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Study of Some Physiological Changes in Sugar Beet cv. 7233 in the Presence of Sugar Beet Cyst Nematode, *Heterodera schachtii* and an Antagonistic Sterile Fungus StFCh1-1 in the Rhizosphere Condition

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Abstract: In this study, several physiological parameters of inoculated sugar beet plants, with the beet cyst nematode, *Heterodera schachtii*, were evaluated in the presence of an antagonistic sterile fungus StFCh1-1 in the rhizosphere condition. The sugar beet plant used in this bioassay was a multigerm cultivar, 7233, which is sensitive to the beet cyst nematode and has been adapted to cultivate in temperate and cool regions of the sugar beet production areas of Iran. In this regard a potent bioassay was conducted in the sterile glass tubes (20×3 cm Ø) containing 30 g autoclaved soil and planted with a sterile germinated seed of sugar beet, totally in 24 tubes. The seedling of sugar beet plant at four leaves stages in two treatments (nematode and nematode + fungus) was inoculated with 50 disinfected beet cyst nematodes. As a standard procedure the bioassay composed of four treatments including: fungus, nematode, nematode + fungus and untreated control. Two months after nematodes' inoculation some physiological parameters of plants were measured including: total chlorophyll, potassium and biomass. The two treated plants with nematode and nematode plus fungus showed significant decrease in biomass and chlorophyll contents but treatment with fungus alone showed no significant differences in the biomass and chlorophyll content of plants in comparison with the control. The potassium content of shoots in the invaded sugar beet plants was lowest, but it was highest in the roots. These changes might be indication of adaptive osmoregulation or acclimation responses in plants due to the nematodes as bio-stressors through the increase of metabolites and solutes. Also, these results confirmed that in plants inoculated with nematode plus fungus, a few number of female of nematodes were developed due to the antagonistic effects of sterile fungus StFCh1-1. Meanwhile, the fungus didn't have any detrimental effect on biomass, chlorophyll content and potassium in leaves of sugar beet cv. 2733 and it is safe to use as a biocontrol agent against *Heterodera schachtii*.

Key words: Antagonistic sterile fungus StFCh1-1, *Heterodera schachtii,* total chlorophyll, biomass, potassium

INTRODUCTION

Beet cyst nematode, *Heterodera schachtii* Schmidt 1871, has been recognized as an important pest of sugar beet and some other crops throughout the temperate zones of the world for over 100 years. It causes serious stand and yields reductions wherever sugar beets is grown. Today, *H. schachtii* is present in 39 sugar beet-growing countries throughout the world including Iran. It is the most serious nematode pest of sugar beet and capable of causing severe losses (Cooke, 1984, 1987, 1992a, b). In Iran the beet cyst nematode is thought to be indigenous (Eshtiaghi, 1988). The incidence of this nematode on sugar beet has been recorded for the first time from Khorassan province (Torbat-Heydareyeh) in the north east of the country by Schifer and Esmailpoor (Kalali and Farivar-Mahin, 1977). This nematode is widespread through the most sugar beet production areas of Iran. Nematode antagonists have been observed in a wide range of organisms including fungi. These wide ranges of opponent against nematodes have been comprehensively reviewed by Stirling (1991) and it may be feasible in the future as a part of integrated pest management (Kerry, 1995). Fungal antagonists as biological control agents have been most extensively studied and appeared to be the most important parameter in regulating nematode populations in soil (Chen and Dickson, 2005). Limited studies have been done to investigate the effect of nematophagous fungi on plant species (Bourne and Kerry, 1999, 2000; Oyekanmi *et al*., 2006).

Recent studies of antagonistic fungi associated with the beet nematode revealed that there were some antagonistic fungi which had potential to control beet

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cyst nematode *in vitro* and *in vivo* conditions (Fatemy, 1993; Hojat-Jalal and Coosemans, 1995, 1997; Ahmadi *et al*., 1995a, b and c). In an attempt to isolate the most promising antagonistic fungi of beet cyst nematodes, an effective sterile fungus designated as StFCh1-1 was isolated from developed females of nematodes. These females were extracted from a soil sample which collected from soil around different roots of sugar beet plants in an infested fields from Khorasan province. The egg parasitic index of extracted females was high (4.7). Results of different bioassay indicated that the sterile fungus StFCh1-1 was established in the rhizosphere in the vicinity of beet cyst nematodes in sterile and non sterile pot soil. A high number of females of nematodes were diseased in pot soil in the growth chamber condition. As an advantage, this fungus produces an obvious syndrome on diseased females, which facilitate the quantification of the disease within a population and fungus had no negative effect on plants in different experiments (Hojat-Jalali and Coosemans, 1997; Hojat-Jalali *et al*., 1998a, b).

Nevertheless, no information is available regarding the interaction effect of an antagonistic fungus and the beet cyst nematode, *Heterodera schachtii*, as a biostressor on some physiological aspect of sugar beet plants.

The aims of this study in the rhizosphere conditions were: to evaluate the influence of sterile fungus, StFCh1-1, on biological control of beet cyst nematode, *Heterodera schachtii*, in a susceptible sugar beet cultivar 7233 in soil. and to evaluate some physiological aspects of sugar beet cultivar 7233 including, total chlorophyll, biomass and potassium content in leaves and roots of plants in the presence of nematodes and opponent sterile fungus.

MATERIALS AND METHODS

The origin of the beet cyst nematodes: A soil sample of west Azarbaijan was used to extract cysts of *Heterodera schachtii* for trials. This sample was collected from an infested sugar beet field with different crops rotation in west Azarbaijan province in east-north part of Iran in late August 2004. The soil was collected from depth of 10 to 30 cm around the roots of sugar beet plants using an auger. The cyst of beet cyst nematodes was extracted from air dried soil by using the Fenwick (1940) method. Then, all cysts were picked up manually from debris by using a fine forceps under a stereomicroscope and collected in an eppendorf microtube. The cysts were disinfected by 0.5% sodium hypochlorite for 4 min and rinsed several times and preserved at 4°C until it was required.

Viability test of cysts nematodes: The viability of cysts was determined by using 30 randomized cysts on a sterile hatching apparatus microtube containing 2 mL zinc chloride (4 mM) as an artificial hatching agent (Hojat-Jalali and Coosemans, 1997; Feyarts and Coosemans, 1992) and the treatment was replicated six times. The tubes were incubated at 20°C in the darkness. The number of second stage juveniles of nematodes emerged from cysts were counted two times by using a counting dish and recorded after one month.

Sterilization of sugar beet seeds cultivar 7233: The seeds of sugar beet were surface-sterilized by using a technique of Sijmons *et al*. (1991). About nine sterilized seeds of cultivar were placed aseptically on 0.7% water agar in each petri plate (9 cm Ø) and replicated ten times. The plates were incubated at 20°C in darkness and checked for development of any contamination of fungi and bacteria to be removed. The healthy seedlings of sugar beet were used in the next step of the rhizosphere bioassay.

Setting a rhizosphere bioassay: A bioassay was conducted in the autoclaved glass tubes (20×3 cm Ø), totally 24 tubes. Each tube was filled with 30 g double autoclaved soils with three days interval. As a standard practice (Stirling, 1992) this bioassay composed of four treatments including: fungus, nematode, nematode + fungus and untreated control. In case of inoculation of soil with sterile fungus, StFch1-1, a plug of ten days old of fungus (5 mm Ø) was removed from the edge of its colony on PDA and push it in 2 cm below the soil surface only in two treatments. The tubes moistened with sterile distilled water and incubated at 25°C for two weeks. The soil used in this experiment composed of clay loamy sand (3, 2, 2).

Afterward, each glass tube was planted with a sterile germinated seed of sugar beet and placed in a growth chamber with artificial light, 5000 Lux, at 25°C for 16 h light and 20°C for dark period. Each treatment replicated six times and arranged in a randomized complete design in the growth chamber condition as it was already described. The plants were moistened regularly with a half Hoagland´s nutrient solution. Two weeks later the seedling of sugar beet plant at four leaves stages in two treatments with nematode was inoculated with 50 disinfected beet cyst nematodes. The inoculum was placed very carefully in a small hole in the vicinity of roots of the plant and covered by soil.

The sugar beet plants were harvested two months after nematodes´ inoculation in to the soil. In order to determine the number of cysts and females of nematodes

in two treatments with nematodes, the roots of sugar beet plant in each tube were washed away from soil particles with vigorous water stream on to a 200 µm aperture sieve. Then after, healthy and diseased cysts of nematodes on the roots were counted and collected under stereomicroscope (Olympus SZX12) by using a fine-tipped forceps and preserved in eppendorf microtube. The roots and leaves of treated and untreated sugar beet plants were preserved at 4°C. These materials were used to measure different physiological aspects of plants.

Fresh leaves and tap roots of treated and untreated sugar beets were weighted, recorded and rinsed with distilled water several times. Plant materials dried by filter papers and incubated in an oven at 70°C for three days until to be dried. The dried leaves and tap roots of plants were powdered separately by using a mortar and pestle and used to measure some physiological aspects of sugar beet plants in the following steps:

To measure potassium contents of each sample, a 0.1 g of powdered material of sugar beet plant was treated with 10 mL of 3% sulfusalicylic acid. After 48 h, the sample was filtered through a Whathman filter paper No. 1. The potassium contents in leaves and roots of samples were measured by using a flame photometer (Corning 400) and it was calculated through the standard curve and recorded.

The amount of chlorophyll of each treatment was measured by Arnon (1949) method with a slightly modification.

Statistical analysis: Analysis variance of data was made by one way ANOVA. The variance homogeneity of mean values of Biomass, potassium in leaves and roots, chlorophyll content in leaves was evaluated by the Tukey test.

RESULTS

Result of cyst extraction from an infested soil of sugar beet field in west Azarbijan indicated that its infestation was 7.5 cysts in 20 g soil. The viability of second stage juveniles of nematode emerged from cyst in hatching test was 9.7±2.4 per cyst. Result of this bioassay indicated that, the beet cyst nematodes could reproduced on sugar beet plants cultivar 7233 in soil in the treatments inoculated with nematodes as well as with the sterile fungus StFCh1-1. The number of developed cysts of nematode on roots of sugar beet plants was 14.3±5.8 and 9.8±3.9 in treatments with nematode and nematode plus sterile fungus StFCh1-1 respectively. The number of infected cysts of nematode in the presence of sterile fungus was 32.2% (Fig. 1). The diseased cysts of nematode were identified only through the presence of a sclerotium-like cluster of fungus on body wall which is referred to as syndrome of the sterile fungi on beet cyst nematodes (Fig. 2).

One way analysis of data measuring fresh weight of sugar beet cv. 7233 in four treatments (untreated control, nematode, fungus and nematode + fungus) revealed a significant difference between fresh weights of treatments. Meanwhile, the fungus had not detrimental effect on decreasing fresh weight of plants in comparison to the control. The treatment of nematode as well as nematode plus fungus showed some reduction in weights of both root and shoot (Fig. 3).

One way analysis of data, measuring the potassium content of sugar beet cv. 7233 in four treatments (control, nematode, fungus and nematode + fungus) indicated that there were significant difference between potassium content of leaves and roots using Tukey test ($p = 0.05$).

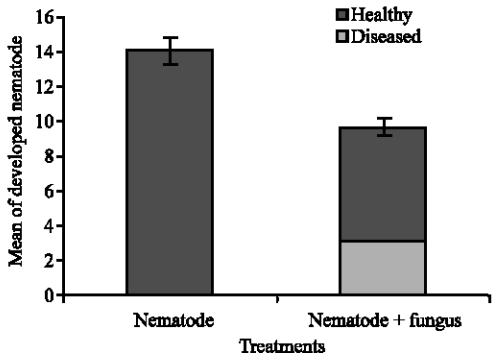

Fig. 1: Comparison between the development of healthy and diseased cyst of nematodes, *Heterodera schachtii*, in control and treated sugar beet plants, cultivar 7233, by an antagonistic sterile fungus, StFCh1-1, in the rhizosphere condition ($p = 0.05$, $n = 6$)

Fig. 2: Syndrom of the diseased beet cyst nematode, *Heterodera schachtii*, produced by a sterile fungus, StFCh1-1, in the rhizosphere condition (original)

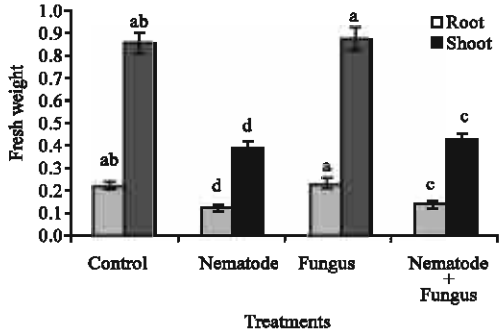

Fig. 3: Comparison between the fresh weights of roots and shoots of sugar beet cv. 7233 in a bioassay with four treatments in the rhizosphere condition. Bars with the same letter(s) are not significantly different, using Tukey test (p = 0.05, n = 6)

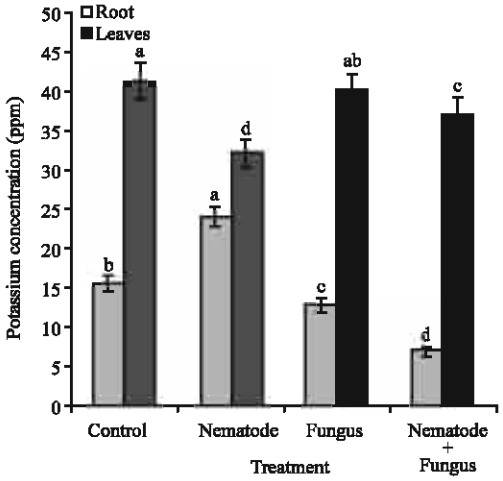

Fig. 4: Comparison between the potassium content of roots and leaves of sugar beet cv. 7233 in a bioassay with four treatments in the rhizosphere condition. Bars with the same letter(s) are not significantly different, using Tukey test (p = 0.05 n = 6)

The potassium concentration in leaves of untreated control and treatment of fungus was the same. Meanwhile, it was the lowest in treatment with nematode alone but not in treatment with nematode + fungus. Also, potassium concentration in roots was highest in plants invaded by nematodes alone neither nematodes plus fungus nor fungus alone (Fig. 4).

One way analysis of data, measuring the chlorophyll a, chlorophyll b and total chlorophyll of sugar beet cv. 7233 in four treatments, including control, nematode,

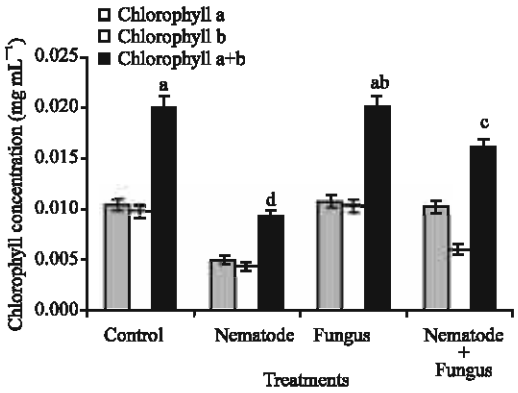

Fig. 5: Comparison between the chlorophyll content of leaves of sugar beet cv. 7233 in a bioassay with four treatments in the rhizosphere condition. Bars with the same letter(s) are not significantly different, using Tukey test (p = 0.05 n = 6)

fungus and nematode + fungus indicated that there were significant difference between total chlorophyll, using Tukey test (p = 0.05). The total chlorophyll concentration was highest in the untreated control as well as in the treatment with fungus alone than that of treated with nematode or nematodes + fungus (Fig. 5).

DISCUSSION

Results of this bioassay confirmed that in the presence of antagonistic sterile fungus StFCh1-1, a few number of beet cyst nematodes was developed on the roots of sugar beet cv. 7233 in the rhizosphere's condition. This result verified previous studies in which the beet cyst nematode, *Heterodera schachtii* and root-knot nematodes, *Meloidogyne incognita* and *Meloidogyne javanica*, were controlled biologically by this fungus in *in vitro* and *in vivo* experiments (Hojat-Jalali *et al*., 1998a, b; Coosemans and Lievens, 2002).

As a biological control agent of the beet cyst nematode this sterile fungus had no detrimental effect on biomass, chlorophyll and potassium concentration in leaves of sugar beet cv. 7233. Meanwhile, potassium concentration in roots was lower than that of the untreated control. May be, this fungus can colonize the roots of host plant and potassium is essential mineral nutrient for enzyme activity, carbohydrate metabolism and ionic imbalance (Griffin, 1994). Therefore, this fungus is safe to use in sugar beet cv. 7233 as a biological control agent of the beet cyst nematodes and it was corroborated by previous studies (Hojat-Jalali *et al*., 1998a, b).

The beet cyst nematode, *Heterodera schachtii*, had destructive effect on biomass and chlorophyll content of sugar beet cv. 7233, which supported the two other studies in sugar beet cv. 7233 as well as Nemakill (Ghasempour *et al*., 2007; Hojat-Jalali *et al*., 2007).

This sedentary endoparasite can disrupt the root system by neoplastic feeding behavior interferes with the physiological processes involved in water and nutrient relations and the phytohormones originating in the root (primary factors), thereby creating a cascade effect on chlorophyll synthesis, photosynthesis and respiration in the shoot (secondary factors). The combination of these primary and secondary effects leads to diminished plant productivity and poor growth compared with uninfected plants (Melakeberhan, 2004). Meanwhile, the destructive impact of nematodes on biomass and chlorophyll was mitigated by sterile fungus StFCh1-1 (Fig. 3 and 4).

In spite of the fact that potassium concentration in roots of invaded plants by the beet cyst nematode was in the highest level, but its concentration was at lowest rate in shoots (Fig. 5). These changes might be indication of adaptive osmuregulation or acclimation responses in plants due to the nematodes as bio-stressors through the increase of metabolites and solutes. Potassium is essential for photosynthesis, starch formation and translocation of sugars within the plant. K is necessary for chlorophyll development, but it is not an actual part of its molecular structure (Olsen and Silvertooth, 2001). Potassium is involved in numerous functions in the plant such as in enzymes activation, cation/anion balance, stomatal movement, phloem loading, assimilate translocation and turgor regulation to name only few. Stomatal resistance decreases and photosynthesis increases with increasing in K content of the leaves (Peoples and Koch, 1979).

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