

Original article (Orijinal araştırma)

Effects of 24-epibrassinolide on root-knot nematode, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) in tomatoes¹

24-Epibrassinolidin domateslerde kök-ur nematodu, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) üzerine etkileri

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Abstract

In this study conducted in 2020, three concentrations (1, 5 and 10 µM) of 24-epibrassinolide were applied to seedlings of *Lycopersicon esculentum* Mill. (Solanales: Solanaceae) cv. H2274, which is susceptible to root-knot nematodes, by immersion, spray and irrigation, and its effects against root-knot nematode *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) were investigated. One-thousand second stage juveniles of *M. incognita*, collected from cucumber roots in a greenhouse located in Çardak, Çanakkale were inoculated on the roots of the plants per pot. After eight weeks, stem fresh weight, stem dry weight, root diameter and longest root length values, in addition to the stem length and stem diameter measured at the beginning and end of the experiment, were recorded. 24-Epibrassinolide, applied by an immersion method, gave similar or better results than the control even in the presence of nematodes. Distilled water plus nematode application showed the highest gall index whereas 5 µM 24-epibrassinolide plus nematode application gave the lowest gall index. The lowest number of egg mass was also obtained from the same concentration of 24-epibrassinolide applied by immersion. As a result, 24-epibrassinolide showed a beneficial effect in terms of reducing the damage caused by the nematodes in tomato plants, depending on the concentration and application method.

Keywords: Biotic stress, brassinosteroids, resistance, root-knot nematode, tomato

Öz

2020 yılında yürütülen bu çalışmada nematoda duyarlı olan *Lycopersicon esculentum* Mill. (Solanales: Solanaceae) H2274 çeşidinin fidelerine daldırma, spreyleme ve sulama şeklinde 24-epibrassinolidin üç konsantrasyonu (1, 5 ve 10 µM) uygulanmış ve kök-ur nematodu, *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae)'ya karşı etkileri araştırılmıştır. Her bir saksıya *Meloidogyne incognita*'nın Çanakkale, Çardak'ta bir seradan alınan hıyar köklerinden elde edilen 1000 adet ikinci dönem larvası bitkilerin köklerine verilmiştir. Sekiz hafta sonunda, denemenin başlangıcında ve sonunda ölçülen gövde boyu ve gövde çapına ilave olarak gövde yaş ve kuru ağırlıkları ile kök çapı ve en uzun kök uzunluğu değerleri kaydedilmiştir. Daldırma şeklinde verilen 24-epibrassinolidin nematod varlığında dahi kontrol bitkilerine yakın veya daha iyi sonuçlara neden olduğu belirlenmiştir. Saf su+nematod uygulaması en yüksek gal indeksini, 5 µM 24-epibrassinolid+nematod uygulaması ise en düşük gal indeksini göstermiştir. Aynı konsantrasyondaki 24-epibrassinolid daldırma şeklinde uygulandığında da en az yumurta paketi sayısı tespit edilmiştir. Sonuç olarak, 24-epibrassinolid nematodun zararlarını azaltma yönünden konsantrasyona ve uygulama yöntemine bağlı olarak destekleyici bir etki göstermiş olup nematodların domates bitkileri üzerindeki etkilerini hafifletmiştir.

Anahtar sözcükler: Biyotik stres, brassinosteroidler, dayanıklılık, kök-ur nematodu, domates

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Introduction

For supplying the nutritional needs of the increasing populations both in the world and in Turkey, it is essential to obtain more production per unit area, and agricultural activities and research are by conducted to achieve this goal. One of the most important of horticultural crops is tomato, which is a strategic and highly profitable crop for humanity. While there are many diseases and pests causing significant losses in tomato production, root-knot nematodes (*Meloidogyne* spp.) are especially known for causing serious damage. *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949, *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949, *Meloidogyne arenaria* (Neal, 1889) Chitwood, 1949 and *Meloidogyne hapla* Chitwood, 1949 (Tylenchida: Meloidogynidae) are the most common species (Moens et al., 2009). *Meloidogyne incognita* can infest almost all plant families, including vegetables (Trudgill & Blok, 2001). These nematodes cause weakening in plants by inhibiting water and nutrients uptake from the soil due to the large or small galls they form in the roots and can eventually lead to death of the plant in severe infestations (Williamson & Hussey, 1996; Karssen & Moens, 2006; Moens et al., 2009).

Nematicides are extensively used against root-knot nematodes all over the world, but the use of pesticides has been steadily declining because of the increasing environmental and human health problems. They are restricted due to toxic effects on both plants and organisms found in soil flora (Nyczepir & Thomas, 2009). Therefore, intensive research is being conducted on alternative methods to control root-knot nematodes. Among these methods, the use of plant-based compounds stands out. Brassinosteroids (BRs) elongate cells and increase cell division in stem, prevent root growth, induce vessel differentiation (Mandava, 1988; Nemhauser et al., 2004).

BRs can have key roles in stress-response systems demonstrated by research on the use of BRs against different types of stresses, such as heat, cold, salinity, heavy metal, and pathogens (including bacteria, fungi, and virus) (Hotta et al., 1998; Yi et al., 1999; De Vleeschauwer et al., 2013; De Bruyne et al., 2014). Research on BRs indicated that they may help overcome stress by inducing reactive oxygen species scavenging enzymes (Jasrotia & Ohri, 2014) or improving plant development (Grace et al., 2009). Lower incidence of infection by late blight, caused by *Phytophthora infestans* (Mont.) de Bary (Peronosporales: Pythiaceae) on potatoes sprayed with BRs was determined (Khripach et al., 1996). In barley, potato tubers and cucumber plants, brassinosteroid-induced disease resistance was also reported (Khripach et al., 2000). Induction of disease resistance to pathogens by BRs has also been shown in tobacco and rice (Nakashita et al., 2003). Effects of BRs on preventing damage caused by nematodes have also been under investigation. For example, *in vitro* tomato plants had better resistance when their seeds were pretreated with 28-homobrassinolide (Kaur et al., 2013) and 24-epibrassinolide (Jasrotia & Ohri, 2017) against *M. incognita*. The results of these studies have warranted further research on BRs for their inducing effects on plant health. However, since much of the research has been conducted *in vitro*, pot and/or field experiments are also needed. Therefore, in this study, tomato plants were raised in pots to observe the effects of 24-epibrassinolide at different concentrations (1, 5 and 10 μ M) and different application methods (immersion, irrigation, and spray) against *M. incognita*.

Materials and Methods

Plant materials and the compounds

Seedlings (3-4 leaf) of tomato [*Lycopersicon esculentum* Mill. (Solanales: Solanaceae)] cv. Heinz 2274 (H2274), reported as susceptible against *M. incognita* (Barker et al., 1985), were obtained from a commercial company. The chemical 24-epibrassinolide (Sigma-Aldrich E1641, Merck, St Louis, MI, USA) was purchased and stored at -20°C until the experiment started.

Preparation of plants and 24-epibrassinolide solutions

The study was conducted in 2020 in plastic pots in a control environment room at 27±2°C. The growing medium, consisting of 70% sand and 30% soil, was sterilized and added to 1.4-l pots, then H2274 seedlings were transplanted to the pots. After 24-epibrassinolide was dissolved in pure ethanol, working solutions were prepared using distilled water at concentrations of 1, 5 and 10 µM. Solutions were stored in dark at +4°C. Prior to the 24-epibrassinolide applications to the seedlings, 0.02% Tween-20 was added only to the spray solutions as a surface wetting agent.

Mass production of *Meloidogyne incognita*

Çanakkale isolate of *M. incognita* was used in the study. Nematode-infected cucumber plants were taken from an infested greenhouse in Çardak, Çanakkale. Infected roots were thoroughly washed and cleaned from soil and other substances. Then the egg masses were extracted from the roots and kept in Petri dishes in distilled water to obtain second stage juveniles (J2s). The distilled water in the Petri dishes was renewed at 24-h intervals and nematode suspension was stored. These J2s, found in suspension, were stored in 10 ml tubes at +4°C until used for the inoculations. The viability of J2s was checked under the stereomicroscope (Leica DM 1000, Leica Microsystems, Germany) before inoculation.

Inoculation of nematodes and application of 24-epibrassinolide

After the seedlings were transplanted into the pots, J2s of *M. incognita* were inoculated as 1000 J2s/pot in all applications. The seedlings were grown at 27±2°C in 18:6 h L:D photoperiod for 8 weeks. In the immersion method, the soil surrounding the roots was carefully washed with water, and the roots were immersed in a container with 24-epibrassinolide solution for 10 min before transplanting followed by nematode inoculation. Control groups of the immersion method were kept in distilled water for the same duration. In spray and irrigation methods, nematode inoculation was performed immediately after the 24-epibrassinolide application. 24-Epibrassinolide was applied three times (1, 7 and 14 d after planting) starting with 10 ml per plant, and increasing to 20 and 40 ml per plant, respectively. Control plants of both methods received the same amount of distilled water.

Collecting data on nematode development and morphological characteristics

At the end of the experiment (56 d), to determine the nematode damage, galls on the roots were evaluated according to the 0-10 scale by Piedra-Buena et al. (2011) adapted from Bridge & Page (1980). The number of egg masses was visually scored under the stereo microscope. Reproduction factor (R0) of the nematode was calculated using the formula of $R0 = \text{final population}/\text{initial population}$.

At the end of the first 24 h following planting, the stem length and the stem diameter was measured. When the plants stopped growing by the end of week 8 (56 d), they were uprooted, and the following data were obtained from the aboveground part: (a) increase in stem length (x fold) = final stem length (cm)/initial stem length (cm), (b) increase in stem diameter (x fold) = final stem diameter (cm)/initial stem diameter (cm), (c) stem fresh and dry weights (g) (after 48 h at 70°C), (d) longest root length (cm), and (e) root diameter (cm).

Statistical analysis

The experiment was conducted with three replicates with one plant per pot in a completely randomized experiment. Comparison of means on both morphological and nematode development was done using analysis of means procedure (Mendeş & Yiğit, 2018; Mendeş, 2019). The analysis was implemented on R statistical package program (version 4.0.2; 2020-06-22) (Pallmann & Hothorn, 2016). The results are presented graphically. Treatments not receiving nematode addition were excluded during the analysis of the nematode development. Data for the root gall index and egg mass number were normalized by square root transformation.

Results

Based on the data analysis, the interaction of 24-epibrassinolide and application method on root gall index, egg mass number and R0 was found significant (Figures 1-3). Significantly higher gall index was found compared to the other applications when T4 (distilled water+J2s) was applied by irrigation to the tomato plants (Figure 1). Although there was no significant difference between other applications, it was observed that T2 (5 μ M 24-epibrassinolide+J2s) decreased the root gall index in all application methods. The highest concentration of 24-epibrassinolide T3 (10 μ M 24-epibrassinolide+J2s) gave the same effect only with the irrigation and spray methods.

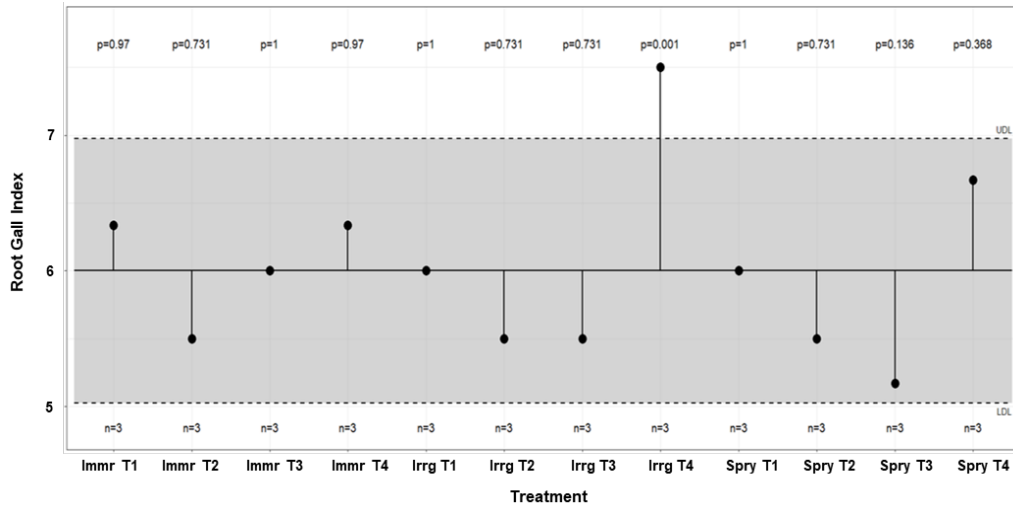


Figure 1. Interaction of application method and 24-epibrassinolide (EBL) rate on root gall index in tomato cv. H2274 (T1, 1 μ M EBL+J2s; T2, 5 μ M EBL+J2s; T3, 10 μ M EBL+J2s; T4, distilled water+J2s; Immr, immersion; Irrg, irrigation; Spry, spray; LDL, lower decision limit; and UDL, upper decision limit).

Like gall index, the number of egg mass on roots reached the highest values with distilled water+J2s application (T4) by the irrigation method (Figure 2). The lowest number of egg masses was with 5 μ M 24-epibrassinolide+J2s (T2) applied by irrigation. The most successful results in reducing the number of egg mass were obtained when 24-epibrassinolide was applied to the plants by the immersion method at all concentrations.

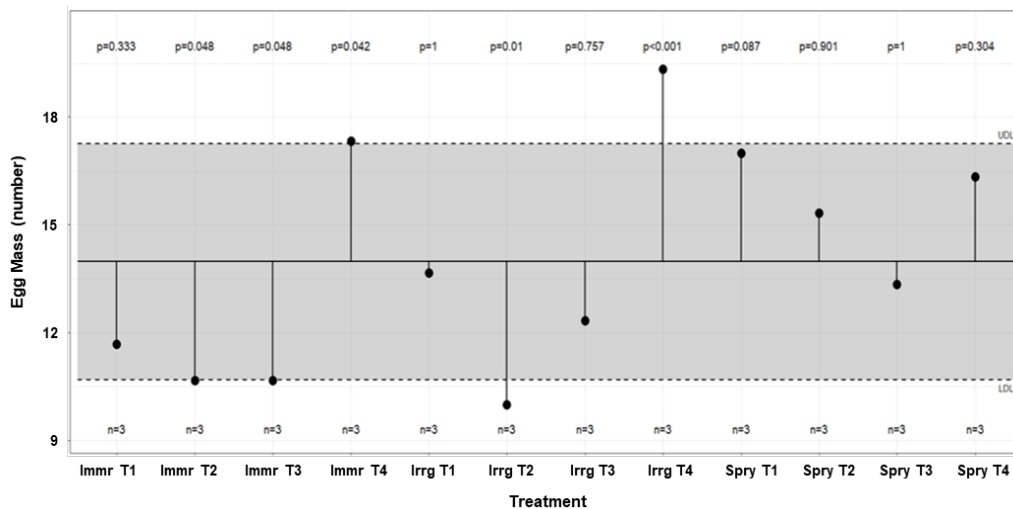


Figure 2. Interaction of application method and 24-epibrassinolide (EBL) rate on egg mass number in tomato cv. H2274 (T1, 1 μ M EBL+J2s; T2, 5 μ M EBL+J2s; T3, 10 μ M EBL+J2s; T4, distilled water+J2s; Immr, immersion; Irrg, irrigation; Spry, spray; LDL, lower decision limit; and UDL, upper decision limit).

Nematode R0 was the highest with the application of distilled water+J2s (T4) by the irrigation method (Figure 3). Nematode R0 was reduced with 1 μM 24-epibrassinolide+J2s (T1) treatment applied by the immersion method. When 24-epibrassinolide was applied to the plants by the immersion and spray methods, even at low 24-epibrassinolide concentrations, R0 remained at a low level compared to other treatments.

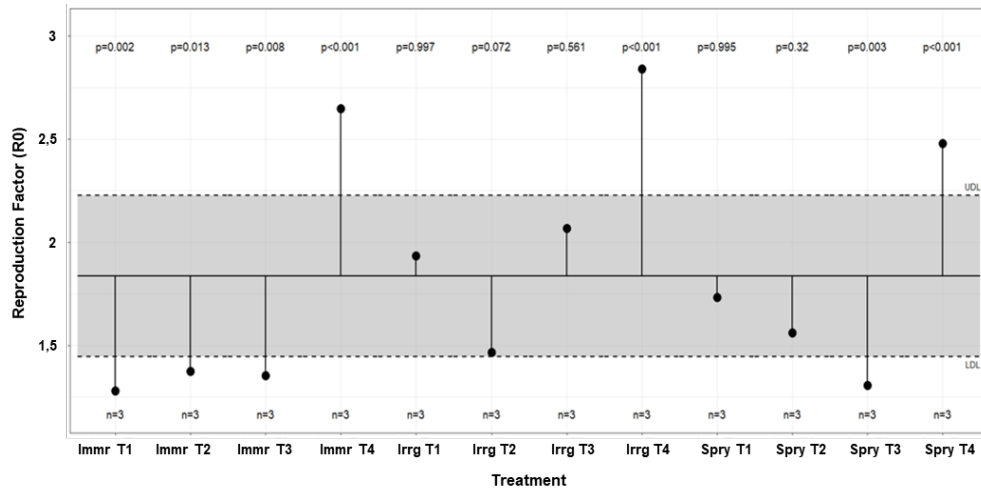


Figure 3. Interaction of application method and 24-epibrassinolide (EBL) rate on nematode reproduction factor in tomato cv. H2274 (T1, 1 μM EBL+J2s; T2, 5 μM EBL+J2s; T3, 10 μM EBL+J2s; T4, distilled water+J2s; Immr, immersion; Irrg, irrigation; Spry, spray; LDL, lower decision limit; and UDL, upper decision limit).

The data analysis revealed that the interaction between the application methods and 24-epibrassinolide rates were significant for changes in stem length and stem diameter, stem fresh and dry weights and the longest root length (Figures 4 to 8). Increase in stem length was highest with the immersion of seedlings in distilled water (T1), 10 μM 24-epibrassinolide (T8), 5 μM 24-epibrassinolide+J2s (T4) and 10 μM 24-epibrassinolide+J2s (T5) (Figure 4). Neither irrigated plants nor sprayed plants were significantly affected.

Increase in stem diameter did not significantly differ between the treatments, apart from with 10 μM 24-epibrassinolide+J2s applied as a spray (T5) (Figure 5). However, Figures 4 and 5 show that when the plants invested more on their longitudinal growth, they had a decreased radial growth.

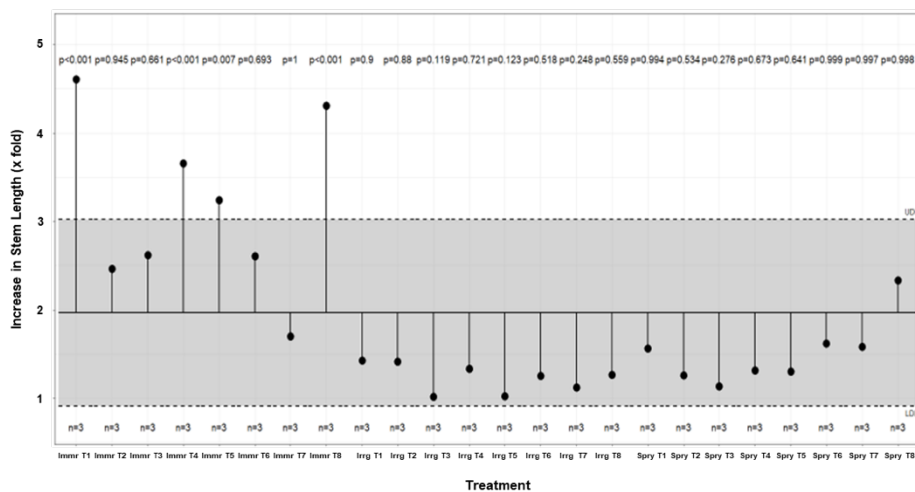


Figure 4. Interaction of application method and 24-epibrassinolide (EBL) rate on increase in stem length (x fold) in tomato cv. H2274 (T1, distilled water; T2, distilled water+J2s; T3, 1 μM EBL+J2s; T4, 5 μM EBL+J2s; T5, 10 μM EBL+J2s; T6, 1 μM EBL; T7, 5 μM EBL; T8, 10 μM EBL; Immr, immersion; Irrg, irrigation; Spry, spray; LDL, lower decision limit; and UDL, upper decision limit).

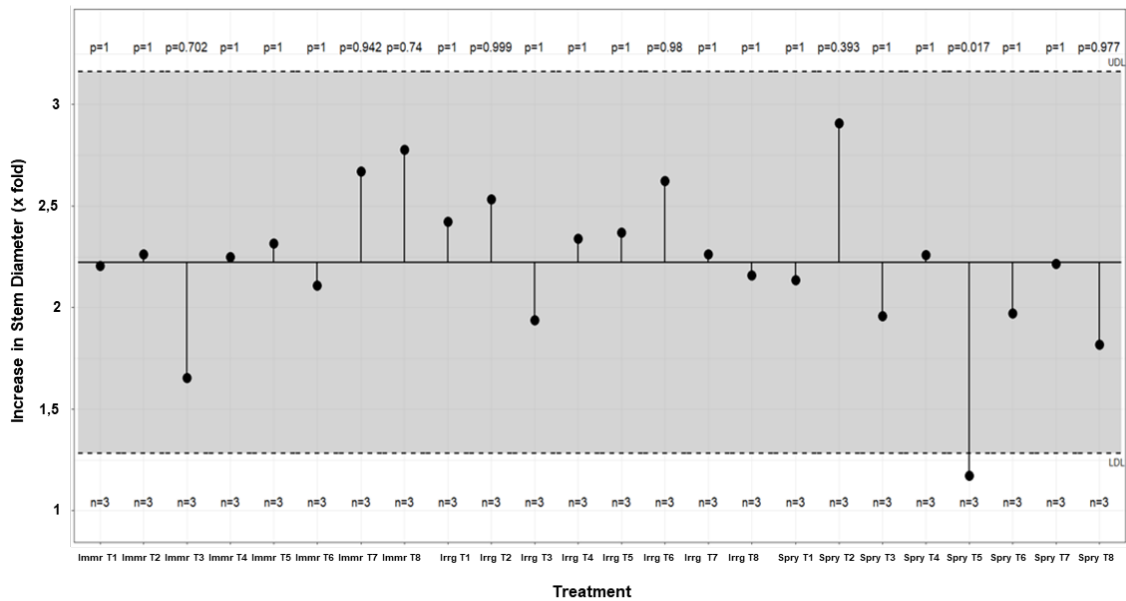


Figure 5. Interaction of application method and 24-epibrassinolide (EBL) rate on increase in stem diameter (x fold) in tomato cv. H2274 (T1, distilled water; T2, distilled water+J2s; T3, 1 μ M EBL+J2s; T4, 5 μ M EBL+J2s; T5, 10 μ M EBL+J2s; T6, 1 μ M EBL; T7, 5 μ M EBL; T8, 10 μ M EBL; Immr, immersion; Irrg, irrigation; Spry, spray; LDL, lower decision limit; and UDL, upper decision limit).

Stem fresh weight of the plants differed with application method for 24-epibrassinolide and/or nematode (Figure 6). For example, immersion in 24-epibrassinolide resulted the highest weight, but the same effect was not observed when this was applied by irrigation or spray, and nematode inoculation decreased fresh weight. Stem dry weight showed similar responses to stem fresh weight (Figure 7).

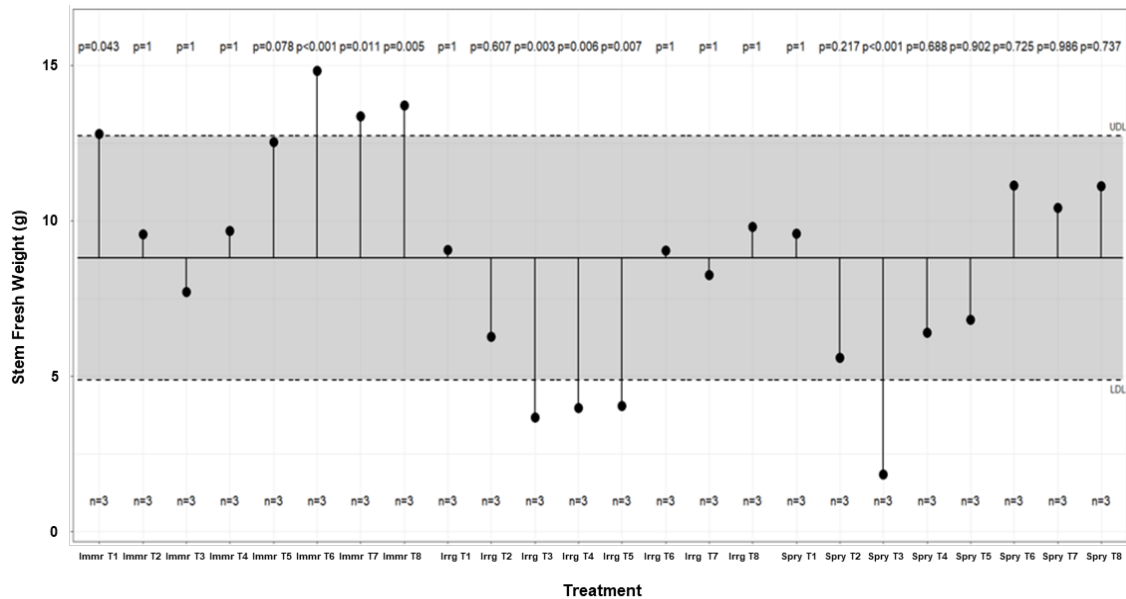


Figure 6. Interaction of application method and 24-epibrassinolide (EBL) rate on stem fresh weight (g) in tomato cv. H2274 (T1, distilled water; T2, distilled water+J2s; T3, 1 μ M EBL+J2s; T4, 5 μ M EBL+J2s; T5, 10 μ M EBL+J2s; T6, 1 μ M EBL; T7, 5 μ M EBL; T8, 10 μ M EBL; Immr, immersion; Irrg, irrigation; Spry, spray; LDL, lower decision limit; and UDL, upper decision limit).

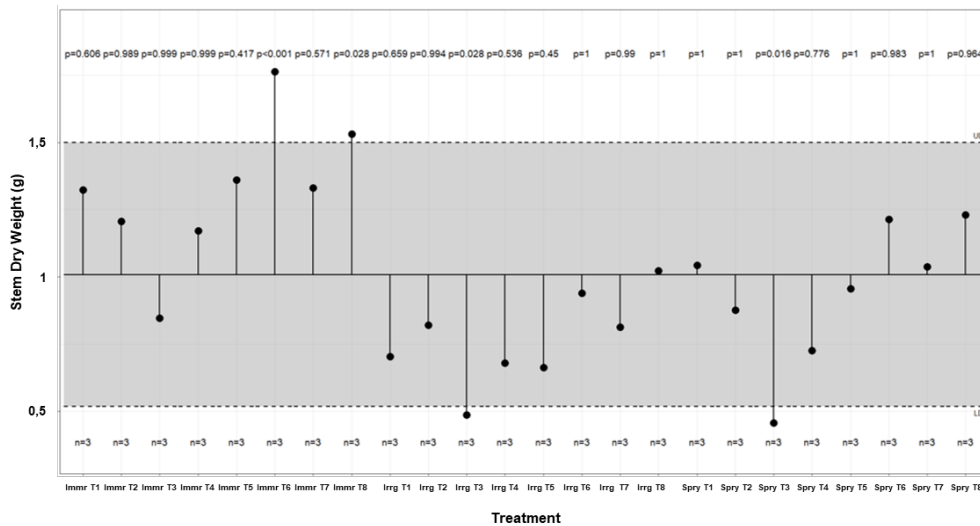


Figure 7. Interaction of application method and 24-epibrassinolide (EBL) rate on stem dry weight (g) in tomato cv. H2274 (T1, distilled water; T2, distilled water+J2s; T3, 1 μ M EBL+J2s; T4, 5 μ M EBL+J2s; T5, 10 μ M EBL+J2s; T6, 1 μ M EBL; T7, 5 μ M EBL; T8, 10 μ M EBL; Immr, immersion; Irrg, irrigation; Spry, spray; LDL, lower decision limit; and UDL, upper decision limit).

Roots were the longest and significantly different with 1 μ M 24-epibrassinolide applied by immersion (T6) and the shortest with 1 μ M 24-epibrassinolide+J2s applied as a spray (T3) (Figure 8). Overall, the figure indicates that nematode infection shortened the roots no matter how 24-epibrassinolide was applied. The data analysis of root diameter revealed that only application methods have a significant effect with immersion giving in longer than irrigation and spray (plot not shown).

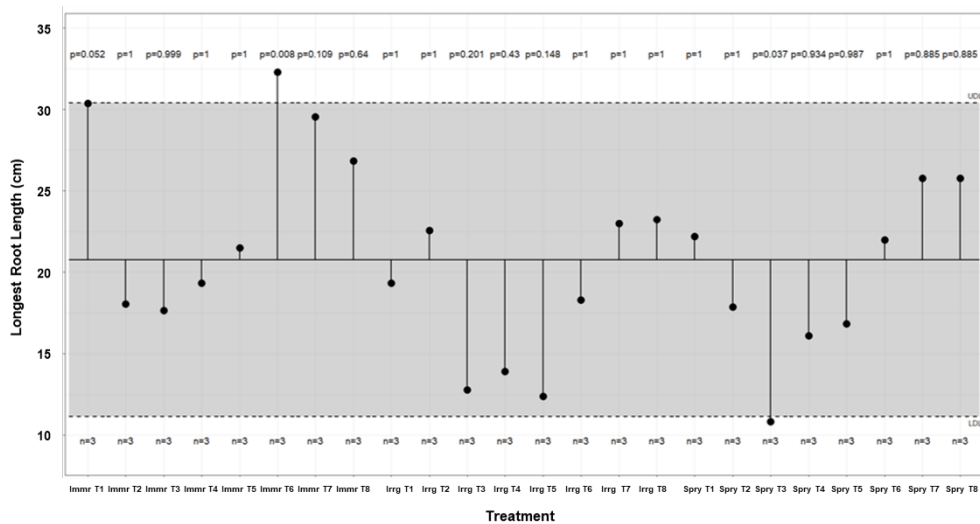


Figure 8. Interaction of application method and 24-epibrassinolide (EBL) rate on the longest root length (cm) in tomato cv. H2274 (T1, distilled water; T2, distilled water+J2s; T3, 1 μ M EBL+J2s; T4, 5 μ M EBL+J2s; T5, 10 μ M EBL+J2s; T6, 1 μ M EBL; T7, 5 μ M EBL; T8, 10 μ M EBL; Immr, immersion; Irrg, irrigation; Spry, spray; LDL, lower decision limit; and UDL, upper decision limit).

Discussion

Although BRs are known to be affect stress response in plants, little is known about their effects against pathogens, particularly plant parasitic nematodes. Nakashita et al. (2003) reported that brassinolide induces disease resistance against tobacco mosaic virus, *Pseudomonas syringae* van Hall, 1902 (Pseudomonadales: Pseudomonadaceae) and *Oidium* sp. (Erysiphales: Erysiphaceae) in tobacco plants and *Pycularia grisea* Cooke ex Sacc., 1886 (Magnaporthales: Pyculariaceae) and *Xanthomonas oryzae*

(Ishiyama, 1922) Swings et al., 1990 (Xanthomonadales: Xanthomonadaceae) in rice. In a similar study by Ding et al. (2009) it was observed that foliar and root 24-epibrassinolide application significantly increased the resistance to *Fusarium* spp. (Hypocreales: Nectriaceae). In our study, root gall index, number of egg mass and nematode R0 were decreased with the application of 24-epibrassinolide depending on the concentration and application method. These results were consistent with the findings of Song et al. (2017), who reported that foliar application of 24-epibrassinolide reduced the gall numbers at different doses in tomato and it had an important effect on root resistance against nematodes. Reduction in number of galls were reported by Kaur et al. (2013, 2014a) with the use of 28-homobrassinolide. Effects of 24-epibrassinolide in reducing the number of galls in tomato were also reported by Jasrotia & Ohri (2017) in plants cultured *in vitro*.

It was observed in the current study that the increase in stem length was higher with the application of 24-epibrassinolide and radial growth of the roots were compromised at the expense of longitudinal growth. This effect of 24-epibrassinolide was also observed in carrot by Que et al. (2017). *Meloidogyne incognita* infected tomato plants had slight increase in plant height when exposed to 28-homobrassinolide (Kaur et al., 2013, 2014 a, b). Jasrotia & Ohri (2017) reported similar effects of 24-epibrassinolide on increasing stress tolerance of *in vitro* tomato plants through inducing antioxidant enzymes. These studies reveal that BRs can support aboveground plant growth and help alleviate nematode-induced stress in plants. Stem fresh weights of the plants differed with the method used to apply 24-epibrassinolide and nematodes. The immersion method resulted the highest weight, but the same effect was not observed with irrigation or spray application and nematode inoculation decreased stem fresh weights. Decreases in plant biomass with nematode infestation of tomato plants were also reported by Opoku-Asiama & Yeboah (2003) and Kaur et al. (2014b). Stem dry weight responded similarly to stem fresh weight. Ali et al. (2006) reported that the plant weight of tomato seedlings immersed in 28-homobrassinolide solution decreased as the concentration increased but was higher than the control. However, a similar effect was not observed in the plants, independent of the application method in the current study. Root length depended on the concentration of the 24-epibrassinolide applied and nematode infection shortened the roots irrespective of the application method. Müssig et al. (2003) found that BRs applied exogenously to *Arabidopsis thaliana* (L.) Heynh. (Brassicales: Brassicaceae) plants were effective in promoting root length. Uzunoğlu & Gökbayrak (2018) also reported that both 28-homobrassinolide and 24-epibrassinolide was successful in improving root characteristics in grapevine.

In conclusion, the results of this study confirm the potential of 24-epibrassinolide in enhancing plant defense against root-knot nematodes, even though any specific concentration or application method was not found optimal. However, it is of note that application of 10 μ M 24-epibrassinolide by immersion was better at modulating of anti-stress responses in plant growth and tolerance to nematode infestation. This confirmation of the benefits of BRs in potted plants justifies the extension of the work to field experiments. In addition, the application of BRs in conjunction with other *Meloidogyne* spp. might provide a better understanding its capacity to induce nematode resistance in plants.

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