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Original Research Article

Jasmonic acid impact on botanical and morpho-physiological characteristics of wheat (*Triticum aestivum* L.) infected by Aphid

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Abstract

Wheat (*Triticum aestivum* L.) is the most essential cereal crop among all other crops of Pakistan and belongs to the family poaceae. The experiment was performed to evaluate the response of exogenously applied jasmonic acid on wheat (*Triticum aestivum* L.) growth parameter against aphid attack. Two wheat varieties i.e., Fareed-2006 and Paktoon-2016 are cultivated in pots and different concentrations of Jasmonic acid (100 µM and 1 mM) were applied. Concluded results showed that aphid infested plants decreased root and shoot length, plant height, total soluble protein and chlorophyll contents but increased the POD and SOD contents, MDA contents, catalase, H_2O_2 concentrations and phenolics of wheat (T*riticum aestivum*) which was further improved through jasmonic acid application. Jasmonic acid treated plants showed better results than control and aphid infested plants. Jasmonic acid spray helped plants to recovery from aphid stress by increasing antioxidant enzyme synthesis, POD (Peroxidase), SOD (Superoxide dismutase) and CAT (Catalase). The results showed that JA sprayed plants have a reduced aphid population than controls plants. When compared to jasmonic acid (100 µM), the greater concentration of jasmonic acid (1mM) showed more effective. Use of jasmonic acid against devastating diseases seems to hold a more promising future in the pest management and agriculture sector.

Keywords: Jasmonic Acid, Physiological, Biochemical Characteristics, Biotic Stress, Devastating.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most essential cereal crop among all other crops of Pakistan as well as an important staple food cereal globally. It contains nearly 12,000 species and almost 668 genera (Christenhusz and Byng, 2016). Wheat is major source of zinc for the population that live in a developing country and contributes 70% calorie for the population that live in rural regions (Cakmak, 2008). However, Pakistan's production is lower than China, India and USA etc (Arain *et al*., 2005). It is a crucial staple crop since it meets 35% of humankind's dietary needs (Miedaner *et al*., 2020). Additionally, JA has been discovered to operate as a stress hormone that regulates how plants respond to environmental stresses such salt, drought, temperature changes and the toxicity of heavy metals (Raza *et al*., 2022).

In response to stressful situations and plant growth, jasmonic acid (JA) and amino acid conjugates (e.g., JA-Ile) have become recognized as crucial phytohormones in recent decades (Browse, 2009; Ghorbel *et al*., 2021; Wasternack, 2007). Jasmonates are lipid-derived signalling molecules that are involved in modifying plant responses to a variety of environmental stressors, including insect pest assault (Ghorbel *et al*., 2021). It's interesting to note that JA participates in a variety of cellular and biological processes (such as molecular, biochemical, and physiological pathways), having a big influence on plant development stages, insect outbreaks, and defence mechanisms against phytopathogenic bacteria (Sun *et al*., 2022). Plant defence responses to diverse biotic stresses are fine-tuned through antagonistic interactions between SA and JA (Gimenez-Ibanez and Solano, 2013).

Plant growth regulators play a critical role in controlling how plants respond to environmental stresses (Upreti and Sharma, 2016). As one of the most significant phytohormones, jasmonic acid plays an important role in plant growth and development as well as the response to biotic and abiotic stresses (Wasternack and Hause, 2013). Endogenous jasmonic acid levels are known to increase in plants subjected to a variety of stress situations (Bankaii *et al*., 2014). Plants stress resistance is increased by exogenous jasmonic acid treatment, which boots antioxidant activity (Qiu *et al*., 2014).

JA is a crucial cellular regulator that actively participates in the maintenance of developmental processes like as germination, root growth, leaf movement, embryo development, sex determination, fruit ripening, senescence, gene expression and regulating stress responses and is also considered as an endogenous growth regulator (Dar *et al*., 2015). Jasmonic acid is regarded as a plant growth regulator and regulating the plant's morphological, physiological and biochemical processes (Mahmood *et al*., 2012). In the process of resisting environmental stress, jasmonic acid has both synergistic and antagonistic actions with ethylene, abscisic acid, salicylic acid and other plant hormones (Wang *et al*., 2020). Auxins, gibberellic acid, and salicylic acid (SA), among other significant phytohormones, have crosstalk networks with JA, which has an impact on a number of signalling processes that control plant development (Wasternack, 2014). Typical physiological responses are activation of the antioxidant (peroxidase, superoxide anion radical, NADPH-oxidase) (Karpets *et al*., 2014), accumulation of soluble sugars and amino acids (Wasternack, 2014), and regulation of stomatal control. Several crops, including tomato (*Solanum lycopersicum*), maize (*Zea mays*), cotton (*Gossypium hirsutum*) and Arabidopsis (*Arabidopsis thaliana*) have been shown to display the defects in floral structure and sterility observed in JA biosynthetic mutant (Li *et al*., 2004; Huang *et al*., 2017; Schubert *et al*., 2019).

MATERIALS AND METHODS

Experimental Treatment:

To avoid from aphids infestation, the control plants were enclosed in a net. Two concentrations of jasmonic acid (100 µM and 1mM) were used, each with three replications. There were five different treatments: control (no aphids, no JA spray) T0, aphid (no JA spray) T1, no aphid plus JA spray (1 mM) T2, aphids plus JA spray (1 mM) T3, no aphid plus JA spray (100 µM) T4 and aphid plus JA spray (100 µM) T5. T2, T3, T4, and T5 were sprayed with their corresponding concentrations of jasmonic acid following a natural aphid infestation. Then studied the physiological changes induced by biotic stress. A count of aphids was made before and after spraying for 1, 3, and 7 days. After one week of the spray, the harvest was taken.

Measurement of Plants Attributes:

All plants were washed with distilled water after harvesting. At the experimental site, fresh weights were measured immediately and dry weights were measured after the plants had dried fully by using digital weighing balance.

Determination of Photosynthetic Pigments:

The concentrations of Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid were calculated using the Arnon protocol (Arnon, 1949). Fresh leaf samples weighing 0.1 g were taken after harvesting. These samples were dipped in 95% acetone (8 ml) for 24 hours in the dark at 4 °C. Using a spectrophotometer, absorbance measurements at 646 nm, 663 nm, and 450 nm were used to determine the amounts of each kind of chlorophyll and their corresponding amounts of carotenoid and total chlorophyll.

Antioxidant Enzymes:

0.5 g samples of the fresh plant were ground and homogenized in 3 mL of phosphate buffer (50 mM) on ice for enzyme extraction. The volume was then increased to 5 mL. Then the samples were centrifuged for 15 minutes at 4 °C at the speed of 15,000 rpm. The supernatant was separated, covered with aluminum foil, and kept at 4 °C and used for the determination of the antioxidants.

Peroxidase (POD):

The activity of POD was evaluated using the Gorin and Heidema (1976) method. The substrate in this procedure was 4-methyl catechol. It combined with H_2O_2 to induced oxidation. The reaction mixture for POD measurement consists of 5 mM 4-methyl catechol, 100 mM buffer of sodium phosphate (Na_3PO_4), 5 mM H_2O_2, and 500 µL of an enzyme extract in a total volume of 3 mL. The absorbance at 420 nm was measured using a spectrophotometer.

Superoxide dismutase (SOD):

Beyer and Fridovich (1987) given a method for the determination of the activity of the superoxide dismutase. Took 2ml plastic cuvette first and the added following solution into it. First added the 400 µl H_2O + 250 µl potassium phosphate buffer (pH 7.8), 100 µl. methionine, 100 µl triton, 50 µl nitrobluetetrazolium (NBT), 50 µl enzyme extraction and then added 50 µl riboflavin. All the solution poured into cuvette then place the plastic cuvette under the lamp light for 15 minutes to proceed the superoxide dismutase reaction. When the time period is completed, measured the absorbance at 560 nm by using spectrophotometer.

Catalase (CAT):

The Kumar method was used to assess the activity of CAT (Kumar *et al*., 2010). Utilizing 3 ml of a combination made up of 2.8 ml of cooled potassium

phosphate buffer (50 mM), pH 7.8, 100 µl of enzyme extraction, and 5.9 mM H_2O_2, and the CAT activity was determined. To begin the procedure, 0.1 ml of enzyme extraction was added to the mixture. After a 20-second period, the absorbance at 240 nm was noticed to be decreasing. The absorbance changed at a rate of 0.01 units per minute.

Phenolic Content:

The Julkkunen-Tiitto (1985) technique was used to calculate the phenolic content. Took 0.1 g of fresh plant material. Grind this substance in 1 ml of 80% acetone. Centrifuge the ground material for 15 minutes at 12000 rpm. Took a supernatant for further process. Added 1ml of distilled water to a 100 µl aliquot in each test tube. After adding the following solutions, thoroughly shake the test tubes with 0.5ml of folin-ciocalteu's Phenol Reagent.

Malondialdehyde (MDA) Content for Lipid Peroxidation Estimation:

Heath and Packer (1968) with negligible modification of Hasanuzzaman *et al*., (2012) was developed a method for the determination of MDA concentration in a tissue leaf. For this process took one gram of fresh leave material were added into 3ml of 0.1 % trichloricacetic acid (TCA) at 4°C. The mixture was centrifuge at 2000 x g. 0.5 % (v/v) thiobarbatric acid (TBA) was added in 20 % prepared solution of TCA.

H_2O_2 content

Velikova, Yordanov, and Edreva (2000) method was used for the determination of the hydrogen peroxidase. Took 0.5 g of fresh plant material put into a chilled pestle and mortar for the grinding by adding 5 ml of 0.1 % (w/v) trichloroacetic acid (TCA). At 12000 rpm for 20 minutes, the homogenate was centrifuge.

Total Soluble Protein (TSP)

To identify the TSP, the Lowry *et al*., (1951) methodology was applied. Frozen plant leaves were crushed in phosphate buffer with a pH of 7.0 and mixed on ice to determine the TSP. After centrifuging the extract, the supernatant was separated. By increasing the volume to 1 ml, the supernatant, which amounted to roughly 0.1 ml, was taken and diluted. The same amount of alkaline copper sulphate was then applied to these samples. For 10 minutes, the samples were vortexed. Folin's reagent was then included into it. The samples were then incubated for a further 30 minutes at 28 ± 2 °C. The absorbance at 650 nm was determined using a spectrophotometer.

Direct Count of Aphids:

When the plant grown to leaf stage, five wheat plants from each pot were selected in a random manner for population counts of wheat aphids. A count of aphids was made prior to spraying as well as on the first, third, and seventh days following spraying.

Statistical Analysis:

The experiment was laid out in a completely randomized design (CDR) with three replicates (Steel and Torrie, 1980). Data was recorded and analyzed statistically by using Statistics 8.1 software. Analysis of variance of all parameters was compiled and CDR was applied to compare the results.

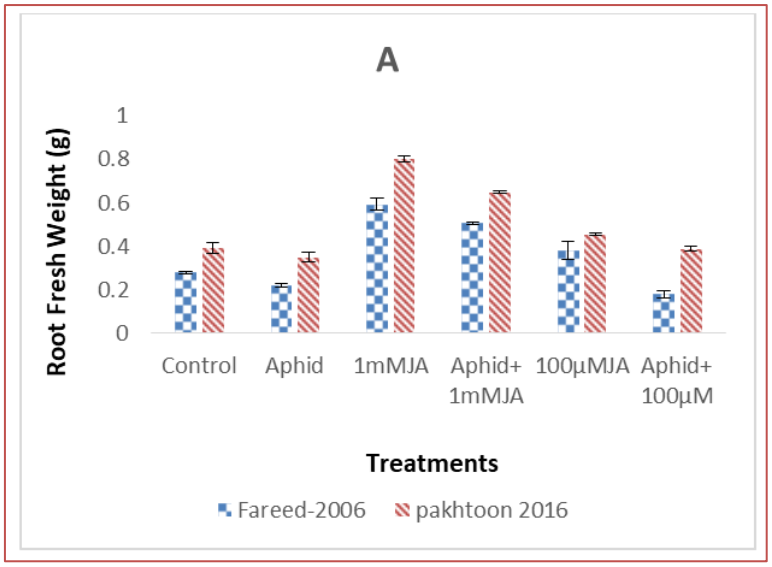
RESULTS

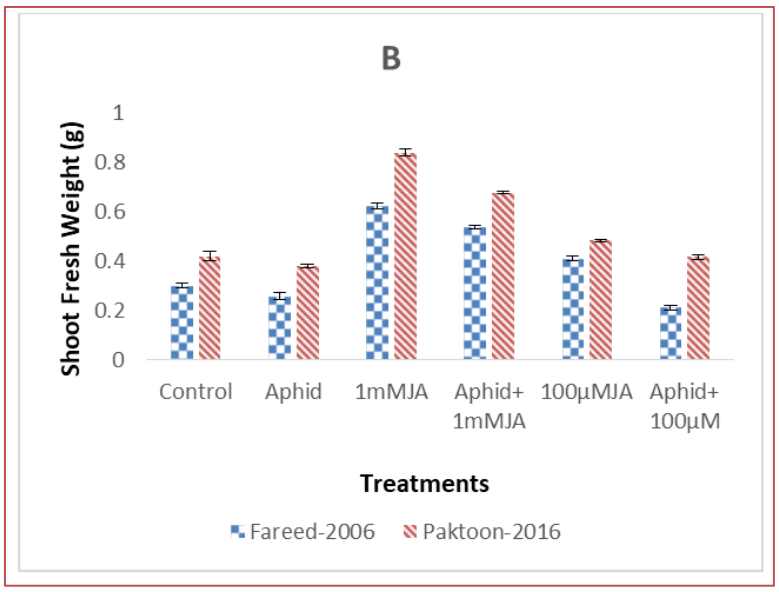
Plant Attributes:

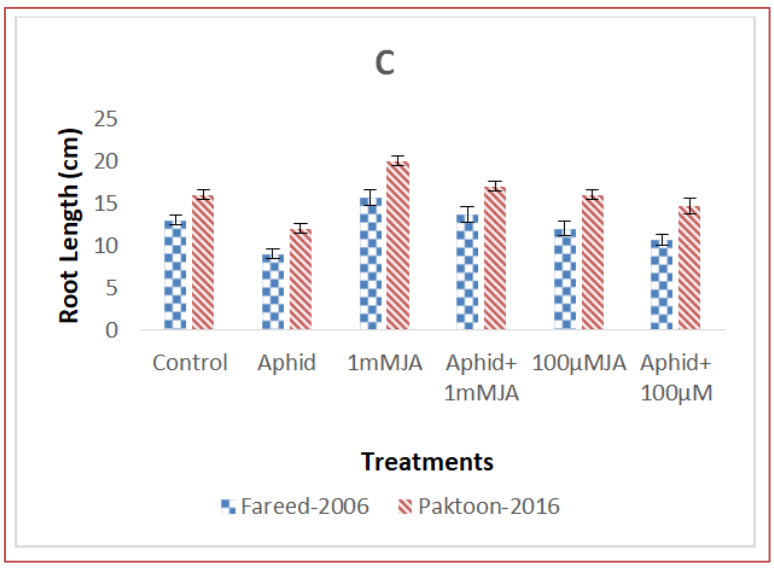
The results showed that fresh root and shoot weight of control plants was higher than the aphid-stressed plants. In aphid-stressed plants, fresh root weight was 0.33-0.35g, which was increased after JA treatment to 0.78- 0.80 g and fresh shoot weight was 0.36-0.38g, which was increased after JA treatment to 0.83-0.85. Aphid stress caused reduction in fresh root and shoot weight of both cultivars. However, maximum reduction in fresh root and shoot weight was observed in cv. Fareed-2006 than in Paktoon-2016 under aphid stress. According to the findings, aphid-stressed plants had shorter plant lengths than control plants. During the aphid infestation, the jasmonic acid spray dramatically increased the plant height. In Fareed-2006 and Paktoon-2016, aphid-stressed plants displayed 38.5 cm and 44.5 cm of overall plant length, respectively. In Paktoon-2016, plant length increased (55–60 cm) after JA spraying. (Fig.1).

Photosynthetic Pigments:

The findings showed that all photosynthetic pigments of control plants were higher than aphid-stressed plants. The concentration of Chlorophyll a, Chlorophyll b, total Chlorophyll, and carotenoid was significantly raised by jasmonic acid spray. After applying JA to plants under aphid stress, Chlorophyll a levels increased from 0.10 to 0.11 mg/g f.wt in Fareed-2006 and from 0.12 to 0.13 mg/g f.wt in Paktoon-2016. Chlorophyll a and chlorophyll b were found to be more abundant in the 1 mM JA treatment than in the 100 µM JA treatment. It was shown that JA treatment enhanced the carotenoid content in both kinds of wheat whereas aphid infestation decreased it. Paktoon-2016 performed better than cv. Fareed-2006 under biotic stress with foliar application of jasmonic acid (Fig.2).


A
Root Fresh Weight (g)
1
0.8
0.6
0.4
0.2
0
Control
Aphid
1mMJA
Aphid+ 1mMJA
100µMJA
Aphid+ 100µM
Treatments
Fareed-2006
pakhtoon 2016


B
Shoot Fresh Weight (g)
1
0.8
0.6
0.4
0.2
0
Control
Aphid
1mMJA
Aphid+ 1mMJA
100µMJA
Aphid+ 100µM
Treatments
Fareed-2006
Paktoon-2016


C
Root Length (cm)
25
20
15
10
5
0
Control
Aphid
1mMJA
Aphid+ 1mMJA
100µMJA
Aphid+ 100µM
Treatments
Fareed-2006
Paktoon-2016

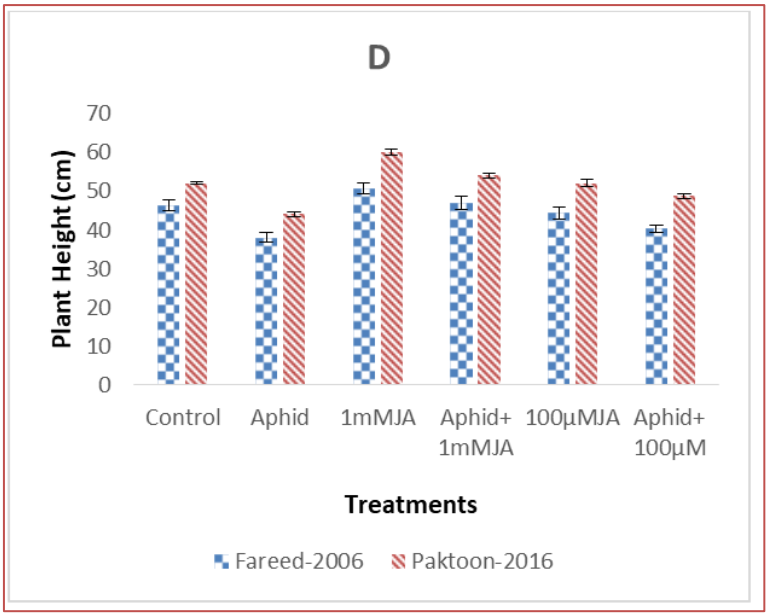
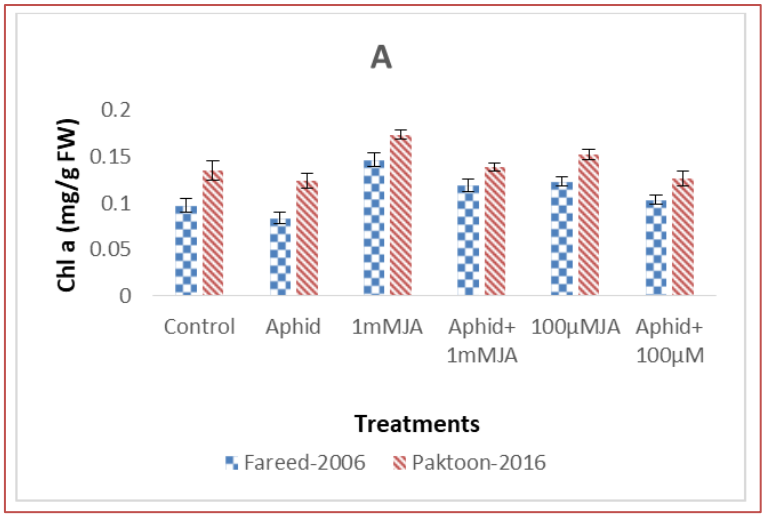
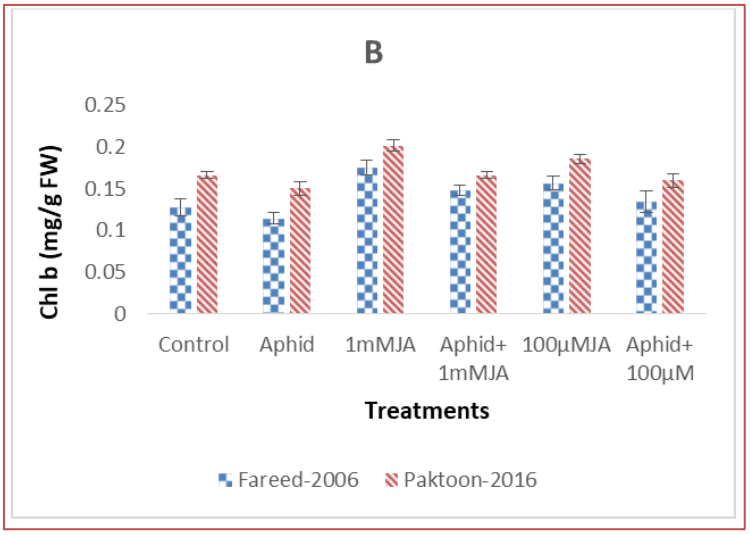


Figure 1: Effect of foliar application of jasmonic acid (100 µM and 1 mM), on root fresh weight (g) (A), shoot fresh weight (g) (B), plant root length (cm) (C), plant height (cm) (D) of two varieties of wheat i.e. Fareed-2006 and Paktoon-2016 grown with and without aphid stress.





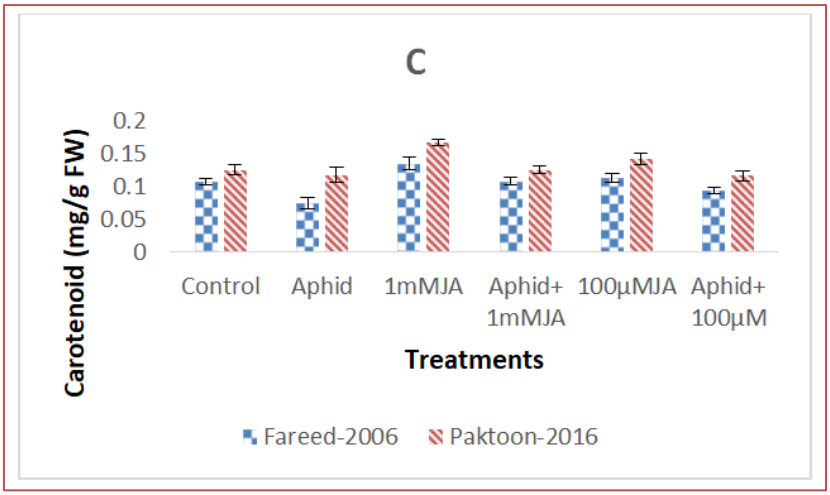
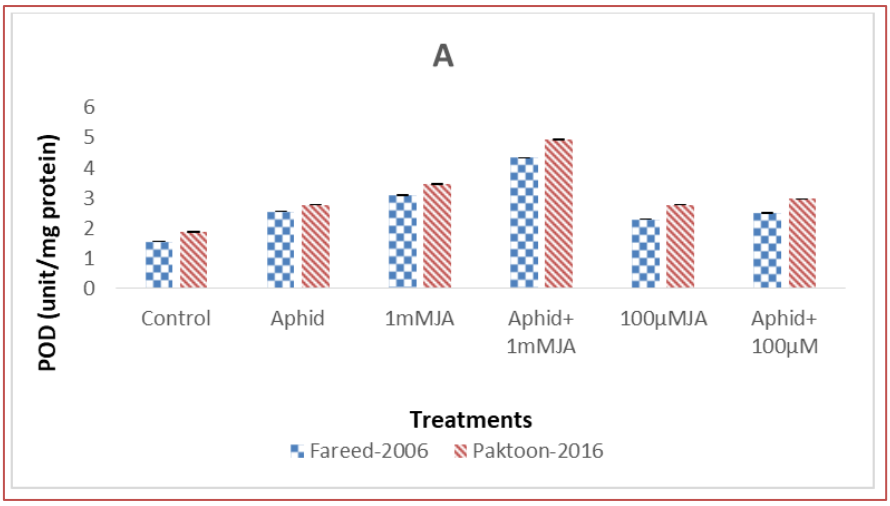
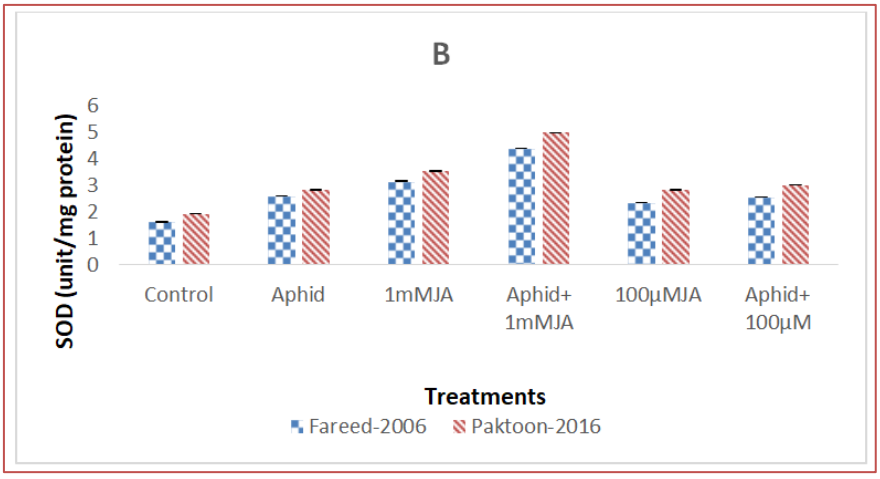


Figure 2: Impact of foliar jasmonic acid treatment (100 µM and 1 mM), on Chlorophyll a (A), Chlorophyll b (B) and Carotenoids content (C) of two varieties of wheat i.e., Fareed-2006 and Paktoon-2016 grown with and without aphid stress.

Antioxidants (SOD, POD and CAT)

Activities of SOD, POD, and CAT differed amongst the treatments. When compared to the control, these activities were significantly increased under aphid stress. The levels of SOD, POD, and CAT were much higher in plants that were not sprayed as well as in plants that were aphid-infested and treated with JA spray. Plants sprayed with 1 mM JA had higher concentrations of SOD, POD, and CAT than plants treated with 100 µM JA (Fig.3).





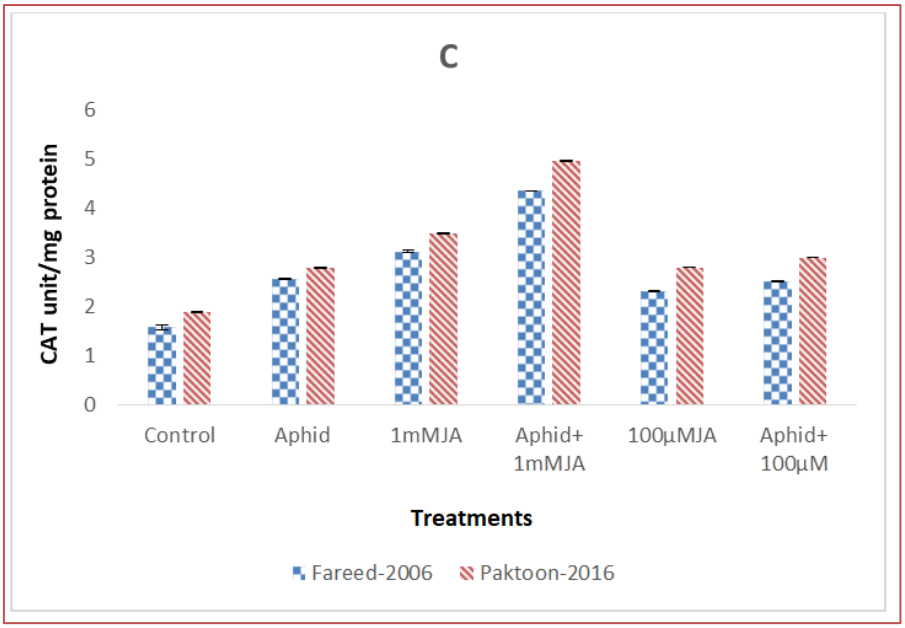
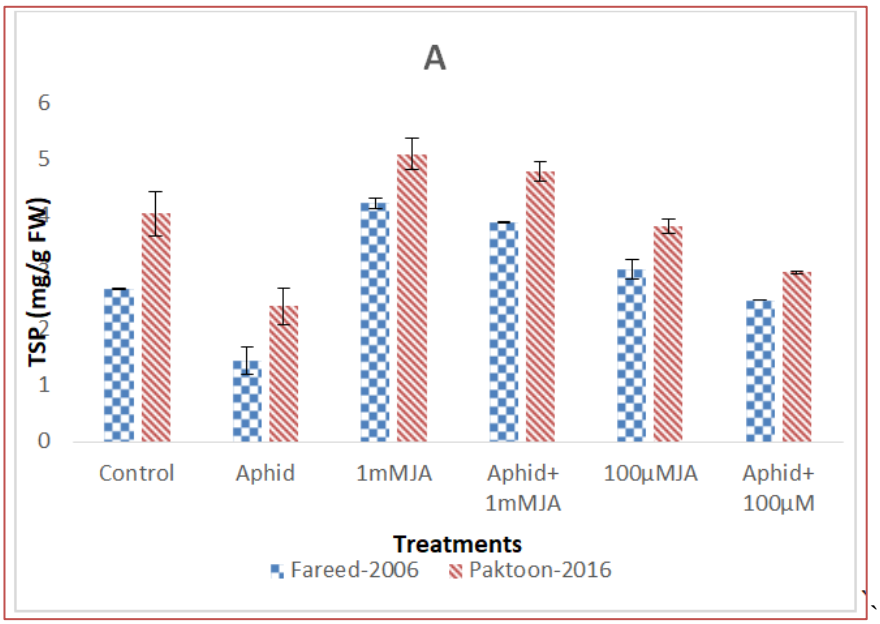
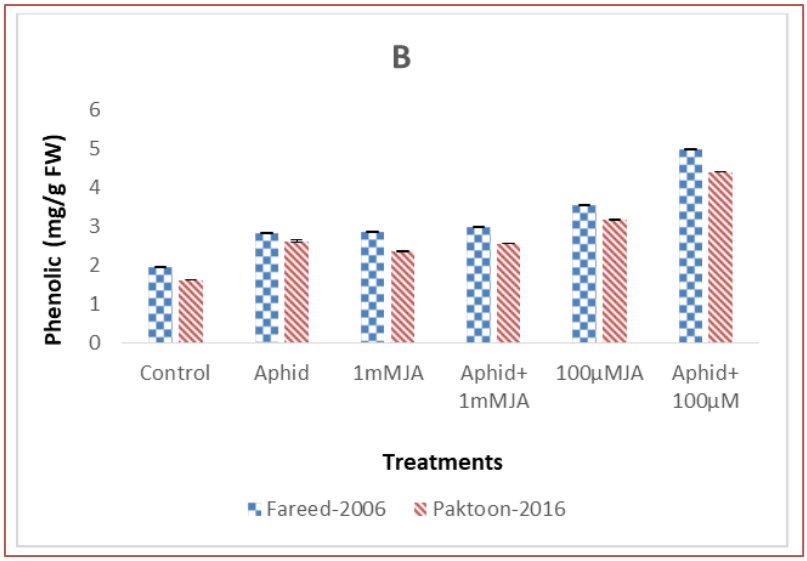


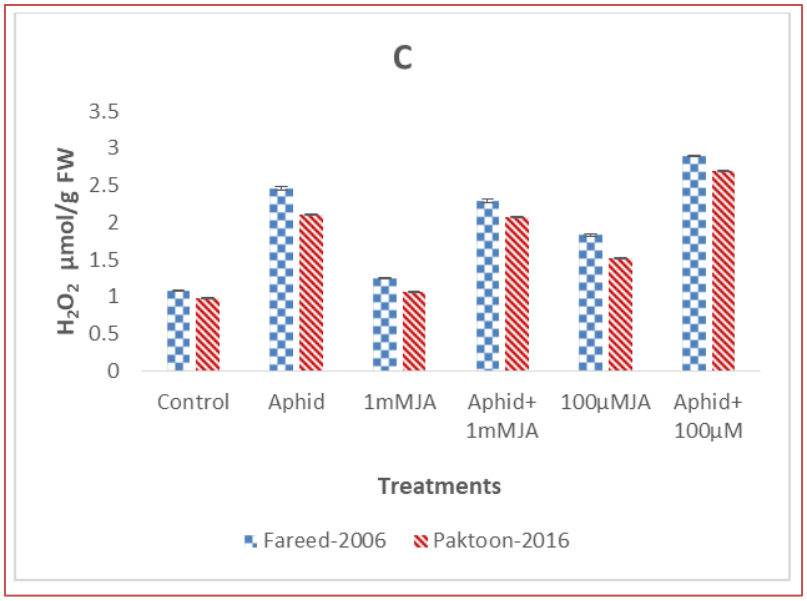
Figure 3: Impact of foliar jasmonic acid application (100 µM and 1 mM), with and without aphid stress, on POD (A), SOD (B) and CAT (C) of two varieties of wheat i.e., Fareed-2006 and Paktoon-2016.

Total Soluble Protein, Phenolics, MDA and H_2O_2:

TSP concentration was lower in aphid-stressed plants than in control plants in both varieties. A substantial increase in TSP was observed in aphid-infested plants following JA treatments. Jasmonic acid spray increased the TSP level in aphid-infested plants from 3.89 to 3.91 mg/g f.wt in Fareed-2006 and from 4.71 to 4.77 mg/g f.wt in Paktoon-2016. The findings showed that aphid-stressed plants had more phenolics than control plants, which declined following JA application. Aphid stress significantly increased the amount of phenolic of both cultivars. Plants that received higher jasmonic concentration 1mM possessed low phenolic content. Aphid stress resulted in a significant increase in H_2O_2 and MDA content in both wheat cultivars. Less H_2O_2 (0.98 mol/g f.wt) and MDA (0.99) was present in control plants than aphid stressed plants. Plants treated with 1 mM JA had lower H_2O_2 and MDA content than plants sprayed with 100 µM JA. (Fig.4).







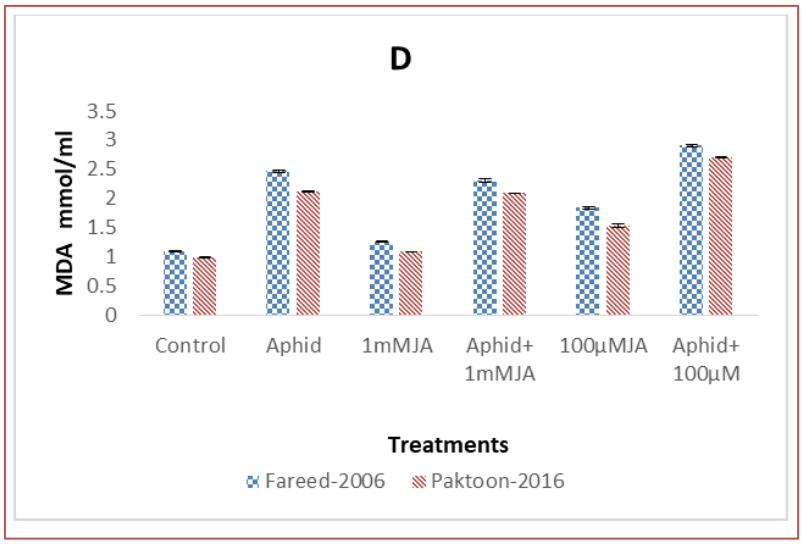


Figure 4: Impact of foliar jasmonic acid application (100 µM and 1 mM), with and without aphid stress, on Total soluble protein (A), phenolics (B), H_2O_2 (C) and MDA content (D) of two varieties of wheat i.e., Fareed-2006 and Paktoon-2016.

Direct Count of Aphids:

Results showed that 1 day after JA application, the aphid population declined. Aphids population significantly decreased after JA application in both varieties. Plants sprayed with a higher concentration of JA (1 mM) had fewer aphids. Plants not treated with JA have the highest aphid population. After a single day of JA application, the aphid population decreased. After 7 days, the aphid population increased in both wheat varieties, with a greater number of aphids in the 100 µM treatment than the 1 mM JA. Comparatively fewer aphids were found in Paktoon-2016 than in Fareed-2006.

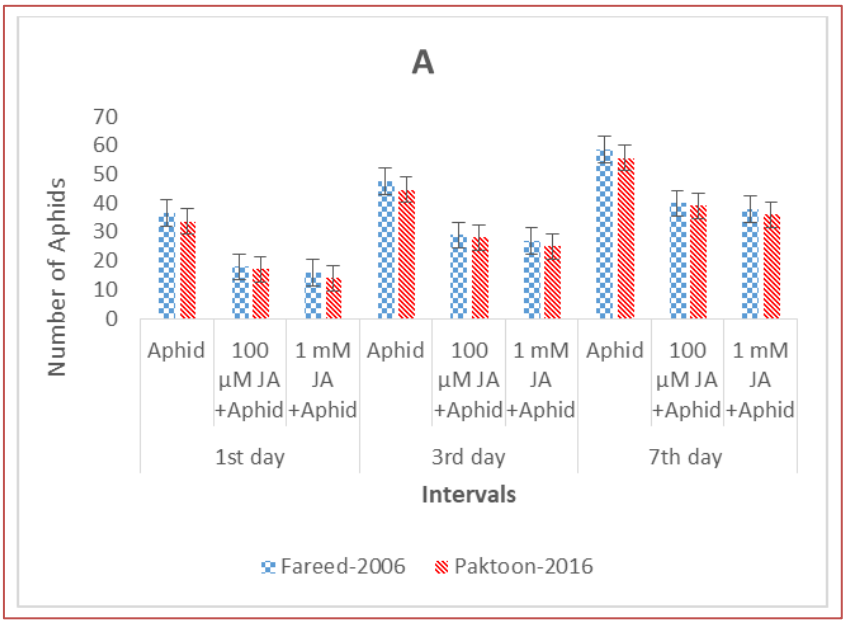


Figure 5: Comparing of average number of aphids between two wheat varieties Fareed-2006 and Paktoon-2016, respectively when 2-month-old wheat seedlings exogenously sprayed with jasmonic acid (100 µM and 1 mM) were infested with aphids and data was collected with intervals.

DISCUSSION

Biotic stress is a serious environmental issues that produced the osmotic stress and reduced the growth in plants and also caused reduction in productivity. Jasmonic acid is regarded as a plant growth regulator and regulating the plant's morphological, physiological and biochemical processes (Mahmood *et al.*, 2012). As one of the most significant phytohormones, jasmonic acid plays an important role in plant growth and development as well as the response to biotic and abiotic stresses (Wasternack, 2013). Endogenous jasmonic acid levels are known to increase in plants subjected to a variety of stress situations (Bankaii *et al.*, 2014). Plant's stress resistance is increased by exogenous jasmonic acid treatment, which boosts antioxidant activity (Qiu *et al.*, 2014).

In the current study, to evaluate the effectiveness of jasmonic acid spray against aphid populations and its impact on plant growth, an experiment was carried out on two wheat types (Fareed-2006 and Paktoon-2016). Important plant characteristics that eventually impact yield include plant biomass, photosynthetic capacity, and pattern.

In wheat, JA application increased wheat shoot and root fresh weight (Ilyas *et al*., 2017; Ali *et al*., 2020). JA applied topically enhanced plant development by lengthening shoots, roots, and plant height (Anjum *et al*., 2016). Similar results shown improved plant growth in maize (Abdelgawad, Khalafaallah and Abdallah, 2014) and soybean (Anjum *et al*., 2011). Similar findings have been found for wheat (Abeed, Eissa and Abdel-Wahab, 2021) as well as safflower (Ghassemi-Golezani and Hosseinzadeh-Mahootchi, 2015) and cotton (Abeed and Dawood, 2020). Exogenous JA treatment can increase plant growth and pest resistance simultaneously (Bhavanam and Stout, 2021). It can lessen the harm that thrips and midges do to wheat (Mouden *et al*., 2017).

In the current study, plants with aphid infestations had lower levels of chlorophyll a, chlorophyll b, and total chlorophyll than the control plants. According to other research, aphid infestation reduces the amount of chlorophyll in wheat (Shahzad *et al*., 2019) (Zhang *et al.*, 2019) as well as in sorghum (Najali *et al*., 2018). In our experiment, jasmonic acid was applied externally, increased the contents of chlorophyll a, b and carotenoids in tomato (Yildirim and Dursun, 2008) as well as in Basil (Gharib, 2006) which is similar with earlier studies (Ali *et al*., 2020; Abbaspour and Rezaei, 2014; Siddiqi and Husen, 2019; Qiu *et al*., (2014)).

The antioxidants whether they are enzymatic or non-enzymatic are produced in plants facing a stress so that they can scavenge the reactive oxygen or oxidative

species and protect it from oxidative damages (Apel and Hirt, 2004). In the present study, an increase in the activities of antioxidant enzymes (SOD, POD and CAT) were seen in plants under aphid infestation than control. The contents of catalase, SOD and POD contents are slightly increased by spraying jasmonic acid. This is because foliar spray of jasmonic acid aid plants to minimize the ROS, thereby increase the scavengers of ROS in the form of antioxidants so the level of SOD, POD and catalase increase to a little level, Ahmad *et al.*, (2008) also found the same results. Additionally, it has been noted that following an aphid infestation, POD and SOD levels in aphid-resistant wheat are higher than those in aphid-susceptible wheat (Qi *et al*., 2020). enzymes like catalase and superoxide dismutase (SOD) may quickly detoxify hydrogen peroxide and other ROS. The latest experiment revealed similar findings, showing that after JA application in aphid-infested plants, Paktoon-2016 (which seemed resistant to aphid attack) had greater levels of both enzymes.

CONCLUSION

The results showed that under the stress of aphids, morphological characteristics declined and improved following JA application. Stress caused a reduction in photosynthetic pigments, but JA treatment helped the plants restore these pigments. When compared to the control, the activities of SOD, CAT, and POD were significantly increased under aphid stress. Aphid-stressed plants had greater amounts of MDA and H_2O_2 than control plants. The findings showed that JA sprayed plants have a reduced aphid population than untreated plants. Plants treated with 1 mM JA had lower H_2O_2 and MDA content than plants sprayed with 100 µM JA.

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