JOURNAL OF RECENT ADVANCES IN APPLIED SCIENCES (JRAAS) 28:100-109, 2013
ISSN 0970 1990, ST CODE 08 ,www.jraas.org. E MAIL: mhcdr@rediff.com
DISPLAYED ON LINE ON JULY. 2012

EFFICACY OF PLANT EXTRACTS AND BIOAGENTS AGAINST THREE FRUIT-ROT FUNGI OF BANANA (*Musa paradisiaca* L.)

SUPRIYA SARKAR*, S.GIRISHAM AND S.M.REDDY
Department of Microbiology, Kakatiya University, Warangal-506009-INDIA
*Corresponding Author Email: supriyasarkar4@gmail.com:

ABSTRACT

Efficacy of aqueous extracts of different plant parts and biocontrol agents was tested against the growth of three post-harvest fungal pathogens of banana (*Macrophomina phaseolina* R-4242, *Fusarium oxysporum* sp. QJC-1403 and *Nigrospora oryzae* NRRL 54030).The plant extracts when applied as pre- and post-inoculation treatments were effective in controlling the growth of the three pathogenic fungi. Extracts of medicinal plants like *Azadirachta indica*, *Eucalyptus globulus* and *Ocimum sanctum* were effective in inhibiting the growth of *N.oryzae*, while the extracts of *Allium cepa* and *Allium sativum* effectively inhibited the pathogenic activity of all three pathogens under investigation. Out of the different bioagents tried, *Penicillium citrinum* and *Streptomyces* spp. could effectively control the growth of the three fruit-rot fungi under *in vivo* conditions.
.

KEY WORDS: Banana, plant extracts, fruit-rot, pathogenic fungi, antagonistic activity.
.

INTRODUCTION

Fruits have been an important part of our food from pre-historic times, as they are a chief source of vitamins, minerals, fats, fibres, trace elements etc. Of various fruit and vegetable crops, bananas are of paramount importance in the human diet. Banana (*Musa paradisiaca* L.) is one of the most popular and staple fruits of India. Banana is the largest fruit crop accounting for 31.7% of total fruit production from 12.6% arable area with a total production of banana, estimated as 16.9 million tonnes /annum from 4.9 lakh ha. Banana, a typical climacteric fruit, softens rapidly once ripening is initiated [1]. Ripe fruits are susceptible to attack, by a variety of microorganisms to which they are resistant during their attachment to the plant. Generally the rot-producing fungi result in spoilage and wastage of fruits even before reaching the consumers.

Though the use of synthetic fungicidal chemicals in plant disease control has been promising in protecting the plants against the pathogens, but many of them proved to be health hazardous [2]. In view of these, the antimicrobial properties of some plant constituents are being exploited [3, 4, 5]. Several workers including- Godara and Pathak [6], Abraham and Prakasan [7], Prasad and Naik [8], Swami and Mukadam [9] and Sumangala *et al* [10] have screened different plant extracts for their antimicrobial properties against fungal pathogens. Mukhopadhyay *et al* [11] and Sheela *et al* [12] regard biological control, using microbial antagonists as a good alternative for the management of diseases in many crop plants. Application of microorganisms or microbial products for the control of fruit pathogens has been widely reviewed [13, 14, 15, 16, 17, 18]. In fruit market, usually more than 10 % of the total banana crop is destroyed due to post-harvest diseases particularly fruit rots [19]. The present investigation was carried out to test the efficacy of some plant extracts and bioagents against the three fruit-rot fungi under *in vivo* condition.

MATERIALS AND METHODS

In vivo evaluation of plant extracts

The present investigation was carried out in the Department of Microbiology, Kakatiya University, Warangal, (A.P.) during the year 2009. Evaluation of plant extracts and bioagents under *in vivo* conditions was assessed as suggested by [20]. Aqueous extracts of different plants viz *Annona squamosa*, *Azadirachta indica*, *Psidium guajava, Ocimum sanctum*, *Vinca rosea*, *Eucalyptus globulus*, *Allium cepa* and *Allium sativum* were prepared to test their antifungal activity. The plant extracts were obtained by grinding 10 gms of freshly collected leaves and bulbs with 100 ml of distilled water and filtering it through double layered muslin cloth. It was diluted with distilled water to 30 % and 50% concentrations.

Healthy fruits of banana were surface sterilized and inoculated separately with the pathogens by scalpel injury method [21]. Plant extracts (30% and 50%) were tested against the rot of banana as pre- and post-inoculation treatment. In the pre-inoculation treatment, the fruits were first dipped in plant extract for 30 minutes and then inoculated with the pathogen, while in the post-inoculation treatment the fruits were first inoculated with the pathogen and then treated with plant extract. Atleast five replicates were maintained. Untreated fruits served as control. The treated fruits were incubated at 25 ± 2°C in a humid chamber for 7 days. At the end of the incubation period the extent

Table -1 : Effect of plant extracts on fruit-rot of banana by three pathogenic fungi

Name of the plant	Parts of the plant	concentration of plant extract (in %)	*M. phaseolina* R-4242				*F. oxysporum* sp.QJC-1403				*N. oryzae* NRRL54030			
			Pre-inoculation dip		Post-inoculation dip		Pre-inoculation dip		Post-inoculation dip		Pre-inoculation dip		Post-inoculation dip	
			Area of infection (in Cm^2)	% of rot	Area of infection (in Cm^2)	% of rot	Area of infection (in Cm^2)	% of rot	Area of infection (in Cm^2)	% of Rot	Area of infection (in Cm^2)	% of rot	Area of Infection (in Cm^2)	% of rot
Allium cepa	B	30	1.2	3.0	0.7	2.5	1.0	4.6	0.4	4.0	0.9	5.3	0.7	5.1
		50	0.8	2.4	0.3	2.3	0.6	3.8	0.2	1.8	0.6	4.8	0.3	3.2
Allium sativum	B	30	0.9	3.4	0.6	3.2	0.7	4.9	0.4	4.0	0.6	4.7	0.5	4.1
		50	0.5	3.0	0.2	2.0	0.4	3.7	0.3	3.2	0.4	3.9	0.3	2.8
Annona squamosa	L	30	1.6	5.9	1.0	6.1	1.4	5.3	1.0	4.9	0.8	5.7	0.4	4.7
		50	1.0	4.4	0.6	3.6	0.8	5.0	0.4	4.1	0.3	3.6	0.2	2.9
Azadirachta indica	L	30	1.0	3.6	0.6	2.9	0.8	5.4	0.4	4.3	0.8	5.3	0.3	4.5
		50	0.7	2.2	0.3	2.0	0.5	4.5	0.2	3.2	0.3	4.3	0.2	1.9
Eucalyptus globulus	L	30	0.9	3.4	0.5	2.8	1.0	4.6	0.8	3.7	0.8	5.4	0.6	3.5
		50	0.4	2.5	0.3	2.6	0.7	3.6	0.3	2.6	0.4	4.4	0.2	1.9
Ocimum sanctum	L	30	1.5	6.7	1.4	5.1	1.6	6.7	1.3	5.4	1.0	5.2	0.9	5.0
		50	0.7	4.7	0.5	3.9	0.9	4.9	0.4	3.5	0.6	3.4	0.5	3.0
Psidium guajava	L	30	3.2	6.9	2.4	4.5	1.6	5.4	1.0	4.9	1.0	5.7	0.7	5.2
		50	2.9	5.3	1.8	3.4	1.1	5.1	0.9	3.4	0.6	4.1	0.3	3.4
Vinca rosea	L	30	0.9	2.8	0.6	2.4	1.2	4.6	0.7	3.6	1.1	5.8	0.9	5.0
		50	0.6	1.6	0.4	0.9	1.0	3.3	0.4	2.0	0.8	3.9	0.7	3.7
Control			5.4	12.2	5.4	12.2	4.9	11.8	4.9	11.8	4.2	10.3	4.2	10.3

L = Leaves;
B = Bulb

Table-1a: ANOVA- Effect of plant extracts on fruit-rot of banana by three pathogenic fungi

Sources of variation	Sum of Squares	df	Mean Square	P-value	F	Result
Between Groups	53930.719	126	428.022	< 0.001	9.230	S
Within Groups	5889.305	127	46.372			
Total	59820.024	253				

Table -2: Effect of different antagonistic microorganisms on three fruit-rot fungi of banana

Name of antagonistic microorganisms	*M. phaseolina* R- 4242		*F. oxysporum* sp.QJC-1403		*N. oryzae* NRRL-54030	
	Area of infection (in Cm^2)	Percentage of rot	Area of infection (in Cm^2)	Percentage of rot	Area of infection (in Cm^2)	Percentage of rot
Penicillium citrinum	0.9	0.8	1.4	0.9	0.5	1.0
Trichoderma viride	1.2	0.9	0.9	0.7	0.6	1.3
Streptomyces spp.	0.8	0.6	0.9	1.8	1.0	0.9
Pseudomonas fluorescens	1.6	1.2	1.5	1.9	0.7	1.4
Control	6.7	15.6	6.2	14.3	4.2	13.2

Table-2a: ANOVA -Effect of different antagonistic microorganisms on three fruit-rot fungi of banana

Sources of variation	Sum of Squares	df	Mean Square	P-value	F	Result
Between Groups	21.967	5	4.393	>0.05	.275	NS
Within Groups	383.440	24	15.977			
Total	405.407	29				

S-significant; NS-not significant

of the fruit-rot was assessed by scraping off the visibly infected part and the percentage of fruit-rot was calculated by the formula:

$$\text{Percentage of rot} = \frac{W - w}{W} \times 100$$

Where
W = Weight of the fruit before inoculation
w = Weight of the fruit after inoculation and incubation (after scraping off the rotted area)

In vivo evaluation of bioagents
Efficacy of antagonistic microorganisms in checking the fruit-rot activity of the pathogens under study was assessed by preparing the spore suspensions of 7 days old cultures of two antagonistic fungi (*Trichoderma viride* and *Penicillium citrinum*) and two bacteria (*Streptomyces* spp. and *Pseudomonas fluorescens*) in sterile water. Rest of the details of inoculation, treatment and incubation are similar to the method of evaluation of plant extracts mentioned earlier.

Statistical Analysis
All the experiments were repeated for five times. The statistical analysis (ANOVA) on the data was performed with the SPSS program version 12.0.

RESULTS AND DISCUSSION
Effect of plant extracts
Table 1 and 1a, Figure 1, 2 reveals that the plant extracts tried could check the fruit-rot caused by the three pathogenic fungi to a significant level. However, the degree of protection varied with the pathogen and plant extract. In general percentage of fruit-rot decreased with the increase in the concentration of plant extract. Pre-inoculation dip was less effective than the post-inoculation dip. Plant extracts of *Azadirachta indica* and *Eucalyptus globulus* were effective against *N.oryzae. F.oxysporum* and *M.phaseolina* were comparatively insensitive. The aqueous extracts (50%) of bulbs (*Allium cepa* and *Allium sativum*) also effectively inhibited the growth of all the three fungal pathogens under study. Though extracts of *Psidium guajava* and *Vinca rosea* were not effective at lower concentration in pre-inoculation dip, but inhibited the fruit-rot by the fungi under investigation to a considerable extent at higher concentration. In contrast to this, post-inoculation dip was very effective. The extracts of *Ocimum sanctum* were selective in action as they were effective only against *N.oryzae* when compared with other two pathogens. The extracts of *V.rosea* could effectively check the growth of *M.phaseolina* at higher concentration. Extracts of *Annona squamosa* were intermediate in its efficacy, as they could check pathogens activity to a limited extent. Thangavelu *et al* [22] and Babu Lal *et al* [19] have also reported the efficacy of plant extracts employed by them against *Colletotrichum musae* and *C. gloeosporioides* causing anthracnose of banana.

The present investigations revealed that the post-harvest diseases of fruits can be checked by the use of phytoextracts. The plant extracts are useful in the plant protection by inhibiting the activity of endo-enzymes or as they produce toxic metabolites which inactivates the pathogens.

Effect of bioagents
From Table 2 and 2a, Figure 3, 4 it is clear that the bioagents tried could control the growth of fruit-rot pathogens by their antagonistic activity which however, varied with the antagonist. *Penicillium citrinum* and *Streptomyces* spp. could control the fruit-rot caused by all the three fruit-rot fungi (*Macrophomina phaseolina* R-4242, *Fusarium oxysporum* sp. QJC-1403 and *Nigrospora oryzae* NRRL 54030) under investigation to a considerable extent. *Trichoderma viride* and *Pseudomonas fluorescens* were next in their degree of inhibition of growth of *N.oryzae*. On the other hand, fruit-rot caused by *F.oxysporum* was checked effectively by *T.viride* followed by *P.citrinum*, while *Streptomyces* spp. and *P. fluorescens* were responsible for protection of banana fruits to a moderate level. Similarly *T.viride* and *Streptomyces* spp. followed by *P.citrinum* and *P. fluorescens* could check the pathogenic activity of *M. phaseolina* in a descending order.
Babu Lal *et al* [19] could control fruit-rot of banana caused by *Colletotrichum gloeosporioides* using *Trichoderma* species. Dutta and Das [14] could control the collar-rot of tomato by using the species of *Trichoderma.* Sastry [20] also reported that *P.citrinum* and *Streptomyces* spp. were effective in checking the tuber rot of carrot caused by *M.phaseolina*.

CONCLUSION
Control of plant diseases by plant extracts is on ascendancy due to failure of fungicide efficacy and intoxicating nature of these chemicals. Fungicides can easily contaminate agriculture product, drinking water and soil and can directly enter food chain of human and animals intoxicating considerably. The management of diseases of fruits including the extensive use of plant extracts particularly of those plants, which have medicinal importance provide a scope for further use of these botanicals or their active compounds to control pathogenic fungi due to their fungistatic or fungicidal activity.

The eco-friendly measures of plant disease control when integrated with potent bioagents can also increase the bioefficacy and ensure complete disease management.

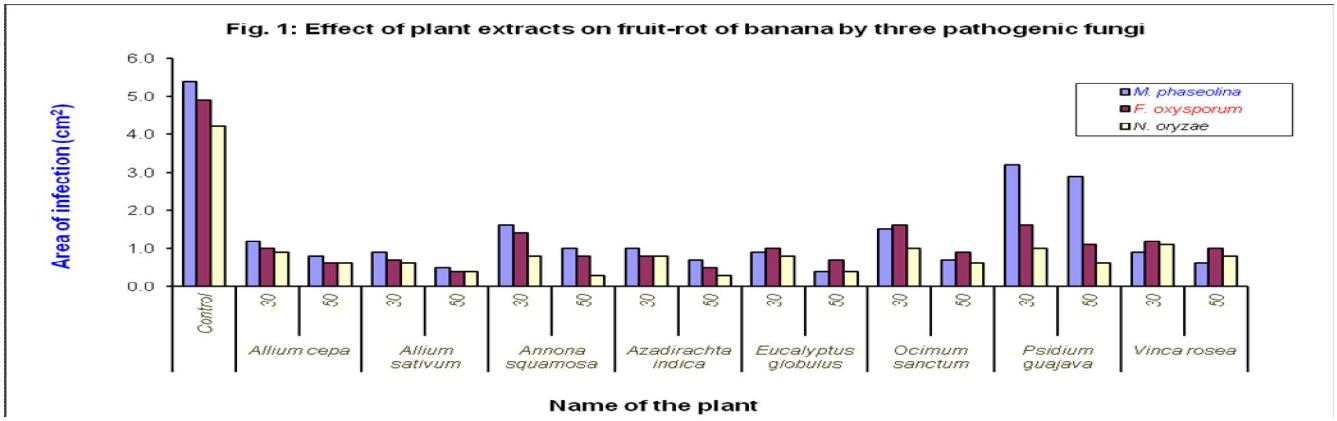
Fig. 1: Effect of plant extracts on fruit-rot of banana by three pathogenic fungi
Area of infection (cm²)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
M. phaseolina
F. oxysporum
N. oryzae
Control
30
50
Allium cepa
Allium sativum
Annona squamosa
Azadirachta indica
Eucalyptus globulus
Ocimum sanctum
Psidium guajava
Vinca rosea
Name of the plant

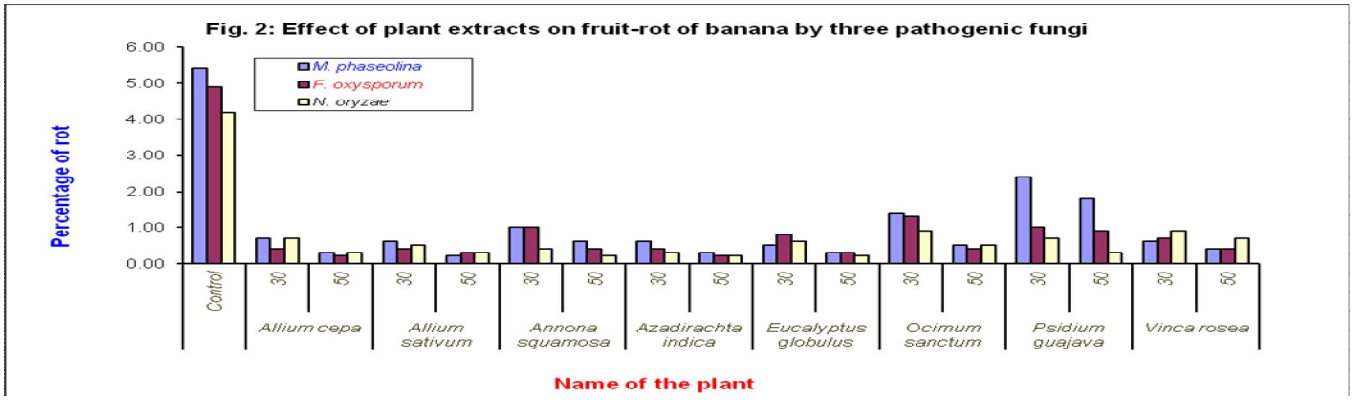
Fig. 2: Effect of plant extracts on fruit-rot of banana by three pathogenic fungi
Percentage of rot
6.00
5.00
4.00
3.00
2.00
1.00
0.00
M. phaseolina
F. oxysporum
N. oryzae
Control
30
50
Allium cepa
Allium sativum
Annona squamosa
Azadirachta indica
Eucalyptus globulus
Ocimum sanctum
Psidium guajava
Vinca rosea
Name of the plant

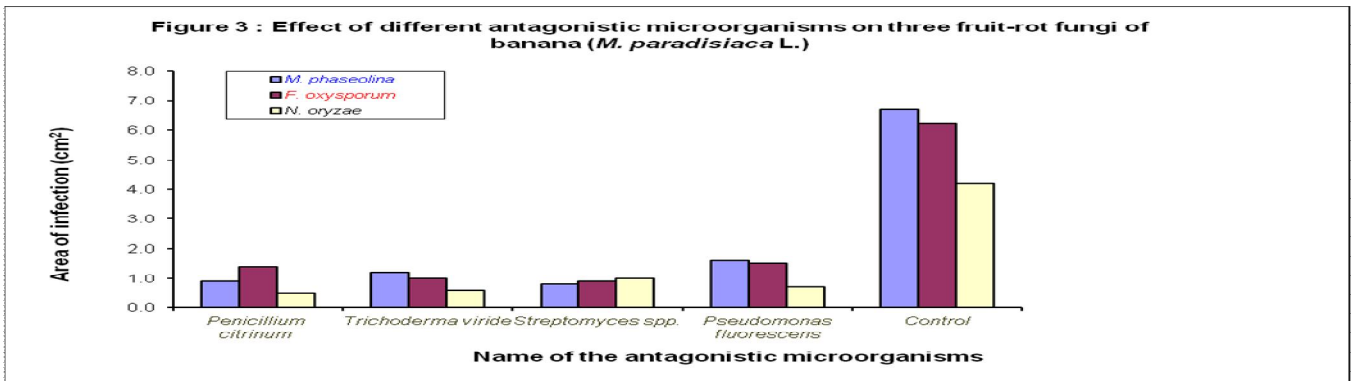
Figure 3 : Effect of different antagonistic microorganisms on three fruit-rot fungi of banana (M. paradisiaca L.)
Area of infection (cm²)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
M. phaseolina
F. oxysporum
N. oryzae
Penicillium citrinum
Trichoderma viride
Streptomyces spp.
Pseudomonas fluorescens
Control
Name of the antagonistic microorganisms

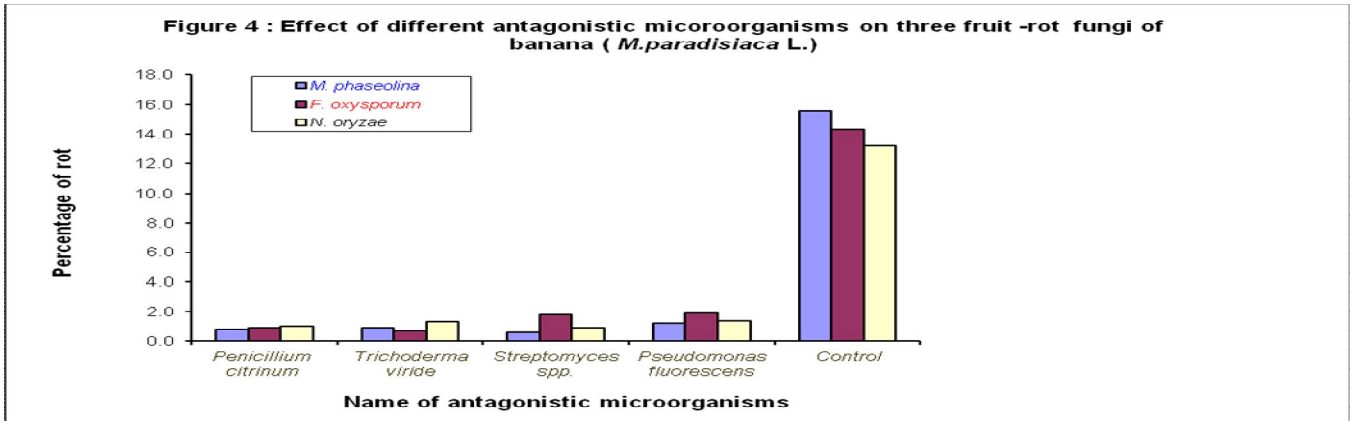
Figure 4 : Effect of different antagonistic micoroorganisms on three fruit -rot fungi of banana (M.paradisiaca L.)
Percentage of rot
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
M. phaseolina
F. oxysporum
N. oryzae
Penicillium citrinum
Trichoderma viride
Streptomyces spp.
Pseudomonas fluorescens
Control
Name of antagonistic microorganisms

ACKNOWLEDGEMENT
The authors thank Head, Department of Microbiology, Kakatiya University, Warangal, Andhra Pradesh for providing necessary facilities.

REFERENCES

1. Jiang YM., Jayce DC. and Macnish AJ. (1999). Extension of shelf life of banana fruit by 1-methylcyclopropene in combination with polyethylene bags. Postharvest Biol and Technol 16:187-193.
2. Jobling J**.** (2000). Essential oils. A new idea for post-harvest disease control. Good fruit and vegetables magazine.pp.11:50.
3. Sharma RR., Gour HN. and Pankaj S. (2005). Effect of plant extracts on growth of *Rhizoctonia solani* and disease development in maize. J Mycol Pl Pathol 35:377-379.
4. Ghangaonkar NM. (2007). Efficacy of plant extracts on the post-harvest fungal pathogens of onion bulbs. Bioinfolet 4:291-294.
5. Deepti Srivastava, Anita K. and Mishra N. **(**2009). Fungal spoilage of some fruits and screening of some leaves for fungitoxicity.Proc Nat Acad Sci India 79: 421-430.
6. Godara SL. and Pathak VN. (1995). Effect of plant extracts on post-harvest rotting of sweet orange fruits. Paper presented in the "Global Conference on Advances in Research on Plant Diseases and their management", held at RCA, Udaipur.
7. Abraham S. and Prakasan V. (2001). Efficacy of botanicals on post-harvest pathogens of carrot. J Mycol Pl Pathol 30:257-258.
8. Prasad Y. and Naik MK. (2003). Evaluation of genotypes, fungicides and plant extracts against early blight of tomato caused by *Alternaria solani*. Indian J Plant Prot 31:49-53.
9. Swami CS. and Mukadam DS. (2006). Antifungal property of some plant extracts against tomato fungi. Geobios 33:261-264.
10. Sumangala K., Patil MB., Nargund VB. and Ramegowda G. (2008). Evaluation of fungicides, botanicals and bioagents against *Curvularia lunata*, a causal agent of grain discoloration in rice. J Pl Dis Sci 3:159-164.
11. Mukhopadhyay AN., Shrestha SM. and Mukherjee PK. **(**1992). Biological seed treatment for control of soil borne plant pathogens. FAO. Pl Prot Bull 40:21-30.
12. Sheela J., Shivaprakasam K. and Seetharaman K. (1998). Effect of soil application of *Pseudomonas fluorescens* using different carriers on groundnut collar rot disease incidence. Madras Agric J 85: 191-192.
13. Sharmarao J., Siddaramaiah AL. and Ramaswamy. (2001). Influence of biocontrol agents and MPG-3 on *Fusarium oxysporum* f.sp.*cubense*, incitant of Panama disease of banana*.* Plant Dis Res 16:68-72.
14. Dutta P. and Das BC. **(**2002). Management of collar rot of tomato by *Trichoderma* spp. and chemicals. Indian Phytopathol 55: 235-237.
15. Gaikwad MS., Gite BD., Saudagar NG. and Kadam PS. 2002. Evaluation of biocontrol agents against charcoal rot (*Macrophomina phaseolina*) of sorghum. Res Crops 3:454-460.
16. Agarwal SC., Sharma S. and Prasad KVV. (2002). Efficacy of biological components in wilt management of chickpea. J Pulses Res 15:177-178.
17. Howell CR. **(**2003). Mechanisms employed by *Trichoderma* species in the biological control of plant diseases, the history and evolution of current concepts. Plant Dis Res 87:4-10.
18. Kapoor AS. (2008). Biocontrol potential of *Trichoderma* spp.against important soil borne diseases of vegetable crops. Indian Phytopathol 61:492-498.
19. Babu LJ., Gour HN. and Pankaj S. (2008). Efficacy of phytoextracts and bioagents against *Colletotrichum gloeosporioides* causing banana (*Musa paradisiaca*) fruit-rot. J Mycol Pl Pathol 38:635-638.
20. Sastry VRSSS. (2002). Studies on post-harvest diseases of fruits and vegetables. Ph.D. thesis, Department of Botany, Kakatiya University, Warangal, Andhra Pradesh,India.
21. Tandon RN. and Mishra AN. (1969). Fruit rot diseases of *Carica papaya* and *Musa paradisiaca* caused by *Rhizopus stolonifer*. Indian Phytopathol 22:334-341.
22. Thangavelu R., Sundararaju P. and Sathiamoorthy S**. (**2004). Management of anthracnose disease of banana caused by *Colletotrichum musae* using plant extracts. J Horti Sci Biotechnol 79:664-668.