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 negative influences of fungicides on environment, using other applicable control measures is a plausible way for the management of the disease. The objective of the study were 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 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 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.

**Keywords:** Disease control, *Malus domestica*, plant growth, root and crown rot,
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 Canlıhoş 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.

### Table 1.

<table>
<thead>
<tr>
<th>2.1. Inoculum preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A virulent strain of <em>P. cactorum</em> [accession number: HM357616 in Genbank (<a href="https://www.ncbi.nlm.nih.gov">https://www.ncbi.nlm.nih.gov</a>)] was obtained from Plant Protection Central Research Institute, Ankara, Turkey. Five agar plugs (5 mm) from 6 day-old colonies of <em>P. cactorum</em> 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.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2.2. Greenhouse trials</th>
</tr>
</thead>
</table>
| 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).

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.

\[
\text{Disease severity} (\%) = \frac{\sum (n_i \times v_i)}{N \times V}
\]

where; \(v_i\) is the scale class, \(n_i\) 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 Tawsend-Heuberger formula below (Karman 1971).

\[
\% \text{ efficacy} = \frac{[\text{Ic} - \text{It}]/\text{Ic}}{\times 100}
\]
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

Figure 2.

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 ($P<0.01$).

**Table 2.**

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* (Kipngen 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 (Hu-Zhe et al. 2004; 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 Phytophthora cactorum. Similarly, in a study, Phytophthora 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 (Dordas 2008). 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; Schalchi 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 heights 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

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 (Dordas 2008). 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. Furthermore, 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

Conflict of interest: The author declares that there is no conflict of interest.

References


avocado display antagonistic activity against Phytophthora cinnamomi through volatile emissions. PLoS ONE 13(3): e0194665. DOI: https://doi.org/10.1371/journal.pone.0194665.


**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

<table>
<thead>
<tr>
<th>Trademark</th>
<th>Active Ingredient/Microorganism</th>
<th>Recommended application doses</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Companion</td>
<td><em>Bacillus subtilis</em> GB03</td>
<td>0.5 mL / m²</td>
<td>Growth products</td>
</tr>
<tr>
<td>Symbion Vam</td>
<td><em>Glomus fasciculatum</em></td>
<td>1 g /m²</td>
<td>Stanes</td>
</tr>
<tr>
<td>Green Miracle</td>
<td>Vegetable oil acid</td>
<td>0.2 mL /m²</td>
<td>Stanes</td>
</tr>
<tr>
<td>Cropset</td>
<td><em>Lactobacillus acidophilus</em>, plant extract, MnSO₄, FeSO₄, CuSO₄</td>
<td>0.06 mL /m²</td>
<td>Ant tarım (Improcrop EU)</td>
</tr>
<tr>
<td>Product</td>
<td>Description</td>
<td>Concentration</td>
<td>Supplier</td>
</tr>
<tr>
<td>------------------</td>
<td>------------------------------------------------------------------------------</td>
<td>---------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>Isr-2000</td>
<td>Lactobacillus acidophilus, plant extract, yeast extract, benzoic acid</td>
<td>0.1 mL/m²</td>
<td>Ant tarım (Improcrop EU)</td>
</tr>
<tr>
<td>Actinovate</td>
<td>Streptomyces lydicus</td>
<td>0.4 mL/m²</td>
<td>Mts agro</td>
</tr>
<tr>
<td>Subtlex</td>
<td>Bacillus subtilis MBI 600</td>
<td>0.5 mL/m²</td>
<td>Bioglobal</td>
</tr>
<tr>
<td>Tricho plus</td>
<td>Trichoderma harzihanum</td>
<td>0.04 g/m²</td>
<td>Bioglobal</td>
</tr>
<tr>
<td>Alexin 95 PS</td>
<td>Phosphorus pentoxide (P₂O₅) 52%, Potassium oxide (K₂O) 42%</td>
<td>0.7 g/m²</td>
<td>Sumitoma</td>
</tr>
<tr>
<td>Combat Plus</td>
<td>Plant activators</td>
<td>0.4 mL/m²</td>
<td>Bioglobal</td>
</tr>
</tbody>
</table>
| Endo Roots Soluble | Glomus intraradices......................21  
                               | Glomus aggregatum.........................20  
                               | Glomus mosseage...............................20  
                               | Glomus clarum................................1  
                               | Glomus monosporus...............................1  
                               | Glomus deserticola............................1  
                               | Glomus brasillianum...........................1  
                               | Glomus etunicatum............................1  
                               | Gigaspora margarita............................1 | 0.5 mL/m² | Bioglobal |
Table 2. Effects of the commercial products on plant growth parameters (trunk diameter, sapling height, number of branches and root dry weight) and disease severity in apple saplings of Red Chief variety grafted onto MM106 rootstock and efficacy rates of the commercial products.

<table>
<thead>
<tr>
<th>Applications</th>
<th>Trunk diameter (mm)*</th>
<th>Sapling height (cm)*</th>
<th>Number of branches*</th>
<th>Root dry weight (g)</th>
<th>Disease severity (%)*</th>
<th>Efficacy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Companion</td>
<td>17.00±0.55 de (13.51-21.27)</td>
<td>153.61±7.38 bcd (105.00-197.00)</td>
<td>1.69±0.18 b (1.00-3.00)</td>
<td>112.34±6.92 abc (83.98-152.05)</td>
<td>6.25 bc</td>
<td>87.5 ab</td>
</tr>
<tr>
<td>Symbion Vam</td>
<td>18.46±0.42 abcd (16.64-23.21)</td>
<td>161.65±5.26 abcd (126.00-202.00)</td>
<td>1.72±0.31 b (0.00-5.00)</td>
<td>120.58±8.22 abc (77.00-159.70)</td>
<td>12.5 bc</td>
<td>62.5 abc</td>
</tr>
<tr>
<td>Green Miracle</td>
<td>17.87±0.34 bcde (15.95-20.53)</td>
<td>172.67±6.92 ab (110.00-215.00)</td>
<td>1.50±0.20 b (1.00-4.00)</td>
<td>110.19±5.49 abc (85.85-134.23)</td>
<td>15.62 bc</td>
<td>58.33 abc</td>
</tr>
<tr>
<td>Cropset</td>
<td>17.29±0.40 cde (13.15-19.67)</td>
<td>171.39±6.21 ab (129.00-221.00)</td>
<td>2.06±0.29 b (1.00-5.00)</td>
<td>99.74±7.68 bc (58.88-126.96)</td>
<td>21.875 b</td>
<td>50 c</td>
</tr>
<tr>
<td>Isr-2000</td>
<td>18.81±0.55 abc (15.06-24.23)</td>
<td>178.06±5.00 a (139.00-212.00)</td>
<td>1.89±0.25 b (1.00-4.00)</td>
<td>113.76±7.43 abc (69.05-141.09)</td>
<td>12.5 bc</td>
<td>62.5 abc</td>
</tr>
<tr>
<td>Actinovate</td>
<td>17.89±0.46 bcde (14.96-20.15)</td>
<td>175.15±5.03 a (151.00-215.00)</td>
<td>2.24±0.22 b (1.00-4.00)</td>
<td>121.24±8.82 abc (70.68-168.64)</td>
<td>12.5 bc</td>
<td>62.5 abc</td>
</tr>
<tr>
<td>Subtilex</td>
<td>19.79±0.43 a (16.79-22.94)</td>
<td>172.17±6.24 ab (113.00-211.00)</td>
<td>1.82±0.20 b (1.00-3.00)</td>
<td>125.16±6.16 a (93.00-151.66)</td>
<td>9.375 bc</td>
<td>75 abc</td>
</tr>
<tr>
<td>Tricho Plus</td>
<td>17.61±0.53 bcde (12.79-20.78)</td>
<td>138.89±6.70 d (90.00-193.00)</td>
<td>1.86±0.23 b (1.00-4.00)</td>
<td>121.65±6.82 ab (97.40-161.51)</td>
<td>18.75 bc</td>
<td>56.25 bc</td>
</tr>
<tr>
<td>Alexin 95 PS</td>
<td>18.92±0.54 ab (14.21-24.74)</td>
<td>181.11±7.57 a (122.00-238.00)</td>
<td>1.88±0.21 b (1.00-4.00)</td>
<td>126.86±5.68 a (95.50-147.65)</td>
<td>3.125 bc</td>
<td>93.75 a</td>
</tr>
<tr>
<td>Combat Plus</td>
<td>17.37±0.33 cde (15.05-20.12)</td>
<td>163.67±7.27 abc (85.00-204.00)</td>
<td>2.22±0.33 b (1.00-6.00)</td>
<td>98.86±7.04 c (72.25-133.68)</td>
<td>21.87 b</td>
<td>50 c</td>
</tr>
<tr>
<td>Endo Roots Soluble</td>
<td>17.85±0.53 bcde (11.30-22.14)</td>
<td>164.83±6.51 abc (112.00-210.00)</td>
<td>1.67±0.14 b (1.00-3.00)</td>
<td>107.82±6.41 abc (66.33-139.38)</td>
<td>6.25 bc</td>
<td>87.5 ab</td>
</tr>
<tr>
<td>C0</td>
<td>17.31±0.34 cde (14.45-19.76)</td>
<td>151.78±5.91 bcd (118.00-205.00)</td>
<td>3.18±0.43 a (1.00-7.00)</td>
<td>110.20±4.94 abc (93.55-140.85)</td>
<td>0 c</td>
<td>-</td>
</tr>
<tr>
<td>C1</td>
<td>16.78±0.54 e (13.05-20.63)</td>
<td>145.22±5.78 cd (113.00-193.00)</td>
<td>2.11±0.24 b (1.00-4.00)</td>
<td>98.80±5.52 c (68.35-119.66)</td>
<td>43.75 a</td>
<td>-</td>
</tr>
</tbody>
</table>

* 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

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