

BIO-MANAGEMENT OF SCLEROTINIA ROT DISEASE ON BEANS USING DIFFERENT MUTUALISTIC FUNGAL ISOLATES AND MARINE ALGAE

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ABSTRACT: *The biological control potential of four different candidate biocontrol agents, three isolates belonging to genus Trichoderma and an isolate of mutualistic entophytic Fusarium oxysporum (Fo162), in addition to four different marine algae (Ulva fasciata, Gelidium crinale, Jania rubens and Coralline elongata) were determined against Sclerotinia Sclerotiorum on bean plants under green house conditions. The influence of either tested mutualistic fungi or algae on the growth criteria i.e. plant height, fresh and dry roots and shoot weight as well as on Sclerotinia disease severity were compared with Uniform fungicide (Mefenoxam and Azoxystrobin). In order to infer size the possible mode of action that can be involved in the possible tri-trophic interactions between S. sclerotiorum, bean plants and the tested bio-control agents, the chemical assessment of some vital enzymes and substances i.e. peroxidase, poly phenoloxidase and total sugar contents had been done. The results revealed that all the tested bio- and chemical- agents affected positively plant heights, fresh weight and dry weight of both roots and shoots of treated plants. In general, marine algae were superior to the other bio-control fungi with regard to their influence on growth parameters. Comparing to control plants, inoculated only with the pathogen, the all scanned bio-agents fungi and algae reduced significantly the disease severity on treated plants. The highest disease reduction was observed with mutualistic fungi in general and with Trichoderma hamatum isolate Thm particularly. The obtained results from the chemical assay revealed the highest total sugar concentration was detected with plants treated with the tested seaweeds (marine algae). In contrary, the results indicated that highest activity for antioxidant enzymes i.e. peroxidase and poly phenoloxidase were recorded mainly with the tested biological mutualistic fungi. In conclusion, the bio-management technique using either specific bio-control isolates i.e. Trichoderma hamatum (Thm), T. viride (Tv), T. harzianum (T.sp.), Fusarium oxysporum (fo162), or promising seaweeds i.e. Ulva fasciata, Gelidium crinale, Jania rubens and Coralline elongata is a useful tool in controlling the Sclerotinia rot disease of bean plants and therefore it can be considered suitable alternative for the traditional chemical control methods. Moreover, different mode of actions including changing of : total sugar contents, the activity of peroxidase and poly phenol oxidase are candidate to be involved in the interactions between bean, S. sclerotiorum and biological control mechanism.*

Key words: *Sclerotinia sclerotiorum, bean, marine algae, mutualistic bio-control fungi.*

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary is known as the main causal pathogen of white mold disease or Sclerotinia rot disease. This fungus attacks a wide range of economic plants all over the world including numerous field and vegetable crops where it causes severe and significant yield losses (Purdy, 1979; Tu, 1985; Tu, 1987). Both dry and green beans, Phaseolus vulgaris L., were found to be among the highly susceptible

host plants to Sclerotinia sclerotiorum invasion (Tu, 1997). The disease incidence percentage of white mold on bean crops reached up to 100% in some locations (Tu, 1989a). Recently in Egypt, green and dry beans cultivations were expanded where green beans occupied nowadays more than 58,000 feddan with estimated production about 251,000 tons while dry beans were grown in more than 59,000 fedden which produce about 69,000 tons annually

(Egyptian Agriculture Ministry statistics, 2012).

To provide reasonable protection for these value cultivations against one of the most hazardous and destructive pathogens i.e. *Sclerotinia sclerotiorum*, different management techniques including resistant cultivars, chemical control and/or biological control were tested (Tu, 1986). However, many researchers reported that using the resistant cultivars to control *Sclerotinia sclerotiorum* in general and on beans in particular was not an adequate tool due to the wide host range of the causal pathogen without known strain specificity in pathogenicity (Steadman, 1979). Moreover, the commercial white beans particularly, have many specific traits that must be maintained which makes attempting to use these materials in breeding programs for white mold disease have limitations and less efficient (Roberts *et al.*, 1982; Tu and Beversdorf, 1982; Tu, 1997).

Regarding to the chemical control, the effective fungicides against *S. sclerotiorum* such as benomyl, chlorothalonil, thiophanate methyl, iprodione and dicloran are expensive rather than their contra effect on environment and human beings.

These difficulties within traditional control methods makes finding new effective and safe alternatives are very relevance. Therefore, biocontrol measures depending on using useful micro- and/or macro-organisms are candidate to play an extensive role in controlling the white mold fungus on his hosts.

Fortunately, soil besides harbouring plant pathogens, also supports many other beneficial organisms. Some of these beneficial organisms also have been shown to colonize plants without causing disease and are known as mutualistic (Petrini, 1991; Wilson, 1995; Stone *et al.*, 2000). These Mutualistic endophytic organisms can be either microscopic i.e. bacteria (Chanway, 1996; van Wees *et al.*, 1999), fungi (Hallmann and Sikora 1994 a,b., Niere *et al.*, 2001., Olivain and Alabouvette, 1997) or macroscopic as marine macro-algae (Angus and Dargie, 2002; Cuomo *et al.*, 1995).

Recent research clear that, mutualistic fungi such as *Fusarium oxysporum* and *Trichoderma* species that have biological control potential are also ecofriendly and have no contra effects on non targeted organisms including human, useful microflora and host plants. Therefore they can be used as an effective alternative measure to control plant diseases in general and white mold disease or *Sclerotinia* rot disease in particular (Tu, 1997; Budge *et al.*, 1995; Gerlagh *et al.*, 1994).

In the present study, the biological control capability of four candidate bio-control agents, three isolates belonging to genus *Trichoderma* v.z. *T. viride*, *T. hamatum* and *T. harzianum* and one isolate of mutualistic endophytic *Fusarium oxysporum*, Fo162, was determined against *Sclerotinia sclerotiorum* on beans under green house conditions. Beside these antagonistic fungi, four different marine algae (seaweeds) i.e. *Ulva fasciata*, *Gelidium crinale*, *Jania rubens* and *Coralline elongate*, were tested in vivo against *S. sclerotiorum* as well. Furthermore, the influence of these tested bio-agents on the growth criteria i.e. fresh weight, dry weight, and plant height of inoculated beans were compared to the standard chemical fungicide (uniform). In order to infer size the possible mode of action which can be involved in such tri-interactions between *Sclerotinia sclerotiorum*, bio-control agents and bean plants, peroxides, poly phenoloxidase and total soluble sugars were also bio-chemically determined.

MATERIALS AND METHODS

Bio-control agents inocula:

Pure cultures of the potential biocontrol fungi, *Trichoderma hamatum* (Thm), *T. viride* (Tv), *T. harzianum* (T sp) and mutualistic endophytic *Fusarium oxysporum* (Fo162) were maintained on Micro-Bank tubes stored at -80 °C. To obtain fresh cultures, biocontrol agents were reared on potato dextrose agar plates (PDA) amended with 150 mg l⁻¹ of chloramphenicol and incubated at 25°C in the dark for two weeks. Five millimeters diameter discs of two weeks old cultures were inoculated into autoclaved 500

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ml flasks contained 200 g of barley grains moisten with 100 ml of distilled water. Inoculated barley grains were incubated in shad at room temperature with interval shake for three weeks. Three weeks after inoculation (when the potential bio-agents almost filled the flasks), inocula were mixed thoroughly into autoclaved sand: field soil mixture (1:1, v/v) with rate of 5 g per kg.

Four different marine macro algae *i.e.* *Ulva fasciata*, *Gelidium crinale*, *Jania rubens* and *Coralline elongate* were collected from Mediterranean sea coast at Alexandria governorate, Egypt. Collected algae were washed gently with tap water to remove debris. Identification of obtained algae was carried out at Genetic Engineering Institute (Sadaat city University, Egypt). Identified fresh algae were then air dried in shade at room temperatures before dehydration was completed in oven at 60 °C for 12 hours. Dried algae were mixed thoroughly into autoclaved sand: field soil mixture (1:1, v/v) with rate of 5 g⁻¹kg (w: w).

Pathogen inoculum:

Pure cultures of *Sclerotinia sclerotiorum* were obtained from Department of Plant Pathology, Faculty of Agriculture, Menoufia University, Egypt. Three discs (five millimeters diameter) of two weeks old PDA pure cultures of *S. sclerotiorum* were inoculated into autoclaved 500 ml flasks contained 200 g of barley grains moisten with 100 ml of distilled water. Inoculated flasks were incubated at room temperature in shad for three weeks with interval hand shake.

In vivo test:

Sclerotinia sclerotiorum inoculum was mixed thoroughly into autoclaved sand: field soil mixture (1:1, v/v) with rate of 1,5 g per kg soil (w:w). Immediately after pathogen inoculation, infested soil was treated with prepared inocula of each individual bio control agent with rate of 5 g⁻¹kg soil (w:w). Plastic pots (15 cm diameter) were filled with sand: field soil mixture (1:1, v/v) which was previously mixed thoroughly with both pathogen and each individual bio control agent. Four replicates (4 pots) were used

within each individual bio-agent as well. However, uniform chemical fungicide (Mefenoxam and Azoxystrobin) was applied with rate of 1 cm³ per 1L into four pots filled with soil that was infested previously only with *Sclerotinia inoculum*. This fungicide was purchased from Syngenta Egypt Company.

Control pots (C+) were filled with soil that infested only with *Sclerotinia inoculum* where no other biological or chemical substrates were present. Healthy control plants (C-) were grown in autoclaved sand: field soil mixture free of any biological or chemical treatments.

Five surface-sterilized seeds of dry beans cv "El-Karnak" were sown in each plastic pot filled with treated soil with either pathogen or individual biological control agents. Six weeks after sowing, experiment was terminated and disease incidence as well as infection percentage was recorded based on (0 to 5) visual disease index scale (Ishikawa *et al.*, 2005):

- 0= healthy plants
- 1= up to 20% infection
- 2= 21-39 % infection
- 3= 40-59 % infection
- 4= 60-79 % infection
- 5= ≤ 80% infection

Disease severity was determined using the following formula:

$$\text{Disease severity (\%)} = \left(\frac{\sum \text{scale} \times \text{number of plants infected}}{\text{Highest scale} \times \text{total number of plants}} \right) \times 100$$

After estimating disease parameters, plants were gently uprooted from the soil and roots were gently washed with tap water before blotted between two filter papers. Plant heights as well as fresh and dry weights of both roots and shoots were recorded

Biochemical assay:

Total sugars in dry leaves were estimated as described by Dubois *et al.* (1956).

Peroxidase and poly phenoloxidase enzymes activity were determined in bean leaves treated with either biological or

chemical agents according to methods described by Johri *et al.* (2005) and Coseteng, & Lee (1978), respectively.

RESULTS

The obtained results from the bioassay that conducted under green house conditions indicated that all the tested chemical and biological substrates significantly reduced the percentages of *Sclerotinia* rot disease on bean plants (Fig.1), compared to the control (infested only with pathogen). The lowest percentage of disease severity (10%) was recorded with the chemical fungicide (uniform) followed by *Trichoderma hamatum* (20%) isolate Thm and *T. harzianum*, *T sp.*, (25%). The results showed that the disease severity reached up to 40% with both *Gelidium crinale* (G) and *Coralline elongate* (CO) marine algae. *Sclerotinia* disease severity recorded 50, 55

and 65% within mutualistic *Fusarium oxysporum* (162), *Jania rubens* (J) and *Ulva fasciata* (U), respectively.

Some growth criteria *i.e.* plant height, roots and shoots fresh and dry weights were determined in order to infer size the influence of the tested biological agents on physiological and morphological status of treated bean plants. The results revealed that both chemical and biological treatments increased significantly plant height compared to control plants (infested only with *S. sclerotiorum*). In general, the top plant heights were recorded with marine algae, *G. crinale* (G), *C. elongate* (CO) and *J. rubens* (J). Among the tested isolates of mutualistic fungi, *T. harzianum* isolate Tsp. and *T. hamatum* isolate Thm were superior to the other isolates (Fig. 2).

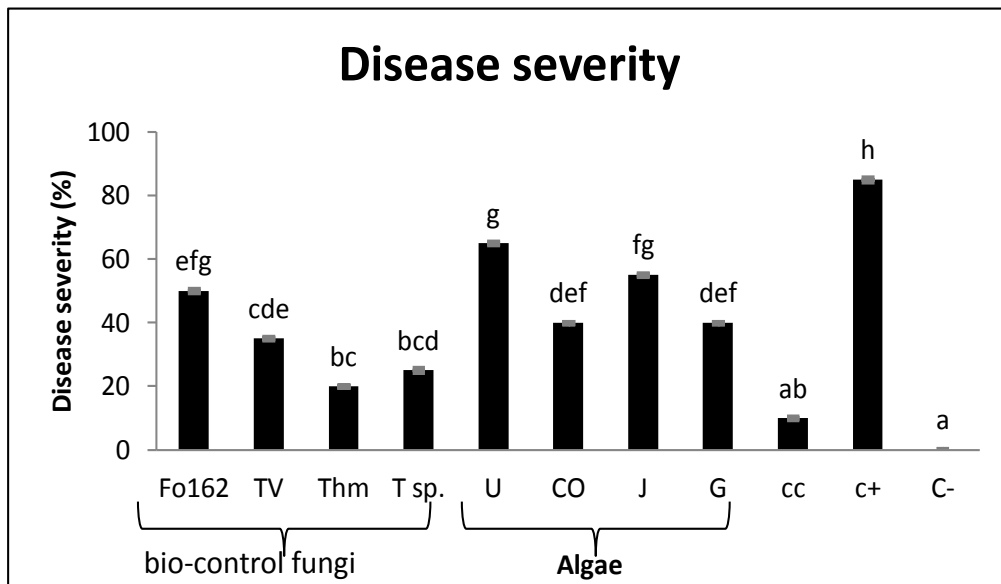


Figure (1): Effects of *Fusarium oxysporum* (Fo162), *Trichoderma viride* (TV), *T. hamatum* (Thm), *T. harzianum* (T. sp), *Ulva fasciata* (U), *Coralline elongate* (CO), *Jania rubens* (J), *Gelidium crinale* (G) and uniform fungicide (cc) on disease severity caused by *Sclerotinia sclerotiorum* compared to control (C+) plants (infested only with *Sclerotinia sclerotiorum*) and to healthy plants, non-inoculated, (C-). Paired means with different letter(s) are significantly varied based on Tukey test ($P \leq 0.05$). Bars indicated the standard error of the mean.

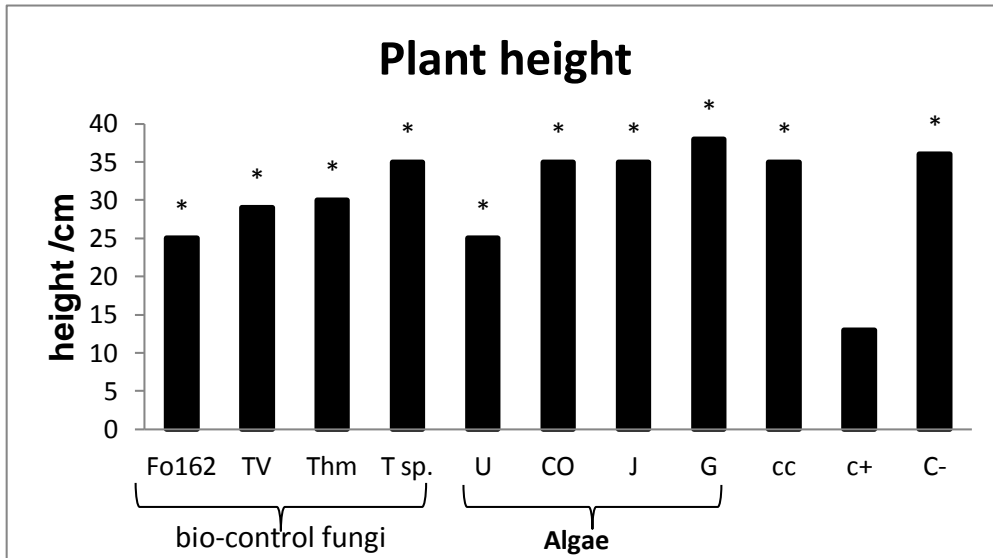


Figure (2): Influence of *Fusarium oxysporum* (Fo162), *Trichoderma viride* (TV), *T. hamatum* (Thm), *T. harzianum* (T. sp), *Ulva fasciata* (U), *Coralline elongate* (CO), *Jania rubens* (J), *Gelidium crinale* (G) and uniform fungicide (cc) on plant height of bean plants compared with infested (C+) and non-infested (C-) plants with *Sclerotinia sclerotiorum*. Paired means with (*) are significantly varied compared to control plants (C+) based on Tukey test ($P \leq 0.05$).

The effect of the tested biological agents and chemical control substrate on fresh matter of bean planted in soil infested with *Sclerotinia sclerotiorum* was evaluated. Results showed that the highest foliar fresh weights were recorded with marine algae, *C. elongate* (CO), *G. crinale* (G) and *J. rubens* (J), respectively (Fig.3). Similar results were observed within roots fresh weights. Thus the highest root weights were observed again with *C. elongate* (CO), *G. crinale* (G) and *J. rubens* (J), algae, respectively (Fig.3).

Similar results were observed again in aspect to the effect of the tested biological and chemical substrates on dry matter of infected beans. The highest foliar dry weight as well as the best roots dry weight were observed with plants treated with *C. elongate* (CO), *G. crinale* (G) and *J. rubens* (J), chemical fungicide, *T. harzianum* isolate Tsp and *T. viride* isolate Tv, respectively (Fig.4).

The obtained results from bio-chemical investigations revealed that the highest enzymatic activity for peroxidase substance was recorded with beans inoculated with *T. hamatum* (Thm), *T. viride* (Tv), *T. harzianum* (T.sp) and the mutualistic *F. oxysporum* isolate 162, respectively (Table. 1). With regard to poly phenoloxidase activity, the results demonstrated that the bio-control fungi, *T. hamatum* (Thm), *T. viride* (Tv), *T. harzianum* (T.sp) and the mutualistic *F. oxysporum* isolate, were superior to the other tested marine algae and to the chemical control substrate (Table 1).

Chemical analysis of sugars concentration accumulated within the leaves of treated plants showed that the highest concentrations were detected within *C. elongate* (CO), *G. crinale* (G) and *Fusarium oxysporum* isolate Fo162 (table 1).

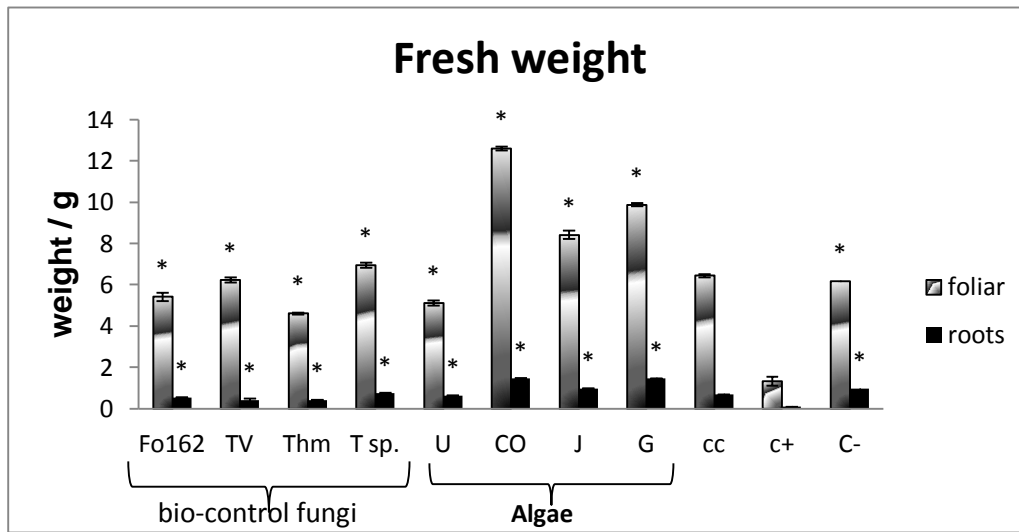


Figure (3): Influence of *Fusarium oxysporum* (Fo162), *Trichoderma viride* (TV), *T. hamatum* (Thm), *T. harzianum* (T. sp), *Ulva fasciata* (U), *Coralline elongate* (CO), *Jania rubens* (J), *Gelidium crinale* (G) and uniform fungicide (cc) on foliar and roots fresh weights of bean plants compared with *Sclerotinia sclerotiorum* infested (C+) and non-infested (C-) plants. Paired means with (*) are significantly different based on Tukey test ($P \leq 0.05$). Error Bars indicated the standard error of the means.

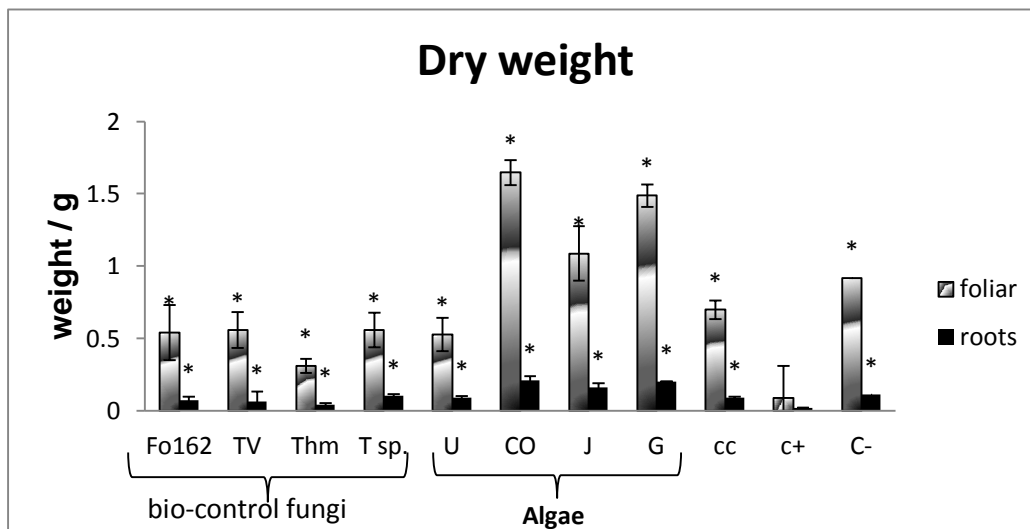


Figure (4): Influence of *Fusarium oxysporum* (Fo162), *Trichoderma viride* (TV), *T. hamatum* (Thm), *T. harzianum* (T. sp), *Ulva fasciata* (U), *Coralline elongate* (CO), *Jania rubens* (J), *Gelidium crinale* (G) and uniform fungicide (cc) on foliar and roots dry weights of bean plants compared to *Sclerotinia sclerotiorum* infested (C+) and non-infested (C-) plants. Paired means with (*) are significantly different based on Tukey test ($P \leq 0.05$). Error Bars indicated the standard error of the means.

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Table 1. Effect of *Fusarium oxysporum* (Fo162), *Trichoderma viride* (TV), *T. hamatum* (Thm), *T. harzianum* (T. sp), *Ulva fasciata* (U), *Coralline elongate* (CO), *Jania rubens* (J), *Gelidium crinale* (G) and uniform fungicide (cc) on peroxidase, poly phenoloxidase enzymes and total sugar content of bean plants infected with *Sclerotinia sclerotiorum* compared to control plants (pathogen only; C+) as well as to the healthy plants (C-).

Treatments	Peroxidase (O.D. after 2 min.)	Poly Phenoloxidase (O.D. after 45 min)	Total sugars (TS) (mg g ⁻¹ DW)
<i>Fusarium oxysporum</i> (Fo162)	1.30 ^c	1.35 ^b	21.88 ^{bc}
<i>Trichoderma viride</i> (TV)	3.00 ^b	1.50 ^{ab}	17.19 ^{cd}
<i>Trichoderma hamatum</i> (Thm)	5.20 ^a	1.60 ^a	14.84 ^d
<i>Trichoderma harzianum</i> (T. sp)	1.75 ^c	1.30 ^b	14.84 ^d
<i>Ulva fasciata</i> (U)	0.60 ^d	0.70 ^d	18.75 ^c
<i>Coralline elongate</i> (CO)	0.90 ^d	1.00 ^c	25.00 ^b
<i>Jania rubens</i> (J)	0.75 ^d	0.70 ^d	18.75 ^c
<i>Gelidium crinale</i> (G)	0.85 ^d	1.00 ^c	22.66 ^b
Uniform fungicide (cc)	0.80 ^d	1.15 ^c	10.94 ^d
Pathogen alone (C ⁺)	0.40 ^e	0.50 ^e	2.00 ^e
Healthy plants (C ⁻)	0,70^d	0,80^d	31.25 ^a

- Paired means with different letter(s) are significantly varied based on Tukey test ($P \leq 0.05$).

DISCUSSION

Sclerotinia sclerotiorum is one of the major plant pathogens that attack wide range of numerous host plants all over the world. This fungus can cause huge damage and significant yield losses on its hosts in general and on beans in particular. The differences in disease severity and the losses of yield among fields and locations can be referred to variations in precipitation, soil drainage, cultural practices, and sclerotial density (Tu, 1987). Recently, it was estimated that the average incidence of Sclerotinia mold disease on bean crops reached up to 100% (Tu, 1989b).

The primary infection of the susceptible hosts can be initiated from the seeds obtained from previously infected plants, Sclerotia formed in the soil and/or the saprophytic mycelium feeding on depresses and remains of infected and died plants. Tu (1988) reported that seedlings from infected seeds subsequently died from white mold at an early stage while seeds that failed to germinate were rotted by *S. sclerotiorum*, and three to six sclerotia were formed in place of each seed.

Due to the lack of resistant varieties especially within bean cultivars and withdraw of many of the effective fungicides rather than their adverse and negative

effects on humans and environment, searching for new safe and functional alternatives becomes quit necessary.

Recently, biocontrol agents attracted the scientist and researcher attentions as promising and effective alternatives for the traditional management techniques.

In the present study, the biological potential of eight different candidate bio-control agents, four fungal isolates belonging to *Trichoderma* and *Fusarium* genera in addition to four different macro marine algae, was investigated under green house conditions against *Sclerotinia sclerotiorum* on bean plants. Moreover, uniform fungicide (Mefenoxam and Azoxystrobin, mixture) was used to compare the efficiency of biological control application with chemical control process. Furthermore, the influence of these tested antagonists on growth criteria and some essential enzymatic activity was evaluated to gain more information about the possible mechanism that can be involved in tri-trophic interactions between beans, *S. sclerotiorum* and bio-control agents.

The obtained results revealed that all the tested biocontrol agents reduced significantly both disease incidence and severity compared to control plants (beans planted in soil infested with *Sclerotinia* inoculum only). The highest disease reduction was observed with chemical control. The results also showed that the best biological disease control was observed in general with the tested mutualistic fungi and in particular with isolates of *Trichoderma hamatum* (Thm), *Trichoderma harzianum* (T. sp) and *Trichoderma viride* (TV). Among the four tested sea weeds, *Coralline elongate* (CO) and *Gelidium crinale* (G) showed relative high bio-control potentials against *S. sclerotiorum* on treated bean plants.

On the other hand, the obtained results demonstrated that the four tested marine algae affected positively the fresh and dry weights of both roots and shoots of treated bean plants more than the other tested bio-control fungi as well as the control plants. Thus the highest fresh and dry roots and

shoots weight was observed on plants treated with *Coralline elongate* (CO), *Gelidium crinale* (G) and *Jania rubens* (J) algae, respectively.

These results are in agreement with Gerlagh *et al.* (1994); Budge *et al.* (1995) and Tu (1997) who investigated the biological activity of various mutualistic fungal strains especially those belonging to the genera of *Trichoderma*, *Gliocladium*, *Fusarium*, *Mucor*, *Penicillium*, *Aspergillus*, *Stachybotrys*, and *Verticillium* against *Sclerotinia* diseases on a wide spectrum of crops.

In such these tri-trophic interactions between the host plant, fungal pathogen and mutualistic biological agents, different mechanisms of action were observed and considered responsible for protecting the host plants from hazardous pathogens and parasites (Alabouvette *et al.*, 2001; Fravel *et al.*, 2003; Fuchs *et al.*, 1997). In 2009 Almagro *et al.* reported that the production of antimicrobial phytoalexins metabolites as well as the induction of the host plant defense proteins including class III peroxidases was involved in the host defence reactions against *Sclerotinia* attack.

In the present study, peroxidase and poly phenoloxidase activities were determined using bio-chemical assay in order to infer size their potential in the biological control process against *S. sclerotiorum* on beans. The obtained results showed that the highest peroxidase induction was observed with the mutualistic fungi in general and particularly with *Trichoderma hamatum* (Thm), *Trichoderma viride* (Tv), *T. harzianum* (T.sp.) and the endophytic *Fusarium oxysporum* (Fo162). Moreover, the results also showed that the other tested marine algae also affected positively the production of peroxidase compared to the control plants which were planted into the soil infested only with *Sclerotinia* pathogen.

Similar results were detected with regard to the poly phenoloxidase enzymatic activity where the mutualistic four tested fungi increased the concentrations of poly phenoloxidase within bean plants more than the other treatments. In deed, the bio-

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chemical analysis showed also that both tested mutualistic fungi and marine algae significantly increased sugar concentrations with bean plants compared to chemical treated plants and to control plants (inoculated with pathogen only).

According to the obtained results from both bioassay and chemical investigation, it seems that the four tested mutualistic isolates of fungi were more effective and specific against *Sclerotinia sclerotiorum* disease and this could be in partial due to their relative high capability to increase the accumulation of certain metabolites *i.e.* peroxidase and poly phenoloxidase enzymes which are involved in host plants defence reactions. This theory is consistent with the study of Ros Barceló and Pomar (2002) who demonstrated that plant peroxidases are able to catalyse the synthesis of bioactive plant products and therefore can play a role in plant defense through their involvement in the synthesis of phytoalexin enzymes. Furthermore, the relevance and significance of the products resulting from peroxidases-mediated reactions and essential physiological process have been addressed several times (Langcake and Pryce, 1977a,b; Langcake, 1981; Waffo-Teguo *et al.*, 2001; Ros Barceló and Pomar, 2002). More recently, El-Mougy *et al.* (2013) noticed that some biological control agents like *Saccharomyces cerevisiae* affected different hazardous pathogens and parasites through inducing the natural defense metabolites *i.e.* peroxidases and poly phenoloxidase on numerous vegetable crops.

On the other hand, many of the marine algae (seaweeds) were found to be natural sources of different bio-active compounds and therefore they can be considered as promising bio-control agents against different pathogens and parasites (Michael *et al.*, 2005; El-gamal, 2010). Remarkable, see weeds were used for long time as organic fertilizers and soil conditioners (Blunden and Gordon, 1986; Temple and Bomke, 1998). Recently, MacArtain *et al.* (2007) proved that seaweeds generally have high concentrations of C, K, Mg, Na, Cu, Fe, I, and Zn. The presence of these valuable

nutrient elements within seaweeds (marine algae) make them excellent organic fertilizers and could explain why the highest fresh and dry weights were observed on beans treated with the tested marine algae.

In conclusion, these results revealed that the tested antagonistic fungi were more effective against *S. sclerotiorum* while the other tested marine algae were superior in increasing the vegetative growth of treated plants.

Finally, it could be concluded that all tested mutualistic isolates of *Trichoderma* and *Fusarium* fungi provided a reasonable biological protection to bean plants against *S. sclerotiorum* disease under green house conditions. This influence could be partially referred to their ability to increase the production of metabolic active compounds such as peroxidase and Poly phenoloxidase. Furthermore, the four tested seaweeds or marine algae which showed remarkable ability to promote the vegetative growth and to increase the sugar contents within treated bean plants make them the perfect choice for the integrated pest management strategies.

REFERENCES

- Alabouvette, C., V. Edel, P. Lemanceau, C. Olivain, G. Recorbet and C. Steinberg (2001). Diversity and interactions among strains of *Fusarium oxysporum*: Application and biological control. In: M.J. Jeger and N.J. Spence (Eds.): *Biotic interactions in plant-pathogen associations*, pp 131-157. CAB International, London, England.
- Almagro, L., L. V. Go´mez Ros, S. Belchi-Navarro, R. Bru, A. Ros Barcelo and M. A. Pedren˜o (2009). Class III peroxidases in plant defense reactions. *Journal of Experimental Botany*, Vol. 60, No. 2, pp. 377–390.
- Angus, S. and T. Dargie (2002). The UK Machair Habitat Action Plan: progress and problems. *Botanical Journal of Scotland*, 54,63-74.
- Blunden, G. and S.M. Gordon (1986). Betaines and their sulphino analogues in marine algae. *Progress in phycological research* 4: 39-79.

- Budge, S. P., M. P. Mc Quilken, J. S. Fenlon and J. M. Whipps (1995). Use of *Coniothyrium minitans* and *Gliocladium virens* for biological control of *Sclerotinia sclerotiorum* in glasshouse lettuce. *Biol. Control* **5**: 513-522.
- Chanway, C. P. (1996). Endophytes: they're not just fungi! *Canadian Journal of Botany* **74**: 321-322.
- Coseteng, M.Y. and C.Y. Lee (1978). Changes in apple polyphenol oxidase and polyphenol concentrations in relation to degree of browning. *Journal of Food Science*, **52**: 985.
- Cuomo, V., A. Perretti, I. Palomba, A. Verde and A. Cuomo (1995). Utilization of *Ulva rigida* biomass in the Venice Lagoon (Italy): biotransformation in compost. *Journal of applied Phycology*, **7**: 479-485.
- Dubois, M., A. Gilles, S. Hamiton, P.R. Rebers and P.A. Smith (1956). Colorimetric method for determination of sugar and related substances. *Analytical Chemistry* **28**, 350.
- El-gamal, A.A. (2010). Biological importance of marine algae. *Saudi Pharmaceutical Journal*, **18** (1): 1-25.
- El-Mougy, N. S., M. M. Abdel-Kader, S. M. Lashin and A.A. Megahed (2013). Fungicides Alternatives as Plant Resistance Inducers against Foliar Diseases Incidence of Some Vegetables Grown under Plastic Houses conditions. *International Journal of Engineering and Innovative Technology (IJEIT)*, Vol. 3 (6), ISSN: 2277-3754.
- Fravel, D., C. Olivain and C. Alabouvette (2003). *Fusarium oxysporum* and its biocontrol. *New Phytol.* **157**: 493-502.
- Fuchs, J.G., Y. Moëne-Loccoz and G. Défago (1997). Non pathogenic *Fusarium oxysporum* strain Fo47 induces resistance to Fusarium wilt in tomato. *Plant Dis.* **81**:492-496.
- Gerlagh, M., M. Kruse, H. M. van de Geijn and J. M. Whipps (1994). Growth and survival of *Coniothyrium minitans* on lettuce leaves in contact with soil in the presence and absence of *Sclerotinia sclerotiorum*. *Europ. J. Plant Pathol.* **100**: 55-59.
- Hallmann, J. and R. A. Sikora (1994a). Occurrence of plant parasitic nematodes and nonpathogenic species of *Fusarium* in tomato plants in Kenya and their role as mutualistic synergists for biological control of root knot nematodes. *Int. J. Pest Manage.* **40**, 321-325.
- Hallmann, J. and R. A. Sikora (1994b). Influence of *Fusarium oxysporum*, a mutualistic fungal endophyte, on *Meloidogyne incognita* of tomato. *J. Plant Dis. Protect.* **101**, 475-481.
- Ishikawa, R., K. Shirouzu, H. Nakashita, H.Y. Lee, T. Motoyama, I. Yamaguchi, T. Teraoka and Arie, T. (2005). Foliar spray of validamycin A and validoxylamine A controls Fusarium wilt. *The American Phytopathological society* (95): pp.1209-1216.
- Johri, S., U. Jamwal, S. Rasool, A. Kumar, V. Verma and G.N. Qazi (2005). Purification and characterization of peroxidases from *Withania somnifera* (AGB 002) and their ability to oxidize IAA. *Plant Science*, **169**: 1014-1021.
- Langcake, P. and R.J. Pryce (1977a). A new class of phytoalexins from grapevines. *Experientia* **33**, 151-152.
- Langcake, P. and R.J. Pryce (1977b). The production of resveratrol and the viniferins by grapevines in response to ultraviolet irradiations. *Phytochemistry* **16**, 1193-1196.
- Langcake, P. (1981). Disease resistance of *Vitis* spp. and the production of the stress metabolites resveratrol, e-viniferin, a-viniferin and pterostilbene. *Physiological Plant Pathology* **18**, 213-226.
- MacArtain, P., C.I.R. Gill, M. Brooks, R. Campbell and I.R. Rowland (2007). Nutritional value of edible seaweeds. *Nutritional Reviews*, **65**(12): 535-543.
- Michael, T.M., M.M. John and P. Jack (2005). *Brock microbiology of microorganisms*. 11th Edition, New Jersey. ISBN: 13-978-0226701479.
- Niere, B. I., R. A. Sikora and P. R. Speijer (2001). Mutualistic endophytic fungi—role in biocontrol and safety of application. In: Sikora, R. A. (Eds.). *Integrated Control of Soil Pests*. IOBC/wprs Bulletin **24**, 117-120.
- Olivain, C. and C. Alabouvette (1997). Colonization of tomato root by a non-

Bio-management of sclerotinia rot disease on beans using different

- pathogenic strain of *Fusarium oxysporum*. New Phytol. **137**, 481-494.
- Petrini, O. (1991). Fungal endophytes of tree leaves. Microbial Ecology of leaves (J. Andrews & S. Hirano, (Eds.), 179-197. Springer Verlag, New York.
- Purdy, L. H. (1979). *Sclerotinia sclerotiorum*: History, diseases and symptomatology, host range, geographic distribution, and impact. Phytopathology **69**: 875_880.
- Roberts, M. E., M. H. Dickson and J. E. Hunter (1982). Heritability of white mold resistance. Annu. Rep. Bean Improv. Coop. **25**: 104.
- Ros Barcelo', A. and F. Pomar (2002). Plant peroxidases: versatile catalysts in the synthesis of bioactive natural products. In: Atta-ur-Rahman, ed. Studies in natural products chemistry, Vol. 27. The Netherlands: Elsevier, 735-791.
- Steadman, J. R. (1979). Control of plant diseases caused by *Sclerotinia species*. Phytopathology **69**: 904:907.
- Stone, J. K., C. W. Bacon and J. F. White (2000). An overview of endophytic microbes: endophytism defined. In *Microbial Endophytes* (C. W. Bacon and J. F. White, (Eds.). Marcel Dekker, New York. pp.3-30
- Temple, W.D. and A.A. Bomke (1988). Effects of kelp (*Macrocystis intergrifolia*) on soil chemical prosperities and crop responses. Plant and soil, 105: 213-222.
- Tu, J. C. (1985). Tolerance of white bean (*Phaseolus vulgaris* L.) to white mold (*Sclerotinia sclerotiorum*) associated with tolerance to oxalic acid. Physiol. Plant Pathol. **26**: 111_117.
- Tu, J. C. (1986). Integrated disease control of white mold (*Sclerotinia sclerotiorum*) in navy bean (*Phaseolus vulgaris*). Int. Symp. Crop Prot. **39**: 731_740.
- Tu, J. C. (1987). Epidemiology of white mold (*Sclerotinia sclerotiorum*) in navy bean (*Phaseolus vulgaris*) Med. Fac. Landbouww. Rijksuniv. Gent. **53**: 787_796.
- Tu, J. C. (1988). The role of white mold-infected white bean (*Phaseolus vulgaris* L.) seeds in the dissemination of *Sclerotinia sclerotiorum* (Lib.) de Bary. Phytopathol. Z. **121**: 40_50.
- Tu, J. C. (1989a). Management of white mold of white beans in Ontario. Plant Dis. **73**: 281_285.
- Tu, J.C. (1989b). Modes of primary infection caused by *Sclerotinia sclerotiorum* in navy bean. Microbios **57**: 85_91.
- Tu, J.C. (1997). An integrated control of white mold (*Sclerotinia sclerotiorum*) of beans, with emphasis on recent advances in biological control. Botanical Bulletin of Academia Sinica. **38**: 73-76.
- Tu, J. C. and W. D. Beversdorf (1982). Tolerance to white mold (*Sclerotinia sclerotiorum* (Lib.) De Bary) in ExRico 23, a cultivar of white bean (*Phaseolus vulgaris*). Can. J. Plant Sci. **62**: 65-69.
- van Wees, S. C. M., M. Luijendijk, I. Smoorenburg, L. C. van Loon and C. M. J. Pieterse (1999). Rhizobacteria-mediated induced systemic resistance (ISR) in *Arabidopsis* is not associated with a direct effect on expression of known defence-related genes but stimulates the expression of the jasmonate-inducible gene *Atvsp* upon challenge. Plant Molecular Biology **41**:537-549.
- Waffo-Teguo, P., D. Lee, M. Cuendet, J.M. Merillon, J.M. Pezzuto and A.D. Kinghorn (2001). Two new stilbene dimer glucosides from grape (*Vitis vinifera*) cell cultures. Journal of Natural Products 64, 136-138.
- Wilson, D. (1995). Endophyte-the evolution of a term, and clarification of its use and definition. Oikos **73**: 274-276.

المكافحة الحيوية لمرض العفن الاسكليروتيني فى الفاصوليا باستخدام عزلات فطرية وطحالب بحرية نافعة

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تم تقدير كفاءة مكافحة الحبيوية لأربعة كائنات تضاد حيوى ، ثلاثة منها تتبع جنس ترايكودرما والرابعة الفطر فيوزاريوم اوكسيسبورم Fo 162 ، بالاضافه الى أربعة طحالب بحرية مختلفه (*Ulva fasciata, Gelidium crinale, Jania rubens and Coralline elongata*) وذلك ضد الفطر *Sclerotinia sclerotiorum* على نباتات الفاصوليا تحت ظروف الصوبة. وقد قورنت كفاءة هذه الكائنات النافعة بكفاءة المبيد الفطرى النموذجى (خليط Mefenoxam and Azoxystrobin) على عوامل النمو مثل ارتفاع النبات ، الوزن الغض والجاف للجذور والمجموع الخضرى وكذلك شدة الاصابه بمرض العفن الاسكليروتيني. كما تم اجراء التحليل الكيمياءى للمعاملات المتباينه لتقدير انزيمات مضادات الاكسده (بيروكسيداز ، بولى فينول اوكسيداز) بالاضافة الى تقدير السكريات الكلية وذلك لدراسة التداخل الثلاثى بين الكائن الممرض ، العائل وكائن التضاد الحيوى. وتشير النتائج الى أن جميع الكائنات المفيدة المستخدمه وكذلك المبيد الفطرى كان لها تأثير معنوى ايجابى على ارتفاع النبات والوزن الغض والجاف لكل من الجذور والمجموع الخضرى للنباتات المعامله وبصفة عامة كانت الطحالب البحرية ذات كفاءة أعلى فى زيادة نمو النباتات. كما انه مقارنة بالنباتات المعده بالكائن الممرض فقط فان جميع كائنات التضاد الحيوى المستخدمه أدت الى النقص المعنوى فى شدة الاصابه بالمرض ، وكان التأثير الأفضل فى ذلك للفطريات تحت الدراسه وبصفة خاصة الفطر ترايكودرما هاماتم. كما اظهرت نتائج التحليل الكيمياءى ان الطحالب البحرية أنتجت اعلى تركيز من السكريات الكلية فى حين أن أعلى نشاط لانزيمى بيروكسيداز ، بولى فينول اوكسيداز قد تم تسجيله مع فطريات التضاد الحيوى موضع الدراسة. والخلاصة ان استخدام تقنية مكافحة الحبيوية من خلال استخدام فطريات التضاد الحيوى *T. viride (Tv)*, *Trichoderma hamatum (Thm)*, *Ulva fasciata*, *harzianum (T.sp.)*, *Fusarium oxysporum (fo162)* او الطحالب البحرية (*Gelidium crinale, Jania rubens and Coralline elongata*) هى وسيلة فعاله لمكافحة مرض العفن الاسكليروتيني فى الفاصوليا وبذلك فانها تعتبر كبديل مناسب لطرق المقاومة الكيمياءية وأكثر من ذلك فان ميكانيكية التأثير المتضمنه التأثير على تركيز ونشاط انزيمى بيروكسيداز ، بولى فينول اوكسيداز وكذا تركيز السكريات الكلية هى ذات أهميه فى التفاعل الثلاثى المشترك بين نباتات الفاصوليا والفطر الممرض (*Sclerotinia sclerotiorum*) وكائنات التضاد الحيوى.