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Research Article

IN VITRO EVALUATION OF BOTANICALS AND PANCHAGAVYA AGAINST LEAF BLAST FUNGUS *PYRICULARIA GRISEA*

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ABSTRACT

Rice blast caused by *Pyricularia grisea* cav. continues to be a major constraint in rice production. Since, the existing chemical control measures being costly and may favour development of resistance in pathogens, the potential alternative methods have been explored in the present studies. Five plant parts extract namely Neem seed kernel extract, Neem oil, *Asafoetida* spp. and *Pongamia* spp. extracts and Panchagavya, were evaluated for their efficacy against blast of rice in in vitro conditions. The results concluded that the Neem seed kernel showed a significantly more mean suppression value.

Keywords: Rice blast, *Pyricularia grisea*, plant parts extract,

INTRODUCTION

Rice (Oryza sativa L.) is one of the most important cereal crop of family Poaceae. It is a staple food crop of 60 percent of the world's population. "Rice is life" the theme of International Year of Rice, 2004 that reflected the importance of rice (*Oryza sativa* L.), which holds the key to our country's ability to produce enough food for our people. Asia accounts for 90 percent of world's production and consumption of rice because of its favorable hot climate .Though, India has the distinction of having the largest area of 44 million hectare in the world, it occupies second position in total production (89 m tons).The productivity of rice in India (2.05 t/ha) is much below the world average (2.62 t/ha).

Growth in population and economic prosperity are the key driving forces of increasing rice demand. The development of need based cost effective plant protection measures for effective management of rice diseases is the need of the future for higher and sustainable rice yield [1].The biological method of plant disease management seems to be a better alternative to chemical fungicides in managing the blast disease. In addition, the biological control of plant pathogens is an attractive preposition as it mimics the nature's own way of balancing of population of living organisms [5].With the aim of controlling the rice blast by using biological control methods the present investigation was undertaken in the field trail experiments against the disease in order to find out suitable biological control for management of blast.

MATERIALS AND METHODS

Fresh infected leaves of the rice plant showing typical symptoms of the leaf blast were collected from the disease prone zones of Nellore-ARS for isolation of the pathogen.

Infected portions of the leaves were cut into small bits with a little healthy leaf tissue around were surface sterilized with 0.1 percent mercuric chloride for 30 sec followed by series of washings with sterilized water. These bits were transferred aseptically into rice polish agar medium contained petriplates. The petriplates were incubated at room temperature (28±2ºc) (fig;1). After 72 hours of incubation, radiating mycelia growth was observed from the edges of the infected bits. Edge of the fungal colonies were transferred to rice polish agar medium slants in a refrigerator at 10ºC and periodical subculturing for all the studies. The virulence of the culture was checked by artificially inoculating the rice cultivars and by reisolation. The causal organism was identified as *Pyricularia grisea* on the basis of morphological and cultural characteristics [11].

Maintenance of isolates *Pyricularia grisea*

The fungus was subcultured on Rice polish agar slants and kept at 28±100 C for 15 days. Subsequent, subculturing of isolates was done at an interval of 20 days. Such isolates were stored in a refrigerator at 50 C. The cultures were revived periodically.

Rice polish agar medium

Rice polish	-	20 gm
Agar	-	17 gm
Distilled water	-	1000ml

Mix 20g rice polish with 500ml water and steam for 15min. Blend the suspension for several minutes and mix with 500 ml water containing 17g agar. Autoclave and pour into culture plates. While pouring, agitate the medium to prevent setling of rice polish. The pH was adjusted to 6.8 prior to sterilization.

Preparation of plant extracts and panchagavya

Fresh plant parts namely neem seed kernel powder, kranja seed powder, hing powder and neem oil were used as treatments. Solvent, ethnol (95%) was used for the phytochemical extraction of various plant parts. For extraction with ethnol, 25g of powdered plant materials was dissolved in enough sterilized ethnol to make 100ml extract (25% w/v). The mixture was kept undisturbed at room temperature for 24 hrs in a sterile flask covered with aluminum foil to avoid evaporation and subjected to filtration through sterilized Whatman no.1 filter paper. After filtration, the extract was evaporated in water bath until 25ml extract was left in the container. Ethnolic extract thus obtained were immediately evaluated for antifungal activities using poisoned food technique [2].

Panchagavya is prepared by mixing cow dung, cow ghee, cow urine, water, cow milk, cow curd, coconut milk, jaggery, ripened bananas with appropriate measurements [9] and extract is prepared and concentrations with 3000-9000 ppm were determined against the test pathogen using poisoned food technique.

Poisioned food technique

Generally antifungal activity is determined by poisoned food technique [6]. Eight days old fungal culture is punched aseptically with a sterile cork borer of generally 5mm diameter. The fungal discs are then put on the gelled agar plate. The agar plates have been prepared by impregnating concentrations from 500ppm-9000ppm

of fungicides and plant extracts at a temperature of 45-50^{0}C. The plates are incubated at temperature 26+/- 1^{0}C for fungi. Colony diameter is recorded by measuring the two opposite circumference of the colony growth. Percentage inhibition of mycelial growth is evaluated by comparing the colony diameter of poisoned plate (with fungicide or plant extract) and non-poisoned plate (with distilled water) and calculated using the formula given below.

$$\% \text{ Mycelial inhibition} = \frac{\text{Mycelial growth (check)} - \text{Mycelial growth (treatment)}}{\text{Mycelial growth (check)}} \times 100$$

RESULTS

Suppression of Pyricularia grisea by botanicals and panchagavya

The data was presented in table-1. All the four botanicals and Panchagavya at all the concentrations tested have suppressed the rice blast pathogen P.grisea very significantly over the no botanical check treatment. Among all the treatments tested Neem seed kernel extract has more suppression value (53.0%) which is followed by Neem oil with a mean suppression value of 43.8%, *Pongamia* spp. with a mean suppression value of 21.6%, Panchagavya with a mean suppression value of 20.8% and *Asafoetida* spp. with a mean suppression value of 18.8%. There is no much difference in the percent of suppression of fungus with each step up increase in concentration from 3000ppm to 9000ppm of medium.

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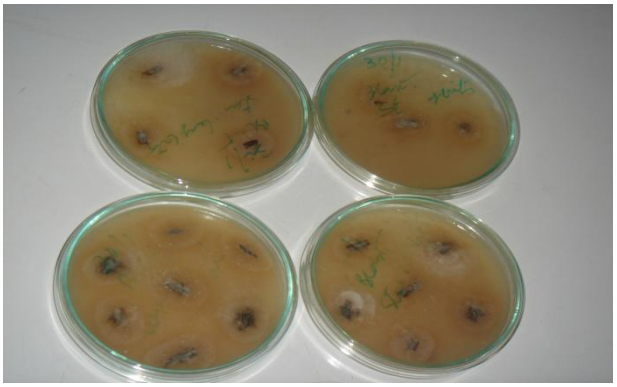

Fig. 1: Isolation of pathogen (leaf bits) from field and raising it on media

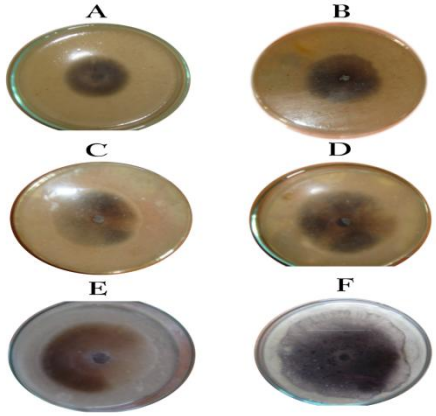

Fig. 2: Effect of Organic products

A –Neem seed kernel extract

B –Neem oil

C –Panchagavya

D –*Asefoetida spp.*

E –*Pongamia spp.*

F –Check

Table1: In vitro evaluation of Organic products against blast fungus *Pyricularia grisea* using Poisoned food technique

S.NO	organic treatments	Concentration quality	Percent suppression of radial growth of fungus *P.grisea* in petri dishes
1	Neem seed kernal extract	3000 ppm	49.6
2		5000 ppm	51.1
3		7000 ppm	52.2
4		9000 ppm	53.0
5	Neem oil	3000 ppm	39.6
6		5000 ppm	41.1
7		7000 ppm	42.3
8		9000 ppm	43.8
9	*Pongamia* sp.	3000 ppm	19.0
10		5000 ppm	19.8
11		7000 ppm	20.1
12		9000 ppm	21.6
13	Panchagavya	3000 ppm	18.0
14		5000 ppm	18.9
15		7000 ppm	19.6
16		9000 ppm	20.8
17	*Asafoetida* spp	3000 ppm	16.3
18		5000 ppm	17.2
19		7000 ppm	18.1
20		9000 ppm	18.8
21	check	-	02.2

F test = Significant

SEM = 0.614

SED = 1.45

C.D = 1.762

C.V (%) = 3.5

DISCUSSION

The extraordinary use of chemical fungicides resulted in environmental pollution and ill health to biotic community as a whole. Therefore, the use of botanicals and biological methods of plant disease management seems to be a better alternative to chemical fungicides in managing the blast disease. The present study was under taken to investigate the effect of botanicals and Panchagavya on management of paddy blast.

The results projected that Neem seed kernel extract has significantly suppressed the rice blast pathogen *P.grisea* which is followed by Neem oil, *Pongamia*, Panchagavya and *Asafoetida* in the decreasing order. Neem contains Nimbicidin or Azadirachtin which has antifungal properties. It might be the reason for the suppression of fungus. The results are in agreement with [4], indicated that *Azadirachta indica* on in vitro studies exhibited the mycelial growth as well as spore germination of *P. grisea* pathogen.

Pongamia pinnata kernel extract, panchagavya *Ferula asafoetida* and (20%) showed 71% inhibition on mycelial growth of *Aspergillus ochraceus* [7]. Panchagavya was found to have the properties of both fertilizer and bio-pesticide and hence, it may be the reason for inhibition of mycelial growth [8]. In *Ferula asafoetida*, the essential oils and their constituents have been found effective as antifungal agent. [3], [10]. Biological control of onion black mold by Indian culinary spices under In vitro conditions [12].In vitro screening of Antimicrobial activity of *Wrightia tinctoria* [13].

The biological method of plant disease management seems to be a better alternative to chemical fungicides in managing the blast disease. In addition, the biological control of plant pathogens is an attractive preposition as it mimics the nature's own way of balancing the population of living organisms [5]. The organism should survive on the leaf surface because *P. grisea* affects the foliar parts of rice.

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