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Full Length Article

Pathogenic Association and Management of *Botryodiplodia theobromae* in Guava Orchards at Sheikhupura District, Pakistan

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Abstract

A survey was carried out in five tehsils of District Sheikhupura for the assessment of guava decline. Maximum disease prevalence (100%) and disease incidence (36%) was recorded in Tehsil Sharaqpur. Samples of plant roots, shoots and soil were randomly collected for the isolation and identification of pathogens. Mean Colonization percentage of the *Botryodiplodia theobromae* Patouillard [*Lasiodiplodia theobromae* (Patouillard) Griffon and Maublanc] from 1987 tissues of 326 samples was counted to be (48.84%) maximum, followed by *Fusarium oxysporum* f.sp. *psidii* (44.10%), *Phytophthora parasitica* (38.10%), *F. solani* (35.10%) *Helminthosporium* spp. (15.20%) and *Curvularia lunata* (11.20%). *Aspergillus flavus* and *A. niger* were also isolated from the samples but mostly from twigs. Isolated fungi were multiplied and purified on PDA. Most dominating isolated fungus (*B. theobromae*) was evaluated for pathogenicity. Management of the *B. theobromae* was done under *in vitro* and *in vivo* conditions. *In vitro* efficacy of seven fungicides viz. carbendazim, thiophanate-methyl, alliette, acrobat MZ 75/667WP, dithan M–45 80% WP, mancozeb 80% WP and metalaxyl plus mancozeb 72% WP was evaluated against *B. theobromae* by poisoned food technique at different doses viz. recommended (R), 0.75R, 0.50 and 0.25R. All the employed doses of the test fungicides significantly reduced the biomass of the test fungal specie but recommended dose rate reduced more significantly. In field experiment, carbendazim was found to be more effective than other fungicides checked, in reducing the fungal infection in guava trees, suppressing the dieback and wilting with significant enhancement in vegetative growth of plants.

Keywords: Guava growing areas survey; Guava decline; *Botryodiplodia theobromae*

Introduction

Guava (*Psidium guajava* Linn.) belonging to the family *Myrtaceae*, is one of the most gregarious fruit trees occupies the third position after citrus and mango in terms of area and fourth in terms of production after citrus, mango and bananas in Pakistan (Pervaiz *et al*., 2008). Guava fruit comprises high amounts of vitamins A, B1 (Thiamin), B2 (Riboflavin) and C since 100 g of fruit contains about 260 mg of vitamin C (Rahman *et al*., 2003), which is 2–5 times higher than the fresh orange. The major guava growing areas in Pakistan are Sharaqpur, Kasur, Lahore, Sheikhupura, Sangla Hills and Gujranwala in Punjab; Kohat, Haripur and Bannu in Khyber Pakhtunkhwa, and Larkana and Hayderabad in Sindh giving annual production of 495 thousand tonnes (Anonymous, 2012). Since last decade the guava production has been adversely affected by decline problem. Guava decline has becoming the national problem in Pakistan and caused in yield reduction from 8920 kg per hectare in 2003–2004 to 8223 kg per hectare in 2008–2009 (Anonymous, 2010). Old planting material, extensive fruit drop and attack of insect pests and diseases are major factors that affect guava production badly (Khushk *et al*., 2009). The initial symptoms of the tree decline include wilting and yellowing of the leaves. The tree can decline rapidly or relatively slowly. Where tree decline is rapid, the leaves tend to remain on the tree, but shrivel and become necrotic, so that the tree has a scorched appearance. Where tree decline is slow, the leaves drop naturally and eventually resulting in the complete defoliation of the tree. Some other symptoms such as chlorosis and reduction of leaf area, reduction in fruit production and leaf rosetting were also associated with affected trees. The development of fruit on such trees ceases and the fruit eventually becomes mummified. Guava decline spread mainly through root infection and the movement of infected plant material. The present study was conducted to investigate the cause of decline in guava and to suggest its management in Sheikhupura district.

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Materials and Methods

Survey for the Assessment of Guava Decline in Different Guava Growing Areas

An extensive survey of guava orchards was done in Sheikhupura district, Punjab province, Pakistan to ascertain the disease severity. The survey was conducted in five tehsils of Distt. Sheikhupura i.e. Muridke, Sharaqpur, Sheikhupura, Ferozewala and Safdarabad in 2011–2012. Randomly 5 orchards were selected from each location. Total of 25 orchards were visited.

The disease prevalence was measured by the following formula: Disease Prevalence (%) = (Number of orchards infected/Total number of orchards inspected) × 100

The disease incidence was measured by the following formula: (Disease Incidence (%) = Number of infected plants/Total number of plants) × 100.

Collection of Samples

Samples were collected from infected tissues to ascertain the association of different pathogens. The samples consisting of bark at collar region, twigs and soil were collected. Trees showing declining symptoms were selected and samples of roots, soil, bark at collar region, and leaves of healthy as well as diseased plants were collected. About three hundred and twenty six samples were collected from guava orchards of 15–25 years old trees.

Isolation, Identification and Purification of the Pathogens

Isolation of fungi was done by usual isolation procedure (Safdar *et al.*, 2010). Infected samples after sterilization with sodium hypochlorite were plated on PDA and incubated at 27±2^{o}C for 3–4 days and data was recorded. The fungi which colonized on these pieces was purified and identified on the basis of their morphological characters (Ellis, 1971). The frequency of each isolated fungus was calculated by using the following formula: Relative density of fungi (%) = (Number of pieces colonized by a pathogen/ Total number of pieces) × 100 and Frequency (%) = (Number of colonies of a particular fungi/ Total number of colonies) × 100.

Pathogenicity of Isolated Fungi

Pathogenicity of isolated fungi was confirmed using Detached Twig Inoculation Technique on guava variety 'Gola' (Shah *et al*., 2010). A moist chamber was prepared by placing a blotter sheet on a plastic tray (50 × 40 × 7.5 cm). Guava twigs of 8–10 cm long and approximately 2–3 cm thick of similar age were taken from healthy branches, washed twice with distilled water, air dried on sterilized blotting paper /, and again surface sterilized with 2% sodium hypochlorite. An injury was produced on the surface of twigs with the help of a cork borer. Inoculation was done by placing 15-day-old 8 mm culture discs of each isolated fungal culture in an inverted position both on injured and uninjured sites of inoculation. A check was maintained in a similar manner on twigs, which was not inoculated. Total three replications were made with five twigs per replication. The trays with twigs were incubated in a growth chamber (at 28 ± 2°C). The observations were recorded regularly for symptom/lesion development.

In vitro Evaluation of Different Fungicides against *Botryodiplodia theobromae*

The efficacy of different fungicides against the most frequently isolated fungal pathogen *B. theobromae* was studied by using poisoned food technique. The calculated quantity of each fungicide for each concentration was weighed and dissolved in 5 mL of distilled water and made up to 100 mL. Then required quantity of fungicide was added in the freshly prepared PDA (potato dextrose agar) separately and allowed to cool to a pouring temperature of 40–45^{o}C. Twenty five milliliters of these PDA amended with different fungicide at different rates was poured into 9 cm diameter sterilized petri dishes. Each plate including the control (without fungicide) on solidification was inoculated in the middle with 14 days old *B. theobromae* culture using sterilized inoculating needle. Each concentration of each fungicide including control has five replications. Labeled petri dishes were placed in an incubator at 28±2^{o}C and observed daily for mycelial growth. Radial mycelial growth was measured at three and six day's interval after inoculation, by measuring the diameter along two perpendicular lines from the underside of the petri dish (Mamza *et al.*, 2008).

Field Application of Fungicides

The field experiment was carried out at a commercial guava orchard situated at Sharaqpur, Sheikhupura district, Punjab province. The orchard was showing increasing decline problem and trees were showing severe disease symptoms. The fungicides that gave the best inhibition of *B. theobromae in vitro* were used in the field trials. Carbendazim @ 2 g L^{-1}, dithane M-45 @ 2 g L^{-1} and alliete @ 2 g L^{-1} were dissolved in water to get a final concentration of recommended dose. There were four trees per replication. Total four replications were selected per fungicide treatment. The plants were systematically sprayed three times with 15 days interval. Before the first spray the plants were tagged and all dead branches were removed by pruning. After the first spray treated and control plants were irrigated with canal water and provided necessary farmyard manure and urea fertilizer. Isolation from the branches of treated and control plants were made before each spray to determine the infection on each plant. The effect of fungicides on guava plants was also evaluated by assessing the diseases severity and disease incidence before and after treatment. Disease incidence and severity were assessed with the help of a model proposed by Cardoso *et al.* (2004) and Khanzada *et al.* (2005) for evaluating disease incidence

and severity of cashew gummosis and mango decline respectively, caused by *L. theobromae*. Incidence and severity were also assessed with same model but after some modification in diseases rating scale. Incidence (*I*) was based on the presence of typical symptoms of the disease. Disease severity (*S*) was estimated by the equation $S = \Sigma (xini)/n$, in which *x* represented modified disease grade (Cardoso *et al*., 1998; Khanzada *et al*., 2005). Some modifications were made in the scale used (0, No symptoms; 1, Drying of small twigs; 2, Drying of small branches, 3, Drying of main branches; or 4, Drying of half of the tree; 5, Drying of more than half of the tree or whole tree), *ni* represented the number of diseased plants on the *i*th grade of the disease scale and *n* was the total number of diseased plants evaluated. Disease incidence ($I = \Sigma x/N$) was the proportion of diseased plants, which consisted of the number of diseased plants (*x*) divided by the total number evaluated (*N*). Both disease severity and incidence were evaluated before the 1st, 2nd and 3rd spray and after two weeks of 3rd spray of each fungicide.

Data Recording

Experiment was harvested after two months. Data was recorded on disease incidence and disease severity and percent infection. Data were subjected to ANOVA by using SAS statistical software (SAS Institute, 1988) and significant difference among the treatments was portioned by Least Significant Difference Test (LSD) at probability levels of P = 0.05 (Steel *et al*., 1997).

Results

Survey for the Assessment of Guava Decline in Different Guava Growing Areas

Disease prevalence: An extensive survey of Sheikhupura district conducted for decline prevalence and data was recorded. At each location five orchards were selected randomly. The result showed that maximum disease prevalence was in Sharaqpur (100%) followed by Sheikhupura, Ferozewala, Safdarabad showing 80%, 60% and 60% prevalence, respectively. This reveals that all of orchards visited in Sharaqpur were infected with decline. Muridke showed the 40% prevalence as shown in Fig. 1.

Disease incidence: During visit total number of trees of each orchard were counted and recorded. Total numbers of trees showing the decline symptoms were recorded. Disease incidence was calculated using this data. It was the maximum at Sharaqpur (3%) followed by Sheikhupura, (2.41%) and Ferozewala, (2%) as shown in Fig. 2. It was low at Safdarabad i.e., 1.92% as shown in Fig. 2.

Isolation, Identification and Purification of Pathogens

The examination of infected parts of guava trees revealed the association of a number of fungi at different

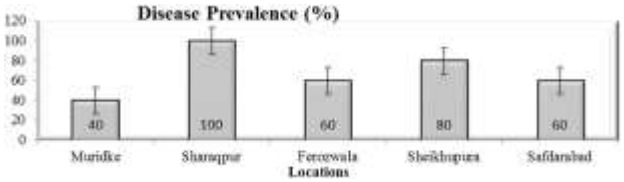

Fig. 1: Disease prevalence in Sheikhupura District

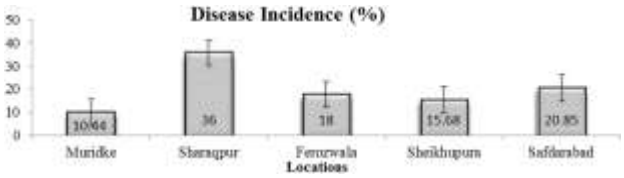

Fig. 2: Disease incidence in Sheikhupura District

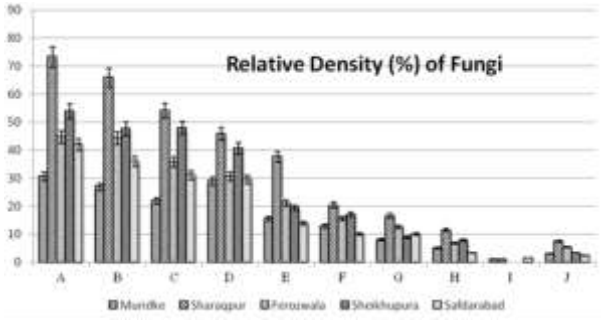

Fig. 3: Relative density (%) of different Fungi isolated from different plant parts of guava trees at different locations
Muridke Sharaqpur Ferozwala Sheikhupura Safdarabad
Where A=*B. theobromae*, B=*F. oxysporum*, C=*P. parasitica*, D=*F. solani*, E=*C. gloeosporioides*, F=*Helminthosporium* spp., G=*Curvularia lunata*, H=*Aspergillus flavus*, I=*A. fumigatus*, J=*A. niger*

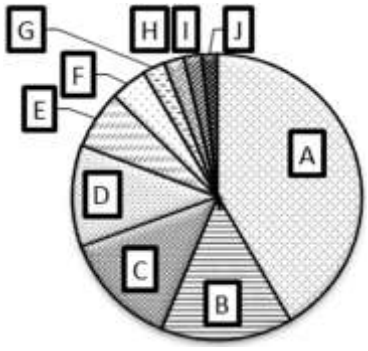

Fig. 4: Frequency percentage (%) of different Fungi isolated from different plant parts of guava trees at different locations
Where, A=*B. theobromae*, B=*F. oxysporum*, C=*P. parasitica*, D=*F. solani*, E=*C. gloeosporioides*, F=*Helminthosporium* spp., G=*Curvularia lunata*, H=*Aspergillus flavus*, I=*A. fumigatus*, J=*A. niger*

frequency viz. *B. theobromae*, *C. gloesporioides*, *F. solani*, *P. parasitica*, *Helminthosporium* spp. *F. oxysporum*, *Curvularia lunata*, *A. flavus*, *A. niger* and *A. fumigatus*.

Majority of the selected samples yielded the fungus *B. theobromae*, which was isolated from all locations as shown in Fig. 4. Colonization percentage of the *B. theobromae* in 1987 tissues of 326 samples was 42%. *C. gloesporioides* (14.78%) was the second frequent fungi. *F. solani* (13.07%) and *Phytophthora parasitica* (11.37%) were the third and fourth frequent fungus. These all fungi were frequently isolated from twigs and collar region. *F. oxysporum, C. lunata, Helminthosporium* spp., *A. flavus* and *A. fumigatus* were also isolated from samples but mostly from twigs. *Aspergillus niger* was the last and least frequent fungus as mentioned in Fig. 4.

Keeping in view the highest isolation frequency of *B. theobromae* from all samples a comparison was studied between the different locations. Maximum colonization of *B. theobromae* was observed at Sharaqpur followed by Sheikhupura, Ferozewala, and Sardarabad. Muridke showed the minimum colonization of *B. theobromae* as mentioned in Fig. 3. Mean relative density of the *B. theobromae* in all locations was counted to be (48.84%) maximum followed by *Fusarium oxysporum* f.sp. *psidii* (44.10%), *Phytophthora parasitica* (38.10%), *F. solani* (35.10%) *Helminthosporium* spp. (15.20%) and *C. lunata*, (11.20%). *A. flavus* and *A. niger* were also isolated from the samples but mostly from twigs.

Pathogenicity of Isolated Fungi

All of the isolated fungi responded variably with respect to the pathogenicity test on guava cultivar 'Gola' under controlled conditions (Table 1). The least similar relationship was observed between pathogenic behaviors of fungi. The symptoms developed on healthy twigs after 3–7 days of inoculation by all the fungi. The highest percent infection was observed by *B. theobromae* (86.66%) and the lowest (13.33%) by *A. niger*. No percent infection was observed in control. The largest lesion size (7.8 × 2.66 cm) was noticed in *B. theobromae* and the smallest (0.43 × 0.1 cm) in *A. niger*.

In vitro* Evaluation of Different Fungicides against *B. theobromae

In vitro efficacy of different fungicides was checked on mycelial growth of *B. theobromae* as given in Table 2 and 3. All of fungicides show significant results with the variable response of tested fungi. It was observed that a significant increase in the inhibition of mycelia growth of the fungi occur with an increase in the concentration. Although none of the fungicide gave 100% inhibition at any of the three concentration rates. The data was recorded after three days and six days interval to check the fungitoxic effect of fungicides with time interval. The results revealed that the carbendazim was most effective at all the concentrations inhibiting the colony growth after three days i.e., 2.970 cm, 2.084 cm, 1.700 cm and 0.330 cm and six days 2.280 cm, 2.260 cm, 1.700 cm and 0.4500 cm at 0.25, 0.5, 0.75 and 0.2 g/mL concentrations, respectively. It inhibits the colony growth maximum at all the dose rates. With an increase in dose rate there was an increase in the inhibition of mycelial growth. Dithane M-45 and acrobat MZ were next moderately effective fungicides against *B. theobromae* significantly reducing the growth of fungi at all dose rates as compared to the control because a significant increase in the colony growth was observed as shown in Table 1 and 2. Minimum inhibition in colony growth was

Table 1: Pathogenic behavior of different Fungi isolated from samples collected from different guava growing areas of Punjab following detached twig Inoculation method on guava variety 'Gola'

Fungi	Percent Infection (%)	Lesion Size (cm)	
		Length(cm)	Width(cm)
B. theobromae	86.66 a	7.8	2.66
P. parasitica	66.66 b	6.16	2.36
C. gloeosporioides	46.66 c	6.23	2.06
Curvularia lunata	40 cd	5.4	1.83
F. oxysporum	33.33 cde	2.4	1.06
Helminthosporium spp.	26.66 def	4.86	1.86
F. solani	20 ef	2.76	0.86
A.fumigatus	20 ef	0.8	0.26
A. flavus	13.33 f g	1.6	0.43
A. niger	13.33 fg	0.433	0.1
Control	0 g	0	0
LSD (p=0.050)	16.35	-	-

Table 2: Effect of different fungicides on mycelial growth (cm) of *B. theobromae* by Poisoned food technique after 3 days

Treatments	0.25 (g/100 mL)	0.5 (g/100 mL)	0.75 (g/100 mL)	0.2 (g/100 mL)
Thiophanate-methyl	3.200bc	2.960b	2.762 bcd	2.614 c
Carbendazim	2.970bc	2.084c	1.700e	0.3300e
Acrobat	3.100bc	2.680b	2.500cd	2.054 d
Dithan M-45	2.800c	2.770b	2.280de	2.000d
Mancozeb	3.480ab	2.924b	2.770bcd	2.602c
Alliette	3.020bc	2.824b	3.200abc	2.126d
Metalaxyl plus Mancozeb	3.528ab	3.648a	3.400ab	3.114b
Control	3.796a	3.796a	3.796a	3.796a
LSD	0.5884	0.5342	0.7653	0.3865

Table 3: Effect of different fungicides on mycelial growth (cm) of *B. theobromae* by poisoned food technique after 6 days

Treatments	0.25 (g/100 mL)	0.5 (g/100 mL)	0.75 (g/100 mL)	0.2 (g/100 mL)
Thiophanate-methyl	6.800ab	6.400de	6.300b	5.400c
Carbendazim	2.280c	2.260f	1.700c	0.4500d
Acrobat	6.548b	6.200e	6.000b	5.900bc
Dithan M-45	6.940ab	6.700cd	6.400b	6.100bc
Mancozeb	7.200ab	7.160b	7.000ab	6.900ab
Alliette	7.004ab	6.900bc	6.180b	6.600abc
Metalaxyl plus Mancozeb	7.400ab	7.200b	6.900ab	7.000ab
Control	7.900a	7.900a	7.900a	7.900a
LSD	1.190	0.3732	1.051	1.307

Means followed by the same letter are not significant from each other at P = 0.05 according to Least Significant Difference Test

observed in case of metalaxyal+mancozeb and thus found to be least effective against the *B. theobromae.* With the increase in time a significant increase in the colony growth was observed as shown in Table 2 and 3. Colony growth was maximum in control, without any fungicide.

Field Experiment

Carbendazim, dithane M-45 and acrobat MZ proved to be the best fungicides suppressing the growth of *B. theobromae* under *in vitro* experiment. These fungicides were further evaluated under field conditions. In field experiment, carbendazim proved to be highly effective for control of decline disease followed by dithane M-45 and acrobat MZ (Fig. 5 and 6). Data recorded reveals that the fungal infection in treated guava plants gradually reduced with the number of fungicidal sprays. It was accompanied with a gradual reduction in the disease severity and disease incidence in treated trees as compared to untreated control trees (Fig. 5 and 6). It was also noted in the trees treated with fungicides that infection reduced with each fungicidal treatment. Carbendazim gave the maximum reduction in disease severity (1.87, 1.25 and 0.37) after 1st, 2nd and 3rd spray respectively and lowest disease prevalence (8.33% after third spray) as compared to control (100%). Similarly maximum fungal infection in treated guava trees gradually reduced with the spray of carbendazim (7.14%) as compared to control (100%) and results in complete disappearance of typical symptoms of the disease after the 3rd spray. However, the plants sprayed with dithane M-45 and acrobat MZ still exhibit little declined symptoms. In untreated control plants, disease severity and percent infection was increased with increase in time.

Discussion

Guava decline is becoming a serious threat day by day in guava growing areas of Pakistan. It is a complex disease and different pathogens have been reported in guava rhizosphere. Considering this aggravated intensity of disease and hazard, a comprehensive survey was conducted on disease incidence in the district Sheikhupura, Punjab. This disease had been reported at almost all of guava growing areas of Pakistan so there is the need of extensive work over its etiology and epidemiology, to avoid the heavy losses expected in future. Disease prevalence and disease incidence was calculated in the district and samples were collected to check its etiology. The result showed that all of the orchards visited were infected with the decline. The samples of leaves, branches, twigs and bark at collar region were taken from each tree. Colonization percentage of the *B. theobromae* in 1987 tissues of 326 samples was the maximum; followed by the other fungi, which were *C. gloesporioides, F. solani, Helminthosporium* spp., *Phytophthora parasitica, F. oxysporum, C. lunata, Helminthosporium* spp., *A. flavus* and *A. fumigatus.* The examination of infected parts of guava trees revealed the

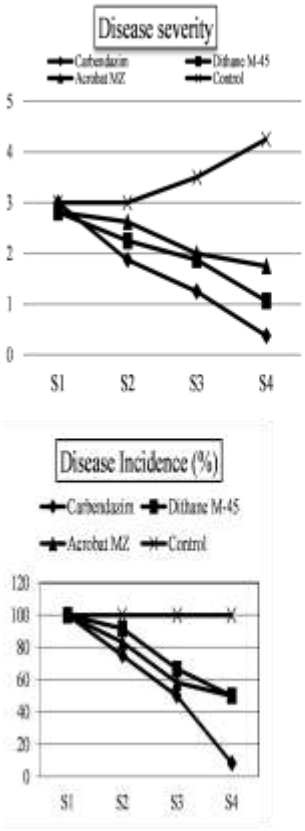

Fig. 5: Effect of Fungicidal sprays on Guava Decline
S1= Before 1st spray, S2= Before 2nd spray, S3= Before 3rd spray, S4= After 2 weeks of 3rd spray

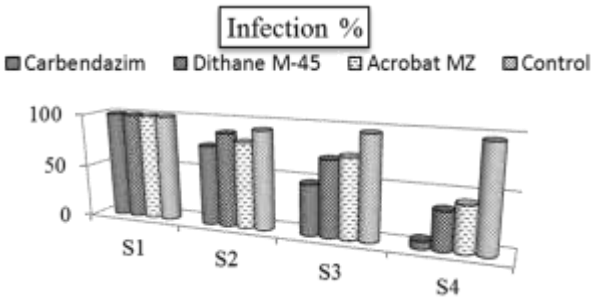

Fig. 6: Effect of Fungicidal sprays on infection% of *L. theobromae*
S1= Before 1st spray, S2= Before 2nd spray, S3= Before 3rd spray, S4= After 2 weeks of 3rd spray

association of these fungi in different frequency at different locations. From majority of the selected samples *B. theobromae* was the most dominant. Maximum colonization of *B. theobromae* was observed at Sharaqpur followed by Sheikhupura, Ferozewala, and Sardarabad. Muridke showed the minimum colonization of *B. theobromae.*

Our results are comparable with other studies describing the association of *B. theobromae* on other tree crops such as *Eucalyptus* spp. (Sharma *et al*., 1984; Roux *et al*., 2000, 2001) and other fruit and tree crops such as mango (Sangchote, 1991); avocado (Darvas and Kotze, 1987); kumquat (Ko *et al*., 2004); eggplant (Woodward, 2005); cocoa (Mbenoun *et al*., 2008); Proteas (*Protea magnifica*) (Denman *et al*., 2003); apricot and peach trees (Li *et al*., 1995); white cedar (Sandrock *et al*., 1999), peanut (Phipps and Porter, 1998). *B. theobromae* is becoming a major constraint to guava production in Sheikhupura-Punjab. *B. theobromae* is a common, widespread pathogen of tropical woody trees, causing shoot blight and dieback of trees and shrubs and blue stain in timber (Mohali *et al*., 2005). It is an important opportunistic pathogen with worldwide distribution causing different types of diseases in a wide range of hosts (Punithalingam, 1976). *B. theobromae* also cause the blight and dieback of many trees including *Pinus* spp. (Mohali *et al*., 2005), *Azadirachta indica* (Cedeno *et al*., 1995), *Citrus* spp. (Cedeno and Pru, 1992) and pear (Shah *et al*., 2010). It is the causal agent of gummosis of branches and trunks of citrus (Fawcett, 1936; Cedeno and Pru, 1992), mango (Narasimhudu and Reddy, 1992; Khanzada *et al*., 2004; Al Adawi *et al*., 2006), cashew (Cardoso *et al*., 2006) and neem (Khalil, 2010). In Sudan the fungus proved to be involved in many diseases (Tarr, 1955; Khalil, 2010).

B. theobromae is a cosmopolitan soil-borne fungus causing a number of diseases both in field and storage conditions on more than 280 plant species including crops, fruits and plantation trees (Domsch, *et al*., 1980; Sutton, 1980). In Pakistan, the fungus has been reported on more than 50 plant species (Ahmed *et al*., 1997). *B. theobromae* have also reported to cause the different diseases such as storage rot of Taro, black-band disease of jute, crown rot diseases of banana fruit, fruit rot of coconut, stem-end rot of mango fruit, soft rot of papaw, guava, litchi, sapodilla fruit and die-back in lemon plant fruits (Alam and Nahar, 1990; Wall and Cruz, 1991; Mortuza and Ilag, 1999; Alam *et al*., 2001; Anthony *et al*., 2004). Pathogenic behavior of *B. theobromae* was further confirmed by pathogenicity test in which all of the isolated fungi developed the lesions of variable size on twigs inoculated with respective fungal culture. The least similar relationship was observed between pathogenic behaviors of different fungi. The highest percent infection was observed by *B. theobromae* and the lowest by *A. niger*. No percent infection was observed in control. Similarly the largest lesion size was noticed in *B. theobromae* and the smallest in *A. niger*. The fungus (*B. theobromae*) during infection remained confining to the current growth and the bark of infected parts shrinks considerably resulting in depressed lesions. Moreover, longitudinal and transverse cracks appear on the affected bark of some older branches (Verma and Cheema, 1984). The pathogen causes distension, disrupts the cell walls and weakens the strength and toughness of wood (Shah, 2007). The fungus imparts also blue stain in timber (Cedeno *et al*., 1996) and cause discoloration, distortion and rotting of tissues (Verma *et al*., 1990).

In vitro effect of different fungicides on mycelial growth of *B. theobromae* showed that all of fungicides show significant results with the variable response of test fungi. Among all of the tested fungicides, carbendazim was most effective at all the concentrations followed by dithane M-45 and acrobat MZ as compared to the control. These fungicides were further evaluated under field conditions. In field experiment, carbendazim proved to be highly effective for control of decline disease followed by dithane M-45 and acrobat MZ. Data recorded reveals that the fungal infection in treated guava plants gradually reduced with the number of fungicidal sprays. It was accompanied with a gradual reduction in the disease severity and disease incidence in treated trees as compared to untreated control trees. Our results are similar to Sultana and Ghaffar (2010) who resulted that carbendazim and topsin-M completely inhibit the growth of *L. theobromae in vitro* at 50 ppm and reduced the recovery of seed borne fungi with enhanced seed germination as seed treatment. Inhibitory effect of carbendazim and thiophanate methyl against *L. theobromae* have also been reported by Khanzada *et al*. (2005) revealing that in field experiment, carbendazim was found to be more effective than thiophanate-methyl and alliete in reducing the fungal infection in diseased plants, suppressing the diseased symptoms resulting in significant enhancement in vegetative growth of plants. Similarly Mishra and Sitansu (2008) during *in vitro* and *in vivo* evaluation of fungicides reported that carbendazim was most effective against all pathogens isolated (*Colletotrichum gloeosporioides* and *B. theobromae*) from infected guava trees except *P. psidii* and *Phytophthora nicotianae* var *parasitica*, which were more sensitive to thiophanate methyl and metalaxyl, respectively.

Rehman *et al*. (2011) reported that *B. theobromae* (12.55%) was most abundantly isolated fungal pathogen isolated from mango declined orchard at Multan and Muzaffargarh. Further they reported that carbendazim proved the best fungicide *in vitro* suppressing the growth of *B. theobromae* showing 1.27 cm, 1.5 cm, and 1.76 cm colony diameter at 100 ppm, 60 ppm and 20 ppm, respectively. Markson *et al*. (2012) reported *B. theobromae* as most virulent pathogen recording a percentage rot severity of 57.5% and suggested that forcelet (carbendazim) and coacobre at 20 g/L cause the highest inhibition of spore germination (81% and 70%, respectively), while reduction in mycelial dry weight dropped to 56.3% and 30%, respectively. Similarly, Hegde *et al*. (2013) used different systemic and combi product fungicides against *B. theobromae* and recommend the carbendazim as predominately effective systemic fungicide to manage the pathogen under *in vitro* conditions. Banik *et al*. (1998) also established that carbendazim at 400 ppm completely inhibited the linear growth of *L. theobromae* followed by thiophanate-methyl at 450 ppm.

Calculation of the effect of synthetic fungicides on the radial growth of the *B. theobromae* showed that increase in the concentration of the fungicide was positively correlated with the growth inhibition. Similar observations have been made by Madunagu *et al*. (2001), Wokocha and Okereke (2005), Madunagu *et al*. (2005) and Chiejina (2005) using plant extracts and Amadioha (2000), Amadioha and Markson (2007) and Amiri *et al*. (2008) using fungicides. The different levels of reductions of radial growth by the synthetic fungicides may possibly be due to fluctuating extent of intrusion of these chemicals with the metabolism of the fungi involved. Deacon (1980) described that in addition to genetic requirement and other factors, the metabolism of fungi depends on the substrate composition which might be the active principle in the synthetic fungicides that affect the qualitative state of the fungus. This effect according to Cooke (1980) is peculiar to each fungus. Agrawal and Mehrota (1988) study the effect of synthetic fungicides on *Phytophthora colocasiae* and described that a positive correlation may exist between mycelial growth inhibition and inhibition in the rate of respiration. Similarly Aluiter *et al*. (2007), while studying the sensitivity of some fungicides to isolates of *Phytophthora cactorum*, resulted that Salicylhydroxamine Acid (SHAM) inhibited the growth of the fungus through inhibition of the alternative pathway which was dependent on the AOX enzyme in mitochondrion respiration operated by plants and fungi. Taking into account the findings obtained from earlier researches in this area, it could be concluded that the chemicals (whether synthetic or of plant origin) effect their actions through interfering with or inhibiting the normal metabolic activities (especially respiration) of the target pathogen. However, the severity of the accomplishment primarily depends on the type and relative amount of the active ingredient in the chemical (fungicide) tested.

Conclusion

Maximum disease prevalence (100%) and disease incidence (36%) was recorded in Tehsil Sharaqpur. Mean colonization percentage of the *B. theobromae* was recorded to be (48.84%) maximum. Most frequently isolated fungus (*B. theobromae*) was evaluated for pathogenicity. *In vitro* and *in vivo* evaluation of fungicides against *B. theobromae* showed that Carbendazim was most effective than other fungicides checked in reducing the fungal infection in guava trees.

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