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Effect of Oxidative Stress on Abscission of Tomato Fruits and its Regulation by Nitrophenols

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ABSTRACT. *Abscission of reproductive parts in tomato cultivar PKM 1 was studied. The plants were sprayed with four different concentrations of Nitrophenols (ATONIK) at flowering and fruit set stage. Observations were recorded in the flowers and developing fruits. Application of Nitrophenols significants increased the activity of antioxidant enzymes namely superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) and auxin content coupled with decreased activity of polyphenol oxidase (PPO) IAA oxidase (IAAO) enzymes over the control significantly. Among the concentrations experimented, application of Nitrophenols at 0.4% during fruit set stage was found to be the most effective in recording higher antioxidant enzymes activity and auxin level which had reflected in an increased number of fruit clusters per plant, fertility co-efficient and yield of tomato.*

INTRODUCTION

A considerable volume of research has been devoted to identify the enzymes that bring about cell separation of flowers and fruits. The culmination of abscission is the physical detachment of the target organ, and thus much work has been focused on the phenomenon of cell wall dissolution at the site of abscission. Although a range of factors have been proposed to contribute to the process of wall softening, it is brought about by an increase in the activity of lipolytic enzymes (Gopinadhan and Jo Droillard, 1992). Increase in lipoxygenase activity causes oxidative injury in the membrane by initiating the chain reaction of lipid peroxidation by forming lipid hydroperoxides and superoxide radicals (Quirino *et al*., 2000). Oxidative stress arises from an imbalance in the generation and metabolism of reactive oxygen species (ROS), with more ROS (such as H_2O_2, $OH^{\cdot}$ and O_2^{-}) being produced than are metabolized (Dhindsa *et al*., 1981). The ROS are able to attack polysaccharides, proteins and nucleic acids (Matysik *et al*., 2002). Plants have evolved enzymatic protection mechanisms that efficiently scavenge ROS and prevent damaging effects of free radicals (Srivalli and Khanna Chopra, 2001). Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) are involved in the scavenging of ROS (Shanker *et al*., 2004). Phenolics are also able to act as radical scavengers or radical-chain breakers, thus extinguishing strongly oxidative free radicals such as the hydroxyl radical yielding products with much lower oxidative capacities as compared to the parent compounds (Grossman, *et al*., 2002). The O_2^{-} produced in the "Mehlar reaction" will be dismutated to O_2 and H_2O_2 by SOD (Bowler *et al*., 1992). Peroxidase catalyses the dehydrogenation of structurally diverse phenolic and endiolic substrates by H_2O_2 and are thus often regarded as antioxidant enzymes (Shigeoka *et al*., 2002). Catalase removes the H_2O_2 produced under adverse situations. The maintenance of this enzyme prevents an increase in cytosolic H_2O_2, which can create toxic conditions in the plant cell leading to oxidative stress and cell death (Prochazkova *et al*., 2001). Whether different isoenzymes contribute to prevention of abscission remains to be

determined. If this proves to be the case, then it could reflect in delayed abscission of fruit through genetic manipulation. Therefore, the objective of the present experiment was to examine variations, if any, in the degree of antioxidant enzyme activity and auxin level in tomato plants sprayed with Nitrophenols and its impact on yield improvement in tomato by controlling abscission of flowers or fruits in tomato.

MATERIALS AND METHODS

Tomato (*Lycopersicon esculentum* Mill.) cultivar PKM 1 was planted in a clay loam textured soil with a pH of 7.6 and EC of 0.31 dS m^{-1} during 2003 in the experimental field of Tamil Nadu Agricultural University, Coimbatore (11°N; 77°E; 426.7m amsl), India. In the experimental site, the maximum and minimum temperature and relative humidity ranged between 33°C and 20 °C and 80 and 60%, respectively. The minimum and maximum irradiance was 800-1100 µM$m^{-2}s^{-1}$ PAR). Tomato seeds were sown into a field nursery in April 2003. Seedlings were transplanted in the field (one seedling per hole), a month later. The soil of the experimental field was low in available N (195 kg ha^{-1}), medium in available P (6 kg ha^{-1}) and high in available K (386 kg ha^{-1}). A net 20 m^2 plot contained 74 plants (3 plants per 1 m^2) planted in a 60 x 45 cm spacing. The experiment was performed in completely randomized block design with six replications. Each replication had 74 plants. The plants were irrigated once in five days. Observations were made in the flowers [60 days after transplanting (DAT)] and developing fruits (70 DAT) in tomato. Nitrophenols (ATONIK) obtained from Asahi Chemicals Limited, Japan was sprayed at flowering [58 DAT (S_1)] and fruit set stage [68 DAT (S_2)] using back pack hydraulic sprayer (ASPEE, Mumbai, India) equipped with a hollow cone nozzle till all leaves were completely drenched with the spray solution. Unsprayed plants served as control. Sampling was done 24 h after spraying.

Six treatment were used, namely T_1 (Control), T_2 (Foliar spray of Nitrophenols 0.1%), T_3 (Foliar spray of Nitrophenols 0.2%), T_4 (Foliar spray of Nitrophenols 0.4%), T_5 (Foliar spray of Nitrophenols 0.8%) and T_6 (Foliar spray of para-chloro phenoxy acetic acid (PCPA) 50 µl L^-).

For all enzyme and auxin (IAA) estimation, sampling was done in duplicate from all the six replication (n=12). Fruit set percentage was calculated by adopting standard procedure of Villareal and Lal (1979). The first formed five flower clusters were observed to represent fruit setting percentage. The yield was estimated from at least twenty plants from each treatment (n=20).

Enzyme assay

For assay of enzymes *viz.* superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), IAA oxidase (IAAO), and polyphenol oxidase (PPO) frozen flower tissue at S_1 and fruit tissue at S_2 were homogenized in ice-cold 0.1M Tris–HCl buffer at pH 7.8 containing 1mM EDTA, 1mM dithiotreitol and 5ml of 4% polyvinyl pyrrolidone per gram fresh weight (g^{-1} FW). The homogenate was filtered through a nylon mesh and centrifuged at 20,000x*g* at 4ºC. The supernatant was used for measuring enzyme activity (Shanker *et al.*, 2004).

SOD was determined by nitroblue tetrazolium (NBT) method of Beyer and Fridovich, (1987) by measuring the photoreduction of NBT at 560 nm. One unit of SOD

activity equaled to the amount required to inhibit photoreduction of NBT by 50%. CAT was estimated according to Teranishi *et al*. (1974). One milliliter of the supernatant was added to the reaction mixture containing 1ml of 0.1M H_2O_2 and 3ml of 0.1M sodium phosphate buffer. The reaction was discontinued by adding 10 ml of 2% H_2SO_4 after 1 min of incubation at 20ºC. The reaction mixture was then titrated against 0.01M $KMnO_4$ to determine the quantity of H_2O_2 used by the enzyme. Enzyme activity was expressed as mg H_2O_2 destroyed g^{-1} FW. POX activity was determined in the homogenates by measuring the increase in absorption at 470 nm and expressed as change in absorbance at 470 nm g^{-1} min^{-1} FW in a reaction mixture that contained extract, 50 mM buffer K-phosphate (pH 7.0), 0.1 mM EDTA, 10 mM guaiacol, 10 mM H_2O_2 (Racusen, and Foote, 1965). PPO was quantified according to the method described by Bateman and Daly (1967) and expressed as change in optical density g^{-1} min^{-1} FW. IAAO was determined according to Parthasarathy *et al*. (1970), and expressed as µg unoxidised auxin g^{-1} h^{-1} FW. Auxin (IAA) was estimated according to the methodology of Ginnet *et al*. (1986) and expressed in ng g^{-1} FW.

Isozyme analysis

Electrophoretic separation of isozymes was achieved with 10% native PAGE, with slight modifications to the method described by Laemmli (1970). The methodology of Nadlony and Sequira (1980), Jayaraman *et al*. (1987), Beau-Champ and Fridovich (1971), and Gurmeet Talwar *et al*. (1985) were followed for POX, PPO, SOD, CAT and IAAO, respectively, for visualizing the isoforms.

The data were analysed statistically according to Sukhatme and Amble (1985) using AGRES statistical package for arriving ANOVA. Duplicate sample from all the six replications were taken for all the enzyme assays (n=12). Mean separation and significance between control and treatments were compared at 0.05 probability levels by LSD method.

RESULTS AND DISCUSSION

Flowering and fruit set in tomato is highly influenced by hormonal balance, antioxidant enzymes activity and source-sink relationship. The effect of applications Nitrophenoles on the activities of ROS scavenging anymes, Auxin catabolizing enyxmes, and fertility and yield componets are discussed below.

Scavenging Enzymes of Reactive Oxygen Species (ROS)

Superoxide dismutase activity (Enzyme Units)

SOD activity in tomato, was increased with Nitrophenols spray at both flowering stage (S_1) and fruit set stage (S_2) (Table 1). At S_2 among the concentrations 0.4% nitrophenols (T_4) recorded the highest enzyme activity (2.065 enzyme units) followed by T_6 (PCPA 50 µl L^{-1}). The increase in enzyme activity in these treatments over control and PCPA spray accounted for 22.1 and 8.4%, respectively. The activity gel of SOD revealed that S_1 and S_2 produced five isoforms each. T_4 (application of Nitrophenols at 0.4%) produced the maximum number of isoforms *i.e.* five and four, respectively (Fig. 1). During S_1, one novel form was established (SOD 1) and during S_2

two novel isoforms were seen (SOD 1 and SOD 2) when compared with control. SOD catalyses the disproportion of superoxide radicals and converts them to molecular oxygen and H_2O_2 (Srivalli and Khanna Chopra, 2001). In the Nitrophenols treated plants, more activity isoforms of SOD were observed compared to control plants. SOD plays an important role in protecting cells against the toxic effects of superoxide radicals produced during oxidative burst (Halliwell and Gutteridge, 2000). This indicates the possible role of Nitrophenols in the retention of reproductive parts through subdued accumulation of ROS. Superoxide radicals are known to inhibit catalase and peroxidase activity (Matysik *et al*., 2002) and thereby efficient scavenging of superoxide is a must for enhanced catalase and peroxidase activity. Such increased catalase and peroxidase activities as observed in Nitrophenols treated plant confirms the role of SOD in protecting CAT and POX enzymes from superoxide radicals during abscission. The defensive function provided by SOD during abscission in plant tissues was reported by Rabinowich and Fridovich (1983). To a great extent, the differences in SOD activity were shown to be related to subcellular localization of SOD isoforms and to the cellular decompartmentalization that results from membrane deterioration during oxidative burst (Droillard and Paulin, 1990). In Nitrophenols treated plants, enhanced expression and forms of SOD indicate the possible participation of nitrophenols in delaying the membrane detoriation during abscission. Increase in SOD activity may have increased the peroxidase activity (Table 1) by providing the substrate, H_2O_2. The combination of hydrogen peroxide, formed by SOD activity and O_2^-, may lead to the formation of very active hydroxyl radicals by the Haber-Weiss reaction (Halliwell and Gutteridge, 1986). Thus, SOD activity and the removal of H_2O_2 by catalase and peroxidase are necessary for an effective defense against the action of free radicals.

Table 1. Effect of Nitrophenols foliar spray on Superoxide dismutase (SOD), Calalone (CAT) and Peroxidase (POX)

Treatments	Superoxide dismutase (Enzyme Unit)		Catalase (mg H_2O_2 g^{-1} FW)		Peroxidase (Δ OD g^{-1} min^{-1} FW)	
	S1	S2	S1	S2	S1	S2
Control	1.566	1.691	0.474	0.372	0.432	0.264
Nitrophenols 0.1%	1.631	1.824	0.497	0.385	0.453	0.314
Nitrophenols 0.2%	1.665	1.887	0.564	0.416	0.506	0.328
Nitrophenols 0.4%	1.953	2.065	0.593	0.437	0.574	0.364
Nitrophenols 0.8%	1.780	1.891	0.561	0.419	0.523	0.330
Foliar spray of PCPA 50 µl L^{-1}	1.828	1.904	0.587	0.426	0.536	0.338
Critical Difference (5%)	0.02*	0.02*	0.01*	0.03*	0.04*	0.03*

* Significance at 5% level of probability by LSD.

Catalase activity (mg H_2O_2 destroyed g^{-1} FW)

Foliar spray of nitrophenols distinctly decreased the H_2O_2 concentration in tomato plant over control (T_1) (Table 1). Among nitrophenols spray concentrations, T_4 (0.4%) was

the best over other treatments by recording a significantly higher value of H_2O_2 destruction during both flowering and fruit set stages of crop growth. During fruit set stage, it recorded an increase of 17.4 and 2.5% over T_1 and T_6, respectively. This was closely followed by T_6 (PCPA 50 µl L^{-1}) by showing an increase of 14.5% over T_1. In activity gel, S_1 and S_2 produced one isoform in total, but the intensity varies with the treatment (Fig. 1). The key enzyme scavenging H_2O_2 is catalase and it has a high reaction rate but a low affinity for H_2O_2. Catalase activity is not limited to peroxisomes, and appears to be crucial for maintaining the redox balance during oxidative stress (Foyer and Noctor, 2000). From the present study, it was noticed that application of Nitrophenols has increased the expression and number of catalase isoforms, indicating that the oxidative stress situation may be converted to normal condition by maintaining the redox potential. Among the treatments, 0.4% Nitrophenols in tomato enhanced the enzyme activity. The maintenance of this enzyme activity at higher level prevents the increase of cytosolic H_2O_2, which creates toxic conditions in the plant cell leading to oxidative stress (Srivalli and Khanna Chopra, 2001). Greater expression and activity of catalase may have contributed to reduced abscission in Nitrophenols treated plants.

Peroxidase activity (Δ OD g^{-1} min^{-1} FW)

An increased peroxidase activity of tomato sprayed with 0.4% Nitrophenols was observed in both S_1 and S_2 (Table 1). A per cent increase of 32.8 and 7.0 and 37.8 and 7.6% over control and PCPA spray at flowering and fruit set stages, respectively, were recorded. During S_1 and S_2, peroxidase showed four forms in total. T_4 had all the four forms (Fig. 1). Different isoforms of peroxidases are found in chloroplasts, mitochondria, peroxisomes and cytosol. The different isoforms are also regulated differentially in response to stress and development (Ye *et al*., 2000). From this investigation, it was clearly established that peroxidase isoforms were lost during abscission. Treatments increased the isoforms in tomato compared to control plants. Among the treatments imposed, 0.4% nitrophenols increased the number of isoforms in tomato compared to other treatments. The increase in peroxidase isoforms in t nitrophenols reated tomato was to scavenge even low concentrations of H_2O_2 as the enzyme has a high affinity to H_2O_2. Orendi *et al*. (2001) reported that an increase in the peroxidase enzyme activity led to decrease of H_2O_2 content and lipid peroxidation leading to increased fruit set.

Auxin Catabolising Enzymes

Polyphenol oxidase (PPO) activity (Δ OD g-1 min-1 FW)

Treatment with 0.4% Nitrophenols had conspicuously decreased the polyphenol oxidase activity at S_2 (Table 2). T_1 and T_6 showed PPO activity value of 0.414 and 0.304, respectively, whereas the treatment T_4 recorded only 0.276, which was 33.3% decrease over control at this stage. In tomato, four and nine isoforms were obtained during S_1 and S_2, respectively (Fig. 1). During S_1, T_5 had three forms, whereas, T_2, T_3, T_4 and T_6 had PPO 2, PPO 3, PPO 4 and PPO 5 forms. During S_2, T_3, T_4 and T_5 had these isoforms each. The co-factor required for the maximal rate of IAA oxidation by IAA oxidase is monosubstituted phenols. These phenolic co-factors act as electron donors to allow recycling of the catalytic Fe^{3+} form. This process is inhibited by polyphenols (Pedreno *et al*., 1990). Reduced polyphenol isoforms, observed in

Nitrophenols treated plants may be favoured accumulation of IAA by inhibiting IAA decarboxylation. Besides this function, PPO is also involved in lignin biosynthesis (Li *et al*., 2003). Decreased isoforms of PPO in Nitrophenols treated plants might be involved in the lignin biosynthesis *i.e.* the oxidation and polymerization of cinnamyl alcohols (Driovich *et al*., 1992) thus altering abscission pattern. Decrease in PPO activity leads to accumulation of auxin protective phenols (polyphenols).

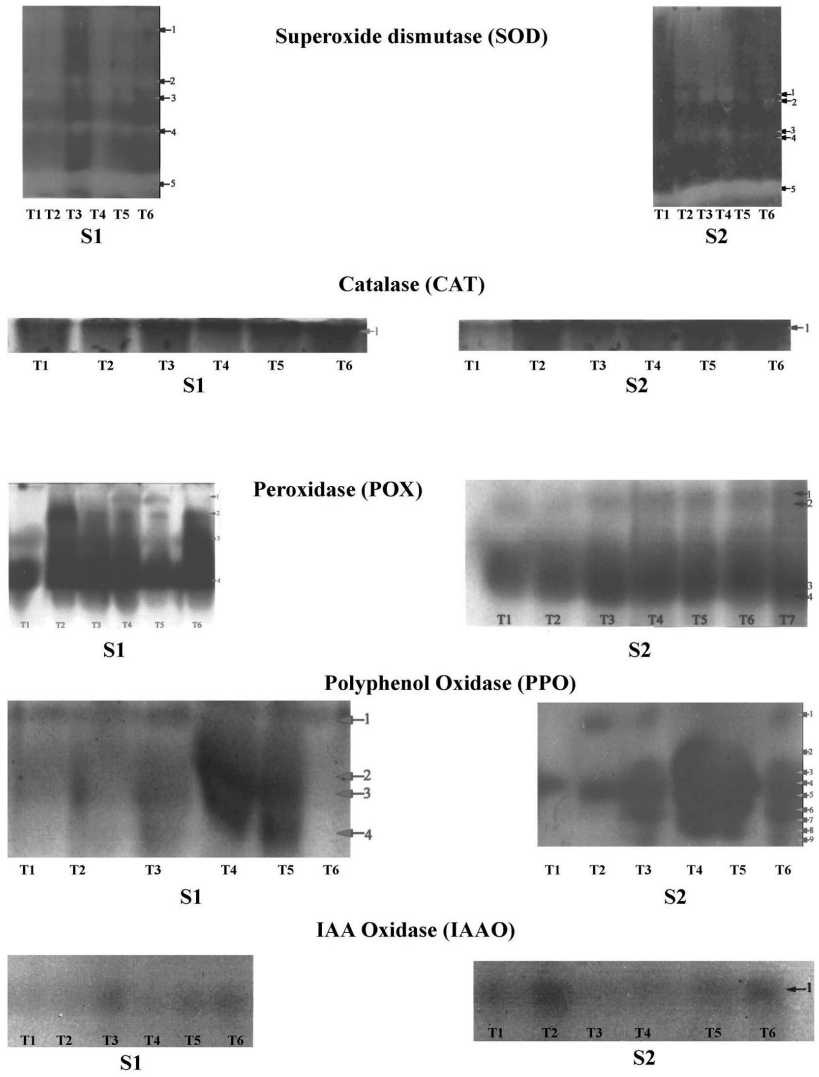

Fig.1. Effect of Nitrophenols on isozyme banding patterns of antioxidant enzymes and auxin catabolic enzymes in tomato.

IAA oxidase activity (µg unoxidised auxin g^{-1} h^{-1} FW)

The highest IAA oxidase activity was recorded for treatment with 0.4% Nitrophenols (T_4) both at S_1 and S_2 stages. It recorded a value of 428.18 at S_1 and 523.59 at S_2 (Table 2). All the treatments at both stages produced only one isoform. Since the staining was negative and the product produced by isoform was not stable for more than half an hour, the bands were faint (Fig. 1). Nitrophenols treatments significantly reduced the IAA oxidase activity. This may be probably due to the decrease in polyphenol oxidase activity (Pedreno *et al*., 1990). Nitrophenols has guaiacol (nitrophenol) as one of its constituents. Guaiacol being a diphenol, may have inhibited the IAA oxidase activity (Li *et al*., 2003).

Table 2. Effect of Nitrophenols foliar spray on Polyphonol Oxidase (PPO) and IAA Oxidase enzyme activity

Treatments	Polyphenol oxidase (Δ OD g^{-1} min^{-1} FW)		IAA Oxidase (µg unoxidised auxin $g^{-1}h^{-1}$ FW)	
	S1	S2	S1	S2
Control	0.526	0.414	300.26	358.65
Nitrophenols 0.1%	0.500	0.364	370.38	443.54
Nitrophenols 0.2%	0.444	0.332	407.63	474.45
Nitrophenols 0.4%	0.356	0.276	428.08	523.59
Nitrophenols 0.8%	0.426	0.312	416.11	494.81
Foliar spray of PCPA 50 µl L^{-1}	0.394	0.304	419.70	501.90
Critical Difference (5%)	0.035*	0.023*	11.37*	11.34*

* Significance at 5% level of probability by LSD

Table 3. Effect of Nitrophenols foliar spray on IAA concentration and yield and yield components

Treatments	IAA concentration (ng g^{-1}FW)		Number of flower clusters $plant^{-1}$		Number of fruit clusters $plant^{-1}$	
	S1	S2	S1	S2	S1	S2
Control	183	262	28.36	28.25	11.42	12.27
Nitrophenols 0.1%	208	317	23.80	24.66	12.99	13.26
Nitrophenols 0.2%	245	371	23.65	24.05	13.95	14.02
Nitrophenols 0.4%	374	644	22.41	22.37	15.76	16.54
Nitrophenols 0.8%	247	428	23.85	24.29	14.23	14.89
PCPA 50 µl L^{-1}	301	520	22.63	22.98	14.63	15.83
Critical Difference (5%)	14*	20*	0.09*	0.09*	0.07*	0.06*

* Significance at 5% level of probability by LS

IAA concentration (µg content (ng g^{-1} FW)

Foliar treatment with 0.4% Nitrophenols (T_4) recorded the maximum IAA concentration followed by T_6 (PCPA 50 µl L^{-1}) at S_2 (Table 3). The next best treatment was T_5 (Nitrophenols 0.8%) followed by T_3 (Nitrophenols 0.2%). The most effective treatment (T_4) recorded an increase of IAA content 145.0 and 23.7% over control and PCPA, respectively, at S_2. The treatments differed significantly among themselves at both growth stages. Increased concentration of auxin in the cell causes increased lignification of cell wall (Ray, 1960). From the present study, it is shown that Nitrophenols regulated the process of abscission by increased production/synthesis of auxin. The enhanced synthesis may be due to the fact that Nitrophenols might have acted as an auxin precursor (Nanda *et al*., 1971), which in turn be reflected in more fruits retention in tomato. According to Upadhyay (2002) decrease in flower and fruit drop may be due to creation of favourable balance of endogenous hormones. Nitrophenols treated plants had more auxin content than control plants, and consequently the abscission process may have delayed. Pedreno *et al*. (1990) have shown that the abscission retarding action of auxin is primarily due to its capacity to maintain IAA oxidase at a low level. The present investigation also reveals that, the increase in auxin content in Nitrophenols plant treated with Nitrophonols might be due to decreased IAA oxidase and polyphenol oxidase enzyme concentrations.

Fertility co-efficient and yield and yield components

Treatment with different concentrations of Nitrophenols showed wide differences of fertility co-efficient ranging from 46.74 in T_1 (control) to 80.11 in T_4 (0.4% Nitrophenols) (Table 4). The superiority of T_4 treatment was significant as the next best treatment T_6 (PCPA 50 µl L^{-1}) recorded only 75.45 fertility co-efficient at S_2. The number of flower clusters per plant of tomato was significantly influenced by various treatments. Spraying Nitrophenols at S_2 produced a lower number of flower clusters per plant than S_1. Among the concentrations of spray, treatment with 0.4% Nitrophenols (T_4) produced comparatively low number of flower clusters, followed by T_6 (PCPA 50 µl L^{1}) than other treatments. Accordingly, the number of fruit clusters per plant could be improved significantly by Nitrophenols spray.

When the number of fruit clusters formed per plant was considered, the treatment T_4 was the best, which followed by T_6 recording values of 16.54 and 15.83, respectively (Table 3). Abscission of flowers is remarkably higher in control plants due to subdued auxin content and reduced activity of antioxidant enzymes. This might have resulted in continued flower production to assure a minimum fruit set. However, in Nitrophenols treated plants higher auxin content along with higher activity of antioxidant enzymes might have reduced abscission of flowers. This may favour the plants to divert the photoassimilates to the already formed flowers rather than investing them in the production of new flowers, resulting in higher fruit set. Application with 0.4% Nitrophenols (T_4) at S_2, recorded the highest yield of 1768 g while the yield per plant in control (T_1) being only 1221 g (Table 4). All the levels of application of Nitrophenols have increased the plant yield compared to control (T_1).

Table 4. Effect of nitrophenols foliar spray on fertility co-efficient (%) and yield plant^{-1} (g)

Treatments	Fertility co-efficient (%)		Yield plant^{-1} (g)	
	S1	S2	S1	S2
Control	46.7	47.5	1230	1221
Nitrophenols 0.1%	59.7	65.2	1395	1565
Nitrophenols 0.2%	64.7	69.6	1425	1640
Nitrophenols 0.4%	73.7	80.1	1606	1768
Nitrophenols 0.8%	62.4	71.3	1455	1712
PCPA 50 µl L^{-1}	68.6	75.4	1512	1755
Critical Difference (5%)	3.5*	2.7*	18*	19*

* Significance at 5% level of probability by LSD

The application of Nitrophenols has increased the yield potential through its effect on antioxidant enzymes and auxin content. The highest yield recorded in the treatment of 0.4% Nitrophenols indicates the optimum influence of Nitrophenols increasing antioxident enzymes and auxin levels.

CONCLUSIONS

The present study clearly indicates that application of Nitrophenols at 0.4% at fruit set stage significantly increased the antioxidant enzymes and decreased auxin catabolic enzymes, which favour the internal auxin content. The plants treated with Nitrophenols show low abscission of flowers due to increased levels of auxin and antioxidant enzymes resulting increased fruit set and yield.

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