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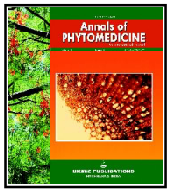

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Inhibitory effect of botanicals and bioagents against *Ustilaginoidea virens* (Cooke) Takah. causing false smut of rice

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Abstract

Ustilaginoidea virens (Cooke) Takah. causing false smut of rice is a destructive disease which causes an economic yield loss ranges from 0.2-49%. The present study is carried out to check inhibitory effect of botanicals and bioagents against *U. virens*. Among botanicals, neem oil at both concentration, *i.e.*, 5 per cent and 10 per cent was found highly effective and inhibit the growth by 66.97% and 70.14%, respectively followed by onion (62.89%, 67.64%), garlic (58.82%, 64.25%), ginger (55.88%, 63.57%) and tulsi (46.40%, 54.98%). Among bioagents, maximum inhibition (53.55%) was observed in *Bacillus subtilis* followed by *Pseudomonas fluorescence* (41.87%), *Trichoderma viride* (40.35%) and *Trichoderma harzianum* (36.29%) in *in vitro* condition.

1. Introduction

Rice (*Oryza sativa* L.) being the most staple cereal food crop, grown in India, providing one third of calories requirement for more than 70 per cent of Indian population. More than 90 per cent of world's rice is grown and consumed in Asia, known as rice bowl of the world. To focus attention on the importance of rice in global food security and necessity into increased rice production and productivity, United Nation General Assembly in 2002, declared to celebrate the year 2004 as "International year of rice.

In India, it is cultivated in an area of 43.77 Mha with a production of 169.14 Mt and has an average productivity 3.86 t/ha (Anonymous, 2019). The major rice producing states are West Bengal, Punjab, Uttar Pradesh, Andhra Pradesh, Bihar and Tamil Nadu. In Uttar Pradesh, it is cultivated in an area of 5.81 Mha with production of 19.91 Mt and average productivity 3.42 t/ha (Anonymous, 2019).

Rice crop is affected by number of biotic and abiotic factors. Among biotic factors, diseases caused by fungi, bacteria, virus and others are the major one. False smut is one of the most calamitous fungal diseases of rice and its occurrence is reported from every corner of the world wherever rice is growing (Ladhalakshmi *et al.*, 2018). Initially, the disease was of minor importance and commonly known as "Welcome/Lakshmi disease" because the disease was considered as an indicator of vigorous crop resulting into good harvest but due to continuous use of high dose of nitrogenous fertilizer extensively, cultivation of hybrid cultivars and climate change, the disease becomes major one (Jehua *et al.*, 2019). The disease was first reported from Tirunelveli district of Tamil Nadu state of India (Cooke, 1878). The False smut pathogen *U. virens* infect the plant during flowering stage where an individual healthy grain converts firstly into whitish, yellowish orange to green velvety spores which later turns into greenish black in colour (Baite *et al.*, 2014).

In Uttar Pradesh, a yield loss was recorded ranges from 5-85 per cent (Singh *et al.*, 2014). Continuous use of fungicides has resulted in accumulation of toxic compounds which is hazardous to humans, animals and environment and also build-up resistance in the pathogens. So, botanicals and bioagents are the best alternative and economical way to manage the disease. Management of diseases and pests by the use of plant-based products was practiced over time until synthetic pesticides were developed (Mahmood *et al.*, 2016). Various researchers reported different effective botanicals from time-to-time. Neem and garlic was found effective as reported by Mahmud *et al.* (2017). Different bioagents are normally dominant components of the soil microflora in extensively varying habitats and are very effective against the *U. virens* (XiaoLe *et al.*, 2011; Kannahi *et al.*, 2016; Nath and Das, 2020). Therefore, the present investigation was carried out to know the appropriate and effective botanicals and bioagents against *U. virens in vitro*.

2. Materials and Methods

The experiments were conducted in the laboratory of Department of Plant Pathology, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, U.P. during 2020-21.

2.1 Isolation of pathogen

Panicles infected with false smut were collected from the NSP-6 farm of the University. The smut balls were brought to the laboratory

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and isolates them on a PDA (Potato Dextrose Agar) medium as described by Ladhalakshmi *et al.*, 2012. The smut balls were thoroughly washed repeatedly in tap water. Surface sterilized in a sodium hypochlorite (1% $NaOCl_2$) solution for 30 sec, followed by three times rinsing in sterilized distilled water. The false smut balls were cut into two halves, the inner layer containing spores was then streaked on PSA medium which was supplemented with 100 ppm streptomycin to avoid bacterial contamination. The inoculated Petri-plates were incubated in a BOD incubator at 25 ± 1°C. After 10 days of incubation, the mycelial growth of *U. virens* was observed. The isolates were purified by hyphal tip method. The culture obtained by this method was maintained on PSA slants and Petri plates.

2.2 Preparation of plant extracts

The plant parts were collected from main Experiment Station of Vegetable Science of the University. The plant parts were washed with tap water followed by sterilized water. In case of water extract of botanicals, 100 g of botanicals were crushed in 100 ml of water separately, grinded with pestle and mortar in water in *in vitro* condition for 10 min. Then, the extracted juice were sieved and taken in conical flask and stored at 4°C.

2.3 Evaluation of botanicals against *U. virens*

Inhibitory effect of five botanical extracts (Table 1) were tested at two concentrations, *i.e.*, 5 per cent and 10 per cent against the mycelial growth of *U. virens*. Required quantity of each treatment was incorporated in 100 ml PDA at luke warm stage and mixed thoroughly by sacking, prior to pouring into Petri plates. After pouring of PDA in Petri plates, the medium was allowed to solidify and these plates were centrally inoculated with the 6 mm diameter disc of *U. virens* at the centre of the Petri plate. The disc is cut by sterilized cork borer taken from the edge of vigorously grown 10 days old culture. Control was used as such without treatment in the medium. Four replications of each treatment incubated at 26 ± 2°C for growth of the pathogen. The efficacy of various chemicals was observed by measuring mycelial growth of the fungus in millimeters (mm). The mycelia growth was recorded after 10 days of incubation.

Table 1: List of botanicals, their scientific name, family and plant parts used

S.No.	Botanicals	Scientific name	Family	Plant parts used
1	Garlic	*Allium sativum*	Amaryllidaceae	Clove
2	Ginger	*Zingiber officinalis*	Zingiberaceae	Rhizome
3	Neem oil	*Azadirachta indica*	Neliaceae	Oil
4	Onion	*Allium cepa*	Liliaceae	Bulb
5	Tulsi	*Ocimum sanctum*	Lamiaceae	Leaf

Table 2: Efficacy of botanicals (after 10 day) at different concentration against *U. virens* causing false smut of rice

S.No.	Botanicals	Radial growth (mm) Conc. (5%)	Inhibition %	Radial growth (mm) Conc. (10%)	Inhibition %
T_1	Garlic (*Allium sativum*)	18.2	58.82	15.8	64.25
T_2	Ginger (*Zingiber officinalis*)	19.5	55.88	16.1	63.57
T_3	Neem oil (*Azadirachta indica*)	14.6	66.97	13.2	70.14
T_4	Onion (*Allium cepa*)	16.4	62.89	14.3	67.64
T_5	Tulsi (*Ocimum sanctum*)	23.6	46.60	19.9	54.98
T_6	Control	44.2	-	44.2	-
	CD (*p*=0.01)	**1.17**		**1.03**	
	CV	**1.61**		**1.51**	

2.4 Evaluation of bioagents against *U. virens*

All the bioagents isolates were collected from the Department of Plant Pathology, ANDUAT, Ayodhya to evaluate their efficacy against the *U. virens, viz.*, *Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescence* and *Bacillus subtilis* through dual culture technique. The 6 mm disc of *U. virens* and bioagents were placed at corresponding to each other in a single Petri plate. The efficacy of various treatments was assessed by measuring the myelial growth of the fungus after 10 days of incubation.

The per cent inhibition of mycelial growth was calculated by using the formula (Mckinney, 1923):

$$\text{Inhibition per cent (I)} = \frac{C - T}{C} \times 100$$

where,

I = Inhibition per cent

C = Colony diameter in control

T = Colony diameter in treatment

2.5 Statistical analysis

The data recorded on radial growth were statistically analyzed using completely randomized block design (Web Agri Stat Package 1.0).

Plate 1: Efficacy of botanicals at 5% on the growth of *U. virens* (after 10 days).

Plate 2: Efficacy of botanicals at 10% on the growth of *U. virens* (after 10 days).

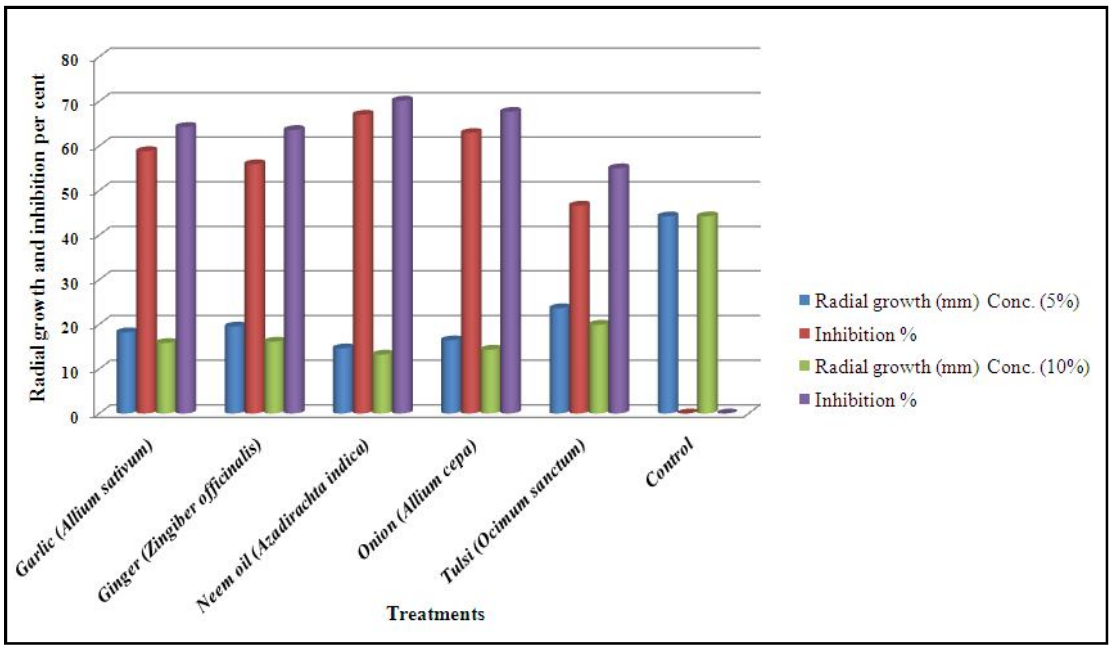

Figure 1: Efficacy of botanicals (after 10 day) at different concentrations against *U. virens*.

Table 3: Efficacy of bioagents (after 10 days) against *U. virens* causing false smut of rice.

S. No.	Bioagents	Colony diameter of pathogen (mm)	Inhibition %
T_1	*Trichoderma viride*	23.5	40.35
T_2	*Trichoderma harzianum*	25.1	36.29
T_3	*Pseudomonas fluorescence*	22.9	41.87
T_4	*Bacillus subtilis*	18.3	53.55
T_5	Control	39.4	-
	CD (*p*=0.01)	**0.65**	
	C V	**0.71**	

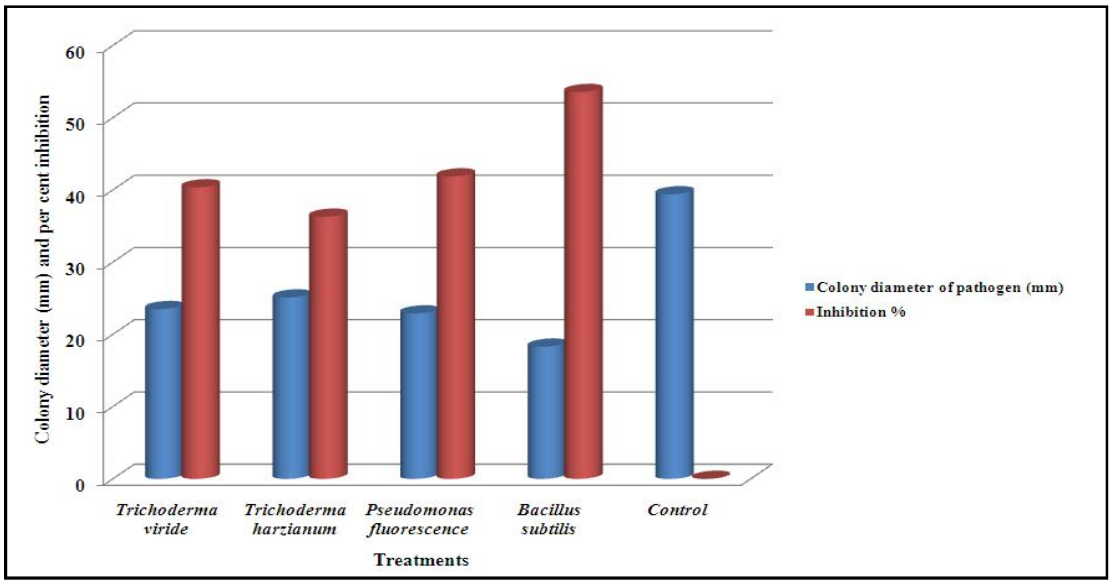

Figure 2: Efficacy of bioagents (after 10 days) against *U. virens* causing false smut of rice.

3. Results

3.1 Effect of botanicals at different concentration on the mycelial growth of *U. virens*

Five botanicals were tested in present study under lab condition through food poison technique in 5 per cent and 10 per cent concentrations. Data were recorded 10 day after incubation on the basis of effect of botanicals on the mycelial growth (mm) of the pathogen is presented in Table 2.

3.1.1 Effect of botanicals on the mycelia growth at 5 per cent concentration

The results presented in Table 2, Figure1 and Plate 1 revealed that the minimum *U. virens* growth (14.6 mm) was observed in neem. Neem is found at par with onion having radial growth 16.4 mm, followed by garlic (18.2 mm), ginger (19.5 mm) and tulsi (23.6 mm). The maximum 44.2 mm mycelial growth was observed in control.

3.1.2 Effect of botanicals on the mycelia growth at 10 per cent concentration

The results presented in Table 2, Figure 1 and Figure 2 revealed that the minimum *U. virens* growth (13.2 mm) was observed in neem. Neem is found at par with onion having radial growth 14.3 mm, followed by garlic (15.8 mm), ginger (16.1 mm) and tulsi (19.9 mm). T_4 was found at par with T_2. The maximum 44.2 mm mycelial growth was observed in control.

3.2 Effect of botanicals at different concentration on the growth inhibition of *U. virens* after 10 days

All the botanicals significantly inhibit the mycelial growth of *U. virens*. Neem oil at 5 per cent and 10 per cent was found most effective and inhibit the growth by 66.97% and 70.14%, respectively followed by onion (62.89%, 67.64%), garlic (58.82%, 64.25%), ginger (55.88%, 63.57%) and tulsi (46.40%, 54.98%).

3.3 Effect of bioagents on the mycelial growth of *U. virens* through dual culture technique.

Inhibitory effects of different bioagents were evaluated through dual culture technique. The results were recorded after 10 days of incubation and presented in Table 3 and Figure 2. It revealed that the minimum radial growth was observed in *Bacillus subtilis* (18.3 mm) followed by *Pseudomonas fluorescence* (22.9 mm), *Trichoderma viride* (23.5 mm) and *Trichoderma harzianum* (25.1 mm). *Pseudomonas fluorescence* and *Trichoderma viride* were found at par to each other. All the treatments were found significantly superior over the control.

All the bioagents notably inhibit the mycelia growth of *U. virens*. Maximum per cent inhibition 53.55 per cent was observed in *Bacillus subtilis* followed by *Pseudomonas fluorescence* 41.87 per cent, *Trichoderma viride* 40.35 per cent and *Trichoderma harzianum* 36.29 per cent.

4. Discussion

Uses of botanicals and bioagents are the best alternative of fungicides for the management of diseases due to its eco-friendly nature and also economically. Many plant extracts gives excellent result against phytopathogens. Here, study identified that neem oil, onion bulb extract and garlic clove extract was found highly effective against the disease. The results found was well supported by Mahmud *et al.* (2017) as he also found that neem and garlic was found effective against *U. virens*. Integration of botanicals in present agriculture system is necessary as it possess many benefit to the farmers including food safety, reduced disease levels, improve food quality which catch higher marketable price (Lengai *et al.*, 2020). It possesses many antimicrobial metabolites which help inhibiting the mycelial growth of pathogen.

All the bioagents significantly inhibit the growth of *U. virens*. Maximum per cent inhibition was observed in *Bacillus subtilis* (53.55), followed by *Pseudomonas fluorescence* (41.87), *Trichoderma viride* (40.35) and *Trichoderma harzianum* (36.29). The results were well supported by XiaoLe *et al.* (2011); Nath and Das (2020) found *Bacillus* sp. most effective. Kannahi *et al.* (2016) studied the antagonistic potential of four isolates of *Trichoderma* spp., *viz.*, *T. viride*, *T. harzianum*, *T. reesei* and *T. virens* obtained from rhizosphere of rice under *in vitro* condition and reported that all the *Trichoderma* isolates are highly effective against *U. virens*. The different species of *Trichoderma* was also found effective and maximum inhibit the growth (Kumar *et al.*, 2014; Mahmud *et al.*, 2017). The antagonistic fungus or bacteria affected or inhibited a plant pathogen by the action of mycoparasitism, antibiosis, competition for nutrients and space. The antagonists produces different extracellular enzymes like glucanase, chitinase, *etc*., to mortify the mycelia growth of pathogen (Kohl *et al.*, 2019). It could be used for sustainable management of disease without harming the environment.

5. Conclusion

The present study identified various plant extracts (garlic, ginger, neem oil, onion, tulsi) and bioagents (*Bacillus subtilis*, *Pseudomonas fluorescence*, *Trichoderma viride* and *Trichoderma harzianum*) against the pathogen *U. virens* at different concentration. Neem oil was found highly effective followed by onion and garlic. Maximum per cent inhibition was observed in *Bacillus subtilis* followed by *Pseudomonas fluorescence*, *Trichoderma viride* and *Trichoderma harzianum*. All the plant extracts and bioagents evaluated significantly control the disease. So, they can be used to manage the disease under natural field condition. The future strategy required to identify plant or bioagents with antimicrobial potential and made the formulation from them with better shelf life and utilize them for eco-friendly and sustainable management of the disease without harming the environment.

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Conflict of interest

The authors declare no conflict of interest related to this article.

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