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BIOCONTROL OF *RALSTONIA SOLANACEARUM* AND ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA* ON POTATO

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ABSTRACT: *The present study was search about the relationship between root-knot nematode and Ralstonia solanacearum on potato (Lady rosseta c.v) and the biological control of them by two plant growth promoting rhizobacteria i.e (Serratia marcescens and Pseudomonas fluorescens), blue green algae (Nostoc muscurum) and compost in a split root system. Results revealed that potato cv. lady rosseta was highly susceptible to bacterial wilt disease and the combinations of M. incognita and R. solanacearum recorded higher bacterial wilt disease rating than those inoculated with both pathogens individually. All bio-control agents significantly increased Percentage of disease reduction with R.solanacearum and M.incognita (alone or combined) specially blue green algae (Nostoc muscurum) and compost by (96-84%), (86-80 %) respectively, reduced significantly all nematode parameters i.e. number of galls /half root system, number of egg masses /half root system and number of juveniles/250g soil when compared to plants treated with nematode alone. The highest reduction of disease and nematode parameters in the first and second experiments were recorded with the blue green algae followed by compost , S.marcescens and Pseudomonas fluorescens, respectively, Results of both experiments revealed that all treatments enhanced the plant growth parameters i.e. stem length, number of main stems, number of branches/plants , number of leaves/plant as well as the chemical constituents i.e. total nitrogen content in leaves.*

Key words: *Biological control, Root- knot nematodes, Soil amendments, Plant growth promoting rhizobacteria , Potato (Solanum tuberosum) .*

INTRODUCTION

Bacterial wilt disease caused by *Ralstonia solanacearum* is one of the most serious soil-borne disease in tropical environments (Burgess *et al.*, 2008). The pathogen has a wide host range representing 44 families (He *et al.*, 1983). Highly susceptible crops are potato, tomato, egg plant, chili, bell pepper and peanut. Bacterial wilt disease has limited both commercial and domestic level production (Somodi *et al.*, 1993). The damage leads to large losses of yield and income, and disease control is difficult (Hartman, 1993; Doan and Nguyen ,2005). Root-knot disease is also soil- borne disease that caused by root-knot nematodes *Meloidogyne* spp. that occur worldwide and affect more than 2000 plant species (Koening *et al.*,1999). In the soil ecosystem *R. solanacearum* coexists with a large number of microorganisms, some favour the pathogen for their own interest, some inhibit them during the competition for space, nutrients and air. The involvement of nematodes in bacterial invasion is usually thought to be caused by wounds on the roots (Hayward, 1991). The concomitant infection by plant parasitic nematodes particularly sedentary endo-parasitic root-knot nematode and *R. solanacearum.* was long been reported to increase the severity of bacterial wilt Napiere and (Quimio ,1980) , (Cadet *et al.*, 1989), (Deberdt, 1999) ,(Hussain and Bora, 2009) , (Singh and Siddiqui , 2012) , (Siddiqui *et al.*, 2013). The combined pathogenic effects of *R. solanacearum* and *Meloidogyne* spp. were greater than the independent effects of each one (Sitaramaiah and Sinha, 1984) , (Ateka *et al.*, 2001) , (Hussain and Bora, 2009). (Chen, 1984) reported changes the physiology of the plants due to nematode infestation predisposed tobacco plants to bacterial wilt. It was also suggested that root knot nematodes infestation greatly reduce the genetic resistance to bacterial wilt (Deberdt ,1999).

The aims of this work is to study : 1) the damage of potato plants (leady rosseta c.v) that caused by *R. solanacearum* and *M.incognita* individually or combined , 2) the relationship between *R. solanacearum* and *M.incognita* in attacking potato plants and 3) evaluations some bio- control agents *Serratia marcescens, Pseudomonas fluorescens* , blue green algae and compost in controlling this damage.

MATERIALS AND METHODS

One potato cultivar, (*Solanum tuberosum* sub sp. *tuberosum* L) lady rosseta was left in the refrigerator (4 °C) for sprouting. Sprouts were removed from the mother tubers and planted in sterilized clay pots (20 cm in diam.), containing 8 kg./ pot an autoclaved mixture soil of 1:2 (v:v) clay : sand. Each pot was planted with one potato sprout and each treatment was replicated six times .Pots without any treatments served as a control . Pots were watered daily or as needed. Experiments were carried out in successes two seasons 2011 and 2012 in split –root system.

Isolation of *R. solanacearum*

Ralstonia solanacearum was isolated from naturally infected potato plants showing wilt symptoms, collected from different locations of Menoufia governorate. Infected potato tubers were cut into small pieces and placed in test tubes containing 5 ml of sterile distilled water for standard isolation (Hildebrand *et al.,* 1988). Bacteria were allowed to flow for 5 to 10 minutes. One loopful of the bacterial suspension was streaked onto Kelman's tetrazolium medium (Kelman ,1954) and incubated at 28°C for 48 hrs. The recovered colonies were harvested and suspended in sterilized distilled water. Inoculum potential was spectrophotometrically adjusted to OD 600 nm = 0.1 (approximately 10^8 CFU/ml) (Grimault *et.al.* 1994). Inoculation was carried out by pouring 5 ml of bacterial suspension around the base of each plant.

Extraction of *M. incognita*

Meloidogyne incognita isolated from potato growing fields in Menoufia governorate. Potato galled roots and tubers were collected from the culture of plants and washed gently with water to remove soil particles. The roots and tubers were cut into 1–2 cm segments and placed in a conical flask containing 200 ml of 0.5% NaOCl solution. The flask was shaken vigorously for 3 minutes. The suspension was passed through 60 and 325 mesh sieves nested over the 500 mm mesh sieve which collected the nematode eggs. The eggs in the sieve were placed quickly under stream of cold water to remove the residual NaOCl. The eggs were then transferred into a beaker with the aid of a wash bottle. The number of eggs per ml was standardized and the desired number of eggs (approximately 2500 eggs/ pot) were placed in separate vials. The egg suspension was introduced by pouring into the sterilized potted soil. *M. incognita* was identified to the species using the morphological characteristics of the perineal pattern of the adult female (Chitwood 1949 and Taylor *et al.* 1955).

Isolation of bio-control agents

-Serratia marcescens: The rizobacterum *Serratia marcescens* was isolated from compost consist of animal wastes and rice straw (1:1) on nutrient agar medium (Difico, 1984) and was identified according to Bergey's Manual . Inoculation was carried out by pouring 5 ml of bacterial suspension (10^{-6} cfu /ml) around the base of each plant.

-Pseudomonas fluorescens : Soil samples were collected from the non-rhizophere region of 5-6 cm away from root base of potato plant. One gram of the soil sample was taken and it was dissolved in 9ml of sterile distilled water to make a dilution of 10^{-1}.One ml of 10^{-1}dilution was pipetted out using a sterile pipette and transferred to another 9ml sterile distilled water in test tubes. It gave a dilution of 10^{-2}.Similarly, serial dilution was continued up to 10^{-6}. One ml of 10^{-6}diluted suspension was transferred to Petri dishes containing King's B medium (King *et al.*, 1954) and incubated at 28 ± 2°C for 5 days

and then colonies of *P. fluorescens* were counted. Inoculation was carried out by pouring 5 ml of bacterial suspension (10^8 cfu /ml) around the base of each plant.

-Blue green algae (Cyanobacteria): *Nostoc muscurum* was obtained from Soil Microbiology Department, Sakha Agricultural Reasearch Station. The identified cyanobacteria inoculated on BG_{11} (Rippka *et al.*, 1979) nutrient agar slants and left in a diffused light at room temperature (28±2°C) to grow for 12 days thereafter, they were kept in a refrigerator at 4°C. Inoculum potential was 10^6 CFU/ml. Inoculation was carried out by pouring 200 ml of bacterial suspension around the base of each plant.

- **Compost :** Animal compost was obtained from Soil Microbiology Department ,Sakha Agricultural Reasearch Station. Compost was applied in pots and incorporated to a depth of 5 cm before sowing at the rate of (200 gm/pot).

Experiment I : The first experiment was carried out with four treatments as follows :

1. Pots inoculated with *M. incognita* only.
2. Pots inoculated with *R. solanacearum only.*
3. Pots inoculated with *M. incognita* + *R. solanacearum* .

4- untreated pots (control).

Wilt disease rating Bacterial

Wilt disease rating on plants was recorded up to 30 days after the inoculation. The wilted plants were tested for bacterial oozes as well as isolation of *R. solanacearum* on a semi selective medium Kelman's tetrazolium medium (Kelman,1954) . Scale of (Kempe and Sequeira 1983) was used as follow:
0 = no symptoms, 1 = up to 25 % of foliage wilted, 2 = 25-50 % of foliage wilted, 3 = 50-75% of the foliage wilted, and 4 = 75-100% of foliage wilted.

Nematode disease rating(Galling index)

Galling index was determined according to follow rating scale: 1= no galling; 2= trace (1–25% galling); 3= slight (26–50% galling); 4= moderate (51–75% galling) and 5= severe (76–100% galling). Population density of nematodes in soil (number of juveniles) (Franklin & Goodey, 1957.) . Number of galls and egg masses were determined in one gram root sample stained with acid fuchsin lactophenol (Byrd *et al.*, 1983) .Egg masses were determined by staining the infected roots with phloxin B solution for 20 minutes as described by (Daykin and Hussey 1985). Counting was done with the aid of a dissecting microscope and a hand tally counter.

Experiment II : The second experiment was carried out as follows:

1- control
2- *R. solanacearum* only
3- *M. incognita* only
4- *R. solanacearum* + *M. incognita*
5- *S. marcescens* + *M. incognita*
6- *S. marcescens* + *R. solanacearum*
7- *S. marcescens* + *R. solanacearum* + *M. incognita*
8- *P.fluorescence*+*M. incognita*
9- *P.fluorescence*+*R. solanacearum*
10- *P.fluorescence*+ *R. solanacearum* + *M. incognita*
11- Blue green algae.+*M.incognita*
12- Blue green algae.+*R. solanacearum*
13- Blue green algae + *R. solanacearum* + *M. incognita*
14- Compost+ *M. incognita*
15- Compost+*R. solanacearum*
16- Compost+ *R. solanacearum* + *M. incognita*

M. incognita (2500 nematode eggs) and *R. solanacearum* (5 ml of bacterial suspension) were applied one week before of sowing. Treatments were arranged in a completely randomized design with six replicates on a bench under greenhouse condition (28 ±2 °C), and watered as needed. Bio-control agents were applied 7 days after sowing . Experiment was ended and data recorded 85 days after *R. solanacearum* and *M. incognita* inoculation.

The recorded data were :

Disease parameters:

- Percentage of disease reduction of treated potato with *R.solanacearum* and *M.incognita*
- Percentage of reduction in number of galls /half root
- Percentage of reduction in egg masses /half root system
- Percentage of juveniles/250 g soil

Vegetation plant growth parameters

- Stem length of potato crop
- Number of main stems
- Number of branches/plants
- Number of leaves/plant
- Dry weight of leaves /plant
- Numbers of tubers /plant

Chemical constituents parameters

- Total nitrogen content in leaves /plant
- Total phosphorus content in leaves /plant
- Total potassium content in leaves /plant
- Starch content in tubers /plant

Statistical Analysis:

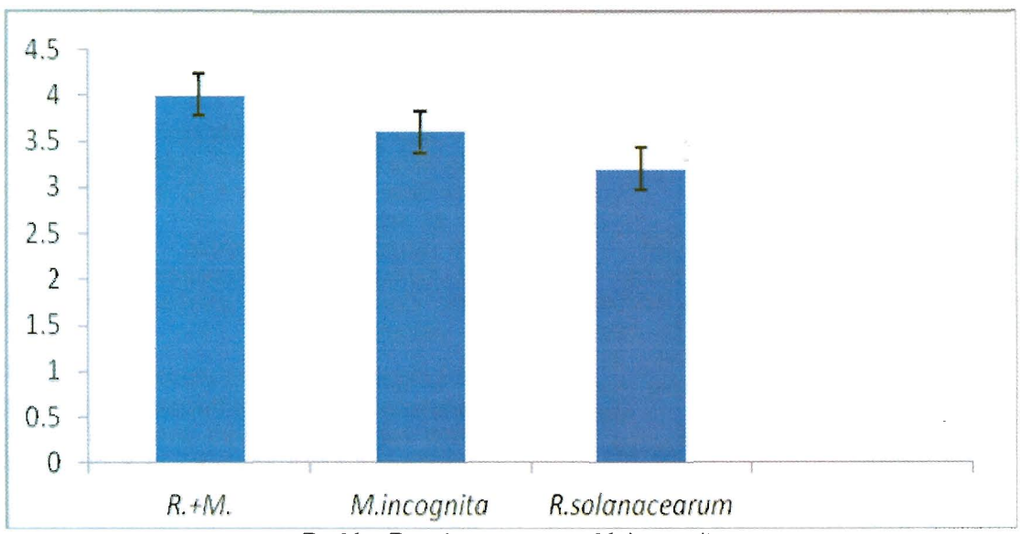
Data were then subjected to analysis of variance (ANOVA) (Gomez and Gomez, 1984) .

Results

Experiment I:

wilt disease rating Bacterial

Results cleared that potato cv. lady rosseta was highly susceptible to bacterial wilt disease compared to untreated control, according to the scale of Kempe and Sequeira (1983) . The rate of bacterial wilt disease recorded 3.2 (50-75%) in case of inoculation with *R. solanacearum* individually and 3.6 (50-75%) in case of inoculation with *M. incognita*. Combinations of both *M. incognita* and *R. solanacearum* showed higher bacterial wilt disease rating 4(75- 100%) than those inoculated with each pathogens separately (Fig 1).



R.+M.= R. solanacearum +M. incognita

Fig (1). Bacterial wilt disease rating on potato plants infected with *R. solanacearum* and *M. incognita* separately and their combinations under greenhouse conditions

Nematode disease rating

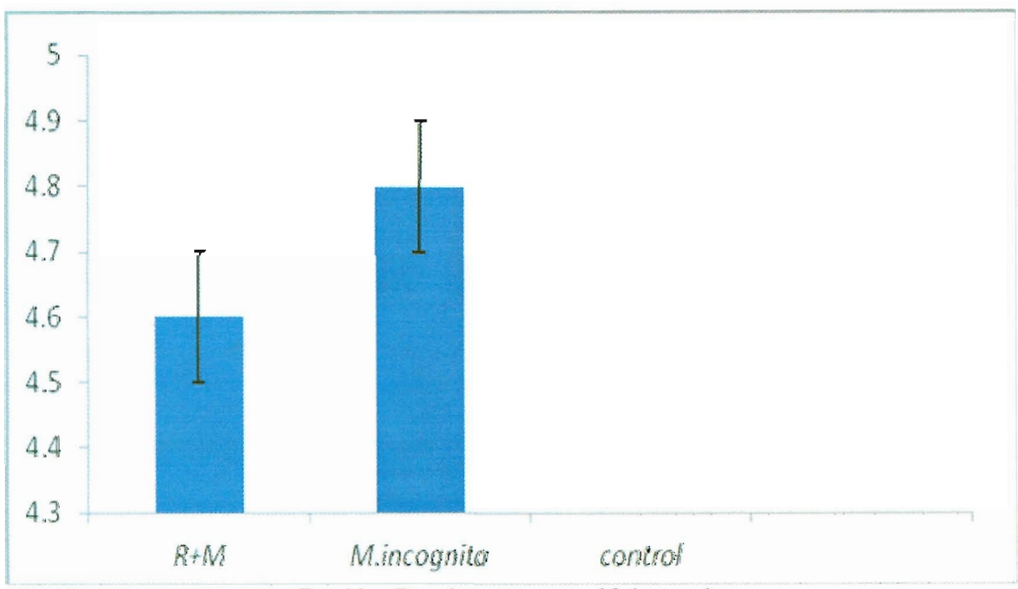
The nematode galling index (Fig 2), recorded at the end of the experiment (85 days of nematode inoculation). All infected plants with *M. incognita* showed gall development on potato roots. The Gall index of the whole root system showed more severe galling with scale number 4.8 by 76 – 100 % , followed by inoculation of both pathogens combined as recorded 4.6 by 76 – 100 % severe galling .

Experiment II :

Results of experiments indicated that, all treatments with the different bio- control agents significantly increased the percentage of disease reduction of potato treated with *R. solanacearum* and *M. incognita* alone or combined . The reduction percentage of disease ranged between 60 and 96% compared to potato treated with *R. solanacearum* and *M. incognita* individual. The highest reduction percentage of nematode disease recorded 96% and 86% with the treatments of both blue green algae , or compost respectively. The same trend of results was obtained with the bacterium disease reduction percentage by 86 and 89% with blue green algae and / or compost respectively as shown in (Fig 3) .

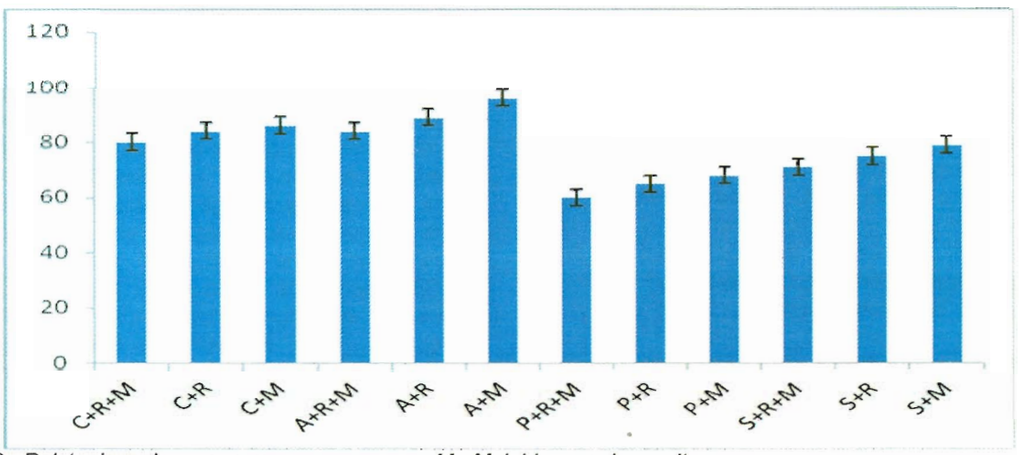
Results also revealed that all bio- control agents significantly reduced all nematode parameters i.e. number of galls, egg masses /half root system and juveniles/250 g soil . The highest percentage reduction recorded with blue green algae and compost treatments . The number of galls reduced by88% and 82% respectively (Fig. 4). Egg masses reduction recorded 91 and 84% respectively (Fig 5) , whereas the reduction percentage of juveniles recorded 82 and 80% respectively as shown in (Fig. 6).

Results showed also that all treatments with bio- control agents markedly encouraged mean of plant growth characters i.e. stem length of potato crop (Fig. 7) , number of main stems (Fig. 8) , number of branches/plants (Fig 9) , number of leaves/plant (Fig. 10) , numbers of tubers /plant (Fig. 11) and dry weight of leaves /plant (Fig. 12) . The highest results were obtained from combination of both blue green algae and compost with *M. incognita* or *R .solanacearum* compared to the plants treated with the other bio- control agents.



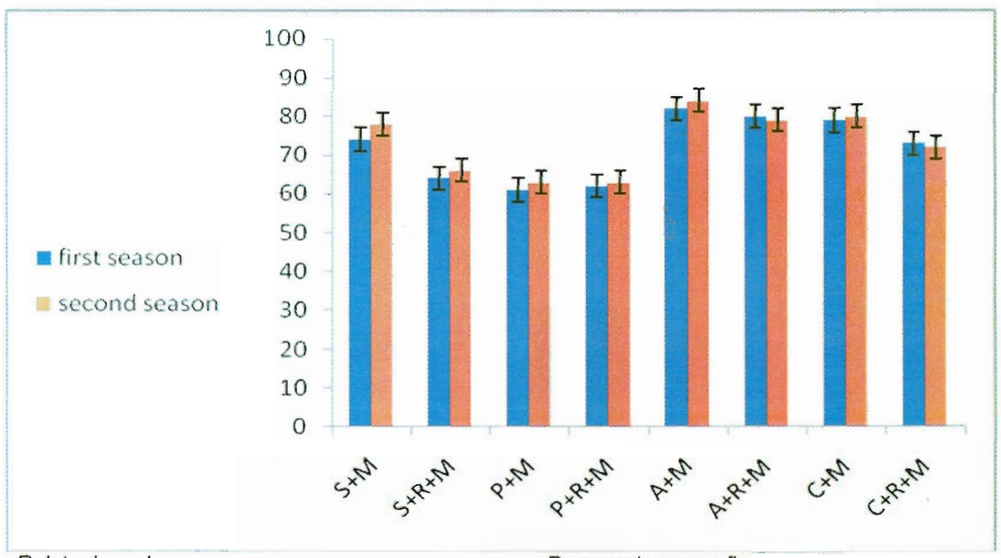
R + M = R. solanacearum+ M. incognita

Fig (2). Root galling index of infected potato with *M. incognita* alone or combined with *R. solanacearum* under greenhouse conditions.



R =Ralstonia solanacearum *M =Meloidogyne incognita*
S =Serratia marcescens *P =Pseudomonas fluorescence*
A= blue green algae *C =compost*

Fig (3). Percentage of disease reduction of potato plants treated with *M. incognita* and / or *R. solanacearum* as affected by bio-control agents inoculation under greenhouse conditions.



R =Ralstonia solanacearum *P =pseudomonas fluorescence*
M =Meloidogyne incognita *A= blue green algae*
S =Serratia marcescens *C =compost*

Fig (4). Effect of bio-control agents on reduction percentage of galls /half root system at two successive seasons (2011-2012).

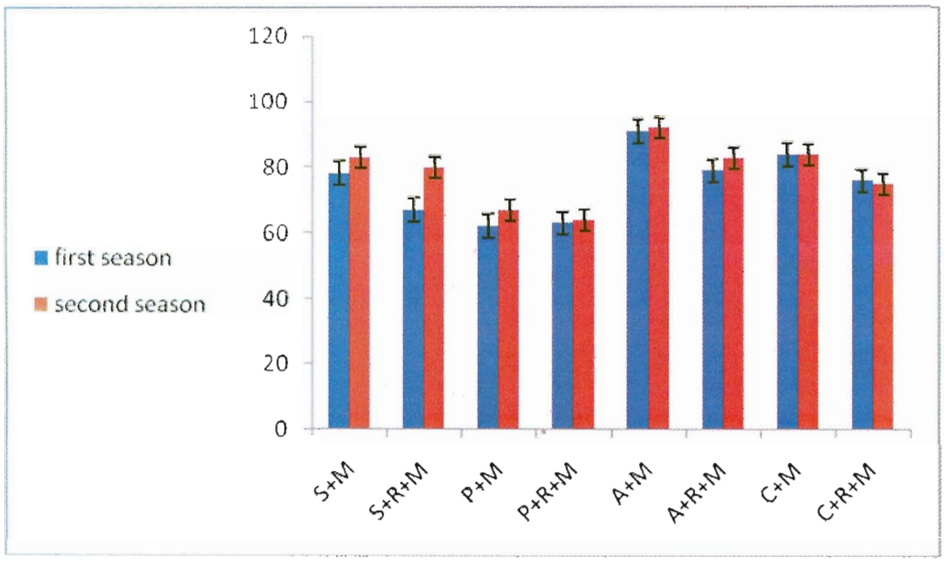


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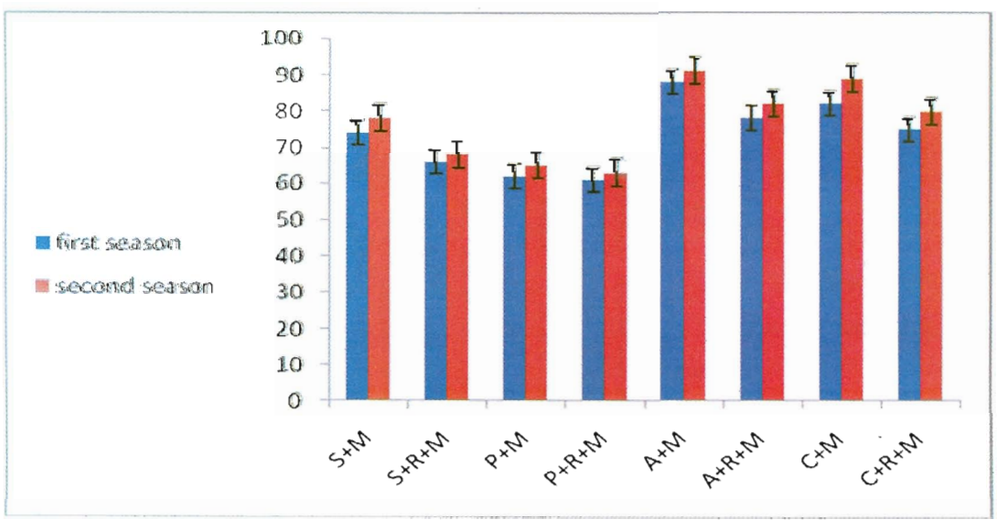
Fig (5). Effect of bio-control agents on reduction percentage of egg masses /half root system at two successive seasons (2011-2012) .



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P =Pseudomonas fluorescence
A= blue green algae
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Fig (6). Effect of bio-control agents on reduction percentage of juveniles/250 g soil at two successive seasons (2011-2012) .

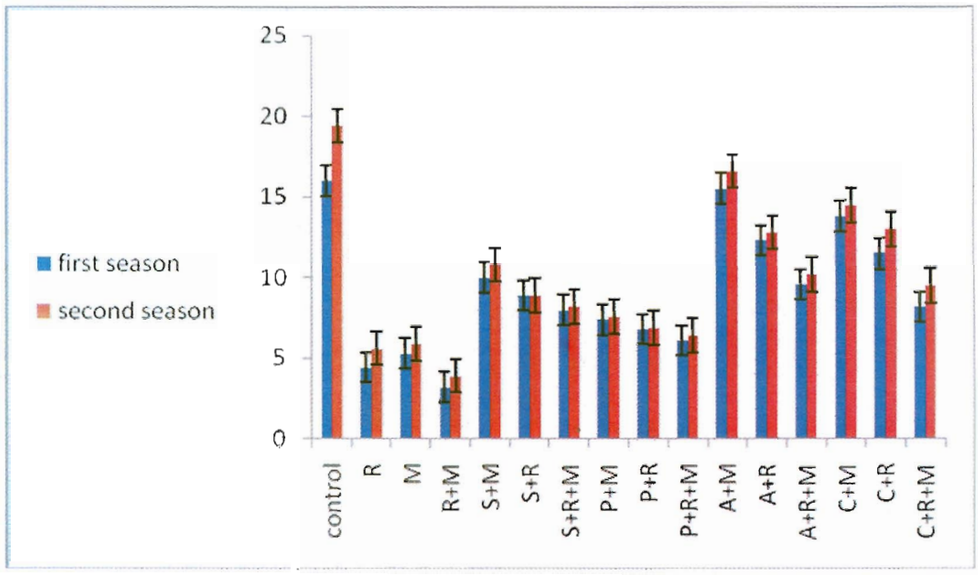

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Fig. (7). Effect of bio-control agents on mean stem length of potato crop at two successive seasons (2011-2012) .

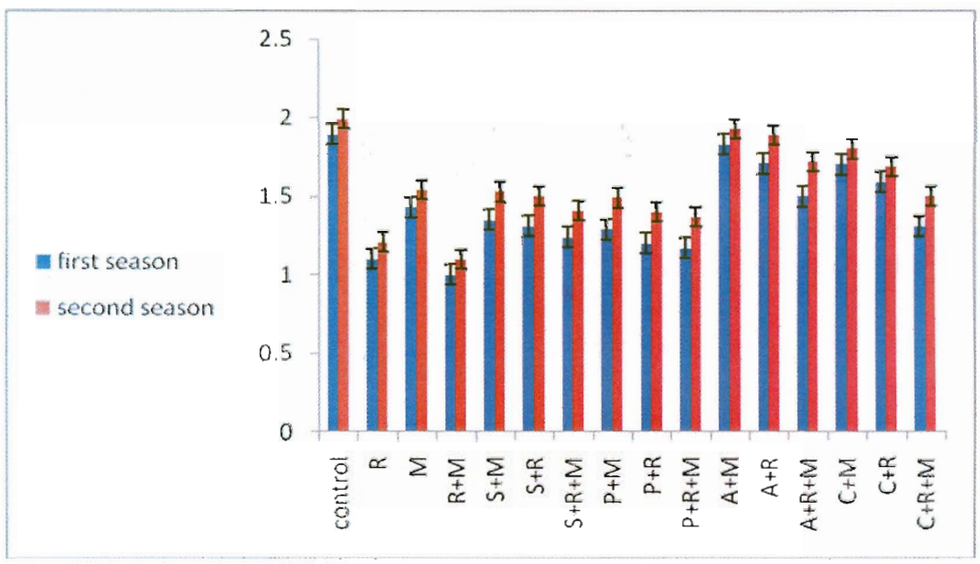

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Fig (8). Effect of bio-control agents on mean number of main stems at two successive seasons (2011-2012) .

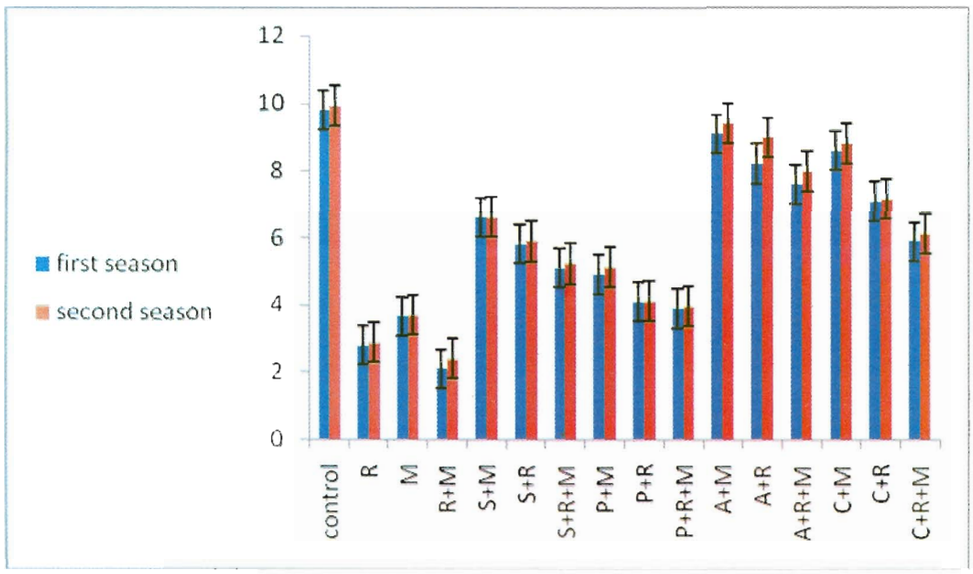

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Fig. (9). Effect of bio-control agents on mean number of branches/plants at two successive seasons (2011-2012) .

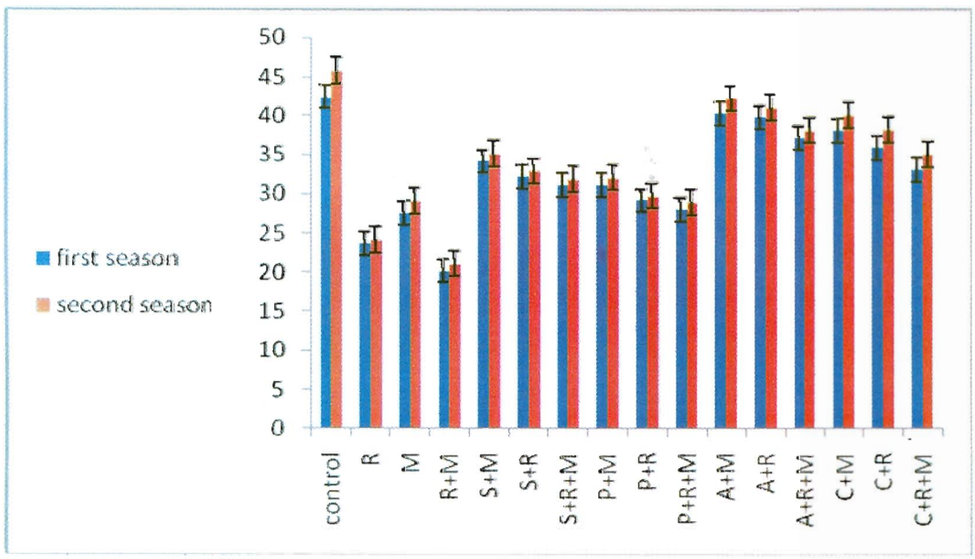

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Fig. (10). Effect of bio-control agents on mean number of leaves/plant at seasons(2011-2012)

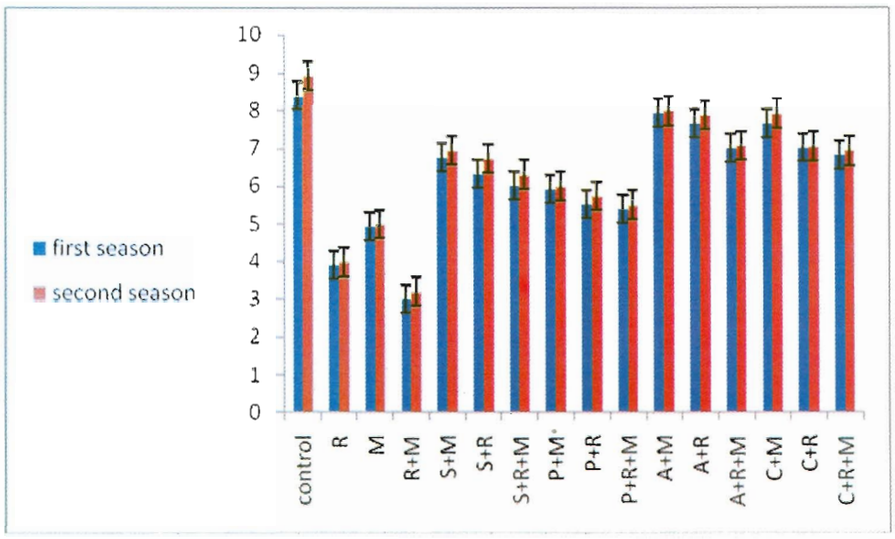

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Fig (11) . Effect of bio-control agents on mean numbers of tubers /plant at seasons(2011-2012) .

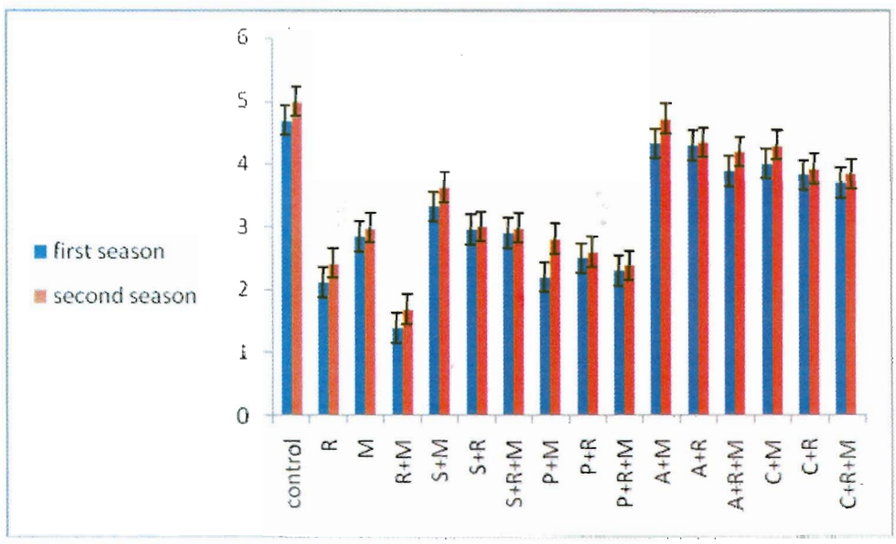

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Fig (12). Effect of bio-control agents on mean of dry weight of leaves (g) /plant at seasons (2011 -2012) .

The same trend results were recorded for chemical characters i.e. total nitrogen content (Fig. 13) , total phosphorus (Fig. 14), total potassium content in leaves /plant (Fig. 15) and starch content in tubers /plant (Fig.16). The lowest results were recorded for combination of *P .fluorescence* or *S. marcescens* with *M. incognita* and *R . solanacearum* respectively compared to the other bio- control agents.

Discussion

In the present investigation, results showed that potato plants inoculated with *M. incognita* or *R. solanacearum* separately showed increased of wilt disease rating ,but the interaction between them showed more increase of wilt disease rating . This result was in agreement with Bekhiet *et al.*,2010 who found that the interaction between root-knot nematode, *Meloidogyne incognita* and the bacterium, *Ralstonia solanacearum* showed higher bacterial wilt disease rating than those inoculated with each pathogens simultaneously.

The present results indicate that all used bio-control agents treatments decreased wilt disease rating of *M. incognita* or *R. solanacearum* and all nematode parameters i.e. number of galls /half root, egg masses /half root system and juveniles/250 g soil and markedly increased plant growth.

The most effective treatments were blue green algae and compost . Algae are one of the chief biological agents that have been studied for the control of plant pathogens (Hewedy *et al.*, 2000). Cyanobacteria were found to be a rich source for various products of commercial, pharmaceutical or toxicological interest: primary metabolites, such as proteins, fatty acids, vitamins or pigments (Borowitzka, 1995), (Mohamed *et al* .,2011),(Piccardi *et al.*,2000) and(El-Sheekh *et al.*2006)

Various strains of cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activities such as antialgal, antibacterial, antifungal and antiviral activity (Noaman *et al.*, 2004). They have received little attention as potential bio-control agents of plant diseases. Kulik (1995) stated that for a number of reasons, cyanobacteria and algae are suitable candidates for exploitation as bio-control agents of plant pathogenic bacteria and fungi: Cyanobacteria and algae produce a large number of antibacterial and antifungal products.

Application of organic matter (compost) to the soil has beneficial effects on soil nutrients, soil physical properties, soil biological activity and crop performance. The nutrient content of the amendments and the large quantities of these materials added to the soil result in increased soil fertility, plant growth and tolerate nematode attack (Rodríguez- Kábana *et al.*, 1987; Ravindra *et al.*, 2014). The enhancement of plant growth by organic amendments in the present study could be due to the combination of the suppressive effect of nematodes with a direct fertilizing effect on the plants.

Compost has beneficial effects in plant disease management. So, it can be included in the integrated disease management of field and horticultural crops (Ravindra *et al.*, 2014). Addition of this organic extracts to growing media encouraged the growth of soil organisms which suppress the plant diseases (Praveena *et al.*, 2013) . Compost show multiple modes of activity in suppressing plant diseases, like induced resistance, antibiosis and competition. Recent results clear that compost reduced percentage of wilt disease caused by *M. incognita* or *R. solanacearum* and this result was in agreement with (Abbasi *et al.*, 2002) , (Tanu 2005) and (Praveena *et al.*, 2013) .

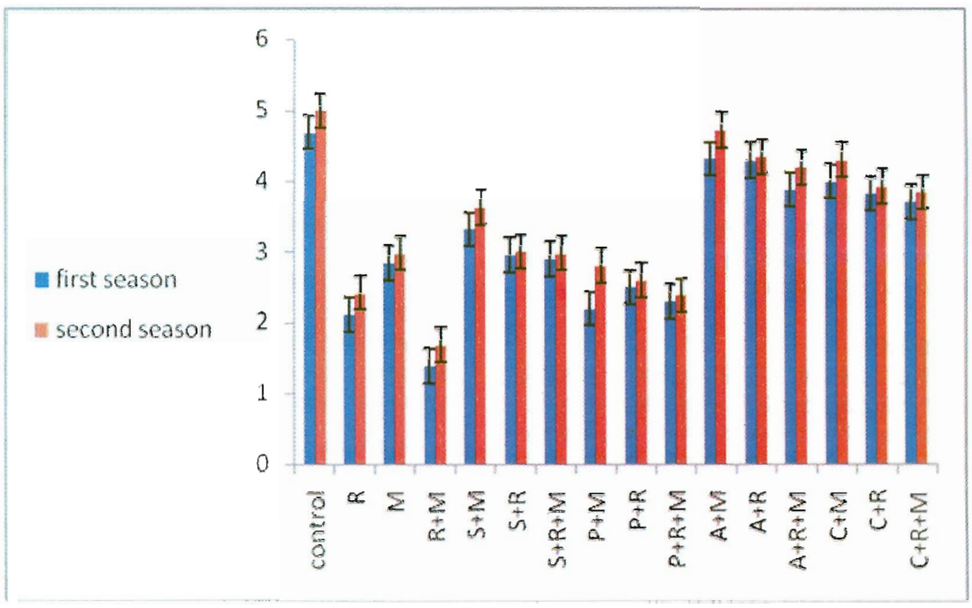

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Fig. (13). Effect of bio-control agents on total nitrogen content in leaves /plant at two successive seasons (2011-2012) .

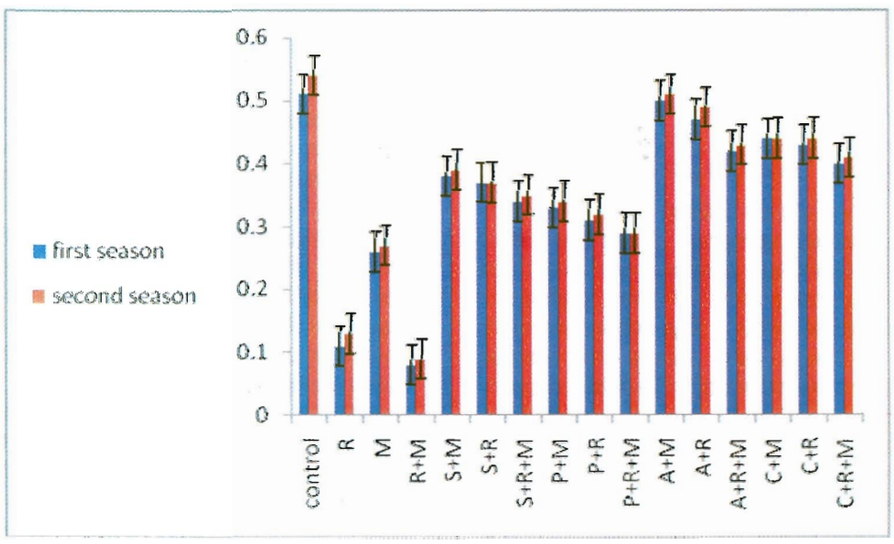

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Fig. (14). Effect of bio-control agents on total phosphorus content in leaves /plant at two successive seasons (2011-2012) .

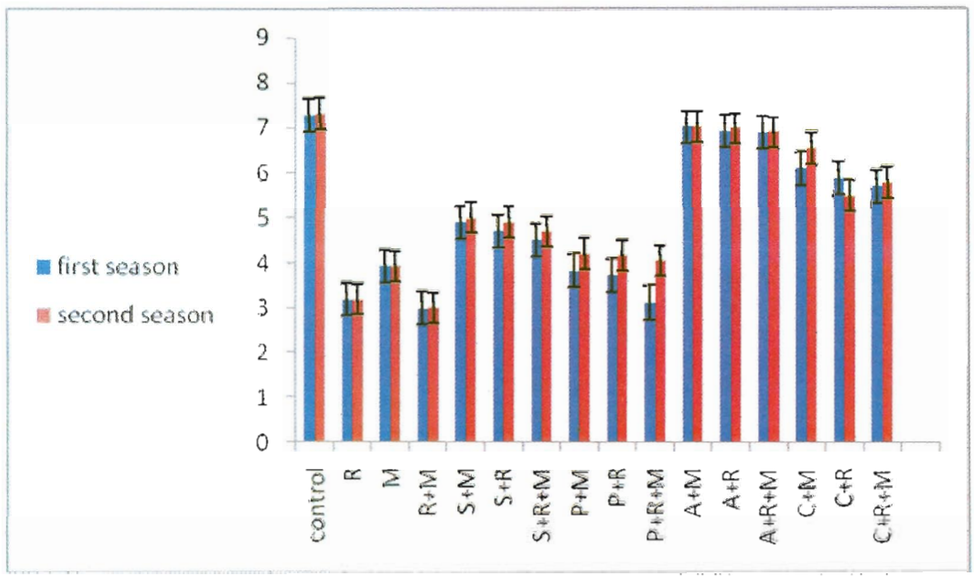

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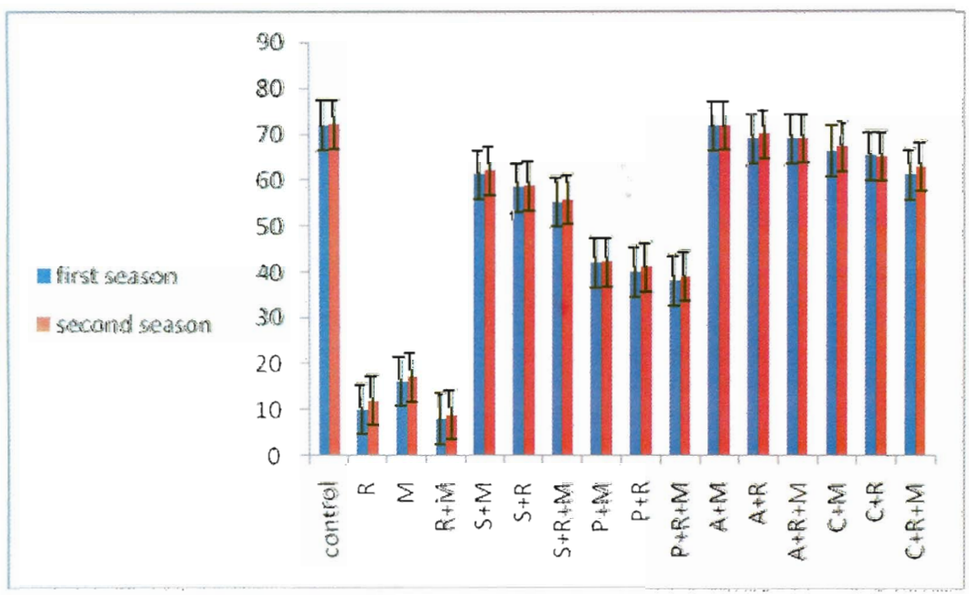
Fig. (15). Effect of bio-control agents on total potassium content in leaves /plant at two successive seasons (2011-2012).



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C =compost

Fig. (16). Effect of bio-control agents on starch content in tubers /plant at two successive seasons (2011-2012).

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المكافحه الحيويه لبكتيريا رالستونيا سولاناسيرم ونيماتودا تعقد الجذور علي نبات البطاطس

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الملخص العربي

في هذا البحث تم دراسة العلاقه بين نيماتودا تعقد الجذور *M. incognita* وبكتريا رالستونيا سولاناسيرم *R solanacearum*. علي حدوث مرض الذبول البكتيري في البطاطس صنف ليدي روزيتا وكذلك المقاومة الحيوية لكل منهما سواء كانت منفرده او مجمعة معا وذلك باستخدام كل من بكتريا *Serratia marcescens,* (*Pseudomonas flurescens*) والطحالب الخضراء المزرقة (*Nostoc muscurum*) والكمبوست عامي ٢٠١١ ، ٢٠١٢.

وقد أظهرت نتائج التجارب حساسية الصنف ليدي روزيتا للاصابة بالمرض بالمقارنة بالكنترول. كما أوضحت الدراسة أنه في حالة التلقيح بالنيماتودا و البكتريا معا تزداد شدة مرض الذبول البكتيري عنها في حالة إضافة النيماتودا أو البكتيريا منفردة.

ومن خلال النتائج تبين ان كل المعاملات المستخدمه في المكافحه الحيويه وبخاصة الطحالب الخضراء المزرقه والكمبوست قد ادت الي انخفاض معنوي في النسبه المئويه لتقليل المرض بنسبة (٨٤-٩٦ %) في حالة الطحالب وبنسبة (٨٠- ٨٦ %) في حالة الكمبوست .

وكذلك انخفاض معنوي في كل الصفات الخاصه بالنيماتودا مثل أعداد العقد النيماتوديه (بنسبة ٨٢ ، ٨٨% علي التوالي) وعدد البيض لكل كيس (بنسبة ٨٤-٩١%) وعدد اليرقات لكل ٢٥٠ جرام تربه (بنسبة ٨٠-٨٢ %) وذلك اذا قورنت بالنباتات المعامله بالنيماتودا فقط .

كما وجد أيضا ان كل المعاملات قد شجعت من الصفات الخضريه لنبات البطاطس مثل عدد السوق الرئيسية للنبات ، عدد الافرع لكل نبات والمكونات الكيميائيه مثل المحتوي النيتروجيني والفسفوري في الاوراق لكل نبات. وقد تم الحصول علي هذه النتائج في موسمي الدراسه ٢٠١١ ، ٢٠١٢ .