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Effect of Phytobiocides in Controlling Soft Rot of Tomato

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ABSTRACT:
The effect of various phytobicides such as Oleander, Chili, Mint, Garlic, Turmeric, and Neem on the control of soft rot of tomato was made both in laboratory and screen house conditions. Significant ($P \leq 0.05$) differences were found among different phytobiocides which were used to produce zone of inhibition (in mm) of *Erwinia carotovora carotovora* (on culture medium LB). Maximum zone of bacterial growth inhibition was achieved by turmeric (9.33 mm) followed by neem (7.33mm) and garlic (6.33mm). The screen house studies indicated that turmeric treated plants gave maximum height (61.67 cm), fresh shoot weight (35.68 g), fresh root weight (9.88 g), dry shoot weight (8.00 g), and dry root weight (3.25 g) followed by Neem and Garlic. These phytobiocides especially turmeric could serve as novel antibacterial agents.
Key words: Phytobiocides, *Erwinia*, Turmeric, Zone of inhibition

INTRODUCTION

Tomato (*Lycopersicum esculentum* Mill) is a red edible fruit having many varieties which are widely grown, often in greenhouses in cooler climates and open fields. It is consumed in diverse ways, including raw, as an ingredient in many dishes and sauces, in drinks and as a vegetable for culinary purposes. Tomatoes are attacked by many kinds of pathogens such as fungi, nematodes, bacteria, viruses and viroids. Among bacterial diseases, bacterial soft rot devastates many significant crops particularly tomatoes and cause a huge decrease in yield. Bacterial soft rots cause a greater loss of produce than any other bacterial disease known. Yield losses may reach upto 100% due to insufficient conditions in a storage facility (Arsenijevic and Obradovic 1996). In turn this impacts customers with reduced quantities of produce for sale, a reduction in quality, and an increase in expense. The soft rottening bacteria of genus *Erwinia* are very important, Among the *Erwinia* species *Erwinia carotovora* subsp *carotovora* (Ecc) is of economic importance because of its ability to cause severe soft rots on tomatoes (Permbelon and Kelman 1980).

Ecc, a rod shaped bacterium, was named after the crop of Carrots from which it was first isolated. The bacterium infects a variety of vegetables and plants including carrots, potatoes, cucumbers, onions, tomatoes, lettuce and ornamental plants like Iris (Wood, 1998). Whereas, *E. carotovora subsp. atrosepticum*'s pathogencity is restricted to potatoes in temperate regions, *E. carotovora subsp carotovora* infects a much broader host of plants, including potatoes, in warmer climates (Bell, 2004). Rottening disease of tomato, caused by *Erwinia carotovora* subsp *carotovora* is a serious problem of tomato in the world. The pathogen causes wilting of the whole tomato plants, water-soaking areas on stem, browning of vascular tissue, hollowing of pith, and soft rotting stem and fruits. The symptom development starts from root or crown region of seedlings in greenhouses.

This research work was conducted to check the effect of different Phytobiocides on the control of soft rot bacterial disease.

MATERIALS AND METHODS

a) *In vitro* effect of phytobiocides and its optimum concentration selection using bacterial zone of inhibition and disc diffusion method

Aqueous extract of easily available plants such as Garlic, Neem, Mint, Turmeric, Oleander, were screened *in vitro*. Dried plants tissues (50 g liter^{-1}) were grounded soaked for 24 hrs and then filtered through whatman (10 mm diameter) filter paper. Paper discs (6 mm) were soaked in each plant extract for 24 hrs and then dried. Bacterial culture lawn was prepared by spreading bacterial culture (100 µl) on growth medium with the help of spreader. The discs were placed in the middle of the bacterial lawn, and incubated at 27°C for 48 to 72 hours. After incubation, the data was taken as inhibition zones in mm around the discs. The effective biocide and concentration were selected for further experiment.

b) *In vivo* effect of phytobiocides on management of bacterial soft rot

In order to check out efficiency of various phytobiocides in controlling tomato soft rot under screen house conditions, a detailed experiment in Completely Randomized Design (CRD) with two factors and three replications was conducted in screen house using original (50 gram of phytobiocide per liter of distilled water) and 12 fold (by taking 11ml of sterile distilled water+1ml of original solution) dilution of the original concentration. Tomato nursery was raised in sterilized soil (sand: clay; 1:2). About five inch tall plants (two weeks old) were transplanted to pots (one plant per pot; 7 x 7 inch each) having sterilized soil. Ten days after transplantation, plants were inoculated with Ecc (chilies isolate) by adding 200 ml/pot of freshly prepared cloudy bacterial suspension (about 10^9cfu/ml). Twenty four hours after inoculation, each plant was treated with 200 ml of original and 12 fold solution of phytobiocides as a soil drench. Plants drenched with 200 ml of 0.85% saline solution served as negative control. Data was recorded and the results were statistically analyzed using Latin Square Design. The following parameters were assessed; plant height (cm), fresh shoot and root weight (g), dry shoot weight and root weight (g).

Disease rating scale used for diseased (soft rot of tomato) plants

Scale	Description
0	No soft rot symptoms
1	Less than 50% of the plant had symptoms
2	More than 50% of the plant had symptoms
3	Plants completely dead

RESULTS AND DISCUSSION

a) Laboratory Studies

Data presented in the Table-1 indicated significant ($P \leq 0.05$) differences among different phytobiocides which were used to produce zone of inhibition (in mm) of *Ecc* (on culture medium LB). Maximum zone of inhibition (minimum bacterial growth) was achieved by turmeric (9.333 mm) followed by neem (7.333 mm) and garlic (6.333 mm), while mint was the least effective among all the phytobiocides (0.4467 mm inhibition zone) followed by oleander which was the 2nd least effective phytobiocide. Significant difference ($P \leq 0.05$) was also found among different concentration, used to reduce the bacterial growth (inhibition zone). Maximum inhibition zone was achieved by using original concentration (undiluted) followed by 12 fold dilution. While the 3rd concentrations i.e. 16 fold dilution of the phytobiocides was not as much as effective as original and 12 fold in inhibiting bacterial growth. The interaction between phytobiocides and concentration was also significant ($P < 0.05$). The zone of inhibition (13.33mm) caused by turmeric was different from that of T_6 (chilies) i.e. 5.66mm when original concentration of phytobiocide was used for inhibition of bacterial growth. Similarly, 16 fold dilution of the original garlic concentration produced 4mm zone of inhibition, whereas the same dilution of neem gave 6 mm zone of inhibition. Like-wise, the difference between some phytobiocides was again significant ($P \leq 0.05$) when 12 fold dilutions were used. A well-known fungicide Copper oxychloride (COC) was used for comparison; however, the inhibition zone (5.72 mm) was smaller than that of turmeric and neem.

Table 1: Effect of different concentrations of phytobiocides on bacterial growth (as measured by zone of inhibition in mm)

Concentrations	Treatments								
	Mint	Oleander	Neem	Turmeric	Garlic	Chilies	COC*	Control	Mean
Original 50g/liter	1.3hij	5 efg	8 bcd	13.3 a	9.7 b	5.7 def	9.2 bc	0.00 j	6.5 a
12fold	0.007 j	2.7 ghij	8 bcd	8 bcd	5.3 defg	3.3 fghi	6.6 cde	6.00 j	4.2 b
16fold	0.00 j	1.0 ij	6 def	6.7 cde	4 efgh	1.7 hij	1.3 hij	0.00 j	2.6 c
Mean	0.5 d	2.9 c	7.3 b	9.3 a	6.3 b	3.6 c	5.7 b	0.00 d	

LSD ($P<0.05$) for phytobiocides = 1.655; *COC = Copper oxychloride

Means followed by same letter (s) do not differ significantly ($P < 0.05$) from each other

b) Screen House studies

Control of plant disease is more successful and economical when integrated disease management (IDM) is followed. Phytobiocide management is one of the components of IDM, although it is not extensively used yet it is the safest and easily available approach (Yasunka *et al.,* 2005). Complete control of every disease is very difficult and bacterial diseases are more difficult than controlling fungal and nematodal diseases. Many such diseases can be controlled with Cupper-based chemicals and antibiotics. However, chemical control causes environmental hazards, whereas antibiotics are expensive and lead to emergence of resistance in bacteria (Yasunka *et al.,* 2005).

A detailed experiment was carried out in order to test the effect of different phytobiocides in controlling soft rot of tomato under screen house conditions. Results indicated that turmeric treated plant showed highest disease resistance over control and showed resistance to soft rot (at both concentration) followed by neem and Garlic (Table 2).

Among all phytobiocides, turmeric showed the best results i.e. plants treated with turmeric gave maximum

height 61.67 cm, followed by neem (57.93cm) and garlic (50.58cm). Chilies treated plants were only 44.88cm tall. In case of fresh shoot weight (Table 2) again turmeric showed the best results by giving 35.68 g shoot weight as compared with neem treated plants giving 30.33 g shoot weight. Significant difference ($P<0.05$) was found among phytobiocides using plants fresh root weight as a parameter. In this case too turmeric treated plants gave best results. As obvious from Table 2, turmeric gave the best result i.e. plants treated with turmeric had maximum dry shoot weight (8.607g). Neem ranked as the second best phytobiocide. Neem treated plants had 7.72g dried shoot weight. The results were also significant ($P<0.05$) in case of dry root weight. Neem and garlic ranked second and third while turmeric remained on top by giving maximum root weight 3.55g In case of dry root weight the interaction among concentration is significant ($P<0.05$). When 12 fold dilutions of turmeric and chilies were used 3.617g, and 2.533g weights of roots were recorded. Similarly when original concentration (50g/liter) of phytobiocides was used the results were different in case of neem and garlic. (Table 2)
Laboratory studies indicated that a number of phytobiocides were effective in controlling soft rot of tomato. Among these phytobiocides, turmeric caused maximum zone of inhibition followed by neem, garlic and chilies. These results were in line with those of **Apisariyakul** (1995) who reported that turmeric could be used as anti-oxidant, antimicrobial, anti-protozoal and anti-allergic. It was found that turmeric treated plants were of maximum height and had the maximum root and shoot weight followed by neem treated plants. Results of neem were in line with those of Bdliya (2006) who showed that aqueous extract of neem leaf and seed aqueous extract significantly reduced the incidence and severity of tuber soft rot. neem has a blend of 3 to 4 related compounds along with over 20 lesser ones, which are equally active. The general class of these compounds is triterpenes and within this category, the most effective are the limonoids, which are abundant in neem. Of these limonoids, azadirachitin has been found to be the main anti-microbial, being up to 90% effective in most instances. It repels and disrupts the life cycles, however it does not kill immediately, but is nonetheless one of the most effective growth and feeding deterrents ever examined. Nimbin and nimbidin, also found in neem, have anti-viral properties and these have been shown to be effective in inhibiting fungal growth as well (Koul *et al.,* 1989).
Garlic extract can be used against a range of microorganisms as Allicin in garlic is an antimicrobial substance. (Alan *et al*., 2007). Selection of chilies with other control measure is also a good choice for control of soft rot, as chilies give comparatively good result both *in vivo* and *in-vitro*. Chilies are also easily available and economical as reported by Claudio *et al.,* (2003).

Table 2: Effect of phytobiocides on the control of soft rot of tomato using plant height, fresh and dry shoot weight, fresh and dry root weight as a parameter.

Treatments	Plant height (cm)	Fresh shoot weight (g)	Fresh root weight (g)	Dry shoot weight (g)	Dry root weight (g)
T1= Turmeric	61.7 a	35 .7 a	9.9 a	8.0 a	3.1 a
T2= Neem	57.9 b	30.7 b	8.5 b	7.7 b	2.4 b
T3= Chilies	44.9 d	27.1 c	6.5 d	6.7 c	1.9 e
T4= Garlic	50.6 c	28.7 bc	7.2 c	7.0 c	2.3 c
T5= plant inoculated with bacteria only	34.5 f	19.3 e	5.1 f	4.3 e	1.8 e
T6= No bacteria no phytobocides	41.8 e	22.2 d	6.1 e	6.1 d	2.1 cd
LSD	2.2	2.2	0.4	0.3	0.3

Means followed by same letter (s) do not differ significantly ($P < 0.05$) from each other

Conclusion
Further research is needed to investigate more phytobiocides for the control of different diseases to safe guard our environment, and to provide healthy food to the growing population of the world.

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16fold	0.00 j	1.0 ij	6 def	6.7 cde	4 efgh	1.7 hij	1.3 hij	0.00 j	2.6 c
Mean	0.5 d	2.9 c	7.3 b	9.3 a	6.3 b	3.6 c	5.7 b	0.00 d	

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