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Physiological and metabolome characterization of *Amaranthus hybridus* L. grown under cypermethrin stress: an insight of Jasmonic acid treatment

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Abstract

The indiscriminate use of pesticides compromises physiology and metabolism in crops, posing health risks through residue accumulation in edible tissues. *Amaranthus hybridus* L., a fast growing, nutritionally and medicinally valuable crop was studied here to assess the impact of cypermethrin (CYP) at recommended (R1, 100 ppm) and double dose (R2, 200 ppm) alongside foliar application of jasmonic acid (JA) at 50 µM, 100 µM, and 200 µM concentrations. CYP at R1 dose induced hormesis, while R2 was toxic, elevating the production of ROS molecules (H_2O_2, SOR, MDA). JA application upregulated the antioxidant activity of SOD, POD, APX, GST, DHAR, GSH, and proline to alleviate oxidative stress and improve growth indicators, including shoot length, leaf area, chlorophyll content, Fv/Fm ratio, and biomass. JA at 100 µM yielded the highest increase in biomass, 11.52% and 13.7% for R1 and R2 treated plants, respectively and also led to reduced accumulation of CYP residues. The UHPLC-MS analysis of leaf tissue revealed increase in the contents of carotenoids, flavonoids, phenolics, phenylpropanoids, steroids content in the plant group combinedly treated with JA and CYP compared to those treated with CYP alone, indicating a protective and growth-promoting role of JA under pesticide stress conditions. Overall, 100 µM concentration of JA proved to be effective against the stress induced by the either dose of CYP in the study. These insights could offer strategies to reduce pesticide-induced damage in vegetable crops, advancing sustainable agriculture.

Keywords Jasmonic acid, Insecticide, Crop growth, Secondary metabolite, UHPLC-MS

Introduction

Pest attacks are reported to reduce the productivity of the crops by 20–40% worldwide every year, according to the Food and Agricultural Organization, costing a combined loss of $290 billion globally. The application of insecticide is an important strategy to reduce the damaging effects of pests on the crops. With the changing climate, the attacks from the pests have increased, and so has been the application of insecticides on the crops. The lack of proper knowledge, imprudent use, and greed to get better production among the farmers leads to overapplication of insecticides on the crops [1]. Overexposure to insecticides has been known to impart negative consequences to the plants as well as to its consumers. High doses of insecticides have been reported to negatively affect the physiology and biochemistry of crops leading to oxidative stress, reduced photochemical activity, hormonal imbalance, changes in enzyme activity and metabolite profile, ultimately leading to reduced stature and productivity of the crops [2]. Along with that, the accumulation of

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residues of the insecticides poses a potential threat to the health of its consumers including humans [1].

Phytohormone application to crops, even under stress condition, is capable of uplifting the intricate physiological and metabolic activities [3]. Jasmonic acid (JA), a plant hormone, has also been applied exogenously to increase growth in different crops under different stressful situations [4, 5]. However, studies regarding JA application to plants under insecticide stress is limited. With this context, the approach of exogenous JA in alleviating cypermethrin toxicity in *Amaranthus hybridus* L. has been studied in the present study. Owing to its nutritional richness, *Amaranthus hybridus* L. is one of the commonly grown vegetable crops in several countries. However, the leaves are also prone to attacks from aphids, moths, caterpillars reducing its productivity and economic value. Cypermethrin (CYP), a pyrethroid insecticide, is widely used on several crops in many different countries, including Amaranthus. It is effective against a broad spectrum of insects including aphids, caterpillars, fleas, ticks, mosquitoes, ants, mites, moths. However, The European Food Safety Authority (EFSA) has reported persistence of CYP residues in Amaranthus at concentrations exceeding the maximum residue level, due to overapplication of the insecticide. Accumulation of CYP residues in plant leads to disturbance in its physiological functions. Borowik et al. [6] have found about 20% reduction in the yield of maize plants with CYP application. CYP toxicity has also been observed in onion, maize and grass pea plants and results show decline of root and shoot length, mitotic index as well as chlorophyll pigments [7]. CYP exposure has also been reported to decrease the root diameter, reduce the area of vascular bundles, and cause cellular injury in *Helianthus annuus* and *Brassica juncea* with CYP exposure [8].

Although CYP toxicity in crops has been studied, research on how its toxicity can be mitigated, particularly by applying JA, remains limited. Hence, we conducted the experiment with the following hypothesis "Jasmonic acid mitigates the adverse effects of cypermethrin stress in *Amaranthus hybridus* L., enhancing growth, enzymatic activity, and secondary metabolite profiles through its role in stress signaling and defense mechanisms". The exogenously applied phytohormones may respond differently at different concentrations in crops, and, the optimum concentration of these phytohormones may also vary among crop to crop. Hence, in this study the optimum dose of JA in providing maximum benefit to the crop under cypermethrin stress has also been evaluated. Unlike most other studies, the experiment was conducted in natural weather condition to get a more realistic and reliable response of the experiment. Additionally, the comprehensive metabolomic analysis detailing change in secondary metabolites (e.g., flavonoids, phenolics, carotenoids) under JA and CYP treatments will add new dimensions to our understanding of the biochemical mechanisms through which JA mitigates pesticide stress.

Materials and methods

Experimental setup

The experiment was performed during the month of February–March. The seeds of *Amaranthus hybridus* L. (Kashi Suhaavani) were obtained from Indian Institute of Vegetable Research, Varanasi. The experiment was conducted in pots in the Botanical Garden of the Department of Botany, Banaras Hindu University, Varanasi, Uttar Pradesh. The dimensions of the pot were 27.5 cm X 21.5 cm X 12 cm. The pot was filled with 4 kg of sandy-loam soil together mixed with farm-yard manure. The initial physico-chemical properties of the soil were measured (provided in supplementary table). The pots were arranged in a completely randomized design, with three replicates assigned to each treatment. The seeds were sown in the month of February and harvested in March, 2024. Initially twenty seeds were sown in the pot and later ten healthy seedlings were allowed to grow in each of them. The treatments were conducted in three rounds, with jasmonic acid (JA) applied first and cypermethrin (CYP) applied three days later. Each round was spaced seven days apart. The leaves were sprayed on both the sides. The crop was harvested after forty-five days of sowing for comparing the different physiological, and biochemical parameters among the different treatments.

Jasmonic acid (purity > 99.00%) was purchased from Sigma-Aldrich Chemicals Private Limited, Bangalore. The different concentrations of JA selected for the experiment were: JA1 (50 µM), JA2 (100 µM), and JA3 (200 µM). The selection of jasmonic acid concentrations was based on a literature survey of commonly reported effective concentrations in Amaranthus. The CYP insecticide used in the study was marketed by Syngenta India Ltd. under the commercial name of Cyper-10EC. Each application of JA and CYP was performed by mixing with 0.05% (v/v) Tween® 20 (obtained from Sigma-Aldrich Chemicals Private Limited, Bangalore) for better adsorption on the leaves. CYP was applied to the crop at two different concentrations: R1 (recommended dose, 100 PPM), and R2 (double dose, 200 PPM). A total of nine different treatments were studied. Each treatment had three replicates. The treatments were:

W: Plant group treated with water.
R1: Plant group treated with recommended dose (100 PPM) of CYP.
R2: Plant group treated with double dose (200 PPM) of CYP.

JA1R1: Plant group treated with 50 µM JA and 100 PPM CYP.
JA2R1: Plant group treated with 100 µM JA and 100 PPM CYP.
JA3R1: Plant group treated with 200 µM JA and 100 PPM CYP.
JA1R2: Plant group treated with 50 µM JA and 200 PPM CYP.
JA2R2: Plant group treated with 100 µM JA and 200 PPM CYP.
JA3R2: Plant group treated with 200 µM JA and 200 PPM CYP.

Estimation of oxidative parameters

Superoxide radical (SOR) was measured as per the method described by Elstner and Heupel [9]. Fresh samples of leaf (200 mg) were homogenized in 2 mL phosphate buffer (65 mM, pH 7.8). 1 mL of supernatant was collected after allowing centrifugation for 10 min at 10,000 rpm. A mixture containing 0.9 mL potassium phosphate buffer, and 100 µL hydroxylamine hydrochloride (10 mM) was added to the supernatant prior incubating it in dark for 30 min at 25 °C. Later, 1 mL each of sulfanilamide (50 mM) and N-(1-naphthyl)-ethylenediamine dihydrochloride was added and again incubated at 25 °C for 20 min. The change in colour was recorded on a spectrophotometer at 530 nm on Shimadzu-2600 (Japan). The absorbance was compared with a standard curve to estimate the rate of SOR production.

Hydrogen peroxide (H_2O_2) production was measured according to Sergiev et al. [10]. 40 mg fresh leaf was extracted in 2 mL trichloroacetic acid (TCA, 0.1% w/v). After centrifugation for 10 min at 15000 g, 0.5 mL supernatant was taken and added to 0.5 mL of potassium phosphate buffer (10 mM) and 1 mL of 1 M potassium iodide. The absorbance was recorded at 390 nm.

Lipid peroxidation in the leaf was estimated by measuring the formation of thiobarbituric acid reacting substances (TBARS) as described by Heath and Packer [11]. Fresh leaf tissue, 100 mg, was homogenized in 2.0 mL of 5% TCA prior centrifugation at 10,000 rpm for 15 min. 2.0 mL mixture comprising 20% TCA and 0.5% thiobarbituric acid was added to 0.5 mL of the leaf extract. The mixture was incubated in boiling water bath for 20 min after which it was cooled immediately on an ice-bath. After centrifuging again at 10,000 rpm for 5 min the absorbance was recorded at 532 and 600 nm.

The dye 2',7'-dichlorodihydrofluorescein diacetate (DCFDA) was used to observe the fluorescence image of the damage caused from the oxidative radicals in the leaf. Fresh whole leaf was taken and immediately soaked in 150 µM DCFDA solution for 40 min. The leaf was then rinsed with phosphate buffered-saline and observed under the fluorescence microscope. The images were taken from Nikon eclipse 90i.

Determination of antioxidant activities

The enzymatic antioxidant activity measured includes catalase (CAT), Superoxide dismutase (SOD), peroxidase (POD), Ascorbate peroxidase (APX), glutathione s-transferase (GST), Dehydroascorbate reductase (DHAR). The non-enzymatic antioxidants measured were proline, ascorbate, and reduced glutathione.

SOD activity was measured according to the method described by Giannopolitis and Reis [12] using nitro blue tetrazolium (NBT). The enzyme was extracted from 100 mg fresh leaf in 2 mL potassium phosphate buffer (50 mM, pH 7.8) containing 100 mM EDTA. After centrifugation, 100 µL of the enzyme extract was added to a 2.9 mL reaction mixture prepared containing potassium phosphate buffer (50 mM, pH 7.8), riboflavin (1.3 µM), EDTA (0.1 mM), methionine (13 mM), NBT (63 µM), and sodium carbonate (0.05 M, pH 10.2). The absorbance was recorded at 560 nm.

CAT activity was determined according to Aebi et al. [13]. The enzyme extract was obtained by homogenizing 100 mg fresh leaf sample in 3 mL potassium phosphate buffer (50 mM, pH 7.0) with 1 mM EDTA. For the reaction, 200 µL of the enzyme extract was mixed with 0.5 mL of H_2O_2 (40 mM) and 1.3 mL of potassium phosphate buffer. The absorbance change at 240 nm was recorded over one minute.

POD activity was measured as per Gahagan et al. [14]. The contents of 40 mg fresh leaf were extracted in 3 mL of potassium phosphate buffer (150 mM, pH 6.1). Following centrifugation 200 µL of enzyme extract was added to 1.8 mL of reaction mixture comprising potassium phosphate buffer (50 mM, pH 6.1), guaiacol (3% w/v), and H_2O_2 (1.2% v/v). The increase in absorbance was recorded for 3 min at 470 nm.

APX activity was measured following the method of Nakano and Asada [15]. The contents of fresh leaf tissue weighing 100 mg was extracted in 2.0 mL potassium phosphate buffer having 1 mM EDTA. The supernatant (0.2 mL) obtained from centrifugation was added to a reaction mixture containing the buffer, H_2O_2 (0.4 mM), and ascorbate (2.0 mM). The decrease in absorbance was recorded at 290 nm for a minute.

The GST activity was assessed according to Habig et al. [16]. 100 mg of leaf tissue was extracted on potassium phosphate buffer (100 mM, pH 6.3). After centrifugation 0.2 mL of enzyme extract was combined with 1.8 mL of reaction mixture which included phosphate buffer, 1-Chloro-2,4-dinitrobenzene (3.0 mM),

and reduced glutathione (120 mM). The absorbance was read at 340 nm for five minutes.

The DHAR activity was assayed following the protocol of Dalton et al. [17]. The assay mixture consisted of 90 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA, 2.5 mM GSH, 0.2 mM dehydroascorbate. The enzyme was extracted by grinding 200 mg leaf tissue in 2.0 mL of the potassium phosphate buffer. After adding 100 µL of the enzyme extract to the assay mixture, and the absorbance was recorded at 265 nM for a minute.

Proline was measured as described in Bates et al. [18]. 200 mg leaf contents were extracted in 3% sulphosalicylic acid. After centrifugation 3% glacial acetic acid and acid ninhydrin was added to the supernatant. The contents were heated in a water bath for one hour at 90°C. After cooling the sample in cold water toluene was added and the reddish pink layer was taken out for measuring the absorbance at 520 nm.

Total ascorbate was measured by grinding 200 mg fresh leaf in 5% phosphoric acid. After centrifuging the suspension for 20,000 g for 15 min the supernatant was collected. Next, 200 µL of supernatant was added to potassium phosphate buffer (150 mM, pH 7.4) containing 5 mM EDTA, 10 mM dithiothreitol. After leaving the mixture for 10 min N-ethylmaleimide (0.5%) was added. The mixture was stirred for 5 min after which 10% TCA, 44% phosphoric acid, 4% bipyridyl in 70% ethanol was added. Finally, 3% $FeCl_3$ was added and the reaction mixture was incubated for 1 h at 40 °C. The absorbance was taken at 525 nm.

GSH content was estimated by incorporating some modifications in the protocol of Salbitani et al. [19]. 200 mg leaf was ground in 1.5 mL sulfosalicylic acid containing 1 mM EDTA. The extract was centrifuged for 10 min at 10,000 RPM and the supernatant was collected and kept on ice. The enzyme extract (50 µL) was added to a reaction mixture containing 20 µL of 5 mM DTNB, 100 µL of sodium phosphate buffer (0.1 M, pH 7.0), 20 µL of 0.3 mM NADPH, 10 µL glutathione reductase. The final volume was maintained to 1 mL and read on spectrophotometer at 412 nm for 5 min. The GSH concentration was measured using a standard curve prepared using known concentrations of GSH.

Chlorophyll content and chlorophyll fluorescence

Chlorophyll *a*, and *b* content in the leaf was estimated using 80% acetone according to the method described in Arnon et al. [20]. Chlorophyll fluorescence, in terms of Fv/Fm was recorded using PAM-2500 (Heinz Walz, Germany) in the plants adapted for 30 min in dark.

Plant length, leaf area, and biomass

The plants were uprooted and the roots were cleaned to remove soil attached to it. The fresh weight of each plant was taken on a weighing balance (Sartorious, model no. BSA 224S-CW). The length of the plant was also measured separately for the root and shoot. The leaf area of different treatments was measured using a leaf area meter (Systronics, model no. 211).

HPLC analysis of Cypermethrin

The level of cypermethrin was detected in the leaf samples in a HPLC instrument. The pesticide was extracted following the QuEChERS protocol. The leaf sample was ground in chilled acetonitrile containing 1% acetic acid. The clean-up was performed using primary secondary amine, followed by graphitized carbon black.

Metabolites estimation

Two treatments showing best results in the oxidative parameters and biomass, JA2R1 and JA2R2, were compared with their respective controls i.e., R1 and R2. 300 mg leaf samples were crushed in liquid nitrogen and incubated in chilled methanol for 72 h at 25°C for extraction of secondary metabolites. The mixture was centrifuged and the supernatant was concentrated to 2 mL on a speed vacuum centrifuge. The elute was analysed on UHPLC system (Dionex ultimate 3000 RS series, Thermo-Fischer Scientific). The analysis was performed by a Q-TOF–MS in ESI mode covering both positive and negative ion methods. The TOF–MS data were acquired in the 100–1000 m/z range with a scan time of 0.1 s. All the analyzed compounds were obtained on a compound discoverer software (3.3.2.31) along with online databases (mzcloud, chemspider).

Statistical analysis

The statistical analysis was conducted using data from three replicates. The mean, standard deviation, and ANOVA were calculated using IBM SPSS software (ver. 21.0). Significant differences between groups are indicated by different superscript letters in the graphs and tables, as determined by Tukey's Honest Significant Difference (HSD) test at a significance level of $P \leq 0.05$.

Results

Effect on oxidative stress

The H_2O_2 level in the untreated control plant was 8.2 µM/g fresh weight. The H_2O_2 level got elevated by 35.5% in the R2 plants and 9.6% in the R1 plants, compared to the untreated control. However, JA application minimised the H_2O_2 level in the CYP-treated plants. Specifically, compared to the R2 plants, JA reduced

H_2O_2 levels by 12.36%, 27.73%, and 21.35% at JA1, JA2, and JA3 concentrations, respectively. Similarly, compared to the R1 plants, JA application reduced H_2O_2 levels by 21.32%, 31.70%, and 29.46% at JA1, JA2, and JA3 concentrations, respectively (Fig. 1).

The level of SOR, compared to the control treatment (4.76 nM/g fresh weight), was seen to increase by 33.4%, and 115.5% with application of CYP at recommended and double dose, respectively. Application of JA2 provided maximum reduction in the SOR level, specifically by 15.41% compared to the R1 plants and 20.5% compared to the R2 plants (Fig. 1).

The MDA level showed insignificant difference between the control and the plant group treated with recommended dose of CYP. Moreover, the application of JA did not result in any notable changes in the R1 plant group. The application of CYP at double dose exhibited increase of 62.86% in the MDA formation, compared to the control treatment. Compared to the R2 plants, JA application led to 14.0%, 17.56%, and 11.4% reduction in the level of MDA at JA1, JA2, and JA3 concentrations, respectively (Fig. 1).

Effect on antioxidative enzymes

The SOD activity in the control plant was recorded to be 127.82 (U/g fresh weight). CYP application resulted increase of 45.3%, and 126.8% in the SOD activity in the R1 and R2 plants, respectively. The application of JA further elevated the SOD activity. The most significant increase in SOD activity was recorded to be 287.37 U/g fresh weight in the JA2R1. In the R2 plant group, the SOD activity was recorded to be equally high with application of JA2 and JA3 concentrations (Fig. 2).

DHAR activity was found to be 2.24 (U/g fresh weight) in the untreated control which increased by 22.3% in the R1 plants while reduced to half in the R2 plants. The application of JA to the CYP-treated plants increased the activity of DHAR. Compared to the R1 plants, the DHAR activity recorded an increase of 11.3%, 39.9%, and 27.8% with the application of JA1, JA2, and JA3 concentrations, respectively. Similarly, compared to the R2 plants the DHAR activity increased by 102.1%, 198.9%, 172% at JA1, JA2, and JA3 concentrations (Fig. 2).

The activity of the POD enzyme in the control was 11,581.3 (U/g fresh weight) whereas treatment with CYP exhibited an increase at either of the doses. The R1 dose showed 15.2% increase in the POD activity, while the R2 dose resulted increase of 26.3%, compared to control treatment. The application of JA to the CYP treated plants increased the POD activity. Specifically, compared to the R1 plants the POD activity increased by 11.9%, 31.0%, and 22.5% upon co-treatment with JA1, JA2, and JA3 concentration, respectively. Compared to the R2 plants, the supplementation of JA1 did not lead to any significant difference in the POD whereas JA2 application increased the POD activity by 11.3%. Application of JA3 to the R2 plants reduced the POD activity by 10% (Fig. 2).

The application of CYP at recommended dose led to increase in the CAT activity compared to the untreated group. However, in R2 plants, a significant reduction in the CAT activity was found. The application of JA to the insecticide treated groups increased the activity of the CAT enzyme. All the three concentrations of JA resulted in statistically similar increase (15.7%) in the CAT activity in the R1 plants. In the R2 plants, JA2 bestowed maximum increase (33%) in the CAT activity (Fig. 2).

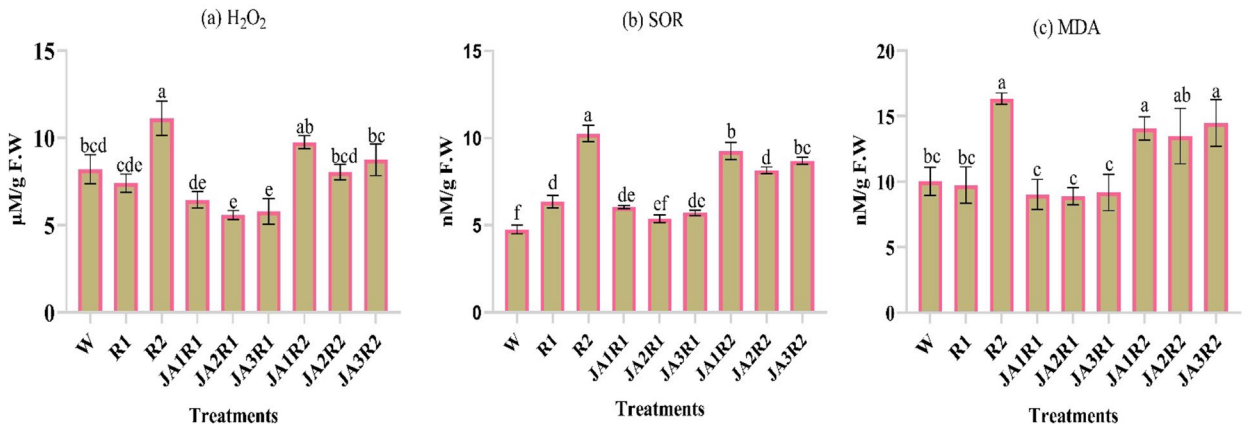


Fig. 1 Effect of different treatments on oxidative parameters (**a**) H2O2 (**b**) SOR (**c**) MDA. The data represent mean of three random samples (*n*=3). The error bars indicate standard deviation. The superscript letters show significant difference according to Tukey's HSD test at a significance level of $P \leq 0.05$

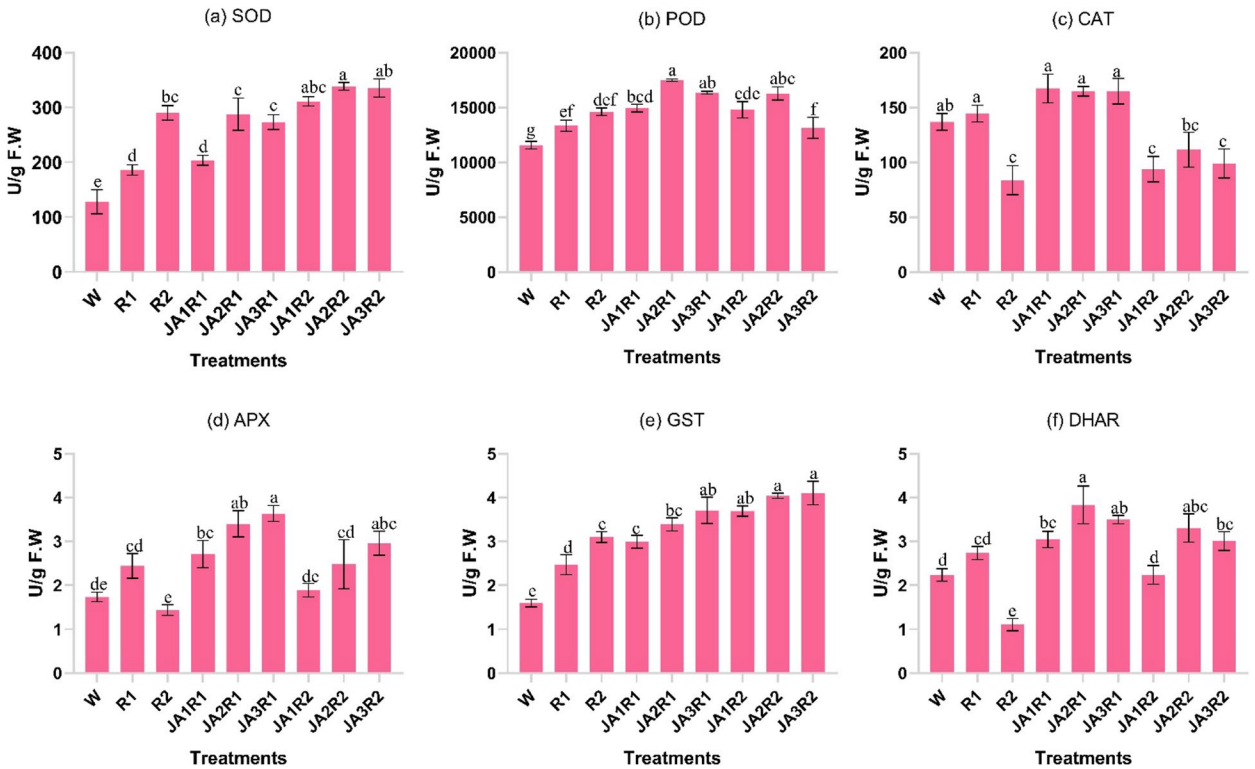


Fig. 2 Response of enzymatic antioxidants (**a**) SOD, (**b**) POD, (**c**) CAT, (**d**) APX, (**e**) GST and (**f**) DHAR to different treatments. The data represent mean of three random samples (n=3). The error bars indicate standard deviation. The superscript letters show significant difference according to Tukey's HSD test at a significance level of $P \leq 0.05$.

The APX activity was seen to increase, by 41.0%, with CYP treatment at recommended dose whereas at higher dose a 17.3% reduction was observed, compared to the control group. The application of JA to the CYP-treated plants increased the APX activity. The APX activity recorded an increase of 11.0%, 39.34%, and 48.77% with the application JA1, JA2, and JA3 to the R1 plants, respectively. In case of R2 plants, JA1, JA2, and JA3 application led to 31.47%, 73.43%, and 107.0% increase in the APX activity, respectively (Fig. 2).

The application of cypermethrin enhanced the GST activity in comparison to the control plants. An increase of 55.3%, and 95% in the GST activity was recorded in the R1 and R2 plants, respectively. Further, the GST activity recorded increase with application of JA to the cypermethrin-treated plants. JA application to R1 plants led to 21.0%, 37.25%, 50.2% increase at JA1, JA2, and JA3 concentrations, respectively, compared to the R1 plants. In the R2 plants, the application of JA1, JA2, and JA3 increased the GST activity by 19.03%, 30.32%, and 32.26%, respectively (Fig. 2).

Effect on non-enzymatic antioxidants

The application of CYP resulted increase in the proline content, more pronounced at the higher dose, compared to the control. Whereas, JA application reduced the level of proline in the crops. The application of JA1, JA2, and JA3 reduced proline levels by 4.4%, 8.05%, and 9.5% in R1 plants, and by 7.14%, 10.5%, and 13.27% in R2 plants, respectively (Fig. 3).

The ascorbate content also showed increase with the application of CYP, compared to control. 100 PPM CYP (R1 dose) application resulted increase in the ascorbate level by 26.1% while the application of 200 PPM CYP (R2 dose) led to 45.6% increase in the level. The ascorbate level in the R1 plants increased progressively at increasing JA concentrations. Specifically, the application of JA1, JA2, and JA3 increased ascorbate levels by 11.2%, 22.3, and 24.0% in the R1 plants, respectively. In the R2 plants, only JA2 resulted in significant increase in ascorbate level (9.9%) (Fig. 3).

Compared to the control group (13.53 U/g fresh weight), the level of reduced glutathione (GSH) increased

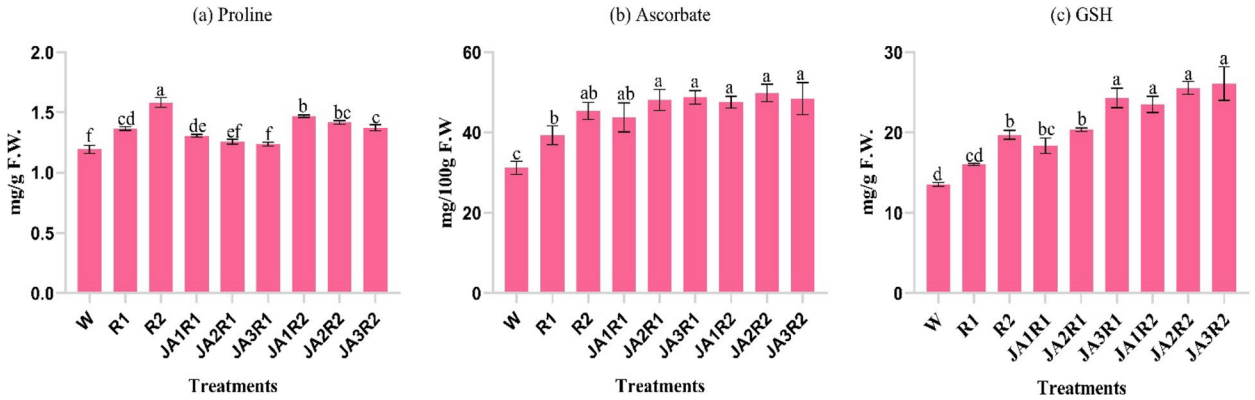


Fig. 3 Response of non-enzymatic antioxidants (**a**) Proline, (**b**) Ascorbate, (**c**) GSH to different treatments. The data represent mean of three random samples (n=3). The error bars indicate standard deviation. The superscript letters show significant difference according to Tukey's HSD test at a significance level of $P \leq 0.05$

progressively with the application of CYP at both the doses. JA application to the CYP-treated plants further increased the level of GSH. The application of JA increased GSH activity by 14.5%, 26.9%, 51.7% in the R1 plants and by 19.1%, 29.6%, 32.3% in the R2 plants at JA1, JA2, and JA3 concentrations, respectively (Fig. 3).

Effect on chlorophyll content and chlorophyll fluorescence

Chlorophyll *a* content showed insignificant change between the control and R1-treated plant group. In the R2 treated plant group the chlorophyll *a* level reduced by 17.7%. However, JA application led to an increase in the chlorophyll a content in the CYP-treated plants. Specifically, chlorophyll *a* increased by 4.63%, 12.74%, and 9.27% in the R1 plants, and by 11.27%, 21.6%, and 21.3% in the R2 plants, with JA1, JA2, and JA3 application, respectively (Table 1).

The chlorophyll *b* content showed insignificant change when the plant was applied with double dose of CYP whereas an increase of 10.2% was recorded when treated with recommended dose of CYP. The application of JA2, and JA3 to R1 plants increased the chlorophyll *b* level by 19.7% whereas JA1 application did not lead to any significant change. In case of R2-treated plants the application of JA showed statistically similar increase of 21% at all the three different concentrations (Table 1).

Chlorophyll fluorescence, Fv/Fm, showed lowest value in the R2-treated plants. R1-treated plants did not show statistical difference for the Fv/Fm value with respect to control-treated plant. The application of JA to the CYP-treated plants showed increase in the Fv/Fm values. The maximum increment in the Fv/Fm value was shared equally between JA2 and JA3 for both the doses of CYP (Table 1).

Effect on plant length, leaf area, and fresh weight

The variations in the growth of the plant in response to different treatments has been shown in Fig. 4. The

Table 1 The effect of different treatments on photosynthetic and growth parameters in Amaranthus crop

Treatment	Chl. a (μM/g F.W.)	Chl. b (μM/g F.W.)	Fv/Fm	Shoot length (cm)	Leaf area (cm^2)	Fresh shoot biomass(g)
W	2.59 ± 0.017^{c}	0.78 ± 0.026^{cd}	0.75 ± 0.004^{bc}	25.2 ± 0.18^{bc}	25.4 ± 1.06^{abc}	8.16 ± 0.09^{def}
R1	2.68 ± 0.028^{bc}	0.86 ± 0.026^{bcd}	0.77 ± 0.005^{bc}	28.8 ± 0.64^{de}	28.5 ± 0.95^{ab}	9.55 ± 0.06^{c}
R2	2.13 ± 0.020^{e}	0.71 ± 0.016^{d}	0.68 ± 0.002^{d}	22.6 ± 0.93^{ab}	18.6 ± 0.49^{c}	7.66 ± 0.06^{f}
JA1R1	2.71 ± 0.017^{abc}	0.88 ± 0.017^{abc}	0.80 ± 0.004^{abc}	30.8 ± 0.71^{ef}	32.5 ± 0.89^{ab}	10.04 ± 0.07^{bc}
JA2R1	2.92 ± 0.026^{a}	1.03 ± 0.038^{a}	0.83 ± 0.004^{a}	34.3 ± 0.36^{gh}	37.2 ± 1.26^{a}	10.65 ± 0.08^{a}
JA3R1	2.83 ± 0.021^{ab}	0.99 ± 0.023^{ab}	0.81 ± 0.004^{ab}	31.0 ± 0.21^{efg}	33.5 ± 0.78^{ab}	9.93 ± 0.05^{ab}
JA1R2	2.37 ± 0.055^{d}	0.84 ± 0.017^{bcd}	0.74 ± 0.011^{c}	24.8 ± 0.68^{abc}	22.3 ± 0.33^{bc}	8.25 ± 0.04^{ef}
JA2R2	2.59 ± 0.063^{c}	0.86 ± 0.025^{bcd}	0.79 ± 0.004^{abc}	26.4 ± 0.47^{cd}	25.1 ± 0.55^{bc}	8.71 ± 0.04^{d}
JA3R2	2.58 ± 0.033^{cd}	0.86 ± 0.028^{bcd}	0.76 ± 0.007^{abc}	25.2 ± 0.31^{bc}	23.4 ± 0.21^{bc}	8.55 ± 0.05^{de}

The data represent mean of three random samples (n = 3) along with the standard deviation. The superscript letters in each column shows significant difference among different treatment according to Tukey's HSD test at a significance level of $P \leq 0.05$

Fig. 4 Growth response of Amaranthus crop to different jasmonic acid treatments (JA1: 50 µM, JA2: 100 µM, JA3: 200 µM) under (**a**) R1 dose (100 ppm) of CYP and (**b**) R2 dose (200 ppm) of CYP

average shoot length of the control treated plant was 25.2 cm which increased to 28.8 cm on R1 application and reduced to 22.6 cm on R2 treatment. JA1, JA2, and JA3 increased average shoot length by 7%, 19%, and 7.6% in R1 plants, and by 9.7%, 16.8%, and 11.5% in R2 plants, respectively (Table 1).

The leaf area increased by 12.2% with the recommended CYP dose but decreased by 26.7% with the higher dose compared to control. The application of JA to the CYP-treated plant led to increase in the leaf area at all the three different concentrations. The maximum increase in leaf area, 30.5%, and 34.9% was recorded with the JA2 application in the R1 and R2 treated plant, respectively (Table 1).

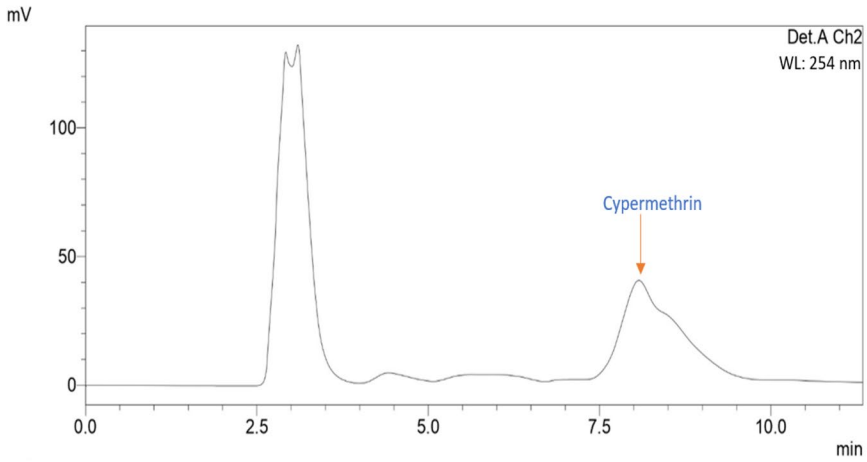
The total fresh weight of the plant showed contrasting results with the different doses of CYP. CYP at recommended concentration increased the fresh weight of the plant by 17.0% while the higher dose resulted in 6.1% decline. The application of JA to the CYP-treated plants resulted increase in the fresh weight. The maximum increase in the fresh weight was observed with the application of JA2 concentration. Compared to its respective control, the maximum increase was found to be 11.5%, and 13.71% in the plants treated with recommended and double dose of CYP, respectively (Table 1).

Effect on CYP residue in leaf

The persistence of CYP in the plant leaves following JA application was measured using HPLC. Plant group treated with CYP alone showed the highest peak area, greater in case of R2 treated plants. The application of JA to the R1 treated plant group reduced the CYP peak area by 0.7%, 20.5%, and 27.7% with the application of JA1, JA2, and JA3, respectively. Similarly, in the R2 treated plants, a reduction of 12%, 13.5%, and 18.6% in the CYP peak area was observed with the application of JA1, JA2, and JA3, respectively (Fig. 5).

Effect on metabolic profile

The CYP-treated plants showing the best response for growth with the application of JA were selected for studying the variations in the metabolic profile. The treatments selected for the analysis were R1, R2, JA2R1, and JA2R2. Compared to the CYP-only treatments (R1 and R2), the combination treatments (JA2R1 and JA2R2) resulted in higher number of total secondary metabolites. R1 plants contained 138 different secondary metabolites which



Treatment	Peak Area
R1	365835
R2	451547
JA1R1	363406
JA2R1	290901
JA3R1	264386
JA1R2	397658
JA2R2	390464
JA3R2	367507

Fig. 5 HPLC chromatogram of cypermethrin along with its peak area under different treatments

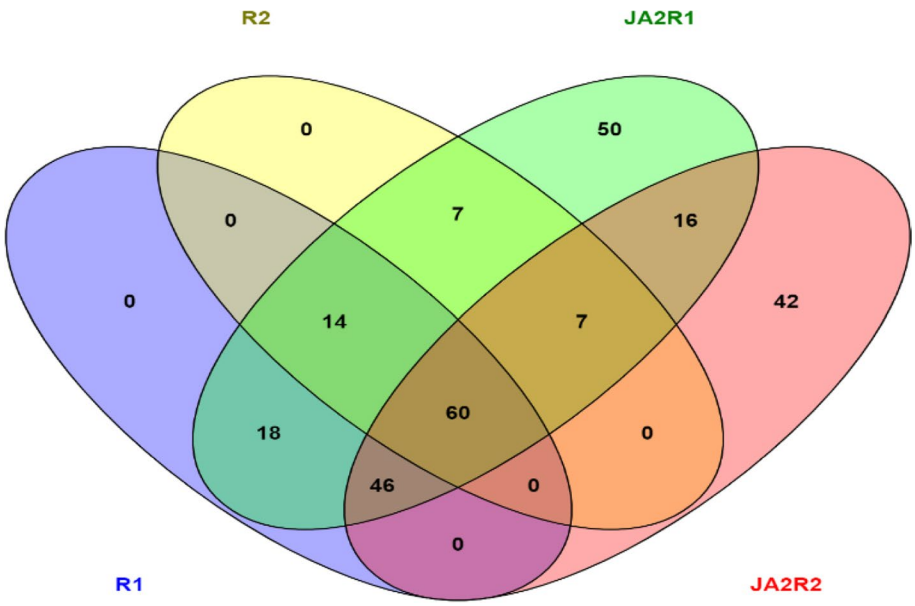


Fig. 6 Venn-diagram depicting the number of secondary metabolites in four different treatments. The figure highlights both common metabolites present across treatments and those that are exclusively found in specific treatments. Data were obtained through UHPLC-MS analysis

increased to 218 with the application of JA. Similarly, in the R2 treated plants 88 different secondary metabolites were found that were increased to 171 with the application of JA (Fig. 6). The UHPLC-MS chromatogram for the treatments is shown in Fig. 7.

In terms of number, a total of 121 metabolites were found to be upregulated, whereas 32 metabolites were down-regulated with the application of 100 µM JA to the R1 treated plant. Sixty-five metabolites did not show significant change (Fig. 8a). The JA2R1 treatment significantly enhanced various metabolite levels compared to the R1-treated group. Carotenoid levels increased by 112%, while flavonoid levels rose by 25%. Indole content showed a remarkable 640% increase. Total phenolic compounds increased by 30%, phenylpropanoids by 80%, and terpenoids by 65%. However, alkaloids showed 12% reduction, coumarins reduced by 4%, quinoline compounds increased by 72% (Fig. 9).

A total of 135 distinct secondary metabolites were upregulated, while 28 secondary metabolites were down-regulated with the application of 100 µM JA to the R2 plants. Twenty-seven secondary metabolites showed

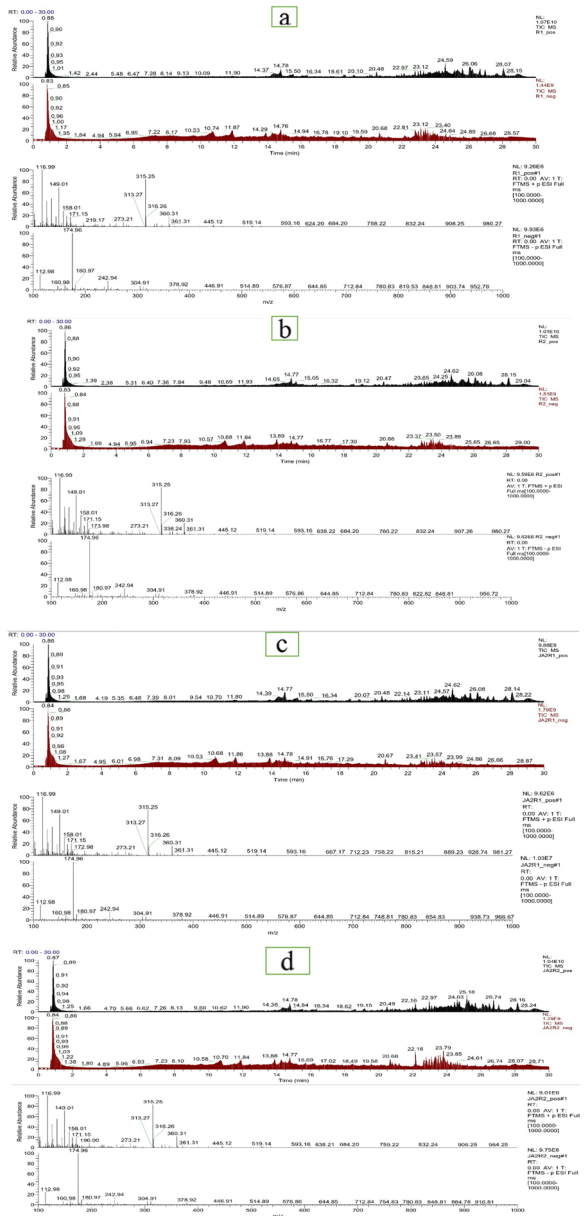


Fig. 7 UHPLC-MS chromatogram of metabolites for (**a**) R1, (**b**) R2, (**c**) JA2R1, (**d**) JA2R2

insignificant difference between the JA2R2 and R2 group (Fig. 8b). In terms of amount, the level of indole compounds reduced by 50%, quinoline compounds by 75.5%, and terpenoids by 1.5%. The other classes of secondary metabolites such as alkaloids, carotenoids, coumarins, fatty acids, phenols, phenylpropanoids, steroids showed 223%, 2105%, 2579%, 223.5% 268%, 300%, 167% increase compared to the R2-treated plants. Flavonoids and sphingolipids were not detected in the R2 plants while they showed-up in the JA2R2-treated plants (Fig. 9).

Discussion

Effect of JA application on stress indices

The results indicate that CYP treatment induces significant oxidative stress in Amaranthus crop, evident from the increase in H_2O_2, and SOR. These ROS cause oxidative damage leading to disruptions in normal cellular functions and membrane leakage, as evident from the increased formation of MDA in the R2 plant group. The R1 plant group, however, showed insignificant increase in MDA level compared to the control plant. This could be reasoned to the threshold level of the ROS in the plant to cause significant lipid peroxidation [21]. The robust antioxidant defense system includes both enzymatic and non-enzymatic components. This system effectively scavenges reactive oxygen species (ROS), preventing them from causing lipid peroxidation. As a result, malondialdehyde (MDA) levels are maintained close to those observed in control plants, as discussed later. Reports from Zhu et al. [22] and Qin et al. [23] have also shown reduction in H_2O_2, SOR, and MDA with the application of JA in plants under stress conditions. This signifies the importance of JA in ameliorating CYP-induced oxidative damage by decreasing ROS accumulation. In our case, the application of 100 µM JA, proved to be more effective in reducing the ROS formation thereby reducing oxidative damage in the CYP-treated plants.

The fluorescence images obtained from DCFDA (2',7'-dichlorofluorescin diacetate) staining revealed ROS levels in Amaranthus plants under various treatments (Fig. 10). Control plants exhibited low fluorescence, indicating minimal ROS production, whereas plants treated with the recommended CYP dose (R1) exhibited high fluorescence intensity indicating elevated levels of reactive oxygen species (ROS). The double-dose group (R2) showed even higher fluorescence. This increase reflects greater oxidative stress induced by the higher CYP dose. The application of JA reduced the ROS levels in CYP-treated plants, with 100 µM concentration being most effective across both the doses of CYP. These images provide visual confirmation of the biochemical findings, highlighting the potential of JA in reducing oxidative stress markers in the plant.

Effect on antioxidant activity

SOD, a key antioxidant enzyme, defends against ROS by converting superoxide radicals into oxygen and hydrogen peroxide. CYP-induced superoxide production led to an increase in SOD activity. This activity was further enhanced by the application of JA. These findings suggest that JA mitigates oxidative stress, likely by upregulating SOD activity. Similar findings were reported by

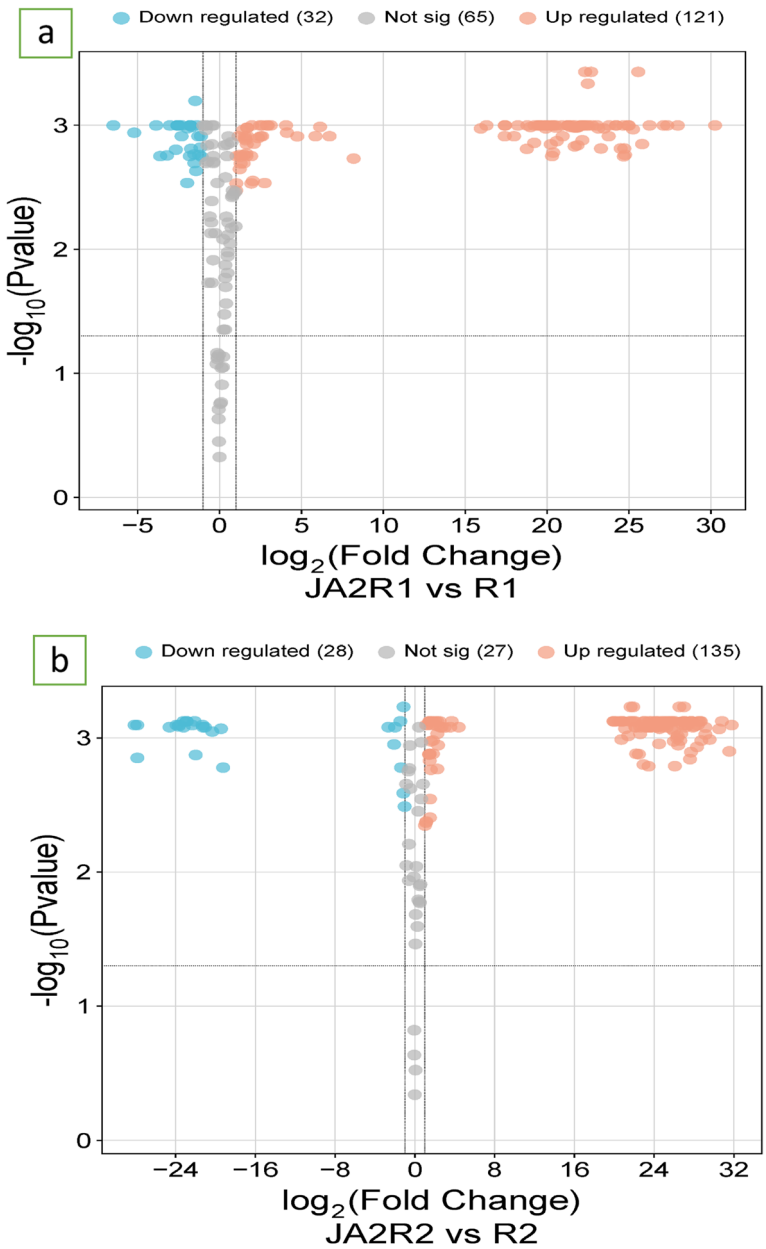


Fig. 8 Volcano plot illustrating the differential abundance of secondary metabolites between (**a**) JA2R1 and R2 (**b**) JA2R2 and R2. The plot represents the $\log_2$ fold change (x-axis) versus the $-\log_{10}$ p-value (y-axis) for each metabolite. Each point corresponds to a specific secondary metabolite, with significantly upregulated metabolites highlighted in red, downregulated metabolites in blue, and non-significant metabolites in gray

Sheteiwy et al. [24] and Farooq et al. [25] in different stress contexts.

DHAR, a key enzyme in the ascorbate–glutathione cycle, regenerates ascorbate and maintains redox balance. Its activity increased by 22.3% with the recommended CYP dose but dropped significantly with the double dose, reflecting stress-level-dependent responses. JA application enhanced DHAR activity in CYP-treated plants, with JA2 showing the most significant effect. Similar increases with JA were reported by Sharma et al. [26] in mustard under imidacloprid stress. Shan et al. [27] linked this increase to nitric oxide synthesis, which phosphorylates MEK1/2 protein kinase, activating DHAR.

The increase in POD activity with CYP application reflects a response to elevated H_2O_2 levels, as POD detoxifies H_2O_2 using electron donors. In R1 plants,

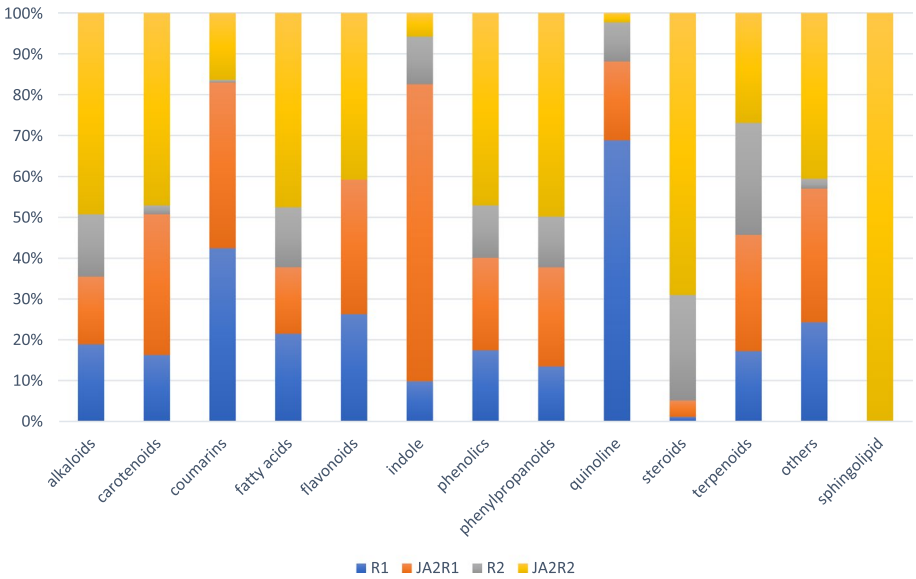


Fig. 9 Proportion of different classes of secondary metabolites under different treatments

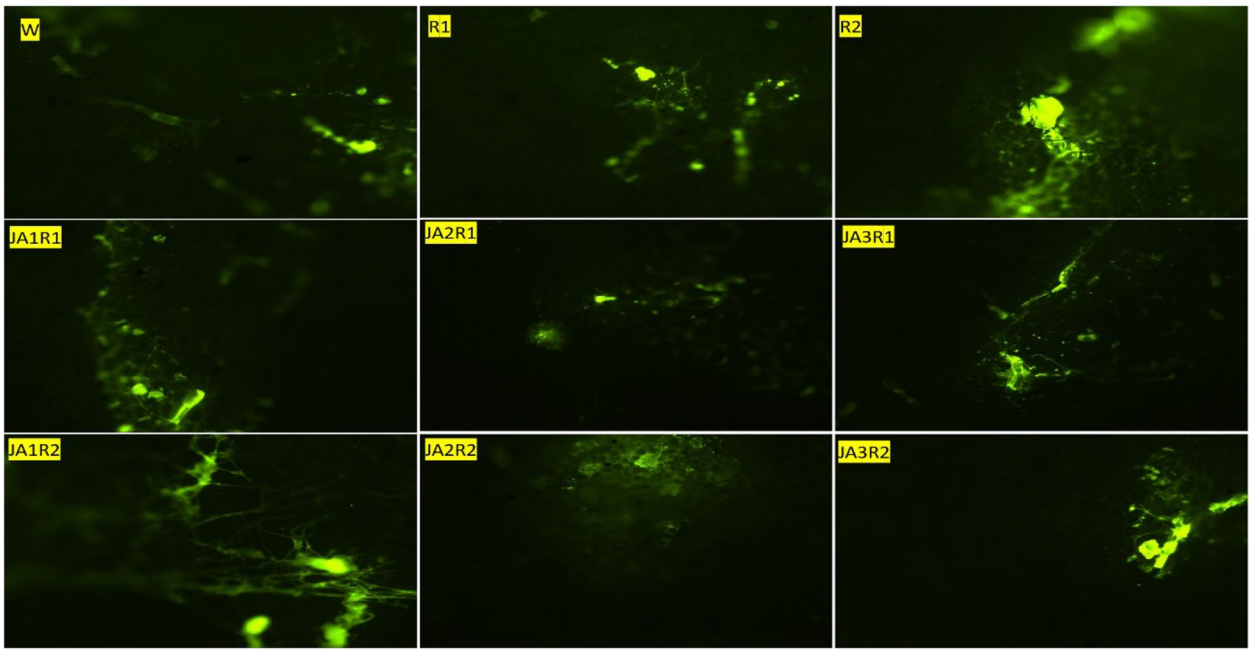


Fig. 10 Fluorescence images of oxidative stress in leaves obtained from DCFDA staining. Higher fluorescence indicates increased ROS production, while lower fluorescence suggests reduced oxidative stress

JA promoted POD activity, with maximum increase at 100 µM. In R2 plants, JA1 had little effect, while JA2 increased POD activity by 11.3%, and JA3 decreased it by 10%. This variability suggests complex interactions between JA and POD, influenced by pesticide and phytohormone concentrations. Similar increases in POD activity with JA were reported by Sheteiwy et al. [24] in soybean and Farooq et al. [25] in mustard.

CAT enzyme breaks down H_2O_2 into water and oxygen, protecting plants from oxidative stress. CYP application at the recommended dose increased CAT activity, indicating enhanced antioxidant defense under

moderate stress. However, at higher CYP doses, CAT activity declined, suggesting that excessive stress overwhelmed the plant's defense system. Similar findings were reported by Zhang et al. [28]. Consistent with other studies [29, 30], JA application boosted CAT activity in CYP-treated plants, likely due to increased CAT gene expression, as observed under various stress conditions [31].

APX, a key enzyme in the ascorbate–glutathione cycle, reduces H_2O_2 to water. The recommended dose of CYP increased APX activity. However, the higher dose showed no significant change in APX activity. This may indicate damage to APX activity under severe stress. JA application enhanced APX activity in CYP-treated plants, mitigating H_2O_2 damage. Similar increases with JA were reported by Sirhindi et al. [31] in soybean under nickel stress. Further, the upregulation of APX gene expression has been observed by Shan et al. [27] and Farooq et al. [25] with the JA application.

Glutathione s-transferase (GST) detoxifies xenobiotics, including insecticides, by conjugating glutathione to foreign molecules, protecting plants from oxidative damage. GST activity increased with CYP application, more prominently at 200 PPM. JA further enhanced GST activity, with higher JA concentrations amplifying the effect, indicating JA's role in boosting detoxification capacity. Ma et al. [32] reported increased GST activity in wheat under isoproturon stress. Similarly, Kaya and Doganlar [33] observed elevated GST activity in tobacco under imazapic stress. This activity was shown to be further enhanced with the application of JA.

Proline acts as an osmoprotectant, maintaining cell turgor and countering osmotic imbalances caused by CYP-induced ROS. In this study, CYP exposure increased proline levels, with higher accumulation at the double dose, consistent with findings in cucumber treated with several pesticides [34]. Proline also supports antioxidant defense by stabilizing enzymes [35]. JA application reduced proline accumulation in CYP-treated plants, likely due to enhanced antioxidant enzyme activity. Duric et al. [36] reported a similar decrease in proline with JA in *Impatiens walleriana* under drought stress.

Ascorbate, a key non-enzymatic antioxidant, scavenges ROS and supports APX in detoxifying H_2O_2. In our study, CYP exposure increased ascorbate levels, highlighting its critical role during stress. Ascorbate also functions as a co-factor in synthesizing hormones, alkaloids, and flavonoids, enhancing the plant's defense against oxidative and chemical stress [37]. JA application further increased ascorbate activity in CYP-treated plants. This aligns with findings by Kamran et al. [3] and Farooq et al. [25]. They reported upregulated ascorbate gene expression and reduced oxidative damage in JA-treated stressed plants.

CYP application led to increased GSH levels in plants, rising significantly at the higher dose. This increase maintains a strong antioxidant defense as GSH scavenges ROS [38, 39] and also regenerates ascorbate within the ascorbate–glutathione cycle defense [40]. JA further elevated the GSH levels, enhancing cellular protection against pesticide stress. GSH also conjugates with pesticide molecules via glutathione s-transferase. This conjugation increases the solubility of the pesticides and facilitates their excretion [41].

Effect on Photosynthetic attributes

Chlorophyll *a* content decreased at the higher CYP dose, while the recommended dose had an insignificant effect. Chlorophyll *b* increased with the recommended dose of CYP but showed no significant change at the double dose. Similar results were reported by Tort and Turkyilmaz [42] with captan fungicide and by Kaur and Kaur [43] in wheat treated with 2,4-D. Chlorophyll *a* is crucial for photosynthesis, capturing light and initiating electron transport, while chlorophyll b broadens light capture [44]. Chlorophyllase, which converts chlorophyll to chlorophyllide [45], may be downregulated by JA, as shown by Sharma et al. [26] in *Brassica juncea* treated with imidacloprid. This suggests that JA application might reduce chlorophyllase activity, helping to restore chlorophyll levels.

The Fv/Fm value represents the maximum quantum efficiency of Photosystem II. A greater reduction was observed in Amaranthus plants treated with the higher CYP dose. This indicates that while the recommended dose was tolerable, the higher dose caused toxicity, damaging Photosystem II and lowering photosynthetic efficiency [46]. JA application alleviated the reduction in chlorophyll fluorescence. Our findings align with previous studies showing that JA improves Fv/Fm under stress [47]. This improvement is likely due to increased secondary metabolites that protect photosynthetic pigments, particularly carotenoids. These metabolites enhance photosynthetic performance and biomass production, as seen in JA2R1 and JA2R2 plants [48].

Plants treated with the recommended dose of CYP showed increased leaf area, likely due to reduced insect damage. This could also be attributed to the phenomenon of 'hormesis,' where low doses benefit plants, while high doses cause negative, irreversible effects [49]. Hormesis has been observed with glyphosate at low doses in various crops, stimulating CO_2 assimilation, transpiration, stomatal conductance, and electron transport in photosystem [50–52].

The higher dose of CYP reduced leaf area, indicating toxicity to the plant, a finding consistent with Huang et al. [53]. JA application alleviated this stress, with 100 µM JA

enhancing leaf area in both CYP treatments. Similarly, JA supplementation has been shown to increase leaf area in sorghum grown under saline soil [54].

Increased leaf area enhances the plant's ability to capture sunlight for photosynthesis, leading to higher growth and biomass. In this study, the recommended dose of CYP increased leaf area and chlorophyll content, boosting photosynthesis and resulting in a 12.5% increase in biomass. However, the higher CYP dose reduced biomass by 5%, likely due to decreased photosynthetic pigments and damage to the photosynthetic apparatus. JA application to CYP-treated plants improved biomass, consistent with findings by Sirhindi et al. [55] and Zhao et al. [56] in *Brassica oleracea* and *Puccinellia tenuiflora*, respectively.

Effect on CYP accumulation

Higher CYP doses led to increased persistence of CYP residues in plant leaves, as detected by HPLC analysis. JA application reduced these residues, with the peak area of CYP decreasing as JA doses increased in both R1 and R2 plant groups. This suggests that JA facilitates CYP detoxification in the plant. The reduction in residues could be attributed to the increased GSH and GST activity with JA supplementation, both key players in detoxification. Similar findings were reported by Ma et al. [32], highlighting the role of GSH and GST in the degradation of isoproturon residues in wheat.

Effect on metabolite profile

The application of CYP resulted in significant metabolic differences. CYP-treated plants showed reduced levels of secondary metabolites. In contrast, plants co-treated with JA and CYP maintained higher levels of these metabolites. In the R1 plants, 138 secondary metabolites were identified, which decreased to 88 in the R2 plant group. However, JA application increased both the count and levels of secondary metabolites. The JA2R1 plants showed a significant increase, with 218 metabolites, while the JA2R2 plants had 171 metabolites. Application of JA to plants has been reported to increase the levels of secondary metabolites [57].

In our case, JA2 application in the R1 group doubled the level of carotenoids, while flavonoids, phenolics, phenylpropanoids, terpenoids, and steroids also increased. However, alkaloids, coumarins, quinoline, and fatty acids showed lower accumulation. In contrast, JA2 application in the R2 plants increased the levels of alkaloids, carotenoids, flavonoids, coumarins, fatty acids, phenols, phenylpropanoids, steroids, and other metabolites. However, compounds from indole, quinoline, and terpenoids were reduced.

Carotenoids like neoxanthin, astaxanthin, antheraxanthin and their derivatives including beta-carotenal, 4-ketozeaxanthin, echinenone were detected in the plant system. The carotenoids are involved in light harvestation and protecting the photosynthetic system from excess light and heat. They are also known to pose antioxidant property protecting cells from damage due to oxidative stress [58]. Hence, the increase in carotenoid level with JA application would certainly improve the photosynthetic efficiency of our plant system under CYP influence.

Flavonoids like chalcone, rutin, quercitol, and maritimetin were identified in the plants. The increase in flavonoid content with JA strengthened the plant tissues from UV-induced damage by absorbing the UV-light [59]. They also have antioxidant property [60]. Chalcone serves as precursor to more complex flavonoids and isoflavonoids, having antimicrobial properties [61]. Rutin also contributes in plant defense by strengthening the cell wall, acting as an antioxidant compound, and UV protectant [62].

Phenolic compounds contribute to the antioxidant and anti-inflammatory properties in plants. Specifically, compounds like gingerol, hydroxyacetophenone, benzoylphenol, hydroxybenzaldehyde, phloroglucinol, vanillin, catechol, hippuric acid, and methoxythiophenol play a key role in antimicrobial activities. These compounds help protect the plant against bacterial and fungal pathogens [63]. Additionally, phenols like 3'-hydroxyacetophenone, isohomovanillic acid, pyrogallol, hydroxybenzaldehyde, and vanillin provide antioxidant defense. They protect the plant from oxidative stress by scavenging free radicals [64].

Phenylpropanoids play a key role in providing structural support to the plant system. They are precursors to lignin, which provides rigidity and structural support to the plant cell wall. This, in turn, aids in water transport and allows plants to grow and withstand stress [65]. Many compounds belonging to this class have been identified in the study. Some of them like hydroxymandelonitrile, cinnamaldehyde, hydroxycinnamoylmethane, phenyl-3-buten-2-one, amylcinnamaldehyde, chavicol, cuminaldehyde, gallicynoic acid, stilbene, eugenol protect the plant from the attacks of microbes and herbivores. Hydroxycinnamoylmethane, coumaryl alcohol functions in the lignin biosynthesis, contributing to the structural integrity of the plant. Eugenol, gallicynoic acid, feruloylglycine also has antioxidant properties [66]. The increase in these metabolites strengthened the plant system internally.

Sterols detected in the study maintain plant cell membrane integrity and fluidity, with compounds like ergosterol peroxide acting as antifungal agents. Specifically, terresterol, certonardosterol play role in maintaining membrane integrity and fluidity in plant cells [67]. Ergosta-5,7,22,24(28)-tetraen-3beta-ol is part of the ergosterol pathway, contributing to membrane structure

[68]. Sterols also play a role in signaling pathways that regulate plant growth and stress responses. 3-dehydro-6-deoxoteasterone, a precursor to brassinosteroids, helps regulate plant development and stress adaptation [69].

The increase in secondary metabolites with JA supplementation could be because of increase in the activity of enzymes such as beta-amyrin synthase, 3-hydroxy-3-methylglutaryl-coenzyme A reductase, 1-deoxy-D-xylulose-5-phosphate reductoisomerase, and hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate reductase. Suzuki et al. [70] has previously reported increase in the activity of these enzymes with JA supplementation in *Medicago tuncatula*. The involvement of these enzymes in the production of secondary metabolites has been reported previously [71].

Statistical inference

The various biochemical and physiological parameters were compared for studying their nature of correlation. High positive correlation was found between H2O2 and MDA ($r=0.91$), SOR and MDA ($r=0.95$) indicating that increase in MDA is strongly associated with increase in H2O2, and SOR. Proline showed strong positive correlation with H_2O_2, SOR, and MDA indicating the protective role of proline against the oxidative agents. Similarly, GSH and GST showed high correlation ($r=0.98$), indicating that both are part of the same antioxidant defense pathway, similar to DHAR and APX. The Chl *a* and Chl *b* pigments showed high correlation, which is expected since both chlorophylls are crucial components of the photosynthetic apparatus. A strong positive interaction between leaf area and fresh weight of the plant was seen ($r=0.97$) indicating increased photosynthesis with increase in the leaf area leading to increased biomass of the plant. A moderate level of correlation was observed between SOD and POD ($r=0.67$), CAT and APX ($r=0.66$) suggesting that these antioxidants may work together in scavenging ROS compounds. A negative

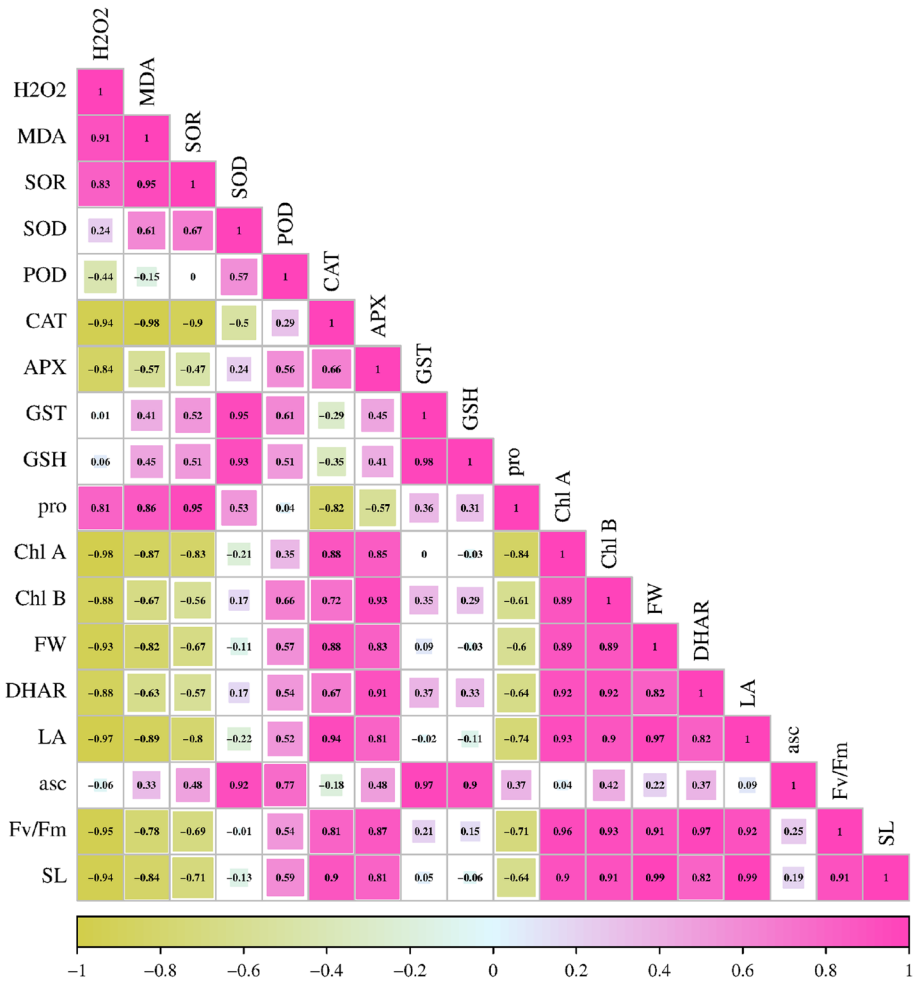


Fig. 11 Correlation matrix highlighting the interdependence between oxidative stress, enzymatic activities, and growth parameters in Amaranthus crop

correlation ($r = -0.57$) between proline and APX was obtained indicating that an increase in APX activity leads to decline in proline level. CAT showed strong negative correlation with H_2O_2, SOR, and MDA. MDA and Chl *a* level also showed negative correlation ($r = -0.87$) indicating damage to the chlorophyll pigment with increase in lipid peroxidation (Fig. 11).

The PCA biplot analysis shows the separation of different treatment groups based on two principal components (PC1 and PC2) and the influence of various physiological and biochemical parameters. For the R1-treated plant group (Fig. 12a), the PC1 axis accounts for the majority of the variance (67.9%) in the data and the PC2 axis (11.7%) explains the secondary variations in the data. The plants treated with water (W) form a separate cluster from the treated plants. This indicates significant differences regarding the various parameters between untreated plants and those exposed to CYP or combinations with JA. The R1 treatment, i.e., the recommended dose of CYP lies to the left of the quadrant indicating it shares some similarities with the control. In contrast, the combined JA and R1 treatments are spread mostly towards the right

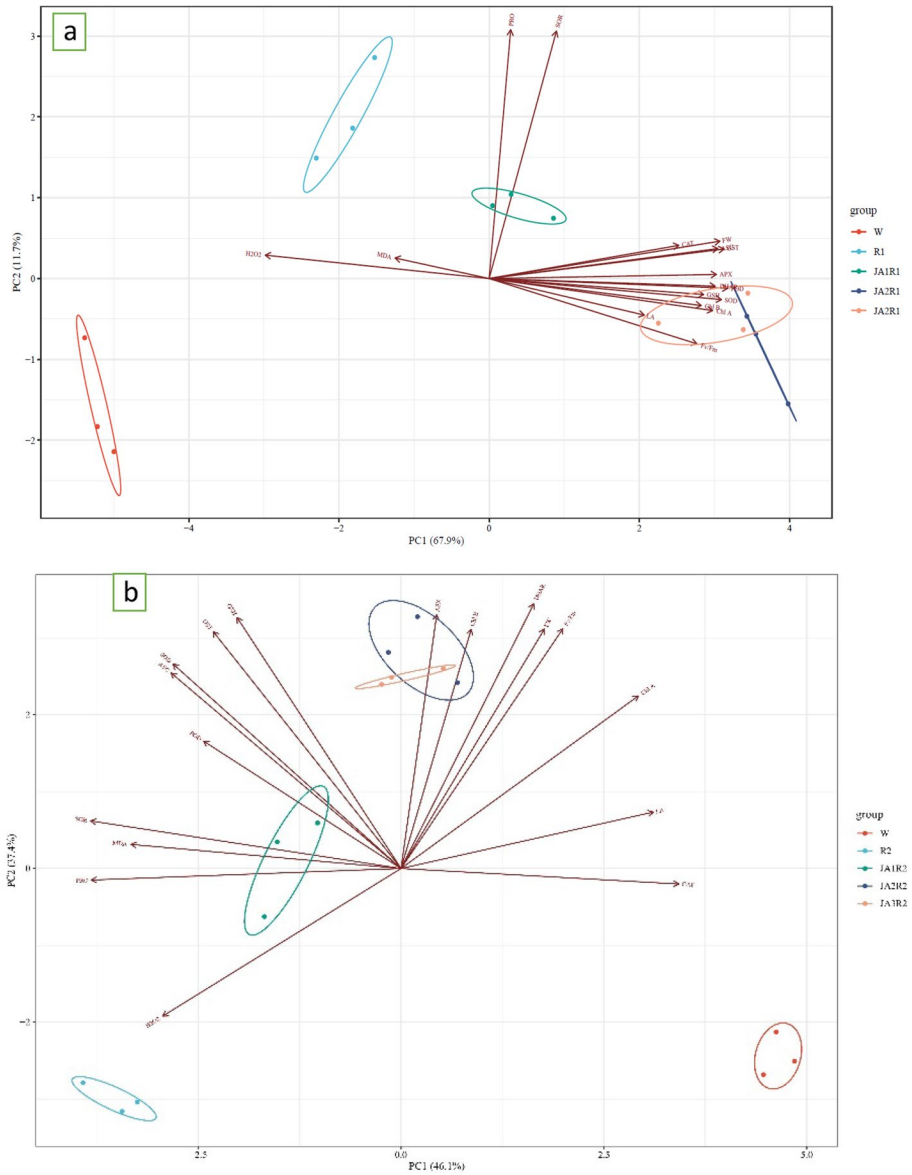


Fig. 12 Principal Component Analysis (PCA) biplot illustrating the distribution of treatments based on metabolic, oxidative, enzymatic, and growth parameters in Amaranthus crop for (**a**) R1 plant group, and (**b**) R2 plant group. The Arrows indicate the direction and strength of correlation with the respective parameters, providing a visual representation of how treatments differ in their effects on plant responses

side of the plot. This suggests distinct physiological and biochemical responses when JA is combined with CYP. The JA2R1 treatment is positioned further to the right, suggesting more pronounced changes. These changes are likely related to higher activities of antioxidant enzymes and growth parameters. The vectors for antioxidant enzymes (CAT, DHAR, APX, etc.) are directed towards the right, indicating that JA application to the R1 plants is associated with increased activities of these enzymes.

The R2 plant group, treated with 200 ppm CYP, the PC1 axis explains 46.1% variance, while the PC2 axis explains 37.4% variation in the dataset (Fig. 12b). The untreated and R2-treated group are positioned at opposite extremes of the plot. This indicates their contrasting influence on the different physiological, and biochemical parameters. The combined JA and CYP treatments (JA1R2, JA2R2, and JA3R2) are dispersed across the top and right sides of the plot, towards the same direction in which the studied parameters point. This alignment suggests that JA application, particularly at JA2 concentration, significantly influences the level of the different physiological, and biochemical parameters.

Conclusion

The nutritive richness of Amaranthus crop makes it worth protection from insect damage. The application of insecticides, however, has increased beyond the recommended level due to imprudent knowledge of the farmers. This excessive dose creates a stress condition in the plant, affecting both growth and productivity. It also leads to accumulation in the plant system. This accumulation becomes a concern for the health of consumers. In this study, the problem has been addressed using foliar application of jasmonic acid. It has been revealed that jasmonic acid at a 100 µM concentration effectively alleviates stress in plants treated with cypermethrin at both recommended and double doses. Additionally, JA promotes plant growth and reduces cypermethrin accumulation in the plant system. Hence, this study deciphers the optimal dose of exogenous jasmonic acid that provides maximum benefit to the plant under cypermethrin stress.

These results pave the way for the development of sustainable agricultural practices. Phytohormones like JA can be integrated into pest management strategies to reduce dependency on chemical pesticides and minimize their environmental impact. Future research, focusing on gene expression, can investigate the molecular mechanisms underlying the synergistic or antagonistic interactions between JA and CYP. This could reveal JA's role in enhancing overall crop productivity and quality. Additionally, exploring JA's broader applications in other crops under cypermethrin or other pesticide stress could contribute to eco-friendly and resilient farming systems.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06131-7.

Supplementary Material 1.

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Authors' contributions
Author AK conducted the experiment, analyzed the data, and wrote the first draft of the manuscript. Author PKY interpreted the data. Author AS designed the experiment, interpreted the results, and revised the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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