Application of melatonin and PGPR alleviates thiamethoxam induced toxicity by regulating the TCA cycle in *Brassica juncea* L

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**Abstract**

Thiamethoxam, a broad spectrum, neonicotinoid insecticide, is used on various crops including *Brassica juncea* L to protect from intruding insects such as leaf-hoppers, aphids, thrips and white-flies. Exposure to thiamethoxam causes acute malady such as tumour development, cell apoptosis, liver damage and neurotoxicity. Melatonin is entailed in umpteen developmental processes of plants, including stress responses. The pleiotropic effects of melatonin in modulating plant growth validate it's imperative contribution as multi-regulatory substance. Exiguous information is known about the role of *Pseudomonas putida* in improving plant growth under thiamethoxam stress. Taking these aspects into consideration the contemporary study investigates the role of melatonin and *Pseudomonas putida* strain MTCC 3315 in alleviating the thiamethoxam induced toxicity in *B. juncea* plant. Fourier Transform Infrared Spectroscopy (FTIR) analysis unclad that thiamethoxam induced stress primarily affects the protein content of plant as compared to lipids, carbohydrates and cell wall components. Organic acid profiling of the treated samples carried-out by High-Performance Liquid Chromatography (HPLC), reported an upregulation in the level of organic acids, malic acid (110%), citric acid (170%), succinic acid (81%), fumaric acid (40%) and ascorbic acid (55%) in thiamethoxam treated plants compared to the investigational untreated plants. The melatonin treated seedlings grown under thiamethoxam stress, exhibit increased level of malic acid, citric acid, succinic acid, fumaric acid and ascorbic acid by 81%, 0.94%, 11%, 21% and 6% respectively. Further, thiamethoxam stressed plants inoculated with *Pseudomonas putida* showed stupendous up-regulation by 161% (malic acid), by 14% (citric acid), by 33% (succinic acid), by 30% (fumaric acid) respectively. Lastly, the combinatorial application of melatonin and *Pseudomonas putida* resulted in prodigious upsurge of malic acid by 165%, succinic acid by 69%, fumaric acid by 42% respectively in contrast to distinct melatonin and *Pseudomonas putida* treatments. The accumulation of organic acids ascertains the defence against thiamethoxam stress and corresponds to meet the energy generation requirement to skirmish thiamethoxam mediated abiotic stress in *Brassica juncea* plant.

1. Introduction

Pesticides are an extensive part of agriculture, ensuring crop fortification and augmenting crop yield (Jan et al., 2020). Plants exposed to pesticides show retardation and mutation in various physiological and biochemical processes thereby causing phytotoxic symptoms such as inhibition of photosynthesis, plant growth, pigment synthesis, mitosis and stomatal conductance respectively (Liu et al., 2021). Thiamethoxam (TMX), a broad-spectrum insecticide, belongs to neonicotinoid family, is used on various crops...
including *Brassica juncea* L. to protect from intruding insects such as leaf-hoppers, aphids, thrips and white-flies (Yadav et al., 2019). Thiamethoxam metabolism has already been reported in various plants including banana (Suganthi et al., 2018) and pepper (Mei et al., 2019). In citrus fruits 0.061 mg/kg thiamethoxam residues were detected (Gui et al., 2019). Thiamethoxam accretion escalates the complications involved during residue removal in crops (Wang et al., 2020a,b). Higher concentration of thiamethoxam induced stress response and caused toxic effect in plants such as sunflower (Georgiaev and Vassilevskova-Ivanova 2021). Exposure to thiamethoxam causes serious health problems such as tumor development, cell apoptosis, liver damage and neurotoxicity (Han et al., 2018). Also, thiamethoxam have been detected in children consuming inorganic vegetables (Wang et al., 2020a). Therefore, developing an effective technique to remove pesticide residues accumulated in internal tissues of edible crops is practically meaningful in the aspect of improving agricultural food safety.

*Brassica juncea*, a member of Brassicaceae family is generally recognized as Indian mustard. The estimated mustard yield is 72.42 million tonnes with productivity of 1980 kg/ha in 2018–19 in India (ICAR, 2018). This oil seed crop is usually invaded by various arthropods such as aphids. The pest manifestation radically decreases the mustard crop-yield (Yadav et al., 2019). More than 43 species of insects infest *Brassica juncea* crop in India causing up-to 96% of yield loss (Pradhan et al., 2020). In order to control pest attack, pesticides are used to protect plant and promote high yield (Xu et al., 2020). Application of pesticide regulates reactive oxygen species (ROS) generation thereby disrupting ROS homeostasis leading to oxidative stress and imposes detrimental impact on plants. To neutralize the oxidative stress generated by pesticide, plants activate their antioxidant defence system (enzymatic and non-enzymatic) (Sharma et al., 2018a) and metabolize pesticides by phase detoxification system, that usually entails conversion, conjugation and compartmentalization (Su et al., 2019).

Plant growth promoting rhizobacteria (PGPR), an omnipresent root microbiome is enormously exploited as biocontrol agent (Sharma et al., 2020). They are capable of enhancing plant growth by colonizing plant roots that can benefit the plant. Various PGPR’s such as *P. aeruginosa*, *B. gladioli*, *P. pseudoalcali* are known to withstand biotic and abiotic stresses (Khanna et al., 2019; Yasin et al., 2020a). To enhance stress tolerance in plants, PGPR has been known to produce metabolites which alter the defense response of plants by mitigating the damage caused by ROS (Rashid et al., 2020). PGPR confer vital functions in host plants for example releasing many phytohormones (IAA, gibberellins, cytokinins, ethylene, salicylic acid), enzymes, sidophore production, phosphate solubilization and nitrogen fixation (Yasin et al., 2020a,b). PGPR also up-regulates the secondary metabolites like organic acids, phenols, amino acid and sugars in plants (Khanna et al., 2019).

Phytohormones including melatonin are well recognized to alleviate aftermath of numerous abiotic stresses in plants (Kaya et al., 2020). Melatonin is a potent anti-stress biomolecule involved in various morpho-physiological processes such as plant growth, redox network, leaf senescence, osmoregulation, photosynthesis and promotes regeneration (Siddiqui et al., 2020), and possess strong free radical scavenging activity under stress conditions (Debnath et al., 2018). Despite its effective function in plant growth, melatonin improves plant tolerance against harsh environmental conditions (Liu et al., 2021). Credible evidence shows that melatonin maintains redox homeostasis by minimizing ROS generation and augments glutathione content (Kaya et al., 2019). Melatonin also regulates the function of other hormones for plant growth and development (Arnao and Hernandez 2018), however, meagre information is available on how it is involved in tolerance of plants to pesticide toxicity. Organic acids are bio-synthetic precursors for various bio-molecules. Profuse evidences demonstrate that organic acids accrue during abiotic stress condition such as salinity (Huangfu et al., 2021), and biotic stress (Khanna et al., 2019) in plants. The PGPR have also been significantly known to regulate the levels of plant metabolites such as organic acids and amino acids (Yasmin et al., 2020a). The intermediates of TCA cycle were found to be up-regulated in the *Oryza sativa* raised under salinity stress and this up-regulation confers the role of increased organic acid levels in neutralization of abiotic stress (Das et al., 2019).

The potential harmful effects of thiamethoxam and its toxic effects on plants and other non-target species need to be understood and elucidated. The interplay between melatonin and *Pseudomonas putida* in enhancing tolerance against pesticide toxicity and underlying biochemical mechanism of this relationship still remains unknown. On this account, present experiment aims to determine the role of exogenously supplemented melatonin and PGPR in *Brassica juncea* seedlings subjected to thiamethoxam stress.

2. Materials & methods

2.1. Microbial strain inoculation

The PGPR strain (*Pseudomonas putida* strain MTCC 3315) was procured from CSIR-IMTECH, Mohali, Punjab (India). The microbial strain was cultured by adding it into sterile 50 mL nutrient broth (NB) (13 g L\(^{-1}\)) medium. The culture flask was kept at 28 °C (24 to 48 h) in BOD incubator for proliferation. After optimum growth sub-culturing was done for future use. For current experiment 1 mL of grown culture was added in 50 mL of NB at 28 °C for 24–48 h. Further, it was centrifuged at 10000 rpm, 4 °C for 20 min to collect pellet. Further pellet was rinsed with double distilled water and therefore resuspended to acquire 10\(^5\) cells/mL.

2.2. Plant material and treatment

Certified seeds of *Brassica juncea* (Variety PBR 357) were sterilized using 0.01% mercuric chloride (HgCl\(_2\)) for 1–2 min followed by thorough rinsing with double distilled water. Melatonin (Himedia, catalog number: PCT0840-1G, ≥ 98% pure) was used as seed priming treatment in our *in-vitro* experiment. Based on earlier studies the effective concentration of melatonin was selected (Liu et al., 2021). Accordingly, 50 μM was chosen for the present study considering its best effect against pesticide toxicity. The surface sterilized seeds were immersed in freshly prepared solution (melatonin dissolved in ethanol followed by dilution with double distilled water [ethanol: water (v: v) = 1:10,000]) for 7 h. The melatonin doused seeds were washed with double distilled water, blotted dried and were kept at room temperature in dark until returning to its initial weight (over-night). Autoclaved petri-plates were layered with Whatman 1 filter paper and added with thiamethoxam 0.6 mM concentration. Subsequently, primed seeds were sown in thiamethoxam supplemented petri-plates and simultaneously microbial suspension was inoculated into petri-plates containing seeds. The petri-plates were kept in seed germinator under controlled condition (light intensity – 175 μmol m\(^{-2}\) s\(^{-1}\); temperature – 25.0 ± 0.5 °C, photo-period 16 h). After 10 days sowing, the seedlings were harvested for further analysis.

2.3. FTIR analysis

The FTIR spectra of powdered samples (control, TMX (0.6 mM), TMX + melatonin, TMX + *P. putida* and TMX + melatonin + *P. putida*) were recorded using Spectrometer (Perkin Elmer, USA). The FTIR spectra were recorded between 4000 to 400 cm\(^{-1}\). The FTIR spectra were collected in triplicates and the average was considered for the present study.
were observed using the Shimadzu FTIR spectrophotometer (Model IR Trace-100) with IR Microscope AIM-9000. The activity of the samples was measured amid 400 – 4000/cm at 4/cm resolution. Apodization was achieved using Happ-Genzel function and 16 scans were performed for each sample to achieve the associated peaks. The region assignment for lipids (3000–2000/cm), proteins (1800–1500/cm), carbohydrates (1500–1200/cm) and cell wall components 1000–600/cm were carried out and accordingly functional groups chemistry were revealed (Nikalje et al., 2019).

2.4. HPLC analysis of the organic acids

HPLC analysis of all the samples was carried out on Perkin-Elmer 200 series HPLC system. The stationary phase of the system constitutes of C-18 reverse phase column and KH2PO4 (pH- 2.5) was used as mobile phase. 15 μl of sample injection volume was used and the respective chromatogram for each sample was obtained at 210 nm wavelength. Organic acids considered in the present study were oxalic acid, malic acid, ascorbic acid, citric acid, succinic acid and fumaric acid. The organic acid standards were prepared using the methodology adopted in (Arnetoli et al., 2008). Detection of the organic acids in test samples was confirmed by matching their retention time with the standard organic acids retention time. The quantification of organic acids was carried out based on comparing the peak area of standard with respective treatments.

2.5. Statistical evaluations

The data obtained from HPLC was subjected to one-way ANOVA (Analysis of variances) to evaluate the impact of each treatment with respect to considered parameters on the Brassica juncea plant. The quantitative data was represented as mean of triplicates ± standard error. The significance of mean difference for each treatment was analysed by Tukey’s HSD (Honestly Significant Difference) test at P < 0.05 significance level. All the analysis were performed by using the SPSS statistical package (version 17.0).

3. Results

3.1. FTIR analysis of the control and treated samples

The basic tenet of FTIR relies on vibration of chemical bonds in the IR region. In IR region, chemical bonds absorb radiation between 4000 and 400 cm \(^{-1}\). As per Griffith and de Haseth (Griffiths and de Haseth, 1986), each functional group in a molecule has its own characteristic absorption frequency in the IR Spectrum. The sensitivity of IR spectroscopy has been successfully applied to in-vitro and in-vivo detection of biological systems. In our study, FTIR peaks obtained for the control and treated (TMX, TMX + melatonin, TMX + P. putida and TMX + melatonin + P. putida) seedlings are mentioned below in Fig. 1 to assess the vibrational frequency of functional groups. All the samples were scanned from 400 to 4000 cm \(^{-1}\) wave number and 3000–2000 cm \(^{-1}\) region was assigned for lipids, 1800–1500 cm \(^{-1}\) for proteins, 1500–1200 cm \(^{-1}\) for carbohydrates and 1000–600 cm \(^{-1}\) for cell wall components. Several absorption regions were identified and the band assignments are depicted in Table 1.

3.1.1. Lipid regions:

For lipid region peak corresponding to wave number 3304.6 cm \(^{-1}\) represents N-H stretching (Primary Amine) which was present in control sample whereas no peak was observed in all treated samples. However, peak corresponding to 3292.56 cm \(^{-1}\) was present in all treated samples except control thereby suggesting presence of C-H stretching alkynes derivatives. Thus, the results indicate partial impact on lipids.

3.1.2. Protein region

In protein region peak corresponding to wave number 1743.65 cm \(^{-1}\) and 1739.79 cm \(^{-1}\) denotes presence of C = O stretching esters were present in control plant, thiamethoxam treated plant and thiamethoxam + P. putida treated plant, whereas absence of ester group was noticed in thiamethoxam + melatonin and thiamethoxam + melatonin + P. putida treated plant, thereby revealing the effect of melatonin to reduce the ester compounds that is aliphatic ketones.

3.1.3. Carbohydrate region

For carbohydrates all treated samples were showing peak corresponding to wave number of 1315.5 cm \(^{-1}\) except control indicating the presence of phenols (O-H bending) group. Similarly carboxylic acids corresponding to 1415.75 cm \(^{-1}\) and 1417.68 cm \(^{-1}\) wave-number were observed in thiamethoxam and thiamethoxam + melatonin treated plants.

3.1.4. Cell wall components

The wave-number 1000–600 cm \(^{-1}\) corresponds to cell wall components. In control sample, the peak at 893.04 cm \(^{-1}\) wave number [characteristic of C-N stretch (Amines), =C-H bend (Benzene, Alkenes) (Xyloglucan)] was absent, whereas, it was present in all treated samples. However the peaks at 696.3 cm \(^{-1}\) and 663.51 cm \(^{-1}\) wave-number were observed in control sample only signifying that cell wall components might get degraded in presence of pesticide, howbeit the effect of melatonin and P. putida in maintaining the integrity of cell wall components was negligible.

3.2. Organic acids detection by HPLC

The present study investigates the relative organic acid content (oxalic acid, malic acid, ascorbic acid, citric acid, succinic acid and fumaric acid) variation observed in B. juncea plant subjected to thiamethoxam stress and to evaluate the role of melatonin and PGPR used as ameliorative in regulating the organic acid levels. With the help of HPLC technique the organic acids were detected in control and treated samples and their relative content were analysed based on peak area percent. The HPLC peaks obtained for the control, pesticide, pesticide + hormone, pesticide + PGPR and pesticide + hormone + PGPR treated plants are shown in Fig. 2 below.

Comparing the relative organic acid content among control (untreated) and pesticide treated plants the relative organic acid (oxalic acid, malic acid, ascorbic acid and citric acid) content of pesticide treated plants were found to be increased as referred in Table 2. Similar trend of increased relative organic acid content was observed for oxalic acid, ascorbic acid and fumaric acid in thiamethoxam + melatonin, thiamethoxam + P. putida and thiamethoxam + melatonin + P. putida treated plants on comparison to control (Table 2). On the other hand, peak area percent of citric acid and succinic acid were increased in control sample as compared to thiamethoxam + melatonin, thiamethoxam + P. putida and thiamethoxam + melatonin + P. putida treated plants (Table 2).

3.2.1. Quantification of organic acids in various treatments

The quantification of organic acids for various treatments carried out in triplicates are shown in Table 3. An increase in the amount of organic acids, malic acid (110%), citric acid (170%), succinic acid (81%), fumaric acid (40%) and ascorbic acid (55%) was observed in thiamethoxam treated plants in comparison to the investigational untreated plants. The exogenous application of
Melatonin in thiamethoxam treated plants resulted in up-regulation of malic acid, citric acid, succinic acid, fumaric acid and ascorbic acid by 81%, 0.94%, 11%, 21% and 6% respectively. Further, the organic acids content of thiamethoxam treated plants inoculated with P. putida were elevated by 161% (malic acid), by 14% (citric acid), by 33% (succinic acid), by 30% (fumaric acid), by 100% (oxalic acid) and decreased by 10% (ascorbic acid). Finally, the combinatorial approach, involving the application of melatonin and P. putida in thiamethoxam treated plants, resulted in substantial upsurge of malic acid by 165%, citric acid by 10%, succinic acid by 69%, fumaric acid by 42%, ascorbic acid by 3% and oxalic acid by 100% respectively.
Fig. 2. HPLC chromatogram obtained for the control untreated plant (a), Thiamethoxam treated plant (b), Thiamethoxam + melatonin treated plant (c), Thiamethoxam + P. putida treated plant (d), Thiamethoxam + melatonin + P. putida (e) treated plant respectively. The horizontal axis in the chromatogram represents the retention time (time taken by the analyte to reach detector), whereas, vertical axis represents milli absorbance units (mAU).

Table 2
Peak area percent for various organic acids detected in B. juncea subjected to numerous treatments (thiamethoxam, thiamethoxam + melatonin, thiamethoxam + P. putida and thiamethoxam + melatonin + P. putida).

| Organic Acid | Control (%) | TMX (%) | TMX + Melatonin (%) | TMX + P. putida (%) | TMX + Melatonin + P. putida (%) |
|--------------|-------------|---------|---------------------|---------------------|---------------------------------|
| Oxalic acid  | 11.27       | 17.06   | 55.32               | 53.44               | 17.24                           |
| Malic acid   | 6.37        | 13.59   | 3.55                | 3.51                | 6.39                            |
| Ascorbic acid| 1.07        | 6.00    | 2.30                | 6.05                | 6.34                            |
| Citric acid  | 39.74       | 53.26   | 0.99                | 5.92                | 2.79                            |
| Succinic acid| 27.01       | 0       | 21.38               | 1.42                | 1.46                            |
| Fumaric acid | 14.53       | 10.08   | 16.45               | 29.66               | 65.78                           |

Table 3
Organic acid quantification of the various treatments subjected to the HPLC analysis. The quantified value represents the mean ± standard error for three replicates of each sample. The * sign represents that mean difference for each treatment are statistically significant at P < 0.05 according to Tukey HSD test.

| Treatment                  | Malic Acid (Mean ± Std error) | Citric Acid (Mean ± Std error) | Succinic Acid (Mean ± Std error) | Fumaric Acid (Mean ± Std error) | Ascorbic Acid (Mean ± Std error) | Oxalic Acid (Mean ± Std error) |
|----------------------------|-------------------------------|--------------------------------|----------------------------------|---------------------------------|----------------------------------|-------------------------------|
| Control                    | 1.48 ± 0.01*                  | 1.95 ± 0.09*                   | 0.53 ± 0.03*                     | 0.30 ± 0.04*                    | 0.20 ± 0.06*                     | 0.01 ± 0.001*                 |
| Thiamethoxam               | 3.11 ± 0.03*                  | 5.27 ± 0.08*                   | 0.96 ± 0.01*                     | 0.42 ± 0.001*                   | 0.31 ± 0.001*                    | 0.01 ± 0.001*                 |
| Melatonin                  | 3.18 ± 0.01*                  | 4.20 ± 0.03*                   | 0.89 ± 0.02*                     | 0.32 ± 0.001*                   | 0.23 ± 0.01*                     | 0.01 ± 0.0007*                |
| P. putida                  | 6.01 ± 0.15*                  | 5.13 ± 0.07*                   | 0.98 ± 0.04*                     | 0.43 ± 0.0007*                  | 0.21 ± 0.006*                    | 0.02 ± 0.0005*                |
| Thiamethoxam + Melatonin   | 5.63 ± 0.16*                  | 5.32 ± 0.09*                   | 1.07 ± 0.01*                     | 0.51 ± 0.004*                   | 0.33 ± 0.008*                    | 0.01 ± 0.0006*                |
| Thiamethoxam + P. putida   | 8.12 ± 0.23*                  | 6.01 ± 0.09*                   | 1.28 ± 0.03*                     | 0.55 ± 0.003*                   | 0.28 ± 0.007*                    | 0.02 ± 0.0004*                |
| Thiamethoxam + Melatonin + P. putida | 8.25 ± 0.39* | 5.81 ± 0.02* | 1.63 ± 0.06* | 0.60 ± 0.003* | 0.32 ± 0.008* | 0.02 ± 0.0004* |
4. Discussion

The immense exploitation of pesticides like thiamethoxam has resulted their omnipresence in plants, soil and water (Wang et al., 2020a,b). Recently, gene expression analysis of maize plant exposed to thiamethoxam supressed the expression of 64 genes including glutathione-s-transferase and peroxidases which combats the oxidative stress (House et al., 2021). Further, this suppression of defence-related genes was also observed in soybean exposed to thiamethoxam which proves that it functions as a suppressor of genes involved in stress mitigation (Wulff et al., 2019). FTIR is becoming faster and sensitive method for evaluation of stress responses in stress biology. In current study, we used FTIR spectroscopy for detection of chemical and conformational changes in thiamethoxam treated seedlings. Bulk chemical analysis of changes in carbohydrates, proteins, lipids and cell wall were performed for seedling under thiamethoxam stress, treated with melatonin, Pseudomonas putida and in combination. The melatonin and Pseudomonas putida treated seedlings generated large number of sharp FTIR peaks indicating rich chemical composition as compared to thiamethoxam treated seedlings. The peak corresponding to wave number 663.51 cm$^{-1}$ and 696.3cm$^{-1}$ were completely absent in all treated seedlings which indicates cell wall components are highly susceptible to stress conditions and no effect of ameliorative substances was observed as reported previously in Atriplex prostrate grown under salinity stress conditions (Nikalje et al., 2019). Another key indicator of stress is the rate of cell wall synthesis and pectin accumulation which was observed by analysing ester at the absorption band 1745 cm$^{-1}$. Study by Hoang et al., (2015) manifested ice plants under stress conditions accumulate the ester. Similar observations were seen in our study for Brassica juncea plant inoculated with PGPR under thiamethoxam stress however, absence of esters was noticed in melatonin treated seedlings exposed to thiamethoxam. Taken together, the FTIR data concludes partial impact on protein, lipids and cell wall components and suggest that under stress conditions Brassica juncea plant is unable to maintain higher order bio-components to some extent.

Previous literature studies which ascertain that enhanced level of organic acids in plant correlates with their defence against the onset of biotic or abiotic stress (Bali et al., 2018) Organic acids also participate in the plant’s defence mechanism thereby controlling the osmotic pressure and ionic balance during abiotic stress (Zeng et al., 2008; Sharma et al., 2017). Similar observations were noticed in current study corresponding to elevated level of organic acids, malic acid, citric acid, succinic acid, fumaric acid and ascorbic acid were observed in thiamethoxam treated plants. In a recent study, exogenous application of melatonin in rice resulted in elevated levels of organic acids which enhances the mitochondrial respiration so as to meet the energy requirement to combat the arsenic stress (Samanta et al., 2020). Application of melatonin in soybean has been found to up-regulate the expression of gene translating acetyltransferase NLS-like, reduced H$_2$O$_2$ generation and upregulated efflux of organic acids to mitigate the Aluminium toxicity (Zhang et al., 2017). The current study also demonstrates the role of melatonin in combating the plant stress, thereby, upregulating the organic acids level (Table 3). Equivalently, thiamethoxam treated B. juncea plants in present investigation by the exogenous application of melatonin were observed to upregulate the malic acid, citric acid, succinic acid, fumaric acid and ascorbic acid by 81%, 0.94%, 11%, 21% and 6% respectively (Table 3), suggesting their role in alleviating the thiamethoxam stress. The plant growth promoting rhizobacteria P. putida has been observed previously in effective degradation of thiamethoxam by utilising its degraded constituents as a source of carbon and energy (Rana et al., 2015). Similar trend of thiamethoxam degradation was observed in the current study on the basis of elevated levels of organic acids (by 161% (malic acid), by 14% (citric acid), by 33% (succinic acid), by 30% (fumaric acid), by 100% (oxalic acid)) in treated seedlings (Table 3). The combinatorial approach involving application of melatonin and P. putida has also proved to effective against alleviating the thiamethoxam stress in B. juncea seedlings as inferred by the upregulated levels of organic acids (malic acid by 165%, citric acid by 10%, succinic acid by 69%, fumaric acid by 42%, ascorbic acid by 3% and oxalic acid by 100%) on comparison to the thiamethoxam treated plants (Table 3).

5. Conclusion

In summation, our findings indicate that Melatonin and Pseudomonas putida alone as well as synergistically boosts the resilience of Brassica juncea seedlings under thiamethoxam stress. The association of Melatonin and Pseudomonas putida with plants in up regulating levels of metabolites under thiamethoxam toxicity has been explored. FTIR analysis clearly asserts that effect of pesticide stress is more on protein and cell wall components of the plant as compared to lipids and carbohydrates and combinatorial approach of melatonin and PGPR with B. juncea can significantly reduce the thiamethoxam induced stress on biomolecules. Under abiotic stress conditions plants produce ROS which causes an imbalance in redox homeostasis and anti-oxidant defence system, regulating oxidative stress and ultimately compromising the growth, development and crop yield (Hasanuzzaman et al., 2020). Organic acids have been recognised to combat the abiotic stress conditions by regulating the osmotic pressure level and ionic balance (Sharma et al., 2018b). In current research endeavour, relative organic acid content of intermediates of TCA cycle were analysed by the HPLC technique. Our findings revealed that relative organic acid content in thiamethoxam, thiamethoxam + melatonin, thiamethoxam + P. putida and thiamethoxam + melatonin + P. putida treated plants was considerably higher as compared to the control plant. The accretion of organic acids (intermediates of TCA cycle) may correspond to meet energy generation requirement to combat the thiamethoxam mediated abiotic stress. This unfolds new areas of research to ascertain precise molecular mechanisms involved in regulatory processes fostered by application of melatonin and Pseudomonas putida in stressed plants. Our study breakthroughs can also be extrapolated to other crops for which melatonin and Pseudomonas putida will be exploited as natural bio-stimulating agents in order to palliate the negative impact of multifarious abiotic stresses.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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