Minufiya J. Agric. Res. Vol.40 No. 6: 1491 – 1505 (2015) "http://www.mujar.net"

BIOLOGICAL AND PHYSIOLOGICAL EFFECTS OF MUTUALISTIC BIO-CONTROL FUNGI AND SEAWEEDS APPLICATION ON INFECTED FABA BEAN PLANTS WITH ROOT-KNOT NEMATODE, *MELOIDOGYNE JAVANICA*

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(Received: Oct. 21 , 2015)

ABSTRACT: *The biological control efficacy of four mutualistic fungal isolates i.e., Fusarium oxysporum 162 (Fo162), Trichoderma viride (TV), T. harzianum (T10) and T. koningii (Tkg) as well as four seaweeds ,Ulva fasciata, Gelidium crinale, Jania rubens and Coralline elongate, against root-knot nematode, Meloidogyne javanica on faba bean plants, Vicia faba, was determined under greenhouse conditions in Egypt. The biological and physiological changes on faba bean plants due to the individual soil application of bio-fungi or seaweeds was evaluated. The results revealed that all tested mutualistic fungi and seaweeds significantly reduced the number of galls and egg masses of root-knot nematode on treated faba bean plants when compared to non-treated plants (control). The highest reduction of galls and egg masses number was observed with mutualistic endophytic fungi, Fusarium oxysporum isolate Fo162 and Trichoderma harzianum isolate T10, respectively. As observed with nematode parameters, all tested bio-agent fungi and seaweeds positively improved the growth criteria of treated faba bean plants. Significant increases in root volumes, plant highest, roots and shoots fresh and dry weights were recorded in treated plants comparing to untreated one (control).*

Remarkable increases in amino acids synthesizing capacity, accumulation of total sugars, production of proline and activity of peroxidase and polyphenol oxidase were detected in plants treated with the tested biological fungi and seaweeds. In conclusion, using specific isolates of mutualistic endophytes as Fusarium oxysporum Fo162 and Trichoderma harzianum isolate T10 represents suitable alternative for the traditional chemical control methods of root-knot nematodes on faba bean plants and therefore can be used successfully in the integrated pest management strategies to reduce the dependency on hazardous synthetic nematicides. In addition, the present study revealed that the tested seaweeds can be considered as natural resources for providing plants with essential elements that can enhance the tolerance of these plants against attack of Meloidogyne species. Moreover, different mode of actions including changes at the physiological and biochemical levels were found to be participated in the tri-trophic interactions between faba bean, root knot nematode and the tested mutualistic fungal isolates and seaweeds. These changes were observed through the activation ability of treated plants to produce large amounts of amino acids, total sugars, anti oxidant enzymes i.e. peroxidase and polyphenol oxidase in addition to proline and NPK.

Key words: *Biological control, marine algae, plant enzymes, nematicidal agents.*

INTRODUCTION

Many soil organisms of macro and micro inhabitants are known as destructive pests and pathogens. These hazardous organisms attack wide spectrum of economically important plants where they can cause massive losses.

Plant parasitic nematodes in general and root-knot nematode in particular are located among the most soil borne predominant and destructive pests that attack many important crops (Noling, 2005). Among the 24 genera of plant parasitic nematodes affecting host plants, Meloidogyne is the most predominant and widely distributed representative genus all over the world. Four species belonging to genus Meloidogyne *i.e. M. incognita, M. javanica, M. arenaria and M. hapla* were found to be able to infect selectively more than 2000 host plants where they can affect both yields and qualities (Taylor and Sasser, 1978; Trudgill, 1997; Manzanilla-Lopez *et al.,* 2004). Remarkable, more than 10 percent of world crop production is lost annually as a result of parasitic nematode attack (Whitehead, 1998). The presence of galls on the root system of infected host plants is the primary symptom associated with root-knot nematode infection. These galls affect root functions and reduce nutrient and water uptake that weakens the plants, causing wilting as well as nutrient deficiency symptoms (Sasser and Freckman, 1987).

Recently, more than 116,000 Fadden of arable lands in Egypt are cultivated with faba bean plants, *Vicia faba,* which produce annually about 223,000 tons (Egyptian Agriculture Ministry statistics, 2013).

Unfortunately, faba bean cultivars in general are among the highest preferable hosts for the root-knot nematodes especially in both tropical and subtropical areas where the crops production is severely limited by this nematode invasion (Sikora and Fernandez, 2005).

Different control methods including pesticides, resistant cultivars and crop rotation were used in order to gain reasonable protection for the valuable crops like faba bean against root-knot nematode. However, the use of these management techniques has significant limitation. For example, chemical control which depends on using fumigants or systemic nematicides is often restricted due to the high costs and/or adverse effects on environment and human health. During the last 20 years no new safer and effective nematicidal compounds have entered the market in addition to withdraw of many effective compounds due to their high toxicity.

Eventually, organic agriculture which mainly depends on replacement of hazardous chemical fertilizers and pesticides with safer alternatives as the biological agents and organic components has a large expansion nowadays. The world organic cultivated area reached up to 30 million hectare in 2010. More than 2,4million hectare were added to the organic area only in 2010 where the global organic agriculture production was increased from 61,4 billion $ in 2006 to 94.2 billion $ (FAO 2012). In Egypt the total area of registered organic farms reached up to 79986 Fadden in 2011 (Egyptian Agriculture Ministry statistics, 2013).

Fortunately, soils, besides harbouring nematodes and plant pathogens, also provide many other beneficial organisms. Some of these beneficial organisms also have been shown to colonize plants without causing disease and therefore are known as mutualistic endophytes (Petrini 1991; Wilson 1995; Stone *et al.,* 2000). Many species of these mutualistic endophytes genera had been screened for their activity against different genera of plant parasitic nematodes on a wide spectrum of crops (Stirling, 1991). The majority of fungal endophytes isolated from over 500 plants belong to a wide array of genera with the most common being: *Acremonium*, *Trichoderma*, *Cladosporium*, *Phoma* and *Fusarium* (Schulz *et al.,* 1998, 2002 and 2005).

Recently, different marine macro-algae, and seaweeds, were also screened and evaluated as promising candidate bio-fertilizers and bio-control agents which can apply into the soils to reduce the dependency on chemical fertilizers and pesticides (Shevananda, 2008; Shah *et al.*, 2013). However, most of these evaluations and determinations were conducted only under laboratory conditions in absence of the tri-trophic interactions between host plant, pathogen and bio-agent (Possinger Angela, 2011).

From the previous preview , the present study aims to determine the bio-efficacy of four mutualistic fungal isolates, three belonging to genus Trichoderma and one isolate of *Fusarium oxysporum*, in addition to four seaweeds (*Ulva fasciata, Gelidium crinale, Jania rubens* and *Coralline elongata*) against root-knot nematode infected faba bean plants under greenhouse conditions. Furthermore, to evaluate the effects of either tested mutualistic fungi or seaweeds on some essential growth criteria. Also, to investigate the possible mode of action which may be involved in the tri-trophic interactions among root-knot nematode, faba bean plants and the screened bio-control agents through measuring some important chemical and physiological parameters.

MATERIALS AND METHODS

Root knot nematode, *Meloidogyne javanica* extraction:

The eggs of root-knot nematode, *Meloidogyne javanica,* were extracted from 2-month-old heavily galled tomato roots using a modified extraction technique described by Hussey and Barker (1973). Roots were rinsed with tap water, cut into 1-cm pieces, macerated for 20 s at high speed in a blender (Molonix, France), and collected in a glass bottle. NaOCl was added to a final concentration of 1.5% and the bottle was manually shaken vigorously for 3 min. To remove the NaOCl, this suspension was then thoroughly rinsed with tap water over a set of sieves with mesh sizes of 250, 100, 45, and 25 µm. The retained nematode eggs on the 25 µm mesh sieve were collected in a glass beaker with the aid of water. The egg suspension was continuously aerated for 7 to 10 days using an aquarium pump to induce the hatching of the second-stage juveniles (J2s). To separate the active juveniles from non-hatched eggs and dead juveniles, the suspension was placed in a modified Baermann funnel for 24 h. The nematode suspension was adjusted to 1,000 active juveniles per 3 ml and immediately used for inoculation purposes.

Bio-control fungi:

Four potential bio-control fungal isolates *i.e.* three isolate belonging to genus Trichoderma and one isolate of *Fusarium oxysporum,* Fo162, were tested against root-knot nematode on faba bean plants. The non-pathogenic mutualistic *Fusarium oxysporum* (Fo162), was originally isolated from the cortical tissue of surface-sterilized tomato roots grown in Kenya by Hallman and Sikora (1994) and kindly provided by Bonn University, INRES-Institute, Germany. Pure culture of the other three Trichoderma isolates *i.e. T. viride* (Tv), *T. harzianum* isolate (T10) and *T. koningii* (Tkg) were obtained from Agriculture Research Center, Institute of Plant Pathology, Department of Vegetable Diseases, Egypt. To obtain fresh cultures of fungi, 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 millimetres 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 shadow 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 sandy clay soil (1:1, v/v) with rate of 5 g per kg and five replicates were treated with each bio-agent isolate.

Seaweeds preparing:

Four marine macro algae (seaweeds) *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 shadow at room temperatures before dehydration was completed in oven at 60 °C for 12 hours. Dried algae were grinding and mixed thoroughly into autoclaved sandy clay soil (1:1, v/v) at the rate of 5 g / kg soil (w: w).

Greenhouse experiments:

In vivo bio-assay test was conducted at the biological greenhouse of the Faculty of Agriculture, Menoufia University, Egypt (2015) to determine the bio-efficacy of four bio-control fungi as well as four seaweeds under greenhouse conditions (23 ± 2 °C, 55 ± 5 % RH) against root-knot nematode infected faba bean plants. Four surface-sterilized seeds of faba bean cv "Giza 843" were sown in plastic pot (20 cm diameter) filled with two kg of autoclaved sandy clay soil (1:1, v/v). Prior to sowing, soil in each pot was mixed thoroughly with one individual bio-control agent inoculum at rate of 5g / kg and five replicates were made for each bio-control agent. Control pots (C+) were filled with only non-treated autoclaved sandy clay soil where no biological agent inoculum was existing. After germination, 6 days after sowing, 1000 second stage of nematode juveniles suspended in 3ml water were inoculated into each pot around plant roots.

Growth characters

Eight weeks after nematode inoculation, experiment was terminated and faba bean plants were gently uprooted, washed with tap water, blotted between two tissue papers. Root volume, root and shoot fresh and dry weights as well as plant height were recorded.

Nematode parameters assay:

Egg masses were stained prior to counting by dipping the nematode infected roots in phloxine-B staining solution (0.015%) for 20 min as described by Daykin and Hussey (1985). Root galling was estimated according to Taylor and Sasser (1978).

Physiological analysis

The following physiological values in treated and non-treated faba bean plants with individual potential bio-control agents were determined as follow:

1- **Membrane integrity % (permeability)**: The percentage of electrolyte leakage % was determined and calculated according to Leopold *et al.* (1981). This parameter was measured in both leaves and roots.
2- **Water Relations**: **Relative Water Content % (RWC)**: was calculated by the equation of Larcher (1995).
3- **Antioxidant Enzymes Activity**: Peroxidase and poly phenoloxidase activities were measured in fresh leaves as described by Fehrman and Dimond (1967) and Broesh (1954) respectively.
4- **Total sugars**: Estimated in the dry leaves as described by Dubois *et al.* (1956).
5- **Total concentration of proline**: Measured in fresh leaves (µg g^{-1} Dr. Wt.) according to Bates *et al.* (1973).
6- **Total amino acid (TAA)**: Determined in dry leaves following the method described by Rosen (1957).
7- **Amount of Nitrogen, Phosphor and Potassium (NPK)** within leaves of faba bean as described by Ling (1963) ,Chapman and Prat (1961) and A.O.A.C.(1990) respectively.

Statistical analysis:

Data were statistically analyzed according to standard analysis of variance by a one-way ANOVA with Stat graphics (Statistical Graphics, Rockville, MD, USA) software. Tukey's test was used to compare differences between different treatments if the F value was significant. Statistical differences referred to in the text were significant at ($P < 0.05$) as given in the Figures (Plake and Kemmerer, 1987).

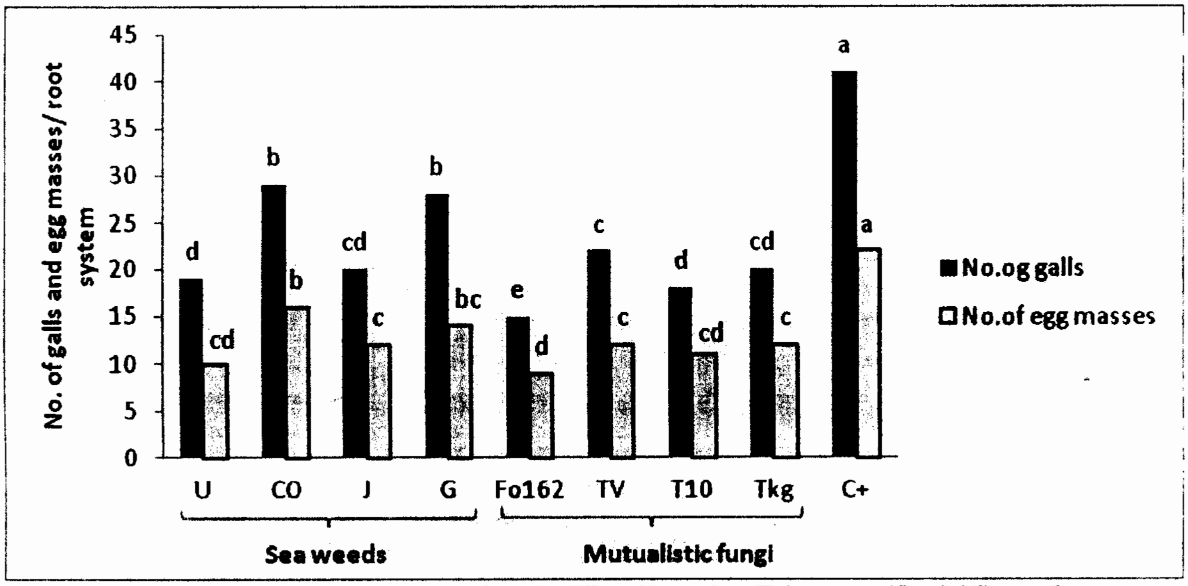
Results

The biological control efficacy of eight potential bio-control agents against root-knot nematode *Meloidogyne javanica* was determined using *in vivo* screen test under greenhouse conditions. The obtained results showed that all tested biological substances significantly reduced the number of galls and egg masses in treated faba bean plants when compared to non-treated plants, control, (Fig.1). The highest reduction of nematode infection expressed as the lowest galls number was observed with faba bean plants treated with mutualistic endophytic, *Fusarium oxysporum* isolate 162. The results revealed also that the other three tested Trichoderma isolates *i.e. Trichoderma viride* (TV), *T. harzianum* (T10) and *T. koningii* (Tkg) inhibited also the nematode infection as they decreased the number of galls and number of egg masses in treated plants when compared to control (Fig.1). Noteworthy, *Trichoderma harzianum* isolate T10 was superior to the other tested Trichoderma isolates. Among the four tested seaweeds, *Ulva fasciata, Gelidium crinale, Jania rubens* and *Coralline elongate,* the *Ulva fasciata* (U) and *Jania rubens* (J) reduced the number of galls in faba bean roots higher than the other two tested marine algae , *Coralline elongate* (CO) and *Gelidium crinale* (G), (Fig.1).

The influence of treating faba bean plants with tested bio-control fungi and seaweeds on growth criteria *i.e.* root volumes, root fresh and dry weights, plant heights as well as foliar fresh and dry weights were determined. The obtained results indicated that, the all tested bio-control agents significantly increased roots volume compared to control plants, inoculated with nematode only, (Fig.2). The results showed also that, the highest root volumes were recorded in treated faba bean plants with *Trichoderma harzianum* isolate T10 and *Coralline elongate*, CO, followed by *Ulva fasciata*, U, (Fig.2).

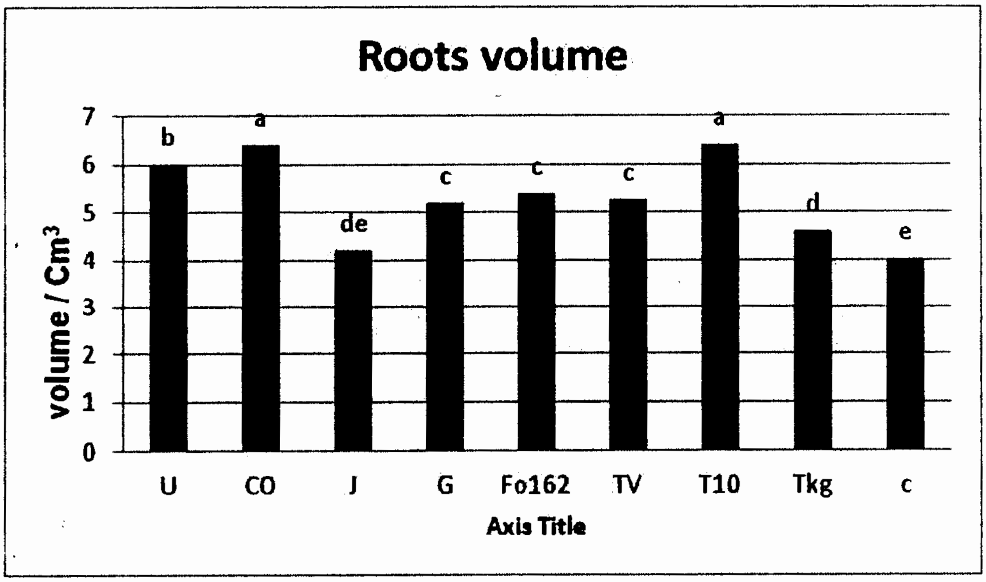
The positive influence of tested bio-control agents on fresh and dry root weights were observed as well in (Fig.3). The results illustrated that the highest increase in both fresh and dry root weights were observed on plants treated with *T. harzianum* (T10) followed by *T. viride* (TV) and *Coralline elongate* (CO), respectively (Fig.3).

Similar results were recorded with regard to the influence of tested bio-control agents on plant heights. The results indicated that the treatments of *T. harzianum* (T10) followed by mutualistic *Fusarium oxysporum* (Fo162) followed by *Coralline elongate* (CO) and *T. viride* (TV) significantly increased faba bean plant heights , more than the other biological substances as well as more than control plants (Fig.4).



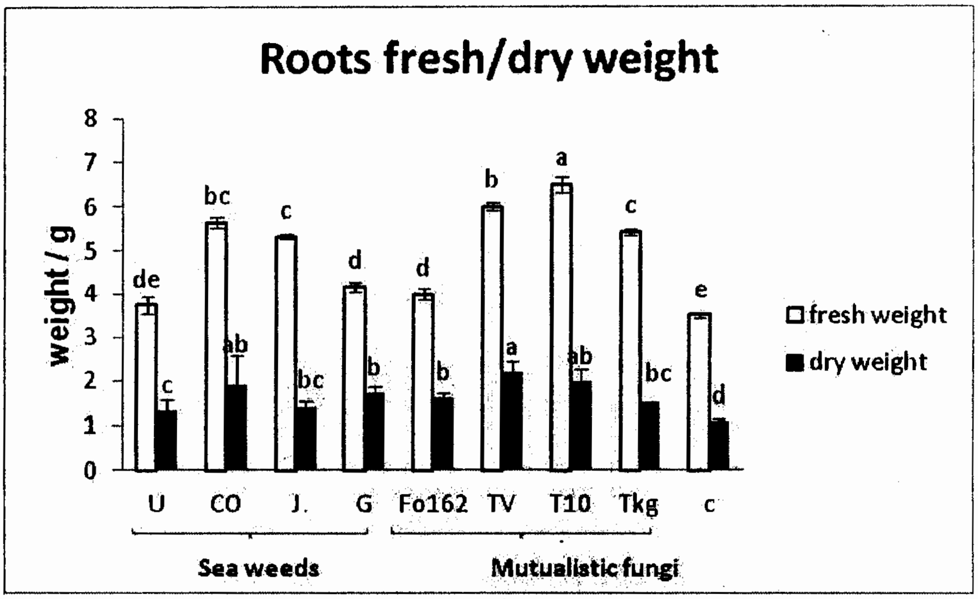
Means with different letters are significantly differed based on Tukey test, ($P \leq 0.05$). n = 5.

Figure (1): Effects of *Ulva fasciata* (U), *Coralline elongate* (CO), *Jania rubens* (J), *Gelidium crinale* (G), *Fusarium oxysporum* isolate 162 (Fo162), *Trichoderma viride* (TV), *T. harzianum* (T10) and *T. koningii* (Tkg) on number of galls and egg masses per root system comparedon nematode infested control plants (C+).



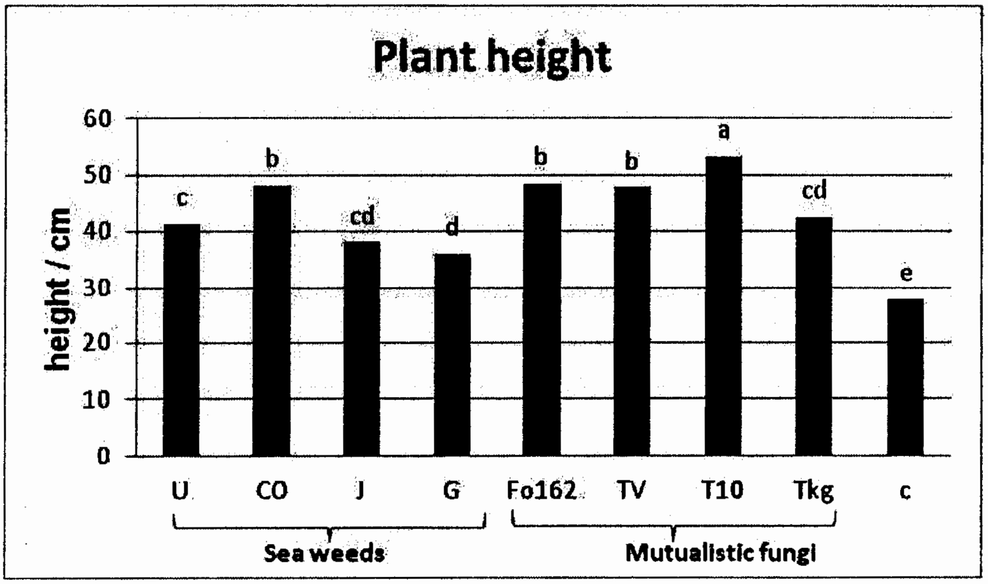
Means with different letters are significantly differed based on Tukey test, ($P \leq 0.05$). n = 5.

Figure (2): Effects of *Ulva fasciata* (U), *Coralline elongate* (CO), *Jania rubens* (J), *Gelidium crinale* (G), *Fusarium oxysporum* isolate 162 (Fo162), *Trichoderma viride* (TV), *T. harzianum* (T10) and *T. koningii* (Tkg) on root volumes of inoculated faba bean plants compared to nematode infested control plants (C).



Means with different letters are significantly differed based on Tukey test, ($P \leq 0.05$). n = 5.

Figure (3): Effects of *Ulva fasciata* (U), *Coralline elongate* (CO), *Jania rubens* (J), *Gelidium crinale* (G), *Fusarium oxysporum* isolate 162 (Fo162), *Trichoderma viride* (TV), *T. harzianum* (T10) and *T. koningii* (Tkg) on fresh and dry root weights of inoculated faba bean plants compared to nematode infected control plants (C).



Means with different letters are significantly differed based on Tukey test, ($P \leq 0.05$). n = 5.

Figure (4): Effects of *Ulva fasciata* (U), *Coralline elongate* (CO), *Jania rubens* (J), *Gelidium crinale* (G), *Fusarium oxysporum* isolate 162 (Fo162), *Trichoderma viride* (TV), *T. harzianum* (T10) and *T. koningii* (Tkg) on plant height compared to nematode infested control plants (C).

The influence of tested biological mutualistic fungi and seaweeds on foliar growth was determined and significant increase was recorded on foliar fresh and dry weight within plants treated with *T. harzianum*,T10, followed by *F. oxysporum*, Fo162 and *Coralline elongate*, respectively, (Fig.5).

The physiological and biochemical analysis of treated plants revealed that treating faba bean plants individually with either bio-control fungi or seaweeds resulted in remarkable changes at the ultra structural level. One of the primary injuries which could be caused by nematode infection is the loss in cell compartmentation due to the disruption of membranes integrity. The obtained results showed that the percentage leakage at nematode infected faba bean roots and leaves was significantly higher than that of treated plants with mutualistic bio-control fungi and seaweeds. The lowest membrane leakage percentage at roots (40%) and leaves (30%) was observed on plants treated with Fo162. Among the four tested seaweeds, *Ulva fasciata* (U) resulted in the lowest damage to roots and leaves membrane, thus the percentage of membrane leakage was reached up to 49% and 35% within roots and leaves of treated faba bean plans, respectively (Table 1).

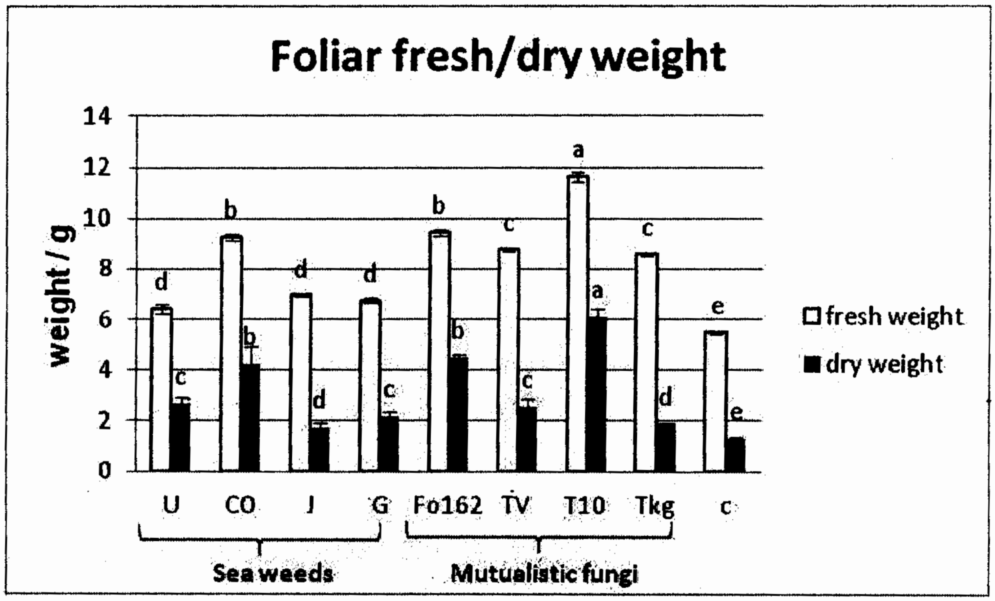
The obtained results from physiological and bio-chemical analysis showed also that the plants inoculated with nematodes only had the lowest relative percent of water content (RWC %), compared to the untreated plants, negative control, (Table 1). No significant differences were recorded among the treatments of potential bio-control fungi and seaweeds (Table 1).

Comparing to infected faba bean plants with nematode, control plants (C+), all tested bio-agents increased significantly the percentage of peroxidase and poly phenoloxidase activities (Table .1). Moreover, the results illustrated that the plants treated with *Trichoderma harzianum*, T10, exhibited the highest significant increase in peroxidase activity followed in intensity by *Trichoderma viride* (TV), and mutualistic endophytic *Fusarium oxysporum* (Fo162), respectively (Table 1). Similar results were recorded with regard to poly phenoloxidase. Thus the highest activity was observed in plants treated with Fo162 followed by *T. viride* (Tv) and *Jania rubens* (J), (Table 1). The obtained results showed also that all tested biological fungi and seaweeds increased the total sugar content within treated faba bean plants compared with control plants, infected with nematode only (C+), (Table 1). The highest increase was recorded with *T. harzianum*, T10, and *J. rubens* (J), respectively.

The bio-chemical investigation demonstrated that treating faba bean plants individually with either bio-fungi or seaweeds affected the bio-synthesis of different osmolytes *i.e.* proline and total amino acids comparing to control plants, infested with nematode only, (Table 2). The results showed also that the amount of macro elements *i.e.* nitrogen, phosphor and potassium were also affected by presence of the tested bio-agents.

The results indicated that the proline concentration significantly increased due to the presence of the nematode. Moreover, the highest proline concentrations in faba bean leaves (1087 µg/g) was detected with plants treated with *F. oxysporum* isolate FO162, followed in intensity (1045 µg/g) by *Coralline elongate* (CO), (Table. 2). Similar results were recorded with regard to concentration of total amino acids. Thus *F. oxysporum* isolate FO162 and *T. harzianum* (T10) resulted in highest accumulation (244 and 216 µg/g) of total amino acids (TAA) in faba bean leaves, respectively (Table 2).

The results illustrated also that, all tested biological agents significantly increased the concentrations of NPK comparing to control plants (C+), infected with nematode only (Table 2).The highest amount of nitrogen was recorded in faba bean plants inoculated by seaweeds *i.e. Coralline elongate* (CO), *Jania rubens* (J) and *Gelidium crinale* (G). Moreover, *Trichoderma harzianum* (T10) followed by *T. viride* (Tv) and *Gelidium crinale* (G) recorded the highest increase in phosphor concentration comparing to the other treatments. Furthermore, results demonstrated that *Coralline elongate* (CO) followed by *Fusarium oxysporum* isolate 162 (Fo162) and *Trichoderma viride* (TV), increased potassium concentrations within treated plants higher than that in all other treatments (Table 2).



Means with different letters are significantly differed based on Tukey test, ($P \leq 0.05$). n = 5.

Figure (5). Effects of *Ulva fasciata* (U), *Coralline elongate* (CO), *Jania rubens* (J), *Gelidium crinale* (G), *Fusarium oxysporum* isolate 162 (Fo162), *Trichoderma viride* (TV), *T. harzianum* (T10) and *T. koningii* (Tkg) on foliar fresh and dry weights of inoculated faba bean plants compared to nematode infested control plants (C).

Table (1): Effects of *Ulva fasciata* (U), *Coralline elongate* (CO), *Jania rubens* (J), *Gelidium crinale* (G), *Fusarium oxysporum* isolate 162 (Fo162), *Trichoderma viride* (TV), *T. harzianum* (T10) and *T. koningii* (Tkg) on leaves and roots membrane leakage %, relative water content %, peroxidase, polyphenol oxidase and total sugars compared to control (C+) and non-infested plants (C-).

Treatment	Membrane Leakage (%)		Relative Water Content (%)	Enzymes		Total sugars (TS) (mg/g DW)
	Leaves	Roots		Peroxidase (O.D. 2 min.)	Poly phenoloxidase (O.D. 45 min.)	
Ulva fasciata (U)	49^{g}	35^{f}	73.11^{b}	15^{d}	7^{ab}	23,75^{a}
Coralline elongate (CO)	64^{d}	52^{c}	71.84^{b}	12^{c}	6,5^{b}	30,63^{d}
Jania rubens (J)	69^{c}	53^{c}	73.20^{b}	17^{e}	7,5^{ab}	37,50^{e}
Gelidium crinale (G)	71^{b}	56^{b}	71.18^{b}	12^{c}	6,5^{b}	23,75^{a}
Fusarium oxysporum isolate 162 (Fo162)	40^{h}	30^{g}	75.38^{b}	19^{f}	8^{a}	23,44^{a}
Trichoderma viride (TV)	51^{f}	43^{e}	71.46^{b}	20^{f}	7,5^{ab}	25,00^{b}
Trichoderma harzianum(T10)	49^{g}	35^{f}	72.77^{b}	25^{g}	6,1^{b}	39,10^{f}
Trichoderma koningii (Tkg)	56^{e}	45^{d}	73.36^{b}	15^{d}	6,2^{b}	23,66^{a}
nematode infected control plants (C+)	85^{a}	64^{a}	64.16^{a}	10^{b}	5,5^{c}	23,44^{a}
non-infested control plants(C-)	38^{h}	35^{f}	72.47^{b}	7,5^{a}	8,8^{a}	28,13^{c}

Means with different letters are significantly differed based on Tukey test, ($P \leq 0.05$). n = 3.

Table (2): Effects of *Ulva fasciata* (U), *Coralline elongate* (CO), *Jania rubens* (J), *Gelidium crinale* (G), *Fusarium oxysporum* isolate 162 (Fo162), *Trichoderma viride* (TV), *T. harzianum* (T10) and *T. koningii* (Tkg) on proline, total amino acids and NPK concentrations compared to control (C+) and non-infested control plants (C-).

Treatment	Proline (µg/g DW)	Total amino acids (mg/g DW)	Macro Elements		
			Nitrogen (%)	Phosphor (ppm)	Potassium (ppm)
Ulva fasciata (U)	230^{f}	44^{e}	4,76^{d}	0,81^{de}	13,4^{f}
Coralline elongate (CO)	1045^{a}	175^{b}	8,6^{a}	1,54^{bc}	36,68^{b}
Jania rubens (J)	217^{f}	66^{d}	8,08^{ab}	1,25^{cd}	22,43^{d}
Gelidium crinale (G)	362^{d}	103^{c}	8,8^{a}	1,78^{b}	14,86^{e}
Fusarium oxysporum isolate 162 (Fo162)	1087^{a}	244^{a}	6,7^{cd}	0,81^{de}	26,43^{c}
Trichoderma viride (TV)	289^{e}	119^{c}	5,08^{d}	1,78^{b}	25,62^{c}
Trichoderma harzianum (T10)	942^{b}	216^{a}	7,0^{bc}	2,31^{a}	45,99^{a}
Trichoderma koningii (Tkg)	471^{c}	84^{cd}	5,32^{d}	0,71^{e}	9,85^{h}
nematode infested control plants (C+)	434^{c}	72^{d}	2,80^{e}	0,18^{f}	5,68^{i}
non-infested control plants (C-)	360^{d}	80^{cd}	7,08^{bc}	0,72^{e}	10,55^{g}

Means with different letters are significantly differed based on Tukey test, ($P \leq 0.05$). n = 3.

Discussion

Faba bean is considering one of most important crops all over the world in general and in Egypt in particular. Unfortunately this valuable crop is attacked by wide array of specific and non-specific plant pathogens and parásites. Among all these hazardous organisms, root-knot nematode represents the main threat for faba bean cultivation. Depending on chemical control rather than the other traditional management methods *i.e.* resistant varieties and agriculture practices failed alone to stop the draining of yield losses which resulted from root-knot nematode invasion especially in the tropical and sub-tropical regions (Sikora and Fernandez, 2005). Therefore, looking for effective and safe alternatives to control root-knot nematodes on their host plants has an increasing importance.

Recently, it was known that environments consistently harbour a variety of macro- and micro-scopic organisms with different potentials to interact with hazardous pathogens and parasites either directly or indirectly. Some can form a tight association up to the level that they migrate into the roots. Many of these invading microorganisms have the capability to colonize roots of various host plants without causing any disease symptoms and therefore known as mutualistic endophytic organisms (Wilson, 1995). Over the last two decades, it has become clear that many endophytic fungal and bacterial species can have beneficial effects on the development and survival of plants (Zum Felde *et al.*, 2005; Olivain *et al.*, 2006).

Certain mutualistic endophytic isolates belonging to specific species of *Trichoderma, Gliocladium, Fusarium, Mucor, Penicillium, Aspergillus, Stachybotrys,* and *Verticillium* genera have the potential for application in agricultural systems besides the traditional chemical pesticides. Some can be beneficial with regard to tolerating biotic and abiotic stress caused by drought, salinity, minerals deficiency and pathogen invasion through modulating an array of physiological, biochemical and morphological processes (Oelmüller, 2009; Sherameti *et al.,* 2005) resulting in the accumulation and translocation of assimilates, osmotic adjustment, maintenance of cell wall elasticity and the increase of the water use efficiency by the host plant. Others can support the plant in coping with biotic stress conditions.

Moreover, many of seaweeds (marine algae) attracted the interest of scientists as natural sources of different bio-active compounds. Therefore they are candidate to be promising bio-control agents against different pathogens and parasites (Michael *et al.*, 2005; El-gamal, 2010). Many bioactive compounds have been identified in seaweeds; antioxidants are often, reducing agents such as thiol, ascorbic acid or poly phenols (Hu, 2008; Li *et al.*, 2011). Of the beneficial antioxidant compounds, phenolic compounds widely exist in plants and have been considered to have high antioxidant capacity and free radical scavenging capacity (Kahkonen *et al.*, 2001).

The obtained results from present study revealed that all tested mutualistic fungi and seaweeds had negative impact on nematode infection as they significantly reduced the number of galls and egg masses of root-knot nematode on treated faba bean plants when compared to non-treated plants (control). The highest reduction of galls and egg masses number was observed with mutualistic endophytic *Fusarium oxysporum* isolate 162 and *Trichoderma harzianum* isolate T10, respectively. As observed with nematode parameters, all tested bio-agents fungi and seaweeds affected positively the growth criteria of treated faba bean plants. For example, the results cleared that *T. harzianum* (T10) resulted in the highest increase in root volumes of treated plants followed by *Coralline elongate* (CO). Similar results were detected with regard to the increase in roots and shoots fresh and dry weights.

These results are consistence with, Hallmann and Sikora (1994) who isolated fungal endophytes from roots of field grown tomato in Kenya and reported that many of these endophytic isolates showed biocontrol activity toward the root-knot nematode *M. incognita*. They added also, the mutualistic endophyte *F. oxysporum* strain 162 (Fo162) was the most promising isolate and produced high levels of antagonistic activity against *M. incognita* on tomato. Moreover, they reported a reduction in gall formation between 50 and 75% within colonized tomato plants by the endophyte in greenhouse trials. Furthermore, the same isolate (Fo162) has been repeatedly shown to have strong activity toward *Meloidogyne species* in other investigations (Dababat *et al.,* 2008; Dababat and Sikora2007a,b).

Specific Trichoderma and Fusarium strains were found to be effective against different plant pathogens and parasites (Sikora, 1992 and 1997; Siddiqui and Shaukat, 2004; Sikora and Dababat 2007; Selim *et al.*, 2014). However, little is known about the tri-trophic interactions between the plant, the fungal endophyte and the root-knot nematode. Benítez *et al.,* (2004) mentioned that some Trichoderma strains exert biocontrol activity against different plant pathogens either indirectly, by competing for nutrients and space, modifying the environmental conditions, or promoting plant growth and plant defence mechanisms and antibiosis, or directly, by mechanisms such as mycoparasitism. The antagonistic ability of Trichoderma spp. against pathogens and parasites has been demonstrated also by Chen *et al.* (2009) and Yang *et al.* (2012)

Noteworthy, through using split root design system, Selim *et al.,* (2014) show that *T. harzianum* isolate (T10) was able to trigger the systemic resistance pathways within tomato plants against *Meloidogyne javanica* by increasing the accumulation of special different antagonistic compounds that affect the nematode invasion and egg mass production on the responder side of treated tomato plants. These findings are in agreements with the present results which demonstrated that T10 was superior to the other tested Trichoderma isolates in controlling root-knot nematodes on faba bean plants under Egyptian conditions.

Remarkable, seaweeds are used at the meanwhile as organic fertilizers and soil conditioners in many agriculture systems. Moreover, they have been found to increase the systemic defense responses through providing host plants with essential elements and biological active compounds related to defense responses (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 value nutrient elements within seaweeds (marine Algae) make them excellent organic fertilizers and could explain the positive influence of the tested seaweeds on faba bean plants to make them more tolerant against root-knot nematode infection and compensate the damage caused by the nematode invasion.

Additionally, seaweeds algae, as biocontrol agents may be increase the protection of faba bean plants against nematodes by increasing RWC, antioxidant enzymes *i.e.* peroxidase and phenoloxidase as well as increasing TAA concentrations. Highly significant increase in proline concentration was also recorded by tested seaweeds. .Moreover increases in faba bean leaves NPK concentrations which reflected highly defence system against nematode disease was demonstrated in present study as well(Bhattacharyya *et. al.*, 2013).

Furthermore, algae organisms are known to be a rich source in biological novel compounds that can be potentially active against phytopathogens (Kulik, 1995).

Moreover, Walter *et al.,* (2005) mentioned that use of algal biomass as a slow release fertilizer is involved in controlling process of NPK concentrations which led to adjustment of aquatic systems.

Acknowledgments

Authors would like to thank Professor Dr. Mohamed El-Ameen Sweelam from Menoufia University, Faculty of Agriculture, Entomology and Zoology Deptr., for ongoing cooperation to support and achieving the present research.

REFERENCES

A.O.A.C. (1990). Official Methods of Analysis of Association of Official Analytical Chemists.15th Ed.Vol.1, pp. :47-57.

Bates, L.R., R.P. Waldren and I.D. Teare (1973). A rapid determination of free proline in water stress studies. Plant and Soil 39, 205-207.

Benítez, T., A.M. Rincón, M.C. Limón and A.C. Codón (2004). Biocontrol mechanisms of *Trichoderma* strains. International Journal of Microbiology 7, 249-260.

Bhattacharyta, S., P. R. Deep, B. Nayak, Monalisa Panigrahi and B. Mohapatra (2013). Antimicrobial activity of two diazotrophic Cyanobacteria against *Staphylococcus aureus* .Int. J. Med .Arom . Plants, 3(2):283-292.

Blunden, G. and S.M. Gordon (1986). Betaines and their sulphino analogues in marine algae. Progress in Phycological Research 4: 39-79.

Broesh, S. (1954). Colorimetric assay of phenoloxidase.Bull.Soc. Chem.Biol. ,36:711-713.

Chapman, H.D. and P.E. Pratt (1961). Methods of Analysis of Soil, Plant and Water .Div.of Agric. Sci. ,Univ. of California.

Chen, L. L., L. J. Liu, M. Shi, X. Y. Song, C. Y. Zheng, X. L. Chen and Y. Z. Zhang (2009). Characterization and gene cloning of a novel serine protease with nematicidal activity from *Trichoderma pseudokoningii*, SMF2. FEMS. Microbiology Letters 2, (299) 135–142.

Dababat, A. A. and R. A. Sikora (2007a). Induced resistance by the. mutualistic endophyte, *Fusarium oxysporum* 162, toward *Meloidogyne incognita* on tomato. Biocontrol Sci. Techn. **17**, 969-975.

Dababat, A. A. and R. A. Sikora (2007b). Influence of the mutualistic endophyte *Fusarium oxysporum* 162 on *Meloidogyne incognita* attraction and invasion. Nematology **9**, 771-776.

Dababat, A. A., M. E. Selim, A. A. Saleh and R. A. Sikora (2008). Influence of Fusarium wilt resistant tomato cultivars on root colonization of the mutualistic endophyte *Fusarium oxysporum* strain 162 and its biological control efficacy toward the root-knot nematode *Meloidogyne incognita*. Journal of Plant Disease and Protection, **115** (6) 273-278.

Daykin, M.E. and R.S. Hussey (1985). Staining and histo-pathological techniques in nematology. In: Barker KR, Carter CC, Sasser JN, editors. An Advanced Treatise on Meloidogyne: Volume II. Raleigh, NC: North Carolina State University Graphics; 1985. pp. 39–48.

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.

Fehrman, H. and A. E. Dimond (1967). Peroxidase activity and Phytophthora resistance in different organs of the potato. Plant Pathology,57:69-72.

Food and Agriculture Organisation, FAO, (2012). Agricultural statistics, Home page available at (http://apps.fao.org).

Hallmann, J. and R. A. Sikora (1994). 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.

Hu, C. C ., J.T. Lin, F. J. Lu, F. P. Chou and D.J. Yang (2008). Determination of carotenoids in Dunaliella Saline cultivated in Taiwan and antioxidant capacity of the algal carotenoid extract. Food Chemistry, 109:439-446.

Hussey, R.S. and K.R. Barker (1973). A comparison of methods collecting inocula of *Meloidogyne* spp. including a new technique. Plant Disease Reporter 57, 1025-1028.

Kahkonen, M. P., A. I. Hopia and M. Heinonen (2001). Berry phenolics and their antioxidant activity .J. Agric. Food Chem., 49: 4076-4082.

Kalapos, T. (1994). Leaf water potential, leaf water deficit relationship for ten species of semiarid grassland community. Plant and Soil 160, 105-112.

Kulik, M. M. (1995). The potential for using cyanobacteria and algae in the biological control of plant pathogenic bacteria and fungi.Eur. J.Plant Pathol. , 101:585-599.

Larcher, W. (1995). Plant water relations.In"Physiological Plant Ecology".3rd ed.Springer, Berlin,pp.215-275.

Leopold, A.C., M.E. Musgrave and K.M. Williams (1981). Solute leakage resulting from leaf desiccation. Plant Physiology 68, 1222-1225.

Li, Y. X., I. Wijesekara, Y. Li and S. K. Kim (2011). Phlorotannins as bioactive agents from brown algae. Process Biochemistry , 46:2219-2224.

Ling, E.R. (1963). Determination of total nitrogen by semimicro-Kgeldahl method. Dairy Chem.,11:23-84.

MacArtain, P., C.I.R. Gill, M. Brooks, R. Campbell and I.R. Rowlamd (2007). Nutritional value of edible seaweeds. Nutritional Reviews, 65(12): 535-543.

Manzanilla-Lopez, R. H., K. Evans and J. Bridge (2004). Plant diseases caused by nematodes. In: Chen, Z. X., Chen, S. Y. and Dickson, D. W. (Eds.). Nematology- Nematode management and utilization. **Vol. 2**, CABI Publishing, 637-703.

Michael, T.M., M.M. John and P. Jack (2005). Brock microbiology of microorganisms. 11th Edition, New Jersey. ISBN: 13-978-0226701479.

Noling, J. W. (2005). Nematode management on tomatoes, peppers and eggplant. Institute of Food and Agricultural Science, University of Florida. http://edis.ifas.ufl.edu/.

Oelmu¨ller, R., I. Sherämeti, S. Tripathi and A. Varma (2009). *Piriformospora indica*, a cultivable root endophyte with multiple biotechnological applications. Symbiosis, 49: 1–17.

Olivain, C., C. Humbert, J. Nahalkova, J. Fatehi, F. L'haridon and C. Alabouvette (2006). Colonization of tomato by pathogenic and non-pathogenic *Fusarium oxysporum* strains inoculated together and separately into the soil. Appl. Environ. Microbiol. 72, 1-9.

Petrini, O. (1991). Fungal endophytes of tree leaves. Microbial Ecology of leaves (J. Andrews & S. Hirano, (Eds.), 179-197. Springer Verlag, New York.

Plake, B. S. and B. K. Kemmerer (1987). Evaluation of Statistical Software for Microcomputers: STATGRAPHICS. American Educational Research Association, Washington, DC.

Possinger, A. R. (2011). Using Seaweed as a Soil Amendment: Effects on Soil Quality and Yield of Sweet Corn (Zea mays L.). Master Thesis in Biological and Environmental Sciences University of Rhode Island. http://digitalcommons.uri.edu/theses.

Rosen, H. (1957). A modified ninhydrin colorimetric analysis. Archives of Biochemistry and Biophysics 67, 10-15.

Sasser, J. N. and D. W. Freckman (1987). A world perspective on nematology: the role of the society. In: Veech, J. A. and Dickson, D. W. (Eds.) Vistas on Nematology. Society of Nematologists, Hyattsville, Maryland, pp. 7-14.

Schulz, B. and C. Boyle (2005). The endophytic continuum. Mycol. Res. **109** (6) 661-686.

Schulz, B., C. Boyle, S. Draeger, A.K. Römmert and K. Krohn (2002). Endophytic fungi: a source of biologically active secondary metabolites. Mycological Research **106**: 996-1004.

Schulz, B., S. Guske, U. Dammann and C. Boyle (1998). Endophyte-host interactions II. Defining symbiosis of the endophyte-host interaction. Symbiosis 25: 213-227.

Selim, M.E. (2010). Biological, chemical and molecular studies on the systemic induced resistance in tomato against *Meloidogyne incognita* caused by the endophytic *Fusarium oxysporum*, Fo162. Ph D Thesis, University of Bonn, Germany.

Selim, M.E., M.E. Mahdy, M.E. Sorial, A.A. Dababat and R. A. Sikora (2014). Biological and chemical – dependant systemic resistance and their significance for the control of root-knot nematodes. Nematology 16 (8) 917-927.

Shah, T.M., S.T. Zodape, D.R. Chaudhary, K. Eswaren and Chikara (2013). Seaweed sap as an alternative liquid fertilizer for yield and quality improvement of wheat. Journal of plant nutrition, 36(2): 192-200, Doi: 10.1080/01904167.2012.737886.

Shevananda (2008). Influence of bio-fertilizers on the availability of nutrients (N, P, K) in soil in relation to growth and yield of *Stevia rebaudiana* grown in South India. International journal of Applied research in natural products, 1(1):20-24.

Sherameti, I., B. Shahollari, Y. Venus, L. Altschmied, A. Varma and R. Oelmu¨ller (2005). The endophytic fungus *Piriformospora indica* stimulates the expression of nitrate reductase and the starch-degrading enzyme glucanwater dikinase in tobacco and Arabidopsis roots through a homeodomain transcription factor which binds to a conserved motif in their promoters. Journal of Biology Chemistry 280: 2641–2647.

Siddiqui, I.A. and S.S. Shaukat (2004). Systemic resistance in tomato induced by biocontrol bacteria against the root-knot nematode, *Meloidogyne javanica* is independent of salicylic acid production. Journal of Phytopathology 152, 48-54.

Sikora, R. A. (1992). Management of the antagonistic potential in agricultural ecosystems for the biological control of plant-parasitic nematodes. Ann. Rev. Phytopathology **30**, 245-270.

Sikora, R. A. (1997). Biological system management in the rhizosphere an inside-out/side-in perspective. Comm. Appl. Biol. Sci. Ghent University, **62**: 151-157.

Sikora, R. A. and E. Fernandez (2005). Nematode parasites of vegetables. In: Luc, M. Sikora, R. A. And Bridge, J. (Eds.). Plant parasitic nematodes in subtropical and tropical agriculture. CABI Publishing: UK, pp. 319-392.

Sikora, R.A., K. Schäfer and A.A. Dababat (2007). Modes of action associated with microbially induced in planta suppression of plant-parasitic nematodes. Australasian Plant Pathology 36, 124-134.

Stirling, G. R. (1991). Biological control of plant parasitic nematodes. CAB International, Wallington, UK. 282 pp.

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

Taylor, A. L. and J. N. Sasser (1978). Biology, identification and control of root-knot nematodes (*Meloidogyne* spp.). North Carolina University Graphics, cooperative publication of Department of Plant Pathology, North Carolina State University and US Agency for International Development, Washington. DC.

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.

Trudgill, D. L. (1997). Parthenogenetic root-knot nematodes (*Meloidogyne* spp.); how can these biotrophic endoparasites have such an enormous host range? Plant pathology **46**, 26-32

Walter, M., K. Shannon and Carolina Pizarro (2006). Biofertilizers from algal treatment of dairy and swine manure effluents: Characterization of Algal Biomass as Slow Release Fertilizer. J. of Vegetable Sci. 12(4):107-125.

Whitehead, A. G. (1998). Plant Nematode Control. CAB International, Uk, pp. 384.

Wilson, D. (1995). Endophyte-the evolution of a term, and clarification of its use and definition. Oikos **73**: 274-276.

Yang, Z., Z. Yu, L. Lei, Z. Xia, L. Shao, K. Zhang and G. LI (2012). Nematicidal effect of volatiles produced by Trichoderma sp. Journal of Asia-Pacific Entomology; ISSN:1226-8615 @ ; VOL.15; NO.4; PAGE.647-650.

Zum Felde, A., A. L. Pocasangre and R. A. Sikora (2005). The potential use of microbial communities inside suppressive banana plants for banana root protection. In: Turner, D. W., and Rosales, F. E. (Eds). In Banana root system: toward a better understanding for its productive management. Proceedings of an international symposium. International network for the improvement of banana and plantain (INIBAP), pp. 169-177. Montpellier, France.

التاثيرات البيولوجية والفسيولوجية لتطبيق فطريات المكافحة الحيوية والطحالب البحرية على نباتات الفول البلدى المصابة بنيماتودا تعقد الجذور , *Meloidogyne javanica*

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الملخص العربى

تم تقدير كفاءة المكافحة البيولوجية لاربعة عزلات فطرية هى الفيوزاريوم اوكسيسبورم (عزله Fo١٦٢), التRايكودرما هارزيانم عزله (T10), الترايكودرما فيريدى عزله (Tv) و الترايكودرما كوننجياى عزله (Tkg) بلاضافه الى ٤ طحالب بحرية هى : *(Ulva fasciata, Gelidium crinale, Jania rubens* and *Coralline elongate)*

وذلك لمكافحة نيماتودا تعقد الجذور *Meloidogyne javanica* على نباتات الفول تحت ظروف الصوبة فى مصر . كما تم تقدير التغيرات البيولوجية والفسيولوجية التى تحدث فى نباتات الفول البلدى نتيجة اضافة الفطريات والطحالب المخيتبره الى التربة.

وقد اظهرت النتائج ان جميع العزلات الفطرية والطحالب البحرية موضع الدراسة فد ادت الى تقليل عدد العقد الجذرية واكياس البيض الخاصة بنيماتودا تعقد الجذور بشكل معنوى مقارنة بالنباتات التى تم تلقيحها فقط بالنيماتودا. هذا وقد تم تسجيل اعلى انخفاض فى عدد العقد الجذرية وكذا اكياس البيض على النباتات التى تم اضافة فطر الفيوزاريوم اوكسيسبورم عزله (Fo١٦٢) لها وكذا الترايكودرما هارزيانم عزله (T10). كما اظهرت النتائج ان جميع الفطريات والطحالب المختبره قد ادت الى تحسين فى صفات النمو فى نباتات الفول المعاملة بها حيث لوحظ حدوث زيادة معنوية فى حجم الجذور, طول النباتات, وكذا الوزن الطازج والجاف للجذور والمجموع الخضرى. هذا وقد ادت الاضافات المنفردة من الفطريات والطحالب البحرية المختبره الى تربة نباتات الفول المعاملة بالنيماتودا الى حدوث زيادة ملحوظة فى تكوين الاحماض الامنية والسكريات والبرولين بالاضافة الى زيادة نشاط انزيمى بيروكيسديز والبولى فينول اوكسيديز.

ويمكن تلخيص النتائج المتحصل عليها من الدراسة بان استخدام عزلات محددة مثل الفيوزاريوم اوكسيسيورم عزله (Fo١٦٢) وكذا الترايكودرما هارزيانم عزله (T10) من الممكن ان يعتبر بديلا مناسبا يؤدى الى تقليل الاعتماد على استخدام المبيدات النيماتودية الضارة لمكافحة نيماتودا تعقد الجذور على الفول البلدى كما ان استخدام بعض الطحالب البحرية والتى تعتبر مصدرا طبيعيا لتزويد النباتات بالعناصر الغذائية الاساسية من الممكن ان يؤدى الى زيادة المقاومة الطبيعية فى هذه النباتات بالشكل الذى يؤدى الى تقليل معدل الاصابة بالنيماتودا ويحسن من نموها.