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In-vivo investigation on antifungal properties of leaf extracts of certain medicinal plants through seed treatment and foliar sprays against rice blast disease (*Magnaporthe grisea*) in Kashmir, India

Mohd Shahijahan Dar*, Showkat Ahmad Ganaie, Waseem Raja, Rafiq Ahmad Teeli

Department of Botany, Government Degree College Pulwama (J&K), India

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ABSTRACT

A field experiment was carried out at Rice Research Station, Khudwani Anantnag (J&K) India during kharif 2008–2009 to study the efficacy of leaf extracts of some medicinal plants viz, *P.communis, M. longifolia, C. officinalis, C. album, C. sativa and D. stramonium* as seed treatment and foliar sprays against rice blast disease *in-vivo*. The experiment was laid down in (RBD) with three replications. The incidence and severity of blast disease was recorded at 15, 20, 25, 30 and 35 days after sowing by using SES (0–9) scale. The results revealed that seeds of rice variety China −1007 treated with leaf extracts of *Pyrus communis* @ 250mlkg^{-1} seeds recorded lowest disease incidence (0.16%), least disease severity (0.10%) and only three days for the germination/sprouting of seeds, superior seedling vigour index (4.0) and remarkably significant increase in grain yield in both the conditions as compared to control followed by *Calendula officinalis* (incidence = 0.60% and severity = 0.20%) and *Mentha longifolia* (incidence = 0.65% and severity = 0.25%) with remarkable 4 days of seed germination/sprouting. Other plant leaves extracts were also found effective but to a lesser extent. The results also indicate that the three sprays of standard solution @ 15% of leaf extract of *Pyrus communis* greatly exterminated disease incidence suppression (3.43%) on tillering, (4.15%) on booting and (0.11%) on neck stages of the crop. Apart of treatments, low grain yield, maximum disease incidence on tillering (20.00%), booting (22.41%) and neck blast stages (2.21%) was observed in unsprayed plots.

Introduction

Among the cereals, rice (*Oryza sativa* L.) is an important food crop and is grown in a wide range of geographic and climatic conditions [1]. It constitutes for most important staple diet for half of the world's population particularly in Asia [2–4]. In India, the productivity is less than those in agriculturally advanced countries because of poor agronomic practices followed in many remote areas and partially because a huge amount of crop being damaged by abiotic and biotic stresses [5]. However, its production has increased over the last decades due to improvements in cultivation practices and the introduction of high yielding cultivars [6]. A major constrain in profitable rice production is the occurrence of the certain fungal diseases and paddy blast caused by *M.grisea* is one of the most important disease of rice worldwide and is generally considered as the principal disease of rice [7]. *M. grisea* has received considerable attention as the causal agent of rice blast disease [8] which leads to 10–30% loss per year [9]. Total destruction of the crop over large areas has been reported from Jammu and Kashmir [10].Sustainable agriculture depends on the use of chemical fungicides, pesticides, herbicides and fertilizers. Repeated use of these chemicals is causing severe concern from the health and environmental point of view. The ultimate aim of recent research in this area has been the development of alternative control strategies to reduce dependency on synthetic fungicides. Many plant diseases caused by fungi have been inhibited by plant extracts [11–13]. Furthermore, the mycelial growth, conidial germination, and appressorium formation of rice blast were significantly inhibited and leaf blast was reduced by rue (*Ruta graveolens*) extract [14].Thus the exploration of plant resources for their antifungal potential against the pathogen is quite inevitable for a sustainable and ecofriendly management of the pathogen. The fungal pathogens of rice, *Sarocladium oryzae* (sheath rot pathogen) and *Pyricularia oryzae* (blast pathogen) were effectively controlled by neem oil and neem seed kernels [15]. Further, these plant extracts could be readily used by the farmers to lessen the impact of the pathogen on their rice crops. Hence, new plants locally available need to be explored for their antifungal property. The main focus of the present study was to

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* Corresponding author.
E-mail addresses: absarjahan13@gmail.com (M.S. Dar), shahidshowkat90@gmail.com (S.A. Ganaie), rajawaseem26@yahoo.in (W. Raja), drjahan96@gmail.com (R.A. Teeli).

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Table 1
Effect of phytoextracts through seed treatment against the blast disease caused by *Magnaporthe grisea* in paddy cv.China-1007.

S.No	Phytoextracts	Concentration of phytoextract (ml) (kg^{-1} seed)	Days after sprouting (DAS)	Leaf Blast										Seedling Vigour Index (1–9 scale)
				Disease Incidence (%)					Severity (%)					
				Days after Sowing (DAS)										
				15	20	25	30	35	15	20	25	30	35	
1	*Pyrus communis*	250	3	0.0	0.0	0.00	0.00	0.16	0.0	0.0	0.00	0.00	0.10	4.0
2	*Mentha Longifolia*	250	4	0.0	0.0	0.40	0.50	0.65	0.0	0.0	0.15	0.20	0.25	5.0
3	*Cannabis sativa*	250	5	0.0	0.0	0.43	0.52	0.67	0.0	0.0	0.17	0.23	0.27	6.0
4	*Chenopodium Album*	250	5	0.0	0.1	0.45	0.55	0.70	0.0	0.1	0.20	0.25	0.30	7.0
5	*Calendula Officinalis*	250	4	0.0	0.0	0.35	0.45	0.60	0.0	0.0	0.10	0.15	0.20	5.0
6	*Datura Stramonium*	250	5	0.0	0.0	0.43	0.51	0.66	0.0	0.0	0.16	0.23	0.26	6.0
7	Untreated	–	6	0.0	7.40	9.70	11.90	13.50	2.0	2.90	4.10	5.50	7.10	9.0
C.D at 5%		———	———	**0.03**	**0.05**	**0.18**	**0.21**	**0.25**	**0.1**	**0.1**	**0.30**	**0.35**	**0.60**	**1.90**

Data represented the mean of two years with three replications.

evaluate efficacy of plant extracts against *M.grisea* in-vivo. Thus six plants locally used in medicinal purposes were selected based on their abundant availability during the growing season and for their ethno-economic use.

Materials and methods

Plant extract preparation

Six plants viz. *Pyrus communis, Mentha longifolia, Calendula officinalis, Chenopodium album, Cannabis sativa and Datura stramonium* were selected for the study. Healthy non infected leaves of these plants were collected from the local area of Anantnag Kashmir, India. Extracts were prepared from these locally and commercially available plants. Fresh leaves of the selected plants presented in (Table-1) were taken and thoroughly washed in tap water to remove the dust, and then leaves of each plant were chopped and macerated with mortar and pestle or by using the automated grinder in equal quantity (1:1) of distilled water. Then this material was taken in a beaker and boiled at 80 °C for 10 min in a hot water bath. The material was homogenized for 5 min and filtered through muslin cloth. The filter was centrifuged at 5000 RPM for 15 min and the clear supernatant was collected and filtration then designated as standard (S). The standard solution(S) was further diluted (15%) by adding required amounts of distilled water. Three sprays of each standard solution (15%) were practiced by foot sprayer with fine nozzle on the transplanted crop at productive stages viz; tillering, booting and neck blast stages.

Field trial

In order to get an effective control of rice blast disease, combating yield losses, the biotic agents those available commercially and locally have been used as seed treatments to paddy variety, China-1007@ 250 ml kg $seed^{-1}$ at high altitude Research Station, Khudwani (SKUAST-K) Anantnag, (J&K) India during kharif 2008–2009. The seeds were soaked in standard solution (250 ml/kg seed) of each plant leaves and distilled water for 12 h, and then the solution was drained off. The seeds were put in cotton bags and incubated overnight in the dark to sprout. The sprouted seeds were then taken and broadcasted uniformly over the prepared nursery beds which were arranged in randomized block design. Sowing was taken on 3rd week of April which is usual sowing time in Kashmir valley for rice nursery. 2–3 cm water level in seed bed was maintained till the establishment of sprouted seeds. One month old healthy seedlings about 20 cm height at 3–4 seedlings per hill were transplanted on last week of May in plot size $2 \times 5m^2$ with three replications. For better tillering, the seedlings were transplanted less than 3 cm deep. Spacing was maintained plant to plant 10 cm and row to row 20 cm.Recommended agronomical practices in vogue were followed for raising healthy seedlings for entire period of crop and 2 to 3 foliar sprays of standard solution @15% of each plant leaf extract was also followed on transplanted crop at tillering, booting and doughing stages and each treatment replicated three times. The incidence and severity of blast disease was recorded at 15, 20, 25, 30 and 35 days after sowing by using SES (0–9) scale [16]. The seedling emergence and vigour was also recorded by adopting 1–9 scale of IRRI [17].

$$\text{Incidence } \% = \frac{\text{No. of samples affected with disease}}{\text{No. of Sample observed}} \times 100$$

$$\text{Severity } \% = \frac{\text{Sum of the score of diseased samples}}{\text{No. of samples scored}} \times \frac{1}{\text{highest score}} \times 100$$

Statistical analysis

The data obtained was analyzed using technique of ANOVA as given by Ronald E Walpole to test the effectiveness of plant extracts and if there is any significant difference in the antifungal properties of the plant extracts.

Results and discussion

Plant fungal pathogens are frequently found as one of limiting factors for crop production. More than 10,000 species of fungi can cause disease in plants. To control the diseases, many farmers still rely on the use of chemical fungicides, however most synthetic fungicides can cause acute toxicity, and some cause chronic toxicity as well. Thus, an appropriate technological improvement towards a more effective use of natural resources is required in agriculture to develop environmental friendly sustainable farming system. This paper highlights the potential of extracts of certain locally available plants as antifungal agents to control rice blast diseases caused by *M.grisea*.

Effect of plant extracts through seed treatment on blast disease and yield traits

Results indicate that all the tested plant extracts are found to be lethal to blast disease and reduced the blast incidence in paddy cultivar, China −1007 by seed treatment. Tested phytoextracts significantly increased the germination/sprouting of the seeds as compared to control. Among all plant extracts, leaf extract of *Pyrus communis* recorded the lowest (0.16%) disease incidence and severity (0.10%) and only