BIOLOGICAL CONTROL OF FUNGAL DISEASES OF POTATO

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ABSTRACT

Solanum tuberosum (Potato) is one of the most important tuber crops with the production of 325 million tons worldwide per year. In the present study an attempt has been made to screen and evaluate the antifungal activity of plant extracts against the fungal pathogens of potato plant. Crude ethanolic extracts of five different plant materials viz. Brassica nigra (cake), Cinnamomum camphora (fruits), Eupatorium adenophorum (twigs), Lantana camara (twigs) and Melia azedarach (fruits) were screened and tested against the three fungal isolates (Phytophthora infestans, Alternaria solani and Fusarium oxysporum) from diseased potato leaf samples. The antifungal activity of the crude extracts obtained was evaluated by agar well diffusion method and two fold broth dilution method. C. camphora gave the highest yield of 70% while M. azedarach had the lowest yield of 9.75% of crude extracts. B. nigra was found most effective against P. infestans with both MIC and MFC values 6.25mg/ml. Similarly, M. azedarach showed the higher antifungal activity against A. solani with both MIC and MFC values 3.125mg/ml. C. camphora was found least effective against P. infestans and A. solani while it was most effective against F. oxysporum with both MIC and MFC values 3.125 mg/ml. Different types of plant extracts with different concentration significantly inhibited the growth of all the fungal pathogens at P (<0.05). The extracts used in this experiment were found to be suitable for the control of these fungal pathogens.

Key Words: *late blight, early blight, fusarial wilt, plant extract*

INTRODUCTION

Potato (*Solanum tuberosum*) is one of the world's most nutritious tuber crops that can meet the food needs of people in a substantial manner (Chadha and Grewal, 1993). Because of its succulent nature, it is susceptible to a number of diseases. Late blight (*Phytophthora infestans*) is one of the most serious diseases of potato worldwide that can result in complete destruction of the crop. Early blight (*Alternaria solani*) and fusarial wilt (*Fusarium oxysporum*) also take a heavy toll of the crop every year (Pushkarnath, 1976). The use of protective chemical fungicides prior to the infection has been one of the common approaches against these diseases. The problems associated with the use of hazardous chemicals for plant disease control has received increasing attention worldwide because, it causes health hazards, environmental pollution, pathogens become resistant to chemical pesticides and ecological imbalances may occur (Fry, 1982). In recent years, there has been a growing movement in the world to reduce the amount of pesticides being applied to the environment. According to a study, only one percent of the total pesticide applied is effective in controlling pests, remaining 99% goes into various environmental systems (Dhaliwal and Arora, 2001). Biological control is an alternative treatment to chemical control that deserves more research.

Most of the plants present in Nepal possess one or more of the chemical properties such as antimicrobial, antifungal, antiviral, antihelmintic, anticancer, sedative, laxative, cardiotonic and diuretic. Different parts of the plants can be successfully used for controlling various insect pests due to the presence of secondary metabolites (Parajuli et al., 1998). The main group of active component in plants is alkaloids, glycosides, saponins, tannin, essential oil, etc. one of the factors for plants having antimicrobial activities is because different types of antimicrobial compounds play a role in plant defense, poliphenolic compounds being known to have multiple functions. These components are extractable with different kinds of solvents (Kruger, 1992). These botanical pesticides are natural products that can even be used in their crude form. They are far low toxic to non target organisms, biodegradable and environmentally safe (Neupane, 2003).This study was therefore designed to investigate the efficacy of the ethanolic extracts of the different fungicidal plant materials against the fungal pathogens isolated from the infected potato plant.

MATERIALS AND METHODS

Collection of fungicidal plant materials

All the selected fungicidal plant materials namely *Brassica nigra* (cake), *Cinnamomum camphora* (fruits), *Eupatorium adenophorum* (twigs), *Lantana camara* (twigs) and *Melia azedarach* (fruits) were collected from Kirtipur.

Preparation of plant materials and their extracts

The collected plant materials were shade dried and grinded to powder using a grinder. The powdered materials were then kept in air tight containers until use. For extraction of bioactive components, 100gm of each of the powdered materials were subjected to continuous soxhlet extraction using ethanol as solvent and the obtained extracts were concentrated under reduced pressure using rotary vacuum evaporator (Tiwari et al; 1992).

Collection of infected potato leaf samples

The leaf samples of infected potato plant were collected from the fields of Kathmandu valley in clean plastic bags.

Isolation and identification of the pathogenic organisms

The infected leaf tissues were surface sterilized using 70% ethanol and placed in two different agar media. For isolation of *P. infestans*, antibiotic amended Rye A Agar medium was used with incubation at 18°C for 5 days in the dark (Sato and Kato, 1993) and for other fungal organisms, Potato Dextrose Agar medium was used with incubation at 27°C for 5 days. The isolated fungal organisms were identified on the basis of their cultural characteristics and microscopic observation.

Evaluation of antifungal activity

The antifungal activity was evaluated by agar well diffusion method as given by Dingle *et al.* (1953). The standard culture inoculum for each fungal species was prepared on Potato Dextrose Broth with adjusting to a range of 1×10^6 - 5×10^6 spores/ml (Aberkene *et al.* 2002). Different concentration (5, 10, 30, 50 and 100 mg/ml) of the various extracts were prepared in Dimethyl Sulphoxide and the solvent itself was tested for its activity as a control at the same time. The zone of inhibition was suggested by the clean area without growth around the well. The MIC and MFC values of each extracts were determined against the fungal pathogens by two fold broth dilution method.

RESULT AND DISCUSSION

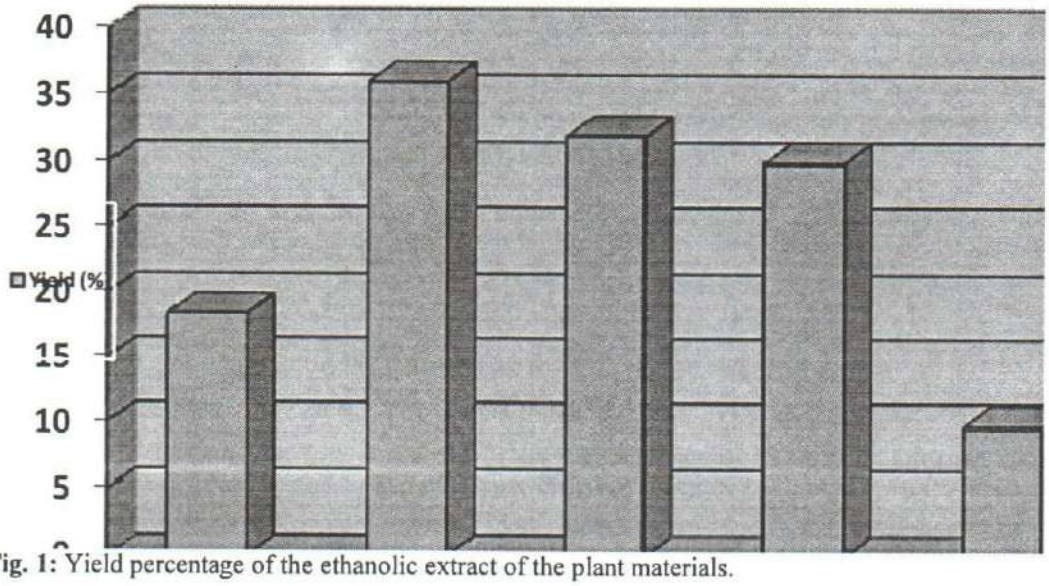

Fig. 1: Yield percentage of the ethanolic extract of the plant materials.

The extraction of crude ethanolic extracts of the selected plant materials dictated the highest yield from C. *camphora* (36%) and lowest from *Melia azedarach* (9.8%). There was a distinct difference in the percentage yield of extracts from different plant materials. The difference in yield might be due to the various factors such as time of extraction, type and part of plant materials, fineness of powder, extent of dryness, etc. Similarly, incomplete extraction results in lesser yield.

The pathogenic fungal organisms were isolated from the infected potato leaf samples using suitable culture media and were identified on the basis of cultural characteristics and microscopic observation. A. *solani* and *F. oxysporum* were isolated on the PDA medium. *P. infestans* does not grow luxuriantly on some of the usual media used for the cultivation of fungi such as cornmeal, PDA or oatmeal agar. The fungus grows slowly and is easily overgrown by other fungi or bacteria (Ingram and Williams, 1991). Hence the antibiotic amended rye A agar medium was used for the cultivation of the fungi and was subcultured on PDA media (Sato and Kato, 1993).

Table 1: Antifungal activity of different concentration of plant extracts against *P. infestans.*

SN	Extract of plant	Zone of inhibition (mm) showed by different concentration of extracts (mg/ml)					MIC (mg/ml)	MFC (mg/ml)
		5	10	30	50	100		
1	*B. nigra*	-	8	9	11	12	6.25	6.25
2	*C. camphora*	-	-	7	8	9	12.5	25
3	*E. adenophorum*	-	7	8	9	11	6.25	12.5
4	*L. camara*	-	8	8	9	10	6.25	6.25
5	*M. azedarach*	-	7	8	10	11	6.25	12.5

Table 2: Antifungal activity of different concentration of plant extracts against *A. solani*

SN	Extract of plant	Zone of inhibition (mm) showed by different concentration of extracts (mg/ml)					MIC (mg/ml)	MFC (mg/ml)
		5	10	30	50	100		
1	*B. nigra*	-	7	9	11	13	6.25	12.5
2	*C. camphora*	-	-	7	9	11	25	25
3	*E. adenophorum*	8	9	10	13	15	3.125	6.25
4	*L. camara*	7	9	10	12	14	3.125	6.25
5	*M. azedarach*	8	9	11	16	20	3.125	3.125

Table 3: Antifungal activity of different concentration of plant extracts against *F. oxysporum*

SN	Extract of plant	Zone of inhibition (mm) showed by different concentration of extracts (mg/ml)					MIC (mg/ml)	MFC (mg/ml)
		5	10	30	50	100		
1	*B. nigra*	-	7	8	10	14	6.25	12.5
2	*C. camphora*	8	10	11	12	13	3.125	3.125
3	*E. adenophorum*	-	8	9	11	15	6.25	12.5
4	*L. camara*	7	9	10	15	17	3.125	6.25
5	*M. azedarach*	-	8	9	10	14	6.25	12.5

Note: (-) indicates no ZOI;
Values are means of three replicates

Different types of plant extracts with different concentration significantly inhibited the growth of all the fungal pathogens at P (<0.05). *B. nigra* was found to be most effective against *P. infestans* with both MIC and MFC values 6.25mg/ml. Similar result was observed by Timila and Ashley (2008) and Timila (2004) in which mustard meal (0.5% amended) significantly reduced the incidence of Phytophthora blight disease of Pepper compared to control.

Melia azedarach showed the higher inhibitory activity against *A. solani* with both MIC and MFC values 3.125mg/ml. *C. camphora* was effective against *F. oxysporum* with both MIC and MFC values 3.125mg/ml. According to Bowers and Locke (2000) 1,5 and 10% aqueous emulsions of formulated extracts of clove oil, neem oil, mustard essential oil, chili pepper extract, cassia tree extracts reduced the population density of *F. oxysporum*.

The plant extracts showed the different antifungal activity. One of the reasons for different fungi toxicity activity of various plant extracts may be due to their different chemical composition (Rao and Srivastava, 1994). The composition of plant oils and extracts is known to vary according to local climatic and environmental conditions (Janssen *et al.*, 1987).

CONCLUSION

Since the selected plant materials under this study were effective against the isolated fungal pathogens in the *in-vitro* test, the field trials should be carried out prior using these extracts for the control of these diseases. The extraction of these plant materials with other solvents, purification of the crude extracts and the identification of the bioactive compound should also be done.

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