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EFFECT OF UREA, PAPAYA MILK AND MINTHOL ON RHIZOCTONIA SOLANI AND MACROPHOMINA PHASEOLINA

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1. INTRODUCTION

In today's scenario loss of crops from fungal plant diseases is resulting in hunger and starvation, especially in developing countries like India where access to disease-control methods is limited and annual losses of 30 to 50 percent are common for major crops. Synthetic chemical used as a fungicide these days poses a great threat to our ecosystem and environment. The alternative of these fungicides are biological controls and natural products which are cheaper and are more environment friendly and will not contaminate the food web as compared to fungicide. *Rhizoctonia solani*, fungus is a pathogen of agricultural crops in the plant family Solanaceae that includes eggplant, pepper, potato and tomato. The most abundant source of protein comes from the crop of common bean worldwide which is also a major part of human consumption and also known for improving the soil fertility through nitrogen fixation. Similarly other important crops such as soyabean, peanut, and corn are infected by Rhizoctonia solani (Kuhn) and Macrophomina phaseolina (Tassi) Goid, which are among the most important fungal pathogens. Rhizoctonia solani has a broad host range and little effective resistance and is an important necrotrophic pathogen. *Rhizoctonia solani*, primarily attacks below ground plant parts such as the seeds, hypocotyls, and roots, but is also capable of infecting above ground plant parts (e.g. pods, fruits, leaves and stems). The most common symptom of Rhizoctonia disease is referred to as "damping-off" characterized by non germination of severely infected seed. Another fungus, *Macrophomina phaseolina* (Tassi) Goid. a necrotrophic soilborne fungus causes charcoal rot infecting about 500 plants. The fungus can infect the root and lower stem of over 500 plant species and is widely distributed.[1-14] *M. phaseolina* causes disease on soyabean, peanut, and corn. In peanut, it causes seed and seedling rots, wilt, root and stem rots, leaf spot, and rotting of developing pods and seed. Charcoal rot on soybean leads to early maturation, chlorosis and incomplete pod filling. While in corn the fungus causes a stalk rot during hot, dry conditions. Continuous use of chemical fungicide may result in extensive deterioration of soil components and other environmental problems.[15-22] Effects of papaya extrudes as a fungicide has never been studied. Three different chemicals (urea, papaya milk and minthol) were tested against the growth of these two pathogens out of which Papaya milk and Urea are used for the first time and it shows a great degree of antagonistic activity against *R solani* and *M. phaseolina* and is very cheap and environment friendly to use.

2. MATERIALS AND METHODOLOGY

The samples were collected from different regions of New Delhi, India. The pure culture of *Rhizoctonia solani* and *Macrophomina Phaseolina* was collected from Department of Pathology, IARI Pusa Campus, New Delhi, India and Urea was obtained from college chemical collection, Papaya milk was collected from Papaya taken from Gole Market New Delhi and Minthol was obtained from the Fertilizer Shop in New Delhi and stored in Microbiology laboratory under sterile condition. Potato Dextrose Agar was used for the cultivation and enumeration of culture.**[23-25]** 4.0 g of Potato Starch , 20.0 g of Dextrose and 15 g of Agar were Suspended in 1 litre of milli Q water and mixed thoroughly than the formed solution was heated with frequent agitation for 1 min to completely dissolve the powder. The PDA media was subject to autoclave at 121°C for 15 minutes. The PDA media was tested by using stable control culture (E. coli).

Three different concentration of Urea (10^{-1} M, 10^{-2} M and 10^{-4} M)were prepared from the stock solution. One ml of each concentration of Urea was added with the 30ml of PDA media in molten stage. Papaya milk was added in the PDA media in microlitre quantity. Three different concentrations were prepared i.e. $4x10^{-7}$ml/100ml, $8x10^{-7}$ml /100ml and $16x10^{-7}$ml /100ml. Similarly three different concentration of Minthol (10^{-5}M, 10^{-6}M, & 10^{-7}M)were prepared from the stock solution. One ml of each concentration was added in the molten stage of PDA media. Five Petri plates were made for every different concentration of Urea, Papaya milk and Minthol, i.e. total 45 Petri plates and 9 controls were prepared.

Inoculation was done after preparing the media and different concentration of samples. The cork borer was used to make the hole in the centre of a Petriplate and for the inoculation, the disc fungal growth of the same area i.e. 1cm in diameter was placed in the centre of the Petri plates and incubated at 25^{o}C of temperature.[27]

3. RESULTS AND DISCUSSION

All the Petri plates were observed for growth or inhibition everyday including holidays at regular intervals. The growth was measured horizontally as well as vertically and average was taken of three replicates .In controls no fungicide was used and data was recorded as in case of treatments. The observations were concluded after five days. The Tables and graphs were generated on the basis of readings obtained and then the result was interpreted accordingly.[27]

Figure legends

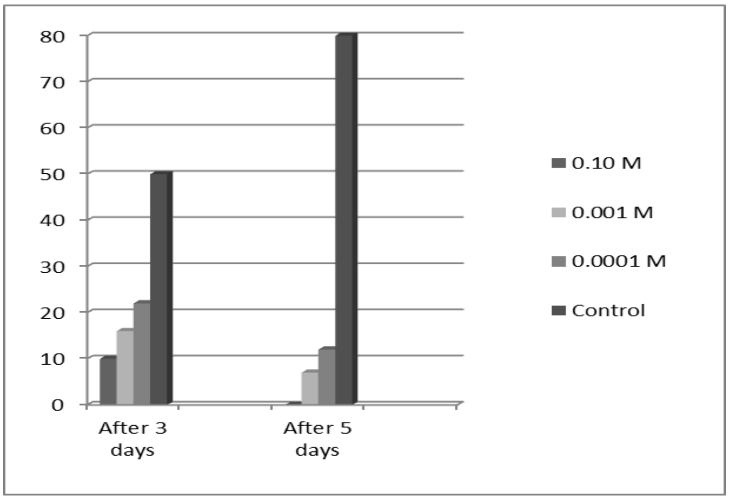

Figure 1 : Effect of Urea on the growth of Rhizoctonia Solani

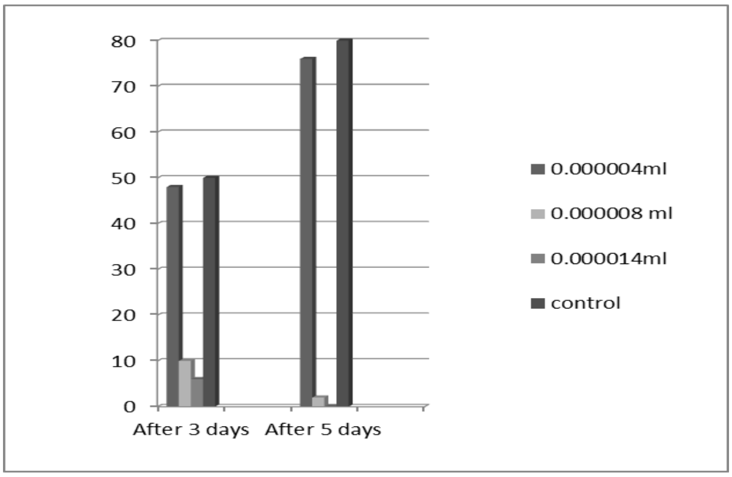

Figure 2 : Effect of Papaya milk on the growth of Rhizoctonia Solani

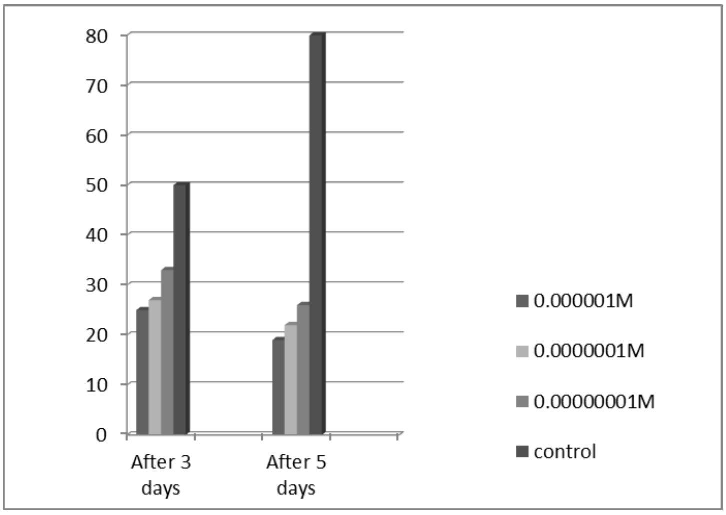

Figure 3: Effect of Minthol on the growth of Rhizoctonia Solani

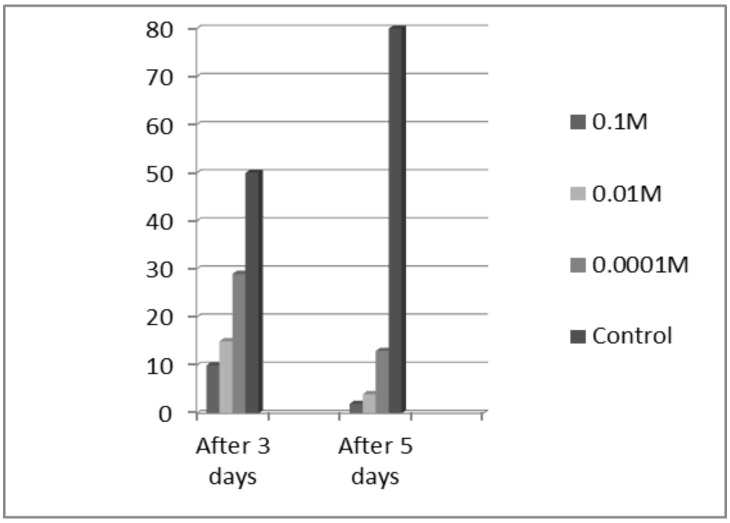

Figure 4: Effect of urea on the growth of Macrophomina Phaseolina

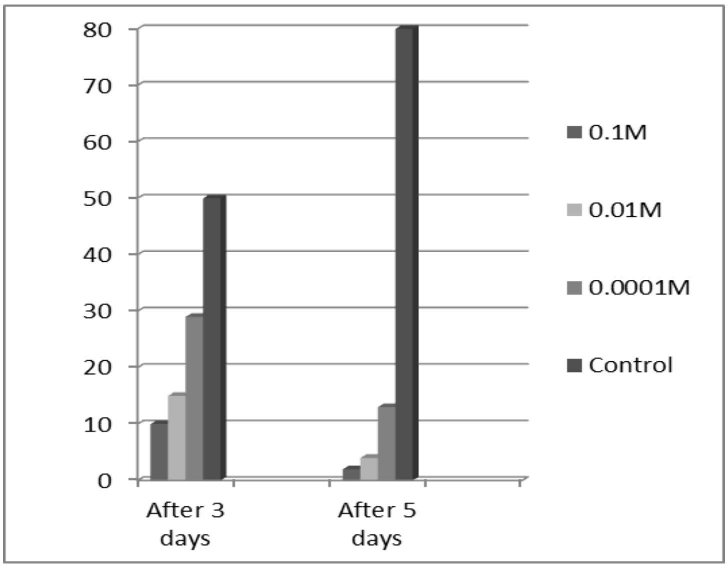

Figure 5: Effect of Papaya milk on the growth of Macrophomina Phaseolina

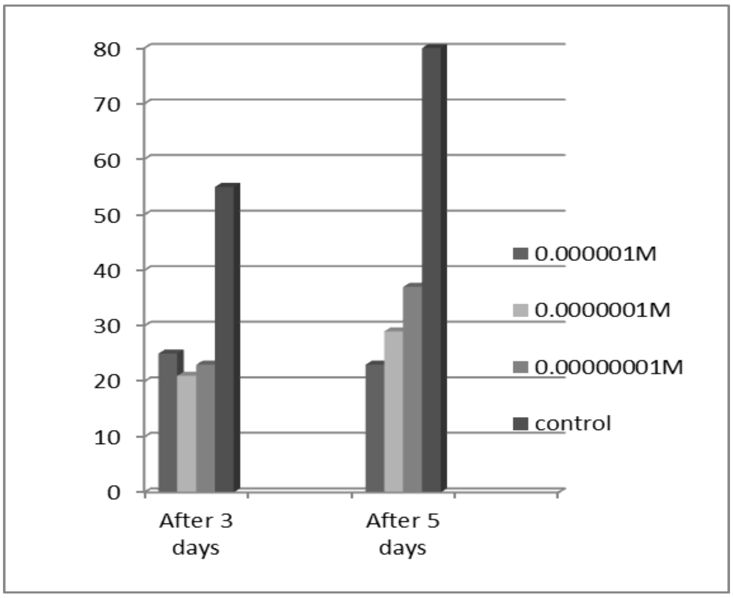

Figure 6: Effect of Minthol on the growth of Macrophomina Phaseolina

TABLE LEGENDS

Table 1: Effect of Papaya milk on *Rhizoctonia Solain*

Urea Concentration	Growth(mm)	
	After 3 days	After 5 days
10^{-1}M	9.5	0.25
10^{-2}M	16.5	6.5
10^{-4}M	22	12
Control	**55**	**80**

Table 2: Effect of Papaya milk on *Rhizoctonia Solani*

Papaya milk Conc (ml/100ml)	Growth(mm)	
	After 3 days (mm)	After 5 days (mm)
$4X10^{-6}$	48	76
$8X10^{-6}$	10	2
$14X10^{-6}$	6	0
Control	50	80

Table 3: Effect of Minthol on growth of *Rhizoctonia Solani*

Minthol conc	Growth(mm)	
	After 3 days (mm)	After 5 days (mm)
10^{-5}M	25	19
10^{-6}M	27	22
10^{-7}M	34	26
Control	50	80

Table 4: Effect of Urea on growth of *M Phaseolina*

Urea conc	Growth(mm)	
	After3 days	After 5 days
10^{-1}M	10	1
10^{-2}M	15	4
10^{-4}M	29	13
Control	50	80

Table 5: Effect of Papaya milk on growth of *M Phaseolina*

Papaya conc ml/100ml	Growth(mm)	
	After 3 days	After 5 days
4 x 10^{-7}	46	67
8 x 10^{-7}	10	2
14 x 10^{-7}	6	0
Control	50	80

Table 6: Effect of Minthol on growth of *M Phaseolina*

Minthol conc	Growth(mm)	
	After 3 days	After 5 days
10^{-6} M	25	23
10^{-7} M	21	29
10^{-8} M	23	37
CONTROL	50	80

Effect on Rhizoctonia Solani

By using urea for *Rhizoctonia solani* at 10^{-1} M , 10^{-2} M, 10^{-4}M concentrations, the inhibition was not at all significant after 3 days but when growth was measured after 5 days significant inhibition (0.5mm) was observed as compared to control(80mm) In case when papaya milk was used for *Rhizoctonia solani* at concentration of 4µl/100ml, the inhibition was nil after 3 days when 8µl/100ml concentration was used,the inhibition was 13mm and 12mm in case of concentration 14µl/100ml the inhibition observed was 9mm and 7mm, whereas the growth in the control was 55mm. At 14µ1/100ml maximum inhibition was observed and there was no growth at all. The information indicates that papaya milk is quite effective in inhibition of *Rhizoctonia solani* growth.But when minthol was used at 10^{-6} M, 10^{-7} M and10^{-8} M concentration it was observed that Rhizoctonia *solani* could not be inhibited completely after three days but after five days, some inhibition(50mm) was observed in contrast to control(80mm) (Figure 1, 2 and 3 ; Table 1, 2 and 3).

Effect on Macrophomina Phaseolina

It was observed that inhibition of *Rhizoctonia bataticola* was significant by some extent by the use of urea at 10^{-1} M , 10^{-2}M, 10^{-4}M concentrations, after 3 days but when growth was measured after 5 days, significant inhibition (15mm) was observed as compared to control (80mm). When papaya milk was used for *Rhizoctonia bataticola* at concentration of 4µl/100ml, the inhibition observed was nil after 3 days but when 8µl/100ml concentration was used, the inhibition observed was 13mm and 12mm respectively, In case of concentration of 14µl/100ml, the inhibition observed was 9mm and 7mm whereas the growth in the control plate was 55mm. At 14µl/100ml, maximum inhibition (7mm, 5mm) was observed. After 5 days, seen that at 14µl/100ml there was no growth at all. The above information indicates that papaya milk is quite effective in inhibition of *Rhizoctonia bataticola* growth. Therefore, it can be stated that both the fungi are inhibited completely at 14µl/100ml. But when minthol was used at 10^{-6} M, 10^{-7} M and 10^{-8} M it was seen that at these three concentrations, *Rhizoctonia bataticola* could not be inhibited completely after three days but after five days, some inhibition(34mm, 40mm) was observed when minthol was used at 10^{-5} M and 10^{-6} M (Figure 4, 5 and 6 ; Table 4, 5 and 6).

As the result shows that Urea in very low concentration i.e. 10^{-1M}, 10^{-2M}, 10^{-4M} can inhibit the growth of Rhizoctonia solani and Rhizoctonia bataticola. so it seems to be very helpful for those crops which are susceptible of these pathogens(*Rhizoctonia solani* and *Rhizoctonia bataticola*). Around 85.0 to 89.2% of consistent ascospore inhibition was provided by Urea depending on the experiment. Several studies have shown the effect of treatment of urea on fungi which is well documented.[38-44] In most of the studies mentioned, urea provided complete eradication of pseudothecia or ascospore production. Urea is also known to stimulate the growth of microorganisms[45], which increases microbial competition, among the microorganisms, some are antagonistic.

Papaya milk is very uncommon and its effectiveness or quality is still unrevealed. So It has been used to check the growth of above mentioned pathogens.it has been reported that the root extracts of carica papaya inhibit the fungal growth, especially Candida albicans. Three different concentrations of Papaya milk were used, i.e. 4µl/100ml 8µl/100ml, and 14 µl/100ml, the results were very positive and it shows a high degree of inhibition to *Rhizoctonia solani* and *Rhizoctonia bataticola* . the mechanism behind this may be the lytic Enzymes which target the cell wall of these isolated fungi, and the mode of action may possibly be by an attack on the sugar residues on the cell of these fungal species. It was observed that few minutes after bleeding for the papain exudates it tend to thicken, thus becoming more gummy which will slow the enzymes activity of papain on the mycelial growth of the fungal isolates.[37] In India, papaya extrudes is treated like waste and it is easily available Everywhere. The main achievement of the experiment relies on the fact that it is used in very low concentration. It is cost effective so it can be used by all the classes of farmers.

Generally chemical fungicides are used these days for controlling primary and secondary infections as biological control strategies aiming to control leaf infection have a limited commercial future. It is causing a great detrimental environmental impact and resistance is also being observed. Minthol has been used from several years as fungicide but never been used against *Rhizoctonia solani* and *Rhizoctonia bataticola*. Minthol was used in very low concentration i.e. 10^{-5} M, 10^{-6} M and 10^{-7} M. The result indicates that Minthol shows no significant effect on the above mentioned fungi.

REFERENCES

1. Adam, G. C. Thanatephorus cucumeris (Rhizoctonia solani) a species of wide host range. In Advances in Plant Pathology, Genetics of Plant Pathogenic Fungi. (G.S. Sidhu, ed.), 1988; 6: 535-552. Academic Press, New York.
2. Nikraftar et al. Journal of Applied Microbiology 118, 704--717 2014 The Society for Applied Microbiology Journal of Applied Microbiology ISSN 1364-5072 in crop plants, 2013.
3. Agrios, G. N. Plant Pathology. 4th Ed., 1997; 606. Academic Press, New York.
4. Alexopoulos, C. J., Mims, C. W. and Blackwell, M. Introductory Mycology., 4th Ed., 1996; 869. John Wiley and Sons, New York.
5. Anderson, N.A. The genetics and pathology of Rhizoctonia solani. Ann. Rev. Phytopathol, 1982; 20: 329-347.
6. Castro, C., Davis, J. R., and Wiese, M.V. Quantitative estimation of Rhizoctonia solani AG-3 in soil. Phytopathology, 1988; 78: 1287-1292.
7. Cubeta, M.A., and R. Vilgalys. Population biology of the Rhizoctonia solani complex. Phytopathology, 1997; 87: 480-484.

8. Guttierez, W.A., H.D. Shew, and T. A. Melton. Sources of inoculum and management for Rhizoctonia solani damping-off on tobacco transplants under greenhouse conditions. Plant Disease, 1997; 81: 604-606.
9. Hawksworth, D. L., Kirk, P. M., Sutton, B. C., and Pegler, D. N. Ainsworth and Bisby's, Dictionary of the Fungi. 8th Ed., 1995; 616. University Press, Cambridge, UK.
10. Hennis, Y., Ghafar, A., Baker, R. and Gillespie, S.L.. A new pellet soil sampler and its use for the study of population dynamics of Rhizoctonia solani in soil. Phytopathology, 1978; 68: 371-376.
11. Khaledi, P. Taheri and S. Tarighi Department of Crop Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran :Antifungal activity of various essential oils against Rhizoctonia solani and Macrophomina phaseolina as major bean pathogens N. Journal of Applied Microbiology ISSN 1364-5072., 2014.
12. Ko, W. H., and Hora, F. K. A selective medium for the quantitative determination of Rhizoctonia solani in soil. Phytopathology, 1971; 61: 707.
13. Ogoshi, A. Ecology and pathogenicity of anastomosis and intraspecific groups of Rhizoctonia solani Kühn. Ann. Rev. Phytopathol, 1987; 25: 125-143.
14. Sneh, B., Burpee, L., and Ogoshi, A. Identification of Rhizoctonia species, 1991; 133. APS Press, St. Paul, MN, USA.
15. Sneh, B., Jabaji-Hare, S., Neate, S. and Dijst, G.. Rhizoctonia species: Taxonomy, Molecular Biology, Ecology, Pathology, and Control, 1996; 578. Kluwer Academic Publishers, Dordrecht, The Netherlands.
16. Vilgalys, R., and M. A. Cubeta. Molecular systematics and population biology of Rhizoctonia. Annu. Rev. Phytopathol, 1994; 32: 135-155
17. Bowen, C. R. and Schapaugh Jr., W. T. Relationships among charcoal rot infection, yield, and stability estimates in soybean blends. Crop Science, 1989; 29: 42-46.
18. Bowers, G. R. and Russin, J. S. Soybean disease management. In Soybean production in the mid-south. L. G. Heatherly and H. F. Hodges, 1999 CRC Press.
19. Cloud, G. L. and Rupe, J. C. Comparison of three media for enumeration of sclerotia of Macrophomina phaseolina. Plant Disease, 1991; 75: 771-772.
20. Dhinga, O. D. and Sinclair, J. B. An annotated bibliography of Macrophomina phaseolina, 1977; 1905-1975. Universidade Federal de Vicosa, Minas Gerais, Brazil.
21. Mehan, V. K. and McDonald, D. Charcoal Rot. In Compendium of peanut diseases, 2^{nd} Edition. N. Kokalis-Burelle et al. eds. 1997 APS Press. St. Paul MN.

22. Olaya, G. and Abawi, G. S. Effect of water potential on mycelial growth and on production and germination of sclerotia of Macrophomina phaseolina. Plant Disease, 1996; 80: 1347-1350.
23. White, D. G. Fungal stalk rots. Compendium of Corn Diseases 3rd Edition. D. G. White ed. 1999 APS Press. St. Paul, MN.
24. Wyllie, T. D. Charcoal rot of soybean-current status. In Soybean diseases of the north central region. T. D. Wyllie and D. H. Scott, eds. 1988 APS Press, St. Paul, MN.
25. Wyllie, T. D. Charcoal rot. In Compendium of soybean diseases, 3rd Edition. J. B. Sinclair and P. A Backman, eds. 1993 APS Press. St. Paul, MN.
26. Anderson, N.L., et al. Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.
27. Jorgensen., et al. Manual of Clinical Microbiology, American Society for Microbiology, Washington, D.C.
28. Isenberg, H.D. Clinical Microbiology Procedures Handbook, Vol. I, II & III. American Society for Microbiology, Washington, D.C.
29. James K. Mitchell, Saber Maleki, Kathy M. Orsted, Richard J. Rawlings, and Catherine Price from *The American Biology Teacher*, April 1997; 59: 4.
30. United States Pharmacopeial Convention. The United States pharmacopeia, 31st ed., Amended Chapters 61, 62, 111. The United States Pharmacopeial Convention, Rockville, 2007 MD.
31. Directorate for the Quality of Medicines of the Council of Europe (EDQM). The European Pharmacopoeia, Amended Chapters 2.6.12, 2.6.13, 5.1.4, Council of Europe, 67075 Strasbourg Cedex, France, 2007.
32. Japanese Pharmacopoeia. Society of Japanese Pharmacopoeia. Amended Chapters 35.1, 35.2, 7. The Minister of Health, Labor, and Welfare, 2007.
33. Vanderzant, C., and D. F. Splittstoesser (eds.). Compendium of methods for the microbiological examination of food, 3 Rd ed. American Public Health Association, Washington, D.C, 1992.
34. Marshall, R. T. (ed.). Standard methods for the microbiological examination of dairy products, 16Th ed. American Public Health Association, Washington, D.C, 1993.
35. www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalmanualBAM/default.htm.

36. Curry, A. S., J. G. Graf, and G. N. McEwen, Jr. (eds.). CTFA Microbiology Guidelines. The Cosmetic, Toiletry and Fragrance Association, Washington, D.C., 1993.
37. Murray, P.R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C., 1995.
38. Mac Faddin, J. F. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol.1. Williams & Wilkins, Baltimore, MD., 1985.
39. Nwinyi, Obinna Chukwuemeka* and Abikoye Busola Anthonia. Antifungal effects of pawpaw seed extracts and papain on post harvest Carica papaya L. fruit rot African Journal of Agricultural Research, 18 June, 2010; 5(12): 1531-1535.
40. Burchill, R. T., and Hutton, K. E. The suppression of ascospore production to facilitate the control of apple scab (Venturia inaequalis (Cke) Wint.). Ann. Appl. Biol., 1965; 56: 285-292.
41. Cross, J. E., Garrett, C. M. E., and Burchill, R. T.. Changes in the microbial population of apple leaves associated with the inhibition of the perfect stage of Venturia inaequalis after urea treatment. Ann. Appl. Biol., 1968; 61: 203-216.
42. Darpoux, H., Lebrun, A., and Tullaye, B. Action de traitement sur la formation des périthèces et la production de l'inoculum primaire de Venturia inaequalis (Cooke) Wint. et de Venturia pirina Adher. Phytiatr.Phytopharm. Rev. Fr. Med. Pharm. Veg., 1975; 24: 3-14.
43. Gupta, G. K. Role of on-season, post harvest and pre leaf fall sprays in the control of apple scab (Venturia inaequalis). Indian J. Mycol. Plant Pathol, 1979: 141-149.
44. Gupta, G. K., and Lele, V. C. Role of urea in suppression of ascigerous stage, and comparative in-vitro efficacy of fungicides against apple scab. Indian J. Agric. Sci., 1980; 50: 167-173.
45. Moller, W. J. Efficacy of autumn urea in reducing spring inoculum of apple scab. Calif. Plant Pathol, 1981; 52.