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Published in:
Journal of Crop Protection

Published: 01/01/2022

Document Version
Publisher's PDF, also known as Version of record

J. Crop Prot. 2022, 11 (3): -

Short Paper

Efficacy of Beltanol (SL 37.5%) on damping-off disease of cucumber

Seyed Reza Fani[1], Hossein Azimi[2], and Claudia Probst[3]

1. Plant Protection Research Department, Yazd Agricultural and Natural Resources Research and Education Center, AREEO, Yazd, Iran.
2. Iranian Research Institute of Plant Protection, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran.
3. University of Applied Sciences, School of Engineering, Department of Agricultural Technology and Management, Wels, Austria.

Abstract: Damping-off disease caused by *Phytophthora melonis* is the most common disease of cucumber seedlings *Cucumis sativus* L.. To study the efficacy of 8-hydroxy quinoline sulfate (Beltanol®) in control of cucumber damping-off disease, glasshouse experiments were carried out with six treatments at research stations in Tehran, Alborz, and Semnan provinces in Iran. Treatments included 0.3, 0.4, and 0.5 $ml \cdot l^{-1}$ of Beltanol as the experimental fungicide, metalaxyl+mancozeb (Rosalaxyl® WP 72%; FRAC code 3 + M03) at 2 $g \cdot l^{-1}$ as the standard fungicide along with inoculated and untreated and non-inoculated (healthy) controls. Cucumbers were cultivated from seed in trays, and treatments were applied twice. Once after seed sowing and second time at the 2-leaf stage. Disease incidence was recorded at the 4-leaf stage. Beltanol at 0.3 $ml \cdot l^{-1}$ had the least effect among fungicides, with nearly 50% of treated plants showing signs of disease. Application of Beltanol at 0.4 and 0.5 $ml \cdot l^{-1}$ decreased disease incidence by 59.55 and 64.47% compared to the inoculated control, respectively. Rosalaxyl® performed better than Beltanol and reduced disease by 83.55%. However, to provide alternatives for proper fungicide rotations, Beltanol at the rate of 0.4 $ml \cdot l^{-1}$ may manage damping-off disease in cucumber.

Keywords: Cucurbits, Mancozeb, Metalaxyl, *Phytophthora*, 8-hydroxy quinoline

Introduction

Damping-off disease caused by the soil-borne Oomycete plant pathogen *Phytophthora melonis* Katsura is one of the most important diseases damaging cucumber (*Cucumis sativus* L. plants at the seedling stage in greenhouses. Currently, there is no suitable single method to control the disease (Babadoost, 2004; McGrath, 2020). Cultivar resistance is currently not commercially available, and crop rotation cannot be used as a suitable and definitive method in disease management programs due to the persistance of the pathogen for years in the soil and its wide host range (Khan *et al*., 2004).

Proper and timely use of appropriate fungicides, ideally in rotation, is considered an effective method in the integrated management of this disease (McGrath, 2020). Introducing new fungicides is important to design appropriate fungicide rotation schedules and prevent the development of resistance against commonly

Handling Editor: Naser Safaie

* Corresponding author: r.fani@areeo.ac.ir
Received: 12 January 2022, Accepted: 21 September 2022
Published online: 08 November 2022

[DOR: 20.1001.1.22519041.2022.11.3.4.9]

used chemical groups. Use of the fungicide Mefenoxam (Apron XL® LS, FRAC code 4, high risks for resistance development) at 0.42 mL·kg^{-1} seed and Metalaxyl (Alginate® FL, FRAC code 4, increased risks for resistance development) at 0.98 mL·kg^{-1} seed, protect cucumber seedlings against disease for up to 5 weeks after planting (Babadoost, 2004; McGrath, 2020). Mefenoxam and Metalaxyl cannot be used in rotation since both are phenyl amides (PA fungicides). Application of Dimetomorph+Mancozeb (Acrobat® WP 50%, FRAC code 40 + M03) at the rate of 448 g·ha^{-1}, copper sulfate (Cuprofix® dispersion, FRAC code M1) at the rate of 2.25 kg·ha^{-1} at 7-day intervals can provide good protection against the disease (Babadoost, 2004; McGrath, 2020). Field disease damage can be minimized using Mefenoxam (FRAC code 4) for seed treatment and Dimethomorph (FRAC code 40) for aerial spraying. Application of Alliet® (FRAC code 33), copper (FRAC code M1), Acrobat® (FRAC code 40+M03), Ridomil® (FRAC code M03 + 4), and Bravo® (FRAC code M5) fungicides has had adequate control on the disease (McGrath, 2020).

Studies have been focused on crown rot disease caused by *Phytophthora* species (Gupta and Thind, 2018); its etiology, pathogenicity (Alavi and Strange, 1979), host range (Alavi, 1990), chemical and non-chemical control methods (Dhingra and Sinclair, 2017), utilizing phytoalexin defense potential within the host plant. The Agri-fos® systemic fungicide (mono- and di-potassium salts of phosphorous acid) has been recommended for control of root, crown, stem rot, and leaf spot caused by *P. melonis* and *Phytophtora capsici* on squash *Cucurbita pepo* L. (Kanaskie *et al*., 2006). Low control of cucumber damping-off by aerial spraying of this fungicide reported by Azimi *et al*. (2010). The efficacy of Metalaxyl + Mancozeb (trade name Rosalaxyl® WP 72%; FRAC code 3 + M03) was evaluated as good in controlling cucumber seedling damping-off (Azimi, 2014).

The fungicide Beltanol® (8-hydroxy quinoline sulfate) (Probelte S. A. U., Murica, Spain) promises good protection against soil-borne pathogens including *Phytophthora*, *Pythium*, *Rhizoctonia*, *Verticillium*, and *Fusarium*. It belongs to the quinoline group of chemicals and has not been assigned a FRAC code by the Fungicides Resistance Action Committee (Anonymous, 2021). The risk of resistance development is currently unknown. A number of authorized uses is recorded in its registration documents in France for up to 2 times with irrigation water. The PHI index (preharvest **i**nterval; the time between a pesticide application and harvest) is at least three days, and the minimum time between applications is 14 days (Anonymous, 2017). Hassan *et al.* (2006) have reported Beltanol to be effective in the control of *Rhizoctonia solani* Kuhn. Beltanol was effective in the control of cucumber damping-off disease caused by the *Phytophthora drechsleri* Tucker (Bani and Samir, 2006). This study evaluated the efficacy of Beltanol (SL 37.5%) for registration as a new fungicide in the control of cucumber damping-off disease caused by *P. melonis*.

Materials and Methods

Pathogen inoculum

P. melonis was isolated from infected cucumbers and identified morphologically by conventional methods (Gerami and Azimi, 2018). The pathogen was purified via hyphal tip transfer and maintained in pure culture on standard laboratory media, including potato dextrose agar (PDA) and corn meal agar (CMA).

Pathogen propagation and experimental set-up

To propagate the pathogen, peat moss enriched with chopped carrots was used in a weight ratio of 9: 1. The prepared mixture was poured into plastic bags. The top of the bag was sealed but with a plastic tube (number 20 with a length of 15 cm) sticking out of the bag connecting the inside with the outside. To moisten the peat moss mixture, 400 ml of distilled water was poured into each bag through the tube, and after thorough mixing, the tube was sealed with cotton and aluminum foil. Bags were sterilized twice (with a 24 hours interval) in an autoclave for 20 minutes at 121 °C at a pressure of 1.5 atmospheres each time. Seven

[DOR: 20.1001.1.22519041.2022.11.3.4.9]

days old *P. melonis* was transferred via agar plugs from PDA to sterilized bags (10 plugs of 10 mm diameter per bag). Bags were thoroughly mixed to distribute the colonized agar plugs in the individual bags, followed by incubation at 22 ℃ in constant darkness. The growth of the pathogen and colonization of peat moss in the bags was examined daily. After 7-10 days, bags were used to inoculate the soil of planting trays, as described by Azimi and Shahriyari (2015). Briefly, the relative humidity in the inoculation bags was adjusted based on the previous experiences by moistening the substrate inside the bag to the extent that, while avoiding dryness of the substrate, sufficient moisture would be provided for the growth and development of *Phytophthora*. As a model, the amount of humidity should be such that the substrate would not stick to the hand, and the humidity of the substrate could be felt by touching it with the hand. In addition, the irrigation method after inoculation was through absorption from the bottom of the cultivation tray. For this purpose, the cultivation trays after planting were placed inside the galvanized metal trays containing water that was made for this purpose. Obviously, the amount of water at the bottom of the trays was also adjusted so that, while avoiding the pathogen from lysis, it would provide enough moisture for seed germination and growth and be suitable for the pathogen. Experiments were carried out with six treatments and four replications (Table 1) using a randomized complete block design. Experiments were conducted in three research greenhouses in Karaj Plant Research Laboratory, Plant Research Institute in Tehran and the Agricultural and Natural Research and Education Centre of Shahroud. For each experimental treatment, two culture trays with 70 planting wells and a capacity of six liters were used. Each tray was divided into 35 wells with hypothetical cross-lines, and every 35 wells were considered a repkicate. The planting mixture consisted of peat moss and perlite at a ratio of 3 to 1, plus a small amount of clay and sand. The soil mixture was sterilized twice with an interval of 48 hours. The inoculum prepared in plastic bags as described above was first mixed in a 1: 9 ratio with sterile peat moss and then in a 1: 10 ratio with the soil mixture of the planting trays (combination of peat moss + perlite + clay + sand) (Strange *et al*., 1981). The substrate-pathogen mixture and the non-inoculated control (soil mixture without the pathogen) were sprayed with sterile distilled water to slightly moisten the soil and then covered with plastic sheets to maintain high moisture for a week at room temperature, which allowed the pathogen to get established in the soil.

Table 1 Doses of Beltanol fungicide for control of cucumber damping-off disease, compared to controls.

Treatments	Dose
Beltanol SL 37.5%	0.3 $ml \cdot l^{-1}$
Beltanol SL 37.5%	0.4 $ml \cdot l^{-1}$
Beltanol SL 37.5%	0.5 $ml \cdot l^{-1}$
Rosalaxyl WP 72%	2 $g \cdot l^{-1}$
Healthy control	
Infected control	

After soil inoculation, one pre-germinated cucumber seed cv. Supersultan (Nunhems, Netherland) was planted per well. Based on the treatment plan (Table 1), a total of 2 liters of fungicide solution (or water) was prepared for each treatment, and one liter of the solution was applied evenly after sowing using a handy sprayer and again at the 2-leaf stage following all precautions when dealing with fungicides. The healthy and infected controls were only treated with water.

Evaluation of treatments was done by counting healthy plants and determining the percentage of diseased plants from seedling emergence up to the 4-leaf stage. Infected plants were removed from the soil, and identification of the causal agent was done by isolation and re-cultivation in CMA. Results were analyzed using Analysis of Variance (ANOVA) and Duncan's multiple range tests in SAS 8.0 (SAS Institute, Cary, NC).

Results and Discussion

Combined results of the three experimental locations showed a significant difference between treatments at $P < 0.01$. Due to significant differences in Treatment × Location interaction at combined ANOVA (Table 2), the results of each studied location were analyzed separately by SAS software, and mean comparisons were made separately for each site (Table 3).

[DOR: 20.1001.1.22519041.2022.11.3.4.9]

Table 2 Analysis of variance of disease incidence (%) for treatments and locations.

Source of variation	df	MS	F value
Location (L)	2	1251.81	20.16**
Block (Location)	9	75.06	1.21ns
Treatment (T)	5	11485.76	158.00**
T × L	10	753.79	12.14**
Error	45	62.09	-

Ns: not significant; **: significant at P < 0.01.

Semnan province (Shahroud)

Analysis of variance of disease incidence in this experiment showed a significant difference between treatments at p < 0.01 (Table 2).

Duncan's multiple range test comparisons of the mean of treatments showed that the treatments were divided into four statistical groups. Plants treated with Beltanol concentrations of 0.4 ml/l and 0.5 ml/l showed an average disease incidence of 53.67 and 51.53% (Table 3). An application concentration of 0.3 ml/l showed significantly less disease control, with 67.96% of plants infected (Table 3). Rosalaxyl, as a reference fungicideat concentration of 0.5 g/l had better effects than all concentrations of the target fungicide and was statistically comparable to the healthy control. Disease pressure in Shahroud experimental station was high, *i.e*. 89.39% in the infected (and untreated) control (Table 3).

Tehran province (Tehran)

Analysis of variance of disease incidence in this experiment showed a significant difference between treatments at P, 0.01 (Table 2).

A comparison of the mean of treatments by Duncan's multiple range test showed that the treatments were divided into four statistical groups. Among the fungicides, Beltanol-L, at concentrations of 0.4 ml/l and 0.5 ml/l, and Rosalaxyl fungicide at concentration of 0.5 g/l performed equally well in this trial. Beltanol-L at 0.3 ml/l still performed better than the infected (and untreated) control but significantly worse than the other treatments (Table 3). Compared to Shahroud, Rosalaxyl treated plants still had a higher disease incidence than the healthy control. Disease pressure in Tehran experimental station was high *i.e*. 91.17% in the infected (and untreated) control (Table 3).

Alborz province (Karaj)

Analysis of the variance of disease incidence in this experiment showed a significant difference between treatments at P < 0.01 (Table 2).

A comparison of the mean of treatments by Duncan's multiple range test showed that the treatments were divided into four statistical groups. Among the fungicides, Beltanol at concentrations of 0.4 ml/l and 0.5 ml/l and Rosalaxyl at 0.5 g/l performed equally well in this trial. Beltanol at 0.3 ml/l still performed better than the infected (and untreated) control but significantly worse than the other treatments (Table 3). Compared to Shahroud, plants treated with Rosalaxil in Tehran and Karaj had higher disease prevalence (27.6 and 17.6% respectively) than the healthy control. Disease pressure in Karaj experimental station was high, with 94.74% in the infected (and untreated) control (Table 3).

Table 3 Mean comparison of the disease incidence.

Treatments	Rep 1		Rep 2		Rep 3	
	DAI[1] (%)	Eff. [2] (%)	DAI[1] (%)	Eff. [2] (%)	DAI[1] (%)	Eff. [2] (%)
Infected, untreated control	89.39a	-	91.17a	-	94.74a	-
Beltanol 0.3 ml·l^{-1}	67.96b	23.97	52.96b	41.91	28.31b	70.12
Beltanol 0.4 ml·l^{-1}	53.67c	39.96	39.39c	56.79	18.31c	80.67
Beltanol 0.5 ml·l^{-1}	51.53c	42.35	30.81c	66.20	15.46c	83.68
Rosalaxyl 2 g·l^{-1}	0.1d	99.89	27.60c	69.73	17.60c	81.42
Uninfected control	0.1d	-	3.67d	-	6.17d	-

Values in the same column followed by the same letter are not significantly different at P < 0.05 according to Duncan's multiple range test mean separation test.

[a] DAI = Disease average incidence,

[b] Eff. = Effectiveness of treatment = % disease reduction compared to infected, untreated control.

Rep 1: Semnan (Shahroud), Rep 2: Tehran (Tehran), Rep 3: Alborz (Karaj).

[DOR: 20.1001.1.22519041.2022.11.3.4.9]

The family Cucurbitaceae, with about 90 genera and more than 750 species, includes a large group of crops like cucumbers, all of which are widely used by humans as food (Zohary *et al*., 2012). Cucurbitaceae, also known as cucurbits, were domesticated in Mexico about 7000 years ago. Humans have used cucumber for more than 5,000 years, and after tomatoes, cabbage and onions, it ranks fourth among vegetables in terms of cultivated area in the world (Zohary *et al*., 2012). One of the most important diseases that damages cucumber seedlings is damping-off. Timely use of fungicides has always been considered one of the effective methods in the integrated management of the disease (McGrath, 2020). This study investigated the efficacy of controlling damping-off disease and subsequent death of cucumber seedlings caused by *P. melonis*. This study showed that the fungicide Beltanol produced by Probelte S.A.U. company effectively controls this disease at any tested application concentration. However, to achieve disease control comparable to the standard fungicide, a minimum concentration of 0.4 g/l is needed. These results confirm the studies by Bani and Samir (2006) who described Beltanol as an effective control agent for cucumber damping-off disease caused by *P. drechsleri*.

Interestingly the reference fungicide containing metalaxyl-mancozeb (Rosalaxyl, also known as Ridomil Gold) outperformed Beltanol in the combined analysis. But due to its good effect in controlling damping-off caused by *P. melonis*, Beltanol can be a suitable alternative to Mancozeb-containing fungicides and be a useful tool for fungicide rotations. The results of this study may be recommended for damping-off diseases of other cucurbits caused by different species of *Phytophthora* and *Pythium*.

Acknowledgments

The authors thank the Iranian Research Institute of Plant Protection (IRIPP) for funding project No. 04-16-16-095-960541.

Disclosure statement

The authors declare no conflicts of interest.

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[DOR: 20.1001.1.22519041.2022.11.3.4.9]

کارایی بلتانول (SL 37.5%) در کنترل بیماری بوته‌میری خیار

سیدرضا فانی[۱]، حسین عظیمی[۲] و کلودیا پروبست[۳]

۱- بخش تحقیقات گیاه‌پزشکی، مرکز تحقیقات و آموزش کشاورزی و منابع طبیعی استان یزد، سازمان تحقیقات، آموزش و ترویج کشاورزی، یزد، ایران.
۲- مؤسسه تحقیقات گیاه‌پزشکی کشور، سازمان تحقیقات، آموزش و ترویج کشاورزی، تهران، ایران.
۳- دانشگاه علوم کاربردی، دانشکده مهندسی، گروه فناوری و مدیریت کشاورزی، ولز، اتریش.
پست الکترونیکی نویسنده مسئول مکاتبه: r.fani@areeo.ac.ir
دریافت: ۲۲ دی ۱۴۰۰؛ پذیرش: ۳۰ شهریور ۱۴۰۱

چکیده: بیماری بوته‌میری که توسط *Phytophthora melonis* Katsura ایجاد می‌شود یکی از مهم‌ترین بیماری‌های گیاهچه‌های خیار (*Cucumis sativus* L.) است. برای مطالعه کارایی ۸- هیدروکسی کینولین سولفات (با نام تجاری بلتانول®) در کنترل بیماری بوته‌میری خیار، آزمایش‌های گلخانه‌ای با شش تیمار در ایستگاه‌های تحقیقاتی استان‌های تهران، البرز و سمنان انجام شد. تیمارها شامل غلظت‌های ۰/۳، ۰/۴ و ۰/۵ میلی‌لیتر در لیتر بلتانول به‌عنوان قارچ‌کش آزمایشی، متالاکسیل + مانکوزب (نام تجاری رزالاکسیل® WP72% کد FRAC 3+M01) در غلظت دو گرم در لیتر به‌عنوان قارچ‌کش استاندارد همراه با شاهدهای مایه‌زنی شده (تیمار نشده با قارچ‌کش) و مایه‌زنی نشده (سالم) بودند. بذر خیار در سینی کشت شد و تیمارها در دو مرحله یعنی بعد از کاشت بذر و در مرحله ۲ برگی اعمال شد. وقوع بیماری در مرحله چهاربرگی ثبت شد. بلتانول با غلظت ۰/۳ میلی‌لیتر در لیتر کم‌ترین اثر را در بین قارچ‌کش‌ها داشت و تقریباً ۵۰ درصد گیاهان تیمار شده علائم بیماری را نشان دادند. استفاده از بلتانول در غلظت‌های ۰/۴ و ۰/۵ میلی‌لیتر در لیتر در مقایسه با شاهد مایه‌زنی شده به‌ترتیب به‌میزان ۵۹/۵۵ و ۶۴/۴۷ درصد بروز بیماری را کاهش داد. قارچ‌کش رزالاکسیل با کاهش ۸۳/۵۵ درصدی بیماری، بهتر از بلتانول عمل کرد. با این حال، برای معرفی سموم جایگزین برای تناوب در استفاده از قارچ‌کش‌ها، بلتانول به‌میزان ۰/۴ میلی‌لیتر در لیتر می‌تواند بیماری بوته‌میری را در خیار کنترل کند.

واژگان کلیدی: فیتوفتورا، کدوییان، مانکوزب، متالاکسیل، ۸- هیدروکسی کینولین

[DOR: 20.1001.1.22519041.2022.11.3.4.9]