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Use of Neem Oil as A Control Measure in Rice Blast in in-Vitro Conditions

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ABSTRACT: The indiscriminate use of chemicals has led to development of fungicide resistance and more importantly, environmental pollution, posing a potential risk to animal and human health. Consequently, some pest management researchers have focused their efforts on developing alternative inputs to synthetic chemicals for controlling pests and diseases. During present investigation, experiments were carried out to test the efficacy of neem oil, under in vitro experiments. At 0.25% concentration, neem baan inhibited the fungal growth to 45.70%, at 0.5% concentration the inhibition percentage increased up to 62.86% and with the increase in concentration to 1.0%, the diameter of mycelia growth also showed a downward trend and resulted in 79.09% inhibition effect.

KEYWORDS: inoculums, fungicide, pest management, neem baan, culture, fungi, oil, pest

INTRODUCTION

During the second half of the 20th century, we have witnessed an unprecedented growth in human population.The population pressure increases demands for basic human needs such as food. Control of plant diseases will remain a high priority as growers adopt new measures for sustainable crop production (Conway 1996). Beyond good agronomic and horticultural practices, growers often rely heavily on chemical fertilizers and pesticides. The indiscriminate use of chemicals has led to development of fungicide resistance [Okigbo 2004 and Carvalho 2004] and more importantly, environmental pollution, posing a potential risk to animal and human health [Lyon *et al* 1995]. Consequently, some pest management researchers have focused their efforts on developing alternative inputs to synthetic chemicals for controlling pests and diseases (Pal 2006).The application of natural products produced by plants and/or animals with antifungal properties or to induce plant defense mechanisms (Tripathi and Dubey, 2004); . This study focused at evaluating the phytotoxic activity of locally available neem, against *Pyricularia oryzae* Neem has been proved to be effective against certain fungi that infect the plants. The Neem Tree has been described as Important, because of Anti Malarial, Anti Tubercular, Anti Viral, Anti Allergic, Anti Enzymeic, Anti Scabic, Anti Dermatic, Anti Gingivitic, Anti Inflammatory, Anti Periodontitic, Amoebicidal, Diuretic, Spermicidal, Anti Pyrrhoeic, Anti Seborrhoeic, Anti Feedant, Anti Fungal, Anti Furuncular, Bactiricide, Insecticidal, Larvicidal, Nematicidal, Piscicidal, Joshep *et al* (2008) tested the *in vitro* efficacy of different plant extracts including *Azardiachta indica*, to control brinjal wilt pathogen *Fusarium solani f. sp. melongenae* and found that among the different extracts 20% of *Azardiachta indica* extract was found to be most effective among the different plant extracts.

Atungwu *et al* (2009) conducted a study to evaluate the efficacy of Neem (*Azadirachta indica A. Juss*) leaf powder and organic fertiliser for management of *Meloidogyne incognita* in soybean (*Glycine max*) production Baan was used in the present investigation. It is composed of neem seed kernel extract (60% w/w) in solvent methanol containing 0.15% Azadirachtin emulsifier, 5% w/w polysorbate20 and polyoxyethylene ether and treated neem oil 35%w/w.

II.MATERIAL AND METHODS

To full fill this objective, during present investigation, experiments were carried out to test the efficacy of neem oil, under in vitro experiments in following steps:

1 Glassware and Cleaning

In all the experimental studies Borosil glass wares were used. The glass wares, for ex. Petri plates, test tubes, conical flask, beaker, measuring cylinder, pipette, cavity block, borer etc. were kept in the cleaning solution containing 60 g of

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potassium dichromate ($K_2Cr_2O_7$), 6 ml of concentrated sulphuric acid (H_2SO_4) in 1000 ml of water for overnight and then rinsed in tap water.

2 Sterilization

All the glassware were sterilized in an autoclave at 1.1 kg/cm2 pressure for 15 minutes and then kept in hot air oven at 550C for one hour. The solid and liquid media were sterilized at 1.1 kg/cm2 pressure for 15 min.

3 Collection, Isolation of the pathogen and Preparation of Inoculums

Infected plant tissues were collected from rice fields of Bundi district, Rajasthan as the source of inoculum. The culture of *Pyricularia oryzae* used throughout the investigation was prepared from blast infected plant parts collected from the farmer's field of Bundi. The plant parts were examined and the pathogen was isolated by following standard tissue isolation procedure (Tuite, 1969). Under aseptic conditions, the infected plant tissue were cut in small section 5-10 mm square from the margin of the infected lesions such that it contained both diseased and healthy looking tissue. The tissues were surface sterilized for two minutes in 90% ethanol, washed with three changes of sterile water and blotted dry on clean sterile paper by use of forceps and finally plated in Petri dishes containing oat meal agar (OMA) supplemented with paddy powder medium **[**Oat flakes 20.0g, Agar– agar 20.0 g, Paddy powder 10g, Distilled water (to make up) 1000.00 ml .For the preparation of the medium first oat flakes and paddy powder were boiled with 500 ml of distilled water for 30 min. and filtered through muslin cloth. Agar–agar was melted in 500 ml water separately. Both the solutions were mixed thoroughly and sterilized plates. The culture media inoculated with diseased tissues were incubated under continuous light at 25°C for 24 hours after which the light was put off and incubation continued for seven days allowing the growth of mycelia and spores and observations were made daily for emergence of culture. Under sterile condition a drop of sterile water was put on the slide and a small piece of mycelia placed on it, covered with a cover slip and placed under a light microscope for observation. After the identification of the fungus, the pathogen was sub-cultured in order to isolate rice blast fungus and accelerate the sporulation. The Petri dishes were re incubated in the laboratory for seven days during which growing fungi were viewed under a light microscope. Further sub-culturing was done to obtain pure cultures. After the development of the fungal colonies stock cultures had been prepared using O M A supplemented with paddy powder in test tubes and stored in refrigerator at 4°C.

4 Preparation of the solutions of control agents

From the commercial product Neem Baan solutions of different concentrations were prepared. For this purpose 2.5ml, 5ml and 10ml pure neem oil was taken in separate container and each was diluted with 1000 ml distilled water and thus the three concentrations prepared were of 0.25%, 0.5% and 1.0% and named as N1, N2 and N3 respectively.

Fully and uniformly grown fungus culture plates were taken and three wells of 8mm were prepared at five cm apart from each other. Two ml each of neem oil of 0.25%, 0.5% and 1.0% concentrations prepared were loaded on separate filter papers of 8mm size under aseptic condition and dried. The process of loading was repeated until the saturation of filter paper. These loaded pieces were placed in wells made in culture plates. Appropriate control with distilled water loaded filter paper was maintained as check experiment. Each treatment was replicated three times. The plates were incubated for seven days at25±2˚C. Zone of Inhibition in mycelial growth was recorded when the growth of the selected pathogen was completed in the control treatment. Mean radial mycelial growth inhibition of each conc. of control agent was measured with scale. Radial mycelial growth inhibition of fungus on different conc. of neem oil were transformed into inhibition percentage by using the following formula (Naz et al 2006):

Where
I = Per cent inhibition
C = Radial growth in control
T = Radial growth in treatment

Observation

Experiment was carried out for the management of *Pyricularia oryzae* infecting rice plant causing blast disease of rice through neem baan, under in *In vitro* conditions. *In vitro* evaluation of control agents was carried out with respect to inhibition of mycelial growth of *P. oryzae* at different concentrations as described before in Methodology. The data was presented in Table and Fig.

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Effect of control agent Neem Baan on inhibition of mycelia growth of *Pyricularia oryzae*

S N	Treatments	Concentrations (%)	Percent Inhibition
1.	Neem Baan	0.25	45.70
		0.5	62.86
		1.0	79.09

IV.RESULT AND DISCUSSION

Neem Baan, solutions had significant effect on inhibition of mycelial growth of *P. oryzae*. In order to quantify the inhibitive effect of neem baan against *P.oryzae*, different concentrations of neem baan were evaluated. The data presented in Table indicates that all the concentrations of neem baan were found to be significantly effective in inhibiting the mycelia growth of *P. oryzae* as compared to control. At 0.25% concentration, neem baan inhibited the fungal growth to 45.70%, at 0.5% concentration the inhibition percentage increased up to 62.86% and with the increase in concentration to 1.0%, the diameter of mycelia growth also showed a downward trend and resulted in 79.09% inhibition effect.The mycelial growth was reduced by 45.70%, 62.86% and 79.09% at 0.25%, 0.5% and 1.0% concentration of neem baan as compared to control. The inhibition rates increased with increasing concentrations of the control agent. The results were in conformation with Sanjeet *et al* (2005) who found that extracts of *A. indica* was highly controlling in leaf spot disease in Faba beans caused by *Alternaria alternata* under laboratory and field conditions. Studies conducted by Niaz *et al* (2008) had also demonstrated the marvelous effects of neem products like neem seed oil against *F. moniliforme, M. phaseolina and R. solani* and neem seed kernel extract (NE) against *Monilinia fructicola, Penicillium expansum, Trichothecium roseum* and *Alternaria alternate* (Wang *et al* 2010). The present results are also in corroboration with Hassanein *et al* (2008) who observed that 5%, 10%, 15% and 20% concentration of neem extract effectively suppressed the mycelial growth of both the species *viz. Alternaria solani* (causing early blight) and *Fusarium oxysporum f.sp. lycopersici* (causing wilt disease) in tomato.The phytoconstituents alkaloids, glycosides, flavanoids and saponins are antifungal principles of neem plant. These principles might be the defensive mechanism of the plants against different pathogens (Hafiza 2000).

The results of the present research work will be useful for devising effective eco-friendly strategies to manage the blast disease of paddy. The knowledge gained during the present investigations will serve as a foundation for further research work on the biology of the pathogen and epidemiology and management of the disease.

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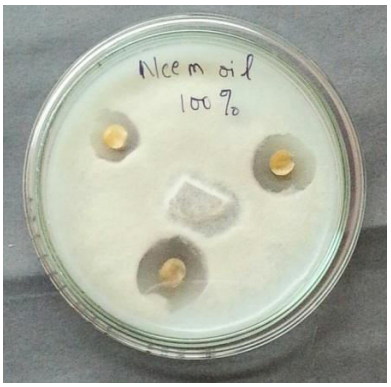

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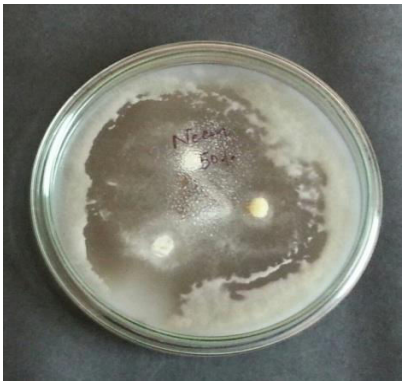

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