjuna (2006) evaluated different oil based liquid formulations for their viability at refrigerated and ambient room conditions and noticed that conidial viability decreased with increased storage period. Further, he reported that most of the oil based liquid formulations except pongamia oil (14.66 %), castor oil (8.33 %), groundnut oil (8.00 %), sesamum oil (5.00 %) and sunflower oil (4.66 %), failed to germinate under both refrigerated and ambient room conditions after 10 days of storage.

Rachappa *et al.*, (2007) studied the influence of carrier material and storage conditions on the viability of *M. anisopliae* conidia under *in vitro* conditions at Dharwad from Jan. 2001 to Jan. 2002. Irrespective of the carrier material used, the storage of conidia under refrigerated conditions 4°C, over a period of 1 year reduced viability to 62% as against 84% at ambient temperature. After 1 year of storage at ambient temperature, the colony forming units (39.6 × 106 cfu/g) were almost 2.4 times lesser than those stored under refrigeration (95 × 106 cfu/g). Among the different carrier materials used for wettable powder formulation, attapulgite and kaolinite retained the

viability of conidia significantly more (33.5 & 31.9% respectively after 1 year) followed by sorghum flour (27.9%) and talc (26.9%).

Pandey *et al.*, (2008) evaluated the influence of different cereal grains as solid substrates on sporulation, viability and pathogenicity of *Metarhizium anisopliae*

(Metsch.) *Sorokin* was studied under laboratory condition. Among the six grains (viz. corn, sorghum, finger millet, bajra, barley and wheat), sorghum as a solid substrate resulted in highest spores 6.3×10^7 conidia/ml production with spore viability of 86.6 per cent. The production and viability was 86.6 per cent. The production and viability of conidia increased when the substrates were supplemented with sucrose compared with conidia produced from substrates alone.

Pandey *et al.*, (2010) tested ten synthetic media for mass production and viability of *Beauveria bassiana* and *Metarhizium anisopliae*, among ten synthetic media sabouraud dextrose medium with yeast extract was significantly superior over all other media for both the entomopathogenic fungi and supported the maximum biomass (1.13g and 100g), conidial count (5.10×10^7, 4.80×10^7 ml^{-1}) and viability of conidia (94.33 and 92 %) for *B. bassiana* and *M. anisopliae*, respectively. Czapek dox medium was the least suitable medium for the growth and sporulation of entomogenous fungi. In all media addition of yeast increased the growth of fungi. Among 8 grain - based media, for growth and sporulation of *M. anisopliae* isolate, green gram was the most suitable grain medium with the highest biomass (0.87g) conidial count (6.77×10^7 conidia ml^{-1}) and viability (90.33 %).

Ibrahim *et al.*, (2015) evaluated that different whole grains of rice (parboiled), wheat (durum) and fine burgul (a form of whole wheat that has been cleaned steamed or parboiled, sizes) for mass multiplication and viability of the entomopathogenic fungi *Metarhizium anisopliae*. The germination for all treatments before desiccation was over 85% and germination after desiccation was over 70%. The highest germinations of

99.98% and 95.3% were recorded for conidia production on burgul before and after desiccation, respectively. The lowest rate of germination was observed for conidia obtained from wheat 86.7% and 70.2%, respectively.

Roshandel *et al.*, (2015) studied the effect of natural grain-based media on the sporulation and germination of conidia and blastospores of *Metarhizium anisopliae*. Experiments were conducted with factorial arrangement in a completely randomized design with nine treatments and three replications. Results also showed a significant difference between the means of the germination rate of conidia one, two and three months of storage at 4°C and 25°C. The highest and lowest germination rates of conidia belonging to SDA (control) and wheat bran medium were 97.03% and 75.87%, respectively, after one month storage in a refrigerator. After two months of storage at room temperature, maximum and minimum germination rates belonged to SDA and crushed rice (62.09%, 60.09%) and rice flour medium (29.4%). After three months of storage in the refrigerator, SDA and crushed corn medium treatments had the most germination percentages and wheat bran had the lowest germination percentage of 24.1%. The germination rate of conidia reduced to 10.9% 35.01% for treatments 3 months stored at room temperature.

Pal *et al.*, (2016) studied the storage and viability of the entomopathogenic fungus, *Beauveria bassiana*, *Metarhizium anisopliae*, and *Verticillium lecanii* in the laboratory using liquid and solid agricultural and industrial waste media. The FYM (25.37×10^7 spores/ml) produced maximum spores followed by FYM liquid + Press mud liquid (1 : 1) + 1 g Dextrose (23.57×10^7 spores/ml) and Jowar grain + 1.0 g Dextrose

(8.5×10^7 spores/ml) were the most suitable media for spore production, viability and storage of *M. anisopliae*.

2.3 To study the bioassay of *Metarhizium anisopliae* against *Helicoverpa armigera*

Varhade (2001) studied the pathogenicity of *M. anisopliae* using different concentrations ranging from 2.26×10^9 to 2.26×10^6 spores/ml of fungal suspension against 2nd instar larva of *H. armigera* and reported 95% larval mortality in 2.26×10^9spores/ml concentrations at 8 DAT.

Pandey and Kanaujia (2003) studied the pathogenicity of two isolates of *M. anisopliae,* cultured on SDA medium supplemented with and without larval extract, using *S. litura* as the test insect. The results indicated that the virulence of both isolates increased when cultures were grown on SDA medium supplemented with the larval extract. The LC_{50} (Median Lethal Concentration) values of Pantnagar isolate and MUCL-8237, grown on SDA medium supplemented with larval extract, were 1.08×10^6, 1.42 - 1.0×10^6, 1.73×10^6 conidia/ml, and 6.42×10^6, 2.65×10^6, 7.58×10^6 conidia/ml against 2nd, 3rd, and 4th instar larvae, respectively, showing higher virulence of the former in terms of LC_{50} values against *S. litura.*

Wadyalkar *et al.*, (2003) conducted experiments with *M. anisopliae* microbial insecticide against *H. armigera,* a potential threat to the successful cultivation of many economically important crops. Pathogenicity with 10^8 spores/ml concentration of fungal suspension revealed that larval mortality recorded 100.00, 90.00, 76.67, and 56.67% against 1st, 2nd, 3rd and 4th instar larvae of *H. armigera,* respectively.

Nguyen *et al.*, (2007) studied the susceptibility of the third instar *Helicoverpa armigera* to seven strains of three entomopathogenic fungal species, i.e., *Metarhizium anisopliae*, *Beauveria bassiana* and *Paecilomyces fumosoroseus*, was tested under laboratory conditions using the larval immersion method. The median lethal concentration (LC_{50}) for L_3 was 6.0×10^5 in *M. anisopliae* 79. This strain was further used to characterize the age-dependent mortality of different larval stages (L_2-L_5) and the effect on pupae of *H. armigera*. Larval stages did not differ in their mortality but differed in median lethal time, with shorter values recorded in the second instar. Tested fungi also caused a high reduction between 74.4 and 100% in the emergence of pupae using the soil inoculation method and the pupal immersion technique.

Nahara *et al.*, (2008) studied the effect of repeated conidial sub-culturing of *Metarhizium anisopliae* on its virulence against *Helicoverpa armigera* (Hubner). The LT_{50} observed against the third instar larvae of *H. armigera* for the first sub-culture was 3.4 days, it increased to 4.5 and 5.6 days for the 20^{th} and the 40^{th} sub-cultures, respectively. The LT_{50} values after the passage of the 40^{th} sub-culture on *H. armigera* decreased to 4.4 and 3.7 days for the 40^{th} (first in vivo) and the 40^{th} (fifth in vivo) passages, respectively. Similarly, the LC_{50} of *M. anisopliae* toward the third instar larvae of *H. armigera* increased from the first sub-culture (0.17×10^4) to (3.0×10^4). The 40^{th} conidial transfer on potato dextrose agar and again decreased to 0.74×10^4 and 0.23×10^4 in the 40^{th} (first in vivo) and the 40^{th} (fifth in vivo) passage, respectively.

Pandey and Wajid (2009) studied the efficacy of the entomogenous fungus *M. anisopliae* in controlling *S. litura* by bioassay test. The LC_{50} of *M. anisopliae* isolate MTCC -4101 were 4.44×10^4, 4.45×10^6 and 1.62×10^8 conidia/ml for 4–5, 10-11, and

15–16 days-old larvae of *S. litura*, respectively, while in case of MTCC-4103 isolate, the corresponding values were 1.95×10^5, 6.71×10^6 and 6.9×10^8 conidia/ml, respectively. The LT_{50} values at the highest concentration of fungus were 101.16, 116.51 and 149.75 h for 4–5, 10-11, and 15–16 days-old larvae *in* MTCC-4101 isolate, while 99.27, 119.53, and 201.03 h for MTCC-4103 isolates, respectively. MTCC-4101 was comparatively more virulent than MTCC-4103.

Rachappa *et al.*, (2009) evaluated different isolates of Metarhizium anisopliae against *Helicoverpa armigera* at the Agricultural Research Station, Malnoor, Gulbarga District, Karnataka, India. They found that isolate Ma2 was the most virulent against *Helicoverpa armigera* (Hübner) with fewer LC_{50} values of 1.77×10^6 conidia per ml.

Gopalakrishnan *et al.*, (2011) evaluated the entomopathogenic fungus *Metarhizium anisopliae* against *H. armigera under* laboratory condition. They recorded that the mortality rate was 73 per cent and 92 per cent weight reduction over control.

Kpindou *et al.*, (2012) observed that the pathogenicity of six isolates of the entomopathogenic fungi, *M. anisopliae* (Met 28, Met 32, Met 92, Met 31, Met 34 and Met 341) and two isolates of *B. bassiana* (Bb11 and Bb 12) was evaluated in the laboratory by applying topically 1 µl of an oil-based formulation of conidia at a concentration of 108 conidia/ml in the third, fourth, fifth and the sixth instars of *H. armigera*. Taken separately, each isolate caused less mortality among the larvae of the sixth stage than in younger stages. The corrected mortality rates varied from 3.6 ± 1.8% (Met 28, sixth instar) to 56.3 ± 0.8% (Met 31, third instar) for *M. anisopliae* and from 3.6 ± 1.8% (Bb 12, sixth instar) to 34.4 ± 4.2% (Bb 11, third instar) for *B. bassiana*. Certain isolates of *Metarhizium* were infective to pupa. The host development stage at

inoculation affected both survival times compared to control. The results of the presented study showed that the isolates of *Metarhizium* (Met 31) and *Beauveria* (Bb 11, known as Bba 5653), the last being tested successfully against *Plutella xylostella* (Lepidoptera: Noctuidae), are virulent isolates and can be promising isolates for the control of the cotton bollworm *H. armigera*.

Wakil *et al.*, (2013) evaluated local isolates of *Metarhizium anisopliae* against six field populations of *Helicoverpa armigera* Hubner in a series of laboratory bioassays. *M. anisopliae* was used at a concentration of 1.3×10^6 conidia/ml. against 2^{nd}, 3^{rd}, 4^{th} and 5^{th} larval instars. The mortality was observed every 24 h until pupation. They recorded significant differences in mortality in all tested populations when treated with *Metarhizium anisopliae*.

Agale *et al.*, (2017) reported that *M. anisopliae* did not cause the mortality of the second instar *Helicoverpa armigera* larvae at 24 h after treatment with different concentrations. The infectivity of *M. anisopliae* increased after 48 h of the treatment and the mortality was 50 per cent with the highest concentration 4.3×10^3 conidia per ml. There was no significant difference from two (3.9×10^4 and 2.9×10^5) concentrations of *M. anisopliae*.

Mantzoukas *et al.*, (2018) examined the susceptibility of *Helicoverpa armigera* population to *Metarhizium robertsii* under laboratory conditions. The *Helicoverpa armigera* larvae were treated individually and along with three conidial suspensions viz. 1×10^3, 1×10^5 and 1×10^7 spores/ml. compared with the untreated check population. The treatments 1×10^7 spores/ml significantly reduced the population of *Helicoverpa armigera*.

Tahir *et al.*, (2018) screened the entomo-pathogenicity of 22 isolates of *Beauveria bassiana* sensu lato and *Metarhizium anisopliae* (Ascomycota: Hypocreales), isolated from soils of different origins and insect cadavers. Twenty-two fungal isolates were screened for their pathogenicity toward 2nd instar larvae of *H. armigera*. Two conidial concentrations (1×10^7 and 1×10^8 conidia ml^{-1}) were applied against 2nd instar larvae by larval dip method. Four isolates of *B. bassiana* and one of *M. anisopliaes* exhibited >80% and >75% larval mortality, respectively. Overall, a higher number of sporulating moth cadavers were observed when inoculated with higher concentrations compared to lower concentrations. Five selected fungal isolates were evaluated at four different concentrations against 2nd and 4th instars larvae causing, 7 days after the treatment, 72.92%–100% and 65.24%–93.96% mortality, respectively. This study showed the fungal conidia concentration-dependent pathogenicity of several *B. bassiana*, and *M. anisopliae* isolates on larvae of the harmful pest *H. armigera.*

Fite *et al.*, (2019) observed the virulence of 14 isolates of *M. anisopliae* against larvae of *H. armigera*. One isolated of *M. anisopliae* strain (DLCO-EA-40) was most effective against third instar larvae of *Helicoverpa armigera* at 10^8 conidia per ml.

Khare and Gupta (2019) evaluated the pathogenicity of the fungus under *in vitro* conditions in the second instar larvae of *H. armigera* to study the effect of spore suspension of *Metarhizium anisopliae. In vitro*, the spore suspension (Direct spray method) at 1.0×10^8 spores/ml recorded the highest mortality (76.6%) after ten days of spraying the spore suspension on the body of *H. armigera.* Lower concentrations resulted in lesser mortality.

Kilic *et al.*, (2019) conducted their experiment to determine the effects of 9 isolates of *Metarhizium anisopliae* against *H. armigera under* laboratory condition. They placed all treated insects in BOD at 26±1^{0}C, 70±5 per cent relative humidity for 12 days. The isolates of *Metarhizium anisopliae* showed 53.3 to 73.3 per cent mortality against *Helicoverpa armigera*. All the isolates were very effective against *H. armigera*.

Lagogiannis *et al.*, (2020) evaluated three fungal species, Beauveria *bassiana*, *Metarhizium roberstsii* and *Isaria fumosorosea* on the survival of *Helicoverpa armigera* larvae in vitro. The fungi were sprayed at six different concentrations (10^3, 10^4, 10^5, 10^6, 10^7, 10^8 conidia/ml) on the second instar larvae of *Helicoverpa armigera*. The fungi *Metarhizium robertsii* treatment shows larval mortality (10^8) ranging between 20 (Three days) and 97 per cent (Nine days). The overall, at nine days doses of 10^7, and 10^8 in all treatment of *M. robertsii* was significantly more pathogenic than all other doses in terms of mortality.

Lawo *et al.*, (2020) reported that greater susceptibility to *M. anisopliae* occurred when susceptible *H. armigera* larvae fed on *Bt* chickpea leaves than when they fed on control leaves. When susceptible *H. armigera* larvae fed on *Bt* chickpea leaves were treated with *M. anisopliae*, an additive effect occurred at a spore suspension (5.7×10^8 spores/ml), with larval mortality between 53 and 97%, whereas at a spore suspension (1.2×10^8 spores/ml), the effect was more than additive in two of four bioassays, resulting in 72 and 87% mortality. Interestingly, this was observed when the untreated *Bt* leaves caused little mortality to susceptible *H. armigera* larvae (9 and 20%).

Taliyan *et al.*, (2020) Studied that the pathogenicity of *M. anisopliae* against 1st - 4th instar *H. armigera* larvae. The effect of *Metarhizium anisopliae* spores/ml on

Helicoverpa armigera larval instars was significantly higher recorded showed that the all treatments were significantly superior and causes larval mortality. The data recorded at 4, 6, 8 11, and 14 days after the pathogencity of various larval instars in the I^{st} instar of *H. armigera* with 1.8 x 10^9 spore suspension, showed the best. The I^{st} instars larval mortality per cent up to 35.63 followed by 73.39, 85.69, 98.37 and 100.0.

2.4 To study the bioefficacy of *Metarhizium anisopliae* against *Helicoverpa armigera* on chickpea crop

Nahar *et al.*, (2004) conducted that the effectiveness of oil-based conidia formulations of indigenous fungal isolates *Metarhizium anisopliae* M34412, *Beallveria bassiana* 83301 and *Nomuraea rileyi* N812 was evaluated against *Helicoverpa armigera* (Hubner) infestation on the pigeon pea under field conditions. The *M. anisopliae* M34412 conidia in the oil formulation (7:3, diesel: Sunflower oil) were found to be most effective in controlling *H. armigera.* The results were compared with other control agents such as, endosulfan and *HaNPV (H. armigera* Nuclear Polyhedrosis Virus). The per cent efficacy of was *M. anisopliae* 66.74.

Rijal *et al.*, (2008) conducted a field study to evaluate the efficacy of two most virulent native isolates of insect pathogenic fungi (*Metarhizium anisopliae* and *Beauveria bassiana*) and compared them with four commercial biopesticides against Chickpea pod borer (*Helicoverpa armigera* Hubner) at Chitwan, Nepal. They reported that *M. anisopliae* was the most virulent ones of all isolates with a concentration of 10^7 conidia per ml. The M1 isolates had the highest mortality rates and required the shortest time to kill the larvae of *H. armigera*. After treatment with M1 isolates for 10 days, the larval mortality exceeded 85%.

Kpindou *et al.*, (2011) evaluated the dose transfer of oil-based ultra-low sprays of *Metarhizium anisopliae* (Metch.) Sorokin to the *Helicoverpa armigera* (Hub.). Two doses of conidia (75 and 50 g/ha, being 3.75×10^{12} and 2.5×10^{12} conidia/ha, respectively) developed in 2 liters (70:30, Kerosene- peanut oil) were applied using a Berthoud Micro Ulva to three groups of cotton bollworms larvae were positioned in three rows downwind of a single spray line. Both the distribution of the droplets and the effect of direct contact with spray droplets were quantified. The mean number of droplets decreased from 416 (±60) droplets per cm^2 (kine1, 0.4m) to 45 (±36) droplets per cm^2 (line 3, 5m). To achieve 50% mortality, > 10 and > 13 days were necessary to 75 and 50 g/ha, respectively. From day 15, the difference in mortality rates was significant between the two treatments. At line 3 (5 m), at 12 days after application, the mortality rate reached 52% for 75 g/ha and 47% for 50 g/ha.

Phukon *et al.*, (2014) revealed that the entomopathogenic fungi *M. anisopliae* (10^9 cfu per ml or 10gm per lit.) and *B. bassiana* (10^6 cfu per gm or 10gm per lit.) could be effectively used under field conditions to reduce the *Helicoverpa armigera* population below the economic threshold level and could be an environmentally safe alternate approach for insect pest management in organic crop production. They recorded that the efficacy of *Metarhizium anisopliae* (10^9 cfu /ml) showed 3.8 larvae/15 plants after 7 DAT at the vegetative stage and 1.2 larvae/15 plants after 7 DAT at the flowering stage and 5.2 larvae/15 plants after 7 DATs at the pod formation stage.

Chaudhary *et al.*, (2014) evaluated different treatments against *Helicoverpa armigera* i.e. *Metarhizium anisopliae* talc @ 3g/liter, *Metarhizium anisopliae* talc @ 4g/liter, *Metarhizium anisopliae* talc @ 5g/liter, *Metarhizium anisopliae* oil emulsion @

4ml/liter and *Metarhizium anisopliae* oil emulsion @ 5ml/liter of water. The highest reduction in the larval population (85.41%) was recorded in the talc formulation of Metarhizium anisopliae with a concentration of 5 ml/l at 10 DAS. Oil emulsion formulation of *M. anisopliae* was found to be less effective in reducing larval reduction compared with talc formulation.

Savita and Paudurngan (2015) observed that the effectiveness of entomopathogenic fungi *Metarhizium anisopliae, Beauveria bassiana* and *Nomureae rileyi* with concentration 1x 10^9 conidia per ml for were evaluated against *Helicoverpa armigera* (Hub.) on chickpea under field conditions. *Metarhizium anisopliae* was found to be the most effective entomopathogenic fung at 1x 10^{10} conidia per ml and a minimum surviving larval population 1.10 larvae, pod damage 9.50 per cent and higher yield 14.50 q/ha.

Sarkar *et al.*, (2015) screened different bio-pesticides i.e., *Metarhizium anisopliae* @ 1× 10^8 cfu/g (5g/l), *Verticillium lecanii*, *Beauveria bassiana* and *Bacillus thuringiensis* var *Kurstaki* against *Helicoverpa armigera*. There was no significant variation among treatments in mean percent fruit infestation after 14 days of the first spray. However, at 7 and 14 days of second spray all the treatment showed significant reduction of borer infestation and showed superior performance over control. Among all bio-pesticides *M. anisopliae* exhibited reducing fruit damage percentage was 18.96 per cent.

Type *et al.*, (2017) conducted that the present work and they isolated 68 *Metarhizium* strains from infected insects using a soil dilution and bait method. The isolates were identified by the amplification and sequencing of the ITS1-5.8S-ITS2 and 26S rDNA region. The most virulent strain of *Metarhizium anisopliae* was selected based

on the median lethal concentration (LC_{50}) and time (LT_{50}) obtained in insect bioassays against III-instar larvae of *Helicoverpa armigera.* Field trials of the formulations for the control of an *H. armigera* infestation in pigeon peas were carried out by randomized block design. During the field trial for the control of *H. armigera* in pigeon peas, 78.0% efficacy was obtained with the *M. anisopliae.* The average yield (q/ha) in the untreated control was 7.31 q/ha, which was less than that after *M. anisopliae* M3444 treatment (14.04 q/ha).

Kelwatkar *et al.*, (2017) assessed the efficacy of *M. anisopliae* and neem derivatives alone and in combination against *Helicoverpa armigera* infesting chickpea. On the basis of the overall mean, the differences in the mean larval population among different treatments were significant. Among all treatments *M. anisopliae* @ 1×10^8 spores/ml showed a mean larval population of 1.50 larvae per meter row length.

Patil *et al.*, (2018) evaluated nine treatments with newer insecticides, botanicals and microbial insecticides against *H. armigera* at Mahatma Phule Vidyapeeth Rahuri, Ahmednagar during 2015–16 and 2016–17. They used the microbial pesticide *M. anisopliae* 1.15 WP at 1×10^8 cfu/g. The treatment show list effective among all treatments with pooled data of both years 1.40 mean larval populations of *H. armigera* and 19.03 mean fruit damage percent.

Kumar *et al.*, (2018) evaluated the bio-efficacy of *Metarhizium anisopliae* 2×10^8 cfu/gram @ 1 kg/ha against chickpea pod borer, *Helicoverpa armigera* in chickpea crop during 2009–10, 2010–11. They recorded that the mean larval population at 3 DAT was 0.79 larvae/10 plants, 0.93 larvae/10 plants at 7 DAT, 1.06 larvae / 10 plants at 10 DAT, and at 15 DAT, 2.03 larvae/ 10 plants after the first application of treatments. The second

bio-pesticide spray was applied 90 days after sowing. After 3 days of application the larval population was 0.68 larvae/10 plants, 0.93 larvae/10 plants at 7 DAT, 1.01 larvae/10 plants at 10 DAT and 1.71 larvae/10 plants at 15 DAT in the first year. 0.63, 0.73, 1.01, 1.52 larvae/10 plants first spray. 0.61, 0.64, 1.03, 1.39 larvae/10 plants in the second spray during both years. Mean pod damage per was 14.23 and 9.26, with a yield of 1511.11 kg/ha and 1567.34 kg/ha in both years. The cost benefit ratio was 1:4.22 & 1:5.35 Rupees.

Kaur Joshi (2018) evaluated different agricultural substrates, viz. corn, rice, sorghum and wheat, for the mass production and viability of *M. anisopliae* isolates at different temperatures. The *M. anisopliae* recorded maximum viable count at temperatures 25 ± 2^0C (60.75×10^5 cfu/g) and 30 ± 2^0C (57.75×10^5 cfu/g), which were also at par with each other and significantly better than lower temperature 20 ± 2^0C ($52.6610^5 \times$ cfu/g). *M. anisopliae* formulation viability in storage at two different temperatures indicated that refrigeration temperature (4 ± 2^0C) was better compared to room temperature (25 ± 2^0C) till six months.

Fite *et al.*, (2019) recorded the virulence of 14 isolates of *Beauveria bassiana* and *Metarhizium anisopliae* and a commercial wettable product of *Bacillus thuringiensis* (Bt) against larvae of *H. armigera*. Three isolates of *B. bassiana*, APPRC-9604, APPRC-T5 and DLCO-EA-56, and one of *the M. anisopliae* strains (DLCO-EA-40) was most effective against the third instar *H. armigera* at 10^8 conidia/ml. Field trials indicated that APPRC-9604 is effective in reducing larval infestations, decreasing pod damage and increasing chickpea yield.

CHAPTER-3 METERIAL AND METHODS

The present investigation on "**Studies on mass production of entomopathogenic fungi *Metarhizium anisopliae* (Metschnikoff) using different media and potential against chickpea pod borer, *Helicoverpa armigera* (Hubner)"**. The details of materials used, experimental procedures followed and techniques adopted have been given below.

3.1 Experimental site and locations

The experiment was conducted at Biological control laboratory and Crop Research Centre (CRC) of Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut during *Rabi,* season 2020-21 and 2021-22 respectively. The University is located just 70 km away from National Capital region of Delhi on National highway No. 58 (Delhi-Haridwar Road). Geographically University is located at 29.01^0North latitudes, 77.75^0East longitudes and altitudes of 237 meter above the mean sea level.

3.2 Climate and weather conditions

The climate of this region is subtropical with extreme hot and cold in summer and winter seasons. The region experiences maximum temperature ranges from 40 to 45 ^{0}C during summer and minimum temperature ranges from 7 to 8 ^{0}C during winter. Occasionally, frost was also occurred during winter season (December - February).

3.3 Materials used

3.3.1 Glassware

The glassware *viz.*, conical flasks, boiling flask, volumetric flask, desiccator, petri plates, beakers, culture tube, glass rod, droppers, funnel, microscopic slide and cover slips were utilized in the current research.

The glassware's sterilized by soaked them overnight with 5 % bleach or by boiling and washed with detergent. The air dried glassware's autoclaved at 121^0C for 20 minutes under the 15 lbs pressure/inch. The steam sterilized glassware dry sterilized at 180^0C for two hours **(Ranjan *et al.*, 2017).**

3.3.2 Equipment

The equipment such as autoclave, oven, laminar air flow cabinet, mechanical blender, mechanical shaker, BOD, household mixer, ocular and stage microscope, neubauer haemocytometer, colony counter, wet and dry bulb thermometer, hygrometer and pH meter were used in current study.

3.3.3 Other materials

Trays, inoculation needles, cup borer, saline glass bottle, tissue papers, blotting paper, whatman No.1 filter paper, sterilized cotton wool, plastic vials, rubber bands, aluminum foils, parafilm, ethyl alcohol, sodium hypochlorite, streptomycin sulphate, dodine were made available in the Biological control laboratory of Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut.

3.4 Preparation of media

3.4.1 Sabouraud's dextrose agar (SDA)

Sixty five grams of synthetic Sabouraud dextrose agar media was used to prepare the growth substrate, which was dissolved in 1000 ml of distilled water and heated to boiling point. It was distributed in conical flasks and autoclaved in the oven for 20 min at 15 bar pressure, at 121^0C.

3.4.2 Potato dextrose broth (PDB)

Twenty four grams of synthetic potato dextrose broth media was used to prepare the growth substrate, which was dissolved in 1000 ml of distilled water and heated to boiling point. It was distributed in conical flasks and autoclaved in the oven for 20 min at 15 bar pressure, at 121^0C.

3.4.3 Sabouraud's dextrose broth (SDB)

Thirty grams of synthetic Sabouraud dextrose broth media was used to prepare the growth substrate, which was dissolved in 1000 ml of distilled water and heated to boiling point. It was distributed in conical flasks and autoclaved in the oven for 20 min at 15 bar pressure, at 121^0C.

3.5 Agronomic practices

A. Land and its preparation

Land preparation for sowing chickpea is based on the soil type and cropping system. In the case of a heavy soil, a rough seedbed is prepared to avoid packing of the cloddy surface due to winter rains and to facilitate soil aeration and easy seedling emergence. It is necessary to deep-plough the field at the beginning of the rainy season.

Deep ploughing also reduces wilting of chickpea that tends to develop due to the presence of hardpans in the root zone.

B. Sowing time

In India, mid - October to mid - November is the ideal period for sowing chickpea.

C. Spacing

Row-to-row spacing 30 cm and plant-to-plant spacing of 10 cm are generally used but in some cases 45 cm row-to-row and 10 cm plant-to-plant can be used.

D. Seed rate (Kg/ha)

Seed rate of 75-100 kg per hectare depending upon seed size may be sufficient for one hectare. The seed should be placed 8-10 cm. deep.

E. Seed treatment

Chickpea seeds can be treated with the talc based formulation of antagonistic microorganisms, whereas combination of these two microorganism seeds were treated first by *Trichoderma* 10g/kg of seed then apply jaggary solution (1%) followed by *rhizobium* culture 205g/kg seed.

F. Nutrient management

Fertilizer requirements depend on the nutrient status of the field, and thus, vary from field to field. Therefore, the doses of fertilizers should be determined based on the results of soil test. The generally recommended doses for chickpea include 20–30 kg nitrogen (N) and 40–60 kg phosphorus (P) per ha. If soils are low in potassium (K), an application of 17 to 25 kg per ha or 60 tonnes of FYM is recommended. Total quantities

Plate 1: Land Preparation of experimental field.

Plate 2: General view of experimental field.

of NPK should be given as a basal dose. Foliar spray of 2% urea at flowering has been found beneficial in rainfed crops **(Annonymous, 2010)**.

G. Water management

Chickpea is mostly sown as a rainfed crop. However, where irrigation facilities are available, give a pre-sowing irrigation. It will ensure proper germination and smooth crop growth. If winter rains fail, give one irrigation at pre-flowering stage and one at pod development stage. In case of only single irrigation is available should be given at flowering stage of gram crop. A light irrigation should be given because heavy irrigation is always harmful to gram crop. Excess of irrigation enhances vegetative growth and depresses Chickpea yield.

3.6 Isolation, characterization and selection of most virulent isolate of *M. anisopliae*

3.6.1 Isolation by baiting methodology

- A random survey was carried out in the Meerut region.
- Composite soil samples were collected from top 10 cm layer
- Collected soil samples were placed in 500 g plastic containers. The culture of *Galleria mellonella* was maintained in the bocontrol laboratory. For this culture, third instar *Galleria mellonella* of 5-10 larvae released into each container. The container was covered tightly with lids containing small holes to facilitate gaseous exchange and was kept at room temperature.

3.6.2 Maintenance of culture

Diseased and mummified larvae were retrieved from the container for isolation of fungi. The isolated fungus from the insect cadavers was placed on an artificial Sobouraud Dextrose Aagar medium (3.4.1). The fungus was full grown within 10-14 days after

inoculation at temperature 25±1^{0}C. Virulence was revived by passing through an insect host after 5-6 sub-culturing. New pure cultures was raised and stored at 25±1^{0}C for two week. The fungi were identified mainly based on the morphological characteristics of reproductive structures with the aid of several taxonomic keys **(Zimmermann, 1986)**. After isolated fungus grown on SDA at room temperature 25-28 ^{0}C for 8-10 days, and complete sporulation, conidia from the medium were harvested by washing them thoroughly with sterilized water containing Tween-80 (0.2%) for immediate use. Otherwise, spores were harvested with sterile metal spatula. Harvested conidia were air dried under laminar air flow and stored in small air tight screw cap vials (10 cm with 2.5 cm diameter) in refrigerator at 4^{0}C before using for further studies. Suspension of spores was made using distilled water withTween-80 (0.2 %) and filtered through a double layered muslin cloth. Spore count was made using a double rolled Neubauer's haemocytometer after necessary serial dilutions under phase contrast microscope.

3.6.3 Identification and characterization of *M. anisopliae*

3.6.3.1 Procedure of DNA isolation

Before starting, prepared fungal lysis buffer, 5mg/ml zymolyase 20T, 1 M sorbitol, 0.1 M EDTA.

- Harvest up to 1 × 10^8 fungal cells in a 1.5 or 2 ml micro centrifuge tube by centrifugation for 5-10 s at maximum speed ≥ 12000 × g. Discard the supernatant.
- Resuspend the pellet in 500 µL of fungal lysis buffer. Incubate for 1 hour at 37^{0}C Centrifuge cells for 10 min at 3000 × g. discard the supernatant.

- Resuspend the pellet in 180 µL of digested solution, add 20 µL of proteinase K solution and mix thoroughly by vortxing or pipetting to obtain a uniform suspension.
- Incubate the sample at 56^0C while vortexing occasionally or use a shaking water bath, rocking platform or thermo mixer until the cells are completely lysed (45 min).
- Add 20 µL of lysis solution, mix by vortexing and incubate the mixture for 10 min at room temperature.
- Add 200 µL lysis solution, mix thoroughly by vortexing for 15 s until a homogeneous mixture was obtained.
- Add 400 µL of 50% ethanol and mix by pipetting or vortexing.
- Transfer the prepared lysate to a gene JET genomic DBA purification column inserted in a collection tube. Centrifuge the column for 1 min at 6000 × g. Discard the collection tube containing the flow through solution. Place the Gene JET genomic DNA purification column into a new 2 µL collection tube (included).
- Add 500 µL wash buffer (with ethanol added) Centrifuge for 1 min. at 8000 × g. Discard the flow-though and place the purification column back into the collection tube.
- Add 500 µL of wash buffer II (with ethanol added) to the gene JET DNA purification column. Centrifuge for 3 min at maximum speed (≥ 12000 × g). If residual solution is seen in the purification column empty the collection tube and re-pin the column for 1 min at maximum speed. Discard the collection tube

containing the flow- through solution and transfer the gene JET genomic DNA purification column to sterile 1.5 mL micro centrifuge tube (not included).

- Add 200 µL of elution buffer to the centre of the gene JET genomic DNA purification column membrane to elute genomic DNA, incubate for 2 min at room temperature and centrifuge for 1 min at 8000 × g.
- Discard the purification column. Use the purified DNA immediately in downstream application or store at 20^0C.

3.6.3.2 Gel-electrophoresis

Agarose electrophoresis of the isolate genomic DNA was performed to know the quality of DNA. As the size of genomic DNA is quite big, a 0.8 % gel was used to visualize the genomic DNA, as it can resoled DNA molecules in the range of 0.7 to 8.5 kb. The amplified DNA samples was mixed with a loading dye (30% glycerol containing 0.25% xylene cyanol and 0.25% bromophenol blue) and electrophoresed on 2.0% agarose gel in IX TAE buffer at 4-5 volt/cm for 2.5-3 hours. The electrophoresed sample of the gel was photographed on the documentation system.

3.6.3.3 Polymerase chain reaction

The Polymerase chain reaction (PCR) for amplification of fungi was standardized and carried out in a final reaction volume of 25 µL. The primers ITS-F (5'-TCCGTAGGTGAACCTGCGG-3') and ITS-R (5'-TCCTCCGCTTATTGATATGC-3') were used for the gene amplification.

Table 1: Preparation of PCR mix for amplification

S. No.	PCR components	Amount (in µl)
1	10X Dream Taq Buffer with $MgCl_2$	2.5 µl
2	25Mm $MgCl_2$ (R0971)	1 µl
3	10 mM $dNTP_s$ (R0191)	1 µl
4	(20mM) Forward primer (0.25 Mm Final)	1 µl
5	(20mM) Reverse Primer (0.25 mM Final)	1 µl
6	Dream Taq	0.5 µl
7	Sterile distilled water	16 µl
8	DNA template	2 µl
	Total	**25 µl**

All the steps were carried out in ice bucket containing crushed ice. Eppendorf tubes were placed in a thermal cycler (Bio-Rad, Mini cycler) for 34 cycles of PCR follow as mentioned in table 2. After completion of the cycles, the samples were stored at -20ºC till electrophoresis.

Table 2: Conditions of PCR reaction cycles

Segment 1	**Step**	**Temperature**	**Time**
Cycle 1	Initial Denaturation	94° C	**3 min**
Segment 2			
Cycle 35	1.Denaturation	94° C	**45 sec**
	2.Annealing	55º C	**45 sec**
	3.Extension	72° C	**1 min**
Segment 3	Repeat from 2 for 34 cycles		
Cycle 1	**Final extension**	**72° C**	**10 min**

3.6.3.4 Analysis of PCR products

Amplified fragments were checked on 0.8 % of agarose gel according to the procedure outlined by **(Sambrook and Russel, 2001)**.

A. Sequencing of selected clones

After PCR amplification, amplified products were sequenced using Sanger sequencing methods at Biokart India Pvt Ltd, Bangalore.

B. In-silico analysis

The sequence data obtained after sequencing was then validated by performing BLAST [www.ncbi.nih.gov/BLAST] **(Altschul *et al.*, 1990)** analysis to ensure that the sequences are true. To identify phylogeny with other *Metarhizium* species, sequences and designation of other fungi resulting from BLAST analysis were obtained from the GenBank **(Benson *et al.*, 2012)** and multiple sequence alignment was done using ClustalW **(Thompson *et al.*, 1994)** and sequence identity matrix was also created. The aligned sequences were used to generate phylogenetic tree based on neighbour-joining method **(Saitou, 1987)** using Mega-X software **(Kumar *el al.*, 2018)**.

3.6.4 Selection of most virulent isolate of *M. anisopliae*

Selection of most virulent strain between five local isolated *M. anisopliae* and isolates were performed against *H. armigera* by dipping assay method.

Plate 3: Pure culture of most virulent isolate (SVPUAT 1) of *M. anisopliae*.

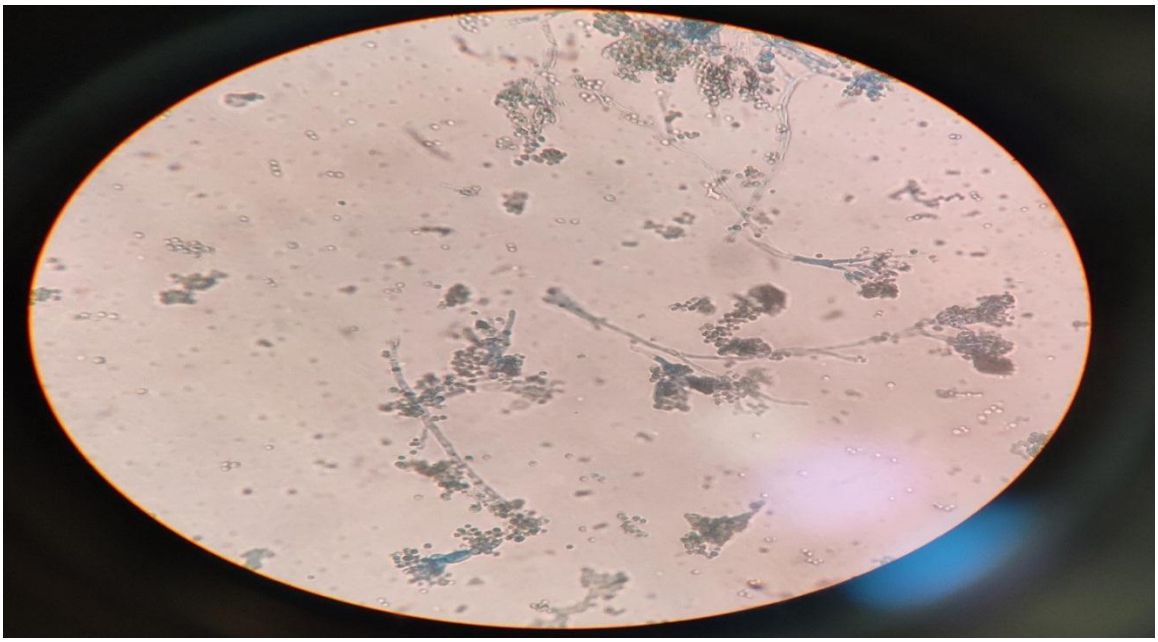


Plate 4: Microscopic view of *M. anisopliae* spores.

Conidia of different isolates were produced in petri dishes (9 cm) containing Sabouraud Dextrose Agar (SDA) and incubated at 25±1^{0}C. After ten days, conidia were harvested using peanut oil and spatula and transferred to conical flasks (250 ml) containing 100 ml sterilized distilled water with 0.02% the speeder sticker (tween, 80). Conidial concentrations in the suspensions were quantified directly under the optical microscope with a haemocytometer. Then the suspensions were standardized until the direct concentration of 2 × 10^8 spores/ml was obtained. The 2rd instars larvae of *H. armigera* dipped in prepared suspension and placed in petri dishes with fresh food. The control was treated with distilled water. Each treatment was replicated three times along with control. Per cent mortality was calculated according Abbott formula **(Abbott, 1925)**. The experiment was carried out under laboratory condition at 26^{0}C±2 and 60-70 % RH.

3.7 To study the economic mass production and viability of *Metarhizium anisopliae* on different substrates

3.7.1 Mass multiplication of *M. anisopliae*

The experiment was laid out in completely randomized block (CRD) design with seventeen treatments which included fifteen solid substrates and two synthetic media, for the identification and standardization of an appropriate economical medium for the development and sporulation of *M. anisopliae.* The experiment details are show in Table 3.

Table 3: Treatments detail for mass multiplication of *Metarhizium anisopliae* on different substrates

SYMBOL OF TREATMENTS	SUBSTRATES	TREATMENTS
		SOLID SUBSTRATES
T_1	CEREALS (WHOLE GRAINS)	RICE, *ORYZA SATIVA* (L.) + 1 % YEAST POWDER + 1.0G. DEXTROSE
T_2		WHEAT, *TRITICUM AESTIVUM* (L.) + 1 % YEAST POWDER + 1.0G. DEXTROSE
T_3		BAJRA, *PENNISETUM TYPHOIDES* (BURN.F.) + 1 % YEAST POWDER + 1.0G. DEXTROSE
T_4		MAIZE, *ZEA MAYS* (L.) + 1 % YEAST POWDER + 1.0G. DEXTROSE
T_5		SORGHUM, *SORGHUM BICOLOUR* (L.) + 1 % YEAST POWDER + 1.0G. DEXTROSE
T_6	PULSES (WHOLE GRAINS)	COW PEA, *VIGNA UNGUICULATA* (L.) + 1 % YEAST POWDER + 1.0G. DEXTROSE
T_7		GRAM, *CICER ARIETINUM* (L.) + 1 % YEAST POWDER + 1.0G. DEXTROSE
T_8		PIGEONPEA, *CAJANUS CAJAN* (L.) + 1 % YEAST POWDER + 1.0G. DEXTROSE
T_9		BLACK GRAM, *VIGNA MUNGO* (L.) + 1 % YEAST POWDER + 1.0G. DEXTROSE
T_{10}		GREEN GRAM, *VIGNA RADIATE* (L.) + 1 % YEAST POWDER + 1.0G. DEXTROSE
T_{11}	DUNG CONCOCTION AND INDUSTRIAL WASTES	PRESS MUD + 1 % YEAST POWDER + 1.0G. DEXTROSE
T_{12}		FYM + 1 % YEAST POWDER + 1.0G. DEXTROSE
T_{13}		VERMICOMPOST + 1 % YEAST POWDER + 1.0G. DEXTROSE
T_{14}		FYM LIQUID + 1 % YEAST POWDER + 1.0G. DEXTROSE
T_{15}		CORCYRA REARING WASTE + 1 % YEAST POWDER + 1.0G. DEXTROSE
		ARTIFICIAL MEDIA
T_{16}	SYNTHETIC MEDIA	POTATO DEXTROSE BROTH (PDB)
T_{17}		SABOURAUD'S DEXTROSE BROTH (SDB)

Fig 5: Soaking of grains media.

Fig 6: Removal of excess moisture from the grains media.

Plate 7: Inoculation of *M. anisopliae* into different substrates.

Plate 8: General view of different substrates in BOD.

3.7.1.1 Methodology

A. Solid substrates

Cereals (rice, wheat, bajra, maize, sorghum) pulses (cowpea, gram, pigeonpea, black gram, green gram) and Dung concoction and industrial wastes (vermicompost, FYM, press mud, FYM liquid, corcyra rearing waste) were used for estimating the biomasss of *Metarhizium anisopliae* at 28^0C. 50 gram of each grain were washed well and soaked for one day prior to use and then mesh properly and filter it. Now these grain mediums were packed separately in individual 250 ml conical flask. They were plugged with cotton wool and autoclaved at 15 psi for 30 minute at 121^0 C. After cooling, two 5 mm of fungal disc culture was inoculated into each flask, separately. All these procedure were done under laminar air flow chamber. They were incubated in BOD at 28^0C for 30 days. To avoid clumping, after 7 days of inoculation, the flasks were shaken vigorously to separate the culture and to break the mycelia mat.

B. Liquid (artificial) substrates

100 ml of each media (i.e. potato Dextrose broth, sabouraud's Dextrose broth) were dispensed into 250 ml conical flask, plugged with non-absorbent cotton and autoclaved at 15 psi for 30 min. Each flask was replicated three times. After that, inoculated with two 5 mm fungal disc of *M. anisopliae* under laminar air flow chamber and incubated at 28^0C for 30 days. Conical flasks were shaken 7 day after inoculation for the uniform growth of the fungus.

3.7.1.2 Estimation of spore of entomopathogenic fungi

Observation on spore counting was done on 10^{th}, 20^{th} and 30^{th} days after inoculation. The counting of spores was according to the method reported by **(Bias *et al.*,**

1990) using haemocytometer with slight modification. For this purpose 10g/10ml of homogenous grain/solution sample was drawn from each replication of uniformly sporulated flasks and was transferred to 100 ml sterilized distilled water containing tween 80 (0.05%) solutions in 250 ml conical flasks. The flasks were shaken in mechanical shaker at 500 rpm for 10 minute. The suspension was filtered through double layered muslin cloth. Counting of spores were made after the serial dilution of the suspension using a drop of conidial suspension made from solid and liquid culture was placed on the engraved grid and the preparation was allowed to stand for 1-2 minutes to allow the conidia to settle at the bottom. A cover glass was placed over the grid carefully to avoid no air bubble enters between the slide and cover glass. The slide was focused until coloured rings were visible as the two surfaces of cover glass and slide come into close contact. The conidia of fungus were counted in the 4 corner large square (I, II, III, IV) which consist of 16 small square each group has 0.2 mm square. The number of spores/cell per ml of suspension were calculated using the following formulas

Total number of spores/ml/gm. = No. of spores in single square $\times 10^4 \times$ Dilution factor.

3.7.2 Economics of mass production of *Metarhizium anisopliae* on different substrates

The economics of mass production of *M. anisopliae* was based on total cost [Fix cost and variable cost *i.e.* cost of substrates per 100g (Rs.) and cost of production of *M. anisopliae* 1×10^7 spores per ml (Rs.)] was calculated and presented in table form.

3.7.3 Viability of *M. anisopliae* on different substrates

The experiment was laid out in completely randomized block (CRD) design with seventeen treatments which included fifteen solid substrates and two synthetic media, for

determination of viability of fungal spores produced on different substrates. The sporulated substrates stored for six month at room temperature. The experiment details are show in Table 3.

3.7.3.1 Methodology

Prepared Sabouraud Dextrose Agar on the basis of above recommended method (3.4.1) as a growth substrate, In order to prepare the suspensions for the needs of the experiments (3.7.1.2), the 1.0 ml of fungal suspension at 1×10^{7} dilution factor inoculated on 9 cm SDA petri dishes and secured with parafilm for protection against contamination and allowed to grow in the incubator for 24 hr., at 28 ± 1^{0}C.

3.7.3.2 Observation to be recorded

After 24 hr., the fungal colonies were counted by Olympus microscope (×1000 magnification) **(Bena-Molaei *et al.*, 2011)** and determined the conidial viability per cent of different substrate at 1, 2, 3, 4, 5 and 6 month after storage.

$$\text{Conidial viability per cent} = \frac{\text{Number of germinated conidia}}{\text{Total number of conidia}} \times 100$$

3.8 To study the bioassay of *Metarhizium anisopliae* against *Helicoverpa armigera*

Studies on efficacy of different colony forming unit of *M. anisopliae* on different instars of *Helicoverpa arimigera* larvae in laboratory condition were carried out by adopting following procedures.

3.8.1 Rearing of insect

To facilitate a pure culture of *H. armigera* huge number of larva were collected regularly from untreated chickpea crops and were reared by using synthetic diet till larva attain the pupation stage. Manual collection of different stages of larva from the plants of chickpea from the SVPUA&T Meerut was done regularly till the standardization of culture in the laboratory. All laboratory experiment conducted for rearing was kept at 25± 1^0C, 65±5 % RH. Field collected larvae were separated from host plant and were placed in artificial diet for *in vitro* rearing. Precautionary measures were followed throughout rearing by elimination of parasites and diseased larva. *H. armigera* larvae were reared individually on trays/ vials with artificial diet. These trays were covered with brown paper to provide dark conditions for larva. *H. armigera* rearing in the laboratory was done on modified semi-synthetic diet **(Hemed *et al.,* 2008)** with slight modifications. The diet was changed regularly with three days interval till completion of all larval stages or until larva attained pupation. The process of pupation occurred by forming a cocoon within the diet itself during rearing. Initial stages of pupae was light green in colour and turns dark brownish upon completion with black head formation. After complete pupation the pupae were separated based on the size an abdominal appearance into male and female and was transferred to glass container (20 cm × 10 cm) covered with brown paper on all sides of glass containers to provide dark condition necessary for pupation and adult emergence. Healthy pupae were selected and separated individually as pairs till eclosion (Emergence from pupa case). After eclosion period the adults were released into cages (32 cm × 32 cm × 32 cm) from the glass containers containing pupae which were placed on sand enclosed with water in plastic trays to maintain humidity. Temperature

was maintained between 25±1 ^{0}C during pupation. A cotton swab prepared was dipped in honey mixed with multivitamin capsule and Protein-X was placed inside the cage on the sixth day after pupation as a food source for adults after emergence. The adults emerge inside the cage was allowed for motion. The cages were covered with white cloth inside essential for egg laying. The eggs were laid in two to three days intervals by female moth on the cloth provided at the top or sides of cloth covered on cages. The eggs laid was clearly notice on white cotton cloth and eggs hatching neonate larva was collected using fine camel hairbrush and was released into fresh diet for feeding. Routinely used apparatus like forceps, containers, brushes, plastic trays, glass containers and cotton cloth for rearing was sterilized frequently with absolute alcohol or decont 0.1 % (commercially available cleaning agent). The stock culture was maintained regularly at the Biocontrol laboratory of SVPU&T Meerut for bioassay of different concentration of *M. anisopliae*. The composition of the diet (Table 4) was mixed thoroughly with boiling distil water and cooled to room temperature before pouring to multiwall diet plates. The larvae of *H. armigera* were allowed to feed on chickpea leaves before two day of treatment so that the larvae may acclimatize with natural diet again.

Table 4: Composition of synthetic diet of *H. armigera*

INGREDIENTS		QUANTITY (GM OR ML)
CHICKPEA FLOUR	:	100GM
YEAST EXTRACT	:	30GM
WESSON'S SALT MIXTURE	:	5GM
METHYL PARA HYDROXYL BENZOATE	:	5GM
DEXTROSE	:	25GM
ASCORBIC ACID	:	10GM
AGAR - AGAR	:	12.75GM
SORBIC ACID	:	6GM

STREPTOMYCIN SULPHATE	:	100MG
CHOLINE CHLORIDE (5%)	:	10 ML
FORMALDEHYDE	:	2 ML
MULTIVITAMIN CAPSULES (250 MG)	:	03
PROTEIN-X	:	2GM
AUTOCLAVED DOUBLE DISTILLED WATER	:	780 ML

3.8.2 Fungi culture

The most virulent isolate of entomopathogenic fungi, *M. anisopliae* strain SVPUAT 1 was obtained and grown on Peptone media (10g Peptone, 40g Dextrose, 2g Yeast extract, 15g Agar and 500 ml. Chloramphenicol and completed to one litre by distilled water). The media was autoclaved at 121^0C for 20 minutes, and poured in Petri-dishes (10 cm diameter x 1.5 cm) then inoculated with the green muscardine fungi and kept at 25 ± 2^0C and 85% ± 5 R.H. The fungal isolates was re-cultured every 14-30 days and kept at 4^0C.

3.8.3 Preparation of suspension of pathogen

The preparation of suspensions for the needs of the experiments, the fungi were cultured on 9 cm SDA petri dishes, secured with parafilm for protection against contamination and allowed to grow in the incubator for 15 days at 25^0C. The Conidia were collected from SDA petri dishes of the cultures after 15 days. The suspensions were prepared by scraping conidia from the surface of the petri dishes using a sterile metal hook. The conidia were transferred to 250 ml conical flask containing 100 ml of sterile distil water and 0.05% Tween 80. The conidial suspension was filtered through several layers of sterile muslin cloth before it was homogenized for 5 min using a magnetic stirrer. Finally, a Neubauer haemocytometer was used in an optical microscope (400x) to

determine the desired doses (2×10^3, 2×10^4, 2×10^5, 2×10^6, 2×10^7, 2×10^8, 2×10^9 and 2×10^{10}).

3.8.4 Bioassay procedure

The assessment of different concentrations (Table 5) of *M. anisopliae* was conducted by dip method. The experiment was conducted with randomized complete block design under *in vitro* conditions. In this method different larval (2^{nd}, 3^{rd} and 4^{th}) instars of *H. armigera* were used to assess the efficacy of *M. anisopliae*. A total of 15 larvae were dipped for 10 second in prepared concentration first and released in fresh chickpea food including tender leaves, shoots and pods. The extra moisture of treated larvae was soaked on sterilized tissue paper. The experiments were conducted in the plastic transparent cage with randomized complete design with three replications. Moreover, the method was repeated twice to further confirm the results.

3.8.5 Observation to be recorded

After application of different concentration of entomopathogenic fungi observations were recorded on daily basis for mortality of *H. armigera* till 120 hrs. The per cent of mortalities were calculated by Abbot's formula as follow:

$$\frac{\text{Survival percent in control} - \text{Survival percent in treatments}}{\text{Survival percent in control}} \times 100$$

Table 5: Different concentration of *M. anisopliae* for bioassay and field application

TREATMENTS	CONCENTRAIONS (CFU/ML)	DOSES
T_1	2×10^3 SPORES/ML + 0.5 % JAGGERY	5ML/LITER
T_2	2×10^4 SPORES/ML + 0.5 % JAGGERY	5ML/LITER
T_3	2×10^5 SPORES/ML + 0.5 % JAGGERY	5ML/LITER
T_4	2×10^6 SPORES/ML + 0.5 % JAGGERY	5ML/LITER
T_5	2×10^7 SPORES/ML + 0.5 % JAGGERY	5ML/LITER
T_6	2×10^8 SPORES/ML + 0.5 % JAGGERY	5ML/LITER
T_7	2×10^9 SPORES/ML + 0.5 % JAGGERY	5ML/LITER
T_8	2×10^{10} SPORES/ML + 0.5 % JAGGERY	5ML/LITER
T_9	CONTROL	-

3.8.6 Experimental detail

Replication : 3

Treatments : 9

Design : CRD

3.9 To study the bioefficacy of *Metarhizium anisopliae* against *Helicoverpa arimgera* on chickpea crop

The field evaluation of *M. anisopliae* to control *H. armigera* infestation on gram, *Cicer arietinum* (L.) was conducted in randomized block design with three replications during the *Rabi* season, 2020-21 & 2021-22 at Crop Research Centre (CRC) of Sardar Vallabhbhai Patel University of Agriculture and Technology Meerut (UP).

3.9.1 Methodology

The spore of most virulent isolate of *M. anisopliae* strain (SVPUAT 1) was obtained from biocontrol laboratory, SVPUA&T, Meerut and cultured in laboratory by adopting recommended procedure (3.8.2). Fungal concentrations were prepared by above recommended procedure (3.8.3). The above concentrations with 0.5 per cent jaggary was sprays with the help of knapsack sprayer at ETL stage (one larva or two eggs per plant) of *H. armigera* in chickpea.

3.9.2 Observation to be recorded

3.9.2.1 Assessment of larval population

- Ten plants per plot was randomly selected and tagged for recording observations.
- The pre count and post count were recorded a day before treatment and 3, 7, & 14 days after the application of the treatment.
- Efficacy of fungi at different concentration was calculated on the basis of larval reduction per cent per ten plant after the treatment.
- The data on the larval reduction per cent was subjected to suitable transformation and then statistical analysis.

$$\text{Larval reduction per cent} = \frac{X_1 - X_2}{X_1} \times 100$$

Where,

X_1 = Larval population in untreated plot

X_2 = Larval population in treated plot

3.9.2.2 Assessment of pod damage

Pod damage was taken at the time of harvesting, total number of pods and number of damage pods taken and per cent pod damaged was worked out by using following formula

$$\text{Per cent pod damage} = \frac{\text{Number of affected pods/plant}}{\text{Total number of pods/plant}} \times 100$$

3.9.2.3 Incremental cost benefit ratio

A. Yield

The yield obtained in individual treatment of chickpea crop was recorded separately for assessing the efficacy of different treatments. Data of yield kg/plot was converted into q ha^{-1} with following formula

$$\text{Grain yield (q/ha)} = \frac{\text{Grain yield (Kg/plot)} \times 100000(m^2)}{\text{Plot size } (m^2) \times 100}$$

Increase in yield over control was worked out by deduction the yield recorded in control plot rom the yield of the respective treated plot. The monetary value of increase yield was computed in rupees using minimum support price of chickpea. A comparison of cost involved in different treatments was also calculated on the basis of maximum retail price printed on the pack taking account of the smallest pack size as reference. Net return for each treatment was calculated by deducting the cost of treatment from the monetary value of increased yield.

Incremental cost benefit ratio, net return per rupees invested, were calculated by using the following formula

$$\text{Incremental cost benefit ratio} = \frac{\text{Net return (Rs/ha)}}{\text{Cost of treatment (Rs/ha)}}$$

The incremental cost benefit ratio for all the treatments was worked out by considering the prevailing price of inputs like concentration of fungi, labour charge, rent of sprayer and market rate of gram etc.

3.9.3 Experimental Layout for bioefficacy of *Metarhizium anisopliae*

Design	:	Randomized Block Design (RBD)
Variety	:	KVA 202
Treatments	:	9
Replications	:	3
Plot Size	:	4.0 X 3.0 m^2
Row to row distance	:	30 cm
Plant to plant distance	:	10 cm
Date of sowing	:	11 Oct. and 4 Oct.

3.9 Statistical analysis

All the data were analysed statistically by **"SPSS version 24.0 2019"** to evaluate with suitable transformation of the different treatments and the mean values were compared at significant level of *P>0.05*. The lethal time at 50 and 90 (LT_{50} and LT_{90}) per cent mortality of *H. armigera* larvae was subjected to probit analysis.

Table 6: Mean weekly weather parameters for the period of experiment October 2020 to first week of April 2021

S.W.	DATE	TEMPERATURE (^{0}C)		RELATIVE HUMIDITY %		RAINFALL (MM)
		MAX.	MIN.	MOR.	EVE.	
43	19 OCT. - 25 OCT.	33.00	16.10	85.40	45.10	0.00
44	26 OCT. - 01 NOV	31.00	13.40	81.60	43.70	0.00
45	02 NOV. - 08 NOV	28.40	11.30	83.40	45.30	0.00
46	9 NOV. - 15 NOV	27.60	9.90	84.30	44.40	1.30
47	16 NOV. - 22 NOV.	25.00	9.50	82.40	54.70	1.40
48	23 NOV. - 29 NOV.	25.80	8.40	83.90	45.70	0.00
49	30 NOV. - 06 DEC	26.30	7.90	85.00	45.00	0.00
50	07 DEC. - 13 DEC	22.90	6.40	85.60	49.90	5.90
51	14 DEC. - 20 DEC	20.30	6.00	87.40	51.30	0.00
52	21 DEC. - 27 DEC	18.70	4.90	92.10	54.40	0.20
1	28 DEC. - 03 JAN.	19.00	6.20	94.10	66.10	24.00
2	04 JAN. - 10 JAN.	18.80	5.70	94.90	63.60	0.00
3	11 JAN. - 17 JAN.	18.10	7.20	93.30	62.30	0.00
4	18 JAN. - 24 JAN.	18.80	6.50	90.00	57.30	0.00
5	25 JAN. - 31 JAN.	21.60	7.10	85.70	56.00	1.10
6	01 FEB. - 07 FEB.	23.90	7.70	86.00	55.30	5.60
7	08 FEB. - 14 FEB.	26.60	9.80	84.30	43.00	0.00
8	15 FEB. - 21 FEB.	29.40	12.00	83.30	40.40	0.00
9	22 FEB. - 28 FEB.	30.50	14.10	76.60	41.30	0.20
10	01 MAR. - 07 MAR.	32.30	14.50	72.60	35.40	0.00
11	08 MAR. - 14 MAR.	31.70	15.70	71.00	32.90	0.10
12	15 MAR. - 21 MAR.	33.00	16.60	75.60	38.00	0.00
13	22 MAR. - 28 MAR.	34.00	16.90	71.90	35.10	0.00
14	29 MAR. - 04 APR.	35.10	17.80	48.60	26.60	0.00

Table 7: Mean weekly weather parameters for the period of experiment October 2021 to first week of April 2022

S.W.	DATE	TEMPERATURE (^{0}C)		RELATIVE HUMIDITY %		RAINFALL (MM)
		MAX.	MIN.	MOR.	EVE.	
43	25 OCT. - 31 OCT.	30.94	18.00	69.86	49.57	12.60
44	01 NOV. 07 NOV.	30.06	14.86	72.00	50.14	0.00
45	08 NOV. - 14 NOV	28.73	12.86	76.86	48.00	0.00
46	15 NOV. - 21 NOV.	29.01	13.86	76.43	49.71	0.00
47	22 NOV. - 28 NOV.	28.11	11.00	80.57	46.86	0.00
48	29 NOV. - 05 DEC	26.76	9.71	81.57	46.29	0.00
49	06 DEC. - 12 DEC	23.07	11.64	84.29	48.14	0.90
50	13 DEC. - 19 DEC	22.40	9.36	83.43	39.43	0.00
51	20 DEC. - 26 DEC	20.74	7.17	82.43	38.43	0.00
52	27 DEC. - 02 JAN.	20.00	6.49	88.63	49.50	2.50
1	03 JAN. - 09 JAN.	20.60	7.50	84.60	61.10	9.90
2	10 JAN. - 16 JAN.	17.70	5.30	91.90	80.60	67.50
3	17 JAN. - 23 JAN.	16.20	4.70	92.60	71.10	3.70
4	24 JAN. - 30 JAN.	16.60	5.30	91.60	67.90	33.90
5	31 JAN. - 06 FEB.	20.10	6.00	88.60	67.00	18.40
6	07 FEB. - 13 FEB.	20.50	7.30	85.90	64.10	4.50
7	14 FEB. - 20 FEB.	24.30	8.30	82.60	57.40	0.00
8	21 FEB. - 27 FEB.	25.90	9.90	82.70	50.30	0.70
9	28 FEB. - 06 MAR.	26.00	10.50	88.60	53.10	31.50
10	07 MAR. - 13 MAR.	30.30	13.40	76.00	43.00	0.00
11	14 MAR. - 20 MAR.	34.20	17.10	71.10	39.60	0.00
12	21 MAR. - 27 MAR.	37.50	20.10	67.40	34.30	0.00
13	28 MAR. - 03 APR.	38.70	20.30	58.90	28.40	0.00
14	04 APR. - 10 APR.	40.10	22.80	52.60	23.20	0.00

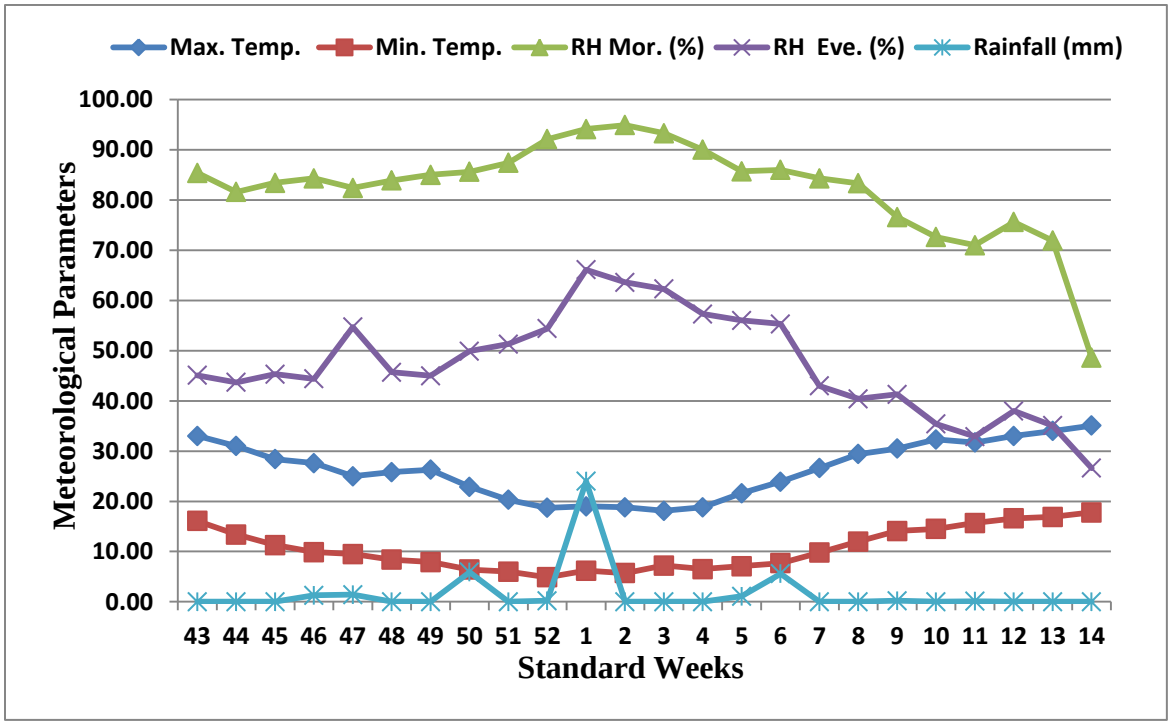


Fig. 1: Standard Weekly meteorological data during crop period of 2020-2021

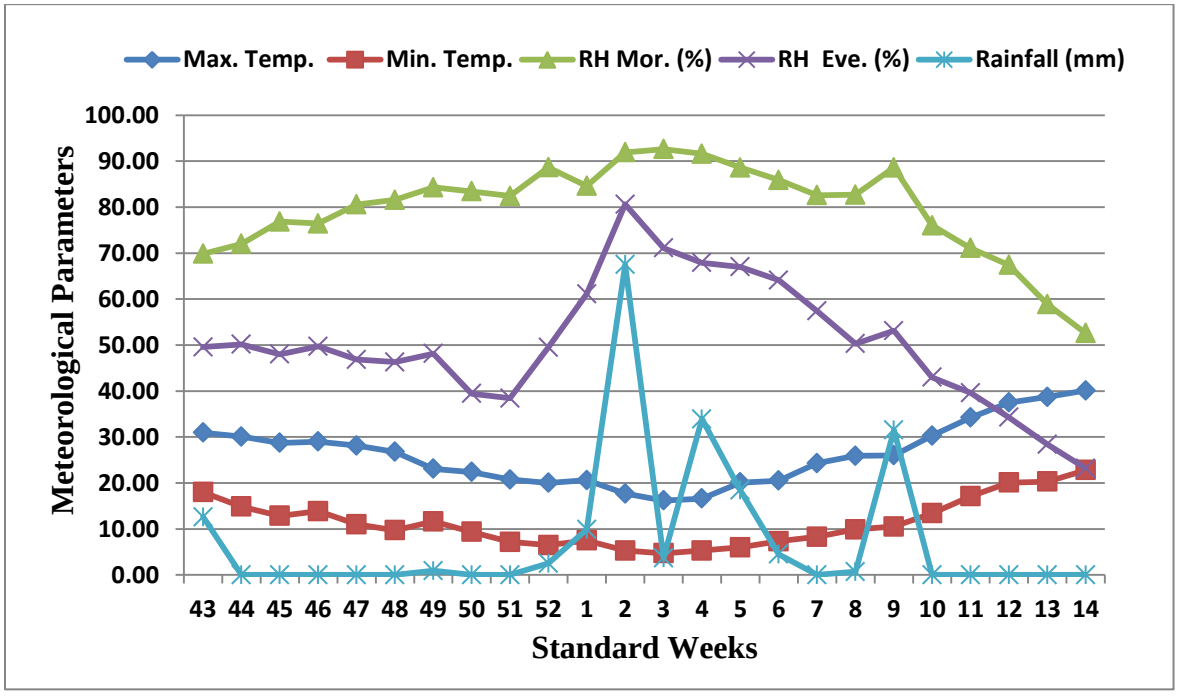
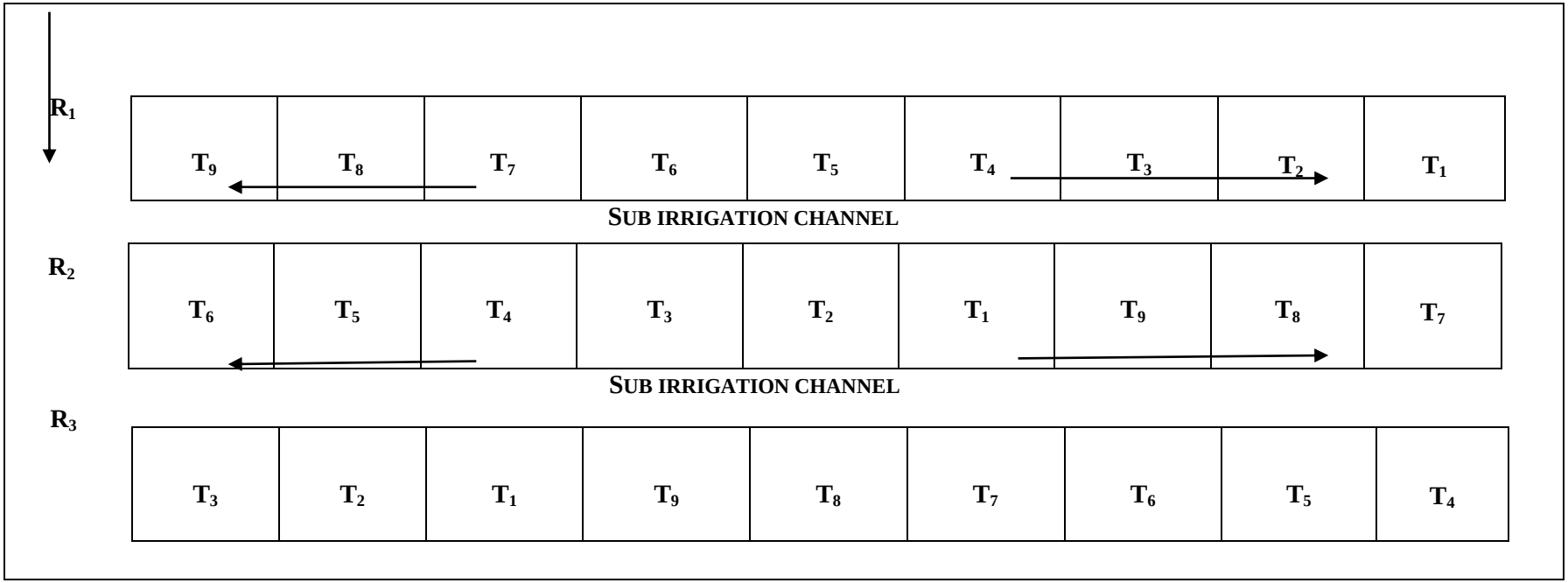


Fig. 2: Standard Weekly meteorological data during crop period of 2021-2022

Main – irrigation channel



Detail of Experiment

Design	:	RBD	Row to row distance	: 30 cm	
Treatment	:	9	Plant to plant distance	: 10 cm	
Replication	:	3	Variety	: KVA 202	
Plot Size	:	4×3 m^2			

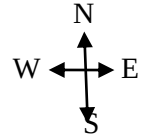


Fig. 2: Layout plan of experiment conducted during *Rabi*, 2020-2021 and 2021-2022.

CHAPTER- 4 RESULTS AND DISCUSSION

The present investigation entitled "Studies on mass production of entomopathogenic fungi *Metarhizium anisopliae* (Metschnikoff) using different media and potential against chickpea pod borer, *Helicoverpa armigera* (Hubner)" was carried out in lab and field condition during 2020-2021 and 2021-2022. The result obtained from various studies are presented under different headings and discussed interpreted with available literature.

4.1 To study the isolation, characterization and selection of most virulent strain of *Metarhizium anisopliae*

4.1.1 Survey and isolation of entomopathogenic fungi *M. anisopliae*

Isolation of the native isolates of entomopathogenic fungi *M. anisopliae* was carried out. Among the 207 soil samples examined from four districts viz. Meerut, Muzaffarnagar, Saharanpur and shamali in western plain zone of Uttar Pradesh, revealed *M. anisopliae* isolated from districts Meerut, Muzaffarnagar, Saharanpur and shamali which is presented in Table 8. The details of places, crops periods of visits mycopathogens and disease incidence of *M. anisopliae* are also presented in Table 8 and Table 10 respectively. The fungus *M. anisopliae* was isolated by insect baiting methodology **(Zimmermann, 1986)**. The isolated fungus cultures were purified by single spore isolation method by making serial dilution spores suspension on Sabouraud Dextrose Agar (SDA) plates and subsequently sub-culturing was done **(Sambrook *et al.*, 1989)**. The microscopic examinations of different native isolates were done for

Table 8: Details of the soil samples collected for isolation of *M. anisopliae* in Western Uttar Pradesh

LOCATIONS	GEOGRAPHICAL LOCATION (LAT. N, LONG. E)	NO. OF SOIL SAMPLES COLLECTED	STANDING CROPS	SOIL TYPE	POSITIVE/ NEGATIVE SAMPLE
MEERUT					
SVP ORCHARD	N. LAT. 28^0 57´ E. LONG.77^0 40´	15	MANGO, GUAVA, POMEGRANATE, LITCHI	SANDY	-
DAURALA		15	MANGO	LOAMY	-
MAVANA		10	MANGO, GUAVA	SANDY LOAM	-
LAWAR		09	MANGO, CITRUS	SANDY LOAM	-
MAUHMADPUR		12	MANGO	LOAMY	+
MUZAFFARNAGAR					
JANSATH	N. LAT. 29^0 97´ E. LONG. 77^0 55´	09	CUCURBITACEOUS CROPS	LOAMY	+
KHATAULI		14	MANGO, PAPAYA	LOAMY	-
SHADPUR		10	MANGO	SANDY LOAM	-
KHANPUR		16	MANGO, PAPAYA, LITCHI	SANDY LOAM	+
JAWAN		05	MANGO, GUAVA	SANDY LOAM	-
SAHARNPUR					
UMARIKALA	N. LAT. 29^0 45´ E. LONG. 77^0 55´	12	MANGO	SANDY LOAM	-
BADGAOV		14	MANGO, POPULAR	SANDY	+
BIDVI		10	MANGO, CITRUS	LOAMY	-
JHABHIRUN		13	MANGO, POPULAR	LOAMY	-
SHAMALI	N. LAT. 29^0 45´ E. LONG. 77^0 32´				
JASALA		10	GUAVA, MANGO	SANDY LOAM	-
GAGERU		20	MANGO, CHILLY	LOAMY	+
KANDHALA		13	MANGO, PAPAYA, POPULAR	LOAMY	-
TOTAL		**207**			

identification of the fungus. The microscopic photograph of the slide of conidia is given in plate 4.

4.1.1.1 Microscopic structure and features of *M. anisopliae*

The radial growth of *M. anisopliae* was high when it grown on Sabouraud Dextrose Agar (SDA). The initial colonies on SDA have a white mycelial margin with clumps of conidiophores which become coloured with the development of spores, varying from olivaceous buff to yellow/green to olivaceous/dark herbage green. Phialides was 6.3 – 13.5 × 1.8 - 3.6 µm. Conidia usually 5-8 µm long by 1.5-3.5 unwire var. *anisopliae.*

4.1.1.2 Molecular characterization

RT-PCR assay using ITS primers mentioned in section 3.6.3.3 yielded an expected amplicon of~650 bp (Fig 4) from five samples and samples were collected from different region of Meerut (Western U.P.). The sequences obtained were deposited to NCBI Gene Bank with accession numbers ON183248. Pairwise sequence identity comparison of the *M. anisopliae* (Table 9) sequence of TuMMoV with similar sequences shared 95.80 – 99.40 per cent nt with the global isolates (Fig 3).

To better understand the genetic variability of the *M. anisopliae* isolates, a phylogenetic tree was constructed based on the ITS region differences of *M. anisopliae* from different geographical locations. In the phylogenetic tree, the Meerut isolate of this study cluster with *M. anisopliae* reported from globally (Fig 3).

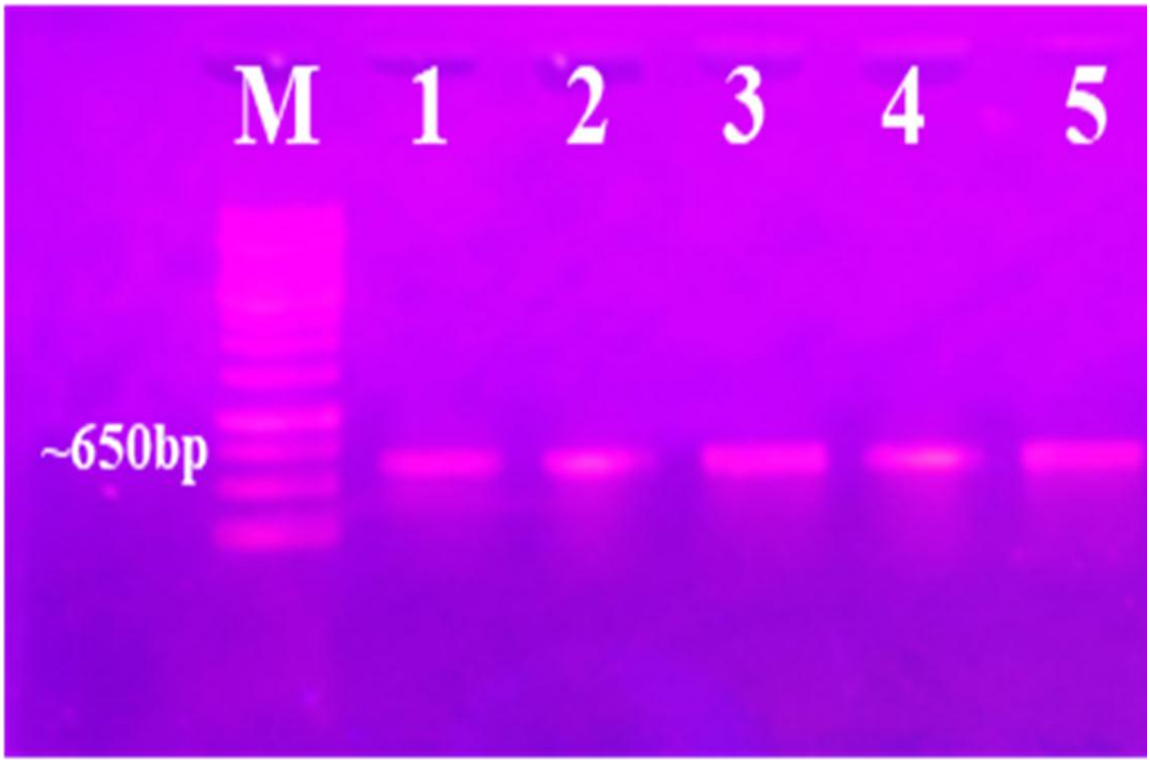


Fig. 4: PCR detection of ITS sequences of *Metarhizium anisopliae*. Expected amplicon size was ~650 bp. Lane M, 1 kb DNA ladder (Cat. No. SM0311; Thermo Scientific); Lanes 2, 3, 4 and 5.

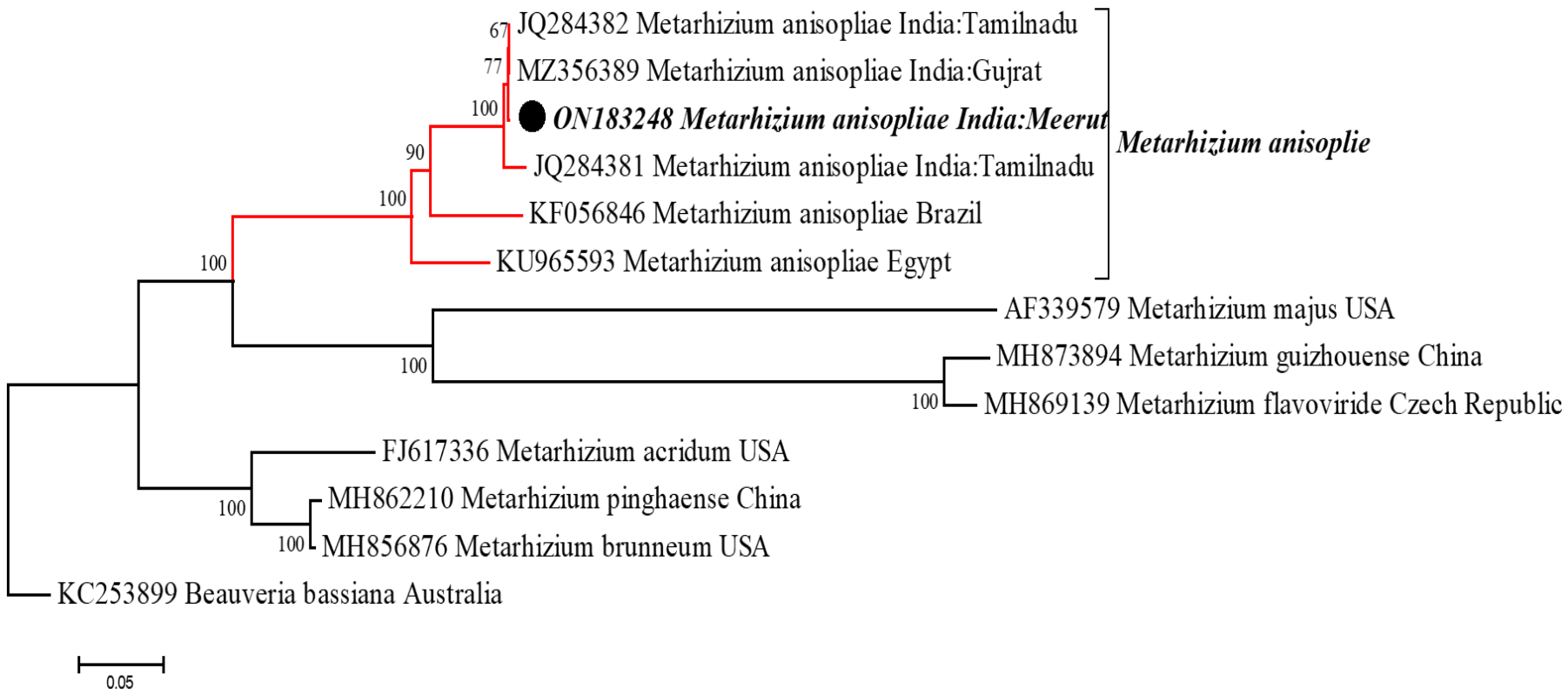


Fig. 3: Phylogenetic tree comparing the ITS sequences of *Metarhizium anisopliae* isolate (ON183248) of study (shown in circle and bold) with other species of *Metarhizium* and a *Beauveria bassiana* (KC253899) outgroup.

Seq-> nts	ON183248 Metarhizium anisopliae India:Meerut	JQ284382 Metarhizium anisopliae India:Tamilnadu	MZ356389 Metarhizium anisopliae India:Gujrat	JQ284381 Metarhizium anisopliae India:Tamilnadu	KF056846 Metarhizium anisopliae Brazil	KU965593 Metarhizium anisopliae Egypt	MH862210 Metarhizium pinghaense China	MH873894 Metarhizium guizhouense China	MH869139 Metarhizium flavoviride Czech Republic	MH856876 Metarhizium brunneum USA	AF339579 Metarhizium majus USA	FJ617336 Metarhizium acridum USA
ON183248 *Metarhizium anisopliae* India:Meerut	ID											
JQ284382 *Metarhizium anisopliae* India:Tamilnadu	**99.8**	ID										
MZ356389 *Metarhizium anisopliae* India:Gujrat	**99.8**	100	ID									
JQ284381 *Metarhizium anisopliae* India:Tamilnadu	**93.0**	93.2	93.2	ID								
KF056846 *Metarhizium anisopliae* Brazil	**84.7**	84.5	84.5	78.9	ID							
KU965593 *Metarhizium anisopliae* Egypt	**86.3**	86.5	86.5	81.0	84.1	ID						
MH862210 *Metarhizium pinghaense* China	**57.7**	57.7	57.7	56.4	54.2	58.4	ID					
MH873894 *Metarhizium guizhouense* China	**63.7**	33.9	33.9	31.3	33.3	33.6	32.9	ID				
MH869139 *Metarhizium flavoviride* Czech Republic	**64.9**	35.1	35.1	32.5	34.0	34.3	33.2	95.2	ID			
MH856876 *Metarhizium brunneum* USA	**59.0**	60.0	60.0	55.2	56..3	60.5	96.2	34.4	34.7	ID		
AF339579 *Metarhizium majus* USA	**62.7**	32.9	32.9	31.4	31.8	33.6	28.3	31.7	30.5	29.2	ID	
FJ617336 *Metarhizium acridum* USA	**69.9**	49.9	49.9	52.8	50.9	50.7	75.9	28.4	28.8	73.7	26.3	ID

Table 9: Comparisons of nucleotide sequence (nts) identity of pairwise combinations of the ITS sequences of *Metarhizium anisopliae* isolate (ON183248) of study with other species of *Metarhizium*.

4.1.1.3 Selection of most virulent isolates of *M. anisopliea* against *H. armigera*

The isolated five local strains of *M. anisoplisea* were examined to test the pathogenicity against second instar larva of *H. armigera* (Table 10 and Fig 5). The pathogenicity of local fungus of *M. anisopliae* isolate SVPUAT 1 Accession no. ON183248 was highest (100 per cent) after seven days of treatment followed by isolate SVPUAT 5 (71.11 per cent), SVPUAT 4 (57.77 per cent) and SVPUAT 2 (44.44 per cent). The minimum mortality per cent recorded in isolate SVPUAT 3 (31.11 per cent). The lowest LT_{50} and LT_{90} value was also recorded in isolate SVPUAT 1 with 3.16 and 5.16 days respectively.

4.2 Mass production of *M. anisopliae* on different substrates

In the present study, several naturally available substrates of both solid and liquid media were tested for mass multiplication of *M. anisopliae*. The success of microbial control of insect pests depends not only on the isolation, characterization and pathogenicity, but also on the successful mass production of the microbial agents in the laboratory. Large-scale availability of the pathogen is a primary requirement in the bio-control programme. For as successful integrated pest management programme, the agents like the entomopathogenic fungi should be amenable to easy and cheap mass production. However, the production of local isolate of *M. anisopliae* in suitable media has not yet been studied, thus the present study was carried out under Bio-control laboratory of SVPUAT, Meerut. The finding of present study is delineated below:

4.2.1 Solid and liquid media

4.2.1.1 Tenth day after inoculation

Table 10: Mortality of *Helicoverpa armigera* after exposure to indigenous *Metarhizium anisopliae* isolates

ISOLATES	% MORTALITY (MEAN ± SE)	LT_{50} (95 % CI)	SLOPE (±SE)	LT_{90} (95 % CI)	SLOPE (±SE)
SVPUAT 1	100.0 ± 0.00A	3.16	5.97 ± 1.07	5.16	5.97 ± 1.11
SVPUAT 2	44.44 ± 3.84E	6.00	2.70 ± 1.20	17.83	2.70 ± 1.60
SVPUAT 3	31.11 ± 3.85D	9.50	2.09 ± 1.48	39.16	2.09 ± 2.49
SVPUAT 4	57.77 ± 7.69C	4.66	3.05 ± 1.13	12.25	3.05 ± 1.38
SVPUAT 5	71.11 ± 3.85B	4.16	3.78 ± 1.10	9.15	3.78 ± 1.24

The mortality of *H. armigera* at six days post-treatment. Each point is the mean of three replicates. Value in the same column followed by different superscripts are highly significantly different ($p < 0.05$) Tukey's HSD test. The LT_{50} and LT_{90} (in days) with 95 % confidence intervals (CI) and the slope are also indicated.

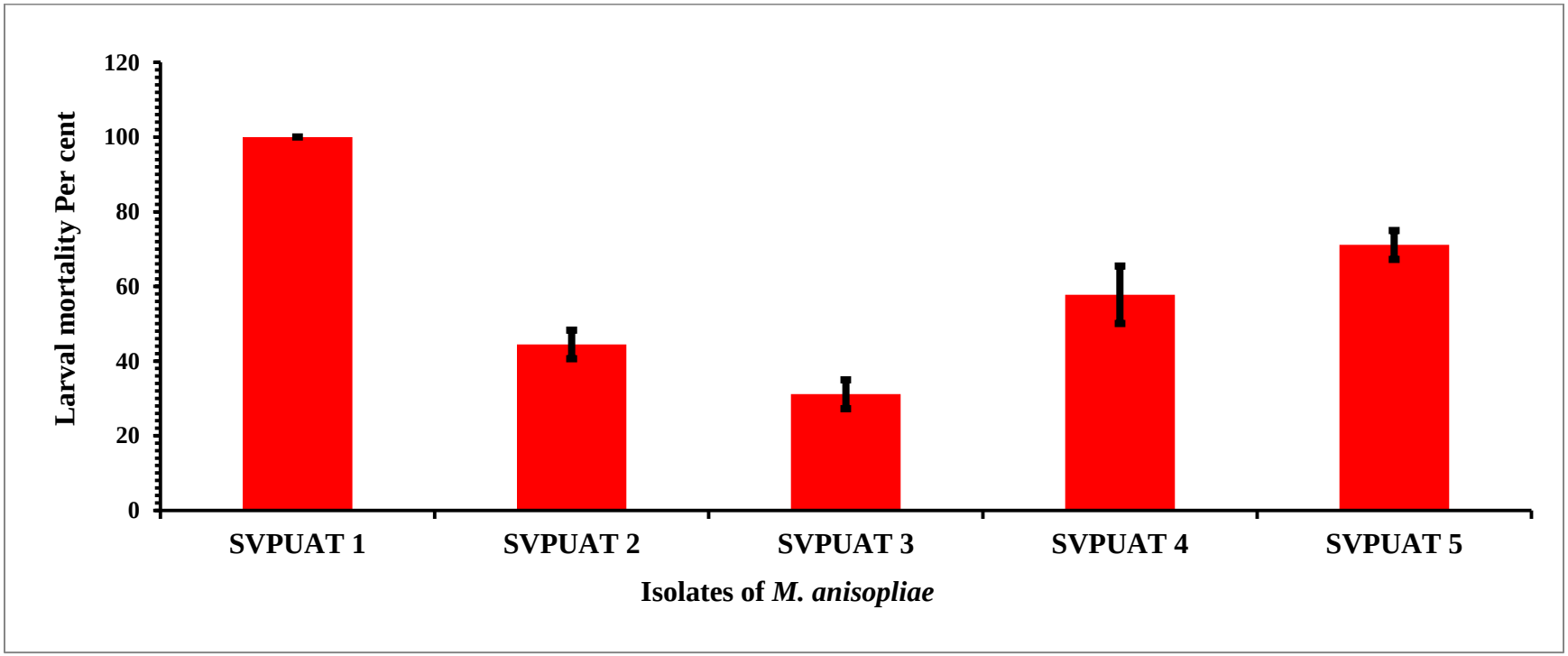


Fig. 5: Mortality of *Helicoverpa armigera* after exposure to indigenous *Metarhizium anisopliae* isolates.

4.2.1.1.1 Cereals

The result indicated in Table 11 and Fig 6 showed that the highest sporulation was recorded in Rice + 1 % Yeast + 1 gm Dextrose (60 × 10^7 spores/gm). That was significantly higher among all the cereal grains media. The next best grain was Bajra + 1 % Yeast + 1 gm Dextrose (48 × 10^7 spores/gm) followed by Sorghum + 1 % Yeast + 1 gm Dextrose (45.33 × 10^7 spores/gm) and Wheat+ 1 % Yeast + 1 gm Dextrose (34.66 × 10^7 spores/gm) respectively. The lowest sporulation was recorded in Maize + 1 % Yeast + 1 gm Dextrose (24.66 × 10^7 spores/gm).

4.2.1.1.2 Pulses

Among all tested pulses grains, the maximum spore production of *M. anisopliae* was recorded on Cow pea + 1 % Yeast + 1 gm Dextrose (53.33 × 10^7 spores/gm) followed by Green gram + 1 % Yeast + 1 gm Dextrose (50.33 × 10^7 spores/gm), Gram + 1 % Yeast + 1 gm Dextrose (49.33 × 10^7 spores/gm), Pigeonpea + 1 % Yeast + 1 gm Dextrose (45.33 × 10^7 spores/gm). The least spore production was observed in Black gram + 1 % Yeast + 1 gm Dextrose (40.00 × 10^7 spores/gm).

4.2.1.1.3 Dung concoction and industrial wastes

Among the dung concoction and industrial wastes, Corcyra rearing wastes + 1 % Yeast + 1 gm Dextrose produced significantly higher spore production (18.30 × 10^7 spores/gm) followed by FYM liquid + 1 % Yeast + 1 gm Dextrose (14.80 × 10^7 spores/ml), FYM + 1 % Yeast + 1 gm Dextrose (4.60 × 10^7 spores/gm) and Vermicompost + 1 % Yeast + 1 gm Dextrose (2.60 × 10^7 spores/gm). There was no conidial production in Press mud + 1 % Yeast + 1 gm Dextrose (0.00 × 10^7 spores/gm).

4.2.1.1.4 Synthetic media

The sabouraud's dextrose broth produced maximum spore concentration (58.20 × 10^7 spores/ml) and the potato dextrose broth were on par to SDB with 56.40 × 10^7 spores/ml.

At tenth day after inoculation the highest sporulation among all the substrates was recorded in Rice + 1 % Yeast + 1 gm Dextrose (60 × 10^7 spores/gm) followed by sabouraud's dextrose broth (58.20 × 10^7 spores/ml). The substrate sobouraud's dextrose broth was on par to rice. The potato dextrose broth (56.40 × 10^7 spores/ml) was also on par to Sabouraud's dextrose broth. The cereals grain Bajra + 1 % Yeast + 1 gm Dextrose (48 × 10^7 spores/gm) showed non-significant difference when compared to Gram + 1 % Yeast + 1 gm Dextrose (49.33 × 10^7 spores/gm). No conidial count observed in Press mud + 1 % Yeast + 1 gm Dextrose. Whereas, the minimum spores count was recorded in Vermicompost + 1 % Yeast + 1 gm Dextrose (2.60 × 10^7 spores/gm).

4.2.1.2 Twenty day after inoculation

4.2.1.2.1 Cereals

The table 5 revealed that among the evaluated different cereals grain as substrates, Rice + 1 % Yeast + 1 gm Dextrose (92.75 × 10^7 spores/gm) yielded highest conidial count followed by Bajra + 1 % Yeast + 1 gm Dextrose (71.25 × 10^7 spores/gm) and Sorghum + 1 % Yeast + 1 gm Dextrose (48.00 × 10^7 spores/gm). The grains of Maize + 1 % Yeast + 1 gm Dextrose (40.00 10^7 spores/gm) was on par to Wheat+ 1 % Yeast + 1 gm Dextrose (40.66 10^7 spores/gm).

4.2.1.2.2 Pulses

Among the pulses highest conidial count recorded in substrate Cow pea + 1 % Yeast + 1 gm Dextrose (72.00 × 10^7 spores/gm) which was on par with Green gram + 1 % Yeast + 1 gm Dextrose (70.66 × 10^7 spores/gm). The next best substrate was Gram + 1 % Yeast + 1 gm Dextrose (67.33 × 10^7 spores/gm) followed by Pigeonpea + 1 % Yeast + 1 gm Dextrose (55.33 × 10^7 spores/gm). The least conidial count among pulses grain was recorded in Black gram + 1 % Yeast + 1 gm Dextrose (52.66 × 10^7 spores/gm).

4.2.1.2.3 Dung concoction and industrial wastes

At twentieth day after inoculation the highest spores was recorded in Corcyra rearing wastes + 1 % Yeast + 1 gm Dextrose (26.84 × 10^7 spores/gm) and the next best substrate was FYM liquid + 1 % Yeast + 1 gm Dextrose (23.33 × 10^7 spores/gm) followed by FYM + 1 % Yeast + 1 gm Dextrose (8.90 × 10^7 spores/gm) and Vermicompost + 1 % Yeast + 1 gm Dextrose (3.00 × 10^7 spores/gm). The minimum conidial count was observed in Press mud + 1 % Yeast + 1 gm Dextrose (0.97 × 10^7 spores/gm).

4.2.1.2.4 Synthetic media

Both the synthetic media significantly differ from each other. The maximum conidial spores was produced by Sabauraud's dextrose agar (78.00 × 10^7 spores/ml) followed by Potato dextrose broth (74.33 × 10^7 spores/ml).

At twentieth day after inoculation Rice + 1 % Yeast + 1 gm Dextrose (92.75×10^7 spores/gm) yielded highest conidial count which was significantly superior over all the seventeen substrates. The second most appropriate substrate for mass production of *M. anisopliae* was Sabouraud's dextrose broth (78.00×10^7 spores/ml). However, the substrate Wheat+ 1 % Yeast + 1 gm Dextrose (40.66×10^7 spores/gm) was on par with substrate Maize + 1 % Yeast + 1 gm Dextrose(40.00×10^7 spores/gm). The least conidial count was recorded in Press mud + 1 % Yeast + 1 gm Dextrose ($0.97\ 10^7$ spores/gm).

4.2.1.3 Thirty day after inoculation

4.2.1.3.1 Cereals

The result in (Table 11 and Fig 6) at thirtieth day after inoculation all the cereals grains differed significantly from each other. The highest conidial count was recorded in Rice + 1 % Yeast + 1 gm Dextrose (94.00×10^7 spores/gm) followed by Bajra + 1 % Yeast + 1 gm Dextrose (74.50×10^7 spores/gm), Sorghum + 1 % Yeast + 1 gm Dextrose (68.00×10^7 spores/gm) and Maize + 1 % Yeast + 1 gm Dextrose (49.33×10^7 spores/gm). The minimum spore production was observed in Wheat+ 1 % Yeast + 1 gm Dextrose (46.66×10^7 spores/gm).

4.2.1.3.2 Pulses

Among the all pulses grains, the highest spore production were recorded in Cow pea + 1 % Yeast + 1 gm Dextrose (82.78×10^7 spores/gm) followed by Green gram + 1 % Yeast + 1 gm Dextrose (78.00×10^7 spores/gm). The substrate Pigeonpea + 1 % Yeast + 1 gm Dextrose (75.00×10^7 spores/gm) on par with Gram + 1 % Yeast + 1 gm

Dextrose (74.33 × 10^7 spores/gm).The least conidial concentration was observed in Black gram + 1 % Yeast + 1 gm Dextrose (56.00 × 10^7 spores/gm).

4.2.1.3.3 Dung concoction and industrial wastes

The same sporulation pattern was noticed in all the substrates as twenty day after inoculation. All the substrates were significantly different to each other. The best industrial wastes for the sporulation of *M. anisopliae* were Corcyra rearing wastes + 1 % Yeast + 1 gm Dextrose (48.52 × 10^7 spores/gm) and the second best one was FYM liquid + 1 % Yeast + 1 gm Dextrose (41.00 × 10^7 spores/ml) followed by FYM + 1 % Yeast + 1 gm Dextrose (11.33 × 10^7 spores/gm) and Vermicompost + 1 % Yeast + 1 gm Dextrose (3.70 × 10^7 spores/gm). The substrate Press mud + 1 % Yeast + 1 gm Dextrose (1.00 × 10^7 spores/gm) showed minimum spore production.

4.2.1.3.4 Synthetic media

Similar growth pattern was observed with highest conidial count in Sabouraud's Dextrose Broth (89.33 × 10^7 spores/ml) and Potato Dextrose Broth (86.66 × 10^7 spores/ml) as twenty day after inoculation.

Among all the seventeen substrates, the most appropriate substrate for mass production of *M. anisopliae* fungi was Rice + 1 % Yeast + 1 gm Dextrose (94.00 × 10^7 spores/gm) followed by Sabouraud's Dextrose Broth (89.33 × 10^7 spores/ml). The cereal grain Maize + 1 % Yeast + 1 gm Dextrose (49.33 × 10^7 spores/gm) was on par with industrial waste substrate Corcyra rearing wastes + 1 % Yeast + 1 gm Dextrose (48.52 ×

10^7 spores/gm). However, the minimum spore production found in Press mud + 1 % Yeast + 1 gm Dextrose (1.00×10^7 spores/gm).

4.2.2 Overall Mean

4.2.2.1 Cereals

The statistically analyzed data shown in table 11 and depicted in fig 6.The highest overall mean of spore was recorded in Rice + 1 % Yeast + 1 gm Dextrose (82.25×10^7 spores/gm) followed by Bajra + 1 % Yeast + 1 gm Dextrose (64.58×10^7 spores/gm), Sorghum + 1 % Yeast + 1 gm Dextrose (53.77×10^7 spores/gm) and Wheat+ 1 % Yeast + 1 gm Dextrose (40.66×10^7 spores/gm). The least conidial count was produced by Maize + 1 % Yeast + 1 gm Dextrose (37.99×10^7 spores/gm).

4.2.2.2 Pulses

On the basis of overall mean value of spores the substrate Cow pea + 1 % Yeast + 1 gm Dextrose (69.37×10^7 spores/gm) was found to be the best one among all the pulses. The second best substrate was Green gram + 1 % Yeast + 1 gm Dextrose (66.33×10^7 spores/gm) followed by Gram + 1 % Yeast + 1 gm Dextrose (63.66×10^7 spores/gm) and Pigeonpea + 1 % Yeast + 1 gm Dextrose (58.55×10^7 spores/gm). The lowest mean spores were observed in Black gram + 1 % Yeast + 1 gm Dextrose (49.55×10^7 spores/gm).

4.2.2.3 Dung concoction and industrial wastes

The highest overall mean of spores was recorded in Corcyra rearing wastes + 1 % Yeast + 1 gm Dextrose (31.22×10^7 spores/gm).The next best treatment was FYM liquid + 1 % Yeast + 1 gm Dextrose (26.37×10^7 spores/ml) followed by FYM + 1 % Yeast + 1

Table 11: Mass production of *M. anisopliae* on different substrates

Tr. Code	Substrates	Spores count (1×10^7 spores/ml or gm) at different DAI			Overall mean
		10 DAI	20 DAI	30 DAI	
	Cereals (Whole grains)				
T_1	Rice + 1 % Yeast + 1 gm. Dextrose	60.00 ±1.80J	92.75 ±2.8A	94.00 ±2.82A	82.25 ± 16.83A
T_2	Wheat+ 1 % Yeast + 1 gm. Dextrose	34.66 ± 1.03T	40.66 ± 1.21S	46.66 ± 1.40QR	40.66 ± 5.30J
T_3	Bajra + 1 % Yeast + 1 gm. Dextrose	48.00 ± 1.44PQ	71.25 ± 2.13H	74.50 ± 2.23FG	64.58 ± 12.63F
T_4	Maize + 1 % Yeast + 1 gm. Dextrose	24.66 ± 0.73UV	40.00 ± 1.20S	49.33 ± 1.48P	37.99 ± 10.83K
T_5	Sorghum + 1 % Yeast + 1 gm. Dextrose	45.33 ± 1.35R	48.00 ± 1.44PQ	68.00 ± 2.04I	53.77 ± 10.82H
	Pulses (Whole grains)				
T_6	Cow pea + 1 % Yeast + 1 gm. Dextrose	53.33 ± 1.59 MN	72.00 ± 2.16GH	82.78 ± 2.48D	69.37 ± 13.03D
T_7	Gram + 1 % Yeast + 1 gm. Dextrose	49.33 ± 1.47P	67.33 ± 2.01I	74.33 ± 2.23FG	63.66 ± 11.29F
T_8	Pigeonpea + 1 % Yeast + 1 gm. Dextrose	45.33 ± 1.35R	55.33 ± 1.65LM	75.00 ± 2.25F	58.55 ± 13.16G
T_9	Black gram + 1 % Yeast + 1 gm. Dextrose	40.00 ± 1.20S	52.66 ± 1.57NO	56.00 ± 1.68KL	49.55 ± 7.42I
T_{10}	Green gram + 1 % Yeast + 1 gm. Dextrose	50.33 ± 1.50OP	70.66 ± 2.11H	78.00 ± 2.34E	66.33 ± 12.53E
	Dung concoction and industrial wastes				
T_{11}	Press mud + 1 % Yeast + 1 gm. Dextrose	00.00 ± 00.00B	0.97 ± 0.03AB	1.00 ± 0 .03AB	0.65 ± 0.49P
T_{12}	FYM + 1 % Yeast + 1 gm. Dextrose	4.60 ± 0.13Z	8.90 ± 0 .27Y	11.33 ± 0.34Y	8.27 ± 2.96N
T_{13}	Vermicompost + 1 % Yeast + 1 gm. Dextrose	2.60 ± 0.08ZA	3.00 ± 0.09ZA	3.70 ± 0.11Z	3.10 ± 0.48O
T_{14}	FYM liquid + 1 % Yeast + 1 gm. Dextrose	14.80 ± 0.44X	23.33 ± 0.69V	41.00 ± 1.23S	26.37 ± 11.59M
T_{15}	Corcyra rearing wastes + 1 % Yeast + 1 gm. Dextrose	18.30 ± 0.54W	26.84 ± 0.80U	48.52 ± 1.46PQ	31.22 ± 13.52L
	Synthetic media				
T_{16}	PDB (Potato Dextrose Broth)	56.40 ± 1.69KL	74.33 ± 2.22FG	86.66 ± 2.60C	72.46 ± 13.31C
T_{17}	SDB (Sabouraud's Dextrose Broth)	58.20 ± 1.74JK	78.00 ± 2.34E	89.33 ± 2.68B	75.17 ± 13.78B

Value presented as mean ± SD

For each column, different superscript (small alphabet) letter indicate significantly different at $p \leq 0.05$, as the measure by Tukey's test between treatments.

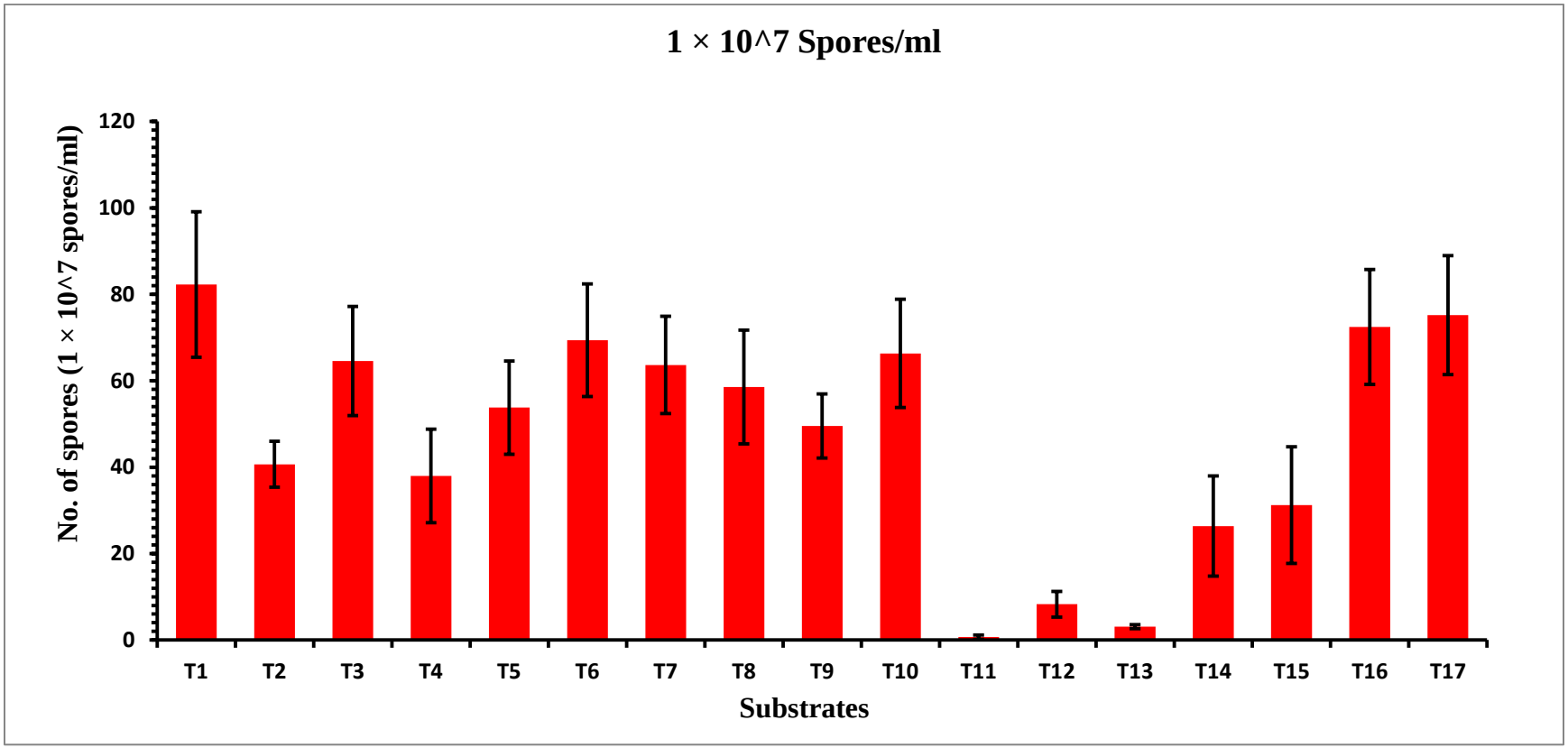


Fig. 6: Mass production of *M. anisopliae* on different substrates

gm Dextrose (8.27×10^7 spores/gm) and Vermicompost + 1 % Yeast + 1 gm Dextrose (3.10×10^7 spores/gm). Whereas, the minimum mean conidial count observed in Press mud + 1 % Yeast + 1 gm Dextrose (0.65×10^7 spores/gm).

4.2.2.4 Synthetic media

The Sabouraud's Dextrose Broth mean conidial count (75.71×10^7 spores/ml) was found significantly superior over Potato dextrose Broth (72.46×10^7 spores/ml).

Among all seventeen substrates, Rice + 1 % Yeast + 1 gm Dextrose (82.25×10^7 spores/gm) was significantly superior over all substrates. The second best substrate was Sabouraud's Dextrose Broth (75.71×10^7 spores/ml). Whereas, the minimum spore production was observed in Press mud + 1 % Yeast + 1 gm Dextrose (0.65×10^7 spores/gm).

4.2.2.3 Rate of increase in growth of *M. anisopliae* at different day after inoculation

2.2.3.1 Ten to twenty day after inoculation

The rate of spore per cent increase presented in table 12 and depicted in fig 7. The per cent increase was found in the range of 0.00 to 93.47 per cent. The highest rate of spore increase between ten to twenty days after inoculation was recorded in FYM + 1 % Yeast + 1 gm Dextrose (93.47 per cent). The substrate Cow pea + 1 % Yeast + 1 gm Dextrose (35.01 per cent) on par with substrate Gram + 1 % Yeast + 1 gm Dextrose (36.48 per cent)

4.2.3.2 Twenty to thirty day after inoculation

The rate at which there was increase in spores ranged between 1.35 to 80.77 per cent. The highest rate of increase was recorded in Corcyra rearing wastes + 1 % Yeast + 1

gm Dextrose (80.77 per cent). The second most rapidly growing substrate was FYM liquid + 1 % Yeast + 1 gm Dextrose (75.45 per cent). The substrates Potato dextrose broth (16.59 per cent), Wheat+ 1 % Yeast + 1 gm Dextrose (14.75 per cent) and Sabouraud's dextrose broth (14.52 per cent) was on par with each other. The per cent increase in sporulation of *M. anisopliae* in substrates Maize + 1 % Yeast + 1 gm Dextrose (23.32 per cent) and Vermicompost + 1 % Yeast + 1 gm Dextrose (23.33 per cent) were also at par with each other. The minimum rate of increase observed in substrate Rice + 1 % Yeast + 1 gm Dextrose (1.35 per cent).

4.2.3.3 Overall mean

On the basis of overall mean the highest rate of increase recorded in FYM liquid + 1 % Yeast + 1 gm Dextrose (66.68 per cent) followed by Corcyra rearing wastes + 1 % Yeast + 1 gm Dextrose (66.68 per cent). The substrates Pigeonpea + 1 % Yeast + 1 gm Dextrose (28.80 per cent) and Rice + 1 % Yeast + 1 gm Dextrose (27.96 per cent) were on par with each other. The substrates Green gram + 1 % Yeast + 1 gm Dextrose (25.39 per cent), Cow pea + 1 % Yeast + 1 gm Dextrose (24.99 per cent), Sabouraud's dextrose broth (24.27 per cent) and Sorghum + 1 % Yeast + 1 gm Dextrose (23.78 per cent) were on par. The lowest per cent increase was noticed in Press mud + 1 % Yeast + 1 gm Dextrose (1.54 per cent).

Table 12: Rate of increase in growth of *M. anisopliae* at different day after inoculation

TR. CODE	TREATMENTS	RATE OF INCREASE OF *M. ANISOPLIAE* AT DIFFERENT INTERVAL AFTER INOCULATION (PER CENT)		OVERALL MEAN
		10 TO 20 DAI	20 TO 30 DAI	
CEREALS (WHOLE GRAINS)				
T_1	RICE + 1 % YEAST + 1 GM. DEXTROSE	54.58 ± 1.63F	1.35 ± 0.04TU	27.96 ± 29.17E
T_2	WHEAT+ 1 % YEAST + 1 GM. DEXTROSE	17.31 ± 0.52N	14.75 ± 0.44OP	16.03 ± 1.46K
T_3	BAJRA + 1 % YEAST + 1 GM. DEXTROSE	48.43 ± 1.45G	4.56 ± 0.14RS	26.49 ± 24.05F
T_4	MAIZE + 1 % YEAST + 1 GM. DEXTROSE	62.20 ± 1.86D	23.32 ± .69M	42.76 ± 21.33D
T_5	SORGHUM + 1 % YEAST + 1 GM. DEXTROSE	5.89 ± 0.18R	41.67 ± 1.25H	23.78 ± 19.61HI
PULSES (WHOLE GRAINS)				
T_6	COW PEA + 1 % YEAST + 1 GM. DEXTROSE	35.01 ± 1.05IJ	14.97 ± 0.45OP	24.99 ± 11.00GH
T_7	GRAM + 1 % YEAST + 1 GM. DEXTROSE	36.48 ± 1.09I	10.39 ± 0.31Q	23.44 ± 14.30I
T_8	PIGEONPEA + 1 % YEAST + 1 GM. DEXTROSE	22.06 ± 0.66M	35.55 ± 1.07IJ	28.80 ± 7.43E
T_9	BLACK GRAM + 1 % YEAST + 1 GM. DEXTROSE	31.65 ± 0.95K	6.34 ± 0.19R	18.99 ± 13.87J
T_{10}	GREEN GRAM + 1 % YEAST + 1 GM. DEXTROSE	40.39 ± 1.21H	10.39 ± 0.31Q	25.39 ± 16.45FG
DUNG CONCOCTION AND INDUSTRIAL WASTES				
T_{11}	PRESS MUD + 1 % YEAST + 1 GM. DEXTROSE	0.00 ± 0.00U	3.09 ± 0.095ST	1.54 ± 1.69L
T_{12}	FYM + 1 % YEAST + 1 GM. DEXTROSE	93.47 ± 02.80A	27.30 ± 0.82L	60.38 ± 36.29C
T_{13}	VERMICOMPOST + 1 % YEAST + 1 GM. DEXTROSE	15.38 ± 0.46OP	23.33 ± 0.70M	19.35 ± 4.38J
T_{14}	FYM LIQUID + 1 % YEAST + 1 GM. DEXTROSE	57.63 ± 1.72E	75.74 ± 2.27C	66.68 ± 10.078A
T_{15}	CORCYRA REARING WASTES + 1 % YEAST + 1 GM. DEXTROSE	46.67 ± 1.40G	80.77 ± 2.42B	63.72 ± 18.76B
SYNTHETIC MEDIA				
T_{16}	PDB (POTATO DEXTROSE BROTH)	31.79 ± 0.95K	16.59 ± 0.50NO	24.19 ± 8.35GHI
T_{17}	SDB (SABOURAUD'S DEXTROSE BROTH)	34.02 ± 1.02J	14.52 ± 0.43P	24.27 ± 10.69GHI

Value presented as mean ± SD

For each column, different superscript (small alphabet) letter indicate significantly different at $p \leq 0.05$, as the measure by Tukey's test.

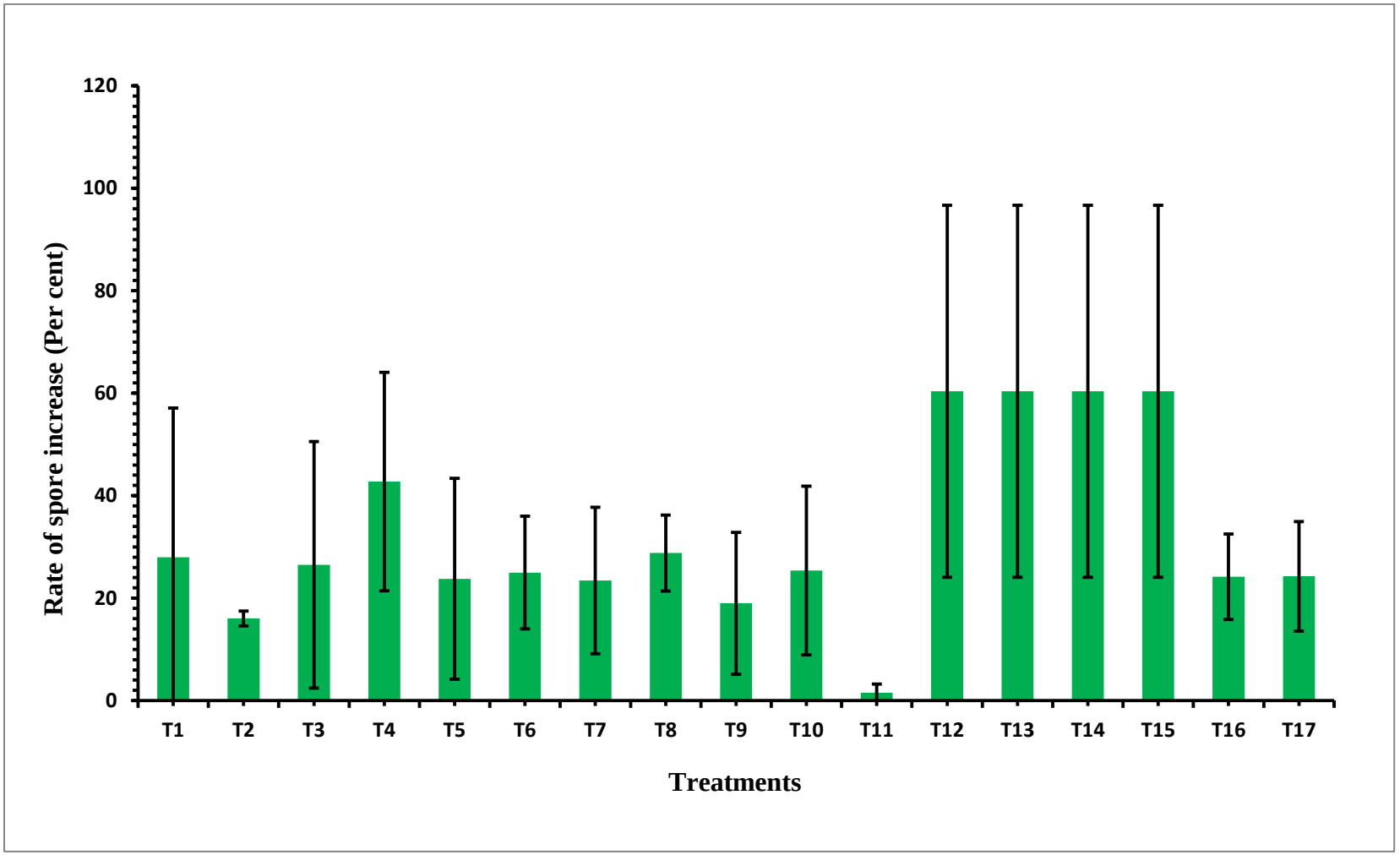


Fig. 7: Rate of increase in growth of *M. anisopliae* at different day after inoculation

The result showed that the rice was one of best substrate among all substrates used for mass multiplication of *M. anisopliae*. The results are accordance with the finding of **Raypuriya *et al.*, (2019), Agal *et al.*, (2019), Bhoosreddy (2014)** the result indicated the highest sporulation recorded in rice. The similar result were founded by **Jagdeesh *et al.*, (2008), Latifian *et al.*, (2014) and Roshandel *et al.*, (2015)** reported that Highest average yields were obtained as 3.1×10^8 and 3×10^8 conidia/g in rice and Sabouraud's Dextrose Agar with 1% yeast extract, respectively. The lowest level of conidia yield was 4×10^6 conidia/g which obtained in the wheat bran medium. Hence rice was best substrates for mass multiplication of *M. anisopliae* which may be due to high carbohydrate content and adequate carbon nitrogen ratio.

In case of pulses, the best substrate for mass multiplication of *M. anisopliae* was Cowpea, Green gram and Gram. The present finding are supported by **Tekam *et al.*, (2018)** they reported that cow pea is the best medium for mass production of this fungus with 12.68×10^7 spores per ml conidial count. This finding is also accordance with **Mehta *et al*., (2012)**. The pulses are good medium for multiplication of entomopathogenic fungus like *M. anisopliae* due to rich protein with sufficient amount of carbohydrate and adequate carbon nitrogen ratio.

The result of industrial waste is corroborated with **Prasad *et al.*, (2014).** There is no related finding of industrial waste that is why the present finding can't be compared with any other.

In case of synthetic media, Sabourud's dextrose agar and Potato dextrose agar are mostly similar for *M. anisopliae*, the findings are supported by **Raypuriya *et al.*, (2019), Agale *et al.*, (2018) and Tekam *et al.*, (2018).** This finding also supported by **Pratap *et al.*, (2012) and Kulat *et al.*, (2002)** they reported 9.43×10^6 spores/ml at 10 day after inoculation.

4.2.4 Economics of mass production of *M. anisopliae* on different substrates

Cost of production of 1×10^7 spores was calculated for all the substrates and the data is presented in table 13. The lowest production cost was recorded on Corcyra rearing wastes + 1 % Yeast + 1 gm. Dextrose (Rs. 0.02) which was on par with substrates Rice + 1 % Yeast + 1 gm. Dextrose, Bajra + 1 % Yeast + 1 gm. Dextrose and FYM liquid + 1 % Yeast + 1 gm. Dextrose with the production cost of Rs. 0.03. The next substrate was Wheat+ 1 % Yeast + 1 gm. Dextrose (Rs. 0.06) which was on par with Maize + 1 % Yeast + 1 gm. Dextrose and Sorghum + 1 % Yeast + 1 gm. Dextrose. The next substrate was Cow pea + 1 % Yeast + 1 gm. Dextrose (Rs. 0.12) showed non-significant difference with Gram + 1 % Yeast + 1 gm. Dextrose, Green gram + 1 % Yeast + 1 gm. Dextrose and FYM + 1 % Yeast + 1 gm. Dextrose (Rs. 0.16). This was followed by Black gram + 1 % Yeast + 1 gm. Dextrose and Press mud + 1 % Yeast + 1 gm. Dextrose (Rs. 0.20) also show non-significant difference with each other. The least economically feasible substrates were SDB (Sabouraud's Dextrose Broth) (Rs. 0.31), Pigeonpea + 1 % Yeast + 1 gm. Dextrose (Rs. 0.32), PDB (Potato Dextrose Broth) (Rs. 0.33) and Vermicompost + 1 % Yeast + 1 gm. Dextrose (Rs. 0.39).

The current findings are accordance with **Tekam *el al.*, (2018)**, **Raypuriya *et al.* (2019)**. It is also supported by **Prasad *et al.*, (2016)**.

Table 13: Economics of mass production of *M. anisopliae* on different substrates

TRE. CODE	SUBSTRATES	MEAN SPORE COUNT (1×10^7 SOPORE/ML)	COST OF SUBSTRATE/100G (RS)	COST OF PRODUCTION OF *M. ANISOPLIAE* 1×10^7 SOPORE/ML (RS)
T_1	RICE + 1 % YEAST + 1 GM. DEXTROSE	82.25	3.00	0.03
T_2	WHEAT+ 1 % YEAST + 1 GM. DEXTROSE	40.66	2.50	0.06
T_3	BAJRA + 1 % YEAST + 1 GM. DEXTROSE	64.58	2.00	0.03
T_4	MAIZE + 1 % YEAST + 1 GM. DEXTROSE	37.99	2.50	0.07
T_5	SORGHUM + 1 % YEAST + 1 GM. DEXTROSE	53.77	4.00	0.07
T_6	COW PEA + 1 % YEAST + 1 GM. DEXTROSE	69.37	8.50	0.12
T_7	GRAM + 1 % YEAST + 1 GM. DEXTROSE	63.66	8.00	0.12
T_8	PIGEONPEA + 1 % YEAST + 1 GM. DEXTROSE	58.55	5.50	0.32
T_9	BLACK GRAM + 1 % YEAST + 1 GM. DEXTROSE	49.55	10.0	0.20
T_{10}	GREEN GRAM + 1 % YEAST + 1 GM. DEXTROSE	66.33	8.00	0.12
T_{11}	PRESS MUD + 1 % YEAST + 1 GM. DEXTROSE	0.65	0.30	0.20
T_{12}	FYM + 1 % YEAST + 1 GM. DEXTROSE	8.27	0.50	0.16
T_{13}	VERMICOMPOST + 1 % YEAST + 1 GM. DEXTROSE	3.10	1.20	0.39
T_{14}	FYM LIQUID + 1 % YEAST + 1 GM. DEXTROSE	26.37	0.70	0.03
T_{15}	CORCYRA REARING WASTES + 1 % YEAST + 1 GM. DEXTROSE	31.22	0.70	0.02
T_{16}	PDB (POTATO DEXTROSE BROTH)	72.46	23.57	0.33
T_{17}	SDB (SABOURAUD'S DEXTROSE BROTH)	75.17	15.76	0.21
CD @ 5 %		**2.758**	**-**	**0.177**
SEM ±		**0.953**	**-**	**0.061**

4.2.5 Viability of *M. anisopliae* on different substrates

There was significant difference ($p < 0.05$) found in the conidial viability per cent of *M. anisopliae* produced from seventeen solid and liquid substrates at one to sixth month after storage (Table 14 and Fig 8). However, conidial viability decreased from the one month up to sixth month in storage, irrespective of the substrates on which they were produced.

4.2.5.1 One month after storage

At one month after storage the viable conidial per cent was ranged in 92.68 to 66.00 per cent. The highest conidial viability per cent was found in the substrates of FYM liquid + 1 % yeast powder + 1.0g Dextrose (92.68 per cent) which was on par with FYM + 1 % yeast powder + 1.0gm Dextrose (88.26 per cent) and Sorghum + 1 % Yeast + 1 gm Dextrose (88.24 per cent). The lowest conidial viability per cent observed in Press mud + 1 % Yeast + 1 gm Dextrose (66.00 per cent) which was also on par with Pigeonpea + 1 % Yeast + 1 gm Dextrose (71.11 per cent).

In case of cereals, the most suitable substrates for one month conidial viability of *M. anisopliae* were Sorghum + 1 % Yeast + 1 gm Dextrose (88.24 per cent). However among pulses the best one was Green gram + 1 % Yeast + 1 gm Dextrose (85.46 per cent) while FYM liquid + 1 % yeast powder + 1.0g Dextrose (92.68 per cent) and Sabouraud's Dextrose Broth (85.45 per cent) found superior in industrial wastes and synthetic media respectively.

4.2.5.2 Two month after storage

The conidial viability per cent was ranged from 89.41 to 27.03 per cent after two month of storage. The highest viable conidial per cent were recorded in FYM liquid + 1 % yeast powder + 1.0g Dextrose (89.41 per cent) which was statistically on par with the substrates of FYM + 1 %

Yeast + 1 gm Dextrose (88.26 per cent). However, the lowest conidial viability per cent was recorded in the substrates of Vermicompost + 1 % Yeast + 1 gm Dextrose (27.03 per cent).

The substrate Sorghum + 1 % Yeast + 1 gm Dextrose (67.15 per cent) was found superior in case of cereals whereas, in case of pulses, the best substrate was Cow pea + 1 % Yeast + 1 gm Dextrose (70.46 per cent). The FYM liquid + 1 % yeast powder + 1.0g Dextrose (89.41 per cent) and Sabouraud's Dextrose Broth (68.66 per cent) were found best in industrial wastes and synthetic media respectively.

4.2.5.3 Three month after storage

The conidial viability for all substrates was ranged from 8.92 to 64.22 per cent after three month of storage. The highest conidial viability per cent was found in the substrates of FYM liquid + 1 % yeast powder + 1.0g Dextrose with 64.22 per cent followed by FYM + 1 % Yeast + 1 gm Dextrose (58.78 per cent) and Sorghum + 1 % Yeast + 1 gm Dextrose (44.60 per cent). However the minimum conidial viability was recorded in the substrates of Vermicompost + 1 % Yeast + 1 gm Dextrose (8.92 per cent) which was on par with the substrates of Press mud + 1 % Yeast + 1 gm Dextrose (10.00 per cent).

The highest conidial viability in cereals was found in the substrates of Sorghum + 1 % Yeast + 1 gm Dextrose (44.60 per cent) which was on par with the substrates of Bajra + 1 % Yeast + 1 gm Dextrose (43.40 per cent). However, in case of pulses, Green gram + 1 % Yeast + 1 gm Dextrose (44.01 per cent) found suitable media for about three month of storage of *M. anisopliae* which was also on par with Bajra + 1 % Yeast + 1 gm Dextrose and Sorghum + 1 % Yeast + 1 gm Dextrose. The best substrate of industrial wastes and synthetic media was FYM

liquid + 1 % yeast powder + 1.0g Dextrose (64.22 per cent) and Sabouraud's Dextrose Broth (38.80 per cent).

4.2.5.4 Four month after storage

At fourth month after storage, the viable conidial per cent was ranged in 0.00 to 38.20 per cent. The highest conidial viability per cent was found in the substrates of FYM liquid + 1 % yeast powder + 1.0g Dextrose (38.20 per cent) which was statistically superior over all the substrates. The second best media was FYM + 1 % Yeast + 1 gm Dextrose (29.39 per cent). However, the lowest conidial viability per cent was recorded in Vermicompost + 1 % Yeast + 1 gm Dextrose (0.00 per cent) and Press mud + 1 % Yeast + 1 gm Dextrose (0.00 per cent).

In case of cereals, substrates Bajra + 1 % Yeast + 1 gm Dextrose (25.05 per cent) found to be the most suitable substrates for four month of conidial viability of *M. anisopliae* which was on par with Sorghum + 1 % Yeast + 1 gm Dextrose (24.01 per cent). However, in pulses, the best one was Green gram + 1 % Yeast + 1 gm Dextrose (29.91 per cent) while FYM liquid + 1 % yeast powder + 1.0g Dextrose (38.20 per cent) and Sabouraud's Dextrose Broth (14.92 per cent) found superior in industrial wastes and synthetic media respectively.

4.2.5.5 Five month after storage

Conidia produced on different substrates showed variation in viability after five month of storage. The conidial viability per cent of different substrates was ranged from 0.00 to 22.76 per cent. The highest conidial viability per cent was found in FYM liquid + 1 % yeast powder + 1.0g Dextrose (22.76 per cent). However, the substrates Press mud + 1 % Yeast + 1 gm Dextrose and Vermicompost + 1 % Yeast + 1 gm Dextrose showed 0.00 per cent conidial viability.

Among the cereal grains the highest conidial viability per cent was recorded in Sorghum + 1 % Yeast + 1 gm Dextrose (11.26 per cent) followed by Rice + 1 % Yeast + 1 gm Dextrose (9.93) which was on par with Bajra + 1 % Yeast + 1 gm Dextrose (9.84 per cent). However, in case of pulses, the most suitable substrates for conidial viability was Green gram + 1 % Yeast + 1 gm Dextrose (17.51 per cent). The FYM liquid + 1 % yeast powder + 1.0g Dextrose (22.76 per cent) and Sabouraud's Dextrose Broth (4.85 per cent) were the best one among all industrial wastes and synthetic media.

4.2.5.6 Six month after storage

The conidial viability for all the substrates was ranged from 0.00 to 13.80 per cent after six month of storage. The highest conidial viability per cent was recorded in the substrates of FYM liquid + 1 % yeast powder + 1.0g Dextrose (13.80 per cent) followed by Green gram + 1 % Yeast + 1 gm Dextrose (6.83 per cent) and Sorghum + 1 % Yeast + 1 gm Dextrose (5.88 per cent). However the maximum substrates i.e. Press mud + 1 % Yeast + 1 gm Dextrose, Vermicompost + 1 % Yeast + 1 gm Dextrose, Maize + 1 % Yeast + 1 gm Dextrose, Corcyra rearing wastes + 1 % Yeast + 1 gm Dextrose, Potato Dextrose Broth and Sabouraud's Dextrose Broth showed zero per cent conidial viability.

Among all the cereals, the highest conidial viability per cent was recorded in substrates Sorghum + 1 % Yeast + 1 gm Dextrose (5.88 per cent) followed by Rice + 1 % Yeast + 1 gm Dextrose (4.96 per cent) and Bajra + 1 % Yeast + 1 gm Dextrose (4.47 per cent). However, in case of pulses, the highest conidial viability per cent were recorded in Green gram + 1 % Yeast + 1 gm Dextrose and Black gram + 1 % Yeast + 1 gm Dextrose with 6.83 and 5.36 per cent respectively. The FYM liquid + 1 % yeast powder + 1.0gm Dextrose (13.80 per cent) and FYM +

1 % Yeast + 1 gm Dextrose (2.91 per cent) were the best one among all the industrial wastes and synthetic media.

The findings are supported by **Pal and Singh (2016)** they reported most suitable media for long term storage of *M. anisopliae* was FYM + 1 gm dextrose with 37.25×10^7 conidia/ml after six month of storage however, in case of FYM liquid + 1.0gm Dextrose recorded 12.62×10^7 conidia/ml. These findings are corroborated with **Pandey and Kanaujiya (2008)** they reported that the most suitable grains media for long term storage of *M. anisopliae* were finger millet and sorghum with 86.6 and 80.6 per cent conidial viability. These findings are also agreement with **Kaur and Joshi (2018)** and **Roshandel *et al.*, (2016)**.

4.3 To study the bioassay of *Metarhizium anisopliae* against *Helicoverpa armigera*

Result obtained in lab condition for *Metarhizium anisopliae* (SVPUAT 1, Accession No. ON183248) at different concentration against individual instars (II^{nd}, III^{rd} and IV^{th}) of *Helicoverpa armigera* larvae are elucidated below:

Table 14: Conidial viability of *M. anisopliae* on different substrates during six month of storage period

TR. CODE	CONIDIAL VIABILITY PER CENT DURING SIX MONTH OF STORAGE					
	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS
	CEREALS (WHOLE GRAINS)					
T_1	81.55 ± 2.44BCDE	42.55 ± 1.27I	30.14 ± 0.90HI	19.85 ± 0.59D	9.93 ± 0.29D	4.96 ± 0.15D
T_2	78.57 ± 2.36DEF	50.00 ± 1.50GH	32.85 ± 0.98GH	16.42 ± 0.49E	7.84 ± 0.23 E	2.85 ± 0.09FG
T_3	85.01 ± 2.55BCDE	62.63 ± 1.88DE	43.40 ± 1.30CD	25.05 ± 0.75C	9.84 ± 0.29D	4.47 ± 0.13E
T_4	81.09 ± 2.43BCDE	54.04 ± 1.62FG	29.05 ± 0.87I	12.83 ± 0.38F	6.75 ± 0.20 F	0.00 ± 0.00I
T_5	88.24 ± 2.64ABC	67.15 ± 2.01BCD	44.60 ± 1.34C	24.01 ± 0.72C	11.26 ± 0.33C	5.88 ± 0.17C
	PULSES (WHOLE GRAINS)					
T_6	85.36 ± 2.56ABCD	70.46 ± 2.11B	40.66 ± 1.22DE	19.73 ± 0.59D	7.65 ± 0.23 E	3.21 ± 0.09F
T_7	80.72 ± 2.42CDE	51.57 ± 1.54GH	37.21 ± 1.11EF	17.61 ± 0.53E	6.27 ± 0.19 FG	2.69 ± 0.08G
T_8	71.11 ± 2.13FG	48.44 ± 1.45H	30.21 ± 0.90HI	18.21 ± 0.54DE	3.11 ± 0.09J	1.33 ± 0.04H
T_9	83.32 ± 2.50BCDE	54.16 ± 1.62FG	33.32 ± 1.00GH	16.66 ± 0.50E	8.32 ± 0.25 E	5.36 ± 0.16D
T_{10}	85.46 ± 2.56ABCD	64.10 ± 1.92CD	44.01 ± 1.32CD	29.91 ± 0.90B	17.51 ± 0.52B	6.83 ± 0.20B
	DUNG CONCOCTIONS AND INDUSTRIAL WASTES					
T_{11}	66.00 ± 1.98G	33.00 ± 0.99J	10.00 ± 0.30J	0.00 ± 0.00G	0.00 ± 0.00K	0.00 ± 0.00I
T_{12}	88.26 ± 2.65AB	88.26 ± 2.65A	58.78 ± 1.76B	29.39 ± 0.88B	5.83 ± 0.17 G	2.91 ± 0.08FG
T_{13}	81.08 ± 2.43BCDE	27.03 ± 0.81K	8.92 ± 0.27J	0.00 ± 0.00G	0.00 ± 0.00 K	0.00 ± 0.00I
T_{14}	92.68 ± 2.78A	89.41 ± 2.68A	64.22 ± 1.93A	38.20 ± 1.14A	22.76 ± 0.68A	13.80 ± 0.41A
T_{15}	77.62 ± 2.33EF	58.39 ± 1.75EF	34.34 ± 1.03FG	13.05 ± 0.39F	5.48 ± 0.16GH	0.00 ± 0.00I
	SYNTHETIC MEDIA					
T_{16}	83.46 ± 2.50BCDE	46.54 ± 1.39HI	28.46 ± 0.85I	11.92 ± 0.59D	4.22 ± 0.12I	0.00 ± 0.00I
T_{17}	85.45 ± 2.56ABCD	68.66 ± 2.06BC	38.80 ± 1.16 E	14.92 ± 0.49E	4.85 ± 0.14HI	0.00 ± 0.00I

MAS – Month After Storage

Value presented as mean ± SD

For each column, different superscript (small alphabet) letter indicate significantly different at $p \leq 0.05$, as the measure by Tukey's test between treatments.

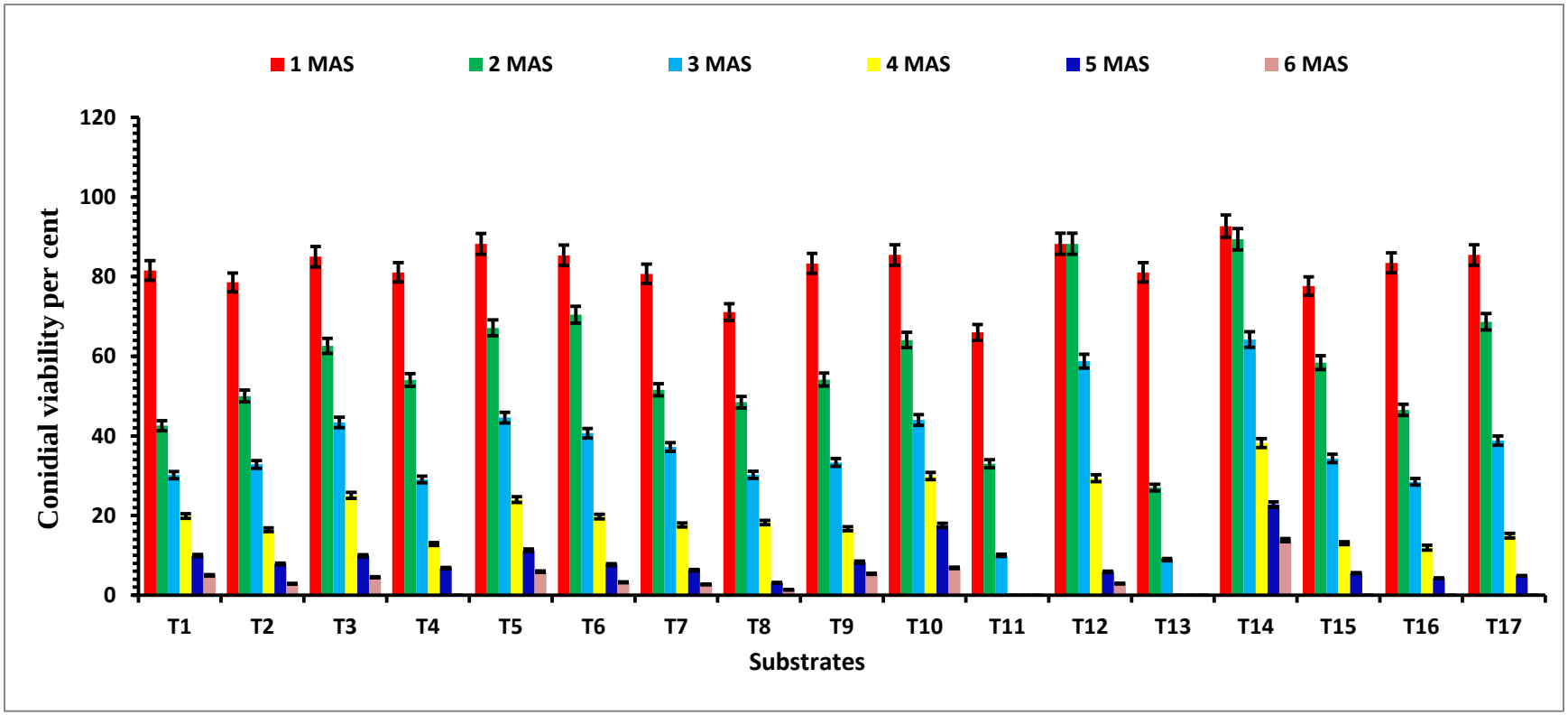


Fig. 8: Conidial viability of *M. anisopliae* on different substrates during six month of storage period

Plate 9: Storage of different sporulated substrates at room temperature.

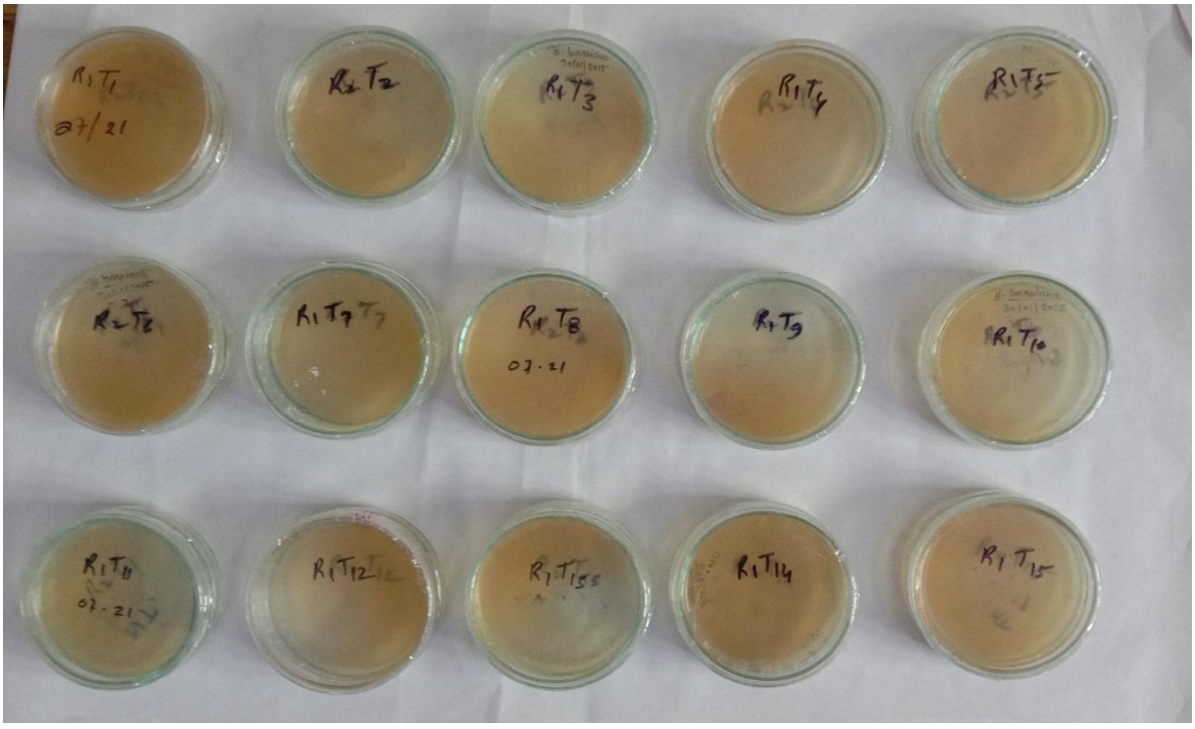


Plate 10: Viability test of *M. anisopliae* spores produced from different substrates at frequent interval.

4.3.1 Bioassay of *M. anisopliae* on *H. armigera* during first year 2020-2021

4.3.1.1 Second instar

The second instar larval mortality of *H. armigera* is presented in table 15 and depicted in fig 9.

4.3.1.1.1 Twenty four hours after treatment

The mortality of second, third and fourth instars larvae of *H. armigera* after twenty four hours of treatment was zero. All the concentration of *M. anisopliae* did not caused any mortality.

4.3.1.1.2 Forty eight hours after treatment

The larval mortality of *H. armigera* at forty eight hours after treatment ranged from 0.00 to 26.66 per cent. The highest larval mortality was 26.66 per cent recorded in the treatment 2×10^{10} spores/ml. The treatment 2×10^{10} spores/ml was significantly superior over all the treatments. The second best treatment was 2×10^{9} spores/ml with 17.77 per cent larval mortality followed by 2×10^{8} spores/ml, 2×10^{7} spores/ml, 2×10^{6} spores/ml and 2×10^{5} spores/ml with 13.33, 8.88, 4.44 and 2.22 per cent larval mortality. The treatments 2×10^{4} spores/ml, 2×10^{3} spores/ml and control were not significantly differing with 0.00 per cent mortality.

4.3.1.1.3 Seventy two hours after treatment

At seventy two hours after treatment the larval mortality per cent ranged from 13.33 to 53.33 per cent. The treatment 2×10^{10} spores/ml was significantly superior over all the treatments with highest larval mortality 53.33 per cent. The next best treatment was 2×10^{9} spores/ml (46.66 per cent). The treatments 2×10^{6} spores/ml (28.88 per cent), 2×10^{5} spores/ml (27.21 per cent)

was on par with each other and the treatments 2×10^4 spores/ml (15.55 per cent) and 2×10^3 spores/ml (13.33 per cent) also showed non-significant relation with each other.

4.3.1.1.4 Ninety six hours after treatment

All the treatments were superior over control. The mortality per cent ranged from 33.33 to 88.88 per cent. The treatment 2×10^{10} spores/ml (88.88 per cent) was significantly superior over all other treatments. The second best treatment was 2×10^9 spores/ml with 73.33 per cent. The treatment 2×10^8 spores/ml (53.33 per cent) was on par with 2×10^7 spores/ml (51.10 per cent). The concentration of 2×10^6 spores/ml (42.22 per cent), 2×10^5 spores/ml (39.99 per cent) and 2×10^4 spores/ml (37.77 per cent) were also found on par with each other. The lowest mortality (33.33 per cent) was recorded in the treatment 2×10^3 spores/ml.

4.3.1.1.5 One hundred twenty hours after treatment

The larval mortality ranged from 53.33 to 95.33 per cent. The highest mortality recorded in the treatment 2×10^{10} spores/ml with 95.33 per cent which was on par with 2×10^9 spores/ml (91.10 per cent). The second best treatment 2×10^9 spores/ml was also on par with the treatment 2×10^8 spores/ml (88.88 per cent). The lowest mortality observed in the treatment 2×10^3 spores/ml (53.33 per cent).

4.3.1.1.6 LT_{50} and LT_{90} value

Table 15 showes that the highest concentration of *M. anisopliae* gives the less LT_{50} and LT_{90} value. The highest LT_{50} and LT_{90} value was at the concentration of 2×10^3 spores/ml with 4.75 and 7.71 days, while the lowest value of LT_{50} and LT_{90} was at the concentration of 2×10^{10} spores/ml with 2.68 and 4.33 days followed by 2×10^9 spores/ml, 2×10^8 spores/ml, 2×10^7

Table 15: Efficacy of *M. anisopliae* strain against second instar larvae of *H. armigera* during 2020-2021

Tr. Code	Concentrations (Spores ml^{-1})	Mortality Per cent at different time interval					LT_{50} (Days)	LT_{90} (Days)
		24 hours	48 hours	72 hours	96 hours	120 hours		
T_1	2×10^3	0.00 ± 0.00	0.00 ± 0.00^{G}	13.33 ± 0.39^{F}	33.33±1.00^{E}	53.33 ± 1.59^{G}	4.75	7.71
T_2	2×10^4	0.00 ± 0.00	0.00 ± 0.00^{G}	15.55 ± 0.46^{F}	37.77 ±1.13^{DE}	59.99 ± 1.79^{F}	4.50	7.08
T_3	2×10^5	0.00 ± 0.00	2.22 ± 0.06^{F}	27.21 ± 0.81^{E}	39.99 ± 1.20^{D}	66.66 ± 1.99^{E}	4.21	7.21
T_4	2×10^6	0.00 ± 0.00	4.44 ± 0.13^{E}	28.88 ± 0.86^{E}	42.22 ± 1.26^{D}	73.33±2.19^{D}	4.02	6.92
T_5	2×10^7	0.00 ± 0.00	8.88 ±0 .26^{D}	33.33 ± 0.99^{D}	51.10 ± 1.53^{C}	82.22 ± 2.46^{C}	3.65	6.25
T_6	2×10^8	0.00 ± 0.00	13.33 ± 0.39^{C}	37.77 ± 1.13^{C}	53.33± 1.60^{C}	88.88± 2.66^{B}	3.42	5.92
T_7	2×10^9	0.00 ± 0.00	17.77 ±0.53^{B}	46.66 ± 1.39^{B}	73.33 ± 2.20^{B}	91.10 ± 2.73^{AB}	3.03	5.08
T_8	2×10^{10}	0.00 ± 0.00	26.66 ± 0.79^{A}	53.33 ± 1.59^{A}	88.88 ± 2.66^{A}	95.33 ± 2.85^{A}	2.68	4.33
T_9	Control	0.00 ± 0.00	0.00 ± 0.00^{G}	0.00 ± 0.00^{G}	0.00 ± 0.00^{F}	0.00 ± 0.00^{H}	-	-

Number denote by same letter within the column are statistically non - significant (Tukey's test, $p < 0.05$) data show the mean of three.

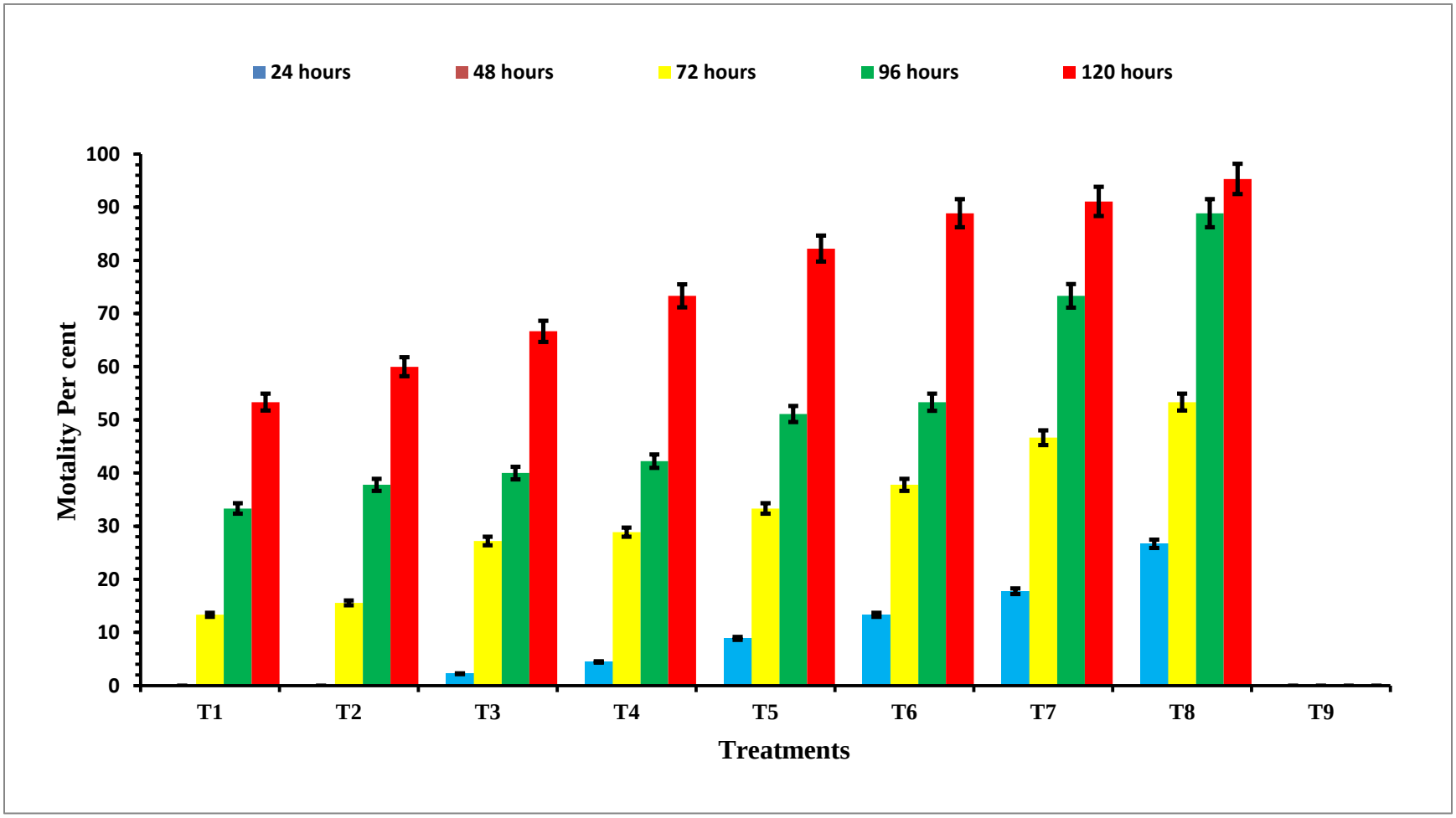


Fig. 9: Efficacy of *M. anisopliae* strain against second instar larvae of *H. armigera* during 2020-2021.

spores/ml, 2×10^6 spores/ml, 2×10^5 spores/ml and 2×10^4 spores/ml with the LT_{50} values of 3.03, 3.42, 3.65, 4.02, 4.21 and 4.50 day and 5.08, 5.92, 6.25, 6.92, 7.21 and 7.08 days LT_{90} values respectively.

4.3.1.2 Third instar

The result of larval mortality of *H. armigera* was shown in table 16 and depicted in fig 10.

4.3.1.2.1 Forty eight hours after treatment

The larval mortality ranged from 0.00 to 20.00 per cent. The highest mortality (20.00 per cent) recorded in the treatment 2×10^{10} spores/ml which was significantly superior over all the treatments followed by 2×10^9 spores/ml (13.33 per cent), 2×10^8 spores/ml (8.88 per cent), 2×10^7 spores/ml (6.66 per cent) and 10^6 spores/ml (2.22 per cent) respectively. Zero per cent mortality recorded at the concentration of 2×10^5 spores/ml, 2×10^4 spores/ml and 2×10^3 spores/ml which showed non-significant relation with control.

4.3.1.2.2 Seventy two hours after treatment

All the treatments were superior over control. The mortality ranged from 8.88 to 48.88 per cent. The concentration 2×10^{10} spores/ml (48.88 per cent) showed highest mortality among all concentration followed by 2×10^9 spores/ml, 2×10^8 spores/ml, 2×10^7 spores/ml, 2×10^6 spores/ml and 2×10^5 spores/ml with 40.00, 35.55, 31.10, 22.22 and 17.77 per cent respectively. The concentration 2×10^4 spores/ml (11.11 per cent) and 2×10^3 spores/ml (8.88 per cent) were on par with each other.

4.3.1.2.3 Ninety six hours after treatment

After ninety six hour of treatment, the third instar larval mortality of *H. armigera* ranged from 35.55 to 75.55 per cent. The highest per cent mortality was recorded in the treatment 2×10^{10} spores/ml (75.75 per cent). This treatment was significantly superior over all treatments followed by treatment 2×10^{9} spores/ml (68.88 per cent). The treatments 2×10^{8} spores/ml (51.10 per cent) and 2×10^{7} spores/ml (48.88 per cent) were on par with each other. The concentrations 2×10^{6} spores/ml (42.22 per cent) and 2×10^{5} spores/ml (39.39 per cent) were also on par with each other. The minimum mortality was recorded in the treatment 2×10^{3} spores/ml (35.55 per cent) which was non-significant with the treatment 2×10^{4} spores/ml (37.77 per cent).

4.3.1.2.4 One hundred twenty hour after treatment

The mortality of third instar larva of *H. armigera* after one hundred twenty hours, among all treatment ranged from 51.10 to 91.10 per cent. The highest mortality was 91.10 per cent recorded in the treatment 2×10^{10} spores/ml. The second most effective treatment was 2×10^{9} spores/ml (88.88 per cent) which was on par with the treatment 2×10^{8} spores/ml (84.44 per cent). However, the lowest mortality was recorded in the treatment 2×10^{3} spores/ml (51.10 per cent).

4.3.1.2.5 LT_{50} and LT_{90} value

At for third instar larvae of *H. armgera* the LT_{50} and LT_{90} value was shown in table 16. The highest LT_{50} and LT_{90} value was recorded at low concentration of *M. anisopliae* 2×10^{3} spores/ml with 4.79 and 7.50 days. The minimum value was recorded at the concentration of 2×10^{10} spores/ml with 2.96 and 5.04 days. The value of LT_{50} and LT_{90} was decreased with increase in concentration of *M. anisopliae*.

Table 16: Efficacy of *M. anisopliae* strain against third instar larvae of *H. armigera* during 2020-2021

Tr. Code	Concentrations (Spores ml^{-1})	Mortality Per cent at different time interval					LT_{50}	LT_{90}
		24 hours	48 hours	72 hours	96 hours	120 hours	(Days)	(Days)
T_1	2×10^3	0.00 ± 0.00	0.00 ± 0.00F	8.88 ± 0.26G	35.55 ± 1.06F	51.10 ± 1.53F	4.79	7.50
T_2	2×10^4	0.00 ± 0.00	0.00 ± 0.00F	11.11 ± 0.33G	37.77±1.13EF	60.00 ± 1.80E	4.50	6.88
T_3	2×10^5	0.00 ± 0.00	0.00 ± 0.00F	17.77 ± 0.53F	39.99±1.19DE	64.44 ± 1.93DE	4.33	6.79
T_4	2×10^6	0.00 ± 0.00	2.22 ± 0.06E	22.22 ± 0.66E	42.22 ±1.26D	68.88 ± 2.06D	4.17	6.88
T_5	2×10^7	0.00 ± 0.00	6.66 ± 0.19D	31.10 ± 0.93D	48.88 ± 1.46C	80.00 ± 2.40BC	3.76	6.33
T_6	2×10^8	0.00 ± 0.00	8.88 ± 0.26C	35.55 ± 1.06C	51.10 ± 1.53C	84.44 ± 2.53B	3.59	6.08
T_7	2×10^9	0.00 ± 0.00	13.33 ± 0.39B	40.00 ± 1.20B	68.88 ±2.06B	88.88 ± 2.66AB	3.23	5.33
T_8	2×10^{10}	0.00 ± 0.00	20.00 ± 0.60A	48.88 ± 1.46A	75.55 ± 2.26A	91.10 ± 2.73A	2.96	5.04
T_9	Control	0.00 ± 0.00	0.00 ± 0.00F	0.00 ± 0.00H	0.00 ±0.00G	0.00 ± 0.00F	-	-

Number denote by same letter within the column are statistically non - significant (Tukey's test, $p < 0.05$) data show the mean of three.

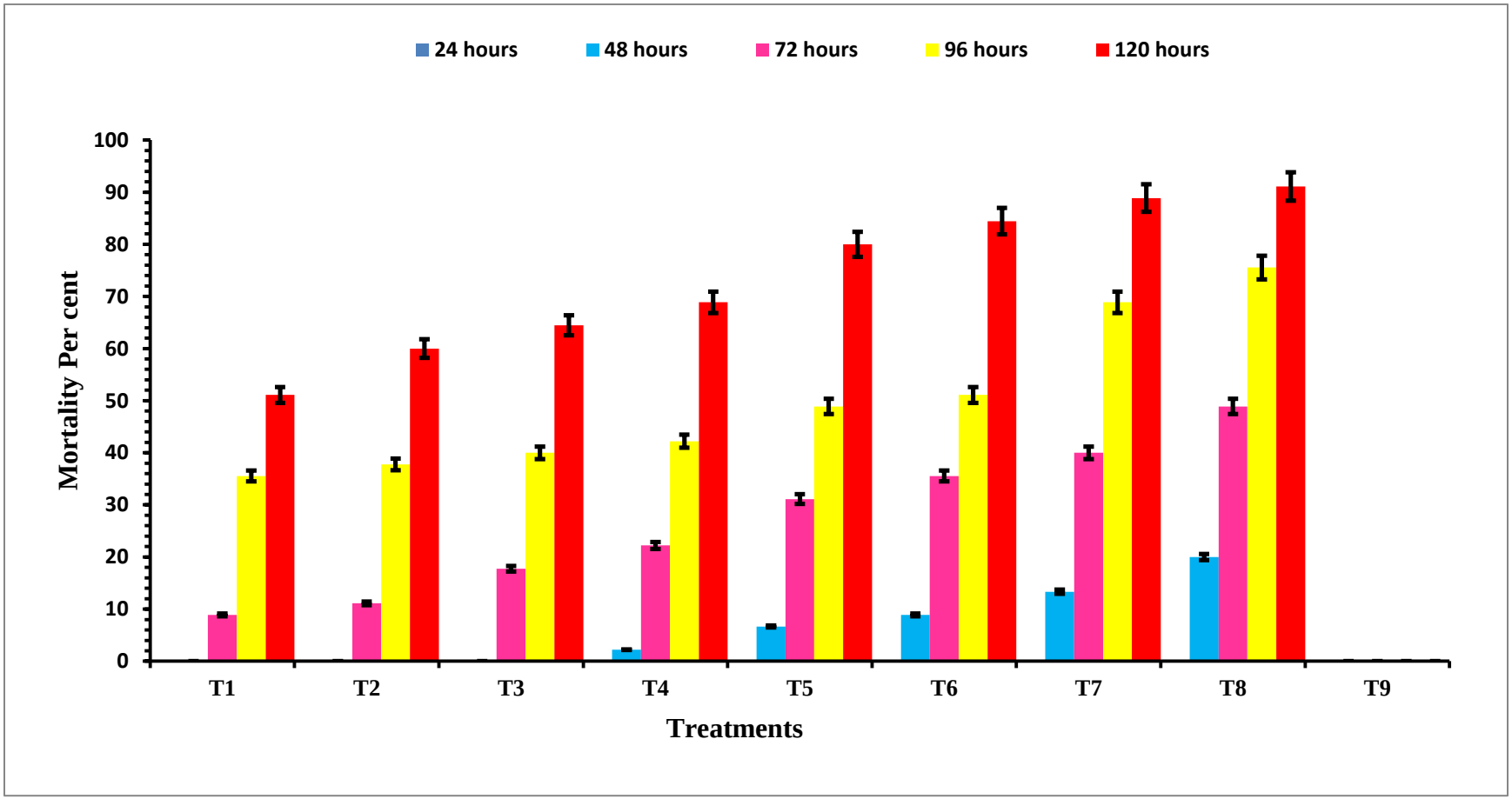


Fig. 10: Efficacy of *M. anisopliae* strain against third instar larvae of *H. armigera* during 2020-2021.

4.3.1.3 Fourth instar

The result was shown in table 17 and depicted in fig 11.

4.3.1.3.1 Forty eight hours after treatment

The mortality was only recorded in the treatment 2×10^{10} spores/ml and 2×10^{9} spores/ml with 8.88 per cent and 6.66 per cent while all rest of the treatments showed zero per cent mortality.

4.3.1.3.2 Seventy two hours after treatment

The larval mortality ranged from 6.66 to 33.33 per cent. The highest mortality per cent was recorded in the treatment 2×10^{10} spores/ml (33.33 per cent) followed by treatment 2×10^{9} spores/ml (28.88 per cent). The treatment 2×10^{8} spores/ml (24.44 per cent) and 2×10^{7} spores/ml (22.22 per cent) were on par with each other. Whereas the treatment 2×10^{5} spores/ml (13.33 per cent) and 2×10^{4} spores/ml (11.11 per cent) was statistically on par with each other. The minimum larval mortality was recorded in the treatment 2×10^{3} spores/ml with 6.66 per cent.

4.3.1.3.3 Ninety six hours after treatment

After ninety six hours of treatment the mortality of larvae ranged from 40.00 to 86.66 per cent. The highest per cent mortality was 86.66 per cent in the treatment 2×10^{10} spores/ml. This treatment was on par with the treatment 2×10^{9} spores/ml (80.24 per cent). The treatment 2×10^{9} spores/ml (80.24 per cent) was also on par with 2×10^{8} spores/ml (75.55 per cent). The lowest mortality was recorded in the treatment 2×10^{3} spores/ml with 40.00 per cent.

Table 17: Efficacy of *M. anisopliae* strain against fourth instar larvae of *H. armigera* during 2020-2021

Tr. Code	Concentrations (Spores ml^{-1})	Mortality Per cent at different time interval					LT_{50}	LT_{90}
		24 hours	48 hours	72 hours	96 hours	120 hours	(Days)	(Days)
T_1	2×10^3	0.00 ± 0.00	0.00 ± 0.00C	6.66 ± 0. 19F	28.88 ± 0.86E	40.00 ± 1.20F	5.29	8.71
T_2	2×10^4	0.00 ± 0.00	0.00 ± 0.00C	11.11 ± 0.33E	31.11 ± 0.93DE	46.66 ± 1.39EF	5.00	8.33
T_3	2×10^5	0.00 ± 0.00	0.00 ± 0.00C	13.33 ± 0.39E	35.55 ± 1.06CD	55.55 ± 1.66DE	4.67	7.42
T_4	2×10^6	0.00 ± 0.00	0.00 ± 0.00C	20.00 ± 0.60CD	37.77 ± 1.13C	62.22 ± 1.86CD	4.42	7.08
T_5	2×10^7	0.00 ± 0.00	0.00 ± 0.00C	22.22 ± 0.66C	42.22 ± 1.26C	68.88 ± 2.06BC	4.17	6.54
T_6	2×10^8	0.00 ± 0.00	0.00 ± 0.00C	24.44 ± 0.73C	48.88 ± 1.46B	75.55 ± 2.26B	3.97	6.00
T_7	2×10^9	0.00 ± 0.00	6.66 ± 0.19B	28.88 ± 0.86B	53.33 ± 1.59B	80.00 ± 2.40AB	3.73	6.17
T_8	2×10^{10}	0.00 ± 0.00	8.88 ± 0. 26A	33.33 ± 0.99A	60.00 ± 1.80A	86.66 ± 2.59A	3.48	5.67
T_9	Control	0.00 ± 0.00	0.00 ± 0.00C	0.00 ± 0.00G	0.00 ± 0.00F	0.00 ± 0.00	-	-

Number denote by same letter within the column are statistically non - significant (Tukey's test, $p < 0.05$) data show the mean of three.

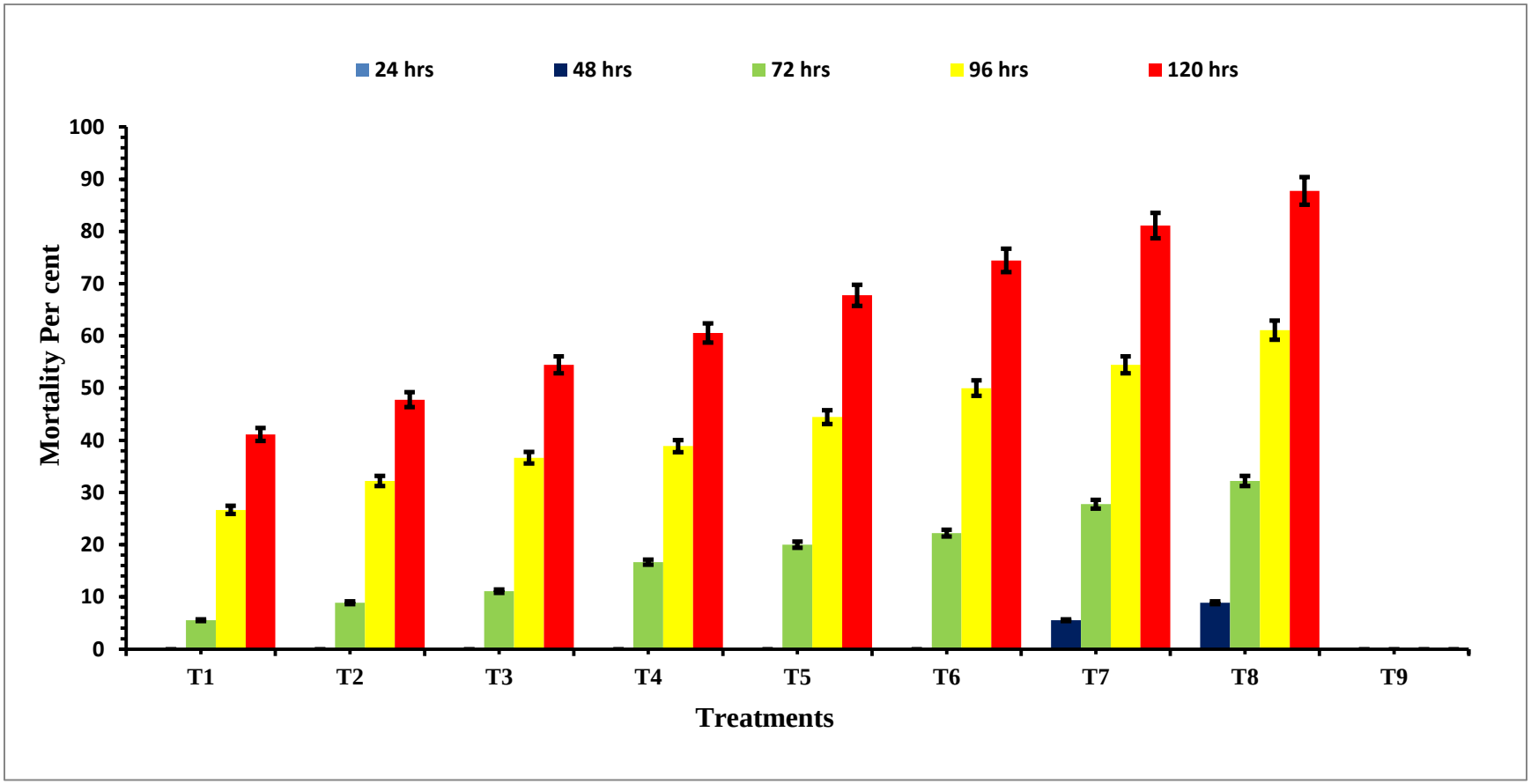


Fig. 17: Efficacy of *M. anisopliae* strain against fourth instar larvae of *H. armigera* during 2020-2021 & 2021-2022 (Pooled).

4.3.1.3.4 LT_{50} and LT_{90} value

The value of LT_{50} and LT_{90} of fourth instar larva of *H. armigera* was slightly greater in comparison of second and third instar larva which was shown in table 17. The similar pattern was recorded with highest LT_{50} and LT_{90} value of 5.29 and 8.71 days at the concentration of 2×10^3 spores/ml. The lowest LT_{50} and LT_{90} value was recorded at the concentration of 2×10^{10} spores/ml with 3.48 and 5.67 days. Whereas, the second lowest LT_{50} and LT_{90} value was 3.73 and 6.17 days at the concentration of 2×10^9 spores/ml.

4.3.2 Bioassay of *M. anisopliae* on *H. armigera* during 2021-2022

4.3.2.1 Second instar

The mortality per cent of second instar larvae of *H. armigera* in different treatment was shown in table 18 and depicted in fig 12.

4.3.2.1.1 Twenty four hours after treatment

The mortality of second, third and fourth instars larvae of *H. armigera* after twenty four hours of treatment was zero. All concentration of *M. anisopliae* did not cause any mortality.

4.3.2.1.2 Forty eight hours after treatment

All treatments were superior over control except 2×10^4 spores/ml (0.00 per cent) and 2×10^3 spores/ml (0.00 per cent). The mortality per cent was ranged from 0.00 to 31.10 per cent. The treatment 2×10^{10} spores/ml (31.10 per cent) showed the highest mortality which was statistically superior over all treatments. The second best treatment was 2×10^9 spores/ml (20.00 per cent). However the minimum larval mortality was recorded in treatment 2×10^5 spores/ml (2.22 per cent).

4.3.2.1.3 Seventy two hours after treatment

The larval mortality ranged from 11.10 to 55.55 per cent. The highest mortality trend of *H. armigera* larvae was observed in the treatment of 2×10^{10} spores/ml (55.55 per cent) which was significantly superior over all other the treatments. The second best treatment was 2×10^{9} spores/ml (48.88 per cent). Whereas, the treatment 2×10^{5} spores/ml (17.77 per cent) was on par with the treatment 2×10^{4} spores/ml (15.55 per cent). However the lowest mortality was observed in the treatment 2×10^{3} spores/ml (11.10 per cent).

4.3.2.1.4 Ninety six hours after treatment

All the treatments were superior over control after ninety six hours of treatment. The mortality ranged from 26.66 to 80.00 per cent. The highest larval mortality was recorded in the treatment 2×10^{10} spores/ml (80.00 per cent) which was on par with second best treatment 2×10^{9} spores/ml (75.55 per cent). The minimum larval mortality was observed in the treatment 2×10^{3} spores/ml (26.66 per cent).

4.3.2.1.5 One hundred twenty hours after treatment

The similar trend was found as first year assay with the range of 55.55 to 93.33 per cent larval mortality. The highest lethal concentration was 2×10^{10} spores/ml with 93.33 per cent mortality which was on par with the concentration of 2×10^{9} spores/ml with 91.10 per cent mortality. However, this concentration was non-significant with 2×10^{8} spores/ml with 84.44 per cent mortality. The minimum larval mortality was found in the concentration of 2×10^{3} spores/ml with 55.55 per cent.

Table 18: Efficacy of *M. anisopliae* strain against second instar larvae of *H. armigera* during 2021-2022

Tr. Code	Concentrations (Spores ml^{-1})	Mortality Per cent at different time interval					LT_{50} (Days)	LT_{90} (Days)
		24 hours	48 hours	72 hours	96 hours	120 hours		
T_1	2×10^3	0.00 ± 0.00	0.00 ± 0.00G	11.10 ± 0.33G	26.66 ± 0 .79E	55.55 ± 1.66F	4.79	7.46
T_2	2×10^4	0.00 ± 0.00	0.00 ± 0.00G	15.55 ± 0.46FG	28.88 ±0.86CDE	60.00 ±1.80E	4.63	7.33
T_3	2×10^5	0.00 ± 0.00	2.22 ± 0.06F	17.77 ± 0.53F	33.33 ± 0.99C	66.66 ± 1.99D	4.42	7.21
T_4	2×10^6	0.00 ± 0.00	6.66 ± 0.19E	26.66 ± 0.79E	37.77 ± 1.13C	71.10 ± 2.13CD	4.15	7.50
T_5	2×10^7	0.00 ± 0.00	11.11 ± 0.33D	33.33 ± 0.99D	46.66 ± 1.39B	80.00 ± 2.40BC	3.72	6.75
T_6	2×10^8	0.00 ± 0.00	13.33 ± 0.39C	37.77 ± 1.13C	51.10 ± 1.53B	84.44 ± 2.53B	3.51	6.29
T_7	2×10^9	0.00 ± 0.00	20.00 ± 0.60B	48.88 ± 1.46B	75.55 ± 2.26A	91.10 ± 2.73AB	2.96	5.04
T_8	2×10^{10}	0.00 ± 0.00	31.10 ± 0.93A	55.55 ± 1.66A	80.00 ± 2.40A	93.33 ± 2.79A	2.69	4.75
T_9	Control	0.00 ± 0.00	0.00 ± 0.00G	0.00 ± 0.00I	0.00 ± 0.00F	0.00 ± 0.00G	-	-

Number denote by same letter within the column are statistically non - significant (Tukey's test, $p < 0.05$) data show the mean of three.

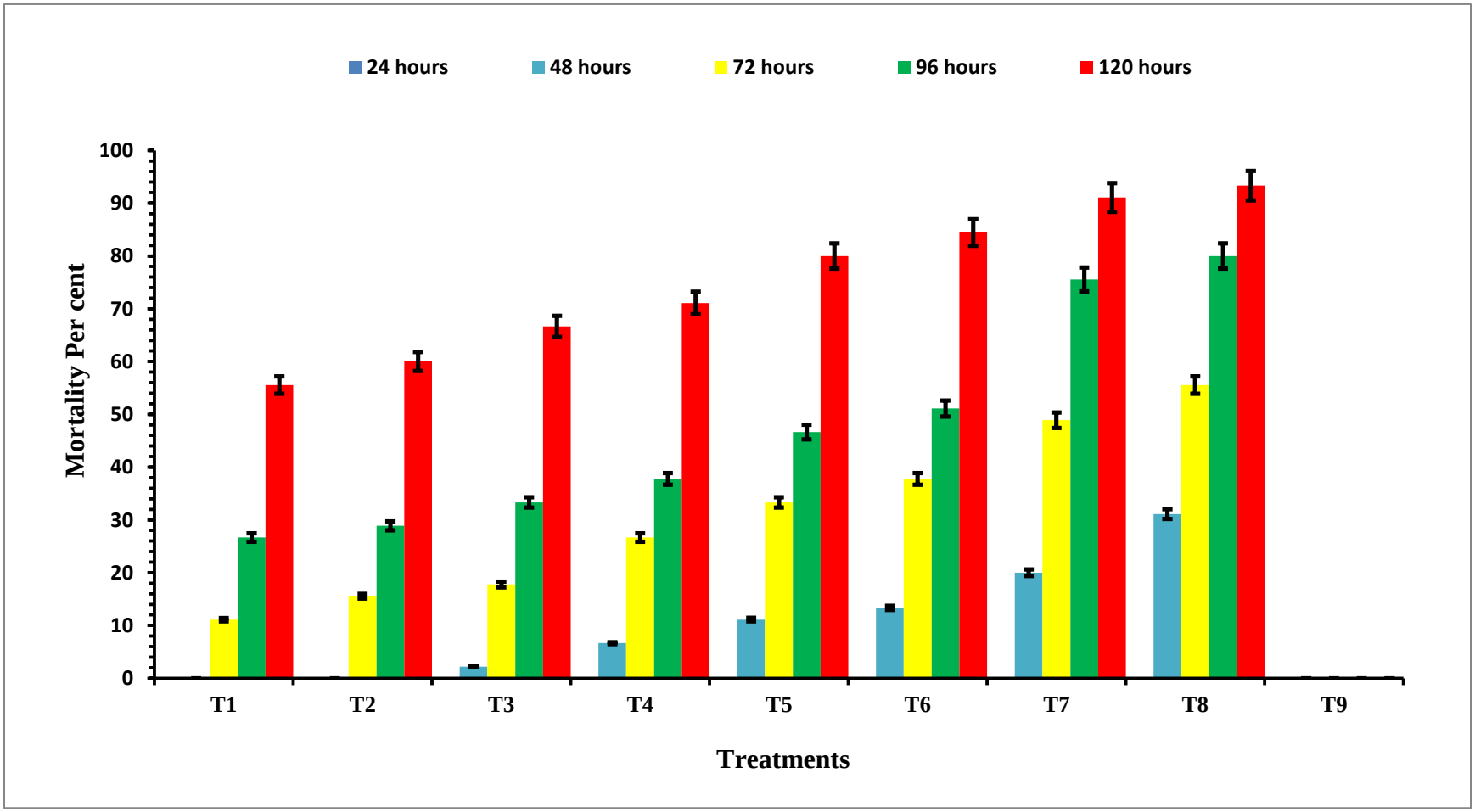


Fig. 12: Efficacy of *M. anisopliae* strain against second instar larvae of *H. armigera* during 2021-2022.

4.3.2.1.6 LT_{50} LT_{90} value

The LT_{50} and LT_{90} value at second instar during 2021-2022 was shown in table 18. The lowest value of LT_{50} and LT_{90} was recorded at the concentration of 2×10^{10} spores/ml with 2.69 and 4.75 days. The second lowest concentration was 2×10^{9} spores/ml with 2.96 and 5.04 days. Whereas, the highest LT_{50} and LT_{90} value was recorded at the concentration of 2×10^{3} spores/ml with 4.79 and 7.46 days.

4.3.2.2 Third instar

The third instar larval mortality of *H. armigera* at different concentration of *M. anisopliae* was presented in the table 19 and depicted in fig 13.

4.3.2.2.1 Forty eight hours after treatment

The mortality per cent ranged from 0.00 to 22.22 per cent. The highest mortality was recorded in the treatment 2×10^{10} spores/ml (22.22 per cent) which was significantly superior over all the treatments. The next most lethal concentration was 2×10^{9} spores/ml (17.77 per cent). Whereas, concentration 2×10^{5} spores/ml, 2×10^{4} spores/ml and 2×10^{3} spores/ml were showed non-significant difference with control (0.00 per cent).

4.3.2.2.2 Seventy two hours after treatment

The larval mortality ranged from 8.88 to 51.10 per cent. The highest mortality was recorded in the treatment 2×10^{10} spores/ml (51.10 per cent) which was significantly superior over all treatments. The treatment 2×10^{5} spores/ml (15.55 per cent) was on par with treatment 2×10^{4} spores/ml (13.33 per cent). The lowest mortality was noticed in treatment 2×10^{3} spores/ml (8.88 per cent).

4.3.2.2.3 Ninety six hours after treatment

The mortality was ranged from 33.33 to 75.55 per cent. The highest lethal concentration was 2×10^{10} spores/ml (75.55 per cent) which was significantly superior over all the treatments. However, the second most lethal concentration was 2×10^{9} spores/ml (66.66 per cent). The concentration of 2×10^{7} spores/ml (42.22 per cent) was on par with the concentration of 2×10^{6} spores/ml (40.00 per cent). The least larval mortality was recorded at the concentration of 2×10^{3} spores/ml (33.33 per cent) which was also on par with the concentration of 2×10^{4} spores/ml (35.55 per cent).

4.3.2.2.4 One hundred twenty hours after treatment

All the tested concentration of *M. anisopliae* against third instar of *H. armigera* after one hundred twenty hours of treatment showed significantly superior over control with the range of 48.88 and 91.10 per cent larval mortality. The highest lethal concentration was 2×10^{10} spores/ml (91.10 per cent) which was on par with the second highest conidial concentration 2×10^{9} spores/ml (86.66 per cent). The lowest larval mortality recorded at the concentration of 2×10^{3} spores/ml (48.88 per cent).

4.3.2.2.5 LT_{50} and LT_{90} value

The value of LT_{50} and LT_{90} at third instar during 2021-2022 was shown in table 19. The highest LT_{50} and LT_{90} value was recorded at the concentration of 2×10^{3} spores/ml with 4.88 and 7.79 days. The lowest value was recorded at the concentration of 2×10^{10} spores/ml with 2.90 and 5.00 days of LT_{50} and LT_{90} value.

Table 19: Efficacy of *M. anisopliae* strain against third instar larvae of *H. armigera* during 2021-2022

Tr. Code	Concentrations (Spores ml^{-1})	Mortality Per cent at different time interval					LT_{50} (Days)	LT_{90} (Days)
		24 hours	48 hours	72 hours	96 hours	120 hours		
T_1	2×10^3	0.00 ± 0.00	0.00 ± 0.00F	8.88 ± 0.26G	33.33 ± 0.99F	48.88 ± 1.46EF	4.88	7.79
T_2	2×10^4	0.00 ± 0.00	0.00 ± 0.00F	13.33 ± 0.39F	35.55±1.06DEF	55.55 ± 1.66E	4.67	7.42
T_3	2×10^5	0.00 ± 0.00	0.00 ± 0.00F	15.55 ± 0.46F	37.77 ±1.13DE	62.22 ± 1.86DE	4.46	6.92
T_4	2×10^6	0.00 ± 0.00	4.44 ± 0.13E	20.00 ± 0.60E	40.00 ± 1.20D	71.10 ± 2.13CD	4.21	7.04
T_5	2×10^7	0.00 ± 0.00	6.66 ± 0.19D	28.88 ± 0.86D	42.22 ± 1.26D	75.55 ± 2.26BC	3.95	6.92
T_6	2×10^8	0.00 ± 0.00	8.88 ± 0.26C	33.33 ± 0.99C	48.88 ± 1.46C	82.22 ± 2.46B	3.68	6.33
T_7	2×10^9	0.00 ± 0.00	17.77 ± 0.53B	44.44 ± 1.33B	66.66 ± 1.99B	86.66 ± 2.59AB	3.16	5.63
T_8	2×10^{10}	0.00 ± 0.00	22.22 ± 0.66A	51.10 ± 1.53A	75.55 ± 2.26A	91.10 ± 2.73A	2.90	5.00
T_9	Control	0.00 ± 0.00	0.00 ± 0.00F	0.00 ± 0.00H	0.00 ± 0.00G	0.00 ± 0.00G	-	-

Number denote by same letter within the column are statistically non - significant (Tukey's test, $p < 0.05$) data show the mean of three.

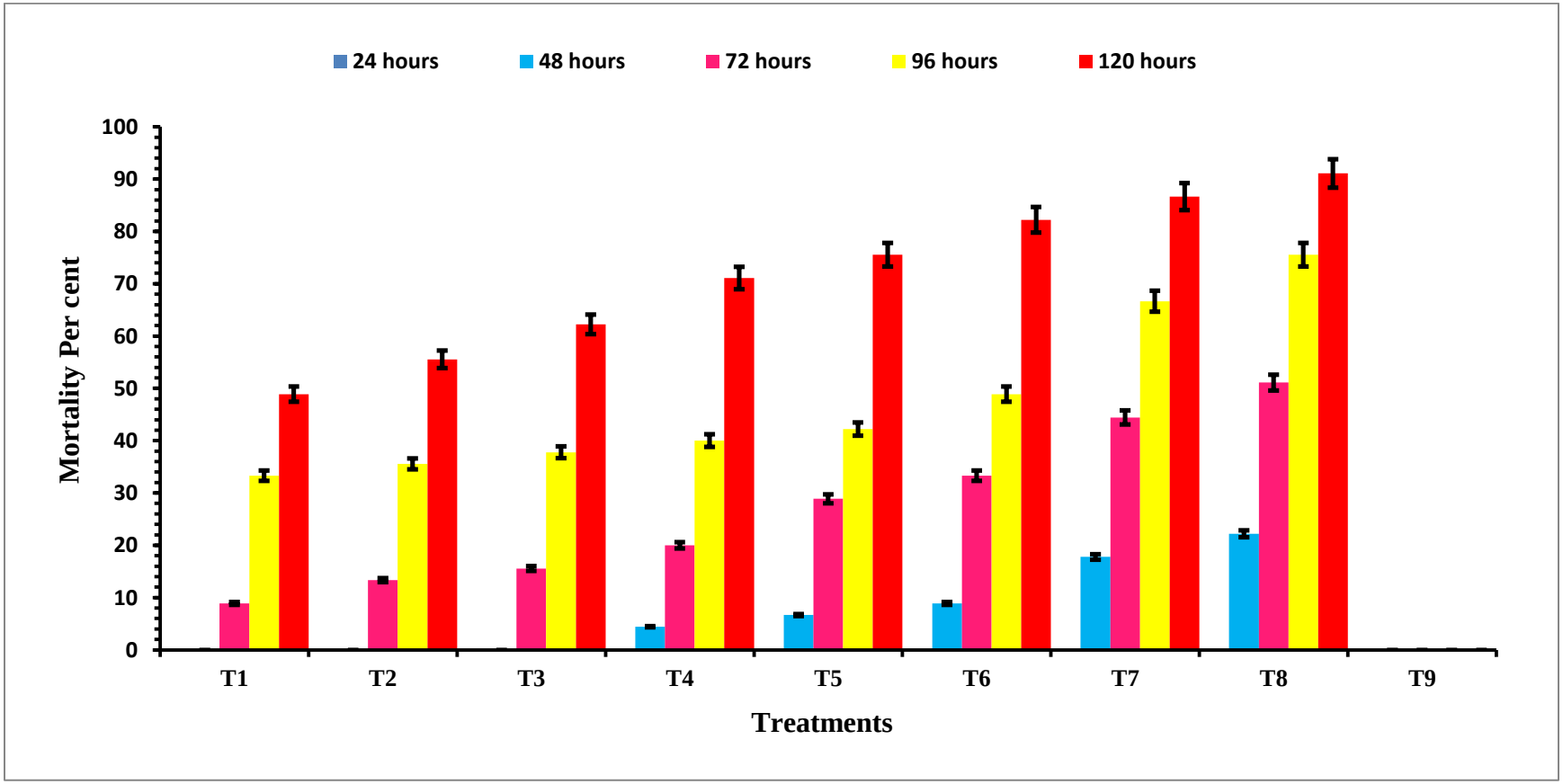


Fig. 13: Efficacy of *M. anisopliae* strain against third instar larvae of *H. armigera* during 2021-2022.

4.3.2.3 Fourth instar

The fourth instar larval mortality of *H. armigera* at different concentration of *M. anisopliae* after different hour's interval presented in table 20 and depicted in fig 14.

4.3.2.3.1 Forty eight hours after treatment

All the treatments were statistically non-significant except 2×10^{10} spores/ml (8.88 per cent) and 2×10^{9} spores/ml (4.44 per cent) after forty eight hours of treatment.

4.3.2.3.2 Seventy two hours after treatment

All the conidial concentration of *M. anisopliae* was superior over control and significantly different to each other with the range of 4.44 to 31.10 per cent. The highest larval mortality was recorded at the concentration of 2×10^{10} spores/ml (31.10 per cent) however the next best concentration of *M. anisopliae* was 2×10^{9} spores/ml (26.66 per cent). The lowest lethal concentration was 2×10^{3} spores/ml (4.44 per cent).

4.3.2.3.3 Ninety six hours after treatment

The larval mortality ranged from 24.44 to 62.22 per cent. The highest mortality was recorded in the treatment of 2×10^{10} spores/ml (62.22 per cent) which was statistically superior over all the treatments. The second best treatment was 2×10^{9} spores/ml (55.55 per cent) and it was on par with the treatment of 2×10^{8} spores/ml (51.10 per cent). The lowest mortality was observed in the treatment of 2×10^{3} spores/ml (24.44 per cent).

Table 20: Efficacy of *M. anisopliae* strain against fourth instar larvae of *H. armigera* during 2021-2022

Tr. Code	Concentrations (Spores ml^{-1})	Mortality Per cent at different time interval					LT_{50}	LT_{90}
		24 hours	48 hours	72 hours	96 hours	120 hours	(Days)	(Days)
T_1	2×10^3	0.00 ± 0.00	0.00 ± 0.00C	4.44 ± 0.13H	24.44 ± 0.73F	42.22 ± 1.26D	5.25	8.08
T_2	2×10^4	0.00 ± 0.00	0.00 ± 0.00C	6.66 ± 0.19G	33.33 ± 0.99DE	48.88 ± 1.46CD	4.88	7.54
T_3	2×10^5	0.00 ± 0.00	0.00 ± 0.00C	8.88 ± 0.26F	37.77 ±1.13D	53.33 ± 1.59C	4.71	7.25
T_4	2×10^6	0.00 ± 0.00	0.00 ± 0.00C	13.33 ± 0.39E	40.00 ±1.20D	58.88 ± 1.76BC	4.50	7.00
T_5	2×10^7	0.00 ± 0.00	0.00 ± 0.00C	17.77 ± 0.53D	46.66 ±1.39BC	66.66 ± 1.99B	4.21	6.46
T_6	2×10^8	0.00 ± 0.00	0.00 ± 0.00C	20.00 ± 0.60C	51.10 ±1.53B	73.33 ± 2.19B	4.02	6.00
T_7	2×10^9	0.00 ± 0.00	4.44 ± 0.13B	26.66 ± 0.79B	55.55 ±1.66B	82.22 ± 2.46A	3.72	5.83
T_8	2×10^{10}	0.00 ± 0.00	8.88 ± 0.26A	31.10 ± 0.93A	62.22 ± 1.86A	88.88 ± 2.66A	3.45	5.46
T_9	Control	0.00 ± 0.00	0.00 ± 0.00C	0.00 ± 0.00I	0.00 ± 0.00G	0.00 ± 0.00E	-	-

Number denote by same letter within the column are statistically non - significant (Tukey's test, $p < 0.05$) data show the mean of three.

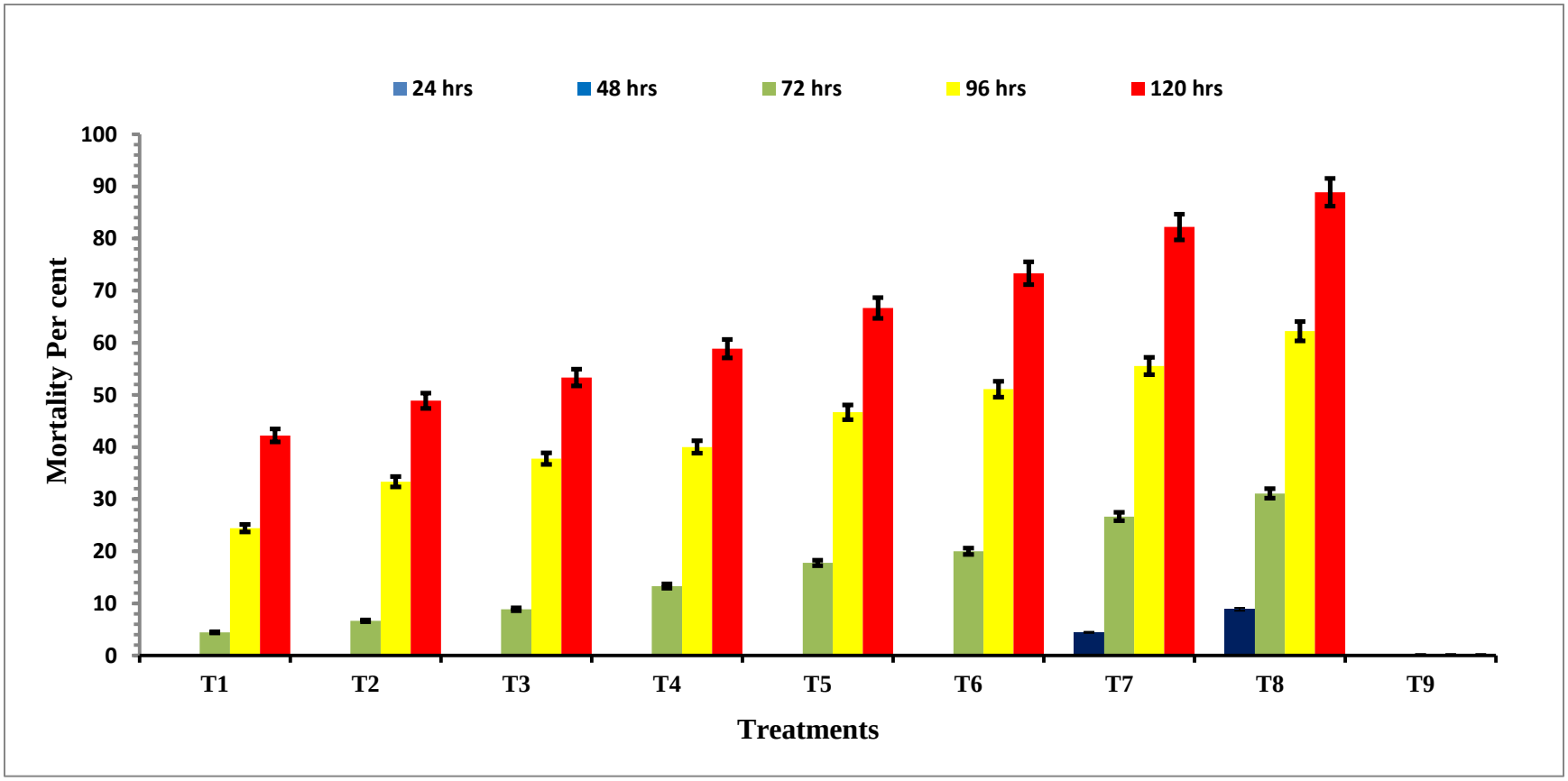


Fig. 14: Efficacy of *M. anisopliae* strain against fourth instar larvae of *H. armigera* during 2021-2022.

4.3.2.3.4 One hundred twenty hours after treatment

All the tested concentration of *M. anisopliae* against fourth instar of *H. armigera* after one hundred twenty hours of treatment showed significantly superior over control with the range of 42.22 and 88.88 per cent larval mortality. The highest lethal concentration was 2×10^{10} spores/ml (88.88 per cent) which was on par with second highest conidial concentration 2×10^{9} spores/ml (82.22 per cent). The lowest larval mortality recorded at the concentration of 2×10^{3} spores/ml (42.22 per cent).

4.3.2.3.5 LT_{50} and LT_{90} value

The value of LT_{50} and LT_{90} at fourth instar during 2021-2022 was shown in table 20. The highest LT_{50} and LT_{90} value was recorded at the concentration of 2×10^{3} spores/ml with 5.25 and 8.08 days. The lowest value was recorded at the concentration of 2×10^{10} spores/ml with 3.45 and 5.46 days of LT_{50} and LT_{90} value respectively.

4.3.3 Bioassay of *M. anisopliae* on *H. armigera* during 2020-2021 and 2021-2022 (Pooled)

4.3.3.1 Second instar

The pooled second instar larval mortality of *H. armigera* at different concentration of *M. anisopliae* presented in table 21 and depicted in fig 15.

4.3.3.1.2 Twenty four hours after treatment

The pooled larval mortality of different concentration of *M. anisopliae* against individual larvae (II^{nd}, III^{rd} & IV^{th}) after twenty four hours was zero.

4.3.3.1.3 Forty two hours after treatment

All the concentrations of *M. anisopliae* were superior over control except the concentration of 2×10^3 spores/ml and 2×10^4 spores/ml which showed non-significant difference with control. The larval mortality ranged from 2.22 to 28.88 per cent. The highest mortality was recorded at the concentration of 2×10^{10} spores/ml (28.88 per cent) which was statistically superior over all the concentrations. The lowest mortality was noted at the concentration of 2×10^5 spores/ml (2.22 per cent).

4.3.3.1.4 Seventy two hours after treatment

All the concentrations were statistically superior over control with mortality range of 12.21 to 54.44 per cent. The most lethal concentration was 2×10^{10} spores/ml (54.44 per cent) which was significantly different over all the treatments. The second most lethal concentration against second instar larvae of *H. armigera* was 2×10^9 spores/ml (47.77 per cent). The lowest mortality was recorded at the concentration of 2×10^3 spores/ml (12.21 per cent) which was on par with the concentration of 2×10^4 spores/ml (15.55 per cent).

4.3.3.1.5 Ninety six hours after treatment

The larval mortality of *H. armigera* ranged from 30.00 to 84.44 per cent. The highest larval mortality was recorded at the concentration of 2×10^{10} spores/ml (84.44 per cent) which was superior over all the concentrations. The second best conidial concentration was 2×10^9 spores/ml (74.44 per cent). The conidial concentration 2×10^8 spores/ml (52.22 per cent) on par with the concentration of 2×10^7 spores/ml (48.88 per cent). The lowest mortality was recorded at

the concentration of 2×10^3 spores/ml (30.00 per cent) which was non-significant with 2×10^4 spores/ml (33.33 per cent).

4.3.3.1.6 One hundred twenty hours after treatment

All the tested concentrations of *M. anisopliae* against second instar of *H. armigera* after one hundred twenty hours of treatment showed significantly superior over control with the range of 54.44 and 94.33 per cent larval mortality. The highest lethal concentration was 2×10^{10} spores/ml (94.33 per cent) which was on par with second highest conidial concentration 2×10^9 spores/ml (91.10 per cent). However the conidial concentration of 2×10^6 spores/ml (72.22 per cent) showed non-significant difference with the concentration of 2×10^5 spores/ml (66.66 per cent). The lowest larval mortality was recorded at the concentration of 2×10^3 spores/ml (54.44 per cent).

4.3.3.1.7 LT_{50} and LT_{90} value

The pooled LT_{50} and LT_{90} value at different concentrations of *M. anisopliae* against second instars larvae of *H. armigera* presented in table 21. The highest LT_{50} and LT_{90} value was recorded at low concentration 2×10^3 spores/ml with 4.79 and 7.58 days followed by 2×10^4 spores/ml, 2×10^5 spores/ml, 2×10^6 spores/ml, 2×10^7 spores/ml, 2×10^8 spores/ml and 2×10^9 spores/ml with 4.58, 4.33, 4.08, 3.68, 3.46 & 2.99 and 7.20, 7.25, 7.20, 6.45, 6.08, and 5.04 days respectively. The highest concentration 2×10^{10} spores/ml exhibited more mortality with 2.68 and 4.54 days of LT_{50} and LT_{90} value.

Table 21: Efficacy of *M. anisopliae* strain against second instar larvae of *H. armigera* during 2020-2021 & 2021-2022 (Pooled)

Tr. Code	Concentrations (Spores ml^{-1})	Mortality Per cent at different time interval					LT_{50} (Days)	LT_{90} (Days)
		24 hours	48 hours	72 hours	96 hours	120 hours		
T_1	2×10^3	0.00 ± 0.00	0.00 ± 0.00G	12.21 ± 0.36H	30.00 ± 0.89E	54.44 ± 1.63E	4.79	7.58
T_2	2×10^4	0.00 ± 0.00	0.00 ± 0.00G	15.55 ± 0.46H	33.33 ± 0.99E	60.00 ± 1.79DE	4.58	7.20
T_3	2×10^5	0.00 ± 0.00	2.22 ± 0.06F	22.49 ± 0.67F	36.66 ±1.09D	66.66 ± 1.99D	4.33	7.25
T_4	2×10^6	0.00 ± 0.00	5.55 ± 0.16E	27.77 ± 0.83E	40.00 ±1.19D	72.22 ± 2.16CD	4.08	7.20
T_5	2×10^7	0.00 ± 0.00	10.00 ± 0.29D	33.33 ± 0.99D	48.88 ± 1.46C	81.11 ± 2.43C	3.68	6.45
T_6	2×10^8	0.00 ± 0.00	13.33 ± 0.39C	37.77 ± 1.13C	52.22 ± 1.56C	86.66 ± 2.59B	3.46	6.08
T_7	2×10^9	0.00 ± 0.00	18.89 ± 0.56B	47.77 ± 1.43B	74.44 ± 2.23B	91.10 ± 2.73AB	2.99	5.04
T_8	2×10^{10}	0.00 ± 0.00	28.88 ± 0.86A	54.44 ± 1.63A	84.44 ± 2.53A	94.33 ± 2.82A	2.68	4.54
T_9	Control	0.00 ± 0.00	0.00 ±0.00G	0.00 ± 0.00I	0.00 ± 0.00F	0.00 ± 0.00E	-	-

Number denote by same letter within the column are statistically non - significant (Tukey's test, $p < 0.05$) data show the mean of three.

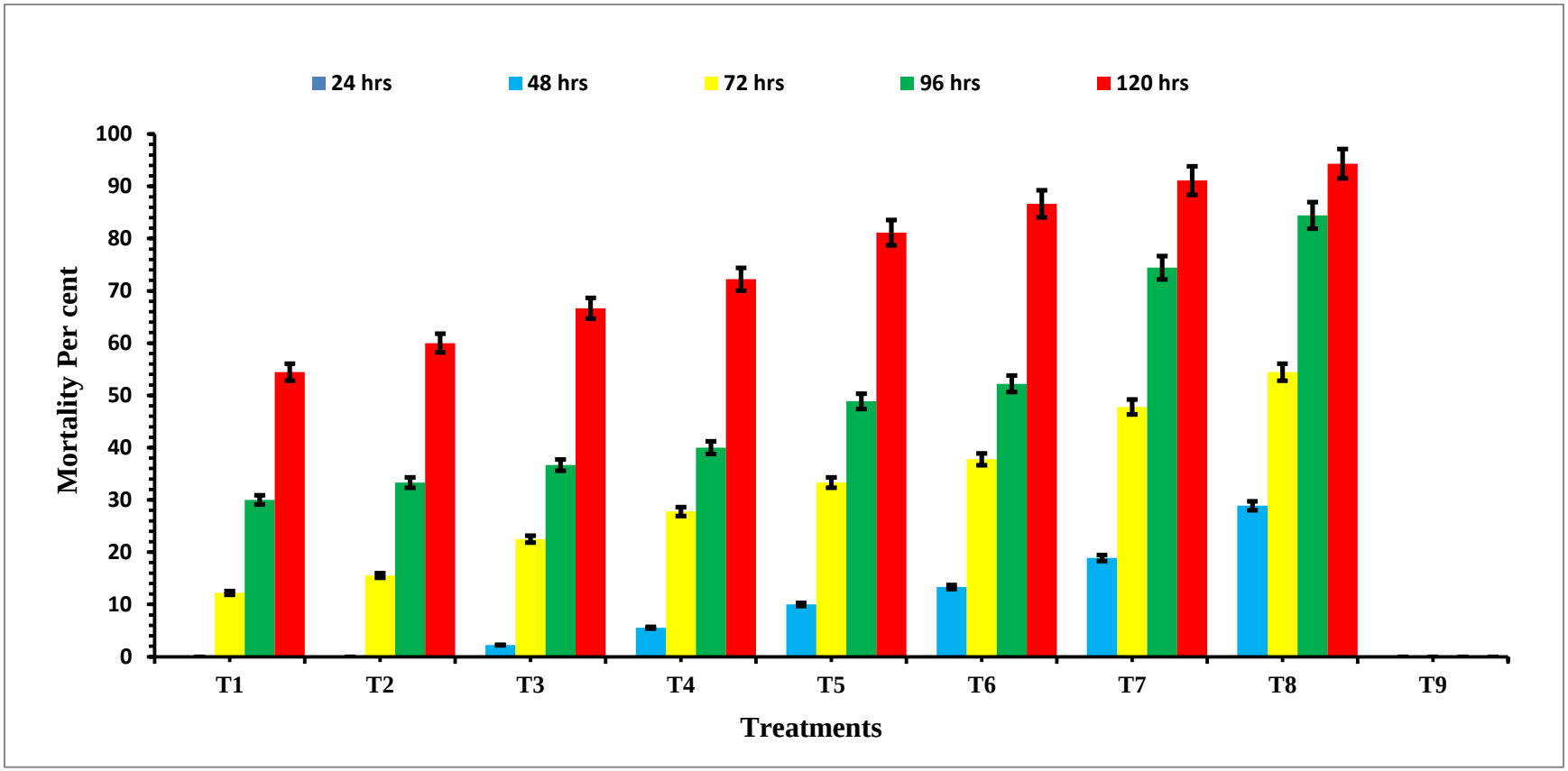


Fig. 15: Efficacy of *M. anisopliae* strain against second instar larvae of *H. armigera* during 2020-2021 & 2021-2022 (Pooled).

4.3.3.2 Third instar larva

The pooled third instar larval mortality of *H. armigera* at different concentration of *M. anisopliae* presented in table 22 and dipicted fig 16.

4.3.3.2.1 Forty eight hours after treatment

The pooled statistical data revealed that the larval mortality of *H. armigera* after forty eight hours of treatment ranged from 0.00 to 21.11 per cent. The maximum larval mortality recorded at the conidial concentration of 2×10^{10} spores/ml (21.11 per cent) which was significantly superior over all the concentrations. However, the second most lethal conidial concentration was 2×10^{9} spores/ml (15.55 per cent). The conidial concentration 2×10^{5} spores/ml, 2×10^{4} spores/ml and 2×10^{3} spores/ml was non-significantly different with 0.00 per cent larval mortality.

4.3.3.2.2 Seventy two hours after treatment

The pooled effect of different concentration of *M. anisopliae* on third instar of *H. armigera* after seventy two hours of treatment ranged from 8.88 to 49.99 per cent. The maximum larval mortality was recorded at the concentration of 2×10^{10} spores/ml (49.99 per cent) which was statistically superior over all the concentrations. The lowest larval mortality was observed at the concentration of 2×10^{3} spores/ml (8.88 per cent) which was on par with the concentration of 2×10^{4} spores/ml (12.22 per cent).

4.3.3.2.3 Ninety six hours after treatment

The pooled larval mortality per cent of *H. armigera* at different conidial concentrations ranged from 34.44 to 75.55 per cent. The highest larval mortality was observed at the

concentration of 2×10^{10} spores/ml (75.55 per cent). The second most lethal conidial concentration was 2×10^{9} spores/ml (67.77 per cent). However, the conidial concentration 2×10^{8} spores/ml (49.99 per cent) was on par with the concentration of 2×10^{9} spores/ml (45.55 per cent). The minimum larval mortality was noticed at the concentration 2×10^{3} spores/ml (34.44 per cent) which was on par with the concentration of 2×10^{4} spores/ml (36.66 per cent).

4.3.3.2.4 One hundred twenty hours after treatment

The pooled effect of all the tested concentration of *M. anisopliae* against third instar of *H. armigera* after one hundred twenty hours of treatment were significantly superior over control with the range of 49.99 and 91.10 per cent larval mortality. The highest lethal concentration was 2×10^{10} spores/ml (91.10 per cent) which was on par with second highest conidial concentration 2×10^{9} spores/ml (87.77 per cent). However the conidial concentration of 2×10^{8} spores/ml (83.33 per cent) showed non-significant difference with the concentration of 2×10^{7} spores/ml (77.78 per cent). The lowest larval mortality recorded at the concentration of 2×10^{3} spores/ml (49.99 per cent).

4.3.3.2.5 LT_{50} and LT_{90} Value

The pooled LT_{50} and LT_{90} value at different concentrations of *M. anisopliae* against third instars larvae of *H. armigera* presented in table 22. The highest LT_{50} and LT_{90} value was recorded at low concentration 2×10^{3} spores/ml with 4.83 and 7.63 days followed by 2×10^{4} spores/ml, 2×10^{5} spores/ml, 2×10^{6} spores/ml, 2×10^{7} spores/ml, 2×10^{8} spores/ml and 2×10^{9} spores/ml with 4.58, 4.38, 4.17, 3.85, 3.63 & 3.20 and 7.13, 6.83, 6.96, 6.63, 6.21 and 5.50 days respectively. The highest concentration 2×10^{10} spores/ml exhibited more mortality with 2.93 and 5.00 days of LT_{50} and LT_{90} value.

Table 22: Efficacy of *M. anisopliae* strain against third instar larvae of *H. armigera* during 2020-2021 & 2021-2022 (Pooled)

Tr. Code	Concentrations (Spores ml^{-1})	Mortality Per cent at different time interval					LT_{50} (Days)	LT_{90} (Days)
		24 hours	48 hours	72 hours	96 hours	120 hours		
T_1	2×10^3	0.00 ± 0.00	0.00 ± 0.00F	8.88 ± 0.26G	34.44 ±1.03E	49.99 ± 1.49DE	4.83	7.63
T_2	2×10^4	0.00 ± 0.00	0.00 ± 0.00F	12.22 ± 0.36G	36.66 ±1.09DE	57.78 ± 1.73D	4.58	7.13
T_3	2×10^5	0.00 ± 0.00	0.00 ± 0.00F	16.66 ± 0.49F	38.88 ±1.16D	63.33 ± 1.89CD	4.38	6.83
T_4	2×10^6	0.00 ± 0.00	3.33 ± 0.09E	21.11 ± 0.63E	41.11 ±1.23CD	69.99 ± 2.09C	4.17	6.96
T_5	2×10^7	0.00 ± 0.00	6.66 ± 0.19D	29.99 ± 0.89D	45.55 ± 1.36C	77.78 ± 2.33BC	3.85	6.63
T_6	2×10^8	0.00 ± 0.00	8.88 ± 0.26C	34.44 ± 1.03C	49.99 ± 1.49C	83.33 ± 2.49B	3.63	6.21
T_7	2×10^9	0.00 ± 0.00	15.55 ± 0.46B	42.22 ± 1.26B	67.77 ± 2.03B	87.77 ± 2.63AB	3.20	5.50
T_8	2×10^{10}	0.00 ± 0.00	21.11 ± 0.63A	49.99 ± 1.49A	75.55 ± 2.26A	91.10 ± 2.73A	2.93	5.00
T_9	Control	0.00 ± 0.00	0.00 ± 0.00F	0.00 ± 0.00H	0.00 ± 0.00F	0.00 ± 0.00	-	-

Number denote by same letter within the column are statistically non - significant (Tukey's test, $p < 0.05$) data show the mean of three.

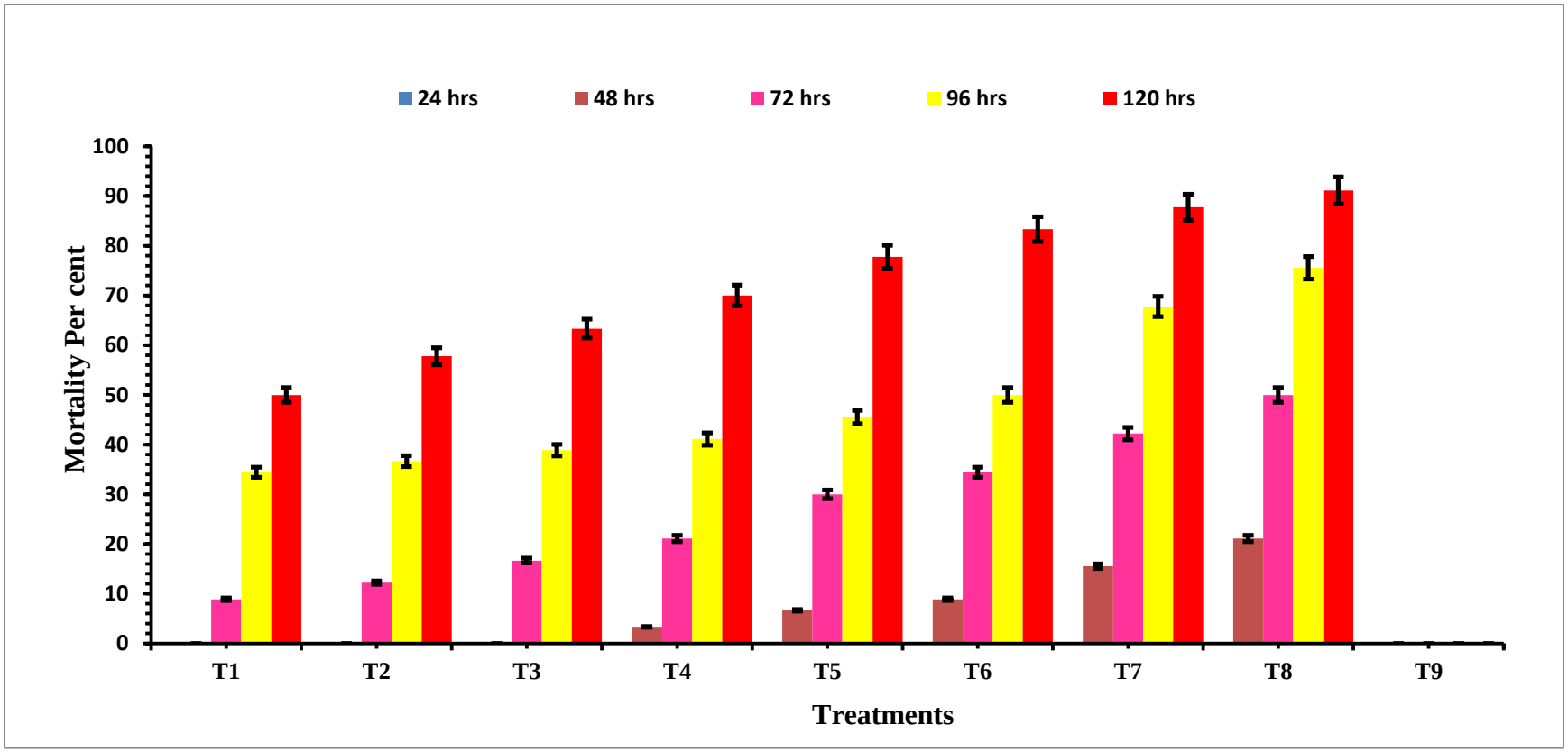


Fig. 16: Efficacy of *M. anisopliae* strain against third instar larvae of *H. armigera* during 2020-2021 & 2021-2022 (Pooled).

4.3.2.3 Fourth instar

The pooled second instar larval mortality of *H. armigera* at different concentration of *M. anisopliae* presented in table 23 and depicted in fig 17.

4.3.2.3.1 Forty eight hours after treatment

The pooled larval mortality after forty eight hours of treatment ranged from 0.00 to 8.88 per cent. The highest larval mortality was recorded at the concentration of 2×10^{10} spores/ml (8.88 per cent) which was significantly superior over all the concentrations. The concentration 2×10^{7} spores/ml, 2×10^{6} spores/ml, 2×10^{5} spores/ml, 2×10^{4} and 2×10^{3} spores/ml spores/ml showed non-significant difference with 0.00 per cent larval mortality.

4.3.2.3.2 Seventy two hours after treatment

After seventy two hours of treatment all conidial concentration showed significant difference over control with the ranged of 5.55 to 32.22 per cent larval mortality. As the third instar the highest mortality was recorded at the concentration of 2×10^{5} spores/ml (32.22 per cent) which was significantly superior over all the concentrations. The other concentration i.e. 2×10^{8} spores/ml (22.22 per cent) and 2×10^{7} spores/ml (20.00 per cent) on par with each other and the concentration 2×10^{5} spores/ml (11.11 per cent) was also on par with 2×10^{4} spores/ml (8.89 per cent). The minimum larval mortality was recorded at the concentration of 2×10^{3} spores/ml (5.55 per cent).

4.3.2.3.3 Ninety six hours after treatment

The pooled larval mortality per cent of *H. armigera* at different conidial concentrations ranged from 26.66 to 61.11 per cent. The highest larval mortality was observed at the

concentration of 2×10^{10} spores/ml (61.11 per cent). The second most lethal conidial concentration was 2×10^{9} spores/ml (54.44 per cent). However, the conidial concentration 2×10^{8} spores/ml (49.99 per cent) was on par with the concentration of 2×10^{9} spores/ml (54.44 per cent). The conidial concentration 2×10^{6} spores/ml (38.89 per cent) and 2×10^{5} spores/ml (36.66 per cent) were also significantly similar. The minimum larval mortality was noticed at the concentration 2×10^{3} spores/ml (26.66 per cent).

4.3.2.3.4 One hundred twenty hours after treatment

The pooled larval mortality per cent of *H. armigera* at different conidial concentration of *M. anisopliae* after one hundred twenty hours of treatment ranged from 41.11 to 87.77 per cent. The highest lethal conidial concentration was 2×10^{10} spores/ml (87.77) was on par with the concentration of 2×10^{9} spores/ml (81.11 per cent). However, the concentration 2×10^{9} spores/ml was also on par with 2×10^{8} spores/ml (74.44 per cent). The minimum larval mortality was recorded at the concentration of 2×10^{3} spores/ml (41.11 per cent) which was statistically similar with the concentration of 2×10^{4} spores/ml (47.77 per cent).

4.3.2.3.4 LT_{50} and LT_{90} Value

The pooled LT_{50} and LT_{90} Value at different concentration of *M. anisopliae* against *H. armigera* were shown in table 23 with range of 5.25 & 8.42 days to 3.46 & 5.54 days. The highest LT_{50} and LT_{90} value was recorded at low concentration of 2×10^{3} spores/ml with 5.25 and 8.42 days followed by 2×10^{4} spores/ml, 2×10^{5} spores/ml, 2×10^{6} spores/ml, 2×10^{7} spores/ml, 2×10^{8} spores/ml and 2×10^{9} spores/ml with 4.96, 4.67, 4.46, 4.21, 3.99 & 3.72 and 7.92, 7.33, 7.08, 6.50, 6.00 and 6.00 days respectively. The highest concentration 2×10^{10} spores/ml exhibited more pathogenic with 3.46 and 5.54 days of LT_{50} and LT_{90} value.

Table 23: Efficacy of *M. anisopliae* strain against fourth instar larvae of *H. armigera* during 2020-2021 & 2021-2022 (Pooled)

Tr. Code	Concentrations (Spores ml^{-1})	Mortality Per cent at different time interval					LT_{50} (Days)	LT_{90} (Days)
		24 hours	48 hours	72 hours	96 hours	120 hours		
T_1	2×10^3	0.00 ± 0.00	0.00 ± 0.00C	5.55 ± 0.16F	26.66 ± 0.79F	41.11 ± 1.23E	5.25	8.42
T_2	2×10^4	0.00 ± 0.00	0.00 ± 0.00C	8.89 ± 0.26E	32.22 ± 0.96DE	47.77 ±1.43DE	4.96	7.92
T_3	2×10^5	0.00 ± 0.00	0.00 ± 0.00C	11.11 ± 0.33E	36.66 ± 1.09D	54.44 ± 1.63D	4.67	7.33
T_4	2×10^6	0.00 ± 0.00	0.00 ± 0.00C	16.67 ± 0.49D	38.89 ± 1.16D	60.55 ±1.81CD	4.46	7.08
T_5	2×10^7	0.00 ± 0.00	0.00 ± 0.00C	20.00 ± 0.59C	44.44 ± 1.33C	67.77 ± 2.03C	4.21	6.50
T_6	2×10^8	0.00 ± 0.00	0.00 ± 0.00C	22.22 ± 0.66C	49.99 ± 1.49B	74.44 ± 2.23B	3.99	6.00
T_7	2×10^9	0.00 ± 0.00	5.55 ± 0.16B	27.77 ± 0.83B	54.44 ± 1.63B	81.11 ± 2.43AB	3.72	6.00
T_8	2×10^{10}	0.00 ± 0.00	8.88 ± 0.26A	32.22 ± 0.96A	61.11 ± 1.83A	87.77 ± 2.63A	3.46	5.54
T_9	Control	0.00 ± 0.00	0.00 ± 0.00C	0.00 ± 0.00G	0.00 ± 0.00F	0.00 ± 0.00F	-	-

Number denote by same letter within the column are statistically non - significant (Tukey's test, $p < 0.05$) data show the mean of three.

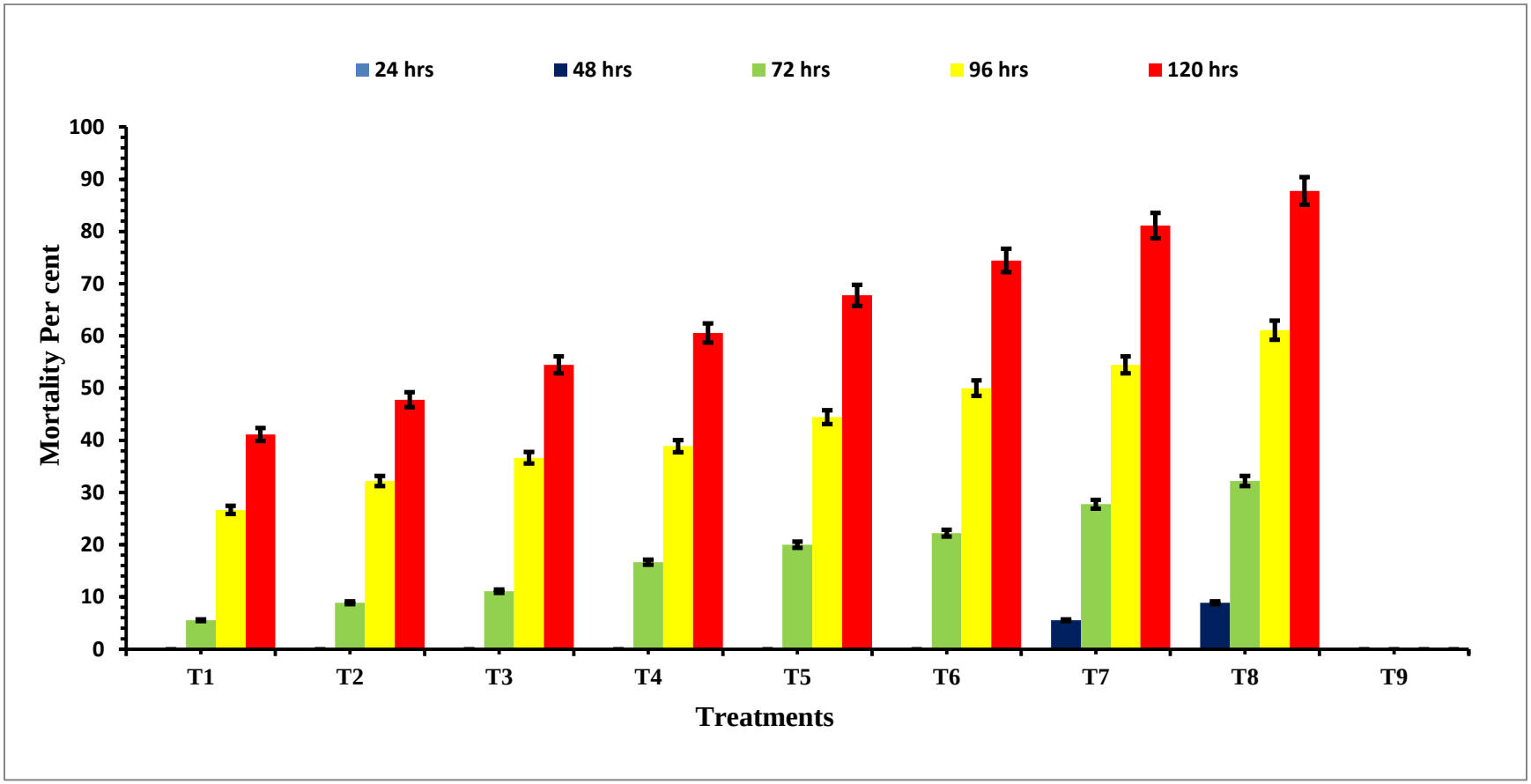


Fig. 17: Efficacy of *M. anisopliae* strain against fourth instar larvae of *H. armigera* during 2020-2021 & 2021-2022 (Pooled).

Plate 11: Rearing of *H. armingera* on artificial diet.

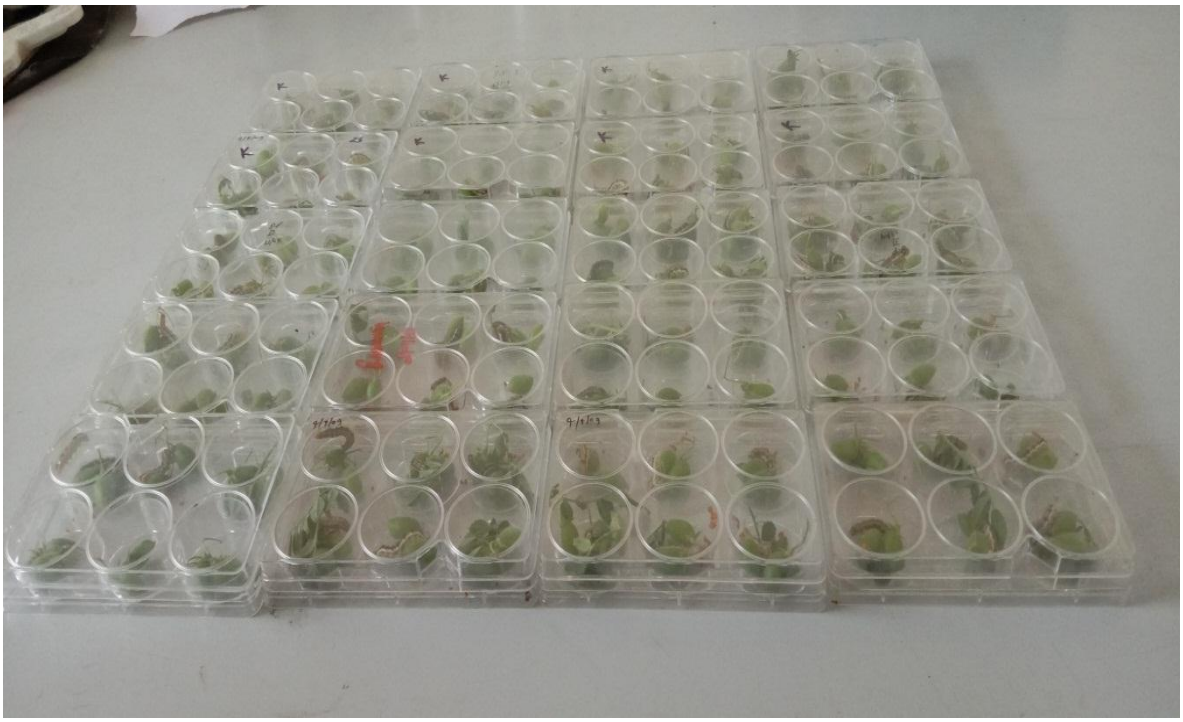


Plate 12: Bioassay of different concentration of *M. anisopliae* against *H. armigera*.

Plate 13:Parasitised larvae of *H. armigera* in lab condition.

Plate 14: Parasitised Pupae of *H. armigera* in lab condition.

The present findings are supported by **Nguyen *et al.*, (2007)** who reported that 90.30 per cent mortality against second instar larvae, 82.00 per cent mortality against third instar larvae and 87.00 per cent mortality against fourth instar larvae of *H. armigera* after two week of exposure. Similar observations made by **Fite *et al.*, (2019)** who reported that higher conidial concentration of entomopathogentic fungi cause more mortality and found highest larval mortality at 1×10^9 spores/ml with 71.00 per cent after eleven days of post treatment. The present findings are also agree with the finding of **Rijal *et al.*, (2008)** who reported that larval mortality at seven days and ten days after treatment was 65.33 and 86.67 per cent with 1×10^7 spores/ml conidial concentration. The present findings are in agreement with **Sabry *et al.*, (2011)** who reported the 60.00 and 80.00 per cent larval mortality after sixth days of treatment on second and third instar larvae of *H. armigera*. The larval stage did not differ in their mortality but differed in median lethal time, with shortest value recorded in the second instar.

The present findings of LT_{50} and LT_{90} supported with the finding of **Rijal *et al.*, (2008)** who reported that the LT_{50} value of best isolate was 5.33 days. The present findings are also in agreement with **Fite *et al.*, (2019)** who reported that the half lethal time (LT_{50}) increased with a decrease in conidial concentration and they found 6.20 days of LT_{50} value at the concentration of 1×10^9 spores/ml. These findings are also supported by **Nguyen *et al.*, (2007)** who mentioned that the LT_{50} value of 3.00 days at the concentration of 1×10^7 spores/ml.

4.4 Bioefficacy of *Metarhizium anisopliae* against *Helicoverpa armigera* on chickpea crop

The data recorded in chickpea crop against pod borer, *H. armigera* at pre-treatment stage of the crop revealed the mean larval population was homogenous and there was non-significant difference between all the treatments. A sharp decline in the larval population density of *H.*

armigera was recorded at third day, seventh day and fourteenth day after application of each spray compared to control. All the treatments were performed at this stage to minimize the pod borer infestation on chickpea crop.

4.4.1 Efficacy of *M. anisopliae* in field condition against *H. armigera* during *Rabi* season 2020-2021

4.4.1.1 First spray

The observation of larval reduction per cent of *H. armigera* were recorded three days after application. All the treatments were superior over control except treatment 2×10^3 @ 5ml/lit water. The larval reduction per cent among all the treatments was found in the range of 0.00 to 34.33 during *Rabi,* 2020 -2021 (Table 24 and Fig 18). The highest larval reduction per cent was recorded in concentration of 2×10^{10} @ 5ml/lit water with 34.33 per cent followed by 2×10^9 @ 5ml/lit water with 28.14 per cent, 2×10^8 @ 5ml/lit water with 21.66 per cent, 2×10^7 @ 5ml/lit water with 18.76 per cent, 2×10^6 @ 5ml/lit water with 15.57 per cent, 2×10^5 @ 5ml/lit with 9.38 per cent and 2×10^4 @ 5ml/lit water with 6.19 per cent.

Seven days after first spray the highest larval reduction per cent recorded in treatment 2×10^{10} @ 5ml/lit water (44.50 per cent). Among all the concentrations the least effective treatment was 2×10^3 @ 5ml/lit water with 19.50 larval reduction per cent of *H. armigera* followed by 2×10^4 @ 5ml/lit, 2×10^5 @ 5ml/lit, 2×10^6 @ 5ml/lit, 2×10^7 @ 5ml/lit, 2×10^8 @ 5ml/lit and 2×10^9 @ 5ml/lit water with larval reduction per cent of 22.25, 25.00, 27.83, 33.33, 36.17 and 38.92 per ten plants, respectively.

All the treatments were superior over control fourteen days after first spray. The reduction per cent of *H. armigera* was ranged from 38.46 to 76.92 per ten plants. The highest reduction per cent was

observed in 2×10^{10} @ 5ml/lit (76.92 per ten plants) followed by 2×10^{9} @ 5ml/lit 2×10^{8} @ 5ml/lit, 2×10^{7} @ 5ml/lit, 2×10^{6} @ 5ml/lit 2×10^{5} @ 5ml/lit 2×10^{4} @ 5ml/lit water with 71.85, 69.23, 66.69, 53.85, 48.77 and 41.08 reduction per cent per ten plants, respectively. The least reduction per cent was observed in 2×10^{3} @ 5ml/lit with 38.46 per ten plants.

4.4.1.2 After second spray

The result presented in table 24 and depicted fig 18 revealed that the mean larval reduction per cent of *H. armigera* ranged from 24.98 to 45.01 per ten plants during *Rabi,* 2020-2021. The data recorded after three days of second spray showed that all the treatments were superior over control. The highest reduction percentage was observed in treatment 2×10^{10} @ 5ml/lit water with 45.01 followed by 2×10^{9} @ 5ml/lit 2×10^{8} @ 5ml/lit, 2×10^{7} @ 5ml/lit, 2×10^{6} @ 5ml/lit, 2×10^{5} @ 5ml/lit, 2×10^{4} @ 5ml/lit and 2×10^{3} @ 5ml/lit water with 42.54, 39.98, 37.51, 35.03, 32.48, 27.53 and 24.98 per ten plants respectively. There was significant difference between all the treatments.

At seven days after second spray, highest reduction per cent 56.08 was recorded in treatment 2×10^{10} @ 5ml/lit water which was significantly superior over the rest treatments followed by 2×10^{9} @ 5ml/lit water with 51.24 per cent. However, the untreated control recorded lowest mortality per cent with 0.00 per ten plants.

After fourteenth days of treatment, similar trend was found such as seventh day. All the treatments were found superior over control the highest reduction per cent was recorded in treatment 2×10^{10} @ 5ml/lit water with 79.06 per cent followed by 2×10^{9} @ 5ml/lit 2×10^{8} @ 5ml/lit, 2×10^{7} @ 5ml/lit, 2×10^{6} @ 5ml/lit, 2×10^{5} @ 5ml/lit, 2×10^{4} @ 5ml/lit water with

74.46, 72.09, 58.13, 55.83, 51.15 and 44.17 per cent respectively. The lowest reduction per cent observed in treatment 2×10^3 @ 5ml/lit water with 29.87 per ten plants.

On the basis of overall mean of first and second spray, the larval reduction per cent of chickpea pod borer, ranged from 25.30 to maximum of 55.98 per cent. On the basis of overall mean the treatment 2×10^{10} @ 5ml/lit water was found to be most effective as it recorded the highest larval reduction 55.98 per cent which was significantly better than all the treatments. The least effective treatment was 2×10^3 @ 5ml/lit water with 25.30 per cent larval reduction of *H. armigera* followed by 2×10^4 @ 5ml/lit, 2×10^5 @ 5ml/lit, 2×10^6 @ 5ml/lit, 2×10^7 @ 5ml/lit, 2×10^8 @ 5ml/lit and 2×10^9 @ 5ml/lit water with 28.82, 33.90, 37.86, 42.64, 47.61 and 51.19 per cent reduction. All the treatments were significantly differing from each other.

4.4.1.3. Pod damage per cent

The statistically analyzed data of pod damage per cent under different treatments are presented in table 27 and depicted in fig 22. The mean pod damage per cent ranged from 36.66 to 6.66 during *Rabi*, 2020-2021. All the treatments were found superior over control and the lowest pod damage per cent 6.66 noticed in plot treated with 2×10^{10} @ 5ml/lit water followed by 2×10^9 @ 5ml/lit 2×10^8 @ 5ml/lit, 2×10^7 @ 5ml/lit, 2×10^6 @ 5ml/lit, 2×10^5 @ 5ml/lit, 2×10^4 @ 5ml/lit water with 8.00, 10.33, 12.00, 14.33, 17.66 and 20.00. The highest pod damage per cent observed after control was in treatment 2×10^3 @ 5ml/lit water with 22.33 per cent.

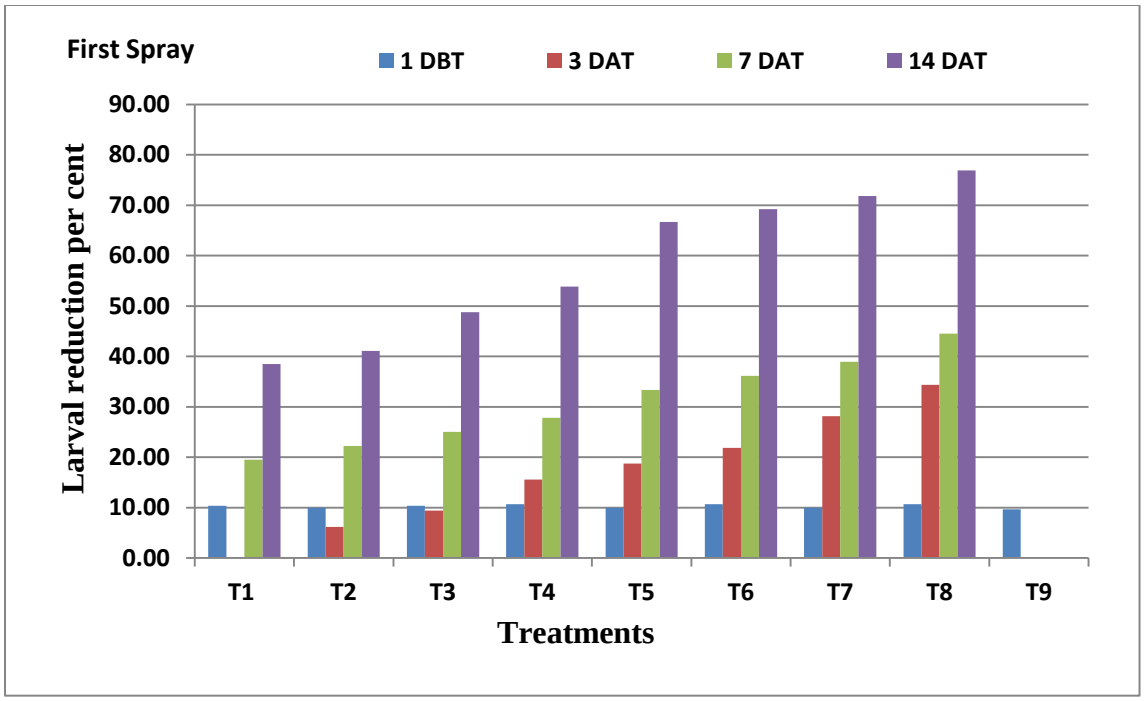
Table 24: Efficacy of *M. anisopliea* in field condition against *H. armigera Rabi*, 2020-2021

TREATMENTS	DOSE ML/LIT WATER	LARVAL REDUCTION PER CENT/10 PLANTS							OVERALL MEAN
		NO. OF LARVA/10 PLANTS	FIRST SPRAY			SECOND SPRAY			
		1 DBT	3 DAT	7 DAT	14 DAT	3 DAT	7 DAT	14 DAT	
$2 \times 10^3 + 0.5\ \%$ JAGGERY	5	10.33	0.00	19.50	38.46	24.98	29.28	39.57	25.30
		(18.71)	(0.00)	(26.19)	(38.31)	(29.96)	(32.75)	(38.98)	(30.18)
$2 \times 10^4 + 0.5\ \%$ JAGGERY	5	10.00	6.19	22.25	41.08	27.53	31.70	44.17	28.82
		(18.42)	(14.41)	(28.14)	(39.83)	(31.63)	(34.23)	(41.63)	(32.46)
$2 \times 10^5 + 0.5\ \%$ JAGGERY	5	10.33	9.38	25.00	48.77	32.48	36.60	51.15	33.90
		(18.71)	(17.84)	(30.00)	(44.27)	(34.74)	(37.21)	(45.65)	(35.59)
$2 \times 10^6 + 0.5\ \%$ JAGGERY	5	10.66	15.57	27.83	53.85	35.03	39.02	55.83	37.86
		(19.05)	(2.22)	(31.82)	(47.19)	(36.27)	(38.62)	(48.33)	(37.96)
$2 \times 10^7 + 0.5\ \%$ JAGGERY	5	10.00	18.76	33.33	66.69	37.51	41.43	58.13	42.64
		(18.42)	(25.65)	(35.22)	(54.74)	(37.74)	(40.05)	(49.66)	(40.74)
$2 \times 10^8 + 0.5\ \%$ JAGGERY	5	10.66	21.86	36.17	69.23	39.98	46.34	72.09	47.61
		(19.05)	(27.86)	(36.97)	(56.29)	(39.21)	(42.86)	(58.08)	(43.60)
$2 \times 10^9 + 0.5\ \%$ JAGGERY	5	10.00	28.14	38.92	71.85	42.54	51.24	74.46	51.19
		(18.42)	(32.01)	(38.57)	(57.93)	(40.68)	(45.68)	(59.63)	(45.66)
$2 \times 10^{10} + 0.5\ \%$ JAGGERY	5	10.66	34.33	44.50	76.92	45.01	56.08	79.06	55.98
		(19.05)	(35.85)	(41.82)	(61.26)	(42.13)	(48.48)	(62.77)	(48.42)

CONTROL	-	9.66 (18.10)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
CD		**NA**	**0.643**	**0.974**	**1.815**	**1.078**	**1.246**	**1.876**	**1.202**
SEM±		**NA**	**0.213**	**0.322**	**0.600**	**0.504**	**0.412**	**0.620**	**0.398**

Angular transformation

DBT – Day Before Treatment, DAT – Day After Treatment.



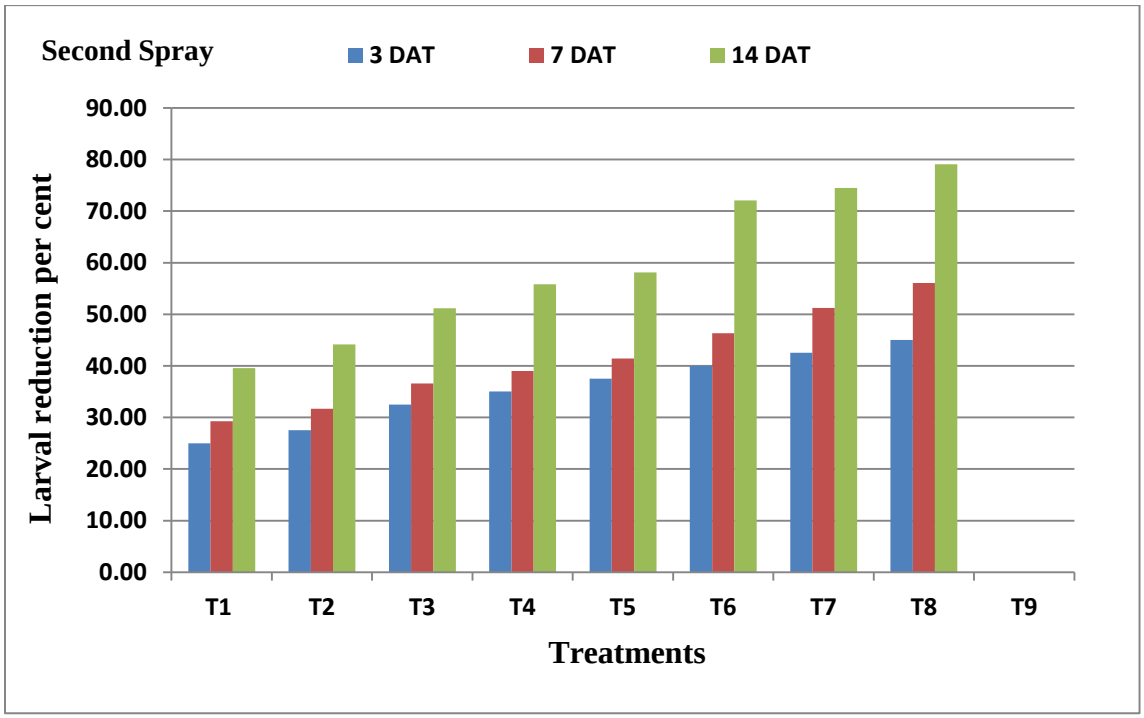


Fig. 18: Effect of different treatments on population of *H. armigera* after first and second spray during *Rabi*, 2020-2021.

4.4.2 Efficacy of *M. anisopliae* in field condition against *H. armigera* during *Rabi*, 2021-2022

4.4.2.1 First spray

The statistically analyzed result presented in table 25 and depicted in fig 19. The result recorded the mean larval reduction per cent of *H. armigera* ranged from 0.00 to 43.40 per cent after three days of first spray. Among all the treatments maximum reduction of *H. armigera* was recorded in treatment 2×10^{10} @ 5ml/lit water with 43.40 per cent. The other treatments were followed by 2×10^{9} @ 5ml/lit, 2×10^{8} @ 5ml/lit, 2×10^{7} @ 5ml/lit, 2×10^{6} @ 5ml/lit, 2×10^{5} @ 5ml/lit, 2×10^{4} @ 5ml/lit water with 40.00, 33.40, 30.00, 23.40, 20.00 and 10.00 per cent respectively. The minimum reduction per cent recorded in the treatment 2×10^{3} @ 5ml/lit water with 3.40 per ten plants.

At seventh day after first spray the statistically analyzed data revealed the maximum reduction per cent and minimum reduction per cent with 59.38 and 28.14. The highest reduction was noticed in higher conidial concentration treatment 2×10^{10} @ 5ml/lit water with 59.38 per cent and the next better treatment was 2×10^{9} @ 5ml/lit, 2×10^{8} @ 5ml/lit, 2×10^{7} @ 5ml/lit, 2×10^{6} @ 5ml/lit, 2×10^{5} @ 5ml/lit and 2×10^{4} @ 5ml/lit water with 53.10, 46.90, 43.71, 37.52, 34.33 and 31.24 per cent respectively. The lowest reduction per cent was observed in treatment 2×10^{3} @ 5ml/lit water with 28.14 per ten plants.

The reduction per cent of *H. armigera* on chickpea at 14 days after first spray was much more similar to that of fourteenth day of first year. The maximum larval reduction was 75.02 per cent noticed in treatment 2×10^{10} @ 5ml/lit water. The next best treatment was 2×10^{9} @ 5ml/lit with 70.00 per cent. Whereas, the minimum reduction per cent was recorded in treatment 2×10^{3} @ 5ml/lit water with 45.01 per ten plants.

4.4.2.2 Second Spray

At three days after treatment the reduction per cent ranged from 0.00 to 57.14. The highest reduction per cent was noticed in treatment 2×10^{10} @ 5ml/lit water with 57.14 followed by 2×10^{9} @ 5ml/lit, 2×10^{8} @ 5ml/lit, 2×10^{7} @ 5ml/lit, 2×10^{6} @ 5ml/lit, 2×10^{5} @ 5ml/lit and 2×10^{4} @ 5ml/lit water with 52.43, 50.00, 47.64, 42.86, 40.50 and 35.71 per cent respectively. Treatment 2×10^{3} @ 5ml/lit water showed minimum reduction per cent with 31.00 per ten plants.

The reduction per cent of *H. armigera* on chickpea crop at seven days after treatment was much more similar to the first spray with the range of 38.61 to 61.39 per cent. The highest reduction was observed in the treatment 2×10^{10} @ 5ml/lit water with 61.39 per cent followed by treatments 2×10^{9} @ 5ml/lit, 2×10^{8} @ 5ml/lit, 2×10^{7} @ 5ml/lit, 2×10^{6} @ 5ml/lit, 2×10^{5} @ 5ml/lit and 2×10^{4} @ 5ml/lit water with 56.82, 54.57, 52.25, 45.43, 43.18 and 40.93 per cent. The minimum reduction per cent was recorded in low conidial concentration treatment 2×10^{3} @ 5ml/lit water with 38.61 per ten plants.

At fourteen days after treatment, the larval reduction was found in the range of 44.70 to 76.63 per cent. The highest reduction per cent noticed in treatment 2×10^{10} @ 5ml/lit water was 76.63 and the next best treatment was 2×10^{9} @ 5ml/lit water with 70.24 per cent. The least larval reduction was observed in treatment 2×10^{3} @ 5ml/lit water with 44.70 per cent.

All the treatments were significantly superior over control. The overall mean of first and second spray executed the larval reduction per cent of chickpea pod borer, *H. armigera* ranging from 31.81 to maximum 62.16 per cent. On the basis of overall mean treatment 2×10^{10} @ 5ml/lit water was found to be most effective as it recorded highest reduction 62.16 per cent which was significantly better than all tested treatments followed by treatment 2×10^{9} @ 5ml/lit, 2×10^{8} @

5ml/lit, 2×10^7 @ 5ml/lit, 2×10^6 @ 5ml/lit, 2×10^5 @ 5ml/lit and 2×10^4 @ 5ml/lit water with 57.10, 52.35, 48.93, 44.09, 40.21 and 35.71 per cent respectively. The treatment 2×10^3 @ 5ml/lit water with 31.81 per cent was found least effective.

4.4.2.3 Pod damage per cent

The statistically analyzed data (table 27 and fig 22) revealed that the mean pod damage per cent was in the range of 7.33 to 37.17. The highest pod damage per cent was noticed in control with 37.17 per cent and least pod damage per cent was 7.33 in 2×10^{10} @ 5ml/lit water treatments followed by 2×10^9 @ 5ml/lit, 2×10^8 @ 5ml/lit, 2×10^7 @ 5ml/lit, 2×10^6 @ 5ml/lit, 2×10^5 @ 5ml/lit and 2×10^4 @ 5ml/lit water with 9.33, 11.33, 13.33, 16.66, 19.00 and 21.33 respectively. The highest pod damage per cent recorded after control was 23.00.

4.4.3 Efficacy of *M. anisopliae* in field condition against *H. armigera* during *Rabi*, 2020-2021 & 2021-2022 (Pooled)

All the treatments were found effective and significantly superior over control when the data of both years were pooled (Table 26 and Fig 20). At one day before spray the mean larval population of *H. armigera* ranged from 9.33 to 10.16 per ten plants.

After three days after first and second spray the highest pooled reduction per cent of *H. armigera* was found at the rate of 38.78 and 51.08 in the treatment 2×10^{10} @ 5ml/lit water. After first and second spray the next effective treatments were 2×10^9 @ 5ml/lit water with 34.07 & 47.48, 2×10^8 @ 5ml/lit water with 27.63 & 44.99, 2×10^7 @ 5ml/lit water with 24.38 & 42.58, 2×10^6 @ 5ml/lit water with 19.49 & 38.95, 2×10^5 @ 5ml/lit water with 14.69 & 36.49 and 2 ×

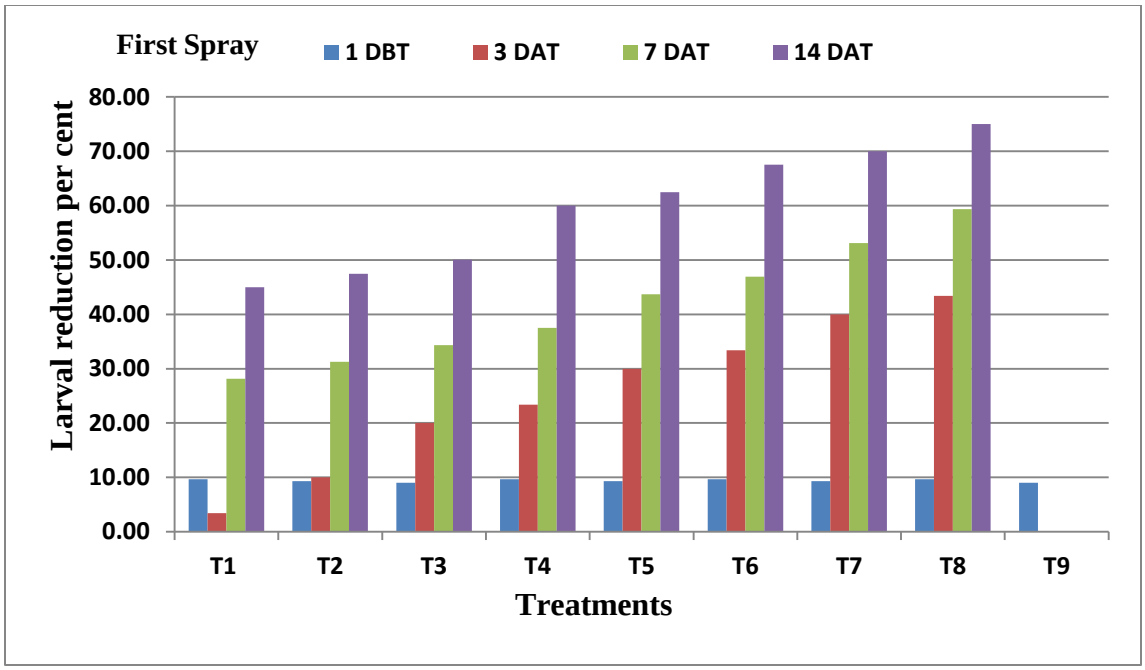
Table 25: Efficacy of *M. anisopliea* in field condition against *H. armigera Rabi*, 2021-2022

TREATMENTS	DOSE ML/LIT WATER	NO. OF LARVA/10 PLANTS	LARVAL REDUCTION PER CENT/10 PLANTS						OVERALL MEAN
			FIRST SPRAY			SECOND SPRAY			
		1 DBT	3 DAT	7 DAT	14 DAT	3 DAT	7 DAT	14 DAT	
$2 \times 10^3 + 0.5\%$ JAGGERY	5	9.66	3.40	28.14	45.01	31.00	38.61	44.70	31.81
		(18.10)	(10.62)	(32.09)	(42.13)	(33.81)	(38.41)	(41.94)	(34.33)
$2 \times 10^4 + 0.5\%$ JAGGERY	5	9.33	10.00	31.24	47.49	35.71	40.93	48.91	35.71
		(17.78)	(18.42)	(33.96)	(43.54)	(36.67)	(39.75)	(44.35)	(36.67)
$2 \times 10^5 + 0.5\%$ JAGGERY	5	9.00	20.00	34.33	50.04	40.50	43.18	53.19	40.21
		(17.44)	(26.55)	(35.85)	(44.98)	(39.50)	(41.07)	(48.81)	(39.33)
$2 \times 10^6 + 0.5\%$ JAGGERY	5	9.66	23.40	37.52	60.02	42.86	45.43	55.30	44.09
		(18.10)	(28.91)	(37.74)	(50.75)	(40.88)	(42.36)	(48.02)	(41.59)
$2 \times 10^7 + 0.5\%$ JAGGERY	5	9.33	30.00	43.71	62.49	47.64	52.25	57.47	48.93
		(17.78)	(33.19)	(41.36)	(52.22)	(43.62)	(46.28)	(49.27)	(44.37)
$2 \times 10^8 + 0.5\%$ JAGGERY	5	9.66	33.40	46.90	67.52	50.00	54.57	61.69	52.35
		(18.10)	(35.28)	(43.20)	(55.22)	(44.98)	(47.60)	(51.72)	(46.31)
$2 \times 10^9 + 0.5\%$ JAGGERY	5	9.33	40.00	53.10	70.00	52.43	56.82	70.24	57.10
		(17.78)	(39.21)	(46.75)	(56.77)	(46.37)	(48.89)	(56.90)	(49.06)
$2 \times 10^{10} + 0.5\%$ JAGGERY	5	9.66	43.40	59.38	75.02	57.14	61.39	76.63	62.16
		(18.10)	(41.19)	(50.40)	(60.01)	(49.08)	(51.55)	(61.06)	(52.02)

CONTROL	-	9.00 (17.44)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
CD @ 5 %		**NA**	**0.848**	**1.247**	**1.825**	**1.307**	**1.434**	**1.795**	**1.365**
SEM±		**NA**	**0.280**	**0.412**	**0.854**	**0.432**	**0.474**	**0.594**	**0.451**

Angular transformation

DBT – Day Before Treatment, DAT – Day After Treatment.



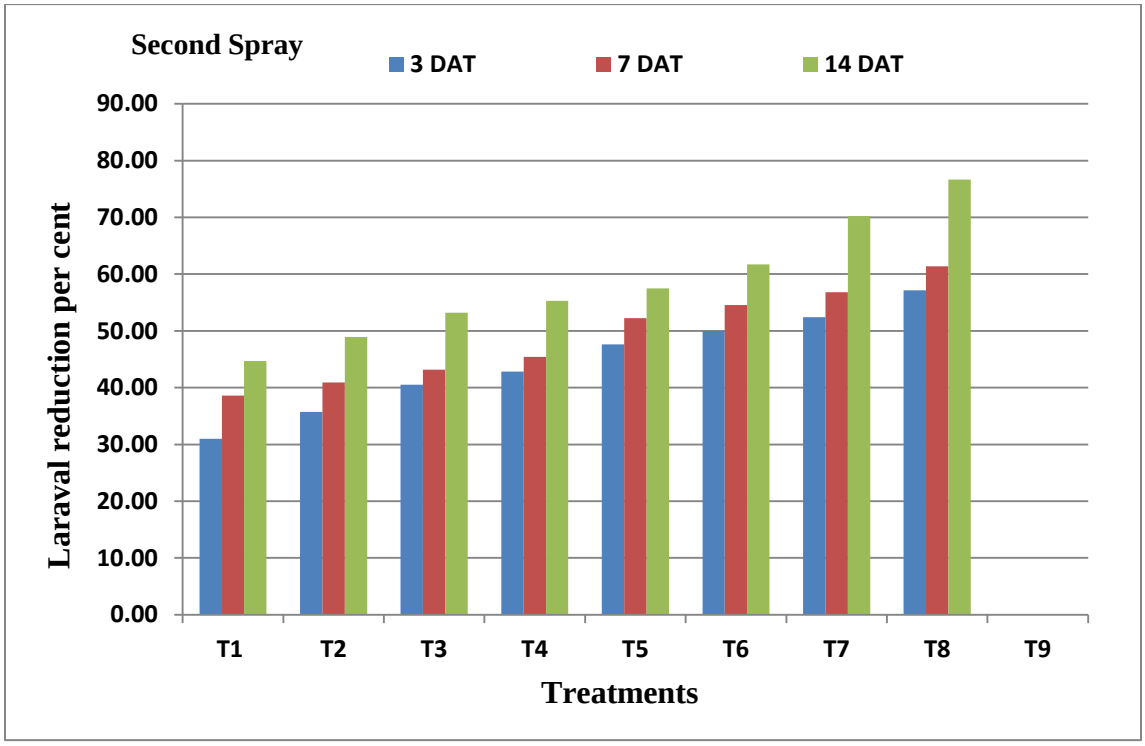


Fig. 19: Effect of different treatments on population of *H. armigera* after first and second spray during *Rabi*, 2021-2022.

10^4 @ 5ml/lit water with 8.10 & 31.61 per cent. The treatments 2×10^3 @ 5ml/lit water showed minimum reduction per cent after and second spray with 1.70 & 27.99 per cent larval reduction.

After seven days of first spray the highest pooled larval reduction of *H. armigera* was 51.94 per cent in the treatment of 2×10^{10} @ 5ml/lit water and the sequence of effectiveness were 2×10^9 @ 5ml/lit, 2×10^8 @ 5ml/lit, 2×10^7 @ 5ml/lit, 2×10^6 @ 5ml/lit, 2×10^5 @ 5ml/lit and 2×10^4 @ 5ml/lit water with 46.01, 41.54, 38.52, 32.68, 29.67 and 26.74 per cent respectively. The lowest larval reduction was observed in treatment 2×10^3 @ 5ml/lit water with 23.82 per cent. The same pattern was found after fourteen days of spray with highest pooled larval reduction 75.97 and the lowest reduction 22.85 per cent in the treatments 2×10^{10} @ 5ml/lit and 2×10^3 @ 5ml/lit water respectively.

After seven days of second spray the pooled larval reduction per cent ranged from 33.95 to 58.73 per ten plants. The highest larval reduction observed in treatment 2×10^{10} @ 5ml/lit water with 5 per 8.73 cent followed by 2×10^9 @ 5ml/lit, 2×10^8 @ 5ml/lit, 2×10^7 @ 5ml/lit, 2×10^6 @ 5ml/lit, 2×10^5 @ 5ml/lit and 2×10^4 @ 5ml/lit water with 54.03, 50.45, 46.84, 42.22, 39.89 and 36.31 per cent respectively. The minimum reduction noticed in treatment 2×10^3 @ 5ml/lit water 33.95 per cent.

Almost same pattern was observed after fourteen days of first and second spray, the highest pooled larval reduction per cent of *H. armigera* was 75.97 and 77.85 per cent recorded in the plots treated with 2×10^{10} @ 5ml/lit water and the next effective treatment was 2×10^9 @ 5ml/lit water with 70.92 and 72.35 per cent followed by treatments 2×10^8 @ 5ml/lit, 2×10^7 @ 5ml/lit, 2×10^6 @ 5ml/lit, 2×10^5 @ 5ml/lit and 2×10^4 @ 5ml/lit water with 68.37, 64.59, 56.93,

49.40, 44.28 and 66.89, 57.80, 55.56, 52.17, 46.54 per cent respectively. The least pooled larval reduction was recorded 41.74 and 41.13 per cent in the plot treated with 2×10^3 @ 5ml/lit water after first and second spray.

The overall mean of pooled larval reduction per cent ranged from 17.00 to 65.49 per cent. The highest reduction was recorded 65.49 per cent in the treatment of 2×10^{10} @ 5ml/lit water followed by 2×10^9 @ 5ml/lit, 2×10^8 @ 5ml/lit, 2×10^7 @ 5ml/lit, 2×10^6 @ 5ml/lit, 2×10^5 @ 5ml/lit and 2×10^4 @ 5ml/lit water with 54.14, 49.98, 45.79, 40.97, 37.05 and 32.27 per cent respectively. The lowest reduction per cent was found in the treatment of 2×10^3 @ 5ml/lit water with 28.55 per cent.

4.4.4 Pooled effect of different treatment on pod infestation by *H. armigera* during *Rabi*, 2020-21 & 2021-22

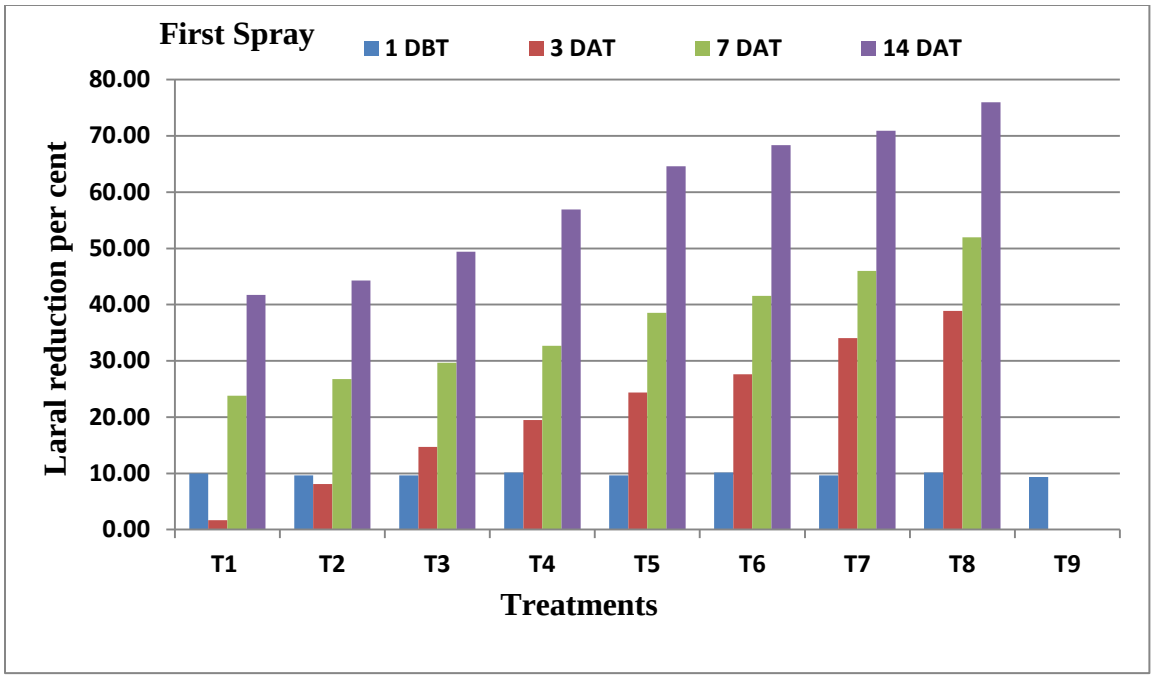
All the treatments were found significantly superior over control when the data of both years were pooled (Table 27 and Fig 22). The statistically analyzed pooled data revealed that the per cent pod damage caused by this pest ranged from 7.33 to 37.17 per cent. The treatment 2×10^{10} @ 5ml/lit water found best among all the treatments with minimum pod damage of 7.33 per cent followed by 2×10^9 @ 5ml/lit, 2×10^8 @ 5ml/lit, 2×10^7 @ 5ml/lit, 2×10^6 @ 5ml/lit, 2×10^5 @ 5ml/lit, 2×10^4 @ 5ml/lit and 2×10^3 @ 5ml/lit water with 8.67, 10.83, 12.67, 15.50, 18.33, 20.67 and 22.67 per cent respectively. The highest pod damage was noticed in control with 37.17 per cent.

Table 26: Efficacy of *M. anisopliea* in field condition against *H. armigera* during *Rabi* 2020-21, 2021-22 (Pooled)

TREATMENTS	DOSE ML/LIT WATER	LARVAL REDUCTION PER CENT/10 PLANTS							OVERALL MEAN
		NO. OF LARVA/10 PLANTS	FIRST SPRAY			SECOND SPRAY			
		1 DBT	3 DAT	7 DAT	14 DAT	3 DAT	7 DAT	14 DAT	
$2 \times 10^3 + 0.5\%$ JAGGERY	5	9.99	1.70	23.82	41.74	27.99	33.95	42.13	28.55
		(18.42)	(7.48)	(29.18)	(40.22)	(31.93)	(35.61)	(40.45)	(32.29)
$2 \times 10^4 + 0.5\%$ JAGGERY	5	9.66	8.10	26.74	44.28	31.62	36.31	46.54	32.27
		(18.13)	(16.52)	(31.09)	(41.70)	(4.20)	(37.03)	(42.97)	(34.59)
$2 \times 10^5 + 0.5\%$ JAGGERY	5	9.66	14.69	29.67	49.40	36.49	39.89	52.17	37.05
		(18.13)	(22.50)	(33.00)	(44.63)	(37.15)	(39.15)	(46.22)	(37.48)
$2 \times 10^6 + 0.5\%$ JAGGERY	5	10.16	19.49	32.68	56.93	38.95	42.22	55.56	40.97
		(18.61)	(26.19)	(34.86)	(48.94)	(38.59)	(40.51)	(48.17)	(39.77)
$2 \times 10^7 + 0.5\%$ JAGGERY	5	9.66	24.38	38.52	64.59	42.58	46.84	57.80	45.79
		(18.13)	(29.56)	(38.35)	(53.47)	(40.72)	(43.17)	(49.46)	(42.57)
$2 \times 10^8 + 0.5\%$ JAGGERY	5	10.16	27.63	41.54	68.37	44.99	50.45	66.89	49.98
		(18.61)	(31.69)	(40.10)	(55.76)	(42.09)	(45.24)	(54.86)	(44.98)
$2 \times 10^9 + 0.5\%$ JAGGERY	5	9.66	34.07	46.01	70.92	47.48	54.03	72.35	54.14
		(18.13)	(35.69)	(42.68)	(57.36)	(43.54)	(47.29)	(58.27)	(47.35)
$2 \times 10^{10} + 0.5\%$ JAGGERY	5	10.16	38.87	51.94	75.97	51.08	58.73	77.85	59.07
		(18.61)	(38.55)	(46.09)	(60.65)	(45.59)	(50.01)	(61.90)	(50.20)

CONTROL	-	9.33 (17.78)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
CD @ 5 %		**NA**	**0.767**	**1.106**	**1.818**	**1.182**	**1.340**	**1.818**	**1.29**
SEM±		**NA**	**0.254**	**0.336**	**0.601**	**0.391**	**0.443**	**0.601**	**0.42**

Angular transformation , DBT – Day Before Treatment, DAT – Day After Treatment.



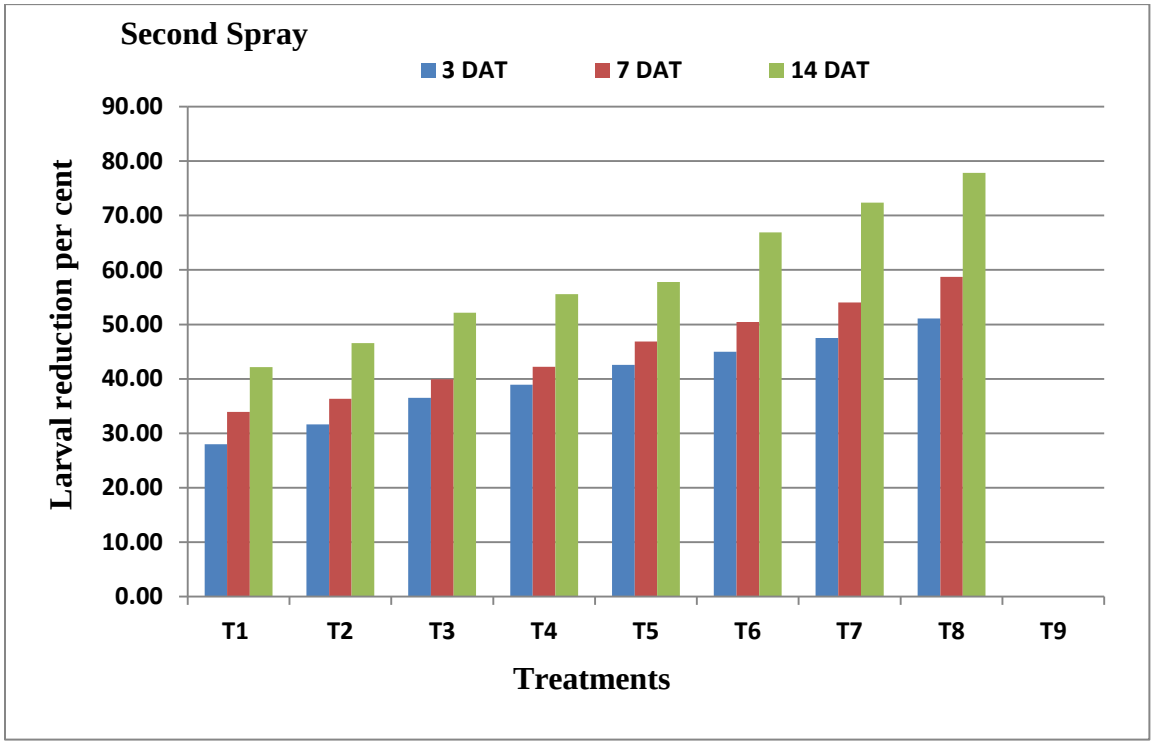


Fig. 20: Effect of different treatments on population of *H. armigera* after first and second spray during *Rabi*, 2020-2021 & 2021-2022 (Pooled).

The present findings also get support from the observations of **Savita *et al.* (2015)** who reported that the mortality of *H. armigera* population gradually increase with conidial concentration of the fungus and mentioned 1.10 larvae/five plants with pod damage 9.50 per cent and 14.50 q/ha yield at 1×10^{10} conidial concentration. Similar observations made by **Agale *et al.* (2017)** who reported that higher conidial concentration of *M. anisopliae* 4.3×10^3 conidia/ml caused 50 per cent larval mortality. The present findings also agree with the finding of **Lagogiannis *et al.* (2020)** who reported that the high yield obtained from the concentrations of 10^7 and 10^8 conidia/ml of all three fungi pathogenic with larval mortality ranging between 87 to 100 per cent at nine days and they also mentioned the lower doses of 10^3, 10^4 and 10^5 which had produced zero mortality by day three, only the higher doses 10^6, 10^7 and 10^8 induced the mortality significantly different from the control. The larval mortality at 13 days 100 per cent @ 10^8 conidial concentration. The present findings are in agreement with **Mohan kumar (2016)** who reported that 68.43 mortality per cent at 2×10^9 conidial concentration at 14 days after treatment. This finding are also in accordance with **Phukon *et al.* (2014)** who reported reduction in fruit damage up to 87.01 per cent over control at 1×10^9 conidial concentration of *M. anisopliae* and 3.8 larvae/15 plants after 7 DAT at vegetative stage and 1.2 larvae/15 plants at fruiting stage.

4.4.5 Effect of different treatments on grain yield in chickpea during *Rabi,* 2020-2021

The data recorded on grain yield during *Rabi*, 2020-2021 indicated that all the concentrations of *M. anisopliae* gave significantly higher yield as compared to control (Table 28 and Fig 23). the maximum grain yield was obtained in the treatment 2×10^{10} @ 5ml/lit water at the rate of 16.22 q/ha and followed by 2×10^9 @ 5ml/lit, 2×10^8 @ 5ml/lit, 2×10^7 @ 5ml/lit, 2×10^6 @ 5ml/lit, 2×10^5 @ 5ml/lit, 2×10^4 @ 5ml/lit and 2×10^3 @ 5ml/lit with grain yield 15.00,

13.98, 13.00, 12.68,12.00, 11.26 and 11.00 q/ha respectively. However, the lowest grain yield was recorded in control with 10.22 q/ha.

4.4.5.1 Effect of different treatments on grain yield in chickpea during *Rabi*, 2021-2022

The statistically analyzed data on grain yield under different treatments are presented in table 28 and depicted in fig 23 all the concentrations gave higher yield and were found superior over control during *Rabi*, 2021-2022. The maximum grain yield with 17.40 q/ha was recorded in the plots treated with 2×10^{10} @ 5ml/lit water while in treatment 2×10^{9} @ 5ml/lit was 16.12 q/ha. The rest treatments i.e. 2×10^{8} @ 5ml/lit, 2×10^{7} @ 5ml/lit, 2×10^{6} @ 5ml/lit, 2×10^{5} @ 5ml/lit, 2×10^{4} @ 5ml/lit and 2×10^{3} @ 5ml/lit produced 15.22, 14.22, 12.90, 12.20, 11.36 and 11.10 q/ha grain yield respectively. The lowest grain yield with 10.24 q/ha was recorded in control.

4.4.5.2 Pooled effect of different treatments on grain yield in chickpea during *Rabi*, 2020-2021 and 2021-2022

All the concentration of entomopathogenic fungus *M. anisopliae* gave higher yield when the data of both years were pooled and found superior over control (Table 28 and Fig 23). The maximum pooled grain yield of 16.18 q/ha was recorded with treatment 2×10^{10} @ 5ml/lit water and 2×10^{9} @ 5ml/lit water was second best treatment with 15.56 q/ha grain yield. The next treatments in order were 2×10^{8} @ 5ml/lit, 2×10^{7} @ 5ml/lit, 2×10^{6} @ 5ml/lit, 2×10^{5} @ 5ml/lit, 2×10^{4} @ 5ml/lit and 2×10^{3} @ 5ml/lit with grain yield 14.49, 13.61,12.79, 12.10, 11.31 and 11.05 q/ha respectively.

Table 27: Pod damage per cent during *Rabi* Season 2020-21, 2021-22 and Pooled

TRE. CODE	TREATMENTS	DOSE /LIT. WATER	POD DAMAGE PER CENT		
			2020-21	2021-22	POOLED
T_1	$2 \times 10^3 + 0.5\ \%$ JAGGERY	5	22.33 (4.83)	23.00 (4.89)	22.67 (4.86)
T_2	$2 \times 10^4 + 0.5\ \%$ JAGGERY	5	20.00 (4.58)	21.33 (4.72)	20.67 (4.65)
T_3	$2 \times 10^5 + 0.5\ \%$ JAGGERY	5	17.66 (4.30)	19.00 (4.47)	18.33 (4.39)
T_4	$2 \times 10^6 + 0.5\ \%$ JAGGERY	5	14.33 (3.95)	16.66 (4.20)	15.50 (4.06)
T_5	$2 \times 10^7 + 0.5\ \%$ JAGGERY	5	12.00 (3.60)	13.33 (3.78)	12.67 (3.69)
T_6	$2 \times 10^8 + 0.5\ \%$ JAGGERY	5	10.33 (3.36)	11.33 (3.51)	10.83 (3.44)
T_7	$2 \times 10^9 + 0.5\ \%$ JAGGERY	5	8.00 (3.00)	9.33 (3.21)	8.67 (3.10)
T_8	$2 \times 10^{10} + 0.5\ \%$ JAGGERY	5	6.66 (2.77)	8.00 (3.00)	7.33 (2.88)
T_9	CONTROL	-	36.33 (6.10)	38.00 (6.24)	37.17 (6.18)
	CD @ 5 %		**0.108**	**0.111**	**0.110**
	SEM±		**0.036**	**0.037**	**0.036**

Square root transformation

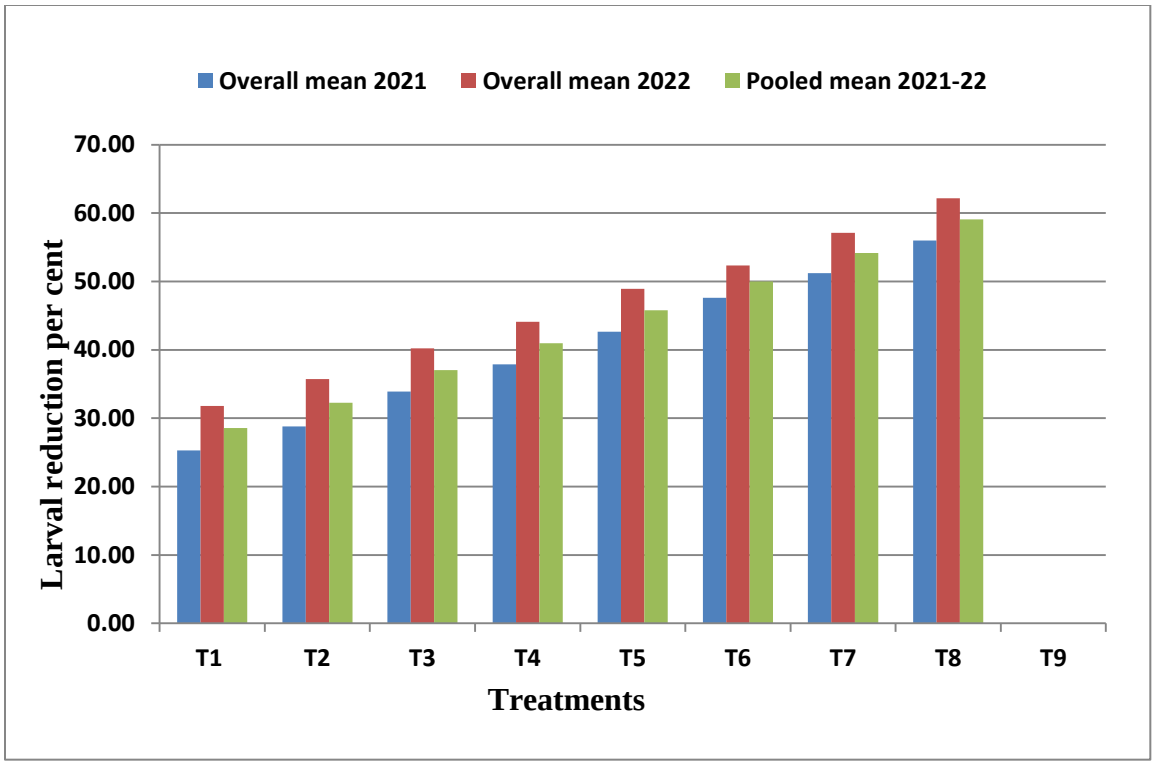


Fig. 21: Effect of different treatments overall mean on *H. armigera* population with pooled during *Rabi*, Season 2021, 2022.

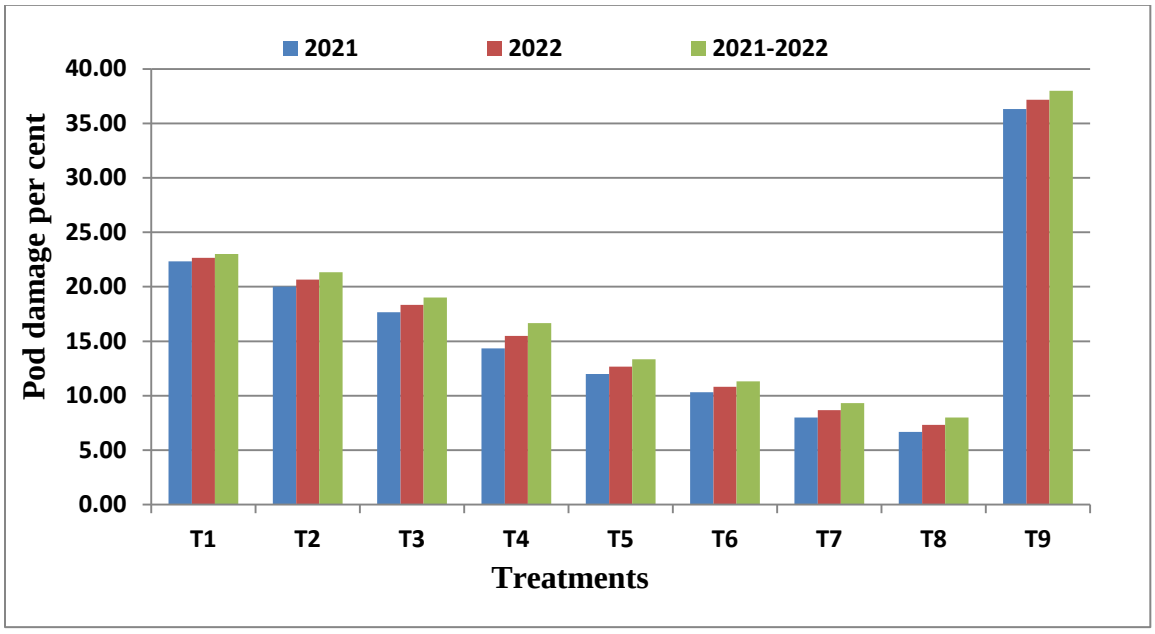


Fig. 22: Pod damage per cent during *Rabi* Season 2021-22 and Pooled.

The present result corroborates with **Savita *et al.* (2015)** who reported that among all the concentration of *M. anisopliae* the treatment 1×10^{10} cfu per ml recorded 9.50 per cent pod damage and produced 14.50 q/ha grain yield. The finding is also in accordance with **Spoorthi *et al.* (2017)** who treated the plot with 2×10^{9} cfu/ml @ 2g/lit water and found 14.58q/ha grain yield. The present finding are also in confirmation with **Tekam *et al.* (2018)** treated plot with 1×10^{9} cfu/ml and found 12.26 per cent pod damage and this finding also supported with the **Lomas *et al.* (2018)** who reported 14.23 per cent pod damage and grain yield 15.11 q/ha plot treated with at the concentration of 2×10^{8} cfu/gram @ 1kg/ha.

The economic evaluation of the treatments was made on the basis of grain yield obtained from experimental plots, treatment cost and marketable value of produce. The statistically analyzed yield data pertaining increase in grain yield revealed that the increase yield varied from 0.78 to 6.43 q/ha and 0.86 to 7.16 during *Rabi*, 2020-2021 and 2021-2022. Maximum increase in yield was 6.43 q/ha during first year and 7.16 q/ha during second year was recorded in the treatment 2×10^{10} @ 5ml/lit water and followed by 2×10^{9} @ 5ml/lit water was second best treatment with 4.78 and 5.88 q/ha increase grain yield during both year. The next treatments in order were 2×10^{8} @ 5ml/lit, 2×10^{7} @ 5ml/lit, 2×10^{6} @ 5ml/lit, 2×10^{5} @ 5ml/lit and 2×10^{4} @ 5ml/lit 3.76, 2.78, 2.46, 1.78 and 1.04 q/ha during *Rabi*, 2021 and 4.76, 3.98, 2.66 1.96 and 1.12 q/ha during *Rabi*, 2022 respectively. However, the minimum increase in grain yield of 0.78 and 0.86 q/ha was found in 2×10^{3} @ 5ml/lit water during *Rabi*, 2021 and 2022, respectively.

When the data of both years were pooled the increase grain yield varied from 0.82 to 6.58 q/ha (Table 31). The maximum increase yield was 6.58 q/ha in 2×10^{10} @ 5ml/lit water followed by 2×10^{9} @ 5ml/lit water with 5.33 q/ha increases in grain yield. The next treatments in order

Table 28: Effect of different treatments of grain yield in chickpea crop during *Rabi*, 2020-21, 2021-22 and Pooled

TRE. CODE	TREATMENTS	DOSE /LIT. WATER	GRAIN YIELD (Q/HA)		
			2021	2022	POOLED
T_1	2×10^3 + 0.5 % JAGGERY	5	11.00 (3.46)	11.10 (3.47)	11.05 (3.47)
T_2	2×10^4 + 0.5 % JAGGERY	5	11.26 (3.50)	11.36 (3.51)	11.31 (3.50)
T_3	2×10^5 + 0.5 % JAGGERY	5	12.00 (3.60)	12.20 (3.63)	12.10 (3.61)
T_4	2×10^6 + 0.5 % JAGGERY	5	12.68 (3.70)	12.90 (3.72)	12.79 (3.71)
T_5	2×10^7 + 0.5 % JAGGERY	5	13.00 (3.74)	14.22 (3.89)	13.61 (3.82)
T_6	2×10^8+ 0.5 % JAGGERY	5	13.98 (3.87)	15.00 (4.00)	14.49 (3.93)
T_7	2×10^9 + 0.5 % JAGGERY	5	15.00 (4.00)	16.12 (4.15)	15.56 (4.07)
T_8	2×10^{10}+ 0.5 % JAGGERY	5	16.22 (4.14)	17.40 (4.28)	16.81 (4.21)
T_9	CONTROL	-	10.22 (3.34)	10.24 (3.34)	10.23 (3.34)
	CD @ 5 %		**0.095**	**0.096**	**0.093**
	SEM±		**0.044**	**0.045**	**0.031**

Squar root transformation

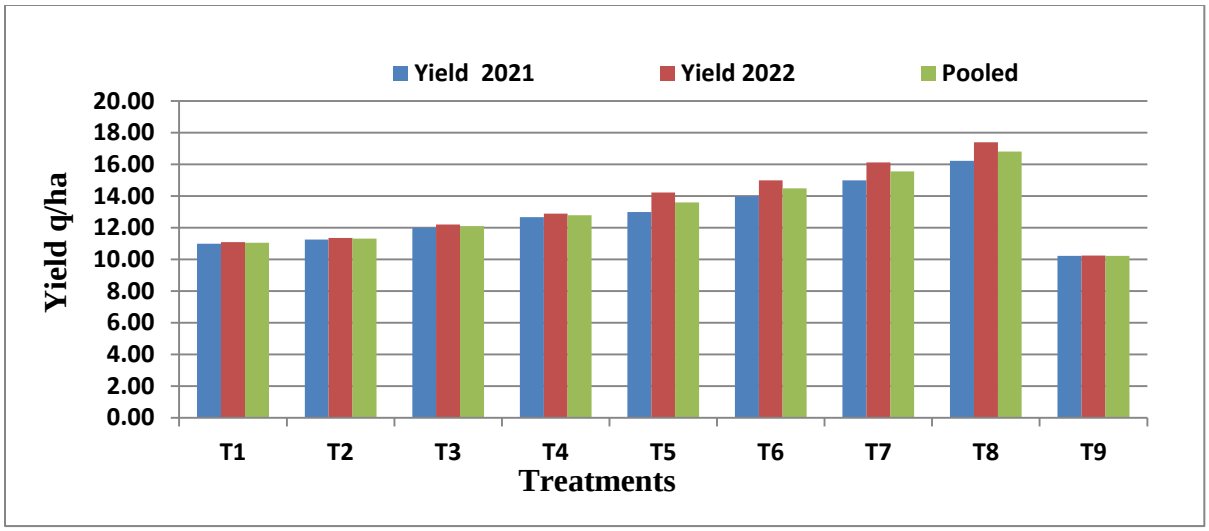


Fig. 23: Effect of different treatments of grain yield in chickpea crop during *Rabi*, 2021, 2022 and Pooled.

Plate 15: Healthy larvae of *H. armigera* in field condition.

Plate 16: Parasitised larvae of *H. armigera* in field condition.

were 2×10^8 @ 5ml/lit, 2×10^7 @ 5ml/lit, 2×10^6 @ 5ml/lit, 2×10^5 @ 5ml/lit and 2×10^4 @ 5ml/lit water with 4.26, 3.38, 2.56, 1.87 and 1.08 q/ha. Whereas, minimum increase in grain yield of 0.82 q/ha was observed in 2×10^3 @ 5ml/lit water.

4.4.6 Effect of different treatments on incremental cost benefit ratio

The labour charge, charge of sprayer and different concentration of *M. anisopliae* charge was uniform for spray in one hectare of crop. The economics of the used treatments were assessed cost of all treatments required for spray in one hectare of land show in table 29 and 30. It was clear form table that the treatment 2×10^3 @ 5ml/lit showed the minimum cost of 2830 Rs./ha. followed by 2×10^4 @ 5ml/lit, 2×10^5 @ 5ml/lit, 2×10^6 @ 5ml/lit, 2×10^7 @ 5ml/lit, 2×10^8 @ 5ml/lit and 2×10^9 @ 5ml/lit water with cost of Rs. 3175, 3520, 3865, 4205, 4550 and 4895 per

hectare. Whereas, the maximum cost of treatment (Rs.5240/ha) was recorded with 2×10^{10} @ 5ml/lit water.

The data regarding net profit revealed that the highest net profit of Rs 26252/ha was obtained with the treatment 2×10^{10} @ 5ml/lit water during *Rabi*, 2021 and followed by 2×10^{9} @ 5ml/lit, 2×10^{8} @ 5ml/lit, 2×10^{7} @ 5ml/lit, 2×10^{6} @ 5ml/lit, 2×10^{6} @ 5ml/lit, 2×10^{5} @ 5ml/lit and 2×10^{4} @ 5ml/lit with net profit of Rs 18407, 1370, 9347, 8127, 5157 and 1895 per hectare respectively. Among all the treatment the lowest net profit (Rs 972/ha) was calculated from the treatment 2×10^{3} @ 5ml/lit water during *Rabi*, 2020-2021.

During *Rabi*, 2022 the highest net profit Rs 31276/ha was observed with the treatment 2×10^{10} @ 5ml/lit water. The net profit of other effective treatments, were found 2×10^{9} @ 5ml/lit, 2×10^{8} @ 5ml/lit, 2×10^{7} @ 5ml/lit, 2×10^{6} @ 5ml/lit, 2×10^{6} @ 5ml/lit, 2×10^{5} @ 5ml/lit and 2×10^{4} @ 5ml/lit were recorded at Rs 25093, 19726, 16093, 9701, 6476 and 2537 per hectare respectively. However, the lowest net profit (Rs 1556/ha) was calculated from the treatment 2×10^{3} @ 5ml/lit water.

The data regarding net profit in pooled data of both year (Table 31) revealed that the highest net profit (Rs 27577/ha) was obtained with the treatment 2×10^{10} @ 5ml/lit water followed by 2×10^{9} @ 5ml/lit, 2×10^{8} @ 5ml/lit, 2×10^{7} @ 5ml/lit, 2×10^{6} @ 5ml/lit, 2×10^{6} @ 5ml/lit, 2×10^{5} @ 5ml/lit and 2×10^{4} @ 5ml/lit with net profit of Rs 21688.38, 16696.75, 12652.75, 8903, 5806.63 and 2211.50 per hectare respectively. The lowest net profit (Rs 1259.75/ha) was calculated from the treatment 2×10^{3} @ 5ml/lit water.

The maximum incremental cost benefit ratio was recorded in the treatment of 2×10^{10} @ 5ml/lit water gave 1:5.01 during *Rabi*, 2020-2021 and 5.97 during *Rabi*, 2021-2022 (Table 29 and

30) and followed by 2×10^9 @ 5ml/lit, 2×10^8 @ 5ml/lit, 2×10^7 @ 5ml/lit, 2×10^6 @ 5ml/lit, 2×10^6 @ 5ml/lit, 2×10^5 @ 5ml/lit and 2×10^4 @ 5ml/lit with incremental cost benefit ratio of 3.76, 3.03, 2.22, 2.10, 1.47 and 060 respectively during first year *Rabi*, 2021 and 5.13, 4.34, 3.83, 2.51, 1.84 and 0.80 respectively during second year *Rabi*, 2021-2022. Whereas, the minimum ICBR of 0.34 during *Rabi*, 2020-2021 and 0.55 during *Rabi*, 2021-2022 was observed in 2×10^3 @ 5ml/lit water.

Working out the cost benefit ratio of both years'on the basis of pooled data (Table 31), revealed that the maximum incremental cost benefit ratio was recorded in the treatment of 2×10^{10} @ 5ml/lit water with 1: 5.26 and followed by 2×10^9 @ 5ml/lit, 2×10^8 @ 5ml/lit, 2×10^7 @ 5ml/lit, 2×10^6 @ 5ml/lit, 2×10^6 @ 5ml/lit, 2×10^5 @ 5ml/lit and 2×10^4 @ 5ml/lit with incremental cost benefit ratio of 4.43, 3.67, 3.01, 2.30, 1.65 and 0.70, respectively. The minimum ICBR of 0.45 was found in the treatment of 2×10^3 @ 5ml/lit water.

The present findings are in agreement with **Spoorthi *et al.*, (2017)** who treated the plot with 2×10^9 cfu/ml @ 2g/lit water and found increased yield over control was 5.23 q/ha and incremental cost benefit ratio 1: 9.01. The present finding also agreed with **Man Mohan Kumar (2016)** who reported that highest cost benefit ratio in the treatment of 2×10^9 @ 2.5ml/lit water 1: 7.68.The present finding corroborate with **Savita *et al.*, (2015)** observed that application of different concentration of *M. anisopliae* increase the yield and incremental cost benefit ratio. This result also corroborate with the finding of **Lomas *et al.*, (2018)** who recorded ICBR 1: 3.79 at 2×10^8 cfu/ml @ 1kg/ha.

Table 29: Yield, economics and incremental cost benefit ratio of different treatments in chickpea during *Rabi*, 2020-21

Table 30: Yield, economics and incremental cost benefit ratio of different treatments in chickpea during *Rabi*, 2021-22

TR. NO.	NAME OF TREATMENTS	DOSE/LIT WATER	YIELD (Q/HA)	INCREASE YIELD OVER CONTROL (Q/HA)	VALUE OF INCREASE YIELD (RS./HA)	COST OF TREATMENTS (RS./HA)	NET PROFIT (RS./HA)	ICBR
T_1	$2 \times 10^3 + 0.5\%$ JAGGERY	5	11.00	0.78	3900.00	2830.00	1070.00	0.38
T_2	$2 \times 10^4 + 0.5\%$ JAGGERY	5	11.36	1.04	5070.00	3175.00	1895.00	0.60
T_3	$2 \times 10^5 + 0.5\%$ JAGGERY	5	12.20	1.98	9976.00	3520.00	6456.00	1.84
T_4	$2 \times 10^6 + 0.5\%$ JAGGERY	5	12.90	2.66	13598.00	3865.00	9727.00	2.51
T_5	$2 \times 10^7 + 0.5\%$ JAGGERY	5	14.20	3.98	20298.00	4205.00	16093.00	3.82
T_6	$2 \times 10^8 + 0.5\%$ JAGGERY	5	13.90	3.76	18270.00	4550.00	13720.00	3.01
T_7	$2 \times 10^9 + 0.5\%$ JAGGERY	5	16.00	5.88	29988.00	4895.00	25093.00	5.13
T_8	$2 \times 10^{10} + 0.5\%$ JAGGERY	5	17.20	6.48	36346.00	5240.00	31106.00	5.94
T_9	CONTROL	-	10.22	-	-	-	-	-

Table 30: Yield, economics and incremental cost benefit ratio of different treatments in chickpea during *Rabi*, 2021-22

TR. NO.	NAME OF TREATMENTS	DOSE/LIT WATER	YIELD (Q/HA)	INCREASE YIELD OVER CONTROL (Q/HA)	VALUE OF INCREASE YIELD (RS./HA)	COST OF TREATMENTS (RS./HA)	NET PROFIT (RS./HA)	ICBR
T_1	$2 \times 10^3 + 0.5\%$ JAGGERY	5	11.10	0.86	4386.00	2830.00	1556.00	0.55
T_2	$2 \times 10^4 + 0.5\%$ JAGGERY	5	11.36	1.12	5712.00	3175.00	2537.00	0.80
T_3	$2 \times 10^5 + 0.5\%$ JAGGERY	5	12.20	1.96	9996.00	3520.00	6476.00	1.84
T_4	$2 \times 10^6 + 0.5\%$ JAGGERY	5	12.90	2.66	13566.00	3865.00	9701.00	2.51
T_5	$2 \times 10^7 + 0.5\%$ JAGGERY	5	14.22	3.98	20298.00	4205.00	16093.00	3.83
T_6	$2 \times 10^8 + 0.5\%$ JAGGERY	5	15.00	4.76	24276.00	4550.00	19726.00	4.34
T_7	$2 \times 10^9 + 0.5\%$ JAGGERY	5	16.12	5.88	29988.00	4895.00	25093.00	5.13
T_8	$2 \times 10^{10} + 0.5\%$ JAGGERY	5	17.40	7.16	36516.00	5240.00	31276.00	5.97
T_9	CONTROL	-	10.24	-	-	-	-	-

able 31: Yield, economics and incremental cost benefit ratio of different treatments in chickpea during *Rabi*, 2020-21, 2021-22 and pooled.

TR. NO.	NAME OF TREATMENTS	DOSE/LIT WATER	YIELD (Q/HA)	INCREASE YIELD OVER CONTROL (Q/HA)	VALUE OF INCREASE YIELD (RS./HA)	COST OF TREATMENTS (RS./HA)	NET PROFIT (RS./HA)	ICBR
T_1	$2 \times 10^3 + 0.5\%$ JAGGERY	5	11.05	0.82	4089.75	2830.00	1259.75	0.45
T_2	$2 \times 10^4 + 0.5\%$ JAGGERY	5	11.31	1.08	5386.50	3175.00	2211.50	0.70
T_3	$2 \times 10^5 + 0.5\%$ JAGGERY	5	12.10	1.87	9326.63	3520.00	5806.63	1.65
T_4	$2 \times 10^6 + 0.5\%$ JAGGERY	5	12.79	2.56	12768.00	3865.00	8903.00	2.30
T_5	$2 \times 10^7 + 0.5\%$ JAGGERY	5	13.61	3.38	16857.75	4205.00	12652.75	3.01
T_6	$2 \times 10^8 + 0.5\%$ JAGGERY	5	14.49	4.26	21246.75	4550.00	16696.75	3.67
T_7	$2 \times 10^9 + 0.5\%$ JAGGERY	5	15.56	5.33	26583.38	4895.00	21688.38	4.43
T_8	$2 \times 10^{10} + 0.5\%$ JAGGERY	5	16.81	6.58	32817.75	5240.00	27577.75	5.26
T_9	CONTROL	-	10.23	-	-	-	-	-

Labour charge Rs. 350/Labour, Rent of sprayer Rs. 100/ sprayer, Cost of produce Rs. 4987.50.

CHAPTER-5 SUMMARY AND CONCLUSION

The present investigation entitled "Studies on mass production of entomopathogenic fungi *Metarhizium anisopliae* (Metschnikoff) using different media and potential against chickpea pod borer, *Helicoverpa armigera* (Hubner)" was carried out in biological control laboratory and Crop Research Center of Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (U.P.) during 2020-2021 and 2021-2022. The findings of investigations were summarized and have been presented as follows:

Isolation of the native isolates of entomopathogenic fungi *M. anisopliae* was carried out. Among the 207 soil samples examined from four districts viz. Meerut, Muzaffarnagar, Saharanpur and shamali in western plain zone of Uttar Pradesh, revealed 5 isolates of *M. anisopliae* isolated from districts Meerut, Muzaffarnagar, Saharanpur and shamali.

RT-PCR assay using ITS primers yielded an expected amplicon of~650 bp from Meerut region sample. The sequences obtained were deposited to NCBI Gene Bank with accession numbers ON183248. Pairwise sequence identity comparison of the *M. anisopliae* sequence of TuMMoV with similar sequences shared 95.80 – 99.40 per cent nt with the global isolates.

The pathogenicity of local fungus of *M. anisopliae* isolates SVPUAT 1 Accession no. ON183248 was highest (100 per cent) after seven days of treatment followed by isolate SVPUAT 5 (71.11 per cent), SVPUAT 4 (57.77 per cent) and SVPUAT 2 (44.44 per cent). The minimum mortality per cent recorded in isolate SVPUAT 3 (31.11 per

cent). The lowest LT_{50} and LT_{90} value was also recorded in isolate SVPUAT 1 with 3.16 and 5.16 days respectively.

Among all seventeen substrates, Rice + 1 % Yeast + 1 gm Dextrose (82.25×10^7 spores/gm) was significantly superior over all substrates. The second best substrate was Sabouraud's Dextrose Broth (75.71×10^7 spores/ml). Whereas, the minimum spore production was observed in Press mud + 1 % Yeast + 1 gm Dextrose (0.65×10^7 spores/gm).

On the basis of overall mean the highest rate of increase recorded in FYM liquid + 1 % Yeast + 1 gm Dextrose (66.68 per cent) followed by Corcyra rearing wastes + 1 % Yeast + 1 gm Dextrose (66.68 per cent). The substrates Pigeonpea + 1 % Yeast + 1 gm Dextrose (28.80 per cent) and Rice + 1 % Yeast + 1 gm Dextrose (27.96 per cent) were on par with each other. The substrates Green gram + 1 % Yeast + 1 gm Dextrose (25.39 per cent), Cow pea + 1 % Yeast + 1 gm Dextrose (24.99 per cent), Sabouraud's dextrose broth (24.27 per cent) and Sorghum + 1 % Yeast + 1 gm Dextrose (23.78 per cent) were significantly similar with each other. The lowest per cent increase was noticed in Press mud + 1 % Yeast + 1 gm Dextrose (1.54 per cent).

The lowest production cost was recorded on Corcyra rearing wastes + 1 % Yeast + 1 gm. Dextrose (Rs. 0.02) which was on par with substrates Rice + 1 % Yeast + 1 gm. Dextrose, Bajra + 1 % Yeast + 1 gm. Dextrose and FYM liquid + 1 % Yeast + 1 gm. Dextrose with the production cost of Rs. 0.03. However the least economically feasible substrates were SDB (Sabouraud's Dextrose Broth) (Rs. 0.31), Pigeonpea + 1 % Yeast + 1

gm. Dextrose (Rs. 0.32), PDB (Potato Dextrose Broth) (Rs. 0.33) and Vermicompost + 1 % Yeast + 1 gm. Dextrose (Rs. 0.39).

At one month after storage the viable conidial per cent was ranged in 92.68 to 66.00 per cent. The highest conidial viability per cent was found in the substrates of FYM liquid + 1 % yeast powder + 1.0g Dextrose (92.68 per cent). Whereas, the lowest conidial viability per cent observed in Press mud + 1 % Yeast + 1 gm Dextrose (66.00 per cent).

The conidial viability per cent was ranged from 89.41 to 27.03 per cent after two month of storage. The highest viable conidial percent were recorded in FYM liquid + 1 % yeast powder + 1.0g Dextrose (89.41 per cent) which was statistically on par with the substrates of FYM + 1 % Yeast + 1 gm Dextrose (88.26 per cent). However, the lowest conidial viability per cent was recorded in the substrates of Vermicompost + 1 % Yeast + 1 gm Dextrose (27.03 per cent).

The conidial viability for all substrates was ranged from 8.92 to 64.22 per cent after three month of storage. The highest conidial viability per cent was found in the substrates of FYM liquid + 1 % yeast powder + 1.0g Dextrose with 64.22 per cent. However the minimum conidial viability was recorded in the substrates of Vermicompost + 1 % Yeast + 1 gm Dextrose (8.92 per cent).

At fourth month after storage, the viable conidial per cent was ranged in 0.00 to 38.20 per cent. The highest conidial viability per cent was found in the substrates of FYM liquid + 1 % yeast powder + 1.0g Dextrose (38.20 per cent). However, the lowest conidial viability per cent was recorded in Vermicompost + 1 % Yeast + 1 gm Dextrose (0.00 per cent) and Press mud + 1 % Yeast + 1 gm Dextrose (0.00 per cent).

Conidia produced on different substrates showed variation in viability after five month of storage. The conidial viability per cent of different substrates was ranged from 0.00 to 22.76 per cent. The highest conidial viability per cent was found in FYM liquid + 1 % yeast powder + 1.0g Dextrose (22.76 per cent). However, the substrates Press mud + 1 % Yeast + 1 gm Dextrose and Vermicompost + 1 % Yeast + 1 gm Dextrose showed 0.00 per cent conidial viability.

The conidial viability for all the substrates was ranged from 0.00 to 13.80 per cent after six month of storage. The highest conidial viability per cent was recorded in the substrates of FYM liquid + 1 % yeast powder + 1.0g Dextrose (13.80 per cent) followed by Green gram + 1 % Yeast + 1 gm Dextrose (6.83 per cent) and Sorghum + 1 % Yeast + 1 gm Dextrose (5.88 per cent). However the maximum substrates i.e. Press mud + 1 % Yeast + 1 gm Dextrose, Vermicompost + 1 % Yeast + 1 gm Dextrose, Maize + 1 % Yeast + 1 gm Dextrose, Corcyra rearing wastes + 1 % Yeast + 1 gm Dextrose, Potato Dextrose Broth and Sabouraud's Dextrose Broth showed zero per cent conidial viability.

All the tested concentration of *M. anisopliae* against second instar of *H. armigera* after one hundred twenty hours of treatment showed significantly superior over control with the range of 54.44 and 94.33 per cent pooled larval mortality. The highest lethal concentration was 2×10^{10} spores/ml (94.33 per cent) which was on par with second highest conidial concentration 2×10^{9} spores/ml (91.10 per cent). However, the lowest larval mortality was recorded at the concentration of 2×10^{3} spores/ml (54.44 per cent).

The highest pooled LT_{50} and LT_{90} value for second instar larva of *H. armigera* was recorded at low concentration 2×10^{3} spores/ml with 4.79 and 7.58 days followed by

2×10^4 spores/ml, 2×10^5 spores/ml, 2×10^6 spores/ml, 2×10^7 spores/ml, 2×10^8 spores/ml and 2×10^9 spores/ml with 4.58, 4.33, 4.08, 3.68, 3.46 & 2.99 and 7.20, 7.25, 7.20, 6.45, 6.08, and 5.04 days respectively. The highest concentration 2×10^{10} spores/ml exhibited more mortality with 2.68 and 4.54 days of LT_{50} and LT_{90} value.

The pooled effect of all the tested concentration of *M. anisopliae* against third instar of *H. armigera* after one hundred twenty hours of treatment were significantly superior over control with the range of 49.99 and 91.10 per cent larval mortality. The highest lethal concentration was 2×10^{10} spores/ml (91.10 per cent) which was on par with second highest conidial concentration 2×10^9 spores/ml (87.77 per cent). However, the lowest larval mortality recorded at the concentration of 2×10^3 spores/ml (49.99 per cent).

The highest pooled LT_{50} and LT_{90} value at different concentrations of *M. anisopliae* against third instars larvae of *H. armigera* was recorded at low concentration 2×10^3 spores/ml with 4.83 and 7.63 days followed by 2×10^4 spores/ml, 2×10^5 spores/ml, 2×10^6 spores/ml, 2×10^7 spores/ml, 2×10^8 spores/ml and 2×10^9 spores/ml with 4.58, 4.38, 4.17, 3.85, 3.63 & 3.20 and 7.13, 6.83, 6.96, 6.63, 6.21 and 5.50 days respectively. The highest concentration 2×10^{10} spores/ml exhibited more mortality with 2.93 and 5.00 days of LT_{50} and LT_{90} value.

The pooled fourth larval instar mortality per cent of *H. armigera* at different conidial concentration of *M. anisopliae* after one hundred twenty hours of treatment ranged from 41.11 to 87.77 per cent. The highest lethal conidial concentration was 2×10^{10} spores/ml (87.77). However, the minimum larval mortality was recorded at the

concentration of 2×10^3 spores/ml (41.11 per cent) which was statistically similar with the concentration of 2×10^4 spores/ml (47.77 per cent).

The highest pooled LT_{50} and LT_{90} value of different concentrations of *M. anisopliae* against fourth instar larva was recorded at low concentration of 2×10^3 spores/ml with 5.25 and 8.42 days followed by 2×10^4 spores/ml, 2×10^5 spores/ml, 2×10^6 spores/ml, 2×10^7 spores/ml, 2×10^8 spores/ml and 2×10^9 spores/ml with 4.96, 4.67, 4.46, 4.21, 3.99 & 3.72 and 7.92, 7.33, 7.08, 6.50, 6.00 and 6.00 days respectively. The highest concentration 2×10^{10} spores/ml exhibited more pathogenic with 3.46 and 5.54 days of LT_{50} and LT_{90} value.

The overall mean of pooled larval reduction per cent ranged from 17.00 to 65.49 per cent. The highest reduction was recorded 65.49 per cent in the treatment of 2×10^{10} @ 5ml/lit water followed by 2×10^9 @ 5ml/lit, 2×10^8 @ 5ml/lit, 2×10^7 @ 5ml/lit, 2×10^6 @ 5ml/lit, 2×10^5 @ 5ml/lit and 2×10^4 @ 5ml/lit water with 54.14, 49.98, 45.79, 40.97, 37.05 and 32.27 per cent respectively. The lowest reduction per cent was found in the treatment of 2×10^3 @ 5ml/lit water with 28.55 per cent.

The pooled data revealed that the per cent pod damage caused by *H. armigera* ranged from 7.33 to 37.17 per cent. The treatment 2×10^{10} @ 5ml/lit water found best among all the treatments with minimum pod damage of 7.33 per cent followed by 2×10^9 @ 5ml/lit, 2×10^8 @ 5ml/lit, 2×10^7 @ 5ml/lit, 2×10^6 @ 5ml/lit, 2×10^5 @ 5ml/lit, 2×10^4 @ 5ml/lit and 2×10^3 @ 5ml/lit water with 8.67, 10.83, 12.67, 15.50, 18.33, 20.67 and 22.67 per cent respectively. The highest pod damage was noticed in control with 37.17 per cent.

When the data of both years were pooled the increase grain yield varied from 0.82 to 6.58 q/ha. The maximum increase yield was 6.58 q/ha in 2×10^{10} @ 5ml/lit water followed by 2×10^{9} @ 5ml/lit water was with 5.33 q/ha increases in grain yield. The next treatments in order were 2×10^{8} @ 5ml/lit, 2×10^{7} @ 5ml/lit, 2×10^{6} @ 5ml/lit, 2×10^{5} @ 5ml/lit and 2×10^{4} @ 5ml/lit water with 4.26, 3.38, 2.56, 1.87 and 1.08 q/ha. Whereas, minimum increase in grain yield of 0.82 q/ha was observed in 2×10^{3} @ 5ml/lit water.

The data regarding net profit in pooled data of both year revealed that the highest net profit (Rs 27577/ha) was obtained with the treatment 2×10^{10} @ 5ml/lit water followed by 2×10^{9} @ 5ml/lit, 2×10^{8} @ 5ml/lit, 2×10^{7} @ 5ml/lit, 2×10^{6} @ 5ml/lit, 2×10^{6} @ 5ml/lit, 2×10^{5} @ 5ml/lit and 2×10^{4} @ 5ml/lit with net profit of Rs 21688.38, 16696.75, 12652.75, 8903, 5806.63 and 2211.50 per hectare respectively. The lowest net profit (Rs 1259.75/ha) was calculated from the treatment 2×10^{3} @ 5ml/lit water.

Working out the cost benefit ratio of both years' pooled data revealed that the maximum incremental cost benefit ratio was recorded in the treatment of 2×10^{10} @ 5ml/lit water with 5.26 and followed by 2×10^{9} @ 5ml/lit, 2×10^{8} @ 5ml/lit, 2×10^{7} @ 5ml/lit, 2×10^{6} @ 5ml/lit, 2×10^{6} @ 5ml/lit, 2×10^{5} @ 5ml/lit and 2×10^{4} @ 5ml/lit with incremental cost benefit ratio of 4.43, 3.67, 3.01, 2.30, 1.65 and 0.70, respectively. The minimum ICBR of 0.45 was found in the treatment of 2×10^{3} @ 5ml/lit water.

CONCLUSION

- Isolation of the native isolates of entomopathogenic fungi *M. anisopliae* was carried out. Among the 207 soil samples examined from four districts viz. Meerut, Muzaffarnagar, Saharanpur and shamali in western plain zone of Uttar Pradesh,

revealed 5 isolates of *M. anisopliae* isolated from districts Meerut, Muzaffarnagar, Saharanpur and shamali.

- The pathogenicity of local isolate of *M. anisopliae* strain SVPUAT 1 Accession no. ON183248 was highest (100 per cent) after seven days of treatment with lowest LT_{50} and LT_{90} value, 3.16 and 5.16 days respectively.
- From the above findings, it can be inferred that the highest spore count of *M. anisopliae* was recorded on Rice + 1 % Yeast + 1 gm Dextrose (82.25×10^7 spores/gm) followed by Sabouraud's Dextrose Broth (75.71×10^7 spores/ml) emerged as the potential medium.
- The highest rate of increase recorded in FYM liquid + 1 % Yeast + 1 gm Dextrose (66.68 per cent) followed by Corcyra rearing wastes + 1 % Yeast + 1 gm Dextrose (66.68 per cent).
- The most economically feasible substrates for mass production of *M. anisopiae* was Corcyra rearing wastes + 1 % Yeast + 1 gm. Dextrose (Rs. 0.02/100 gm) with the production of 31.22×10^7 spores/gm.
- The most suitable substrates for long duration storage of *M. anisopliae* were FYM liquid + 1 % Yeast + 1 gm Dextrose and FYM + 1 % Yeast + 1 gm Dextrose at room temperature.
- The mortality effect of different concentration of *M. anisopliae* on several instars (II^{nd}, III^{rd} and IV^{th}) of *H. armigera* gradually increases with spore concentrations. The highest mortality recorded at highest conidial concentration of 2×10^{10} conidia/ml. The LT_{50} and LT_{90} value was also lower at this concentration.

- The larval reduction per cent was also highest at higher concentration of 2×10^{10} conidia/ml with highest yield and incremental cost benefit ratio.

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Abstract

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Thesis Title: "Mass production of entomopathogenic fungi *Metarhizium anisopliae* (Metschnikoff) using different media and potential against chickpea pod borer, *Helicoverpa armigera* (Hubner)"

The present investigation entitled **"**Mass production of entomopathogenic fungi *Metarhizium anisopliae* (Metsh.) using different media and potential against chickpea pod borer, *Helicoverpa armigera* (Hub.)**"** was carried out at Biological Control Laboratory and Crop Research Centre (CRC) of Sardar Vallabhbhai Patel University of Agriculture & Technology**,** Meerut during 2020-2021 and 2021-2022. Out of 207 soil samples examined from four districts viz. Meerut, Muzaffarnagar, Saharnpur and Shamli in western plain zone of Uttar Pradesh and isolated five isolates of *M. anisopliae* from these samples. RT-PCR assay using ITS primers yielded an expected amplicon of~650 bp from five samples. The sequences obtained were deposited to NCBI Gene Bank with accession numbers ON183248. The pathogenicity of local fungus of *M. anisopliae* isolate SVPUAT 1 Accession no. ON183248 was highest (100 per cent) after seven days of treatment and LT_{50} and LT_{90} value was also low in isolate SVPUAT 1 with 3.16 and 5.16 days respectively. The most suitable media for mass production of *M. anisopliae* was Rice + 1 % Yeast + 1 gm Dextrose (82.25×10^7 spores/gm) whereas, Corcyra rearing wastes + 1 % Yeast + 1 gm. Dextrose (Rs. 0.02/100 gm) was economically best. The highest rate of conidia pre cent increase in FYM liquid + 1 % Yeast + 1 gm Dextrose as well as best for long term storage. The mortality effect of different concentrations of *M. anisopliae* on several instars (II^{nd}, III^{rd} and IV^{th}) of *H. armigera* gradually increases with spore concentrations. The highest mortality recorded at highest conidial concentration of 2×10^{10} conidia/ml. The LT_{50} and LT_{90} value was also lower at this concentration. The larval reduction per cent was also highest at higher concentration of 2×10^{10} conidia/ml (59.07 Per cent) with highest yield (16.81q/ha) and incremental cost benefit ratio (Rs. 5.26).

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