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Management of Fusarium Wilt and Improving the Productivity of Snap Bean using Brassinosteroid, Glycinebetaine and Seaweed Extract

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Fourteen fungal isolates were isolated from naturally infected been exhibiting typical wilt symptoms. On the basis of cultural, morphological and microscopic examinations of the isolates, they were classified into *Fusarium oxysporum* (seven isolates), *F. solani* (two isolates), *Macrophomina phaseolina* (two isolates), *Aspergillus niger*, *Fusarium* sp. and *Mucor* sp. (one isolate of each). Pathogenicity test revealed that *F. oxysporum* isolates were more pathogenic than other ones of *Fusarium* spp. and *M. phaseolina*, whereas *Aspergillus niger* and *Mucor* sp. proved to be non-pathogenic. Best to relatively good growth of *F. oxysporum*, *F. solani* and *M. phaseolina* occurred when media contained either seaweed extract (SWE) at 2ml/l or 28-homobrassinoid (HBR) at 0.004 µg/l, whereas glycinebetaine (GB) at 10mM mostly favoured fungal growth. In field experiment, foliar spraying, at 18DAS, with HBR, SWE and GB at two concentrations, reduced wilt incidence. The highest reduction of wilt incidence was showed by application of HBR, followed by SWE, whereas GB application caused the lowest effect against incidence of wilt infection. Under artificial inoculation with *F. oxysporum* in pot experiment, disease reduction percentages were differed according to the substance, concentration and the season of the experiment. Disease reduction significantly increased with increasing the concentration of the used compound. Bean cultivated in *F. oxysporum* infested soil showed significant decrease in plant parameters, contents of Chlorophyll and carotenoids, concentrations of proline and TSS compared with plants cultivated in non-infested soil, whereas the activities of catalase, peroxidase and superoxide dismutase were higher in plants cultivated in infested soil. Also, concentrations of total carbohydrates and proteins were significantly decreased in pods of plants cultivated in infested soil. Foliar application of bio-regulators HBR, SWE and GB led to increase plant growth, number of pods/plant, the content of photosynthetic pigments, proline and TSS concentrations, activities of CAT, POX and SOD in leaves of treated plants in comparison with untreated plants. So, total carbohydrates and proteins significantly increased in pods of plants cultivated in both soil types.

Keywords: Brassinosteroid, common bean, *Fusarium oxysporum*, Glycine betaine, *Phaseolus vulgaris*, seaweed extract, vegetative growth and wilt.

Snap bean (*Phaseolus vulgaris* L.) is one of the most important and popular vegetable crops all over the world and in Egypt. It is utilized for various purposes,

i.e. fresh bean, dry pulses and edible podded type. It is valued for its beneficial effects in improving soil fertility and thus sustainability and profitability of production systems. It has been described as the first most important pulse crop (Stevenson *et al*., 1995). Nutritional value of beans can't be denied as these are an excellent source of protein, carbohydrates, water-soluble fibers, vitamins and antioxidants. As much as 60% of bean production in the developing world occurs under conditions of drought and salinity stresses (Franca *et al*., 2000). Fusarium wilt in common bean caused by *Fusarium oxysporum* (Schlecht.) f.sp. *phaseoli* Kendrick & Snyder is prevalent on most bean producing areas in the world. Various methods for controlling wilt disease of bean have been investigated including the use of cultural practices and chemical control (Punja *et al*., 1986), plant extracts (Kumar and Tripathi, 1991), resistant varieties (Brisa *et al*., 2007), plant volatile compounds (El-Mougy *et al*., 2007), and biological control (Ristaino *et al*., 1994 and Sallam *et al*., 2008). Brassinosteroids (BRs) are a family of hormones, involved in many cellular processes, including cell expansion and division, tissue differentiation, flowering, senescence and responses to abiotic stress (Bajguz and Hayat, 2009 and Yang *et al*., 2011).

24-epibrassinolide (BL) was found to induce disease resistance in plants (Nakashita *et al*., 2003).They found that wild-type tobacco treated with BL exhibited enhanced resistance to the viral pathogen tobacco mosaic virus (TMV), the bacterial pathogen *Pseudomonas syringae* pv. *tabaci*, and the fungal pathogen *Oidium* sp. and also induced resistance in rice to rice blast and bacterial blight diseases caused by *Magnaporthe grisea* and *Xanthomonas oryzae* pv. *oryzae*. Glycinebetaine (GB) is an osmoprotectant accumulated by barley (*Hordeum vulgare*) plants in response to high levels of NaCl, drought, and cold stress. Věchet *et al*. (2005) reported that GB and other compounds of natural origin showed protection against powdery mildew (*Blumeria graminis* f.sp. *tritici*) on winter wheat (cv. Kanzler), susceptible to this disease, and this slightly effected the synthesis of new proteins (PR-proteins) that were localized in extracellular space. The bulk of the investigations pertaining to bioactive compounds from seaweeds deals with phytopathogens are being restricted to pathogens of commercial crops such as tobacco (Caccamese *et al*., 1980), citrus trees (Kulik, 1995) and rice (Sultana *et al*., 2005).

The present study was designed to isolate, identify the pathogens associated with wilted bean plants and to evaluate the possible role of HBR, GB, and seaweed extract in modifying infection stress imposed by infested soil with the pathogen on yield attributes and plant and pod biochemical aspects of bean drought cultivar (cv. Paulista).

Materials and Methods

Naturally wilted snap bean (cv. Paulista) plants collected from Orabi Agricultural Operation Farms, Qaluybiya Governorate, during 2011/12 growing season, and used for isolation the pathogen(s) as described by Sallam *et al*. (2008). Identification of the isolated fungal pathogen(s) was carried out depending on the basis of cultural and microscopic morphological characters according to the Keys given by Gilman (1957), Booth (1971) and Burgess *et al*. (1994). Inoculum

preparation, soil infestation and pathogenicity of isolated fungi toward bean plants (cv. Paulista) were carried out as described by Sallam *et al*. (2008). Sterilized pots (25-cm-diam.) containing non-infested soil were used as check. Five surface sterilized seeds were sown in each pot, and six pots were used as a replicate. The percentages of seedling damping-off and wilt incidence were calculated, 21 and 70 days after sowing (DAS), respectively. The percentage of seed germination was tested, separately, in Petri dishes as described by (Anonymous, 2005) and it was found 100%.

To study the effect of bioregulators, *i.e*. 28-homobrassinoid (HBR, obtained from Fisher Scientific, UK limited, Bishop Meadow Road, Loughborough), seaweed extract (SWE; a concentrated solution packed by UAD company, Egypt) and glycinebetaine (GB; a powder produced by Fisher Scientific, UK limited), on fungal growth, *i.e.* linear and total growth, the techniques described by Hassan *et al*. (2013) for measuring and calculating the inhibition percentages of mycelial growth on Czapek's medium was used. Three plates (9-cm-diam.) or flasks (100 ml) were used for each treatment as replicates. Linear growth was recorded, 6 day after inoculation, whereas mycelial dry weight was recorded after 18 days of incubation at 25^{o}C.

Concerning the effect of HBR, SWE and GB on disease incidence and growth parameters, two experiments were carried out either under field conditions or in pots as follows:

1- Field experiment was carried out using seeds of cv. Paulista; dwarf French bean, obtained from Bakker brothers, Holland. Seeds were sown on November, 1St in two consecutive winter growing seasons, 2012/13 and 2013/14, under tunnels, using clear polyethylene sheet tunnels (thickness 120µ manufactured by Himaplast Company), in a commercial bean field with previous history of bean wilt disease, *i.e*. the field had been cultivated with bean for successive many years in the no-tillage system and it was observed to be naturally infested with, *i.e*. *Fusarium oxysporum* Schlecht ex Fries, in Orabi Agricultural Operation Farms, Qaluybiya Governorate. The field trials (24 plots) were designed in split-split with three replicates. The area of the experimental plot was 14 m^2, consisted of 5 rows of 4 m length and 0.75 m width. The plant distance was 25 cm apart on one side, an alley (1m wide) was left as boarder between different treatments. Two seeds/hill were sown and thinned to one uniform seedling at 15 DAS. All cultural managements and fertilization were followed according to the recommendations of the Egyptian Ministry of Agriculture (Anonymous, 2000) and plants were irrigated every two days with 75% of field capacity. Soil samples from experimental site were collected at random at depth of 5-20 cm from soil surface before soil preparation for mechanical and chemical analysis (Table 1). Plants were sprayed, three times, *i.e*. at 18 (fist full expanded leaf), 33 and 48 days after sowing (DAS) with aqueous solutions of 28-homobrassinloid (HBR) at 0.004, 0.002 µg/l, seaweed extract (SWE) at 1.0 and 5.0% and Glycine betaine (GB) at 5 and 10 mM/l. Tween 20 (01%) was used as wetting agent. Plants sprayed with distilled water were served as check. The disease incidence was calculated as percentages of wilted plants, 70 days after sowing (DAS).

Table 1. Mechanical and chemical analysis of the experimental soil

Particle size distribution (%)			Soil texture	EC(ds/m) (meq./l)	pH	Soluble anions			Soluble cations (meq/l)			
						HCO_3^-	Cl^-	SO_4^-	Ca^{++}	Mg^{++}	Na^+	K^+
Sand	Silt	Clay	Sandy soil	1.5 mmhos/cm	7.2	2.2	4.3	27.2	20.3	10.6	2.0	1.2
97	2	1										

2- Pot experiment was carried out to study the effect of cultivation in infested soil, HBR; at 0.004, 0.002 µg/l, SWE; at 2.0 and 1.0 %, and GB; at 10 and 5 mM/l, applications on leaves and pod chemical components. For soil infestation, 30 ml of fungal suspension containing $5x10^5$ spores/ml were added in pots (30-cm-diam) filled with sandy soil (Table 1), 7 days before planting. Bean seeds (cv. Paulista) were surface sterilized with sodium hypochlorite solution (1%) for 3 min. and rinsed several times with sterilized water. Pots containing sterilized soil were used as check. Six bean seeds were sown in each pot and the seedlings were thinned after the 8^{th} day to uniform 4 ones. The pots were kept under tunnels. The experimental design was split-split. The experiment was composed of 6 pots as replicates for each treatment.

Growth parameters and physiological studies:

Four bean plants were randomly selected from each treatment, at 70 DAS, to study the following parameters: Plant height, leaf area, shoot dry weight/plant and number of pods/plant.

Samples of leaves and pods were taken from plants grown either in disinfected or fungal inoculated soils and used for chemical analysis. Total chlorophylls (Chl), carotenoids, proline, total soluble sugars concentrations and antioxidant enzyme, *i.e*. catalase (CAT, EC 1.11.1.6).

Peroxidase (POX, EC 1.11.1.7) and superoxide dismutase (SOD, EC 1.15.1.1) activities were determined in fully expanded fifth leaf from the top of plants, 70 DAS. Also, total proteins and carbohydrates concentrations were determined in pods at end of the experiment.

A) Total chlorophylls (Chls) and carotenoids were extracted using the recently full expanded mature upper leaf of three plants by N, Ndimethyl formamide (Noran, 1982). The resultant extracts were incubated in a dark fridge overnight. The total Chls and carotenoids concentrations were determined colorimetrically, using CT 200 spectrophotometer) at 647, 664 and 470 nm, and the concentrations were expressed in $mg^{-1}g$ of fresh weigh.

B) Free proline was extracted using 3% (w/v) aqueous sulphosalisylic acid and estimated by ninhydrin reagent according to the method of Bates *et al.* (1973). Proline concentration was determined colorimetrically, at 520 nm. The proline calibrated concentration was standardized by the standard curve of L-proline as µg proline g^{-1} fresh weight.

C) For total soluble sugars (TSS) extraction, Anonymous (2005) methods were applied, and were determined according to Dubois *et al*. (1956) methods, using phenol 4% , sulphoric acid 96% and distilled water (1: 2: 9 v/v/v) for 1 ml of

soluble sugars extract. Total soluble sugars concentration was determined colorimetrically, at 490 nm, using standard curve of glucose (10-100 µg) and expressed as mg g^{-1} fresh weigh.

D) Determination of antioxidant enzyme activities:
According to the methods described by Bradford (1976), leaf tissues were homogenized in 100 mM chilled sodium phosphate buffer (pH 7) 1:4 w/v, containing EDTA (0.1 mM) and 1% polyvinyl pyrrolidone at 4ºC. The homogenate was centrifuged at 15000 rpm for 15 min at 4^{o} C. Supernatant was used to measure the activities of catalase, peroxidase and superoxide dismutase activities. Enzyme activities were calculated as mg protein/ min. Protein concentration was determined according to the method described by Bradford (1976) by using the standard curve of bovine serum albumin.

D.1. Catalase activity was determined according to Aebi (1984) method. It was expressed as unit min^{-1} mg^{-1}protein.

D.2. Peroxidase activity was assayed by the method of Hammerschmidt *et el*. (1982) as unit min^{-1} mg^{-1}protein. One international unit (iu) of enzyme activity was expressed as Δ OD=0.01.

D.3. Superoxide dismutase (SOD) was assayed according to Beauchamp and Fridevich (1971) by measuring the ability of enzyme to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). The absorbance was recorded at 560 nm. One unit of SOD activity is the amount of protein required to inhibit 50% initial reduction of NBT under light. The activity of SOD was expressed as unit min^{-1} mg^{-1}protein.

E) Determination of total soluble carbohydrates and total proteins in pods of bean cultivated either in infested or non- infested soils:

E.1. Protein concentration was determined as described before, using the methods of Bradford (1976) and expressed as mg protein g^{-1} fw.

E.2. Total soluble carbohydrates (TSC) were extracted from pod of bean as described in Anonymous (2005), and their concentration assay was carried out according to Chow and Landhäusser (2004). The absorbance was recorded at 490 nm using UV-vis spectrophotometer (CT 200) and expressed as mg^{-1} g^{-1} fresh weight, using a standard curve of glucose (10-100µg).

Statistical analysis:

All obtained data were subjected to the statistical analysis of variance and means were compared using the L.S.D. procedure as described by Gomez and Gomez (1984). The MSTATC program software (version 4.0) was used to analyse all data of this study.

Results

Isolation, identification and pathogenicity tests of bean wilt pathogen:

Fourteen isolates of fungi belonging to four different genera were isolated from naturally infected bean plants showing typical symptoms of wilt. The isolated fungi were identified as *Fusarium oxysporum* (seven isolates), *F. solani* (two isolates),

Macrophomina phaseolina (two isolates), *Aspergillus niger*, *Fusarium* sp. and *Mucor* sp. (one isolate from each). *Fusarium oxysporum* exhibited the highest frequency, being 50% whereas all other fungi caused lower frequency, being about 50 %, whereas all other isolated fungi recorded lower frequency (50%).

Pathogenicity tests indicated that *F. oxysporum* caused the most infection to bean plants (40-86.7%) recorded at 70 DAS (Table 2), followed by *M. phaseolina* which caused 30-46.7% infection and *Fusarium*. sp. (23.3%) whereas *F. solani* caused 13.3-20% infection. Both of *Mucor* sp. and *A. niger* failed to infect bean plants until 70 DAS. Isolate No. 3 of *F. oxysporum* appeared to be the highest pathogenic isolate, whereas isolate No. 4 was the lowest pathogenic one.

The artificial infected plants were used to re-isolate the causal pathogens to meet Koch's postulates. Re-isolation from the interior of roots revealed the presence of the concerned fungus in each case.

Table 2. Pathogenicity test of isolated fungi on snap bean plant (cv. Paulista)

Fungus	Isolate number	Damping off (%) 21 DAS	Wilt plants (%) 70 DAS	Survived plants (%)
F. oxysporum	1	10.0	46.7	53.3
	2	13.3	56.7	43.3
	3	20.0	86.7	13.3
	4	10.0	40.0	60.0
	5	13.3	83.3	16.7
	6	13.3	70.0	30.0
	7	13.3	63.3	36.7
F. solani	8	10.0	13.3	86.7
	9	10.0	20.0	80.0
Fusarium sp.	10	3.3	23.3	76.7
M. phaseolina	11	6.7	30.0	70.0
	12	10.0	46.7	53.3
A. niger	13	0.0	0.0	100.0
Mucor sp.	14	0.0	0.0	100.0
Check	-	0.0	0.0	100.0

The effect of HBR, SWE and GB on fungal growth:

Data obtained and presented in Table (3) point to the following: The three tested fungi varied regarding response to different growth parameters, *i.e*. radial and total growth, were variably affected by a given compound. However, best to relatively good growth of the three tested fungi occurred when media amended with either SWE at 2ml/l or HBR at0.004 µg/l. Glycine betaine at 10mM mostly favoured fungal growth, but sometimes its effect varied depending on the fungal species, *i.e. F. oxysporum* showed best linear growth than check, whereas no significant differences were recorded for *F. solani* and *M. phaseolina* linear growth if compared with check. Furthermore, the total growth of *F. solani* and *M. phaseolina* was more than check when GB (10 mM) was supplied.

Table 3. Effect of HBR, SWE and GB on the growth of three bean wilt fungi

Compound[1]	Conc.	Growth at 25°C					
		F. oxysporum		*F. solani*		*M. phaseolina*	
		Linear growth (mm)[2]	Dry weight (mg)[1]	Linear growth (mm)	Dry weigh t (mg)	Linear growth (mm)	Dry weight (mg)
HBR	0.004 µg/l	85[3]	458[3]	90	530	81	570
	0.002 µg/l	80	325	78	490	75	560
SWE	2 ml/l	88	550	90	575	88	570
	1ml/l	73	510	81	510	77	530
GB	10mM	79	321	71	431	65	510
	5mM	67	351	70	450	65	430
Check		73	330	70	450	72	410
L.S.D. at 5% for: Linear growth A= 27.44 B= 4.00 AB= N.S				Dry wt. A= 20.37 B= 11.98 AB= 19.35			

[1] Basic medium was Czapek's solution,
[2] agar 2% was added to study the radial growth.
[3] Average of 4 plates or flasks, data recorded after 6 or 18 DAI to determine linear growth or dry weight, respectively.
- A for treatments and B for fungi.

Effect of HBR, SWE and GB applications on disease incidence:

In field experiment, plants of bean, exposed to natural infection, were sprayed at 18 DAS with HBR, SWE and GB at two concentrations from each substance as shown in Table (4). Data indicate that all treatments reduced the incidence of wilt infection with comparison to check treatment. The highest reduction of wilt incidence was showed by application of HBR at both tested concentrations in the two experimental seasons. This was followed by SWE, whereas GB application caused the lowest effect against incidence of wilt infection.

Table 4. Percentages of wilt incidence, under natural infection, on bean plants treated with HBR, SWE and GB, 70 DAS

Compound	Conc.	2012/13 season			2013/14 season		
		Wilt incidence (%)	Disease reduction (%)	Survival plants (%)	Wilt incidence (%)	Disease reduction (%)	Survival plants (%)
HBR	0.004 µg/l	5.6	91.1	94.4	9.7	90.3	90.3
	0.002 µg/l	8.3	86.7	91.7	11.1	86.2	88.9
SWE	2 ml/l	11.1	82.2	88.9	13. 9	82.8	86.1
	1ml/l	16.7	73.3	83.3	15.3	81.0	84.7
GB	10mM	20.8	66.7	79.2	23.6	70.0	76.4
	5mM	25.0	60.0	75.0	23.6	70.0	76.4
Check		62.5		37.5	80.6		19.4

In pot experiment, bean plants were sown in artificially infected soil with *F. oxysporum* (isolate 3) and sprayed with HBR, SWE or GB, 18 DAS. Wilt incidence was determined at 70 DAS. Data in Table (5) indicate that all treatments led to reduce the percentage of disease incidence in the two years of experiments. The percentages of disease reduction were varied according to the substance that was tested, concentration of the substances and the season of performance of experiment. Generally, the percentage of disease reduction significantly increased with increasing the concentration of the used compound. The percentages of disease reduction ranged between 33.3-77.8 in the 1st season and 45-65 in the 2nd season. The most effective compound in controlling bean-wilt induced by *F. oxysporum* appeared to be HBR at 0.004µg/l; since the percentages of disease reduction were 77.8 and 65% in the two seasons, respectively. SWE at1ml/l and GB at 5mM appeared the least effective to control wilt incidence.

Table 5. Effect of applications of HBR, SWE and GB on snap bean wilt incidence under artificial *F. oxysporum* soil infestation

Substance	Conc.	2012/13 season			2013/14 season		
		Wilt incidence (%)	Disease reduction (%)	Survived plants (%)	Wilt incidence (%)	Disease reduction (%)	Survived plants (%)
HBR	0.004 µg/l	16.6	77.9	83.4	29.2	65.0	70.8
	0.002 µg/l	25.0	66.7	75.0	38.7	53.5	61.3
SWE	2 ml/l	25.0	66.7	75.0	38.7	53.5	61.3
	1 ml/l	50.0	33.3	50.0	41.6	50.0	58.3
GB	10 mM	29.2	61.1	70.8	41.7	50.0	58.3
	5 mM	37.5	50.0	62.5	45.8	45.0	52.2
Check-1		75.0		25.0	83.3		16.7
Check-2*		0.00	0.00	100.0	0.00	0.00	100.0

* Plants cultivated in non-infested soil, either untreated or treated by foliar spraying compounds, showed no disease incidence; the percentage of survived plants was 100%. These plants were used in plant parameters studies.

Vegetative growth characteristics:

Data presented in Tables (6 and 7) show that plant parameters were lower in plants cultivated in infested soil compared with plants cultivated in non-infested soil. Application of tested chemicals led to increase plant growth and number of pods/plant in comparison with check (untreated plants). Application of SWE or HBR resulted in increasing plant height, leaflet area, shoot dry weight and number of pods/plant, in plants cultivated in both tested soils. GB was the least effect compound in this respect. The data obtained in 2012/13 are generally has similar trend with those obtained in 2013/14.

Effect of treatments on total chlorophyll and carotenoids:

The contents of photosynthetic pigments in fresh plant leaves were determined as total chlorophyll (Chl) and carotenoids as shown in Table (8). Data indicated that Chl and carotenoids contents were decreased in leaves of bean cultivated in soil infested with *F. oxysporum*, either in bioregulators treated or untreated plants, if

Table 6. Effect of HBR, SWE and GB on plant height, leaflet area, of snap bean cultivated under tunnels in *F. oxysporum* infested or non- infested soil

Compound	Conc.	Plant height(cm)				Leaflet area (cm^2)			
		1st season 2012/13		2nd season 2013/14		1st season 2012/13		2nd season 2013/14	
		i.s.*	Non**	i.s.	Non	i.s.	Non	i.s.	Non
HBR	0.004µg/l	42.0	50.5	43.5	52.0	43.8	60.8	53.8	62.5
	0.002µg/l	36.3	41.8	38.8	44.3	40.3	52.5	48.5	58.3
SWE	2%	46.8	58.5	44.8	52.8	42.8	57.0	48.5	61.8
	1%	37.5	50.3	41.8	48.3	38.3	60.5	52.5	57.8
GB	10mM	41.8	43.0	38.0	44.8	42.8	57.0	44.8	54.5
	5mM	40.5	45.3	36.3	44.3	40.8	59.3	43.0	51.3
Check		30.5	38.8	26.3	42.3	28.8	53.5	25.3	51.8
L.S.D. at 5% for:		A= 4.66 B= 3.80 AB= N.S		A= 2.25 B=3.67 AB= N.S		A= 4.47 B= 6.36 AB=9.0		A=21.0 B= 7.1 AB= N.S	

* Infested soil, ** Non-infested soil, Conc. (Concentration), A= (Type of soil), B= (Treatment).

Table 7. Effect of HBR, SWE and GB on shoot dry weight and number of pods/plant of snap bean cultivated under tunnels in *F. oxysporum* infested or non-infested soil

Compound	Conc.	Shoot dry weight (g)				Number of pods/plant			
		1st season 2012/13		2nd season 2013/14		1st season 2012/13		2nd season 2013/14	
		i.s.*	Non**	i.s.	Non	i.s.	Non	i.s.	Non
HBR	0.004µg/l	19.6	24.8	21.0	25.2	28.0	46.5	24.8	47.0
	0.002µg/l	18.1	24.0	20.6	23.0	26.3	43.3	24.5	43.0
SWE	2%	22.0	25.9	19.6	24.8	31.5	46.3	22.3	45.8
	1%	18.0	25.5	19.1	22.4	23.3	45.5	20.3	38.5
GB	10mM	19.1	24.4	18.1	24.0	24.5	39.0	19.8	44.0
	5mM	19.3	24.6	17.2	24.0	19.3	41.8	19.0	41.5
Check		16.7	23..2	14.5	23.2	12.5	38.0	13.0	40.3
L.S.D. at 5% for:		A= 5.30 B= N.S AB= N.S		A=5.34 B= N.S AB= N.S		A= 5.54 B= 7.10 AB= 10.05		A= 8.79 B= 5.46 AB= 7.72	

* ; ** As described in footnote of Table (6).

compared with plants cultivated in non-infested soil. Foliar application of different chemicals increased the concentrations of both Chl and carotenoids in bean leaves compared with the check (untreated plants). The highest concentration of Chl was recorded with application of HBR (0.002 and 0.004µg/l) in plants cultivated in non-infested soil. At the second season, no significant differences were recorded when SWE at 1% or HBR (0.002 and 0.004 µg/l) were used. Also, Chl concentration was significantly higher in leaves of plants cultivated in infested soil, treated with HBR, SWE and GB more than that recorded in untreated plants. The highest concentration of total Chl was recorded with HBR (0.002%), whereas the highest concentration of carotenoids was recorded, in most cases, with HBR or GB applications.

Table 8. Effect of HBR, SWE and GB in chlorophyll and carotenoids concentrations in leaves of snap bean cultivated under tunnels in *F. oxysporum* infested or non-infested soil

Compound	Conc.	Total chlorophyll *				Total carotenoids			
		1st season 2012/13		2nd season 2013/14		1st season 2012/13		2nd season 2013/14	
		i.s.**	Non***	i.s.	Non	i.s.	Non	i.s.	Non
HBR	0.004µg/l	3.5	3.9	3.6	4.0	0.5	0.6	0.5	0.6
	0.002µg/l	3.8	3.9	3.8	3.5	0.5	0.6	0.4	0.5
SWE	2%	3.1	3.1	3.0	3.1	0.5	0.5	0.5	0.5
	1%	3.0	3.3	3.3	3.5	0.5	0.6	0.5	0.4
GB	10mM	3.1	3.7	3.5	3.5	0.5	0.6	0.5	0.6
	5mM	2.8	3.0	2.7	3.3	0.5	0.5	0.4	0.5
Check		2.6	3.0	2.5	3.1	0.4	0.5	0.4	0.4
L.S.D. at 5% for:		A= 0.404 B= 0.19 AB= N.S		A= 0.06 B= 0.1 AB= 0.14		A= 0.06 B= 0.05 AB= N.S		A= N.S B= N.S AB= N.S	

* mg^{-1} gfw, ** Infested soil, *** Non-infested soil, Conc., A and B: As described in footnote of Table (6).

Effect of bioregulators on proline and total soluble sugars concentrations:

Data in Table (9) show that proline concentration was higher in plants cultivated in non-infested soil than that cultivated in contaminated soil in both tested seasons of performing experiment. The application of bioregulator compounds increased the concentration of proline in leaves of bean cultivated in both soil types. Glycinebetaine (10mM) and HBR (0.002 µg/l) were the most effective compounds, followed by SWE, in both seasons.

Table 9. Effect of HBR, SWE and GB in proline and total soluble sugar (TSS) concentrations in leaves of snap bean cultivated under tunnels in *F. oxysporum* infested or non-infested soil

Compound	Conc.	Proline conc. (μg^{-1}gfw)				TSS (mg^{1} gfw)			
		1st season 2012/13		2nd season 2013/14		1st season 2012/13		2nd season 2013/14	
		i.s (1).	Non	i.s.	Non (2)	i.s.	Non	i.s.	Non
HBR	0.004µg/l	7.9	10.2	8.1	9.7	14.8	14.5	16.5	15.9
	0.002µg/l	9.0	9.1	7.7	9.3	14.7	14.1	15.8	15.1
SWE	2%	7.7	8.1	7.3	7.9	14.5	14.4	15.1	14.6
	1%	7.5	7.9	7.1	8.0	14.7	13.7	15.7	14.7
GB	10mM	8.4	8.7	7.9	9.9	13.9	13.5	14.5	14.1
	5mM	7.1	7.4	7.3	9.7	13.4	13.1	14.5	14.2
Check		6.5	7.1	6.1	7.4	11.2	14.3	10.7	12.8
L.S.D. at 5% for:		A= 0.52 B= 0.42 AB= 0.60		A= 0.61 B= 0.50 AB= 0.7		A= 0.73 B= 0.91 AB= 1.2		A= 1.58 B= 0.74 AB= 1.05	

* ; ** As described in footnote of Table (6).

Data in Table (9) reveal also that application of bio-regulators significantly increased the concentration of TSS in comparison with the check treatment in both seasons. The most pronounced effect was achieved when HBR was applied at the highest level. The lowest TSS concentration was shown with GB. Total soluble sugars (TSS) concentration was decreased in plants cultivated in infested soil. But, a slightly increase in TSS concentration was recorded in plants cultivated in infested soil in comparing with plants cultivated in non-infested soil after application of bioregulator compounds, in both seasons.

Effect of HBR, GB and SWE on activities of oxidative enzymes:

Data in Table (10) show that cultivation of bean plants in soil infested with wilt-inducing-pathogen led to increase the activities of catalase and peroxidase enzymes. The increase in enzymes activities were more than 1.5 to about 2 times in plants cultivated in infested soil than that cultivated in non-infested soil. Also, the application of HBR, SWE and GB, with all tested concentrations, significantly increased the activities of both CAT and POX in plants cultivated in both tested soils in comparison with untreated plants (check). The highest activities of both determined enzymes were observed in leaves treated with either SWE (2%) or HBR at 0.004µg/l or 0.002µg/l, whereas GB was the least affected one. Also, cultivation of bean plants in infested soil led to increase the activity of superoxide dismutase (SOD) (Table 11). The enzyme activity was significantly increased by application of bioregulators either in plants cultivated in infested or in non-infested soils. Seaweed extract at 2 or 1% recorded the maximum mean values of SOD activity, followed by application of HBR at 0,004 and 0.002µg/l. The least values of SOD activity were recorded by application of GB. In most cases, the similar trend of results was recorded for activities of antioxidant enzymes (CAT, POX and SOD) in the two successive seasons of experiment.

Table 10. Effect of HBR, SWE and GB on activities of catalase and peroxidase enzymes in leaves of snap bean cultivated under tunnels in *F. oxysporum* infested or non-infested soil

Compound	Conc.	Catalase activity (unit/min/mg)				Peroxidase activity (unit/min/mg)			
		1st season 2012/13		2nd season 2013/14		1st season 2012/13		2nd season 2013/14	
		i.s.*	Non**	i.s.	Non	i.s.	Non	i.s.	Non
HBR	0.004µg/l	0.97	0.82	1.31	0.74	0.98	0.52	0.97	0.52
	0.002µg/l	0.81	0.61	0.93	0.65	0.91	0.50	0.98	0.63
SWE	2%	1.31	0.85	1.41	0.97	0.93	0.72	0.97	0.69
	1%	0.92	0.86	1.01	0.91	0.72	0.65	0.89	0.59
GB	10mM	0.83	0.41	0.82	0.51	0.63	0.39	0.67	0.42
	5mM	0.76	0.41	0.72	0.51	0.61	0.41	0.63	0.41
Check		0.73	0.37	0.65	0.42	0.53	0.38	0.61	0.39
L.S.D. at 5% for:		A= 0.106 B= 0.061 AB= 0.08		A= 0.46 B= 0.20 AB= 0.29		A= 0.061 B= 0.13 AB= N.S		A= 0.184 B= 0.112 AB= 0.158	

* ; ** As described in footnote of Table (6).

Table 11. Effect of HBR, SWE and GB in activities of superoxide dismutase (SOD) in leaves of snap bean cultivated under tunnels in *F. oxysporum* infested or non-infested soil

Compound	Conc.	SOD activity (unit/min/mg)			
		1st season, 2012-2013		2nd season, 2013 -2014	
		i.s.*	Non**	i.s.	Non.
HBR	0.004µg/l	0.72	0.69	0.95	0.72
	0.002µg/l	0.79	0.63	0.93	0.71
SWE	2%	1.13	0.91	0.97	0.79
	1%	0.95	0.71	0.93	0.75
GB	10mM	0.75	0.53	0.83	0.63
	5mM	0.61	0.41	0.82	0.59
Check		0.57	0.40	0.71	0.41
L.S.D. at 5% for:		A= 0.087 B= 0.070 AB= N.S		A= 0.246 B= 0.162 AB= 0.229	

* ; ** As described in footnote of Table (6).

Data in Table (12) show the effect of HBR, SWE and GB on total carbohydrates and total protein concentrations in pods of bean cultivated in sterilized or artificially inoculated soil with *F. oxysporum*; bean-wilt-inducing pathogen. The concentrations of total carbohydrates and proteins were significantly decreased in pods of plants cultivated in infested soil. Application of bioregulators and GB led to significant increase in both total carbohydrates and proteins in pods of plants cultivated in both soil types. In most cases, GB at10 mM, especially in plants cultivated in non-infested soil, followed with HBR or SWE increased both carbohydrate and protein concentrations in pods of bean comparing with check. The lowest concentration in carbohydrates was recorded in pods of plants sprayed with HBR (0.002µg/l) in the 1st season or that sprayed with SWE (1%) in the 2nd season, whereas lowest protein concentration was recorded in plants treated with GB (5mM) at the two seasons.

Table 12. Effect of HBR, SWE and GB in total carbohydrates and proteins in pods of snap bean cultivated under tunnels in *F. oxysporum* infested or non-infested soil

Compound	Conc.	Carbohydrates (mg/gfw)				Proteins (mg/gfw)			
		1st season 2012/13		2nd season 2013/14		1st season 2012/13		2nd season 2013/14	
		i.s.*	Non**	i.s.	Non	i.s.	Non	i.s.	Non
HBR	0.004µg/l	103.7	122.5	112.7	125.2	12.7	16.8	14.9	16.5
	0.002µg/l	101.1	110.2	95.8	115.3	10.3	15.7	13.8	15.8
SWE	2%	107.5	127.3	103.8	117.2	15.3	14.7	16.7	18.9
	1%	103,2	117.2	91.7	112.7	12.3	14.8	11.3	16.8
GB	10mM	112.7	127.3	97.1	122.9	15.7	16.8	16.1	18.9
	5mM	110.5	112.2	92.7	121.3	10.0	13.7	12.9	14.5
Check		93.5	110.3	89.5	102.7	10.1	13.9	9.5	14.8
L.S.D. at 5% for:		A= N.S B= N.S AB= N.S		A= 24.5 B= 9.53 AB= N.S		A= 1.97 B= 2.79 AB= 3.95		A= 3.33 B= 2.32 AB= N.S	

* ; ** As described in footnote of Table (6).

Discussion

Wilt disease of snap bean, caused by *Fusarium oxysporum*, is a serious disease causes severe losses in yield under different environmental conditions. The obtained results in this investigation indicate that *F. oxysporum* consider the main pathogen causing wilt disease in snap bean plants. These results are in general agreement with those obtained by many researchers (Rusuku *et al*., 1997; Buruchara and Camacho, 2000; Dhingra and Coelho-Neto, 2001 and El-Mougy *et al*., 2007).

Several applications for controlling the disease were carried out. Application of the fungicides is not economical in the long time because they pollute the environment, leave harmful residues and can lead to the development of resistant strains of the pathogens with repeated use (Vinale *et al*., 2008). Plant disease control with the environmental concern, several promising modern approaches have been developed recently away from fungicides use. Among them the induced resistance approach, which could be induced in plants by applying chemical elicitors (Elad, 1992). Seaweeds are generally classified as a "bio-stimulant," due to seaweed extracts which contain natural plant growth regulators (PGR) which control the growth and structural development of plants. Seaweed extracts have been proven to accelerate the health and growth of plants. The actions of these extracts are many, particularly in the pockets of soil around the feeder roots; rhizosphere, where the mycorrhiza make their home, resulting in a substantially larger root mass. The rhizosphere activity improves the plants ability to form healthier, stronger roots. Having many actions it also enhances the plants own natural ability to ward off diseases, especially that caused by soil borne fungi. At the same time it works within the soil to make more nutrients available to the plant. Another action seaweed has on the roots in the rhizosphere is due again to the increased mass and depth of the roots the plant is able to draw more moisture from the soil increasing the drought tolerance level. The root mass also allows the plant to more effectively absorb and use fertilizers that are applied to the plant and soil. The overall stronger root structure may help plants physically resist certain types of root diseases. The bulk of the investigations pertaining to bioactive compounds from seaweeds deals with human pathogens; studies related to phytopathogens are being restricted to pathogens of commercial crops such as tobacco (Caccamese *et al*., 1980), citrus trees (Kulik, 1995) and rice (Kumar and Regnasamy, 2000 a & b and Sultana *et al*., 2005). A seaweed; namely *Sargassum wightii*, has been an excellent source of antibacterial principles in controlling the bacterial blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* (Kumar and Regnasamy, 2000 a & b). Kulik (1995) indicated the significance of evaluating algae for use in the biological control of plant pathogenic bacteria and fungi. Kumar *et al*. (2008) found that the brown seaweed extracts *Sargassum wightii* and *Turbinaria conoides* proved to inhibit the gram negative bacteria *Pseudomonas syringae* causing leaf spot disease of the medicinal plant *Gymnema sylvestre*. In the present study, HBR, SWE and GB were tested, as a foliage treatment, for controlling *F. oxysporum*; the major bean-wilt-inducing pathogen.

In laboratory experiment, all tested compounds appeared to be stimulated the growth of three tested fungi, causing wilt symptoms on bean, *i.e*. best to relatively

good growth of the three tested fungi occurred when media contained either SWE (2ml/l) or HBR (0.004 µg/l). Glycine betaine at 10mM mostly favoured fungal growth, but sometimes its effect varied depending on the fungal species, *i.e. F. oxysporum* showed best linear growth, whereas no significant differences were recorded for *F. solani* and *M. phaseolina* linear growth in comparison with check, whereas the total growth (dry weight) of all tested fungi was more than check when GB (10 mM) was supplied.

The present study showed that foliage spraying with HBR, SWE or GB considerably reduced the percentage of infection induced by *F. oxysporum*, either under natural or artificial infection. Percentage reduction of wilt incidence ranged between 60-91.1% in the 1st season and 70.0-90.3% at the 2nd season, under field conditions, whereas under artificial inoculation, the percentages of disease reduction ranged between 34.6-78.2 in the 1st season and 48.11-66.7 in the 2nd season. HBR was the most effective among the used compounds; it caused a percentage reduction of infection 78.2 and 69.6 at the 1st season and 66.7 and 59.2 at the 2nd season at 0.004 and 0.002 µg/l, respectively. Seaweed extract and GB appeared the least effective to control the disease.

According to the obtained results a reduction in both Chl and carotenoids contents in leaves of bean cultivated in soil infested with *F. oxysporum*, the pathogen induced wilt of bean, was recorded. Foliar application with HBR, SWE, or GB increased the concentrations of both Chl and carotenoids in bean leaves cultivated in infested or sterilized soils, in comparison with untreated plants. The highest concentration of Chl was recorded with SWE (1%) and HBR (0.002 and 0.004µg/l) applications in plants cultivated in non-infested soil. Also, the treatment of bean plants with any of the tested compounds led to increase the concentrations of both Chl and carotenoids in leaves of plants cultivated in infested soil than that recorded in untreated plants. In most cases, the highest concentration of Chl was recorded when HBR (0.002µl/l), but for carotenoids when HBR or GB were applied.

New discoveries of the physiological properties of Brassinosteroids (BRs), seaweed extracts (SWEs) allow us to consider them in future as highly promising, environmentally-friendly, natural substances suitable for wide application in plant protection and yield promotion in agriculture. Nakashita *et al*. (2003) were the first to demonstrate that BR hormones function in disease resistance in both tobacco and rice. Brassinolide (BL) is the end product of the BR biosynthetic pathway and in rice its application enhanced resistance to blast and bacterial blight diseases caused by *Magnaporthe grisea* and *Xanthomonas oryzae*, respectively. In tobacco, BL induced resistance to Tobacco Mosaic Virus, the bacteria *Pseudomonas syringae* pv. *tabaci* and the fungus *Oidium* sp. The steroid hormone-mediated disease resistance (BDR) plays part in defence response in tobacco; they suggested that BDR functions in the innate immunity system of higher plants including dicotyledonous and monocotyledonous species.

Brassinosteroids hormones sequentially bind to the extracellular domains of the leucine rich repeat receptor BR Insensitive 1 (BRI1) and the co-receptor BRI1-associated Kinase 1 (BAK1) (Santiago *et al*., 2013). Trans phosphorylation between BRI1 and BAK1 activates the former, which in turn leads to a downstream BR

signalling cascade (Yan *et al*., 2012). Exogenous application of BLs to plants at seed level or as foliar spray enhances antioxidant defence activities, and accumulation of osmoprotectants such as proline and glycine betaine under stress conditions illustrated anti stress properties of brassinosteroids (Sirhindi, 2013). BLs reported to play a regulatory role in the control of cell-cycle progression and differentiation in the Arabidopsis, and other plants may offer a novel therapeutic strategy for various diseases. BL induced molecular changes that are related to stress tolerance including enhanced expression of stress responsive genes (Kagale *et al*., 2007), protection of translational machinery (Dhaubhadel *et al*., 2002), potentiated accumulation of osmoprotectants (Sirhindi *et al.*, 2011), NADPH-oxidase-mediated accumulation of hydrogen peroxide and enhanced photosynthetic efficiency (Xia *et al*., 2009).

Obtained results revealed that plant parameters, *i.e.* plant height, leaflet area, shoot dry weight and number of pods/plant, were lower in plants cultivated in infested soil compared with that cultivated in non-infested soil but increase in plant growth was recorded in bean plants sprayed with any of the tested chemicals in comparison with check (untreated plants). SWE and/or HBR were the available compounds under this investigation for increasing plant growth parameters in plants cultivated in both tested soils, whereas, GB was the least effective compound in this respect.

Glycinebetaine (GB) is a quaternary ammonium compound (QAC) that functions as a compatible solute in many plant species (Rhodes and Hanson, 1993). There is a wealth of evidence suggesting that GB plays a role in stress tolerance in some plant species. Additionally it plays in a number of stress conditions, high salt (Arakawa *et al*., 1992), cold (Kishitani *et al*., 1994), and drought by either withholding water (Hitz *et al*., 1982) or using polyethylene glycol (*Arakawa et al*., 1992), lead to induction of glycinebetaine. All of them will inhibit photosynthesis and when photosynthesis is inhibited, excited-state chlorophylls cause the formation of reactive oxygen species (ROS). It was, therefore, of interest to test other, more direct producers of ROS for glycinebetaine induction. Furthermore, exogenous application of GB may have other effects, such as increased resistance to insects (Manninger *et al.*, 1998) or disease (Karjalainen *et al*., 2002). So, the obtained results are, generally, in harmony with those obtained by previous investigators and others; Blunden *et al*. (1997) who have concluded that glycinebetaine acts to increase the concentration of chlorophyll, or to protect against its degradation under stress (Gadallah, 1999).

The present results pointed to increase the activities of CAT, POX and SOD in bean plants cultivated in infested soil than their activities in plants cultivated in non-infested soil. Data showed also that SWE, HBR and GB application led to increase the activities of the tested enzymes in treated plants cultivated either in infested or non-infested soils. In most cases the highest levels of enzyme activities were recorded with application of SWE, followed by HBR (0.004 µg/l), whereas GB showed the lowest activity of all tested enzymes, in both types of soils. Also, the concentrations of total carbohydrates and proteins were significantly decreased in pods of plants cultivated in infested soil, whereas application of HBR, SWE and GB led to significant increase in both total carbohydrates and proteins in pods of plants

cultivated in both soil types. In most cases, GB at10 mM, especially in plants cultivated in non-infested soil, was the best one if compared with check. The lowest concentration in carbohydrates was recorded in pods of plants sprayed with HBR (0.002µg/l) in the 1st season or that sprayed with SWE (1%) in the 2nd season, whereas, the lowest protein concentration was recorded in plants treated with GB (5mM) at the two seasons. These results are in close agreement with that reported by Kumar *et al*. (2012) who found that *Brassica juncea* L. (RCM 619) treated with 24-EBL (10-6, 10-8, 10-10 M) before exposing to heat shock afforded the seedlings tolerant to heat shocks (40oC) by elevating the antioxidant enzyme activity such as SOD, CAT, and APOX to higher levels. Total proteins also increased in such seedlings as compared to untreated heat-exposed seedlings.

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مكافحة الذبول الفيوزاريومى فى الفاصوليا الخضراء وتحسين صفات النبات والمحصول باستخدام مركبات البارا سيناستيرويد والجلايسين بيتايين ومستخلص الأعشاب البحرية

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تم عزل اربعة عشر عزلة من الفطريات تنتمى إلى أربع أجناس (٧ عزلات من الفطر *F. oxysporum*، عزلتان من كل من *Fusarium solani* و *Macrophomina phaseolina* وعزلة واحدة من الفطريات *Aspergillus niger*, *Fusarium* sp. and *Mucor* sp.) من نباتات فاصوليا مصابة طبيعيا بأعراض الذبول. وأظهرت دراسات القدرة المرضية أن عزلات الفطر *F. oxysporum* أكثرها قدرة مرضية وبالأخص العزلة رقم ٣. وقد تحسن نمو الفطريات المختبرة عند إضافة مستخلص الأعشاب البحرية ومركب HBR و الجلايسين بيتايين بتركيزات ٢مل/لتر، ٠,٠٠٤ ميكروجرام/لتر و ١٠ ملليمول على الترتيب. أدى رش المجموع الخضرى للفاصوليا عند عمر ١٨ يوم ، فى تجربة حقلية، بتركيزين من تلك المركبات إلى حدوث نقص ملحوظ فى نسبة الإصابة بالذبول. وكان المركب HBR أكثرها تأثيرا بينما كان مركب الجلايسين بيتايين أقلها. وفى تجربة أصص تم رش نباتات فاصوليا مزروعة فى تربة معقمة غيرمعدية أو معدية بالعزلة رقم ٣ للفطر *F. oxysporum* - عند عمر ١٨ يوم- بالمركبات المختبرة السابقة كل على حدة وكانت النتائج كالآتى: قلت نسبة إصابة النباتات بالذبول عند رشها بالمركبات المختبرة وقد تباينت نسبة النقص فى الإصابة باختلاف المركب والتركيز والموسم، وقد قلت قياسات نمو النباتات والمحتوى من الكلوروفيل والكاروتينات وتركيزى البرولين والسكريات الكلية الذائبة فى النباتات المزروعة فى التربة المعدية بالفطر بالمقارنة بالتربة غير المعدية، كما زاد نشاط كل من الإنزيمات كتاليز، بيروكسيديز وسوبراكسيد ديزموديز وظهر نقص معنوى فى تركيزى البروتينات والكربوهيدرات الكلية فى القرون الناتجة من نباتات مزروعة فى التربة المعدية بالفطر. وقد أدى رش المجموع الخضرى للنباتات بالمركبات المختبرة إلى زيادة نمو النباتات وعدد القرون/نبات، وزيادة المحتوى من صبغات التمثيل الضوئى وتركيزى البرولين والسكريات الكلية ونشاط إنزيمات كتاليز، بيروكسيديز وسوبراكسيد ديزموديز فى أوراق النباتات المعاملة سواء المزروعة فى التربة المعدية أو غير المعدية ، وكذلك زاد المحتوى البروتينى والكربوهيدرات الكلية زيادة معنوية فى قرون النباتات المعاملة فى كلا نوعى التربة.