

Antagonistic Effect of *Beauveria bassiana* against *Rhizoctonia solani* Causing Damping-Off of Cotton

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Abstract— *Rhizoctonia solani* is a soil-borne pathogen that causes significant yield and quality losses in cotton fields. Considering the environmental and economic costs of chemical pesticides, the development of alternative disease control strategies is necessary. *Beauveria bassiana*, a white muscardine fungus, has recently drawn attention worldwide not only as a potential biocontrol agent against insect pests but also for plant disease. In the study, four native isolates of *B. bassiana* were evaluated for antagonistic effect against *R. solani* (test pathogen) in vitro conditions. In the first experiment, *B. bassiana* was plated two days before *R. solani* on Petri plates, and in the second experiment, *B. bassiana* and *R. solani* were plated concurrently on plates. Experiments were performed with five replicates in a completely randomized plot design. All *B. bassiana* isolates inhibited mycelial growth of *R. solani* at different rates. The highest percent inhibition against test pathogen was determined in ET 101 (78.1%) and ET 10 (77.1%) isolates, respectively, when applied two days ago. The highest percentage of inhibition against test pathogen was found in ET 101 (69.0%), ET 10 (67.7%), and Bb1 (67.7%) isolates, respectively, when *B. bassiana* and the test pathogen were applied at same time. ET 101 and ET 10 isolates have shown hope against *R. solani*. The antagonistic effect of *B. bassiana* was found to be more developed in the plate two days before the test pathogen. However, *B. bassiana* isolates need to be investigated under field conditions for their roles in disease management, plant growth, and yield.

Keywords— *Beauveria bassiana*, *Rhizoctonia solani*, Antagonism, Biocontrol, Cotton.

I. INTRODUCTION

Cotton is one of the most significant crops with substantial economic value worldwide (Rai et al., 2022). Cotton is the raw material of approximately 50 industrial sectors, including cotton processing for ginning, fiber for textiles, seeds for oil, pulp for feed, and linter for paper (Ozyigit, 2009). The genus *Gossypium* comprises approximately 50 species grown to arid and semi-arid regions with tropical and subtropical climates, with *Gossypium hirsutum* L., commonly known as upland cotton, and representing 97% of production (Wendel and Grover, 2015). Globally, cotton is cultivated across ~90 countries, covering a total area of 31.92 million ha, and an average of 26.4 million tons of fiber cotton is produced (Vitale et al., 2024). Türkiye ranks seventh in the world cotton production after India, China, the USA, Brazil, Pakistan and Uzbekistan (ICAC, 2023). The total cotton cultivation area in the Southeastern Anatolia, Aegean, Çukurova and Antalya regions of Türkiye is approximately 467.000 ha, the seed cotton yield is 2.2 million tons and the fiber cotton yield is 777.000 tons (TSI, 2024).

Cotton diseases significantly damage both yield and quality, posing a major problem to the economic sustainability of farmers (Chi et al., 2021). Cotton damping-off disease caused by *Rhizoctonia solani*, is an important soil-borne and seedling disease (Mikhail et al., 2010). In the United States, average annual losses from cotton diseases over a 10-year period were reported as 3.1% and losses in fiber production from seedling diseases as 27%. According to the National Cotton Disease Association of the United States, the disease caused more than 109.000 bales of loss in 2004 (Blasingame

and Patel, 2005). *R. solani* is one of the most primitive members of class Basidiomycetes, and exists in its vegetative form in many all agricultural fields (Zaki et al., 2021). *R. solani* Kühn. the anamorphic of *Thanatephorus cucumeris* (Frank.) Donk, pre-or postemergence damping-off, sore shin, and root rot of cotton seedlings (Watkins, 1981). In cotton, the primary seedling disease-causing group of *R. solani* is AG-4 (Rothrock and Buchanan, 2017). The disease is seen in two ways: firstly, the death of the seed between germination and emergence to the soil surface and secondly, the death of the cotton seedling emerging to the soil surface. The disease symptoms and damage of the damping-off agent vary depending on the age and development period of the plant. Seeds of susceptible plants germinate in disease-contaminated soil and become soft, brown, shrivel, and rot. Diseased seeds in the soil are identified by the lack of emergence in certain areas. In damping-off disease, the primary damage occurs in the roots, and the damaged root bark then changes color, softens, and begins to rot. The roots and root collars of diseased seedlings germinating on the soil surface become brown, thin, and the plant can not stand, topples over and dries (Agrios, 2005). This pathogen does not produce asexual spores; instead, it survives overwinters as sclerotia in the soil and plant debris (Ahmad and Hasanuzzaman, 2020). Disease causes seedling loss, leaving some empty fields. In such cases, farmers use more seed than necessary (Rothrock et al., 2015).

Today, cultural and chemical control methods are used to control the disease. Fungicides cause phytotoxicity, environmental and soil pollution, resistance to disease agents, and harmful effects on human health (Ramamoorthy et al., 2002). The pathogen forms resistant structures in the soil and

fungicides become ineffective against soil-borne diseases over time. Therefore, natural, eco-friendly, alternative control methods should be explored. One of the alternative control methods against soil-borne diseases is the use of the antagonistic *Beauveria bassiana* entomopathogenic fungi (EPF) (Tomilova et al., 2020). EPF have been used as biological control agents since approximately the 1900s (Van Driesche and Bellows, 1996). Currently, 700 EPF species belonging to 90 genera have been identified. *B. bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae), *Lecanicillium* (= *Verticillium*) *lecanii* (Zimm.) Zare & Gams (Hypocreales: Clavicipitaceae), and *Purpureocillium lilacinum* (Thom.) Luangsaard, Hou-braken, Hywel-Jones, and Samson (Hypocreales: Ophiocordycipitaceae) EPF are used commercially in many countries to control many pests (Rath, 2000). Currently, *B. bassiana* has been reported to have 707 different hosts, including 521 genera, 149 families and 15 orders (Zimmermann, 2007). Recent studies shows that *B. bassiana*, often exclusively considered as insect pathogens, is also effective against plant pathogens (Jaber and Enkerli, 2016). In several studies, reports on *B. bassiana* as antagonistic effect against several phytopathogens were published viz., *Pythium ultimum*, *P. debaryanum*, *Septoria nodorum*, *P. irregulare*, *Phoma betae* (Vesely and Koubova, 1994), *Armillaria mellea* and *Rosellinia necatrix* (Reisenzein and Tiefenbrunner, 1997), *Rhizoctonia solani* and *Pythium myriotylum* (Ownley et al., 2004; Griffin, 2007; Ownley et al., 2008; Azadi et al., 2016), *Alternaria porri* (Gothandapani et al., 2015), *Plasmopora viticola* (Jaber, 2015), *Fusarium oxysporum* f. sp. *lycopersici* (Culebro-Ricaldi et al., 2017), *Botrytis cinerea* (Yun et al., 2017; Barra-Bucarei et al., 2020), *Verticillium dahliae* and *P. megasperma* (Lozano-Tovar et al., 2017; Erdoğan and Sağlan, 2023), *Colletotrichum kahawae* (Serrato-Diaz et al., 2020), *Curvularia lunata* (Deb et al., 2021), *F. oxysporum* f. sp. *cubense* (Mascarin et al., 2022). Possible mechanisms of plant disease suppression by *Beauveria* spp. are employing direct mechanisms such as mycoparasitism, competition and antibiosis or indirect interaction such as induced systemic resistance (Vega et al., 2009). The aim of study was to determine the antagonist effect of four native isolates of *B. bassiana* (ET 10, ET 101, Bb1, and Bb18) isolated from different hosts in Turkey against *R. solani* AG4 under *in vitro* conditions.

II. MATERIALS AND METHODS

A. Fungal Materials

B. bassiana (EPF) and *R. solani* AG4 (test pathogen) fungal isolates used in the experiment are shown in Table I. Four native isolates of *B. bassiana* were obtained from different hosts and locations in Turkey. All *B. bassiana* isolates were grown in the dark at 25±1°C for 7 days and then subcultured on potato dextrose agar (PDA-Difco, 39 g). Pure culture of *R. solani* was obtained from the collection of fungal cultures at Aydın Adnan Menderes University, Faculty of Agriculture, Department of Plant Protection and subcultured in PDA at 25±1°C for 7 days.

B. Antagonistic effect of *B. bassiana* against *R. solani*

The antagonistic interactions between the *B. bassiana* and the *R. solani* were evaluated using the dual-culture assay on PDA in the 90 mm Petri dishes (Skidmore and Dickinson, 1976). In the first experiment, 5-mm mycelium plugs from 7-day-old cultures of *R. solani* AG4 and *B. bassiana* were placed in the Petri plates 20 mm from each other. *B. bassiana* was plated two days before *R. solani* due to high growth rate of *R. solani* growth. In the second experiment, *B. bassiana* and *R. solani* mycelium plugs (5 mm in diameter, 7-day-old) were placed concurrently at a distance of 20 mm from the corner of Petri plates. Plates inoculated with *R. solani* alone were used as controls. Inoculated plates were incubated at 25±1°C until control plates with test pathogen attained full growth. The experiment was carried out with five replicates in a completely randomized plot design. The Bell scale (Bell et al., 1982) used to determine the relationship between *B. bassiana*, and the test pathogen is given in Table II. Percent inhibition (%) on mycelial growth of test pathogen was calculated as per the formula given by Sundaramoorthy et al. (2012).

$$\text{Percent inhibition (\%)} = (C - T / C) \times 100$$

Where C was the radial growth of the pathogen in control (mm), and T was the radial growth of the pathogen in the presence of entomopathogen fungus (mm).

C. Statistical Analysis

Data were analyzed by using one-way analysis of variance (ANOVA) performed in the JMP IN statistical program (SAS Institute, Cary, NC, 13.0 PC version). Significant differences between treatment means were determined with the LSMean Differences Student's test at $P \leq 0.01$.

III. RESULTS AND DISCUSSION

The antagonistic effects of native isolates of *B. bassiana* on mycelial growth and the percent inhibition (%) of *R. solani* AG4 isolates are shown in Table III. This study had statistically significant ($P \leq 0.01$) mycelial growth and inhibition rates of *B. bassiana* isolates against test pathogen compared to the control Petri plate. Mycelial growths of *R. solani* AG4 isolate (test pathogen) was measured as 82.1 mm in the control Petri plate (in both experiments). The lowest mycelial growth against test pathogen was in ET 101 (17.9 mm) and ET 10 (18.8 mm) isolates, when *B. bassiana* isolates were applied, two days before *R. solani* AG4, and these isolates were included in the same statistical group. The highest mycelial growth was determined in Bb18 (28.0 mm) and Bb1 (25.0 mm) isolates, and these isolates were found in the same statistical group. The highest percentage of inhibition against test pathogen was found in isolates ET 101 (78.1%) and ET 10 (77.1%), with a scale value of 1 for these isolates (Fig. 1). The lowest percentage inhibition was determined in Bb18 (65.0%) and Bb1 (69.0%) isolates, and these isolates were included in the same statistical group. When both *B. bassiana* and *R. solani* AG4 were applied simultaneously, the lowest mycelial growth was found in isolates, ET 101 (25.3 mm), ET 10 (26.6 mm), and Bb1 (26.6 mm) and these isolates were included in the same statistical group. The highest mycelial growth was measured as the 31.0 mm Bb18 isolate.

The highest percentages inhibition against test pathogen was determined at isolates ET 101 (69.0%), ET 10 (67.7%), and Bb1 (67.7%), with a scale value of 2 for these isolates. The lowest inhibition rate was observed with the Bb18 isolate at 62.2% (Table III).

In this study, the highest percent inhibition was recorded in Petri plates of *B. bassiana* isolates applied, days before *R. solani*. This may be because *B. bassiana* possesses mechanisms such as competition, antibiotics, and mycoparasites. This result was confirmed by the earlier reports of Griffin (2007) *B. bassiana* strain Bb 11-98 was observed around the hyphae of *Pythium*, inhibition was taken place by hydrolysis of cell wall of the pathogen. Beauvericin was suppressed the damping-off caused by *R. solani* and *Pythium myriotylum* in tomatoes (Ownley et al., 2008). Ownley et al. (2010) reported that *B. bassiana* suppresses plant pathogens through direct mechanisms such as mycoparasitism, competition and antibiotics and exhibits multiple mechanisms of antagonistic interactions. In the study, *B. bassiana* isolates showed varying degrees of mycelial growth and percent inhibition of *R. solani*. Similar to our results, antagonistic effect against *R. solani* with 22 different *B. bassiana* isolates was investigated on PDA plates. Three *B. bassiana* isolates were inhibitory against *R. solani*, there are differences *B.*

bassiana isolates and plant pathogen biocontrol capacities (Lee et al., 1999). Shternshis et al. (2014) reported that mycelial growth inhibition of *R. solani* during cocultivation with entomopathogenic fungi. Tomilova et al. (2020) reported that *R. solani* growth was also suppressed by *B. bassiana*. *B. bassiana* also began to grow on the *R. solani* mycelium after 10 days of dual cultivation. Deb and Dutta (2021) antagonistic potential of 22 native isolates of *B. bassiana* were evaluated against damping-off disease of tomato caused by *Pythium* sp. All *B. bassiana* isolates were inhibit mycelial growth of *Pythium myriotylum* to the extent of 68-82%. Deb et al. (2023) 53 native isolates of *B. bassiana* were tested for antifungal activity against *R. solani*. *B. bassiana* was found antagonistic effect against *R. solani* with a percent inhibition of 71.15%. Bb18 and Bb1 from *B. bassiana* isolates included in the study showed a high antagonistic effect against both pathotypes of *V. dahliae*. Notably, percent inhibition for Bb18 and Bb1 isolates was found high in experiments performed, 2 days before the pathogen (Erdoğan and Sağlan, 2023). Pachoute et al. (2024) reported that *B. bassiana* showed greater inhibition of *Fusarium* sp. isolate JP1 growth, growth of *Alternaria burnsii* isolate JP2 inhibited by 18.54 to 35.67% and that of *Epicoccum* sp. by 22.73 to 25.23%.

TABLE I. Listed of the fungal materials used in the experiment.

EPF isolate	Isolated from	Origin	Reference
<i>Beauveria bassiana</i> /ET10	<i>Sphenoptera antiqua</i>	Erzurum, Turkey	Tozlu et al. (2017)
<i>Beauveria bassiana</i> /ET101	Coleoptera larvae	Erzurum, Turkey	Erdoğan and Sağlan (2023)
<i>Beauveria bassiana</i> /Bb1	Forest soil	Düzce, Turkey	
<i>Beauveria bassiana</i> /Bb18	Field soil	Düzce, Turkey	
Test pathogen fungus isolate	Isolated from	Origin	Reference
<i>Rhizoctonia solani</i> AG4	<i>Gossypium hirsutum</i>	Aydın, Turkey	Erdoğan et al. (2016)

TABLE II. The scale from Bell et al. (1982) used in the study.

Scale value	Descriptions
1	The antagonist completely overgrew the pathogen
2	The antagonist overgrew at least 2/3 rd of the growth of the pathogen
3	The antagonist colonized half of the growth of the pathogen
4	The pathogen overgrew 2/3 rd of the growth of the antagonist and resisted invasion
5	The pathogen completely overgrew the antagonist

TABLE III. Suppression of *Rhizoctonia solani* by *Beauveria bassiana* in dual-plate assay.

EPF isolate Code	Mycelial growth (mm) ^a	Percent Inhibition (%)	Bell's scale ^{**}
Application of <i>B. bassiana</i> to the medium, two days before the test pathogen			
ET 10	18.8 c	77.1	S1
ET 101	17.9 c	78.1	S1
Bb1	25.5 b	69.0	S2
Bb18	28.0 b	65.0	S2
Control	82.1 a	0.0	-
CD _(p=0.01)	7.7		
Application of <i>B. bassiana</i> and test pathogen to the medium, at concurrently			
ET 10	26.6 c	67.7	S2
ET 101	25.3 c	69.0	S2
Bb1	26.6 c	67.7	S2
Bb18	31.0 b	62.2	S2
Control	82.1 a	0.0	-
CD _(p=0.01)	4.8		

^aData are means of five replicates, ^{*}Means followed by different letters within the column are significantly different according to LSD test (P≤0.01), CD: Critical difference, ^{**}Bell's Scale: S1: The antagonist completely overgrew the pathogen, S2: The antagonist overgrew at least 2/3rd of the growth of the pathogen, S3: The antagonist colonized half of the growth of the pathogen, S4: The pathogen overgrew 2/3rd of the growth of the antagonist and S5: The pathogen completely overgrew the antagonist.



Figure I. Antagonistic effect of *B. bassiana* applied to PDA plates two days before *R. solani*: (a, b) High activity from *B. bassiana* ET 101 and ET 10 to *R. solani* (scale value 1) (c) Control Petri plate (*R. solani* AG4 isolate)

IV. CONCLUSION

ET 101 and ET 10 from *B. bassiana* isolates were found best with respect to biocontrol potential against *R. solani* under *in vitro* conditions. Notably, percent inhibition for ET 101 and ET 10 isolates was high in experiments performed, two days before the pathogen. The reason for this is due to competition in *B. bassiana* and the mechanism of effect of antibiotics. Effects of antagonists against soil-borne pathogens are related to environmental conditions, soil, and population of antagonists. The antagonistic activity of these isolates must be further investigated in field conditions for their role in disease management, plant growth promotion and crop yield.

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