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INVESTIGATION ON THE EFFECT OF BIOPESTICIDES ON MUSTARD APHID *LIPAPHIS ERYSIMI*, KALT.

Longjam Boris Singh*, S. Zeshmarani, Chuwang Hijam, Salam Prabin Singh, and R.K. Lembisana

Krishi Vigyan Kendra, Thoubal District, Department of Agriculture, Govt. of Manipur-795138, India
(ICAR ATARI, Zone-VII, Umiam, Meghalaya)
*Corresponding Author E-mail: borislongjam1@gmail.com; Mob.8974852548
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ABSTRACT

The mustard aphid is a highly destructive pest that significantly impacts the productivity of Indian Mustard (*Brassica juncea* L.). The current experiment aimed to evaluate the effectiveness of biopesticides *Metarhizium anisopliae*, *Beauvaria bassiana* and *Bacillus thuringiensis* in controlling mustard aphid (*Lipaphis erysimi* Kalt.). Biopesticides have a specific target, inhibit insect growth and metabolic processes, and have a lower toxicity to mammals. Several bioassays were conducted using five different concentrations of insecticide. The mortality data was collected over a three-day period, with measurements taken every 12 hours. According to the research findings, *M. anisopliae* demonstrated the highest efficacy (83.11%) in controlling mustard aphid, followed by *B. bassiana* (77.89%) and *B. thuringiensis* (74.01%). Furthermore, the results demonstrated the beneficial effects of the biopesticides on a number of plant parameters, including plant height, the number of leaves, branches, and siliques on the plant as well as the total number of seeds per silique, 1000 seed weight, germination percentage, shoot and seedling length, seed yield per plot, and overall seed yield. The findings of this experiment indicate that bio-pesticides have potential as a viable approach for pest management in the context of mustard aphid.

***Key words*:** Biopesticides, efficacy, mustard aphid, yield.

Introduction

In India, agricultural crop losses caused by insect pests amount to INR 2650 billion (Anon., 2022). India ranks first globally in the cultivation of oilseed *Brassicas*, although the average yield per hectare is hindered by frequent aphid infestations and cultivation in rainfed areas (FAO Stat, 2022). Mustard aphid is considered a significant pest in India, leading to yield losses between 35.4% to 96% and a decrease in oil content of 5% to 6% (Patel, 2004, Shylesha 2006, Kular and Kumar, 2011). An aphid infestation resulted in substantial harm to different parts of the plants, such as leaves, inflorescences, stems, and seeds, and led to a significant decrease in nutritional components like carbohydrates, lipids, nitrogen, and protein levels during various growth stages of the mustard crops (Raikes and Burpee, 1988). Typically, once a pest infestation is observed on the farm, pesticides are used to prevent a decrease in crop yield. Additionally, the expense of pesticides creates an additional challenge for farmers, as well as contributing to environmental pollution (Kumar and Patel, 2017). Chemical insecticides are frequently utilised to manage aphids because of their persistent and harmful effects. Farmers use various pesticides without discrimination to control pests, leading to environmental pollution, insecticide resistance in pests, pest resurgence, and harm to beneficial organisms like pollinators. This disrupts the natural balance and poses health risks to humans. There is a necessity to decrease chemical usage in agricultural practices and explore eco-friendly and environmentally safe methods for pest control (Lanting, 2007). Entomopathogenic fungi are frequently utilised as bio-control agents to environmentally manage mustard aphids (Deka *et al*., 2017). Various strains of *Metarhizium anisopliae* and *Beauveria bassiana* fungi

create metabolic chemicals that can be harmful to insects and are useful for pest management (Vey *et al*., 2001). This can help reduce agricultural losses and protect farm productivity to ensure food security for the increasing population (Jeger *et al*., 2021). Thus, it is beneficial to embrace new technology to create an innovative, efficient, and cost-effective approach that can advance and improve traditional knowledge, practices, and techno-traditions

Material and Methods

Site selection

A field experiment was conducted at the field of Krishi Vigyan Kendra, Thoubal from 2021 to 2022 both in Rabi season. The experimental site is located in Thoubal district with altitude of 790 m above mean sea level and possess coordinates latitude 24.614404 and longitude 94.01693.

Experimental design and treatment details

The experiment was taken up in randomized complete block design (RCBD) with 4 treatments and 5 replications. The different treatments are T1: *Metarhizium anisopliae* (25%) 5.39×10^{8} CFU, T2: *Beauveria bassiana* (25%) 4.78×10^{8} CFU, T3: *Bacillus thuringinesis* (25%) 6.49×10^{7} CFU and T4: Untreated control .

Plot size was $2m^2 \times 2m^2$. Recommended fertilizer dose of 80:40:30 (N: P: K) kg/ha was applied.

Agronomic practice

The land was tilled thoroughly to remove the weeds and attain fine tilth. The seeds were directly sowed in the field manually with the spacing of 30cm × 10cm with recommended packages of practice excluding plant protection.

Preparation of treatments

Concentration preparation

Five conidial suspensions (dilutions) *i.e.*, 5, 10, 15, 20 and 25% of each bio-pesticide were prepared. The determined quantity of each was mixed in water up to the required volume to prepare 5, 10, 15, 20 and 25% dilutions. The Colony-forming unit CFU counted by using hemocytometer.

Calculation of colony forming unit of bacteria and fungi

Colony-forming unit (CFU) is a measure of viable bacterial or fungal cells. Serial dilutions, plating and counting of live bacteria was used to determine the number of bacteria and fungi in a given population. Serial dilutions were made of bacteria and fungi and compared them to the dilution factor. Each colony forming unit represents a bacterium and fungus that were present in the diluted sample. The numbers of colony forming units (CFU's) divided by the product of the dilution factor and the volume of the plated diluted suspension to determine the number of bacteria and fungi per mL that were present in the original solution.

Calculating the number of bacteria per mL of serially diluted bacteria

The number of bacteria and fungi per mL of diluted sample was calculated by using the following equation:

$$\frac{\text{Number of CFU}}{\text{Volume plated (mL) x total dilution used}} \rightarrow \frac{\text{Number of CFU}}{\text{mL}}$$

Application of treatment and Data recording

The observations on population of sucking pest were recorded visually using a magnifying lens early on top 10 cm central apical twig per plant from five randomly selected and tagged plants in each plot. Aphid count was taken 24 hours before spraying at 5 tagged plants per treatment, which was further converted into per plant population and subsequent observation was recorded at 3, 7 and 14 days after spraying on same plants. The formula used for the calculation of percentage reduction of pest population over control using following formula giving by Henderson and Tilton (Henderson and Tilton, 1955) referring it to be modification of Abbott, (1925).

The average percent reduction of pest population of all two sprays was worked out by using Henderson and Tilton formula described as under:

$$\text{Percent reduction} = 1 - \frac{Ta}{Tb} \times \frac{Cb}{Ca} \times 100$$

Where,

Ta = Number of insects in treated plot after insecticides application

Tb = Number of insects in treated plot before insecticides application

Ca = number of insects in Untreated check after insecticide application

Cb = Number of insects in untreated check before insecticide application (Dotasara *et al*., 2017)

Result

Impact of *Metarhizium anisopliae* on mortality

Analysis of varianceshowed that the effects of all concentrations of *Metarhizium anisopliae* were significantly different against adults of *Lipaphis erysimi*. The highest mortality was obtained (83.11) at 25%

Table 1: Effect of *Metarhizium anisopliae* treatment on the per cent mortality of *L. eryisimi*.

Metarhizium anisopliae	**Mean Per cent mortality**					
Concen-tration	**12 hours**	**24 hours**	**36 hours**	**48 hours**	**60 hours**	**72 hours**
25 %	19.850	31.220	45.110	54.110	69.290	83.110
20%	14.770	27.560	37.990	51.890	61.870	67.010
15%	10.440	21.010	34.450	46.220	54.010	57.890
10%	7.020	14.010	21.090	34.900	38.880	47.760
5%	1.770	11.090	14.880	17.780	23.550	29.880
Concen-tration	**12 hours**	**24 hours**	**36 hours**	**48 hours**	**60 hours**	**72 hours**
C.V.	8.717	6.789	5.624	7.145	6.729	5.701
F Prob.	0.000	0.000	0.000	0.000	0.000	0.000
S.E.M.	0.420	0.637	0.772	1.309	1.490	1.457
C.D. 5%	1.259	1.909	2.315	3.926	4.468	4.367

Table 2: Effect of *Beauvaria bassiana* treatment on the per cent mortality of *L. eryisimi*.

Beauvaria bassiana	**Mean Per cent mortality**					
Concen-tration	**12 hours**	**24 hours**	**36 hours**	**48 hours**	**60 hours**	**72 hours**
25 %	17.010 Aa	28.450 Aa	37.990 Aa	46.700 Aa	61.090	77.890
20%	12.580 Bb	21.660 Bb	26.770 Bb	37.880 Bb	47.880	59.890
15%	4.670 Cc	15.040 Cc	21.110 Cc	25.110 Cc	41.100	49.770
10%	1.780 Dd	16.110 Dd	21.130 Dd	29.110 Dd	34.110	41.090
5%	0.000 Ee	1.000 Ee	6.760 Ee	12.010 Ee	16.010	27.010
Concen-tration	**12 hours**	**24 hours**	**36 hours**	**48 hours**	**60 hours**	**72 hours**
C.V.	17.865	13.965	8.086	9.202	7.041	7.433
F Prob.	0.000	0.000	0.000	0.000	0.000	0.000
S.E.M.	0.480	0.856	0.710	1.073	1.087	1.477
C.D. 5%	1.416	2.526	2.094	3.165	3.208	4.358

concentration followed by 20%, 15%, 10% and 5% with respectively as shown in Table 1.

Mortality effect of *Beauveria bassiana*

All concentrations of *Beauveria bassiana* were significantly different against adults of *Lipaphis erysimi*. The maximum mortality (77.89%) was obtained at 25% concentration of B. bassiana followed by 20%, 15%, 10%, and 5% with 59.89%, 49.77%, 41.90% and 27.01% mortality, respectively as compared to control (10.98%)

Mortality effect of *Bacillus thuringiensis*

Effects of *Bacillus thuringiensis* were significantly differing against adults of *Lipaphis erysimi*. The supreme mortality (74.01%) was obtained at 25% concentration of *B. thuringiensis* followed by 20%, 15%, 10%, and 5% to 57.11%, 44.45%, 35.01% and 19.87% mortality, respectively as compared to control (8.89%)

Plant height (cm)

Plant height of mustard varied significantly at 40, 55 and 70 days after sowing (DAS) due to different treatment. At 70 DAS, the longest (85.77cm) plant was produced from T1 treatment and the shortest (69.98) was found from T4 (control) treatment.

Number of leaves plant^{-1}

Number of leaves is directly related to the mustard yield. Number of leaves per plant of mustard varied significantly at 40, 55 and 70 days after sowing (DAS) due to different treatment. At 90 DAS, the highest number of

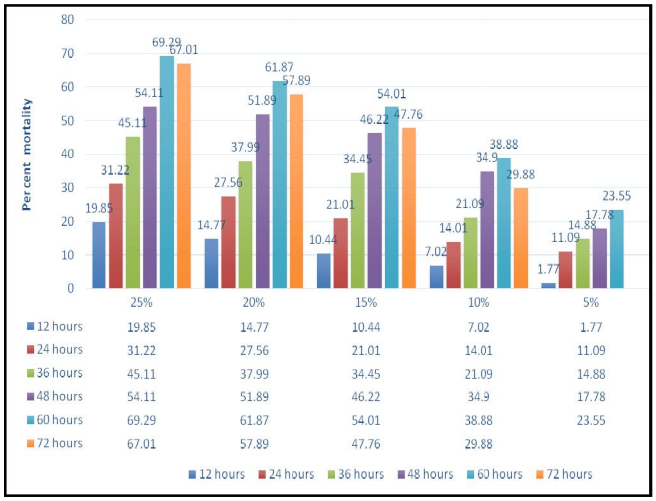

Fig. 1: Effect of *Metarhizium anisopliae* treatment on the per cent mortality of *L. eryisimi*.

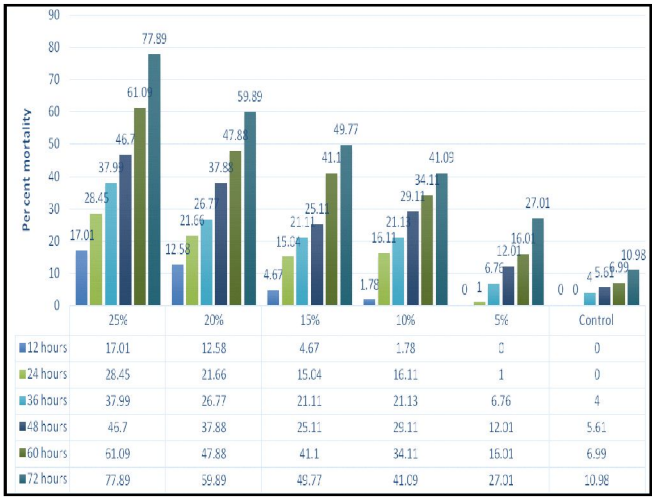

Fig. 2: Effect of *Beauvaria bassiana* treatment on the per cent mortality of *L. eryisimi*.

Table 3: Effect of *Bacillus thuringiensis* treatment on the per cent mortality of *L. eryisimi*.

Bacillus thuringiensis	Mean Per cent mortality					
Concentration	**12 hours**	**24 hours**	**36 hours**	**48 hours**	**60 hours**	**72 hours**
25%	8.220 Aa	21.440 Aa	34.010 Aa	40.010 Aa	57.010 Aa	74.010 Aa
20%	4.990 Bb	13.990 Bb	24.590 bb	32.980 Bb	47.010 Bb	57.110 Bb
15%	3.450 Cc	11.010 Cc	18.770 Cc	25.010 Cc	31.220 Cc	44.450 Cc
10%	1.780 Dd	5.010 Dd	11.980 Dd	16.010 Dd	27.990 Dd	35.010 Dd
5%	0.000 Ee*	0.000 Ee	5.220 Ee	6.010 Ee	12.890 Ee	19.870 Ee
Concentration	**12 hours**	**24 hours**	**36 hours**	**48 hours**	**60 hours**	**72 hours**
C.V.	14.913	10.443	9.965	12.376	7.860	5.386
F Prob.	0.000	0.000	0.000	0.000	0.000	0.000
S.E.M.	0.205	0.400	0.717	1.132	1.067	0.961
C.D. 5%	0.605	1.181	2.116	3.339	3.148	2.834

*Means of replicates followed by the same small letter in a column are not significantly different

Table 4: Effect of treatment on plant height at different days after sowing of mustard.

Treatment	Plant Height (cm)		
	40DAS	**55DAS**	**70DAS**
T1	29.110	60.430	85.770
T2	28.990	55.780	84.760
T3	24.000	50.110	81.330
T4	19.780	46.010	69.980
C.V.	7.418	9.041	10.200
F Prob.	0.000	0.000	0.000
S.E.M.	0.696	1.732	3.009
C.D. 5%	2.143	5.336	9.271

*DAS= days after sowing

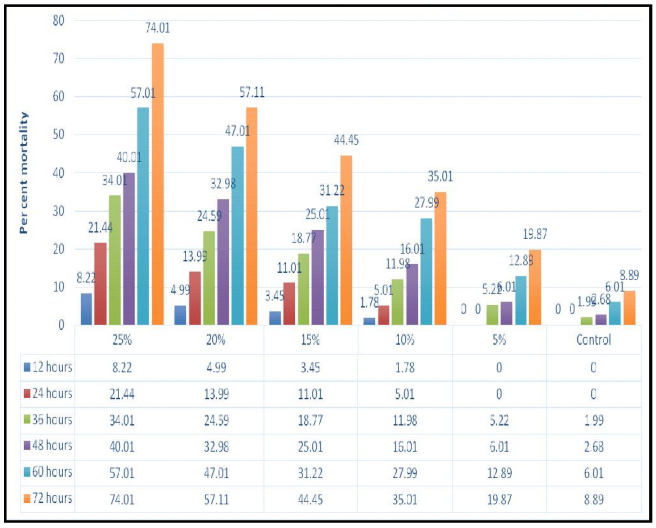

Fig. 3: Effect of *Bacillus thuringiensis* treatment on the per cent mortality of *L. eryisimi*.

leaves (25.83) per plant was obtained from T1 treatment and the lowest (18.01) from (control) T4 (treatment) as shown in Table 5 and Fig. 5 .

Number of branch plant^{-1}

Number of branches plant^{-1} in mustard showed significant difference where the number of branches (9.20) was found in T1 followed by T2 (8.18) and T3 (7.580). Minimum number of branch plant^{-1} was recorded (6.00) in T4 as shown in Table 6 and Fig. 6.

Number of silique plant^{-1}

Silique number plant^{-1}was observed maximum in T1 *i.e.* 81.11 closely followed by T2 (78.11) and minimum number of silique plant^{-1}was found 54.11 in T4 shown in Table 6 and Fig. 6

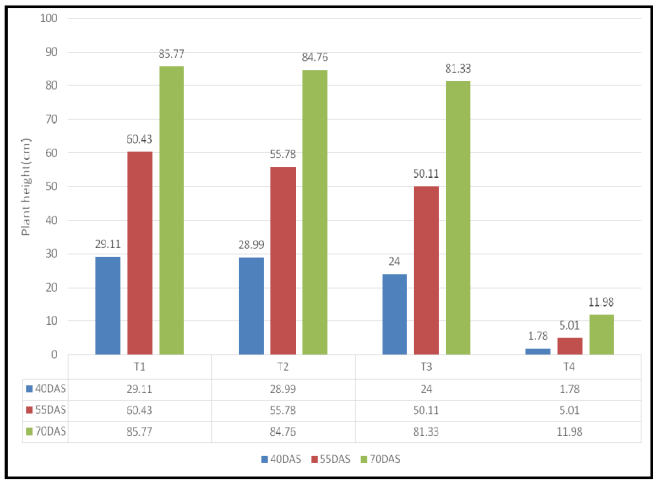

Fig. 4: Effect of treatment on plant height at different days after sowing of mustard.

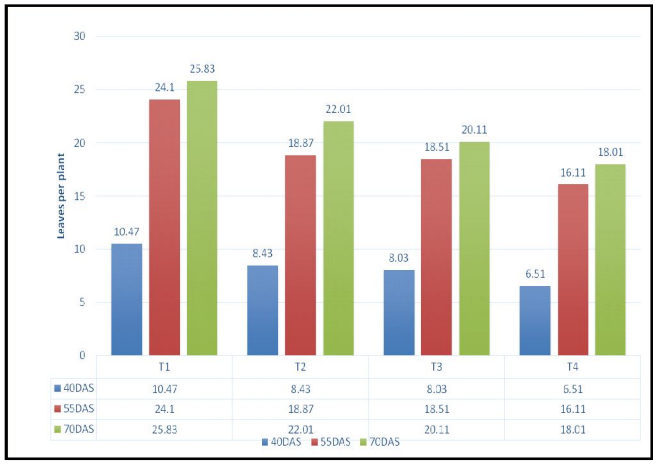

Fig. 5: Effect of treatments on number of leaves per plant at different days after sowing of mustard.

Table 5: Effect of treatments on number of leaves per plant at different days after sowing of mustard.

Treatment	Plant Height (cm)		
	40DAS*	55DAS	70DAS
T1	10.470	24.100	25.830
T2	8.430	18.870	22.010
T3	8.030	18.510	20.110
T4	6.510	16.110	18.010
C.V.	6.336	7.091	5.082
F Prob.	0.000	0.000	0.000
S.E.M.	0.237	0.615	0.488
C.D. 5%	0.730	1.895	1.505
*DAS= days after sowing			

Total number of seed silique^{-1}

Maximum number of seed silique^{-1} was found 22.56 in T1 which is closely followed by T2 (18.70) and minimum number of seed silique^{-1} was found 14.51 in T4 shown in Table 6 and Fig. 6.

Length of silique

Height length of silique was found 8.43 in T1 which is closely followed by T2 (7.81) and lowest number of silique per plant was found 5.44 in T4 (Control) shown in Table 6 and Fig. 6.

1000 seeds weight

There is no significant difference found in 1000 seed weight though higher 1000 seeds weight 3.52 found in T1 and lower 1000 seeds weight 3.30 found in T4 as shown in Table 7 and Fig. 7.

Germination %

Different treatment application significantly influenced the germination of harvested mustard. The highest (95.33%) germination was found from T1 treatment. The lowest (77.67%) germination from was observed in T4 (control) treatment as shown in Table 7 and Fig. 7.

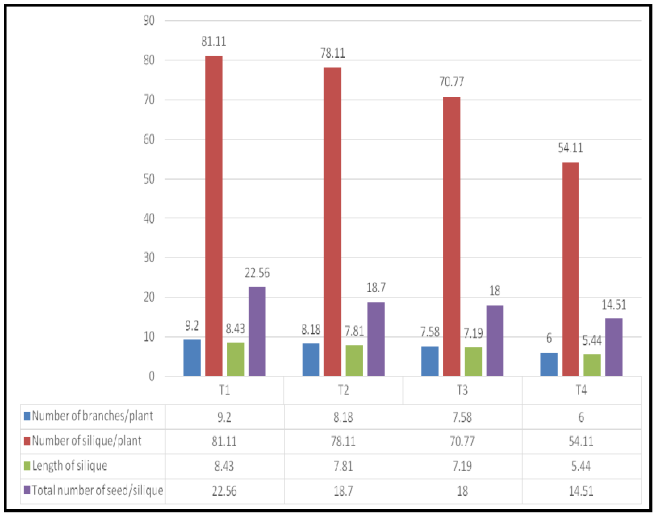

Fig. 6: Effect of treatments on number of branches per plant, silique/plant, length of silique and total no. of seed/ silique of mustard.

Table 6: Effect of treatments on number of branches per plant, silique/plant, length of silique and total no. of seed/ silique of mustard.

Treat-ments	Number of branches/ plants	Number of silique/ plants	Length of silique	Total number of seed/siliques
T1	9.200	81.110	8.430	22.560
T2	8.180	78.110	7.810	18.700
T3	7.580	70.770	7.190	18.000
T4	6.000	54.110	5.440	14.510
C.V.	8.046	7.689	5.558	5.284
F Prob.	0.000	0.000	0.000	0.000
S.E.M.	0.279	2.442	0.179	0.436
C.D. 5%	0.858	7.525	0.553	1.343

Shoot length (cm)

A significant variation was found in shoot length of mustard due to application of bio insecticide. The highest (6.77 cm) shoot length of mustard was found from T1 treatment. The lowest (5.88 cm) shoot length of mustard was observed in T4 (control) treatment as shown in Table 7 and Fig. 7.

Root length (cm)

The highest (7.33 cm) root length of mustard was found from T1 treatment. The lowest (5.80 cm) root length of mustard was found from T4 (control) as shown in Table 7 and Fig. 7.

Seedling length (cm)

The maximum (15.67 cm) seedling length of mustard

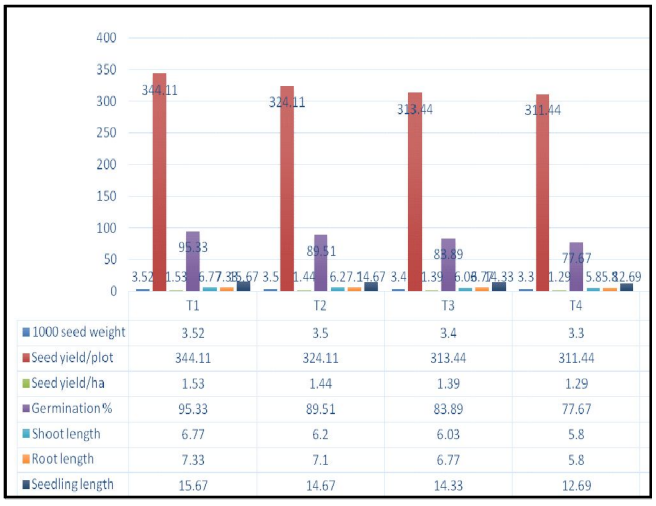

Fig. 7: Effect of treatments on 1000 seeds weight , seed yield/ plot, seed yield/ ha, germination of mustard, shoot length, root length, seedling length.

Table 7: Effect of treatment on 1000 seeds weight, seed yield/plot, seed yield/ ha, germination of mustard, shoot length, root length, seedling length.

Treat-ments	1000 seed weight	Seed yield/ plot	Seed yield/ ha	Germi-nation %	Shoot length	Root length	Seed-ling length
T1	3.520	344.110	1.530	95.330	6.770	7.330	15.670
T2	3.500	324.110	1.440	89.510	6.200	7.100	14.670
T3	3.400	313.440	1.390	83.890	6.030	6.770	14.330
T4	3.300	311.440	1.290	77.670	5.800	5.800	12.690
C.V.	4.770	9.814	5.961	8.814	4.770	7.897	5.923
F Prob.	0.002	0.019	0.005	0.019	0.002	0.003	0.001
S.E.M.	0.132	3.414	0.038	3.414	0.132	0.238	0.380
C.D. 5%	0.408	10.518	0.116	10.518	0.408	0.735	1.170

was found from T1 (*Metarhizium anisopliae*) treatment. The minimum (12.69 cm) root length of mustard was found from T4 (control) treatment as shown in Table 7 and Fig. 7.

Seed yield plot^{-1}

Different levels of bio pesticide application significantly effect on the seed yield per plant of mustard. Highest seed yield per plot was revealed 344.11 Kg in T1 and lowest seed yield per plot was found 289.67 Kg in T4 (Control) as shown in Table 7 and Fig. 7.

Seed yield (ton hectare^{-1})

Seed yield ton per hectare was showed statistically significant variation due to different treatment application. The maximum seed yield ton per hectare (1.530) was found in T1. On the other hand, the minimum seed yield ton per hectare (1.29) was found in T4 (control) treatment as shown in Table 7 and Fig. 7.

Discussion

In the current study, the effects of various biopesticides on the mortality of mustard aphids following the application of all concentrations were analysed and it was discovered that, *Metarhizium anisopliae* was found to be the most effective followed by *Beauvaria bassiana* and *Bacillus thuringiensis* against *Lipaphis erysimi*. The findings aligned with the research conducted by Sajid *et al*., 2017 who reported that *M. anisopliae* was the most effective biopesticide against mustard aphid, followed by *B. bassiana* and *B. thuringiensis*. Saranya *et al.*, (2010) investigated the proportion of aphid death at 12-hour intervals over seven days and discovered that as the concentration grew, so did the aphid mortality rate. At high concentrations, the mortality rate reached between 53 to 60 percent after 72 hours. Never the less, the study found mortality rates of 78% for *B. bassiana* at high concentration and 60 to 70% for M. anisopliae aphids, with the current study showing an 83.11% mortality rate for *M. anisopliae*, 77.89% mortality for *Beauvaria bassiana* and 74.10 % for *Bacillus thuringiensis*.

The ascomycetes *Metarhizium anisopliae* and *Beauvaria bassiana* are best characterized entomopathogenic fungi.Fungi infect vulnerable hosts by directly penetrating through the cuticle,conidia germination and development and hydrophobic interactions with the host cuticle.

Infection continues with hyphae differentiation into blastospores/hyphal bodies in the haemolymph, host colonisation, extrusion to the host corpse surface, and conidiophore and conidia generation. Acids such as trehalase are produced by the fungi inside the insects body

Fungi produces significant amounts of acid *viz*. trehalose in the insect's blood. The fungus utilises sugar while depleting trehalose, reducing the amount of sugar available to the host. After colonising the host, the nutrients are depleted, forcing the fungus to form hyphae that emerge and produce conidia on the deceased host's surface. Among the three biopesticides, the low toxicity of Bt toxins against hemipteran pests has resulted in limited effectiveness against sap-sucking pests (Baum J. *et al.*, 2012). The reasons for the low toxicity of Bt toxins in aphids are diverse, including toxin instability in the aphid gut and low levels of binding (Chougule and Bonning, 2011; Walter and English, 1995; Walters, Kulesza , Phillips and English *et al*., 1994). Attachment of a Bt toxin to the target insect's stomach is a crucial stage for its toxicity (Soberon *et al*., 2007). A synthetic peptide has been shown to enhance toxin binding to the aphid gut. Utilising specific cytolytic toxins to attach peptides to the toxin of an aphid gut has been discovered to enhance toxin binding and its related toxicity (Chougule and Bonning, 2011). Cry proteins are significant because of the poisonous properties that they produce after being consumed by insects belonging to different orders (Crickmore). The order of the toxicity of cry proteins is as follows: Lepidoptera, Diptera, Coleoptera, Hymenoptera, Hemiptera, Isoptera, Orthoptera, Siphonoptera, and Thisanoptera.

According to Mukherjee *et al*., (2003), the method by which entomopathogensstimulate plant growth is believed to be multifactorial. The plant growth enhancing impacts include antibiosis, parasitism, induction of host plant resistance, and competition (Mukherjee *et al*., 2003). The colonisation of the rhizosphere has the potential to

create a barrier that is repellent surrounding the roots of the plant, which would provide the plant with effective protection against insect pests that feed on the plant. *Metarhizium* species have also been shown to have the potential to act as a biofertilizer, according to O'Brien, (2009) findings. *Metarhizium* spp. and *Beauvaria bassiana* also managed to kill insect larvae, endophytically colonise plants, and transfer nitrogen from insects to plants. In the case of arbuscular mycorrhizal fungi, the process of phosphate transfer from fungi to plants has been well investigated and characterised (Bargaz *et al*., 2018; Bitterlich *et al*., 2018). Entomopathogens are responsible for the production of auxins that promote growth on the roots. As a result of the early development of roots, the plant is able to establish itself earlier, and as a result, it has the capacity to outperform the biotic and abiotic sensors in its environment. It was reported by Mayara *et al*., 2022 that the inoculation of coffee seedlings with entomopathogens resulted in a rise in the total leaf area of the seedlings.

This serves as evidence that the entomopathogens operates as a growth promoter in plant seedlings. According to Behie *et al*., (2012) and Bamisile *et al*., (2018), the colonisation of plants by entomopathogenic fungi may either facilitate the intake of nutrients through the root system of the plant or facilitate the transfer of nitrogen from insect cadavers in the soil to the plant in exchange for carbon. Both of these mechanisms have the potential to increase plant growth. In a study that was carried out in 2017, Dara and colleagues investigated the influence that entomopathogenic fungi have on the growth, development, and health of plants that are subjected to water stress. They found that the length of the shoots and roots increased, and the ratio of the shoots to the roots indicated that the root growth was proportional to the shoot growth. According to the findings of the study, the biomass of plants that had been inoculated with entomopathogenic fungi became significantly higher.

Conclusion

The primary objective of this study was to assess and compare the effectiveness of several entomopathogens in controlling mustard aphids. The findings indicate a considerable decrease in the population of mustard aphids subsequent to the application of each treatment, as compared to the control group. The treatment modalities employed in the study exhibit potential applicability in the management of mustard aphids. The longer period of time between application and target killing still favour the chemical pesticides. It is anticipated that there will be a significant transformation in the coming decade as a result of enhanced understanding of the molecular underpinnings of host infections and the growing societal apprehension regarding the adverse consequences associated with pesticide usage. It is already possible to generate more efficient strains for biological control.

Conflict of interests: The authors declare no competing interests.

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