Effects of some commercial products on root and crown rot caused by *Phytophthora cactorum* in apple cultivation

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Abstract

Root and crown rot caused by Phytophthora cactorum (Lebert & John) Schröeter is one of the most common diseases of apple (Malus domestica) in the world. Due to the negative influences of fungicides on the environment, using other applicable control measures is a plausible way for the management of the disease. The objective of the study was to evaluate the effects of eleven commercial products (bio-pesticides, fertilizers and activators) on P. cactorum by comparing plant growth parameters (trunk diameter, plant height, number of branches and root dry weight). Trials were conducted according to a randomized complete block design with three replicates in a greenhouse in two consecutive years. In the trials, saplings of Red Chief variety grafted onto MM106 rootstock were planted into pots containing soil mixture inoculated with P. cactorum. Two control groups (negative and positive) were also included in each treatment. Tested commercial products generally had significant ($P < 0.01$) positive effects on plant growth parameters (trunk diameter, plant height and root dry weight) by reducing the severity of the disease (50-93%). Of the tested commercial products, Alexin 95 PS, Companion, Endo Roots Soluble and Subtilex had the highest efficacies. There were negative correlations between plant growth parameters (trunk diameter, plant height and root dry weight) and severity of the disease, but root dry weight had significant ($P < 0.01$) correlation ($r = -0.56$) with the severity of the disease. Present findings revealed that some commercial products could reliably be used against P. cactorum as an alternative to pesticides in apple cultivation.

Key words: Disease control, *Malus domestica*, plant growth, root and crown rot,

1. Introduction

Apple (*Malus domestica*) is one of the most economically important pome fruits that is widely grown in different ecological regions around the world. In recent years, apple production of the world has reached to 86142197 tons and Turkey is the fourth leading producer accounting for 3625960 tons of the world's production (FAO, 2020). However, *Phytophthora* species are among the most destructive soil-borne pathogens causing economic losses in orchards in fruit production (Erwin and Ribeiro, 1996; Sánchez et al., 2019). For example, in Bulgaria, it was reported that *Phytophthora* species caused drying the plants up to 10% in some orchards (Nakova, 2010), while they caused considerable losses, 2.5 - 24.5% death in nurseries and 0.2 - 77.5% in apple orchards in India (Sharma et al., 2014). Among *Phytophthora* species, *Phytophthora cactorum* (Lebert & John) Schröeter is the most frequently reported species in apple-growing regions (Jones and Aldwinckle, 1997; Ebrahimzadeh and Dolar, 2019).

Most of the apple orchards in our country were established with seedlings, and also M9, MM106, and M111 rootstocks were preferred as clone rootstocks (Bayav and Armağan, 2007). According to the findings obtained so far, all of these commercial rootstocks have been determined to be susceptible to *P. cactorum*. An integrated approach including chemical control and cultural practices has been recommended for root and crown rot management in apple orchards (Carisse and Khanizadeh, 2005). In this sense, Maden et al., (1995) reported that *P. cactorum* was the most widespread pathogen causing deaths in apple nurseries in Eğirdir county of Isparta Province, Turkey. Kurbetli and Demirci (2015) also found that *P. cactorum* caused significant yield losses in apple orchards in Turkey.

Root and crown rot symptoms caused by *P. cactorum* include reddish-brown color in inner bark and wood tissue. Other symptoms of the pathogen are chlorosis of leaves, reduction of tree vitality and growth. As a result of the infections, infected trees slowly weaken and then collapse in a couple of years (Ellis, 2008; Nakova, 2010). In management of the root and crown rot caused by *Phytophthora* species including *P. cactorum*, some pesticides such as fosetyl-al and phosphorous acid were found to be effective (Utkhede and Smith,1991; Flett, 1996; Erkılıç and Canıhoş, 1999; Sharma et al., 2014; Kurbetli and Demirci, 2015; Türkölmez and Derviş, 2017). However, widespread use of those and other pesticides cause negative effects on ecosystem and agriculture. Therefore, using resistant rootstocks and applying commercial products that have not negative influences on ecosystem are the other applicable methods for control of *Phytophthora* species in apple cultivation. Some of the Commercial products are consisted of various active ingredients including biological agents, plant nutrients and activators. For example, PGPR (Plant Growth Promoting Rhizobacteria), PGPF (Plant Growth-Promoting Fungi) and AMF (arbuscular mycorrhizal fungi) have numerous benefits to plants such as nutrient intake, protection against soil-borne pathogens and resistance to environmental stresses (Berendsen et al., 2012; Mendes et al., 2013; Pérez-Jaramillo et al., 2015; Paliwoda and Mikiciuk, 2020). PGPR, PGPF and AMF can protect plants against pathogenic microorganisms such as bacteria, fungi, viruses and nematodes and eliminate their harmful effects by activating induced systemic resistance (ISR) in plants (Pieterse et al., 2014; Hossain and Sultana, 2015; Etesami and Maheshwari, 2018). However, existing commercial products should be tested specifically against pathogens causing serious problems in agriculture. Therefore, developing alternative efficient and environmentally friendly methods to control this disease is urgently needed. Due to its environmentally friendly and nontoxic characteristics, biological control is one of the most desired methods for managing root and crown rot disease.

In this study, a potential biocontrol agent was tested for antifungal activity and effective plant growth-promoting traits. The aims of this study were (i) to determine the effects of eleven commercial products (biopesticides, fertilizers, and activators) on severity of *P. cactorum in vivo* and (ii) to examine the effects of commercial products on plant growth parameters (trunk diameter, plant height, number of branches and root dry weight) of apple saplings.

2. Materials and Methods

Commercial products used in the trials

Information about eleven commercial products used in present trials are provided Table 1.

2.1.Inoculum preparation

A virulent strain of *P. cactorum* [accession number: HM357616 in Genbank (https://www.ncbi.nlm.nih.gov)] was obtained from Plant Protection Central Research Institute, Ankara, Turkey. Five agar plugs (5 mm) from 6 day-old colonies of *P. cactorum* were added to jars (1 L) containing a mixture of volumes [carrot juice (400 mL) + vermiculite (560 mL) + oat seeds (40 ml)] and incubated at 23 °C for 21 days.

2.2.Greenhouse trials

The inoculum mixture in each jar was transferred to a pot (25-L) containing soil mixture [orchard soil and sand (1:1)] and homogeneously mixed into the pot. The inoculum constituted 4% of the total volume of each pot (Latorre et al., 2001). Two-month-old saplings of Red Chief variety grafted onto MM106 rootstock were used as test plants. Inoculations were carried out at the Fruit Research Institute (Eğirdir, Isparta province, Turkey). One sapling of the variety was planted per pot and commercial products were applied at the recommended doses to the pots. In positive controls, the inoculum was applied to the pots without the commercial products. However, in negative controls, sterile water was only used. The trials were conducted according to a randomized complete block design with 3 replicates in two consecutive years (Figure 1).

The saplings were grown in the pots for 4 months in the greenhouse in June-September. In order to promote growth of *P. cactorum*, the pots were kept wet by drip irrigation.

2.3.Evaluation of the trials

2.3.1. Plant growth parameters

Trunk diameter was measured from 10-cm above the grafting point with the use a caliper, while sapling height was measured from the soil level to the top with a meter stick. Number of branches per sapling was recorded by counting branches that are longer than 20 cm.

To determine root dry weights of the saplings, immediately after removing plants, the roots were washed under running tap water and consequently separated from the soil particles. They were kept at 25 °C for a few hours and then cut at the beginning of the root collars. The roots of each sapling were dried at 70 °C for 4 days and then weighed with the use of a digital scale.

2.3.2. Disease severity and efficacy of the commercial products

Considering percentage of stems with lesion, disease severity was determined using 0-4 scale, where: 0 = no visible, 1, 2, 3 and 4 = 1-25, 26-50, 51-75, and 76- 100% with lesions, respectively (Tidball and Linderman 1990) and the formula below.

Disease severity (%) = $\Sigma i\ 1(ni \times vi) / N \times V$

where; vi is the scale class, ni is the number in one class, N is the total number, V is the highest class, i is the number of classes.

To quantify disease severity values, percentage efficacy of each commercial product was determined according to the Tawnsend-Heuberger formula below (Karman, 1971).

% efficacy = $[(Ic - It)/Ic] \times 100$

where; Ic is the disease severity of untreated control, It is the disease severity of the treatment.

2.3.3. Data analysis

Data of the study were analyzed using SPSS 16 (IBM Corp., Armonk) software package. Means of each growth parameter, disease severity and efficacy of each commercial product were categorized according to Duncan's multiple range test ($P < 0.01$).

3. Results

According to average of two years, differences in growth parameters (trunk diameter, plant height and root dry weight) of the trails were found to be significant ($P < 0.01$).

The highest mean trunk diameter (19.79 mm) was found in Subtilex trails, while the lowest one (16.78 mm) was determined in pathogen inoculated control. Mean trunk diameter of non-inoculated control group was 17.31 mm. In addition to Subtilex, as compared to the pathogen inoculated control, mean trunk diameters, of Alexin 95 PS, Isr-2000 and Symbion Vam (18.92, 18.81 and 18.46 mm, respectively) were also found to be significant ($P < 0.01$).

Mean sapling heights of the non-inoculated and pathogen inoculated control groups were 151.78 and 145.22 cm, respectively. However, mean sapling heights in experimental trails ranged from 138.89 to 178.06 cm. As compared to the pathogen inoculated control group, mean sapling heights of Alexin 95 PS, Isr-2000, Actinovate, Green Miracle, Subtilex and Cropset trails (181.11, 178.06, 175.15, 172.67, 172.17 and 171.39 cm, respectively) were found to be significant ($P < 0.01$).

Mean number of branches of the pathogen inoculated control was 2.11, while it was 3.18 in the non-inoculated control. The difference was significant ($P < 0.01$), confirming destructiveness of the pathogen in the apple saplings. On the other hand, mean number of branches of the experimental trails were not significantly different from the pathogen inoculated control group. One example for evaluations of the results of the study is presented in Figure 2.

Mean root dry weights of the non-inoculated and pathogen inoculated controls were 110.20 and 98.80 g, respectively. However, as compared to the pathogen inoculated controls, mean root dry weights of Alexin 95 PS, Subtilex and Tricho plus trials (126.86, 125.24 and 121.65 g, respectively) were significantly different ($P < 0.01$).

Mean disease severity of the pathogen inoculated control was 43.75%, while disease severity values of experimental trails ranged from 3.125 to 21.875%. The highest efficacy (93.75%) was detected in Alexin 95 PS trials (Table 2).

There was a negative correlation ($r = -0.43$) between trunk diameter and disease severity. Similarly, plant height negatively correlated ($r = -0.35$) with disease severity, but the correlation between root dry weight and disease severity ($r = -0.56$) was significant ($P < 0.01$).

4. Discussion

Antifungal and plant growth-promoting effects of various strains of *Bacillus subtilis* were revealed in previous studies. For example, application of *B. subtilis* to tomato seedlings not only enhanced plant growth but also suppressed soil-borne pathogens including *Pythium aphanidermatum* (Kipngeno et al., 2015; Qiao et al., 2017). Plant growth bacteria excrete volatile compounds were found to be effective against soil-borne oomycetes including *Phytophthora cinnamomi* (Méndez-Bravo et al., 2018). In addition to volatile compounds, *B. subtilis* has functional metabolites such as IturinA that causes damages to cell structure of *Phytophthora infestans* by inducing disruptions in cell membrane and organelle formation. For these reasons, *Bacillus subtilis* is capable of inhibiting mycelium growth of *P. infestans* (Wang et al., 2019). In a greenhouse study, *B. subtilis* decreased mortality of pistachio seedlings up to 80% against *Phytophthora pistaciae* (Moradi et al., 2018). Likewise, in the present study, as compared to positive control group, applications of Subtilex (*Bacillus subtilis* MBI 600) caused significant increases in trunk diameter, plant height and root dry weight of the saplings by reducing severity of *Phytophthora cactorum*, indicating antifungal and plant growth-promoting effects of the commercial product.

As related to the other contents of the commercial products used in the present study, Symbion Vam (*Glomus fasciculatum*) and Endo Roots Soluble (*Glomus* spp.) had other bio-pesticides with their Glomus contents. Applications of Symbion Vam (*Glomus fasciculatum*) caused significant increases in trunk diameter and root dry weight by decreasing severity of the disease, while effects of Endo Roots Soluble (*Glomus* spp.) on the plant growth parameters were not significant but its application caused significant reductions in severity of the disease. It is known that arbuscular mycorrhizal fungi (AMF) are potentially protective agents with their ability of forming symbiotic associations with root systems of various crops. For example, in a study, arbuscular mycorrhizal fungus *Glomus mosseae* decreased infections of *Phytophthora parasitica* in tomato by providing a systemic resistance for host plant through excreting hydrolytic enzymes (chitinase, chitosanase, β□1,3□glucanase and superoxide dismutase) (Pozo et al., 2002). In another study, *Glomus* application increased plant height, pseudo-stem diameter and root weight of banana seedlings by suppressing *Fusarium oxysporum* f. sp. *cubense* (FocR4) (Castillo et al., 2019). Likewise, four commercial products (Bacto_Prof, Endomyk_Basic, Endomyk_Conc and Endomyk_Prof mycorrhizal) with mycorrhizal content decreased *Fusarium oxysporum* infections in tomato plants by significantly enhancing plant height (Al-Hmoud and Al-Momany, 2015). With regard to *Phytophthora*, applications of mycorrhizal fungi including *Glomus intraradices* increased plant height, stem diameter, number of leaves and fresh aerial biomass and root growth of pepper and strawberry by suppressing *Phytophthora capsici* and *Phytophthora cactorum*, respectively (Hautsalo et al., 2016; Tena et al., 2016).

Apart from arbuscular mycorrhizal fungi, *Trichoderma* species in particular *Trichoderma harzianum* is closely associated with plant roots. In the present study, Tricho plus (*Trichoderma harzianum*) caused significant increases in root dry weight by reducing severity of *Phytopthora cactorum*. Similarly, in a study, *Phythium ultimum*, *Phytopthora capsici* and *Phytophthora infestans* decreased fresh and dry shoot weight of tomato, while *Trichoderma harzianum* improved entire plant growth including root weight in treatment plots, indicating efficacy of application of *T. harzianum* against the soil-borne pathogens. The mechanism was related to inhibition of entrance of both pathogens to vascular bundle of tomato tissues, which provided reduction in size of infection areas of the pathogens (Uddin et al., 2018; Kariuki et al., 2020). In a 3-year-field experiment, *Trichoderma* spp. decreased soil populations of *Phytophthora cactorum* by reducing disease incidence up to 76.6% in strawberry cultivation (Porras et al., 2007). In another 2-year-field study, *Trichoderma harzianum* reduced severity of *Phytophthora capsici* up to 42% in pepper cultivation (Timila and Manandhar, 2020). All these supported the results of those commercial products tested in the present study.

In addition, of the tested commercial products, some of which had nutrient contents. In fact, plant nutrients may affect tolerance/resistance of plants to pathogens (Gupta et al., 2017). Fertilizers have significant physiological effects on plant growth (Li et al., 2012). For example, potato yields and infection caused by *Phytophthora infestans* are associated with supply of potassium. Application of potassium provided a protection for potato plants against *P. infestans* by enhancing phytoalexins and phenols in tubers, which stimulates production of biochemical compounds, pathogenesis-related enzymes and antioxidant enzyme activities in the host (Kowalska and Drożdżyński, 2018; Mohammadi et al., 2019). In a field study, applications of potassium phosphonate reduced incidence of root rot of papaya caused by *Phytophthora palmivora* by 47% (Vawdrey et al., 2004). Commercial product Phytogard that contains potassium phosphite led to preventively control of *Phytophthora plurivora* in beech plants (Rezende et al., 2020). Likewise, in the present study, of the tested commercial products, Alexin 95 PS (Phosphorus pentoxide + Potassium oxide), considered as fertilizer with its content, caused significant increases in trunk diameter, plant height and root dry weight by reducing severity of the disease. Moreover, of the tested commercial products, the highest efficacy was detected in this commercial product. In addition to nutrients, plant extracts may play a role in plant resistance. Demirci and Dolar (2006) reported that extracts of dry cabbage, garlic, and alfalfa were effective in decreasing severity of *Phytophthora capsici in vitro* and *in vivo*. Similarly, in the present study, Isr-2000 (*Lactobacillus acidophilus* + plant extract + yeast extract +benzoic acid) led to significant increases in trunk diameter and plant height by reducing severity of *Phytophthora cactorum*, while other commercial product Cropset

(*Lactobacillus acidophilus* + plant extract + MnSO4 + FeSO4 + CuSO4) increased plant height by reducing severity of *P. cactorum*.

Plant activators capable to improve plant health through antifungal, antibacterial, oomyceticidal, and as elicitors of plant immunity through the routes of salicylic acid (SA) and jasmonic acid (JA) (Pye et al., 2013; Bitas et al., 2013; Schalchli et al., 2016). In this regard, some plant activators like HarpinEa and Salicylic acid might reduce infections of *Phytophthora infestans* and *Rhizoctonia solani* in tomato and chili by enhancing specific enzyme activities that provide systemic acquired resistance (SAR) in the host (Tosun et al., 2003; Baloch et al., 2018). For example, another plant activator, so called as called PPA (pyrimidin-type plant activator), increased fresh weight of rice and Arabidopsis plants. In addition, PPA also promoted lateral root development and plant defense against pathogen invasion (Sun et al., 2015). In the present study, Combat Plus (plant activator) significantly reduced the severity of *P. cactorum*, which is in agreement with the results of the previous studies aforementioned.

As compared to positive and negative controls, sapling height significantly increased in Actinovate (*Streptomyces lydicus)* applied plots, indicating suppression of *Phytophthora cactorum*. In this regard, Ezziyyani et al., (2007) reported that *Streptomyces rochei* had a specific activity against *P. capsici*. Likewise, in another study, *Streptomyces* spp. was found to be effective in controlling root rot of alfalfa (*P. medicaginis*) and soybean (*P. sojae*) (Xiao et al., 2002). Besides, *Streptomyces* species were effective against *Rhizoctonia solani, Pythium aphanidermatum*, and *P. ultimum* in various crops (Hamdali et al., 2008; El-Tarabily et al., 2009; Goudjal et al., 2014). Suppression of plant pathogens by *Streptomyces* species is related to antibiotic production, volatile components, hydrolytic enzymes, and siderophore production (Palaniyandi et al., 2013; Wang et al., 2013; Sadeghi et al., 2017).

5. Conclusions

Pesticides used for disease control may generate serious consequences for environment and food safety, so there is a need for alternative management methods (Gupta et al., 2017). Therefore, alternative methods should be applicable and environment-friendly and they should be presented to farmers for management of plant diseases in agriculture. As a result of the present study, of the tested commercial products, Alexin 95 PS (fertilizer), Companion (*Bacillus subtilis GB03*), Endo Roots Soluble (*Glomus* spp.) and Subtilex (*Bacillus subtilis* MBI 600) had the highest efficacies against *Phytophthora cactorum*. Applications of these products to newly planted saplings can protect them from soil-borne pathogens like *Phytophthora cactorum* in apple cultivation.

In this study, a potential biocontrol agent was tested antifungal activity and effective plant growth-promoting traits. The conclusions achieved in this work help to understand the current state of the research field, and reveal new insights on the development of efficient biocontrol strategies for the control of *P. cactorum*. Futhermore The present study also suggests that existing commercial products should be tested specifically against plant pathogens causing serious problems in agricultural production.

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Compliance with ethical standards

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Figure 1. Setting up the trials with saplings of Red Chief variety grafted onto MM106 rootstock.

Figure 2. Evaluation of negative control (left), treatment (middle) and positive control (right) in companion (commercial product) application to the Red Chief variety grafted onto MM106 rootstock in the trials.

Table 1. Trademark, ingredients, recommended application doses and manufacturers of commercial products used in present trials

Trademark	**Active Ingredient/Microorganism**	**Recommended application doses**	**Company**
Companion	*Bacillus subtilis* GB03	0.5 mL / m^2	Growth products
Symbion Vam	*Glomus fasciculatum*	1 g /m^2	Stanes
Green Miracle	Vegetable oil acid	0.2 mL /m^2	Stanes
Cropset	*Lactobacillus acidophilus*, plant extract, MnSO4, FeSO4, CuSO4	0.06 mL /m^2	Ant tarım (Improcrop EU)
Isr-2000	*Lactobacillus acidophilus*, plant extract, yeast extract, benzoic acid	0.1 mL /m^2	Ant tarım (Improcrop EU)
Actinovate	*Streptomyces lydicus*	0.4 mL /m^2	Mts agro
Subtilex	*Bacillus subtilis* MBI 600	0.5 mL /m^2	Bioglobal
Tricho plus	*Trichoderma harzihanum*	0.04 g /m^2	Bioglobal
Alexin 95 PS	Phosphorus pentoxide (P_2O_5) 52%, Potassium oxide (K_2O) 42%	0.7 g /m^2	Sumitoma
Combat Plus	Plant activators	0.4 mL /m^2	Bioglobal
Endo Roots Soluble	*Glomus intraradices*...................21 *Glomus aggregatum*................................20 *Glomus mosseage*...................................20 *Glomus clarum*..1 *Glomus monosporus*.................................1 *Glomus deserticola*..................................1 *Glomus brasilianum*................................1 *Glomus etunicatum*.................................1 *Gigaspora margarita*..................................1	0.5 mL /m^2	Bioglobal

Table 2. Effects of the commercial products on plant growth parameters and disease severity in apple saplings of Red Chief variety grafted onto MM106 rootstock and efficacy rates of the commercial products.

Applications	**Trunk diameter (mm)***	**Sapling height (cm)***	**Number of branches***	**Root dry weight (g)**	**Disease severity (%)***	**Efficacy (%)**
Companion	17.00 ± 0.55 de (13.51-21.27)	153.61 ± 7.38 bcd (105.00-197.00)	1.69 ± 0.18 b (1.00-3.00)	112.34 ± 6.92 abc (83.98-152.05)	6.25 bc	87.5 ab
Symbion Vam	18.46 ± 0.42 abcd (16.64-23.21)	161.65 ± 5.26 abc (126.00-202.00)	1.72 ± 0.31 b (0.00-5.00)	120.58 ± 8.22 abc (77.00-159.70)	12.5 bc	62.5 abc
Green Miracle	17.87 ± 0.34 bcde (15.95-20.53)	172.67 ± 6.92 ab (110.00-215.00)	1.50 ± 0.20 b (1.00-4.00)	110.19 ± 5.49 abc (85.85-134.23)	15.62 bc	58.33 abc
Cropset	17.29 ± 0.40 cde (13.15-19.67)	171.39 ± 6.21 ab (129.00-221.00)	2.06 ± 0.29 b (1.00-5.00)	99.74 ± 7.68 bc (58.88-126.96)	21.875 b	50 c
Isr-2000	18.81 ± 0.55 abc (15.06-24.23)	178.06 ± 5.00 a (139.00-212.00)	1.89 ± 0.25 b (1.00-4.00)	113.76 ± 7.43 abc (69.05-141.09)	12.5 bc	62.5 abc
Actinovate	17.89 ± 0.46 bcde (14.96-20.15)	175.15 ± 5.03 a (151.00-215.00)	2.24 ± 0.22 b (1.00-4.00)	121.24 ± 8.82 abc (70.68-168.64)	12.5 bc	62.5 abc
Subtilex	19.79 ± 0.43 a (16.79-22.94)	172.17 ± 6.24 ab (113.00-211.00)	1.82 ± 0.20 b (1.00-3.00)	125.16 ± 6.16 a (93.00-151.66)	9.375 bc	75 abc

Table 2. (continued).

Applications	Trunk diameter (mm)*	Sapling height (cm)*	Number of branches*	Root dry weight (g)	Disease severity (%)*	Efficacy (%)
Tricho Plus	17.61 ± 0.53 bcde (12.79-20.78)	138.89 ± 6.70 d (90.00-193.00)	1.86 ± 0.23 b (1.00-4.00)	121.65 ± 6.82 ab (97.40-161.51)	18.75 bc	56.25 bc
Alexin 95 PS	18.92 ± 0.54 ab (14.21-24.74)	181.11 ± 7.57 a (122.00-238.00)	1.88 ± 0.21 b (1.00-4.00)	126.86 ± 5.68 a (95.50-147.65)	3.125 bc	93.75 a
Combat Plus	17.37 ± 0.33 cde (15.05-20.12)	163.67 ± 7.27 abc (85.00-204.00)	2.22 ± 0.33 b (1.00-6.00)	98.86 ± 7.04 c (72.25-133.68)	21.87 b	50 c

Endo Roots Soluble	17.85 ± 0.53 bcde (11.30-22.14)	164.83 ± 6.51 abc (112.00-210.00)	1.67 ± 0.14 b (1.00-3.00)	107.82 ± 6.41 abc (66.33-139.38)	6.25 bc	87.5 ab
C0	17.31 ± 0.34 cde (14.45-19.76)	151.78 ± 5.91 bcd (118.00-205.00)	3.18 ± 0.43 a (1.00-7.00)	110.20 ± 4.94 abc (93.55-140.85)	0 c	-
C1	16.78 ± 0.54 e (13.05-20.63)	145.22 ± 5.78 cd (113.00-193.00)	2.11 ± 0.24 b (1.00-4.00)	98.80 ± 5.52 c (68.35-119.66)	43.75 a	-
	F = 3.493, P = 0.000	F = 4.194, p =0.000	F = 2.589, p = 0.003	F = 2.09, p = 0.020	F = 3.862, p = 0.002	

* Mean values of two years, the same letters are not significantly different according to Duncan's multiple range test (P < 0.01). Mean ± Std. Error (min-max.), C0: Negative control, C1: Positive control