The Effect of *Trichoderma viride* and *Pseudomonas fluorescence* on Tobacco (*Nicotiana tabacum* L) Wilt Disease.

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ABSTRACT

Wilt is an extremely damaging disease of Tobacco (*Nicotiana tabacum* L) caused by bacteria and fungi. A considerable loss in both the yield and quality of the produce is observed owing to this problem. Existing chemical control methods do not give appropriate results and cause serious hazards to the environment. Therefore, this experiment was carried out to develop an effective biocontrol mechanism, which is more economical and environmentally friendly. The effect of two Biocontrol Agents (BCAs), *Trichoderma viride and Pseudomonas fluorescence* were tested in deep and shallow ploughing levels under local field conditions. Three combinations of BCA and organic fertilizers (BCA + Cow Dung, BCA + Compost and BCA alone) were applied along with another two soil drench applications of the BCAs with the presence of natural incidence of the disease. According to the results, no significant difference was observed between two BCAs. The results indicated that, deep ploughing reduced the disease considerably. Application of the BCAs with compost gave favorable results. Further, combine effect of deep ploughing and application of *Trichoderma viride* with compost followed by a soil drench application of the *Trichoderma* gave best results.

KEYWORDS: Biocontrol Agents, Biocontrol Mechanism, *Nicotiana tabacum, Pseudomonas fluorescence* Soil Drench Application, *Trichoderma viride,* Wilt.

INTRODUCTION

Tobacco (*Nicotiana tabacum.*L) is an important cash crop which belongs to family Solanacea and it is unique with its characteristic commercial component, "Nicotine", contained in its leaves and stems. Tobacco crop is grown for its leaves which are processed and consumed around the world as cigarettes, cigars, chewing or smoking tobaccos and snuff (Singh, 1983). The value of industrial production of tobacco products in Sri Lanka in 2004 was Rs. 52912 million (Anon, 2004).

Year after year wilt continues to be one of the most destructive diseases of tobacco. The disease may occur at any stage from newly planted seedlings to harvesting stage of the crop (Bilgrami and Dube, 1983). As a result of this, a considerable loss is observed in every season. Some times the disease leads to complete destruction of the crop. Although growers have adopted better management practices for disease control, losses due to the disease remain high. The disease reduces both the yield of the leaf and quality of the produce (Dicson, 1992). The most conspicuous symptom of wilt disease is yellowing and drying of leaves and is more pronounced on one side of the plant. Leaves on the affected side are usually stunted with unequal growth in size. The top of the affected plant eventually wilts and gets drawn towards affected side (Bilgrami and Dube, 1983).

Causal agent of wilt disease may be bacteria, fungi and viruses. However, bacteria and fungi have been the most aggressive (Bilgrami and Dube, 1983). Among the bacterial wilt pathogens of tobacco, *Pseudomonas solanacearum* and *Pseudomonas putida* cause higher damages, while *Fusarium oxysporum* is the most prominent fungal wilt pathogen in local conditions (CABI, 2003).

Management of this disease by chemical means is not satisfactory and not ecofriendly. Abused employment of the chemical compounds has favored the development of pathogens resistant to fungicides. Further, fungicides of broad spectrum produce undesirable consequences on non-target organisms (Tjamos et al., 1992). Alternative approaches like biological control of the disease are more appropriate and effective compared to the existing chemical controls which involve high cost and higher degree of environmental pollution. There has been a progress in selecting strains of fungi and bacteria that have given good control of some diseases when applied to soil, seed or plants (Cook and Baker, 1983).

The genus *Trichoderma* comprises a great number of fungal strains that act as biological control agents of *Fusarium, Pythium, Rhizoctonia and Sclerotium* (Benitez et al., 2004). Among those *Trichoderma viride* is used to arrest diseases caused by *Fusarium spp. Trichoderma* is antagonistic to these fungi through production of siderophores and antibiotics (Chet, 1987). Instances of biological control have been reported for species of all five major genera of phytopathogenic bacteria including *Pseudomonas spp. Pseudomonas fluorescence,* which is a naturally and widely occurring bacterium, can be used to suppress the bacterial and *fungal* phytopathogens including *Ralstonia solanacearum* and *Pseudomonas putida.* (Cook and Baker, 1996).

Identifying the potential agents of biocontrol mechanism and their survival under field conditions can provide valuable information to develop a

biocontrol mechanism against tobacco wilt disease, which is more effective, economical and environmentally friendly.

MATERIALS AND METHODS

This experiment was conducted at the Ceylon Tobacco Company (CTC) from April to September 2006. The field experiment related to this study was carried out at Mahawali system "C" area (Dehiattakandiya) in selected paddy fields where the tobacco wilt disease had been observed in previous five cropping seasons.

The experiment was arranged in factorial design with three factors. The factors were biocontrol agent, ploughing depth and the type of organic fertilizer. *Trichoderma viride* and *Pseudomonas fluorescence* were the two levels of the biocontrol agent, two levels of the ploughing depth were deep ploughing and shallow ploughing and the three levels of the organic fertilizer were cow dung, compost and the absence of both fertilizers. Altogether six treatments were applied with each treatment having three replicates. Initially 70 plants were raised per plot with the spacing of 2 ft × 3 ft.

Two Biocontrol Agents (BCAs), namely, *Trichoderma viride,* which is an antagonistic fungus and *Pseudomonas fluorescence,* which is a soil colonizing bacteria were tested under field conditions. The field was prepared into two ploughing depths; deep ploughing (8"-10") and shallow ploughing (6", *normal farmer practice*) by using a disk plough. Other agronomic practices like bed preparation, fertilization and pest control were followed as recommended by the CTC.

The field was separated by bunds into main four blocks that received each BCA under both ploughing levels. In each isolated block nine plots were raised and different treatments containing the same BCAs were applied in these plots by replicating randomly. The main blocks were separately irrigated throughout the experiment to minimize the contamination of two BCAs by each other.

The original form of the BCAs used in the first application was talc based Wettabal Powder (WP) (2×10^6cfu/g, talc-98.5% w/w and Carboxy Methyl Cellulose-0.5% w/w). They were incorporated to the planting holes in three methods by mixing with two types of organic fertilizers and without mixing any fertilizer (WP mixed with cow dung, WP mixed with compost and the WP alone) before planting the seedlings. From each BCA a weight of 5 kg of the WP was mixed with either 200 kg of cow dung or 200 kg compost. Water was added to obtain 40% moisture and kept for two days under shade. This amount was used for applying to the planting holes covering 1ha of land. Three types of mixtures were used to apply the two BCAs as six treatments in each ploughing level.

Tobacco seedlings of variety K326 were planted in planting holes (one per hill), by irrigating the field up to field capacity, one week after the first treatment application. Thereafter, 50%-60% soil moisture level was maintained by flooding the field once in 3-4 days.

Treatments

T_1. *Trichoderma* + Cow dung
T_2. *Trichoderma* + Compost
T_3. *Trichoderma*
T_4. *Pseudomonas* + Cow dung
T_5. *Pseudomonas* + Compost
T_6. *Pseudomonas*

The second application of the BCAs was done as a Soil Drench application with the incidence of the disease and it was applied two times as once in two weeks. This was applied at the rate of 30 ml of the pure solution (2×10^6cfu/ml) for 1000 plants by diluting with water. These were applied using a sprayer by loosening the nozzle. The diluted solution was applied (30 ml/plant) to the base of the plant as to drain through the root system.

Natural infection by the pathogen was the method of inoculation. Disease incidence was measured and number of wilted plants per each plot was recorded weekly. Data were analyzed by Minitab statistical package.

RESULTS AND DISCUSSION

No significant difference was observed in the number of wilted plants under the application of two BCAs.

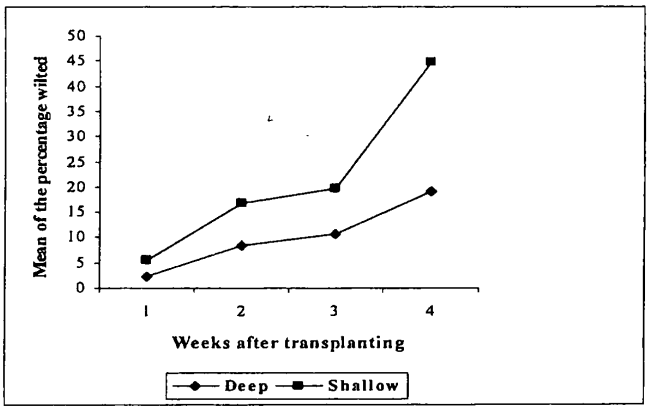

Figure 1. Disease development in each ploughing levels:

A significant variation was observed in number of wilted plants in different ploughing levels. The least number of wilted plants were observed in deep ploughing compared to the shallow ploughing (Figure 1). Deep ploughing may reduces the disease by exposing the dormant structures of the pathogen which are in deep soil levels to the direct sun and by washing off from run off water.

There was a significant difference in number of wilted plants among three methods of application for both the BCAs, but this variation was shown only in the ploughing level one.

Table 1 -Means of the wilted plants percentage under different methods of application in different ploughing levels:

Metod of application	Ploughing level	
	Deep	Shallow
1	16.19[b]	24.17[c]
2	5.71[a]	19.11[c]
3	8.45[a]	22.02[c]

Treatment means in a column having common letters are not significantly different at p=0.05

Out of three methods of application the second and third application methods within the deep ploughing level showed a lesser number of wilted plants compared to the first method of application (Table 1). The application of cow dung, compost improves soil texture and increase the activity of both biocontrol agents. The second method of application showed the least number of wilted plants with both BCAs under deep ploughing level.

Lowest number of wilted plants was obtained in the interaction of first ploughing level, first BCA and second method of application, while the highest number of wilted plants was obtained in the interaction of second ploughing level, first BCA and the first method of application (Figure 2).

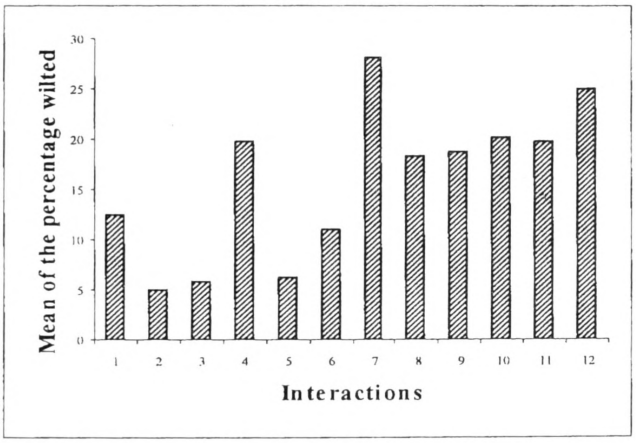

Figure 2 - Mean percentage wilted plants in all three way Interactions:

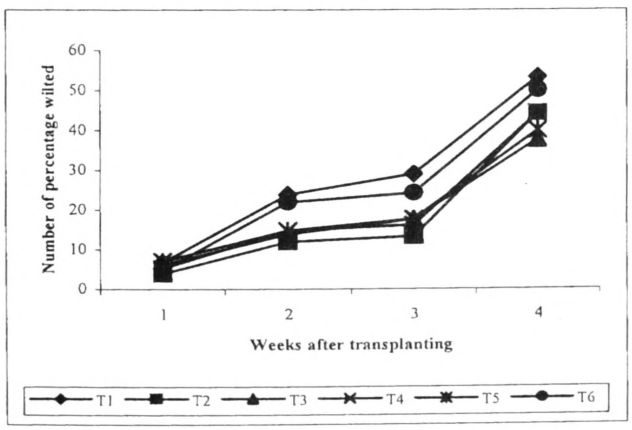

Figure 3 - Disease development with different treatments in deep ploughing:

In general, the disease increased with the time, but a slight decline of the increase rate of disease incidence could be seen at third week with the soil drench application of BCAs (Figure 3 and 4). This may be due to the increased strength of the BCAs in the soil as a result of the second application.

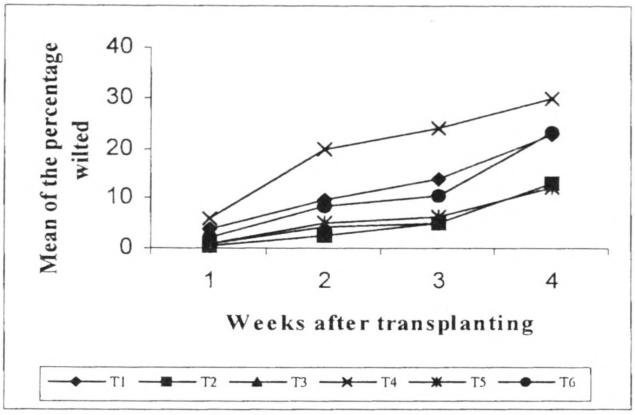

Figure 4 - Disease development with different treatments in shallow ploughing:

Main practical difficulty in conducting this experiment was the contamination of two biocontrol agents by each other. As a result of this, it was difficult to have a control without any BCAs. Due to this comparisons were made with other interactions instead of comparing with a control. To minimize the contamination big bunds (2"×3") were established to separate four blocks and only one BCA were applied to each block. Blocks were separately irrigated to avoid the contamination of BCAs by each other with irrigation water.

CONCLUSIONS

The experiment reveals that the Tobacco wilt incidence can be manage by the deep ploughing. The application of two biocontrol agents tested are not significantly different from each other. Although the application of compost with *Trichoderma* or *Pseudomonas* not eliminates the disease, it can be managed with a very low incidence level that is not causing a significant economic injury. It can be concluded that, application of *Trichoderma* or *Pseudomonas* with compost under deep ploughing can effectively suppress the disease. The soil drench application of *Trichoderma* and *Pseudomonas* effectively control the disease, but frequent application (once a fortnight) is needed. Further studies should be carried out to test the impact of different strengths of the biocontrol agent.

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