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ORIGINAL ARTICLE

Antioxidant enzymatic activities and profiling of gene expression associated with organophosphate stress tolerance in *Solanum melongena* L.cv. Longai

Priyadarshani Yengkokpam[1] · Pranab Behari Mazumder[1]

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Abstract
The tolerance mechanism of chemical pesticide is necessary to combat the pest infestation challenges. This study intended to analyze the responses of enzymatic activity and expression level of an antioxidant gene to organophosphate pesticide stress. The alteration of anti-oxidative correlated with pesticide treatment in eggplant (*S. melongena* L.cv. Longai) using varying concentrations (0, 50, 100, 150 and 200 ppm) of malathion (PM) and tatafen (PTF) each. The enzyme activities of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) were observed to be elevated with pesticide treatment in eggplant seedling. *FeSOD* (iron SOD), *CAT* and *APX* genes associated in defense mechanisms were significantly expressed under PM and PTF stress which contributed to stress tolerance to the plant. The different concentration of both pesticide stresses altered the expression level of mRNA, *FeSOD*, *CAT* and *APX* genes in comparison to the non-treated plant. While mRNA level of three antioxidant genes were evaluated and found to be *APX* gene expression was more potent than the *CAT* and *FeSOD* gene subjected to different concentrations of PM and PTF in eggplant. The current experiment highlights the presence of minimum level of pesticide concentration impacted positively towards the plant growth and metabolism, while high level of pesticide concentration impacted negatively. In summary, antioxidant enzymes activity responded to both pesticide stresses at an early stage of exposure and their gene expression profiles provided more details about their complex interaction and effectively scavenge reactive oxygen species. This allows the plant to maintain growth under pesticide stress.

Keywords Enzyme activity · Pesticide stress · *Solanum melongena* L.cv. Longai · Stress tolerant genes · Transcript level

Introduction

Eggplant (*Solanum melongena* L., 2n = 24) is generally termed as brinjal, aubergine or Guinea squash which is mostly benign in the warm-temperate region, cultivated in Asian tropical and sub-tropical regions across the globe (Taher et al. 2017). It is an abatement-growing perennial solanaceous crop, behaves as an annual crop that can be prolonged under preserved cultivation. Eggplant is contemplated as a day-neutral plant with a height of 50 to 150 cm and flowering starts at the 6th to 10th leaf phase lasting for an extended period, its fruits bear in different shapes, sizes, and skin color as well as weight ranging from 200 to 500 gm (Taher et al. 2017). It is considered as one of the vegetables with the highest capacity of antioxidant (Hurtado et al. 2012). Its extracted tissue has been used in traditional medicine treatment of several diseases, such as bronchitis, asthma, dysuria, cholera as well as curing diabetes (Quamruzzaman et al. 2020).

Eggplant is contemplated as a native of the Indian subcontinent and a credible center of origin, whereas China, Japan and Indo-Burma is the inferior center of origin (Kumar et al. 2020). In Assam, eggplant is propagated to a considerable extent, but it curtails productivity of crops in many countries. Its production affords an imperative source of livelihood, especially in poor resource farmers (Hautea et al. 2016). The preeminent constraint to the fruits of eggplant throughout Asia, is chronic and extensive infestation of the noxious pest, fruit and shoot borer (FSB, *Leucinodes*

✉ Pranab Behari Mazumder
assamuni2018@gmail.com

Priyadarshani Yengkokpam
pydarshani@gmail.com

[1] Plant Molecular Biology and Biotechnology Laboratory, Department of Biotechnology, Assam University, Silchar 788011, India

orbonalis Guenee) (Hautea et al. 2016). The larvae are catastrophic in yield and quality production of fruits by boring into the leaves of midrib and petiole, tender shoots resulting in desiccation and wilting of the stem and it has been active in moderate humidity throughout the year (Javed et al. 2017). Till date, its management practices to combat insect and pest are still restraint to frequent sprays of various pesticides which are detrimental to human health, environment and livestock (Challa et al. 2021). The survey of eggplant cultivators in the major cultivation area of India (Rai 2015) revealed that the yield loss ranges from 11 to 93% of total production due to the attack of pests. Almost all farmers use chemical pesticides, such as malathion, cypermethrin, carbyl, fenvalerate, and imidacloprid, etc., for the elimination of EFSB damaged fruits. Residues of these pesticides were distinguished in the harvested crops as well as eggplant farms soil (Hautea et al. 2016).

Like many other toxic chemicals, pesticides persuade the production and accumulation of free radicals viz. hydroxyl radicals, singlet oxygen, hydrogen peroxide and superoxide (Ara et al. 2013). Reactive Oxygen Species (ROS) are mostly provoked via photorespiration in peroxisomes, photosynthesis in chloroplasts and aerobic respiration in mitochondria (Zaid and Wani 2019). The enormous production of ROS scavenging can result in irreversible oxidation of proteins, lipids, nucleic acid and chloroplastic pigments (Li et al. 2012). Injury in plants caused by excess ROS production is termed "oxidative stress". Plants are incorporated with an enzymatic and non-enzymatic antioxidant defense mechanism to cope and detoxify the enormous generation of ROS. This system consists of numerous enzymatic antioxidants, such as Superoxide dismutase (SOD, EC 1.15.1.1), Ascorbate peroxidase (APX, EC 1.11.1.11), Dehydroascorbate reductase (DHAR), Guaiacol peroxidase (GPX, EC 1.11.1.7), Catalase (CAT, EC 1.11.1.6), Monodehydroascorbate (MDHAR), and Glutathione Reductase (GR), where non-antioxidants such as reduced glutathione, proline and Ascorbic Acid (ASA) work in cascade to sustain the subcellular redox homeostasis by scavenging various ROS production (Wani et al. 2018). The antioxidant enzymes were detected in eggplant subjected to organophosphate pesticides and levels of the enzymes were dependent on the concentration of pesticides. SOD is considered as the first line of defense against the production of ROS, which converts O_2^- free radicals to H_2O_2. Then H_2O_2 reduced to H_2O and molecular O_2 by enzyme APX, POD and CAT, thus averting further damage to the plant cell membrane (Semida et al., 2021). Understanding the involvement of both antioxidant activity and gene expression levels in pesticide tolerance is necessary for the detection of the main antioxidant defense system. The analyses of antioxidant genes in several plant species will increase the comprehensive insight of their specific roles in the defense of plant mechanisms. In this study, the functions of *FeSOD, CAT* and *APX* gene expression levels in the defense system of eggplant exposed to various concentrations of PM and PTF. The expression of mRNA levels of *FeSOD*, *CAT* and *APX* genes was established by qRT-PCR in a pesticide treated plants.

Materials and methods

Experimental design

The experimental materials were cultivars that are currently cultivated in Karimghanj, Assam, India. Due to the pesticides stress trigger a wide variety of plant responses ranging from altered antioxidant metabolism and gene expression to changes in growth rate and crop yield. Under normal growth conditions, the ROS production in cell is very low while stress conditions, elevate the levels of ROS in plant cells. It is highly lethal and can oxidize most of nucleic acids, lipids and protein subsequently causing cell death due to lipid peroxidation and inactivation of antioxidant enzymes as well as membrane damage. Thus, to cope with oxidative stress, plants develop a complex antioxidative defense mechanism consisting SOD, APX, CAT and POD etc., that scavenge peroxide and free radicals (Gill and Tuteja 2010; Shakir and Rehman 2018). Among the key antioxidant enzymes, SOD leads the frontline defense in the antioxidant defense system by $O_2^{\bullet-}$ into H_2O_2 and reducing the possibility of $^{\bullet}OH$ formation. Then, APX and POD are associated in the scavenging of H_2O_2 (Gajewska and Skłodowska 2007). CAT is tertrameric heme-containing enzyme for detoxification of ROS which converts more than 25 million H_2O_2 molecules into H_2O in 60 s. Peroxidase mainly oxidizes PhOH for producing phenoxyl radical (PhO•), where H_2O_2 accepts electron and is converted to H_2O (Gill and Tuteja 2010; Hasanuzzaman et al. 2020). These changes in the activity of these antioxidant enzymes indicate redox alterations related to oxidative stress.

Pesticide treatment

The eggplant seedlings were propagated in plastic pots filled with farm manure about 2 kg of soil per pot. The primary characteristic of the soils is sand particle 30.2%, silt 39.4%, and clay 30.4%. Seedlings were grown outdoors in polybag placed under a defensive condition. Four pesticide treatments via 0, 50, 100, 150 and 200 ppm each for PM (Malathion, EC-50%; accessed from ASAMAL-5, Assam Chemical Industries, Bongaigaon, Assam) and PTF (Tatafen, 10%; Rallis India Limited, Mumbai, India) were selected for the treatment of plants with control samples (without pesticides leveled as 0 ppm). Stock solutions of the PM and PTF were assembled with DMSO due to their insolubility in

water (concentrations ranging between 50 and 200 ppm) and stored in the dark condition at 4 °C. Treatment concentration of PM and PTF was standardized following related studies conducted earlier studies by Shakir et al. (2018). Treated and control seedling was uprooted at 20 days of growth and analyzed for the antioxidant assay and gene expression analysis.

Determination of antioxidant stress markers

Frozen leaf was ground to a fine powder with ice-cold $NaPO_4$ (pH 7.0) (50 mM) containing polyvinylpyrrolidone (1%, w/v) (2:1 buffer volume/FW) and EDTA (0.1 mM). The homogenate was centrifuged for 20 min at 4 ºC at 10,000 rpm and the supernatant was used for spectrophotometric determination of antioxidant activity (Kaur et al. 2016). SOD activity: The activity was evaluated according to Vuleta et al. (2016) based on the measurement of inhibition of the photochemical reduction of nitroblue tetrazolium (NBT; HiMedia) spectrophotometrically at 560 nm. The cocktail contained PO_4 (50 mM) (pH 7.4), EDTA (0.1 mM) to which molecular O_2 generating system containing NBT (82.5 µM), riboflavin (2.2 µM) and methionine (14.3 mM) and required amount of crude enzyme/sample extract (25 µl). The reaction was initiated by adding riboflavin and placing the tubes under the light source (below 30 cm) (40 W fluorescent tube-light: Philips, Kolkata, India) for 30 min. A complete reaction mixture without enzyme which gave the maximal color served as blank. A non-pesticides, treated complete reaction mixture served as a control. One unit of SOD enzyme is inhibited by 50% of maximum reduction of NBT under specific assay conditions as recorded at 560 nm. The CAT enzymatic assay was determined according to Azarabadi et al. (2017) with a slight modification. To determine the H_2O_2 decomposition by monitoring the decrease in absorbance was recorded at 240 nm.

APX enzyme assay: The supernatant subjected to analyze for spectrophotometric determination of enzymatic activity was termed by the process of Moura et al. (2018). It was assayed from the decrease in absorbance as ascorbate was oxidized.

Total RNA isolation and complementary DNA (cDNA) synthesis

The possible variation among the antioxidant genes encoding some scavenger enzymes that is necessary for the direct detoxification of ROS by three well-documented genes, such as *FeSOD, CAT* and *APX*. The steps are as follow: Total RNA was isolated from eggplant leaves treated with different concentration of PM and PTF using Trizol (Life Technologies, USA) protocol followed by RNA BR (Broad-Range) Assay Kit (Qubit®) following the manufacturer's directives. To abolish genomic DNA, the extracted RNA was treated with RNase-free DNase I supplemented provided with the assay kit. To evaluate the RNA purity and quality was measured by Qubit® 2.0 Fluorometer (Life Technologies, USA) and gel electrophoresis which contains agarose (1.5%) as described by Sambrook et al. (1989). The synthesis of the first-strand cDNA was based on reverse transcription reactions and which was achieved with total RNA (2 µg) using 5 mM oligo (dT)18 primers and reverse transcriptase (M-MLV Reverse Transcriptase) 1 mM dNTP solution, 20 µl 3 mM Mg^{2+}, 4 µl 5X FS Buffer and 0.1 M DTT according to the manufacturer's directives (Invitrogen, USA). The thermo-cycling conditions were carried out at 65 °C for 5 min, 37 °C for 60 min and 70 °C for 10 min.

Analysis of quantitative real time-polymerase chain reaction (qRT-PCR)

The experiment includes three biological and technical replicates each for PM and PTF treated and non-treated plants sample. The primer sequences employed in the experiment are shown (Table 1). The primer sequences of the target antioxidant genes are *FeSOD* (GenBank Accession No: KU240391.1), *CAT* (GenBank Accession No: X71653.1), *APX* (GenBank Accession No: AB828071.1) and housekeeping or internal control gene actin (*ACT*, GenBank Accession No: GU984779.1) as reference genes used for normalization in eggplant. The primers were designed based on the eggplant gene sequences available in the Genebank database

Table 1 Nucleotide sequences of designed primers SOD, CAT, APX and ACT utilized for Real-Time PCR assay

GenBank Accession No	Antioxidant Gene	Primer sequence (5'→3')	Amplicon length (bp)	Tm (°C)
KU240391.1	*FeSOD*-F	TATGAAGCCCAATGGAGGAG	20	60
	FeSOD-R	GGCAAGCTTTTTGTCCTCAG	20	60
X71653.1	*CAT*-F	GTGCCAAGGGATTCTTTGAA	20	60
	CAT-R	CCGCGAATATCCCTGATAGA	20	60
AB828071.1	*APX*-F	CATCCTCTCCCATGCTGATT	20	60
	APX-R	TTCACGAACACATCCCTCAA	20	60
GU984779.1	*ACT*-F	CCAAGGCCAACAGAGAGAAG	22	60
	ACT-R	AAACGAAGAATGGCATGAGG	22	60

(https://www.ncbi.nlm.nig.gov/genbank). The transcription levels of all the antioxidant genes were performed using the qRT-PCR with Thermo Fisher Scientific QuantStudio-5–384 Real-time PCR (Applied Biosystems). The PCR reaction mixtures consisted of 1 µl of each primer, 1 µl of cDNA and ddH_2O to final volume of 20 µl and amplification product were observed via SYBR Green dye which is an intercalator-based method. The reaction mixture was initially denatured for 2 min at 94 °C was set to 1cycle and then, denaturation at 94 °C for 30 s followed by 40 cycles and annealing for the 30 s, extension at 60 °C for 30 s were allowed for completion of polymerization. The relative expression levels of antioxidant genes were calculated using the $\log_{10}$ function (Gébelin et al. 2013) and normalized to the relative transcript level of the *ACT* gene. Each primer was composed of about 20 nucleotides with the melting temperatures around 60 °C. Melt-curve analysis was performed to confirm the specificity of the selected primers and primer–dimer absorbance. For quantification, data collection was done during the stage of annealing as well as copy numbers of transcript *FeSOD, APX, CAT* and *ACT* genes under PM and PTF stress treatment was established using amplification curves.

Statistical analysis

All the assays were observed with three independent replicates and were analyzed using the SPSS Statistical Software (IBM SPSS 21.0, IBM Corporation) windows version 16.0 and Microsoft Excel 2007. The data are presented as mean ± SE of three replicates. Differences between each pesticide treatment were analyzed by Least Significant Difference (LSD) ($p \leq 0.05$).

Results

Activities of enzymatic antioxidant

The antioxidant enzyme activities were measured to evaluate the mechanisms of pesticide tolerance in the leaves of the pesticide-treated *S. melongena.* The activity of SOD in PM and PTF treated samples was significantly higher than those of the control plants. The activity of SOD increased up to 40.93 U/mg protein at 100 ppm and suddenly decreased as the treatment concentration increased to 150 ppm (31.56 U/mg protein) and 200 ppm (24.12 U/mg proteins) of PM treatment (Fig. 1a). Similarly, SOD activity was found to be gradually increased at 50 ppm (26.35 U/mg protein) and 150 ppm (35.01 U/mg protein) but a sudden decrease occurred at 100 ppm (23.2 U/mg protein) and 200 ppm (7.32 U/mg protein) of PTF exposure compared with the control plant (12.32 U/mg protein). As shown in Fig. 2a, the activity of CAT was higher in the PM-treated plants and peaked at 200 ppm (16.58 U/mg protein) followed by 100 ppm (7.98 U/mg protein), 150 ppm (7.22 U/mg protein) and 50 ppm (6.59 U/mg protein). In PTF treated plant, the maximum increase was found in 150 ppm treatment (13.43 U/mg protein) followed by 50 ppm (4.87 U/mg protein), whereas activities of CAT significantly decreased at 100 ppm (2.3 U/mg protein) and 200 ppm (1.7 U/mg protein) as compared to the control plant (3.79 U/mg protein). The activity of APX showed a maximum increase under pesticides stress and peaked at 200 ppm (6.04 U/mg protein) followed by 100 ppm (4.47 U/mg protein), 50 ppm (2.5 U/mg protein) and 150 ppm (2.45 U/mg protein) of PM treatment, whereas in PTF treated plant showed maximum activity of APX at 150 ppm (4.66 U/mg protein) followed by 100 ppm (3.3 U/

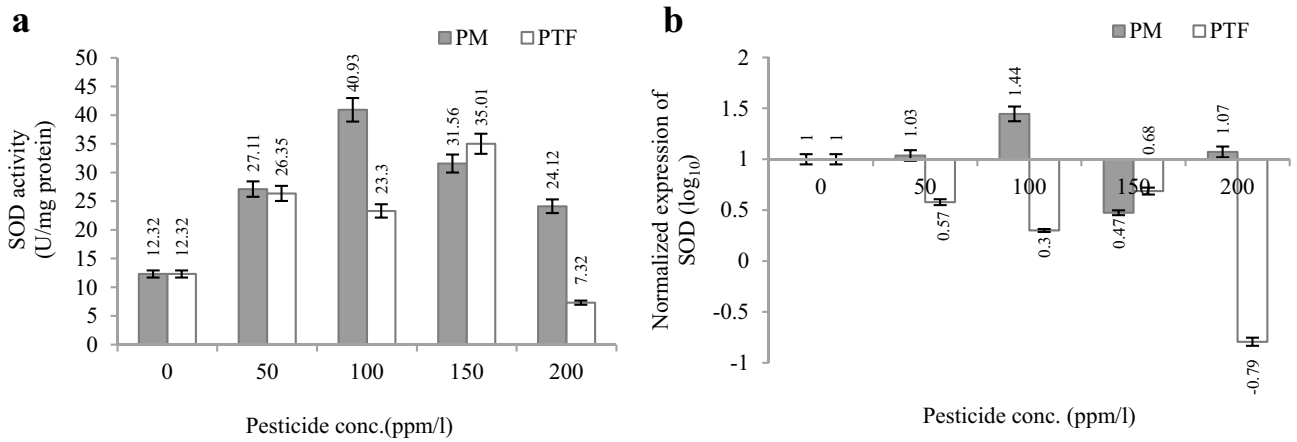

Fig. 1 Effect of different concentration of PM and PTF stress on the activity and transcript expression level of superoxide dismutase (SOD) enzymes in eggplant leaves. **a** SOD activity in leaf. **b** Relative expression of antioxidant gene *FeSOD* transcript established through qRT-PCR in PM and PTF at 50, 100, 150 and 200 ppm using *ACT* as housekeeping gene. The values are mean ± SD ($n = 3$)

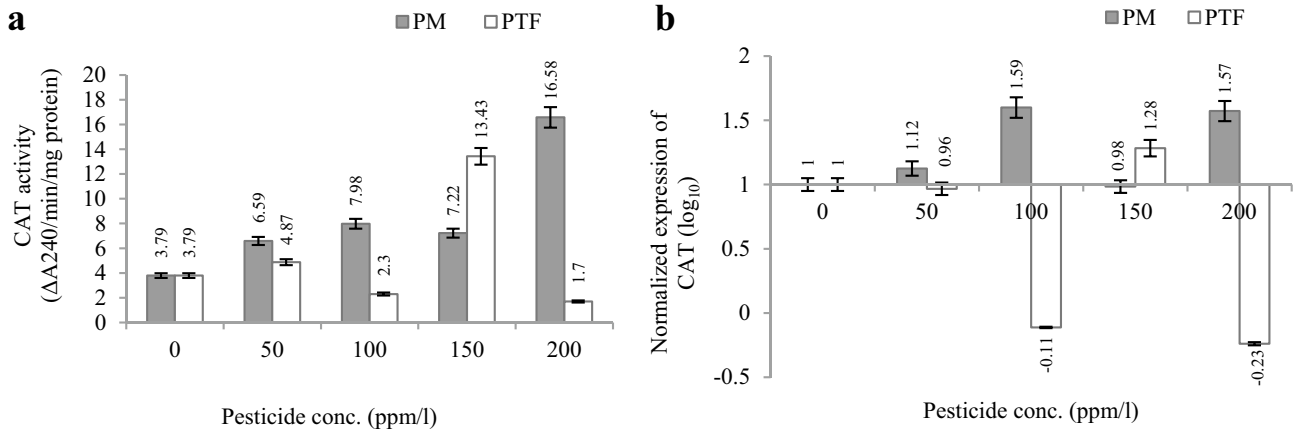

Fig. 2 Effect of different concentration of PM and PTF stress on the activity and transcript expression level of catalase (CAT) in eggplant leaves. **a** CAT activity in leaf. **b** Relative expression of antioxidant gene *CAT* transcript established through qRT-PCR in PM and PTF at 50, 100, 150 and 200 ppm using housekeeping *ACT* gene. The values are mean ± SD ($n = 3$)

mg protein), 50 ppm (3.24 U/mg protein). When the treatment concentration raised to 200 ppm the APX activity was maximum compared to the control plant (0.90 U/mg protein) (Fig. 3a).

Gene expression

The expression levels of *FeSOD, CAT* and *APX* genes were evaluated by qRT-PCR (Thermo Fisher QuantStudio-5-384 qRT-PCR platform, Applied Biosystems) in eggplant treated with different concentrations of PM and PTF. The qRT-PCR data are normalized with the *ACT* as an internal control gene. To analyze the stability results of the transcript level of *FeSOD, CAT, APX* and *ACT* of two pesticides samples were measured 3 times for each concentration exposure. Concerning the control and to each other, different expression levels were recorded in all the concentrations (50, 100, 150 and 200 ppm) of PM and PTF exposure.

In the first treatment (50 ppm), the expression level of *FeSOD* was increased under both PM and PTF. Subsequently, the maximum *FeSOD* expression level was found up-regulated in 100 ppm of PM, which was recorded as a 1.44-fold change followed by 200 ppm (1.071 fold change) and 50 ppm (1.036 fold change) compared to the control level. A decrease was observed again at 150 ppm (0.474 fold change). Then, the expression was shown slightly up-regulated at a concentration of 200 ppm (1.071 fold change) PM treatment. At PTF stress, it was observed that the expression level of the *FeSOD* gene was moderately up-regulated in 50 ppm (0.578 fold change) and 150 ppm (0.686 fold

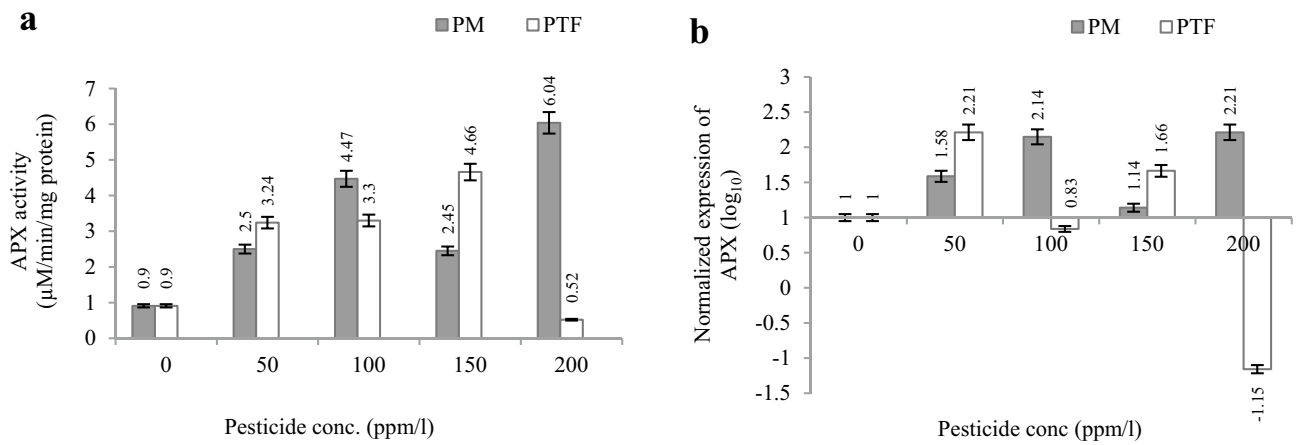

Fig. 3 Effect of different concentration of PM and PTF stress on the activity and transcript expression level of ascorbate peroxidase (APX) in eggplant leaves. **a** APX activity in leaf. **b** Relative expression of antioxidant gene *APX* transcript established through qRT-PCR in PM and PTF at 50, 100, 150 and 200 ppm using housekeeping *ACT* gene. The values are mean ± SD ($n = 3$)

change) and slightly decreases in 100 ppm (0.3 fold change) treated plants. At 200 ppm treated eggplant *FeSOD* expression level was moderately down-regulated in PTF stress, which was a −0.79-fold change decrease compared to the control (Fig. 1b). During the increased concentration of pesticide, plasmolysis started in eggplant leaves. The expression levels of the *FeSOD* gene were under control levels in all the concentrations of PM treatment, but slightly similar in exposed to 150 ppm of both PM and PTF treatment. Expression of *CAT* was up-regulated in all the concentrations of PM treated eggplant, the highest expression level was found in 100 ppm which was 1.599 fold change, but at the treatment of 200 ppm was observed 1.572 fold change followed by 50 ppm (1.124 fold change) and slightly decreased in 150 ppm (0.983 fold change) as compared to control (1.0 fold change). The expression of the *CAT* gene was not affected by different concentrations of PM treatment. In PTF stress treatment, *CAT* gene expression was observed up-regulated in 150 ppm which was 1.283 fold change in 50 ppm it was 0.966 fold change and it was slightly down-regulated at 100 ppm (− 0.11 fold change) and in 200 ppm (− 0.23 fold change) compared to control (1.0 fold change), but the level of *CAT* gene was not expressed under the concentration of 100 ppm and 200 ppm of PTF treatment (Fig. 2b). The expression of *APX* was observed highly up-regulated in all concentrations of PM stress. But, the highest expression levels were found in eggplant exposed to 200 ppm PM concentration which was a 2.21-fold change followed by 100 ppm (2.14 fold change) and 50 ppm (1.58 fold change), whereas slightly decreased in 150 ppm with 1.14 fold change as compared to control. During PTF treatment, the expression level of the *APX* gene was highly up-regulated in 50 ppm, which was 2.21 fold change and followed by 150 ppm (1.66 fold change) and subsequently suppressed the expression level at 100 ppm (0.83 fold change). Down-regulated *APX* was observed in 200 ppm PTF which 1.15-fold change in treating eggplant (Fig. 3b).

Discussion

Plants respond to different insects/pests through several biological, morphological and molecular mechanisms to combat the effects of the attack (Zaid et al. 2021). The mechanisms of defense against pest attacks are wide-ranging, highly dynamic and mediated by direct and indirect defenses. In addition, to attract the natural enemies of the pest/insect, plants also liberate volatile organic compounds, either act in conjugation or independently with each other. Therefore, this defensive mechanism is still limited and could be developed as an essential implement for pest management to curtail the amounts of chemical insecticides/pesticides used for pest control (War et al. 2012). Under oxidative stress conditions, uncontrolled free radicals, enhance, and plants use enzymatic and non-enzymatic antioxidants decrease oxidants and regulate cellular homeostasis (Kamran et al. 2020). Several stress factors are continuously exposed to plants which affect their yield production. ROS accumulation in plants can cause severe oxidative stress (Wani et al. 2018). To resist the excess ROS production in plants, the enzymatic antioxidant enzymes cooperate thereby protecting the functions and structures of cellular components. In general, the activity of more than one antioxidant enzyme in plants increases under different abiotic stress conditions, such as pesticides, salt, etc. (Zaid et al. 2020, 2019; Zaid and Mohammad 2018). This increase in enzymatic antioxidant activity correlates with increased stress tolerance (Heidari et al. 2021). The enzymatic antioxidant activity was differently altered in both the pesticide treatment and control samples. At the lower concentration of pesticide, the SOD activity was elevated in both PM and PTF treated plants, whereas decreased with the pesticide increases. The results are inconsistent with Sharma et al. (2012) that enzymatic antioxidant activity such as SOD, CAT, APX, MDHAR, and GPX increased after chlorpyrifos (CPF) (0.02%, 0.04%, and 0.06%) and 24-epibrassinolide (EBL) (10^{-11}, 10^{-9} and 10^{-7} M) treatments alone and in combination with Indica rice variety Pusa Basmati-1. Accumulation of ROS can cause severe oxidative damage as well as toxic molecules found in various subcellular compartments of plants (Ahmad et al. 2019). Detoxification of ROS is a most vital role in any defense mechanism, this is why plants possess a complex antioxidant system that includes non-enzymatic molecules (such as ascorbate, phenols, proline, tocopherols), and antioxidant enzymatic components (such as ascorbate peroxidase, catalase, superoxide dismutase) and components of the ascorbate–glutathione cycle (Apel and Hirt 2004). Therefore, the equilibrium between ROS production and detoxification is sustained by different enzymatic and non-enzymatic antioxidants (Caverzan et al. 2016; Ahmad et al. 2019). The results are also in the same line with Bashir et al. (2007), where the enzymatic antioxidants, activity of SOD, APX and GR increased significantly markedly in relation to increasing concentration of deltamethrin exposed I Glycine max (L.) Merr (soybean). Their results indicated that a higher concentration of deltamethrin produces oxidative damage in the soybean. These changes observed were dose-dependent, showing a strong correlation with the concentration of treatment. Singh and Roy (2017) also studied *Allium cepa* were treated with different concentration of malathion (50, 125, 250 and 375 ppm) under different periods (3, 9 and 18 h) showed significant ($p \leq 0.05$) elevated levels of SOD and CAT than the control sample, while the activity of POD was significantly ($p \leq 0.05$) low at 375 ppm. Their finding suggested that antioxidant enzyme activity could serve as the useful indicators for monitoring the effects of

malathion exposures in plants. SOD and CAT enzyme play an important role in the antioxidant defense mechanisms under malathion toxicity.

CAT activity had significantly increased in PM treated plants but at PTF treated plants showed decreased activity at the higher concentration of the stress. CAT is considered the main H_2O_2 scavenging an enzyme is also widely involved in plant immunity from various abiotic stresses (Yan et al. 2021). Therefore, a various pattern of the antioxidant activity was shown in the intermediate concentration. It has been reported that APX plays a vital role in combating various biotic and abiotic stresses in plants (Koussevitzky et al. 2012). In particular, APX is one of the important enzymes in the defense mechanism of plants that can detoxify H_2O_2 into H_2O and O_2 using ascorbic acid as an electron donor (Foyer and Noctor 2005). The activity of APX in PM treated plants was gradually increased with increasing concentration of pesticides, whereas PTF treated plants showed gradual increases at 150 ppm and then decreased at 200 ppm compared to the control plant.

APX is also an important enzyme that is involved in the diverse developmental and physiological processes and stress responses by scavenging H_2O_2 in plants (Xiao et al. 2021). It is considered an important ROS-scavenging enzyme, which catalyzes the removal of H_2O_2 to prevent oxidative damage (Kaur et al. 2021). At lower concentrations, the activities of SOD, CAT, and APX have gradually elevated in PM-treated plants and were similar to PTF treated plants and decreased the activity at the higher concentration. From the overall results, the activity of SOD showed maximum fold change of expression among the entire enzymatic antioxidant assayed at 100 ppm PM followed by 150 ppm PTF treated eggplant. A similar result was reported by Mishra et al. (2009) on effect of dimethoate and UV-B on antioxidant response of *Momordica charantia* L. seedling. ROS accumulated considerably in leaves due to high concentration of dimethoate and UV-B. Furthermore, the stresses alone and together also caused the increase activity of SOD, CAT and POD. Thus, high concentration of dimethoate and UV-B accelerated the ROS accumulation particularly H_2O_2 in leaves, causing heavy damage to photosynthetic pigments and growth of bitter gourd seedlings.

Shakir et al. (2018a) also reported that the excessive application of pesticides, such as emamectin benzoate, alpha-cypermethrin, and imidacloprid, can adversely affect the antioxidant activities of SOD, GR, CAT, POD, APX and proline compared with non-treated plants. At higher doses of pesticide application, a significant enhancement in antioxidant levels was found and their results strongly suggested that the above recommended dose of pesticide application can provoke the state of oxidative stress and can damage in non-target host plants. Wang et al. (2014) reported that the various levels of pesticides such as IPP in plant growth and physiological condition of a wheat plant showed a significantly delayed decrease, whereas the antioxidant activity of SOD, CAT, PO, PPO and MDA was increased. Wu et al. (2010) also reported that the effect of fluroxypyr (0–0.8 mg l^{-1}) triggers oxidative damage in *Oryza sativa* plants after 6 days of exposure by producing hydrogen peroxide and superoxide radical. The fluroxypyr-induced oxidative stress activated significant changes in activity of SOD, CAT APX and POD. These antioxidant enzymes activities show a general increase at low fluroxypyr concentrations while a decrease at high fluroxypyr levels (except for POD). Wang et al. (2014) also studied that effect of pesticide 1-[6-chloro-3-methyl-pyridyl-8-nitro-7-methyl-1 2 3 5 6 7-hexahydro imidazo (1,2a)]-pyridine (IPP) to wheat plants. At lower dose rate caused stress effects and modified the activities of SOD, CAT, POD and PPO. Thus, different patterns of biomarker responses were observed by an increase in SOD and MDA and differential effects for antioxidant enzymes with a decrease in CAT, POD and PPO.

Gene expression

At the molecular level, the expression profiling of *FeSOD, CAT* and *APX* genes was specific to each pesticide PM and PTF concentration was found to be correlated with the equivalent enzyme activity. Our results indicated that the initial concentration of pesticide treatment, the SOD activity were increased with the increasing concentration of PM and PTF exposure. In PM-treated plants, the expression of the *FeSOD* gene was highly up-regulated in 100 ppm. With the increasing concentration of PTF stress, the expression of *FeSOD* was observed to be down-regulated at 200 ppm compared to the non-treated plants. The current experiment suggested that the enzyme activity response of eggplant due to PM and PTF treatment could be reflected as a change in gene transcripts. The changes in enzyme activity and *FeSOD* gene transcript in eggplant under stress are shown (Fig. 1b). Similar results were reported by Sharma et al. (2015) that elite rice variety Pusa Basmati-1 was exposed under different salt and pesticides (chlorpyrifos and imidacloprid) stress condition which results in reduction in lipid peroxidation, reduced ROS accumulation and enhanced the enzyme activity as well as expression of antioxidative defense gene. Seedlings treated with IMI stress manifested a pronounced enhancement in the *FeSOD* transcript level in comparison to stress conditions alone. In case of *APX* gene, stress resulted in a selective up-regulation (1.5 fold) under the effect of IMI samples. The expression of *GR* and *CAT* was also enhanced under the effect of IMI stress. Their results suggested that increased activity and expression of chloroplastic genes *Cu/Zn-SOD, APX* and *GR* under various stresses points toward increased ROS production in chloroplasts under these stresses. Aydin et al. (2014) that activity

of SOD in tomato plants could be reflected as alters in gene transcripts, but *SOD* gene expression patterns revealed no positive correlation with antioxidant activity. Consequently, stressful conditions led to treatment in tomato plants and triggered the levels of the *SOD* gene expression. The silencing and over-expression of the *SOD* gene in tomatoes under PEG and NaCl treatment remain to be identified in further studies. The gene expression analysis patterns during PM and PTF treatments illustrated a complex profile. Therefore, the expression level of the *FeSOD* genes in the treated samples was exposed to 100 ppm PM stress showed the maximum among all the pesticide concentrations. These results are also inconsistent with Wu et al. (2017) that the effects of malathion on activity of total SOD and MnSOD their transcriptional levels in *Oxya chinensis* showed the highest at the concentration of 0.8 μg μL^{-1} malathion treatment and elevated significantly about 1.81 and 2.48 fold compared with the control, while CuZnSOD activity has no significant changes after malathion treatments. The increased mRNA expression of *ecCuZnSOD1*, *MnSOD* and *ecCuZnSOD2* were observed after malathion treatment showed the change of *MnSOD* transcript was similar to the profiles of MnSOD activity. The up-regulation expression of *MnSOD* transcript led to the elevate of MnSOD activity to eliminate the excessive ROS caused by malathion. The results suggested that MnSOD exerted a vital role in defense oxidative stress caused by malathion.

During PM treatment, APX activity was elevated gradually at the higher concentration of pesticide treatment, whereas the expression of the *APX* gene was up-regulated gradually in three concentrations (50, 100, 200 ppm) but it was slightly decreased at 150 ppm. Likewise, in PTF treated eggplant, the *APX* gene was observed up-regulated, but highly down-regulated at 200 ppm as compared to the control. The CAT activity also increases with an increased concentration in both pesticide treatments, but gene expression of *CAT* showed gradual up-regulated in all two concentrations (50 and 100 ppm) under PM stress, but it was slightly down-regulated in two concentrations (100 ppm and 200 ppm) of PTF treatment.Harb et al. (2015) also studied the presence of abiotic stress treatment of Rum and Yarmouk genotype, *CAT2*, *SOD*, and *APX* gene was up-regulated after drought stress in Yarmoukk genotype after 2 days. Therefore, under the same drought conditions, each genotype employs various biochemical and molecular responses (Harb et al. 2015). This can be elucidated by Sharma et al. (2013) that the expression of some important antioxidant genes such as *FeSOD*, *Cu/ ZnSOD*, *MnSOD*, *CAT*, *APX* and *GR* were investigated under the various treatments of IMI (imidacloprid) and EBL alone showed an up-regulation in the expression of most of the genes. Though, transcriptional changes for both EBL and IMI treated pusa basmati-1 seedlings indicate their interactions and this could have a potential connection in offering EBL induced IMI stress tolerance in rice.

Conclusions

To improve crop yield under PM and PTF treatments, it is vital to analyze the antioxidant enzyme and gene expression studies to understand the molecular mechanisms of different stress responses in eggplants. Therefore, to reduce the detrimental impacts of pesticide-induced oxidative stress, the results indicated a different antioxidants pattern, where stimulated the activity of SOD, APX and CAT, which was observed to be more efficient in PM than PTF stress. The expression of *FeSOD*, *CAT*, and *APX* genes were observed to have differential expression pattern. The selected antioxidant genes might be induced to stress-resistant capacity and developed several mechanisms to cope with abiotic stress in plant and they contribute in the increase activity of related enzymes activity of eggplant. Therefore, an in-depth mechanistic approach based on molecular breeding and crop improvement of eggplant for tolerance to pesticide stress. The high throughput sequencing method can also be used to expand insight into stress-responsive antioxidant genes.

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Author contributions PBM and PY designed the experiment. PY produced the *Solanum melongena* L.cv. Longai seeds, analyzed the data, and drafted the manuscript. PBM supervised the research study. All authors have read and approved manuscript.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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