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EFFICACY OF BIO-AGENTS AND BOTANICAL EXTRACTS IN CONTROL OF *RHIZOCTONIA SOLANI* CAUSING AERIAL OR WEB BLIGHT OF SOYBEAN

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Abstract

A pot experiment was conducted at the College of Agriculture in C.S.A.U.A & T, Kanpur (U.P.), India; during the *Kharif* season of 2012 and 2013 to study the Aerial blight of soybean caused by *Rhizoctonia solani* Kuhn. The efficacy of seven botanical extract *viz.,* Garlic, Madar, Ginger, Aloevera, Neem, Makoy, Datura *i.e.* application @ 5 per centand six bio-agents *viz.*, *Trichoderma viride, T. atroviride, T. harzianum, T. longibrachiatum, T. koningii & Aspergillus niger* were tested *in vitro* and *in vivo* conditions were against *Rhizoctonia solani*, the causal organism of aerial blight of soybean. Results revealed that the under *in vitro* conditions, botanical extracts of Garlic was found highly effective showed 88.47 per cent inhibition in radial growth of the fungus at 5 per cent concentration followed by Madar at 5 per cent concentration. Datura was found least effective than rest of the plant extract. The use of bio-agents is risk free and the best alternative to sustain plant protection. *Trichoderma viride* was the most significantly effective 70.42 per cent as it inhibited the mycelial growth of *Rhizoctonia solani* after 7 days of incubation followed by *T. atroviride* and *T. harzianum* where 62.47 per cent and 47.68 per cent growth of fungus was observed, respectively. However, minimum effect on growth 8.62 per cent was observed in *Aspergillus niger.*

Key words : *Rhizoctonia solani,* botanical extracts, bio-control agents and Aerial blight.

Introduction

Soybean [*Glycine max* (L.) Merill] is a leguminous oilseed crop having worldwide adaptation. It is known as "Golden bean" or "Miracle crop", as it is the richest source of protein (40%) and oils (20%). Its protein has a good balance of amino acids except methionine and its oil is rich in unsaturated fatty acids. Soybean belongs to the *fabaceae* or *leguminosae* (legume) family. Seeds from these varieties were used primarily as pulses by the local population and the green and dried vegetative parts were used as forage for cattle (Saxena, 1976). The native of soybean is Eastern Asia. Soybean was introduced to India during 1880. This is economically important pulse crop suffers from a number of diseases which is caused by fungi, bacteria, viruses, nematodes and other physiological disorders also disturbs the normal physiological process of the plants. Among the various diseases, the incidence of aerial blight of Soybean disease has been observed with varying degrees of disease in several districts of Uttar Pradesh in 2012-13. Foliar/leaf blight of soybean caused by *Rhizoctonia solani* Kuhn is a severe disease and causes heavy loss in soybean production (Anwar *et al*., 1995). Soybean [*Glycine max* (L.) Merril] plants are infected by the pathogen at any stage of development, which causes very rapid defoliation and frequent crop failure (Wrather *et al*., 2001). Fenille *et al*. (2002) have reported 31-60% yield losses due to foliar blight of soybean. The symptoms of aerial blight of soybean caused by *R. solani*, as leaf and pod spots, leaf blight, defoliation, stem and petiole lesions, cob web like mycelium and sclerotia developed over infected leaves were described by Atkins and Lewis (1954). The *R. solani* produces

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sclerotia as survival structure, which is brown to black composed of clusters of melanin encrusted, thick walled cells, formed by repeated branching from short, thick, lateral hyphae, when produced on plant parts, it is difficult to separate the sclerotia from their surrounding embedded sclerotia. Temperature is more considerable parameter for their growth and development along with sclerotia production. Under certain conditions young plants were found heavily affected with the disease leading to premature death of plants and substantial losses in yield. Due to this disease, the vigorousness of the affected plants is reduced there by production decreased. The disease attacks all the aerial part of the plants *i.e.* leaves, stem, petioles and pods, when the disease occurred in severe form. The leaves and host plant has given blighted appearance. The mycelium has found web like appearance on the leaves so that disease has been named as web blight of Soybean. It also inhibited root elongation causing seedling root rot, yellowing and shredding of cotyledons and leaves in soybean.

Materials and Methods

Evaluation of bio-agents against the pathogen *in vitro*

For this study, the pathogen was isolated from diseased plants of soybean from the aerial blight sick plot of Glass house of Plant Pathology C.S.A. University of Agriculture and Technology, Kanpur (U.P.), India. One week old culture of *Rhizoctonia solani* maintained on PDA Petri plates at 27±1^{0}C was used for study. The culture of all the bio-agents were isolated from the rhizosphere of soybean plants of aerial blight sick plot of this university, Antagonistic activity of these bio-agents against test pathogen was determined by dual culture technique.

The bio control agents used for testing were :

S. no.	Bioagents	Isolate
1.	*Trichoderma harzianum*	Kanpur
2.	*Trichoderma viride*	Kanpur
3.	*Trichoderma longibrachiatum*	Kanpur
4.	*Trichoderma atroviride*	Kanpur
5.	*Trichoderma koningii*	Kanpur
6.	*Aspergillus niger*	Kanpur

Five mm disc of pathogen was taken from the actively growing colonies of the test pathogen and antagonist with help of sterilized cork borer. The discs of the pathogen were placed on one side in agar plates aseptically and the discs of antagonists were placed opposite side of the pathogen in same Petri plates. Each treatment was replicated three times and incubated at 27±1^{0}C. Growth of antagonists and pathogen were recorded after 7 days of incubation.

Screening of different botanical extracts against *Rhizoctoni solani invitro*

The presence of naturally occurring substances in plants with anti-fungal properties has been recognized and tested against the pathogen. In present investigation, the efficacies of botanical extracts of seven plants were tested against *Rhizoctonia solani* causing aerial blight of soybean.

S. no.	Botanical extracts	Part	Dose per cent
1.	Aloe (*Aloe borbandesis*)	Leaf	5
2.	Madar (*Calotropis gigantean*)	Leaf	5
3.	Ginger (*Zingiber officinale*)	Rhizome	5
4.	Garlic (*Allium sativum*)	Clove	5
5.	Neem (*Azadirachta indica*)	Leaf	5
6.	Datura (*Datura fastussa*)	Leaf	5
7.	Makoy (*Solanum nigrum)*	Leaf	5
8.	Control		

Fresh leaves of plant species were collected washed in tap water followed by sterile water crushed in sterile distilled water with mortar and pestle and filtered through 2 layers of muslin cloth to obtained extract. The 100 ml sterilized potato dextrose agar medium mixed with 5 ml of botanical extract contained in 150 ml conical flask then sterilized in an autoclave at 1.1 kg pressure/cm^2 for 5 minutes. Then sterilized media poured in three sterilized Petri plates and after solidification, 5 mm disc of 5 days old culture of pathogen was transferred into the centre and incubated at 27±1^{0}C. PDA not amended with botanical extract served as control. Observation on radial growth of the test fungus was recorded after 7 days of incubation. Five mm disc of pathogen was taken from the actively growing colonies of the test pathogen and antagonist with help of sterilized cork borer. The disc of the pathogen was placed on one side in agar plates aseptically and the disc of antagonist was placed on opposite side of the pathogen in same Petri plates. Each treatment was replicated three times and incubated at 27$\pm$1^{0}C. Growth of antagonists and pathogen were recorded after 7 days of incubation.

Mechanism of interaction was observed and the data were expressed as percent inhibition of growth by the following formula (Bliss, 1934).

$$\text{Percent inhibition (P.I.)} = \frac{\text{Growth in control (mm)} - \text{Growth in treated plates (mm)}}{\text{Growth in control (mm)}} \times 100$$

Table 1 : Inhibitory effect of different bio agents on the growth of *Rhizoctonia solani in vitro* incubated at 27 ± 1°C after 7 days.

S. no.	Bio-agents	Average radial of fungal colony (mm)	Percent inhibition over control
1.	*Trichoderma viride*	26.33	70.42
2.	*Trichoderma atroviride*	33.40	62.47
3.	*Trichoderma harzianum*	46.56	47.68
4.	*Trichoderma longibrachiatum*	51.46	42.17
5.	*Trichoderma koningii*	79.66	10.49
6.	*Aspergillus niger*	81.33	8.62
7.	Control	89.00	
	CD at 5% level	1.5278	

Table 2 : Evaluation of plant leaf extracts against the pathogen *in vitro*after 7 days of incubated at 27 ± 1°C.

S. no.	Botanical extract	Dose (%)	Average diameter of fungal colony (mm)	Percent inhibition
1.	Garlic (*Allium sativum*)	5	9.33	88.47
2.	Madar (*Calotropis gigantea*)	5	10.66	87.98
3.	Ginger (*Zingiber officinales*)	5	42.00	52.62
4.	Aloevera (*Aloe barbandis*)	5	46.66	47.37
5.	Neem (*Azadirachta indica*)	5	56.33	36.47
6.	Makoy (*Solanum nigrum)*	5	68.20	23.07
7.	Datura (*Datura fastussa*)	5	69.89	21.17
8.	Control		88.66	
	C.D. at 5%		1.958	

Results

Evaluation of bio-agents against the pathogen *in vitro*

Bio-agents were evaluated for their inhibiting effect against the pathogen *in vitro* by dual culture technique as described in "Material and Methods". The results of average diameter of fungal colony incubated at 27 ± 1°C after 7 days are presented in table 3 (fig. 3). The results presented in table 1 and its histogram, revealed that all the bio-agents suppressed colony growth of *Rhizoctonia solani*.

The suppression of the growth of pathogen was maximum with *Trichoderma viride* (70.42%) and *Trichoderma atroviride* (62.47%). The other bio-agent were found effective *viz. Trichoderma harzianum* (47.68%), *Trichoderma longibrachiatum* (42.17%), *Trichoderma koningii* (10.49%) and *Aspergillus niger* (8.62%), respectively. Among the biocontrol agents, *Trichoderma viridae* and *Trichoderma atroviride* are the very efficient in controlling the *Rhizoctonia solani*. *Trichoderma koningii* and *Aspergillus niger* are very less effective in controlling the web blight of soybean.

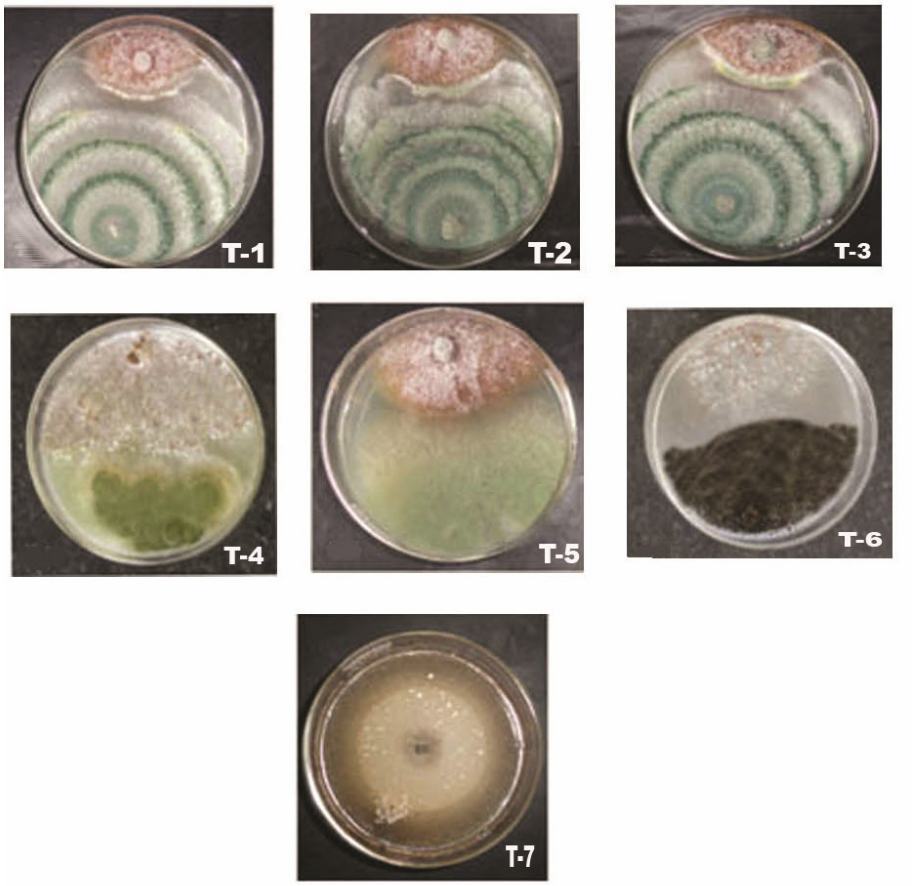
B - screening of botanical extracts against the pathogen *in vitro*

Seven botanical extracts were evaluated for their inhibitory effect against the pathogen *in vitro* (fig. 3). The results of the average diameter of fungal colony after 7 days of incubation at 27 ± 1°C are given in table 2.

All the botanical extracts were effective in the inhibiting the growth of the fungus when compared to control. It is evident from the table 2 and its histogram, that out of nine different plant extracts tested in laboratory, Garlic (88.47%) and Madar (87.98%) are effective against *Rhizoctonia solani* followed by Ginger (52.62%), Aloevera (47.37%). The percent inhibition of the fungal growth is very less in the case of Neem (36.47%), Makoy (23.07%) and Datura (21.17%) as compared to the remaining botanical extracts.

Discussion

Soybean is an important source of protein and edible oil in India during last decade. Soybean subjected to attack by a large number of pathogens. Sinclair (1982) listed over one hundred pathogens infecting soybean crop. In India 40 different species of fungi have been identified to infect this crop, but only few are considered of economic importance and the aerial blight is also one of them. If our farmers could safe guard soybean crop against the



T-1 = *Trichoderma viride* T-5 = *Trichoderma koningii* T-2 = *Trichoderma atroviride* T-6 = *Aspergillus niger*
T-3 = *Trichoderma harzianum* T-7 = Control T-4 = *Trichoderma longibrachiatum*

Fig. 1: Bioassay of bio-agents.

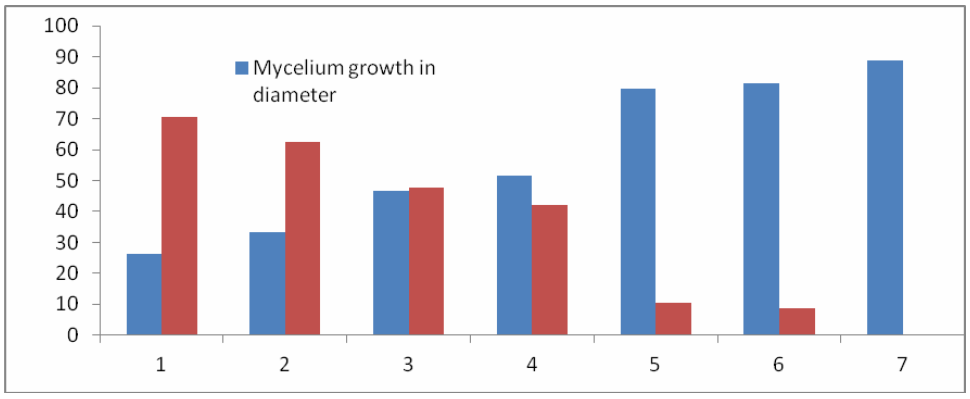
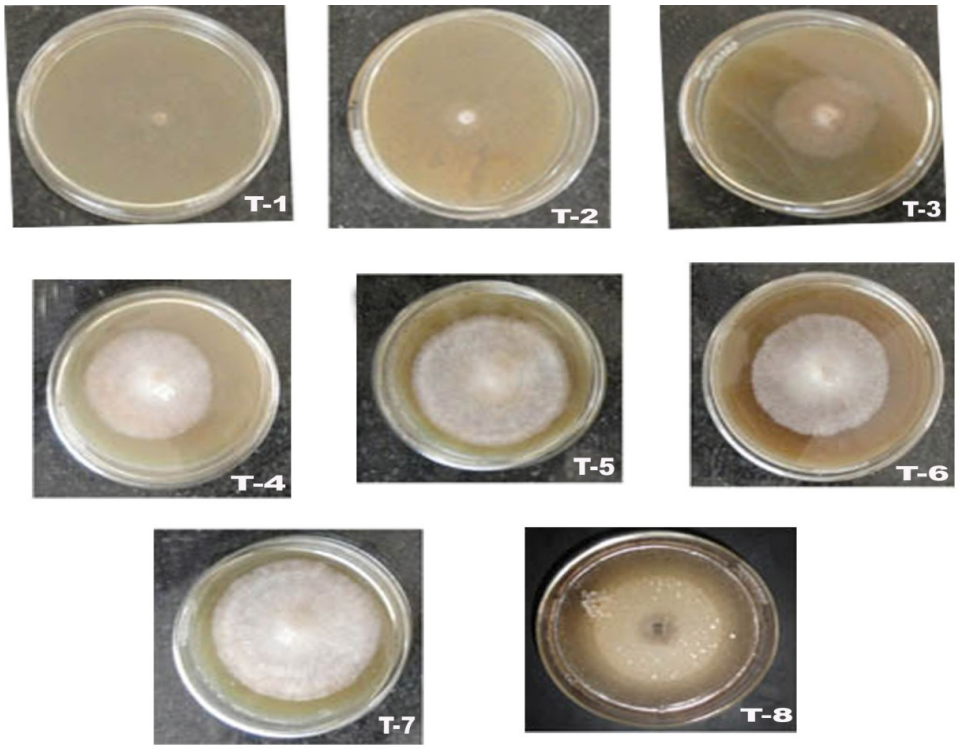


Fig. 2 : Efficacy of different bio-agents against *Rhizoctonia solani.*

disease surely they themselves and country as a whole would be much better off than at present in the pulse and oil production.

Six bio-agents were evaluated *in vitro* condition against the pathogen, amongst these bio-agents *viz.*, *Trichoderma viride* was the most significantly effective (70.42%) as it inhibited the mycelial growth of *Rhizoctonia solani* after 7 days of incubation followed by *T. atroviride* and *T. harzianum* where 33.40mm and 46.56 mm growth was observed, respectively. All bio-agents treatment recorded significantly less disease incidence as compared to pathogen check However, minimum effect on growth (8.62%) was observed in *Aspergillus niger.* Similar finding were also reported by Suruli Rajan (2003), Deeksha and Bhatt and Singh and Verma (2008).



T-1 = Garlic (*Allium sativum*) T-5 = Neem (*Azadirachta indica*) T-2 = Madar (*Calotropis gigantean*)
T-6 = Makoy (*Solanum nigrum*) T-3 = Ginger (*Zingiber officinales*) T-7 = Datura (*Datura fastussa*)
T-4 = Aloevera (*Aloe barbadensis*) T-8 = Control

Fig. 3 : Bioassay of plant extract.

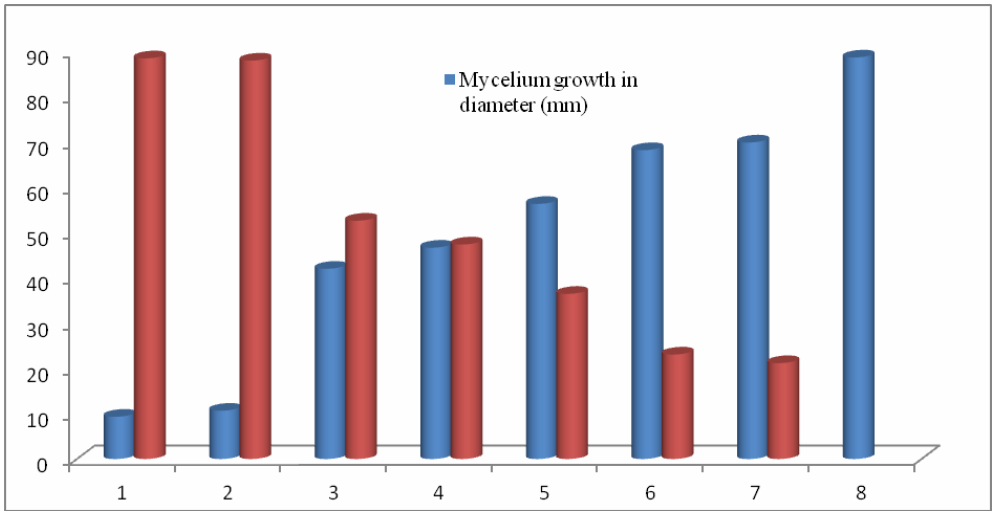


Fig. 4 : Efficacy of different botanical extracts against *Rhizoctonia solani.*

Botanical extract of plant species were also evaluated *in vitro* against the pathogen, amongst these botanical extract *viz.*, Garlic (88.47%) and Madar (87.98%) are effective against *Rhizoctonia solani* followed by Ginger (52.62%), Aloevera (47.037%) inhibited the growth of fungus. Other plant extract, which proved to be effective were Neem (36.47%), Makoy (23.07%), Datura (21.17%), this is similar to the result reported by Ray and Kumar (2008).

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