Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(3): 312-315
Received: 09-03-2020
Accepted: 12-04-2020

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Efficacy of botanical plant product and extracts against *Rhizoctonia solani* Kuhn causing sheath blight disease of rice under *in-vitro* Condition

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Abstract
Rice (*Oryza sativa* L.) is second most important cereal and the staple food for more than half of the world's population. It provides 20% of the worlds dietary energy supply followed by Maize and Wheat. Sheath blight is one major biotic constraints that affects rice production in India and is considered economically important disease of rice in the world The disease is caused by *Rhizoctonia solani* Kuhn (teleomorph: *Thanetophorus cucumeris* (Frank) Donk), a fungal pathogen of both rice and soyabeans. The yield loss due to this disease is reported to range from 5.2-50 per cent depending on the environmental conditions, crop stages at which the disease occurs, cultivation practices and cultivars used. The disease has been named as "sheath blight" because of primary infection on leaf sheath. The fungus attack the crop from tillering to heading stage and leaf blade symptoms also observed. Initial symptoms are noticed on leaf sheath near water level. As the spot enlarge, the centre become grayish with irregular brown blackish border. The fungus *Rhizoctonia solani* produced usually long cells of septate mycelium which are hyaline within young, yellowish brown. It produced large number of globose sclerotia which initially turn white, late turn brown to purplish brown. Sclerotia as a major source of primary inoculum. An experiment was conducted in ivtro condition during 2017-18 at experimental field of IGKV, Raipur to management the sheath blight disease of rice by application of different botanical plants were evaluated for their antifungal activity against *R. solani.* The six botanical plants product and extract namely, Neem oil + Teepol, Neem powder, Karanj oil, Karanj powder + Teepol, Chili + Garlic + Teepol, Chli + Teepol + sarf. Poisoned food technique was employed for the evaluation of botanical plant product and extracts in the laboratory. The all treatments significantly reduced the mycelial growth of *Rhizoctonia solani* over untreated treatment. Hexaconaxole was recorded with zero mm mycelial growth (100% growth inhibition) is followed by Neem oil treatment with 10.33 mm mycelial growth and 88.52% growth inhibition, Chili+Garlic 15.33 mm mycelial growth and 82.92% growth inhibition, Karanj oil 16.33 mm mycelial growth and 81.85% growth inhibition and Chili treatment was recorded 80.55 mm mycelial growth and 80.55% growth inhibition over control treatment. The maximum mycelial growth was recorded under control treatment (90.00 mm).

Keywords: *Rhizoctoia solani*, botanical, extract

Introduction
Rice (*Oryza sativa* L.) is second most important cereal and the staple food for more than half of the world's population. It provides 20% of the worlds dietary energy supply followed by Maize and Wheat. The production of rice to be achieved by 2020 is 128 Mt to feed the growing population in India. To meet the global demand, it is estimated that about 114 Mt of additional milled rice needs to be produced by 2035 with an increase of 26% in next 25 years. In the world at present the area of rice is 162.26 Mha. with production of 483.80 million metric ton and productivity of 2.98 Mt ha^{-1} In India the area of rice is 44.50 Mha^{-1} with production of 106.50 million metric ton and productivity 3.59 Mt ha^{-1}. (Anonymous, 2016) [1].
Sheath blight is one major biotic constraints that affects rice production in India and is considered economically important disease of rice in the world The disease is caused by *Rhizoctonia solani* Kuhn (teleomorph: *Thanetophorus cucumeris* (Frank) Donk), a fungal pathogen of both rice and soyabeans. The yield loss due to this disease is reported to range from 5.2-50 per cent depending on the environmental conditions, crop stages at which the disease occurs, cultivation practices and cultivars used. Significant grain yield losses were reported due to sheath blight when susceptible varieties were grown. The disease has been named as "sheath blight" because of primary infection on leaf sheath. The fungus attack the crop from tillering to heading stage and leaf blade symptoms also observed. Initial symptoms are noticed on leaf sheath near water level. As the spot enlarge, the centre become grayish with irregular brown blackish border. The presence of several large lesions on leaf sheath causes death of whole leaf and in several causes all the leaf of a plant blighted.

The infection spreads to inner sheath resulting death of entire plant. Older plants are highly susceptible, plants heavily infected by in the only heading and grain filling growth stage produced poorly filled grain. The fungus *Rhizoctonia solani* produced usually long cells of septate mycelium which are hyaline within young, yellowish brown. It produced large number of globose sclerotia which initially turn white, late turn brown to purplish brown. Sclerotia as a major source of primary inoculum. Wide host range of the pathogen *Rhizoctonia solani* makes management of the disease a different tast. Hence the disease is being managed by changing the cultural practices by one of chemical fungicide and limited extend with a biological control and biopesticide. The systematic search of higher plants for antifungal activity has shown that plant extracts have the ability to inhibit spore germination and mycelia growth in many fungal species. During recent years, use of plant extracts particularly neem derivatives for the control of plant diseases is gaining importance due to their antifungal and antibacterial properties. Many plant extracts are reported to specifically inhibit the germination of fungal spores. Since, *Rhizoctonia solani* is a typical soil borne fungus and its management through chemicals is expensive and not feasible, because of the physiological heterogeneity of the soil, other edaphic factors *etc*. might prevent effective concentrations of the chemical reaching to the pathogen. Integrated approaches for the disease management are paying more dividences in terms of sustainability. This approach mainly emphasizes on the host plant resistance, cultural practices, eco-friendly means i.e. through the use of botanicals and bio-pesticides *etc*. with a need based application of chemical molecules for disease management. Integrated disease management (IDM) blending to the traid *viz.*, cultural, biological, Bio-pesticide and use of resistance source in the right manner could be adopted. Looking to the above figure and facts, an attempt was made through this investigation to study the different methods contributing for the effective management of sheath blight of rice. Upmanyu and Gupta (2002) [6] evaluated the 16 plant species against *R. solani* and found that extracts of *Ocimumsanctum*, *Allium cepa* and *Phyllanthus emblica* completely inhibited the growth of *R. solani* on 25, 50 and 75% concentration at 24, 48 and 72 hours of inhibition. Mishra *et al.,* (2005) [3] studides the efficacy of 7 aqueous plant extracts (*Calotropis gigantea*, *Vinca rosea*, *Ocimum sanctum*, *Azadirachata indica*, *Eucalyptus citriodora*, *Allium cepa* and *Zingiber officinale*) against *R. solani* in green gram *in vitro* and found that highest inhibitory action (86.11%) on mycelia growth was recorded in ginger.

Materials and Methods

Materials

All the *in vitro* and *in vivo* studies were conducted in the laboratory and fields of Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.). During the course of investigation, glasswares of Borosil and Vensil make and chemicals of standard grade (SD fine, Hi Media, Qualigens, Emarck etc.) were used.

Instruments used

Autoclave, BOD incubator, Compound microscope, Hot air oven, Forceps, Laminar air flow, Spirit lamp Water bath, Micro wave oven.

Cleaning and sterilization of materials

Whenever required, the glasswares were cleaned with detergent powder and finally washed by cleaning solution, rinsed with tap water and/ or distilled water. The dried glass wares were sterilized in hot air oven at 180ºC for two hours. The forceps and other metallic instruments were sterilized by dipping them in alcohol and heating over the flame of spirit lamp during inoculation and purification. Sterilization of the media was done by an autoclave at 1.02 kg/cm2 for 20 minutes.

Media used:

Potato Dextrose Agar (Riker and Riker, 1936) with the following composition was prepared and used during *in vitro* studies.

Potato (peeled and sliced)	- 200 g
Dextrose	- 20 g
Agar	- 20 g
Distilled water	- 1000 ml
pH	- 6.0-7.0

Experimental site

The field experiment was conducted during Kharif 2016 at the experimental field of the Department of Plant Pathology situated in the Research farm of Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.). Besides the field experiment, all the *in vitro* studies were conducted in the laboratory of Department of Plant Pathology.

Statistical analysis

The data obtained in the present study were subjected to analysis of variance (ANOVA) and comparison of treatment means was made using Duncan‘s multiple range test (DMRT) (Little and Hills, 1978) [2].

Isolation of pathogen

The diseased samples were washed thoroughly with tap water. Small portion of infected parts containing healthy as well as diseased tissues were cut in to 0.5 cm pieces with the help of sterilized scalpel blade. These pieces were then surface sterilized with 1 percent sodium hypochlorite solution for 1 minute with 3 subsequent changes in sterilized water to remove traces of the chemical. The pieces were then transferred aseptically to petri dishes containing sterilized Potato Dextrose Agar (PDA) and incubated at 28±2 ºC under BOD incubator. The petri dishes were examined at regular time intervals for fungal growth radiating from the infected pieces.

Purification

In each petri dish about 20 ml PDA medium was poured after supplementing with pinch of streptomycin sulphate, to avoid bacterial contamination. One 8 mm mycelial disc from a freshly isolated culture was transferred aseptically to the solidified PDA in each petri dish. The dishes were incubated at 28±2 ºC in BOD incubator. Adequate numbers of sub culture transformation were separately made for further purification.

Mass multiplication of inoculums

Stems of 35-40 days old rice plants were cut in to small pieces of about 2 cm size and filled in to 500 ml Erlenmeyer flasks upto one third. Flasks were autoclaved at 15 pound per square inch for 30 minutes. Mycelial discs of 5 mm diameter cut from the margin of 48 hrs old culture of the pathogen were inoculated into the flask and incubated at 28±2 ºC up to

fifteen days for full growth of fungus and sclerotia formation. For artificial inoculation, rice plants at maximum tillering stage were taken for inoculation.

Efficacy of Botanical plant product and extracts against *R. solani* under *in vitro* condition

In vitro, extract of different botanical plants Were evaluated for their antifungal activity against *Rhizoctonia solani.* The six botanical plants namely, Neem oil, Neem powder, Karanj oil, Karanj powder, Chilli+Garlic and Chilli were collected from, IGKV, Raipur (C.G.). The extract of each plant species was prepared in cold water by different botanical plant and solvent in 1:1 ratio (w/v)

Cold water extract

Botanical plant were thoroughly washed with distilled water and crushed in 1:1 ratio of distilled water in a pastle and mortar individually. Extract was passed through a double layer muslin cloth and then through Whatman's filter paper No.1. This filtrate was considered as stock solution.

The extracts were mixed aseptically in molten PDA to have final dilutions of 10% and then poured in sterilized petri plates. Sterile distilled water mixed in same dilutions in PDA served as control. Each petri plate was inoculated with 48-72 hrs old sclerotia of *Rhizoctonia solani* and four replications were maintained. The inoculated plates were incubated at 28±20ºC. The mycelial growth and sclerotial formation were recorded every 3 days intervasl up to 9das of the inoculation.

The percent inhibition of mycelia growth was calculated as per the following formula described by Vincent (1947) [7].

$$\text{Inhibition (\%)} = \frac{C-T}{C} \text{ x } 100$$

Whereas

C = Diameter of fungus colony (mm) in control plate,

T = Diameter of fungus colony (mm) in treated plate

Results and Discussion

Efficacy of Botanical plant product and extracts against *R. solani* under *in vitro* condition

The extracts of six botanical plant product *viz.*, Neem oil, Neem powder, Karanj oil, Karanj powder, Chili+Garlic extract and Chili extract where assayed under *in vitro* are evaluated for their antifungal activity on the mycelial growth *Rhizoctonia solani* at 10% concentration.

The all treatments significantly reduced the mycelial growth of *Rhizoctonia solani* over untreated treatment. Hexaconaxole was recorded with zero mm mycelial growth (100% growth inhibition) is followed by Neem oil treatment with 10.33 mm mycelial growth and 88.52% growth inhibition, Chili+Garlic 15.33 mm mycelial growth and 82.92% growth inhibition, Karanj oil 16.33 mm mycelial growth and 81.85% growth inhibition and Chili treatment was recorded 80.55 mm mycelial growth and 80.55% growth inhibition over control treatment. The maximum mycelial growth was recorded under control treatment (90.00 mm).

Present findings are in support to the study of Seint and Masaru (2011) [5] that sixteen naturally available phytoextracts were tested *in vitro* for their potential to control phytopathogens of rice, such as *R. solani*, *R. oryzae*, *R. oryzae sativae* and *Sclerotium hydrophilum.* All the tested fungus pathogens were suppressed 100% by using clove extracts, Neem leaf, Rosemary and pelargonium extracts were found to give the second best suppression against the fungi. Archana *et al.* (2014) also in agreement of the above finding that the essential oil of Mentha, Citronillanad pipermint showed 100% inhibition in the mycelial growth of *R. solani* at both the concentration of 4 µl and 8 µl.

Table 1: Efficacy of Botanical plant product and extracts against *R. solani* under *in vitro* condition

Treatment	Commone name	Mycelial growth mm	Percent growth inhibitioin
T1	Neem oil	10.33	88.52
T2	Neem powder	25.33	71.85
T3	Karanj oil	16.33	81.85
T4	Karanj powder	26.33	70.74
T5	Chili+Garlic	15.33	82.92
T6	Chili	80.55	10.5
T7	Hexaconazole	0	100
T8	Control	90	
SEm ±		0.58	
CD at 5%		1.76	

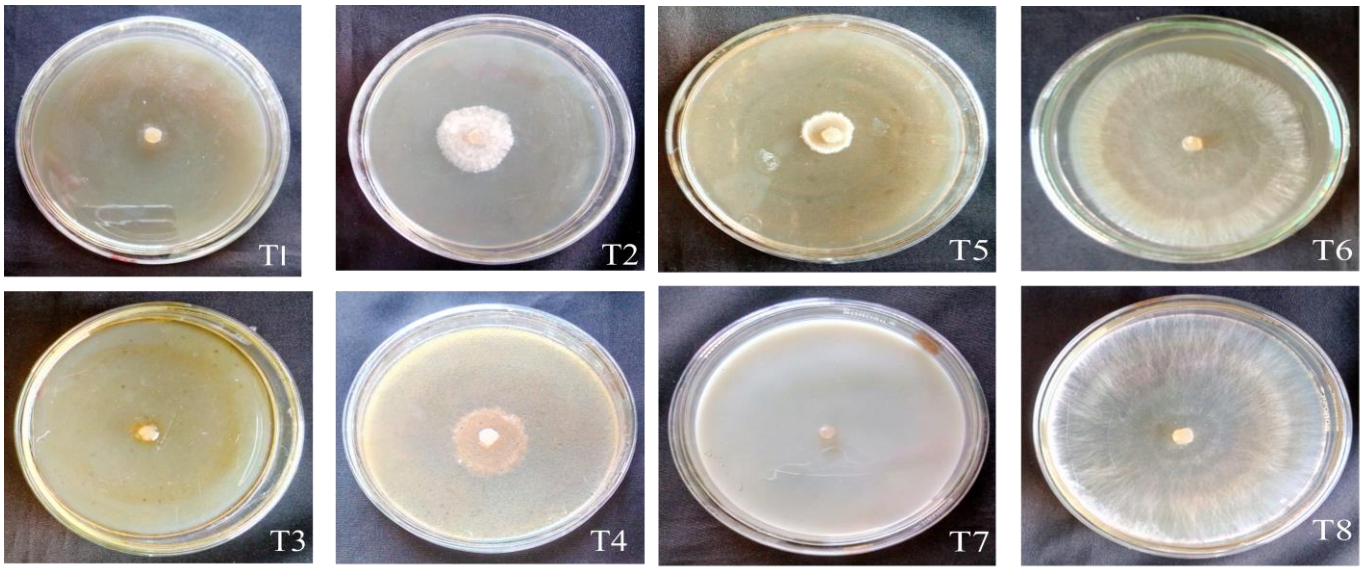

Fig 1: Efficacy of botanical plant extracts against *R. solani* under *in vitro* condition

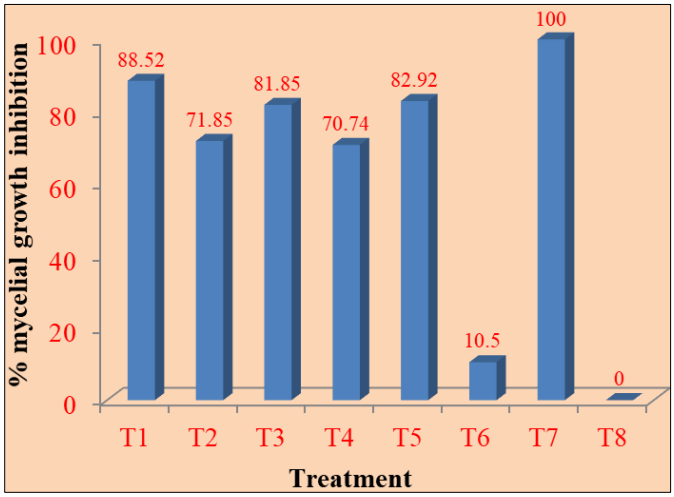

Fig 2: Effect of botanical plant leaf extracts on *R. solani*

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