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Wilting of Bell Pepper (*Capsicum annuum* L.) Causal Organism Isolation and a Successful Control Approach

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ABSTRACT

An experiment was conducted at "Green House Project Farm", Watagoda, Sri Lanka from April 2011 up to August 2011 to identify the causal organisms and suggest an integrated control approach for wilting of bell pepper plants in green houses. Pathogens were isolated from infected plant parts and soil media. Six media treatment methods including Carbendazim only, Metham Sodium only, Plastic potted, Thiram and Carbendazim alternatively, Thiram only and Steam, each with two levels of irrigation including full and half irrigated were tested in field in a seven in to two factorial RCBD design. Statistical analysis of data was done by Minitab 15. Under stereo microscopic observation *Fusarium* spp., *Pythium* spp. and *Phytophthora* spp. were the prominent pathogenic species. Oomycetes (*Pythium* spp. and *Phytophthora* spp.) were significantly detected in the roots and fungi *Fusarium* spp. were detected in stem parts and soil. It was appeared that the primary infection of *Pythium* spp. and *Phytophthora* spp. killed the root cells resulting root rot. Secondary infection of *Fusarium* spp. through broken root cells develops up to the stem and above ground parts, hindering the vascular flow and finally causing withering of the plant in green houses. The lowest wilt percentage was recorded in Metham and Steamed treated plants. The successful media sterilization was by Steaming and followed by Metham. Irrigation levels were not significantly correlated with plant wilting. However, the results of the study suggested that wilt suppression could be achieved by placing the steam treated plants disconnecting the contact with ground.

Key words: Bell pepper, *fusarium* spp., plant wilting, *pythium* spp., *phytophthora* spp.

INTRODUCTION

Intensive bell pepper cultivation in green houses is the most popular method worldwide. Bell pepper (*Capsicum annuum* L.) also called sweet pepper belongs to family Solanaceae. It is native to central and South America. Pepper cultivation is spread throughout Europe and Asia during the 16th century (Tewari, 2001). Fleshy bell shape fruits are prepared and consumed in many ways. Peppers are cultivated mostly for exportation and for local market.

Green house pepper produces are confronted with a serious constrain of wilting of bell pepper plants. Young plants at vegetative stage are more susceptible for wilting and that infected plants show leaf wither off and a dark streak develop upwards from collar region and also xylem tissues dry out resulting plant die. This has badly affected the productivity which is lead to 20-25% income loss annually.

There are several possibilities of wilting. It is due to bacteria, nematodes or fungi. Bacterial infection symptoms are mostly similar to fungal infection symptoms. Leaves wither off and brown colour lesions appear at root area. But plants with bacterial infection develop ooze in the rotted root parts. It is the only visual method to differentiate bacterial infection from fungal infection. Nematode infection results in developing root knots (Lowell, 1991). *Fusarium* spp., *Pythium* spp., Phytophthora spp., *Rhizoctoniya solani* and *Sclerotium* spp. are the most important plant pathogens that found in decaying plant residues in saprophytic nature (Agrios, 1997).

Commercial level produces use poly bags with soil based media. It consists of coir, sand and previously used old plant media. There is a higher possibility of harboring numerous soil borne pathogens in the media. Symptoms of soil borne pathogen infection are referred to as no germination of severely infected seeds or as damping off characterized by death of infected seedlings either before or after their emergence from the soil. It can also cause root rot and seedling blight (Johnston and Booth, 1983). Past research work on wilting of pepper plants shows that *Phytophthora capsici* and *Verticillium dahlia* are associated in wilting (Biles *et al*., 1992; Sanogo and Carpenter, 2006).

The necessity of new commercial methods on preventing and controlling wilting of bell pepper plants are realized. The experimental results are useful for the bell pepper cultivators. Therefore, the objective of this research was to identify the pathogens for bell pepper wilting and suggest an integrated control approach for wilting of bell pepper plants in green houses.

MATERIALS AND METHODS

Experimental site: The laboratory tests of the experiment were carried out at the Faculty of Agriculture and Plantation Management of Wayamba University. Field experiments were conducted in a green house tunnel at "Green House Project Farm", Watagoda, Sri lanka from April 2011 up to August 2011, situated in the Up Country Wet Zone (WU2a) with an average rainfall of 2400 mm and average relative humidity of 58%.

Nematode identification: The infected root and stem parts were cut in to small pieces and placed in Baemann funnel overnight (Peters, 1985). Then suspension was examined under light microscope.

Pathogen identification

Isolation from soil and water

Sample collection: Inoculums of soil from poly bags containing wilted plants, healthy plants and water samples from the main water source were randomly collected.

Isolation: Initially 10 g from sampled soil was added in to 90 mL of sterilized distilled water (10^{-2} dilution). After that it was shaken for 30 min at 165 rpm. Then 1 mL was pipetted in to 9 mL of sterilized distilled water (10^{-3} dilution). Potato Dextrose Agar media (PDA) was autoclaved under 121°C and 15 psi pressure for 20 min. Glassware and other equipment were sterilized by oven at 160°C for 2 h (Duggar, 1989).

From the diluted solution (10^{-3} dilution) 0.05 mL was pipetted in to agar plates in laminar flow. Then it was spread by a spreader. Finally cultured plates were kept at 25°C for 3 to 4 days (Jayathilaka *et al.*, 2006).

Colony colour was observed and slides were prepared from the most prominent fungal colonies. Then they were examined through light microscope. Spore shapes were compared with standard keys as illustrated by Barnett and Hunter (1972).

Pathogen identification from plant parts

Ooze test (bacteria): A cross section of an infected stem part was dipped in clear beaker of water for few hour. Final clearness of water was observed.

Identification of pathogens in root: Rotted root parts were chopped in to pieces and dipped in a petri dish of water. After five to seven days slides of root epidermis (outer layer) were prepared and examined through light microscope (Kelaniyangoda, 2003).

Culturing in PDA media: Diseased, healthy stems and root parts were cultured on PDA media. The culture plates were incubated at 25°C for 48-72 h.

Disease management

Treatments: Seven levels of media and two levels of water were tested in the field. Treatments are tabulated in Table 1.

All other cultural practices such as pest management, fertilizing, pruning etc., were uniform throughout the test as recommended by the Department of Agriculture.

Crop establishment: Planting media was prepared by bulking one part of coir and one part of sand. Five leaf stage bell pepper healthy seedlings were transplanted in to poly bags (12×8 inch) filled with media according to treatments and irrigated manually as to the levels I1 and I2 as shown in Table 1. Irrigation intervals were adjusted according to prevailing weather conditions.

Lay out of experiment: Treatments were arranged in a 7×2 Factorial Randomized Complete Block Design (RCBD) with three replications (blocks) and each having 14 plots (treatments). Each plot had 16 plants. Planting spacing was 0.76 m between rows and 0.15 m within rows.

Pathogen analysis: Media samples from each treatment were analyzed twice a month by *in vitro* culturing for existing pathogenic microbes in soil.

Table 1: Media treatments and their levels tested for management of the disease

Treatment	Levels	Levels	Description
M_1	M_1I_1	M_1I_2	50 % Carbendezim (0.7 g L^{-1}), 400 mL per plant drenched in three weeks gap
M_2	M_2I_1	M_2I_2	Normal media without any treatment (Control)
M_3	M_3I_1	M_3I_2	Metham Sodium (20 g m^{-2}), kept for two weeks moisten
M_4	M_4I_1	M_4I_2	Plastic pots (12×8inch) instead of poly bags
M_5	M_5I_1	M_5I_2	Thiram and Carbendezim drenched alternatively at three weeks intervals
M_6	M_6I_1	M_6I_2	80% Thiram (1.4 g L^{-1}), 400 mL per plant drenched in three week intervals
M_7	M_7I_1	M_7I_2	Steamed for three hour

I_1: Fully irrigated, 400 mL plant day, I_2: Half irrigated, 200 mL plant day, Trt. B Treatments, M B Media treatments

Data recording: The number of plants wilted under each treatment (plot) and the average wet and dry temperatures at the green house tunnel were recorded by a wet and dry bulb thermometer.

Statistical analysis: The data generated from the experiment were statistically analyzed using MINITAB version 15 packages.

RESULTS AND DISCUSSION

Nematode identification: When the suspension was observed under stereo microscope there were no pathogenic nematodes detected. It reveals that nematodes were not associated with the wilting.

Pathogen identification

Isolation from soil and water

Isolation: White fungal colonies were visible in cultures isolated from infected soil media. But it was not present in healthy soil media and irrigated water samples (Table 2). Under microscope, hyaline and sickle shaped conidia were observed. Macroconidia of *Fusarium* are sickle shaped and it is a prominent soil born pathogen. This reveals the association of *Fusarium* spp. in wilting. Also reproductive structures of *Phytophthora* spp. and *Pythium* spp. were detected.

Pathogen identification from plant parts

Ooze test: Water was clear and not termed. It confirms that bacteria were not associated in wilting. Therefore, wilting was not due to bacterial wilt.

Identification of pathogens in root: Fungus with coenocytic hypae was observed in slides. Reproductive structures of Oogonium and anthridium were detected that indicated special characters for *Pythium* spp. identification. In addition to that oval shaped *Phytophthora* sporangia were clearly observed. Rotted roots were highly infected with *Pythium* and *Phytophthora* spp. compared to healthy roots (Table 2).

Culturing in PDA media: Colonies in white to pink colour were observed in culture plates and under stereo microscope, hyaline and sickle shaped conidia were detected that indicated characters of *Fusarium* spp. They were the most detected among above ground plant part cultures (Table 2). *Fusarium* spp., *Pythium* spp. and *Phytophthora* spp. could be seen significantly in infected media. While *Fusarium* spp. has shown a noticeable existence in diseased stem parts, *Pythium* spp. and

Table 2: Presence of prominent fungal species in soil media and plant parts

	Pathogen			
Sample	Fu.	Py.	Phy.	Rhy.
Diseased stem	Yes	-	-	-
Healthy stem	-	-	-	-
Rotted roots	-	Yes	Yes	-
Healthy roots	-	-	-	-
Infected media	Yes	Yes	Yes	-
Healthy media	-	-	-	-
Irrigated water	-	-	-	-

Fu: *Fusarium* spp., Py: *Pythium* spp., Phy: *Phytophthora* spp., Rhy: *Rhizoctoniya solani*, Yes: Significant

Phytophthora spp. are significant in infected root parts, but none of the above species were detected in water used for irrigation (Table 2). Therefore, it is clear that source of infection of the disease pathogen was plant media.

Rhizosphere is the zone rich with plant exudates and moisture. It is the playground and infection court where the pathogen establishes a parasitic relationship with the plant (Raaijmakers *et al.*, 2009). *Pythium* and *Phytophthora* are oomycetes that are not true fungi. As to the findings of Kelaniyangoda *et al.* (2003) root rot in *Codiaeum* spp. had resulted with combination of both *Pythium* sp. and *Fusarium* sp. Therefore, infection by *Fusarium* sp. may be supportive of *Pythium* sp.

Pythium and *Phytophthora* produce swimming spores (zoospores) that chemotactically grow towards plant. Zoospores can attach to root surface, penetrate and infect the epidermal cells of the root tips (Raaijmakers *et al.*, 2009). After the roots have been killed (rot) pathogenic fungi can easily penetrate in to root tissues. A specialized group of pathogens (e.g., *Fusarium oxysporum*) that cause wilt diseases can penetrate through the endodermis into the vascular tissue and move up the xylem to above ground parts of the plant, impeding the flow of water (Beckman, 1987). This causes killing of vascular tissues, hindering water and food supply to the plant and gradually withering the plant to death.

Disease management: Wilting was significantly shown in main treatment factors M_1, M_2, M_4, M_5 and M_6 except M_3 and M_7. Although, interaction with half irrigated showed lower wilted percentage over interaction with full irrigated, results were not significant (Table 3).

Sometimes poor drainage in poly bags can remain high moisture levels in media. That results in pathogen development at base level of bags and gradually getting infested in to rhizosphere. Chemical treatment Carbendezim (M_1) had shown high wilted percentage, Thiram (M_6) low wilted percentage compared to control (M_2). But metham (M_3) had significantly reduced wilting. Similarly nonchemical method steaming (M_7) had shown significant reduction in wilting. But plastic potted (M_4) results were not significant from control (Table 3).

Somehow wilt, was progressed rapidly except in Metham (M_3) and steam (M_7) treated, but between days 46 and 64 overlap of M_7 over M_3 could be seen (Fig. 1). Relative humidity showed a sudden increase between days 41 and 46 and a gradual decrease after day 46 (Fig. 1). The increase in wilting is associated with relative humidity in the tunnel.

Table 3: Percentage of plants wilted in each treatment

	Wilting percentage			
Treatment	I_1	A. S.	I_2	A. S.
M_1 (Carbendazim)	44^{a}	41.55^{a}	39^{a}	38.65^{a}
M_2 (Control)	40^{ab}	39.23^{ab}	32^{ab}	39.23^{ab}
M_3 (Metham)	4^{c}	11.54^{c}	8^{c}	16.43^{c}
M_4 (Plastic pot)	31^{abd}	33.83^{abd}	29^{abd}	32.58^{abd}
M_5 (Thiram and Car.)	41^{abe}	39.82^{abe}	34^{abe}	35.67^{abe}
M_6 (Thiram)	31^{abde}	33.83^{abde}	30^{abde}	33.21^{abde}
M_7 (Steam)	9^{c}	17.46^{c}	7^{c}	15.34^{c}

Percentages followed by the same letter are not significantly different at 0.05 levels. I_1: Fully irrigated, 400 mL plant/day, I_2: Half irrigated, 200 mL plant/day, A.S: Arc sine percentage transformation

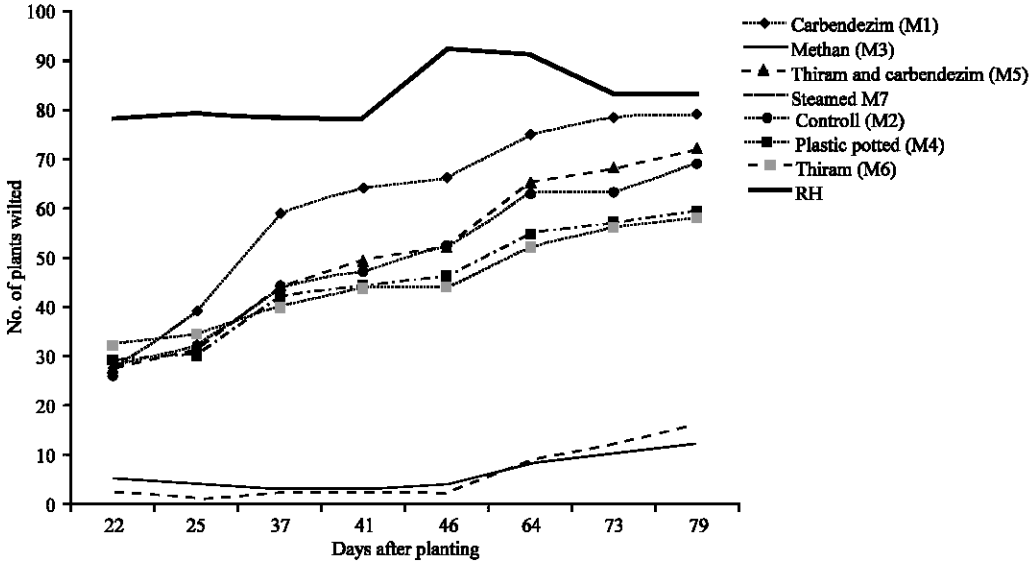

Fig. 1: Treatment effects on wilting and change in relative humidity

Table 4: Presence of prominent fungi in treated media

	Fungi	
Treatment	Fu.	Phy.
M_1 (Carbendazim)	Yes	Yes
M_2 (Control)	Yes	Yes
M_3 (Mthan)	Yes	Yes
M_4 (Plastic pot)	Yes	Yes
M_5 (Thiram and Carbendazim)	Yes	Yes
M_6 (Thiram)	Yes	Yes
M_7 (Steam)	-	-

Fu: *Fusarium* spp., Phy: *Phytophthora* spp., Yes: Significant

Higher moisture levels are favorable for pathogen propagules to disseminate. As the poly bags were placed touching the ground, there is a higher possibility of pathogen contamination in to healthy plants through ground in high moist conditions.

In the experiment, Thiram and Carbendazim were drenched in to soil and they percolated through coir media slowly where it did not reach each and every soil particle. In addition chemicals soon disappear from root zone due to absorption through roots. The chemicals actions are reduced at the deep root zones. Then pathogen can easily reach the root system (Silva and Singh, 1974).

Under favorable temperature and moisture levels pathogens get active and attack tissues. By controlling environmental factors growth of pathogens can be controlled. Pots facilitate good drainage and reduce moisture content. But temperature increase in plastic pots may have resulted pathogen multiplication in media.

Pathogen analysis: Soil fumigants and heat generated by steam kill all kinds of microorganism existing in media. Though appeared that Metham (M_3) was used as a fumigant in the experiment it did not sterilize soil particles completely. However, sterilizing was successfully achieved by steaming (Table 4). According to Table 3 Steam treatment had failed to show lesser wilting

percentage than Metham due to quick growth of pathogens in a lesser competitive microorganism condition in steam media resulted by cross contamination (Roger and Yepsen, 1976). Therefore, preventing cross contaminations can boost positive results. Further studies on *Trichoderma* spp. and use of antioxidents in wilt control should be carried out.

CONCLUSIONS

It appears that wilting of bell pepper plants are due to a collaborative association of *Pythium* spp., *Phytophthora* spp. and *Fusarium* spp. Primary infection caused by oomycetes (*Pythium* spp. and *Phytophthora* spp.) is not visible to naked eyes. But secondary infection caused by fungi (*Fusarium* spp.) is visible and result in wilting. Dark brown water soaked marks developing upwards from stem crown is the symptom of *Fusarium* infection. Media sterilizing by fumigants (Metham Sodium) or by steaming was successful in controlling the infection. Complete control of wilting of bell pepper was difficult to achieve in this experiment. However, the results of the study suggest that wilt suppression could be achieved by placing the steam treated plants disconnecting the contact with ground.

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