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ORIGINAL ARTICLE

Comparing the Efficacy of Two Copper Fungicides in Controlling Peach Leaf Curl Disease

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KEYWORDS

Chemical control;
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ABSTRACT: Peach leaf curl disease, caused by *Taphrina deformans* (Berk.) Tul., is one of the most serious peach diseases, causing quantitative and qualitative damage to crops across Iran each year. In current study, the efficacy of two new fungicides, Badge®WG280 and Barzidox®WP85%, was evaluated in controlling peach leaf curl disease. The experiment was conducted in Golestan, Semnan and North Khorasan using a randomized complete block design with ten treatments and four replications. Treatments included 0.75, 1, and 1.2 ml l^{-1} of Badge®WG280 (copper hydroxid+copper oxychloride), 2, 4, and 6 g.l^{-1} of Barzidox®WP85% (copperoxychloride), 2.5 ml l^{-1} of Nordox®WG75% (copperoxide), 3 g l^{-1} of Captan, and controls (with and without water spraying). Treatments were applied at three physiological stages (the first in autumn after the leaves had fallen, the second at the end of March before the bud had swollen, and the third after the petals had dropped). Disease incidence and severity percentage were determined. Duncan's multiple range test was used to compare the mean disease severity and incidence percentages. The results showed that 6g l^{-1} of Barzidox® had the highest efficacy in reducing disease severity compared to the control in Golestan and Semnan provinces, with 81% and 80% respectively. Furthermore, the best performance of two indicator fungicides, Nordox® and Captan were 73% and 72% in Golestan province and 67% and 68%, in Semnan province, respectively. According to the results, it is concluded that 1.2ml.l^{-1} of Badge® and 4g.l^{-1} of Barzidox® have the best efficiency in controlling the peach leaf curl disease.

INTRODUCTION

The disease known as peach leaf curl, which is caused by *Taphrina deformans* (Berk.) Tul. is widespread throughout the world and causes varying amounts of economic damage depending on the environmental

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conditions [1]. The first reports of diseases date back to 1821 in England [2]. The disease agent overwinters in the form of spores or mycelium on the surface of the tree bark. When spores are spread on buds by water in late winter, primary infection occurs during bud swelling. Overwintering mycelium also grows and develops in the cold and humid spring and spread in the leaf and form a white layer called hymenium between the epidermis and the cuticle which formed asci. The symptoms will appear three weeks after primary infection. Ascospores are released from the ascus in the spring and spread by the wind and infect many leaves. Germination of ascospores produces mycelium and conidia that cause new infections and also are responsible for overwintering of the fungus. Secondary infection can occur when the bud blooms and petals fall [3, 4]. The first symptoms appear in the form of yellow or reddish spots on the growing leaves in spring [1]. When the pathogen enters the leaf tissue, it stimulates the rapid division of cells and increases their size; as a result leaves become thick, wrinkled and twisted. In this way, tumor-like areas, which are usually red in color, appear on the surface of the leaf [3]. A white powder consisting of fungal spores may cover the leaf’s epidermis. Infected leaves may fall early and if they remain on the tree, they turn dark brown in severe infections [1]. This will negatively affect tree nutrition and fruit growth. The contamination of fruits is less severe, but in severe infections, red wrinkled spots cover 30 to 50% of the fruit surface. In this case, fruits drop when they are still very small and the diseased tree may never produce any crop [5]. Young branches are occasionally swollen, stunted and stop growing. Numerous reports from different areas of peach cultivation in the world show that the leaf complication disease occurs only when the weather is cold and humid or frequently rainfall at the beginning of bud development [1, 5, 6]. The occurrence of the disease and its severity depend on the rainfall, the duration of the wet period and the temperature. Since *T. deformans* spores grow in relative humidity of 95% or more, at least three millimeters of rainfall with a wet period of 12.5 hours and a temperature below 16°C is necessary for the disease to occur [7]. The severity of the disease also depends on the sensitivity of the variety [8].

For the chemical control of peach leaf curl, one spraying is done in the autumn season after the leaves fall and the next one in late winter or early spring just before bud swelling, and if necessary, repeated spraying may be done depend on spring rainfall [7]. For a successful control time and sufficient coverage of the fungicides are critical, otherwise disease control will fail [9]. Protective fungicides with multiple mode of action such as chlorothalonil, captafool and copper fungicides such as Burdeaux mixture, copper hydroxide, sulfur, thiram and ziram have been introduced to control peach leaf curl disease, among which chlorothalonil and copper fungicides are the most common and effective for the disease control [3, 10-12].

The first use of copper for grain treatment occurred in 1761. Copper sulfate was first recognized as a fungicide in 1880, and the use of the Burdoaux mixture spread quickly thereafter. At that time, in a region called Bordoaux in France, it was seen that the mixture of copper sulfate and lime (Burdoaux mixture) can control the downy mildew of grape. Burdoaux mixture is the first fungicide that has been used on a large scale in the world [12]. Copper, by penetrating the cells of the target organism and bonding with its enzymes, stops the cell's activity. Copper pesticides are made based on more than 40 different active copper substances, the most famous of which are copper sulfate, oxide, hydroxide and chloride [13]. Copper ions possess protective and to some extent therapeutic properties. Burdoaux mixture is a common copper fungicide in Iran, which was registered for the first time in December 1968 and is one of the oldest registered fungicides in the country [14]. Burdoaux mixture has been registered in Iran against the causes of canker disease, shot hole of stone fruit trees and leaf curl of peach. In the present project, the effectiveness of two fungicides Copper hydroxide + copperoxychloride (Badge ® 280 WG) and copperoxychloride (Barzidox ® 85% WP) has been evaluated in the control of peach leaf curl. Badge fungicide is manufactured by Isagro Company in Italy, while Barzidox fungicide is produced by Agro Life Science Corporation in India.

MATERIALS AND METHODS

This research was carried out in Golestan (Gorgan, northeast of Iran), Semnan (Shahrood County) and North Khorasan

(Khorasan-e Shomali, Bojnord) provinces, Iran. One peach orchard with natural infection history of leaf curl disease was selected from each of provinces. Three peach cultivars including Zaafarani, Sorkhabi and Ahvana were studied in each province. Experiments were carried out using randomized complete block design with 10 treatments and three replications in three regions (Gorgan, Semnan and Bojnord). Fungicides treatments including: (Copper hydroxide + Copper oxychloride; Badge® WG 280 at 0.75, 1 and 1.2:1000, Copper oxychloride; Barzidox® WP 85% at 2, 4 and 6:1000, Copper oxide; Nordox WG 75 at 2.5:1000 and Captan WP 50 at 3:1000 concentrations). The controls were with and without water spraying. In this study, each experimental plot included two peach trees that were approximately 5 years old. Among the trees, one untreated tree was considered to avoid the side effects of the fungicidal treatments. The trees were sprayed using a motorized sprayer with a lance in three times during plant growth: once at the end of autumn after the defoliation, once at the beginning of spring before the buds burst, and once after the petal fell. Since peach fruits are rarely infected [1], to determine the efficacy of fungicides, only the disease symptoms on the leaves were evaluated [9, 11, 15, and 16]. Hundred leaf samples were collected randomly from four geographical directions and center of canopy of each tree. Leaf samples transferred to laboratory in plastic bags.

The data for disease incidence and severity began two weeks after the symptoms had appeared in the control treatments.

Disease assessment in the field

Estimation of disease incidence (DI)

Diseased leaves, as well as the total number of leaves for each tree, were counted. The information recorded in the laboratory was used to calculate the percentage of the proportion of leaves infected per tree using the following formula for disease incidence [15]:

$$DI\% = (n_d/N) \times 100$$

DI=Disease incidence (%)

n_d=Number of leaves infected

N=total leaves sampled

Determination of disease severity

To calculate the percentage of disease severity, symptoms of the disease on each leaf were classified based on 0 to 5 scale described by Keymak *et al.* [15].

0: no symptoms, 1: 0.1% to 0.3%, 2: 1.3% to 0.10%, 3: 10.1% to 25%, 4: 25.1% to 50%, 5: 50.1% to 100%

Using the following formula, the percentage of disease severity was determined.

$$PDS= \sum(xini)/5n\times100$$

In this formula, PDS (disease severity in percent) is obtained based on the degree of disease severity in each tree (xi) compared to all the evaluated infected trees (ni).

Analysis of variance and comparison of means

SAS software was used to analyze the data after determining the percentage of occurrence and severity of leaf curl disease for each plot. Duncan's multiple range test was used for comparison of the means at the probability level of 1%. Because the tree varieties differed between provinces, the data was analyzed separately for each one.

RESULTS

Golestan province

The variance analysis of the data obtained from the evaluation of the treated trees in Golestan province revealed that the treatments had a significant effect on lowering the percentage of disease severity and occurrence when compared to controls (Table 1). The comparison of the means of disease severity percentage (P=1%) showed that Barzidox at doses of 6 and 4 per thousand with an average of 17.00 and 19.50%, respectively, Nordox with an average of 24.00% and Captan with an average of 25.00% had the greatest effect on reducing the severity of the disease, all without statistically significant difference. Barzidox at dose of 2 and Badge at dose of 1.2 per thousand were ranked next with an average of 35.25 and 42.25%, respectively. Badge fungicide at doses of 1 and 0.75 per thousand were the least effective among the fungicide treatments on reducing the severity of the disease, although they showed a statistically significant difference compared to the controls. The lowest rate of disease occurrence was observed in the trees treated with Barzidox at doses of 2, 4, and 6 per thousand, respectively with an average of 5.75, 6.50, and 12.25%, Captan with an average of 11.25% and Nordox with an average of 14.75% (Table 2).

Semnan province

The variance analysis of the data obtained from the evaluation of the treated trees in Semnan province showed that the treatments had a significant effect on lowering the percentage of disease severity and occurrence when compared to controls (Table 1). The comparison of the means of disease severity percentage (*P*=1%) showed that Barzidox at doses of 6 and 4 per thousand with an average of 12.75 and 14.75%, respectively, and Badge at dose of 1.2 per thousand with an average of 25.00% had the greatest effect on reducing the severity of the disease, without statistically significant difference. Badge at dose of 1 per thousand, Captan, Nordox and Barzidox at dose of 2 per thousand were placed in the next rank. In terms of the occurrence of the disease, Barzidox at doses of 6 and 4 per thousand with an average of 6.50 and 9.25%, respectively, and Captan with an average of 9.00% had the greatest effect on reducing the incidence of the disease compared to the controls. Brezidox at dose of 2 per thousand and Nordox with an average of 13.50 and 14.50%, respectively, were ranked next (Table 2).

North Khorasan province

Variance analysis of the data obtained from the evaluation of treated trees in North Khorasan province revealed that all treatments, with the exception of Captan, had a significant effect on reducing the disease severity and occurrence percentages when compared to controls (Table 1). The comparison of the means of disease severity and disease incidence percentages (*P*=1%) showed that the lowest disease severity and incidence occurred in trees treated with Badge fungicide at dose of 1.2 per thousand with an average of 7.75% and 23.75%, respectively; and the doses of 1 and 0.75 per thousand of this fungicide were placed in the next group with an average of 18.35 and 24.70%, respectively. All three doses of Barzidox and Nordox fungicides were ranked next, without statistically significant differences from each other.

The trees treated with Captan fungicide had the highest rates of disease incidence and severity among the fungicide treatments, with averages of 93.75% and 77.50%, respectively, and the incidence of disease was not statistically significantly different from the controls (Table 2).

Table 1. Analysis of variance of percent disease incidence and disease severity of peach leaf curl on leaves in Golestan, North Khorasan and Semnan provinces

S.O.V.	df	Mean squares											
		Golestan				North Khorasan				Semnan			
		D. I. (%)	F Value	D. S. (%)	F Value	D. I. (%)	F Value	D. S. (%)	F Value	D. I. (%)	F Value	D. S. (%)	F Value
Replicate	3	9.13^{ns}	0.37	677.00^{ns}	10.89	2.82^{ns}	0.2	7.86^{ns}	0.33	3.29^{ns}	0.7	32.80^{ns}	7.25
Treatment	9	4531.73**	183.00	3163.88**	50.91	2468.78**	173.91	3367.01**	140.49	1909.72**	411.27	1582.21**	349.87
Error	27	24.76	-	62.14	-	14.19	-	23.96	-	4.64	-	4.52	-
C.V.	-	14.59%	-	16.95%	-	5.15%	-	9.45%	-	8.18%	-	7.54%	-

** Significant at 1% level, D. I. = Disease Incidence, D. S. = Disease Severity, ns: not significant.

Table 2. Mean comparison of percent disease incidence and disease severity of peach leaf curl on leaves in Golestan, North Khorasan and Semnan provinces.

Treatment	**Golestan**			**North Khorasan**			**Semnan**		
	MDI%	**MDS%**	**Efficacy%****	**MDI%**	**MDS%**	**Efficacy%****	**MDI%**	**MDS%**	**Efficacy%****
Badge 0.75 ml L^{-1}	43.50 b	60.25 b	33	52.50 c	24.70 d	72	31.25 b	25.50 b	60
Badge 1 ml L^{-1}	36.25 b	58.50 b	35	44.00 d	18.35 d	79	28.50 b	20.00 c	68
Badge 1.2 ml L^{-1}	23.75 c	42.25 c	50	23.75 e	7.75 e	91	21.25 c	15.50 d	75
Barzidox 2 g L^{-1}	12.25 d	35.25 dc	61	81.00 b	52.85 c	41	13.5 d	21.75 cb	66
Barzidox 4 g L^{-1}	6.50 d	19.50 ed	78	79.25 b	50.00 c	44	9.25 e	14.75 d	77
Barzidox 6 g L^{-1}	5.75 d	17.00 e	81	77.75 b	49.05 c	45	6.50 e	12.75 d	80
Nordox 2.5 ml L^{-1}	14.75 dc	24.00 ed	73	84.00 b	55.15 c	38	14.50 d	21.25 cb	67
Captan 3 g L^{-1}	11.25 d	25.00 ed	72	93.75 a	77.50 b	14	9.00 e	20.00 c	68
No spraying control	93.25 a	91.00 a	-	96.25 a	90.25 a	-	64.50 a	64.25 a	-
Water spraying control	93.75 a	92.25 a	-	98.50 a	92.05 a	-	65.00 a	66.25 a	-

* The means of each column followed by common letters are not significantly different (Duncans multiple range test α= 1%), MDI= Mean of Disease Incidence, MDS= Mean of Disease Severity.
** Compared to the control without spraying.

DISCUSSION

In the present study, the effectiveness of two copper fungicides named Copper Hydroxide + Copper Oxychloride (Badge ® WG280) and Copper oxychloride (Barzidox® WP85%) in controlling peach leaf curl disease was evaluated. Barzidox fungicide at a dose of 6 per thousand was 81% effective in Golestan province and 80% effective in Semnan province when compared to the control (no treatment), while these values were 78 and 77%, respectively, at a dose of 4 per thousand. Badge fungicide at dose of 1.2 per thousand in North Khorasan, Semnan and Golestan provinces reduced the severity of the disease by 91, 75 and 50%, respectively, compared to the control (without treatment). Doses of 1 and 0.75 per thousand of Badge fungicide were also effective with 79 and 68% effectiveness in North Khorasan province and 72 and 60% effectiveness in Semnan province, but these treatments were in the lowest statistical group in Golestan province and were about 30% efficient. The pathogen's resistance to existing fungicides accounts for the fungicide's high effectiveness in North Khorasan and Semnan provinces. Nordox and Captan fungicides were about 70% effective in Golestan and Semnan provinces.

Khabaz Jolfaei *et al.* evaluated the efficiency of several brands of Copper Oxychloride fungicide available in the Iranian market and Nordox fungicide in controlling peach leaf curl disease, all of which were effective in controlling this disease [17]. Rabbani Nasab *et al.* also confirmed the efficacy of Nordox fungicide in controlling the disease [18]. The results obtained from the present investigation confirm the effectiveness of Barzidox ® with a dose of 6 and 4 per thousand and Badge ® with a dose of 1.2 per thousand in controlling peach leaf curl disease. Since the dose of 6 and 4 per thousand of Barzidox ® fungicide were placed in the same statistical group in all the studied areas, therefore, the dose of 4 per thousand of this fungicide is recommended to control peach leaf curl disease.

The effective ingredient of Barzidox ® fungicide is Copper Oxychloride and the active ingredient of Badge ® fungicide is Copper hydroxide + Copper Oxychloride. Copper compounds are broad-spectrum, nonsystemic, protective and have contact activity with multiple sites of action. The risk of resistance to this group of fungicides is very low and rare, and despite more than a century of their use in many countries, there is little evidence of resistance in fungi or the emergence of resistant strains [19]. In recent years, several fungicides based on copper compounds have been investigated and registered in Iran to control peach leaf curl disease, and all have proven effective. Some of these fungicides are Bergandy® (Bordoux mixture) [17], Copper-Volk (Volk oil + Copper Oxide) [20] and Nordox (Copper Oxide) [18].

Since copper fungicides have undesirable effects on the environment, the limit of their consumption should be respected. Especially the side effects caused by the accumulation of these compounds in the soil and their toxicity for fish and aquatic invertebrates should not be neglected [21, 22].

Captan is one of the oldest registered fungicides in Iran, which was registered in 1968 [14]. This fungicide belongs to the Phthalamides class, which has a broad spectrum of action, is non-systemic, and has both protective and curative properties. This fungicide has chronic side effects for humans and because it causes cell multiplication (=reproduction), in 1986, the EPA international agency raised the possibility of carcinogenicity of this fungicide for humans [23]. Although Captan has low acute toxicity, it is harmful when in contact with living tissues and can cause eye or respiratory damage to the user.

The formulations of Badge ® and Nordox® fungicides are water-dispersible granules (WG). Water-dispersible granules are a relatively new formulation that have been introduced as a safer and more economical alternative to wettable powders and concentrated (=dense) suspensions. This formulation's important features include ease of application, increased safety, and proper dispersibility in water and spray tanks. This formulation forms a fairly stable suspension in water.

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Conflict of interests

All the authors declare that there is no conflict of interest in the study.

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