IN VIVO STUDIES ON THE BIOLOGICAL AND CHEMICAL CONTROL OF GUAVA DECLINE CAUSED BY DIFFERENT SOIL BORNE PATHOGENS

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An experiment was conducted to study the effect of antagonist *Trichoderma harzianum* and different fungicides for the control of guava decline (*Psidium guajava*) *in-vivo* at the research area of Department of Plant Pathology University of Agriculture Faisalabad. Guava decline is a serious disease caused by different pathogens *Botryodiplodia theobromae, Fusarium oxysporum* f. sp. *Psidii, Phytophthora parasitica* and *Fusarium solani* f. sp. *psidii.* The disease can be controlled effectively through antagonists but effectiveness increases when fungicides are used as soil drenching alongwith antagonists. This paper reports *in vivo* evaluation of fungal antagonist *T. harzianum* and the fungicides Topsin-M, Alert plus and Reconil-M for the control of guava decline. Various experiments were conducted to analyze the effectiveness of antagonist and fungicides for the control of disease. It was found that in control, where no fungicide and antagonist was added in soil, it showed ample disease intensity i.e. 24-57% and 44.15% in sterilized and unsterlized soil respectively. Statistical analysis revealed that disease intensity was zero and 0.74% when treated with *T. harzianum* and Topsin-M as drenches in sterilized and unsterlized soil and showed maximum control of disease.

Keywords: Guava decline, *Psidium guajava*, *Trichoderma harzianum*, antagonism, biological control, chemical control

INTRODUCTION

Guava (*Psidium guajava* L.) is an arborescent shrub or small tree and is one of the popular fruit of the Punjab in Pakistan. It belongs to the family Myrtaceae and is one of the most gregarious of fruit trees. In Pakistan it is grown on an area of 61.6 thousand hectors with a total annual production of 549.5 thousand tones and yield per hectare is 8920Kg (Anonymous 2005). Guava is liked by fruit growers due to the high adoptability and higher return per unit area (Khan 1985). But its successful cultivation is hampered by a number of biotic and abiotic factors. Among biotic factors, decline is important and is a complex disease syndrome in Punjab.

Guava decline caused by different pathogens *Botryodiplodia theobromae*, *Fusarium oxysporum* f. sp. *Psidii, Phytophthora parasitica* and *Fusarium solani* f. sp. *psidii* is a serious disease and causes considerable losses. Among the pothgens, *Botryodiplodia theobromae* and *Fusarium oxysporum* f. sp. *psidii* are predominant pathogen which are mainly responsible for decline. The control of the disease by using only antagonist is effective, but its effectiveness increases when fungicides are used as soil drenching along with the antagonist. This paper reports *in vivo* evaluation of fungal antagonist *T. harzianum* and the fugicides Topsin M, Alert plus and Reconil-M for the control of guava deadline.

MATERIAL AND METHODS

Various experiments were conducted to select effective antagonist and chemicals for the control of guava decline. Having obtained the evidence of effective antagonistic activity by some microorganisms *in vitro* against *B. theobromae*, *F. oxysporum* f.sp. *psidii*, *P. parasitica* and *F. solani* f. sp. *Psidii.* The experiments were prepared in the earthen Pots (15 x 15cm size) containing sterilized and unsterlized soils separately.

Trichoderma harzianum was used as antagonistic organism and Topsin-M (70% WP), Alert plus (70% WP) and Reconil-M (70% WP) was used as fungicides for the control of the disease. Stem inoculation was done by *B. theobromae* separately. Seven days old culture of *F. oxysporum* f. sp. *psidii* was added at the rate of ½ petri dish per pot in the form of small agar blocks placed in the soil at 2.5cm, 5.00 cm and 7.5 cm depth. The pots were supplied water regularly and after seven days old cultures of the antagonist were added in the soil at the rate of one percent w/w. The pot without antagonistic culture were kept as control. The soil temperature was maintained at 30-32 ^{0}C.

RESULTS AND DISCUSSION

In the experiment, the range of disease intensity remained between 0.00 to 3.86% due to the effect of antagonistic organisms and fungicidal soil drenching on the control of guava decline in sterilized soil,

(Table1). In the treatment where *T. harzianum* was added in the soil, the disease intensity was 1.41 percent and in the treatment where Topsin-M was used as soil drenches along with *T. harzianum*, the disease intensity was 0.00 percent (Table 1).

In case of unsterilized soil the range of percent disease intensity was 0.74 to 5.19 (average being 2.75). In the treatment where *T. harzianum* was added directly in the soil, the disease intensity was 1.33% and where Topsin-M was applied as soil drenching in addition to *T. harzianum*, the disease intensity was reduced to 0.74% (Table 2).

When the data were subjected to the statistical analysis, it was found that the control where no fungicide and antagonist were added showed maximum disease intensity 24.57 and 44.15% in sterilized and unsterilized soil respectively (Table 1 and 2).

Statistical analysis revealed that the disease intensity was zero and 0.74% when treated with *T. harzianum* and Topsin-M as soil drenches in sterilized and unsterilized soil respectively and showed the maximum control of the disease. Similar results were obtained when following treatments were applied i.e. Alert Plus, Reconil-M, *Trichoderma harzianum &* Alert Plus and *Trichoderma harzianum* & Reconil-M showed 2.11, 3.18, 2.79 and 3.86% disease intensity respectively in sterilized soil (Table 1). These treatments when applied in unsterilized soil also exhibited similar results with disease intensity 4.37, 5.19, 2.31 and 3.35% respectively (Table 2).

The present study indicates that the control of soil borne plant disease is possible through the use of antagonistic microorganism as well as with the use of fungicides in the form of soil drenches. Investigations on the control of guava decline caused by *B.*

Table 1. Effect of antagonistic organism and fungicidal soil drenching on the control of guava decline in sterilized soil

Treatments	% disease intensity
Control (Treated) SS + B.T (Stem inoculation) + F.O (Soil inoculation)	24.57a ± 0.44
SS + B.T (Stem inoculation) + F.O (Soil inoculation) +Topsin-M	1.76ef ± 0.88
SS + B.T (Stem inoculation) + F.O (Soil inoculation) +Alert plus	2.11de ± 0.08
SS + B.T (Stem inoculation) + F.O (Soil inoculation)+ Reconil-M	3.18c ± 0.28
SS + B.T (Stem inoculation) + F.O (Soil inoculation) + *Trichoderma harzianum*	1.41f ± 0.74
SS + B.T (Stem inoculation) + F.O (Soil inoculation) + *Trichoderma harzianum* +Topsin-M	0.00g ± 0.00
SS + B.T (Stem inoculation) + F.O (Soil inoculation) + *Trichoderma harzianum* + Alert plus	2.79cd ± 1.41
SS + B.T (Stem inoculation) + F.O (Soil inoculation) + *Trichoderma harzianum* +Reconil-M	3.86b ± 0.17
LSD	0.677

SS = Sterilized soil BT =*Botryodiplodia theobromae* F.O. = *Fusarium oxysporum* f.sp. *Psidii*.

Table 2. Effect of antagonistic organism and fungicidal soil drenching on the control of guava decline in unsterilized soil

Sr. No.	Treatments	% disease intensity
1	Control (Treated) US+B.T (Stem inoculation)+F.O (Soil inoculation)	44.15a ± 15.65
2	US + B.T (Stem inoculation) + F.O (Soil inoculation) +Topsin-M	1.97de ± 1.11
3	US + B.T (Stem inoculation) + F.O (Soil inoculation) +Alert plus	4.37b ± 0.70
4	US + B.T (Stem inoculation) + F.O (Soil inoculation)+ Reconil-M	5.19b ± 0.23
5	US + B.T (Stem inoculation) + F.O (Soil inoculation) + *Trichoderma harzianum*	1.33ef ± 0.70
6	US+B.T (Stem inoculation)+F.O (Soil inoculation)+*Trichoderma harzianum*+Topsin-M	0.74f ± 0.74
7	US+B.T (Stem inoculation)+F.O (Soil inoculation)+*Trichoderma harzianum*+Alert plus	2.31d ± 1.22
8	US+B.T (Stem inoculation)+F.O (Soil inoculation)+*Trichoderma harzianum*+Reconil-M	3.35c ± 0.84
	LSD	0.823

US =Unsterilized soil BT = *Botryodiplodia theobromae* F.O. = *Fusarium oxysporum* f.sp.*psidii.*

theobromae, *F. oxysporum* f. sp. *psidii*. *Phytophthora parasitica* and *F. solani* f. sp. *psidii* through the use of antagonistic organisms gave encouraging results. Regarding the mechanism of the biological control of the fungi, *Trichoderma harzianum* control mainly by being mycoparasitic and an aggressive competition with pathogens. Growth of mycelia of *Tricoderma* spp. alone coated around hyphae of the host fungi has been separated by many workers (Chet *et al*., 1981; Liu and Baker 1980; Weindling 1932).

It was observed that *T. harzianum* caused severe vacuolation, shrinkage and coagulation of the cytoplasm of the pathogen hyphae (Pandy and Upadhyay 2000). *T. harzianum* was found highly antagonistic organism against the soil borne fungi. *Trichodrema harzianum* was commercially available as microbial plant protection agent by the trade name "Trichopak". The active ingredient of *T. harzianum* was sesquiterpene trichoderma 1, 2, 3 which is antagonistic against Fusarium spp. *Rhizoctonia solani* and *Sclerotium rolfsii*. The hyphal coiling and production of inhabitrarty substance by different species of *Trichoderma* resulting in dieback and disintegration of Pythium spp. were also reported by Raju (1991).

Biological control of plant disease with antagonists is accomplished by destroying existing pathogen inoculum, excluding the pathogen from the host plant or suppressing or displacing the pathogen after infection has occurred (Cook and Baker 1983). *Trichoderma harzianum* is a parasite of other fungi and can rapidly colonize plant roots, thereby competing pathogens for nutrient and space. *Trichoderma harzianum* also promotes plant growth in the absence of pathogens.

Trichoderma harzianum is formulated as a granule that is incorporated into soil or mixed in the soil prior to planting (Mcspadden Gardener and Fravel 2002). Biological control of plant disease is gaining momentum in recent years. (Brent 1995 Sharma *et al*., 1998). Biological control by antagonistic organism was a potential non-chemical tool for crop protection against phytopathogens (Papavizas 1985). *Trichoderma harzianum* showed best antagonistic effect for the control of guava decline. Topsin-M, Alert plus and Reconil-M showed good results *in vivo*, alone and with *T. harzianum*, wherease *T. harzianum* alongwith Topsin-M showed best results for the control of guava decline.

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