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Research Article

The Investigation of Effect of Bacteria in Biological Control of Red Spider Mite (*Tetranychus* spp.) and Plant Yield Parameter in Cotton (*Gossypium hirsutum* L.)

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Red spider mite

Abstract: The purpose of this study was to assess the usability of two bacterial strains, namely *Bacillus subtilis* PA1 and *Paenibacillus azotofixans* PA2, for the biological control of red spider species, and their effects on plant quality and yield in cotton under field conditions. The experiments were conducted at three different locations with multiple replications. As a control, a commercial preparation containing Lambda-Cyhalothrin as the active ingredient was used. The obtained results from the study revealed that the application of the bioagent formulation led to a significant decrease in the density of *Tetranychus* spp. at different biological stages, ranging from 59.22% to 61.07%, when compared to the control group. Additionally, several important plant growth parameters showed remarkable improvements. The number of fruit branches increased by 130.20%, plant crown diameter by 88.16%, plant height by 40.15%, the number of flowers by 21.25%, the number of wood branches by 18.13%, the average number of cocoons by 126.53%, and cocoon weights by 54.65% significantly across all three trial parcels. The successful implementation of the bacterial application for pest control had a positive impact on cotton yield. Bulk cotton yield increased by 80.03%, and fiber yield increased by 82.17%. Consequently, the bacterial formulation containing these two bacteria demonstrated its potential as a biopesticide in cotton cultivation, effectively controlling pests while also playing a crucial role in enhancing productivity. Overall, the study suggests that using the bioagent formulation consisting of Bacillus subtilis PA1 and *Paenibacillus azotofixans* PA2 could be an effective and environmentally friendly approach for pest control in cotton farming, leading to increased productivity.

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1. Introduction

Cotton (*Gossypium hirsutum* L.) is a very important fiber plant with a very long vegetation period of 5-6 months, adapted to the tropical and subtropical climate zone of the *Gossypium* type of the Malvaceae family of the Malvales order systematically (Anonymous, 2018). Approximately 35% of the fibers produced in the world are obtained from the cotton plant (Gündüz et al., 2020). As the world's

population continues to grow, alongside the industrialization and development of societies, the rising living standards have increased the consumption and demand for cotton fiber (Mızrak et al., 2020).

Various factors negatively affect cotton agriculture. Among these factors, yield and quality losses due to diseases, pests, and weeds have an important place. Among the pests, cotton aphids (*Aphis gossypi* Glov.), tobacco thrips (*Thrips tabaci* Lind.), red spider mites (*Tetranychus cinnabarinus* (Boisd.), and *T. urticae* Koch), and leafhoppers (*Empoasca decipiens* Paoli, *Asymmetrasca decedens* (Paoli.)) cause significant yield loss by stinging and sucking plant leaves during the basic development period of cotton plant (Güneş, 2005). Among these species, *T. urticae* is one of the pests that threaten plant production in general, the leaves that it causes damage by sucking turn yellow, the assimilation regresses because the amount of chlorophyll of the plant decreases, the leaves curl and fall, and the quality and quantity of the product taken from the damaged plants decreases (Toros, 1992) and thus it is stated that product loss is between 20-45% (Premalatha et al., 2018).

The control of red spider mites is mostly performed in the form of chemical control (acaricide/acaricide+insecticide practices), as in other agricultural pests. The value of the specific acaricide market in the world in 2013 was determined as 900 million Euros and it is noted that this value corresponds to 7% of the insecticides (excluding Fumigants) with a market value of approximately 13.3 billion Euros (Van Leuween et al., 2015). When we look at the active substance basis today, it is seen that some active substances that were put on the market in very old years are still used, as well as the latest acaricides with different effect mechanisms (spirodiclofen, spiromesifen) occupying a large place in the market share (Van Leuween et al., 2015). In Türkiye, the use of acaricide was 2 452 275 tons in 2019, constituting 4.53% of the total pesticide use of 54 098 000 tons (Anonymous, 2019). In addition to fecundational fertilization, mites can reproduce parthenogenetically, lay a large number of eggs, have a short life span, and give many offspring during the year (the development period can be as short as one week at high temperatures of about 32 °C), long term intensive use of acaricides, chemical pesticides lead to the dominance of resistant population which is not affected and will be reflected in plant yield (Fraulo and Liburd, 2007). It is stated that *T. urticae* has developed resistance to 93 different active substances and is the most resistant pest among arthropods (Anonymous, 2015). A mite population that develops resistance to an acaricide with any effect mechanism may also develop resistance to other chemicals with the same effect mechanism. Considering that the development of resistance will be delayed and the duration of use of acaricides will be prolonged if drugs with different action mechanisms are used, it is obvious that there is a need to continuously develop new drugs with action mechanisms (Dekeyser, 2004). Today, the development of new pesticides is in decline due to the high costs and the need for very long and intensive research (Sparks, 2013). In addition, the fact that the intensive use of pesticides causes negative effects on the environment and human health and the deterioration of the natural balance should not be ignored (Topakcı and Göçmen, 2008). In addition, in this respect, the biological control method, which brings plant diseases and pests under control by using microorganisms, is seen as a strong alternative to the use of synthetic chemicals (Kotan et al., 2010). Recently, important studies have been conducted on the biological control against many diseases and pests and effective results have been obtained. The fact that they have been prepared and used in many countries over time has been the best proof of how much these products contribute to biological control studies. In addition, considering the awareness of producers and consumers and the increasing trends towards organic products, it is clear that the importance of biological control in integrated control will gradually increase (Uygun et al., 2010). In agricultural production areas where chemical control is not carried out, the natural balance is preserved, although the density of diseases and pests varies from year to year. For this reason, producers should be made aware of biotechnical methods and cultural struggles to protect human and environmental health (Kaplan and Bayram, 2021). Farmers generally prefer the chemical warfare method to solve plant protection problems, and unconscious pesticide applications bring about many negativities in terms of human and environmental health (Kaplan and Saltuk, 2021).

In recent times, the use of entomopathogens in biological control against mite species in cotton fields has been considered within the framework of IPM (Integrated Pest Management) strategies. The utilization of these environmentally friendly entomopathogens, in addition to being a key component of IPM to reduce pesticide loads in the cotton ecosystem, is steadily increasing as a safer biopesticide in cotton fields.

This study, it was aimed to determine the usability of bacteria isolates which is environmentally friendly and do not threaten the health of humans and other living things that can be used as a hopeful

biological control agent against red spider mite species that cause yield losses in cotton fields both in the world and in our country. Moreover, as a result of previous studies on the yield and yield parameters of cotton plants, it is aimed to reveal how the formulation of two bacterial isolates (*Bacillus subtilis* strain PA1 and *Paenibacillus azotofixans* strain PA2) with known PGPR characteristics will have an effect on plant growth and yield in cotton under field conditions.

2. Material and Methods

2.1. Bacterial strains used in this study

In the study; *Bacillus subtilis* PA1 and *Paenibacillus azotofixans* PA2 bacterial strains isolated from the root zone of wild wheat plants, which were determined to have high potential in terms of nitrogen-fixing and phosphate dissolving properties, were used as a mixture (Table 1). This mixture has been tested in terms of its effectiveness on cotton yield in different regions of Türkiye and has been licensed as SS Super Green and offered for commercial use as a microbial product due to its high efficiency. Diagnosis of bacteria was performed using MIS (Microbial Identification System) and BIOLOG system. Bacterial cultures are preserved in the Microbial Culture Collection of Atatürk University, Faculty of Agriculture, Department of Plant Protection, Bacteriology Laboratory. The stomyl cotton variety of May Seed Company was used in the experiments.

Table 1. MIS and BIOLOG diagnostic results and some biochemical properties of bacterial strains

	Strain PA1	**Strain PA2**
MIS Identification Results	*Bacillus subtilis*	*Paenibacillus azotofixans*
MIS Similarity Indexed	0.786	0.845
BIOLOG Identification Results	*Bacillus subtilis*	*Paenibacillus azotofixans*
BIOLOG Similarity Indexed	0.56	0.87
Nitrogen Fixation	K+	+
Phosphate Solubilizing	+	+

2.2. Pathogenicity tests of bacterial strains on cotton

One-month-old cotton seedlings were used for pathogenicity tests of bacterial strains. Fresh bacterial cultures grown on Nutrient Agar (NA) for 24 hours were transferred to Nutrient Broth (NB), developed at 27 ºC in a horizontal shaker incubator rotating at 90 rpm for 48 hours, suspended in sterile distilled water (sdH_2O) and their density was arranged as 10^8 cell/ml on BIOLOG turbidimeter. The seedlings immersed in the prepared bacterial solutions were grown in pots filled with sterile soil for 1 month and then removed. Roots and ground surface parts that were thoroughly washed in tap water were examined to see if there was an infection. Infection occurrence was evaluated as + and no infection was evaluated as –.

2.3. Biological control studies

2.3.1. Establishment of field experiments

The field experiments were set up in 3 replications and each plot of 500 m^2 on 3 volunteer farmer lands selected in Şanlıurfa province, Ceylanpınar, and Harran districts. In the selected experiment areas, attention was paid to the fact that the red spider mite damage was intense in the previous year.

2.3.2. Preparation of the formulation of bacterial strains for field experiments

Bacterial strains (*B. subtilis* PA1 and *P. azotofixans* PA2) purely stored in long-term storage were inoculated into petris containing NA growth culture and left for incubation for 24 hours at 27°C. Colonies taken from the growing fresh cultures to the core were transferred to NB growth culture, and after they were grown in a horizontal shaker incubator for 24 hours, they were inoculated into a fluid growth culture containing NB, which was previously prepared in a fermenter and sterilized in an autoclave at 121 °C for 20 minutes. Bacteria were developed at optimum pH, oxygen, and temperature values for 24 hours and inoculated by mixing the developing cultures into the carrier fluid consisting entirely of organic substances and sterilized by steam at a ratio of 1:10. The content of this carrier

formulation consists of water, various organic substances (seaweed, whey, and herbal extracts) and various substances (Carboxymethylcellülose, Calcium carbonate, Glycerin, Magnesium sulfate) that protect and homogenize the bacterial isolate in its content. Bacteria inoculated organic fluid carrier was left for incubation at 27°C in the bioreactor. Live bacteria countings per milliliter were made and after 48 hours, when the bacterial concentration exceeded $1x10^8$ cell ml^{-1}, it was packaged under completely sterile conditions and stored in a cold room at 5°C for later use (Trinh and Lee, 2022).

2.3.3. Application of formulation of bacterial strains to cotton plant in the field

The seeds coded by soaking in the formulation prepared with bacterial strains for 24 hours, were sown in the field on 27, 28, and, 29 April 2015, respectively, at Şanlıurfa, Ceylanpınar, and Harran locations. On the 30th, 45th, and 60th days after the sowing date, bacteria were sprayed on the soil surface and the ground surface part of the plant for a total of 3 times and reapplied. Applications were made in the late afternoon hours when the weather was cool, with 250 cc of bacterial suspension in 100 liters of water, with a top-back pulverizator so that the leaves were fully covered. A commercial drug containing the active ingredient of Lambda-Cyhalothrin was used as a control.

2.3.4. Counting of red spider mite eggs, nymphs, and adults

In the evaluations made 50 days after cotton sowing, 20 leaves attached to the main stem were taken from each plot, one leaf from the upper and middle parts of the 10 plants selected randomly in the diagonal direction, and one leaf from the middle and lower parts, and the leaves were brought to the laboratory. Here, live eggs, nymphs, and adults were dropped on the vaseline glass surface with the help of a brushing device and counted under a stereo microscope. The average of the values obtained as a result of the counting was given as the average of that location, and the bacterial application was also calculated as the percent effectiveness by comparing it with the control.

2.3.5. Evaluation of plant growth and yield parameters

The efficacy of bacterial application in terms of some plant growth parameters 60 days after cotton sowing was evaluated, in 20 randomly selected plants from each parcel; average plant height (cm) was measured, wood branch (piece $plant^{-1}$), fruit branch (piece $plant^{-1}$), cocoon (piece $plant^{-1}$) and flower (piece $plant^{-1}$) countings were made. Again, in 3 plants selected randomly from each parcel and deracinated; parameters such as average number of lateral roots (number $plant^{-1}$), plant crown diameter (cm), main root length (cm), cocoon diameter (mm), and root collar diameter (mm) were also evaluated. In the evaluations in terms of yield and yield parameters made 3 days before September 24, 2015, the harvest date, the average opened and unopened cocoons were counted in 20 randomly selected plants from each parcel (pieces $plant^{-1}$), the average cocoon weight (gr) was calculated, and the cotton un-seed yield in 60 plants (gr) was taken, then the number of plants per decare and cotton un-seed yield per decare (kg $decare^{-1}$), ginning yield (%) and cotton gin yield (kg $decare^{-1}$) were determined.

2.4. Analysis of the results

All data were analyzed using the SPSS statistical package software program. The differences between the averages were determined according to the Duncan Multiple Comparison Test (Julie, 2007).

3. Results and Discussion

As a result of the pathogenicity test performed to determine whether bacterial strains are pathogenic in the cotton plant, it was determined that both strains were not pathogenic.

50 days after sowing; the results obtained in the evaluations made to determine the effectiveness of bacteria applications on red spider mite eggs, nymphs, and adults in the cotton plant are given in Table 2.

According to the counts made on the 50th day of the experiment, it was observed that bioagent bacteria applications caused significant decreases in egg, nymph, and adult numbers of red spider mites in all locations compared to control applications, and these decreases were statistically significant. In control applications, the highest number of eggs were counted in Şanlıurfa (20.25%), followed by Harran (20.05%) and Ceylanpınar (17.7%) locations. In the plots where bacteria were applied, the number of eggs decreased in Harran (8.65%), Ceylanpınar (7.95%), and Şanlıurfa (6.95%) locations,

respectively, unlike the control. In control applications, the most nymphs were detected in Harran (20.30%) compared to the nymph average, followed by Şanlıurfa (17.30%), and Ceylanpınar (15.45%) locations, respectively. In the plots where bacteria were applied, a decrease in nymph numbers was detected in Şanlıurfa (7.55%), Harran (7.25%), and Ceylanpınar (6.85%) locations. 50 days after sowing, the highest number of adults were counted in the control applications in the Center (29.05%), followed by the control applications in Ceylanpınar (26.85%) and Harran (26.80%) locations, respectively. In the plots where bacteria were applied, the number of adults decreased in Ceylanpınar (11.09%), Şanlıurfa (10.55%), and Harran locations (9.75%), respectively. On the same counting date, nymphs and adults were seen the most in Harran control application with 47.05%, Şanlıurfa with 45.35%, and Ceylanpınar control applications with 41.30% followed this location. In bacterial applications, nymph and adult countings were made at Ceylanpınar, Şanlıurfa, and Harran locations at 18.75%, 18.10%, and 17.00%, respectively (Table 2).

Table 2. Efficacy of bioagent formulation tested against Tetranychus cinnabarinus and *Tetranychus urticae* in cotton field experiments

Applications-Locations	**Egg*** **(number leaf^{-1})**	**Nymph (number leaf^{-1})**	**Adult (number leaf^{-1})**	**Nymph + Adult (number leaf^{-1})**
Control-Şanlıurfa	20.25 a	17.30 b	29.05 a	45.35 a
Control-Ceylanpınar	20.05 a	15.45 b	26.85 a	41.30 b
Control-Harran	17.7 a	8.65 a	26.80 a	47.05 a
Bioagent formulation-Şanlıurfa	6.95 b	7.55 c	10.55 b	18.10 c
Bioagent formulation-Ceylanpınar	7.95 b	6.85 c	11.9 b	18.75 c
Bioagent formulation-Harran	8.65 b	7.25 c	9.75 b	17.00 c
LSD	**33.55**	**30.57**	**19.34**	**15.82**
CV	**2.86**	**2.39**	**1.15**	**5.09**
Average of bioagent formulation	7.85 A	7.21 A	10.73 A	17.95 A
Average of control	19.33 B	17.68 B	27.56 B	44.56 B
LSD	**1.66**	**1.42**	**1.36**	**1.88**
CV	**33.73**	**31.41**	**19.6**	**16.6**

*There is no statistical difference between the values expressed with a similar letter in the same column (P<0.01).

The effect of applications 60 days after sowing on some plant growth and yield parameters in cotton plants is given in Table 3.

When the plant growth and yield parameters were evaluated in the control plots and the plots where the bioagent formulation was applied in the experiments established in three different locations, an increase was observed in the plots where the bioagent formulation was applied (Table 3). According to the location averages, in the control parcels; the average plant height is 82.06 cm, the plant crown diameter is 37.02 mm, the number of wood branches is 1.82 piece plant^{-1}, the number of fruit branches is 7.68 piece plant^{-1}, number of flowers is 6.35 piece plant^{-1}, cocoon diameter is 11.10 mm, number of cocoons is 12.36 piece plant^{-1}, root collar diameter is 4.91 mm, main root length is 29.33 cm, number of lateral roots is 9.03 piece plant^{-1}, number of opened cocoons is 35.58 piece plant^{-1}, number of unopened cocoons is 33.70 pieces and average cocoon weight is 10.10 g plant^{-1}. In the parcels where bioagent formulation is applied; the average plant height is 115.01 cm, plant crown diameter is 69.66 mm, number of wood branches is 2.15 piece plant^{-1}, the number of fruit branches is 17.68 piece plant^{-1}, number of flowers is 7.70 piece plant^{-1}, cocoon diameter is 16.14 mm, number of cocoons is 28.00 piece plant^{-1}, root collar diameter is 7.99 mm, main root length is 37.33 cm, number of lateral roots is 26.70 piece plant^{-1}, the number of opened cocoons is 69.68 piece plant^{-1}, the number of unopened cocoons is 88.71 piece plant^{-1} and average cocoon weight 15.62 g plant^{-1} (Table 3). In all three experimental areas, increases were observed in terms of all parameters evaluated in bacterial applications, and these increases were found to be statistically significant compared to the control plots. In bioagent formulation applications, increase of 40.15% in plant height, 88.16% in plant crown diameter, 18.13% in the number of wood branches, 130.20% in the number of fruit branches, 21.25% in the number of flowers, 45.40% in the cocoon diameter, 126.53% in the number of cocoons, 62.72% in the diameter of the root collar,

195.68% in the number of lateral roots, 163.23% in the number of unopened cocoons, 95.84% in the average cocoon weight and 54.65% in the number of opened cocoons were observed (Table 3).

In the evaluation made at the end of the harvest; in control parcels; the average cotton un-seed yield is 57.36 g plant^{-1} and 573.66 kg decare^{-1}, the cotton gin yield is 39.50% and fiber yield is 226.20 kg decare^{-1}. In the parcels where bioagent formulation was applied; cotton un-seed yield is 103.27 g plant^{-1} and 1032.76 kg decare^{-1}, cotton gin yield is 39.83% and the fiber yield is 412.34 kg decare^{-1}. In all three experimental areas, increases were observed in terms of cotton un-seed yield and fiber yield in bioagent formulation applications, and these increases were found to be statistically significant compared to control plots. These increases in cotton plants in bioagent formulation applications; seed cotton yield was 80.03% and fiber yield was 82.17% (Table 3).

Table 3. The effect of bioagent formulation on plant growth and yield parameters in cotton field experiments

Applications-Locations	**Plant height (cm)**	**Plant crown diameter (cm)**	**The number of**		
			Wood branches	**Wood branches**	**Wood branches**
Control-Şanlıurfa	93.05±23.16b	37.07±4.86ab	1.68±0.72a	8.00±2.53b	5.05±2.76a
Control-Ceylanpınar	89.05±23.86b	41.05±7.50b	2.10±0.78ab	9.00±2.53b	6.00±2.80ab
Control-Harran	64.10±21.28a	32.95±10.47a	1.68±0.72a	6.05±2.35a	8.00±2.61c
General average (Control plots)	**82.06**	**37.02**	**1.82**	**7.68**	**6.35**
Bioagent formulation-Şanlıurfa	120.05±26.71c	75.05±11.62d	2.15±0.66ab	17.05±2.30d	8.05±3.08c
Bioagent formulation-Ceylanpınar	118.05±20.23c	69.00±11.87cd	2.31±0.79c	21.00±3.17e	7.05±2.56bc
Bioagent formulation-Harran	106.95±19.89c	64.95±11.27c	2.00±0.56ab	15.00±3.64c	8.00±2.44c
General average (Plots with bioagent formulation applied**)**	**115.01**	**69.66**	**2.15**	**17.68**	**7.70**
Average increase (%)	**40.15**	**88.16**	**18.13**	**130.20**	**21.25**
Applications-Locations	**Diameter of cotton boll (mm)**	**The number of cotton boll (per plant)**	**Root collar diameter (mm)**	**The main root length (cm)**	**The lateral root number (per plant)**
Control-Şanlıurfa	10.29±3.15a	12.10±4.27a	4.72±1.22a	30.00±4.25b	6.00±0.79a
Control-Ceylanpınar	11.99±2.76a	13.05±3.48a	5.00±1.38a	33.00±4.53b	12.05±3.89b
Control-Harran	11.04±2.91a	11.95±3.88a	5.02±1.30a	25.00±6.83a	9.05±3.54ab
General average (Control plots)	**11.10**	**12.36**	**4.91**	**29.33**	**9.03**
Bioagent formulation-Şanlıurfa	17.53±4.68b	33.00±10.51c	7.94±2.51b	38.05±8.19c	28.05±8.56c
Bioagent formulation-Ceylanpınar	15.95±4.94b	26.00±9.53b	8.05±2.46b	42.00±7.97c	27.00±7.31c
Bioagent formulation-Harran	14.95±4.65b	25.00±6.64b	8.00±2.45b	31.95±8.99b	25.05±6.59c
General average (Plots with bioagent formulation applied**)**	**16.14**	**28.00**	**7.99**	**37.33**	**26.70**
Average increase (%)	**45.40**	**126.53**	**62.72**	**27.27**	**195.68**
Applications-Locations	**The number of**		**Mean boll weight (g plant^{-1})**		
	Boll opening (per plant)	**Not boll opening (per plant)**			
Control-Şanlıurfa	29.60±6.02b	40.00±13.02b	10.91±2.30b		
Control-Ceylanpınar	60.10±16.59c	50.10±13.65c	11.07±2.27b		
Control-Harran	17.05±11.94a	11.00±5.67a	8.02±3.13a		
General average (Control plots)	**35.58**	**33.70**	**10.10**		
Bioagent formulation-Şanlıurfa	82.05±15.44d	106.05±17.24f	17.36±3.12d		
Bioagent formulation-Ceylanpınar	75.00±13.34d	93.05±18.21e	15.52±3.11c		
Bioagent formulation-Harran	52.00±13.69c	67.05±15.85d	13.98±2.38c		
General average (Plots with bioagent formulation applied**)**	**69.68**	**88.71**	**15.62**		
Average increase (%)	**95.84**	**163.23**	**54.65**		
Applications-Locations	**Seed cotton yield (kg da^{-1})**	**Ginning yield (%)**	**Lint yield (kg da^{-1})**		
Control-Şanlıurfa	610.00b	39.50	240.95		
Control-Ceylanpınar	630.50b	39.00	245.89		
Control-Harran	480.50a	40.00	192.20		

Table 3. The effect of bioagent formulation on plant growth and yield parameters in cotton field experiments (continued)

General average (Control plots)	**573.66**	**39.50**	**226.34**
Bioagent formulation-Şanlıurfa	1049.90d	40.00	422.32
Bioagent formulation-Ceylanpınar	1120.70 d	40.00	448.28
Bioagent formulation-Harran	927.70 c	39.50	366.44
General average (Plots with bioagent formulation applied)	**1032.76**	**39.83**	**412.34**
Average increase (%)	**80.03**	**0.83**	**82.17**

*The differences between the averages indicated by different letters in the same column were found to be significant at the p=0.05 level.

The appearance of some plant growth parameters of the plants that were applied bioagent bacteria formulation and control application in cotton field experiments is given in Figure 1.

Figure 1. Appearance of the plants with bioagent bacteria formulation and control application in terms of some plant growth parameters.

In recent years, there has been a notable increase in the number of studies focusing on the utilization of bacteria for biological control, not only in Türkiye but also worldwide. A new approach in biological control is to promote plant systemic resistance by using plant growth-promoting bacteria (PGPR). Especially with the use of PGPRs for biological control, the protection of plant health and an increase in production can be achieved. Promoting plant resistance to infection by pathogen or acquired systemic resistance (SAR: Systemic Aquaried Resistance), which can be defined as the reaction of the plant against the infection of the pathogen, which is not found naturally in the plant but is physiologically

acquired by the stimulus of biocontrol organisms, is the most studied subject. In particular, SAR, promoted by bacteria is the most effective control method used against plant diseases of soil, seed, or leaf origin. With the use of PGPR for biological control, an increase in production can be achieved along with the protection of plant health. The most commonly used microorganisms in this field are different species of *Bacillus, Pseudomonas, Azotobacter, Burkholderia, Mycorrhyzae, Streptomycetes*, *Enterobacter*, *Verticillium*, *Agrobacterium*, *Aspergillus* and *Trichoderma,* especially those such as *Bacillus subtilis*, *B. cereus*, *B. pumilus*, *B. megaterium* and *B. sphaericus, Pseudomanas cepacia, P. putida, P. fluorescens* and *P. polymyxa,* and they can be used effectively in disease and pest control. (Halos and Zorilla, 1979; Guo et al., 1996; Aysan et al., 1999; Altın and Bora, 2001; Aysan et al., 2003; Guo et al., 2004; Özaktan and Bora, 2006; Akgül and Mirik, 2008).

Compant et al. (2005), emphasized that the use of bacteria in sustainable agriculture has a special status for sustainable agriculture because some of these bacteria have a characteristic that promotes plant growth as well as the ability to control pests. It has been determined that *Bacillus sphaericus* is used effectively in the control of many pests, including mites (Falcon, 1985). It has also been noted that biopesticides based on metabolites of another *Bacillus* type *B. thuringiensis* (Bt) and *Streptomyces avermitilis* bacteria have been used to control *T. urticae* in recent years (Chapman and Hoy, 2009; Brown et al., 2017). Dutton et al. (2003), reported that when Dipel obtained from *B. thuringiensis* subsp. *kurstaki* (HD-1) was applied to the corn plant in spray form, there was a slight decrease in the growth rate of *T. urticae* compared to the control. The researchers concluded that this was due to the decrease in the number of eggs laid by females in plants sprayed with Dipel. In another study, Alper et al. (2013), in their study examining the effect of a spore-crystal mixture of 31 natural *B. thuringiensis* isolates on *T. urticae* nymphs, noted that some isolates may have a certain potential in the development of biopesticides that especially retard the growth of nymphs. Moreover, Neethu et al. (2016), stated that toxin-producing bacteria such as *B. thuringiensis* have been widely used as bio-acaricide in recent years.

Abou Zaid et al. (2018), tested the efficacy of *Lysinibacillus sphaericus* and *Bacillus amyloliquefaciens* bacterial isolates, which are used as bio-control agents as well as promoting plant growth, on the harmful *T. urticae* in beans, found that 3 days after the application as a spray on the leaves, there was a 37% decrease in the *T. urticae* population. Similarly, it has been documented that *Pseudomonas aeruginosa* caused a 100% mortality among adult female *T. urticae* after 72 hours of application at a concentration of 10^7 cfu mL−1 via spray method. For *Bacillus subtilis*, the application resulted in approximately 80% mortality, while the treatment involving *Lysinibacillus sphaericus* led to slightly over 95% mortality (Emam, 2021; Jakubowska et al., 2022). In another study, Zenkova et al. (2020) conducted laboratory experiments to determine the impact of two bacterial species (*Streptomyces avermitilis* and *Bacillus thuringiensis*) on *T. urticae*. They obtained maximum mortality rates of 90% to 100% for adults and 91% to 99% for nymph stages, respectively, using biopesticides based on both *S. avermitilis* and *B. thuringiensis*. The effect of *Acinetobacter* sp., *Bacillus subtilis,* and *B. qassimus* species against *T. urticae*, which causes damage to eggplant, was tried to be determined under laboratory and greenhouse conditions, and the highest mortality rate was found in *Acinetobacter* sp. (87.15% in the laboratory, 77.29% in the greenhouse) after three days of spray application and when *B. subtilis* and *B. qassimus* were used, the mortality rates seven days after the application were 72.22% and 67.11% in the laboratory, and 70.74% and 65.19% in the greenhouse, respectively (Al-Azzazy et al., 2020).

In another study, the efficacy of *Pseudomonas putida* Biotype B belonging to the Pseudomonas type, which has been used successfully in biological control, was investigated against *T. urticae*, and for this purpose, newly emerged mated females were administered *P. putida* biotype B (108-109 colonies/ml) suspensions were applied by spraying and immersion. In the bacterial spraying process, a 100% effect on the mites was determined, similarly, the minimum number of viable eggs was also determined in the spraying process, and it was noted that this process was more effective than the immersion method (Aksoy and Yılmaz, 2008).

The use of high levels of insecticides causes the pest to show resistance to these chemicals and causes problems such as phytotoxicity caused by these insecticides (Ioannidis et al., 1991; Stewart et al., 1997; Mota-Sanchez et al., 2000). For these reasons, especially in recent years, researchers have focused on the use of bacterial and fungal pathogens in the biological control of many pests, including red spider mites. In this research; a bioagent formulation mixture consisting of free nitrogen fixer and phosphate solvent bacteria *Bacillus subtilis* PA1 and *Paenibacillus azotofixans* PA2, which were isolated from the root rhizosphere of wild and cultivated plants in various previous studies, was applied

to cotton by seed coding and ground surface hitch spray method. In this way, it has been determined that the mixture can be used successfully in the control against red spider mites that damage cotton plants in field conditions. It was determined that there was an increase in the number of cocoons in the cotton plant, the number of cocoons opened, and the average cocoon weight in bioagent formulation practices. These large increases in the number of opened and unopened cocoons show that the plant is well-fed in terms of nitrogen thanks to nitrogen fixation of the bacteria. Since this good nutritional state also prolongs the vegetative development process of the plant, it has also caused a great increase in the number of unopened cocoons. In bioagent formulation applications, an 80.03% increase in cotton un-seed yield and an 82.17% increase in fiber yield in cotton plants is because the increase in cocoon number and weight is reflected in the yield, which is of great importance.

Conclusion

This study has tried to determine the usability of the bacterial formulation used as a biological control agent that does not threaten human and environmental health in the control of *T. urticae* and *T. cinnabarinus*, which cause significant damage to cotton, and it has been determined that it can be used successfully in the control. It was also determined that this formulation significantly increased yield and quality by encouraging the development of cotton. It is envisioned that the obtained bioagent formulation can be used successfully in cotton cultivation and at the same time, it will be an alternative product against chemical pesticides and fertilizers that are used excessively in cotton. It is necessary to focus on such studies and to introduce more conscious disinfection and pest management practices. Thanks to more detailed studies on this subject that will be conducted later, alternative methods to chemicals will be put into practice.

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