INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2005/07–1–39–44
http://www.ijab.org

Abscission of Tomato Fruit Follows Oxidative Damage and its Manipulation by ATONIK Spray

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ABSTRACT

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

Key Words: ATONIK; Antioxidative enzymes; ROS; Abscission; Yield

INTRODUCTION

A considerable body of research has been devoted to identifying 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 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, most researchers believe that it is brought about by an increase in the activity of lipolytic enzymes (Gopinadhan & Droillard, 1992). Increase in lipoxygenase activity have been contributed to 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^- & O^-) being produced than are metabolised. The ROS are able to attack polysaccharides, proteins and nucleic acids. Plants have evolved enzymatic protection mechanisms that efficiently scavenge AOS and prevent damaging effects of free radicals. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) are involved in the scavenging of AOS. 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. Peroxidase catalyses the dehydrogenation of structurally diversed phenolic substrates by H_2O_2 and are thus often regarded as antioxidant enzymes (Shigeoka *et al*., 2002). Catalase remove the H_2O_2 produced under adverse situation (Foyer & Noctor, 2000). 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 ATONIK (nitrophenols) and its impact on abscission and yield.

MATERIALS AND METHODS

Tomato (*Lycopersicon esculentum*, Mill.) cv. PKM 1 was planted under normal conditions during 2003 in the experimental field of Tamil Nadu Agricultural University, Coimbatore (11°N; 77°E; 426.7m MSL), India. Tomato seeds were sown into a field nursery in April 2003. Seedlings were transplanted to the field, a month later. Seedlings were planted in a 60 x 45 cm pattern and a net 20 m^2 plot contained 74 plants (3 plants per 1 m^2) was maintained. The soil of the experimental field was well drained clay loam in texture having pH of 7.6 and EC of 0.31 dSm^{-1}, 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}). The experiment was performed with six replications. The plants were irrigated once in five days. Observations were made in the flowers (60 DAT) and developing fruits (70

DAT) in tomato ATONIK, a nitrophenolic compound was applied at flowering (S_1) and fruit set stage (S_2). Unsprayed plants served as control. Sampling was done after 24 h of spray.

Treatment details	
T_1	Control
T_2	Foliar spray of ATONIK 0.1 %
T_3	Foliar spray of ATONIK 0.2 %
T_4	Foliar spray of ATONIK 0.4 %
T_5	Foliar spray of ATONIK 0.8 %
T_6	Foliar spray of PCPA 50 ppm

For the estimation of auxin (IAA) and enzymes duplicate samples were taken from all the six replication (n=12). Fruit set percentage was arrived by adopting standard procedure of Villareal and Lal (1979). The first five clusters were observed to represent fruit setting percentage. The yield was arrived from the at least twenty plant from each treatment (n=20).

Enzyme assay. For activity estimations superoxide dismutase, catalase, peroxidase, IAA oxidase, and polyphenol oxidase, frozen tissue was homogenized in ice-cold 0.1 M Tris–HCl buffer at pH 7.8 containing 1mM EDTA, 1 mM dithiotreitol and 5ml of 4% polyvinyl pyrrolidone. The homogenate was filtered through a nylon mesh and centrifuged at 20,000 x*g* at 4ºC. The supernatant was used for measuring enzyme activity.

Superoxide dismutase (SOD) was determined by nitroblue tetrazolium (NBT) method of Beyer and Fridovich, (1987) 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%. Catalase (CAT) was estimated according to Teranishi (1974). One milliliter of the supernatant was added to the reaction mixture containing 1 mL of 0.1M H_2O_2 and 3 mL of 0.1 M 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. Peroxidase (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 K-phosphate buffer (pH 7.0), 0.1 mM EDTA, 10 mM guaiacol, 10 mM H_2O_2 (Racusen & Foote, 1965). Polyphenol oxidase (PPO) was quantified and expressed as change in optical density/g/min FW. The method described by Bateman and Daly (1967) was followed. IAA oxidase (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.

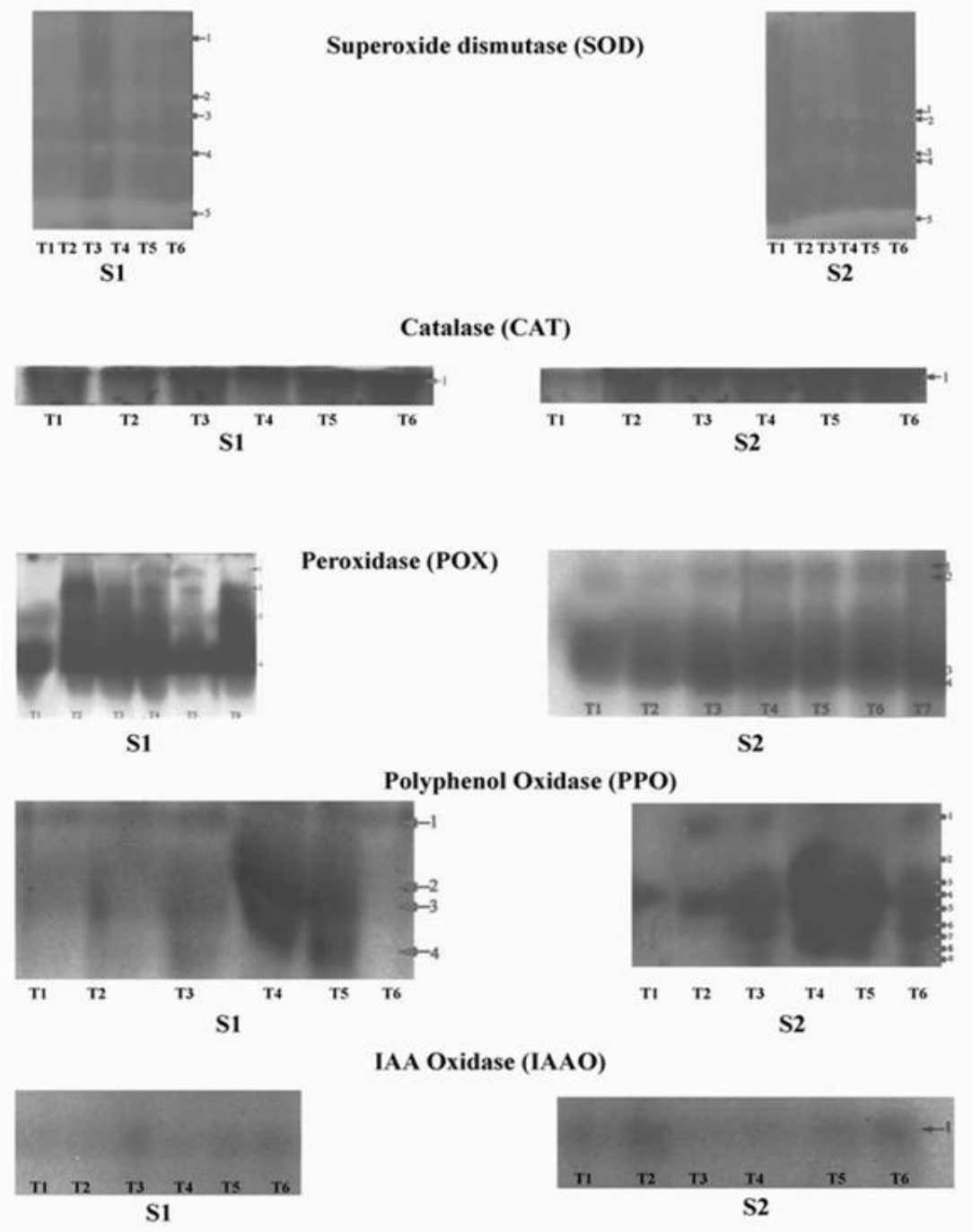
Isozyme analysis. Electrophoretic separation of isozymes was achieved with 10% native PAGE, as described by Laemmli (1970), with slight modifications as required. The methodologies of Nadlony and Sequira (1980), Jayaraman *et al*. (1987), Beau-Champ and Fridovich (1971) and Gurmeet Talwar *et al*. (1985) were followed to visualize the isoforms of peroxidase, polyphenol oxidase, superoxide dismutase, catalase and IAA oxidase, respectively.

The data were analysed statistically according to Sukhatme and Amble (1985). Duplicate sample from all the six replication were taken for all the enzyme assays (n=12). Significance between control and treatment was compared at 0.05 probability levels.

RESULTS AND DISCUSSION

Superoxide dismutase activity. In tomato, ATONIK sprayed at fruit set stage (S_2) was found superior over flowering stage (S_1) (Table I) and S_2 among the tested concentrations 0.4% ATONIK (T_4) recorded the highest enzyme activity (2.065 enzyme unit) followed by T_6 (PCPA 50 ppm). 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 superoxide dismutase revealed that S_1 and S_2 produced five isoforms each. In S1 and S_2, T_4 (application of ATONIK at 0.4%) produced maximum isoforms namely five and four, respectively (Fig. 1). During S_1, one novel form was established (SOD 1) and during S_2 two isoforms were seen (SOD 1 & SOD 2) as novel when compared with control. Superoxide dismutase (SOD) catalyses the disproportion of superoxide radicals and converts them to molecular oxygen and H_2O_2 (Srivalli & Chopra, 2001). SOD plays an important role in protecting cells against the toxic effects of superoxide radicals produced during oxidative burst (Halliwell & Gutteridge, 2000). In the ATONIK treated plants, more activity isoforms of superoxide dismutase were observed compared to control plants. This indicates the possible role of ATONIK in the retention of reproductive parts by subdued accumulation of ROS. Superoxide radicals are known to inhibit catalase and peroxidase activity and so efficient scavenging of superoxide is must for enhanced catalase and peroxidase activity. Such increased catalase and peroxidase activities as observed in ATONIK treated plants confirms role of SOD in protecting these 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 & Paulin, 1990). In ATONIK treated plants, enhanced expression and forms of SOD indicate the possible role of ATONIK in delaying the membrane detoriation during abscission. Increase in SOD activity increased the peroxidase activity by providing substrate namely H_2O_2.

Fig. 1. Effect of ATONIK on isozyme banding patterns of antioxidant enzymes and auxin catabolism enzymes in tomato



The combination of hydrogen peroxide, formed by SOD activity and O_2^-, may lead to the formation of the very active hydroxyl radicals by the Haber-Weiss reaction (Halliwell & 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.

Catalase activity. Foliar spray distinctly decreased the H_2O_2 concentration in tomato plant (Table I) over control. Among the ATONIK spray concentrations, (0.4%) was the best over other treatments by recording a significantly higher value of H_2O_2 destruction during 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 PCPA 50 ppm by showing an

Table I. Effect of ATONIK spray on SOD, CAT, POX, PPO and IAAO enzyme activity

Treatments	Superoxide dismutase (Enzyme Unit)		Catalase (mg H_2O_2 g^{-1} FW)		Peroxidase (Δ OD g^{-1} min^{-1} FW)		Polyphenol oxidase (Δ OD g^{-1} min^{-1} FW)		IAA Oxidase (µg unoxidised auxin g^{-1} h^{-1} FW)	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
T_1	1.566	1.691	0.474	0.372	0.432	0.264	0.526	0.414	300.26	358.65
T_2	1.631	1.824	0.497	0.385	0.453	0.314	0.500	0.364	370.38	443.54
T_3	1.665	1.887	0.564	0.416	0.506	0.328	0.444	0.332	407.63	474.45
T_4	1.953	2.065	0.593	0.437	0.574	0.364	0.356	0.276	428.08	523.59
T_5	1.780	1.891	0.561	0.419	0.523	0.330	0.426	0.312	416.11	494.81
T_6	1.828	1.904	0.587	0.426	0.536	0.338	0.394	0.304	419.70	501.90
CD (5%)	0.02*	0.02*	0.01*	0.03*	0.04*	0.03*	0.035*	0.023*	11.37*	11.34*

* Significance at 5% level of probability

Table II. Effect of ATONIK spray on IAA content and yield and yield components

Treatments	IAA content (ng g^{-1} FW)		Number of flower clusters $plant^{-1}$		Number of fruit clusters $plant^{-1}$		Fertility co-efficient (%)		Yield $plant^{-1}$ (g)	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
T_1	183	262	28.36	28.25	11.42	12.27	46.7	47.5	1230	1221
T_2	208	317	23.80	24.66	12.99	13.26	59.7	65.2	1395	1565
T_3	245	371	23.65	24.05	13.95	14.02	64.7	69.6	1425	1640
T_4	374	644	22.41	22.37	15.76	16.54	73.7	80.1	1606	1768
T_5	247	428	23.85	24.29	14.23	14.89	62.4	71.3	1455	1712
T_6	301	520	22.63	22.98	14.63	15.83	68.6	75.4	1512	1755
CD (5%)	14*	20*	0.09*	0.09*	0.077*	0.063*	3.5*	2.7*	18*	19*

* Significance at 5% level of probability

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 with 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 & Noctor, 2000). From the experiment, it was noticed that application of ATONIK increased the expression, indicating that the oxidative stress situation may be converted to normal condition by maintaining the redox potential. Among the treatments, ATONIK 0.4% in tomato enhanced the enzyme activity. Maintenance of catalase enzyme activity at higher level prevents the increase of cytosolic H_2O_2 or otherwise it will create toxic conditions in the plant cell leading to oxidative stress (Srivalli & Chopra, 2001). Because of greater expression and activity of catalase in ATONIK treated plants, the abscission may be reduced.

Peroxidase activity. Clear trends were noticed by ATONIK spray at 0.4% (T_4) in increasing the peroxidase activity of tomato at both S_1 and S_2 (Table I). It recorded a per cent increase of 32.8 and 7.0 and 37.8 and 7.6 over control and PCPA spray at flowering, fruit set stages, respectively. It was closely followed by T_6 (PCPA 50 ppm). 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 the experiment, it was clearly established that peroxidase activity was reduced during fruit set stage. However, ATONIK treatments could decrease the decrement compared to control treatment. Among the treatments imposed, ATONIK 0.4% in tomato increased the number of isoforms compared to other treatments (POX 3). The increase in isoforms in ATONIK treatment 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) also reported that an increase in the enzyme activity led to decrease of H_2O_2 content and lipid peroxidation thus assured fruit set.

Polyphenol oxidase activity. Among the concentrations of ATONIK, T_4 enormously decreased the polyphenol oxidase activity at fruit set stage (S_2) of tomato (Table I). When $T_1$and T_6 showed a value of 0.414 and 0.304, respectively, 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. The above said process is inhibited by polyphenols (Pedreno *et al*., 1990). Reduced polyphenol isoforms, observed in ATONIK 0.4% treated plants may 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 ATONIK 0.4% 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. The accumulation of auxin protective phenol (polyphenols) is due to the decrease in PPO activity in ATONIK 0.4% treated plants.

IAA oxidase activity. In tomato, among the concentrations of spray, ATONIK 0.4% (T_4) recorded a value of 428.08 at flowering, and 523.59 at fruit set stage (Table I). While comparing the stages of spray application of ATONIK at fruit set stage (S_2) was found to be the best by recording a higher value (unoxidised auxin) than other treatment. All the treatments at all 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). ATONIK treatments significantly decreased the IAA oxidase activity and this might be due to lower activity of low polyphenol oxidase activity (Pedreno *et al*., 1990). ATONIK has guaiacol (nitrophenol) as one of its constituent. Guaiacol being a diphenol may inhibit the IAA oxidase activity (Li *et al*., 2003).

IAA content. Among the concentrations of ATONIK foliar treatments, ATONIK 0.4% (T_4) recorded the maximum IAA content followed by T_6 (PCPA 50 ppm) at fruit set stage (Table II). The next best treatment was T_5 (ATONIK 0.8%) followed by T_3 (ATONIK 0.2%). The best treatment (T_4) recorded an increase of 145.0 and 23.7% over control and PCPA respectively at fruit set stage. The treatments differed significantly among themselves at all growth stages. Increased concentration of auxin in the cell causes increased lignification of cell wall (Ray, 1960). From the experiment, it is evident that ATONIK regulated the process of abscission by the production/synthesis of auxin. The enhanced synthesis may be due to the fact that ATONIK might have acted as an auxin precursor (Nanda *et al.*, 1971), which in turn might be reflected in more fruits retention in tomato. Upadhyay (2002) concluded that decreased flower and fruit drop may be due to creation of favourable balance of endogenous hormones. ATONIK treated plants had more content of auxin than control plants, and consequently the abscission process may have delayed. Pedreno *et al.* (1990) have shown that the abscission retarding action of auxin was primarily due to their capacity to maintain the reduced IAA oxidase. The present investigation also reveals that, the increase in auxin content in ATONIK treatment might be due to decreased IAA oxidase and polyphenol oxidase enzymes.

Fertility co-efficient and yield and yield components. The concentration of ATONIK spray treatments showed wide differences ranging from 46.7 in T_1 (control) to 80.1 in T_4 (ATONIK 0.4%) for fertility co-efficient of tomato (Table II). The superiority of T_4 treatment was very significant as the next best treatment T_6 (PCPA 50 ppm) recorded 75.4 fertility co-efficient at S_2. The number of flower clusters per plant of tomato was significantly influenced by various treatments. Spraying ATONIK at fruit set (S_2) produced a lower number of flower clusters per plant than S_1. Among the concentrations of spray, ATONIK 0.4% (T_4) produced comparatively lower number of flower clusters, followed by T_6 (PCPA 50 ppm) than other treatments. That the number of fruit clusters per plant, could vastly be improved by ATONIK spray. The treatment, T_4 was the best followed by T_6 and they recorded a value of 16.54 and 15.83, respectively. Application of ATONIK at 0.4% (T_4) at S_2, recorded significantly the highest yield of 1768 gram among ATONIK treatments, while the yield per plant in control (T_1) being only 1221. Next to T_4, PCPA 50 ppm (T_6), ATONIK 0.8% (T_5) and ATONIK 0.2% performed better over control with yields of 1755, 1712 and 1640 g, respectively. From the present study it was inferred that the yield was found to be strongly influenced by the application of ATONIK and thus, indicating the importance of this compound in increasing the yield potential through its effect on antioxidant enzymes and auxin content (Nanda *et al.*, 1971). The yielding ability in the present study has been brought out by fertility co-efficient which can able to enhance yield appreciably. The higher yield noticed in the effective treatment of ATONIK 0.4% might be due to higher anti-oxidant enzymes and auxin level.

CONCLUSION

The present study clearly indicates that application of ATONIK at 0.4% at fruit set stage (S_2) significantly increased the antioxidant enzymes *viz.* superoxide dismustase, peroxidase and catalase and lower auxin catabolic enzymes (polyphenol opxidase & IAA oxidase) which may favoured the internal auxin content that favoured increased fruit set and yield.

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(Received 28 June 2004; Accepted 29 August 2004)