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IN-VITRO EVALUATION OF SELECTED FUNGICIDES AND BOTANICAL EXTRACTS AGAINST *Botryodiplodia theobromae,* RESPONSIBLE FOR GUAVA DECLINE

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ABSTRACT

There are numerous diseases which cause slow growth of plants. *Botryodiplodia theobromae* is one of the chronic diseases which generate guava decline. It is a destructive fungal pathogen, which brings considerable economic losses in the yield of guava in tropical regions of Sindh, Pakistan. The management of guava decline is very difficult; however, it can be managed up to satisfactory level through certain chemical and botanical practices. Here we studied the efficacy of some selected fungicides, such as Topsin-M, Cabriotop and Acrobat with three concentrations (25, 50, 75 ppm) and botanical extracts, such as tobacco (*Nicotiana tabacum*), Akk (*Calotropi sprocera*), Neem (*Azadirachta indica*) and Tooh (*Citrullus cololcynthis*), for which the concentrations were kept as (2.5, 5.0, 7.5 ml) against *B. theobromae*. The results manifested that between all of the fungicides Topsin-M was profoundly effective in dropping the linear colony growth of the fungus, whereas Cabriotop appeared moderately helpful, and Acrobat was less effective. While *in-vitro* evaluation of the botanical extracts showed high variation in reducing the fungus growth. The botanical extracts extensively decreased the linear colony growth of *B. theobromae.* Between them, tobacco was found to be the most effective botanical extract followed by akk, whereas, neem was moderately effective, and tooh was found less effective against *B. theobromae*. The present study revealed that *B. theobromae* could be effectively managed through Topsin-M and Tobacco, fungicide and botanical extract, respectively, which could be future prospective in controlling the guava decline under field conditions. Also, further different fungicides and botanical extracts must be evaluated *in-vitro* and then applied under field conditions.

Keywords: Management, guava decline, *Botryodiplodia theobromae*.

INTRODUCTION

Guava (*Psidium guajava* L.) is a delicious fruit in *Myrtaceae* family. This fruit stands the third position in Pakistan, in conditions of the region and fourth in conditions of production behind citrus, bananas and mango (Pervaiz *et al*., 2008). Some researchers named it the common man's fruit and also called it" apple of the tropics." Pakistan has various agro-ecological zones where enormous vegetables and fruits are planted which gives efficient yield to utilized. The fruits and vegetables are the major sources of food and drinks (Shakeel *et al*., 2013). It is a useful source of calcium, vitamins, nicotinic acid, phosphorous and soluble fiber like other agriculture crops and fruits according to (Rahman *et al*., 2003; Adnan *et al.* 2018). It has a high quantity of vitamin C as compared to other fruits (Divya and Kumari, 2009).

Guava is also greatly affected by numerous biotic and abiotic factors. In biotic factors, some important diseases are quite profound in causing heavy losses which include; guava decline, anthracnose, fruit decompose, Phoma rot, Rhizopus decay, neck rot, Cercospora leaf spot, stem canker, and seedling disease. Among these diseases, guava decline is a severe disease in the Punjab province of Pakistan which has seriously decreased the production of guava for the last few years. The disease spread out frequently due to the root infection along the plant materials; was found substantially infected by this syndrome. It was noted that the plants with the phase of one or above five were more at risk to this disease. Matured planting materials are the major cause of decline disease (Anonymous, 2010). The decline of guava is caused by unusual pathogens such as, *Botryodiplodia theobromae*, *Fusarium oxysporum* f. sp. *psidii*, *Phytophthora parasitica* and *F. solani* f. sp. *psidii*, which carry vast victims to the guava tree.

Along with these, *B. theobromae* and *F. oxysporum* f. sp. *psidii* are major pathogens, which are the prime reasons for guava decline (Bokhari *et al.,* 2008). The growing tip develops into gloomy brown color and the black necrotic branch shrinks backward and dies back. Contaminated trees show defoliation, and eventually, loss of twigs take place, and the roots of contaminated plants are also start rotten. The

tallness and girth of the diseased plants are reduced with an increase in disease sternness. Separately from the externally visible symptoms of the drought of the plants, tissues of the contaminated stem, extending to the cambium region, show a dark discoloration (Gomes *et al*., 2012). Keeping in vision the losses of the guava tree, the study was carried out to observe the effect of some selective fungicides and botanical extracts against the mycelial enlargement of *B. theobromae in-vitro* conditions.

MATERIALS AND METHODS

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

Evaluation of selected fungicides: The evaluation of the selected fungicides (Topsin-M, Cabriotop, and Acrobat) was done according to the Completely Randomized Design (CRD) with three treatments and three replications against *B. theobromae* through food poisoning technique. The typical solution was arranged according to the active ingredients of the fungicides. In the present experiment, the following steps were taken; Three drops of solutions (25, 50, 75ppm) of every fungicide were added to Potato Dextrose Agar (PDA), later diluted into a beaker of 9cm Petri plates. The solutions were kept till they transformed into the solid shape. Later the seven-day-old culture of *B. theobromae* was put in the center of the Petri plates. Then we kept it under close observation for seven days. Petri dishes holding PDA medium lacking fungicides were applied as a control. To observe the growth of the fungus, the Petri plates were kept in the incubator at the suitable temperature 25±1 °C for at least seven days. The growth of the fungus was observed and measured closely after every twenty-four hours for a complete week.

Evaluation of selected botanical extracts: To counter the disease *B. theobromae*, four different botanical extracts, i.e. Tobacco (*Nicotiana tabacum*), Akk (*Calotropi sprocera*), Neem (*Azadirachta indica*) and Tooh (*Citrullus cololcynthis*) were evaluated under *in-vitro* conditions through food poisoning technique. To prove the above-mentioned experiment, the Completely Randomized Design (CRD) system was utilized. Three treatments and three replications were tested. The first medium was adjusted with three identical doses of every extract (2.5, 5.0, 7.5ml). For the preparation of aqueous extract; 75 gm clean leaves of every plant were macerated in 25 ml of sterilized water, then mashed with the pestle and mortar. The crushed plant extract was first passed through a thin sterilized muslin cloth and filtered through Whitman's sieve paper. The sterilized PDA medium was added into the sterilized Petri plates. These Petri plates were kept in low temperature into the refrigerator for freezing, and later on different doses of plant extracts were diluted into the PDA media with the use of a sterilized pipette. Than inoculation of the vigorously growing culture of *B. theobromae* (7 days old) were done in each Petri plate by the using of the sterilized inoculating needle. All these Petri plates were next moved into the incubator at 25±1°C and records of mycelial growth of test fungus and inhibition region by test plant extracts were noted after 24 hours till 7 days of days of inoculation. Control was similarly carried out with the only dissimilarity that plant extracts renovate by PDA media.

RESULTS

Evaluation of selected fungicides against *B. theobromae in-vitro:* From the evaluation the following findings were observed; the tested fungicides used in the experiment became the sole agent to stop the growth of the fungus *Botryodiplodia theobromae* ($p<0.000$). From these fungicides, Topsin-M was found mainly effective in suppressing the mycelial growth of *B. theobromae* up to 03.10 mm, whereas Cabriotop was found moderately effective (15.86 mm), and Acrobate was not as much of effectual in dropping the linear colony growth of the fungus (45.16 mm) as compared to other two fungicides and control. Each fungicide at their highest dosage considerably reduced the linear colony growth of fungus as measured up to control (84.66 mm) (Table.1). Consequently, our results substantially proportionate the experiment of Sahi *et al.* (2012) who experimented *in-vitro* evaluation by using different fungicides like Topsin-M, Daconil, Copper oxychloride, and Mancozeb; he found out the result that the valuable majority fungicide in inhibiting the mycelial growth of *B. theobromae*. However, we tested Topsin-M fungicide and came with the similar results that the fungicide Topsin-M controls the mycelial growth of *B. theobromae*.

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

S. NO	Fungicides tested	Dose (PPM) / 100 ml. Medium	Radial colony growth (mm)
1	**TOPSIN-M**	i. 25 ii. 50 iii. 75	09.03 h 06.83 h 03.10 i
2	**CABRIOTOP**	i. 25 ii. 50 iii. 75	25.43 e 21.50 f 15.86 g
3	**ACROBAT**	i. 25 ii. 50 iii.. 75	53.66 b 49.50 c 45.16 d
4	**CONTROL**	-	84.66 a
	LSD (P<0.0000)		P = 0.0000

Data was analyzed by utilizing the computer application Statistics 8.1. The ultimate results prove that the previous fungicides extensively controlled the mycelial colony growth of *B. theobromae* (p<0.000).

Evaluation of selected botanical extracts against *B. theobromae in-vitro:* The botanical extracts significantly retarded the linear growth of test fungus (p<0.000). At their highest doses as compared to control (84.66 mm). Among these, the most effective botanical extract was tobacco which reduced the linear colony growth of the fungus (13.83 mm) with akk (40.63 mm), whereas, neem reduced the linear growth of the fungus up to (56.50 mm), and tooh was less effective (55.10) against *B. theobromae* as compared to control (83.36) (Table.2). Our results are in agreement with the results of Amienyo and Ataga (2007) who studied the effect of extracts of some indigenous plants and found that *A. cordifolia* reduced the growth of *B. theobromae* by 45.64%. Another researcher Nweke, (2015) evaluated the petroleum ether extracts of the leaves and stemmed bark of *Moringa oleifera* Lam. against some fungal pathogens and concluded that the petroleum ether extracts of the plant parts were effective against *B. theobromae*.

Table: 2. Effect of different botanical extracts on mycelial colony growth of *Botryodiplodia theobromae*

S. NO.	Botanical extracts tested	Dose (100ml) Medium	Radial colony growth (mm)
1	**TOBACCO**	i. 2.5 ii. 5.0 iii. 7.5	35.76 j 31.16 k 13.83 l
2	**AKK**	i.2.5 ii. 5.0 iii. 7.5	51.86 gh 48.33 h 40.30 i
3	**NEEM**	i. 2.5 ii. 5.0 iii. 7.5	69.41 b 62.63 cd 56.50 ef
4	**TOOH**	i. 2.5 ii. 5.0 iii. 7.5	64.56 c 59.70 de 55.10 fg
5	**CONTROL**		83.36 a
	LSD(P<0.0000)		P = 0.0000

DISCUSSION

Keeping in view the economic importance of guava, its export value and the losses caused by the *Botryodiplodia theobromae* (decline) disease in guava. We were determined to find out the major causes which primarily generate the disease *Botryodiplodia theobromae.* The following factors were also under considerations; the solution to counter the fungi should be effectual and eco-friendly. In this context, different management practices like chemical and botanical were carried out under *in-vitro* conditions against the *B. theobromae*. It is obvious that chemical fungicides are quite expensive and leave drastic implications for human health and the environment; so we have alternatively chosen the botanical extracts under

in vitro conditions to find out the control of the disease *B. theobromae.* To substantiate the discussion, the samples were collected from the effected and stunted trees of guava and were passed through various tests *in vitro* conditions in particular.

In the concerned study, the focus on isolation and identification of the effected samples proved the relativity of three fungi: *Botryodiplodia theobromae, Fusarium oxysporium,* and *Aspergillus niger*. Among these fungi, *B. theobromae* was found the most severe in devastating the guava plant and making the plant decline in agreement with the results noted by (Pitt and Hocking, 2009; Khanzada *et al*., 2006). The isolated fungus was then identified by morphological individuality and dye of the colonies of fungi. The identification of the fungus was also possessed the identical characteristics and close agreement of the research of Philips (2007). Our results are also in close relevance with the studies of Godfried, (2012), who observed that 25 isolates on PDA at 28°C and resultantly after 48 hours the culture of isolates turned into black colors.

Seven-day close observation, we came up with some concrete results which can profoundly control *B. theobromae.* To prove the findings, the doses (2.5, 5.0, 7.5, ml) were used. Over all four botanical extracts were used to manage the concerned disease, among them tobacco was found the most effective. Whereas in the chemical aspect, three fungicides (25, 50, 75 ppm) were used to control the *B.theobromae;* from them, Topsin-M was found highly effective to stop the growth of the fungus *B.theobromae.*

Our results were found in close relevance compared to the results of Adnan *et al.* (2017) whose studied focused predominantly on fungicides; thus the both studies came up with the same results (fungicides stops the growth of the B. *theobromae*). Furthermore, our results resemble the findings of Safdar *et al*. (2015) experimented on the same pattern and came up the relevant results that under close evaluation *in-vitro* efficacy of seven fungicides; Dithan M-45, Mancozeb, Metalaxyl plus, Carbendazim, Alliette, Thiophanate-methyl, and Acrobat MZ. These outcomes are in close relevance to our research. Moreover, there is another study which was carried out by Bukhri *et al.* (2008) his study initially found out the growth ratio of the disease which was 0.74%, and he adopted a method of treating it with sterilized and unsterilized soil. The ultimate result showed that *B. theobromae* were controlled by Topsin-M.

CONCLUSION

The present study was carried out under the *in-vitro* condition to find out the most effective and eco-friendly approach to manage the guava decline caused by *B. theobromae*. The studies showed that the disease could be managed significantly through certain management strategies through the use of chemical fungicides and botanical extracts. The fungicides were more effective as compared to botanical extracts. However, the botanical control is a safer method as compared to chemical control through fungicide.

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