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Effect of Methyl jasmonate (MeJA) on Biochemical Constituents of Seed Treated Tomato

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ABSTRACT

Tomato (*Solanum lycopersicum*) plant is often attacked by numerous insect pests which lead to severe crop loss. To reduce the crop loss naturally, plants have evolved with variety of defensive hormone namely jasmonic acid (JA) which protect the plants from the insect pests. A study was carried out at Kittur Rani Channamma College of Horticulture, Arabhavi in 2023-24 to investigate the effect of methyl jasmonate (MeJA) on biochemical constituents such as phenol and tannin content in leaves of seed treated tomato. The experiment followed a Completely randomized design included nine treatments and three replications [T_1- MeJA at 0.5 mM, T_2- MeJA at 0.75 mM, T_3- MeJA at 1 mM, T_4- MeJA at 2 mM, T_5- MeJA at 2.5 mM, T_6- MeJA at 3 mM, T_7- Ethyl alcohol, T_8- UHSB - POP and T_9- Untreated control (Water)]. The results revealed that among treatments seeds treated with MeJA at 3 mM recorded highest phenol and tannin content as 4.86 and 2.85 mg/g, respectively which was statistically similar to MeJA at 2.5 mM concentration (4.76 and 2.78 mg/g, respectively) and lowest phenol and tannin content (2.72 and 0.91 mg/g, respectively) was recorded in ethyl alcohol treatment. However, These results highlighted tomato seeds treated with methyl jasmonate has positive effect in increasing phenol and tannin content in leaves and potentially helps in inducing resistance in plants.

Keywords: Methyl jasmonate, Phenol, Tannin,Tomato

INTRODUCTION

Plants have developed a wide range of adaptations over time to protect themselves against herbivores including lowering their overall performance or disrupting the digestive system of the insect pests. One notable defense mechanism is plants have ability to produce secondary metabolites and defense associated proteins that alter the quality of food for herbivores. This, in turn, can decrease the herbivores fitness and fecundity (Kant *et al*, 2015). The association between plants and herbivores involves key molecular processes, particularly the production of Jasmonic acid (JA), which plays a crucial role in plant defense and it also involved in indirect defense mechanism by emitting plant volatiles that attract different parasitoids, which lay eggs inside the herbivore and once the eggs hatch, the herbivore die. This indirectly protects the plant from herbivore damage. Furthermore, the precursor of JA triggers the activation of defensive genes, which are essential for synthesis of methyl jasmonate (MeJA) through the terpenoid biosynthesis pathway (Wang, 2009; Sripontan and Hwang, 2016). Remarkably, JA can be induced within minutes of herbivore attack, leading to rapid defense responses that either affect early-colonizing herbivores or result in delayed responses that hinder herbivore establishment (Kant *et al*, 2004; Wu and Baldwin, 2009). Therefore, the current investigation was carried out to assess the biochemical constituents in methyl jasmonate (MeJA) treated seeds of tomato.

MATERIALS AND METHODS

The research was conducted at K. R. C. C. H, Arabhavi, Belagavi district, Karnataka, India, during the 2023-24 period. The experiment followed a Completely randomized design included nine

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treatments and three replications [T_1 - MeJA at 0.5 mM, T_2 - MeJA at 0.75 mM, T_3 - MeJA at 1 mM, T_4 - MeJA at 2 mM T_5 - MeJA at 2.5 mM, T_6 - MeJA at 3 mM, T_7 - Ethyl alcohol, T_8 - UHSB - POP and T_9 - Untreated control (Water)].

Methodology of seed treatment

For the preparation of one millimolar MeJA solution, 229 micro litres of MeJA (95%) was dissolved in five ml of ethyl alcohol and volume was adjusted to one litre by using deionized water (Pushpalatha *et al*, 2021). Here, Ethyl alcohol was used as soluble solvent. A total of 200 hybrid seeds of tomato (Arth - 2075) were soaked in 50 ml of aqueous methyl jasmonate solution in the petri dishes (35 mm diameter) later these were wrapped in aluminium foil and incubated for 24 hr in the dark at 4°C. Prior to sowing, seeds were washed twice with deionized water for ten minutes and later allowed to dry on a paper towel (Worrall *et al*, 2012). Then seeds were sown in portrays containing autoclaved cocopeat mixed with vermicompost as the growing media. For biochemical estimation fourth youngest leaves from the top were taken. The biochemical constituents such as phenol and tannin were assessed twenty days after seed sowing by following below mentioned procedures.

Estimation of total phenols (mg/g)

For the estimation of phenol content Folin Ciocalteu Reagent (FCR) method was followed. One gram dried leaf sample was grinded in ten ml of 80 per cent ethanol with pestle and mortar. The obtained solution was filtered and filtrate was then placed in a test tube and heated at 100 °C untill the solution was evaporated. one ml of deionized water was added to the test tube. From that 0.5 millilitre of solution was transferred to another test tube, where 2.5 ml solution of sodium carbonate (Na_2CO_3) was added. The mixture was allowed to cool and absorbance was taken at 650 nm by spectrophotometer. Total phenol level was quantified by using standard graph and represented in milligram of dried leaf (Sadasivam and Manickam, 2008).

Assessment of total tannin content (mg/g)

The leaves from the fourth node was collected randomly from each replication in every treatment. These leaf tissue was subsequently dried in a hot air oven at 70°C temperature for 48 hours. One-gram sample of the dried leaves were weighed and ground using pestle and mortar in 10 ml of 80% ethanol. From each treatment, 0.1 millilitre of the extracted sample was transferred to individual test tubes and added with 7.5 ml of deionized water. The test tubes were then placed in boiling water for 30 minutes to extract tannins later, cooled at room temperature and volume was adjusted to 10 ml after that we obtained tannin extract from the clear solution. One milliliter of this tannin extract was transferred to a test tube containing one ml of (35%) sodium carbonate and 200 µl of FCR (1N) was added and these mixture was kept in the dark for 20 minutes. Absorbance was then measured in spectrophotometer at 700 nm wavelength and instead of tannin extract deionized water is used as blank. A standard graph was drawn using standard tannic acid solution ranging from 0-1000 µl in 200 µl intervals, corresponding to 0-100 µg of tannic acid (Sadasivam and Manickam, 2008).

To interpret the results, comparison between treatment means were made using Duncan's multiple range test (DMRT). The standard error mean (SE. m ±) and critical difference (CD) were calculated at one per cent and five per cent level of significance.

RESULTS AND DISCUSSION

Effect of MeJA on total phenols in leaves

Among treatments, significantly highest (4.86 mg/g) phenol content was recorded in the treatment MeJA at 3 mM (T_6) which was statistically on par with MeJA at 2.5 mM (T_5) (4.76 mg/g). However, significantly lowest (2.72 mg/g) amount of phenol content was recorded in ethyl alcohol (T_7) which was statistically on par with UHSB - POP (T_8) and untreated control (T_9) (2.84 and 2.92 mg/g, respectively). The total phenol content in leaves plays important role in defense mechanism where it will interfere with feeding deterrence and growth of the herbivores also helps the plants from oxidative damage. The presence of higher concentration of phenols had direct toxicity effect on herbivores and the phenol content in leaves is increases with increasing concentration of MeJA this is may be due to JA signalling enhanced phenylpropanoid pathways and cross talks of jasmonic acid with salicylic acid and ethylene will amplifies the phenol production in response to rising MeJA levels.

The present findings were supported by Kim *et al* (2006), Moreira *et al* (2009) and Asghari *et al* (2020) who found MeJA at 0.1 and 0.5 mM concentrations increased phenolic contents in the leaves of sweet basil, pine seedling and pomegranate, respectively. Malekpoor *et al* (2016) reported the foliar application of JA at (200 and 400 µL) had significant effect on total phenol in the sweet basil. Ahmadi *et al* (2018) found exogenous spraying of MeJA in addition to NaCl has

Table 1. Impact of MeJA on phenol and tannin content of leaves.

Treatment	Dose/l	Total Phenol (mg/g)	Total Tannin (mg/g)
T_1 - MeJA	0.5 mM	3.62^c	1.90^c
T_2 - MeJA	0.75 mM	3.71^c	1.98^c
T_3 - MeJA	1 mM	3.84^c	2.01^c
T_4 - MeJA	2 mM	4.55^b	2.63^b
T_5 - MeJA	2.5 mM	4.76^{ab}	2.78^a
T_6 - MeJA	3 mM	4.86^a	2.85^a
T_7 - Ethyl alcohol	5 ml	2.72^d	0.88^d
T_8 - UHSB - POP	-	2.84^d	0.91^d
T_9 - UTC	-	2.92^d	0.96^d
S. Em±		0.078	0.04
CD (1%)		0.31	0.16
CV (%)		3.56	3.48

*Note: * DAS = Days after sowing. * Values with letters are significant at (P=0.01 by DMRT).*

significantly increase the phenolic contents in *Brassica* plants. Ehsanpour and Maleki (2018); Miclea *et al* (2020) showed increasing concentration of salicylic acid (SA) has increased the total phenol content in tomato and Lavandula plants. Yolanda *et al* (2013) and Pushpalatha (2021)found that external application of methyl jasmonate had significantly highest total phenol level in grapes.

Effect of MeJA on tannin content of leaves

It was evident that a significant difference was found between the treatments with respect to total tannin levels in leaves (Table 1). Significantly highest (2.85 mg/g) tannin content was recorded in MeJA at 3 mM (T_6) which was statistically on par with MeJA at 2.5 mM (T_5) (2.78 mg/g) followed by the treatment MeJA at 2 mM (T_4) (2.63 mg/g). Further, significantly lowest (0.91 mg/g) amount of tannin content was recorded in ethyl alcohol (T_7) which was statistically on par with UHSB - POP (T_8) and untreated control (T_9) (0.91 and 0.96 mg/g, respectively).

Total tannin contents in plant plays key role in the defense mechanism against insect pests. The use of methyl jasmonate increases the tannin content in leaves with increasing concentration of MeJA was possibly due to jasmonic acid activating the genes involved in the production of secondary metabolites like phenols and tannins. This study was in conformity with Arnold *et al* (2004) who reported JA application stimulate the tannin production in poplar seedlings. War *et al* (2015) found spraying of groundnut plants with jasmonic acid and salicylic acid showed significantly high level of tannin in leaves of groundnut plant. Present findings were also in accordance with Thiruvengadam *et al* (2016) who reported increased tannin content in turnip due to application of JA. Also, Yan *et al* (2021) found application of jasmonic acid (JA) recorded higher tannin level in leaves of wild rose. Hussain *et al* (2023) reported that application of methyl jasmonate showed higher levels of tannin content in grape plant.

CONCLUSION

The current study concluded that treatments exhibited a notable variation in total phenol and tannin levels. Specifically, the highest concentrations of phenol and tannin were observed in the MeJA treatments at 3 mM (T_6), 2.5 mM (T_5) and 2 mM (T_4). In contrast, the lowest levels were found in the ethyl alcohol treatment (T_7), UHSB - POP (T_8) and the untreated control (T_9).

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