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Study on fungicidal effect of plant extracts on plant pathogenic fungi and the economy of extract preparation and efficacy in comparison to synthetic/chemical fungicides

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Abstract
Providing food security to devastatingly increasing population with limited natural resources along with destruction caused by pre- and post-harvest pathogens are the foremost concerns for the developing countries. Numerous pesticides, herbicides and chemical fertilizers are being applied by the farmers to deal with the existing situation but leave very disastrous and undesirable after effects on ecosystem as non-degradable molecules.. Botanicals can be utilized as an ecofriendly and effective alternative against chemical as they are of natural origin. In this context, two chemical/synthetic fungicides namely Manzate and Nystanin in three different concentrations namely 500ppm, 1000 ppm and 1500 ppm were evaluated against *Sclerotium rolfsii, Alternaria alternata, Fusarium monilifrome, Rhizoctonia solani* and *Aspergillus niger in vitro* to compare them with ethanolic botanical extracts of spices (clove, cinnamon, thyme) and weeds (parthenium and calotropis) at 5%, 10%, 15%, 20% and 25%. Results revealed the high efficacy of botanicals i.e. clove extracts showed maximum inhibition (100%), followed by reduced inhibition in cinnamon, thyme, *Parthenium* and *Calotropis* treated plates against all five pathogens even at 5% concentration in comparison to chemical of 500 ppm concentration i.e. 100% in case of *S.rolfsii* only. Hence the herbal products can be further analyzed and applied as a potent, ecofriendly and economical substitute to chemicals.

Keywords: Agrochemicals, Botanical extracts, Food security, Phytopathogens

INTRODUCTION

Agriculture is an important sector of Indian economy as it contributes about 17% of total GDP and provides employment to about 60% of population (GOI, 2011). Nowadays a considerable issue faced by country is production of quality crop to feed enormously growing population with limited natural resources. About 10-30% crop loss caused by microbes, pests, insects, etc. is enhancing the pressure on existing problematic situation (Kumar and Gupta, 2012). Various type of direct and indirect losses caused by plant diseases include, reduced quality and quantity of crop produce, increased cost of production, threat to animal health and environment, loss of natural resources and less remunerative alternatives adopted (Kumar and Saxena, 2009). Fungi are the most important cause of plant disease (Persley, 1993), since they are the most widespread and destructive parasites of plants (Ingold and Hudson, 1993). Farmers started the immense application of synthetic fungicides for quick and effective management of diseases and to deal with the serious damage in agriculture, resulting in critical losses of yield, quality, and profit. But these chemical solutions came up with lots of precarious after effects on living organisms and ecosystems due to their high and acute toxicity, long degradation periods, accumulation in food chains, and an extension of their power to destroy both useful organisms and harmful pests (Tarr, 1962). Due to public awareness about residual effects and development of resistance in many pathogenic microorganism and insect pests, there is a an urgent need and even challenge for plant pathologist to search and promote development of novel, ecofriendly and economical antifungal drugs as a replacement for the existing chemical/ synthetic fertilizers in one of many steps toward the goal of sustainable agriculture (Satish *et al.,* 1999; Strange, 1993).

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Herbal products are again gaining attention due to relatively lower incidence of adverse reactions to plant preparations compared to modern conventional pharmaceuticals and their even their reduced cost (Lulekal *et al*., 2008). Extracts isolated from several plants have reported to posses biological activity such as antimicrobial, antifungal and anti-inflammatory abilities due to the presence of bioactive compounds like alkaloids, glycosides, resins, volatile oils, gums and tannins, etc. (Yusuf *et al*., 2001). Natural products, either as pure compounds, or standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity in the plant kingdom (Cos *et al*., 2006; Maregesi *et al*., 2008). Over 50% of all modern clinical drugs are of natural origin and natural products play an important role in drug development programs in the pharmaceutical industry (Farombi *et al*., 2003; Nair and Chandra, 2005). Spices and herbs have been used as food additives since ancient times as flavouring agents and also as natural food preservatives. Previous studies revealed very strong antifungal activity of essential oils from spices and herbs, such as garlic, mustard, cinnamon, cumin, clove, bay, thyme, basil, oregano, pepper, ginger, sage, rosemary etc., against most common fungi that contaminate food (*Aspergillus* spp., *Cladosporium* spp. and many others) (Gupta, 2013; Skrinjar and Nemet, 2009). Yet in spite of the scale of this research enterprise, only a handful of botanical insecticides are in commercial use on vegetable and fruit crops today, with significant commercial development of only two new sources of botanicals in the past 20 years (Dreistadt, 2003). Keeping in view the importance of disease management for crops in an economical and environmental friendly mode, the present study was conducted to compare the fungicidal efficacy and cost of production between plant extracts and synthetic/chemical fungicides.

MATERIALS AND METHODS

The experiment was conducted in the laboratory of Department of Commercial Biotechnology, College of Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut (Uttar Pradesh). The university is situated on Dehradun – Delhi NH-58 at a distance of 10.0 km. away in the North of Meerut city between 73 and 74 km. on National highway. Geographically, Meerut is located at 29^0 05'50'' North latitude, 77^0 41'50'' East longitudes and at an altitude of 237 meter above the mean sea level. The plant pathogenic fungal strains of *Sclerotium rolfsii*, *Alternaria alternate, Fusarium moniliformae, Aspergillus niger* and *Rhizoctonia solani* were collected from Department of plant pathology, Recombinant techniques and Agriculture Biotechnology.

Preparation of botanical extracts and synthetic fungicide solution: Clove bud, Cinnamon bark, Thyme seeds, *Parthenium hysterophorus and Calotropis procera* were surface sterilized by using 0.01% $HgCl_2$, dried hot air oven (40±2ºC) for 24 hours and were coarsely powdered. To obtain the defined concentrations of 5%, 10%, 15%, 20% and 25%, mentioned amount of 50mg, 100mg, 150mg, 200mg and 250mg botanical powder was immersed separately in 50 ml ethanol (50%) in 100 ml conical flask respectively and were kept in shaker incubator at (40±2ºC) for 24 hours. The extracts were obtained by filtering through Whatman filter paper No. 1 and kept at 50ºC in water bath thus allowing ethanol to evaporate. Then the remains were weighed and diluted with 10% DMSO according to the weight (5ml/gm) for preparing final concentrations which were preserved in a refrigerator at 4^oC till use (Barreto *et al.,* 2002; Chen *et al.,* 1987).

The synthetic fungicides namely Nystatin (Srivastava and Singh, 2011) and Mancozeb (Jagtap *et al*., 2013) were procured from market in powdered form. To prepare solution of three different concentrations of 500ppm, 1000ppm and 1500ppm, solvent were mixed with 0.5 mg/ml, 1.0 mg/ml and 1.5 mg/ml respectively (w/v).

Screening of antifungal activity of extracts: Both botanicals extracts and synthetic fungicidal solution were evaluated against plant pathogenic fungi through poisoned food technique by mixing them with cooled (45ºC) molten Potato Dextrose Agar medium in concentration of 1:9 (Ilyas *et al.*, 1982). Petri-plates with different concentration of ethanolic extracts were inoculated with mycelial disc of 6mm diameter from the periphery of 7 day old cultures of *S.rolfsii, A. alternata, F.monilifrome, R.solani* and *A.niger* and incubated at 25^oC for *in-vitro* studies. PDA plates with 100µl of DMSO were used as negative control whereas plates without any solvent were positive control (Georgii and Korting, 1991, McCutcheon *et al*., 1994). The evaluation was performed by measuring the colony diameter of pathogenic fungi at interval of 24 hour from the inoculation (Florl *et al*., 2003, Rasooli and Abyanek, 2004). The results were expressed in terms of the percent inhibition in diameter of mycelia. Radial mycelia growth was recorded and was further converted to percent inhibition. Variation of each strain on test plate as compared to control plate for every extract and fungicide has been calculated by the formula given:

Percent growth inhibition over control = C –T / C X 100 …...Eq.1

Where: C = Growth of fungus in control, T = Growth of fungus in treatment

Comparative study of chemical and biological fungicides production cost: The comparative study of synthetic and botanical fungicides was also made to evaluate them on economical basis. The cost of defined amount of spices that include clove, cinnamon and thyme, utilized in extract preparation of different concentration was compared to the cost of same amount of synthetic fungicides Nystatin and Manzate (Mancozeb).

RESULTS AND DISCUSSION

Effect of different botanical extracts on plant pathogenic fungi in *in-vitro*: The results of investigation for testing the efficacy of ethanolic extracts of spices and weeds showed that the ethanolic extracts of clove, cinnamon, thyme, *Parthenium* and *Calotropis* at different concentrations i.e. 5%, 10%, 15%, 25% and 25% respectively, showed antifungal activity against *S.rolfsii, A.alternata, F.monilifrome, R.solani* and *A.niger* under *in-vitro* conditions. After three days of inoculation in control plates (i.e. without extracts) and test plates (i.e. with extracts), the inhibition in growth of mycelium of plant pathogenic fungal strains under the influence of various treatments of botanical extracts is presented in table 1.

Table clearly indicate the radius of mycelial growth pattern of *S. rolfsii, A.alternata, F.monilifrome, R.solani* and *A.niger* under *in-vitro* conditions on control plates and on the test plates with different concentration of the ethanolic extracts. The plates with clove extracts showed maximum inhibition i.e. no growth, followed by reduced inhibition in cinnamon, thyme, *Parthenium* and *Calotropis* treated plates with little variation in some. It depicts that all the botanical extracts show antifungal activity against plant pathogenic fungal strains.

Effect of different synthetic fungicides on plant pathogenic fungi *in-vitro*: A comparison of the fungicidal activity of synthetic fungicides viz. Mancozeb and Nystatin at three different concentrations i.e. 500ppm, 1000ppm and 1500ppm against plant pathogenic fungi viz. *S.rolfsii, A.alternata, F.monilifrome, R.solani* and *A.niger* under *in-vitro* conditions after three days is mentioned in table 2 and 3.

The data recorded in bioassay experiment done with poisoned food technique shown in tables revealed that all fungicides were found significantly superior over the control (untreated) due to the inhibition of growth of test pathogens under *in-vitro* conditions. Results depicted that Nystatin was the best fungicide which completely inhibited the radial growth of *S.rolfsii* and *A.niger* i.e. zero growth at all three concentrations followed by *A.alternata* and *R.solani* whereas Mancozeb mainly inhibited *F. monilifrome*. These fungicides inhibited the pathogenic fungal strains to a nearby range as compared to botanical extracts, varying to some extent in case of *Calotropis*, in different concentrations except clove which showed zero growth at low concentration. It can be clearly depicted that clove extract can be used as an effective biological fungicides because 100% inhibition was observed for all the five plant pathogenic fungi viz. *S.rolfsii, A.alternata, F.monilifrome, R.solani* and *A.niger* under *in-vitro* conditions. Cinnamon extract was found to be more effective inhibitor of *R.solani* whereas thyme extract was found to be most effective for *A.alternate*. The extracts obtained from *Parthenium* and *Calotropis* showed considerably less inhibition as compared to the extracts of spices and synthetic fungicides. *A. niger* was found to be least sensitive to *Calotropis* extract whereas *R.solani* was found to be least sensitive to *Parthenium* extract.

Data presented in table clearly indicate that *S.rolfsii* is highly sensitive with 100% inhibition to both of the synthetic fungicides viz. Mancozeb and Nystatin in all three concentrations. Nystatin inhibited *A.niger* completely in all the three concentrations. Experimental observations witness that Nystatin is more effective to all plant pathogenic strains viz. *S.rolfsii, A.alternata, F.monilifrome, R.solani* and *A.niger* under *in-vitro* conditions whereas Mancozeb show significant inhibition of *F.monilifrome* with relatively low inhibitory effect on other fungal strains as compared to Nystatin. *Calotropis* leaf extracts were screened by Sasode and Singh for antifungal activity against fungal pathogen and were further compared with Mancozeb. Results were in favour of *Calotropis* ex-

Table 1. Radial mycelial growth of fungal strains in control and test plates (in cm).

S.N.	Fungal Strains	Control	Clove	Cinnamon	Thyme	*Parthenium*	*Calotropis*
1.	*Sclerotium*	3.5	-	2.1	2.3	2.8	2.9
2.	*Alternaria*	2.8	-	1.7	1.5	1.8	2.2
3.	*Fusarium*	2.5	-	1.3	1.7	2.0	1.9
4.	*Rhizactonia*	3.9	-	1.0	2.5	3.6	3.5
5.	*Aspergillus*	3.9	-	2.6	3.4	3.5	3.6

Table 2. Radial mycelia growth of fungal strain in control and test plates on treatment of Mancozeb (in cm).

S.N.	Fungal Strains	Control	500ppm	1000ppm	1500ppm
1.	*Sclerotium*	4.2	0	0	0
2.	*Alternaria*	2.9	1.1	0.8	0.7
3.	*Fusarium*	2.8	1.3	0.8	0.6
4.	*Rhizactonia*	4.2	2.7	1.7	0.8
5.	*Aspergillus*	4.3	2.8	2.4	2.1

Table 3. Radial mycelia growth of fungal strain in control and test plates on treatment of Nystatin (in cm).

S. N.	Fungal Strains	Control	500ppm	1000ppm	1500ppm
1.	*Sclerotium*	4.2	0	0	0
2.	*Alternaria*	2.9	0.3	0.3	0.2
3.	*Fusarium*	2.8	1.7	1.5	1.2
4.	*Rhizactonia*	4.2	1.4	0.5	0.5
5.	*Aspergillus*	4.3	0	0	0

Table 4. Economical study of biological fungicides.

S. N.	Extracts	Unit cost (Rs./kg)	Concentration (in %)	Amount used (gm in 50 ml)	Total cost (in Rs.)
1.	Clove	1400	5	2.5	3.50
2.	Cinnamon	1200	10	5.0	6.00
3.	Thyme	600	15	7.5	4.50
4.	*Parthenium*	No cost	25	12.5	No cost
5.	*Calotropis*	No cost	25	12.5	No cost

Table 5. Economical study of synthetic fungicides.

S. N.	Extracts	Unit cost (Rs./kg)	Concentration (in ppm)	Amount used (gm in 50 ml)	Total cost (in Rs.)
1.	Nystatin	22,16,000	500	0.025	55.40
2.			1000	0.050	110.80
3.			1500	0.075	166.20
4.	Mancozeb	340	500	0.025	0.01
5.			1000	0.050	0.02
6.			1500	0.075	0.03

tracts as an ecofriendly alternative for management of disease (Sasode and Singh, 2013). Srivastava and Singh tested the antifungal potential of *Parthenium* extract against *Alternaria* sps. in comparison to Nystatin. The results revealed that extracts can be used as alternative for the development of biofungicides (Srivastava and Singh 2011). Shafique and Shafique applied *Parthenium* extracts against *Fusarium solani* which exhibited prominent reduction in the radial growth of colony (Shafique and Shafique, 2012). Similarily, the antimicrobial efficacy of cinnamon extracts were tested against both bacteria and fungi by Gupta and co-worker and results were found effective (Gupta *et al.,* 2008).

Economical comparison between synthetic and biological fungicides: Today, farm level cost includes the cost of the pesticides and their application. This study was conducted under *in-vitro* conditions to analyse the farm level economics of synthetic fungicides and its comparison to the biological fungicides. In this study, estimation, after excluding the cost of labour and energy, will be the same for both synthetic and biological fungicides. The quantity and costs of materials were entered into study based on prices from local suppliers.

By studying the data presented in table 4 and 5, it is evident that Mancozeb is a low cost fungicide and was found effective under *in-vitro* conditions only against *F. monilifrome* as compared to Nystatin. Nystatin was found to be effective against *S. rolfsii, A. alternata, R. solani* and *A. niger* under *in -vitro* conditions but the price range was quite high. Clove was found to be most effective against all the five plant pathogenic fungi with 100% inhibition and its cost value was moderately less than Nystatin. Though clove is a costly spice but it proved to be effective for deriving profitable economic returns. The present study clearly reflects that *Parthenium* and *Calotropis* tested against plant pathogenic fungi shown antifungal activity at concentration of 25% w/v. So weeds can also be utilized as biological fungicide in higher concentrations as they cost nothing and on the other hand, our useful cereal crops will get rid of these weeds. This study will provide a useful foundation for further inquiry and more extensive analysis.

Conclusion

The present study concluded that among five botanical extracts *viz.* bud of Clove, bark of Cinnamon, seed of Thyme, leaves of *Parthenium* and *Calotropis* in different concentrations *i.e.* 5%, 10%, 15%, 25% and 25% evaluated for antifungal activity against the test pathogens like *S.rolfsii, A.alternata, F.monilifrome, R.solani* and *A.niger* under *in-vitro* conditions, The spices had inherent ability to induce antifungal effects on mycelia growth rate and consequently on proliferation of these fungi. The clove possessed better antifungal activity as compared to other spices and weeds even at very low concentration and it represented an alternative source of natural biological antifungal substance which will be helpful in preventing the environment from being contaminated. However, further analysis of clove could be done to isolate the antifungal agents present in the spices so that they can use commercially as bioagents. The cost value of spices was higher and was the main setback in using them as fungicide, but if health hazards and environment are considered as our priority, then the administration of phytochemicals can be easily adopted. By increasing the concentration of weeds in ethanolic extracts, better results were obtained which will benefit the crops against the allelopathic effect of weeds which are responsible for antifungal activity and will be useful in the formulation of safer and more economical bio-pesticides leading to integrated diseases management.

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