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Evaluation of Biofungicides in the Control against Powdery Mildew Disease [*Leveillula taurica* (Lev.) Arm.] in Pepper

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ABSTRACT

In this study, Tea Tree Oil, (Timorex Gold), Orange Oil (Prev-Am), Reynoutria spp. extract (Regalia), *Bacillus subtilis* QST 713 (Serenade SC), *Lactobacillus acidophilus*, *Lactobacillus paracasei* (Vitanal) and a reference product Fluopyram+Tebuconazole (Luna Experience) were applied to pepper plants to determine the efficacy against powdery mildew disease caused by *Leveillula taurica* (Lev.) Arm on pepper. The disease on plants was evaluated based on the 0-5 scale when the disease reached to 20% on untreated plots. Trials were established as a randomized plot design with four replications. The disease severity was assessed using Towsend-Heuberger's formula and the percentage effect of the applications was calculated using the Abbott formula. The conducted experiments were revealed that the highest % efficacy values of the preparations against powdery mildew were obtained from Timorex Gold (80%), Prev-Am (45%), Regalia (44.7%), Serenade (26.3%) and Vitanal (26.2%), respectively. The efficacy of the reference product, Luna Experience, against powdery mildew in pepper was found 89%. It has been concluded that Timorex Gold preparation performed enough efficacy against *Leveillula taurica*, and can be safely used and offered against powdery mildew.

Research Article

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Biberde Külleme [*Leveillula taurica* (Lev.) Arm.] Hastalığına Karşı Mücadelede Biyofungisitlerin Değerlendirilmesi

ÖZET

Bu çalışmada Çay Ağacı Yağı (Timorex Gold), Portakal Yağı (Prev-Am), Reynoutria spp. extrakt (Regalia), *Bacillus subtilis* QST 713 (Serenade SC), *Lactobacillus acidophilus*, *Lactobacillus paracasei* (Vitanal) ve ruhsatlı preperat olarak Fluopyram+Tebuconazole (Luna Experience) *Leveillula taurica* (Lev.)'nın neden olduğu külleme hastalığına karşı etkinliğini belirlemek için biber bitkilerine uygulanmıştır. Hastalık şiddeti, kontrol parsellerinde en az %20 hastalık ortaya çıktığında 0–5 skalasına göre değerlendirilmiştir. Serada tesadüf blokları deneme deseninde 4 tekerrürlü bir deneme yürütülmüştür.. Sayım sonucu elde edilen skala değerlerine Towsend-Heuberger formülü uygulanarak hastalık oranları (%), Abbott formülüne göre de preperatların etkileri hesaplanmıştır. *Leveillula taurica* hastalığına karşı kullanılan preperatların etkinlikleri sırasıyla Timorex Gold (%80), Prev-Am (%45), Regalia (%44.7), Serenade (%26.3), Vitanal (%26.2) olarak belirlenmiştir. Ruhsatlı fungisit olarak kullanılan Luna Experience ise %89 oranında etkili bulunmuştur. Sonuç olarak, Timorex Gold'un biberde külleme hastalığı etmeni *Leveillula taurica*'ya karşı yüksek etki gösterdiği, güvenle kullanılabileceği ve önerilebileceği kanısına varılmıştır.

Araştırma Makalesi

Makale Tarihçesi
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Anahtar Kelimeler
Bacillus subtilis QST 713
Lactobacillus spp.
Portakal yağı
Reynoutria spp. Ektrakt
Çay ağacı yağı

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INTRODUCTION

Pepper (*Capsicum annuum* L.) is a native plant in South America. It belongs to the aubergine (Solanaceae) family and is able to grow in tropical, subtropical and temperate climate areas. The pepper is not only popular worldwide but also important foodstuff in Turkey. Pepper growing is carried out in both greenhouses and open fields in Turkey and the world (Koç et al., 2014). Crop losses caused by biotic factors including fungal, bacterial, and viral microorganisms are important for vegetable farming. Many soil-borne pathogens lead diseases in greenhouses, and are often difficult to control on pepper plants (Yıldız et al., 1990).

Some of important commonly seen diseases in greenhouse-grown pepper plants are white rot (*Sclerotinia sclerotiorum*), fusarium wilt (*Fusarium* spp.), early blight (*Alternaria solani*), leaf mold (*Botrytis cinerea*), phytophthora blight (*Phytophthora capsici*) and powdery mildew (*Leveillula taurica*). Powdery mildew [*Leveillula taurica* (Lev.) Arm.] which is one of the most important pepper fungal diseases can result in serious losses in pepper. In Turkey, more than 75 chemicals have licensed for powdery mildew, *Leveillula taurica,* in Solanacea plants. Despite the widespread use of cultural and chemical control to manage the disease, it cannot be fully controlled. In addition, control methods used for powdery mildew are based on genetic resistance. However, resistant cultivars are not always successfully developed (Tsror et al., 2004; Yücel, 2008). Until recent years, there were no pepper cultivars resistant to *L. taurica*.

The use of fungicides increases the cost of production lower the market value, causing big economic losses and posing tremendous risk to the environment and human health. The use of pesticides has a negative impact on the environment and human health. Also, the use of pesticides against target organisms leads to the formation of resistant races (Aydın, 2015). For this reason, it has to be necessary to reduce chemical pesticides in agriculture and introduce environmentally friendly, sustainable and cheaper control methods. The most promising method to achieve this goal is biological control. The use of biological organisms alone or in combination to manage plant diseases can minimize the impact of chemicals on the environment. Furthermore, promoting plant growth and the importance of microorganisms in biological control are increasing (Sülü et al., 2016). Using biofungicides (*Bacillus* spp., *Trichoderma* spp., *Aspergillus* spp., Tea Tree Oil extract, *Reynoutria* spp. extract etc.) under favorable conditions resulted positive changes in crop plants including yield, durability, quality, earliness, and adaptability (Bora and Özaktan, 1998; Hermosa et al., 2012; Sing et al., 2012; Shao et al., 2013; Ben Abdallah 2015; Aydın, 2015; Zalila-Kolsi et al., 2016; Sing et al., 2019). In this study, the biological activity of three plant extracts, one bacterium and one organic plant activator were investigated against powdery mildew disease caused *L. taurica* on pepper. Tea Tree Oil extract (222.5 g L^{-1} in Timorex Gold), Orange Oil extract (60 g L^{-1} in Prev-Am), *Reynoutria spp.* extract (in 224 g L^{-1} Regalia), *Bacillus subtilis* [Serenade SC, i.e 1.34 % *B. subtilis* QST 713 strain (min. $1x10^9$ cfu ml^{-1})], *Lactobacillus acidophilus* and *L. paracasei* (in 75% Vitanal), as well as a licensed fungicide namely Fluopyram + Tebuconazole (200 g L^{-1} Fluopyram + 200 g L^{-1} Tebuconazole in Luna Experience) were applied against *L. taurica*.

MATERIALS and METHODS

The application of preparations

In this study, the experiments were conducted with naturally occurred powdery mildew disease in a greenhouse located Hacıaliler, Aksu District, Antalya, Turkey. The greenhouse was used for pepper growing in Antalya in 2016 and 2017. *Leveillula taurica* susceptible Kanyon F1 pepper cultivar was used in the study. In both years of application, pepper plants were planted with 150 cm row spacing and 40 cm plant spacing. In both years, the first fungicide application was started when the fruits ripening and the flowering occurred in the plants. The experiments showed that before the first treatment, plant height was 65 cm in 2016 while it was 55 cm in 2017. The study was established with a randomized block design with 4 repetitions containing 20 plants in each repetition.

The pesticides were applied based on the doses written on the preparations according to producers' suggestions in the trial (Table 1).

The treatments in the experiment (Timorex Gold, Prev-Am, Regalia, Serenade, Vitanal) were sprayed by covering all the plants. As a comparative (licenced) fungicide, a chemical fungicide named Luna Experience (200 g L^{-1} Fluopyram + 200 g L^{-1}Tebuconazole) by Bayer Crops licensed in Turkey was applied. Applications were started when powdery mildew disease conditions occurred in the greenhouse. Applications were started before plants sustained disease and continued with 7 days intervals until at least 20% disease symptoms seen on control plots. Plants were sprayed 6 and 8 times in 2016 and 2017, respectively.

Disease assessment in pepper plants

Disease assessment in plants was performed once the disease was reached at least a 20% level in the untreated plots. The assessment was conducted at

03.12.2016 and 09.12.2017. Five old leaves of each of the 20 pepper plants in each plot were examined based on the scale of 0-5 (Table 2).

Table 1. Trade name, producer company, name, and amount of active substance, formulations, and doses of the preparations used in the experiment

Çizelge 1. Denemede kullanılan preperatların ticari ismi, firması, aktif madde adı ve miktarı, formülasyon şekli ve dozları

Trade Name	Company	Name and Amount of the Active Substance	Formulation	Dose
Timorex Gold	Nufarm	222.5 g L^{-1} Tea Tree Oil (Tea Tree Oil)	EC	200 ml 100 L^{-1} water
Prev-Am	Nufarm	60 g L^{-1} Orange Oil	SL	200 ml 100 L^{-1} water
Regalia	Syngenta	224 g L^{-1} *Reynoutria* spp.	SC	200 ml 100 L^{-1} water
Serenade	Bayer	%1.34 *Bacillus subtilis* QST 713 Irkı	SC	1000 ml 100 L^{-1} water
Vitanal	Doğa Organik	*L. acidophilus and L. paracasei*	SL	300 ml 100 L^{-1} water
Luna Experience	Bayer	200 g L^{-1} Fluopyram+ 200 g L^{-1} Tebuconazole	SC	30 ml 100 L^{-1} water

Table 2. Scale of 0-5 evaluating powdery mildew disease (Anonymous, 2018)

Çizelge 2. Külleme hastalığının değerlendirildiği 0-5 skalası (Anonim, 2018)

Assessment scale for powdery mildew disease in cultivated species from solanaceae family	
Scale Value	Definition
0	No disease on the leaf
1	0-1% of the leaf area is infected
2	2-5% of the leaf area is infected
3	6-20% of the leaf area is infected
4	21-40% of the leaf area is infected
5	More than 41% of the leaf area is infected

Data assessment

The severity of the disease in each repetition (%) was calculated with the Townsend-Heuberger formula (Townsend and Heuberger, 1943). Based on the determined disease severity values, the effectiveness of the chemicals in percentage was calculated with Abbott formula (Abbott, 1925).

Towsend-Heuberger Formula

$$Disease\ Severity\ (\%): \frac{\text{Total } (n \times V)}{Z \times N}\ X\ 100$$

Abbott Formula

$$Effect\ (\%): \frac{X-Y}{X}\ X\ 100$$

In the above formulas, n refers to the number of root-root collars or tubers of the plant in different damage groups, V to levels of damage classified into groups, N to total number of root-root collar or tubers under control, Z to the highest scale value, X to the mean disease severity in positive untreated plots (%), Y to the mean disease severity in the treated plots (%), X to the disease severity in the untreated plots, Y to the resultant disease severity of each application.

Statistical analyses

Factorial analysis of variance was separately applied to the obtained data. Obtained disease rates in the experiment were analyzed within variance analysis in factorial order and subjected to angle transformation. The obtained data were evaluated by Tukey multiple comparison test using IBM® SPSS® 22 for the statistical analyses.

RESULTS

Disease severity in percentage (%) against *L. taurica* was obtained with the lowest rate of Timorex Gold (8.3%) application. Among the treatments, the highest disease severity was observed in Serenade (29.4%). A statistical difference was found between Timorex Gold and other applications (Figure 1). Disease severity in control plants was determined as 38.8%. Disease severity was found as 5.1% in Luna Experience application used as a comparison fungicide (Table 3) ($P \leq 0.05$).

In this study, the highest biological effect against *L. taurica* was determined in Timorex Gold (78.7%) and the lowest in Vitanal application (24.9%). The biological effect was determined as 87.0% in the application of Luna Experience (Figure 1).

In 2017, the lowest disease severity in percentage was obtained from Timorex Gold (6.0%) application. This was followed by Regalia (16.6%) and Prev-Am (16.5%) applications, respectively. The highest disease severity percentage was observed in Vitanal (22.2%) and Serenade (22.1%) (Table 4).

A statistical difference was found between Timorex Gold and other applications. ($P \leq 0.05$). Disease rate in control plants was determined as 30.0%. Disease severity was found as 3.3 % in the application of Luna Experience and a statistical difference was found between Timorex Gold and Luna Experience applications. In this study, the most effective biological effect among the bio fungicides based on the

study was determined in Timorex Gold (80%) and the lowest in Vitanal application (26.2%). In the Luna Experience fungicide (Luna Experience), comparison treatments, the biological effect was determined as 89.0% (Figure 2). In this study, it was concluded that Timorex Gold, Prev-Am and *R. sachalinensis* extract (Regalia) can be used in the control of the powdery mildew disease.

Table 3. Effect of preparations applied to a pepper plants in greenhouse against Leveillula taurica in 2016.
Çizelge 3. 2016 yılında serada biber bitkisne uygulanan preparatların L. taurica'ya karşı etkisi

Name of the Treatment	DiseaseSeverity (%)*	Biological Effect (%)*
Timorex Gold	8.3 ±0.21 b	78.7 ±0.53 b
Prev-Am	22.5 ±0.35 c	41.9 ±0.52 c
Regalia	23.2 ±0.22 c	40.1 ±0.59 c
Serenad	29.4 ±0.33 d	24.1 ±0.79 d
Vitanal	29.1 ±0.25 d	24.9 ±0.42 d
Luna Experience[1]	5.1 ±0.13 a	87.0 ±0.32 a
Control	38.8 ±0.29	

* Mean values with different letters in the same columns denote significant differences(Tukey's Multiple Range Test; P ≤0.05. [1] Positive control

Figure 1. Pepper leaves in the greenhouse experiment in 2016 A) Control B) Timorex Gold C) Prev-Am D) Vitanal E) Seranade F) Regalia G) Luna Experience
Şekil 1 2016 yılında sera denemesinden biber yaprakları A) Kontrol B) Timorex Gold C) Prev-Am D) Vitanal E) Seranade F) Regalia G) Luna Experience

Table 4. Effect of preparations applied in the pepper greenhouse against L. taurica in 2017
Çizelge 4. 2017 yılında biber serasında L. taurica'ya karşı uygulanan preparatların etkisi

Name of the Pesticide	Disease Severity (%)*	Biological Effect (%)*
Timorex Gold	6.0 ±0.14 b	80.0±0.45 b
Prev-Am	16.5 ±0.21 c	45.0±0.59 c
Regalia	16.6 ±0.16 c	44.7±0.53 c
Serenade	22.1 ±0.21 d	26.3±0.41 d
Vitanal	22.2 ±0.24 d	26.2±0.55 d
Luna Experience[1]	3.3 ±0.13 a	89.0±0.39 a
Control	30.0±0.14	

* Mean values with different letters in the same columns denote significant differences (Tukey's Multiple Range Test; P ≤0.05. [1] Positive control

In both trials, Timorex Gold, which is one of the commercial biofungicides, was found to be more effective than other biological preparations. Timorex Gold showed a biological effect close to fungicide (Luna Experience) used against powdery mildew disease. The result showed that all applications suppressed powdery mildew disease in pepper, and better values were observed in the suppression of the disease in 2017 compared to 2016. It is thought that this is due to climatic factors, differences in humidity, and temperature values between 2016-2017. In 2016, preparations had difficulty in controlling the disease because the disease was more intense (Figure 3).

Figure 2. General view of the pepper plants in greenhouse A) Control B) Timorex Gold
Şekil 2. Seradaki biber bitkilerinin genel görünümü A) Kontrol B) Timorex Gold

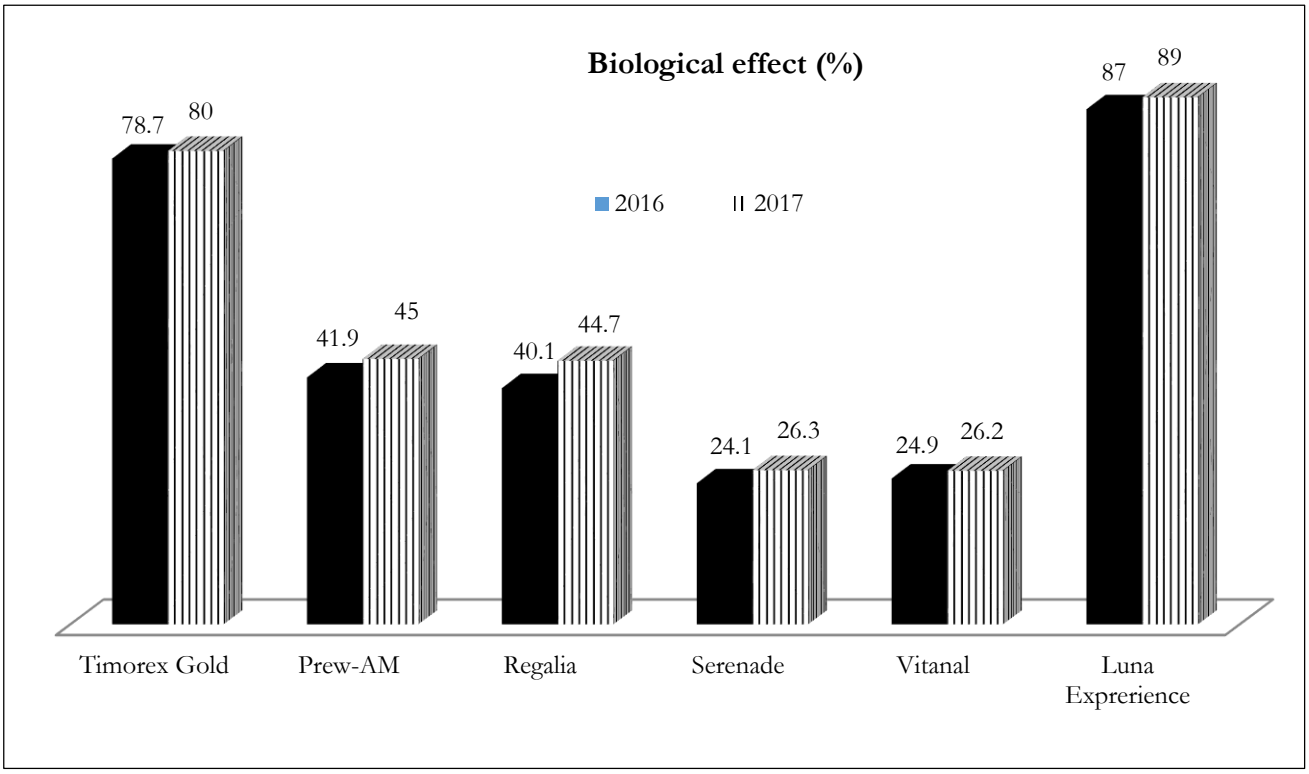

Figure 3. Efficacy of commercial biofungicides against L. taurica in the years of application
Şekil 3. Ticari biyofungisitlerin uygulama yıllarında L. taurica'ya karşı etkinliği.

DISCUSSION

In this study, the use of biological preperations showed strong antagonistic potential which inhibited >50% mycelial growth of *L. taurica* in pepper plants. Timorex Gold (Tea Tree Oil) (80%), Prev-Am (45%) and Regalia (44,7%) were the most effective suppressants of powdery mildew of *L. taurica* in pepper plants. Similar results have been reported by other researchers. Shao et al. (2013) examined the effects of Tea Tree Oil (TTO) on mycelial growth of *Botrytis cinerea* and cell wall through steam and contact. The results have shown that TTO inhibited the mycelial growth of *Botrytis cinerea* and damaged the cell wall of the fungus. The most effective result was observed in the steam application. Terzi et al.

(2007) studied the effects of TTO and its basic components on the cereal diseases on barley leaves infected by *Fusarium graminearum, Fusarium culmorum, Pyrenophora graminea,* and also *Blumeria graminis in vitro* conditions. They concluded that TTO and its components could be considered as natural alternative fungicides). Dagostin et al. (2011) carried out 112 different applications including biological control elements, animal materials, homeopathic remedies, inorganic substances, microbial extracts, natural products, plant extracts as well as physical methods and synthetic materials under the conditions of greenhouses and fields against the mildew (*Plasmopara viticola*) in wine grapes in Italy and Switzerland At the end of these studies, the efficiency of Timorex Gold (TTO) in greenhouse conditions was found to be 39.6% in 50 ml 100 L^{-1} of water dose, 90.5% in 100 ml 100 L^{-1} of water dose, and 93.3% in 500 ml 100 L^{-1} of water dose. Reuveni et al. (2009) reported that Timorex Gold prevented the breathing of fungi, germination tube formation and mycelial growth

Although Orange Oil and *B. subtilis* suppressed powdery mildew in pepper plants in this study, it was not as effective as a fungicide. Similar results were reported by Moyer and Peres (2008). The effect of Orange Oil, *B. subtilis*, acibenzolar-S-methyl, phosphoric acid, potassium bicarbonate, calcium silicate and potassium silicate against powdery mildew disease (*Podosphaera fusca* (Fr.)) in gerbera were investigated in this study, While calcium silicate, and potassium silicate did not suppress the powdery mildew in gerbera, Orange Oil, *B. subtilis*, acibenzolar-S-methyl, phosphoric acid, potassium bicarbonate applications suppressed the powdery mildew disease but were not as effective as fungicide. Dagostin et al. (2011) reported that the effectiveness of Orange Oil in the field part of the study was found to be 20% in leaves in 300 ml 100 L^{-1} water dose, while the effect on bunches of grapes was 28%. In another study, *B. subtilis* QST 713 Race (Serenade) had 50.99% biological activity against the *Alternaria alternata* in tomato, while it showed 24.1% biological effect against the powdery mildew in pepper and it was not effective against the powdery mildew disease in pepper (Ünsal, 2010). Dagostin et al. (2011) found 48% biological effect within 400 ml 100 L^{-1} litres of water dose of Serenade. *Bacillius subtilis* has positive environmental effects, is attractive for the control of plant diseases and slows the development of fungicide resistance (Jochum et al., 2006; Grosu et al., 2015). *Bacillus subtilis* produces antagonistic activities against fungal and bacterial pathogens (Ben Abdallah et al., 2015; Zalila-Kolsi et al., 2016). *Bacillus subtilis* is a producer of Mycosubtilin containing antifungal peptide antibiotic potential.

In this study, *R. sachalinensis* extract (Regalia) used against *L. taurica*, provided 40.1% and 44.7% biological effect in 2016-2017. Although thid extract is not very effective against powdery mildew disease in pepper, it is thought that it can be used with other applications within the scope of integrated pest management. Regalia, which is used for plant diseases, activates the defence system in the plant and increases the secretion of phenolic compounds (Ünsal 2010). Schmidt et al. (2002) reported that extracts of *R. sachalinensis* plant significantly reduced powdery mildew infections in cucumber, tomato, and grapevine plants. Malathrakis et al. (2002) investigated the effect of *R. sachalinensis* against *L. taurica* in tomato in different doses. The most effective dose with 0.0 5% of *R. sachalinensis* was reported against *L. taurica*. On the other hand, Konstantinidou-Doltsinis et al. (2006) tried to determine the effect of *R. sachalinensis* against powdery mildew disease (*L. taurica*) in tomato greenhouses. According to the research results, *R. sachalinensis* has been found to reduce powdery mildew disease (*L. taurica*) by 64.6 % in tomato greenhouses. Walters and Fountaine (2009) reported that *R. sachalinensis* controlled the disease of powdery mildew (*Uncinula necator*) in grape.

In the present study, Vitanal (*Lactobacillus acidophilus* and *Lactobacillus paracasei*) did not give a very effective result against powdery mildew disease (*Leveillula taurica*). Similar results were reported by Lipinska et al. (2016). In their study, it was investigated the effectiveness of *Lactobacillus* bacteria against *Alternaria alternata, Alternaria brassicicola, Aspergillus niger, Fusarium latenicum, Geotrichum candidum, Mucor hiemalis* and *Candida vini* ag. It was reported that, *L. acidophilus* and L. *paracasei* certainly did not prevent the development of *Candida vini* and an antifungal effect was not detected. Also, *L. acidophilus* and *L. paracasei* lowly prevented the development of the other 6 fungi in the experiment and showed a low antifungal effect. Besides, another researcher reported a different result. Daranas et al. (2019) investigated the efficacy of *Lactobacillus* bacteria against *Pseudomonas syringae* pv *actinidiae* in kiwi, *Xanthomonas arboricola* pv *pruni* in plum and *Xanthomonas fragariae* in strawberry. It was reported that PM411 and TC92 breeds of *L. plantarum* completely suppress 3 bacterial pathogens causing disease in plants.

CONCLUSION

According to the results, Timorex Gold was the most effective in suppressing *L. taurica* in pepper plants.This was followed by Prev-Am and Regalia (*R. sachalinensis* extract) in the suppression of powdery mildew disease in pepper. It is concluded that Timorex Gold (Tea Tree Oil) can be safely used and recommended against *L. taurica* disease in pepper.

Timorex Gold with the zero day PHI value (Pesticide Residues in Food) can be used safely in biological control. Timorex Gold can be used alternately with chemical fungicides in the integrated pest manegement program to reduce the number of fungicides, and the amount of fungicide residue. Biological control is considered an alternative to chemical control due to its positive aspects in terms of both human health and environment and food safety. The results of this study will be a source for future studies against powdery mildew (*L. taurica*) disease. Considering the resluts of the present and similar studies, the importance of biological control against powdery mildew pepper caused by *L. taurica* should be more emphasized, and new antagonists and alternative options should be developed further investigated for disease control.

Abbreviations

TTO: Tea Tree Oil
g L^{-1}: g/L
EC: Emulsifiable Concentrate
SC: Suspension concentrate
SL: Soluble liquid
ml: mililiter
L: Liter
spp: Species

Araştırmacıların Katkı Oranı Beyan Özeti

Yazarlar makaleye eşit oranda katkı sağlamış olduklarını beyan eder.

Çıkar Çatışması Beyanı

Makale yazarları aralarında herhangi bir çıkar çatışması olmadığını beyan ederler.

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