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

M. E. Selim* and Abeer H. Makhlouf
Agricultural Botany Department, Faculty of Agriculture, Menoufiya University, Egypt.
**Corresponding author E-mail : m_elwy76@yahoo.com*

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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 entophytic 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

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 cost 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 $^{-1}$kg (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 $^{-1}$kg 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 cm^3 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:

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

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).

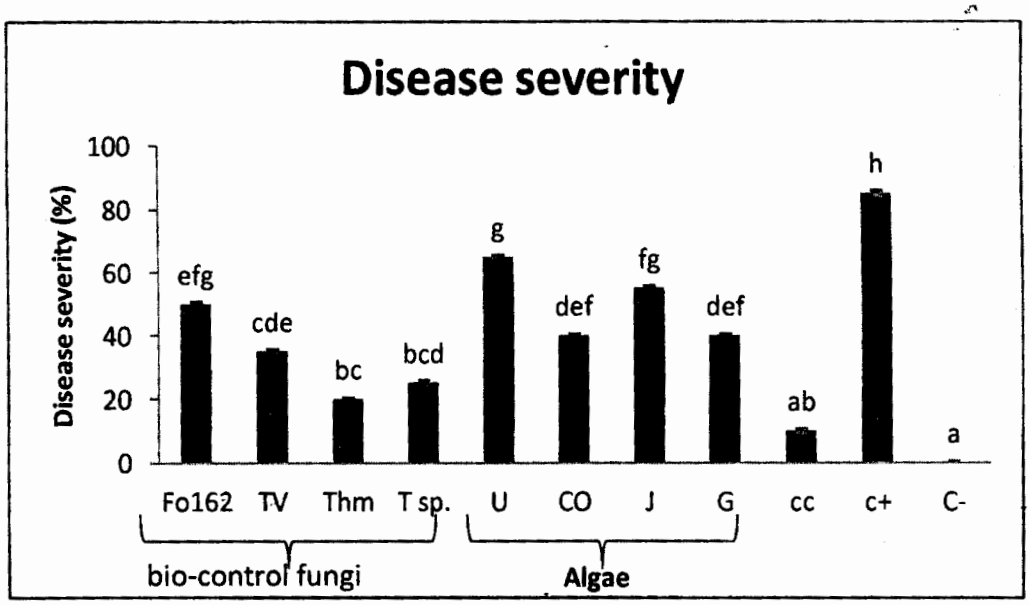

Figure (1): Effects of *Fusarium oxysporum* (Fo162), *Trichoderma viride* (TV), *T. hamatum* (Thm), *T. harzianum* (T. sp), *Ulva fasciata* (U), *Coralline elongate* (CO), *Jania rubens* (J), *Gelidium crinale* (G) and uniform fungicide (cc) on disease severity caused by *Sclerotinia sclerotiorum* compared 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.

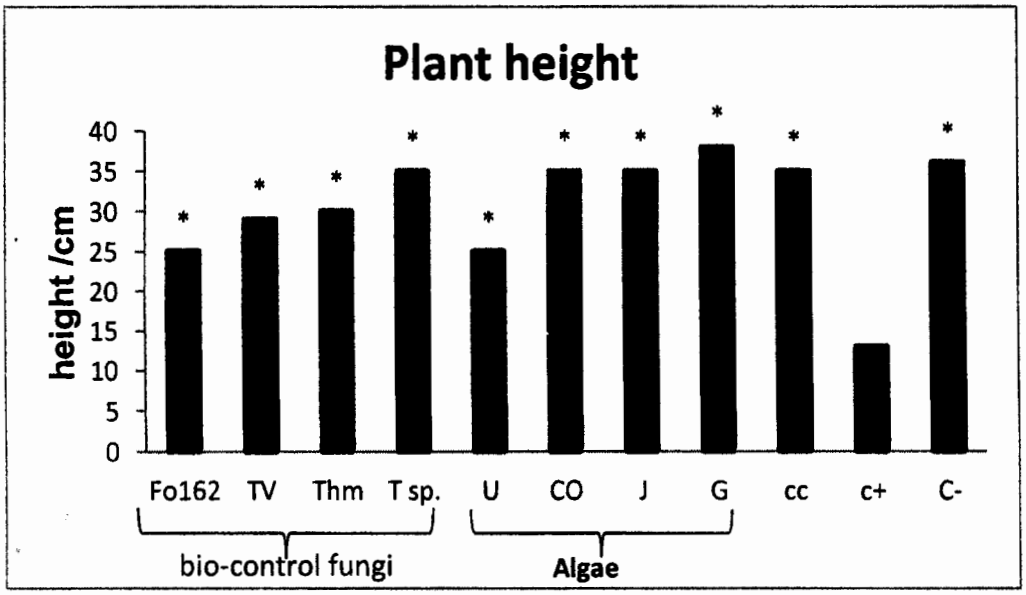

Figure (2): Influence of *Fusarium oxysporum* (Fo162), *Trichoderma viride* (TV), *T. hamatum* (Thm), *T. harzianum* (T. sp), *Ulva fasciata* (U), *Corallina elongate* (CO), *Jania rubens* (J), *Gelidium crinale* (G) and uniform fungicide (cc) on plant height of bean plants compared with infested (C+) 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).

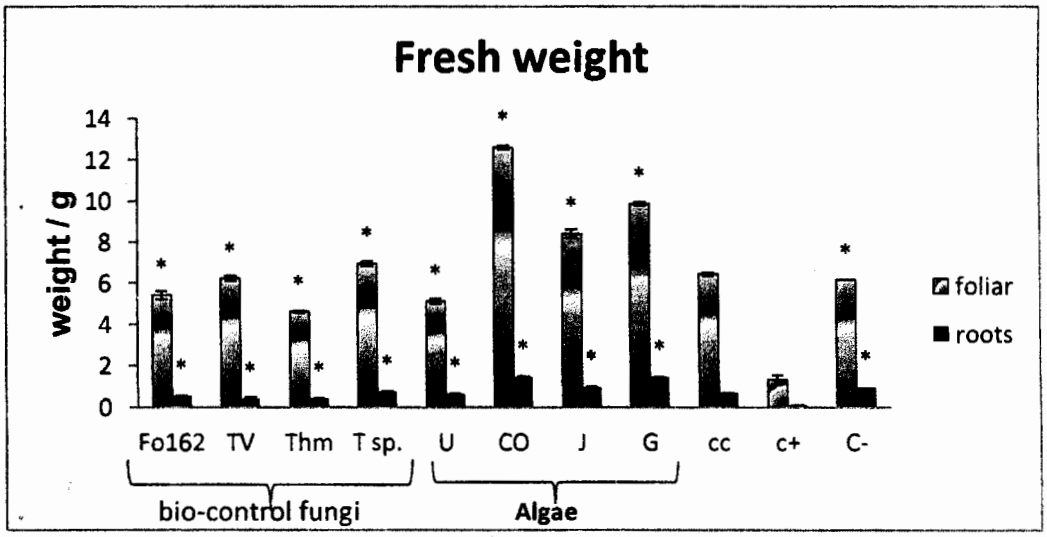

Figure (3): Influence of *Fusarium oxysporum* (Fo162), *Trichoderma viride* (TV), *T. hamatum* (Thm), *T. harzianum* (T. sp), *Ulva fasciata* (U), *Coralline elongate* (CO), *Jania rubens* (J), *Gelidium crinale* (G) and uniform fungicide (cc) on foliar and root fresh weights of bean plants compared 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.

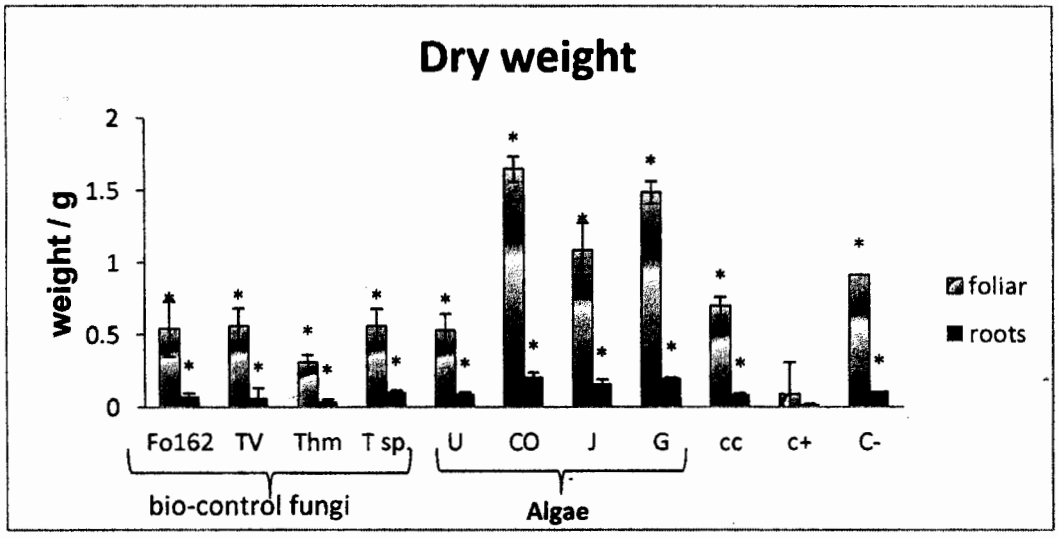

Figure (4): Influence of *Fusarium oxysporum* (Fo162), *Trichoderma viride* (TV), *T. hamatum* (Thm), *T. harzianum* (T. sp), *Ulva fasciata* (U), *Coralline elongate* (CO), *Jania rubens* (J), *Gelidium crinale* (G) and uniform fungicide (cc) on foliar and roots dry weights of bean plants compared to *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.

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)
Fusarium oxysporum (Fo162)	1.30^{c}	1.35^{b}	21.88^{bc}
Trichoderma viride (TV)	3.00^{b}	$1.^{50ab}$	17.19^{cd}
Trichoderma hamatum (Thm)	5.20^{a}	1.60^{a}	14.84^{d}
Trichoderma harzianum (T. sp)	1.75^{c}	1.30^{b}	14.84^{d}
Ulva fasciata (U)	0.60^{d}	0.70^{d}	18.75^{c}
Coralline elongate (CO)	0.90^{d}	1.00^{c}	25.00^{b}
Jania rubens (J)	0.75^{d}	0.70^{d}	18.75^{c}
Gelidium crinale (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 word. 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 save 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 see 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-

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 form 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.

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

محمد علوى سليم ، عبير حمدى عبد الغفار مخلوف

قسم النبات الزراعى- كلية الزراعة - جامعة المنوفية- مصر

الملخص العربى

تم تقدير كفاءة المكافحة الحيوية 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 الاسكليروتينى. كما تم اجراء التحليل الكيميائى للمعاملات المتباينه لتقدير انزيمات مضادات الاكسده (بيروكسيداز ، بولى فينول اوكسيديز) بالاضافة الى تقدير السكريات الكلية وذلك لدراسة التداخل الثلاثى بين الكائن الممرض ، العائل وكائن التضاد الحيوى. وتشير النتائج الى أن جميع الكائنات المفيده 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 المعداه 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 الكيميائى ان الطحالب البحرية أنتجت اعلى تركيز من السكريات الكلية فى حين أن أعلى نشاط لانزيمى بيروكسيداز ، بولى فينول اوكسيديز قد تم تسجيله مع فطريات التضاد الحيوى موضع الدراسة. والخلاصة ان استخدام تقنية المكافحة الحيوية من خلال استخدام فطريات التضاد الحيوى (*Trichoderma hamatum (Thm), T. viride (Tv), T. harzianum (T.sp.), Fusarium oxysporum* (fo162)) او الطحالب البحرية (*Ulva fasciata, Gelidium crinale, Jania rubens and Coralline elongata*) هى وسيلة فعاله لمكافحة مرض العفن الاسكليروتينى فى الفاصوليا وبذلك فانها تعتبر كبديل مناسب لطرق المقاومة الكيميائية وأكثر من ذلك فان ميكانيكية التأثير المتضمنه التأثير على تركيز ونشاط انزيمى بيروكسيداز ، بولى فينول اوكسيديز وكذا تركيز السكريات الكلية هى ذات أهميه فى التفاعل الثلاثى المشترك بين نباتات الفاصوليا والفطر الممرض (*Sclerotinia sclerotiorum*) وكائنات التضاد الحيوى.