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IN -VITRO EVALUATION OF DIFFERENT FUNGICIDES AND THE BIO-CONTROL AGENT *TRICHODERMA HARZIANUM* AGAINST *BOTRYODIPLODIA THEOBROMAE*, THE CAUSE OF GUAVA DECLINE

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ABSTRACT

Guava decline disease, caused by *Botryodiplodia theobromae* is becoming the countrywide threat in Pakistan and annually it brings severe economic losses to the guava production. The management of this disease is very essential. *In-vitro* evaluation of four different fungicides viz. Nativo, Antracol, Aliette and Topas, with different concentrations (30, 50, 100 ppm) and the bio-control agent *Trichoderma harzianum* (5mm mycelial disk) were tested against *B. theobromae*. The result revealed that the effectiveness of the fungicides increased with increased dosage rate. Nativo was proved to be the most effective fungicide; Antracol was moderately effective, whereas Aliette and Topas were found to be less effective in reducing the mycelial growth of *B. theobromae*. Similarly, *in-vitro* evaluation of the bio-control agent *Trichoderma harzianum* against *B. theobromae* was found to be very effective, resulting maximum inhibition of 40.34% in the colony growth of the test fungus within seven days. Thus, farther different fungicides and bio-control agents must be evaluated under *in-vitro* conditions and then applied in field conditions

Key words: Guava decline, *Botryodiplodia theobromae*, fungicides, *Trichoderma harzianum*.

INTRODUCTION

Guava (*Psidium guajava* L.) belongs to the family *Myrtaceae* is commonly known for its food and nutritional values throughout the world. The place of origin of guava extends from the Southern part of Mexico up to the Central part of America (Morton, 1987). It is as well called common man's fruit" and can be termed as "apple of the tropics." Pakistan is a country with miscellaneous agroecological conditions where a huge number of different fruits and vegetables are grown which as well as yield the best. Fruits and vegetables are considered the main sources of drinks (Shakeel *et al.,* 2013). Among all prominent fruits, guava also covers a vital place in the fruit industry of Pakistan, after citrus and mango it has the third position with respect to area while it has the fourth position after citrus, mango and bananas (Pervaiz *et al.*, 2008). It is grown on an area of 63 thousand hectares with a total annual production of 495 thousand tons in Pakistan (Anonymous, 2012) It has outstanding nutritive value, medicinal properties and taste and can be used effortlessly for processing into valuable products. The fruit is sweet and eaten raw or cooked. It makes good jam and is universally known for its jelly. The fruit of guava contains enormous number of vitamins i.e. A, B1 (Thiamin), B2 (Riboflavin) and C. 100 grams of fruit has approximately 260 mg of vitamin C which is 2 to 5 times more than the fresh orange as reported by Rahman *et al.*, (2003). This is a rich source of vitamin C as compared to ber, citrus and apple (Divya and Kumari, 2009). It produces fruit twice a year i.e summer and winter however the top-quality fruit is produced in the winter season (Bal and Dhaliwal, 2004). Guava is an enormously fruitful and to a great extent commercial fruit crop, but the successful cultivation of guava is becoming an alarming problem for the growers in the world as the tree is at risk to many diseases. There are approximately 177 pathogens that affect the guava tree, among them 167 are fungal, 3 are bacterial, 3 are algal, 3 are nematodes and one is epiphyte. Due to its perishable nature, a large number of pathogens have been reported on guava fruit which cause different types of rots of the fruits (Misra, 2004). Some important guava diseases include Guava decline, Wilt, Anthracnose, Botryodiplodia rot, Fruit rot, Phoma rot, Rhizopus rot, Collar rot, Pestalotia leaf spot, Cercospora leaf spot, Stem canker and Seedling blight (Khushk *et al*., 2009). Among these all diseases, guava decline is a complex disease syndrome in Pakistan. In the country, a large number of orcha-

rds have declined and become unproductive. It is becoming the countrywide threat in Pakistan and the yield has been decreased from 8920 kg/hac in 2003 and 2004 to 8223 kg/hac in 2008 and 2009 (Anonymous, 2010). The first symptoms of guava tree decline are leaf wilting, leaf yellowing, leaf shriveling and then become necrotic and later on drop down as a result whole defoliation of the tree occurs. There are several other symptoms associated with the affected trees such as chlorosis and a rapid reduction of leaf area and decrease in fruit production. On such trees, the fruit development stops and the fruit ultimately turns to be mummified. The growing tip turns dark brown. The black necrotic area extends backward causing the die-back (Gomes *et al*., 2012).

The disease scatters mostly via the infection of root and the movement of plant materials which are already infected by this disease. It was reported that the plants with the age of one or above five were more susceptible to this disease (Prasad *et al.,* 1952). Guava decline caused by different pathogens *Botryodiplodia theobromae, Fusarium oxysporum* f. sp. *psidii, Phytophthora parasitica* and *Fusarium solani* f. sp. *psidii* which bring considerable losses to the guava tree. Among the pathogens, *B. theobromae* and *F. oxysporum* f. sp. psidii are predominant pathogens which are mainly responsible for decline (Bokhari *et al.,* 2008). Keeping in view the losses caused by *Botryodiplodia theobromae,* studies were conducted to see the effect of some selective fungicides and a biological control agent on the mycelial colony growth of the fungus *in-vitro* conditions.

MATERIAL AND METHODS

The experiment was carried out during the year 2016 in Department of Plant Pathology, Sindh Agriculture University Tandojam.

Collection of samples: Samples were collected from the diseased trees, showing typical symptoms of decline from various guava orchards of Hyderabad, Sindh, to determine the association of pathogen that caused the decline disease. The samples which were having the bark at collar areas, twigs and the branches were collected. Healthy plants' leaves as well as diseased plants' leaves were also collected. They were kept in polythene bags and after proper labeling brought to the laboratory for further process.

Isolation, identification and purification of the pathogen: Fungi isolation was carried out by standard isolation procedure (Safdar *et al.,* 2010). The samples were washed thoroughly, and then cut into small pieces about 3 to 5mm along with some healthy portion and surface sterilized with 5% sodium hypochlorite. The samples were placed on PDA and then incubated at 28°C for 7 days. The observations for the fungal growth were monitored on daily basis. The fungi that developed colonies on the pieces were later purified and identified on the basis of the morphological characters that they produced (Khanzada *et al.,* 2006).

Culture of *Trichoderma harzianum*: Culture of *T. harzianum,* previously isolated from irrigated and non-irrigated rhizospheric soils of Hyderabad region were obtained from Plant Pathology Laboratory, Sindh Agriculture University Tandojam, maintained on Potato Dextrose Agar (PDA) at room temperature for further experiments.

***In -vitro* evaluation of fungicides:** Four different fungicides (Nativo, Antracol, Aliette and Topas) were tested for checking their efficacy against *Botryodiplodia theobromae*. The standard solution was prepared according to the active ingredients of the fungicides and was tested against the fungus under *in-vitro* condition. The experiment was conducted in Complete Randomized Design (CRD) with 4 treatments, and 3 replications were kept for each treatment (30, 50, 100 ppm). Petri dishes containing amended PDA medium were inoculated with 5 mm disk, taken with sterilized cork borer from the growing edge of 7 days old mycelial culture of *B. theobromae.* Petri dishes containing PDA medium without fungicides were used as control. All the plates were kept at 28°C in the incubator and the mycelial growth of the fungus was recorded in mm after 48 hours of inoculation till 7 days.

***In-vitro* evaluation of bio-control agent:** The most prominent bio-control agent *Trichoderma harzianum* was evaluated against *Botryodiplodia theobromae,* using dual culture method as described by Sivakumar *et al*., (2000). The 5 mm mycelial colony disc of test antagonist (*Trichoderma harzianum)* was taken from 7 days old culture, was paired against same sized mycelial disc of (*B. theobromae)* at opposite ends of Petri dishes and the plates were incubated at 28°C temperature to observe the colony growth inhibition percentage of the pathogen. The inhibition growth % of *B. theobromae* in the presence of *T. harzianum* was calculated over control. Radial growth reduction was calculated by the following formula (Kunz, 2007).

Inhibition of radial mycelial growth

$$\frac{A-B}{A} \times 100 = \%$$

Where,

A= Radial colony growth measurement of the pathogen in control.

B= Radial colony growth of the pathogen in presence of *T. harzianum*.

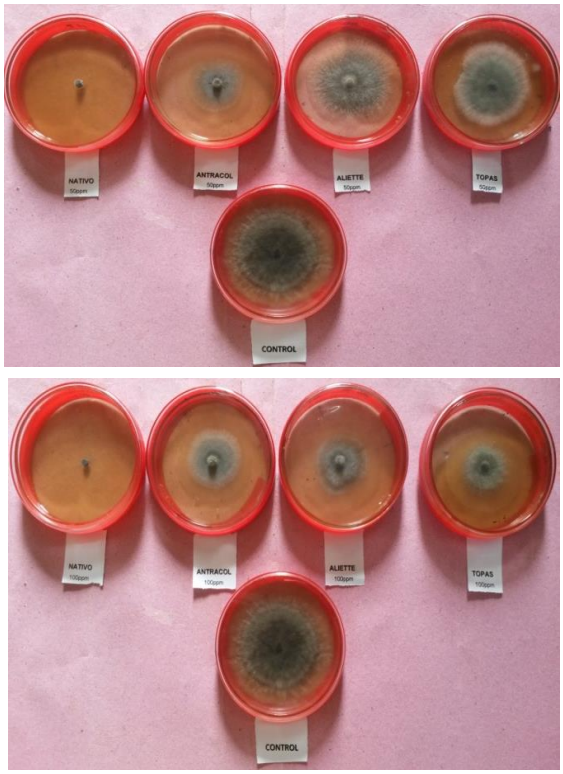
RESULTS AND DISCUSSIONS

Isolation and identification of the fungi causing guava decline: The inspection of infected guava tree parts showed the association of many fungi. Among all the isolated fungi, *Botryodiplodia theobromae* remained most frequent and pre-dominant fungus and was identified on the basis of its morphological characteristics and color of the colonies of fungi in accordance with the results reported by (Pitt and Hocking, 2009; Khanzada et al., 2006). The colony characteristics were also with accordance to the studies of Philips (2007), where he studied that *B. theobromae* colonies were often grayish sepia to mouse grey to black, fluffy with plentiful aerial mycelium. Matured cultures had black pigmentation. The results are also linked with the studies of God fried, (2012), who compared 25 isolates on PDA at 28°C, and all the isolates produced black pigments in culture after 48 hours of inoculation.

***In-vitro* evaluation of fungicides against *Botryodiplodia theobromae*:** The results concerning with the efficacy of fungicides showed that the colony growth of *Botryodiplodia theobromae* was reduced with increasing concentration of all the fungicides. Data was statistically analyzed and revealed that among all the fungicides, Nativo was proved to be the most effective fungicide in reducing the mycelial colony growth of the fungus showing 2.03 mm, 3.50 mm and 4.80 mm colony diameter at 100, 50 and 30ppm respectively. Antracol was moderately effective against the fungus showing 35.90 mm, 44.90 mm and 35.90 mm colony diameter at 100, 50 and 30ppm respectively, whereas, Aliette and Topas were less effective against the test fungus as compared to Nativo and Antracol. Aliette showed 41.06 mm, 47.40 mm and 54.93 mm colony diameter at 100, 50 and 300ppm respectively and Topas showed 42.46 mm, 50.36 mm and 57.53 mm colony diameter at 100, 50 and 30ppm respectively. All the fungicides at their respective doses considerably deterred the growth of fungus as compared to control (87.13 mm) (Fig.1, Tab.1). These studies are in comparison with the reports of previous researchers. Safdar *et al.* (2015) evaluated *in- vitro* efficacy of seven fungicides i.e. Carbendazim, Acrobat MZ, Alliette, Dithan M–45, Thiophanate-methyl, Metalaxyl plus and Mancozeb against *B. theobromae*. All used fungicides considerably decreased the biomass of the experimental fungal specie, where Carbendazim was most effective in inhibiting the colony growth of the fungus. Sahi *et al,* (2012) tested *in-vitro* evaluation of the effect of Topsin-M, Daconil, Copper oxychloride and Mancozeb fungicides against the mycelial growth of *B. theobromae*. The results revealed that effectiveness of he fungicides increased with increased dosage rate. Topsin M, and Daconil were found to be most effective fungicide, Copper oxychloride was intermediate while Mancozeb was the least effective fungicide in inhibiting the mycelial growth of the fungus.

***In-vitro* evaluation of bio-control by dual culture method:** The observation a bio-control agent *Trichoderma harzianun* against *B. theobromae* showed that the *T. harzianun* was highly effective in reducing the mycelial colony growth of *B. theobromae* within seven days. The colony diameter of mycelial growth of *B. theobromae* in dual-culture with *T. harzianum* was 51.46 mm as compared to the fungus colony diameter in control 86.26 mm, resulting maximum inhibition of 40.34% in the colony growth of the test fungus within seven days (Fig.2, Tab.2). The manner of action of *Trichoderma* spp. in dual culture showed mycoparasitism, competed for nutrients and covered a huge space as compared to *B. theobromae,* which are in agreement with Kotze (2008). Sivakumar *et al.* (2000) studied that the *Trichoderma harzianum* is found to be an effective antagonistic agent against *Botryodiplodia theobromae*. It showed a strong antagonism against *B. theobromae* by minimizing the mycelial growth significantly. The antagonistic potentiality of *Trichoderma* spp. against *Botryodiplodia theobromae* was as well previously confirmed by a researcher (Kunz, 2007). Mortuza and Ilag (1999) mentioned that *T.harzianum* revealed the best inhibition in the dual-culture.

Photographs showing the effect of selected fungicides on the mycelial colony growth of *B. theobromae*



Photographs showing the Effect of *Trichoderma harzianum* on mycelial colony growth of *B.theobromae*

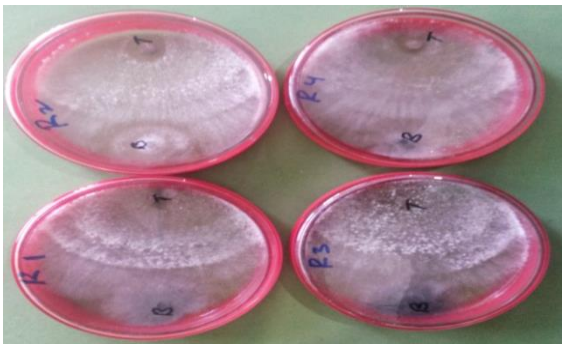


Table: 1. Effect of selected fungicides on the mycelial colony growth of *B. theobromae*

S. NO	Fungicides tested	Dose (PPM) / 100 ml. Medium	Colony growth (mm)
1	**NATIVO** (Trifloxystrobin+tebuconazole)	i. 30.0 ii. 50.0 iii.100.0	4.80 I 3.50 ij 2.03 j
2	**ANTRACOL** (Propineb)	i. 30.0 ii. 50.0 iii.100.0	49.00 de 44.90 f 35.90 h
3	**ALIETTE** (Fostyl-Aluminum)	i. 30.0 ii. 50.0 iii.100.0	54.93 c 47.40 e 41.06 g
4	**TOPAS** (Penconazole)	i. 30.0 ii. 50.0 iii.100.0	57.53 b 50.36 d 42.46 g
6	**CONTROL**	-	87.13 a
	LSD ($p<0.05$)		P = 0.0000

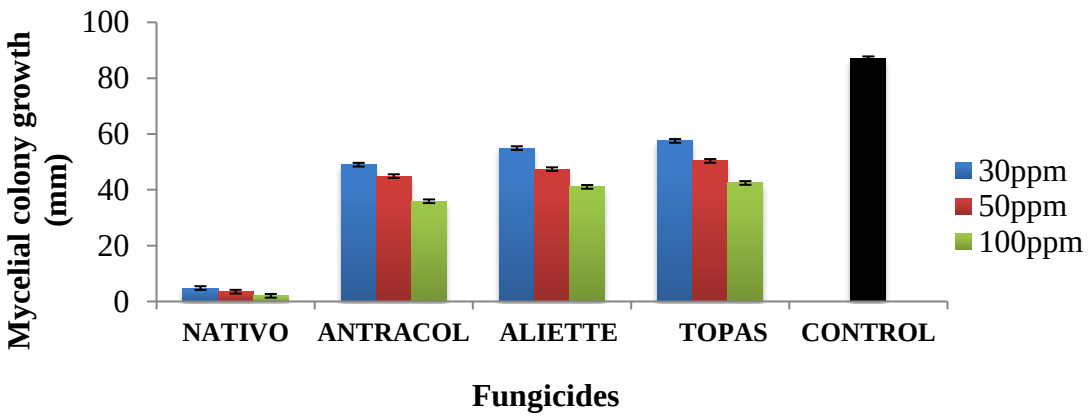


Figure: 1: Effect of selected fungicides on the mycelial colony growth of *B. theobromae*

Table: 2: Effect of *Trichoderma harzianum* on mycelial colony growth of *B. theobromae*

Days after inoculation	Control *(B. theobromae)* Radial colony growth (mm)	Dual-culture *(B. theobromae + Trichoderma harzianum)* Radial colony growth (mm)
Day-1	09.33	05.04 h
Day-2	16.66	09.30 g
Day-3	31.00	15.02 f
Day-4	42.00	27.14 e
Day-5	60.00	35.99 d
Day-6	72.00	43.97 c
Day-7	86.26 a	51.46 b
LSD (p<0.05)	-	P = 0.0000

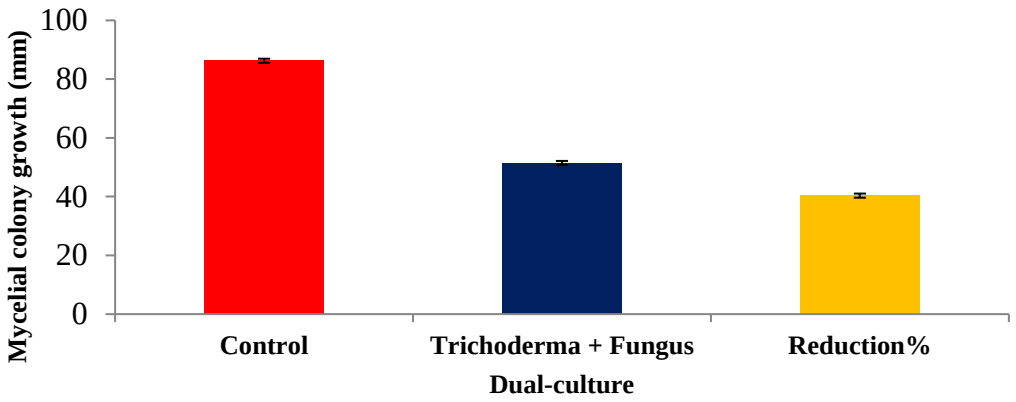


Figure: 2. Effect of *Trichoderma harzianum* on mycelial colony growth of *B. theobromae*

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