J. Crop Prot. **2021, 10 (3): 523-533**

Research Article

Efficacy of copper oxychloride base fungicides to control cucumber downy mildew in greenhouse conditions in Iran

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Abstract: The use of a multi-site fungicide in cucumber downy mildew protection programs are recommended to ensure crops are adequately protected and delay a possible resistance development of high-risk groups of single-site fungicides. Commercially available dicopper chloride trihydroxide (also known as copper oxychloride) based fungicides (M FRAC Group) were assessed for their efficacy against cucumber downy mildew in comparison to a commonly used phosphonate (Fosphite® 53 WSL, P7 FRAC Group) and untreated control. Foliar treatments started with the onset of disease symptoms and were repeated weekly. Disease severity was calculated twice during crop development. Significant differences between the treatments were detected. Fosphite® was the most effective among other treatments, with a reduction in disease severity of 82.6%. Among the copper oxychloride-based fungicides, statistically significant differences were detected. Copertox® and Oksavit® were significantly effective than the other products at the first disease assessment, and Copertox® being the most efficient fungicide at the second disease assessment. Significant differences were also detected among control plots. The efficiency of commercial brands of copper oxychloride in control of cucumber downy mildew was 53-67%. This efficiency is acceptable in normal disease conditions but not desirable in an epidemic situation. If the conditions are favorable for a severe disease epidemic, it is necessary to combine them with more effective fungicides such as Fosphite.

Keywords: Cucurbits, Foliar diseases, Oomycetes, Potassium phosphite, Multi-site fungicides

Introduction

Cucumber downy mildew (CDM) is one of the most critical cucumber (*Cucumis sativus*) diseases in the world (McGrath, 2006). The disease was first reported from Cuba by Berkeley and Curtis in 1868 (Colucci and Holmes, 2010). The causal agent, *Pseudoperonospora cubensis* (Berk. et Curt.) Rostovzev, is an obligate biotrophic Oomycetes with the ability to infect various cucurbit plants, including cucumber, cantaloupe, squash, and melon, at all stages of growth (Colucci and Holmes, 2010). CDM occurs in warm, tropical, and even semi-arid regions such as the Mediterranean basin, but its damage is most significant in temperate areas (Palti and Cohen, 1980; Colucci and Holmes, 2010). Temperatures between 16-22 °C and periods of high leaf wetness (2 hours or longer) favor disease development by enabling zoospore movement on

Handling Editor: Naser Safaie

* Corresponding author: r.fani@areeo.ac.ir
Received: 19 November 2020, Accepted: 07 June 2021
Published online: 18 July 2021

[DOR: 20.1001.1.22519041.2021.10.3.9.7]

the abaxial leaf surface. Long periods of darkness (6 hours or longer) combined with a high relative humidity of more than 90% favor the production of zoosporangia (Lindenthal *et al.*, 2005). Zoospores infect leaves through stomata, and infection reduces plant vigor and yield by increasing respiration and reducing photosynthetic activity and transpiration (e.g., through inhibition of stomatal closure and defoliation), especially in the early stages of host growth. Leaf symptoms include chlorotic spots on the adaxial leaf surface. Leaf spots are restricted by veins and may become necrotic over time. Sporulation of the downy mildew pathogen occurs in the transition zone at the margins of necrotic lesions (Savory *et al.*, 2011). Spores (zoospores or overwintering oospores) are often transmitted by air from infected areas or areas where the pathogen can overwinter. Wind direction and environmental conditions favoring spore production play an essential role in the occurrence of the disease. Therefore, information about these two factors predicts disease onset and progression (McGrath, 2006; Pouzeshimiyab and Fani, 2020). In Iran, CDM was first observed by Eskandari on cucumber farms located in Guilan and Mazandaran in 1964 and has since been reported from all over the country, especially from the greenhouse and under-plastic production systems due to the provision of favorable environmental conditions (Etebarian, 2006). It has been suggested that resistant cultivars should be used in conjunction with contact or systemic fungicides to manage the disease. Although resistance levels in cucumbers are higher than in other members of the Cucurbitaceae family, most commercial cucumber cultivars do not have sufficient resistance to fight off disease effectively. Cultural control can also be effective. These measures include: sufficient ventilation between planting rows, non-shaded plantings, adapted irrigation strategies, and balanced fertilizing regimes (McGrath, 2006). Spraying is the most common way to reduce CDM. The main reasons are the difficulty of reducing humidity due to the structures of greenhouses and the lack of suitable resistant cultivars. When dealing with fungicide-based control of cucumber downy mildew, significant areas of concern are declining fungicide efficacy, increasing pathogen resistance, and pesticide residues on a product with almost daily harvest intervals.

In recent years, various commercial compounds of Cyazofamide (Fani *et al.*, 2014; Pouzeshimiyab and Fani 2017, Fani *et al.*, 2018; Fani *et al.*, 2020), potassium phosphite (Fani *et al.*, 2015), and Bordeaux mixture (Fani *et al.*, 2019) have been assayed in different parts of Iran and registered to control of CMD.

Various fungicides are available to control CDM (McGrath, 2006), but availability varies by country. Limited access or knowledge about resistance development may lead to inappropriate use of pesticides. Inappropriate control time or fungicide with the improper mode of action may all result in poor disease control. Producers are always the ones to suffer by diminishing income and consequently reduced livelihood. Fungicides used to control plant diseases such as CDM are registered and recommended based on assays in the country. Organic fungicides and copper-based mineral fungicides are frequent choices to ensure that *P. cubensis* is effectively managed. Farmers must have access to effective fungicides with a different mode of action (MOA) to reduce the economic damage caused by the disease and to prevent fungal resistance.

Copper oxychloride is a widely used copper-based fungicide. It is recommended to control gummosis disease of citrus, pistachio, stone fruits, red leaf blotch disease of almond *Polystigma amygdalinum*, walnut anthracnose *Gnomonia leptostyla*, cucumber angular leaf spot *Pseudomonas syringae* pv. *lachrymans*, potato late blight *Phytophthora infestans*, citrus blight *Neoscytalidium dimidiatum,* and date palm flower rot (Sheikhi *et al.*, 2017). Copper oxychloride has gained popularity, and a variety of products are available for farmers in Iran. Although these products have similar active ingredients, they differ in trade name, formulation, label terminology, uses, and price.

In this study, the efficiency of 10 commercially available copper oxychloride-based fungicides for CDM control was evaluated in two different locations in Yazd province and compared to the most popular fungicide potassium phosphate.

Materials and Methods

Experimental sites

Experimental greenhouses were located in Dehno, a village at the outskirts of Yazd Province, and Mehriz, a region located 40 km southeast of Yazd. Both areas differ in climate. Dehno has hot and dry weather, and Mehriz is at a foothill with a temperate climate. Although most of the greenhouses in the province are infected with CDM, the relevant experiments were performed in a place with a history of high disease pressure. Cucumber planting in Dehno and Mehriz started in October and December 2017, respectively (Table 1).

Treatments

Experiments were conducted in commercial greenhouses similar to open field conditions located in two distinct areas (Dehno and Mehriz) located in the Yazd province. Cultivar Negin (Nunhems, Netherland) was used for planting. Seeds were grown in seedling trays containing peat moss and transferred to the mainland at the cotyledon leaf stage (BBCH Stage 100) (Feller *et al.*, 1995). BBCH is derived from the Biologische Bundesanstalt, Bundessortenamt, and Chemical Industry and is the system used for uniform coding of phenologically similar growth stages of all mono- and dicotyledonous plant species. Experiments were conducted in a randomized complete block design with 13 treatments and four replications each. Each experimental plot consisted of 10 plants at a distance of 30 cm with an inter-row spacing of 50 cm. Plants were maintained using standard production practices (irrigation, fertilization, etc.). The experimental treatments are described in Table 2.

Table 1 Greenhouse trial conditions for evaluating copper oxychloride (WP 35%) brands against cucumber downy mildew.

Location	Geographic position	Climatic condition	Temperature (°C)		Relative humidity (%)		Annual rainfall (mm)	Altitude	Fungicide application interval	Number of sprays applied	Evaluation time
			Day	Night	Day	Night					
Dehno	South of Yazd city	Hot, dry desert	70	64	10-17	18-25	71.6	1230	10 days	2	10 days after second spraying
Mehriz	Southeast of Yazd city	Semi-desert	72	65	12-18	20-25	102	1470	7 days	2	7 days after second spraying

Table 2 Fungicide treatments in Yazd province.

Treatment No.	Active ingredient (trade name)	Manufacturing company	Application rate
1	Copper oxychloride (Coprox® WP 35%)	Saraye Sepand Pars	2 Kg/ha
2	Copper oxychloride (Oxavit® WP 35%)	Khazar Sam Kood	2 Kg/ha
3	Copper oxychloride (Koosha® WP 35%)	Ghazal Shimi	2 Kg/ha
4	Copper oxychloride (Coprex® WP 35%)	Hezareh Sevom	2 Kg/ha
5	Copper oxychloride (Copertox® WP 35%)	Kimia Gohare Khak	2 Kg/ha
6	Copper oxychloride (Oxytex® WP 35%)	Arian Teb Parto	2 Kg/ha
7	Copper oxychloride (Oksavit® WP 35%)	Arya Shimi	2 Kg/ha
8	Copper oxychloride (Coper® WP 35%)	Samiran	2 Kg/ha
9	Copper oxychloride (Oxyazarin® WP 35%)	Zarin Dasht Pars	2 Kg/ha
10	Copper oxychloride (Behcop® WP 35%)	Alborz Behsam	2 Kg/ha
11	Potassium phosphite (Fosphite® 53 WSL)	JH Biotech	3 ml/l
12	Control (water, sprayed)	-	-
13	Control (no water sprayed)	-	-

[DOR: 20.1001.1.22519041.2021.10.3.9.7]

Treatments were applied twice with an interval of 7 or 10 days, starting with the onset of disease symptoms (BBCH stage 709) in at least one plant.

Disease evaluation

Disease severity was assessed twice: after the disease severity of the control treatment reached 50% (first evaluation, BBCH stage 802) in at least one plot and again at 90% (second evaluation, BBCH stage 805).

At each evaluation, the disease was assessed for ten plants from each plot by scoring ten random leaves of each plant (100 leaves in total) for symptom development. Each leaf was scored from 0 to 9 according to Thomas *et al.* (1987) with some modifications (Table 3, Fig 1). Then the severity of the disease was calculated based on the following formula:

$$\mathrm{DS} = \frac{\sum (ni \times vi)}{N \times V} \times 100$$

Table 3 The scale used to assess disease severity based on Thomas *et al*. (1987).

Score	Symptoms description
0	No symptom
3	Visual spots without sporangium formation (incompatible)
5	Visual spots with a few sporangia (compatible)
7	Visual spots with scattered sporangium (5×10^3 spores per square cm of spot)
9	Spots covering the leaf surface (highly compatible) with a lot of sporangium (5×10^4 spores per square cm of spot)

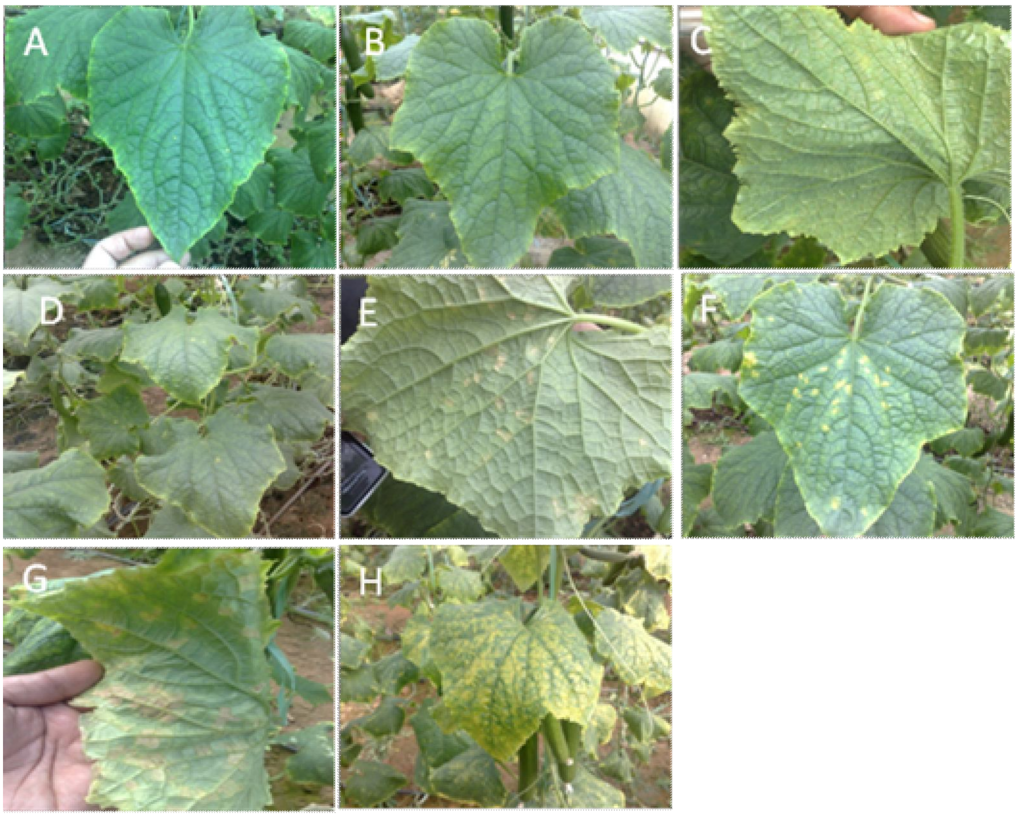

Figure 1 Determination of disease severity of cucumber downy mildew based on Thomas *et al*. (1987) with some modifications. A and B: symptomless leaf; C and D: Visual spots without sporangium formation (incompatible); E and F: Visual spots with a few sporangia (compatible); G and H: Spots covered the leaf surface (highly compatible) with a lot of sporangium.

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DS: disease severity; ni: number of leaves with a similar score; vi: disease score from 0-9 per leaf; N: total number of leaves evaluated; V: highest disease score.

The effect of experimental treatments on the severity of disease infection after data conversion was evaluated using SAS statistical software. The mean data were compared using Duncan's multiple range tests ($P \leq 0.05$). If no disease symptoms were seen on the surface of the studied leaves (score zero), the calculated disease severity was zero. If the surface of each leaf was completely infected (score 9), the severity of the disease was equal to 100%.

Evaluation of control efficiency: The following equation was used to evaluate the effect of treatments on disease control (Mitani *et al*., 2003):

$$CV = (1 - T/C) \times 100$$

CV (Control Value) shows the control value, T percent of fungicide-treated infected leaves, and C percent of infected leaves of untreated plants.

Data analysis

Data were analyzed with the SAS statistical analysis system and submitted to analysis of variance according to a randomized complete block design. Means were separated using Duncan's multiple range tests at $P \leq 0.05$.

Results

Combined analysis of variance for treatments and location

A statistically significant difference between fungicides treatments was detected (Table 4). In the first evaluation (control plants were 50% severely diseased), a significant difference was observed between locations ($P < 0.05$, Table 4), but in the second evaluation, there was no difference (Table 4). In both assessments, interactions between location and treatment (Location × Treatment) were not significant (Table 4).

Table 4 F statistics for cucumber downy mildew disease severity percentages in Dehno and Mehriz regions in the first and second assessment.

Assessment	Variation resources	df	MS	F-value
First assessment	Location	1	70.020	6.107*
	Treatment	12	1293.788	112.846**
	Treatment × Location	12	16.297	1.421 ns
	Error 1 (Block)	6	4.078	0.356 ns
	Error 2 (remained)	72	11.465	
	Total	103		
	Coefficient of Variation (CV)	15.51%		
Second assessment	Location	1	41.042	2.567
	Treatment	12	3348.415	209.397**
	Treatment × Location	12	8.755	0.547
	Error 1 (Block)	6	23.412	1.464
	Error 2 (remained)	72	15.991	
	Total	103		
	Coefficient of Variation (CV)	10.00%		

MS: mean squares; *, **are significantly different at 0.05 and 0.01 probability levels, respectively.

Treatments evaluation

Statistical analysis in the first evaluation (control plants reaching 50% disease severity) showed that in the phosphonate treatment and all copper-oxychloride fungicides tested, and the disease severity was 25-35% lower than the control (with or without water treatment). Data also showed that foliar spraying of water increased the mean disease severity by 19.01% compared to the control without foliar application (Fig. 2, Table 5).

All of the copper oxychloride fungicides controlled disease and phosphonate fungicide, Fosphite®, with the efficacy of 79.39%. Among the copper-oxychloride products, Copertox® and Oksavit®, with efficacy of 67.49 and 63.8%, performed significantly better than Coprox® with an efficacy of 53.8%, but equally well as the other tested products (Table 5).

Statistical analysis in the second evaluation (control plants reaching 90% disease severity) showed that Fosphite® was the highest performing fungicide with an efficacy of 82.6%. Still, with increasing disease pressure, the tested copper-oxychloride fungicides were further separated into distinct performance groups. Copertox® (32% DS) controlled disease significantly better than Oksavit® (37% DS). The performance of Coprox significantly increased, and disease control was equal to Copertox® (32% DS) (Fig. 3, Table 5).

The results also showed that water spraying increases disease severity by 7.53% compared to the control without water spraying. The mean disease severity of the control treatment without water spraying (81.11%) indicates high disease pressure (Table 5).

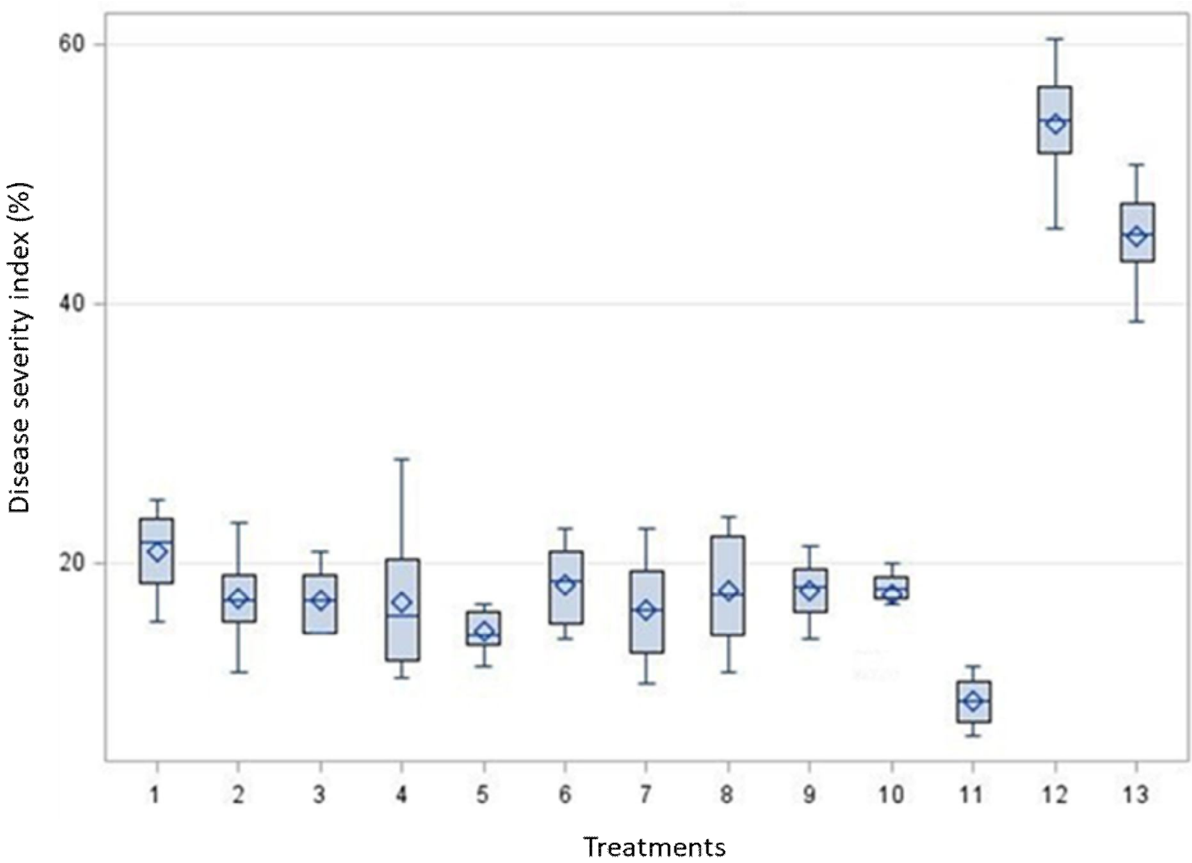

Figure 2 Box plot of the first assessment of fungicides treatments on cucumber downy mildew disease. For treatments, refer to table 2.

Table 5 Comparison of average cucumber downy mildew disease severity and fungicide efficacy percentages of treatments in the first and second assessments.

No.	Treatment	First assessment		Second assessment	
		Disease severity (%)[1]	Fungicide efficacy (%)[2]	Disease severity (%)[1]	Fungicide efficacy (%)[2]
1	Fosphite®	9.33 e	79.39	14.11 f	82.60
2	Copertox®	14.72 d	67.49	31.61 de	61.03
3	Oksavit®	16.39 d	63.80	36.94 c	54.46
4	Coprex®	17.06 cd	62.32	30.89 e	61.92
5	Koosha®	17.17 cd	62.08	34.00 cde	58.08
6	Oxavit®	17.28 cd	61.84	33.11 cde	59.18
7	Behcop®	17.61 cd	61.11	33.94 cde	58.16
8	Coper®	17.89 cd	60.49	35.78 cd	55.89
9	Oxyazarin®	17.94 cd	60.38	33.11 cde	59.18
10	Oxytex®	18.33 cd	59.52	36.28 cd	55.27
11	Coprox®	20.89 c	53.86	31.61 de	61.03
12	Control (no water applied)	45.28 b	-	81.11 b	-
13	Control (water applied)	53.89 a	+ 19.01	87.22 a	+ 7.53

[1] Data represent the means of pooled data (n = 30) at each sampling time. Within each column, different letters indicate significant differences at each assessment time according to Duncan's multiple range test at P = 0.05.
[2] Efficacy is compared to the control (without water application).
+ indicates an increase of disease severity percentage when compared to the control (without water application).

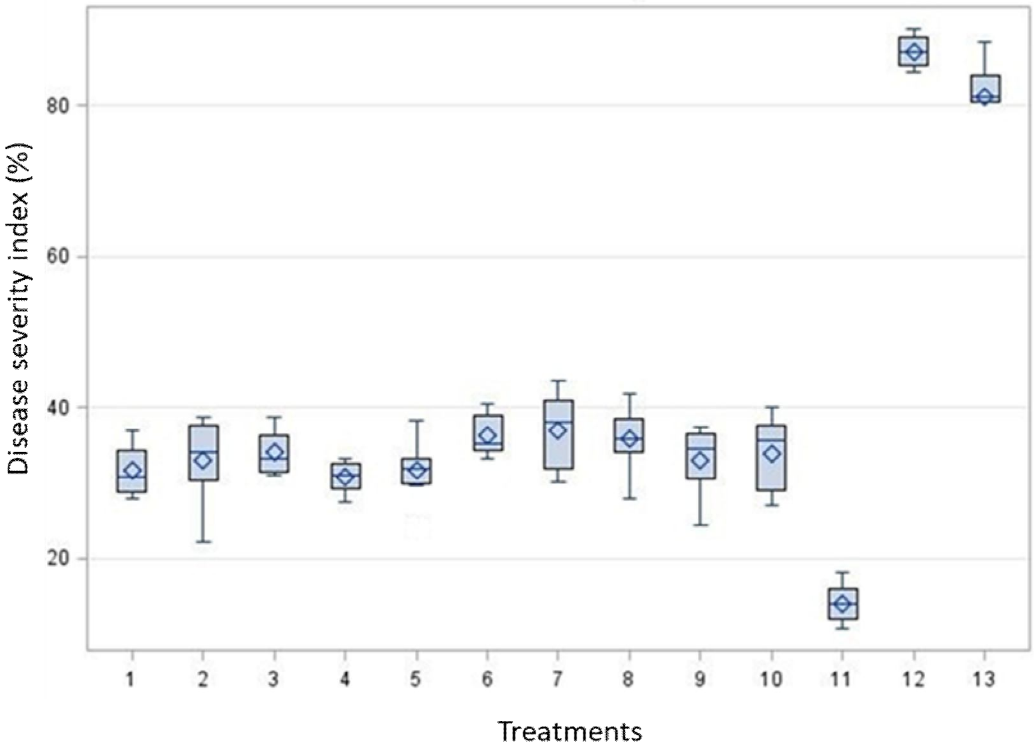

Figure 3 Box plot of the second assessment of fungicides treatments on cucumber downy mildew disease. For treatments, refer to table 2.

[DOR: 20.1001.1.22519041.2021.10.3.9.7]

Discussion

CDM is one of the most critical diseases in the greenhouse and open field cultivation systems in the country, so the disease was widespread at the site of the experiments of this study. The pathogen that causes disease in most cucurbits has changed a lot over the past decade. Reports of high disease severity and widespread epidemics, the emergence of genotypes, races, pathotypes, and compatible types of pathogens have increased (Cohen *et al*., 2015).

Chemical control is very effective in the management of CDM. However, *P. cubensis* is a high-risk pathogen and might pose a challenge for high-risk single-site fungicides, according to FRAC (Fishel and Dewdney, 2012). Decreased efficacy of Mefenoxam, Metalaxyl, and Strobilurin-based fungicides has been reported previously (Colucci, 2008). These fungicides should be used cautiously to conserve their effectiveness, following proper fungicide rotation programs, including fungicides with different MOA (Colucci, 2008).

Comparison of different fungicides in the control of CDM in Faisalabad, Pakistan, showed the prevalence of CDM in copper oxychloride treatment of 62.4% (Chauhdry *et al.,* 2009). In India, copper oxychloride controlled the disease at a rate of 62.54% (Gupta and Jarial 2014). This study also showed that the efficiency of copper oxychloride in the greenhouse of cucumber production in Yazd is 67% at the best condition. The efficiency of different brands of copper oxychloride in the first and second evaluations in the trial sites showed no significant differences in CDM control. Maximum efficiencies of 67 and 61% at the severity of 50 and 90% of disease control treatment may not be desirable for the farmer. However, when there is a risk of resistance in the pathogen population to systemic fungicides, it is logical to use mineral compounds to reduce the risk of developing resistance because of multiple points of action and the elimination of possible resistant strains.

Fungicidal resistance to copper oxychloride-based compounds, which are protective, has been rare. These fungicides are typically able to control a wide range of different pathogens. Copper is not degraded as heavy metal in the environment and should not be used as the only source of protection against disease but should be used in rotation or as a component in the fungicide basket (Lebeda and Cohen, 2011).

Copper oxychloride-based fungicides have a low chance for fungicide resistance to develop and are considered protectant fungicides. These fungicides typically offer broad-spectrum control for many different pathogens. Copper compounds such as copper oxychloride to control downy mildew diseases have been used every day in grape orchards for many years (Agrios, 2005).

Copper ions inhibit the germination of pathogenic fungal/Oomycete spores by acting on enzymes. When using copper oxychloride, complete foliar coverage is essential, and application should be repeated after each rain. Copper oxychloride is also used for winter spraying. This study shows the relative effectiveness of copper oxychloride-based fungicides available from different manufacturers in controlling CDM, consistent with the findings of other researchers. To preserve Fosphite® as a premium pesticide against CDM, copper oxychloride-based fungicides should be added to the spray rotations. The position of copper oxychloride-based fungicides in such rotations should be investigated further. It is further necessary to pay attention to soil contamination and possible toxic effects on the soil microbiome by accumulating copper ions.

Acknowledgments

The authors are thankful to the Iranian Research Institute of Plant Protection (IRIPP) for funding project No. 24-64-16-142-961030. Thanks to Yazd Agricultural and Natural Resources Research and Education Center for assistance in this project.

Declaration of conflicting interests

The authors state that there is no conflict of interest.

[DOR: 20.1001.1.22519041.2021.10.3.9.7]

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کارایی قارچ‌کش‌های حاوی اکسی‌کلرور مس در کنترل سفیدک داخلی در گلخانه‌های خیار کشور

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دریافت: ۲۹ آبان ۱۳۹۹؛ پذیرش: ۱۷ خرداد ۱۴۰۰

چکیده: استفاده از یک قارچ‌کش با چند نقطه اثر در برنامه‌های کنترل بیماری سفیدک داخلی خیار برای اطمینان از محافظت کافی از محصول و به تأخیر انداختن مقاومت احتمالی به گروه‌های پرخطر قارچ‌کش‌های با یک نقطه اثر توصیه می‌شود. کارایی ترکیبات تجاری از قارچ‌کش‌های بر پایه دیکاپرکلرید تری‌هیدروکسید (که به‌عنوان اکسی‌کلرور مس نیز شناخته می‌شود) (از گروه M FRAC) در کنترل بیماری سفیدک داخلی خیار در کنار قارچ‌کشی رایج از فسفونات‌ها (فسفیت® ۵۳٪ WSL، از گروه FRAC P7) با تیمار شاهد بدون سمپاشی ارزیابی شد. سمپاشی اندام‌های هوایی با ظهور علایم بیماری شروع و به‌صورت هفتگی تکرار شد. شدت بیماری دو مرتبه در طول رشد گیاه محاسبه گردید. نتایج نشان داد بین تیمارها اختلاف معنی‌دار وجود دارد. فسفیت با کاهش شدت بیماری به میزان ۸۲/۶ درصد، مؤثرترین ترکیب در بین تیمارهای دیگر بود. در میان ترکیبات تجاری اکسی‌کلرور مس نیز از نظر آماری اختلاف معنی‌دار مشاهده شد. کوپرتوکس® و اکساویت® در ارزیابی اول شدت بیماری از بقیه ترکیبات بهتر و در ارزیابی دوم نیز کوپرتوکس نسبت به بقیه برتری داشت. اختلاف معنی‌دار بین کرت‌های تیمار شاهد نیز دیده شد. کارایی ترکیبات مختلف تجاری اکسی‌کلرور مس در کنترل بیماری سفیدکی داخلی خیار ۶۷-۵۳٪ بود. این میزان کارایی در شرایط عادی بیماری، قابل‌قبول ولی در شرایط اپیدمی مطلوب نیست. چنان‌چه شرایط مناسب برای اپیدمی شدید بیماری فراهم باشد، تلفیق آنها با قارچ‌کش‌هایی کاراتر مانند فسفیت ضروری است.

واژگان کلیدی: کدوییان، بیماری‌های شاخه و برگ، اُاُمیست‌ها، فسفیت پتاسیم، قارچ‌کش‌های با چند محل اثر

[DOR: 20.1001.1.22519041.2021.10.3.9.7]