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# Effect of Plant Growth Promoting Rhizobacteria (PGPR) and Plant Growth Regulators (PGR) on Antioxidant Properties of Chickpea (*Cicer arietinium* L.) in Presence of Toxic Effect of Thiamethoxam

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## ABSTRACT

**Background:** Agriculture have focused on the role of Plant Growth-promoting Rhizobacteria (PGPR) in mitigating the negative impacts of pesticides on crops. PGPR, a diverse group of beneficial soil bacteria, establishes symbiotic relationships with plants, influencing various aspects of plant growth and stress tolerance.

**Methods:** This study underscores the potential of integrating PGPR (Plant growth promoting rhizobacteria) with plant growth regulators (PGR) like melatonin and Strigolactone to mitigate the adverse effects of thiamethoxam exposure on biochemical and antioxidant parameters in chickpeas, thereby contributing to sustainable agricultural practices. Four different combinations of Treatments were used at School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India during 2022-24 to study the effect of thiamethoxam (TMX) on antioxidant properties (TMX, TMX+PGPR, TMX+PGPR+Melatonin and TMX+PGPR+ Melatonin+Strigolactone).

**Result:** The findings presented in current study provide a foundational understanding of the role of plant growth- promoting rhizobacteria (PGPR) and Plant growth regulator in enhancing the growth of Chickpea (*Cicer arietinum* L.) and minimizing the pesticide stress on the plant. The results have shown a significant increase in total chlorophyll (0.0083±0.0021), chlorophyll a (0.003±0.0005) and chlorophyll b (0.0056±0.0025) using PGPR and melatonin compared to the control, with the effect being more pronounced after the addition of Strigolactone. Similarly, oxidative stress markers such as superoxide ions and hydrogen peroxide showed significant values in the PGPR and PGR consortium, indicating their ability to combat pesticide stress.


**Key words:** Oxidative stress, Pesticide, Plant growth promoting rhizobacteria, Plant growth regulator.

## INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a leguminous crop, belonging to Fabaceae family. It is a self-pollinated with chromosomal number 2n = 14. India has a total area of 9.55 million hectares dedicated to chickpea production, with a production of 9.94 million tons and productivity of 806 kg/ha. The productivity of chickpea in Punjab is 700 kg/ha and in Rajasthan, it is 680 kg/ha. Chickpea seeds are composed of carbohydrates (50-58%), protein (15-22%), moisture (7-8%), fat (3.8-10.20%) and micronutrients (<1%) (Tutlani *et al.,* 2023). Chickpea is a vital legume crop valued for its nutritional benefits and economic importance worldwide. As the demand for increased food production grows, it is crucial to develop strategies that not only enhance crop yields but also promote environmental sustainability and human health. Recent studies in agriculture have focused on the role of Plant Growth-promoting Rhizobacteria (PGPR) in mitigating the negative impacts of pesticides on crops (Zahedi and Abbasi, 2015). PGPR, a diverse group of beneficial soil bacteria, establishes symbiotic relationships with plants, influencing various aspects of plant growth and stress tolerance (Resmi *et al.,* 2024). They enhance plant health through mechanisms like improved nutrient uptake, systemic resistance induction and regulation of plant hormones (Bhattacharyya *et al.,* 2012).

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The drive for higher crop yields in modern agriculture has led to the extensive use of chemical pesticides, with Thiamethoxam being particularly prominent due to its

efficacy against numerous pests. Introduced as a second-generation neonicotinoid, thiamethoxam is effective in controlling a wide array of pests, including lepidopterans, thrips, hoppers, flea beetles, aphids and whiteflies. However, increasing crutiny has been placed on thiamethoxam because of its persistence in oil and accumulation in plants, posing risks to non-target species (Barnes *et al.,* 1992; Wang *et al.,* 2020). Its success in managing plant diseases like powdery mildew, rusts and fungal rots has further solidified its usage in pre-and post-harvest treatments (Kaur *et al.,* 2022). The continuous use of such pesticides raises concerns about their long-term environmental impacts, necessitating a reassessment of sustainable pest management practices.

At the same time, melatonin, traditionally recognized for its role in regulating circadian rhythms in animals, has emerged as a versatile plant growth regulator (PGR). Recent research has demonstrated its significant role in plant stress responses, including oxidative stress reduction, gene expression control and enhancing overall resilience to environmental stresses. This study aims to explore the potential synergistic effects of PGPR and melatonin in aiding the degradation of carbendazim and thiamethoxam in chickpea plants. Microorganisms, including soil biota, are known to degrade various pesticides and certain bacteria use pesticides as their sole carbon source, providing opportunities for bioremediation (Qiu *et al.,* 2007).

PGPR, by altering the rhizosphere, boosting plant biomass and improving nutrient uptake, helps plants adapt to harsh conditions (Backer *et al.,* 2018). Specifically, the root microbiome *Pseudomonas putida* plays a critical role in protecting plants from stress by minimizing ROS-induced cellular damage (Srivastava *et al.,* 2017). *P. putida* has also been shown to degrade thiamethoxam without producing harmful metabolites (Rana *et al.,* 2011) and it exhibits high resistance to and degradation capacity for this pesticide (Jan *et al.,* 2021). Therefore, supplementing chickpea plants with exogenous *P. putida* may alleviate pesticide-induced stress. By investigating the interactions between beneficial microbes, plant regulators and pesticide degradation pathways, this study aims to contribute to sustainable pest management practices that align with ecological balance and agricultural resilience. Building on previous research that highlights the complex interplay between PGPR, melatonin and pesticide breakdown, we will explore these relationships in the specific context of chickpea to advance sustainable agricultural practices. The study aimed to evaluate the impact of plant growth promoting rhizobacteria (PGPR) and plant growth regulators (PGR) on the antioxidant properties of chickpea (*Cicer arietinum* L.). It specifically assessed their effectiveness in mitigating the toxic effects of the insecticide thiamethoxam. The objective was to identify treatments that enhance plant defense and antioxidant potential under chemical stress.

## MATERIALS AND METHODS

### Collection of microbial culture (PGPR)

The IDMT-CC3314 accession of the lyophilized strain of *Pseudomonas putida* was obtained from CSIR- IMTECH in Mohali, India. It was cultivated for 48 hours at 28°C in 50 millilitres of fresh nutrient broth (NB) media. The culture was centrifuged (Plasto Crafts, Rota 4R-V/Fm) for 20 minutes at 4°C to extract the pellet. After that, the water was double-distilled and the bacterial population was raised to 109 cells/ml by re-suspending (Jan *et al.,* 2020).

### Plant growth regulators (PGR)

#### Melatonin and strigolactone

Melatonin and Strigolactone was purchased from Himedia laboratories Pvt. Ltd, Mumbai, India. A stock solution of 1 mM was prepared by dissolving melatonin in analytical grade methanol. Different concentrations of melatonin (50 μM and 100 μM) were prepared by serial dilution of stock. For current study, the concentration of melatonin was selected on the basis of effective concentration. Accordingly, 50 μM concentrations was chosen for the experimental work.

### Plant growth promoting rhizobacteria (*PGPR*)

*Pseudomonas putida* (MTCC-1194), a plant growth-promoting rhizobacterium (PGPR), was obtained from CSIR-IMTECH in Mohali, Punjab, India. 50 milliliters of sterile nutrient broth medium (NB media; 13 $gL^{-1}$) were used to cultivate the lyophilized strain. For the purpose of proliferation, the culture flask was maintained at 28°C (24 to 48 hours) in a BOD incubator (Calton Deluxe Automatic, New Delhi, India). Using 50 mL of NB media and 1 mL of growing culture, the experiments were conducted at 28°C for 24 to 48 hours in a BOD incubator. In order to collect pellets, it was centrifuged (Plasto Crafts, Rota 4R-V/Fm) for 20 minutes at 10000 rpm and 4°C. Pellet wasre-suspended to acquire $10^9$ cells/ml.

### *In vitro* germination of seedlings

Surface sterilized seeds were immersed in freshly prepared melatonin solution (50 μM) for 7 hours. The melatonin and Strigolactone dosed seeds were swilled with double distilled water, blotted dried and placed in dark at room temperature, until returning to their initial weight (over-night). Autoclaved petri-plates were layered with Whatman (Grade 1) filter paper and added with thiamethoxam solution (0.6 mM). Subsequently, primed seeds were sown in thiamethoxam supplemented petri-plates and simultaneously microbial suspension ($10^9$ cells/ml) was inoculated into petri-plates containing seeds. The petri-plates were kept in seed germinator (Caltan, NSW 191-192) under controlled condition (light intensity-175 $\mu molm^{-2}s^{-1}$; temperature-25±0.5°C, photo-period-16 hours). After 10 days sowing, the seedlings were harvested for further analysis. Fig 1 showed the seedling growth after applying percentage treatment of thiamethoxam in chickpea.

**Photosynthetic parameter evaluation**

The content of anthocyanin, total flavonoid, carotenoid, chlorophyll-a and chlorophyll-b was determined using a Shimadzu UV-1800 UV-Vis Spectrophotometer.

**Estimation of chlorophyll and carotenoid content**

After homogenizing 0.2 g of fresh plant material in a cooled pestle-motor with 4 mL of 80% acetone, the sample was centrifuged at 12,000 RPM for 20 minutes at 4°C and the absorbance was recorded. The following wave lengths: 480 and 510 nm for carotenoid concentration and 645 and 663 nm for chlorophyll. The procedure was adhered to in order to determine the content (Arnon *et al.,* 1949; Maclachlan *et al.,* 1963).

**Anthocyanin content estimation**

In order to estimate the anthocyanin concentration, 0.35 g of fresh plant tissue was ground up in a iced pestle-motor using a 3 ml extraction mixture that contained 0.03 mL, 2.37 mL, 0.6 mL and 0.03 mL of methanol, HCl and D.$H_2O$, respectively. The absorbance measurements were taken at 657 and 530 nm (Mancinelli *et al.,* 1984).

**Total flavonoid content estimation**

Using a chilled pestle-motor, a fresh plant sample weighing 0.35 g was crushed in 3 mL of absolute methanol. After centrifugation at 4°C, 12,000 rpm and 20 minutes, the resulting supernatant was diluted by adding 0.3 mL, 0.3 mL and 4 mL of $NaNO_2$, $AlCl_3$ and D.$H_2O$, respectively. Following the development of a pink hue during incubation, 2 milliliters of NaOH were added and the optical density at 510 nm was measured (Kim *et al.,* 2021).

**Osmolyte content estimation**

**Trehalose content**

After crushing 10 mg of oven-dried plant material in 80% ethanol, the mixture was centrifuged at 4°C, 5000 rpm and for 15 minutes. Four milliliters and two milliliters of TCA anthrone reagent were added to the 0.1 milliliter supernatant, resulting in the production of a yellow complex with an absorbance measured at 620 nm (Trevelyan *et al.,* 1956). Trehalose was measured using D-glucose as a reference and the result was represented in mg/gm DW.

**Glycine-betaine content**

10 mg oven dried plant material was crushed in 5 mLD.$H_2O$ containing 0.05% of toluene followed by filtration after incubation (24 hours). Following an ice-cold treatment, 10 mL and 2 mL of 1, 2-dichloromethane and D.$H_2O$, respectively, were added to the mixture containing 0.1 mL potassium tri-iodide, 1 mL 2N HCl and 0.5 mL filtrate. Thorough mixing of reaction tubes was done till two separate layers were formed. Upper layer was removed and absorbance of pink coloured lower layer was recorded at 365 nm. Standard curve of betaine hydrochloride was plotted and used for assessing the glycine-betaine content (Grieve *et al.*, 1983).

**Proline content**

A 250 mg plant sample was ground up in 10 mL of sulpho salicylic acid (3%) and centrifuged at 4°C for 10 min at 10,000 rpm. Two milliliters of ninhydrin and glacial acetic acid were then added to two milliliters of supernatant. This was treated with a water bath for one hour at a temperature of 100°C. The reaction was then terminated by moving it to an ice bath. In addition, 4 mL of toluene was added and agitated for 50-60 seconds. After the toluene layer was removed, absorbance was measured at 520 nm and quantified in mg/gm using a standard plot of L-proline (Bates *et al.,* 1982).

**Estimation of oxidative stress markers**

**Quantification of superoxide anion (O.-2) content**

After homogenizing 500 mg of fresh plant tissue in 4 ml of phosphate buffer (65 mM, pH-7.8) with 1% PVP, the mixture was centrifuged at 4°C for 15 minutes at 12,000 rpm. The supernatant was then combined with 0.1 mL and 0.5 mL of hydroxylamine hydrochloride and phosphate buffer, respectively. At room temperature, the amalgam was incubated for thirty minutes. After incubating the combination containing 1 mL of 1-napthylamine and 1 mL of 3-amino benzene sulphonic acid, absorbance was finally measured at 520 nm. By using sodium nitrate, the quantification was given in µmole/g FW (Wu *et al.,* 2010).



**Fig 1:** Seedling growth after applying percentage treatment of thiamethoxam in chickpea.

**Hydrogen peroxide ($H_2O_2$) content**

Fresh plant material (500 mg) was pulverized in 2 mL trichloroacetic acid (1 M) and subjected to centrifugation (Time= 15 min; Temperature= 4°C rpm= 5000) followed by addition of 0.5 mL of KI (Molarity= 1 M) and 1 mL of potassium phosphate buffer (PPB) (Molarity= 10 mM) into 0.5 mL supernatant. The absorbance (wavelength = 390 nm) was recorded for quantification in μmole/g FW by taking hydrogen peroxide (Velikova *et al.,* 2011).

## RESULTS AND DISCUSSION

The results offer a comparative evaluation of several biochemical and physiological parameters across various treatment combinations. Each comparison is classified as highly significant (HS), moderately significant (MS), low significant (LS), or non-significant (NS). The key comparisons focus on treatments involving TMX (thiamethoxam), MEL (melatonin), PGPR (plant growth-promoting rhizobacteria) and Strigolactone, both in relation to the control group and between the different treatment combinations. (Table 1 and 2).

Thiamethoxam elevates oxidative stress, whereas the application of melatonin and PGPR mitigates its levels in chickpea seedlings exposed to thiamethoxam. The TMX+MEL+PGPR treatment results in the highest overall chlorophyll content, while the inclusion of STGR significantly reduces total chlorophyll (T-Chl) levels. However, the combination of TMX+STGR+MEL+PGPR leads to the highest chlorophyll-a (Chl-a) production, suggesting a positive interaction between these components. TMX+MEL alone exhibits the greatest impact on chlorophyll-b (Chl-b), indicating that melatonin may have a specific role in boosting Chl-b levels, though the addition of STGR diminishes this effect. These findings indicate that different treatments influence chlorophyll synthesis in distinct ways, with TMX+MEL and TMX+STGR combinations having different impacts on chlorophyll type balance (Fig 2).

A highly significant increase in total chlorophyll is observed with TMX alone compared to the control and when MEL or PGPR is added, chlorophyll levels rise further, highlighting their supportive role in boosting chlorophyll content. However, the addition of strigolactone causes a highly significant decrease, indicating a possible antagonistic effect between STRG and other components in chlorophyll synthesis. Similar trends are seen with chlorophyll-a, where the addition of STRG shows no significant improvement. Previous research has shown that melatonin can counteract the decline in total chlorophyll synthesis, potentially due to increased uptake of magnesium and nitrogen, as well as reduced chlorophyll degradation (Alharbi *et al.,* 2021). Additionally, melatonin has been linked to enhanced photosynthesis and PSII activity (Kaya *et al.,* 2019).

The treatments with TMX+MEL and TMX+PGPR result in significantly higher anthocyanin levels compared to the control, indicating these combinations may enhance the plant's stress defense mechanisms. However, the combination of TMX+MEL+PGPR or TMX+STGR+MEL+

**Table 1:** Mean differences and (P<0.05) between various treatments through one-way anova.

| Parameters | Combinations | | | | | | |
|---|---|---|---|---|---|---|---|
| | Control vs TMX | Control vs TMX+MEL | Control vs TMX+PGPR | Control vs TMX+MEL+ PGPR | Control vs TMX+MEL+ PGPR+STREG. | TMX vs TMX+MEL | TMX vs TMX+ PGPR |
| Total chlorophyll | 0.02163 | 0.03053 | 0.02880 | 0.02500 | 0.01843 | 0.004400 | 0.002667 |
| | HS | HS | HS | HS | HS | LS | NS |
| Chlorophyll A | 0.02370 | 0.02370 | 0.02377 | 0.02320 | 0.02167 | 0.000 | 6.667005 |
| | HS | HS | HS | HS | HS | NS | NS |
| Chlorophyll B | 0.007600 | 0.01150 | 0.01173 | 0.006700 | 0.001267 | 0.003900 | 0.00413 |
| | HS | HS | HS | MS | NS | LS | LS |
| Flavonoid | -0.004467 | -0.03447 | 0.01887 | -0.02113 | -0.04180 | -0.03000 | 0.02333 |
| | NS | LS | NS | NS | MS | LS | HS |
| Anthocyanin | 0.06170 | -0.6952 | -0.7967 | 0.09633 | -0.2585 | -0.7569 | -0.8584 |
| | NS | HS | HS | NS | MS | HS | HS |
| Trehalose | -0.5372 | -0.9779 | -0.8112 | -0.5225 | -0.3335 | -0.4470 | -0.2740 |
| | HS | HS | HS | HS | HS | HS | MS |
| Proline | 0.02800 | -0.8983 | -0.4343 | -1.787 | -1.769 | -0.9263 | -0.4623 |
| | LS | HS | HS | HS | HS | HS | HS |
| Glycine betaine | 0.001300 | -0.4277 | -0.05837 | -0.1964 | -0.3470 | -0.4290 | -0.05967 |
| | NS | HS | HS | HS | HS | HS | HS |
| Super oxideanion | -0.04753 | -0.3309 | -0.1635 | -0.005533 | -0.001200 | -0.2833 | -0.1160 |
| | HS | HS | HS | NS | NS | HS | HS |
| Hydrogen peroxide | 0.02640 | -0.05527 | -0.1606 | -0.4426 | -0.09960 | -0.08167 | -0.1870 |
| | HS | HS | HS | HS | HS | HS | HS |

PGPR leads to a decrease in anthocyanin content, which could suggest an interaction that negatively affects anthocyanin production. A substantial reduction in anthocyanin levels observed with TMX+MEL suggests that melatonin may inhibit its production. Similarly, the comparison between TMX+PGPR+MEL and TMX+PGPR+MEL+STRG shows a notable decrease, indicating that STRG may interfere with the anthocyanin-enhancing effects of PGPR and MEL (Fig 2).

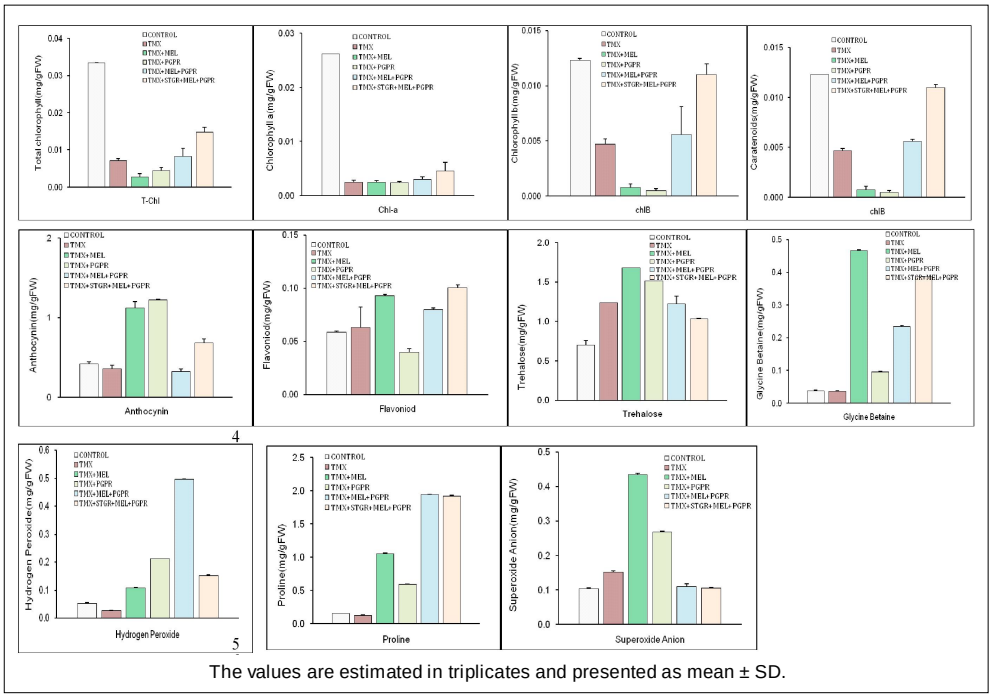
*P. putida* treatment upregulates the antioxidant enzymes activity in TMX dosed (*Cicer arietinium* L.) seedlings while strigolactones have less effect to reduce toxicity of thiamethoxam. The TMX+MEL treatment results in the highest flavonoid content, suggesting asynergistic interaction between melatonin and thiamethoxam. In contrast, the TMX+PGPR combination leads to are duction in flavonoid levels, though adding MEL to TMX+PGPR brings the levels closer to those seen in the TMX+MEL treatment. The TMX+STGR+MEL+PGPR combination significantly reduces flavonoid content, indicating potential antagonistic effects (Fig 2). TMX+MEL increases trehalose levels more than the control and other treatments, indicating melatonin's role in enhancing stress tolerance *via* trehalose accumulation. While TMX+PGPR also elevate trehalose levels, the TMX+MEL+PGPR combination shows a slight decrease in comparison (Fig 2).

In the pursuit of protecting and enhancing crop yields, pesticides are often used excessively (Jan *et al.,* 2020). While thiamethoxam is effective in controlling insect populations, it also leaves behind significant toxic residuesin crops, posing potential risks to living organisms (Saran *et al.,* 2018 and Riascos-Flores *et al.,* 2021). In recent times, microorganisms have been successfully introduced into plants to aid in the remediation of pesticides (Nurzhanova *et al.,* 2021 and Jan *et al.,* 2023). Various studies have shown that PGPRs (plant growth-promoting rhizobacteria) enhance plant growth by activating defense mechanisms under stress conditions (Seth *et al.,* 2021; Bibi *et al.,* 2024 and Zahedi and Abbasi, 2015). This study explores the ability of *Pseudomonas putida* to alleviate the adverse effects of thiamethoxam in chickpea seedlings. Morphological analysis indicated that thiamethoxam negatively affected seedlings, resulting in reduced seedling length, fresh weight and dry weight. Our findings align with earlier research, which reported significant growth inhibition in germination rate, root length and weight in *Allium cepa* seedlings treated with thiamethoxam (Cavuşoğlu *et al.,* 2012). The reduction in plant biomass and growth can be attributed to disruptions in chlorophyll synthesis, photosynthesis, nutrient absorption and imbalances in hormones and water regulation (Jan *et al.,* 2020).

The exposure of thiamethoxam leads to decrease in chlorophyll content as well as pigments like anthocyanins, carotenoids and flavonoids, whereas all these parameters showed a marked increase in *P. putida*-treated seedlings exposed to thiamethoxam. The reduction in chlorophyll content may be due to increased chlorophyllase activity, chloroplast degradation and chlorophyll oxidation caused

**Table 2:** Mean differences and significant values (P<0.05) between various treatments through One-way Anova.

| Parameter | Combinations | | | | | |
|---|---|---|---|---|---|---|
| | TMX vs TMX+ MEL+PGPR | TMX vs TMX+ MEL+PGPR+ STRG | TMX+MEL vs TMX+PGPR | TMX+MEL vs TMX+MEL+ PGPR | TMX+MEL vs TMX+MEL+ PGPR+STRG | TMX+PGPR vs TMX+MEL+ PRPR |
| Total Chlorophyll | -0.001133 | -0.007700 | -0.001733 | -0.005533 | -0.01210 | -0.003800 |
| | NS | HS | NS | MS | HS | NS |
| Chlorophyll a | -0.0005000 | -0.002033 | 6.667-005 | -0.0005000 | -0.002033 | -0.0005667 |
| | NS | NS | NS | NS | NS | NS |
| Chlorophyll b | -0.0009000 | -0.006333 | 0.0002333 | -0.004800 | -0.01023 | -0.005033 |
| | NS | MS | NS | LS | HS | LS |
| Flavonoid | -0.01667 | -0.03733 | 0.05333 | 0.01333 | -0.007333 | -0.04000 |
| | NS | LS | HS | NS | NS | MS |
| Anthocyanin | 0.03463 | -0.3202 | -0.1015 | 0.7915 | 0.4367 | 0.8930 |
| | NS | HS | NS | HS | HS | HS |
| Trehalose | 0.01467 | 0.2037 | 0.1667 | 0.4553 | 0.6443 | 0.2887 |
| | NS | LS | NS | HS | HS | HS |
| Proline | -1.815 | -1.797 | 0.4640 | -0.8887 | -0.8707 | -1.353 |
| | HS | HS | HS | HS | HS | HS |
| Glycine betaine | -0.1977 | -0.3483 | 0.3693 | 0.2313 | 0.08067 | -0.1380 |
| | HS | HS | HS | HS | HS | HS |
| Super oxide anion | 0.04200 | 0.04633 | 0.1673 | 0.3253 | 0.3297 | 0.1580 |
| | HS | HS | HS | HS | HS | HS |
| $H_2O_{2-}$ | -0.4290 | -0.1260 | -0.1053 | -0.3873 | -0.04433 | -0.2820 |
| | HS | HS | HS | HS | HS | HS |




The values are estimated in triplicates and presented as mean ± SD.

**Fig 2:** Total chlorophyll, chlorophyll a and chlorophyll b, Carotenoid, Flavonoid, Anthocyanin, Trehalose, Glycine- betaine and Proline, Superoxide anion, Hydrogen peroxide content estimation of Thiamethaxom treated chickpea seedlings enriched by melatonin, *P. putida* and Strigolactone alone as well as in combination respectively.

by reactive oxygen species (ROS) (Harpaz-Saad *et al.,* 2007). Additionally, previous research has documented that thiamethoxam reduces chlorophyll levels in algae (Al-Badri *et al.,* 2020) and negatively affects photosynthetic pigments and the functionality of the photosynthetic apparatus in *Zea mays* (Todorenko *et al.,* 2020).

Abiotic stressors, including drought, heat, ultraviolet rays and cold temperatures, significantly hinder plant growth through various mechanisms, leading to reduced crop yields (Yilmaz and Kulaz, 2018). Among these factors, drought is particularly complex, as its recurring natured adversely impacts agriculture, the economy, water resources and ecosystems (Zhou *et al.,* 2021). Similarly, addition of pesticides results in water deficiency, protein degradation and a decrease in the accumulation of organic compounds within plants. These changes lead to osmotic stress, which negatively affects plant growth, development, biomass and chlorophyll production. Consequently, the present study demonstrates that co-inoculation of Pseudomonas putida and melatonin significantly enhances growth and development, increases biomass and chlorophyll content and lowers reactive oxygen species (ROS) levels to mitigate pesticide stress (Rangecroft *et al.,* 2019 and Huseynova *et al.,* 2016).

## CONCLUSION

The current study reveals that thiamethoxam negatively impacts the growth characteristics and photosynthetic pigment levels in seedlings, leading to excessive reactive oxygen species (ROS) production and significant redox imbalance. However, the addition of Pseudomonas putida and plant growth regulators (PGRs) such as melatonin and strigolactone enhances the plant's defense mechanisms by increasing the activity of ROS- scavenging antioxidative enzymes and antioxidants. Thiamethoxam also promotes the accumulation of osmolytes, which is further amplified by *P. putida* inoculation, helping to alle*via*te oxidative stress in seedlings. In conclusion, *P. putida* demonstrates strong potential to mitigate the stress caused by thiamethoxam. Thus, applying *P. putida* externally in combination with PGR could be a promising strategy for managing pesticide-related stress from an agronomic perspective. Given the concerns surrounding pesticide-induced toxicity in crops, it is crucial to develop effective strategies to address this issue. Further research utilizing biotechnological and genetic approaches could uncover the mechanisms behind *P. putida*-induced tolerance to thiamethoxam.

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### Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent

the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

### Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish or preparation of the manuscript.

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