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SECONDARY METABOLISM, ENZYMATIC ANTIOXIDANTS AND ANTIBACTERIAL ACTIVITIES AS SIGNALING TO SOME STRESS ELICITORS

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ABSTRACT

Faba bean (*Vicia faba*, Giza 40) seedlings were subjected to some stress elicitors such as biotic due to infection with *Botrytis cinerea* ($5x10^5$ spores ml^{-1}) and abiotic due to the herbicide metribuzin (1.0 kg ha^{-1}) or sludge (150 g l^{-1}). During the subsequent 11 days, there were differential decreases in fresh and dry weights and in protein content of faba shoots and roots; the magnitude of decrease augmented with the progress of time. The decreases were greatest with *B. cinerea* while sludge was the least reductive. At the same time, there were significant increases in anthocyanin content as well as in activities of phenylalanine ammonia lyase (PAL), tyrosine ammonia lyase (TAL) and chalcone isomerase (CI). On the contrary, the activities of superoxide dismutase (SOD), guaiacol peroxidase (GPX) and glutathione S-transferase (GST) were significantly increased in shoots and roots of treated seedlings. On the other hand, the crude extracts of treated seedlings exhibited an inhibitory action towards some bacterial species; the inhibition was most pronounced towards *Bacillus cereus* followed by *Klebsiella pneumonia*, *Bacillus subtilis* and finally *Micrococcus roseus*. Nonetheless, the antibacterial activities were more efficient for extracts derived from seedlings stressed with *B. cinerea* than sludge while metribuzin had a negative effect. These findings suggest that stress elicitors, particularly biotic stress, could induce faba bean to produce some metabolites with antibacterial activities to withstand these harsh conditions.

INTRODUCTION

Faba bean (*Vicia faba* L.) is a major food crop in Egypt. It contains high concentrations of the isoflavones and polyphenols include anthocyanins (Aparicio-Fernandez *et al.*, 2005). This strategic crop is suffering from many destructive diseases among which chocolate spot disease. It is caused mainly by *Botrytis fabae* and, to some extent, by *B. cinerea* (Rahman *et al.*, 2002) resulting in considerable yield losses. Various stress elicitors either biotic or abiotic are known to stimulate production of secondary metabolites in plants. Anthocyanin is the most common secondary metabolites stimulated under stress. It belongs to the most common class of polyphenolic compounds (Aparicio-Fernandez *et al.*, 2005) and shows potent antioxidant properties (Jean, 2006).

Anthocyanins are derived from flavonoids via the shikimic acid pathway and may have a wide range of biological activities, including anti-inflammatory, antiallergic, anticarcinogenic, antiviral, antibacterial and fungicidal properties (Wrolstad 2004). Anthocyanins and other flavonoids are metabolized under the control of the secondary metabolism enzymes

phenylalanine ammonia lyase (PAL), tyrosine ammonia lyase (TAL) and chalcone isomerase (CI) (Nemat Alla and Younis, 1995). Stress conditions enhance the cellular production of reactive oxygen species (ROS) (Paradiso, 2005). However, stress elicitors have been shown to enhance the manufacture of anthocyanins in leaves. The generation of anthocyanins in leaves under stress serves to abate oxidative damage by scavenging and sequestering active oxygen species before they can attack cell macromolecules. Oxidative stress resulting from the imbalance between enhanced ROS production and detoxification is a common feature of a variety of environmental stresses including pathogen attack (Smirnoff, 1998).

Protection against ROS and peroxidation reactions is provided by antioxidant enzymes such as superoxide dismutase (SOD), guaiacol peroxidase (GPX) and glutathione S-transferase (GST) (Nemat Alla *et al.*, 2008a; Nemat Alla and Hassan, 2011). Plants have also developed enzymatic antioxidant system to cope with various stresses and to avoid oxidative damage. *B. cinerea* causes chocolate spot disease to faba bean. Metribuzin [4-amino-6-tert-butyl-4,5-dihydro-3-methylthio-1,2,4-triazin-5-one] is a triazinone herbicide. It is a photooxidative pigment destructor generates ROS inhibiting photosynthesis by binding to a protein in the electron transport system thus effectively blocking energy transport (Kuzniak, 2002; Nemat Alla and Hassan, 2006; Nemat Alla *et al.*, 2008b). Although sludge improves physical, chemical and biological properties of soils, its application in soil can lead to some problems. The present work aimed to check some of the physiological aspects induced in faba bean due to biotic (*B. cinerea*) and abiotic (the herbicide metribuzin or sludge) elicitors as signaling of stress such as secondary metabolism enzymes and enzymatic antioxidants. Moreover, the capabilities of extracts from treated seedlings were checked for their antibacterial activities.

MATERIALS AND METHODS

Growth onditions: Broad bean seeds (*Vicia faba*. Giza 40) were kindly supplied by the Agriculture Research Center in Cairo. Seeds were immersed in water for 9 h then allowed to germinate in plastic pots containing sand/clay soil (1/1, v/v). The pots were kept in greenhouse under 24/16°C day/night temperature, with 14 h photoperiod, 450 μmol m^{-2} s^{-1} photosynthetic photon flux density and 75-80% relative humidity. Each pot contained about 6 germinating seeds and water was applied day after day. When the seedlings were ten days old, the pots were divided into four sets: one was left to serve as control and one for each of the stress treatments (*Botrytis cinerea*, metribuzin and sludge). All stress elicitors were applied only once. Each pot received 100 ml of the different elicitors enough to cover the seedling parts. *B. cinerea* was applied at a concentration of 5 x 10^5 spores ml^{-1}. Metribuzin dosage was equivalent to the field dose (1.0 kg ha^{-1}). Sludge was used at 150 g of dried sludge l^{-1}. At harvest, samples were collected just before treatments (zero time) and also after 3, 5, 7 and 11 days from treatment. The collected samples were rinsed with copious amount of water and washed

well, then separated into shoots and roots. Fresh weights of shoots and roots were recorded and dried in oven at 80°C for 2 days and the dry weights were then recorded.

Isolation, purification and inoculums preparation of *Botrytis cinerea*: *B. cinerea* was isolated from infected pepper leaves. Leaf samples were microscopically examined, washed, surface sterilized with 0.1 % sodium hypochlorite for two minutes then washed thoroughly three times. Samples were then left to be dried in laminar flow bench between two layers of sterilized filter paper. Pieces of infected leaves were placed with their upper surface upwards on PDA media (17 g agar, 200 g potatoes, 20 g dextrose in 100 ml water) in moist chamber at 20-22°C. Plenty of conidiophores were recognized after one week. The developed fungi were carefully transferred into PDA plates and incubated at 20-22°C for one week. Inoculum of *B. cinerea* was prepared from cultures grown on PDA for 10-12 days by adding 5-10 ml sterilized water on the surface of pure colonies in petri dishes for 2-3 h and shaken gently in a round motion. The concentration of spores was adjusted to be 5×10^5 spores ml^{-1}.

Determination of anthocyanin content: Anthocyanin content was determined according to the method adopted by Hoagland (1980). Fresh tissues (5 g) were homogenized in acidic methanol (HCl, 1% v/v) for 5 minutes using a homogenizer. The homogenates were centrifuged for 20 minutes at 5000 xg. Anthocyanin was quantitated by measuring the difference in absorbency at 525 nm and 585nm (Δ525-585) in 10 ml extract.

Assay of enzyme activities: PAL, TAL and CI were extracted by homogenizing tissues (5 g) in Tris-HCl (0.05 M, pH 8.4) containing 15 mM β-mercaptoethanol. The homogenates were centrifuged at 24000 xg for 20 minutes and the supernatants were used as enzyme preparations. The activities of PAL and TAL were assayed following the method of Beaudoin-Egan and Thorpe (1985) in 1 ml reaction mixture containing 500 µmol of Tris-HCl (pH 8), 100 µl of "enzyme preparation", and either 6 µmol of L-phenylalanine (for PAL) or 5.5 µmol of L-tyrosine for (TAL). After incubation at 30°C, the reactions were stopped by 50 µl of 5 N HCl. The amounts of t-cinnamic acid and *p*-coumaric acid formed were determined at 290 and 333nm, respectively. CI activity was assayed in 1 ml reaction mixture containing 500 µmoles of Tris-HCl buffer (pH 8), and 10 µg of chalcone (dissolved in 10 µl of ethylene glycol monomethyl ether) and 100 µl of enzyme preparation (Hahlbrock *et al.*, 1970). After incubation at 37°C, the decrease in absorption at 375 nm was plotted against time.

SOD activity was assayed by using the photochemical nitroblue tetrazolium (NBT) method in terms of SOD's ability to inhibit reduction of NBT to form formazan by superoxide (Beyer and Fridovich, 1987). Plant tissues (5 g) were homogenized in 50 mM phosphate, pH 7.8, 0.1% (w/v) BSA, 5.5 mM ascorbate, and 8 mM β-mercaptoethanol. SOD was assayed in 50 mM phosphate, pH 7.8, 9.9 mM L-methionine, 0.057 mM NBT, 0.025% (w/v) Triton X-100, and 0.1 mM riboflavin. The photoreduction of NBT (formation of purple formazan) was measured at 560 nm and an inhibition curve was made against different volumes of extract. One unit of SOD was defined as that

being present in the volume of extract that caused inhibition of the photoreduction of NBT by 50% of control. GPx was extracted in 220 mM Tris-HCl, pH 7.4, 250 mM sucrose, 50 mM KCl, 1 mM $MgCl_2$, 160 mM β-mercaptoethanol, and 0.57 mM PMSF and centrifuged at 12000 xg for 30 min at 4°C. The reaction mixture contained 20 mM Na acetate, pH 5, 30 mM H_2O_2, 2 mM guaiacol and plant extract. The rate of guaiacol oxidation was recorded at 470 nm and the activity was calculated using the extinction coefficient of 26.6 mM^{-1} cm^{-1} for tetraguaiacol (Ranieri *et al.*, 1997). GST was extracted in 100 mM Tris-HCl, pH 7.5, 2 mM EDTA, 14 mM β-mercaptoethanol and 7.5% polyvinylpolyprollidone (Dixon *et al.*, 1995). After centrifugation at 15000 g for 15 min, ammonium sulfate was added to 80% saturation and the protein pellets were collected. GST assay was performed in 100 mM phosphate, pH 6.5, containing 5 mM GSH and 1 mM CDNB dissolved in 2.5% ethyl alcohol (Ando *et al.*, 1988). The absorbance at 340 nm was measured and the activity was calculated by the extinction coefficient E=9.6 $mM^{-1}cm^{-1}$.

Protein content was determined spectrophotometrically by reaction with Commassie Brilliant Blue G (Bradford, 1976). Each experiment was repeated at least twice in triplicates, so that the mean was obtained from at least six replicates. The full data were first subjected to analysis of variance (ANOVA) followed thereafter by least significant differences (LSD) at 5% level.

Antibacterial activities of faba extraction: The acidic methanol (HCl, 1% v/v) extracts used for anthocyanin determination were tested for the antibacterial activities against some selected bacteria species (*Bacillus cereus, Bacillus subtilis, Klebsiella pneumonia* and *Micrococcus roseus*), kindly supplied by Dr. Abou-Dobara, Faculty of science at Damietta, Mansoura University. These species were cultured on nutrient agar and incubated at 37°C for 24 h. Antibacterial activities of faba extracts were tested by the gar disc method using the selected bacteria species. 200 µl of methanol extracts were loaded into holes in the inoculated agar medium. The inhibition zone was measured after incubation at 37°C for 24 h.

Results

Faba bean growth was significantly reduced by either of the stress elicitors (*B. cinerea*, metribuzin and sludge) during the subsequent 11 days following treatment (Fig. 1). There were great reduction in fresh and dry weight of both shoots and roots; the reduction was most pronounced with *B. cinerea* followed by metribuzin while sludge was least reductive or non significant at all. As the time elapsed, the magnitude of reduction augmented. On the 11^{th} day following treatments, *B. cinerea* induced a reduction in shoot fresh and dry weights by about 33% and 47%, respectively whereas metribuzin caused a reduction by about 16% and 19% meanwhile sludge led to some increases in shoot fresh weight but reduced shoot dry weight by only 7%. Similar decreases were also detected in root fresh and dry weight; *B. cinerea* induced 53% and 48% reduction, metribuzin caused 17% and 22% while the decreases by sludge did not exceed 7%.

In figure 2, anthocyanin contents were greatly increased in shoots by each of the applied stress elicitor; however the increase in roots was

restricted to only *B. cinerea* and sludge while metribuzin resulted in decreases.

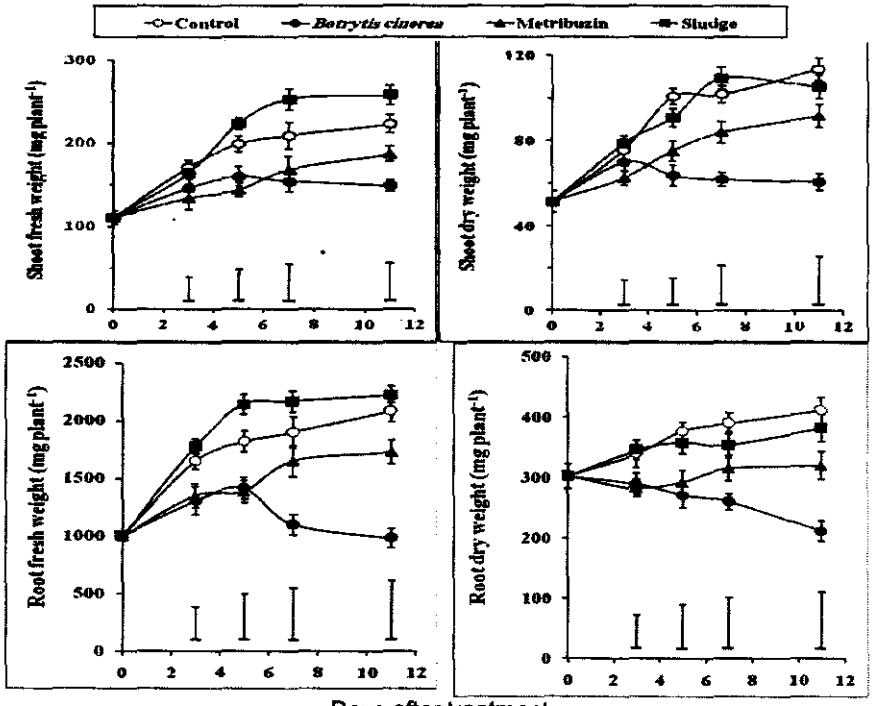

Fig. 1. Effect of treatment with *Botrytis cinerea*, metribuzin or sludge on fresh and dry weight of shoot and root of faba bean for the subsequent 11 days. Means±SE, n=6. Vertical bars represent LSD at p < 0.05.

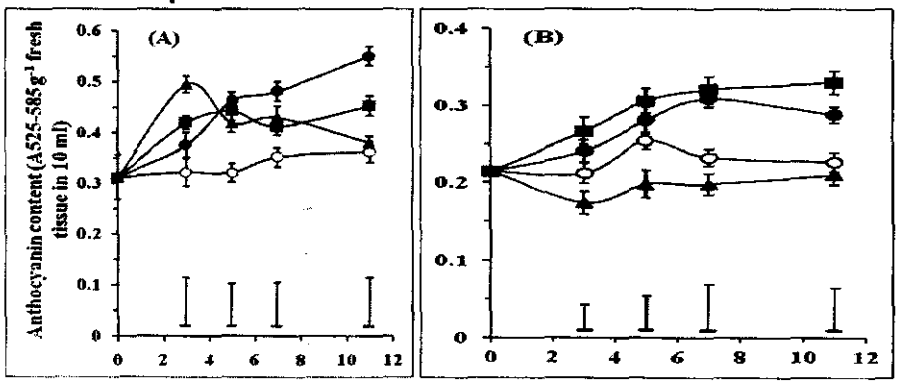

Fig. 2. Effect of treatment with *Botrytis cinerea*, metribuzin or sludge on anthocyanin content in shoot (A) and root (B) of faba bean for the subsequent 11 days. Means±SE, n=6. Vertical bars represent LSD at p < 0.05.

On the 11th day following treatments, anthocyanin in shoot and roots increased by *B. cinerea* by about 52% and 27%, respectively whereas sludge caused a reduction by about 25% and 45% but metribuzin had a slight decreasing effect, an increase in shoots by 5% and a decrease in roots by 7%.

All the stress elicitor significantly enhanced activities of PAL, TAL and CI either in shoots or in roots (Fig.3). The magnitude on enhancement was highest for CI. Anyway, sludge was more inductive for the activities of these enzymes relative to *B. cinerea* and metribuzin. However, the effects of *B. cinerea* were augmenting or at least consistent while those of metribuzin were withdrawn.

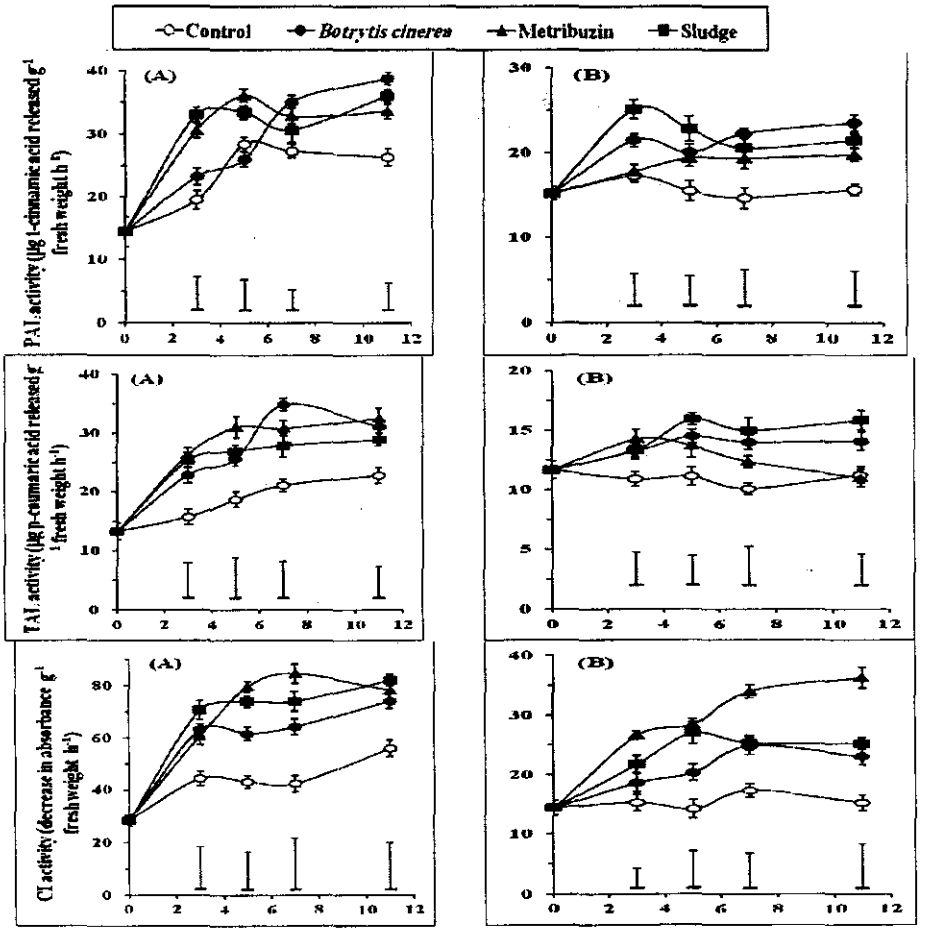

Fig. 3. Effect of treatment with *Botrytis cinerea*, metribuzin or sludge on activities of phenylalanine ammonia lyase (PAL), tyrosine ammonia lyase (TAL) and chalcone isomerase (CI) in shoot (A) and root (B) of 10-d-old faba bean for the subsequent 11 days. Means±SE, n=6. Vertical bars represent LSD at $p < 0.05$.

On the 11th day following treatments, PAL activity was stimulated in faba shoots by *B. cinerea*, metribuzin and sludge by about 47%, 28% and 37%, respectively and in roots by about 50%, 26% and 37%. In the same manner, *B. cinerea*, metribuzin and sludge stimulated TAL activity in shoots by about 36%, 43% and 27%, respectively and in roots by about 25%, 0% and 41%. On the other hand, CI in shoots increased by about 32%, 40% and 47%, respectively and in roots by about 51%, 139% and 66%.

As shown in figure 4, the activities of the enzymatic antioxidants SOD, GPX and GST were differentially changed; particularly stimulated significantly by sludge. SOD activity was increased by *B. cinerea* after 5 days from treatment in roots and after 7 days in shoots.

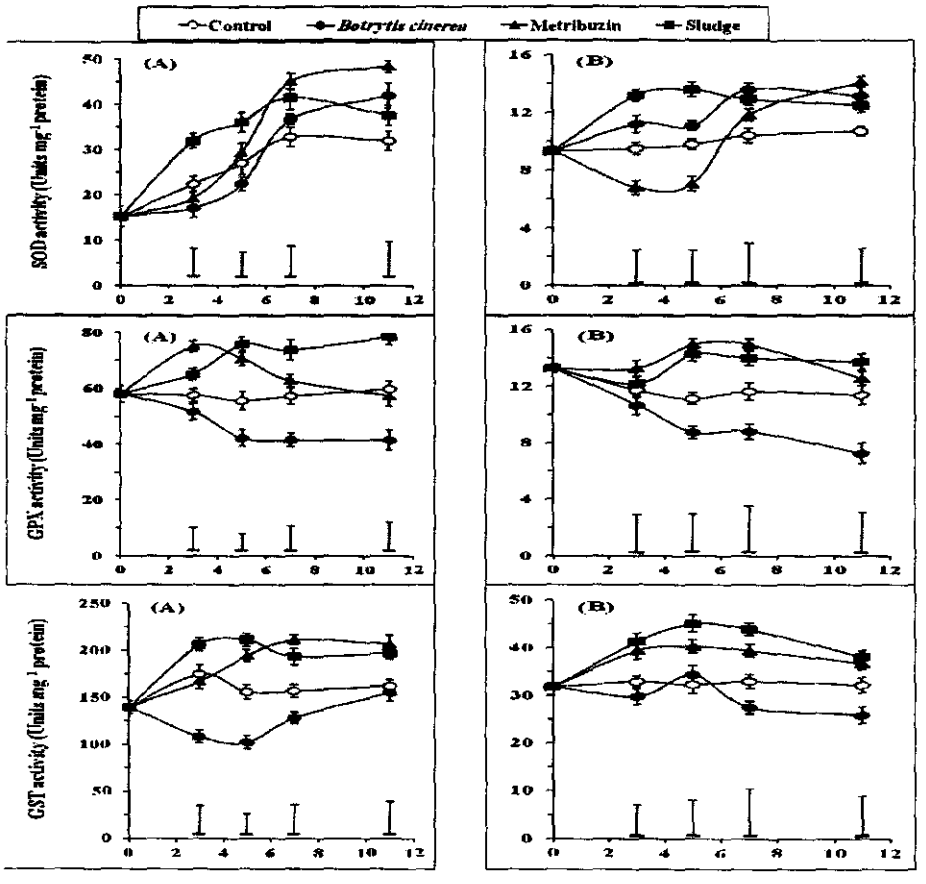

Fig. 4. Effect of treatment with *Botrytis cinerea*, metribuzin or sludge on activities of superoxide dismutase (SOD), guaiacol peroxidase (GPX) and glutathione S-transferase (GST) of faba bean for the subsequent 11 days. Means±SE, n=6. Vertical bars represent LSD at $p < 0.05$.

Similarly metribuzin increased SOD activity in shoots after 5 days from treatment and in roots and after 7 days. Nonetheless, sludge turned ineffective upon SOD activity of shoots and roots after 7 and 5 days, respectively. On the other hand, GPX activity in shoots was significantly inhibited by *B. cinerea* after the 3rd day onwards in shoots but insisted in roots up to the 11th day.

In response to metribuzin, GPX activity was increased in shoots and roots during the first 5 and 7 days, thereafter activity level became comparable to those of control. Although *B. cinerea* seemed to have reductive effects on the activity of GST in shoot and roots, thereafter activity level became comparable to those of control. Metribuzin and sludge induced a significant increase in GST activity in shoots up to the end of the experiment in shoots and during the first 5 days in roots.

In response to metribuzin, GPX activity was increased in shoot and roots during the first 3, 5 and 7 days, thereafter activity level became comparable to those of control. Also, sludge increased the enzyme activity in shoots and roots. Although *B. cinerea* seemed to inhibit the activity of GST in shoot and roots, thereafter activity level became comparable to those of control. Metribuzin induced a significant increase in GST activity in shoots up to the end of the experiment in shoots and during the first 5 days in roots. The effect of sludge upon the stimulation of the enzyme activity was mostly pronounced during the entire experiment in shoots and up to the 7th day in roots.

Methanolic extracts derived from shoots and roots of treated faba bean with *Botrytis cinerea*, metribuzin or sludge showed variable antibacterial activities (Table 1). However, more activity was detected for extracts derived from shoots relative to those from roots. These extracts were more efficient towards *B. cereus* relative to the other tested bacterial species. It seemed that *K. pneumonia* and *B. subtilis* were also inhibited to a great extent by the extracts while *M. roseus* appeared the most resistant species towards the activities of these extracts. More activities towards the most sensitive species were observed for extracts derived from shoots of faba stressed with sludge and those derived from roots of faba stressed with *B. cinerea*.

Table 1: Antibacterial activities of methanolic extracts from shoots and roots of 10-d-old faba bean treated with *Botrytis cinerea*, metribuzin or sludge for the subsequent 11 days.

Treatment	Days of	inhibition zone increase difference [a]			
		K. pneumonia	*B. cereus*	*B. subtilis*	*M. roseus*
Shoot					
B. cinerea	3	2.5	3.3	1.5	5.5
	5	ND	1.5	ND	ND
	7	0.5	3	0.5	ND
	11	ND	3.8	0.5	ND
Metribuzin	3	0.5	3.3	ND	ND
	5	1.5	2.8	ND	ND
	7	1	2.8	ND	ND
	11	2	3	1	ND
Sludge	3	2	2.3	0.5	ND
	5	1	3.8	0.5	ND
	7	1	3.8	0.5	ND
	11	1	3.9	ND	ND
Root					
B. cinerea	3	ND	3.8	0.05	ND
	5	ND	2.8	0.1	ND
	7	1.5	2.8	0.1	ND
	11	ND	4.3	ND	3.5
Metribuzin	3	0.5	2.8	ND	ND
	5	1	2.5	0.05	ND
	7	0.5	2.8	ND	ND
	11	ND	3.8	0.15	2.5
Sludge	3	1.5	2.8	0.05	ND
	5	1	2.8	ND	ND
	7	1	2	ND	ND
	11	0.5	2.2	ND	ND

[a] Values are mean differences in mm between the inhibition zone of the tested extract and blank (acidic methanol). ND means no difference between the inhibition zone of the extract and the blank

DISCUSSION

Sludge was the least inhibitory additive as compared with metribuzin and *B. cinerea* and sometimes induced some positive effects. This might be due to the great contribution of nutrients. This beneficial effect could be contrasted with high salinity of sludge causing osmotic stress and the heavy metal cations uptake leading thus to growth inhibition. Heavy metals cause disturbances in cell membrane functioning in the photosynthetic and mitochondrial electron transport and in the inactivation of many enzymes active in the basic cell metabolism regulation (Rout and Das, 2003). On the other hand, metribuzin might produce ROS because it blocks the electron transport chain from PSII to PSI in photosynthesis (Nemat Alla, 2008b). ROS production would cause a cessation of plant growth (Kuzniak, 2002; Nemat Alla and Hassan, 2006; Nemat Alla *et al.*, 2008a;b; Nemat Alla and Hassan, 2011). On the other hand, *B. cinerea* decreased faba growth may be due to the production of the plant hormone abscisic acid (ABA), which plays a major role in several steps of plant growth and development, such as stomatal closure, embryo and seed dormancy, seed germination and the adaptation to environmental stress (Tudzynski and Sharon, 2002).

The synthesis of anthocyanins in plants can be influenced by stress (Close *et al.*, 2002). Some stress elicitors enhance anthocyanin accumulation in plant cells (Nemat Alla and Younis, 1995) whilst others reduce anthocyanin accumulation (Nemat Alla and Younis, 1995; Lo and Nicholson, 1998). This probably relates to regulation of the phenylpropanoid pathway ensuring allocation of resources from less essential metabolic activities to those of immediate concern for survival (Lo and Nicholson, 1998). Anthocyanins increased in faba bean due to either of the employed treatments particularly sludge and *B. cinerea* although metribuzin induced some decreases in roots. Similar results were also observed in Roselle treated with sludge (Tsaipi and Huang, 2004). The present results corroborate those of other researchers indicating that pathogen infection and fungal elicitors have been also found in relation with increased anthocyanin in different plants (Ferreres *et al.*, 1997).

Anthocyanin and other products of secondary metabolism are controlled by the enzymes PAL, TAL and CI. PAL is the first enzyme of phenylpropanoid metabolism in higher plants (Nemat Alla and Younis, 1995) and it has been suggested to play a significant role in regulating the accumulation of phenolics and phytoalexins in response to infection. It catalyses the reductive deamination of L-phenylalanine resulting in trans-cinnamic acid, the first step in the biosynthesis of plant phenylpropanoid compounds such flavonoids. PAL activity varies during the plant development and cell differentiation. PAL produces phenylpropanoid precursors for chalcone biosynthesis and isomerization through the action of CI, from which flavonoids are synthesized. Thus, any change in the activities of these enzymes would contribute to a modification in the formation of such products of secondary metabolism. Stress conditions increase PAL activity in various plant species (Slatnar *et al.*, 2010). In the present work, metribuzin, *B. cinerea* and sludge increased the activities of PAL, TAL, CI. In this accordance, CI activity was found to be

increased after sludge treatment (MacDonald and D'Cunha, 2007). Zhi-Guo *et al.* (1995) found a positive correlation between PAL and anthocyanin synthesis in apples. It is similar to Challenging wheat leaves with *B. cinerea* conidia resulting in a localised, increase of PAL and peroxidase expression (Mitchell *et al.*, 1994). MacDonald and D'Cunha (2007) indicated that TAL and CI are also involved in plant defense during abiotic stress caused by sludge.

The enzymatic antioxidants have been reported to be important in plant tolerance to biotic and abiotic stresses. SOD is an important enzyme in protecting the cell from oxidative damage. SODs form the first line of the antioxidative defence protecting of plant against cells damage (Møller, 2001). An increase in SOD activity reduced the lesion formation and delayed tumor development. SOD activity increased in leaves of alfalfa plants in the presence of sludge (Mckersie *et al.*, 1993). Thus increasing SOD by stress elicitors might be a plant response to withstand these harsh conditions.

For GPX, Asada (1992) concluded that the physiological function of peroxidase is defensive and the enzyme plays a role in cell wall lignification and tannin production. The present results showed an increase in the activity of GPX in faba bean following treatment with metribuzin and sludge but *B. cinerea* reduced it. Kortekamp and Zyprian (2003) established a strong correlation between peroxidase activity and resistance to *Plasmapora viticola*. On the other hand, Tiedemann (1997), studying bean *B. cinerea* interactions, showed that elevated levels of ROS during early stages of infection in leaf tissue supported fungal infection. Moreover, GSTs are a family of multifunctional detoxification enzymes that catalyse the conjugation of a wide variety of xenobiotics to glutathione (Nemat Alla *et al.*, 2008a). GSTs function in xenobiotic detoxification, hormone homeostasis, vacuolar sequestration of anthocyanin, tyrosine metabolism, hydroxyperoxide detoxification, and regulation of apoptosis and in plant responses to biotic and abiotic stresses (Dixon *et al.*, 2010).

Stress would induce plant defense responses mainly as secondary metabolites (Hahn, 1996). Therefore, the acidified methanol extracts of faba bean were tested for their antibacterial activities against some bacterial strains: *K. pneumonia, B. cereus, B. subtili,* and *M. roseus*. The activities of the extracts were more efficient against *B. cereus* than the other tested species. *B. cereus* is a Gram-positive, a common food poisoning organism. It is associated with immunologically compromised patients, neonates, drug addicts and patients with a history of traumatic or surgical wounds or catheters (Drobniewski, 1993). Wang *et al.* (2000) concluded that acidified methanol extracts of faba bean leaves gave a perfect result against *B. cereus*. Similarly, Ates and Erdourul (2003) reported antibacterial activities of *Glycyrrhiza glabra* (Fabaceae) roots extracts against *B. cereus*. *K. pneumonia* is also influenced by the extracts but to a lesser extent. *Klebsiella pneumonia* is a Gram-negative enteric bacillus that forms large mucoid colonies this organism has been associated with bacterial liver abscesses and metastatic infections. It causes urinary tract infections, pneumonia, intraabdominal infections, septicemias and soft tissue infections (Tsay *et al.*, 2002). Similar results were detected for ethanol and methanol leaf extracts of

Spathodea campanulata against *K. pneumonia* (Parekh and Chanda, 2007). The extracts, particularly from leaves, also affected *M. roseus.* It is a Gram positive bacterial cell. The growth of *B. subtilis* was also influenced by the extracts. Similar findings were detected for methanolic extracts of the pomegranate leaves against *B. subtilis* (Rabe and Van, 1997). Further, Shekhawat (2010) showed that the acidified methanol extract of *Apium graveolens* inhibited the growth of *B. subtilis.*

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مركبات الايض الثانوية ومضادات الاكسدة ومقاومة البكتيريا كعلامات لبعض مسببات الاجهاد

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هدفت الدراسة إلى امكانية استحثاث بعض المركبات فى الفول البلدى "جيزة ٤٠" مثل مركبات الايض الثانوية شاملة الأنثوسيانين وإنزيمات الأيض الثانوية ومضادات الأكسدة نتيجة لبعض مسببات الإجهاد الحيوية مثل فطر البوتريتس سينيريا وغير الحيوية مثل مبيد الميتربيوزين والحماة. وقد أوضحت النتائج نقص وزن المجموع الخضرى والجذر بعد معالجته بمبيد الميتربيوزين وفطر البوتريتس سينيريا وزيادته بعد استخدام الحماة، كما نقص الوزن الجاف للمجموع الخضرى والجذر. ومن ناحية أخرى فقد أدت المعاملات المختلفة إلى زيادة انتاج صبغة الأنثوسيانين فى المجموع الخضرى بعد استخدام الميتربيوزين والحماة وفطر البوتريتس سينيريا ونقصها فى الجذر بعد المعالجة بمبيد الميتربيوزين فقط. كما زادت أنشطة إنزيمات الأيض الثانوية (فينيل ألانين أمونيا لايز، تيروزين أمونيا لايز، كالكون أيزوميريز) وكانت الزيادة أكثر وضوحا فى المجموع الخضرى عن الجذر. أما إنزيم مضاد الأكسدة سوبر اوكسيد ديسميوتيز فقد زاد زيادة واضحة بعد استخدام الحماة وفطر البوتريتس سينيريا وكان استحثاثه أكبر بفعل الميتربيوزين ومن ناحية اخرى أدت المعالجة بالبوتريتس سينيريا إلى تثبيط نشاط الجواياكول بيروكسيديز والجلوتاثيون ترانسفيريز. ولقياس مدى مقدرة بعض نواتج الأيض كمواد مقاومة للبكتيريا فقد تم استخدام مستخلص المجموع الخضرى والجذر المقاس فيه الأنثوسيانين ضد بعض أنواع البكتيريا وأعطى هذا المستخلص نتائج جيدة ضد بكتيريا الباسلس سيرس والكلابسيلا نيومينيا ثم الباسلس ساتلص لكن الميكروكوكس روزس كانت مقاومة لهذا المستخلص.

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