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Assessment of leaf extracts of three medicinal plants against late blight of potato in Kaghan valley, Pakistan

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This study was undertaken to assess sun-dried aqueous leaf extracts of three medicinal plants (*Podophyllum hexandrum*, *Withania somnifera* and *Xanthium strumarium)* against late blight of potato caused by *Phytophthora infestans*. Foliar sprays of 25 % (w/v) leaf extracts of the three medicinal plants at a 3 day interval significantly reduced disease severity and resulted in higher tuber yield and biological yield compared to control. Among the tested plants, leaf extracts of *Podophyllum hexandrum* were more effective in minimizing the disease incidence and producing better biological yield/plant (24.92 g) and tuber yield (14.93 t/ha) than other treatments. Results of this study recommends that foliar application of the tested plants extracts (at 3 d) in the order of efficiency (*P. hexandrum*> *X. straumerium*> *W. somnifera*) against late blight could be used for minimum disease incidence and better yields.

Key words: bioactive compounds, late blight incidence, aqueous leaf extracts, *Phytophthora infestans*

Introduction

Late blight of potato (*Phytophthora infestans* Mont. de Bary) is a serious threat to potato production all over the world which not only causes severe crop damages but also contributes to considerable monitory losses due to excessive use of fungicides for its control. It is estimated that annual crop losses caused by late blight of potato exceeds 2.75 billion US dollars in addition to another 100 million (US dollars) which is spent on different fungicide programs for the control of the disease (Anonymous, 1997). Humid and cold conditions are key contributing factors for *P. infestans'* infection and the disease outbreak

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(Johnson *et al*., 1997). In Pakistan, the occurrence of *P. infestans* was firstly reported by Khan *et al*. (1985) from District Swat, a valley which provides ideal conditions for late blight pathogen. Since then, the occurrence of late blight pathogen and application of fungicides for control of the disease from other potato growing regions of the country have been repeatedly demonstrated (Farooq *et al*., 2002; Khan *et al*., 2003).

Routinely, late blight disease is managed by proper agricultural practices, manipulation of host resistance and using chemical fungicides at high frequency (Olanya *et al*., 2001; Kuhl *et al*., 2007). However, frequent uses of fungicides have raised problems of resistance development in the pathogen, especially to metalaxyl (Phenylamide) containing compounds (Kato *et al*., 1997; Lambert and Currier, 1997; Daayf *et al*., 2001). The occurrence of phenylamide resistant isolates of *P. infestans* with increased virulence and aggressiveness in several countries across the globe has been widely reported in literature (Koh *et al*., 1994; Judelson and Roberts, 1999; Gisi and Cohen, 1996). Moreover, fungicides and pesticides application also poses serious threats to environment in the form air, soil and even water pollution because many of these chemicals may contain copper and other hazardous compounds. Such environmental threats may not be detected directly; however, their long term effects on ecosystem cannot be ignored.

Growing concerns about the problems (environmental and pathogen resistance) have aroused from the frequent uses of late blight fungicides. Researchers are opting for alternative strategies to conventional fungicides for late blight management. In that context, plant extracts (Krebs *et al*., 2006), and biological antagonists (Ghorbani *et al*., 2005) could be used as valuable alternatives to synthetic fungicides for controlling late blight. Previously, extracts of several plant species have been reported to be effective against late blight pathogen (*P. infestans*) in laboratory bioassays (Goufo *et al*., 2008; Shutong *et al*., 2007; Rashid *et al*., 2004) as well as under field conditions (Bassin and Forrer, 2001; Abd-El-Khair and Haggag, 2007). Wang *et al*. (2004) achieved 90 % inhibition of some fungal diseases including late blight of potato by foliar application of 1 % (w/v) acetone and n-hexane or aqueous leaf extracts of *Inula viscose*. In a similar study, Abd-El-Khair & Haggag, (2007) evaluated aqueous extracts of different parts (leaves, fruits and seeds) of nine medicinal plants in laboratory and field conditions against *Phytophthora infestans* and *Alternaria solani*. They reported that among the tested plants, 10 % leaf extract of grass lemon (*Cymbopogon citratus*) were effective in reducing spore germination and disease incidence of both pathogens.

Major compounds of plant extracts are phenols, flavonoids, alkaloids, quinones, saponines, tannins and sterols (Halama and van Haluwin, 2004) and

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their fungicidal or fungistatic properties against various plant pathogens have been established (Scheuerell and Mahaffee, 2002). These products may either have direct inhibitory effects on pathogens, exhibiting fungicidal or fungistatic properties or they can help in establishment of favorable conditions for antagonistic microbes (Scheuerell and Mahaffee, 2002).

In Pakistan, the occurrence of *P. infestans* was firstly reported by Khan *et al*. (1985) from Swat Valley. Since then, applications of fungicides for control of the disease have been repeatedly demonstrated; however, plant extracts for late blight control in field experiments have not been investigated so far. This study was conducted with the objectives of investigating the efficacy of leaf extracts of three medicinal plants (*Podophyllum hexandrum, Withania somnifera*, and *Xanthium strumarium*) against late blight of potato.

Material and methods

Experimental site and design

The experiment was conducted during 2008-09. Certified seeds of potato cv Desiree were obtained from Hazara Agriculture Research Station Abbottabad and were sown in the experimental farm at Sharan (Kaghan Valley) 48 Km away from District Mansehra. Potato seeds were grown in four row plots, 3 meter long with spacing of 70 cm between rows and 30 cm within rows. Experiment was laid out under natural field conditions in a randomized complete block design (RCBD) with four replications.

Preparation of plant extracts

Sun dried leaves of *Podophyllum hexandrum*, *Xanthium strumarium*, and *Withania somnifera* were ground with an electric chopper. In order to prepare 25 % aqueous leaf extract of each plant, 250 gram of each ground sample was added to one liter distilled water and stored for 24 hours at room temperature, then filtered through Wattman No. 1 filter paper and stored at room temperature (10-15 C) for future use.

Application of plant extracts and disease assessment

Prepared plant extracts were applied with a hand sprayer to potato leaves at a 3 day interval soon after the late blight symptoms were visible. Disease

incidence (%) was calculated on weekly basis on a scale from 0-5 (Mantecon, 2009). At harvesting, 10 plants from each plot were randomly selected and oven dried in paper bags at 70 ºC for four days in order to record biological yield/plant. Total tuber yield (tons/hectare) were calculated by collecting and weighing tubers from each experimental plot. Data on recorded parameters was statistically analyzed by applying analysis of variance (ANOVA) and means for different treatments were separated by LSD.

Results and discussion

Data on disease incidence, biological yield/plant and tuber yield as affected by foliar application of 25% water soluble leaf extracts is represented in Table 1. Late blight disease incidence (%) was assessed three times (at 50, 70 and 90 days after planting) during the experimental period. At first evaluation (at 50 DAP), 26.52 % of the leaves were found infected in control plots. There after proportion of infected leaves progressed with increase in days after planting, reaching to a maximum (99.90 %) at 90 DAP. However, in every assessment, disease incidence in plots treated with plant extracts was lower than control. After 90 DAP lowest number of leaves (62.93 %) was deteriorated by *P. infestans* in plots treated with the extracts of *Podophyllum hexandrum* followed by *Xanthium strumarium* (73.93 %) and *Withania somnifera* (85.37 %) when compared to control (99.90 %).

Table1. Effect of three plants extract on disease incidence, biological yield and tuber yield of potato.

Treatment	Disease incidence (%)				
	50^{dap}	**70^{dap}**	**90^{dap}**	**Biologic yield/plant (g)**	**Total tuber yield (t/ha)**
Control	26.52^{a}	83.40^{a}	99.9^{a}	16.92^{d}	11.78^{d}
W. somnifera	15.48^{b}	68.71^{b}	85.37^{b}	19.37^{c}	12.05^{c}
X. strumarium	9.18^{c}	59.13^{c}	73.93^{c}	21.67^{b}	13.49^{b}
P. hexandrum	9.017^{c}	44.31^{d}	62.93^{d}	24.92^{a}	14.93^{a}
LSD (0.05)	0.1959	0.07154	0.05058	0.0016	0.05058
Coefficient of variation (%)	0.82	0.07	0.04	0.23	0.07

dap= days after planting
Values in each column followed by similar letters are non significant at P=0.05

Biological yield and total tuber yield of potato crop was also significantly affected by the tested plant extracts. Due to greater disease incidence in control plots, biological yield/plant and tuber yield were minimized to 16.92 g and

11.78 t/ha respectively. Water soluble leaf extracts of *P. hexandrum* exhibited maximum efficiency against late blight of potato and resulted in enhanced biological yield/plant (24.92 g) and total tuber yield (14.93 t/ha). Plots treated with *X. strumarium* and *W.somnifera* also produced significantly greater biological and tuber yield than control but lesser than *P. hexandrum* treated plots.

Due to heavy rainfall (25.8 inches) and cold temperature (10-12 C), the experimental site provides ideal and conducive environments for late blight pathogen (*P. infestans*) to infect potato crop and spread the disease more rigorously. In our study, lower biological and tuber yield in control plots could be the cause of highest level of blight incidence in control and conducive environment of the experimental site for *P. infestans* to incite the epidemic.

Application of plant extracts significantly lowered disease severity level which corresponded to better biological and tuber yield. Beneficial effects of plant extracts on biological and tuber yield were due to lower level of disease incidence which could have increased the availability of photosynthetic leaf area (Olanya *et al*., 2001) and greater amount of photosynthate being transferred to tubers for storage. Extract of *P. hexandrum* showed excellent activity against the disease incidence reducing the disease severity level by 37.01 % as compared to *X. strumarium* and *W. somnifera* which contributed to reduction in disease severity by 25.99 and 14.56 % respectively (Fig 1). Low level disease severity in treated plants may be attributed to inhibitory effects of bioactive compounds such as withanolides from *W. somnifera* (Rahman *et al*., 1991), xanthanolides from *X. strumarium* (Omar *et al*., 1984) and podophyllotoxins from *P. hexandrum* (Giri and Narasu, 2000) on mycelia growth of *Phytophthora infestans*. This could have increased photosynthetic activity of leaves resulting in better yields.

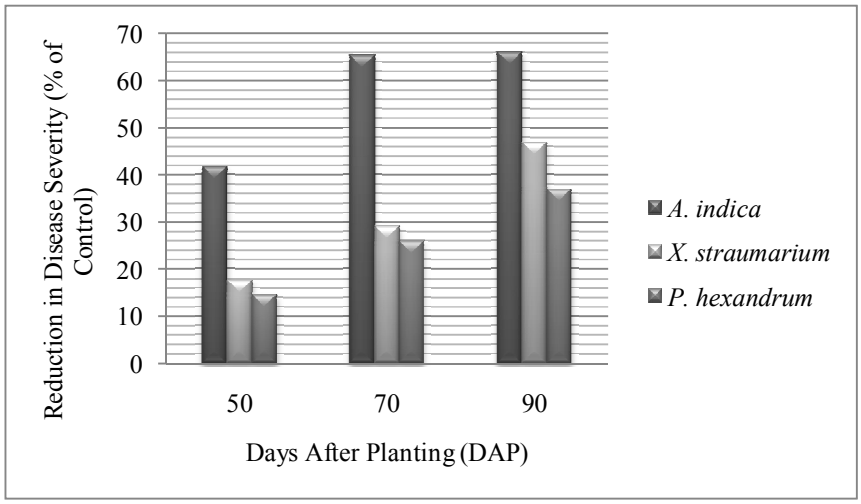

Fig. 1. Reduction in disease severity of potato leaves as influenced by plant extracts.

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