THE PLANT DEFENCE ACTIVATOR BENZO-(1,2,3)-THIADIAZOLE-7-CARBOTHIOIC ACID *S*-METHYL ESTER INDUCES SYSTEMIC RESISTANCE TO POWDERY MILDEW IN OKRA

Emeran A. A.[1]
Agric. Botany, Fac. of Agric, Kafr El-Sheikh University
E-mail: emeranaa@yahoo.com

ABSTRACT

Okra powdery mildew is one of the most destructive foliar disease of malvaceae. Systemic acquired resistance in okra (*Abelmoschus esculentus* (L.) Moench) is induced by benzo-(1, 2, 3)-thiadiazole-7-carbothioic acid *s*-methyl ester (BTH). The response is manifested by an increase in the protective effect against the virulent pathogen *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll., by increasing peroxidase, polyphenol oxidase, superoxide dismutase and phenylalanine ammonia lyase activities, and by increasing accumulation of total phenolics. In greenhouse, best protective effect (89.51%) by BTH treatment was achieved by using BTH at 0.1 mM. An increase in protective effect was apparent when okra plants were inoculated 5 days after BTH treatment, but it was more pronounced when inoculation was performed 30 days after BTH treatment. In field experiments, the results were similar and in the same trend in the both seasons 2005 and 2006. Results revealed the high protective effect of BTH was similar to the wettable sulphur 0.3% (80 WP, Ciba). Due to the protective effect of BTH, pods okra yield reached 7.6 ton feddan^{-1} in 2006.

Keywords; Okra, powdery mildew, BTH

INTRODUCTION

Okra (*Abelmoschus esculentus* (L.) Moench, family Malvaceae)) is a heat-loving vegetable that originated in the Nile basin in Egypt (Ames & MacLeod 1990). The world-wide production is estimated at round 4 million tons (Siemonsma 1982). In Egypt, a total of 16819 feddan are cultivated with okra, producing about 11238 tons marketable pods (Mousa *et al.*, 2007). Powdery mildew caused by *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll., is considered the most important disease attacking okra in Delta (Ghoniem, 1985). Its epidemic form covers all parts of the plant with white floury patches;

thereby adversely affecting the photosynthetic activity of the plants which in turn reduces yield as well as its market price thus causing enormous economic loss to farmers. Till now, there is no source of resistance to powdery mildew in *A. esculentus* (Joi & Shende, 1979: Neeraja *et al.,* 2004 and Raj *et al.*, 1993). Since okra is harvested every alternate day, the dependence on fungicides results in several ecological problems (Parveen & Dhandapani, 2002).Therefore, in addition to existing disease strategies, new approaches are being explored to suppress diseases through eco-friendly means as systemic acquired resistance inducers.

Systemic acquired resistance (SAR) is an inducible defence mechanism that plays a central role in disease resistance (Hammerschmidt & KucH 1995). Certain safety chemicals such as phosphate salts, isonicotinic acid, jasmonic acid, salicylic acid (SA), 2, 6-dichloroisonicotinic acid (INA), and b-aminobutyric acid, can induce SAR in many pathogens-plant system (Cohen *et al.*, 1994; Hammerschmidt & KucH, 1995; Lawton *et al.*, 1996 and Siegrist *et al.*, 1997).

Recent studies have shown the effectiveness of benzo-(1,2,3)-thiadiazole-7-carbothioic acid *S*-methyl ester (BTH) as a novel crop protecting agent which does not itself have anti-microbial properties, but instead increases crop resistance to disease by activating the SAR signal transduction pathway (Benhanou & Belanger, 1998; Cole 1999 and Gorlach *et al.*, 1996). BTH was able to prime key defense genes such as phenylalanineammonia lyase (PAL); PR proteins viz., chitinase (CHI), 1,3 glucanase (GLU) and peroxidase (POD) as well as signal transduction pathway genes in cucumber against anthracnose disease (Bovie *et al.*, 2004 and Narusaka *et al.*, 2001) and activates oxidative burst enzymes in pear against scab disease (Faize *et al.*, 2004). Although BTH is highly effective as inducing enhanced disease resistance, it's mode of action and cellular targets are unclear.

The objective of the present study was to determine the effect of BTH on okra infection by the biotrophic fungus *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll., and to study the activity of enzymes or metabolites that might be involved in induced resistance.

MATERIALS AND METHODS

Pathogen material

An isolate of *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll., was obtained from infected okra plants at Kafr El-Sheikh, Egypt in summer 2004. This isolate was maintained on young okra plants of cv. Balady (landrace in Kafr El-Sheikh). One day before conidia were used as inoculums, heavily sporulating leaves were shaken to displace the old conidia and ensure high conidial viability. Freshly formed conidia were harvested using a suction pump.

Plant material

Okra *Abelmoschus esculentus* (L.) Moench, cv. Balady (Kafr El-Sheikh landrace), was used in all the experiments. For green house experiments, seeds were surface sterilized, pre-germinated in Petri dishes and sown in 1l pots filled with sterilized soil.

In field experiments, sowing occurred during growing seasons (2005 and 2006) in private farm at Kalen, Kafr El-Sheikh, Egypt. Five replications for each treatment were laid out in a complete randomized block design in a clay loam soil, with an alluvial substratum. . Treatment plot consisted of five ridges and each ridge was 3 m long and 0.6 m wide. Seeds were sown on 16th March 2005 and 2006 directly on the ridges with spacing of 30 cm between the plants. To achieve the maximum stand, three–four seeds sown per hill, and thinned to one seedling per hill when seedlings were 10-20 cm tall. No fertilizer was added and weeds around the plants were controlled manually. Those various growth characters and yield component of okra were assessed, i.e. days lapsed to 50% flowering and to 50% fruiting plants, node of the first flower, plant height (cm) at flowering and at the end of season, number of pods/plant and total marketable pods per feddan (calculated from size of plot) . The experimental area was surrounded on all sides by three ridges of the same cultivars to act as spreaders of the disease and to maintain a higher relative humidity within the plot.

Inoculation, incubation and sampling

For greenhouse, plants were inoculated over a period ranging from 0 to 30 days after BTH treatments by blowing approx. 20 mg *Sphaerotheca fuliginea*-conidia. The spore density of approx. 10 conidia/mm^{-2} was determined by counting the number of spores per unit area on cover slips placed in the dusted area. Plants were inoculated 30 days after BTH treatment. After homogeneous inoculation, plants were maintained at 26±5°C and about 14light:10dark photoperiod and relative humidity 75±10% for 10 days .Field plants were not artificially inoculated since the naturally infection were high and homogeneous.

The incidence of powdery mildew was visually evaluated on the second leaf and ranked as a percentage infected area using a rating (r) of 0, 1, 3, 5, 7, or 9, denoting proportions of disease over the whole leaf area of 0%, <1%, 2–5%, 6–20%, 21–40%, and >44%, respectively (Liang *et al.*, 2005). Disease index was calculated with the following equation:

Disease index (%) = [∑ (the number of diseased plants in each range X the disease range)/total plants investigated X the highest range)] X 100.

The protective effect was measured according to disease index by the following equation: Protective effect (%) = $[(D_c - D_T)/D_c] \times 100$

Where D_T is the disease index of treatment and D_c the disease index of control (untreated and not inoculated artificially).

BTH-treatment

To induce resistance, fresh solution of (BTH, 50% wettable granule formulation, Bion®, Novartis Ltd., Basel, Switzerland) at concentrations of 0 (control), 0.05, 0.1 and 1.00 mM in sterile deionized water plus 0.05% Tween 80 was applied using pump sprayer delivering a volume of 10 ml pot^{-1} . All plants were maintained in a greenhouse at 22-30 °C. For plant greenhouse experiments, BTH was applied when the plants had two expanded leaves. However, for field experiments; the BTH solution at the best concentration ٠,١ mM was sprayed over okra plants until run-off. Five ridges were treated with the BTH solution and five treated only with water. The first treatment was performed every 30 days for three times. Fungicide protection was carried in the same time as the other treatment by wettable sulphur 0.3% (80 WP, Ciba) .Untreated plants were only sprayed by water as a control.

Biochemical analysis:

Total Phenolics:

Leaves from the control and BTH-treated plants were sampled at different intervals 5-30 days after the treatment, weighted, frozen in liquid nitrogen and stored at -20°C until used in the same day. Frozen leaves tissue (0.5 g fresh weight) was ground in 5 ml acetone (-20°C) by using a pre-chilled mortar and pestle. After filtering off the solvent extract, the residue was furthers sequentially extracted twice with a similar volume of acetone methanol (1:1, v/v). The combined solvent extract was concentrated on a rotavapor, redissolved in 2 ml of pure methanol and cleared by centrifugation. Total phenolics were determined by using the Folin-Ciocalteu reagent (Prats *et al.*, 2002).

The absorbance of the developed blue colour was measured using a Spectrophotometer at 725 nm. Chlorogenic acid was used as standard. The amount of phenolics was expressed as μg chlorogenic g^{1} tissue.

Enzymatic activities:

To analyze peroxidase (POD, EC 1.11.1.7), polyphenol oxidase (PPO, EC 1.14.18.1) and phenylalanine ammonia lyase (PAL, EC 4.3.1.5) activities, 5 g of the sampled tissue were well crushed into a fine powder with liquid nitrogen and homogenate with 1mL by using a pre-chilled mortar and pestle in extracting buffer (pH 8.8, 0.2M boric acid buffer containing 10% (w/v) insoluble polyvinypolypyrolidone (PVPP), 1mM EDTA, 50 mM b-mercaptoethanol for PAL; pH 7.5, 50 mM phosphate buffer containing 8% (w/v) PVPP, 1mM polyethylene glycol, 1mM phenylmethylsulfonyl fluoride and 0.01% (v/v) Triton X-100 for (PPO and POD). Thereafter, the homogenate was ultrasonicated with a Sonorex RK 510 for 1 min, then filtered through cheesecloth and centrifuged at 25 000 x g for 20 min at 4°C and finally, the supernatant (crude enzyme extract) was collected.

PAL activity was determined as the rate of conversion of L-phenylalanine to transcinnamic acid at 290 nm (Koukol & Conn, 1961). Samples containing 300µL of enzyme extract were incubated with 1mL 0.02M L-phenylalanine and 2mL of the PAL extracting buffer at 20 °C for 2 min. For the POD assay, (Hammerschmidt *et al.*, 1982), 100L of the crude enzyme extract was incubated with 2.5mL of 25mM guaiacol (2-methoxyphenol) and 200µL 250mM H_2O_2 at 2· °C . Changes in the absorbance at 470 nm were recorded at 30 second intervals for 3 min. For the PPO assay, method of Galeazzi *et al.*, 1981 was conducted. 100µL of the crude enzyme extract was incubated with 2mL of 0.05M phosphate buffer (pH 7.0) and 0.5mL of 0.5M catechol at 20 °C for 2 min. Changes in the absorbance at 420 nm were recorded at 30 second intervals.

For the superoxide dismutase (SOD, EC 1.15.1.1), activity assay, 10 g of tissue was ground with 1mL of cold 50mM potasium phosphate buffer (pH 7.8) and then centrifuged at 4°C for 20 min at 13,000×*g*, the supernatant then being immediately assayed for SOD activity according to the method of Gay & Tuzun (2000). The volume of enzyme corresponding to 50% inhibition of NBT reduction was considered as one enzyme unit.

Protein was determined according to the method of Bradford (1976) using bovine serum albumin as a standard.

Statical analysis

All the experiments were repeated once with similar results. Data were subjected to analysis of variance using SPSS Computer

Program. Means were compared using Duncan range test (Duncan, 1955).

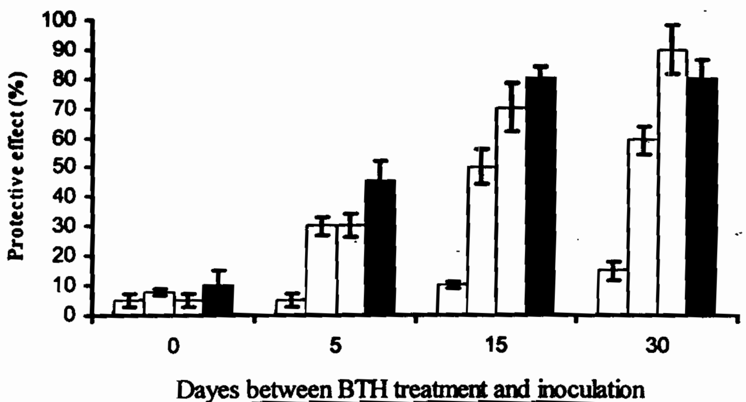
RESULTS AND DISCUSSION

This study investigates the ability of aqueous BTH to protect okra plants from powdery mildew, so greenhouse and field experiments were carried out.

In greenhouse (Figure 1), BTH had no significant protective effect in all used concentrations at 0 days, suggesting that the BTH had not antifungal activities that are dose dependent. However, results revealed that BTH treatment had far significant protective effect when it applied 5-30 days before inoculation by *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll. So, it allows us to conclude that the protective effect observed is not as a consequence of anti-microbial activity of BTH but due to its defense-triggering responses. This effect has also been seen in others plants (Friedrich *et al.*, 1996; Ruess *et al.*, 1996 and Tosi *et al.*, 1999).An increased in protective effect was apparent when okra plants were inoculated 5 days after BTH treatment, but it was more pronounced when inoculation was performed 30 days after BTH treatment. The best protective effect (89.51%) by BTH treatment achieved by using BTH at 0.1 mM, although there was no significant difference at 15 and 30 days before inoculation so we used this concentration in all the experiments. The lower doses of BTH didn't harm the plants. However, at the higher doses tested (1.00 mM), light chlorosis was observed on the edges of leaves, accompanied by necrosis. Several previous studies have reported that BTH could activate the development of systemic acquired resistance and result in disease resistance against virus and fungal attack in wheat (Gorlach *et al.*, 1996), tobacco (Benhanou & Belanger, 1998), *Arabidopsis* (Lawton *et al.*, 1996) and tomato (Anfoka, 2000).

In the field experiments (Table 1), the results were similar in the both seasons 2005 and 2006. The fungicide and BTH treatment had a far significant protective effect against powdery mildew reach to be 90.4 % in the case of BTH treatment in 2006. Number of days lapsed to 50% flowering, the position of the node of first flower, days to 50% fruiting, node of the first fruit and position of node for the first okra pod set were not affected with any treatments. This means that powdery mildew didn't affect those plant development parameters. Regardless of protective effect for fungicide and BTH treatments, untreated okra plants grown was significantly shorter at flowering stage in both years than the others treatments. The maximum plant height reaches to be 301.1 cm in BTH treatment in 2005.

Figure (1): The protective effect (%) of BTH against *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll.



Both the fungicide and BTH treatments significantly increased the number of pods per plant, average pod weight and total okra pod yield. Total pods yield reach to be 7.6 ton per feddan in the case of BTH treatment in 2006.

Table (1) Effect of BTH and wettable sulphur 0.3% (80 WP, Ciba) on the protective effect against *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll., and okra development and yield in tow seasons (2005 and 2006).

Seasons	2005*			2006		
Treatments	untreated	wettable sulphur 0.3% (80 WP, Ciba)	BTH	untreated	wettable sulphur 0.3% (80 WP, Ciba)	BTH
Protective effect (%)	10.9 b	70.9 a	80.1 a	5.2 b	78.2 a	90.4 a
Days to 50% flowering	60.9 a	61.3 a	60.08 a	63.4 a	63.0 a	62.0 a
Node of the first flowers	6.1 a	6.0 a	5.7 a	6.5 a	6.1 a	6.4 a
Plant height at flowering (cm)	60.5 b	67.9 a	68.1 a	62.1 b	72.1 a	73.9 a
Plant height at season end (cm)	251.2 b	298.5 a	301.1 a	270.1 c	300.1 a	289.9 b
Days to 50% fruiting	64.1 a	65.9 a	66.8 a	67.1 a	64.9 a	66.5 a
Node of the first fruit	7.0 a	7.0 a	6.9 a	7.0 a	6.8 a	7.0 a
Number of pods plants^{-1}	59.1 b	70.4 ab	79.3 a	64.2 b	85.0 a	83.9 a
Average pods weight(gram) plants^{-1}	46.1 b	58.9 a	60.1 a	41.1 b	60.3 a	61.4 a
Total pods yield (ton feddan^{-1})	4.9b	6.9 a	7.3 a	4.2 b	7.2 a	7.6 a

*In the same row and in the same season. Means followed by the same letter are not significantly different according to Duncan multiple range test P≤0.05

Total Phenolics:

In order to evaluate whether the effect of BTH is mediated by the production of toxic compounds, total soluble phenolics accumulated in okra leaf tissue were analysed (Figure 2). Results in Figure 2 showed that soluble phenolics (expressed as µg equivalent of Chlorogenic acid) of BTH-treated plant tissues, despite of small fluctuations, showed no dramatically changes. However BTH-treated leaves had higher soluble phenolics ($P < 0.05$) than untreated leaves at all time points observed except at 0 and 12 hours after inoculation. The antifungal properties of total soluble phenolics should be regarded as a possible mechanism inhibiting the development of powdery mildew. Fungitoxicity cited in different reports against different fungi (Mayama & Shishiyama 1978 and Nicholson & Hammerschmidt 1992). An example, the resistance against powdery mildew in cucumber plants treated with an elicitor leaf extract of *Reynoutria sachalinensis* was shown to be accompanied by increased formation of antifungal total soluble phenolics as in our study (Daayf, et *al.*,1995

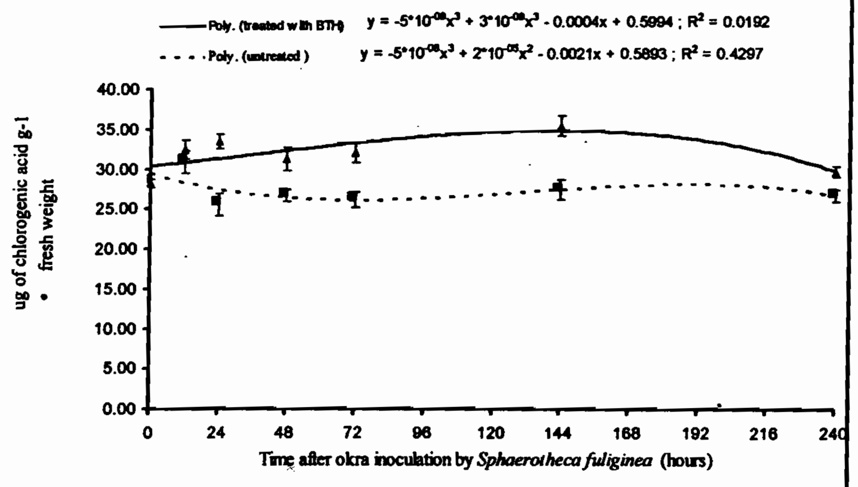


Figure (2): Effect of BTH on the accumulation of total soluble phenolics in okra plants. Values expressed as the µg equivalent of chlorogenic acid per gram of fresh weight, are the means of five independent replicates ± (standard error).

The measured enzymes are considered as key enzymes in control of plant disease in resistance systems especially POD, PPO, PAL and SOD. Results in Figure (3) revealed that POD activity in both untreated and BTH treated increased continuously after inoculation, although it was markedly enhanced by BTH treatment.

POD activities reach to be the maximum at 72 hours after inoculation in which was about 533.3% ($P < 0.01$) higher than that in untreated. Enhancement of peroxidases activity in okra inoculated with powdery mildew suggests polymerization of phenolic compounds during wall modification (strengthening) and papilla formation as in barley-powdery mildew (Kerby & Somerville, 1989). Roles, frequently cited, for a subset of peroxidases, in the presence of hydrogen peroxide, are the catalysis of terminal steps in lignin biosynthesis and esterifcation of hydroxycinnamic acids as well as hydroxynnamoyl-CoAs to form gels that can be accumulated in the cell wall matrix and act as an efficicient physical barrier (Fry, 1986; Gregersen *et al.*, 1997 and Zhang, *et al.*, 1997). So, it seems probable that wall bound esterified phenolic acids play an important role in the BTH-induced resistance to prevent penetration of the powdery mildew pathogens.

PPO activity in untreated leaves declined gradually during after inoculation, although it was markedly enhanced by BTH treatment. As shown in Figure (4), PPO activity in BTH-treated fruit was about 65.3, 302.3, 90.09 and 124.4 % higher than that in untreated at 12, 24, 48 and 72 hours ($P < 0.01$) after the inoculation, respectively. PPO is involved in the oxidation of polyphenols into quinones (antifungi compounds) and lignifications of plant cells during pathogen penetration so its increasing means increasing plant resistance (Mohammadi & Kazemi 2002).

Untreated plants showed no significant changes in PAL activity after inoculations (Figure 5), it means that pathogen penetration alone has no affect on PAL activity. On the other hand, in BTH-treated plants, PAL activity increased only within 24-72 hours after inoculation and this in agreement with Jamali-Zavareh *et al.* (2004).

PAL is a rate-limiting enzyme in the activation of the phenylpropanoid pathway, and an increase in PAL activity, as in our studies is normally associated with biosynthesis of active metabolites, such as phytoalexins, phenols, lignins and salicylic acid in plant defense pathways (Milosevic & Slusarenko, 1996). Similar results had been obtained in wheat, in which the enhanced resistance by BTH is associated with increases in PAL and POD activities and accumulation of phenolic compounds (Gorlach *et al.*, 1996)

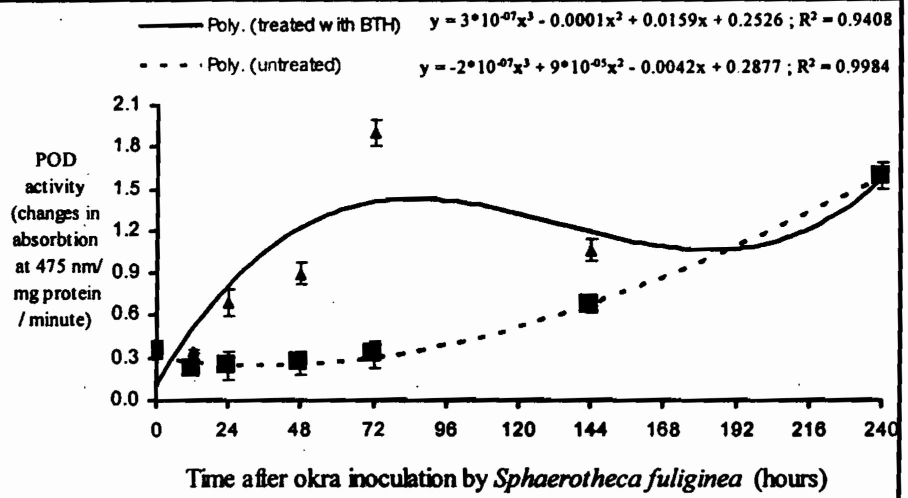


Figure (3): Time-course of peroxidase specific activity of okra leaves extracts after BTH treatment.

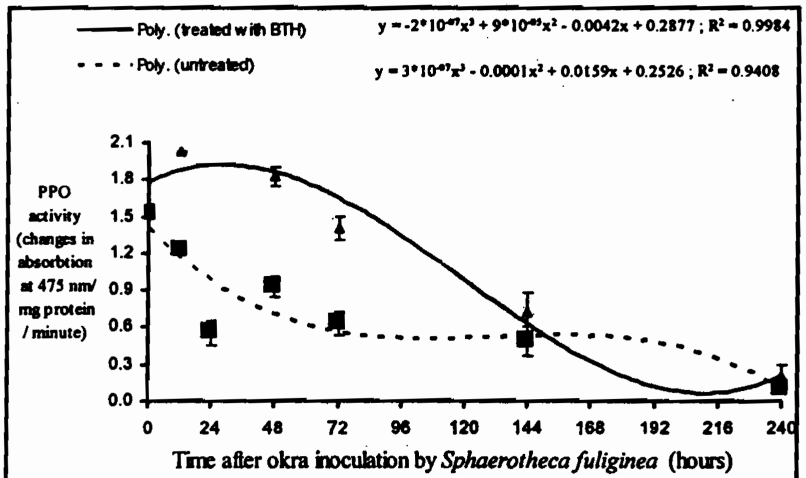


Figure (4): Time-course of polyphenol oxidase specific activity of okra leaves extracts after BTH treatment.

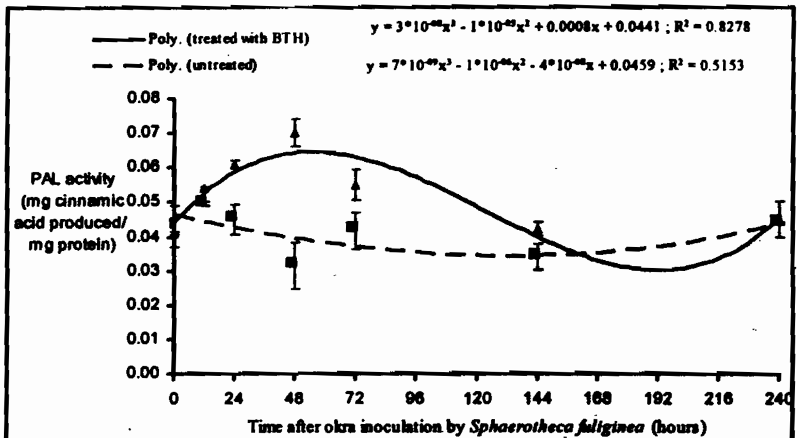


Figure (5) :Time-course of PAL specific activity of okra leaves extracts after BTH treatment.

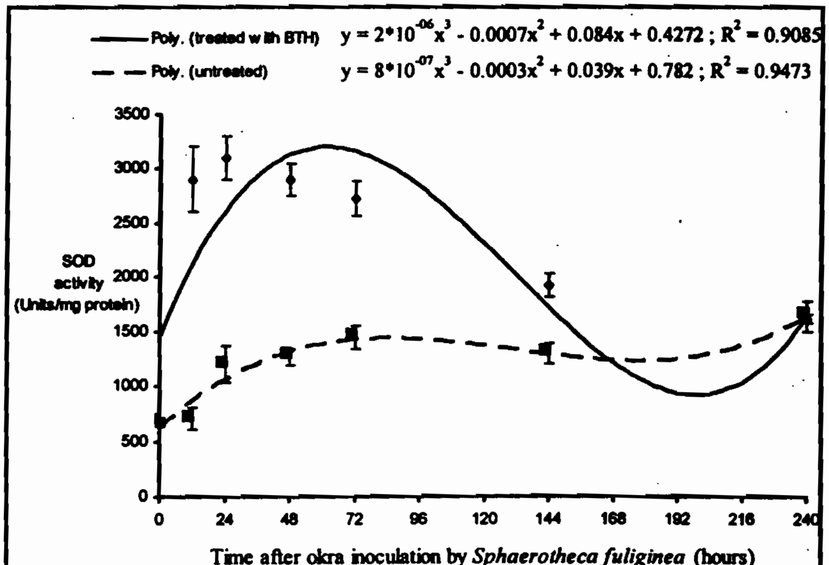


Figure (6): Time-course of SOD specific activity of okra leaves extracts after BTH treatment.

SOD activity dramatically increased with the time in general and tended to decrease after 72 h in BTH treatment (Figure 6). SOD activity reach to be the maximum (3100±200 units/mg protein) at 24 hours then start to decrease. The enhanced SOD activity was clearly evident in BTH treatment during time-course after inoculation except at 0 and 24hours. In BTH treatment okra plants, the highest responses of POD and SOD activities appear only after 48 h (Figs. 3 and 6). This evidence seems to verify a delayed state of reactive oxygen species (ROS) production and hypersensitivity reaction failure. Therefore, it could hypothesize that antioxidant enzymes work after three days as scavengers of reactive oxygen species generated by the increase of powdery mildew lesions caused by the forward advance of pathogen colonization on susceptible okra leaves

ROS production in plants as subsequent reaction to infection with pathogens is well studied (Doke, *et al.*, 2001). Enhancement of SOD activity has been previously reported in cucumber-fungi host pathogen interaction during BTH-induced resistance (Deepak *et al.*, 2006). Ogawa *et al.*, (1997) demonstrated the importance of SOD by dismutating super oxide anion to oxygen and hydrogen peroxide which used for peroxidase enzymes to polymerize lignin precursors. In addition, production and accumulation of hydrogen peroxide could also lead to increase defense responses by activation of PAL and accumulation of pathogen-related protein (Hu *et al.*, 2003; Narusaka *et al.*, 2001 and Lewis 1999).

In conclusion, the present study shows that BTH treatment can significantly enhance disease resistance of okra against powdery mildew both in greenhouse and field, and suggests that BTH application is a promising new technology, substituting fungicidal control in vegetables.

REFRRENCES

Ames, J.M. and G. MacLeod (1990). Volatile components of okra. Phytochemistry, 29: 1201–1207.

Anfoka, G.H. (2000). Benzo-(1,2,3)-thiadiazole-7-carbothioic acid –*S*-methyl ester induces systemic resistance in tomato (*Lycopersicon esculentum*. Mill cv.Vollendung) to cucumber mosaic virus. Crop Prot. 19: 401–405.

Benhanou, N. and R.Belanger (1998). Benzothiazole-mediated induced resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomato. Plant Physiol. 118: 1203–1212.

Bovie, C; M. Ongena; P. Thonart and J. Dommes (2004). Cloning and expression analysis of cDNAs corresponding to genes activated in cucumber showing systemic acquired resistance after BTH treatment. BMC Plant Biol. 4:15.

Bradford, M.M. (1976). A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem 72:248–54.

Cohen, Y.; T. Niderman; E.M.O.K. Singer and R. Fluhr (1994) .β-aminobutyric acid induces the accumulation of pathogenesis-related proteins in tomato (*Lycopersicon esculentum* L.) plants and resistance to late blight infection caused by *Phytophthora infestans*. Plant Physiol. 104: 59-66.

Cole, D.L. (1999). The efficacy of acibenzolar-S-methyl, an inducer of systemic acquired resistance, against bacterial and fungal diseases of tobacco. Crop Prot. 18, 267-273.

Daayf, F.; A. Schmitt and R.R. BeÂlanger (1995). The effects of plant extracts of *Reynoutria sachalinensis* on powdery mildew development and leaf physiology of long English cucumber. Plant Disease 79: 577-580.

Deepak, S.A.; H. Ishii and P. Park, (2006). Acibenzolar-S-methyl primes cell wall strengthening genes and reactive oxygen species forming/scavenging enzymes in cucumber after fungal pathogen attack. Physiological and Molecular Plant Pathology 69: 52–61.

Doke, N.; H.J. Park; S. Katou; H. Komatsubara; T. Makino and S. Ban, (2001).The oxidative burst in plants: mechanism and function in induced resistance. In: Keen, N.T.; S. Mayama; J.E. Leach and S. Tsuyumu, editors. Delivery and perception of pathogen signals in plants. St. Paul, MN: APS Press; p. 184–93.

Duncan, D.B. (1955). Multiple range and multiple F-test. Biometrics. 11: 1-242.

Faize, M.; L. Faize; N. Koike; M. Ishizaka and H. Ishii (2004) Acibenzolar-Smethyl-induced resistance to Japanese pear scab is associated with potentiation of multiple defense responses. Phytopathology. 94: 604–12.

Friedrich, L.; K.A. Lawton; W. Ruess; P. Masner; N Specker; M. Gut-Rella; P. Meier; S. Dincher; T. Staub; S. Uknes; H. Kessmann and J. Ryals (1996). A benzothiadiazole derivative induces systemic acquired resistance in tobacco. Plant Journal 10: 61-70

Fry, S.C. (1986). Cross-linking of matrix polymers in the growing cell walls of angiosperms. Annual Review of Plant Physiology 37: 165-186.

Galeazzi, M.A.M.; N.Sgarbieri and S.M.Costantinides (1981). Isolation, purification and physiochemical characterization of polyphenol oxidase from dwarf variety of banana (*Musa cavendishii*). J. Food. Sci. 46: 150–155.

Gay, P.A. and S. Tuzun (2000). Temporal and spatial assessment of defense responses in resistant and susceptible cabbage varieties during infection with *Xanthomonas campestris* pv. Campestris. Physiol. Mol. Plant Pathol. 57: 201–210.

Ghoniem, K.E.A.E. (1985). Studies on powdery mildew diseases in Egypt. M.Sc. Thesis, Fac. of Agric., Tanta Univ., Kafr El-Sheikh branch.82 p.

Gorlach, J.; S, Volrath; G. Knauf-Beiter; G. Hengy; U. Beckhove; K.L. Kogel; M. Oostendorp; T. Staub; E. Ward; H. Kessmann and J. Ryals (1996). Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. Plant Cell. 8: 629–643.

Gregersen, P.L.; H.Thordal-Christensen; H. Förster and M. Collinge (1997). Differential gene transcript accumulation in barley leaf epidermis and mesophyl in response to attack by *Blumeria graminis* f. sp. *hordei* (syn. *Erysiphe graminis* f. sp. *hordei*). Physiological and Molecular Plant Pathology 51: 85-97.

Hammerschmidt, R. and J. KucH (1995). Induced resistance to disease in plants. Kluwer Academic Publishers, Dordrecht, Netherlands.

Hammerschmidt, R.; E.M. Nukles and J. Kuc (1982). Association of enhanced peroxidase activity with induced resistance of cucumber to *Colletotrichum lagenarium* Physiological Plant Pathology 20: 73-82.

Hu, X.Y.; S.J. Neill; W.M. Cai and Z.C. Tang. (2003) Activation of plasma membrane NADPH oxidase and generation of H_2O_2 mediate the induction of PAL activity and saponin synthesis by endogenous elicitor in suspension-cultured cells of *Panax ginseng*. Acta Bot Sin 45:34–41.

Jamali, Zavareh A.H.; A.Sharifi Tehrani, and M. Mohammadi (2004).Changes in phenolic content and specific activities of peroxidase, phenylalanine ammonia lyase and chitinase in cucumber leaves inoculated with *Podosphaera fusca*, the causal agent of powdery mildew. Proceedings of 56th International Symposium on Crop Protection, Faculty of Agricultural and Applied Biological Sciences, Ghent University, Belgium, 69 : 555 – 563

Joi, M.B. and R.V. Shende (1979). Varietal reaction to powdery mildew (*Erysiphe cichoracearum* DC.) in okra. *Veg. Sci.*, 6: 126-129.

Kerby, K. and S. Somerville (1989). Enhancement of specifc intercellular peroxidases following inoculation of barley with Erysiphe graminis f. sp. hordei. Physiological and Molecular Plant Pathology 35: 323-337.

Koukol, J. and Conn, E.E., (1961). The metabolism of aromatic and properties of the phenylalanine deaminase of *Hordeum vulgare*. J. Biol. Chem. 236: 2692–2698.

Lawton, K.A.; L. Friedrich; M. Hunt; K. Weymann; T. Delaney; H. Kessmann; T. Staub and J. Ryals (1996). Benzothiadiazole induces disease resistance in *Arabidopsis* by activation of the systemic acquired resistance signal transduction pathway. Plant J. 10: 71–82.

Lewis, N.G. (1999). A 20th century roller coaster ride: a short account of lignification. Curr. Opin. Plant Biol 2:53–62.

Liang, Y.C.; W.C. Sun; J. Si and V. Römheld(2005). Effects of foliar- and root-applied silicon on the enhancement of induced resistance to powdery mildew in *Cucumis sativus*. Plant Pathol. 54: 678–685.

Mayama, S. and J. Shishiyama (1978). Localized accumulation of fluorescent and UV-absorbing compounds at penetration sites in barley leaves infected with *Erysiphe graminis* f. sp. *hordei*. Physiological Plant Pathology 13: 347-354.

Milosevic, N. and A.J. Slusarenko (1996). Active oxygen metabolism and lignification in the hypersensitive response in bean. Physiol. Mol. Plant. pathol. 49: 143–157.

Mohammadi, M. and H. Kazemi (2002). Changes in peroxidase and polyphenol oxidase activities in susceptible and resistant wheat heads inoculated with *Fusarium graminearum* and induced resistance. Plant Sci. 162: 491–498.

Mousa, A.A.M.; Mohamed; F. Mohamed; M.H. Dokashi and F. E.E. Elnobi (2007). Intra-row intercropping of cowpea and cucumber with okra as influenced by planting date of secondary crops. Ass. Univ. Bull. Environ. Res.. 10: 13-34.

Narusaka, Y.; M. Narusaka and H. Ishii. (2001). Searching by differential display for new genes implicated in induced disease resistance of cucumber treated with acibenzolar-S-methyl. J. Gen Plant Pathol. 67: 207–11.

Neeraja, M.; C.H. Vijaya; Chiranjeevi and B. Gautham (2004). Screening of okra hybrids against pests and diseases. Indian J. Pl. Protection, 32: 129-131.

Nicholson, R.L. and R. Hammerschmidt (1992). Phenolic compounds and their role in disease resistance. Annual Review of Phytopathology 30: 369-389.

Ogawa, K.; S. Kanematsu and K. Asada (1997). Generation of superoxide anion and localization of Cu/Zn-superoxide dismutase in the vascular tissue of spinach hypocotyls: their association with lignification. Plant Cell Physiol 38:18–26.

Parveen P. M. and N. Dhandapani (2002).Eco-friendly management of major pests of okra (*Abelmoschus esculentus* (L.) Moench). Journal of Vegetable Crop Production 7: 3-11.

Prats, E.; D. Rubiales and J. Jorrin (2002). Acibenzolar-S-methyl-induced resistance to sunflower rust (*Puccinia helianthi*) is associated with an enhancement of cumarins on foliar surface. Phsiological and molecular plant pathology 60: 155-162.

Raj, H.; M.L. Bhardwaj; I.M. Sharma and N.K. Sharma (1993). Performance of commercial okra varieties in relation to diseases and insect pests, Ind. J. Agric. Sci., 63 : 747-748.

Ruess, W.; K. Muller; G. Knauf-Beiter; W. Kunz and T. Staub. (1996). Plant activator CGA245704: an innovative approach for disease control in cereals and tobacco. Proceedings of Brighton Crop Protection Conference, 2: 53-60.

Siegrist, J.; D. Glenewinkel; C. Kolle and M. Schmidtke (1997). Chemically induced resistance in green bean against bacterial and fungal pathogens. J. Plant Dis. Prot. 104: 599-610.

Siemonsma, J.S, (1982). La culture du gombo (*Abelmoschus sp.*), legume-fruit tropical. Univ de Wageningen.

Tosi, L.; R. Luigetti and A. Zazzerini (1999). Benzothiadiazole induces resistance to *Plasmopara helianthi* in sunflower plants. Journal of Phytopathology 147: 365-370.

Zhang, L.; M.P. Robbins; T.L.W. Carver and R.J. Zeyen (1997). Induction of phenylpropanoid gene transcripts in oat attacked by *Erysiphe graminis* at 20°C and 10°C. Physiological and Molecular Plant Pathology 51: 15-33.

الملخص العربي

استحثاث المقاومه الجهازيه بمنشط دفاع النبات المسمي ببينزو (3.2.1) ثيادازول-٧- كاربوثايوك أسد-اس-ميثيل ايستر لمرض البياض الدقيقي في الباميا

د. عمرو علي عمران

قسم النبات الزراعي-كلية الزراعة-جامعة كفرالشيخ-٣٣٥١٦ كفرالشيخ-مصر

E-mail; emeranaa@yahoo.com

يعتبرمرض البياض الدقيقي في الباميا واحدا من أكثر أمراض المجموع الخضري تدميرا في العائله الخبازية. ولقد تم استحثاث المقاومه الجهازيه في الباميا(*Abelmoschus esculentus* (L.)) ببينزو (3.2.1) ثيادازول-٧-كاربوثايوك أسد-اس-ميثيل ايستر(BTH). وظهرت الأستجابه بزياده التأثير الواقي ضد المسبب المرضي (*Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll.) وكذلك بزيادة النشاط الأنزيمي للبيروكسيديز و البوليفينول أوكسيديز و الفينيل الانيين أمونيا لاييز وكذلك بزيادة تراكم الفينولات الكلية.

وفي الصوبه كان أفضل تأثير واقي (٨٩,٥١%) تم الحصول عليه من معامله ال BTH بتركيز 0.1 mM .وظهرت زياده واضحه في التأثير الواقي عند عدوي نباتات الباميا بعد المعاملة بال BTH ب٥ أيام الا أن تلك الزياده كانت أوضح بعد المعامله بال BTH ب٣٠ يوما.

وفي الحقل كانت النتائج متشابهه وفي نفس الاتجاه خلال موسمي الزراعه ٢٠٠٥-٢٠٠٦. وأظهرت النتائج التاثير الواقي العالي للBTH كما للمبيد وكان نتيجه التأير الواقي لل BTH أن زاد محصول القرون ووصل الي ٧,٦ طن / فدان في عام ٢٠٠٦م