Bitki Koruma Bülteni / Plant Protection Bulletin

http://dergipark.gov.tr/bitkorb

Orijinal araştırma (Original article)

Management of Egyptian broomrape [*Phelipanche aegyptiaca* (Pers.) Pomel] using biofungicide (*Trichoderma* spp.) in tomato

Domateste biyofungisit (*Trichoderma* spp.) kullanılarak Mısırlı canavar otu [*Phelipanche aegyptiaca* (Pers.) Pomel]'nun mücadelesi

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A R T I C L E   I N F O

Article history:
DOI: 10.16955/bitkorb.1657350
Received : 13-03-2025
Accepted : 22-05-2025

Keywords:
tomato, *Phelipanche aegyptiaca*, *Trichoderma* spp., biological control agent

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A B S T R A C T

Phelipanche aegyptiaca (Pers.) Pomel, commonly known as Egyptian broomrape, is a root parasitic plant that causes significant yield losses in tomato production. This study aimed to evaluate the efficacy of a commercial bioproduct containing *Trichoderma asperellum* strain ICC012 and *Trichoderma gamsii* strain ICC080, against *P. aegyptiaca* in tomato. The experiment was conducted under controlled greenhouse conditions using three concentrations of the bioproduct (N: recommended dose, N/2, and 3N/2) in a randomized block design with four replications. Applications were made in two programs: Program A (one week before and one day after planting) and Program B (an additional application 15 days after planting). Results showed that the biological control agent (BCA) applications significantly increased disease severity in *P. aegyptiaca* shoots, with the values ranging from 69.5% to 79.6%, compared to 40% in the control. The number of shoots exhibiting the highest scale value on necrotic area increased significantly from three in the control to 17-19.5 in BCA-treated pots. Additionally, the average number of dead tubercles on tomato roots was 32 in BCA-treated pots, compared to 16 in untreated controls. The fresh weight of BCA-treated broomrape shoots was significantly different from the control, while there was no significant difference in the dry weight of the shoots among treatments. BCA did not significantly alter the weights of aerial parts and the roots of tomato compared to the control. These results suggest that *Trichoderma* species can control broomrape through multiple mechanisms. Further field trials are recommended to validate these findings under natural conditions.

INTRODUCTION

Broomrape species (*Phelipanche* spp. and *Orobanche* spp.) are obligate parasitic plants that establish direct connections with the vascular systems of their host plants, causing significant economic losses in various crops (Dörr and Kollmann 1995). Among these, *Phelipanche aegyptiaca* (Pers.) Pomel (Egyptian broomrape) is particularly damaging to Solanaceous crops, including tomato, with yield losses ranging from 5% to 100% depending on infestation density and environmental conditions (Joel et al. 2013, Parker 2009). In Türkiye, where tomato production accounts for approximately 7% of global output, ranking it as the world's third-largest producer after China and India (FAOSTAT 2022), broomrape-induced yield losses have been reported at 24% (Aksoy and Uygur 2008).

The life cycle of broomrape enters the parasitic phase when the plant establishes a connection to the host's vascular system via the haustorium, enabling it to extract water and nutrients. This parasitic relationship severely compromises the host plant's growth and productivity, leading to significant yield losses (Yoshida et al. 2016). Following attachment, the broomrape develops a tubercle, which serves as the foundation for the emergence of shoots. These shoots eventually break through the soil surface, flower, and produce seeds, completing the life cycle (Rispail et al. 2007).

Conventional control methods are often ineffective or economically unfeasible, prompting the exploration of biological control strategies. *Trichoderma* spp. are widely recognized as effective biocontrol agents, playing a significant role in suppressing plant pathogens (Shoresh et al. 2010). *Trichoderma* spp. exhibit a broad spectrum of biocontrol activity, effectively managing a wide range of foliar (Elad 2000), root (Amira et al. 2017), and fruit pathogens (Li et al. 2025), as well as invertebrates such as nematodes (Poveda et al. 2020). They are known for their ability to antagonize plant pathogens, induce systemic resistance, and enhance plant growth and development, making them effective biological control agents (Harman et al. 2004, Howell 2003, Vinale et al. 2008). Additionally, *Trichoderma* species have been shown to mitigate a wide range of abiotic stresses, such as drought (Shukla et al. 2012), salinity (Rawat et al. 2011), extreme temperatures (Montero-Barrientos et al. 2010), and cold stress (Afrouz et al. 2023). They are widely used in vegetable crops and the most useful strains exhibit the ability to colonize plant roots, a feature known as 'rhizosphere competence' (Harman et al. 2004). For instance, *Trichoderma gamsii* has been shown to enhance systemic resistance in crops, such as maize, against fungal pathogens like *Fusarium verticillioides* (Galletti et al. 2020), while long-term field studies have demonstrated the efficacy of *T. asperellum* and *T. gamsii* in protecting perennial crops (Di Marco et al. 2022). Additionally, *Trichoderma asperellum* ICC 012 and *T. gamsii* ICC 080 strains have been reported to up-regulate key defense-related genes, such as *pr1*, *sod*, *pgip2*, and *pal1*, in wheat, enhancing resistance against *Fusarium* pathogens (Cesarini et al. 2025).

Recent studies have also highlighted the potential of *Trichoderma* species in controlling parasitic weeds. *Trichoderma harzianum* significantly inhibits *Striga hermonthica* seed germination and haustorium initiation, while also enhancing host plant growth, highlighting its potential as a biocontrol agent against parasitic weeds (Azarig et al. 2020). Studies have shown that *Trichoderma viride* can significantly influence *S. hermonthica* seed germination, with higher concentrations (75%) completely inhibiting germination, while also enhancing the growth and vigor of millet varieties, such as increased shoot length, root length, and dry weight (Hassan et al. 2013). For example, *T. asperellum* significantly reduces the germination of *Orobanche cumana* seeds in sunflower (Maširević et al. 2014), while *Trichoderma* spp. (*T. harzianum*, *T. viride*, and *T. virens*) significantly enhance growth parameters in faba bean plants, such as shoot length, shoot fresh weight, shoot dry weight, and leaf number, even in the absence of *Orobanche crenata* infection, demonstrating their potential as effective bio-control agents (El-Dabaa and Abd-El-Khair 2020). Recent studies have also demonstrated that *T. harzianum* suppresses *Phelipanche ramosa* infestation in tomatoes by inhibiting tubercle formation and enhancing antioxidant defenses (Fidan and Tepe 2024). Furthermore, different application methods of *Trichoderma* culture filtrates, such as foliar sprays and soil drenches, have been shown to significantly reduce *P. aegyptiaca* infection in tomatoes. For instance, foliar application of *T. virens* reduced the number of aboveground stalks and underground juveniles by 83% and 66%, respectively, while increasing tomato fruit fresh and dry weights by 86% and 90%. Similarly, soil drench application of *T. brevicompactum* reduced the fresh and dry weights of *P. aegyptiaca* stalks and juveniles by 77%, 52%, 75%, and 49%, respectively (Jalali et al. 2024). Despite the demonstrated efficacy of *Trichoderma* species against various parasitic weeds, the potential of *Trichoderma asperellum* ICC 012 and *T. gamsii* ICC 080, formulated in a commercial product, to control *P. aegyptiaca* in tomato has not been previously investigated.

The aim of this study is to evaluate the efficacy of *Trichoderma asperellum* ICC 012 and *T. gamsii* ICC 080, present in a commercial formulation, against *P. aegyptiaca* in tomato under *in vivo* conditions.

MATERIALS AND METHODS

Plant material

The experiment was conducted using the commonly grown commercial tomato (*Solanum lycopersicum* cv. Bizimköy F1) variety. The seeds of *P. aegyptiaca* used in the experiments were obtained from a prior study (Cignitas and Kitis 2022).

Biological Control Agent (BCA)

The commercial biological fungicide was used *Trichoderma asperellum* strain ICC012 and *Trichoderma gamsii* strain

ICC080 at a concentration of 10^8 CFU/g, presented in a wettable powder (WP) formulation. It is registered for use at a rate of 300 g/da for root rot fungal pathogens (*Fusarium oxysporum*, *Macrophomina phaseolina*, *Pythium* spp., *Rhizoctonia solani*) in strawberry, and 250 g/da for root rot fungal pathogens (*Rhizoctonia solani*, *Fusarium oxysporum*, *Fusarium* spp.) in pepper, and for root rot fungal pathogens (*Pythium* spp., *Rhizoctonia* spp., *Alternaria* spp., *Fusarium* spp.) in tomato.

Preparation of the BCA inoculum

The BCA suspensions were prepared at three different concentrations (N: recommended dose, N/2, 3N/2). An amount of 250 g (registered dose for pepper and tomato) and 300 g (registered dose for strawberry) of the BCA formulation was dissolved in 3 liters of sterile distilled water for N dose. The other doses, N/2 and 3N/2, were adjusted by calculating according to recommended dose. To stimulate the sporulation, the suspensions were placed on a circular shaker for 24 hours at room temperature (25 ± 1°C) before applying the treatments.

Greenhouse experiment

The experiment was conducted in a greenhouse at Batı Akdeniz Agricultural Research Institute (BATEM), Antalya province in Türkiye, during the fall season in 2023. The *P. aegyptiaca* seeds (20 mg/kg soil) were blended with sterilized soil mixture of silt: peat: perlite (1: 1: 1) in 3-liter plastic pots. Thirty-day-old tomato seedlings were used for the host plant of the broomrape. The BCA applications were applied at 100 ml per seedling and performed in two programs: A, B. Program A included two different application times: the first one is seven days before planting the seedlings, and second is one day after planting. Program B included a third one in addition to these two applications, which is 15 days after planting the seedlings. As a negative control, the pots left without BCA, and as a non-infested control, the pots contained neither BCA nor broomrape seeds. The experiment was set up with four replications, with each replication consisting of one plant per pot, under 14 h natural light at 23 ± 1 °C. The study was conducted as a randomized complete block design. The experimental design was shown in Table 1.

Assessment of the experiment

Ninety days after planting the seedlings, the shoots and tubercles of the broomrapes, as well as the roots and aerial parts of the tomato plants, were carefully harvested to evaluate efficacy parameters (Figure 1). Re-isolation of the BCA was performed from the infected parts of the broomrape, fulfilling Koch's postulates.

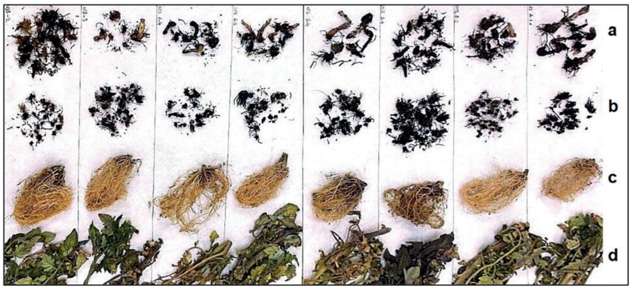

Figure 1. Harvested parts of the Egyptian broomrape and tomato plants. Shoots (a) and tubercles (b) of the *Phelipanche aegyptiaca*, roots (c) and aerial parts (d) of the tomato

Assessment of the effect of the BCA on broomrape

To evaluate the effect of the BCA on the shoots, the necrotic area was scored from 0 to 4 (Figure 2). Based on the scales, percentages of disease severity (DS) were calculated for each treatment according to Townsend-Heuberger formula:

$$DS (\%)=\sum((n\times v))/((Z\times N))\times 100$$

Table 1. The experimental design of the applications

BLOCKS			
I	II	III	IV
3N/2 A	Negative control	N A	N/2 B
N A	N/2 A	Non-infested control	Negative control
N/2 A	3N/2 A	N/2 B	N B
Non-infested control	N/2 B	3N/2 B	N/2 A
3N/2 B	Non-infested control	3N/2 A	N A
N B	3N/2 B	N/2 A	3N/2 A
N/2 B	N A	Negative control	Non-infested control
Negative control	N B	N B	3N/2 B

where n is the number of shoots in the disease scale, v is a numerical value of the disease score, Z is the highest score value, and N is the total number of shoots (Townsend-Heuberger 1943).

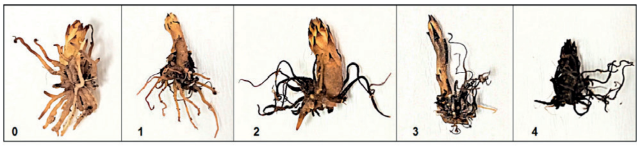

Figure 2. Scale used for evaluation of necrotic area of *Phelipanche aegyptiaca* shoots. 0=Healthy shoot, 1=1-25% necrosis of shoot, 2=26-50% necrosis of shoot, 3=51-75% necrosis of shoot, 4=76-100% necrosis of shoot, dead

To evaluate the effect of the BCA on the tubercles, the average number of healthy and dead tubercles was calculated in per pot both with and without BCA treatments. Tubercles that showed blackening caused by necrosis were considered as dead.

Assessment of the effect of the BCA on biomass

Fresh and dry weights of the parts of broomrape and tomato plant were measured to evaluate the effect of the BCA treatments on biomass. After evaluating regarding necrosis parameters, the shoots and tubercles were weighed, subsequently incubated at 65 °C for 48 h, and then weighed again. The process was also performed for the roots and aerial parts of tomato plants.

Statistical analysis

The data were subjected to ANOVA analysis using the SAS statistical software (SAS Institute, Cary, NC, USA). Differences between treatments were determined using Tukey's test at $P < 0.05$.

RESULTS

Re-isolation of the BCA

As a result of BCA re-isolation, morphological and microscopic observations were confirmed that the causal agent was *Trichoderma* spp. (Figure 3).

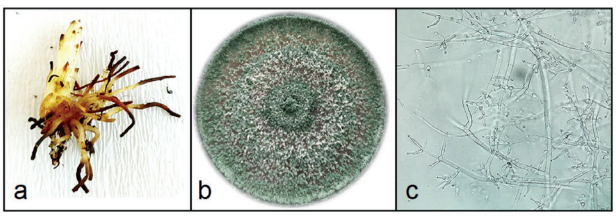

Figure 3. Necrotic roots of the *Phelipanche aegyptiaca* shoots (a), fourteen-day-old *Trichoderma* spp. culture on PDA (b), mycelial structures of the *Trichoderma* spp. at 20x magnification (c)

Effects of the BCA on broomrape

According to the evaluation of the shoots, DS (%) values in the pots treated with BCA were significantly different from the control. The DS values ranged from 69.5% to 79.6% across treatments (Figure 4). There was no statistical difference between the doses and the programs.

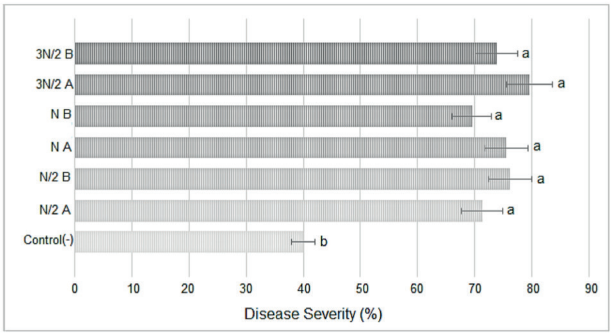

Figure 4. Disease severity (%) values of the treatments

The number of the shoots scored as 0 was 12 in the control, while none of the BCA-treated shoots were scored with 0. As expected, the number of the shoots treated with BCA in N, N/2 and 3N/2 doses peaked at scale 4, while for the control, this scale value was lowest, with the values of 19.5, 17, 18.5 and 3 shoots per pot, respectively (Figure 5).

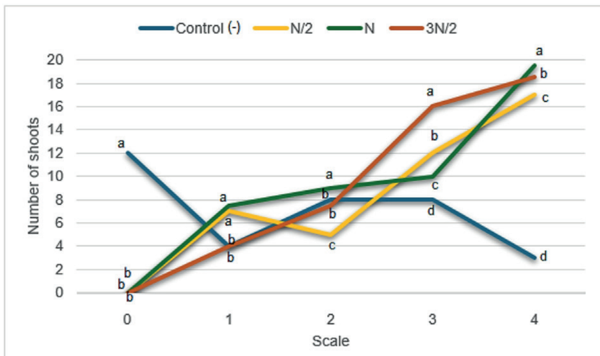

Figure 5. Number of the shoots per pot in treatments on a scale of 0-4

There was a significant difference both with and without BCA in terms of healthy and dead tubercles. Average number of healthy and dead tubercles in the non-treated and BCA-treated BCA pots was 2, 16 and 1.3, 32.1 per pot, respectively (Figure 6).

Effect of the BCA on biomass of broomrape and tomato plants

Fresh weight of the shoots treated with BCA was significantly different from the control, ranging from 3.80 g to 5.71 g. Dry weight of the shoots treated with BCA ranged from 0.56 g to 0.73 g, and there was no difference between any treatments. On the other hand, mean fresh weight of the tubercles

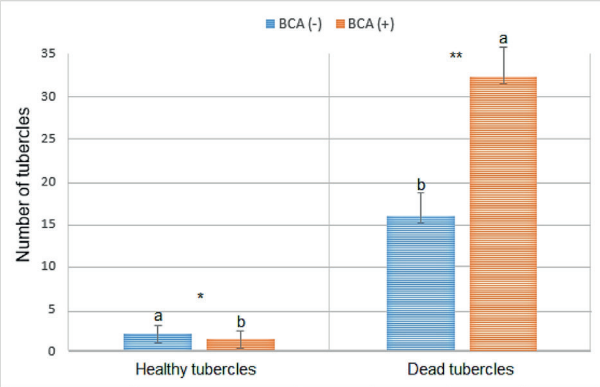

Figure 6. Average number of healthy and dead tubercles per pot in the treatments

ranged from 1.98 g to 4.30 g, and dry weight of the tubercles ranged from 0.29 g to 0.54 g in pots treated with BCA (Table 2).

Fresh weight of the roots of BCA-treated plants ranged from 2.91 g to 4.53 g, with no significant difference from the negative control. Dry weight of the roots of these treatments ranged from 0.54 g to 0.59 g. On the other hand, fresh weight of the aerial parts of BCA-treated plants ranged from 12.7 g to 16.6 g, and dry weight ranged from 1.89 g to 3.32 g. There was no significant difference between the BCA-treated and negative control pots. Non-infested control treatment had the highest value in terms of all the parameters (Table 3).

DISCUSSION

The results showed that applying the biological control agent (BCA) containing *Trichoderma asperellum* ICC 012 and *T. gamsii* ICC 080 significantly increased disease severity (DS) in *Phelipanche aegyptiaca* shoots on tomato plants, with DS values ranging from 69.5% to 79.6% compared to 40% in the control. All treatments nearly doubled the disease severity in *P. aegyptiaca* shoots compared to the control. Furthermore, while the number of shoots naturally exhibiting the highest disease severity (scale 4) averaged 3 in the control, this number significantly increased to 17-19.5 in pots treated with the BCA. These findings indicate that BCA application not only enhances disease severity but also substantially increases the proportion of severely affected

Table 2. Effect of the BCA on biomass of shoot and tubercle of *Phelipanche aegyptiaca*

Treatment	Shoots		Tubercles	
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
Negative control	7.38 a ± 0.97	0.94 a ± 0.09	1.54 a ± 1.45	0.18 b ± 0.13
N/2 A	5.55 ab ± 2.17	0.73 a ± 0.39	2.73 a ± 2.31	0.34 ab ± 0.20
N/2 B	5.71 ab ± 1.19	0.71 a ± 0.08	2.59 a ± 1.12	0.42 ab ± 0.10
N A	4.31 b ± 1.04	0.56 a ± 0.14	3.50 a ± 2.61	0.38 ab ± 0.17
N B	3.80 b ± 2.64	0.64 a ± 0.49	1.98 a ± 1.10	0.29 ab ± 0.16
3N/2 A	4.82 b ± 1.23	0.67 a ± 0.27	2.09 a ± 1.47	0.34 ab ± 0.16
3N/2 B	4.49 b ± 0.86	0.60 a ± 0.13	4.30 a ± 2.90	0.54 a ± 0.26

Mean ± standard deviation (n = 4).
Means with the same letter are not significantly different from each other (Tukey's test, P < 0.05).

Table 3. Effect of the BCA on biomass of root and aerial parts of tomato

Treatment	Shoots		Tubercles	
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
Non-infested control	6.56 a ± 0.43	0.68 a ± 0.11	25.6 a ± 0.62	4.56 a ± 0.11
Negative control	4.13 b ± 1.31	0.60 a ± 0.16	11.7 b ± 0.99	1.93 b ± 0.24
N/2 A	3.34 b ± 1.26	0.57 a ± 0.09	12.7 b ± 1.67	1.89 b ± 0.51
N/2 B	4.13 b ± 0.49	0.59 a ± 0.13	15.1 b ± 5.81	1.89 b ± 0.63
N A	4.53 b ± 0.12	0.55 a ± 0.04	16.6 b ± 3.47	2.66 b ± 0.31
N B	2.91 b ± 0.40	0.57 a ± 0.19	13.3 b ± 3.50	3.32 b ± 0.33
3N/2 A	4.17 b ± 0.32	0.54 a ± 0.05	14.1 b ± 1.30	2.13 b ± 0.40
3N/2 B	3.48 b ± 0.67	0.57 a ± 0.02	13.2 b ± 1.31	2.25 b ± 0.14

Mean ± standard deviation (n = 4).
Means with the same letter are not significantly different from each other (Tukey's test, P < 0.05).

shoots and inhibits tubercle formation. When the tubercles formed on the host root were examined, the average number of dead tubercles was 32 in pots with BCA application, compared to 16 in those without BCA. This suggests that after *P. aegyptiaca* attaches to the host root, *Trichoderma* species likely trigger the plant's defense responses at an early stage, leading to necrosis in the tubercles and preventing successful parasitism. These results highlight the potential of BCA in disrupting both the shoot development and tubercle formation of *P. aegyptiaca*, contributing to its overall suppression. In support of these findings, Azarig et al. (2020) investigated the effects of *T. harzianum* on the early developmental stages (seed germination) and incidence of *S. hermonthica*. They reported that *T. harzianum* reduced *S. hermonthica* seed germination and incidence by approximately 50% compared to the control. These findings align with previous studies showing that *Trichoderma* species can effectively reduce parasitic weed infestation by inhibiting tubercle formation and enhancing host plant resistance (Fidan and Tepe 2024, Maširević et al. 2014).

The observed effects may be attributed to the BCA's ability to induce systemic resistance in tomato plants, as evidenced by the up-regulation of defense-related genes such as *pr1*, *sod*, *pgip2*, and *pal1* (Cesarini et al. 2025). This mechanism is consistent with previous findings that *Trichoderma* species can enhance plant defense mechanisms against various pathogens and parasitic weeds (Galletti et al. 2020, Howell 2003). Additionally, the ability of *Trichoderma* strains to colonize plant roots and exhibit rhizosphere competence (Harman et al. 2004) likely contributed to their effectiveness in reducing *P. aegyptiaca* infestation.

On the other hand, the results indicated that the BCA did not significantly alter the weights of tomato shoots and roots compared to the control in the 90-day pot experiment. However, under field conditions, it is likely that the BCA applications will reduce the number of tubercles and shoots through enhanced parasitism, thereby improving the growth parameters of tomato plants. Biomass of reproductive tissues, which is one of the most important growth parameters for crops, is negatively affected by the presence of *P. aegyptiaca* (Fernández-Aparicio et al. 2016). Furthermore, other studies have also demonstrated that *Trichoderma* species can improve plant growth and development, even under parasitic weed pressure (El-Dabaa and Abd-El-Khair 2020, Hassan et al. 2013). For instance, *T. viride* has been shown to enhance the growth and vigour of millet varieties by increasing shoot length, root length, and dry weight (Hassan et al. 2013), while *T. harzianum* has been reported to suppress *P. ramosa* infestation in tomatoes by inhibiting tubercle formation and enhancing antioxidant defenses (Fidan and Tepe 2024).

Despite these promising results, it is important to note that this study was conducted under controlled conditions, which may not fully replicate field environments. The lack of significant differences in some growth parameters between BCA-treated and control plants suggests that further optimization of application methods and dosages may be necessary. Future studies should investigate the long-term effects of BCA applications under field conditions and explore the potential synergistic effects of combining BCA with other biocontrol agents, such as mycorrhiza or plant growth-promoting rhizobacteria. Additionally, the impact of environmental factors, such as soil type and climate, on the efficacy of *Trichoderma* strains should be evaluated.

In conclusion, the findings demonstrated that BCA formulation containing *Trichoderma asperellum* ICC 012 and *T. gamsii* ICC 080 has potential as an effective bioproduct against *P. aegyptiaca*, offering a sustainable solution for managing parasitic weeds in tomato production. The ability of these strains to reduce parasitic weed infestation underscores their value in integrated pest management systems. Further research is needed to fully exploit their potential and develop practical applications for sustainable agriculture.

Author's Contributions

Authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The author declared no conflict of interest.

ÖZET

Mısırlı canavar otu olarak bilinen *Phelipanche aegyptiaca* (Pers.) Pomel, domates üretiminde önemli verim kayıplarına neden olan kök paraziti bir bitkidir. Bu çalışmada, *Trichoderma asperellum* ICC012 ve *Trichoderma gamsii* ICC080 suşlarını içeren ticari bir biyolojik ürünün domateste canavar otu üzerindeki etkinliği değerlendirilmiştir. Deneme, ürünün üç farklı konsantrasyonunda (N: tavsiye dozu, N/2 ve 3N/2) uygulanmasıyla, tesadüf blokları deneme desenine göre dört tekerrürlü olarak kurulmuş ve kontrollü sera koşullarında yürütülmüştür. Uygulamalar iki programda gerçekleştirilmiştir: Program A (fide dikimden bir hafta önce ve bir gün sonra) ve Program B (fide dikiminden 15 gün sonra ek bir uygulama). Sonuçlar, biyolojik kontrol ajanı (BCA) uygulamalarının *P. aegyptiaca* sürgünlerinde hastalık şiddetini önemli ölçüde artırdığını, bu değerlerin kontroldeki %40'a kıyasla %69.5 ile %79.6 arasında değiştiğini göstermiştir. Nekrotik alandaki en yüksek skala değerini alan sürgün sayısı, kontrolde ortalama üç iken BCA uygulaması yapılan saksılarda ortalama 17-19.5 ile önemli ölçüde artmıştır. Ayrıca, BCA uygulanan saksılarda ölü tüberküllerin ortalama sayısı 32 iken,

uygulama yapılmayan saksılarda 16 olarak belirlenmiştir. BCA uygulanan canavar otu sürgünlerinin yaş ağırlığı kontrole göre önemli bulunurken, kuru sürgün ağırlığında hiçbir uygulama arasında önemli bir fark bulunmamıştır. Domates bitkisinin büyüme parametrelerinde kontrole kıyasla önemli bir fark gözlemlenmemiştir. Bu sonuçlar, *Trichoderma* türlerinin çoklu mekanizmalarla canavar otunu kontrol edebileceğini düşündürmektedir. Bu bulguların doğal koşullarda doğrulanması için saha denemelerinin yapılması gerekmektedir.

Anahtar kelimeler: domates, *Phelipanche aegyptiaca*, *Trichoderma* spp., biyolojik kontrol ajanı

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Cite this article: Çiğnitaş, E., & Başbağcı, G. (2025). Management of Egyptian broomrape [*Phelipanche aegyptiaca* (Pers.) Pomel] using biofungicide (*Trichoderma* spp.) in tomato. Plant Protection Bulletin, 65-3. DOI: 10.16955/bitkorb.1657350

Atıf için: Çiğnitaş, E., & Başbağcı, G. (2025). Domateste biyofungisit (*Trichoderma* spp.) kullanılarak Mısırlı canavar otu [*Phelipanche aegyptiaca* (Pers.) Pomel]'nun ücadelesi. Bitki Koruma Bülteni, 65-3. DOI: 10.16955/bitkorb.1657350