

Efficacy of Seaweed Concentrate from *Ecklonia maxima* (Osbeck) and Conventional Fungicides in the Control of Verticillium Wilt of Pepper

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SUMMARY

In order to control the causal agent of Verticillium wilt of pepper (*Verticillium dahliae*), the efficacy of two conventional fungicides, thiophanate-methyl and carbendazim, and seaweed concentrate (SWC) from *Ecklonia maxima* was evaluated in greenhouse conditions. Pepper plants were inoculated with selected *V. dahliae* isolate in the stage of more than nine fully developed leaves on primary stem. The tested fungicides and SWC were applied three days before inoculation of pepper plants. Carbendazim was the most efficient fungicide among tested substances (69.64%). SWC proved to be more effective when applied at 1.0% concentration (41.96%). The use of thiophanate-methyl provided good Verticillium wilt control in pepper (60.71%). SWC was less efficient than thiophanate-methyl and carbendazim, but still significantly better compared to the disease control plot.

Keywords: *Ecklonia maxima*; Carbendazim; Thiophanate-methyl; Pepper; *Verticillium dahliae*

INTRODUCTION

Verticillium wilt is one of the most important diseases of pepper. It is present in all commercial pepper-growing areas. The disease is also known as the “green wilt of pepper” because of its characteristic symptoms. Its causal pathogens are fungi of the genus *Verticillium* (Santamarina and Roselló, 2006).

Chemical treatment of soil and cultivation of resistant cultivars are the most common means of controlling Verticillium wilt of pepper. Methyl bromide has been widely used for soil disinfection, but its use will be withdrawn by the year of 2015 (in developing countries) because of its harmful impact on human health and the environment. (Watson et al., 1992). In developed countries the ban on methyl bromide has been in force since 2005. Other fungicides commonly used to control Verticillium wilt of pepper are benzimidazoles - carbendazim and thiophanate-methyl (Talboys, 1984; Tian et al., 1998). However, these fungicides have not shown sufficient efficacy because of the pathogen ability to survive in the environment for a long time and its rapid spreading by various agricultural practices. Numerous research efforts have been focused on finding alternative ways to control Verticillium wilt. A significant amount of research work is currently focused on the use of mycopathogenic fungi (such as *Pythium oligandrum*, *Trichoderma harzianum*, etc.) and chemicals which stimulate plant defense mechanisms or a plant growth (Santamarina and Roselló, 2006; Rekanović et al., 2007; Jayaraj et al., 2008; Ślusarski and Pietr, 2009).

Kelpak is the trade name of a seaweed concentrate (SWC) prepared by a cell burst process from the brown algae *Ecklonia maxima* (Osbeck) Papenfuss (Kelp Products Ltd). This species has a prolific growth rate mainly due to the presence of the plant hormone groups known as auxins and cytokinins. The auxin-dominated extract stimulates prolific adventitious root formation when Kelpak is applied to almost any plant. The improved nutrient status, together with the higher level of cytokinin in the plant, improves the top growth, which increases the yield and quality of crops. The beneficial effects of SWC on the growth and yield of plants are well documented (Beckett et al., 1994; Kowalski et al., 1999). The improved root system also makes the plant more resistant to stresses such as drought, water logging, nematode infection and soil-borne diseases. Crouch and Van Staden (1993) showed that, in tomato, not only seedling growth, yield and fruit ripening were improved, but also the resistance to infection with *Meloidogyne incognita* was recorded.

The aim of this study was to evaluate the efficacy of SWC in Verticillium wilt control as an alternative strategy to chemical control measures.

MATERIAL AND METHODS

Verticillium dahliae isolates

Verticillium dahliae were isolated from infected pepper plants from several localities in Serbia: Padinska Skela (V-PS), Leskovac (V-L), Smederevo (VSM), Smederevska Palanka (V-SP), Čačak (V-C), Subotica (V-SU), Kikinda (V-KI) and Kraljevo (V-KV), using the method described by Dhingra and Sinclair (1995). Small fragments of diseased xylem tissue were placed aseptically on potato dextrose agar (PDA) and incubated at $25 \pm 1^\circ\text{C}$ for 10 days. The mycelium was transferred to fresh PDA medium to obtain a pure culture.

Inoculum preparation

A pure culture of *Verticillium* sp., grown on PDA at 25°C for two weeks, was used as a source of inoculum. The medium from petri dishes, each containing 15-day-old fungal cultures, was blended with 100 ml of distilled water until complete homogenization (D'Ercole et al., 2000).

Testing of the pathogenicity

Pathogenicity of the isolates was tested by transplanting pepper plants (*Capsicum annuum* L. cv. ‘Soroksari’) at four-leaf stage, previously dipped in the inoculum suspension for two to three minutes. After transplanting, the plants were first watered with the remaining inoculum suspension (5 ml per plant) and then with water. Visible changes on the inoculated plants were recorded daily. The most virulent isolate was used in the efficacy testing.

Testing of the efficacy of fungicides and biofungicide in Verticillium wilt control

The efficacy of two fungicides with similar modes of action were tested (inhibition of mitosis by binding to beta-tubuline) and a SWC in controlling Verticillium wilt using a method proposed by D'Ercole et al. (2000). The basic data for the tested compounds are shown in Table 1.

Table 1. Compounds teste

Comercial name	Active ingredient	Manufacturer	Amount of active ingredient	Concentration rate
Galofungin	Carbendazim	Galenika-Fitofarmacija a.d.	500 g/kg	0.05%
Funomil	Thiophanate-methyl	Agromarket d.o.o.	700 g/kg	0.05%
Kelpak	Seaweed concentrate	Kelp Products	100% liquid seaweed concentrate	0.5%, 0.1%

Five-week-old pepper plants (cv. Šorok-šari) were transplanted into 20 cm diameter plastic pots containing sterile growing substrate (Floragard[®], Germany). Two hundred millilitres of each fungicide and SWC were added to each pot three days before inoculation. The plants in the controls were watered with 200 ml of sterile distilled water. Then, treated plants were inoculated by watering with 5 ml of inoculum suspension, prepared as described under inoculum preparation section. The plants inoculated and watered with 200 ml of sterile distilled water served as a positive control (K-1). Uninoculated pepper plants, watered with 200 ml of sterile distilled water, served as a negative control (K-2). The pots were kept in a greenhouse ($T=24\pm2^{\circ}\text{C}$). Plants were fertilized weekly with a 1% 20:20:20 (= N:P₂O₅:K₂O) commercial soluble fertilizer. The experiment was complete randomized block with four replicates per treatment and five plants per replicate.

Parameters recorded

Verticillium wilt disease was assessed 90 days after transplanting when disease severity in the untreated control was the highest. In order to evaluate disease severity in an appropriate way, we used the method described by Wilhelm et al. (1974) which eliminates other „wilting factors” (drought, insufficient nutrition etc). The main stem of each pepper plant was cut near the ground and approximately 10 cm above. Then, the obtained part of the stem was cut vertically and rated on a scale of 0-4 based on the intensity and pattern of vascular discoloration. The score of 0 indicated the absence of discoloration; (1) very slight streaking in the wood nearest the pith; (2) slight streaking distributed sporadically throughout the wood; (3) distinct dark discoloration throughout the wood; and (4) intense uniform discoloration and wood deterioration. Disease severity (infection

degree, ID) was evaluated using Townsend-Heuberg-er's formula (Puntner, 1981):

$$\text{ID} = \frac{\sum(nv)}{NV} \times 100$$

where: n = degree of infection rated on a scale of 1–4, v = number of plants in a category, N = highest degree of infection rate, and V = total number of plants screened.

Fresh pepper plant heights and weights, and fruit weights were also measured 90 days after the transplanting.

The effectiveness of fungicides was calculated by Abbott (1925) formula (% effectiveness = control-treatment/control x 100). The data were analysed separately for each parameter using ANOVA and the means were separated by Duncan's multiple range test.

RESULTS

Altogether, eight isolates were obtained from the diseased pepper plants. The isolates, grown on PDA, formed white mycelium, later becoming black with the formation of microsclerotia (50-100 μm) (Figure 1 and 2). Conidiophores were abundant, hyaline, verticillately branched. Conidia were hyaline, ellipsoidal to irregularly sub-cylindrical, with average size 4.9 μm (2.7 μm - 7.5 μm) x 2.6 μm (2.0 μm - 3.2 μm) (Figure 3).

The isolates differed in their pathogenic characters. All isolates caused marked wilting symptoms on pepper plants 40 days after inoculation. However, wilting symptoms were observed on pepper plants inoculated with the isolate V-PS, originating from Padinska Skela, 14 days after inoculation. Since V-PS isolate showed the highest virulence, it was used for inoculation in the tests of the efficacy of fungicides and SWC against Verticillium wilt (Figure 4).

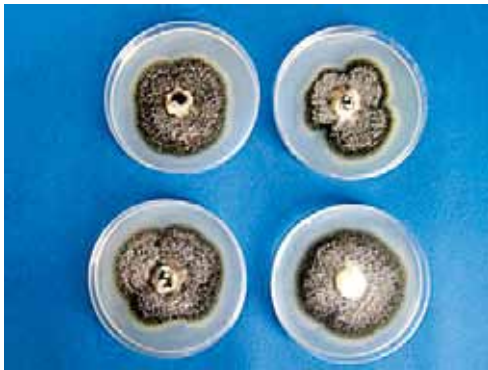


Figure 1. *V. dahliae* – white and black micelium on PDA medium



Figure 2. *V. dahliae* – microsclerotia (40x)



Figure 3. *V. dahliae* – hyphae, conidiophores and coniclia



Figure 4. Wilting symptoms on pepper plants 14 days after inoculation V-PS (P. Skela); V-K2 (uninoculated plants)

Table 2 summarizes the results of the disease severity and efficacy of the fungicides and SWC. The results showed a significant difference in the efficacy between SWC (0.5%) (30.36%) and the fungicide carbendazim (69.64%). There was no significant difference between the efficacy of SWC applied at both concentrations.

Table 2. Disease severity on pepper plants and efficacy of fungicides and SWC

Treatments	Conc. (%)	Disease severity index	Efficacy (%)
SWC	0.5	1.95 c ¹	30.36
SWC	1.0	1.63 bc	41.96
Carbendazim	0.05	0.85 a	69.64
Thiophanate-methyl	0.05	1.1 ab	60.71
K-1 – inoculated plants	–	2.8 d	–
K-2 – untreated and uninoculated plants	–	0.0 a	–

¹ Mean values within columns followed by different letters are significantly ($p < 0.05$) different according to Duncan's test.

The results also showed a significant difference, in terms of plant height, between the SWC (33.88 cm and 35.1 cm) and the conventional fungicide carbendazim (46.95 cm). There was no significant difference between the efficacy of SWC applied at both concentrations (Table 3).

Regarding plant weight, there was no significant difference between SWC (128.73 g and 130.27 g) and the

conventional fungicides - carbendazim (137.43 g) and thiophanate-methyl (136.18 g) (Table 3).

Regarding fruit weight, there was a significant difference between the efficacy of SWC (98.53 g) and conventional fungicides (100.97 g and 109.47 g) when the SWC was used at lower concentration of 0.5% (Table 3).

Table 3. Effect of applied fungicides and SWC on pepper plant heights, plant weights and fruit weights

Treatments	Conc. (%)	Plant height (cm)	Plant weight (g)	Fruit weight (g) ^a
SWC	0.5	30.58 b ¹	128.73 a ¹	98.53 c ¹
SWC	1.0	33.88 b	130.27 a	100.97 bc
Carbendazim	0.05	46.95 a	137.43 a	109.47 b
Thiophanate-methyl	0.05	41.60 ab	136.18 a	108.68 b
K-1 – inoculated plants	–	30.52 b	128.55 a	97.8 c
K-2 – untreated and uninoculated plants	–	47.7 a	140.98 a	119.32 a

¹Mean values within columns followed by different letters are significantly ($p < 0.05$) different according to Duncan's test.

DISCUSSION

Because of the longevity of microsclerotia and a broad host range of *V. dahliae*, crop rotation and chemical control are usually not a feasible option for Verticillium wilt control in pepper.

For effective management, an integrated approach is required. It usually involves a combination of cultural practices aimed at minimizing the disease, such as crop rotation, manipulation of fertility and irrigation, with the planting of pathogen-free seeds or stock, and the use of available resistant cultivars and sometimes pre-plant soil treatments, such as soil fumigation or solarization, so as to reduce microsclerotia viability in soil (Powelson and Rowe, 1993; Jeger et al., 1996).

The conventional fungicides proved to be more efficient than the SWC in our experiments. However, the efficacy of SWC was acceptable, especially considering its ecological and toxicological advantages.

The research also showed that there was a significant difference in the efficacy of the treatments studied, as well as in plant heights and fruit weights. However, in the terms of plant weights there was no significant difference among all treatments, including inoculated and untreated pepper plants (K-1).

Our results confirmed that SWC, when applied as a soil drench to pepper plants, has the ability to reduce Verticillium wilt of pepper. It has already been shown that SWC stimulates root growth and uptake of nutrients from soil, thus indirectly causing faster growth and development of plants (Beckett et al., 1994). According to the research conducted by Crouch and Van Staden (1993), plants treated with seaweed concentrate that stimulate root growth were able to develop resistance to pathogens.

Although the results of our experiment showed that the efficacy of the investigated fungicides was higher than of SWC in the control of Verticillium wilt, it is

necessary to continue research in order to determine optimal timing of treatment, environmental conditions and application rate.

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Efikasnost ekstrakta morske alge *Ecklonia maxima* (Osbeck) i konvencionalnih fungicida u suzbijanju prouzrokača verticilioznog uvenuća paprike

REZIME

U cilju suzbijanja prouzrokača verticilioznog uvenuća paprike (*Verticillium dahliae*) u uslovima staklenika ispitivana je efikasnost dva konvencionalna fungicida, karbendazima i tiofanat-metila, i ekstrakta morske alge *Ecklonia maxima*. Biljke paprike su inokulisane odbaranom izolatom *V. dahliae* u fenofazi kada je na glavnom stablu bilo potpuno razvijeno devet listova. Ispitivani fungicidi i ekstrakt morske alge su primenjeni tri dana pre inokulacije biljaka paprike. Karbendazim je bio najefikasniji u odnosu na ispitivane preparate (69,64%). Ekstrakt morske alge je ispoljio veću efikasnost pri višoj koncentraciji (41,96%). Tiofanat-metil je takođe ispoljio dobru efikasnost u suzbijanju *V. dahliae*. Ekstrakt morske alge je ispoljio manju efikasnost u odnosu na karbendazim i tiofanat-metil, ali je ipak statistički značajno bio bolji u odnosu na kontrolnu inokulisanu varijantu.

Cljučne reči: *Ecklonia maxima*; karbendazim; tiofanat-metil; paprika; *Verticillium dahliae*