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Field application of bio compost to control *Fusarium* dry rot disease of potato in newly Reclaimed lands

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Fusarium dry rot caused by *Fusarium sambucinum* could infect all cultivated potato cultivars at different degrees of disease infections. Laboratory and field trails were carried out for controlling the causal organism and disease incidence. *Fusarium sambucinum* isolate No.3 was highly pathogenic to all potato cultivars. *Trichoderma harzianum* was antagonistic to all tested isolates with different degrees of inhibition. *Trichoderma harzianum* inhibited the linear growth of all tested isolates of *F. sambucinum* by overcoming their growth. Drenching soil before tuber sowing with bio compost 4 (composted bagasse + rice straw + soybean straw + *T. harzianum*) and/or bio compost 1(composted bagasse + *T. harzianum*) significantly reduced Fusarium dry rot disease incidence and its severity .Compost 1 and compost 4 reduced disease incidence and severity by 58.2, and 68.7% and 65.9 , 68.1% during the first cultivation seasons 2010 and by 60.7, 71.4 % and 73.1, 78.0% during the second cultivation season 2011. These treatments increased the obtained tuber yield/ feddan by 64.7 and 55.6 % in the first plantation 2010 and by 77.7 and 59.2% in the second plantation 2011.Therefore, the usage of composted agricultural wastes fortified with bio control agents could be suggested as easily soil bio-treatment for controlling dry root rot as well as soil borne pathogens of potato especially under organic system.

Key words: Potato, Dry root rot , Bio-compost, Control, *Fusarium sambucinum*

Introduction

Soil borne diseases are still a major threat to potato cultivation in Egypt. Several important potato pathogens in Egypt originated from soil borne inoculums. Potato plants are susceptible to devastation by various diseases such as Black scurf caused by *Rhizoctonia solani* and dry rot caused by *Fusarium sambucinum* (Agrios, 1997; Yogen *et al.*, 2006; Peters *et al.*, 2008). Fusarium dry rot disease is considered the most major yield limiting factors for potato production (Daami-Remadi and El-Mahjoub, 2006). Fusarium dry rot of seed tubers can reduce the plant establishment by killing the developing potato spouts. Also, it can greatly affect tuber quality and subsequently, can severely reduce its market value (Sadfi *et al.,* 2002; Peters *et al*., 2008).

Control of soil borne pathogens such as *R. solani* and *F. sambucinum* is difficult because these pathogens survive for many years as sclerotia in soil or as mycelim in organic matter under several environmental conditions (El-Kot, 2008). Control of Fusarium diseases has commonly relied on culture practices and on the use of fungicidal treatments However, culture practices alone are not efficient and at the present time. Chemical control was massively applied, however, for the increasing public concern over the fungicides usage. Recently, alternative control methods are strongly desired for sustainable agriculture. Organic amendments play an important role as environmentally friendly and sustainable alternative approach to protect plants against soil borne pathogens. Soil amendments, using composted agricultural wastes fortified with bio control agents could be acceptable approaches in this regard. The use of organic agricultural wastes in this respect can be an advantageous both in soil fertility, recycling of agricultural residues and could provide a powerful tool for management of plant diseases. It has been reported that several composts and /or composts fortified with bio control agent used as soil amendments reduced pathogens propagules density and protected plants from soil borne plant pathogens (Huang, 1991; Yogen *et al*., 2006; Khalil and El-Maghrabia 2010).

Using agricultural wastes, domestic food wastes or some grains as substrates for *T. harzianum* growth formulation and directly delivery in soil for controlling soil borne pathogens on some crops were recorded (Godwin and Arinze, 2000; Liu and Huany, 2000; El-Kot, 2008). Amendment of compost with *Trichoderma harzianum* also was reported to accelerate agricultural wastes composting and improved its diseases suppressive effect (Mitra and Nandi, 1994; Nemec *et al.,* 1996; Ravelo *et al*., 2000; El-Mohamedy, 2004; El-Mohamedy *et al*., 2006 and 2010).

The present investigation was designed to investigate the potential of manipulating soil with bio composts made from composted agricultural wastes fortified with *Trichoderma harzianum* to reduce the potato dry rot diseases caused by *Fusarium sambucinum* under field conditions.

Materials and methods

Causal organism

Samples of potato plants and tubers showing typical symptoms of dry rot were collected from different farms at El-Nobaria provience, El-Beharia Governorate, Egypt. Samples pieces were kept in a moist chamber for 72 h, then the appeared hyphal tips of growing fungus was picked up and transferred to sterilized potato dextrose agar plates (PDA). After 3 days of incubation at 25°C, the fungal isolates were purified and identified according to cultures morphological and microscopically characteristics as described by Booth (1977), Barnett and Hunter (1972). The fungal pure cultures were maintained on PDA slants at 4°C for further studies.

Pathogenicity test

The evaluation of pathogenic ability of different isolates of *Fusarium sambucinum* was performed in pot experiments under greenhouse conditions of Plant Pathology Dept., NRC, Egypt. Experiments were carried out in a sandy loam soil artificially infested individually with *Fusarium sambucinum* isolates the causal pathogen of potato dry rot. Fungal mass production used for soil infestation was obtained by growing the tested isolates on autoclaved sand barley medium (Abdel-Kader, 1997) [sand: barley (1:1 w:w) + 40% water]. Soils were amended individually at a ratio of 5% (w:w) with pathogenic fungal cultures and mixed thoroughly to ensure equal distribution of fungal inocula, then filled in plastic pots (25-cm-diameter) and irrigated every second day for 1 week before sowing. Seeds of potato cultivars Diamont, Picasso and Sponta were surface sterilized using 3% sodium hypochlorite for 5 min, then picked up and air-dried. Surface sterilized potato seeds were sown, three seeds per pot, five pots per replicates in each treatment. The average percentage of dry rot incidence at both pre-, and post –emergence growth stages was recorded up to 60 days of sowing (the experimental period).

In vitro Antagonistic studies

An isolate of *Trichoderma harzianum* kindly obtained from Plant Pathology Dept., National Research centre was used in this test. This isolate proved its highly antagonistic character against wide spectrum of various plant pathogenic soil borne fungi.

The *in vitro* antagonistic ability of *T. harzianum* was tested against *Fusarium sambucinum* the causal pathogens of dry rot disease of potato using dual culture technique (Ferreria *et al*., 1991). A 5-mm disk of *Trichoderma harzianum* growth culture was placed onto the PDA, 10mm from the edge of the Petri dish. Another disk of the same diameter of *Fusarium sambucinum* fungal growth culture was placed on the opposite side of the dish at the same distance. The control treatment was inoculated with a culture disk of either pathogenic or antagonistic culture alone at the same conditions. Both experimental and control dishes were assigned to a completely randomized design, with five replicates per treatment. All inoculated Petri dishes were incubated at 28±2^{o}C and the fungal growth diameter away from and towards the antagonist agent was measured after the pathogenic fungal growth in the control treatment had reached the edge of the Petri dish. The radial growth of the all treatments was measured after 4 and 8 days of incubation periods and the percentage of reduction in fungal growth was calculated.

This test was repeated three times and the inhibition was calculated as the percentage reduction in colony diameter growth compared with the control for each particular tested bio-agent.

Bio composts formulations

Compost materials

Three available agricultural residues, *i.e.* sugarcane bagasse (industrial wastes of sugarcane), rice straw and soybean straw were used as raw materials for compost formulation according to the rapid composting method described by Cuevas (1993); El-Mohamedy (2004) and El-Mohamedy *et al.* (2010).

Bio composts Preparation

For types of compost were prepared for soil amendment as follows:
Bio compost 1 (sugarcane bagasse + *T. harzianum*).
Bio compost 2 (rice straw + *T. harzianum*).
Bio compost 3 (sugarcane bagasse + rice straw + *T. harzianum*).
Bio compost 4 (sugarcane bagasse + rice straw + soybean straw + *T. harzianum*).

A mixture of each previous bio-compost was ground to small pieces, then, 8.0g ammonium sulphate, 20.0g super phosphate, 20.0g potassium sulphate were added to each 1 kg of ground mixture. The chemical fertilized mixture were moistened by adding 400 mL water per 1 kg and mixed thoroughly the packed into polyethylene bags. All bags were autoclaved for 1 hr at 121^{o}C. The autoclaved mixture was inoculated with spore suspension of *Trichoderma harzianum* 3x10^{6} spore/mL at the rate of 50 mL/bag. All bags were incubated for 14 days at room temperature (25-28^{o}C). Then composted agricultures wastes fortified with *T. harzianum* were used for direct introduction into the field soil. Bio composts soil treatments were applied at the rate of 750g/m^{2} during soil preparation 10 days before planting. The applied bio composts were incorporated in the top of 20 cm of the soil surface at planting row sites.

Field experiments

The efficiency of manipulation soil with different formulations of bio composts on the incidence of dry rot disease of potato caused by soil borne pathogen *Fusarium sambucinum* were studied.

Potato seeds cv. Diamond were planted in the Researches and experimental station (NRC) in Nubaryia region, Behera Governorate, Egypt during two successive cultivation seasons 2010 and 2011. Field experiment consisted of plots 21 m^2 (3x14m) each with three replicates for each particular treatment as well as control. Each plot comprised of 3 lines with 60 cm between holes. The experiment was designed in complete randomized complete block design. Whole potato tubers were sown (one tuber/hole) in winter growing season. The experiment was carried out using a five treatments as follow, T_1= Bio compost 1, T_2= Bio compost 2, T_3= Bio compost 3, T_4= Bio compost 4 , T_5 = control (without any soil treatment) . All plots received the traditional agricultural practices. Average percent of disease incidence of Fusarium dry rot was determined as percentage of infected tuber in a 200 tuber sample.

After harvest, total tuber yield per plot (g) and per faddan (ton) at different applied soil treatments comparing with untreated control was determined.

Statistical analysis

Tukey test for multiple comparison among means was utilized (Neler *et al.*, 1985).

Results and discussions

Isolation, identification and pathogenicity tests

The most dominant identified fungi which isolated from samples of both infected tubers and plants with dry rot disease were belonging to the genus Fusarium, as shown by microscopic examination. Fusarium isolates were identified as *Fusarium sambucinum*. In this respect many researchers recorded that dry rot disease caused by *Fusarium sambucinum* have limiting effects on potato cultivations (Peters *et al*., 2008; El-Kot, 2008).

Moreover, the ability of five isolates of *Fusarium sambucinum* to induce dry rot disease of potato was studied in pot experiment under greenhouse conditions. Presented results in Table (1) and Fig. (1) indicate that all tested isolates *Fusarium sambucinum* were pathogenic to all tested potato cultivars at different degrees of disease infection. *Fusarium sambucinum* isolate No. 3

proved to be highly pathogenic to all potato cultivars when compared with other tested isolates. Data also clearly show that Picasso cv. and Sponta cv. were the most susceptible, while Diamont cv. showed less susceptibility. This finding is in agreement with those of (Peters *et al*., 2008). They reported that all the commonly grown potato cultivars are susceptible to *Fusarium sambucinum*.

Unfortunately all the commonly potato cultivars are susceptible to *R. solani* and *F. sambucinum*, although some are less susceptible than others but several breeding lines have been reported to have a higher of resistance to dry rot).

Table 1. Pathogenicity test of *Fusarium sambucinum* on different potato cultivars in pot experiment

Fungal isolate	Disease incidence %					
	Pre-emergence growth stage			Post-emergence growth stage		
	Diamont cv.	Sponta cv.	Picasso cv.	Diamont cv.	Sponta cv.	Picasso cv.
F. sambucinum 1	4.8 b	8.0 c	8.8 c	8.0 b	11.0 c	13.0 d
F. sambucinum 2	3.0 d	4.4 d	5.4 d	4.8 d	8.0 d	9.4 e
F. sambucinum 3	5.2 b	11.0 b	12.8 b	7.8 b	15.2 b	17.0 b
F. sambucinum 4	4.2 c	7.4 c	9.0 c	6.4 c	11.4 c	15.2 c
F. sambucinum 5	3.0 d	5.8 d	8.2 c	5.0 d	9.0 d	13.0 d
Control	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a

Figures with the same letters are not significant ($P \leq 0.05$).

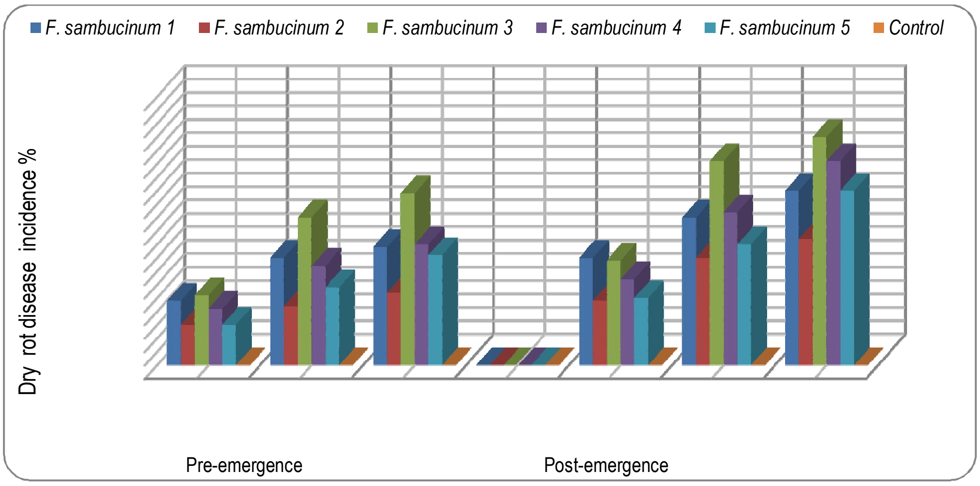

Fig. 1. Pathogenic ability of different *Fusarium sambucinum* isolates to cause dry rot disease on various potato cultivars at pre-, and post-emergence growth stages in pot experiment

***In vitro* Antagonistic studies**

The antagonistic ability of *T. harzianum* was tested against *F. sambucinum* isolates on PDA medium under *in vitro* conditions. Results in Table (2) and Fig. (2) reveal that *T. harzianum* proved its ability to antagonize all tested isolates of *F. sambucinum* at different degrees of growth inhibition. The highest reduction in the linear growth was found after 8 days of incubation with *F. sambucinum* No.1 (88%), where the least reduction was recorded with *F. sambucinum* No.3 (70%) *T. harzianum* inhibited the linear growth of all tested isolates of *F. sambucinum* by overcoming their growth in Peri-dishes.

Similar results were also reported by many investigators (Sadfi *et al*., 2002; Khalil and Al-Mughrabia, 2010). They reported that the inhibitory effect of antagonistic fungal and bacterial such as *Trichoderma* spp., *B. subtilis* and *P. fluorescen*s against growth reduction of *P. ultimum* and *R. solani* under *in vitro* conditions. The inhibition in the growth of the pathogen could be attributed to antibiosis, hyperparasitism or production of chitinase and *ß*-1,3 glucanase enzymes which degrade the cell wall leading to lyses of mycelium of the pathogen (Windham *et al*., 1986; Ahmed and Baker, 1987).

Table 2. Linear growth (mm) of *F. sambucinum* isolates against *T. harzianum in vitro*

Pathogenic fungal isolate	**Linear growth of *F. sambucinum* (mm)**	
	4 days	**8 days**
	Linear growth	Linear growth
Fusarium sambucinum 1	45.0 cd	10.8 e
Fusarium sambucinum 2	46.8 c	16.2 d
Fusarium sambucinum 3	52.2 b	27.0 b
Fusarium sambucinum 4	54.0 b	21.6 c
Fusarium sambucinum 5	48.6 c	14.4 d
Control	90 a	90 a

Figures with the same letters are not significant ($P \leq 0.05$).

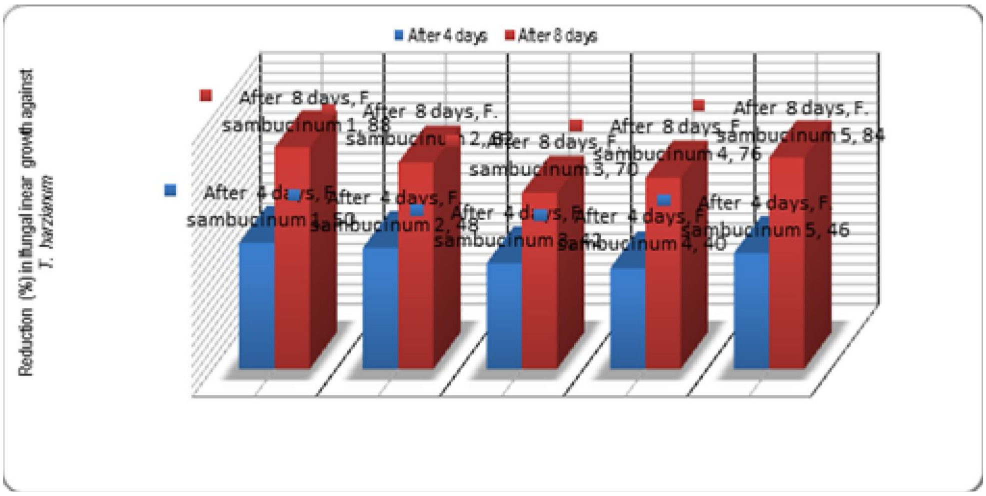

Fig. 2. Reduction (%) in the linear growth of different *F. sambucinum* isolates against *T. harzianum in vitro*

Field experiments

The field experiments were carried out to evaluate the effect of applied different bio composts as soil treatment on Fusarium dry rot incidence of potato under naturally infested soil at Researches and experimental station (NRC) in Nubaryia region, Behera Governorate, Egypt during two successive winter cultivation seasons 2010 and 2011.

Data in Table (3) and Fig (3) show that all applied soil treatments reduced the incidence and severity of Fusarium dry rot disease during the two cultivation seasons 2010 and 2011. The highest reduction in disease incidence and severity were observed with the treatment, bio compost 4 (T_4) followed by bio compost 1 (T_1). Meanwhile, soil treatments with bio compost 2 (T_2) and 3 (T_3) showed considerable effect in reducing disease comparing with control treatment. Drenching soil before tuber sowing with bio compost 4 (T_4) [sugarcane bagasse +rice straw+ soybean straw + *T. harzianum*] and/or bio compost 1(T_1) [sugarcane bagasse + *T. harzianum*] significantly reduced Fusarium dry rot incidence and diseases severity .Compost 1 and compost 4 reduced disease incidence and severity by 58.2, and 68.7% and 65.9, and 68.1% during the first cultivation seasons 2010 and by60.7, 71.4 % and 73.1, 78.0% during the second cultivation season 2011. Moreover, bio compost 2 (T_2) [rice straw + *T. harzianum*] and bio compost 3 (T_3) [sugarcane bagasse + rice straw + *T. harzianum]* reduced the disease incidence by 37.6, 43.7% and disease severity by 45.4, 56.8%, as well as by 50, 50% and 51.1, 60.9% in respective order during the first and second cultivation seasons 2010 and 2011.

In this regards, several researchers have been recorded that bio compost application as soil amendment could suppress diseases caused by *R. solani* and Fusarium spp. on many economic crops (El-Mohamedy, 2004; Godwin and Arinze, 2000; Liu and Huany, 2000). Using agricultural wastes, domestic food wastes or some grains as substrates for *T. harzianum* growth formulation and directly delivery in soil for controlling soil borne pathogens on some crops were also recorded (El-Mohamedy, 2004; El-Mohamedy *et al*., 2006 and 2010 ; Yogen *et al*., 2006; Khalil and El-Maghrabia 2010).

Such means comprise elimination of pathogens density in the soil and maintaining soil condition, favorable for root development and enhancement the competitive ability of bio agents against pathogens. Therefore, these methods introduced efficient disease control and incearsing yield of many crops (Huang, 1991; Ceuster et al., 1999 ; Davis *et al.,* 1996; Lazarovites, 2001; Yogen *et al*.,2006). El-Mohamedy *et al*., 2010 found that amended soil around stems of diseased mandarin trees by bio compost (BCAW) and Topsin-M (1 g/L) treatments as twice applications per season resulted in recovering great number of diseased trees and decreased the disease severity on others. Population density of *Fusarium* spp. were highly decreased, where population density of *Trichoderma* spp. were increased in rhizosphere soil of treated trees by bio compost (BCAW). Control of root rot pathogens through amended soil with organic materials formulated with bio-control agents may be attributed to : i) increasing the activity of indigenous microflora resulting in suppression of pathogens population through competition or specific inhibition, ii) releasing degradation compounds such carbon dioxides, ammonia, nitrites, saponine or enzymes which are generally toxic to the pathogens, iii) inducing plant defense mechanisms, iv) cellulose and glucanese are prevalent to high concentration in soil as a result of biodegradation of cellulose and lignin (Windham *et al*., 1986; Mitra and Nandi,1994; Liu and Huany, 2000).

Table 3. Influence of soil treatment with different bio compost formulations on the incidence of potato dry rot disease under field conditions

Soil treatment	**First season 2010**		**Second Season 2011**	
	Disease incidence (%)	Disease severity (%)	Disease incidence (%)	Disease severity (%)
Bio compost 1 (T_1)	7.0 c	3.0 c	5.5 c	2.2 c
Bio compost 2 (T_2)	10.0 b	4.8 b	7.0 b	4.0 b
Bio compost 3 (T_3)	9.0 b	3.8 c	7.0 b	3.2 b
Bio compost 4 (T_4)	5.0 d	2.8 d	4.0 c	1.8 c
Control	16.0 a	8.8 a	14.0 a	8.2 a

Figures with the same letters are not significant ($P \leq 0.05$).

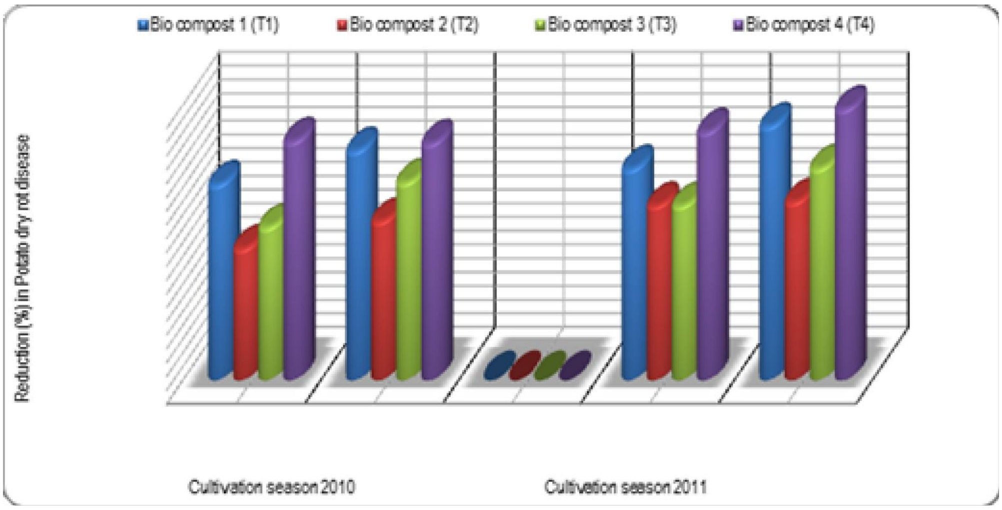

Fig. 3. Reduction in potato dry rot incidence and severity in response to soil treatment with different bio compost formulations under field conditions

Effect of soil amendment with different bio compost on potato tuber yield

Drenching soil with different bio compost before sowing potato seeds resulted in reducing dry rot disease under field conditions and improving the final tubers yield of potato. Results in Table 4 and Figure 4 showed that all soil treatments cause an increase in the average tuber yield/plot. The best soil treatment was drenching soil with bio compost 4 (T_4) followed by bio compost 1(T_1), as these treatments increased the obtained tuber yield/ feddan by 64.7 and 55.6 % in the first plantation 2010 and by 77.7 and 59.2% in the second plantation 2011.Meanwhile drenching soil with bio compost 2 or bio compost 3 caused increasing in total feddan yield by 37.3,44.31% and 40.7,48.1% in the first and second cultivation seasons 2010 and 2011 respectively.

Similar results have cleared that the suppression of black scurf, scab and Verticillium wilt of potato as a result of soil amendment with organic manure (Davis *et al*., 1996; Yogen *et al*., 2006). Soil amendment with agricultural wastes alone or in combination with bio-control agents was recommended for controlling soil borne pathogens and increasing the yield of many crops. Sugar can bagasse degraded by *Trichoderma* spp. was used as soil amendment to improve growth and yield of rice and pea (Mitra and Nandi, 1994). In newly cultivated soil organic material is frequently recommended to prevent the increase of pathogens and this was attributed to unfavorable conditions that produce by organic and bio compost soil amendments as well, such soil treatments enhance toxicity and antagonistic ability of bio control agents against soil borne plant pathogens. These probably contributed to the higher nutrient contents, which could be found with organic amendments (Tsao and Oster 1981; Ceuster *et al*., 1999; Yogen *et al*., 2006).

Table 4. Influence of soil treatment with different bio compost formulations on tuber yield of potato plants under field conditions

Treatment	First cultivation season 2010	Second cultivation season 2011
	Tuber yield /plot (Kg)	Tuber yield /plot (Kg)
Bio compost 1 (T_1)	44..2 b	43.0 b
Bio compost 2 (T_2)	39.0 c	38.0 c
Bio compost 3 (T_3)	41.0 c	40.0 c
Bio compost 4 (T_4)	46.8 b	48.0 b
Control	28.4 a	27.0 a

Figures with the same letters are not significant ($P \leq 0.05$).

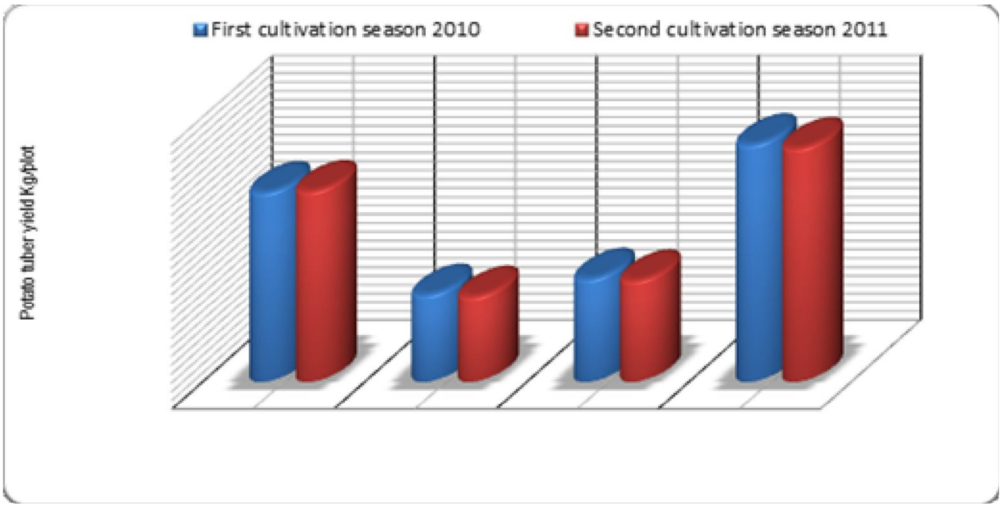

Fig. 4. Increase in potato tuber yield in response to soil treatment with different bio compost formulations under field conditions

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