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In Vitro Evaluation of Inhibitory Effect of Some Plant Activators against Cotton Seedling Diseases

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Abstract Seedling root rot disease causes yield loss in cotton cultivated areas in the world. The aim of this study was to determine *in vitro* inhibitory effect of plant activators against seedling root rot pathogens utilizing dual culture technique. For this purpose, inhibitory effect of the plant activators on colony growth in potato dextrose agar (PDA) medium containing its plant activators at various concentrations (5, 25, 100, 250, 500 ppm) was investigated under *in vitro* conditions. PDA plates without plant activators were used as a negative control, and PDA plates mixed with licensed fungicide (Azoxystrobin 75 g l^{-1}+ Metalaxyl-M 37.5 g l^{-1} + Fludioxonil 12.5 g L^{-1}-Astranova company) were used as a positive control. Petri dishes were incubated in the dark at 24±1°C for 10 days. *In vitro* experiments were carried out with three replicates depending on a completely randomized plots design. Doses of plant activators inhibited both pathogen isolates to varying degrees under *in vitro* conditions. The highest inhibition effect against *Rhizoctonia solani* (AG4) and *Fusarium* spp. isolates was obtained from ASTRADYN 125 FS application (100% and 61.5%). After ASTRADYN 125 FS application, in the high dose (500 ppm) application of Green Miracle plant activator, the lowest colony diameter and the highest effect were determined against *R. solani* (AG4) as 11.67 mm and 72.2%, respectively. In the high dose application of auxiGRO plant activator, the lowest colony diameter and the highest inhibition effect were found against *Fusarium* spp. as 19.67 mm and 55.0%, respectively.

Keywords Seedling disease, plant activator, inhibitory effect, alternative control

1. Introduction

Cotton (*Gossypium* spp.) is an industrial crop grown in tropical and subtropical warm-climate regions of the world [1]. Although there is no certainty about the homeland of cotton, it is estimated that it spread to the world from the warm regions of Asia, America and Africa [2, 3]. Cotton has an important place in the Turkish and world economy as it constitutes the raw material of more than fifty industrial branches from the textile industry to the war industry, from the feed industry to the oil industry [4]. Cotton is grown on ~35 million hectares in more than 90 countries around the world, producing an average of 26.7 million tons of lint cotton yield in these regions [5]. China, India, USA, Pakistan, Brazil, Uzbekistan and Turkey provide 87 per cent of the world's production of cotton, which is a strategic product [6]. In Turkey, cotton is cultivated on a total area of 477 thousand ha in four main regions and yielding 2.2 million tons of seed cotton yield [7].

Seedling root rot disease of cotton was first described by Atkinson in 1892 [8]. The disease is known to be caused by soil-borne fungi [9]. Among the diseases causing economic loss in cotton cultivation, the most destructive one is cotton seedling root rot disease (*Rhizoctonia solani*, *Pythium* spp., *Fusarium* spp.,

Thielaviopsis basicola) [10]. In particular, *R. solani* has been reported to be a widespread and important pathogen as a post-emergence wilt disease wherever cotton is grown [11]. In the USA, the average annual seedling loss from seedling root rot in cotton over a 10-year period was calculated as 3.1% and it was reported that 27% of the yield loss in lint production was due to seedling root rot [12].

Disease symptoms and damage of seedling root rot agents vary according to the age and developmental stage of the plant. When the seeds of susceptible varieties are sown in the contaminated field, the seeds germinate under the soil, soften, then turn brown and brown and finally rot. The fact that the seeds are infected with pre-emergence smut is only recognised by the emergence of seedlings [13]. The first symptoms of the disease are seen in the newly formed root. The bark tissue of the root changes colour, softens and then rots. The root and root collar of the diseased seedlings turn brown, become thinner, the plant becomes unable to stand, then falls on the soil and dries up. In the years when the climate is cool and the moisture content in the soil is high, the disease is more severe and can catch the seedlings at an older age. At such times, the diseased seedlings develop dark brown coloured sunken spots just below the soil level and then these seedlings dry up [14]. In rainy and cool years, especially in contaminated and moisture retaining soils, the disease causes great damage and causes the root and root-throat of all seedlings in the cotton field to rot and die, requiring the field to be replanted. When the disease is not at a level that requires replanting, it causes some empty areas to remain in the field due to seedling loss. In order to meet this possibility, the farmer uses more seed than necessary. In this way, it causes great economic damages by causing an increase in seed, pesticide and processing costs and crop loss due to late sowing [15].

Fungicides are highly effective chemical substances used in the control of damping-off diseases. However, resistance, phytotoxicity, human and environmental health problems have emerged due to chemicals used unconsciously and intensively for years [16]. Nowadays, with the increasing awareness of producers and consumers, the demand for synthetic fungicides applied to disease agents is decreasing day by day and the interest in alternative control methods is gradually increasing. In this context, plant activators, which are known to promote resistance in cultivated plants, are used to control seedling root rot disease. Systemic induced resistance (SIR) studies started in the early 1960s and SIR studies have reduced or increased diseases caused by soil-borne plant pathogens [17].

Unlike pesticides, plant activators do not directly affect the disease agent, plant activators provide resistance by stimulating genes that activate the resistance mechanism in the plant [18]. The active component of AuxiGro, gamma aminobutyric acid (GABA), was developed by Emerald Bio (USA) in the 1990s. GABA, regulates mineral uptake in plants, speeds up photosynthesis and increases disease resistance of plants by reducing stress factors against pathogens [19]. Plant activators with *Lactobacillus acidophilus* active ingredient increase microbial activity and fertilizer performance, improve soil structure, vitamin and micronutrient uptake. These activators are used in potatoes, tomatoes, cotton, grapes, citrus fruits, vegetables and many product groups [20]. The development and practical use of new substances that stimulate the physiological activity of plants has gained momentum [21]. Messenger ™ (Eden Bioscience), Crop-Set (Improcrop), Bion (Syngenta) and ISR 2000 (Improcrop) are some commercially available products developed for this purpose [22]. Seaweed plant extracts have shown an inhibitory effect on the growth of a wide range of phytopathogenic fungi [23, 24]. Zhang et al. [25] reported that Hcm1 containing harpin protein suppressed the growth of *Verticillium dahliae* and *Fusarium oxysporum* and Hcm1 can activate innate immunity and prevent Verticillium and Fusarium wilt in cotton. Green Miracle plant activator is a long chain fatty acid based new generation stress alleviator for improving the plant health [26]. This work was performed in order to evaluate *in vitro* inhibitory effect of some plant activators against seedling root rot pathogens (*R. solani* AG4 and *Fusarium* spp.) by means of direct confrontation (dual culture) technique.

2. Materials and Methods

Fungal isolates and plant activators

Two pathogenic isolates of *R. solani* (AG4) and *Fusarium* spp. used in the experiment were originally isolated from the roots of cotton seedlings infected with damping-off disease. Isolation, purification, and identification of these fungi were carried out at Adnan Menderes University, Mustafa Kemal University, Faculty of Agriculture,

Department of Plant Protection. Fungal isolates were propagated on potato dextrose agar (PDA-Difco) medium and subcultured into fresh medium as needed.

The contents and doses of plant activators and licensed fungicide used in dual culture tests conducted under *in vitro* conditions are given in Table 1.

Table 1: Plant activators and licensed fungicide active ingredients and application doses used in the study

Brand name	Company	Active ingredients	Formulation	Dose (100 l)
auxiGRO	Boyut Foreign Trade Inc.	29.2% gamma aminobutryric acid (GABA)+ 29.2% l-Glutamic acid	WP	30 g
Green Miracle	Agrobest	80% vegetable fatty acid	EC	200 ml
Maxicrop	Valagro	*Ascophyllum nodosum* seaweed	WP	30 g
ProAct Plus	AMC-TR	Harpin protein 0.6%+am	WG	10 g
Sojall Vitanal	Adana Nature Organic Farming	*Lactobacillus acidophilus*	EC	60 ml
ASTRADYN 125 FS*	Astranova	Azoxystrobin 75 g l^{-1}+Metalaxyl-M 37.5 g l^{-1}+ Fludioxonil 12.5 g l^{-1}	FS	250 ml

*ASTRADYN 125 FS licensed fungicide

***In vitro* inhibitory effect of plant activators on *Rhizoctonia solani* (AG4) and *Fusarium* spp.**

Colony diameter of *R. solani* (AG4) and *Fusarium* spp. isolates was measured on PDA medium containing different concentrations (5, 25, 100, 250 and 500 ppm) of plant activators under *in vitro* conditions utilizing dual culture technique. For this purpose, different concentrations of plant activators were mixed in autoclaved (121ºC at 15 min.) PDA medium, and 25 ml was transferred to sterile plastic Petri dishes (90 mm diameter). PDA medium containing plant activator were allowed to solidify at room temperature. A mycelial plug (5 mm diameter) of *R. solani* (AG4) and *Fusarium* spp. isolates was taken from the margin of 7-day-old colony growing on PDA and placed in the centre of the Petri plates containing plant activator + PDA. PDA plates without plant activators were used as a negative control. PDA plates containing licensed fungicide (Azoxystrobin 75 g l^{-1} + Metalaxyl-M 37.5 g l^{-1} + Fludioxonil 12.5 g l^{-1}-Astranova company) were used as a positive control. Petri plates were incubated at 24±1ºC for a 10 day after inoculation. Colony diameters of *R. solani* (AG4) and *Fusarium* spp. were measured separately and per cent inhibition was calculated using the formula (Equation 1) by Deans& Svoboda [27]. *In vitro* experiments were performed using three replicates in a completely randomized plots design.

$$\text{Per cent inhibition (\%)}:[dc\text{-}dt\ /dc] \times 100 \quad (1)$$

Where; dc: Average diameter (mm) of fungal colony in negative control, dt: Average diameter (mm) of fungal colony in treatment or licensed fungicide (positive control).

Statistical analysis

One-Way ANOVA (analysis of variance) was carried out to determine the effects of the treatments. The Least Significant Differences (LSD) test was used to examine the significance level (P) of 0.01 for the differences. All statistical analyses were performed using JMP software version 13 (SAS Institute Inc., Cary, NC, USA).

3. Results and Discussion

Inhibitory effects of 5, 25, 100, 250 and 500 ppm doses of auxiGRO, Green Miracle, Maxicrop, ProAct Plus and Sojall Vitanal plant activators on colony diameter and inhibition rates (%) of *R. solani* (AG4) and *Fusarium* spp. isolates under *in vitro* conditions are given in Table 2. The inhibitory effect of plant activators on colony diameter of *R. solani* (AG4) and *Fusarium* spp. isolates was found to be statistically significant ($p \leq 0.01$) compared to the negative control. The plant activators inhibited colony growth of both pathogen isolates at different levels depending on the doses. Maximum effects (100% and 61.5%) against *R. solani* (AG4) and *Fusarium* spp. were obtained from the positive control application (ASTRADYN 125 FS). After ASTRADYN 125 FS application, in the high dose (500 ppm) application of Green Miracle, auxiGRO, Sojall Vitanal, ProAct

Plus and Maxicrop plant activators, the lowest colony diameter was determined against *R. solani* (AG4) as 11.67, 13.50, 14.50, 17.08 and 20.67 mm, respectively and the highest inhibition rate was measured as 72.2%, 67.8%, 65.5%, 59.2% and 50.7%, respectively. After ASTRADYN 125 FS application, in the high dose of auxiGRO, Green Miracle, Maxicrop, Sojall Vitanal and ProAct Plus, the lowest colony diameter was found against *Fusarium* spp. as 19.67, 20.67, 28.67, 29.50 and 33.75 mm, respectively and the highest inhibition rate was detected as 55%, 52.8%, 34.5%, 32.6% and 22.9%, respectively (Table 2). In a study, Yildirim & Yapici [28] reported that harpin protein had a 76% effect against the mycelial growth of *Botrytis cinerea* in strawberry at a concentration of 1000 µg ml^{-1}, and the effect continued to decrease at low doses. The another research by Lucon et al. [29] determined that 1 and 2 mg ml^{-1} concentrations of Messenger did not have an inhibitory effect on the mycelial growth of the citrus black spot disease *Guignardia citricarpa*. Galdeano et al. [30] found that the harpin protein at concentrations of 7.5-15-30-60-120 µg ml^{-1} was not effective on conidial germination and mycelial growth of *Cercospora coffeicola* in the coffee plants under *in vitro* conditions. Kadıoğlu [31] reported that gamma amino butyric acid, acibenzolar-S-methyl had no inhibitory effect on mycelial growth of pepper phytophthora blight (*Phytophthora capsici*), but the harpin protein, extract of *Reynoutria* spp. and chitosan have inhibitory effects. Seaweed extract made from a combination of *Durvillaea potatorum* and *Ascophyllum nodosum* has been reported to inhibit the growth of *Sclerotinia minor* in lettuce by 18-100% *in vitro* conditions [32]. Şahbaz & Akgül [33] determined that the products promoting plant resistance (Aliette WG, Bion MX 44 WG, ISR-2000 and salicylic acid) had no effect on the mycelial growth of Fusarium and Verticillium pathogen *in vitro* conditions. In another study, salicylic acid and fosetyl-Al from plant activators (salicylic acid, acibenzolar S-methyl, messenger, ISR 2000, Crop Set and fosetyl-Al) inhibited of *R. solani* at doses above 700 µg ml^{-1} and other plant activators had no effect on pathogens [34]. In a similar study, Delisoy & Altınok [35] reported that plant activators such as AuxiGro, Crop-Set and ISR-2000 inhibited the growth of *Fusarium oxysporum* f. sp. *melonis* by 49.25%, 41.80% and 35.82% respectively. In a study conducted under *in vitro* conditions, the highest fungicidal effect was obtained from Aliette with 31.5% inhibition at 1000 ppm among plant activators (ISR-2000, Crop-Set, Aliette and Messenger Gold) against stem canker and black scurf diseases in potato [36]. Sağlan [37] found that in the high dose of Maxicrop, Sojall Vitanal, ProAct Plus, Green Miracle and auxiGRO inhibited mycelial growth of isolate of PHCVd3 (*V. dahliae* Kleb.) by more than 90.00% and in the high dose of Maxicrop also inhibited 91.00% of isolate of PHCVd47 (*V. dahliae* Kleb.).

Table 2: Inhibition rates of some plant activators against *R. solani* (AG4) *and Fusarium* spp. *in vitro*

Plant activators	Doses (ppm)	*R. solani* (AG4)		*Fusarium* spp.	
		Colony diameter (mm)*	Per cent inhibition (%)	Colony diameter (mm)*	Per cent inhibition (%)
auxiGRO	0 (N-Control)[2]	41.92 a[1]	0.0	43.75 a[1]	0.0
	5	29.67 b	29.2	37.50 b	14.3
	25	26.25 c	37.4	34.08 c	22.1
	100	22.58 d	46.1	29.67 d	32.2
	250	20.58 d	50.9	26.92 e	38.5
	500	13.50 e	67.8	19.67 f	55.0
	P-Control[3]	0.00 f	100.0	16.83 g	61.5
	$CV_{(0.01)}$	8.66		2.11	
Green Miracle	0 (N-Control)[2]	41.92 a	0.0	43.75 a	0.0
	5	29.50 b	29.6	35.00 b	20.0
	25	23.25 c	44.5	27.50 c	37.1
	100	17.67 d	57.9	24.00 d	45.1
	250	15.67 d	62.6	21.17 e	51.6
	500	11.67 e	72.2	20.67 e	52.8
	P-Control[3]	0.00 f	100.0	16.83 f	61.5
	$CV_{(0.01)}$	10.42		2.92	

Maxicrop	0 (N-Control)[2]	41.92 a	0.0	43.75 a	0.0
	5	34.83 b	16.9	39.08 b	10.7
	25	31.08 bc	25.9	38.58 b	11.8
	100	26.17 cd	37.6	38.50 b	12.0
	250	24.33 d	42.0	36.83 c	15.8
	500	20.67 e	50.7	28.67 d	34.5
	P-Control[3]	0.00 f	100.0	16.83 e	61.5
	$CV_{(0.01)}$	11.59		5.79	
ProAct Plus	0 (N-Control)[2]	41.92 a	0.0	43.75 a	0.0
	5	34.75 b	17.1	39.42 b	9.9
	25	29.83 c	28.8	38.83 b	11.2
	100	25.58 cd	39.0	37.83 c	13.5
	250	21.42 de	48.9	37.42 c	14.5
	500	17.08 e	59.2	33.75 d	22.9
	P-Control[3]	0.00 f	100.0	16.83 e	61.5
	$CV_{(0.01)}$	10.56		1.08	
Sojall Vitanal	0 (N-Control)[2]	41.92 a	0.0	43.75 a	0.0
	5	34.42 b	17.9	39.33 b	10.1
	25	29.25 c	30.2	37.58 c	14.1
	100	23.83 d	43.1	35.67 d	18.5
	250	19.25 e	54.1	34.25 e	21.7
	500	14.50 f	65.4	29.50 f	32.6
	P-Control[3]	0.00 g	100.0	16.83 g	61.5
	$CV_{(0.01)}$	7.24		1.50	

*Data are means of three replicates, [1]Means followed by different letters within a column are significantly different according to LSD test ($p \leq 0.01$), [2]Negative Control, [3]Positive Control: ASTRADYN 125 FS-Astranova company, CV: Coefficient of variation.

4. Conclusion

Today, the use of synthetic fungicides in the control against plant pathogens is decreasing day by day and the tendency to alternative control methods is increasing. In the study, no mycelial growth was found in *R. solani* (AG4) isolate compared to *Fusarium* spp. isolate and 100% inhibition was observed in the positive control application (ASTRADYN 125 FS). After ASTRADYN 125 FS application, in the high dose of Green Miracle plant activator, the highest effect was determined against *R. solani* (AG4) isolate as 72.2%. In the high dose of auxiGRO plant activator, the highest inhibition rate was found against *Fusarium* spp. isolate as 55%. After ASTRADYN 125 FS application, the highest inhibitory effect of Green Miracle and auxiGRO plant activators can be attributed to the 80% vegetable fatty acid and GABA+29.2% l-Glutamic acid content of these plant activators against both pathogen isolates. However, aiming to estimate the efectiveness of plant activators under natural environmental conditions, it is suggested to fully carry out biocontrol trials in the feld. Because, in the future, there will be a need for plant activators that do not disturb the natural balance, do not have harmful effects on human and environmental health, and are more easily degradable unlike synthetic fungicides.

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