Journal of Yeast and Fungal Research Vol. 4(1), pp. 5-11, February 2013
Available online at http://www.academicjournals.org/JYFR
DOI: 10.5897/JYFR12.029
ISSN 2141-2413 ©2013 Academic Journals

Full Length Research Paper

Antagonistic confrontation of *Trichoderma* spp against fruit rot pathogens on Sapodilla (*Manilkara zapota* L.)

U. N. Bhale[1]*, P. M. Wagh[2] and J. N. Rajkonda[3]

[1]Research Laboratory, Department of Botany, Arts, Science and Commerce College, Naldurg, Tq. Tuljapur, Osmanabad District, 413602 (M.S.) India.
[2]Department of Biology, S. S. and L. S. Patkar College of Arts and Science, Goregaon (W), Mumbai- 400063 (M. S.) India.
[3]Department of Botany, Yeshwantrao Chavan College, Tuljapur, Osmanabad District, 413601 (M. S.) India.

Accepted 20 December, 2012

Antagonistic potentials of five *Trichoderma* species that is *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma pseudokoningii* and *Trichoderma virens* were tested against fruit rots pathogens of sapodilla (*Manilkara zapota* L.) under laboratory conditions. Dual culture experiment of tested pathogens and *Trichoderma* spp revealed that, the percent inhibition of *T. koningii* (57.70%) and *T. harzianum* (54.40%) proved to be more than 50% antagonistic over control in case of *A. niger*. Similarly, in case of *R. solani*, *T. koningii* (67.07%) showed eloquent antagonistic activity as compared to others. In *G. candidum*, *T. pseudokoiningii* (75.07%) and *T. viride* (74.40%) have highly inhibited the radial growth over control followed by others. In case of *R. solani*, only *T. koningii* overgrew beyond 60% (R_3 scale). The results of this study identify *T. koningii* and *T. pseudokoningii* as promising biological control agents for further testing against post harvest disease in fruits.

Key words: *Manilkara zapota,* dual culture, fruit rot pathogens*, Aspergillus niger, Rhizoctonia solani, Geotrichum candidum, Trichoderma viride*, *T. harzianum*, *T. koningii*, *T. pseudokoningii, T. virens.*

INTRODUCTION

Sapodilla (*Manilkara zapota* L.) is one of the edible fruits cultivated all over India. In India it ranks fifth position in production and consumption next to mango, banana, citrus and grapes. It is also commercially important because it is a source of chicle, the principle ingredient in chewing gum. It is a rich source of sugar, protein, phenol, carotenoids, amino acids, pectin, vitamin C and mineral like Phosphorus, Calcium, Iron and Magnesium (Moore and Stearn, 2007). At least 50% of total production of fruits and vegetables in the country is lost due to wastage and value destruction, and the cost of this wastage is estimated to be Rs. 23,000 crorers each year. As per the specifications of National Institute of Nutrition (NIN), at least 300 g of fruits and vegetables are to be consumed by an individual per day for balanced diet (Roy, 2001). Sapodilla fruits are highly sensitive due to soft texture, therefore exogenous agents especially fungi, that affect physiology, morphology and biochemistry of fruits and thus ultimately causes severe loss to the fruit seller (Arya, 2011). Chemical control of pathogens provides certain degree of control but at the same time have adverse effects on environmental pollution (Charaya, 1993; Sankaram, 1999; Sokhi et al., 2000; Miller, 2004). In recent years the need to develop biologically ecofriendly disease control measures as an alternative to chemicals has become a priority of scientists worldwide. Therefore, it is important to find a practical, economic and non-toxic method to prevent fungal deterioration of stored food. Biological control of phytopathogens is an eco-friendly and cost effective approach. Hence, it should become an

*Corresponding author. E-mail: unbhale2007@rediffmail.com.

important component of plant disease management practices. Fungal antagonist that is, *Trichoderma* was evaluated as potential bio-control agent against number of fungal phytopathogens. Species of the genus *Trichoderma* are well documented fungal biocontrol agents (Papavizas, 1985; .Elad and Kapat, 1999; Howell, 2002). The antagonistic action of *Trichoderma* species against phytopathogenic fungi might be due to either by the secretion of extracellular hydrolytic enzymes (Chet, 1987; Di Pietro et al., 1993; Schirmbock et al., 1994) or by the production of antibiotics (Dennis and Webster, 1971a; Dennis and Webster, 1971b; Claydon et al., 1987; Howell, 1998). The effectiveness of biocontrol with *Trichoderma* spp. has also been shown by other investigators against *Penicillium digitatum* on citrus fruit (Borras and Aguilar, 1990), *B. cinerea* on grape berries (Elad, 1994), *Monilinia fructigena* on stone fruit (Hong et al., 1998), *B. cinerea*, *M. fructigena* and *P. expansum* on apple (Falconi and Mendgen, 1994), and *B. cinerea* and *P. expansum* on yams (*Dioscorea* spp.) (Okigbo and Ikediugwu, 2000).

The present investigation was made to evaluate *Trichoderma* spp against fruit rot pathogens such as *Aspergillus niger, Rhizoctonia solani* and *Geotrichum candidum* of sapodilla under laboratory conditions.

MATERIALS AND METHODS

The experiments of this work were carried out in the period 2008 to 2011.

Isolation and identification of test pathogen

Fruits showing symptoms of fungal infection were collected and symptomatology of the disease was studied under natural and *in vitro* conditions. Isolation of the pathogen was done from each of the distinct soft rots type of symptoms observed on fruits. Infected fruit parts (1 to 2 mm) were cut into small pieces by sterilized blade then surface sterilized with mercuric chloride (0.1%) for 1 min. The pieces were then washed thrice with sterilized distilled water and dried by sterilized blotting paper. These pieces were placed on Petri dishes (90-mm diameter) containing 20 mL potato dextrose agar (Peeled potato –200 g, Dextrose –20 g, Agar– 20 g and distilled water – 1000 ml, pH – 6.5) (PDA; Sd fine-CHEM Limited Mumbai, India) medium and incubated at 28 ± 2°C. The fungi namely, *Aspergillus niger, Rhizoctonia solani* and *Geotrichum candidum* were isolated and identified with the aid of standard literature available (Ellis, 1971; Barnett, 1960). The pathogenicity test of fungi was performed by the method of Thompson (1996).

Isolation of *Trichoderma* spp

Rhizospheric soils of irrigated and non irrigated plants were collected from different parts of Marathwada region of Maharashtra, India. From the rhizosphere soil samples, *Trichoderma* spp were isolated by using PDA and *Trichoderma* selective medium (TSM) by dilution plate technique (Johnson, 1957). The isolated species were identified up to species level based on colony characters, growth, structure of mycelium, conidiophores, phialides and conidia (Kubicek and Harman, 2002). All *Trichoderma* spp were purified by hyphal tip technique (Tuite, 1996). The isolated *Trichoderma* spp were maintained throughout the study by periodical transfers on PDA and TSM slants under aseptic conditions to keep the culture fresh and viable.

Dual culture experiment

Antagonistic efficacy of *Trichoderma* spp namely, *T. viride*, *T. harzianum*, *T. koningii*, *T. pseudokoningii* and *T. virens* were tested against the isolated pathogenic fungi by dual culture experiment (Morton and Stroube, 1955). *Trichoderma* spp and test fungi were inoculated 6 cm apart. Three replicates were maintained for each treatment and incubated at 28 ± 2°C for 7 days. Monoculture plates of both served as control. Seven days after incubation (DAI), radial growth of test fungi and *Trichoderma* spp were measured. Colony diameter of test fungi in dual culture plate was observed and compared with control. Percentage of radial growth inhibition (%RGI) was calculated by using the formula: 100 × [C - T / C], Where C = growth in control and T = growth in treatment (Vincent, 1947).

The degree of antagonism between each of the *Trichodema* species and test pathogens in dual culture was scored on scale of R1 - R5 that is, R1=*Trichoderma* completely overgrew pathogens (100% over growth); R2= *Trichodma* overgrew at least two-third pathogens (75% over growth); R3= *Trichoderma* colonizes on one half of the pathogens (50% over growth); R4= *Trichoderma* and the pathogens contact point after inoculation and R5= Pathogens overgrow bioagent - *Trichoderma* (Bell et al., 1982).

Statistical analysis

Data describing *in vitro* antagonisms were statistically analysed using the main factor was the *A. niger, R. solani* and *G. candidum* isolates of fruit rots pathogen and the sub-factors were the *Trichoderma* species. Arcsine transformation of biological control (*Trichoderma* species) percentage was calculated by using the following formula:

$$Y = \arcsin \sqrt{p} = \mathrm{Sin}^{-1}\sqrt{p}$$

Where, p is the percentage of inhibition and Y is the result of transformation.

Statistical analysis of the experiments was performed using the Handbook of Biological Statistics (Mungikar, 1997; McDonald, 2008).

RESULTS AND DISCUSSION

Isolation and identification of test pathogens

Infected soft rots fruits showed light brown coloured patch in the centre surrounded by white or creamish boundary and at severity complete rottening of fruit took place. These fruits with symptoms were collected from different locations of Thane District of Maharashtra. The fruit rot pathogens such as *Aspergillus niger* V. Tieghem, *Rhizoctonia solani* Kuhn and *Geotrichum candidum* Link ex Fries, were isolated following protocols (Ellis, 1971).

Isolation of *Trichoderma* spp

Five species of *Trichoderma: T. viride* Pers. ex. Gray, *T. harzianum* Rifai, *T. koningii* Oudemans *T. pseudokoningii* Rifai and *T. virens* J. Miller, Giddenand Foster A.A., were isolated from irrigated and non-irrigated rhizospheric soils of Marathwada region of Maharashtra. Isolates were deposited at Department of Botany, Arts, Science and Commerce College, Naldurg.

Taxonomical and morphological characters

Trichoderma viride Pers. ex.

Colony grows rapidly, white to greyish or rarely yellowish, surface smooth becomes hairy, typical coconut odour is emitted in old culture. Mycelium hyaline smooth, branched and septate. Chlamydospores intercalary, globose, rarely ellipsoidal, 10 to 15 µm in diameter. Conidiophores arise in compact or loose tuft, main branches produced several side branches. Phialides are in false whorls beneath each terminal phialides, usually more than 2 to 3 phialides, 8 to 15 × 2 to 3 µm in size, curved, pin shaped, narrower at the base. Conidia are Globose or short ovoid broadly ellipsoidal with minute roughing at their wall, 3.5 to 4.5 µm in size, accumulated at the tip of each phialides, pale green, smooth.

***Trichoderma harzianum* Rifai**

Colony growing rapidly, white green, bright green to dull green. Mycelium is septate, colourless, smooth, 1.5 to 2.5 µm. Chlamydospores are mostly globose, smooth, 6 to 12 µm in diameter. Conidiophores are loose, tuft, main branch produced numerous side branches specially in lower portion. Phialides arise in false verticillate up to five in numbers, short, skittle shaped, narrow at the base, and attenuate abruptly sharp, pointed neck, 25 to 75 × 3 to 4 µm. Conidia are acuminated at the tip of the phialides, subglobose short, obovoid, often broad truncate base, smooth, pale green, much darker in mass, 2.8 to 3.2 × 2.5 to 2.8 µm.

***Trichoderma koningii* Oudemans**

Colony fast growing, greenish white, dull to dark green.Mycelium is hyaline, highly ramified, 2 to 5 µm in size. Chlamydospores is formed in submerged hyphae, globose, ellipsoidal to barrel shaped, up to 12 µm in diameter. Conidiophores are branched, compact or in loose tuft, main branch produced several side branches, in group of 2 to 3 at wide angles. Phialides are pin shaped, narrower at the base, attenuate towards apex, 7.5 to 12 × 2.5 to 3.5 µm. Conidia are elliptical, oblong, truncate base and rounded apex, pale green appear much darker in mass, 3 to 5 × 1 to 2 µm in size.

***Trichoderma pseudokoningii* Rifai**

Colony grows rapidly with very poor aerial growth. Mycelium is septate, smooth, colourless, 1 to 5 µm in size. Chlamydospores are infrequently in medium, globose, smooth, hyaline, 7 to 10 µm in diameter. Conidiophores are loosely tuft, may appear hairy at maturity, somewhat powdery with numerous long branches. Conidiophores branches irregularly formed, single or in opposite pairs or group of three. Phialides are in false whorls, opposite pair in group of four in apical portion, pin shaped, narrower at the base than middle, attenuated distinctly, abovate or spindle shaped, 5.5 to 8 × 2.5 to 3.5 µm in size. Conidia are short, sub cylindrical, almost oblong, ellipsoidal usually rounded, distally attenuate below, short, truncate base, green mass, 3.5 to 2 × 2 µm.

***Trichoderma virens* J. Miller, Gidden and Foster A. A.**

Colony grows rapidly, floccose, white to grayish colouration. Mycelium is whitish in colour, turning grey at maturity, irregularly branched. Chlamydospores are mostly globose to subglobose, smooth, 7 to 12 µm in diameter. Conidiophores are conidiophores sub hyaline, 30 to 300 µm long, 2.5 to 4.5 µm in diameter, towards base frequently unbranched for about half of the length, towards the apex, branching irregular. Phialides are ampulliform to lageniform, 4.5 to 10 × 2.8 to 5.5 µm, swelling in the middle, mostly arising in closely verticils of 2 to 5 or terminal branches. Conidia are broadly ellipsoidal to obovoid, 3.5 to 6.0 × 2.8 to 4.1 µm, dark green.

Dual culture experiment

Trichoderma spp effectively inhibited the mycelial growth of the fruit rot pathogens. Table 1 illustrates that, in case of *A. niger*, *T. koningii* (57.70%) and *T. harzianum* (54.40%) spp were found to be more than 50% antagonistic over control. In *R. solani, T. koningii (67.7%)* showed highest mycelial growth inhibition, but others showed below 50% antagonism. Similarly, *T. pseudokoningii (75.07%)* and *T. viride* (74.40%) showed significant results followed by others in *G. candidum.* Among the three fruit rot pathogens, only *G. candidum* showed better inhibition by *Trichoderma* spp. (Figures 1 and 2).

According to modified Bell's scale, *T. harzianum and T. koningii* did not progress beyond 60% (R3 scale) but remaining species failed to overgrow *A. niger.* In case of

Table 1. Evaluation of *Trichoderma* spp against fruit rots pathogens of sapodilla.

Trichoderma spp	Test Pathogens					
	Radial growth of *A. niger* (mm)	% Inhibition	Radial growth of *R. solani* (mm)	% Inhibition	Radial growth of *G. candidum* (mm)	% Inhibition
T. viride	50	44.40 (50.57)	50	44.40 (51.43)	23	74.40 (88.12)
T. harzianum	41	54.40 (62.58)	55	38.80 (42.19)	35	61.10 (72.02)
T. koningii	38	57.70 (67.31)	29	67.07 (80.00)	30	66.07 (79.18)
T. pseudokoiningii	52	42.20 (48.06)	50	44.40 (51.00)	22	75.55 (88.90)
T. virens	50	44.40 (50.98)	50	44.40 (50.57)	28	68.9 (80.44)
Control	89.22		90		89	
SEm± CD(p=0.05)	7.53 19.35		8.11 20.83		10.42 26.76	

Radial growth and percent inhibition values are means of three replicates. Figures in parentheses are arcsine transformed values of % inhibition. ± = Standard Error

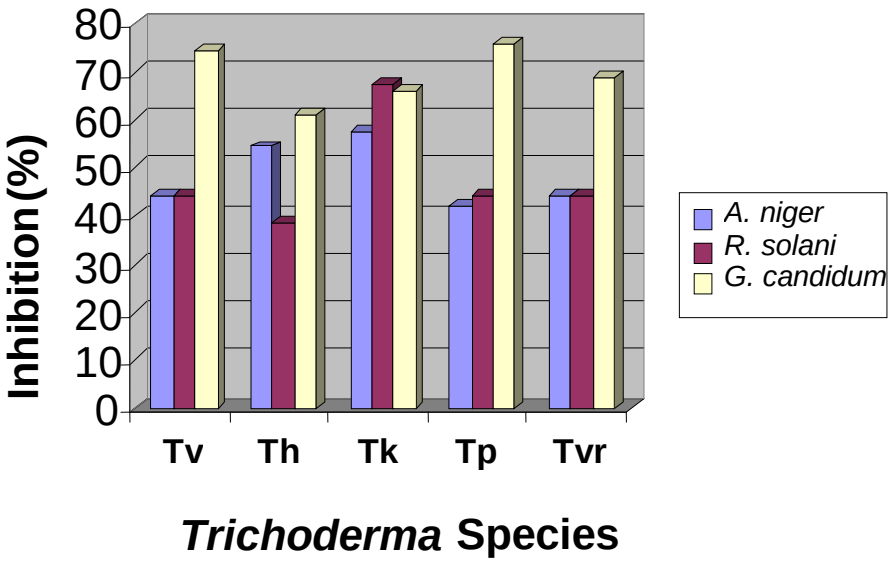

Figure 1. Evaluation of *Trichoderma* spp against fruit rots pathogens of sapodilla.

R. solani, only *T. koningii* overgrew beyond 60% (R_3 scale). In *G. candidum, T. pseudokoningii* and *T. viride* overgrew at least two third of pathogen (R_2 scale) but others were beyond 60% (R_3 scale) (Table 2).

Dual culture of pathogens and *Trichoderma* spp revealed that *T. viride* (Tv-2) (71.41%) highly inhibited the mycelia growth over control (Faheem et al., 2010). *T. viride* (86.2%) inhibited maximum growth of test fungus inciting collar rot of groundnut followed by *T. harzianum* (80.4%) (Harsukh et al., 2011). Seventeen *Trichoderma* strains were screened against *R. solani in vitro*, all strains including *T. harzianum, T. viride* and *T. aureoviride,* inhibited the growth of *R. solani* (Shalini, 2007). The antagonistic activity of the genus *Trichoderma* to *F. solani* and *R. solani* has been widely demonstrated (Lewis, 1998). The species of *Trichoderma* significantly inhibited the mycelial growth of plant pathogenic fungi (Rajkonda et al., 2011). Efficacy of *Trichoderma* species were reported against *Fusarium oxysporum* sp. *carthami* causing wilt of safflower and isolates no. 29 and 33 were found to minimize the growth of the pathogen as compared to others (Waghmare and Kurundkar, 2011).

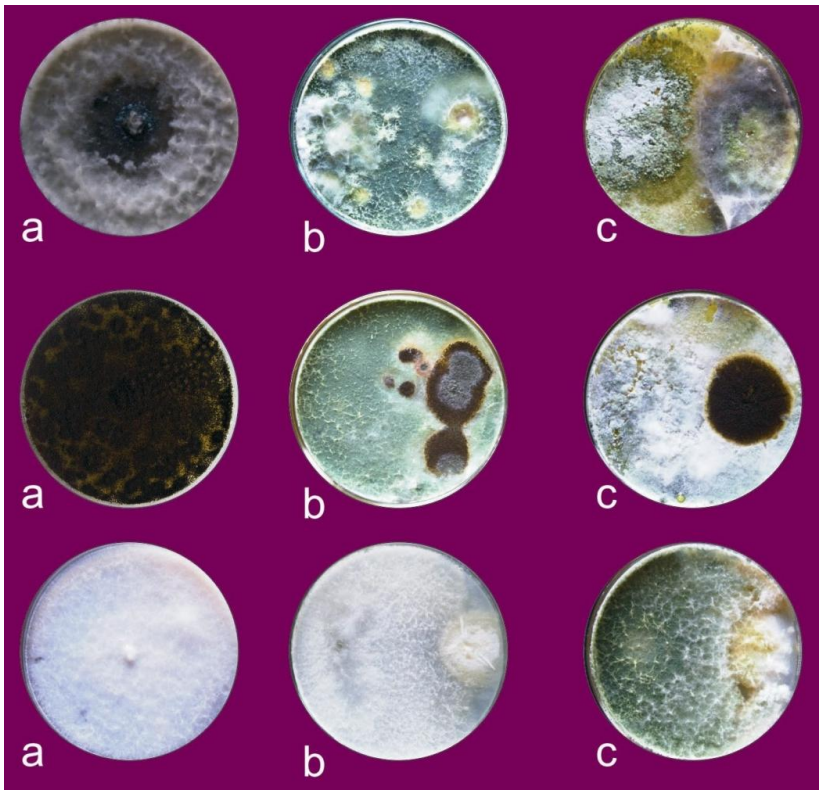

Figure 2. Antagonistic effects of *Trichoderma* spp on fruit rot pathogens of sapodilla. Row 1: *Rhizoctonia solani* (a-control, b-*T. koningii*, c-*T. harzianum*); Row 2: *Aspergillus niger* (a- control, b- *T.koningii*, c- *T.viride*); Row 3: *Geotrichum candidum* (a- control, b- *T.virens*, c- *T.koningii*).

T. harzianum was isolated from rambutan orchards in Sri Lanka and proved its antogonistic effect against *Botryodiplodia theobromae* (Sivakumar et al., 2000). *T. pseudokoningii* and *T. harzianum* have good antagonistic potentials against *C. destructivum* of cowpea *(*Akinbode and Ikotun, 2011). The results indicated that the treatment with the invert emulsion formulation of *T. harzianum* protected fruit from infection by the primary postharvest pathogens (*Rhizopus stolonifer*, *Botrytis cinerea*, and *Penicillium expansum)* of the fruits (grape, pear, apple, strawberry, and kiwifruit) tested for up to 2 months and reduced the diameters of decay lesion up to 86% and is a promising treatment to prolong the postharvest shelf-life of fresh fruit (Batta, 2007). Haran et al. (1996) reported dual culture experiments in which *T. harzianum* was overgrown by *R. solani* but hardly overgrown by *S. rolfsii* under the same conditions.

Conclusion

Trichoderma grows rapidly on a culture medium which should be beneficial during the confrontation. Our results concluded that the tested *Trichoderma* spp reduced the growth of all the tested three pathogens. *Trichoderma* spp showed significantly reduced the mycelial growth in *G. candidum*. We found an inhibition of mycelial growth of the pathogen tested. If there is direct contact between the two fungi, *Trichoderma* spp invaded colonies of fungal isolates sporulated there even after six days of confrontation. Therefore it can be incorporated for integrated disease management of fruit rot pathogens. Future research in this area should include *in vivo* studies on the effectiveness of the *Trichoderma* species as biocontrol agents. This could be done by *Tricoderma* into the soil of sapodilla plantations or by dipping the fruits into a suspension of *Trichoderma* after harvest. The effect of *Trichoderma* against other microorganisms especially against those that are beneficial to crops should also be investigated. Our future strategy will be to treat the soil by *Trichoderma* spp during plantation and after harvesting, sapodilla fruits can be dipped into a suspension of *Trichoderma* can be suggested.

ACKNOWLEDGEMENTS

Authors are thankful to the Director, Agharkar Research

Table 2. Evaluation of Trichoderma spp against fruit rots pathogens of sapodilla by dual culture,using Bell's scale*(R).

Trichoderma spp	Test pathogen		
	A. niger	*R. solani*	*G. candidum*
T. viride	R_4	R_4	R_2
T. harzianum	R_3	R_4	R_3
T. koningii	R_3	R_3	R_3
T. pseudokoiningii	R_4	R_4	R_2
T. virens	R_4	R_4	R_3

*Degree of antagonism. R1=Trichoderma completely overgrew pathogens (100% over growth); R2=Trichoderma overgrew at least two-third pathogens (75% over growth); R3=Trichoderma colonizes on one half of thepathogens (50% over growth); R4=Trichoderma and the pathogens contact point after inoculation; R5= Pathogens overgrow bioagent - Trichoderma.

Institute (ARI) Pune for the identification of *Trichderma* spp. and also thankful to UGC, New Delhi for financial assistance of major research project. The authors also thankfully acknowledged Principal Dr. S. D. Peshwe for providing laboratory facilities.

REFERENCES

Akinbode OA, T Ikotun (2011).Potentials of two *Trichoderma* species as antagonistic agents against *Colletotrichum destructivum* of cowpea. Afr. J. Microbiol. Res. 5(5):551-554.

Arya A (2011). Tropical Fruits- Diseases and Pests, KalyaniPublishers, New Delhi.

Barnett HL (1960). Illustrated genera of imperfect fungi. Burgress Publishing Company II eds, West Virginia.

Batta Yacoub A (2007). Control of postharvest diseases of fruit with an invert emulsion formulation of *Trichoderma harzianum* Rifai. Postharvest Biol. Technol. 43:143-150.

Bell DK,Wells HD, Markham CR (1982). *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens. Phytopathology 72(4):379-382.

Borras D, Aguilar RV (1990). Biological control of *Penicillium digitatum* on postharvest citrus fruit. Int. J. Food Microbiol. 11:179-184.

Charaya MU (1993). From DDT to Microbial pesticides. In: Pesticides pollution,(Eds.Kudesia, V.P. and Charaya, M.U.), Pragati Prakashan, Meerut, India pp. 207-220.

Chet I (1987). *Trichoderma* - application, mode of action and potential as a biocontrol agent of soil borne plant pathogenic fungi. In: Chet, I. (Ed.), Innovative Approaches to Plant Disease Control, John Wiley and Sons, New York, pp. 137-160.

Claydon N, Allan M, Hanson JR., Avent AG (1987). Antifungal alkyl pyrones of *Trichoderma harzianum*. Trans. Br. Mycol. Soc. 88:503-513.

Dennis C, Webster J (1971a). Antagonistic properties of species groups of *Trichoderma*. I. Production of non-volatile antibiotics. Trans. Br. Mycol. Soc. 57:25-39.

Dennis C, Webster J (1971b). Antagonistic properties of species groups of *Trichoderma*. II. Production of volatile antibiotics. Trans. Br. Mycol. Soc. 57:41-48.

Di Pietro A, Lorito M, Hayes C, Broadway K, Harman GE (1993). Endochitinase from *Gliocladium virens*. Isolation, characterization, synergistic antifungal activity in combination with gliotoxin. Phytopathology 83:308-313.

Elad Y (1994). Biological control of grape gray mold by *Trichoderma harzianum*. Crop Prot. 13:35-38.

Elad Y, Kapat A (1999). The role of *Trichoderma harzianum* protease in the biocontrol of *Botrytis cinerea*. Eur. J. Plant Pathol. 105:177-189.

Ellis MB (1971). Dematiaceous Hypomycetes. Commonwealth Mycological. Institute, Kew, Surrey, England.

Faheem Amin, Razdan VK, Mohiddin FA, Bhat KA, Saba Banday (2010). Potential of *Trichoderma* species as biocontrol agents of soil borne fungal propagules. J. Phytol. 2(10):38-41.

Falconi J, Mendgen K (1994). Epiphytic fungi on apple leaves and their value for control of the postharvest pathogens: *Botrytis cinerea*, *Monilinia fructigena* and *Penicillium expansum*. J. Plant Dis. Prot. 101:38-47.

Haran S, Schickler H, Oppenheim A, Chet I (1996). Differential expression of *Trichoderma harzianum* chitinase during mycoparasitism. Phytopathology. 86:980-985.

Harsukh G, Kalu Rakholiya, Dinesh Vakharia (2011). Bioefficacy of *Trichoderma* isolates against *Aspergillus niger* Van Tieghem inciting collar rot in Groundnut (*Arachis hypogaea* L.). J. Plant Prot. Res. 51(3):240-247.

Hong CX, Michailides TJ, Holtz BA (1998). Effects of wounding, inoculums density, and biological control agents on postharvest brown rot of stone fruits. Plant Dis. 82:1210-1216.

Howell CR (2002). Cotton seedling pre-emergence damping-off incited by *Rhizopus oryzae* and *Pythium* spp. and its biological control with *Trichoderma* spp. Phytopathology 92:177-180.

Howell CR (1998). The role of antibiosis in biocontrol. In: Harman GE, Kubicek CP(eds) *Trichoderma* & *Gliocladium*, vol. 2.Taylor & Francis, Padstow, pp. 173-184.

Johnson LA (1957). Effect of antibiotics on the number of bacteria and fungi isolated and fungi isolated from soil by dilution plate method. Phytopathology 47:21-22.

Kubicek CP, Harman GE (2002). *Trichoderma* and *Gliocladium* (vol. I). Basic biology, taxonomy and genetics. pp. 14-24.

Lewis JA, Larkin RP, Rogers DL (1998). A formulation of *Trichoderma* and *Gliocladium* to reduce damping-off caused by *Rhizoctonia solani* and saprophytic growth of the pathogen in soil less mix. Plant Dis. 82:501-506.

McDonald JH (2008). Handbook of Biological statistics. Sparky House Publishing, Baltimore, Maryland. pp. 160-164.

Miller GT (2004). Sustaining the Earth, 6th edition. Thompson Learning, Inc. Pacific Grove, California. Chapter 9, pp. 211-216.

Moore HE, Stearn (2007). Post harvest physiology and technology of Sapote mamey fruit. Post-harvest Biology and Technology.45:285-297.

Morton DJ, Stroube WH (1955). Antagonistic and stimulating effects of soil microorganisms upon sclerotium. Phytopathology 45:417-420.

Mungikar AM (1997). An Introduction to Biometry. Saraswati Printing Press, Aurangabad, pp. 57-63.

Okigbo RN, Ikediugwu FE (2000). Studies on biological control of postharvest rot in yams (*Dioscorea* spp.) using *Trichoderma viride*. J. Phytopathol. 148:351-355.

Papavizas GC (1985). *Trichoderma* and *Gliocladium*: biology, ecology and potential for biocontrol. Ann. Rev. Phytopathol. 23:23-54.

Rajkonda JN, Sawant VS, Ambuse MG, Bhale UN (2011). Inimical potential of *Trichoderma* species against pathogenic fungi. Plant Sci. Feed. 1(1):10-13.

Roy SK (2001). Strategic post harvest Management of fruits and vegetables. Indian Hortic. 45(4):4-7.

Sankaram A (1999).Integrated pest management: Looking back and forward. Curr. Sci. 77:26-32.

Schirmbock M, Lorito M, Wang YL, Hayes CK, Arisan-Atac I, Scala F, Harman GE, Kubicek CP (1994). Parallel formation and synergism of hydrolytic enzymes and peptaibolantibiotics, molecular mechanisms involved in the antagonistic action of *Trichoderma harzianum* against phytopathogenic fungi. Appl. Environ. Microbiol. 60:4364-4370.

Shalini S, Kotasthane AS (2007). Parasitism of *Rhizoctonia solani* by strains of *Trichoderma* spp. EJEAF Chem. 6:2272-2281.

Sivakumar D, Wilson Wijeratnam RS, Wijesundera RLC, Abeyesekere, M (2000). Antagonistic effect of *Trichoderma harzianum* on Postharvest Pathogens of Rambutan (*Nephelium lappaceum*). Phytoparasitica 28(3):240-247.

Sokhi SS, Singh PP, Grewal RK (2000). Environmental impact of

Fungicides in agroecosystem. In Pesticides and Environment, (Eds. Dhaliwal,G.S. and Balwinder Singh),Commonwealth Publishers, New Delhi,India. pp. 254-278.
Thompson AK (1996). Post -harvest technology of fruits and vegetables. Blackwell Science Ltd.London.
Tuite J (1996). Plant Pathological Methods. Fungi and Bacteria Burgess Pub. Co. Minneapolis, Minn. USA. 293 pp.
Vincent JM (1947). Distortion of fungal hyphae in the presence of certain inhibitors. Nature 150:850.
Waghmare SJ, Kurundkar BP(2011). Efficacy of local isolates of *Trichoderma* spp against *Fusarium oxysporum sp. carthami* causing wilt of safflower. Adv. Plant Sci. 24(1):37-38.