Plant steroidal hormone 24-epibrassinolide regulate pathogen stress tolerance in *Solanum lycopersicum* L. by modulating antioxidant defense expression

Shummu Slathia

ABSTRACT

The present investigation illustrated antifungal efficacy of 24-epibrassinolide in the management of *Alternaria alternata* rot inflicting tomato fruits. Application of 24-epibrassinolide completely inhibited the growth of *A. alternata in vitro* as well as *in vivo*. There was a significant reduction in mycelial growth as well as conidial count of *A. alternata* after addition of 24-epibrassinolide in PDA medium as compared to control. An increase in malondialdehyde content was recorded after pathogen inoculation suggesting oxidative stress. A significant decrease in malondialdehyde content was observed in tomato fruits affected with *A. alternata* and treated with 24-epibrassinolide, with maximum decrease recorded for 10^{-9} M epibrassinolide concentration both at pre as well as post inoculation. Enhanced activities of antioxidant enzymes like superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase were recorded in response to pathogen inoculation, activities got further stimulated by the application of epibrassinolide when compared with pathogen-inoculated tomato fruits only. Similarly, non enzymatic antioxidants *viz*., ascorbic acid, glutathione and total phenols were enhanced in tomato fruits inoculated with *A. alternata* and treated with -epibrassinolide, revealing its positive role in disease resistance as observed by a decrease in rot percentage. Results, thus, demonstrate practical efficacy of 24-epibrassinolide as a plant-based antifungal product for post harvest application.

Keywords: Solanum lycopersicum, 24-epibrassinolide, malondialdehyde, antioxidant enzymes and non-enzymatic antioxidants.

Abbreviations –ASA, ascorbic acid; CAT, catalase; FW, fresh weight; GPOX, guaiacol peroxidase; GR, glutathione reductase; IN, inoculated; MDA, malondialdehyde; PDA, potato dextrose agar; ROS, reactive oxygen species;; SOD, superoxide dismutase; TBA, thiobarbituric acid; TCA, trichloroacetic acid; TPC, total phenol content

I INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops cultivated all over the world for its fleshy fruits (Beckles 2012). However, tomato fruits are also highly perishable and susceptible to a number of

micro-organisms that causes post harvest decay and are nearly ubiquitous in nature (El Katatny and Emam 2012). Harvested tomato fruits carry heavy spore loads while in the field. As a result of poor packaging and improper arrangement, the fruits get bruised and squeezed allowing different types of rots to develop when the favourable growth conditions are available (Sharma et al., 2009). Of the entire range of fungi that have been isolated as causal agents of tomato fruit rot *A. alternata* (Fr.) Keissler is a major storage decay agent (Barkai-Golan 1973; Chary et al., 1980; Samyal and Sumbali, 2002; Waller et al., 2002). At times 80% of the overall decay in the stored tomatoes is caused by this particular fungus (Zilter and Ween 1984).

Generally, to restrict or prevent pathogen incursion by the fungal pathogens, host elicits a broad spectrum of innate immunity. Upon sensing the invading pathogen, plants mount a set of general defense reactions, such as cell wall reinforcement, accumulation of antimicrobial proteins (pathogenesis-related proteins) and production of phytoalexins (Kombrink and Kaburaki 1995). Plants also produce diverse secondary metabolites including phenolics with a prominent function of curbing the microbial pathogens (Sudha and Ravishankar 2002). The generation of and protection against reactive oxygen species (ROS) are intrinsic traits of any living cell. In normal conditions, ROS appear as inevitable by-products, formed as a result of reduction of molecular oxygen in chloroplasts and mitochondria (Asada 2002). The low levels of ROS inside the cells are maintained by the relevant mechanisms using compartmentalized isozymes of catalase (CAT), superoxide dismutase (SOD) and/or peroxidase (POX) (Mehlhorn et al., 1996). Improvement in CAT and SOD activities occurs in *Fusarium*-infected chickpea as compared with control (Garcia-Limones et al., 2002). Similarly, Zhu et al. (2010) also observed enhancement in the activity of SOD, CAT, polyphenol oxidase and phenyl ammonia lyase in jujube fruits inoculated with *Penicillium expansum*. An increase in total phenols in tomato roots infected with *F. oxysporum* also occurred (Mandal and Chakraborty 2010).

Several researchers have engaged themselves to control post harvest diseases of fruits and vegetables. One of the most effective measures to control the disease caused by various pathogens is the effective application of fungicides (Omar et al., 2006). However, there are two major concerns: the increasing consumer concern over pesticide residues on foods, the predominance of fungicide-resistant strains due to excessive use of fungicides (Naseby et al., 2000). Moreover, some synthetic fungicides are non-biodegradable and hence can accumulate in the soil, plants and water and consequently effects the humans through food chain (Tapwal et al., 2011). Therefore, there is a need for new effective means of post-harvest disease control that poses less risk to human health and environment.

Brassinosteroids (BRs) have recently been recognized as a new class of phytohormones occurring ubiquitously in the plant kingdom (Clouse and Sasse 1998). They control a broad range of processes, including seed germination, stem elongation, cell division and expansion, xylem differentiation, plant growth and apical dominance (Sasse 2003). In addition to its critical role in growth regulation and photomorphogenesis, BRs can induce plant response to a variety of abiotic stresses, such as high and low temperature, drought and salinity injury (Brosa 1999; Slathia et al., 2012). The potential role of BRs in pathogen defense has also been the topic of recent studies. BR-induced disease resistance was noted in barley, potato tubers, and cucumber plants (Khripach et al., 2000). Field application of 24-

epibrassinolide to barley plants significantly decreased the extent of leaf disease induced by mixed fungal infection, along with an increase in crop yield (Pshenichnaya et al., 1997). Recently Zhu et al. (2010) studied the effect of BRs against blue mould rot caused by *Penicillium expansum* in jujube fruits. BRs effectively inhibited development of blue mould and enhanced the activities of defense related enzymes such as phenyl ammonia lyase (PAL), polyphenol oxidase (PPO), catalase (CAT) and superoxide dismutase (SOD). However, to our knowledge, little information is available on the effects of BRs on resistance of fruits against pathogenic fungi.

The aim of the present study was to evaluate the effect of 24-EBL application to tomato fruits infected with *A. alternata* and changes in antioxidant system of tomato fruits.

II MATERIAL AND METHODS

2.1 Survey of vegetable markets and isolation of *A. alternata* from diseased tomato fruits

The various vegetable markets were surveyed for the collection of visibly cracked and bruised tomato fruits in pre-sterilized polythene bags. *A. alternata* was isolated from visibly infected fruits after incubating them at 28 ± 2°C for three days. The purified cultures were maintained in duplicates on sterilized potato dextrose agar (PDA). The Koch's postulates were performed for testing the pathogenicity of *A. alternata*.

2.2 Antifungal efficacy of 24-epibrassinolide (24-EBL)

Brassinosteroid used in present study was 24-epibrassinolide (24-EBL). The different concentrations of EBL were prepared from stock solution of 10^{-5} M, previously prepared from 10^{-3} M EBL prepared in DMSO (Dimethyl sulphoxide). The concentration of 10^{-7}, 10^{-9} and 10^{-11} M EBL were prepared by serial dilution of the parent stock solution.

2.3 *In vitro* antifungal efficacy of 24-EBL

The antifungal efficacy of 24-EBL was performed against *A. alternata* by the poisoned food technique (Perucci et al., 1994). Requisite amounts of 24-EBL were added separately to plates containing 0.5 ml 5% Tween 20 and 9.5 ml molten PDA. Likewise, control sets were prepared using equal amount of distilled water replacing EO. The prepared plates were inoculated aseptically with discs of test fungus and incubated at 28±2°C for 7 days. The percentage of mycelial inhibition was calculated by using the formula

$$\text{Percentage mycelial inhibition} = \frac{dc-dt}{dc} \times 100$$

Where dc is the mean mycelial growth diameter of colony of control sets

dt is the mean mycelial growth diameter of colony of treatment sets

Calculation of percent reduction in number of spores

Reduction in the number of spores by application of EBL was done by counting the number of spores in haemocytometer.

2.4 *In vivo* antifungal efficacy of 24-EBL

2.4.1 Preparation of spore suspension of *A. alternata*

Five-day-old cultures of *A. alternata* were flooded with 10 mL of sterilized distilled water. The spores were rubbed from the surface of Petri dish and spore density was calculated by using haemocytometer to obtain a uniform suspension of $1x10^5$ spores mL^{-1}.

2.4.2 Pre inoculation treatment

To analyse the efficacy of various treatments by pre inoculation treatment, healthy fruits were weighed and surface sterilized with 1% sodium hypochlorite solution and then dried under aseptic conditions and rinsed with 70% alcohol. Pre-weighed tomato fruits prior to inoculation were dipped in the respective solutions for 30 mins. Treated fruits were then incubated at 25±2°C for 12hrs and thereafter inoculated with 5µl of spore suspension ($1x10^5$spores/ml) of *A. alternata*. Inoculated tomato fruits were then incubated in sterilized polythene bags for 3 days 28±2°C, R.H. 90%.

2.4.3 Post inoculation treatment

To analyse the efficacy of various treatments by post inoculation treatment, healthy fruits were weighed and surface sterilized with 1% sodium hypochlorite solution and then dried under aseptic conditions and rinsed with 70% alcohol. Pre-weighed tomato fruits prior to treatment were inoculated with 5µl of spore suspension. Inoculated fruits were then incubated at 25±2°C for 12hrs and thereafter treated with respective solutions. The tomato fruits were then incubated in sterilized polythene bags for 3 days 28±2°C, R.H. 90%.

2.4.4 Calculation of percent rot development and percent rot control

After 3 days of incubation percentage rot was calculated by using the formula:

$$\text{Percentage rot} = \frac{(W-w)}{W} \times 100$$

Where, W= Weight of the fruit before inoculation

w= Weight of the fruit after removal of rotten tissue

Similarly, percentage rot control was evaluated by using the formula:

Percentage control= % decay in untreated fruit- % decay in treated fruit x 100

% decay in untreated fruit

2.5 Biochemical estimation of various physiological parameters

2.5.1 Lipid peroxidation

The peroxidation of lipids was estimated according to the method of Heath and Packer (1968). Briefly, tomato fruits [0.5 g fresh weight (FW)] were homogenized in 3 mL of 0.1% trichloroacetic acid (TCA) as previously described in Choudhary *et al.* (2011).

2.5.2 Protein content and antioxidant enzymes

2.5.2.1 Preparation of fruit extract

The fruit tissue (0.5 g FW) was homogenized in 3 mL of 0.1 M potassium phosphate buffer using pre-chilled pestle and mortar, then centrifuged at 15,000 rpm for 20 min. Supernatants obtained were used for the estimation of protein contents and activities of the antioxidant enzymes.

2.5.2.2 Protein content

The protein content was estimated according to method of Lowry et al. (1974).

2.6.2.3 Estimation of enzymatic activities

The activities of superoxide dismutase, guaiacol peroxidase, catalase, ascorbate peroxidase and glutathione reductase were estimated by following the methods of Kono (1978), Putter (1974), Aebi (1983), Nakano and Asada (1981), and Nordhoff et al. (1993) respectively.

2.7 Non-enzymatic antioxidants

The estimation of glutathione, total phenols, ascorbic acid and proline contents was done by following the methods of Sedlak and Lindsay (1968), Ragazzi and Veronese (1973) and Cakmak and Marschner (1992) and Bates et al. (1973) respectively.

2.8 Statistical analysis

All the experiments were performed in triplicate. Data shown are the means of three replicates along with standard error (n = 3). Student's t-test was carried out and data were presented at $p \leq 0.05$. All the statistical calculations were performed using IBM SPSS 20.0 software.

III RESULTS

3.1 Survey of fruit and vegetable markets for isolation of *A. alternata*.

The various vegetable markets were surveyed for the collection of visibly cracked and bruised tomato fruits in pre-sterilized polythene bags. The *A. alternata* was isolated from visibly infected fruits after incubating them at 28 ± 2°C for three days. A total of 22 isolates were recovered from bruised tomato fruits. The purified cultures were maintained in duplicates on sterilized potato dextrose agar (PDA). The Koch's postulates were performed for testing the pathogenicity of *A. alternata*. All the isolates were able to cause rot in healthy tomato fruits with A19 (CHA) being the most destructive one (Fig. 1). Thus, this particular isolate was used for further experiments.

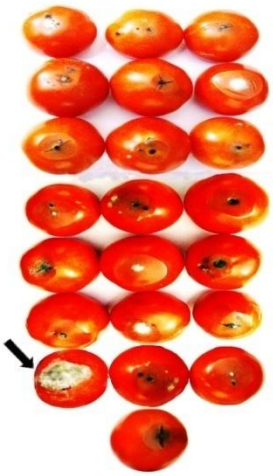

Figure-1 Tomato fruits inoculated with different isolates of *Alternaria alternata*

3.2 *In vitro* antifungal efficacy of 24-EBL

24-EBL significantly decreased mycelial growth of *A. alternata* at all applied concentrations with maximum reduction of at 10^{-7} M. Moreover, there was also a reduction in number of conidia of tested pathogen after application of 24-EBL (Fig. 2).

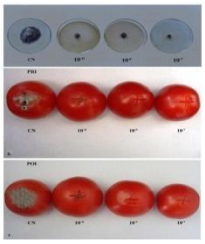

Figure-2 Effect of different concentrations (10^{-7}, 10^{-9} and 10^{-11} M) of 24-epibrassinolide (24-EBL) on mycelial inhibition of *Alternaria alternata* (a and b) and severity of black rot caused by *A. alternata* (c and d) in tomato fruits.

3.3 *In vivo* antifungal efficacy

3.3.1 Effect of 24-EBL on Percent rot development and percent rot control of tomato fruits

Inoculation of *A. alternata* caused a significant rot development in tomato fruits as compared with CN fruits. Treatment with 24-EBL both at PRI and POI caused a significant reduction in rot development. The results related to percent rot development and control are given in Fig. 2. Maximum reduction was recorded at 10^{-7} M followed by 10^{-9} M and 10^{-11} M as compared with IN fruits after 72 hours after inoculation (hi) in PRI. Similarly, after POI application there was a significant reduction at all applied concentrations over IN. Moreover, rot development at 10^{-11} M was significant from 10^{-7} M and 10^{-9} M which were insignificant from each other. Revealing a positive role of 24-EBL in reducing rot development, there was a significant rot control with maximum protection at 10^{-7} M (9.58-fold) followed by 10^{-9} M (9.42-fold) and 10^{-11} M (8.42-fold) as compared with inoculated control in pre inoculation application. Similar trend of rot control was observed after POI application (Fig. 3).

3.3.2 Effect of 24-EBL on Lipid peroxidation (malondialdehyde (MDA).

Application of 24-EBL caused a significant reduction in MDA at PRI as well as POI application. Maximum reduction of 9.24-fold was recorded at 10^{-9} M followed by 10^{-7} M (8.58-fold) and 10^{-11} M (8.43-fold) when compared with fruits inoculated with *A. alternata*. Similarly, there was a reduction in MDA content after POI application but trend was different with maximum reduction at 10^{-9} M (9.33-fold) as compared with inoculated fruits after 72hi (Fig. 3).

3.3.3 Effect of 24-EBL on Protein content (PR)

A significant reduction in PR content was observed in IN fruits over CN. Insignificant changes were recorded at 10^{-7} M both at PRI as well as POI application as compared with IN fruits after 72hi. On the other hand, PR content at PRI as well as POI significantly increased at two concentrations. Maximum enhancement 5.82- and 6.20-fold was recorded at 10^{-9} M followed by 10^{-11} M (2.54- and 4-fold) at PRI and POI respectively after 72hi (Fig. 3).

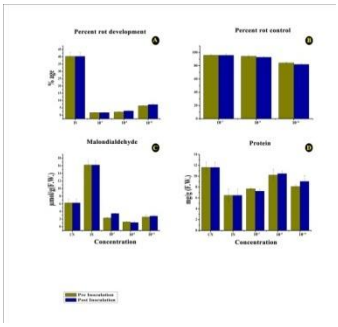

Figure-3 Effect of different concentrations (10^{-7}, 10^{-9} and 10^{-11} M) of 24-epibrassinolide (24-EBL) on percent rot development (A), percent rot control (B), malondialdehyde (C) and protein (D) in tomato fruits with (IN) or without (CN) inoculation of *Alternaria alternata*.

3.3.4 Effect of 24-EBL on various antioxidant enzymes

3.3.4.1 Superoxide dismutase (SOD)

There was an enhancement in SOD activity in inoculated tomato fruits that suggests its role in detoxification of ROS that are produced in stressed conditions. EBL acted as enhancer of SOD activity and the maximum increase was recorded at 10^{-9} M (12.37-fold) followed by 10^{-7} M (6.76-fold) as compared with IN tomato fruits after 72hi. The results were similar after POI application with maximum increase at 10^{-9} M (10.90-fold) followed by 10^{-7} M (9.64-fold) when compared to IN fruits after 72hi (Fig. 4).

3.3.4.2 Guaiacol peroxidase (GPOX)

Application of 24-EBL significantly enhanced GPOX activity at all applied concentrations with maximum value at 10^{-7} M (124.58-fold) followed by 10^{-11} M (96.88-fold) and 10^{-9} M (87.5-fold) when compared with IN fruits. Statistically, there was a significant difference within different concentrations of EBL tested. Likewise, there was an increase in GPOX activity after POI application with maximum enhancement at 10^{-7} M (87.12-fold) and minimum increase at 10^{-9} M (72.5-fold) after 72hi over IN fruits (Fig. 4).

3.3.4.3 Catalase (CAT)

CAT activity in tomato fruits (CN and IN) and exposed to different concentrations of 24-EBL are given in Fig. There was a significant enhancement in CAT activity in tomato fruits inoculated with *A. alternata* when compared with CN. Application of EBL further elevated CAT activity at all applied concentrations with maximum increase at 10^{-7} M (7.15-fold) followed by 10^{-11} M (5.92-fold) and 10^{-9} M (5.09-fold) over IN fruits in PRI application after 72hi. On the other hand, after POI application there was a significant enhancement in CAT activity only at 10^{-7} M (8.81-fold) (Fig. 4).

3.3.4.4 Ascorbate peroxidase (APOX)

Inoculation of *A. alternata* enhanced APOX activity over CN. In PRI, maximum enhancement was recorded at 10^{-9} M (10.21-fold) followed by 10^{-11} M (6.03-fold) and 10^{-7} M (5.25-fold) after 72hi as compared with IN fruits. After POI application significant changes were noticed only at 10^{-9} (8.26-fold) as compared to IN fruits (Fig. 4).

3.3.4.5 Glutathione reductase (GR)

No significant changes were recorded for GR activity after inoculation of *A. alternata* as compared to CN fruits. Further application of EBL showed significant enhancement in GR activity after PRI as well as POI. Maximum increase 18.86-fold was recorded at 10^{-9} M followed by 9.02-fold at 10^{-7} M as compared IN after 72hi. There was a significant difference between different concentrations of EBL tested. Similar trend of GR was observed after POI application (Fig. 4).

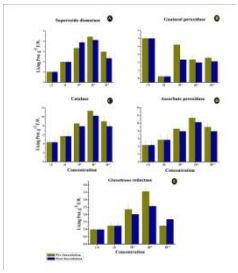

Figure-4 Effect of different concentrations (10^{-7}, 10^{-9} and 10^{-11} M) of 24-epibrassinolide (24-EBL) on the activities of superoxide dismutase (A), guaiacol peroxidase (B), catalase (C), ascorbate peroxidase (D) and glutathione reductase (E) in tomato fruits with (IN) or without (CN) inoculation of *Alternaria alternata*.

3.4.5 Effect of 24-EBL non-enzymatic antioxidants

3.4.5.1 Ascorbic acid (ASA)

There was a stimulatory effect of EBL on ASA content after both PRI as well as POI applications. A 10.89-fold increase was recorded at 10^{-9} M followed by 4.97-fold at 10^{-7} M over IN fruits. Significance was also checked within different concentrations tested. There was a significant in between all the concentrations. A similar trend in GR activity was recorded after POI application of EBL with maximum increase recorded at 10^{-9} M (7.47-fold) (Fig. 5).

3.4.5.2 Total phenols (TPC)

There was a rapid accumulation of TPC content after inoculation of *A. alternata* as compared with CN. Furthermore, application of EBL significantly enhanced TPC content at all applied concentrations of EBL. Maximum rise was recorded at 10^{-7} M (6.04-fold) followed by 10^{-9} M (4.80-fold) when compared to IN fruits after 72hi. In POI application there was a significant enhancement only at 10^{-7} M (9.15-fold) as compared with IN fruits (Fig. 5).

3.4.5.3 Proline (PL)

Significant rise in PL content in tomato fruits inoculated with *A. alternata* in comparison to healthy control fruits was observed which further significantly increased at 10^{-9} M (10.89-fold) and 10^{-7} M (4.97-fold). After POI application there were significant changes only at 10^{-9} M (7.64-fold) as compared to IN fruits (Fig. 5).

3.4.5.4 Glutathione (GSH)

GSH content in inoculated tomato fruits reduced while as application of 24-EBL significantly and remarkably enhanced GSH content as compared to IN, with maximum increase in GSH content (33.50-fold) at 10^{-9} M followed by 22.15-fold at 10^{-11} M and 12.79-fold at 10^{-7} M as compared to IN fruits. POI application of EBL also enhanced GSH with highest increase for 10^{-9} M (25.98-fold) followed by 10^{-11} M (16.15-fold) and 10^{-7} M (14.67-fold) (Fig. 5).

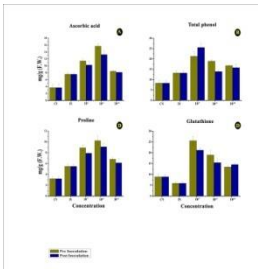

Figure-5 Effect of different concentrations (10^{-7}, 10^{-9} and 10^{-11} M) of 24-epibrassinolide (24-EBL) on ascorbic acid (A), total phenol (B), proline (C) and glutathione (D) in tomato fruits with (IN) or without (CN) inoculation of *Alternaria alternata*.

IV DISCUSSION

Although BRs have been implicated in a broad range of stress responses in plants relatively less is known about their role in pathogen response (Zhu et al., 2010). In our study, EBL application to fruits of tomato significantly increased the resistance to *A. alternata* as evidenced by a decreased severity of *Alternaria* rot. This result confirmed the role of BRs in plant response to pathogen attack. Our results were in consistent with the results of Nakashita et al. (2003) BRs induce resistance in tobacco plants infected with tobacco mosaic virus, *Pseudomonas syringaem* and *Oidium* sp. In rice, BRs induced resistance to *Magnaporthe grisea* and *Xanthomonas oryzae*, which cause rice blast and bacterial blight, respectively Zhu et al. (2010) also noticed that application of BRs caused a significant attenuation in decay in jujube fruits infected with *P. expansum*.

We also noticed a significant modulation in antioxidant enzymes (SOD, GPOX, CAT, APOX and GR) in tomato fruits after application of EBL at PRI as well as POI which are the main detoxifying enzymes in plant cells (Gara et

al., 2003). Enhancement in the activities antioxidant enzymes were suggested as a factor contributing to EBL-induced resistance (Khirpach et al., 2000). These antioxidant enzymes works synergistically to promote the scavenging of ROS produced during abiotic and/or biotic stress (Kuzniak and Sklodowska 2001). Among the antioxidant enzymes, SOD forms the first line of defense against ROS as it dismutates superoxide anion to hydrogen peroxide. Thereafter, the harmful hydrogen peroxide is neutralized by CAT, POD and APX to water and oxygen (Choudhary et al., 2012). Zhu et al. (2010) noticed an enhancement in activities of CAT and SOD enzymes in jujube fruits infected with *P. expansum*. However, in the study of Ding et al. (2009) there was significant enhancement in activities of SOD, CAT, GPOX and APOX along with MDA content in cucumber plants infected with *F. oxysporum* which were greatly attenuated by the application of EBL.

Likewise, various non-enzymatic antioxidants (ASA, GSH and TPC) evaluated in tomato fruits inoculated with *A. alternata* and treatment with 24-EBL, showed a rising trend. Presumably, their accumulation helped in decimating the negative effects of oxidative stress as previously observed in the studies of Noctor and Foyer (1998). Amongst non-enzymatic antioxidants, rapid production of phenols at infection site depicts initial activation of defense mechanism in plants thereby restricting or slowing down pathogen growth (Matern and Kneusal 1988) In addition, phenols also restrict the growth of invading pathogen by binding with hydrolytic enzymes released by fungal pathogens during cell division (Schlosser, 1994; Dai et al., 1995). Protective activities of BRs against plant diseases have been indicated based on evaluations from field trials and greenhouse experiments, but its mechanism at the molecular level remains to be clarified (Ding et al., 2009).

In conclusion, application of 24-EBL at all applied concentrations reduced post harvest decay of tomato fruits caused by *A. alternata*. The mechanism by which 24-EBL induced resistance in tomato fruits might be due to the innate antioxidant system which was elevated after application of 24-EBL. Moreover, the present study is of importance not only for basic understanding of the role of the hormone, this conventional approach of using BRs to manage various post harvest losses without any adverse effects on the consumer's health can be extended to other post-harvest rots of fruits and vegetables.

Perusal of literature did not reveal any such information on use 24-EBL to control *A. alternata* rot of tomato fruits. Thus, this report constitutes first report of use of 24-EBL to control pathogen stress in tomato fruits.

REFERENCES

[1.] Aebi, H.E., (1983). Catalase In: Methods of enzymatic analysis. Verlag Chemie, Weinhan 4, 273-282.

[2.] Akhtar, K.P., Matin. M., Mirza. J.H., Shakir. A.S., Rafique, M., et al., (1994). Some studies on the post harvest diseases of tomato fruits and their chemical control. Pakistan Journal of Phytopathology 6: 125-129.

[3.] Asada, K., (1992). Ascorbate peroxidase- a hydrogen peroxidase- scavenging enzymes in plants. Physiologia Plantarum, 85, 235-241.

[4.] Barkai-Golan, R., (1973). Postharvest heat treatment to control *Alternaria alternata* Auct. rot in tomato. Phytopathology Mediterririan, 12, 108-111.

[5.] Bates, L.S., Waldren, R.P., Teare, I.D., et al., (1973). Rapid determination of free proline for water stress studies. Plant Soil, 39, 205-208.

[6.] Beckles, M.D., (2012). Factors affecting the postharvest soluble solids and sugar content of tomato (*Solanum lycopersicum* L.) fruit. Postharvest Biology and Biotechnology, 63, 129-140.

[7.] Brosa, C., (1999). Biological effects of brassinosteroids. Journal of Biochemical and Molecular Biology, 34, 339-358.

[8.] Cakmak, I., & Marschner, H., (1992). Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase and glutathione reductase in bean leaves. Plant Physiology, 98, 1222-1227.

[9.] Chary, S.I., Kumar, P.B.K., Reddy, S.M., et al., (1980). Hitherto unrecorded postharvest diseases of tomato. Indian Phytopathology, 33, 625-629.

[10.] Choudhary, S.P., Bhardwaj, R., Gupta, B.D., Dutt, P., Gupta, R.K., Kanwar, M., Biondi, S., et al., (2011). Enhancing effects of 24-epibrassinolide and putrescine on the antioxidant capacity and free radical scavenging activity of *Raphanus sativus* seedlings under Cu ion stress. Acta Physiologia Plantarum, 33, 1319-1333.

[11.] Choudhary, S.P., Yu, J.Q., Shinozaki, K.Y., Shinozaki, K., Tran, L.S.P., (2012). Benefits of brassinosteroid crosstalk. Trends in Plant Science, 17, 594-605.

[12.] Clouse, S.D., & Sasse, J.M., (1998). Brassinosteroids: essential regulators of plant growth and development. Annual Review of Plant Physiology and Plant Molecular Biology, 49, 427-451.

[13.] Dai, G.H., Andary, C., Modolot-Cossan, L., Boubals, D., et al., (1995). Involvement of phenolic compounds in the resistance of grapevine callus to downy mildew (*Plasopara viticola*). European Journal of Plant Pathology, 101, 541-547

[14.] Ding, J., Shi, K., Zhou, Y.H., et al., (2009). Effects of root and foliar applications of 24-epibrassinolide on *Fusarium* wilt and antioxidant metabolism in cucumber Roots. Horticulture Science, 44, 1340-1345.

[15.] El-Katatny, M.H., & Emam, A.S., (2012). Control of postharvest tomato rot by spore suspension and antifungal metabolites of *Trichoderma harzianum*. Journal of Microbiology Biotechnology and Food Science, 1, 1505-1528.

[16.] Gara, L.D., Pinto, M.C., Tommasi, F., et al., (2003). The antioxidant systems vis-a-vis reactive oxygen species during plant-pathogen interaction. Plant Physiology and Biochemistry, 41, 863-870.

[17.] Garcia-Limones, C.A., Hervas, J.A., Navas-Cortes, R.M., Jimenez-Diaz, M., et al., (2002). Induction of an antioxidant enzyme system and other oxidative stress markers associated with compatible and incompatible interactions between chickpea (*Cicer arietinum* L.) and *Fusarium oxysporum* f. sp. Ciceris. Physiology and Molecular Plant Pathology, 61, 325-337.

[18.] Heath, R., & Packer, L., (1968). Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics, 196, 385-395.

[19.] Khirpach, V., Zhabinskii, V., De Groot, A., et al., (2000). Twenty years of brassinosteroids: steroidal plant hormones warrant better crops for the XXI century. Annals of Botany, 86, 441-447.

[20.] Kono, Y., (1978). Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. Archives of Biochemistry and Biophysics, 186, 189-195.

[21.] Kuzniak, E., & Sklodowska, M., (2001). Ascorbate, glutathione and related enzymes in chloroplasts of tomato leaves infected by *Botrytis cinerea*. Plant Science, 160, 723-731.

[22.] Lowry, O.H., Rosebrough, N.J., Farr, A.L., et al., (1951). Protein measurement with the folin phenol reagent. Journal of Biological Chemistry, 193, 265-275.

[23.] Mandal, S.M., Chakraborty, D., Dey, S., et al., (2010). Phenolic acids act as signaling molecules in plant-microbe symbioses. Plant Signal Behavior 5: 359-368.

[24.] Mehlhorn, H., Lelandaism M., Korth, H., Foyer, C., et al., (1996). Ascorbate is the natural substrate for plant peroxidases? FEBS Letters, 378, 203-206.

[25.] Nakano, Y., & Asada, K., (1981). Hydrogen peroxide is scavenged by ascorbate- specific peroxidase in spinach chloroplast. Plant Cell and Physiology, 22, 867-880.

[26.] Nakashita, H., Yasuda, M., Nitta, T., Asami, T., Fujioka, S., Arai, Y., Sekimata, K., Takatsuto, S., Yamaguchi, I., Yoshida, S., et al., (2003). Brassinosteroid functions in a broad range of disease resistance in tobacco and rice. Plant Journal, 33, 887-898.

[27.] Naseby, M.A.S., Abo-Elyousr, K.A.M., Hassan, M.A.E., et al., (2008). Evaluation of *Trichoderma* species as biocontrol agents for damping-off and wilt diseases of *Phaseolus vulgaris* L. and efficacy of suggested formula. Egyptian Journal of Phytopathology, 36, 81-93.

[28.] Noctor, G., & Foyer, C., (1998). Ascorbate and glutathione: keeping active oxygen under control. Annual Review of Plant Physiology and Molecular Biology, 49, 249-279.

[29.] Nordhoff, A., Buecheler, U.S., Werner, D., Schirmer, R.H., et al., (1993). Folding of the four domains and dimerization are impaired by the Gly446. Glu exchange in human glutathione reductase. Implications for the design of antiparasitic drugs. Biochemistry, 32, 4060-4066.

[30.] Omar, R., Neil, T.M.O., Rossall, S., et al., (2006). Biological control of *Fusarium* crown and root rot of tomato with antagonistic bacteria and integrated control when combined with the fungicide carbendazim. Plant Pathology, 55, 92-99.

[31.] Perrucci, S., Mancianti, F., Ciont, P.L., Flamini, G., Morelli, I., Macchioni G., et al., (1994). *In vitro* antifungal activity of essential oils against some isolates of *Microsporum canis* and *M. gypseum*. Planta Medica, 60, 184-187.

[32.] Pshenichnaya, L.A., Khripach, V.A., Volynets, A.P., Prokhorchik, R.A., Manzhelesova, N.E., Morozik, G.V., et al., (1997). Brassinosteroids and resistance of barley plants to leave diseases. In: Problems of Experimental Botany, Belorussian Science, Minsk, pp. 210-217.

[33.] Putter, J., (1974). Peroxidase In: Bergmeyer HU (ed), Methods of Enzymatic Analysis (Ed. HU Bergmeyer) Verlag Chemie, Weinhan pp. 685-690.

[34.] Ragazzi, E., & Veronese, G., (1973). Quantitative analysis of phenolics compounds after thin-layer chromatography separation. Journal of Chromatography, 77, 369-375.

[35.] Samyal, S., & Sumbali, G., (2002). Post-harvest mycopathogenic loss of tomato fruits in Jammu. Journal of Indian Botanical Society, 81, 105-108.

[36.] Sasse, J.M., (2003). Physiological actions of brassinosteroids: an update. Journal of Plant Growth Regulation, 22, 276-288.

[37.] Schlosser, U.C., (1994). SAG-Sammlung von Algenkulturen at the University of Gottingen. Catalogue of strains 1994. Botanica Acta, 107.

[38.] Sedlak, J., & Lindsay, R.H., (1968). Estimation of total protein, protein bound and non-protein sulphahydral groups in tissue with Ellmans reagent. Analytical Biochemistry, 25, 192-205.

[39.] Sharma, R.R., Singh, D., Singh, R, et al., (2009). Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review. Biological Control, 50, 205-221.

[40.] Slathia, S., Sharma, A., Choudhary, S.P., et al., (2012). Influence of exogenously applied epibrassinolide and putrescine on protein content, antioxidant enzymes and lipid peroxidation in *Lycopersicon esculentum* under salinity stress. American Journal of Plant Sciences, 3, 714-720.

[41.] Sudha, G., & Ravishankar, G.A., (2002). Involvement and interaction of various signaling compounds on the plant metabolic events during defense response, resistance to stress factors, formation of secondary metabolites and their molecular aspects. Plant Cell, Tissue Organ Culture, 71, 181-212.

[42.] Tapwal, A., Garg, N.S., Gautam, N., Kumar, R., et al., (2011). *In vitro* antifungal potency of plant extracts against five phytopathogens. Brazilian Journal of Biology and Technology, 54, 1093-1098.

[43.] Waller, J.M., Lenne, J.M., Waller, S.J., et al., (2002). Plant pathologist's pocketbook, 3rd edition. pp.156.

[44.] Zhu, Z., Zhang, Z., Qin, G., Tian, S., et al., (2010). Effects of brassinosteroids on post-harvest disease and senescence of jujube fruit in storage. Post Harvest Biology and Biotechnology, 56, 50-55.

[45.] Zilter, T.A., & Wein, H.C., (1984). Outbreak of *Alternaria alternata* causing fruit rot of tomatoes in upstate New York. Plant Disease, 68, 628.