Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; SP2: 309-311

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National Conference on Conservation Agriculture
(ITM University, Gwalior on 22-23 February, 2018)

Evaluation of phytoextracts and bioagents against stem rot disease of paddy caused by *Sclerotium oryzae* Catt.

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Abstract
Rice (*Oryza sativa* L.) is the most important sources of food which provide diet more than 50% of the world population. Stem rot disease caused by *Sclerotium oryzae,* decline the yield of crops. The concern about climate change, environment pollution and sustainable crop production disease management on sustainable basis is crucial. Thus, phytoextracts and bioagents use as an alternative to chemical fungicides for the management of diseases. Effect of phytoextracts and biocontrol agents (BCAs) has evaluated against pathogen *Sclerotium oryzae* causing stem rot of rice in vitro. The interval of five days at different concentration of phytoextracts revealed that *Allium sativum* exhibited maximum inhibition of pathogen around 57-75%. Meanwhile *Allium cepa* and *Gingiber officinale* show almost equal effect which inhibit 44% of mycelium growth of pathogen at concentration of 20%. The minimum inhibition shown by neem leaf extract was found 28-38% at different concentration. Moreover, BCAs also inhibit pathogen by 35-52% at different interval of days. *Trichoderma harzianum* and *Trichoderma viride* exhibited better result among different BCAs, inhibited pathogen around 39-52% at different interval of days. While *Trichderma koningii* and *Trichoderma longibractum* inhibited 35-47%. The best result shown by *Trichoderma harzianum* which inhibit pathogen by 52%, 43% and 40% at consecutive interval of three, five and seven days respectively.

Keywords: Environment, fungicide, phytoextracts, bioagents, rice

Introduction
Rice (*Oryza sativa* L.) is one of the most important sources of food for the world population. Around 3 billion people about 50% of human population uses rice as food and nutrients source. Globally rice occupies an area of 166 million hectares with a production of 758.8 metric tonnes of paddy (FAO, 2017). Stem rot of rice is a serious threat to rice production in North India (Prasant and Puri, 2012), 50-75% loss in yield was estimated in badly affected crops and 5-15% loss reported almost every area in Punjab (Sharma and Mehrothra, 1985). Stem rot caused by *Sclerotium oryzae* Catt. affect the stem of rice crops. The stem rot diseases found during 2014-2015 near water lodging field around Aligarh region, where moderate loss of crops and their yield occurs. Sclerotia are produced on infected rice tissues as the rice plants mature (Ou, 1972). The concern about climate change, environment pollution for sustainable crop production disease management is crucial. Thus, phytoextracts and bioagents as an alternative for the chemical fungicides for the management of diseases.

Materials and Methods
Four available phytoextracts were evaluated against the mycelial growth of *Sclerotium oryzae* by Poisoned Food Technique described by (Vincent, 1927 and Khongsai *et al.*, 2015) [8, 4]. Phytoextracts were measured and mixed separately with sterilized potato dextrose agar medium to give the concentration of 10% and 20% poured aseptically in sterilized petriplates. The commonly used bioagents viz. *Trichoderma harzianum, T. viride, T. longibractum,* and *T. Koningii* were evaluate the antagonistic activities against *S. oryzae* under *in vitro* conditions through dual culture plate technique (Bell *et al*., 1982) [1]. Radial growth of the pathogen was measured and percent inhibition was calculated by the following formula I%= C-T/C x 100; where I = inhibition of mycelial growth, C= growth of pathogen in the control plate (mm) and T= growth of pathogen cultures (mm).

Isolation of fungal pathogen
The infected stem of paddy plants were washed thoroughly with tap water and were cut into

small pieces of 2-4 mm in size using sterilized blades. These pieces were surface sterilized by dipping in mercuric chloride solution (1:1000) for one minute and were washed by sterilized water for several times. The sterilized pieces were placed in the petriplates containing 20 ml solidified potato dextrose agar (PDA).

Preparation of aqueous phytoextracts

100 grams of each plant materials was taken, washed under running tap water then thoroughly washed in distilled water and then dried at room temperature for one day. Surface of plant material was sterilized with 1% NaOCl (sodium hypochlorite) solution and then crushed with mortar and pestle by adding 100 ml of sterilised distilled water, the crushed material was then filtered through double layer muslin cloth then filtrate was centrifuged at 5000 rpm for 15 minutes. Solvent was further placed on Whatman's No. 1 filter paper (Bisht and Khulbe, 1995) [2]. Prepared phytoextracts were heated to 40°C for 5 minutes to avoid contamination (Jaganthan and Narsimhan 1988) [3]. For experiments 10% and 20% of concentration of each plant extract was poured in sterilized petriplates containing 20ml of PDA. After the solidification of the medium mycelial disc of 3 mm diameter was plugged from 7 days old colony of *Sclerotium oryzae* and was incoculated aseptically in the centre of each petriplate and incubated at 25±2°C. Three replicates of each treatment were maintained. The observation were taken after interval of every 24 hours.

Procurement of biocontrol agents (BCAs)

The BCAs *Trichoderma harzianum, T. viride, T. longibractum,* and *T. koningii* procure from Indian type culture collection from ITCC, New Delhi.

Table 1: Plant used for management of stem rot diseases of rice caused by *Sclerotium oryzae* in vitro

S No.	Plant used	Scientific Name	Family	Part used
1	Zinger	*Zingiber officinale*	Zingiberaceae	Rhizome
2	Garlic	*Allium sativum*	Amaryllidaceae	Clove
3	Onion	*Allium cepa*	Amaryllidaceae	Bulb
4	Neem	*Azodirachta indica*	Meliaceae	Leaves

Analysis of Data

The data of the experiments have been subjected to analysed statistically using MINITAB-14 statistical software. The level of significance was P≤0.05.

Results and Discussion

The effect of antimicrobial activity of plant extracts on mycelial growth of *Sclerotium oryzae* presented in Table 2. The result revealed after five days of inoculation clove of *Allium sativum* (garlic) extract @ 20% concentration was found most effective in minimizing radial growth of the *Sclerotium oryzae* (5.60 mm) against control (22.5 mm), which gradually increases to 9.60 mm @ concentration of 10%. Meanwhile, *Zingiber officinale* and *Allium cepa* inhibited 31% and 41% respectively concentration of 10% meanwhile, it showed equal effect around 44.44% at concentration 20%. Moreover Neem found least antagonist to *Sclerotium oryzae* at both concentration. Dual culture of bioagents against pathogens *Sclerotium oryzae* also revealed antagonistic behaviour in Table 3. Among bioagents *Trichoderma harzianum* and *Trichoderma viride* found most effective followed by *Trichoderma koningii* and *Trichoderma longibractum*. After three days of inoculation inhibition of *T. harzianum*, *T. viride*, *T. konigii* and *T. longibractum* was found 52.08%, 52.70%, 47.67%, and 45.79% respectively. Moreover, after five days *T. harzianum*, *T. viride*, *T. konigii* and *T. longibractum* was found 43.62%, 42.50%, 39.24%, and 37.61% respectively. Interestingly, after seven days *T. harzianum*, *T. viride*, *T. konigii* and *T. longibractum* was found 40.11%, 39.59%, 36.60%, and 35.10% respectively. The results of antagonist result reveals that *T. harzianum* inhibit maximum 40-52%, while *T. longibractum* inhibit minimum 35-45%. The data revealed that BCAs were found significant (P≤0.05) against mycelia growth over untreated check. However the rate of antagonism decreases with passage of time.

Table 2: Effect of phytoextracts on the radial growth of mycelium against *Sclerotium oryzae*

Phytoextracts (Treatments)	Plant parts used	10% Concentration		20% Concentration	
		Growth (mm)	Percent Inhibition (%)	Growth (mm)	Percent Inhibition (%)
Zingiber offcinale	Rhizome	15.60	30.66	12.50	44.44
Allium sativum	Clove	09.60	57.33	05.60	75.11
Allium cepa	Bulb	13.40	40.44	12.50	44.44
Azadirachta indica	leaves	15.99	28.93	13.99	37.82
Control		22.50		22.50	
CD(P≤0.05)		00.42		00.40	

Table 3: Effect of Biocontrol agents (BCAs) on the radial growth of mycelium against *Sclerotium oryzae*

Biocontrol Agents (BCAs)	3 Days of Inoculation		5 Days of Inoculation		7 Days of Incolution	
	Growth (mm)	Percent Inhibition (%)	Growth (mm)	Percent Inhibition (%)	Growth (mm)	Percent Inhibition (%)
Tichoderma harzianum	12.70	52.08	17.31	43.62	20.00	40.11
Trichodema viride	12.53	52.70	17.65	42.50	20.18	39.59
Trichoderma koningii	13.87	47.67	18.65	39.24	21.18	36.60
Trichoderma longibractum	14.37	45.79	19.15	37.61	21.68	35.10
Control	26.50		30.70		33.40	
CD(P≤0.05)	01.08		00.39		00.81	

Conclusion

For sustainable management of stem rot of rice, timely spraying of phytoextracts and BCAs used in the field conditions to manage diseases results increasing the yield of rice grains. This combination could be used as eco-friendly management of the disease and as a component of integrated disease management. This method may be an alternate to the chemical fungicide.

Acknowledgement

The author is grateful to The Chairperson of Department of Plant Protection, AMU, Aligarh is gratefully acknowledged for permitting us to conduct in vitro experiment. I highly thankful to University Grant Commission (UGC), Government of India for providing Non-NET fellowship during the course of this research.

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