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EFFECT OF FUNGICIDES AND MICROBIAL ANTAGONISTS IN THE CONTROL OF *LASIODIPLODIA THEOBROMAE*, THE CAUSE OF SEED ROT, SEEDLING AND ROOT INFECTION OF BOTTLE GOURD

Nasreen Sultana* and A.Ghaffar**

ABSTRACT: The effect of fungicides and microbial antagonists in the control of *Lasiodiplodia theobromae* , the cause of seed rot, seedling and root infection on bottle gourd was studied in vitro and in vivo. Carbendazim and Topsin-M completely inhibited the growth of *L. theobromae* in vitro at 50 ppm whereas Aliette, Benlate, Mancozeb, Ridomil and Vitavax showed complete inhibition of colony growth at 100 ppm. Invariably all fungicides at three different concentration viz., 1, 2, 3 g (a.i.) kg^{-1} seeds reduced the recovery of the seed borne fungi. The most effective seed treatments were Benlate, Topsin-M, Carbendazim and Aliette @ 3 g kg^{-1} seeds which enhanced seed germination and reduced seed infection in bottle gourd. *Trichoderma harzianum, T. viride, Gliocladium virens, Stachybotrys atra* and *Bacillus subtilis* showed better results in the control of pre- and post-emergence infection of *L. theobromae* in seedling of bottle gourd in vitro and in vivo. *G. virens* has been found most effective to reduce seed and root infection in vivo whereas *B. subtilis* performed best to reduce seed and seedling infection of bottle gourd in vitro.

Key Words: *Bottle Gourd; Lasiodiplodia theobromae, Seed Root; Diseases; Fungicides; Microbial Antagonists; Pakistan.*

INTRODUCTION

Lasiodiplodia theobromae (Pat) Griffon & Moube (Syn. *Botryodiplodia theobromae*) has been reported to attack various cucurbitaceous fruits such as cantaloupes, bottle gourd, squash and fluted pumpkin (Beraha et al., 1976; Laxminarayan and Reddy, 1976; Maholay, 1988; Neufo and Emebiri, 1990). The fungus is also reported to be seed borne in various cucurbits viz., watermelon, bottle gourd and squash, reducing seed germination and produce seedling mortality up to 40% in bottle gourd and squash (Sohi and Maholay, 1974; Maholay and Sohi, 1982; Maholay, 1989). *L. theobromae* invade all parts of the rubber seeds, causing discolouration, distortion and rotting of tissues (Verma et al., 1990). There have been reports of internal infection of the fungus in both symptomatic and asymptomatic seed of pine (Ciliers et al., 1995) in bottle gourd and squash (Maholay and Sohi, 1982). The fungus is seed transmitted and caused pre- and post-emergence death of maize seedling (Kumar and Agarwal, 1997).

There are numerous reports of potentially valuable biological control microorganisms, some of which have been used as seed treatment and function across a broad range of environment as most successful chemical pesticides (Cook, 1993). *Trichoderma* spp. has been widely used as antagonistic fungal agents against several pests as well as plant growth enhancers. Faster metabolic rates, anti-microbial metabolites, and physiological conformation are key factors which chiefly contribute to antagonism of these fungi (Verma et al., 2007). There are few reports where fungicides have been used for the control of disease caused by *L. theobromae* under laboratory condition (Ahmad et al., 1996, Hong-Ye Li, et al., 1995, Sheler et al., 1997, Banik et al., 1998, Shahbaz et al., 2009). In the present study the effect of fungicides and microbial antagonists in the control of *L. theobromae*, the cause of seed rot, seedling

*PARC-Crop Diseases Research Institute, Karachi University Campus, Karachi-75270 , Pakistan.
**Department of Botany, University of Karachi, Karachi-75270, Pakistan.

and root infection of bottle gourd has been studied.

MATERIALS AND METHODS

Seven fungicides viz. Carbendazim (Benzimidazole), Topsin-M (Thiphanate-Methyl), Aliette (Fesetyl-Al 80% up), Benlate (Triadimenol), Vitavax (Carboxin), Ridomil (Metalaxyl acylatenine) and Mancozeb (Dithane M-45) were evaluated against colony growth of *L. theobromae*. Fungicides were used @ 10, 50, 100, 500, 1000 and 10,000 ppm concentration in autoclaved PDA medium by poisoned food techniques (Borum and Sinclair, 1968). Agar disk (5mm dia) of test fungi were cut from 8-10 days old culture plate by using sterile cork borer and placed in the centre of Petri plates containing different concentration of fungicides. There were four replicates of each treatment. The plates without fungicides served as control. The inoculated plates were incubated at 28°C. The radial growth was recorded after 7-10 days of incubation when the fungus covered the plates completely in control. The percent inhibition (PI) of the fungus over control was calculated by using the following formula

$$PI = \frac{(A-B)}{A} \times 100$$

where A is colony growth of the fungus in control plate and B is colony growth of the fungus in treated plate.

Seeds naturally infected with *L. theobromae* were treated with fungicides and microbial antagonist. Fungicides viz., Aliette, Benlate, Carbendazim, Ridomil, Topsin-M and Mancozeb were used @ 1, 2 and 3 g kg^{-1} of seeds. Microbial antagonist viz., *T. harzianum* @ 8 x 10^9 conidia ml^{-1}, *T. viride* @ 6.5 x 10^7 conidia ml^{-1}, *G. virens* @ 1.7 x 10^8 conidia ml^{-1}, *B. subtilis* @ 1 x 10^9 conidia ml^{-1} and *S. atra* @ 5.8 x 10^9 conidia ml^{-1} adjusted by means of haemocytometer and 1% gum arabic solution was used as sticker. Naturally infected seeds treated with 1% gum arabic solution prior to seed treatment with fungicides and microbial antagonists and were sown in 20 cm diam, pots containing sterilized soil @ 10 seeds per pot for each treatment. Pots were placed in screen house benches and were regularly observed for the development of symptoms. After 40 days plants were removed and infection percentage as well as root colonization were recorded. Data were subjected to Duncan's multiple range tests at P= 0.05 depending upon the experimental design by SPSS version 12.

RESULTS AND DISCUSSION

In*Vitro* Effect of Fungicides

Carbendazim and Topsin-M completely inhibited the growth of *L. theobromae* @ 50 ppm whereas Aliette, Benlate, Mancozeb, Ridomil and Vitavax showed complete inhibition of colony growth at 100 ppm (Table 1). Mycelial growth of *L. theobromae* has been reported to be inhibited by Carbendazim and Thiophanate-methyl when used @ 1 ppm and at relatively high concentration i.e., 1000 ppm and 10000 ppm by Alliete (Khanzada et al., 2005).

Seeds of bottle gourd with > 70% seed

Table 1. Mean percent inhibition of *Lasiodiplodia theobromae* on potato dextrose agar (PDA) by seven fungicides

Concentr-	% inhibition by fungicides						
ation (ppm)	Aliette	Benlate	Carbendazim	Mancozeb	Ridomil	Topsin-M	Vitavax
10	68.6a	51.4a	56.4a	47.2a	44.0a	52.5a	38.2a
20	87.6b	79.3b	89.5b	78.2b	73.3b	88.2b	67.5b
50	93c	98.5c	100 c	89.6c	90.5c	100 c	96.1c
100	100d	100 d	100 c	100 d	100 d	100 c	100 d
500	100d	100 d	100 c	100 d	100 d	100 c	100 d
1000	100d	100 d	100 c	100 d	100 d	100 c	100 d

Means followed by the same letter do not differ significantly at (P=0.05) according to Duncan's multiple range test

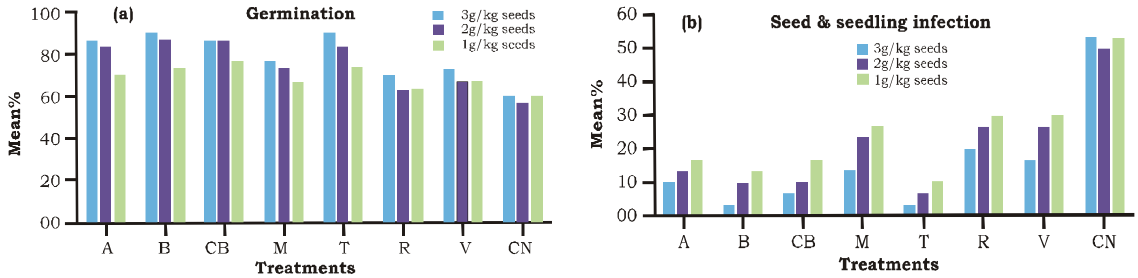

Figure 1. Effect of seed treatment with the fungicides on (a) germination and (b)infection of *Lasiodiplodia theobromae* on bottle gourd. (A= Aliette, B=Benlate, CB= Carbendazim, M=Mancozeb, T= Topsin-M, R=Ridomil, V=Vitavax, CN= Control)

infection of *L. theobromae* were treated with fungicides showed significant effect on seed germination, seed and seedling infection. The effects of fungicides were more pronounced where fungicides were used @ 3 g (a.i.) kg^{-1} seeds but complete control of seed and seedling infection was not achieved. Maximum increase in germination occurred in seeds treated with Topsin-M, Benlate , Carbendazim and Aliette (Figure 1). Seeds and seedling infection were significantly controlled by all fungicides as compared to control. Topsin-M, Benlate, Carbendazim and Aliette @ 3g (a.i.) kg^{-1} seeds significantly reduced seed and seedling infection. Similarly Solanka et al. (1996) have reported that seed germination was increased and seed infection of *L. theobromae* on cotton was decreased in seed treatment with Thiram +Carbendazim.

In*Vivo* Effect of Fungicides

All the fungicides significantly reduced seedling mortality and root infection by *L. theobromae* in bottle gourd over untreated control (Figure 2). The most effective fungicides in reducing root infection were Topsin-M and Carbendazim followed by Aliette, Benlate and Vitavax. Mancozeb and Ridomil were the least effective as they produced 20-22% root infection. Maximum reduction in seedling mortality was observed where Benlate, Aliette, Carbendazim and Topsin-M were used which produced 6-14% seedling mortality as compared to control with 52% seedling mortality. Mancozeb and Vitavax treatments reduced seedling mortality by 18-28% whereas Ridomil was found least effective giving 34% mortality. Similarly seed germination was significantly increased where seeds were treated with fungicides. Best germination was observed where the seeds were treated with Benlate and Topsin-M (86%) followed by Carbendazim and Aliette (80%), Vitavax (76%), Mancozeb (74%) and Ridomil (72%). Significant increase in plant size was observed where seeds were treated with fungicides. Plant achieved maximum size where Benlate, Aliette, Carbendazim and Topsin-M were used.

In the present study, invariably all fungicides at three different concentration viz., 1, 2, 3 g (a.i.) kg^{-1} seeds reduced the recovery of the seed borne fungi. The most effective seed treatments were with Benlate, Topsin-M, Carbendazim and Aliette @ 3 g kg^{-1} seeds which enhanced seed germination and reduced seed infection in bottle gourd. Goulart (1992) reported that Benomyl reduced seed borne infection of *L.theobromae* in cotton and also improved crop stand and controlled damping off. Thiophanate-methyl consistently inhibited mycelial growth and conidial germination *L. theobromae* and controlled disease development in Japanese apricot trees (Hong-Ye Li, 1995).

In Vitro Effect of Microbial Antagonists

All the antagonists viz., *T. harzianum*, *T. viride*,*G. virens*, *B.subtilis* and *S. atra* provided significant reduction in seed infection by *L. theobromae* in bottle gourd. Seed germination was significantly enhanced by

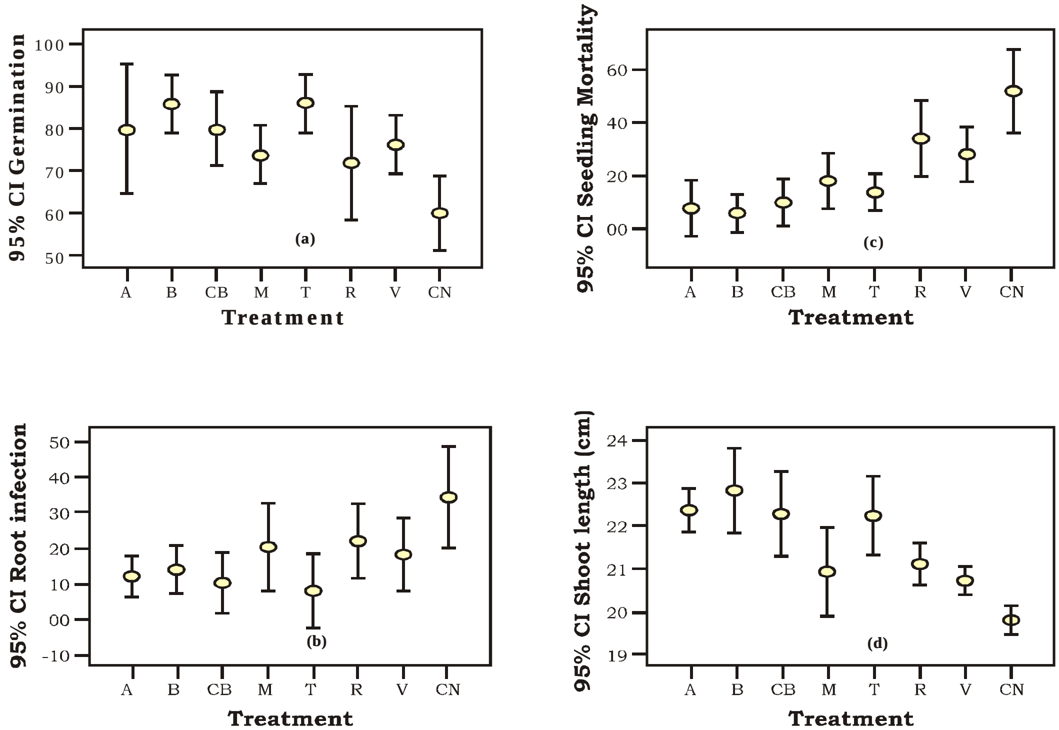

Figure 2. Effect of seed treatment with fungicides on a) germination, b) shoot length, c) seedling mortality and (d) root infection by *Lasiodiplodia theobromae* on bottle groud. (A= Aliete, B= Benlate, CB= Carbendazim, M= Mancozeb, T= Topsin, M,R= Ridomil, V= Vitavax, CN= Control)

G. virens (Figure 3a). *B. subtilis* performed best in reducing the seed and seedling infection followed by treatments with *T. harzianum*, *T. viride*. *G. virens* and *S. atra*. Microscopic investigation demonstrated direct parasitism and coiling of *T. harzianum* and *T. viride* around hyphae of *L. theobromae*, causing swollen, deformed, shortened, or rounded cells of the pathogen. Granulation of cytoplasm and disintegration of the hyphal walls of *L. theobromae* also were noted in dual culture. *T. viride* reduced rotting by 29.07-65.06% in artificially inoculated banana fruits (Mortuza and Ilag, 1999).Treatment of fruits of banana with conidia and culture filtrates of *Trichoderma* sp., prior to inoculation with the pathogens (*L. theobromae*, *Fusarium oxysporum* and *Colletotrichum musae*) provided a better control than their simultaneous application (Adebesin et al.,2009).

In Vivo Effect of Microbial Antagonists

All the antagonist viz., *T. harzianum*, *T. viride*, *G. virens*, *B. subtilis* and *S. atra* provided significant reduction in seedling mortality and root infection caused by *L. theobromae* on bottle gourd. *G. virens* caused maximum reduction in seedling mortality and root infection followed by *T. harzianum* and *T. viride*. In contrast to in vitro effect, seed germination was significantly increased to 82% where *T. harzianum* and *T. viride* were used. Similarly plants achieved maximum size where *G. virens*, *T. harzianum* and *T. viride* were used (Figure 3b).

The role of microorganisms as biocontrol agents is very significant and some of them provide long term natural control. In the present study use of *T. harzianum*, *T. viride*, *G. virens*, *B. subtilis* and *S. atra* against *L. theobromae* showed better results in the control of pre- and post-emer-

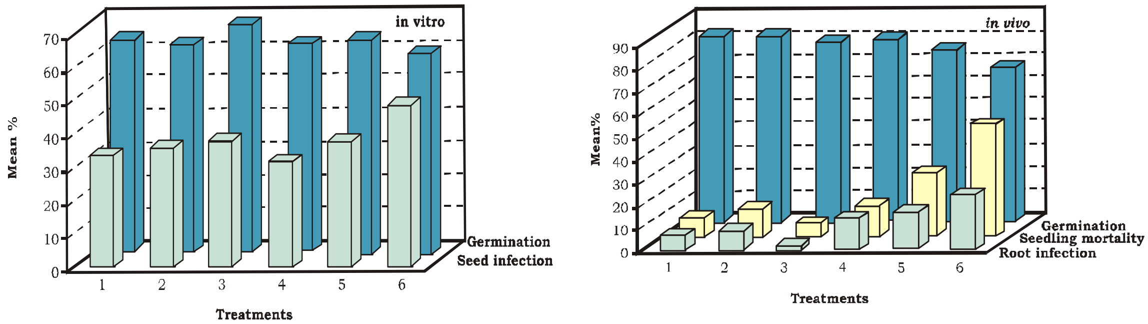

Figure 3. Effect of microbial antagonists on in vitro and in vivo seedling and root by *Lasiodiplodia theobromae* on bottle gourd. (1= *Trichoderma harzianum*, 2= *T. viride*, 3= *Gliocladium virens*, 4= *Bacillus subtilis*, 5=*Sachybotrys atra*, 6= Control)

gence infection in seedling of bottle gourd both in vitro and in vivo. Antagonist *G. virens* has been found most effective to reduce seed and root infection in vivo whereas *B. subtilis* performed best to reduce seed and seedling infection of bottle gourd in vitro. *B. subtilis* has been reported to produce antibiotics which act as surfactants and destroy the selective permeability of the hyphal cell membranes (Olsen, 1965; Swinburne et al., 1975).

The efficacy of bioagent varied with the host, the pathogen and field locations (Shahzad, 1994). Microscopic investigation demonstrated direct parasitism and coiling of *T. harzianum* and *T. viride* around hyphae of *L. theobromae*, causing swollen, deformed, shortened, or rounded cells of the pathogen. Granulation of cytoplasm and disintegration of the hyphal walls of *L. theobromae* also were noted in dual culture. *T. viride* reduced rotting by 29.07 to 65.06% in artificially inoculated banana fruits (Mortuza and Ilag,1999).

Treatment of fruits of banana with conidia and culture filtrates of *Trichoderma* sp., prior to inoculation with the pathogens (*L. theobromae*, *F. oxysporum* and *C. musae*) provided a better control than their simultaneous application (Adebesin et al.,2009). A broad spectrum of antifungal compounds by *Bacillus* spp., have been identified and described (Pusey, 1990) and mode of action of the well known antagonist *B. subtilis* has been attributed to antibiosis (Nandi and Sen, 1953; Vaseduva and Chakravarti, 1954). However in nature more than one type of interaction can occur between microbial populations, depending on microbial density (Atlas and Barthe, 1987). Blakemen and Brodie (1977) postulated five basic mechanisms viz., direct parasitism, the production of extracellular antibiotics or other substances, competition on the host and stimulation of host defenses.

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