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EFFECT OF PRE- EMERGENCE HERBICIDES TREATMENT ON *SCLEROTINIA SCLEROTIORUM* IN *VITRO* AND STEM BLIGHT DISEASE SEVERITY IN COMMON BEAN(*)

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ABSTRACT

In pot experiments, both herbicides butralin and pendimethalin were applied as pre-emergence treatment at three concentrations (0.5X, 1X, 1.5X) of the recommended rate to investigate their effect on the infection of common bean (*Phaseolus vulgaris* L. cv Nebraska) with two isolates (Ss_1, Ss_2) of *Sclerotinia sclerotiorum* (Lib.) de Barry, Data showed that both compounds suppressed the infection by both isolates at all concentration compared with the control. In the first season 2013 at the highest concentration (1.5X) of both herbicide disease severity mean (%) recorded were 45.14, 46.92 and 19.67, 18.78 compare to control treatment 58.34, 24.33, for Ss_1 and Ss_2, respectively. However the reduction values were not significant at the highest concentration mean value about 45.14, 19.67 for Ss1 and Ss2 compared with the control 58.34 and 24.33, respectively. In the second season the highest concentration (1.5X) of both herbicide disease severity mean (%) recorded for Ss_1, Ss_2 were 47.22, 39.28 and 21.33, 20.10 compare with 52.00, 27.44 to the control. Laboratory experiments showed that both compounds had an adverse effect on the linear growth (mm) and mycelial dry weight (mg/50 liquid medium). Data, also, showed that sclerotial formations of both isolates were lowered in the media amended with the tested compounds. The germination of sclerotia "mycleigenic and carpogenic" were also negatively affected by tested herbicide. Oxalic acid production was reduced by both tested herbicides. However no visible root injury was observed due to using both herbicides tested

Key words: *S. sclerotiorum*, herbicide, pendimethalin, butralin and oxalic acid

INTRODUCTION

Common bean is the most important legumes used for direct human consumption in Africa, Caribbean, and Latin America where it provides a cheap source of protein, calories, vitamins, fiber, and minerals for low income populations (Broughton *et al*, 2003; Beebe, 2012). The crop represents a cheap source of proteins and carbohydrate in human diets for relatively abroad sector of the Egyptian inhabitants, mostly for those who cannot afford the high cost of animal protein, especially in the rural area (HEIA, 2003; Mostafa *et al*., 2014). Moreover the total cultivated area in 67,596 Fedan for green bean production with a yield of about 2,820,897 ton (Mostafa *et al*., 2014). Under Egyptian conditions, bean plants are liable to infection by different pathogenic (i.e. fungal, bacterial, virus and nematodes and Physiological disorder). However, fungal diseases, especially damping-off and root-rot diseases are the constrain ones Amongst the major fungal pathogens *Sclerotinia sclerotiorum* (Lib.) de Bray, the causal organism of Sclerotinia rot (SR) is the most ubiquitous, omnivorous, soil-borne and destructive plant pathogen, inciting disease on more than 500 plant species as well as more than 408 host species of *Sclerotinia sclerotiorum* are known, also the pathogen is a widespread fungal and causes dangerous diseases on many economically important vegetables and field. (Saharan and Mehta, 2008; Boland and Hall, 1994). Approximately 90% of its life cycle is spent in soil as sclerotia and their high persistence makes *S. sclerotiorum* very successful pathogen (Adams and Ayars, 1979). This fungus produces and secretes concentrations of oxalic acid ($C_2H_2O_4$) into their surrounding media (Cessna *et al.,* 2000). The production of oxalic acid was considered as a pathogenicity determinant in *S. sclerotiorum* (Goody *et al.,* 1990; Li *et al*., 2008 and Williams *et al*., 2011). Manual injection of culture filtrate containing oxalic acid induces brown necrotic lesions similar to the symptoms caused by Sclerotinia disease (Dai *et al.,* 2006). Oxalic acid has been reported to disturb guard cell function during infection by *S. sclerotiorum* by inducing stomatal opening and inhibiting stomatal closure by abscisic acid (Guimaräes and Stotz, 2004).

Substituted dinitroanaline compounds were first reported by eli lily company in 1960 (Alder *et al*., 1960). They are used as selective herbicide for weed control in many vegetable and field crops (Ashton and Crafts, 1981). As with other many pesticide, the biological activity of these compounds may extend beyond their effect on weeds to other microorganisms in the surrounding environment. Generally, Altman (1991) stated that herbicide may affect

plant diseases through their effect on the pathogen, plant resistance or on the microorganism in the rhizosphere. It has been reported that dinitroanaline exert a direct fungi toxic effect on plant pathogen (Neubauer and Avizohar-Hershenson 1973; Zidan *et al.*, 1998; Osman and El-Khadem, 1989 and Postor & March 1991). Previous reports indicated that using increase the severity of some plants diseases (Standifer *et al*., 1966; Neubauer and Avizohar-hershenson, 1973; Gilbertson *et al*., 1987 &Fitter *et al*., 1999).

In Egypt, there is a growing increase in using herbicide in recent years for weed control by some growers. Also, there is no much information in the literature concerning the effect of herbicides on the causal agent of plant diseases. Thus this work was conducted to

1) Isolate the fungal pathogen associated with the stem blight of beans,
2) Test the pathogenic capability of the obtained isolates,
3) Investigate the effect of tested herbicide on stem blight a diseases development in bean plants,
4) Study *in vitro* the effect of the tested herbicides on growth parameters of the pathogen and,
5) Test the effect of both herbicides on some metabolite products related to pathogenesis.

MATERIALS AND METHODS

All pathogenicity and herbicides treatment experiments were conducted in plastic pots (15 cm diameter) under greenhouse of Plant Pathol. Dept., Fac. Agric., Minia Univ.

1- Sample collection:

Samples of naturally infected bean plants showing root rot , stem blight and wilt symptoms were collected from different locations of Mania Governorate**,** Soil was carefully removed from around the roots and basal stems then they were placed in a paper bag and stored in a cooler and brought to the laboratory.

2- Isolation and identification of the causal agents:

The diseased tissues were washed in running tap water and cut into small pieces about 0.5cm long, and surface disinfected with 0.5% $NaOCl_3$ for 3 min, rinsed with sterile distilled water three times, dried on sterile towel paper, The prepared small pieces (5/mm) were placed onto Petri dishes (90 mm) containing potato dextrose agar (PDA, Difco, Detroit, MI) amended with 30 mg/L streptomycin (streptomycin sulfate, Sigma, St Louis, MO). The plates were incubated at (25± 2 C°) and observed for fungal growth; colonies on PDA were sub cultured with hyphal tips technique until pure. Pure cultures of all isolated fungi were maintained on PDA slants till identification. All fungi isolated were examined microscopically and identified according to the description giving by (Barnett and Hunter, 1998). Only isolates belonging to the fungus *Sclerotinia* were considered for the purpose of this study and other fungal isolates were stored for other studies. Five isolates of the target fungus were

designed Ss_1, Ss_2, Ss_3, Ss_4, and Ss_5 were used for this study.

3- Pathogenicity tests:

3.1- **Preparation of inoculua and inoculation:**

The inoculum used in the foregoing studies for all isolates consisted of uniform agar discs of 1 mm in diameter bearing 7- days' old growth of the desired pathogenic fungus. Inoculum discs were manipulated to the desired medium under aseptic conditions. The inoculum was grown in 250 ml. glass bottles containing sterilized 100 g of natural medium containing mixture of {(100g barely grain), (50g washed sand) and (100 ml water) } . The bottles were inoculated with uniformed agar disc of desired fungal and incubated at 25±2 ºC for two weeks to obtain sufficient. The isolate inoculum was then mixed with mixture soil (Nile salt+ sand) at the rate of 5g/ kg of soil weight and then mixed thoroughly. In check treatment equal amount of the uninoculated substrate was added. Healthy apparent common bean (Nebraska) Seeds were sowed in pot (5 seeds/each pot) and three replicates were used for each particular treatment**.** . Re-isolation was carried out from some of the artificially diseased plants to fulfill Koch's postulations and the developed fungi were confirmed with the original isolates.

3.2. Diseases assessment:-

To assess the Sclerotinia rot intensity, the following modified disease rating (0-4) scale **(Lesovoi** et ***al.*, 1987 and Sansford, 1995)** was followed:

Numerical scale description/ lesion length on stem

0 = Healthy (no visible lesion), 1= 0.1 – 2 cm lesion length on stem, 2= 2.1 – 4.0 cm lesion length on stem, 3=4.1 – 6 cm lesion length on stem, 4= > 6.1 cm lesion length on stem or complete dried plant.

The length of lesion on infected stem was considered for recording the disease intensity (Sharma, 1987). The infected area was calculated from plants in each plot and then average for each treatment was worked out. The per cent disease severity was calculated using the formula of Wheeler (1969):

$$\text{Disease severity \%} = \frac{\text{Sum of individual ratings}}{\text{No. of plants observed x Maximum disease rating}} \times 100$$

4- Effect of herbicides on stem blight development:

The experiment consisted of the following herbicides: The tested herbicides butralin { N-sec-butyl-4-tert-butyl-2,6-dinitroanilin (Amex® 48 % Nufarm Company)} and Pendimethalin { N-(1ethylpropyl)-2,6-dinitro-3,4-xylidine][Stomp Extra® 45% BASF Company} which used in this study were chosen for study because they are registered for pre-emergence application on soil were applied three rates, This study was carried out with two independent experiments. The desired concentrations were prepared based on

the recommended rate (X) for both herbicide pendimethalin (1.5L/300L/fedan) and Butralin (2.5L/ 300L/fedan). These were applied (0.5X, 1X, 1.5X) which are 0.75L, 1.5L, and 2.25L per fedan for pendimethalin an 1.25L, 2.5L and 3.75L/ fdan for Butralin expressed as (0.5X, 1X and 1.5X of recommended rate) according to the recommendations of Agriculture Ministry. The prepared concentrations were uniformly incorporated in to the soil to deliver equal amount for each pot hand sprayer was used for this purpose. Soil was infested with tested fungi as previously described, sown with healthy seeds (Nebraska cultivation) (5seed / pots), and then the herbicides were applied until covered the soil surface. Eight weeks after sowing rot and wilt were examined. Disease assessment was conducted as described above in pathogenicity tests.

5- Effect of herbicides on *S. sclerotiorum* linear growth *in vitro*

The herbicides were added to Czapek's media, while the control was on Czapek's without herbicide. The herbicide-Czapek's agar medium was prepared by adding the tested herbicide into sterilized (121^{o}C, 15 min) Czapek's medium, and mixed thoroughly before pouring into the Petri-dishes. The Petri-dishes were covered and allowed to dry for 1 h in sterile condition. Fungus subculture of 8 days old was transferred aseptically to the center of the herbicide-Czapek's agar medium and control plates (marked by perpendicular lines). The plates were then covered and sealed, followed by incubation at 25ºC in darkness. The effect of herbicides on the fungal isolates was measured by the liner growth of fungal colony in both control and herbicides-Czapek's agar plates, when the mycelium of control reached the edge of plate. Three replicates were used per each treatment.

6 - Effect of herbicides on *S. sclerotiorum* mycelial dry weight:

Three different concentrations (0.5X, 1X, and 1.5X) of three herbicide were amended to 100 ml-flasks containing 50 ml of autoclaved (121ºC for 20 min) Czapek's liquid medium after sterilization. The herbicide- Czapek's medium or flasks were inoculated with disks of 7-day-old cultures of each isolate, after 7 days of incubation, the culture medium was passed through filter paper (Whatman No 1) and fungal mass was dried at 75^{o} C for 16 h and then weighed. Three replicates were used in this study.

7- Effect of herbicides on *S. sclerotiorum* reproduction:

7.1. Sclerotia formation:

Isolates of *S. sclerotiorum* when their mycelial growth approached the edge of a plate of Czapek's agar medium, were amended with the three herbicides concentration as described above and were then inoculated with a 5mm- diam agar plug from cultures of the pathogen. Inoculated plates were incubated for two weaks at 25 ± 2 C. Sclerotia from each plate were harvested, and then air dried overnight at room temperature (25 ± 2C°). Sclerotia numbers were recoded.

7.2. Sclerotial mycleigenic germination

Sclerotia selected randomly from each treatment were surface sterilized in 0.5% $NaOCl_3$ solution followed by two washes in sterile distilled water. Herbicide-amended Petri dishes were seeded in the center with a 3 Sclerotia from the respective isolates agar medium. Seeded plates were then incubated at 25 ± 2 C with until sclerotia germinated. A sclerotium was counted viable if it produced visible mycelium. And the diameter of visible mycelium was measured.

7.3. Carpogenic germination:

Plastic pots were used for sclerotial germination experiments and apothecial development, conducted pots were filled with 1 kg autoclaved soil and 100 Sclerotia which had developed on barley grains were evenly spread out according to origin and then covered with a thin layer (1 cm) of soil. Then water were sprinkled evenly onto the soil , the pots were treated by concentration (0.5 x , 1x , 1.5 x)of tested herbicides All pots incubated in a greenhouse underwent a day/night , until the appearance of stripes. (**Lalfakawma, 2012**)

7.4. Effect of herbicides on oxalic acid secretion by S. sclerotiorum:

Culture media:

The production of oxalic acid from isolates was carried out in medium which had been described by (Kumar 2013). Both isolates were cultured in conical flasks 100 ml which containing 30 ml of the medium PDA broth, which amended by concentration of two herbicides (0.5X, 1X, 1.5X), flasks were inoculated with 5mm fungal discs of isolates. The flasks were incubated at 25 °±2 for 15 days. Mycelium of isolates was harvested by filtration through known weight Watman No.1 filter paper and the dry weight was estimated as previously described. The obtained culture filtrates were used for determination.

Estimation of oxalic acid by titration:

Dilute a sample 5ml in a 45ml volumetric flask using water and pipette 5ml of that diluted sample into a boiling flask and add 50% sulfuric acid heat it 65-70◦C then titrate with N/10 KMnO4. Volume of KMnO4 (N/10) x 6.3= g/l of oxalic acid, where 6.3 is factor (Kumar 2013).

8- Statistically analysis :

Data were carried out in complete randomized design; data were subjected to analysis of variance (ANOVA) using the Statistical Analysis System (MSTAT-C statistical) .the protected least significant difference (L.S.D) values at 5 % ($P< 0.05$) was used to test the differences between the treatment means Snedecor and Cochran (1967).

RESULTS

1 - Pathogenic capability of *S. sclerotiorum*:

Result in Table (1) revealed that five isolates, designated as Ss_1, Ss_2, Ss_3, Ss_4 and Ss_5, belong to the fungus *Sclerotinia sclerotiorum* were isolated from diseased plants of common bean a having typical symptoms of stem

blight, collected from different locations at El-minia governorate . Pathogenicity test of obtained isolates showed that all the tested isolate were pathogenic to bean plants (*Phaseolus vulgaris*) means of measured parameters (i.e. damping off and both incidence and severity (%) of basal stem rot were significantly differed among the five tested isoltes. Both Ss_1 and Ss_2 showed the highest values for both damping-off and stem blight assessment; both isolates gave 40.67, 32.00 and 79.33, 58.72 & 54.78 & 26.79 for damping off and stem blight incidence and severity, respectively.

Table (1): Damping-off, Stem blight diseases incidence and disease severity for five isolate of *Sclerotinia sclerotiorum* on bean plants:

Isolate No	Damping-off (%)	Stem blight	
		Incidence %	Severity %
Ss_1	40.67	79.33	54.78
Ss_2	32.00	58.27	26.79
Ss_3	32.67	52.31	25.82
Ss_4	22.67	48.00	24.33
Ss_5	29.33	55.50	26.07
L.S.D at 5%	1.020	2.923	3.009

umber are on average = reading (5 plants / pot, and 3 pots).

2 - Effect of herbicides treatment on stem blight development:

Results in table (2) showed the stem blight severity on bean plants grown in pots field With soil treated with both tested herbicide at three different concentration and then infested with both tested isolates Ss_1 and Ss_2 of *S. sclerotiorum*, results indicated all tested concentrations of both herbicides reduced the severity of the diseases compare with the control , however the reduction values were not significant at the highest concentration mean value about 45.14, 19.67 for Ss_1 and Ss_2 compared with the control were 58.34 and 24.33, respectively. While concerning both isolated showed a significant difference. Means given by both isolates Ss_1, Ss_2 showed a significant difference for each tested concentration of both herbicides (Table2). Data in this table represented for the seasons 2014. Showed the same trend as they were observed in the first season 2013.

3 - Effect of herbicides on growth parameters of *S. sclerotiorum:*

Linear growth: Obtained results in table (3) showed that there were highly significant reductions in value of liner growth of both isolates for each tested concentration compared with the control. The highest value reduction reached 39.00, 30.67 mm for Pendimethalin to Ss_1, Ss_2 in Czapek Dox agar Media amended with Pendimethalin, while at the same concentration in Butralin inhibited completely both isolates grown on Czapek Dox agar. Difference among values for Ss_1 isolate compared with

the value of the isolate Ss_2 showed less significant difference.

Mycelial dry weight: Data in table (3) showed a highly significant difference in reduction of mycelium of dry weight for all tested concentrations compared with the control Reduction in dry weight reached to completely in height concentration of butralin compare with the control 630, 648 to $Ss_{1,}$ Ss_2 respectively.

Table (2) Basal stem blight severity in potted bean plants infected with two isolates of *Sclerotinia sclerotiorum* and pretreated with herbicide:

			Disease severity (%)							
			First season (2013)				Second season (2014)			
		Isolates	Control	Ss_1	Ss_2	M.	Control	Ss_1	Ss_2	M.
Herbicides treat		zero	0.0	58.34	24.33	41.33	0.0	52.00	27.44	39.72
	Pendimethalin	0.5X	0.0	52.31	23.67	37.99	0.0	49.67	26.45	38.06
		1X	0.0	45.17	21.23	33.25	0.0	47.89	23.33	35.61
		1.5X	0.0	45.14	19.67	32.40	0.0	47.22	21.33	34.28
		M	0.0	50.24	22.26		0.0	49.20	24.64	
	butralin	0.5X	0.0	51.97	23.00	37.49	0.0	44.44	24.78	34.61
		1X	0.0	43.78	20.33	32.06	0.0	40.64	21.22	30.93
		1.5X	0.0	46.92	18.78	32.85	0.0	39.28	20.10	29.69
		M	0.0	50.25	21.61		0.0	44.09	23.9	
L.S.D at 0.5%		A		7.841				9.756		
		B		4.396				5.215		
		AB		11.09				13.80		

Each value represent mean of 5 plants in replicate and 3 replicates in treatment.).

4 - Effect of herbicides on sclerotia formation and germination:

Result in Table (4) indicated that incorporation of both herbicides to the medium on which the two isolates were grown significantly reduced sclerotial production. The difference between each tested concentration (i.e. 0.5X, 1X, 1.5X) were highly significant compared with the control treatment for example, at (1X) concentration Pendimethalin. The mean values of sclerotial production were 0.33 and 0.0 while the same concentration with Butralin gave 0.0 for Ss_1 and Ss_2 and respectively compared with the control treatment which showed mean values of 17.00 and 14.33 for Ss_1 and Ss_2, respectively. Differences between the two isolates were significantly differenced. The effect of both tested herbicides i.e. Pendimethalin and Butralin on mycelial germination of sclerotia is presented in table (4). Result showed that both compounds at any each of three tested concentration significantly reduced mycelogenic germination of sclerotia originated from each of the two isolate and plated out in petri dishes contained Czapek Dox agar media amended with both compounds

with Pendimethalin the mean values for numbers of germinated sclerotia were 1.33 and 2.33 for Ss_1, Ss_2 respectively, when the compound was tested at 1X concentration while the same concentration of butralin gave mean value 0.33 and 1.00 for Ss_1, Ss_2 respectively. The mean value of control treatment was 3.00 and 3.00 for Ss_1 and Ss_2 respectively.

Table (3) Effect of herbicide treatment on growth (linear growth mm – dry weight mg/50ml) of *Sclerotinia sclerotiorum*:

			Growth parameter					
			Linear growth (mm)			Dry weight (mg/50ml)		
		Isolates	Ss_1	Ss_2	M.	Ss_1	Ss_2	M.
Herbicides treat.		zero	90.00	90.00	90.00	630	648	639
	Pendimethalin	0.5X	55.83	65.33	60.58	506	513	509
		1X	50.17	47.67	48.92	423	412	418
		1.5X	39.00	30.67	34.83	130	210	170
		M	58.75	58.14		422	445.75	
	butralin	0.5X	37.77	42.33	40.05	513	480	496
		1X	20.00	18.67	19.33	223	207	215
		1.5X	0.0	0.0	0.0	0.0	0.0	0.0
		M	36.94	37.75		341.5	333.75	
	M.		43.25	43.76		346.8	353.1	
L.S.D at 0.5%		A		4.478			40.63	
		B		2.394			21.72	
		AB		6.333			21.72	

Each value represent mean of 3 replicates (plates/ flasks) in treatment.

Effect of herbicide on carpogenic germination:-

Data in Table (5) represented the means of carpogenic germination of sclerotia originated from both tested isolates. Sclerotium was considered to be germinated by giving both stipes and apothecia. Data indicated that both compounds (i.e. Pendimethalin and Butralin reduced the carpogenic germination of sclerotia in soil at each of any tested concentration compound with the control treatment. There was almost a proportional relationship between the concentration and the lowest reduction was recorded with Butralin as it was applied at the highest concentration. The reduction was about 9.33 and 7.00 compared with the control treatment which were 44.00 and 38.00 for Ss_1 and Ss_2 of *Sclerotinia sclerotiorum*, respectively.

Table (4) Effect of herbicides concentration of sclerotia reproduction (formation – direct germination) for *S. sclerotiorum* isolates.

			Scl. Reproduction								
			Scl. Formation			Number of germinated Sclerotia			Mycelial diameter mm		
		Isolates	Ss_1	Ss_2	M.	Ss_1	Ss_2	M.	Ss_1	Ss_2	M.
	zero		17.00	14.33	15.67	3.000	3.000	3.000	30.23	30.32	30.27
Herbicides treat	Pendimethalin	0.5X	4.333	2.667	3.500	3.000	3.000	3.000	14.11	13.95	14.03
		1X	2.333	1.000	1.667	2.333	1.333	1.833	10.19	10.61	10.40
		1.5X	0.333	0.0	0.1667	1.000	1.000	1.000	5.233	5.300	5.267
		M	6.00	4.50		2.333	2.083		14.94	15.05	
	butralin	0.5X	0.333	0.666	0.5001	2.000	1.667	1.833	10.50	9.000	9.748
		1X	0.0	0.0	0.0	0.3333	1.000	0.6667	9.600	1.100	5.350
		1.5X	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		M	4.33	3.75		1.333	1.416		12.53	10.11	
	M.		3.476	2.667		1.667	1.571		11.41	10.04	
	L.S.D at 0.5%	A		1.016			0.5770			3.875	
		B		0.5431			0.3084			2.071	
		AB		1.437			0.8159			5.480	

Each value represent mean of 3 replicates (plates) in treatment.

Table (5) Effect of herbicides treatment on carpogenic germination to sclerotia of *S. sclerotiorum*

Isolate		Scl. carpogenic							
		pendimethalin				Butralin			Mean A
	Zero	0.5X	1X	1.5X	0.5X	1X	1.5X	
Sc_1	44.00	23.33	19.33	9.33	14.33	10.67	9.33	18.62
Sc_2	38.00	13.33	9.333	9.33	13.00	10.00	7.00	14.29
Mean B	41	18.17	14.33	9.33	13.67	1034	8.17	
L.S.D at 0.5%	A				1.318			
	B				1.744			
	AB				3.488			

Each value represent mean of 3 replicates (pots) in treatment.

6- Effect of herbicide treatment on oxalic acid (g/l) secretion by *S. sclerotiorum*:

Data in table (6) registed that the cultivated medium which supplement with the selected herbicides decreased the secretion of oxalic acid of both fungal isolates (Ss_1, Ss_2) compared with control as a metabolic product. This decrease in oxalic acid production was proportional to the increasing of herbicide concentration. The herbicide Butralin was the more effect on the production of oxalic acid by the fungal isolates than Pendimethalin.

Table (6) Effect of herbicides concentrations of oxalic acid secretion in culture filtrate

Isolate	Oxalic acid g/l							
		Pendimethalin			Butralin			Mean A
	Zero	0.5X	1X	1.5X	0.5X	1X	1.5X	
Ss_1	4.333	3.400	2.933	2.033	3.267	1.900	0.0	2.905
Ss_2	3.833	3.030	2.433	1.967	2.933	1.567	0.0	2.551
Mean B	4.083	3.215	2.683	2.000	3.100	1.733	0.0	
L.S.D at 0.5%	A				0.1661			
	B				0.3107			
	AB				0.4393			

Each value represent mean of 3 replicates (flasks) in treatment.

DISCUSSION

In Minia Governorate, there is extensive cultivation of common bean (*Phaseolus vulgarisl L*) as promising cash crop either for local consumption or exportation. In thus governate *Sclerotinia sclerotiorum* (lib) de Bary has been reported to be a major soil borne fungus to cause basal stem blight in cucurbits vegetable. (Abdel-Gawad, 2002). However the fungus is a widespread fungal pathogen that causes diseases on many economically important vegetables and field crops (Boland and Hall, 1994). Approximately 90% of its life cycle is spent the soil as sclerotia and their persistence makes the fungus a very successful pathogen (Adams and Aycs, 1979). Certainly germination of sclerotia under natural conditions will be affected by different biotic and abiotic agents. Among the abiotic agent using of soil herbicide to control weeds. Generally the relationship between herbicide and plant pathogen still are not well studied. Although, many reports indicated that substituted dinitroanaline herbicide exert a direct fungi toxic effect on plant pathogens (Osman and El-Khadam, 1989, Postor and March 1991, and Zidan *et al*., 1998). Recently, in Egypt it is observed that these are a growing increase in using herbicide for control weeds. In this study, results indicated that *S. sclerotiorum* (Lib) de Bary was isolated from common bean plants a having typical basal stem blight. Five isolates were purified and tested for their pathogenicity, All of the five isolated were pathogenic to common bean (Nebraska). Diseases assessment for artificially inoculated plants varied among the five tested isolates. However isolates *Ss1* and *Ss2* showed the highest diseases incidence and severity which were 79.33,58.27 and 54.78, 26.65% for Ss_1 and Ss_2, respectively similar finding was reported by (Abdel-Gawad, 2002) who found a variation among *S. sclerotiorum* tested on some cucurbits vegetable. It has been reported that *S. sclerotiorum* produces and secrets concentration of oxalic acid ($C_2H_2O_{4)}$

into surrounding media (Cessne *et al*., 2000). Production of oxalic acid is considered as critical pathogenicity factor in pathogens process by S. *sclerotiorum* (Goody *et al*., 1990, Li *et al*., 2008 and Williams *et al* 2011., and El-Argawy, 2012). Generally, a work of El-Argawy (2012) confirmed the association of oxalic acid with pathogenicity of *Sclerotinia sclerotiorum* where the highly virulent isolate produced high amount of oxalic acid while the less virulent isolates produced low amount of the oxalic.The influence of herbicide on plant diseases has previously been studied on different crops and pathogens, with a wide range of conclusion including effect that increase, decrease damage, depending on the herbicide plants species and pathogen (Altman and Rovira, 1989).

In this study the effect of both dinitroanaline herbicides (Pendimethalin, Butralin) were tested at three different concentration on the development of basal stem blight diseases on common bean plants grown in soil pretreated with herbicide and then infected with the two isolates of *Sclerotinia sclerotiorum*. Results showed that these was a decreases in disease severity with each of tested concentration for both herbicide, but different a many all concentrations were not significant the highest decrease in disease severity was recorded with the highest concentration of Butralin herbicide which reached about 45.14, 19.67 compared with 58.34, 24.33 for Ss_1 and Ss_2 respectively. In case of the herbicide Pendimethalin suppression in diseases severity, with application of the highest concentration reached about 46.92, 18.78 compare with the control 58.34 and 24.33 for Ss_1 and Ss_2 respectively. However results between values of both isolate were significant. This results are in line with (Summer and Dowler 1984) and Abdel-Gawad (2002) found that application of some herbicide including Pendimethalin caused an increases in root diseases severity, of corn (*Zea mayz*) caused by *Rhizoctonia solani*. Further studies to interpret the ways that the two tested herbicide extent there effects, through suppression of diseases severity, therefore both herbicide were tested their direct effect on growth and reproduction of *S. sclerotiorum* of production the oxalic acid by the tested isolates.

Growths of both isolates Ss_1, Ss_2 were conducted with as linear growth (mm) or mycelial dry weight (mg). Results indicated that all tested concentrations exert an adverse effect on the growth of both isolates. A significant reduction in both liner growth and dry weight compared with the control treatment.

The amount of oxalic acid secreted and produced by both *S. sclerotiorum* isolates was significantly reduced when isolates were grown in a medium amended with both tested herbicide (i.e. butrlain and pendimethalin) mean value of the oxalic acid for each tested concentration was significantly reduction compared with the control for example. The mean values of

oxalic acid at concentration (1X) for Pendimethalin and Butralin were 2.933, 2.433 and 2.467, 2.093and for the control were 4.333 and 3.833 for isolates Ss_1 and Ss_2, respectively as previously reported the production of oxalic acid was considered as pathogenicity determinant in *S. sclerotiorum* (Goody *et al*., 1990, Li *et al*., 2008, Williams *et al*., 2011 and El-Argawy, 2012). Result of this study suggest that reduction in the amount of oxalic may partly explained the suppression in the diseases severity of the stem blight caused by both isolates of *S. sclerotiorum* on common bean plants (Nebraska). Also, result of this study confirmed the previous finding by other authors which stated that production of oxalic acid is pathogenicity determinant factor in *S. sclerotiorum* since it has been observed that Ss1 was the higher production of oxalic. The higher in diseases severity while Ss2 was the less in oxalic acid that less in diseases severity.

In conclusion, fortunately both tested herbicide compounds have positive effect on bean plants via soil treatment as they suppressed the stem blight diseases severity caused by both isolates of *S. sclerotiorum* this may be due to the direct effect on the fungal isolates causing reduction in growth and reproduction also the reduction of oxalic acid was significantly reduced. Also no visible injury were detected when the root of treated plant by both herbicide, were observed.

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الملخص العربى

تاثير المعاملة بمبيدات حشائش ماقبل الانبات على الفطر *Sclerotinia sclerotiorum* فى المعمل و على شدة مرض لفحة الساق فى الفاصوليا البيضاء

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اجريت هذه الدراسة لقياس تاثير المعاملة بمبيدى الحشائش بنديمثالين و بيوترالين بثلاثة تركيزات هى (0.5X , 1X, 1.5X) من التركيز الموصى به (X) على مرض لفحة قاعدة الساق نباتات الفاصوليا حيث ادت المعاملة بكلا المبيدين الى انخفاض الاصابة بكلا العزلتين و ذلك لكل التركيزات المختبرة ، مقارنة بالكونترول المعدى وغير المعامل . ففى الموسم الاول 2013 كانت النسبة المئوية لشدة الاصابة للعزلتين Ss_1, Ss_2 على التوالى هى 45.33 و 46.92 عند المعاملة بالتركيز 1.5 X للمبيد بنديمثالين و كانت 19.67 و 18.78 للمبيد بيوترالين مقارنة ب 58.34 و 24.33 للكونترول المعدى و غير المعامل بالمبيدات وتم اجراء هذه التجربة فى الاصص تحت ظروف الصوبة .

سجلت الدراسة ان كلا من المبيدين لهما تاثير قوى على تثبيط النمو لكلا العزلتين Ss1, Ss2 . ايضا لوحظ ان تكوين الاجسام الحجرية قد انخفض على وسط النمو (البيئة المضاف اليها كلا المبيدين) و ايضا وجد المعاملة بكلا المبيدين ادى الى انخفاض انبات الاجسام الحجرية (الانبات الثمرى – الانبات الميسليومى). ايضا تاثر انتاج كلا من العزلتين Ss1, Ss2 لحامض الاوكساليك فى الراشح الناتج من المزرعة الفطرية سلبيا عند تنميتهم على وسط غذائى مضافة اليه كلا من المبيدين بنديمثالين و بيوترالين بالتركيزات السابق ذكرها حيث لوحظ انخفاض كمية الحامض كلما زاد التركيز فى الوسط الغذائى وذلك عن طريق التقدير الكمى للحامض.