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Effect of Paclobutrazol, Plant Growth Retardant, on Some Soil- Borne Fungal Pathogens *in vitro* Conditions

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Abstract: The present study aimed to determine the effect of growth retardant Paclobutrazol (PBZ) on the mycelial growth of soil-borne fungal pathogens such as *Macrophomina phaseoli* and *Fusarium oxysporum f.* sp., against which chemical control is rather difficult. In the study, the mycelial growth of the two pathogens decreased through increase in the dose of PBZ. The inhibiting dose (ED_{50}) was 40 ppm for *M. Phaseoli*, while it was 50 ppm for *F. oxysporum f.* sp. *Lentis*. At the end of the study, the effect of PBZ, on the two fungi was observed as fungistatic

Key words: Paclobutrazol, *Fusarium oxysporum f.* sp. *lentis*, *Macrophomina phaseoli*

INTRODUCTION

The human being needs to be fed in order to maintain his life and due to this obligation, he has learned to process the soil and animal breeding since the first ages. While getting crops from the soil, he has always aimed to obtain those of quality and plentiful amount from the unit area. Producers perform cultural proceedings such as soil processing, irrigation, running, fertilizing, control with disease and pests in order to reach this aim. Besides these obligatory practices, the "Plant Growth Regulators" discovered at the beginning of the 20th century in the World have started to be used in Turkey especially over the last 40 years in horticulture and field crops and significant results to aimed at practice have been obtained.

Of these substances that are mostly of synthetic nature; Paclobutrazol (PBZ), Growth retardant, comes to the fore. This chemical can be applied both through soil and foliar sprays. Since it is effective for a long time, it is mostly used in perennial plants rather than annual plants (Aron *et al*., 1985., Delgado *et al*., 1985; Harty and Van Staden, 1988; Smeirat and Qrunfleh, 1989).

PBZ reduces vegetative growth in the plants since it inhibits Gibberellin biosynthesis (shortening in axis and node intervals), accelerates generative growth and causes yield increase in the unit area. With altered physiological and morphological structure, PBZ has been shown to enhance extreme environmental conditions (Wheaton, 1989; Harty and Vanstaden, 1988.). As a result, its usage against some fungal pathogens has led to the decrease in disease (Cohen *et al*., 1987; Cimen *et al*., 1994; 1996; 2004*)*. Non-formation of residue in lemon fruit in a study performed, as well as these characteristics, is a satisfactory event (Cimen *et al*., 1999).

In this study, *in vitro* conditions, we aimed to determine the effect of PBZ on some soil- borne fungal pathogens such as *Fusarium oxysporum f.* sp. *lentis* and *Macrophomina phaseoli*, against which chemical control is difficult.

MATERIALS AND METHODS

Materials: The study was carried out at the Plant Protection Department laboratory, Agriculture Faculty, Dicle University in Diyarbakır of Turkey, in 2002. The chemical material Paclobutrazol (PP-333, Cultar, $C_{15}H_{20}ClN_{30}$) used in the research is a concentrated suspension containing 250 g active substance (B-Chlorophenyl) methyl-(l.1-dimethyl)-1-H-1, 2,4 triazole-1 ethanol) in a liter. The disease pathogens used in the study are; *Fusarium oxysporum f.* sp. *lentis* and *Macrophomina phaseoli*. They were obtained from Diyarbakır Plant Protection Institute.

Methods: The studies related to the efficiency of paclobutrazol on the mycelial growth of *F. oxysporum f.* sp and *M. phaseoli* were conducted *in vitro* conditions. To that end, from the stock solution of the previously prepared chemical, besides applications of PBZ to 250 mL Erlenmeyer, containing 100 mL PDA media, at wide intervals as 0, 50, 100, 200 and 400 ppm, it was added in

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increasing doses as 0, 10, 20, 30, 40 ppm until ED_{50} value was found. These obtained "PDA+ Paclobutrazol" media were sterilized in an autoclave at 121°C temperature and under 1 atm. pressure for 15 min and then they were transferred to sterilized petri with 90 mm diameter as in 20 mL.

Each petri plate was set up with five repeats according to the trial pattern of randomized parcel. The mycelial disc of 6mm diameter, obtained from the cultures of 10 days grown in incubator at 22°C for pathogens was inoculated into the center of petri plates containing PBZ+ Potato Dextrose Agar (PDA) and left incubation at 22°C. At the end of incubation, the colony diameters of disease pathogens were measured at one week intervals until they filled the petr plates in the controls (the measurement days were changed according to growth of the pathogens). The mycelia growth of the pathogens during the measurement was seen to be in two different directions and the average of these values were taken.

After these measurements, in order to determine whether PBZ had fungicide or fungistatic effect, the mycelia discs that were previously placed in control and "PBZ+ PDA" media were transferred to petri plates containing only PDA and left for incubation; then, the measurements continued until the mycelial growth filled the petri.

RESULTS

In the study carried out under laboratory conditions, the averages of the result of the trial performed for the growth of mycelial disc, with 6mm diameter and taken from *M. phaseoli* culture of 10 days in PDA media containing PBZ are given in Table 1 and the images in Fig. 1a. When 12 days of growth of *M. phaseoli* was considered, the mycelial growth decreased significantly ($p = 0.05$) with an increase in the dose of PBZ (Table 1). While the mycelial growth reached to 90.0mm in the controls, it was 36.6 ppm at 50 ppm PBZ dose; in 100, 200 and 400 ppm, it remained 29.6, 20.2 and 11.8 mm.

When the rate of inhibition of mycelial growth by Paclobutrazol was considered, 12 days measurements at 50, 100, 200 and 400 ppm were found as 59.3, 67.1, 77.5 and 86.8%, respectively (Table 1). ED_{50} value remained between 0-50 ppm and hence, a new trial was set up (Fig. 1b), where PBZ's 0, 10, 20, 30 and 40 ppm doses were used and the results are added to the Table 1. As a result of the measurement and assessments, ED_{50} value was determined as 40 ppm.

In PBZ doses are shown in Table 1 and Fig. 1. The initial mycelial disk of *M. phaseoli* was removed to medium including only PDA. Here, the previously decreasing mycelial growth of pathogen in PBZ doses

Table 1: Influence of various doses of Paclobutrazol on the mycelial growth of *Macrophomina phaseoli*

PBZ doses	Mycelial growth (mm)		Inhibition of mycelial growth (%)
	6 day	12 day	12 day
0	60.6a	90.0a	
10	38.6b	53.2bc	40.8
20	32.8c	51.2cd	43.1
30	28.2de	50.4de	43.8
40	26.2e	46.6e	48.2
50	25.0f-g	36.6f	59.3
100	22.4g	29.6g	67.1
200	15.4h	20.2h	77.5
400	8.4ı	11.8ı	86.8

The averages showing the same letter are not statistically different from each other according to the LSD ($p = 0.05$) test

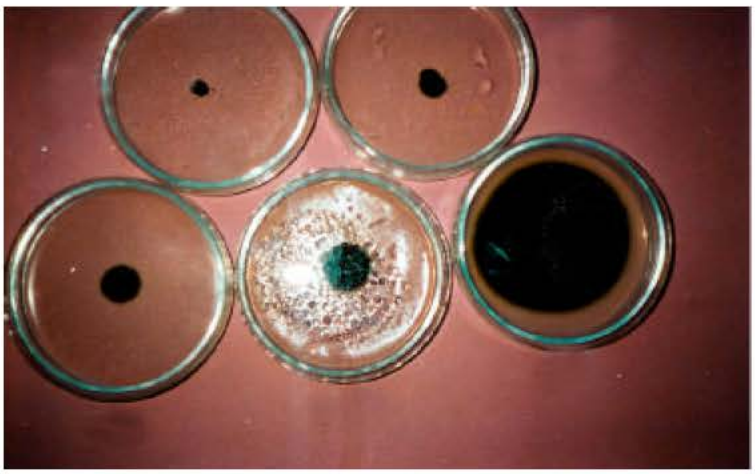

Fig. 1a: The effect of various of dose of paclobutrazol on the mycelial growth of *Macrophomina phaseoli*

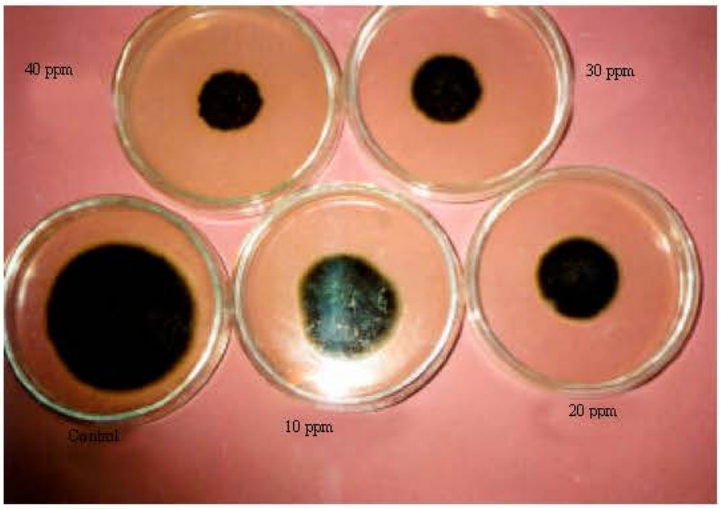

Fig. 1b: The effect of various does of paclobutrazol on the mycelial growth of *Macrophomina phaseoli*

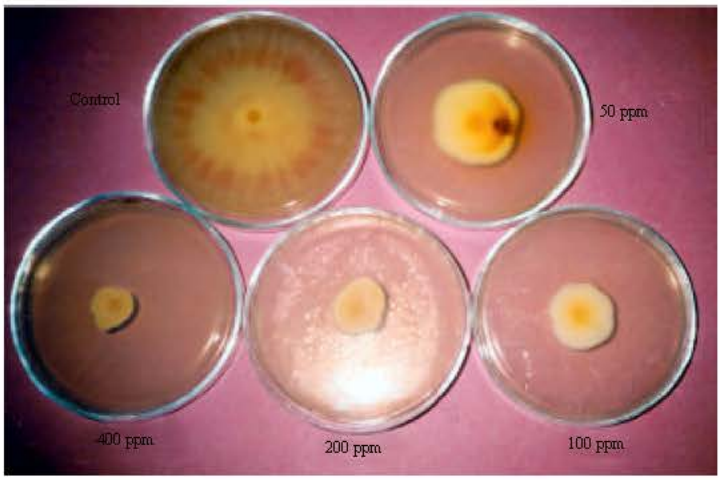

Fig. 2: The effect of various does of paclobutrazol on the mycelial growth of *Fusarium oxysporum f.* sp. *Lentis*

Table 2: Influence of various doses of Paclobutrazol on the mycelial growth of *Fusarium oxysporum f.* sp. *lentis*

Paclobutrazol doses (ppm)	Average mycelia growth (mm)		Inhibition rate (%)
	9 day	12 day	12 day
0	78.85a	90.00a	
50	38.78b	44.33b	50.7
100	29.21c	34.71c	61.4
200	21.14de	24.41d	72.8
400	18.28e	22.28e	74.6

The averages showing the same letter are not different from each other as statistically according to the LSD (p = 0.05) test

accelerated in PDA media. With this result, the effect of the chemical has been accepted to be fungistatic.

As in *Macrophomina phaseoli*, mycelial growth of the *F. oxysporum f.* sp. *lentis* was decreased significantly (p = 0.05) with increase in PBZ dose (Table 2, Fig. 2). In Table 2, the mycelial growth reached to 90.00 mm in control of 12 days, while mycelial growth of *Fusarium oxysporum f.* sp. *lentis* reached to 34.71, 24.41 and 22.28 mm at 100, 200 and 400 ppm, respectively; however, PBZ reached 44.31 mm at 50 pmm.

When inhibition rates of PBZ on the mycelial growth is taken into consideration, it was calculated that, during 12 days measurement, the inhibition rate was over 50 % at 50ppm and more. The inhibition rate at 50 ppm was found as 50.7%, while at 100, 200 and 400 ppm doses, it was calculated as 61.4, 72.8 and 74.6%, respectively (Table 2).

DISCUSSION

In the studies related to the efficiency of PBZ *in vitro* conditions, it has been shown that the mycelial growth of

F. oxysporum f. sp. *lentis* and *M. Phaseoli* has been inhibited. When the initial of mycelial disks on PDA with PBZ of these fungal pathogens were inoculated on PDA medium again, it was seen that the mycelial growth accelerated more than the medium containing PBZ, but the mycelial growth was inhibited compared to the control. This indicates that, the two pathogens exposed to PBZ have been slightly inhibited again later, which caused by the fungistatic effect of PBZ against both pathogens. The results we have obtained may result from the inhibition of biosynthesis of ergosterol, which is an obligatory component of fungal membrane since it is within the Triazole group, besides the inhibition of gibberellin's biosynthesis with 4 enantiomers within the PBZ (Burden *et al*., 1987). We have not encountered any study about the relationship between the same pathogens; *F. oxysporum f.* sp. *lentis* and *M. Phaseoli* with PBZ. However, a study related to the efficiency of PBZ *in vitro* conditions performed in this respect has indicated that PBZ 25-100% inhibits mycelial growth of eight fungal pathogens of woody plants: *Armillariagallica*, *Botryosphaeria dothidea*, *Ceratocystis fadacearum*, *Fusarium roseum, Ophiostoma novo-ulmi*, *Sirococcus clavigignenti-juglandacearum*, *Sphaeropsis sapinea and Verticillium dahliae* (Jacobs and Berg, 2000).

The results obtained *in vitro* conditions were transferred to soil conditions and, it was suggested that PBZ could be used against the disease pathogens (Cohen *et al*., 1987; Cimen *et al*., 1996; 2004).

With the application of PBZ in horticulture and field crops in soil conditions, it causes yield increase and can be applied easily both through soil and foliar sprays (Aron *et al*., 1985; Delgado *et al*., 1985; Harty and Van Staden, 1988; Smeirat and Qrunfleh, 1989), without any residue (Cimen *et al*., 1999). Morphological modifications might occur on the plant (Wheaton, 1989; Harty and Van Staden, 1988.) and thus, thanks to mechanic obstacle formed such as smaller stomatal pores, thicker leaves and increased number and size of surface appendages on leaves, it may inhibit the penetration of fungal pathogens.

Even though these structural barriers can be overcome by the fungal pathogens owing to the fungistatic effect of PBZ, discovered previously, the invasion of pathogens in host tissue might be slowed down or inhibited.

Under the light of all these studies, PBZ can easily be applied against two fungal pathogens: *F. oxysporum f.* sp. *lentis* and *M. Phaseoli,* against which chemical control is very difficult. Against Dry root rot disease of melon, which is very common in our region, caused by *M. phaseoli*, or other assoicated with vascular wilts, it would be useful to start field studies immediately.

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