*Available online at* www.ijpab.com

**ISSN: 2320 – 7051**

*Int. J. Pure App. Biosci.* **3(3):** 318-323 (2015)

*Research Article*

International Journal of Pure & Applied Bioscience

Peer-Reviewed, Refereed, Open Access Journal

# Antagonistic Activity of Fungal and Bacterial Biocontrol Agents against Postharvest Pathogens of Major Fruits from Dharwad, Karnataka Region

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Received: 13.05.2015 | Revised: 14.06.2015 | Accepted: 26.06.2015

**ABSTRACT**

*Postharvest diseases cause huge losses to harvested fruits and vegetables during transportation and storage. Synthetic fungicides are primarily used to control postharvest decay losses. Biological control agents involving fungi and bacteria are known to control many postharvest pathogens. In the present study, 2 bacterial antagonists and 6 fungal antagonists were tested for their efficacy to inhibit the mycelial growth of six postharvest pathogens viz., Alternaria alternata, Aspergillus niger, Botryodiplodia theobromae, Colletotrichum gloeosporioides, Colletotrichum musae and Fusarium musae. Maximum mycelial growth inhibition of Alternaria alternata (75.21% & 74.45%) by Trichoderma harzianum and Trichoderma virens isolate 2 respectively, Aspergillus niger by Bacillus subtilis (77.00%) and Trichodenna harzianum (76.72%), B. theobromae by Trichodenna viride and T. virens isolate 2 (74.34%). C. gloeosporioides, was inhibited by Bacillus subtilis (88.88%), Pseudomonas fluorescens (86.67%), Trichoderma viride (83.32%) and Colletotrichum musae by Bacillus subtilis (84.45%), F. moniliforme by B. subtilis ( (83.40%). Biocontrol agents can be further used in integrated disease management programmes against postharvest pathogens.*

***Key words:*** *Postharvest pathogens - Biocontrol agents - Inhibition - Trichoderma - Bacillus- Pseudomonas.*

## INTRODUCTION

Postharvest diseases cause considerable losses to harvested fruits during transportation and storage. Postharvest losses in mango (17-36%), banana (12-14%), citrus (8.30-30.70%), grapes (23-30%) have been reported from India (Madan and Ullasa, 1993). Fungicides are a primary means of controlling postharvest diseases (Eckert and Ogawa, 1985).

**Cite this article:** Venkata Ramesh, K., Antagonistic Activity of Fungal and Bacterial Biocontrol Agents against Postharvest Pathogens of Major Fruits from Dharwad, Karnataka Region, *Int. J. Pure App. Biosci.* **3(3):** 318-323 (2015).

However, the recent trend is shifting toward safer and more eco-friendly alternatives for the control of postharvest decays of various biological approaches, the use of antagonistic microorganisms is becoming popular throughout the world (Sharma *et. al.*, 2009). Basic approaches are available for using microorganisms to control postharvest diseases: use and management of the beneficial microflora that already exist on fruit and vegetable surfaces or the artificial introduction of antagonists against postharvest pathogens. In the present study, 2 bacterial biocontrol agents and 6 fungal biocontrol agents were tested *in vitro* against 6 postharvest pathogens for their effectiveness in inhibiting the mycelial growth.

## MATERIALS AND METHODS

Fruits mango, banana, citrus, grapes and pomegranate showing symptoms of postharvest diseases) were collected from Dharwad market and nearby fields. Fungi were isolated by following standard tissue isolation method. Pathogenicity of the organisms was proved by proving Koch's postulates. Biocontrol agents viz., *Trichoderma harzianum* Rifai, *T. viride* Pers. ex. Gray, *T. pseudokoningi* Rifai, *T. virens* (Miller, Giddens & Foster) v. Arx isolates 1 and 2, *T. reesei* Simmons, *Bacillus subtilis* Cohn and *Pseudomonas fluorescens* Migula were tested for their efficacy in inhibiting the mycelial growth of six postharvest fungal pathogens *Alternaria alternata, Aspergillus niger, Botryodiplodia theobromae, Colletotrichum gloeosporioides, Colletotrichum musae* and *Fusarium moniliforme.*

Antifungal activity of biocontrol agents in inhibiting the mycelial growth of postharvest fungal pathogens was tested using the poisoned food technique. Both the biocontrol agents and test fungus were cultured on potato dextrose agar medium in order to get fresh and active growth. Bacterial agents were maintained on nutrient agar. About 20 ml of PDA was poured into sterile petriplates and allowed to solidify. From the previously grown young cultures of bioagents and test pathogens, a 0.5 cm mycelial discs of fungal pathogens and respective biocontrol agents were transferred aseptically to petriplates simultaneously by leaving sufficient space in between the two discs. In case of bacterial biocontrol agents, mycelial discs of the test fungus were kept at opposite ends and bacterium was streaked at the center. A pathogen disc alone placed at the center of the plates served as control. Colony diameter of both the test fungus and bioagents were measured after the control plate is fully covered and per cent inhibition is calculated by following the method given by Vincent (1927).

## RESULTS AND DISCUSSION

Antifungal activity of biocontrol agents was tested against six postharvest fungal pathogens *Alternaria alternata, Aspergillus niger, Botryodiplodia theobromae, Colletotrichum gloeosporioides, Colletotrichum musae* and *Fusarium moniliforme* was tested. The results on the effect of biocontrol agents on inhibition of mycelial growth and spore germination are presented below (Table 1 and Fig. 1).

*In vitro* evaluation of biocontrol agents against *Alternaria alternata* revealed that all the biological control agents evaluated were found to inhibit the mycelial growth. *Trichoderma harzianum* (75.21%) *Trichoderma virens* (=*GIiocladium virens*) isolate 2 (74.45%) were found to be most effective. *Pseudomonas fluorescens* (70.78) was also found to b*e* effective as a bacterial antagonist against *A. alternata* (Plate 1). *Bacillus subtilis* was most effective against *A. niger* by inhibiting up to 77 percent followed by *Trichodenna harzianum* (76.72%), *T. viride* (76.65 %,), *T. virens* isolate 2 (74.00%) were also found to be effective.

*Trichodenna viride* and *T. virens* isolate 2 were equally effective in inhibiting *Botryodiplodia theobromae* (Plate 2) to the extent of 74.34 per cent. Among the bacterial antagonists *Bacillus subtilis* inhibited up to 55.55 per cent while *P. fluoroscens* was not found to show any inhibitory effect on *B. theobromae* as the pathogen was observed to grow freely inspite of the presence of the bacterial culture. Evaluation of biological control agents against *Colletotrichum gloeosporioides* also gave promising results. *Bacillus subtilis* (88.88%), *Pseudomonas fluorescens* (86.67%), *Trichoderma viride* (83.32%), *T. reesei* (74.42%) were found to be more effective against *C. gloeosporioides* (Plate 3).

In case of *Colletotrichum musae, Bacillus subtilis* (84.45%) was found to be more antagonistic when compared to others. *Pseudomonas fluorescens*, (79.30%), *Trichoderma viride* (77.02%), *T. virens* isolate 2 (71.10%), *T. harzianum* (70.30%) were also found to be effective.

*Bacillus subtilis* was also found to be most effective antagonistic organism against *Fusarium moniliforme* inhibiting up to 83.40 per cent followed by *Trichoderma harzianum* (77.41%), *T. viride* (73.20%), *T. virens* isolate 1 (72.12%) and *T. virens* isolate 2 (71.10%). *Pseudomonas fluorescens* (6.71%) was found to be least effective (Plate 4).

Antagonistic nature of *Trichoderma spp*. against plant pathogens has been well documented by Cook and Baker (1983). Antagonistic nature of *P. fluorescens* has also been well documented. *P. fluorescens* is known to produce a variety of antagonistic secondary metabolites like production of siderophores (Fravel, 1988) and antibiotics like phenozines, pyroluterin. pyrolnitrin (Vidyasekharan and Muthamilan, 1995). Antagonistic effect of *Trichodermaa spp*. against *A. niger* has been reported by earlier workers. Bharat Rai *et al.* (1980) reported that *T. viride* was found to be parasitic on *Aspergillus.* Similar reports of effectiveness of *Trichoderma spp*. against *Aspergillus* were reported by Prabhu and Urs (1998), Calistru *et al*. (1997). Antagonism of *Trichoderma viride* against *B. theobromae* has been reported by Bhuvaneswari (1999) and Shirshikar (2002). Several other workers, Patil (1992), Aurangueren *et al.* (1994), Majumdar and Pathak (1995) have also reported the antagonistic nature of *Trichoderma spp*. against *B. theobromae*.

Antagonistic property of *B. subtilis* against *Colletotrichum gloeospohoides* has been reported by Arras (1993). Koomen and Jeffries (1993) reported that *Bacillus cereus* and *Pseudomonas fluorescens* inhibited *C. gloeosponoides*. The present findings were also in agreement with the reports of Bhuvaneswari (1999) and Shirshiknr (2002) who reported that *T. viride* exhibited more antagonistic activity than *Trichoderma harzianum* and *T. viride* over grew the colonies of *C. gloeosporioides* culture *in vitro*. In the present investigations, only *T. viride* was found to overgrew the *C. gloeosporioides* colony. This difference in the antagonism may be attributed to the variability in the isolates of antagonists or the pathogen itself.

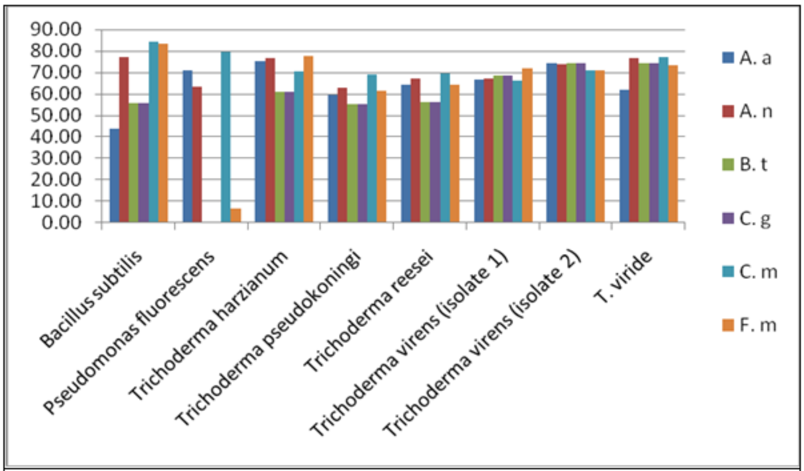
Different workers have reported the effectiveness of *B. subtilis* and *P. fluorescens* against *Colletorichum spp*. Arras (1993) reported the antagonistic nature of *B. subtilis* on *C. gloeosporioides*. Chidanandaswamy (2001) reported that, *Pseudomonas fluorescens* was effective in inhibiting *C. capsici*. He also reported that, *T. viride, T. harzianum*, were also effective against *C. capsici*. Efficacy of *T. viride, T. harzianum, B. subtilis* against *F. moniliforme* has been reported by Deshmukh (1997). Calistru *et al*. (1997) also reported the efficacy of *Trichoderma spp*. against *F. moniliforme*.

**Table 1: Antagonistic activity of bacterial and fungal biocontrol agents against postharvest pathogens**

| S. No | Biocontrol agent | Percent inhibition of mycelial growth | | | | | |
|---|---|---|---|---|---|---|---|
| | | ***A. a*** | ***A. n*** | ***B. t*** | ***C. g*** | ***C. m*** | ***F. m*** |
| 1 | *Bacillus subtilis* | 43.99 (40.51) * | 77.00 (61.35) | 55.55 (47.52) | 88.88 (70.46) | 84.45 (66.75) | 83.40 (65.94) |
| 2 | *Pseudomonas fluorescens* | 70.78 (57.25) | 63.10 (52.28) | 0.00 (1.00) | 86.67 (68.56) | 79.30 (62.93) | 6.71 (14.97) |
| 3 | *Trichoderma harzianum* | 75.21 (60.13) | 76.72 (61.12) | 61.09 (47.88) | 73.30 (58.89) | 70.30 (56.98) | 77.41 (61.62) |
| 4 | *Trichoderma pseudokoningi* | 59.60 (50.50) | 62.82 (51.41) | 55.40 (47.51) | 74.40 (59.60) | 68.89 (56.07) | 61.40 (51.59) |
| 5 | *Trichoderma reesei* | 64.41 (53.37) | 66.91 (54.92) | 56.30 (47.57) | 74.42 (59.60) | 69.29 (56.32) | 64.13 (53.15) |
| 6 | *Trichoderma virens* (isolate 1) | 66.68 (54.74) | 67.12 (54.98) | 68.38 (56.33) | 67.80 (55.43) | 66.05 (54.31) | 72.12 (58.18) |
| 7 | *Trichoderma virens* (isolate 2) | 74.45 (59.63) | 74.00 (59.32) | 74.34 (58.68) | 73.34 (58.89) | 71.10 (57.44) | 71.10 (57.48) |
| 8 | *T. viride* | 62.13 (51.57) | 76.65 (61.08) | 74.34 (58.68) | 83.32 (65.89) | 77.02 (61.14) | 73.20 (58.82) |
| 9 | SEm ± | 0.43 | 0.39 | 0.09 | 0.47 | 0.39 | 0.64 |
| 10 | CD at 1% level | 1.82 | 2.53 | 1.39 | 1.99 | 1.64 | 2.71 |

*A. a - Alternaria alternata, A. n - Aspergillus niger,*
*B. t - Botryodiplodia theobromae, C. g - Colletotrichum gloeosporioides,*
*C. m - Colletotrichum musae, F. m - Fusarium moniliforme*

*Figures in the parentheses are angular transformed values




*A. a - Alternaria alternata, A. n - Aspergillus niger,*
*B. t - Botryodiplodia theobromae, C. g - Colletotrichum gloeosporioides,*
*C. m - Colletotrichum musae, F. m - Fusarium moniliforme*

**Fig. 1:** Percent inhibition of mycelial growth of postharvest pathogens by biocontrol agents


**Plate 1: Antagonistic activity of biocontrol agents against *A. alternate***

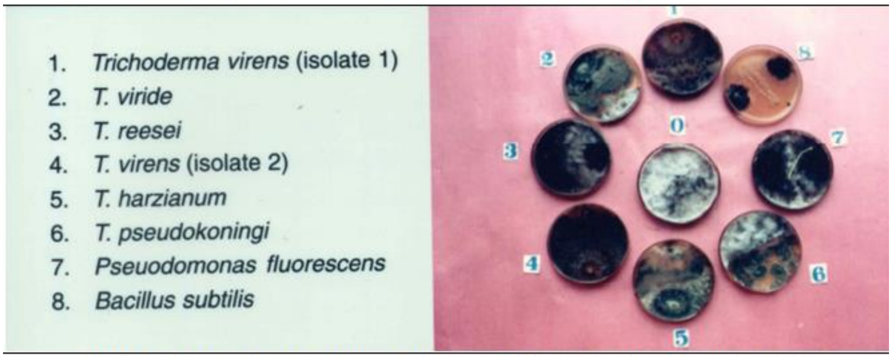



**Plate 2: Antagonistic activity of biocontrol agents against *B. theobromae***

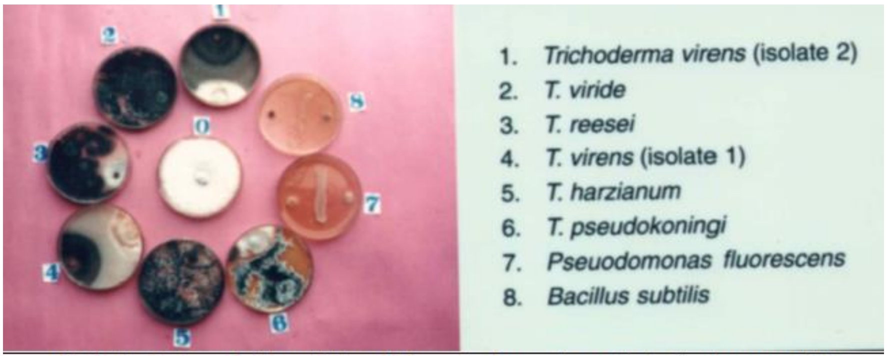



**Plate 3: Antagonistic activity of biocontrol agents against *C. gloeosporioides***

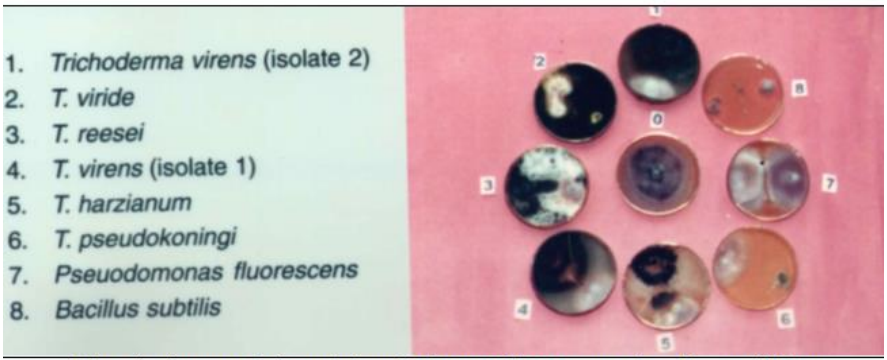



**Plate 4: Antagonistic activity of biocontrol agents against *F. moniliforme***

## CONCLUSION

It can be concluded from the present investigations that, biocontrol agents are effective against postharvest pathogens and there are variations in their effectiveness which can be attributed to the variation in isolates or the factors associated with the ability of the pathogen to overcome the antagonism.

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