

***In vitro* Evaluation of antagonistic effect of Bio Control Agents (BCA) against mango dieback incited by *Lasiodiplodiatheobromae*(Pat.) Griffon &Maubl**

Abstract

Mango (*Mangifera indica* L.) is one of the most important tropical fruit crops, belongs to family anacardiaceae. Mango is infected by number of diseases at all stages of its development, among them dieback caused by *Lasiodiplodiatheobromae* (Pat.) Griffon &Maubl. is considered to be the most destructive disease, leading to significant yield loss and low fruit quality of mango due to slowly wilting of tree. The present investigation was carried out to evaluate the inhibitory activity of different bio-control agents (BCA) against *L. theobromae*. Different bioagents viz., *Trichoderma viride* NAU isolate, *T. harzianum*, NAU isolate, *Pseudomonas fluorescens* NAU isolate, *Bacillus subtilis* NAU isolate and Rhizospheric actinobacteria were tested by dual culture technique. Among them, highest per cent growth inhibition was found in *T. viride* followed by *T. harzianum* and Rhizospheric actinobacteria.

P. fluorescens was found least effective against pathogen. The results indicated that among all the BCA, *T. viride* was most effective against *L. theobromae*. Hence, it can be explored further for management of mango dieback.

Key words: Mango, Die back, Bio-control agent, *Lasiodiplodia*, *Trichoderma*

Introduction:

Mango (*Mangifera indica* L.), is a highly significant tropical fruit that is a member of the anacardiaceae family. The mango has earned the title of the "King of fruit" due to its delectable exotic taste, delightful aroma, and its abundance of valuable nutritional compounds (Torres-León *et al.*, 2016). A single medium-sized mango, around 200 grams in weight, supplies an adult with more than their daily vitamin 'A' requirement and three-fourths of the needed vitamin 'C'. This recognition establishes it as one of the top-quality fruits.

While it is cultivated in at least 87 countries, nowhere is the value of mango as pronounced as in India, where it accounts for 40% of the total fruit production in the country. Mango farming is leading fruit crop farming in India where area and production is about 2.325 million ha and 20.822 million tons, respectively (Anon., 2020). Basically, mango is a tropical crop but it grows in wide range of soils, climate and altitude and is relatively easy to cultivate. Due to wide ranged adaptability of climatic conditions, mango is affected by a number of diseases at all stages of its development, right from the seedling in the nursery to the fruits in storage or transit such as dieback, anthracnose, malformation, powdery mildew etc. Among them, mango dieback is known to be caused by *Lasiodiplodiatheobromae* (Pat.) Griffon &Maubl. It is also called as tree decline disease. *L. theobromae* is an aggressive and vigorous pathogen and is able to infect exceptionally large number of host plants, causing various types of disease symptoms viz. black rootrot, fruit rot, dry rot, wood stain, dieback, stem end rot, seedling rot, graft union blight, twig blight and tip dieback, brown rot of panicle (Ragab *et al.*, 1971; Ploetz and Prakash, 1997). The organism is a wound parasite and capable of causing great damage under favourable conditions. Dieback is considered to be the most destructive disease, leading to significant yield loss and low fruit quality of mango (Ploetz,

2003). By considering the severity of the disease, this experiment has been carried out to find out effective and environment friendly management strategy.

Material and Methodology

Antagonistic effect of different bioagents viz. *Trichoderma viride*, *T. harzianum*, *Pseudomonas fluorescens*, *Bacillus subtilis* and rhizospheric actinobacteria was tested by dual culture technique for their antagonism against pathogen (Dennis and Webster, 1971). The rhizospheric actinobacteria were isolated using serial dilution technique (Reynolds, 2005) for which soil samples were collected from mango rhizosphere. The remaining bio-control agents were collected from laboratory of Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari.

Seven days old culture of the bio agents and the pathogen were employed by following dual culture method. Mycelial disc of 5 mm diameter cut from the periphery of both antagonist and test pathogen were placed at 50 mm apart from each other in Petri plates. In case of bacterial bio agents, mixed media of NA (Nutrient Agar) and PDA (Potato Dextrose Agar) were used in the ratio 50:50. Half portion of plates streaked with bacterial bio agent and 5 mm diameter mycelial disc of pathogenic fungi placed on the other side of Petri plates. In control, only test pathogen was kept in the centre of Petri plate. The Petri plates were incubated at $27 \pm 1^{\circ}\text{C}$ in BOD incubator. The observations on mycelial growth (mm) and per cent growth inhibition of test fungi were recorded after 7 days of incubation (Asalmolet. al., 1990).

$$I = \frac{C-T}{C} \times 100$$

Where,

I=Inhibition per cent

C=Colony diameter (mm) in control plate

T=Colony diameter (mm) in treated plate

Result and discussion:

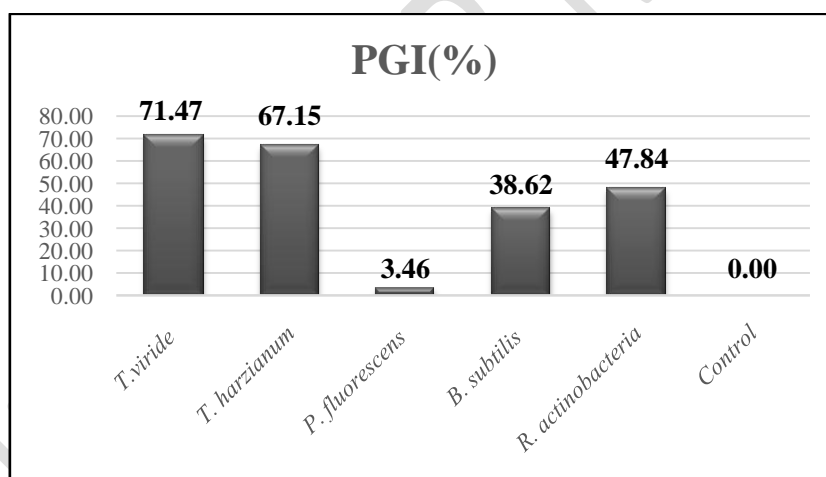
The result of present investigation revealed that, all the antagonists significantly inhibiting the mycelial growth of *L. theobromae*. The lowest mycelial growth (24.80 mm) was recorded in *T. viride* with highest per cent growth inhibition (71.47 %) as compared to other treatments at seven days after inoculation which is followed by *T. harzianum* (28.50 mm) with 67.15 per cent growth inhibition (PGI). The next best bio control agent in order of merit was rhizospheric actinobacteria (45.30 mm) 47.84 PGI. Remaining, bio control agents were found least effective in inhibiting the mycelial growth of *L. theobromae* with 38.62 PGI in *B. subtilis* and 3.46 PGI in *P. fluorescens* at 7th day after incubation.

Thus, all the bioagents were found antagonistic against *L. theobromae*. significantly superior over control. The lowest mycelial growth and highest per cent growth inhibition was observed in *T. viride* which was found at par with *T. harzianum* seven days after incubation. Significantly next best treatment was rhizospheric actinobacteria. The least inhibitory activity was found in *B. subtilis* and *P. fluorescens* having significant difference in their antagonism.

Table 1: Bio-efficacy of antagonists on the mycelial growth of *L. theobromae* in vitro

	Name of Antagonists	Mycelial growth (mm)	Per cent growth inhibition (%)
T ₁	<i>Trichoderma viride</i> NAU isolate	1.72 (24.80)	71.47
T ₂	<i>Trichoderma harzianum</i> NAU isolate	1.83 (28.50)	67.15
T ₃	<i>Pseudomonas fluorescens</i> NAU isolate	2.98 (83.80)	3.46
T ₄	<i>Bacillus subtilis</i> NAU isolate	2.41 (53.30)	38.62
T ₅	Rhizospheric actinobacteria	2.24 (45.30)	47.84
T ₆	Control	3.03 (86.80)	0.00
S. Em. ±		0.04	
C.D. at 5%		0.11	
C.V. %		3.15	

(Figures outside the parentheses are \sqrt{X} transformation values whereas figures in parentheses are original values DAI: Days after inoculation.)

**Fig 1: Per cent growth inhibition of *L. theobromae* by various bio control agents in vitro**

The similar results to this investigation reported by Adeniyi *et al.* (2013). They tested *T. viride* and *Aspergillus niger* against *L. theobromae* causing inflorescence blight of cashew by different methods dual culture technique *in vitro*. In all techniques, *T. viride* exhibited antagonism against *L. theobromae*. In the pathogen at the centre technique, *T. viride* had 90.48 per cent growth inhibition. In case of effectivity of rhizospheric actinobacteria, Kamil *et al.* (2018) found that rhizosphere actinobacterial isolates were capable of producing strong anti-fungal metabolites active against *L. theobromae* which have produced large zones of pathogen inhibition (>30mm), and were considered as the most promising BCA candidates. Sinha *et al.* (2018) also revealed that *Trichoderma* isolates produce various volatile and non-volatile compounds which play an important role in inhibition of *L.*

theobromae and
L.pseudotheobroma.

Summary and conclusion:

Based on present investigations, it is concluded that *T. viride* and *T. harzianum* have highest inhibitory property against *L. theobromae*. Therefore, they can be further explored under *in vivo* condition. Moderate antagonistic activity was seen in Rhizospheric actinobacteria and *B. subtilis*, *P. fluorescens* having lowest effect on the *L. theobromae*.

References:

- Adeniyi D. O., Adedeji, A. R., Oduwaye, O. F. and Kolawole, O. O. (2013). Evaluation of biocontrol agents against *Lasiodiplodiatheobromae* causing inflorescence blight of cashew in Nigeria. *J. Agric. Vet. Sci*, **5**(3):46-48.
- Anonymous (2020). Third advance estimates of area and production of horticulture crops. Department of Agriculture and Farmer's Welfare, GOI, pp. 1.
- Asalmol, M. N., Sen, B. and Awasthi, J. (1990). Role of temperature and pH in antagonism of *Aspergillus niger* and *Trichoderma viride* against *Fusarium solani*. *Proc. Indian Phyto-Pathological Society (Western Zone). On Bio-control of Plant Pathogen. M.P.A.U.*, Pune. pp. 11-13.
- Dennis, J. C. and Webster, J. (1971). Antagonistic properties of species groups of *Trichoderma*. Production of non-volatile antibiotics. *Trans. Br. Mycol. Soc*, **57**: 25-39.
- Kamil, F. H., Saeed, E. E., El-Tarabily, K. A. and Abu Qamar, S. F. (2018). Biological control of mango dieback disease caused by *Lasiodiplodiatheobromae* using streptomycete and non-streptomycete actinobacteria in the United Arab Emirates. *Front. microbiol.*, **9**:829.
- Ploetz, C.R.L. and Om, Prakash. (1997). Foliar, floral and soil borne diseases. In: The Mango (eds. Litz, R.E). *CAB, International, Wallingford, UK*, pp. 281-325.
- Ploetz, R. C. (2003). "Diseases of mango," in *Diseases of Tropical Fruit Crops*, ed R. C. Ploetz (St. Paul, MN: APS Press), 327–363.
- Ragab, M. M., Sabet, K. A. and Dawood, N. A. 1971. *Botryodiplodiatheobromae* Pat. The cause of fruit rot and die back of mango in A.R.E. *Agric. Rev.*, Cairo **49**: 81-97.
- Reynolds, J.(2005). Serial dilution protocols., *American Society for Microbiology: Washington*,1-7.
- Sinha, B., Bui, R., Sobita Devi, Ph., Salam, R., Dinesh, K. and Chakma, T. (2018). *In vitro* Studies on efficacy of some plant extracts and biocontrol agents against *Lasiodiplodiatheobromae* and *Lasiodiplodiapseudotheobromae*. *Int.J.Curr.Microbiol.App.Sci*, **7**(7): 448-457.
- Torres-León, C., Rojas, R., Contreras-Esquivel, J. C., Serna-Cock, L., Belmares-Cerda, R. E., and Aguilar, C. N. (2016). Mango seed: Functional and nutritional properties. *Trends in Food Sci. & Tech.*, **55**:109-117.