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The Effect of Paclobutrazol, Growth Retardant, on Cotton Growth and Verticillium Wilt (*Verticillium dahliae* Kleb.)

I. Cimen, [1]S. Başbag, [1]M. Temiz and A. Sagır
Faculty of Agriculture, Plant Protection Division, University of Dicle, Diyarbakır, Turkey
[1]Faculty of Agriculture, Field Crop Division, University of Dicle, Diyarbakır, Turkey

Abstract: In the Tigris Basin, since the profit obtained from cotton is relatively higher with respect to other field plants, cotton growing has been preferred more and continued without crop rotation. Due to lack of crop rotation, Verticillium wilt of cotton (*Verticillium dahliae* Kleb.) becomes a significant problem. In this study, through inhibiting or declining Verticillium wilt of cotton, we aimed to increase crop yield by Paclobutrazol (PBZ), which is a growth retardant. The dose and time of application of PBZ were determined at Nazilli 87 in 1998 and Sur-Grow SG 501 cotton varieties in 1999. In the research, it was determined that the retardant was effective in declining the disease. It was also established that, in case of early application and dose increase, the inhibitor was more effective in declining the disease, however leading to yield lose due to excessive stocky and dwarfing. It was considered to arrange more plants per unit area in order to benefit from dwarfing of the retardant mentioned and therefore, the yield was increased about 48% at Maraş 92 variety in 2000, through its 0.05 g m^{-2} of application dose after the second irrigation without decreasing density 20 plant m^{-2}, which is a significant for cotton labour work. In conclusion, the application of Paclobutrazol at a dose of 0.05 g m^{-2} was considered suitable after the second irrigation without decreasing the density (through more plants per unit area), when the plant height reaches 40-50 cm.

Key words: Cotton, cotton wilt disease, paclobutrazol, plant growth regulator, *Verticillium dahliae*

INTRODUCTION

Cotton is of utmost importance as an industrial crop in Turkey. Its sown areas have been constantly increasing because of irrigation possibilities in the South-eastern Anatolia Region of Turkey and this increasing rate has become as 30% in the Diyarbakır province. One of the most important problems that have adversely affected cotton farming is Verticillium Wilt (*Verticillium dahliae* Kleb.), when there was no crop rotation. The disease was first established in the Manisa province of Turkey. Since then, it has fast spread in the Aegean and the Mediterranean Region of Turkey and the proportion of infected plant has attained 87% in the South-eastern Anatolia Region[1-3].

In the Tigris Basin, due to the fact that the profit obtained from cotton is relatively more with respect to other field plants, its farming has been preferred more and continued without crop rotation. As a result of this, Verticillium wilt of cotton (*Verticillium dahliae* Kleb.) becomes a significant problem.

As a result of using growth retardant Paclobutrazol (PBZ) in growing, it stimulates mechanical obstacle (tolerant mechanism) against fungal diseases, especially, in host plant besides benefit the obtained for growing. On this subject, decreasing in the disease incidence was obtained by application of PBZ in order to increase the resistance against *Fusarium* causing wilt in melon in Israel[4].

Also, the relationship between Paclobutrazol and Mal secco (*Phoma tracheiphila* (Petri) Kanc.Et.Ghik) disease which is another vascular disease and a major problem in lemon growing in Turkey was studied with reasonable success. *In vitro* stage of this study, the effect of PBZ on the mycelial growth of the agent mentioned above was found to be fungistatic and ED_{50} was established as its 6 ppm and plus doses[5]. In greenhouse stage of the same study, the disease incidence was prevented by 71.6% after artificial inoculation of *Phoma tracheiphila* at the young lemon trees on which Paclobutrazol had been performed earlier[6]. In the natural inoculation, this proportion was found to be 52.6% with the same application[7]. It was also reported that soil + leaf application could be recommended to growers and that there was not any death with this application, whereas the death rate of control trees was 18.7% in the fourth year. In

Corresponding Author: Ismail Cimen, Faculty of Agriculture, Plant Protection Division, University of Dicle, Diyarbakır, Turkey

the forth year, increasing yield was obtained with the same application by 47% per tree and by 55% per cross sectional area[8]. In addition to a study conducted, while there was no adverse affect of PBZ on lemon fruit quality, no residue detected as well[9].

Mycelial growth *in vitro* of eight plant pathogenic fungi of woody plants on was inhibited 25-100% by PBZ in USA[10].

In this study, through inhibiting or declining of Verticillium wilt of cotton, we aimed to increase crop yield by Paclobutrazol, which is a growth retardant.

MATERIALS AND METHODS

Chemical material used *in vitro* and field study was Paclobutrazol (PBZ) (PP-333, Cultar; 25% a.i) (B-Chlorophenyl) methyl-(l.1-dimethyl)-1-H-1, 2,4 triazole-1 ethanol) which is effectively transported systematically via xylem. Studies on PBZ were conducted both in laboratory and field conditions.

Laboratory study: Bioactivity study of PBZ was carried out *in vitro* conditions. Different doses of chemical, stock solution of which had previously been prepared, were added into 250 ml Erlenmayer flask, which contained 100 ml Potato Dextrose Agar (PDA) medium. This 'PDA+PBZ' media was autoclaved for 15 min at 121°C, under 1 atm pressure. Later, 20 ml of this media sterilized was emptied into 90 mm diameter petri dish and then, 6 mm diameter mycelial disk of *Verticillium dahliae* from which 15 day culture on PDA was inoculated in the center of this petri dish. After then, these petri dishes were incubed under 22°C. Mycelial growth of *Verticillium dahliae* had been measured from two different directions at intervals of one week up to complete cover of petri dish. Then, ED_{50} was calculated.

Field studies: In the experiments conducted under field conditions, of three plant materials, Nazilli 87 and Maraş 92 were varieties, which were tolerant to Verticillium wilt, whereas Sure-Grow SG 501 variety was susceptible. It was impossible to study with the same variety in the consecutive years seeing that grower had changed variety every year because of damage of Verticillium wilt. The experiment field soil was of heavy structure near the Tigris. In the first experiment (1998), at the wheat sown in the field the previous year, according to randomised blocks of experiment design with the four replicates, 0.05, 0.1, 0.2, 0.3 and 0.4 g m^{-2} dozes of PBZ were applied into the soil with the Nazilli 87 variety after the first irrigation. The second experiment (1999) was established with Sure-Grow SG 501 cotton variety in the infected soil with *Verticillium dahliae* and cotton sown in the consecutive years. Factorial experiment design with three replicates, 0.05 and 0.1 g m^{-2} doses of the retardant mentioned were treated after the first and second irrigation once for growing season. These dozes and application way were decided according to the results obtained in experiment in 1998. In the third study in 2000, prepared with the same experiment design but with four replicates, 0.05 and 0.1 g m^{-2} doses of PBZ were applied to Maraş 92 variety at the decreasing plant density plots 5 plant m^{-2} and without those 20 plant m^{-2} after second irrigation. The modifications of the method were done due to second year experiment results.

The establishment of plant growth and determination of yield characteristics: Plant height (cm) was determined as length which was measured between the soil surface and the highest shoot tip, while the crown width was fixed as length, which was considered between the largest two branches at the plants in the plot at the end of vegetation. Yield per unit area kg ha^{-1}, per unit plant g $plant^{-1}$ and per crown sectional area g m^{-2} were calculated after the wholly opened bolls had been collected and weighed.

The determination of the rate of infected plant and disease incidence: According to discoloration in the trunk section taken at the fifth nodium from the soil surface, the proportions of infected plants and disease incidence were determined in 30 samples of plant from two rows in the middle of plot. Disease incidence was evaluated as follow scale: 0=no discoloration xylem on trunk sectional area; 1=1-33% discoloration xylem; 2=34-67% discoloration xylem; 3=68-100% discoloration xylem[11].

RESULTS

Results of laboratory study showed decreasing mycelial growth of *Verticillium dahliae* is seen as long as the supplemental doses of PBZ increase PDA. The inhibiting rate of mycelial growth by PBZ were calculated over 50% at the doses of 20 ppm and above (Table 1).

According to the results of the first year studies (1998), the plant height (cm) decreased and crown width (cm) narrowed at the Nazilli 87 cotton variety, which were found to be significant (P:0.01). Also, the rate of infected plants (P:0.05) and disease incidence (P:0.01) was observed to have been reduced significantly compare to control (Table 2). While the lint yield per unit area kg ha^{-1} and plant g $plant^{-1}$ were decreased, whereas the lint per crown sectional area g m^{-2} increased by PBZ through diminishing plant appearance. The yield was found to be significant (P: 0.01) per unit area and per plant.

Table 1: The effect of Paclobutrazol on the mycelial growth of *Verticillium dahliae*

PBZ	Mycelial growth (mm)[a]			Effect of mycelial growth (%)
	10 days	14 days	24 days	
Control	32.3	40.1	53.3	-
10 ppm	21.6	29.4	40.0	24.9
20 ppm	13.3	15.5	24.5	54.0
40 ppm	11.6	12.8	15.5	70.9
80 ppm	8.1	8.5	10.4	80.4

[a]Mycelial growth was measured for 10, 14 and 24 days

Table 2: The effect of different doses of PBZ on plant growth, Verticillium wilt (*Verticillium dahliae* Kleb.) and yield lint on the Nazilli 87 cotton variety (1998)

PBZ treatment[a] ($g\ m^{-2}$)	Plant growth		Disease		Yield		
	Plant height (cm)	Crown width (cm)	Infected plant (%)	Disease severity (%)	Per unit area ($kg\ ha^{-1}$)	Per plant ($g\ plant^{-1}$)	Per crown sectional ($g\ m^{-2}$)
Control	105.5a**	47.0a**	18.32a*	11.38a**	2218.2a**	38.52a**	220.50
0.05	66.9b	28.2b	3.30b	2.22b	1968.9a	34.49a	554.75
0.1	62.6bc	29.2b	4.15b	2.22b	1592.8bc	31.77a	486.75
0.2	53.6cd	21.7c	0.82b	0.27b	1183.5cd	15.95b	483.25
0.3	46.9d	20.7c	3.30b	1.66b	794.7d	13.25b	311.25
0.4	48.3d	23.0bc	3.30b	1.66b	874.0d	15.08b	351.25

*, **;Significantly at 5% and 1%, respectively. Means within columns followed by the same letter are not significantly different at the 5% level according to (LSD). [a] PBZ was treated to inter-rows after the first irrigation

Table 3: The effect of different doses of PBZ on the plant growth, Verticillium wilt (*Verticillium dahliae* Kleb.) and yield lint at the various times on Sure Grow ST 501 cotton variety (1999)

PBZ treatment[a] ($g\ m^{-2}$)		Plant growth		Disease		Yield		
		Plant height (cm)	Crown width (cm)	Infected plant (%)	Disease severity (%)	Per unit area ($kg\ ha^{-1}$)	Per plant ($g\ plant^{-1}$)	Per crown sectional ($g\ m^{-2}$)
Control		99.4a*	66.20a*	92.13	64.26	2709.8	56.95	165.39
0.05	1st[b]	86.7b	47.90bc	76.10	47.80	2499.8	62.18	344.91
	2nd	80.0b	54.90ab	89.46	60.16	2320.7	52.43	221.39
0.1	1st	78.4b	42.50c	70.46	41.93	2482.8	53.40	376.26
	2nd	76.3b	55.60ab	87.40	59.63	2871.3	56.97	234.54

*: Significantly at 5%. Means within columns followed by the same letter are not significantly different at the 5% level according to (LSD). [a] :PBZ was treated to inter-rows after the first and second irrigation. [b] : The timing of PBZ treatment (after the first and second irrigation)

Table 4: The effect of different doses of PBZ on plant growth, Verticillium wilt (*Verticillium dahliae* Kleb.) and yield lint at the various plant density at the Maraş 92 cotton variety (2000)

PBZ treatment[a] ($g\ m^{-2}$)		Plant growth		Disease		Yield
		Plant height (cm)	Crown width (cm)	Infected plant (%)	Disease severity (%)	Per unit area ($kg\ ha^{-1}$)
Control	Decreasing density[x]	78.30	34.58	60.55a**	41.90	3211.8ab*
	No decreasing density[y]	88.50	41.05	30.15c	38.33	2523.4b
0.05	Decreasing density	62.90	26.20	48.68b	35.65	2298.7b
	No decreasing density	61.10	29.20	40.65bc	27.43	4765.6a
0.1	Decreasing density	54.74	26.15	36.00c	31.53	1801.9b
	No decreasing density	59.43	26.35	36.78c	23.45	1970.0b

*, **;Significantly at 5% and 1%, respectively. Means within columns followed by the same letter are not significantly different at the 5% level according to (LSD). [a] :PBZ was treated to between rows after the second irrigation according to decreasing plant density or not. [x],5 plants per square meter (plants m^{-2}). [y], 20 plants per square meter (plants m^{-2})

In the second year studies, only 0.05 and 0.1 g m^{-2} doses of PBZ were treated at the Sure-Grow ST 501 cotton variety plots after the first and second irrigation once a growing season. In the results of the combination formed between the dozes of PBZ and its application time, the plant height (cm) and crown width (cm) decreased with respect to control and the difference between the two data was found to be significant (P:0.05), as in the previous year (Table 3). Also, the proportion of infected plant (%) and disease incidence (%) reduced throughout PBZ treatments. This decreasing became more effective after the first irrigation than the second one. As for evaluating yield, per crown sectional area g m^{-2} increased as long as plant habitués became smaller. However, unlike the previous year, the yield per unit plant g $plant^{-1}$ was close to control. The yield increase was obtained by 6% for the lint yield per unit area at 0,1 g m^{-2} dose of PBZ after the second irrigation with respect to control (Table 3).

In the third year, the effect of the doses of PBZ (0.05 and 0.2 g m^{-2}) on the plant growth, Verticillium wilt and lint yield at the plots of Maraş 92 cotton variety with

decreasing plant density or without (Table 4). The reducing effect of plant habitués by PBZ continued in 2000 as in the previous years. In disease criteria, the results of combinations formed between PBZ and plant density were found to be significant on the disease infection rate (P:0.01), whereas they were not significant on the disease incidence. As regards the results between combinations, the yield was found to be significant (P:0.05). The highest yield kg ha^{-1} was obtained at the plots of 0.05 g m^{-2} dose of PBZ, where density decrease was not performed. The yield increase was obtained by 48% by this treatment (4765.6 kg ha^{-1}) with respect to control with decreasing density (3211.8 kg ha^{-1}), which is being used in practice (Table 4).

DISCUSSION

If we summarize the results of three years on plant growth, PBZ is seen to have resulted in decreasing the vegetative growth of cotton by means of its diminishing plant height and crown width (Table 2,3 and 4). This effect of the retardant arises from inhibition of Gibberellins (GA) biosynthesis, which plays a part in elongation of cell in plants. The primary mode of action of PBZ is inhibition of *ent*-kaurene oxidase, which catalyzes the sequential oxidations from *ent*-kaurene to *ent*-kaurenoic acid in the early sequence of GA biosynthesis and this reaction is formed in microsomes[12]. As a result of this study with PBZ which has realized this biochemical reaction, many studies, aimed at the main deciduous fruit trees and the others, have been conducted and important results as regards its practice were obtained.

Mycelial growth of *Verticillium dahliae in vitro* condition was reduced by PBZ and ED_{50} was obtained as 20 ppm and plus doses of its (Table 1). This result may have stemmed from fungistatic effect of PBZ against pathogen. We have not encountered so far any study concerning bioactivity of PBZ on *Verticillium dahliae*. However, in a study carried out in Turkey, PBZ was stated to have had fungistatic effect on agent Mal secco (*Phoma tracheiphila* (Petri) Kanc.Et.Ghik) disease, which is another vascular disease for lemon and ED_{50} was determined as 6 ppm and plus its doses[5]. In the bioactivity studies concerning the retardant, since the chemical was of four enantiomers (fungicide and PGR) in its constitution, it was expressed that it could inhibit GA as well as the sterol biosynthesis involved in Triazole group[13].

The laboratory experiment results were parallel to field results and consequently, the proportion of infected plant and disease incidence for Verticillium wilt were decreased by PBZ. The possibility that first comes to mind is that, because of a result of its decreasing plant height (Table 2, 3 and 4) through its contain PGR feature of enantiomers in advance[13] there may have become a mechanical obstacle in tissues and that this situation may have inhibited the growth of *Verticillium dahliae*. As a matter of fact, this view has been confirmed with the studies carried out previously. In a study carried out in Israel, decrease in disease incidence was obtained by PBZ treatment in order to increase the resistance to *Fusarium* leading to wilt at melon. A relation between resistance to wilt and decreasing elongation of seedlings was observed. Disease incidence was observed to have increased again after GA_{4+7} which has an antagonistic effect on PBZ sprayed to seedlings[4]. Correspondingly, reduction in the elongation of flushes at Kutdiken lemon variety was obtained and consequently, incidence of Mal secco causing an important problem for lemon was decreased by 71.6% in Turkey. Furthermore, it was determined that there was a relation between susceptibility to disease and internodes elongation when the flushes on susceptible and tolerant lemon varieties to disease mentioned were measured[5].

The second possible occurrence is that, even if agent of Verticillium wilt has overcome the mechanical obstacle, its growth may have been inhibited by PBZ, involved in xylem tissue. The chemical involved in Triazole group, owing to its enantiomers that can inhibit sterol biosynthesis[13] growth of *Verticillium dahliae* may have been inhibited in vascular tissue. As a matter of fact, growth of agent is inhibited at PDA+PBZ media *in vitro* conditions (Table 1).

The retardant's effective at early treatment arises from the fact that the host grows slowly and pathogen grows fast under field conditions. When treated early, its residue has increased in plant and hence, its fungistatic and PGR features have come out more effectively.

During the three years, we have tried to determine the convenient application doses, treatment timing and suitable application way of PBZ in order to get the highest lint yield per unit area. According to the first two years results, the lint yield per crown sectional area g m^{-2} was seen to increase, whereas the yield per plant g $plant^{-1}$ and in the same way per unit area kg ha^{-1} decreased to the extent of making plant appearance small by PBZ. As a result of this, with a view to getting more lint yield per unit area kg ha^{-1}, more plants were arranged in order in a unit area and the yield was obtained. On this point, many studies were carried out primarily over deciduous fruit trees and then on the other cultural plants, although not in cotton, similar results were obtained consistent with ours. Among these, the most remarkable feature is that the vegetative growth has been slowed down because of expansion of branch angle with trunk, although shortening effect of PBZ was not evident when it was treated in low doses[14].

When PBZ was applied at early and increasing dose, it was observed that Verticillium wilt decreased the disease more effectively, but leading to yield loss due to

excessive dwarfing and degeneration. It was considered to arrange more plants per unit area in order to benefit from dwarfing of retardant mentioned and thereby, the yield was increased about 48% at Maraş 92 variety in 2000, through its 0.05 m^{-2} application dose after the second irrigation without decreasing density, which is for cotton labour work.

In conclusion, the application of Paclobutrazol at a dose of 0.05 m^{-2} was considered to be suitable after the second irrigation without decreasing the density through more plants per unit area, when the plant height reaches 40-50 cm.

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