Efficacy Of *Beauveria Bassiana* And *Metarhizium Anisopliae* For The Management Against *Pieris Brassicae* And *Pieris Rapae* Larvae On Crucifers' Crop Under Field Conditions.

Leimapokpam Martina[a*], Mohsin Ikram[b], Sandeep Kumar[c], Rajendra Prasad[d]

[a*] School of Agriculture, Uttaranchal University, Dehradun, Uttarakhand, India,
Email: leimapokpammatry12345@gmail.com
[b] School of Agriculture, Uttaranchal University, Dehradun, Uttarakhand, India; Email: mohsin3757@gmail.com
[c] School of Agriculture, Uttaranchal University, Dehradun, Uttarakhand, India; Email: skpatho93@gmail.com
[d] School of Agriculture, Uttaranchal University, Dehradun, Uttarakhand, India; Email: rajenpd@gmail.com

[*]**Corresponding Author:** Leimapokpam Martina
Email: leimapokpammatry12345@gmail.com

Abstract
The aim of the present study was the evaluation of different entomopathogenic fungi against *Pieris brassicae* larvae (Cabbage butterfly) and *Pieris rapae* larvae (imported cabbage worm) in RBD with 5 treatments and 4 replications. The efficacy of different entomopathogenic fungi, viz., *Beauveria bassiana*, *Metarhizium anisopliae* was tested in different treatments i.e., 3g/L, 5g/L. The survey was conducted in different areas of Dehradun farm sites during February and March 2023. Periodic field visits were conducted in different regions of Dehradun. Various insect pests from different field sites were collected in boxes with help of hand-picking methods and an aerial net. The field-collected larvae were brought to the laboratory, reared on cabbage mustard leaves and observed for mortality and development of mycosis. Cadavers' larvae were isolated by cutting into small pieces with the help of a sterile blade, and the bits were aseptically transferred onto the PDA. The media were kept at 25±1°C in a BOD incubator for mycelial growth. The radial growth of entomopathogenic fungi at every observation. *B. bassiana* showed the most effective of all treatments, whereas the minimum mycelial growth was done by *M. anisopliae*. For mass production of entomopathogenic fungi on solid substrates, viz., wheat and sorghum, *B. bassiana* showed the highest mycelial growth on sorghum, whereas *M. anisopliae* showed the highest mycelial growth on wheat.

Keywords: Entomopathogenic fungi, *B. bassiana*, *M. anisopliae*, *Pieris brassicae*, *Pieris rapae* larvae.

Introduction

Among the vegetables, crucifers are important winter crops and consist of cabbage, cauliflower, mustard, broccoli, Brussels sprouts, kale, bok choy and radish. The cruciferous vegetables belong to the family Brassicaceae. Cruciferous vegetables are one of the dominant food crops worldwide. These vegetables play an important role in balancing the vegetarian diet and also serves as important sources of minerals, vitamins and crude fibre. Among vegetable crops, more than 48.2 percent of the area under this cop is contributed by the Asian continent, which accounts for 42.3 percent of total world production. India is one of the most important cabbage-growing countries in Asia, with an arear of 369 thousand ha. and a production of 7,949 thousand metric tonnes with a productivity of 21.4 metric tonnes per ha. India is the second leading producer of cabbage in the world, followed by China. **(Anonymous, 2011).**

Insect pests are the most important crop limiting factor for crucifers **(Bhavani *et al.*, 2009).** The cabbage and other crucifer crops are attacked by a number of different insect pests, among them the cabbage cluster caterpillar, *Crocidolomia pavonana* (F.) (Lepidoptera: Pyralidae) (**Dadang *et al.*, 2009)**, Cabbage webworm, *Helulla undalis* (F.) (Lepidoptera: Crambidae), Cabbage aphids (*Brevicoryne brassicae* L.), imported cabbage worm *(Pieris rapae)*, cabbage butterfly, *Pieris brassicae* (L.) , Tobacco caterpillar, *Spodoptera litura* (F.), and cabbage looper, Trichoplusia ni (Hubner) (Lepidoptera, Noctuidae) **(Chalfant *et al.*, 1979)**. The cabbage butterfly, *Pieris brassicae* (Linnaeus) (Lepidoptera: Pieridae), is a destructive pest of crucifer crops in India **(Shanker *et al.*, 2016)** as well as around the world **(Hasan, 2008).** A single larva can consume about 74 to 80 cm^2 leaf area **(Younas *et al.*, 2004).** In cruciferous vegetables, this pest alone causes 40 percent yield loss annually in India **(Hasan and Ansari, 2010.** The imported cabbage worm, *Pieris rapae* (L.) (Lepidoptera: Pieridae) is another serious pest of cruciferous crops, mainly cabbage and mustard. The damage caused by *P. rapae* was slight, but it can be severe in years with high infestation **(Hem *et al.*, 1996).** *P. rapae* is an economically important pest of brassica crops the world over. Entomopathogenic fungi (EPF) are fungal species that are pathogenic to insects. The divisions of fungi are Ascomycota, Zygomycota, Deuteromycota, Oomycota, and Chytridiomycota **(Samson *et al.*, 1988).** Entomopathogenic fungi are among the first organisms to be used for the biological control of pests. Alternatively, entomopathogenic fungi (EPF), like *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Ascomycota: Hypocreales), *Metarhizium anisopliae* (Metschn.) Sorokin (Hypocreales: Clavicipitaceae), *Isaria fumosorosea* Wize (Hypocreales: Cordyciptaceae), *Verticillium lecanii* (Zimm.) (Deuteromycotina: Hyphomycetes), and *Nomuraea rileyi*

(Farl.), have been found to be promising tools for controlling several agricultural insect pests **(Trdan *et al.*, 2020).** The present study was done understand the efficacy of entomopathogenic fungi used as a biological control agent against *Pieris brassicae* larvae and *Pieris rapae* larvae under field conditions.

Materials and methods

Survey methodology

The survey was conducted in different areas of Dehradun farm sites during February and March 2023. Periodic field visits were conducted at three farm sites i.e., Lakhanwala, Sahaspur, and Kotra Santaur at Dehradun had investigated for cabbage, mustard and radish crops, as well as nearby Sudhowala sites as shown in Fig.

Figure 1. Field survey of various farm sites in Dehradun.

Identification and collection of various insect pests on the crucifer crop

Various insect pests attacking or feeding on the crucifer crop were identified with the help of farmers in the field at Dehradun and also with the help of entomologists and references from internet browsing. Various insect pests from different field sites were collected in boxes with the help of hand-picking method and an aerial net. The field collected larvae were brought to the laboratory, reared on cabbage, mustard leaves and observed for mortality and development of mycosis. *Pieris brassicae* eggs and -1st instar larvae were also collected from filed sites for rearing. The list of plate for the collection of various insect pests on the crucifer crop as shown in Fig. 2

Cabbage cluster caterpillar larvae

Imported cabbage worm larvae

Cabbage looper larvae

Eggs of cabbage butterfly

Figure 2. Identification and collection of various insect pests on the crucifer crop

Rearing of major insect pests in the crucifer crop

The lepidopteran species of *Pieris rapae* larvae (imported cabbage worm), Cabbage cluster caterpillar, *Pieris brassicae* larvae (Cabbage butterfly), Diamond back moth (DBM) larvae, Cabbage looper, and Turnip sawfly larvae were reared at 21±1°C in different boxes (60 x 60 x 60 cm) covered with muslin cloth at the entomology laboratory as shown in Fig 3.

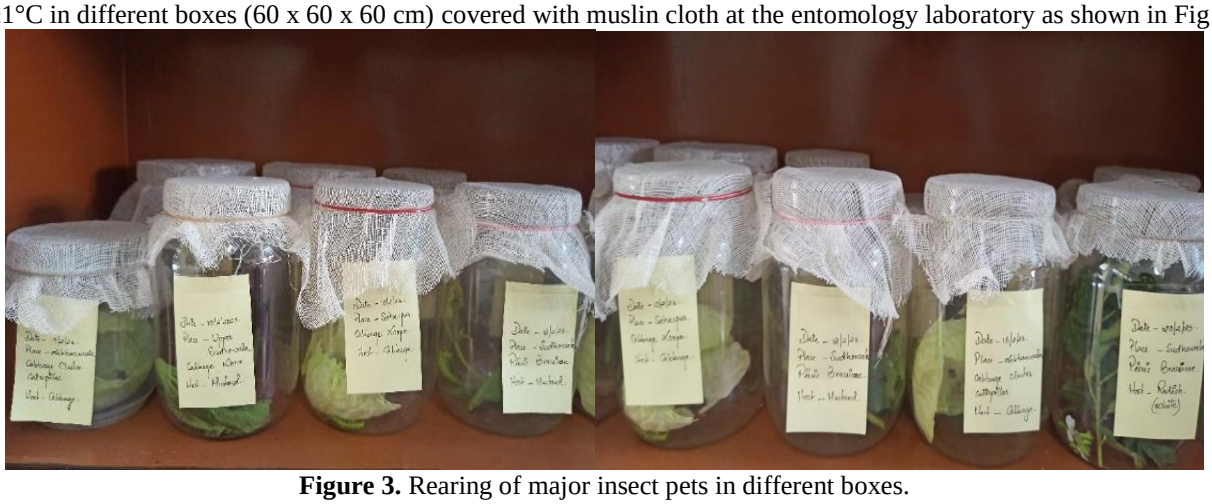

Figure 3. Rearing of major insect pets in different boxes.

Isolation and identification of entomopathogenic fungi (*B. bassiana* and *M. anisopliae)* from insect samples

Entomopathogenic fungi were isolated in pure form from the mycosed cadavers of *Pieris brassicae* larvae (Cabbage butterfly) and *Pieris rapae* larvae (imported cabbage worm) that died in the college entomology lab in Dehradun. The infected *Pieris brassicae* larvae showed a white colour for *B. bassiana* as shown in Fig 4 (a), and a slight whitish to yellowish mycelial surface growth for *M. anisopliae* infected with *Pieris rapae* larvae as shown in Fig 4 (b). The infected larvae were surface sterilized with 3% sodium hypochloride for 30 seconds and then thoroughly washed with sterilized doubled- distilled water. The infected larvae were then cut into small pieces with the help of sterile blade and the bits were aseptically transferred onto the PDA media. The media were kept at 25±1°C in a BOD incubator for mycelial growth and sporulation.

Identification of *B. bassiana*

From the study, it was found that *B. bassiana* has abundant aerial mycelium and pure white colonies. The mycelium was globose or oval with septate hyphae. Each spore was clustered in a group to form conidiophores. The hyphae are about 2-3 µm in size and group on conidiogenous cells with 4-8 µm in size. Then hyphae formed conidiogenous cells with bottle-like forms and branches that were up to more than 25 µm and 2 µm wide. The fertile hyphae were found on branches, circular and swollen whereas mycelium is aggregate hyphae that form white-colour hyphae as shown in Fig 4 (a).

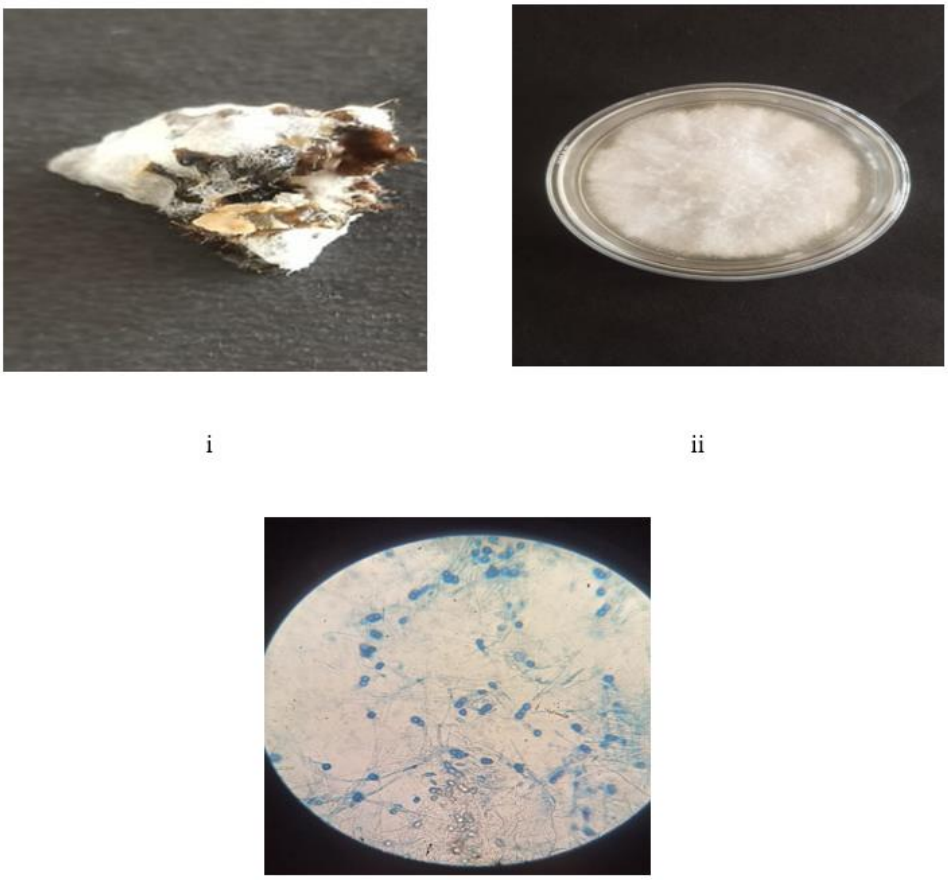

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Figure 4 (a) i. *Pieris brassicae* larvae (Cabbage butterflies) infected with *Beauveria bassiana* isolate. ii. Growth of *B. bassiana* and iii. Round and oval. Conidia at 10x.

Identification of *M. anisopliae*

The fungal isolate formed thick and cottony colonies of whitish to yellowish mycelia with a grey colour on the lower surface. It had radial growth of 12 days of incubation on PDA. Initially the culture produces a white mycelial with branching conidiophores. The elongated branches of conidiophores form cylindrical conidia, sometime single or clustered phialides. The colours vary from olive green to dark green. The conidial were cylindrical, slightly narrowing in the centre, and the conidial width (2- 3.5 µm) and length (4 – 7 µm) as shown in Fig 4 (b).

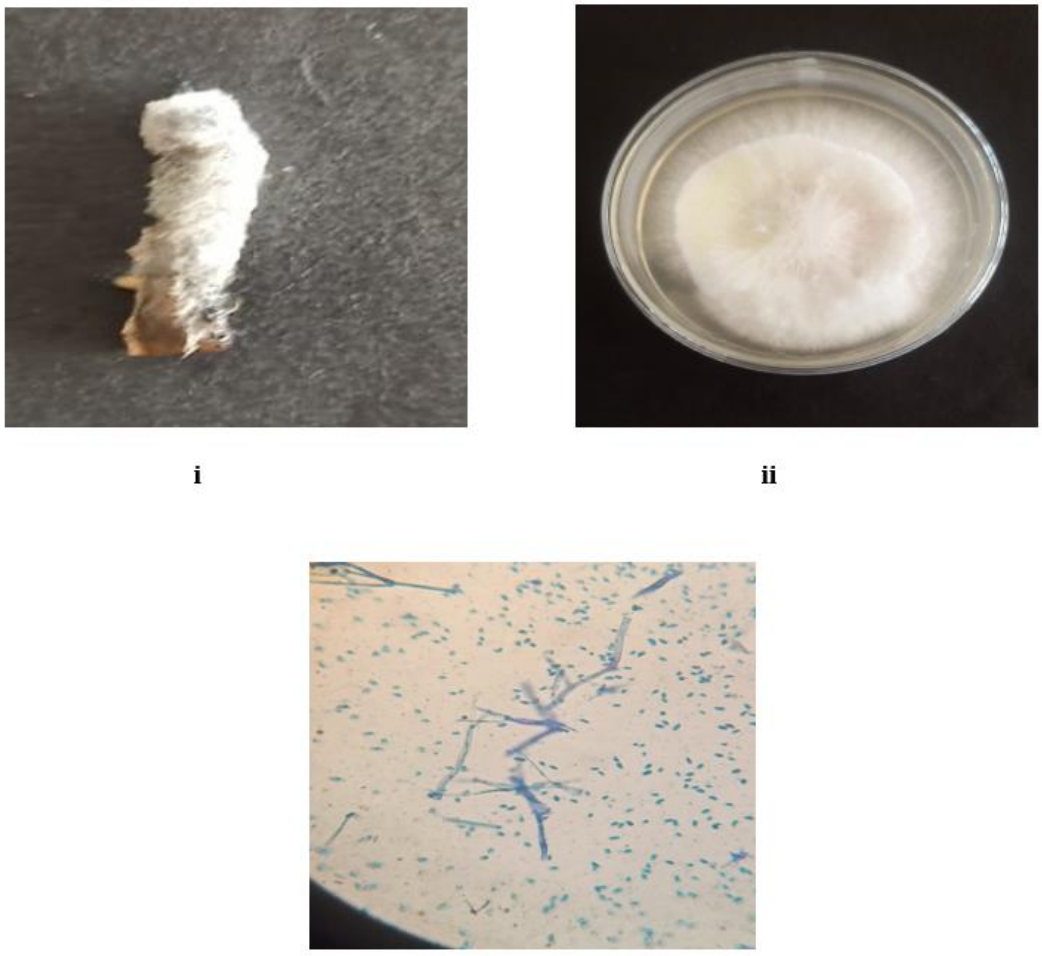

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Figure 4 (b) i. *Pieris rapae* larvae (imported cabbage worm) infected with *Metarhizium anisopliae* isolate. ii. Growth of *M. anisopliae* and iii. Cylindrical shape conidia at 10x

Maintenance of pure culture

The entomopathogenous fungus isolated from dead insects was identified by observing the standard characters of the specific fungus and are cross-checked. Then it was subcultured for 4-5 times to get the purest form of the culture. The cultures of entomopathogenic fungi were grown in sterilised petri dishes on Potato Dextrose Agar (PDA) medium for 12 days. Then the culture was preserved in the test tubes. Then the slant cultures are kept in the refrigerator at 4°C for further use.

Mass production of entomopathogenic fungi (*B. bassiana* and *M. anisopliae*) on different grain media, viz, wheat and sorghum.

Mass production of *Beauveria bassiana* and *Metarhizium anisopliae* on various whole grains, viz., wheat and sorghum, was used. 500 g of each grain was washed well and soaked in water overnight. The excess water was drained by decanting and shade drying it for 10-20 minutes to further remove the excess moisture. The grains were packed separately in individual 1000 ml glass bottles for both *B. bassiana* and *M. anisopliae*. They were plugged with cotton and autoclaved at 15 psi for 1 hour. After cooling, each glass bottles were inoculated with a 5 mm diameter fungal disc cut from the edge of twelve-day-old culture of *B. bassiana* and *M. anisopliae* using a sterilised cork borer under a laminar air flow chamber. Glass bottles were incubated in a BOD incubator at 25±1°C and 95± 5 percent relative humidity for 15-20 days in BOD incubator. To avoid clumping, after 7 days of inoculation, the glass bottles were shaken vigorously to separate the grain and to break the mycelial growth.

3.12. Testing of entomopathogenic fungi (that mass multiply in the lab) of major insect pests of cabbage crops under field conditions.

Fully mycelium growth of *Beauveria bassiana* and *Metarhizium anisopliae* glass bottles were taken for testing against *Pieris brassicae* and *Pieris rapae* larvae under field conditions. Remove the mycelial growth with the help of a long wooden stick and put it into the grinder for powder formation. After grinding, the entomopathogenic fungi of *Beauveria*

bassiana and *Metarhizium anisopliae* broken grains were dried in the hot air oven and later mixed with calcium carbonate for testing under field conditions. Entomopathogenic fungi powder was mixed with distilled water at different dose and sprayed with a hand compression sprayer on major insect pests of the cabbage crop in field, as shown in Table 1.

Table 1. Details of the treatment with different doses

S. No.	Treatments Entomopathogenic agents	Dose g/L
1.	*Beauveria bassiana*	3g
2.	*Beauveria bassiana*	5 g
3.	*Metarhizium anisopliae*	5g
4.	*Metarhizium anisopliae*	3 g
5.	*Untreated control*	Water spray

Statistical analysis

Data was analysed by using CRD in lab conditions with the help of an analysis of variance table (ANOVA) wherever required. The F value will be calculated and critical difference (CD) was tested at five per cent level of significance.

Result and Discussion

Efficacy of entomopathogenic fungi on mycelial growth on PDA media and solid substrates, viz., wheat and sorghum.

Evaluation of Entomopathogenic fungi for mycelial growth

Entomopathogenic fungi from cadaver samples were taken and grown on PDA media. Then the plates are checked for mycelium growth at different days, such as the third, sixth, ninth, and twelfth days after inoculation (Table 2) and Fig 5. On the 3rd day after inoculation, T2- *B. bassiana* was the most effective of all entomopathogenic fungi (24.75 mm), followed by T3- *M. anisopliae* (23.75 mm) and T1 *B. bassiana* (19.50 mm). The minimum mycelial growth was T4- *M. anisopliae* (18.50 mm), as shown in table 1. However, on the 6th day after inoculation, T2- *B. bassiana* was the most effective of all entomopathogenic fungi (43.75 mm), followed by T3- *M. anisopliae* (41.50 mm) and T1- *B. bassiana* (39.75 mm). The minimum mycelial growth was observed in T4- *M. anisopliae* (38.75 mm). On the 9th day after inoculation, T2- *B. bassiana* was the most effective of all entomopathogenic fungi (64.25 mm), followed by T3- *M. anisopliae* (62.25 mm) and T1- *B. bassiana* (58.00 mm). The minimum mycelial growth was observed inT4- *M. anisopliae* (57.00 mm). On the 12th day after inoculation, T2- *B. bassiana* was the most effective of all entomopathogenic fungi (85.50 mm), followed by T3- *M. anisopliae* (83.75 mm) and T1- *B. bassiana* (81.00 mm). The minimum mycelial growth was observed in T4- *M. anisopliae* (79.75 mm).

Table 2: Effect of different entomopathogenic fungi on the growth of mycelium "mm," i.e., *Beauveria bassiana* & *Metarhizium anisopliae*.

S. No.	Treatments	3DAI	6DAI	9DAI	12DAI
1.	*B. bassiana*	19.50	39.75	58.00	81.00
2.	*B. bassiana*	24.75	43.75	64.25	85.50
3.	*M. anisopliae*	23.75	41.50	62.25	83.75
4.	*M. anisopliae*	18.50	38.75	57.00	79.75
	C.D	1.043	1.492	1.371	1.441
	SEM (±)	0.16	0.33	0.28	0.30

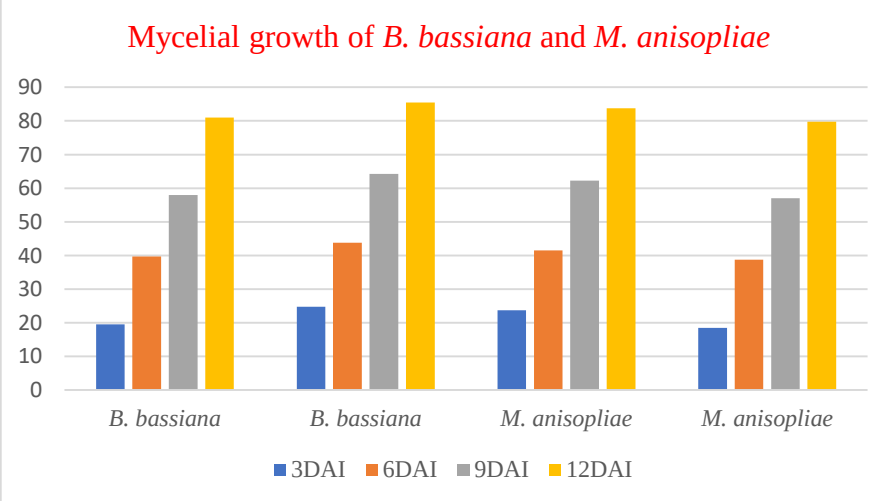

Figure 5. Mycelial growth of different entomopathogenic fungi, viz., *B. bassiana* and *M. anisopliae* (mm).

Evaluation of different grains (Wheat and sorghum) for mass production of entomopathogenic fungi

The mycelial growth of *B. bassiana* and *M. anisopliae* were cut into small pieces of 5 mm size with the help of a cork borer and filled into different grain bottles with the help of an inoculating loop. They were incubated in a BOD incubator at 25±1°C for 15-20 days. To avoid clumping, after 7 days of inoculation, the glass bottles were shaken vigorously to separate the grain and break the mycelial growth. The mycelial growth is recorded on different days, namely fifth, tenth, fifteenth, twentieth, and twenty-fifth days after inoculation.

The following table shows the amount of mycelial growth (mm) at different grains in table 3 and Fig 6.

Among the different grains used, on 5th day after inoculation, it was proved that maximum mycelial growth was required for mass production of entomopathogenic fungi T2- *B. bassiana* of sorghum (64.25 mm), followed by T3- *M. anisopliae* of wheat (60.75 mm) and T1- *B. bassiana* of wheat (54.75 mm) whereas, minimum mycelial growth was shown in T4- *M. anisopliae* of sorghum (51.25 mm) as shown in table 3. On 10th day after inoculation, it was proved that maximum mycelial growth was required for mass production of entomopathogenic fungi T2- *B. bassiana* of sorghum (85.00 mm), followed by T3- *M. anisopliae* of wheat (80.25- mm) and T1- *B. bassiana* of wheat (62.00 mm) whereas, minimum mycelial growth was shown in T4- *M. anisopliae* of sorghum (60.50 mm). On 15th day after inoculation, it was proved that maximum mycelial growth was required for mass production of entomopathogenic fungi T2- *B. bassiana* of sorghum (96.50 mm), followed by T3- *M. anisopliae* of wheat (92.25 mm) and T1- *B. bassiana* of wheat (76.50 mm) whereas, minimum mycelial growth was shown in T4- *M. anisopliae* of sorghum (75.50 mm). However, on the 20th day after inoculation it was proved that maximum growth for mass production of entomopathogenic fungi T2- *B. bassiana* of sorghum (101.75 mm) and it was followed by T3- *M. anisopliae* of wheat (97.25 mm) and T1- *B. bassiana* of wheat (87.50 mm) whereas, minimum mycelial growth was shown in T4- *M. anisopliae* of sorghum (85.25 mm). On 25th day after inoculation it was proved that maximum mycelial growth for mass production of entomopathogenic fungi was shown in T2- *B. bassiana* of sorghum (104.50 mm), followed by T3- *M. anisopliae* of wheat (101.75 mm) and T1- *B. bassiana* of wheat (93.25 mm) whereas, minimum mycelial growth was shown in T4- *M. anisopliae* of sorghum (91.00 mm).

Table 3: Efficacy of different grains (Wheat and sorghum) for mass production of *Beauveria bassiana* and *Metarhizium anisopliae* in "mm"

S. No.	Treatment	5DAI	10DAI	15DAI	20DAI	25DAI
1	*B. bassiana* (Wheat)	54.75	62.00	76.50	87.50	93.25
2	*B. bassiana* (Sorghum)	64.25	85.00	96.50	101.75	104.50
3	*M. anisopliae* (Wheat)	60.75	80.25	95.25	97.25	101.75
4	*M. anisopliae* (Sorghum)	51.25	60.50	75.50	85.25	91.00
	CD.	1.475	1.525	1.389	1.492	1.441
	SEM (±)	0.32	0.34	0.28	0.33	0.30

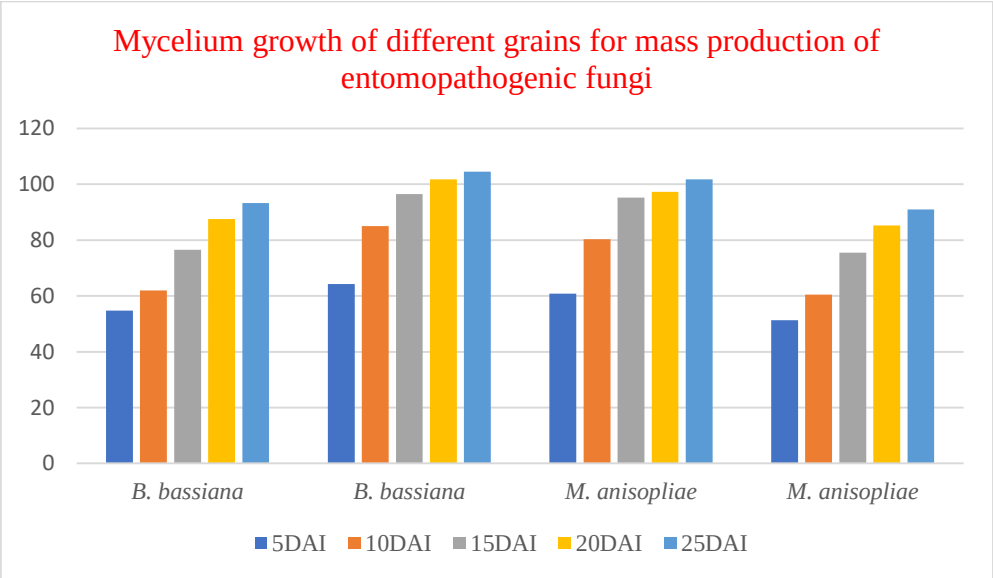

Figure 6. Mycelial growth of different grains for mass production of entomopathogenic fungi in "mm".

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CONCLUSION

- Among the isolates *B. bassiana* found *higher* mycelial growth (85.50 mm) followed by *M. anisopliae* (83.75mm) was promising in laboratory as well as field against *Pieris brassicae* larvae (Cabbage butterfly) and *Pieris rapae* larvae (Cabbage worm).
- The isolation and mass production of solid substrates for *B. bassiana* and *M. anisopliae* was superior against both *P. brassicae* and *P. rapae* larvae.
- We have found results of isolation and mass production of entomopathogenic fungi i.e., *B. bassiana* and *M. anisopliae* shows higher efficacy against *Pieris brassicae* larvae and *Pieris rapae* larvae (imported cabbage worm) under field conditions. So, we can use these entomopathogenic fungi against respective crucifer pests as biological control agents.

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