BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, December 2011. Vol. **8**(2), p. 783-787

Studies on Fruit Rot Pathogens in Grapes and its Control by *Trichoderma* Spp

N. Uma Maheswari* and R. Saranya

PG and Research Department of Microbiology, Sengamala Thayaar Educational Trust Woman's College, Mannargudi - 614 001 (India).

(Received: 25 September 2011; accepted: 18 November 2011)

Biological control of plant pathogens utilizes one microorganism to eliminate or reduce the disease caused by another. A Grape (*Vitis vinifera* L) is a one of the most economic fruit crops. The grapes are mostly infected by fungal pathogens. In grapes fruit rot disease caused by *Botrytis cinerea* and *A.niger*. The present study was undertaken to isolate and identification of fruit rot pathogens. The *T.viride* and *T.harzianum* were used to control the pathogens by in vitro method and compared with chemical fungicide. Among this study *T.harzianum* showed high inhibitory property against *A. niger* (52mm in 288 hours) and *T.viride* (63mm in 288 hours) showed high inhibitory property against *Botrytis cinerea*. In culture filtrates of *T.viride* and *T. harzianum* (5%, 10%, and 15%) were tested against pathogens. The maximum growth inhibition percentage of test pathogens was at 15% culture filtrate of *T. harzianum* (24.86%) and *T.viride* (27.10%). In disc diffusion method, Thiophanate methyl was used as chemical fungicide against test pathogens. *B.cinerea was* produced clear zone within 72 hrs. The *A. niger* does not inhibited by chemical fungicides. The growth of test organisms in PDA medium with different pH concentrations (4, 5, 6, 7 and 8) were also tested. In that, the optimum growth of test pathogen such as *B. cinerea* were noticed in pH5 (35mm) and *A. niger* pH6 (53mm). Application of bio control agent's was sprayed in grapes to control these pathogens.

Key words: Chemical fungicide, Fruit rot pathogen, Grapes, Thiophanate methyl, *Trichoderma spp.*

A grape is a non-climatic fruit that grows on the perennial and deciduous woody vines of the genus *vitis*. Grapes are small round or oval berries that feature semi- translucent flesh encased by a smooth skin. Some contain edible seeds while others are seedless. Grapes are covered by a protective, whitish bloom. Grapes can be eaten raw or they can be used for making jam, juice, jelly, vinegar, wine, grape seed extracts, rasins, and molasses and grape seed oil. Approximately 71% of world grape production is used for wine, 27% as fresh fruit, and 2% as dried fruit. Grapes excellent sources of manganese and good sources of vitamin B6, thiamin (vit B1), potassium, vitamin C [1].

Botrytis cinerea Pers, is a destructive pathogen of grapes and other small fruits throughout the world [2]. Early *botrytis* rot of grapes is a new development in an old disease caused by *botrytis cinerea*. A brown rot of grapes starts in mid season and may continue to develop until harvest in the absence of rain. The disease may affect only a few grapes or most all the grapes in a cluster. Infection takes place during bloom. The fungus invades the stigma and style and then becomes latent in the necrotic stigma and style tissue at the stylar end of the grape [3]. Additionally,

* To whom all correspondence should be addressed.

pre and post harvest decay caused by *Aspergillus niger* [4]. It is a post harvest disease. High storage temperature and humid conditions favour the development of the disease. The fungus enters the berries through the injuries caused due to poor post harvest handling operations

Chemical control of soil borne pathogens provides certain degree of control but at the same time have adverse effect on environment affecting the beneficial soil microorganisms. Biological control using either natural products or antagonistic microorganisms proved to be successful for controlling various plant pathogens in many countries [5]. *Trichoderma* is the most commonly used fungal biological control agent and have long been known as effective antagonists against plant pathogenic fungi.

The present study was aimed at *Trichoderma* species namely *T.viride* and *T.harzianum* was tested against the fruit rot pathogens of grapes.

MATERIAL AND METHODS

Sample collection

The sample was collected from fruit stall of Thanjavur district, Tamil Nadu, India. The sample was brought to the laboratory and stored in refrigerator at 4°C for further biological analysis. The *Trichoderma viride* and *Trichoderma harzianum* were collected from Sri Amman Biocare. A production of biofertilizer and biocontrol unit. Thirukkanurpatti, Thanjavur.

Isolation and identification of fungi from grape samples

After sample collection, serial dilution was performed for isolating microbial growth from the collected samples. Isolated fungi were identified based on cultural and morphological characteristics using lactophenol cotton blue staining [6,7].

Culture filtrate method

Antibiotic interaction was carried out the culture filtrate of *T.viride* and *T.harzianum* and added separately to the cooled PDA medium to give the concentration of 5%, 10% and 15%. The PDA medium was dispersed in petriplates and allow to solidify. After solidification the pathogens were cut (6 mm) and inoculated at the centre of the plates. The plates were incubated at 27°C for 7 days [8].

The percent inhibition of growth was calculated as follows.

$$\% \text{ of inhibition of growth} \frac{\text{Growth in control-growth in treatment}}{\text{Growth in control}} \times 100$$

Antagonistic activity

The bio control agent *Trichoderma viride* and *Trichoderma harzianum* were selected to study the antagonistic activity against *Botrytis cinerea* and *Aspergillus niger* isolated from test sample. The Potato dextrose agar medium was prepared and poured in to the Petriplate. After solidification 6mm diameter of pour culture of *Trichoderma viride* against *Botrytis cinerea* and *Aspergillus niger* was placed on the PDA medium in opposite direction. Another set of plates *Trichoderma harzianum* against *Botrytis cinerea* and *Aspergillus niger* was placed on the PDA medium in opposite direction. The plates were incubated at 27± 2^0 C for 15 days and the results were noted at every 72 hours on 3, 6, 9, 12 and 15th days respectively [9].

Disc diffusion method

The PDA medium was prepared and sterilized at 121^0 C for 15 minutes and allow it to cool approximately 50^0C. Then the medium was poured into the sterile Petriplate. After solidification the isolated pathogens *Botrytis cinerea* and *Aspergillus niger* were swabbed on the agar plate with help of sterile cotton buds [10].

Effect of pH

The PDA medium was prepared and pH was set at different level such as 4, 5, 6, 7 and 8 respectively by adding 1% NaOH and concentrated HCl. Then the media was autoclaved and poured into Petriplates. The pathogen nearly 6 mm diameter range was inoculated and incubated at 27^0 ±2^0Cfor 72hours [7].

Application

The 2% of *Trichoderma viride* and *Trichoderma harzianum* solution was prepared and foliar spray over the harvested grapes and stored in 30°C.Control was maintained separately. The results were observed and recorded [11].

Statistical analysis

All the experiments were conducted at least thrice. The results were statistically analysed by using arithmetic mean and average [12].

RESULTS

Isolation and identification of fungi from infected Grapes

The infected grape sample was collected from fruit stall of Thanjavur district, Tamil Nadu, India. The infected grape sample was inoculated into potato dextrose agar medium and incubated. After incubation period, the plates were examined for the fungal colonies were observed and identified based on the morphological and spore characters by Lacto phenol Cotton Blue staining method.

Culture filtrate method

The sterilized culture filtrate of *Trichoderma viride* and *Trichoderma harzianum* was used to determine the potential to inhibit the mycelia growth of *Aspergillus niger* and *Botrytis cinerea*. The culture filtrates (non volatile) from two *Trichoderma* species inhibit the growth of test microorganisms. *Trichoderma harzianum* which is inhibitory the growth of test fungi (*Botrytis cinerea*) by 26.50% and *Aspergillus niger* by 23.22% at 15% concentration. *Trichoderma viride* which inhibitory the growth of test fungi *Botrytis cinerea* by 24.44% and *Aspergillus niger* by 29.77% at 15% of concentration.(Table-1)

Antagonistic activity

Trichoderma viride and *Thichoderma harzianum* were growing quickly and dominate the pathogen *Botrytis cinerea* within 12 days. After 15 days the pathogens completely inhibited by antagonist of *Trichoderma viride* and *Thichoderma harzianum*. *T. harzianum* wa inhibited the growth of *A.niger* within 21 days.(Table-2)

Disc diffusion method

After incubation period, the plates were examined the pathogen *Botrytis cinerea* inhibited in disc diffusion method by commercially available systemic fungicide Thiophanate methyl 70%WP. The 25mm clear zone was observed in 72 hrs, the zone was maintained till 120 hrs but the pathogen tolerate the chemical fungicide and grew on the zone respectively 3mm on 168 hours, 6mm on 216 hours and 8mm on 264 hours. The *A.niger* was does not inhibited by chemical fungicide. The results were recorded. (Table 3)

Table 1. Culture filtrate method

	Trichoderma harzianum				Trichoderma viride			
% of culture iltrate	*B.cinerea* (mm)	% of growth inhibition	*A.niger* (mm)	% of growth inhibition	*B.cinerea* (mm)	% of growth inhibition	*A.niger* (mm)	% of growth inhibition
0	63	-	90	-	63	-	90	-
5	54.3	13.80	79.1	12.11	55.7	11.58	76.2	15.33
10	49.5	21.42	74.3	17.44	51.2	18.73	69.5	22.77
15	46.3	26.50	69.1	23.22	47.6	24.44	63.2	29.77

· Values are mean.
·The experiment was performed by maintaining three replicate pertreatment

Table 2. Dual culture method

hours	T.viride (in mm)		*T.harzianum* (in mm)		*B.cinerea* (in mm)		*A.niger* (in mm)	
72	24	24	26	26	16	17	47	45
144	46	37	49	37	23	25	53	49
216	60	45	66	49	25	25	45	35
288	63	51	69	52	21	22	39	34

·Values are mean.
·The experiment was performed by maintaining three replicate per treatment

Effect of pH

The organisms were cultivated in various concentration of pH like 4, 5, 6, 7 and 8. Maximum mycelial growth of *B.cinerea* was noticed in pH5 (35mm) and minimum growth in pH 4 and 8 (24mm).Maximum mycelial growth of *A.niger* was noticed in pH 6 (53mm) and minimum growth in pH 4 (35mm) (Table-3).

Application

The bio control agents *Trichoderma viride* and *Thichoderma harzianum* treated grapes and control grapes were stored separately upto 12 days. The treated post harvested grapes were observed without fungal infection and spoilage. Whereas the untreated control grapes were observed fungal infection and spoiled with sour smell on 7th day.

Table 3. Disc diffusion method (Thiophanate methyl 70% WP) and effect of pH

Hours	*B.cinerea* (zone in mm)	*A.niger* (zone in mm)
72	25	6
168	22	6
216	19	6
264	17	0
pH	*B.cinerea* (in mm)	*A.niger* (in mm)
4	24	35
5	35	37
6	29	53
7	26	46
8	24	36

· Values are mean.
· The experiment was performed by maintaining three replicate per treatment

DISCUSSION

In our study findings *Trichoderma harzianum* and *Trichoderma viride* were used as a biocontrol agent against fruit rot pathogens of *Aspergillus niger* and *Botrytis cinerea.*

In antagonistic activity, *T.harzianum* was found very effective in inhibiting the growth suppression on *A.niger* and control the *B.cinerea.*In our results were correlated with volatile and non-volatile compounds produced by microorganisms may be involved in the suppression of plant pathogen[13]. The volatile compounds from *T.viride* suppress the mycelial growth of *B.cinerea* and control the *A.niger*. This work is in conformity with [14].

In culture filtrate method, the maximum percentage of inhibition of the growth of the test pathogens were at 15% culture filtrate of *T.viride* and *T.harzianum*. In our study similar to *B.cinerea* was suppressed by *Pseudomonas antimicrobica* in vitro and strawberry leaves. Antifungal compounds were detected in cell-free filtrates of the bacterium [15].

In disc diffusion method, both the test pathogens *B.cinerea* and *A.niger* resistant the chemical fungicides thiophanate methyl 70% WP. The present research was correlated with disease management has been dependent upon synthetic chemicals, with new compounds replacing old chemical groups as fungicide resistance develops[16].

Biological control of post harvested diseases (BCPD) has emerged as an effective alternative. Because wound – invading necrotrophic pathogens are vulnerable to biocontrol, antagonists can be applied directly to the targeted area (fruit wounds), and a single application using extending delivery systems (drenches, line sprayers, on-line dips) can significantly reduce fruit decays [17].

Finally it was concluded that *T. viride* and *T. harzianum* used to control the fruit rot disease causing fungal species from grapes. *Trichoderma* isolates reduce the growth of the pathogens significantly and, therefore, can be incorporated for integrated disease management of plant pathogens. The degree of antagonism was varied between and within species of *Trichoderma* against the tested plant pathogens.

CONCLUSION

Biological control is a promising tool to maintain current level of agricultural production while reducing the release of polluting chemical pesticides to the environment. It is a complex process made up from several successive steps generally initiated by a remote sensing of host which stimulates directed growth, subsequently contact is made between fungal antagonist and host (pathogen) surface. The mechanisms

employed by biocontrol agents to effect biological control of plant diseases are many and complex, and their use varies with the kind of biocontrol agent, pathogen and host plant involved in the interaction. As with so many other aspects of science, basic knowledge about the mechanisms involved in the biocontrol process will be of immense value to those intent on developing new methods for utilizing biocontrol agents.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. V. Dhivaharan, Dean, Department of Life Sciences for providing tireless help for the course of the study.

REFERENCES

1. (http:// en. Wikipedia.org)
2. Ciccarone, A.. Current knowledge about *B.cinerea* Per on grape. *Vine academia Italiana Della vite e Del vino.* **22:**3-33 (1970).
3. McClellan, W.D and Hewitt, W.B.. Early Botrytis Rot of Grapes:Time of infection and latency of *Botrytis cinerea* Pers in *Vitis vinifera* L. *Phytopathology*. **63:** 1151-1157 (1973).
4. Zahavi, T.,.cohe,L.,weiss,B.,Schena, L., Dalls, A., Kaplunou, T., Zutkh,. J., Ben- Arie, R and Droby, S. Biological control of *Botrytis, Aspergillus* and *Rhizopus* rots on table and wine grapes in Israel. *Postharvest Biology and technology*. **20:** 115-124 (2000).
5. Papavizas, G.C and Lumsden, R.D. Biological control of soil borne fungal propagules. *Ann. Rev. Phytopathol*., **18:** 389-413(1980).
6. Gilman, J.C. *A manual of soil fungi*, Revised 2nd edition, Oxford and IBH publishing company (Indian Reprint) Calcuta, Bombay, New Delhi. 450 (1957).
7. Aneja, K.R. Experiments in Microbiology, Plant pathology and Biotechnology. New age international publishers, New Delhi. 119- 228 (2002).
8. Narasima Rao, S., Anahasur, K.H and Naik, K.S. Effect of culture filtrates of antagonists on the growth of *Sclerotium rolfsii. Indian J. Plant production* **29:**127-130 (2001).
9. Dennis,C., Webster,S. Antagonistic properties of species group of *Trichoderma* II production of non- volatile antibiotics. *Trans. Br. Mycol.Soc.* **57:**41-48 (1971).
10. Kirby Bauer, A.W., Kirby,W.M.M., Serris, J.C and Turek., M. Antibiotic susceptibility testing by a standardized single disc method. *Am. J. clinic. Pathol.* **45:** 493-496 (1966).
11. Krishan Chandra, Greep,S and Srivathsa, R.S.H. Biocontrol agents and biopesticides (liquid formulations). Published by: Regional Director, Regional centre of organic farming, No:34, 5th main road, Hebbal, Bangalore. 19-20 (2005).
12. Gupta, S.P.. *Statistical methods*. Sulthan Chand and Sons, Educational publishers, New Delhi 180-181 (2004) .
13. Gupta, V.K and Utkhede, R.S.. Factors affecting the production of antifungal compounds of *Enterobacter aerogenes* and *Bacillus subtilis,* antagonists of *Phytophthora cactorum*. *J. Phytopathol.* 177:9-16 (1986).
14. Maheswari, D.K., Drobey, R.C and Sharma, V.K. Biological effects of *Trichoderma virens* on *Macrophomina phaseolina* causing charcoal rot of peanut. *Indian J Microbial*.**41:**251-256 (2001).
15. Walker, R., Emsile,K, A and Allan, E.J. Bioassay methods for the detection of antifungal activity by *Pseudomonas antimicrobica* against the gray mould pathogen *Botrytis cinerea*. *J.Appl. Bacterial.* **81:**531-537 (1996).
16. Janisiewicz, W.J and Korsten, L. Biological control of post harvest diseases of fruits. *Ann.rev. Phyto pathol*. **40:** 411-441 (2002).
17. Rosslenbroich, H.J and Stuebler, D. *Botrytis cinerea* history of chemical control and novel fungicides for its management. *Crop protection.* **19:** 557-561 (2000).