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EFFICIENCY OF DIFFERENT NEEM (*AZADIRACHTA INDICA* A.JUSS) PRODUCTS AGAINST VARIOUS LIFE STAGES OF *PHYTOPHTHORA INFESTANS* (MONT.) DE BARY

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Abstract

Efficacy of different neem (*Azadirachta indica* A.Juss) products was investigated against *Phytophthora infestans* using two isolates viz., NR-971 and BN-971 differing in aggressiveness. NR-971 was virulent against 0,1,3,4,7,10,11 resistant genes while BN-971 showed virulence against 0,1,2,3,4,7 resistant genes. Three products from neem viz., neem leaf diffusate, neem leaf powder and neem seed cake were evaluated for their effect on mycelial growth, sporangial production and sporangial germination of the 2 isolates. Neem leaf diffusate and neem leaf powder completely inhibited the mycelial growth of both isolates at 2.0% v/v and 80% w/v concentration, respectively while neem seed cake showed 100% mycelial growth inhibition in NR-971 isolate at 1.0% and that of BN-971 isolate at 0.8% w/v concentration. Neem leaf diffusate completely inhibited sporangial production of NR-971 isolate at 1.0% and that of BN-971 isolate at 0.4% w/v concentration. Both neem leaf powder and neem seed cake completely inhibited sporangial production sporangial germination of NR-971 and BN-971 isolate at 0.6% and 0.4% w/v concentrations, respectively. The more virulent isolate NR-971 was found more sensitive to neem products than less virulent isolate BN-971.

Introduction

Late blight caused by *Phytophthora infestans* (Mont.) de Bary is an important disease of potato crop. The disease was reported for the first time in Pakistan in 1984 from Kalam and Malam Jabba Valleys in Swat district by Khan *et al.*,(1985).Since then the disease has been reported from potato growing plains of Punjab and North West Frontier Province. More recently, it has been found in the area of Balochistan and Northern Pakistan where macroclimate is unsuitable for late blight (Ahmad *et al*.,1995).

Late blight of potato can be successfully controlled by a combination of sanitary measures, resistant varieties, and well-timed fungicidal sprays. However, the realization of the harmful effects caused by the chemical pesticides has forced the scientists and farmers to search for the alternative materials to avoid the ecological hazards. Among the several materials available, plant product offered greater scope than the rest, as they are safe, easily biodegradable and eco-friendly. Neem (*Azadirachta indica* A. Juss) has been reported to have insecticidal, antifungal, antibacterial and antiviral properties. During the present study, three products viz., neem leaf diffusate, neem leaf powder and neem leaf cake were used against *Phytophthora infestans*, the late blight fungus

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Materials and Methods

Two isolates NR-971 and BN-971 of *Phytophthora infestans* were obtained from Mycology Laboratory of CDRP, NARC, Islamabad. On the basis of virulence against potato differentials, NR-971 was categorized as belonging to race 0,1,3,4,7,10,11 while BN-971 from race 0,1,2,3,4,7. These isolates were grown and maintained on rye agar medium (Caten & Jinks, 1968).

Fresh neem leaves obtained from local plantation in Punjab were dried on laboratory tabletop. Dried leaves were finally ground in electric chopper to get fine leaf powder.

For preparing the neem leaf diffusate, 1 kg of leaf powder was soaked overnight in 1 litre of distilled water. After soaking, the suspension was boiled for five minutes and passed through muslin cloth and then filtered through Whatman No.1 filter paper. Filterate thus obtained was used as neem leaf diffusate. Neem seed cake was obtained from Grain Storage Laboratory, Tropical Research Institute, Karachi. It was finely ground in an electric chopper to get fine powdered neem seed cake which was later used for experimental purposes.

Evaluation of neem products: Efficacy of neem products was evaluated against mycelial growth, sporangial production and sporangial germination of *P. infestans*.

Mycelial Growth: Neem leaf diffusate was tested at 0.2, 0.4, 0.6, 0.8 1.0 and 2.0% v/v. Neem leaf powder and neem seed cake were studied at 0.2, 0.4, 0.6, 0.8 and 1.0% w/v in rye agar medium. Rye agar medium without any neem amendment served as control. Sterilized amended and unamended media were poured in 90 mm Petri plates under sterilized conditions. There were 3 replications of each treatment. Plates were inoculated with 5.5 mm diameter mycelial plugs from the edge of actively growing 14 days old cultures of Bn-971 and NR-971 isolates of *P. infestans.* Two plugs one from each isolates (Bn-971 and NR-971) were placed on opposite margin of each Petri plate. Inoculated plates were incubated at 18 ± 2^{o} C in darkness. Radial mycelial growth was measured after three weeks of incubation.

Sporangial production: After measuring the radial mycelial growth in the above mentioned experiment, two mycelial discs of 5.5 mm diameter each were taken from culture growing on treated and untreated plates. First disc was taken from periphery of the fungal growth and the second disc was taken immediately next to first one towards centre of the culture. Mycelial discs were placed on glass slide and pressed under a cover slip. Number of sporangia was counted under a compound microscope at 40 x magnification. Sporangial counts on both plugs were averaged to get number of sporangia per plug.

Sporangia germination: For studying the different effect of neem products on sporangial germination, sporangial suspension was prepared by surface washing of 14 days old cultures of *P. infestans* with sterilized distilled water. Concentration of sporangial suspension was adjusted to 5×10^4 sporangia/ml of water by haemocytometer (Tool *et al.,* 1989). Two millimeters of the sporangial suspension was poured in pre-sterilized test tubes and amended with pre-sterilized neem products to investigate their effect on sporangial germination.

For neem leaf diffusate, stock solution of required quantities was added in tubes to get 0.2, 0.4, 0.6, 0.8 and 1.0% v/v concentration. Neem leaf powder and neem seed cake were used at 0.2, 0.4, 0.6, 0.8 and 1.0% w/v concentrations. Weighed amounts of pre-sterilized neem leaf powder and neem seed cake were added directly in beaker to get desired concentration. Beaker without any amendment served as control. Each treatment was replicated three times. Final volume of 100 ml was maintained in each amended as well as unamended beaker by adding sterilized distilled water.

Sporangial germination was recorded after 1, 3, 5 and 7 day of the experiment. In order to count germinated sporangia one drop from each amended and unammended test tube was poured on a glass slide. After covering with a cover slip germinated sporangia were counted under compound microscope at 40 x magnifications. Germinated sporangia appeared shrunk with growing germ tube. Percentage inhibition in mycelial growth, sporangial production and sporangial germination was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Growth in control - Growth in treatment}}{\text{Growth in control}} \times 100$$

Mean mycelial growth inhibition, inhibition in sporangial production and inhibition in sporangial germination by different neem products were compared by standard error bar method on bar diagram of their respective means (Shattock, 1988).

Results and Discussion

All the products used in the study proved highly efficient in inhibiting the growth of *P. infestans* at various life stages. Efficacy of neem products varied not only at different growth stages but also between isolates. Among the three neem products, neem leaf powder was most effective at almost each growth stages of *P. infestans* studied. Maximum inhibition of mycelial growth occurred at 0.8 and 0.6% w/v concentration and sporangial germination ceased (100 %) at 0.6 and 0.4% w/v concentration of neem leaf powder in NR-971 and BN-971 isolates of *P. infestans* respectively (Fig. 1b & e). During sporangial germination BN-971 showed greater sensitivity than BN-971 but 100% inhibition of sporangial germination occurred at 1.0% w/v concentration in both the isolates (Fig. 1h). Neem leaf diffusate was capable to inhibit 100% mycelial growth and sporangial germination at 2.0 and 1.0% v/v concentration, respectively (Fig 1a & g). Complete inhibition of sporangial production occurred at 1.0 and 0.8% v/v concentration of neem leaf diffusate in NR-971 and BN-971 isolates of *P. infestans*, respectively (Fig. 1d).

Since both neem leaf diffusate and neem leaf powder are leaf-based products, the results showed that neem leaves contain some antifungal compound that is effective against various growth stages of *P. infestans.* Sadri *et al*., (1983) reported that neem leaves contain gedunin which possess antifungal properties. Hameed (1997) and Mirza e*t al.,* (2000) also reported effectiveness of neem leaf based products against *P. infestans* where decoction of neem leaves was used. Neem leaf decoction was very effective in inhibiting mycelial growth, sporangial production and sporangial germination. It is interesting to note that neem leaf powder was capable of fungal inhibition at lower concentration as compared to neem leaf diffusate indicating that different concentrations

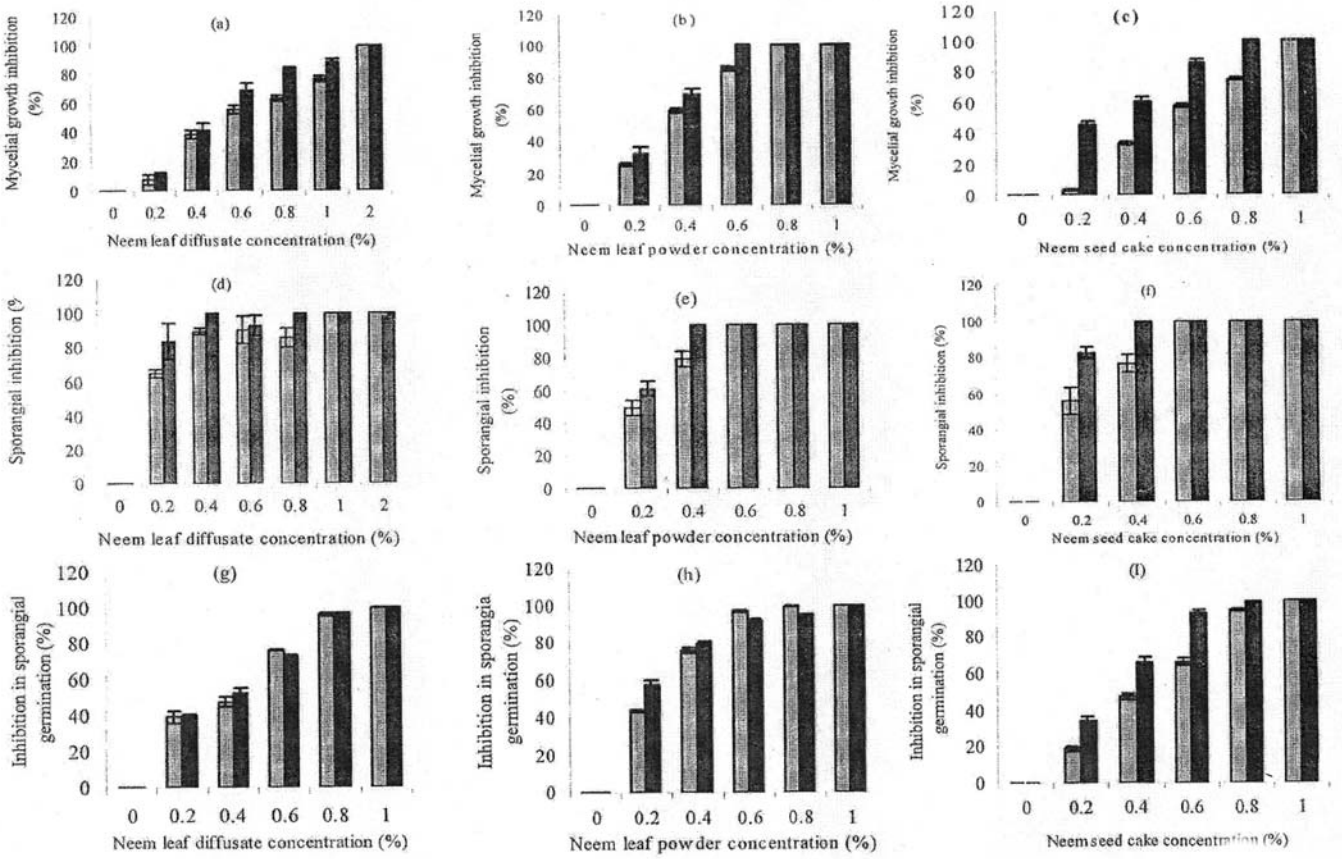

Fig. 1. Effect of different neem products against various life stages of NR-971 and BN-971 isolates of *Phytophthora infestans* (Eroor Bar = ± Standard Error) NR-971 (Race 0, 1, 3, 4, 7, 10, 11) BN-971 (Race 0, 1, 2, 3, 4, 7).

of antifungal agent are available in neem leaf powder and neem leaf diffusate. Active ingredients from neem leaves may be less available in aqueous solutions but when powdered leaves were used, active ingredient is utilized in greater quantity. Neem seed cake showed different antifungal effect against both NR-971 and BN-971 isolates of *P.infestans.* Mycelial growth of BN-971 was completely inhibited at 0.8% w/v concentration while that of NR-971 at 1.0% w/v concentration (Fig. 1c). Sporangial production of BN-971 isolate was completely inhibited at 0.4% concentration and that of NR-971 at 0.6 % w/v concentration (Fig.1f). This variation in effectiveness of neem seed cake between isolates continued during sporangial germination. At 0.2% w/v concentration 34.32% and 19.0% reduction in sporangial germination in BN-971 and NR-971 isolate of *P.infestans* respectively was observed. But sporangial germination in both isolates was completely inhibited at 1.0% w/v concentration of neem seed cake (Fig. 1i). Hameed, (1997) and Mirza *et al.,* (2000) reported that antifungal activity of neem seed oil is due to Azadirachtin. As neem seed cake is also a seed based product so azadirachtin could also be responsible for antifungal activity against *P. infestans.*

Behavior of both isolate to neem products was almost different at each growth stage. Reason for different behavior of both isolates could be their different generic makeup. Both isolates belonged to different races on the basis of virulence pattern. BN-971 is more virulent and showed greater sensitivity to neem products while NR-971 was less virulent and showed less sensitive to neem products. These results are also supported by metalaxyl sensitivity test in which NR-971 was graded as resistant and BN-971 was graded as moderately resistant (Anon., 1998). Similarly Yang *et al.,* (1996) while working on *Sclerotinia sclerotiorum* reported that isolates resistant to carbendazim were less virulent than isolates sensitive to carbendazim. Results of the study suggests that neem has the ability to check growth and propagation of *P.infestans.* Potential of neem and neem based formulations in plant disease management can be very healthy addition, though complete suppression of plant diseases is not possible. Hence use of neem products for the control of different pathogens and diseases should be considered.

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