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Efficacy of the Plant Growth Promoting Rhizobacterium and Lufenuron for Reducing Insect-associated Yield Losses in Cauliflower

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Abstract **Original Research Article**

Insect attacks on plants are a severe problem in the agricultural sector. The damages caused by insects have an adverse impact on plant growth which leads to slow economic growth, particularly in countries dependent on agriculture. Plant growth-promoting rhizobacteria (PGPR) are widely utilized in agriculture because they offer a viable alternative to pesticides and artificial fertilizers. The growth and life cycle of insects/pests is inhibited by the insect growth regulators (IGRs), which belong to third-generation insecticides. Lufenuron is an IGR having a broad-spectrum insecticidal activity. Lufenuron inhibits insect growth by reducing chitin synthesis. A field experiment was carried out at Ayyub Agricultural Research Institute, Faisalabad, to evaluate the effect of MUR-4 (a potential PGRP) and lufenuron on cauliflower under insect attack. Cauliflower (Naran F1) seedlings were grown with and without a potential PGPR (MUR-4) using a randomized complete block design (RCBD). Attacks of cutworms, diamondback moths, armyworms and aphids were recorded periodically. Lufenuron was applied as a foliar spray, to inoculated as well as non-inoculated plants, upon insect attack. A foliar spray of lufenuron exerted a positive effect and reduced the insect attack. Plant biochemical analysis manifested the higher activities of phenolics and proline (secondary metabolites) in MUR-4 PGPR inoculated plants. Increased levels of malondialdehyde (MDA) and H_2O_2 indicated oxidative stress in plants. Peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD) enzymes play a very important role in defence against insect-induced biotic stress. MUR-4 PGPR inoculation enhanced the activity of the mentioned enzymes i.e. POD, CAT and SOD. We concluded that lufenuron spray can positively assist farmers in insect control strategies. Furthermore, MUR-4 PGPR inoculation improved the plant defence system against insect herbivory.

Keywords: Plant Growth-Promoting Rhizobacteria (PGPR-MUR-4), Insect Growth Regulator/ IGR (Lufenuron), *Brassica Oleracea* L. Var Botrytis (Cauliflower), Plant-Microbe Interaction, Plant-Insect Interaction.

INTRODUCTION

The popular winter vegetable cauliflower (*Brassica oleracea* L. var. botrytis) is grown for its delicious white curd. It developed from wild cabbage (*Brassica oleracea* L. var. capitata), formerly known as colewort, through processes of selection, adaption, and mutation (Purugganan *et al*., 2000). Records from the 6th century BC show that it was a member of the Brassicaceae family when it first originated in the Mediterranean region around 2,000 years ago (Fatima *et al*., 2024). As early as the 16th century, European researchers in Egypt and Turkey reported the health advantages of its high glucosinolate content (Sahito *et al*., 2021). About 200 years ago, in 1822, cauliflower was brought to the Indian subcontinent (Tavolacci, 2020).

The top producers of cauliflower worldwide are Italy, Spain, India, China, and the United States. With an average production of 17 tons per hectare, Pakistan is in the top ten producers worldwide (Organization, 2017); Sahito *et al*., 2021). Cauliflower is low in calories but high in minerals, dietary fibre, and important vitamins C and A *(Anwar et al*., 2023) (Silvosa-Millado *et al*., 2021). It is well-liked in diets for weight loss and is known to

lower the incidence of breast cancer due to its nutritional composition (Uuh-Narvaez & Segura-Campos, 2021).

For more than 350 million years, insects and plants have coexisted (Howe & Jander, 2008); (Hare, 2011) War *et al*., 2012). Nonetheless, insect pests still result in substantial agricultural losses, harming over 40% of crops globally and costing $220 billion yearly (Canton, 2021). Armyworm (*Spodoptera litura* F.), diamondback moth (*Pluttella xylostella* L.), black cutworm (*Agrotis epsilon*), and cauliflower aphid species (*Bevicoryne brassicae* L.) are the main pests of cauliflower, affecting plant growth and causing farmers to suffer significant financial losses *(Kumar et al*., 2023).

Because its larvae harm young plants by cutting plant stems at ground level, the black cutworm is very deadly (Devi, 2020) (Joshi *et al*., 2020). The diamondback moth is another destructive insect that may reduce agricultural output by up to 80% (Zalucki *et al*., 2012). It is notoriously difficult to manage due to its rapid reproduction and tolerance to conventional pesticides (Furlong *et al*., 2013). According to (Ahmad *et al*., 2013) and (Zhou *et al*., 2012), the armyworm is a polyphagous pest that decimates cauliflower and other crops, resulting in output losses ranging from 31% to 100% (Simon & Peccoud, 2018). Cauliflower crops are severely weakened by aphids that transmit plant viruses and drain plant resources, such as the green peach aphid (*Myzus persicae*) and cabbage aphid (*Brevicoryne brassicae*) (Chen *et al*., 2020).

Despite their previous effectiveness in eradicating these pests, synthetic pesticides have caused environmental pollution, resistance, and resiliency (Ngegba *et al*., 2022). As a result, several methods of controlling pests are being researched, such as biopesticides derived from plant extracts and microbial agents (Dotasara *et al*., 2017). Insect growth regulators, or IGRs, are third-generation insecticides that manage pests without endangering the environment (Gad *et al*., 2021) (Williams, 1967). By disrupting the production of chitin, the broad-spectrum IGR lufenuron stops insects from producing larvae. In insects that lack a healthy exoskeleton due to a lack of chitin, dehydration usually results in mortality (Mayer *et al*., 2013). Plants have evolved several defensive strategies to combat insect pests (Fatima *et al.*, 2024). These include structural defences, the capacity to attract natural pest foes, and the production of secondary compounds and proteins that discourage or damage herbivores (War *et al.*, 2012). These defences include both direct mechanisms, such as producing poisons and physical barriers (Karban, 2011), and indirect approaches, such as attracting predatory insects (Howe *et al*., 2008).

The plant growth-promoting rhizobacteria (PGPR) are starting to show promise as a substitute for conventional pest control methods. To aid in plant development, these soil bacteria fix nitrogen, solubilize phosphate, and produce phytohormones such as cytokinin and indole acetic acid (IAA) (Pineda *et al*., 2013). Additionally, to fight disease and pests, PGPR encourages induced systemic resistance (ISR), a defense mechanism that produces phytohormones (Serteyn *et al*., 2020). Another important plant defence mechanism that offers long-term disease resistance is systemic acquired resistance (SAR) (Kamle *et al.*, 2020). Chemicals like reactive oxygen species (ROS) and salicylic acid (SA), which release signals in response to plant-pathogen interactions, are the driving forces behind SAR. SA may boost H_2O_2 levels and fortify plant defenses by inhibiting catalase and ascorbate peroxidase (Saberi Riseh *et al*., 2022).

PGPR enhances plant pest defenses and prevents disease penetration by colonizing root surfaces and generating systemic resistance (Kannojia *et al*., 2019). Lipopolysaccharides, flagellar proteins, antibiotics, volatile organic compounds, quorum-sensing molecules, and siderophores are examples of bacterial determinants that cause ISR in plants (Bakker *et al*., 2007). This increased resilience allows plants to better fight subsequent invaders (De Vleesschauwer & Höfte, 2009). Additionally, siderophores—specialized iron-chelating molecules necessary for biological processes including respiration, DNA synthesis, nitrogen fixation, and photosynthesis are produced by PGPR (Mustaine *et al*., 2017). Additionally, PGPR generates gibberellins, IAA, and cytokinin, which help fix nitrogen and give plants ammonium while shielding them from pest attacks and disease (Meena *et al*., 2020).

The current study expected that cauliflower plants treated with MUR-4 PGPR or foliar spray of the IGR lufenuron would have improved defensive mechanisms and less insect-related damage (Ullah, Qasim, *et al.*, 2024). The study aimed to assess the growth and metabolic changes in cauliflower caused by the putative PGPR strain MUR-4, determine its role in activating plant defence mechanisms against insect pests, and investigate the potential value of lufenuron as an IGR in controlling cauliflower insect infestations. Furthermore, the comparative efficiency of the PGPR and IGR in reducing insect-associated damage was studied to obtain insight into long-term pest management approaches for cauliflower cultivation.

MATERIALS AND METHODS

Materials

The experiment was carried out using cauliflower seedlings of the Naran-F1 variety that were bought from the vegetable section of the Ayyub Agricultural Research Institute (AARI) in Faisalabad, Pakistan. A previously identified strain of plant growth-promoting rhizobacterium (PGPR), known as "MUR-4," was obtained from the Plant Biotechnology Laboratory at Government College University Faisalabad's Department of Bioinformatics and Biotechnology. In this

investigation, the insecticide used was lufenuron (Match EC50), an insect growth regulator (IGR).

Experimental Design

The field experiment aimed to determine the effects of lufenuron and the PGPR strain MUR-4 on cauliflower production and growth under biotic stress caused by insect infestation at AARI in Faisalabad. This experimental arrangement had three replications and four treatments, implemented through a randomized complete block design (RCBD). The treatments used were:

1. **T1**: No treatment (control)
2. **T2**: Cauliflower seedlings inoculated with MUR-4 PGPR
3. **T3**: Foliar spray of lufenuron on non-inoculated plants
4. **T4**: Foliar spray of lufenuron on MUR-4 inoculated plants

The seedlings were transplanted into the field during the last week of October 2021, and they were allowed to grow under natural environmental conditions. Insect infestations were monitored periodically, and lufenuron was applied as needed based on the observed presence of pests.

Insect Infestation Monitoring

From December 21, 2021, until the last harvest on February 15, 2022, insect infestation was routinely observed. To record the occurrence and intensity of insect attacks, especially by important pests like aphids, cutworms, armyworms, and diamondback moths, observations were conducted every two to three days.

Foliar Application of Lufenuron

To control insect infestations, lufenuron was sprayed on leaves at a concentration of 10 mL/5 L (0.2%). The application was performed as required, based on the observed insect presence and the potential threat to the plants.

Growth Attributes Measurement

The growth parameters of cauliflower were assessed based on methodologies outlined in previous studies by (Širić *et al*., 2022). The measured attributes included:

Measurement of Plant Height, Weight, and Curd Weight

Fifteen plants from each treatment were marked at harvest time to record the fresh and dry weights of the plants as well as their average height (cm). After a growth period of 180 days from sowing, the cauliflower curds were harvested, and their fresh weights were measured following the methods detailed in Siric *et al*., (2022).

Measurement of Root Length and Fresh and Dry Weights of Leaves and Roots

To determine the fresh and dried weights of the leaves, five plants per treatment were chosen. Roots and leaves trim at the base. An electronic balance was used right away to record the leaf's fresh weight. After the leaves were dried for 72 hours at 70°C, their dry weight was measured. The identical plants' roots were meticulously cleansed under flowing water to remove any dirt particles. After washing, excess water was absorbed using filter paper, and the length of the roots and fresh weight were recorded. The identical root samples were then dried in an oven until a constant weight was achieved to determine the root dry weight.

Biochemical Attributes Measurement

Post-harvest, various biochemical attributes of cauliflower were analyzed to assess the physiological effects of the treatments. The methods employed for each biochemical measurement are detailed below.

Chlorophyll Content

A reaction solution made of pure ethanol, pure acetone, and distilled water combined in a ratio of 4.5:4.5:1 was used to measure the amount of chlorophyll. Fresh leaf samples were chopped into small pieces and placed into 10 mL of this reaction solution in each test tube. The tubes were stored in darkness until the leaf pieces turned white. A spectrophotometer was used at three different wavelengths: 480 nm, 645 nm, and 663 nm, and chlorophyll readings were obtained.

Total Malondialdehyde (MDA) Content

The total MDA content was calculated using the procedure described by (Dhindsa *et al*., 1981). Fresh cauliflower leaves were ground in a 7.5% trichloroacetic acid (TCA) solution, and the resulting mixture was centrifuged at 8000 rpm for 10 minutes. The top layer was combined with 1 milliliter of 0.6% thiobarbituric acid (TBA) in 10% TCA. Spectrophotometric measurements were performed at 530 nm and 600 nm following 30 minutes of heating this solution at 100°C in a water bath.

Total Phenolic Content

The (Bray & Thorpe, 1954) technique was used to calculate the total phenolic content. 2 mL of sodium carbonate (Na_2CO_3), 2.5 mL of Folin-Ciocalteu reagent, and a 1-mL sample of the supernatant were mixed. After adding distilled water to bring the mixture's volume to 10 mL, it was left to remain at room temperature in the dark for 30 minutes. A spectrophotometer was then used to test the solution's absorbance at 750 nm.

Proline Content

The (Bates *et al*., 1973) method was used to determine the proline content. 3% sulfosalicylic acid (w/v) was used to grind fresh cauliflower leaves. A 2 mL sample of glacial acetic acid and 2 mL of ninhydrin reagent were mixed with 2 mL of the leaf extract. After

30 minutes of incubation at 100°C in a water bath, the test tubes holding the reaction mixture were allowed to cool to room temperature. Two separate layers developed after 4 mL of toluene was added, and the lower layer was examined at 520 nm using a spectrophotometer.

Hydrogen Peroxide (H_2O_2) Content

The hydrogen peroxide concentration was estimated using the method outlined by (Velikova *et al*., 2000). A 0.1 g sample of leaf material was ground in 2.5 mL of a 0.1% TCA solution. The mixture was supplemented with 0.5 mL of plant extract and 0.5 mL of phosphate buffer (pH 7). 0.5 mL of plant extract and 0.5 mL of phosphate buffer (pH 7) were added to the mixture as supplements.

Catalase (CAT) Activity

CAT activity was measured using the technique developed by (Chance & Maehly, 1995). A 3 mL reaction mixture was created by mixing 0.1 mL of enzyme extract, 5.9 mM H_2O_2, and phosphate buffer (pH 7.0). The enzymatic extract initiated the process, and variations in absorbance at 240 nm were recorded over 20 seconds using a spectrophotometer.

Superoxide Dismutase (SOD) Activity

The (Giannopolitis & Ries, 1977) approach was used to measure the superoxide dismutase (SOD) concentration. We evaluated the SOD activity in a 3 mL reaction mixture containing 20–50 µL of enzyme extract, 1.3 µM riboflavin, 50 µM nitroblue tetrazolium (NBT), 75 µM ethylene diamine tetra-acetic acid (EDTA), 13 µM methionine, and 50 µM phosphate buffer (pH 7.8). The reaction mixture was placed within a chamber with aluminium covering the inner surface and subjected to a 30 W fluorescent light source for 15 minutes. After that, the light was turned off to halt the reaction. The absorbance was measured using a UV-visible spectrophotometer at 560 nm.

Peroxidase (POD) Activity

To estimate POD activity, the method outlined by Chance and Maehly (1995) was applied. 0.1 mL of plant buffer extract, 2.6 mL of buffer solution, 0.1 mL of guaiacol, and 0.1 mL of H2O2 were all added to the reaction mixture until the volume was 3 mL. For 120 seconds, the change in absorbance was monitored at 20-second intervals.

Soil Analysis

Soil samples were collected before and after the application of MUR-4 PGPR and analyzed at the Soil Department of the Ayyub Agricultural Institute in Faisalabad to determine soil properties and microbial activity.

Statistical Analysis

The data were statistically analyzed using CoStat's analysis of variance (ANOVA), and treatment means were compared using the Least Significant Difference (LSD). Differences were considered significant at $p < 0.05$.

RESULTS

Insect Infestation Rate

During the second week of December 2021, when the cauliflower plants were in their vegetative growth stage, the insects first appeared. First came the cutworms, followed by the armyworm and diamondback moth. The flowering stage was when the most aphid attacks were noted. The non-inoculated groups experienced the highest levels of diamondback moth attack. Attacks from diamondback moths were somewhat reduced in the MUR-4 PGPR inoculated group. There were no appreciable differences in cutworm attacks across all groups. The percentage of armyworm attacks was nearly the same across all groups. Aphid attack was maximum in non-inoculated groups (28% in the control group, and 17% in non-inoculated plants which received lufenuron later) however, plants inoculated with MUR-4 PGPR exhibited only 1% infestation.

Table 1: The proportion of insects that infested cauliflower plants before lufenuron spraying

Treatment	Diamondback moth	Cutworms	Armyworm	Aphid
Control	23%	0.67%	86%	28%
MUR-4 inoculation	15%	0.27%	81%	1%
Lufenuron	22%	1.17%	84%	17%
MUR-4 inoculation+ lufenuron spray	10%	0.50%	89%	3%

After the lufenuron spray, cutworms vanished entirely. Diamondback moth infestation was zero after 24 hours of spraying. After spraying, armyworm attacks decreased, but seven days later, they resumed their appearance. Following a lufenuron spray, the aphid infestation also decreased, but it worsened after 20 days.

Table 2: Infestation percentage of insects attacked on cauliflower plants after lufenuron spray

Treatment	Diamondback moth				Cutworms	Armyworm				Aphid			
	24 hr	48 hr	72 hr	7 day	24hr-7 day	24 hr	48 hr	72 hr	7 day	24 hr	48 hr	72 hr	7 day
Control	19%	20%	25%	34%	0%	25%	28%	30%	35%	27%	31%	36%	48%

MUR-4 inoculation	16%	17%	13%	16%	0%	20%	15%	18%	25%	1.3%	5%	7%	3%
Lufenuron	10%	0%	0%	4%	0%	15%	10%	4%	9%	6%	0%	0%	5%
MUR-4 inoculation+ Lufenuron spray	6%	0%	0%	1%	0%	11%	5%	2%	10%	0%	0%	0%	4%

3.1. Plant Growth Attributes

Compared to control plants, stressed PGPR-treated plants had a large (90%) increase in plant height, a measure of plant growth. The lufenuron showed a substantial impact when combined with PGPR, but not when administered alone. The plant's height was 9% lower than the control due to the insect infestation ($p \leq 0.05$) (Table 3). By lessening the inhibitory effects of insect infestation on plant leaves and root length, the PGPR-inoculated plants considerably (86%) enhanced the root length in comparison to the control. The bug almost ate the leaves, and the curd weight of the control group drastically decreased. Higher yields were obtained by plants infected with PGPR, and the curd weight rose by 21% when PGPR and lufenuron were combined (Table 3).

Table 3: Growth Attributes

Treatments	Plant.height	Number.of.leaf	root.length	Curd. weight
T1	13.40 ± 0.43a	12.20 ± 0.31a	8.66 ± 0.23a	104.50 ± 0.80a
T2	24.20 ± 0.51b	18.13 ± 0.42b	11.86 ± 0.28b	112.85 ± 0.71b
T3	14.47 ± 0.62a	15.13 ± 0.26c	9.34 ± 0.15a	105.75 ± 0.62a
T4	25.20 ± 0.58b	20.60 ± 0.32d	16.05 ± 0.37c	126.14 ± 1.14c

In plants inoculated with PGPR, the fresh weights of the leaves and roots increased significantly (68% and 85%, respectively). Both the fresh weights of the root and the leaf were reduced by insect infestation, but the fresh weight of the leaf was more negatively impacted. The growth in fresh weight of the roots and leaves was enhanced by the PGPR inoculation. The dry weight of the root and leaf was also higher ($p \leq 0.05$) in PGPR-inoculated plants (Figure 1). The dry weight of the leaf and root increased by 66% and 80%, respectively, in comparison to the control group (Figure 1).

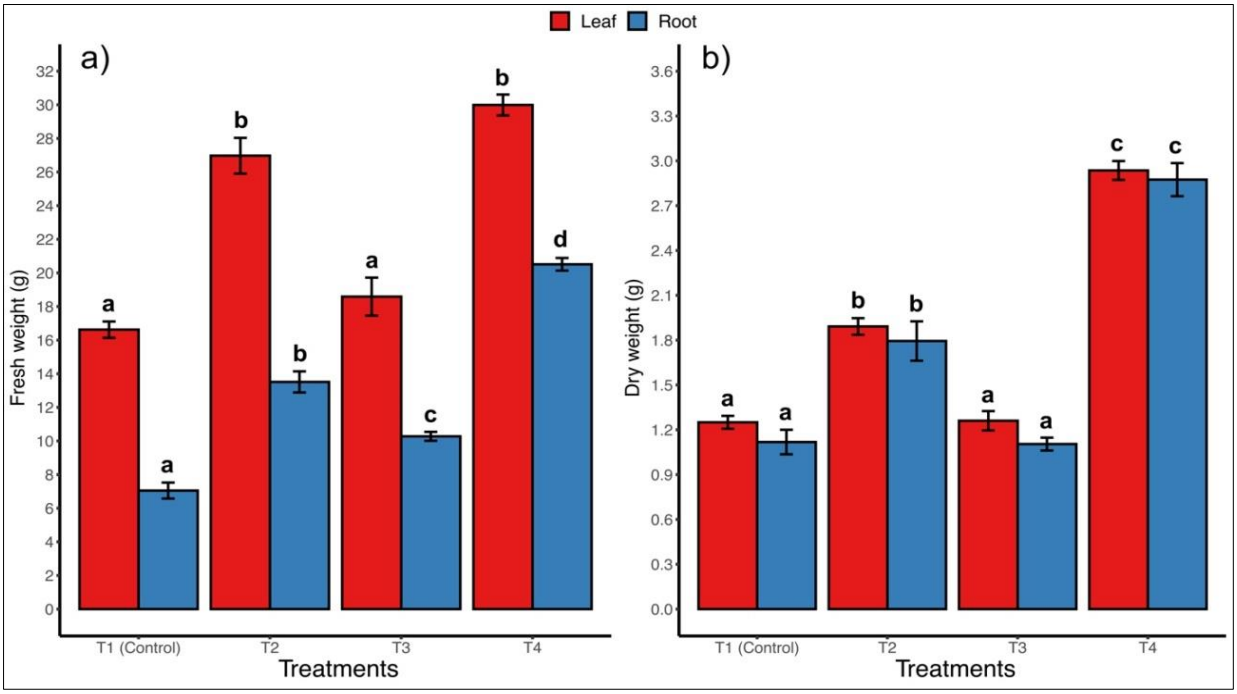

Figure 1: Effect of MUR-4 PGPR inoculation and lufenuron spray on the fresh and dry weight of leaf and root in cauliflower under insect-associated biotic stress

T1: Control group, no treatment
T2: MUR-4 PGPR inoculated plants
T3: Plants grown with Lufenuron
T4: Plants grown with MUR-4 PGPR inoculation and Lufenuron spray

3.2. Physiological Parameters

Plants infected with PGPR produced more phenolics ($p \leq 0.05$). The PGPR combined treatment with lufenuron increased the amount of phenolics in the leaves by 70% compared to the control under stressful conditions (Figure 2). Likewise, PGPR had a four-fold higher proline content and lufenuron combined treatment (Figure 2).

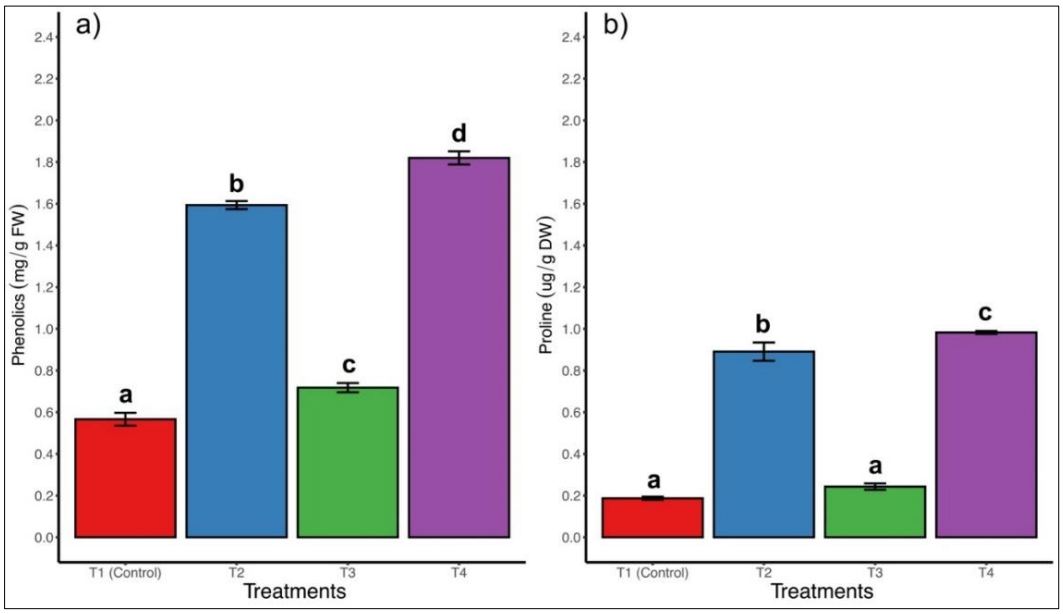

Figure 2: Effect of MUR-4 PGPR inoculation and lufenuron spray on phenolics and proline in cauliflower under insect-associated biotic stress

T1: Non-treated, Control group
T2: MUR-4 PGPR inoculated plants
T3: Plants grown with Lufenuron
T4: Plants grown with MUR-4 PGPR inoculation and Lufenuron spray

The PGPR-inoculated plants exhibited a marked increase in Chlorophyll a, b and carotenoids treatment. Lufenuron and PGPR combined treatment depicted an 85% increase in chlorophyll a, 78% in chlorophyll b and 75% in carotenoid content of leaves (Figure 3). The response of PGPR was 75% higher ($p \leq 0.05$), particularly for Malondialdehyde content.

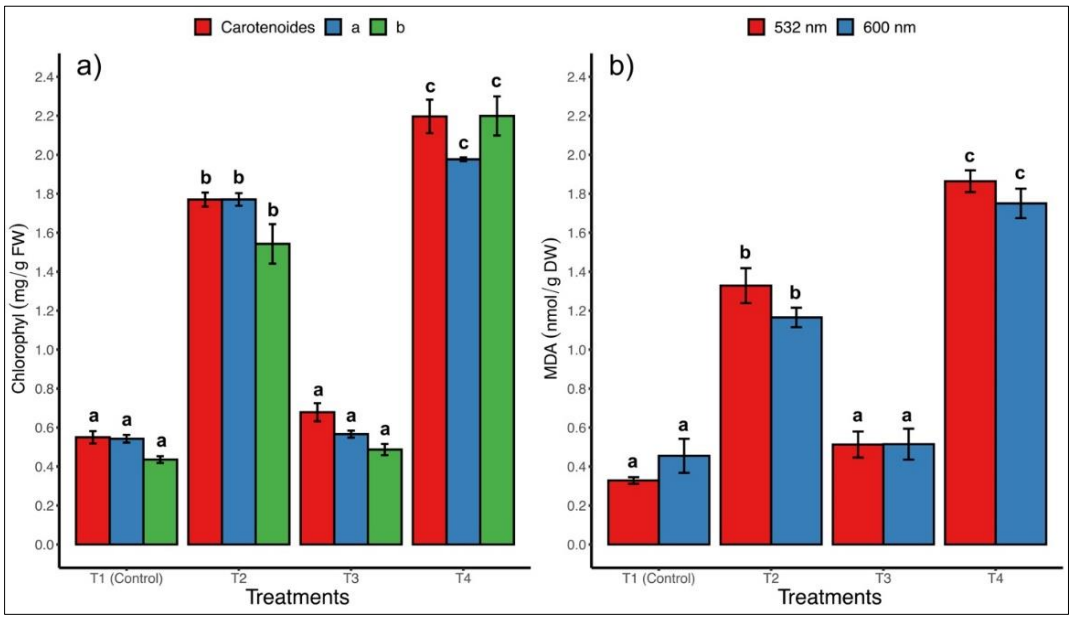

Figure 3: Effect of MUR-4 PGPR inoculation and lufenuron spray on Chlorophyll a, b, carotenoids and MDA content in cauliflower under insect-associated biotic stress

Plants infected with PGPR under stress had increased levels of the protein enzymes SOD and POD ($p < 0.05$) (Figure 4, 5). SOD, POD, and CAT activity were all 79%, 73%, and 71% lower in inoculated plants, respectively. When compared to untreated control plants, the inoculated plants that were infested with insects showed a hydrogen peroxide concentration rise of up to 53% (Figure 5).

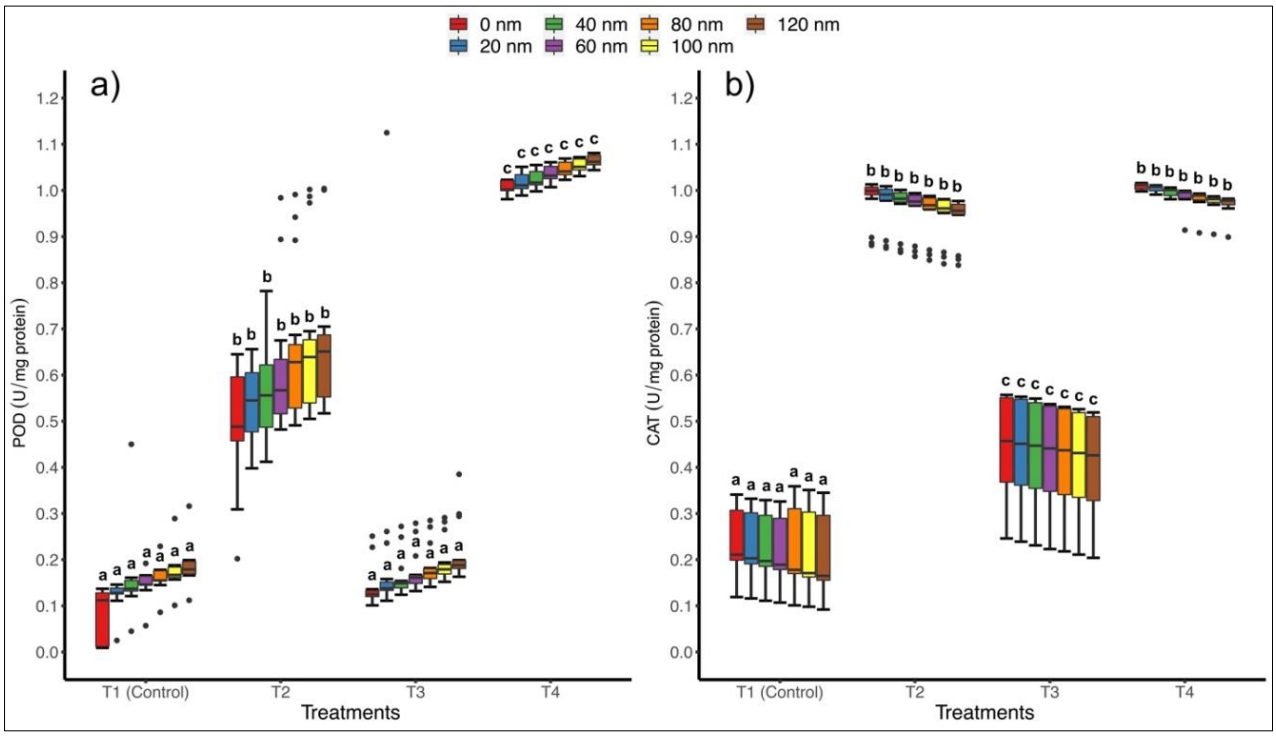

Figure 4: Effect of MUR-4 PGPR inoculation and lufenuron spray on peroxidase and catalase activity in cauliflower under insect-associated biotic stress

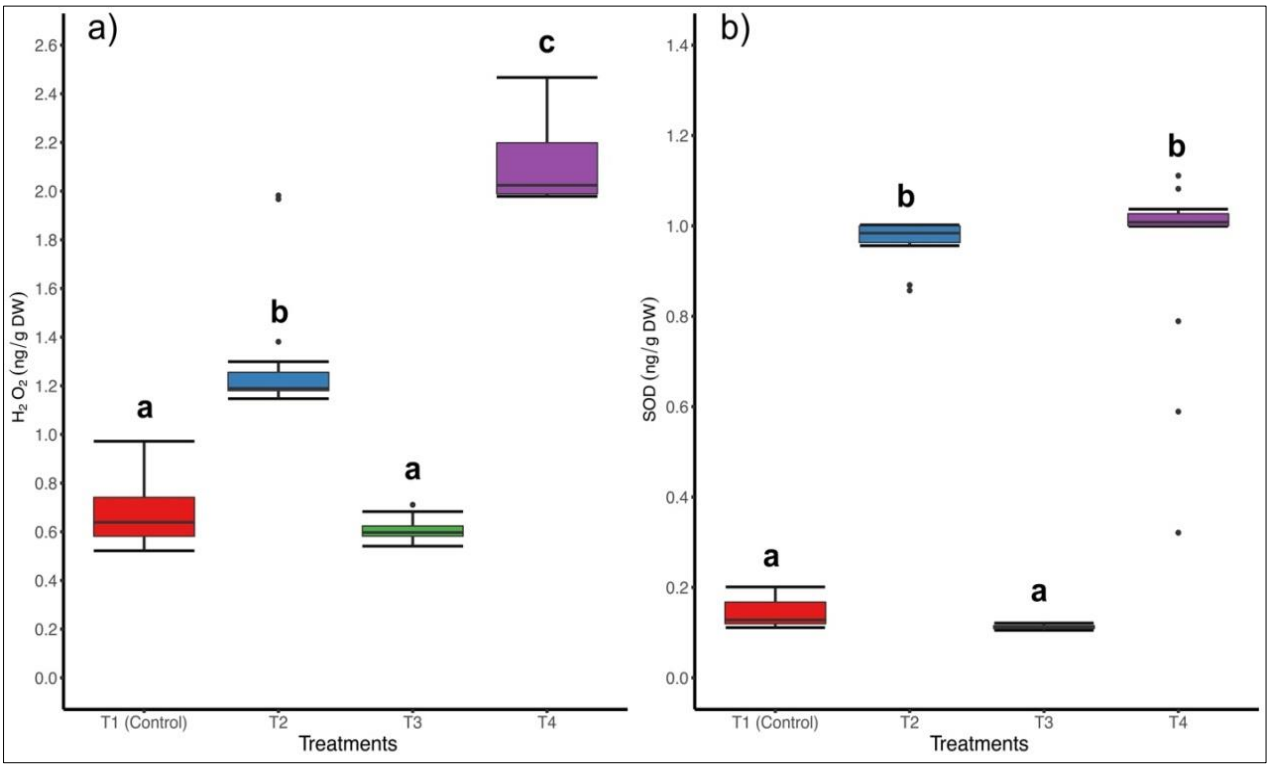

Figure 5: Effect of MUR-4 PGPR inoculation and lufenuron spray on hydrogen peroxide content and SOD activity in cauliflower under insect-associated biotic stress

Soil Analysis

A sample of soil had been sent to the Ayyub Agricultural Research Institute's soil department in Faisalabad, both before and after the MUR-4 PGPR application. According to the analysis, the soil had a loamy texture, a pH of 8.7, and a saturation level of 38%. The results revealed that the phosphorus and potassium considerably increased after MUR-4 PGPR treatment.

Table 4: Available nutrients status in rhizospheric soil of cauliflower without/ with MUR-4 PGPR application

pH		EC mScm-1		Organic Matter (%)		Total Nitrogen (%)		Available Potassium (mg kg-1)		Available Phosphorus (mg kg-1)	
Before	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**
8.7	8.7	1.10	1.33	0.98	1.26	0.49	0.63	145	166	15.5	17.5

DISCUSSION

The effectiveness of PGPR as a growth stimulant and biocontrol agent is examined in this article. According to Anwar *et al*., (2023), cauliflower (*Brassica oleraceae* L. var. botrytis) is a vital winter meal that contains substantial amounts of antioxidant phytochemicals (Haidri *et al.*, 2024). Both young and elderly cauliflower plants are attacked by a variety of insects and pests (Qamar *et al*., 2018). Several strains of plant growth promoting rhizobacteria (PGPR) have been applied as biofertilizers since they have both a direct and indirect impact on insect pest resistance (Hussain *et al.*, 2016);(Naeem *et al*., 2018). Some of the products believed to be suitable to replace conventional pesticides include insect growth regulators or IGRs (Ullah, Munir, *et al*., 2024). These chemicals have their own and selective mode of action, non-toxic to the environment, and has the lowest risk to any species other than the targeted one (El-Zahi *et al*., 2021) (Kai *et al*., 2009).

Some beneficial bacteria residing in the soil as plant growth promoting Rhizobacteria (PGPR) penetrate into the root tissues and boost plant growth (Remans *et al*., 2008) ; (Yadegari *et al*., 2010). Even in the presence of insect-associated biotic stress, MUR-4 PGPR-inoculated plants in this study showed an increase in height and the overall number of leaves (Yıldırım, 2022). An efficient technique for assessing photosynthetic activity and plant health in the face of biotic or abiotic stress invasion is chlorophyll fluorescence (Sahito *et al*., 2021).

When insects invade plants, the most effective changes are made(ALKahtani *et al*., 2020). Due to insect PGPR, non-inoculated cauliflower plants displayed a decrease in chlorophyll content, including changes in chlorophyll content (Kousar *et al*., 2020). When compared to non-inoculated plants, cauliflower plants treated with MUR-4 PGPR inoculation exhibited a 75% increase in carotenoids, a 78% increase in chlorophyll b, and an 85% increase in chlorophyll (Ullah, Qasim, *et al*., 2024). Through chelation growth, PGPRs generate phytohormones that improve mineral and iron solubility, increase insect resistance, and increase nitrogen and phosphorus intake (Bowen & Rovira, 1999); Naeem *et al*., 2018).

The increase in the leaf fresh weight by 68.76% also pointed to improved moisture uptake and plant vigor; the MUR-4 PGPR treatment marginally enriched the soil P and K content (Ullah, Qasim, *et al*., 2024) . Maximum bacterial colonization of cauliflower roots during insect-induced biotic stress resulted in a substantial 21% increase in fresh leaf weight, fresh root weight, and yield (David *et al*., 2018) observed similar results. Similar to the results of Kousar *et al*., (2020), MUR-4 PGPR inoculation produced a noteworthy 66% increase in leaf dry weight. MUR-4 PGPR improved secondary metabolite production and boosted cauliflower plant growth and yield under biotic stress. Another form of plant defense is the secondary metabolites which involves chemicals that actually reduce palatability of the tissues in which they are manufactured (Reise & Waller, 2009) War *et al*., 2012). Flavonoids are the most populous and important secondary metabolites present in the plants and they are responsible for many defense mechanisms, antibiotic synthesis and metabolisms in plants (Ullah, Munir, *et al*., 2024). In order to reduce the negative impact that insect-created stress has on the subsequent growth and yielding of plants, PGPR enhance phenolic production on the plants. Cauliflower plants infected with MUR-4 PGPR had 70% higher phenolics than the control group. These findings were quite consistent with those of (Bano & Muqarab, 2017), and the main cause of this increase seemed to be MUR-4 PGPR. However, there was no noticeable difference in plants treated with lufenuron, indicating that it did not affect phenolic production (Ullah, Munir, *et al*., 2024).

Proline, which helps with ROS detoxification, stops membrane breakdown and maintains protein structure, is accumulated by plants under biotic or abiotic stress (Abd-ur-Rahman *et al*., 2017). There was no statistically significant difference between the control group and the cauliflower plants cultivated with MUR-4 PGPR. Proline levels, however, were four times greater when MUR-4 PGPR and lufenuron were administered together than when MUR-4 PGPR was alone (Haidri *et al.*, 2024).

Since plants use oxidative stress as a defense strategy, oxidative stress caused by insects typically results in the rapid and temporary production of ROS

(Maffei *et al.*, 2007); (Tavolacci, 2020). Oxidative damage is indicated by malondialdehyde (MDA), a result of the oxidation of polyunsaturated fatty acids (Haidri *et al*., 2024). The increased MDA content in insect-infested cauliflower plants was indicative of increased oxidative stress and ROS production. Host plant resistance (HPR) to insects has been linked to the oxidative state of the host plants, which generates ROS that are then removed by antioxidative enzymes (He *et al*., 2011); (Zhao *et al.*, 2009)Zhao *et al*., 2009). The first enzyme to scavenge ROS, superoxide dismutase (SOD) (Bano & Muqarab, 2017), was significantly increased (79%) in MUR-4.

SOD activity was higher in crops treated with pesticides and herbicides (Abd-ur-Rahman *et al*., 2017; *(Parween et al*., 2012). When compared to the control group, the lufenuron foliar spray reduced SOD activity by 25%. When plants are attacked by insects, their ROS synthesis increases, releasing negative free oxygen radicals that SOD converts to hydrogen peroxide (H_2O_2) (Torres, 2010; War *et al*., 2012). The rate of H_2O_2, a readily diffusible molecule that is less harmful than free oxygen radicals and an essential component of the triggered defense response in plants to various stresses, was 53% higher in cauliflower plants grown with MUR-4 PGPR inoculation and treated with lufenuron foliar spray (Abd-ur-Rahman *et al*., 2017).

Catalase (CAT), a soluble hemoprotein that converts hydrogen peroxide to hydrogen and water, is another essential defense enzyme (Akhtar & Azam, 2014). Cauliflower plants inoculated with MUR-4 PGPR showed 69% more CAT activity than non-inoculated plants to fight insect infestation stress. CAT activity increased by 71% when lufenuron foliar spray was used in conjunction with MUR-4 PGPR inoculation. Peroxidase (POD) is necessary for plants to respond quickly to insect attacks because it detoxifies H_2O_2, scavenges reactive oxygen species (ROS), and performs defensive functions (Bano & Muqarab, 2017; (Usha Rani & Jyothsna, 2010); War *et al*., 2012). The plants treated with MUR-4 PGPR and lufenuron exhibited the greatest POD activity, 73% higher than the untreated ones. Numerous processes linked to plant defense, including suberization, lignification, auxin metabolism, somatic embryogenesis, and wound healing, are regulated by peroxidase enzymes (He *et al*., 2011; (Sethi *et al.*, 2009) ; War *et al*., 2012).

CONCLUSIONS

Attacks by armyworms, aphids, and diamondback moths reduced cauliflower plant growth (such as plant height, root length, fresh and dry weight of leaf and root, and curd weight) by altering numerous physiological and biochemical characteristics. On the other hand, MUR-4 PGPR inoculation improved the physiological, biochemical, and growth characteristics of cauliflower plants. MUR-4 PGPR stimulated the plants' defensive mechanism and improved their ability to absorb nutrients. Although lufenuron foliar spray did not change the physiology of the plant, it was very successful in controlling insect attacks. There was no discernible difference between the combined treatment of MUR-4 PGPR inoculation and lufenuron spray and MUR-4 PGPR alone.

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