



Isolation and Studies of seed mycoflora under the different varieties of green gram (*V radiata* L.) Wilczek

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ABSTRACT

Vigna radiata L. popularly known as mung bean or golden gram is an important pulse crop which is widely cultivated and consumed throughout India. Seed borne mycoflora affect the germination and vigour of seeds. Thus, due to seed borne diseases, there is a reduction in the production, resulting in failure of fulfilling the demand for mung bean seeds. In the present studies the most common fungi found to be growing on all untreated seeds were Al- *Alternaria* spp, As- *Aspergillus* spp, Cha - *Chaetomium* spp, Cla- *Cladosporium* spp, Co- *Colletotrichum* spp, Cu- *Curvularia* spp, Dre- *Drechslera* spp, Fu- *Fusarium* spp, Pe- *Penicillium* spp, Rhi- *Rhizopus* spp, and Rho- *Rhizoctonia* spp. The observation with seed treated with the plant extract reveals that a short treatment of 5 min to 2 h had almost no effect over the seed mycoflora. Many of the fungi grew when the seeds were treated for a short period. The inhibition of fungi growth was observed when the seeds were soaked in the plants extract for 1.30 h. The result indicated that the longer duration of seed treatments with plants extracts was effective in controlling the growth of the entire surface borne seed mycoflora. Application of plants extracts for the control of seed borne disease is method devoid of any health hazard problem. Leaf extracts of *A. alliaceum* showed a wide spectrum of fungicidal activity, which indicates that the active principle could possibly be an ester. Results signify the potentiality of *A. alliaceum* as a source of antifungal therapies and hence further work is necessary to evaluate its active principle potentiality in *in vivo* studies on other pathogens as this biofungicidal botanics is environmentally safe and could replace the toxic and hazardous synthetic compounds.

Key words: *Vigna radiata*; seed mycoflora; *Adenocalymma alliaceum*; fungicidal activity

1) INTRODUCTION

Green gram (*Vigna radiata* (L.) Wilczek) is an annual plant with herbaceous bushy appearance which is one of the most important pulse crops of the arid and semiarid tropics among all crops [1, 2]. It is an excellent source of easily digestible protein. Green gram is nutritious as it contains 24 g protein/100g seed, carbohydrates 56.7 g/100g of edible part of the seeds, thiamin (0.47mg/100 g seed), and riboflavin (0.27mg/100g seed), iron (7.3mg/100g seed) [3]. Several factors are responsible for low production of Green gram. Among them, diseases play important role [3]. Many fungal pathogens, some of which are seed transmitted, often reduce the germination ability or kill the infected plants or substantially reduce the productive capacity. However, published reports of seed-borne fungi of Green gram in India are very few. Various scientist studied seed mycoflora of Green gram and other pulses.

Drawback of synthetic chemical methods have increased interest in developing further alternatives control measures particularly those that are environmentally social and biodegradable [4].

Thus, replacement of synthetic fungicides by natural products particularly of plant origin, which are non-toxic and specific in their action, is gaining considerable attention. The rationale for exploiting plants for their antibiotic capabilities stems from the ability of plants to produce a wide array of secondary metabolites which presents a large and relatively untapped source of antifungal drugs [2, 4, 5].

Several herbal extracts have been reported to express antimicrobial activities [6]. Inhibition of pathogens causing plant disease by the use of plant extracts and essential oils having antimicrobial properties have been observed [7] from *Acalypha willensiana*, *Azadirachta indica*, *Datura metel*, *Eucalyptus comadulensis*, *E. citridora*, *Embllica officinales*, *Allium sativum*, *Allium cepa*, *Lecus aspera*, *Ranunculus scleratus*, *Ocimum sanctum*, *Calotropis pocera* [2, 8].

In this case Medicinal creeper (*Adenocalymma alliaceum*) of family Bignoniaceae, is a widespread climber shrub in North Brazil known as "Ipod alho" (garlic bush) because it

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has pungent garlic like smell. The leaves as well as the flower smell alliaceous and are used as substitute for garlic by natives, when the latter is not accessible in the interior regions [9]. *A. alliaceum* is one of the most important commercial cultivars because it contains a mixture of several compounds which are used as antispasmodics, diuretics, anesthetics, and narcotics [7, 9, 10,].

So, the present investigation was designed to isolation and studies of seed mycoflora under the different varieties of green gram (*V. radiata* L.) Wilczek which is one of the economically important crops of Asian countries. The damage *V. radiata* seeds due to fungi is as high as 40 % which gives the country a great economic loss. Hence, keeping in view the adverse effect of the fungicides on the agro- ecosystem, emphasis was given to find out the environmentally safe biocide from *A. alliaceum* to manage diseases.

2) MATERIALS AND METHODS

Seed source: - Seed samples were obtained from the Daliganj market, Lucknow. A total number 5 varieties of *V. radiata* were used viz. ADT 3, VBN (Gg) 3, VRM (Gg) 2, Co (Gg) 7 and K1.

Isolation Technique: - Two generalised isolation procedures were employed for the isolation of pathogenic and saprophytic fungi [11]. The two methods were the moist blotter and the potato dextrose agar (PDA) method.

Isolation on Moist Blotting Paper: - Ten non-sterilized seeds were evenly placed on three layers of moistened 9 cm diameter filter paper (Whatman No.1) in plastic petri dishes to allow for the penetration of light. A total of 10 seeds were used for each sample. Total 50 seeds are taken. The plates were incubated at $27 \pm 2^\circ\text{C}$ for 4 to 5 days in an alternating cycle of 12-hour s NUV (near ultraviolet light) and 12 hours darkness regime. Fungi developing on seeds were examined and transferred to PDA for identification and pathogenicity studies.

Incubation on PDA: - Ten seeds surface sterilized for 10 minutes in 1 percent solution of sodium hypochlorite as a pre- treatment were evenly spaced on PDA plate. The plates were incubated at $27 \pm 2^\circ\text{C}$ for 4 to 5 days in an alternating cycle of 12 hours NUV and 12 hours darkness. A total of 10 seeds per sample were used. Fungi developing on seeds were identified as in the previous experiment.

Plant Material: - During the month of January and February the fresh leaves (100g) of *A. alliaceum* Miers were collected from the Botany Department, Lucknow University, Lucknow. The *in vitro* study was carried out in the Mycology and Plant Pathology research Laboratory, Lucknow.

Preparation of Aqueous Extract of Leaves of Garlic Creeper: - Collected leaves were properly washed with 70% ethanol and water separately. Final wash with distilled/sterilized water removes the trace of ethanol. An aqueous extract was prepared by blending fresh leaves with distilled water (10ml/g fresh weight) i.e.,10% in a blender for 2 minutes at $27 \pm 2^\circ\text{C}$. The homogenate was filtered through a layer of muslin cloth. The filtrate was centrifuged at 12000 rpm for 20 minutes. The supernatant was sterilized through a Millipore filter (0.2 μm) and served as

the stock solution. All dilution were made with sterile distilled water when necessary [12].

Seed treatment: -The different soybean seed cultivar was treated with leaf extract of *A. alliaceum* (2 %) by soaking seed in it for different time leave as 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1hour, 1.15-hour, 1.30 hour,1.45 hour, and 2 hour.

Study of mycoflora of treated seeds: - Treated seed with plant extract for above time intervals were incubated in Potato dextrose agar (PDA) at $27 \pm 2^\circ\text{C}$ for 4 to 5 days in an alternating cycle of 12-hour s NUV (near ultraviolet light) and 12 hours darkness regime. They were studied for the growth of fungal from the seed surface.

3) RESULTS AND DISCUSSION

The most common fungi found to be growing on all untreated seeds were Al- *Alternaria* spp, As- *Aspergillus* spp, Cha - *Chaetomium* spp, Cla- *Cladosporium* spp, Co- *Colletotrichum* spp, Cu- *Curvularia* spp, Dre- *Drechslera* spp, Fu- *Fusarium* spp, Pe- *Penicillium* spp, Rhi- *Rhizopus* spp, Rho- *Rhizoctonia* spp (Table 1).

Table 1: Mycoflora of *V. radiata* variety on untreated seeds

Name of pathogens	Varity of <i>V. radiata</i>				
	ADT 3	VBN (Gg)3	VRM (Gg)2	Co (Gg)7	K1
<i>Alternaria alternata</i>	+++	++	+	-	-
<i>Alternaria tenuis</i>	+	+	++	-	+
<i>Aspergillus carbonarius</i>	++	++	++	+	+
<i>Aspergillus flavus</i>	+	+	++	++	-
<i>Aspergillus fumigatus</i>	++	+	-	-	+
<i>Aspergillus nidulans</i>	+	-	++	++	++
<i>Aspergillus niger</i>	++	+	++	+++	++
<i>Chaetomium globosum</i>	++	-	-	+++	+++
<i>Cladosporium</i> sp	-	+++	++	+	-
<i>Colletotrichum truncatum</i>	++	+++	-	++	-
<i>Curvularia lunata</i>	+	++	-	+	+
<i>Drechslera tetramera</i>	-	-	+	-	++
<i>Fusarium moniliforme</i>	-	+	+	++	+
<i>Fusarium oxysporum</i>	+	++	-	+++	+
<i>Penicillium itaalicum</i>	-	-	+	-	++
<i>Penicillium</i> sp	-	+	++	++	-
<i>Rhizopus stolonifer</i>	++	++	++	+	+
<i>Rhizoctonia solani</i>	+++	++	-	+	+

Symbols used; '+' = presence on 10 % seeds; '++'= presence on 20% seeds; '+++ '= presence on 30 % seeds; '-' = absence on seeds

The observation with seed treated with the plant extract reveal that short treatment of 5 mint to 2 h had almost no effect over the seed mycoflora. Many of the fungi grew when the seeds were treated for short period. The inhibition of fungi growth was observed when the seeds were soaked in

the plants extract for 1.30 h. The result indicated that the longer duration of seed treatments with plants extracts was effective in controlling the growth of the entire surface borne seed mycoflora (Table 2). Application of plants extracts for the control of seed borne disease is method devoid of any health hazard problem. Hill bunt of Wheat (*Tilletia foetida*) was effectively controlled by seed

treatment with plants extract of *Datura stramonium*, *Thuja sp.* and *Eucalyptus* [8, 13].

The antifungal effect of selected medicinal extracts can be applied at a larger scale to treat the seed before sowing them in the field. The extract being of plants origins has hazardous effects on the seeds as well as on soil. The seed treatment with plants extract does not have any adverse effect on the germination of seed even after the treatments

Table 2: Effect of the Duration of Exposure of the Leaf Extract on seed of *V. radiata* microflora.

S. No.	<i>V. radiata</i> variety	Mycoflora on treated seed with <i>Adenocalymma alliaceum</i> Miers leaf extracts									
		Exposure Time									
		5 mit	15mit	30mit	45mit	1 h	1.15h	1.30h	1.45h	2h	
1	ADT 3	Al	Al	Al	Al	Al	Al	Al	Al	Al	-
		As	As	As	As	As	As	-	-	-	-
		Cha	Cha	Cha	Cha	Cha	Cha	Cha	Cha	-	-
		Co	Co	Co	-	-	-	-	-	-	-
		Cu	Cu	Cu	Cu	Cu	Cu	-	-	-	-
		Vu	Dre	Dre	Dre	Dre	-	-	-	-	-
		Fu	Fu	Fu	Fu	Fu	Fu	Fu	Fu	-	-
		Rhi	Rhi	Rhi	Rhi	Rhi	Rhi	Rhi	Rhi	-	-
		Rho	Rho	Rho	Rho	Rho	Rho	Rho	Rho	Rho	-
2	VBN(Gg)3	Al	Al	Al	Al	Al	Al	Al	Al	Al	-
		As	As	As	As	As	As	-	-	-	-
		Cla	Cla	Cla	Cla	Cla	-	-	-	-	-
		Co	Co	Co	-	-	-	-	-	-	-
		Cu	Cu	Cu	Cu	Cu	Cu	-	-	-	-
		Fu	Fu	Fu	Fu	Fu	Fu	Fu	Fu	-	-
		Pe	Pe	Pe	Pe	Pe	Pe	Pe	Pe	Pe	-
		Rhi	Rhi	Rhi	Rhi	Rhi	Rhi	Rhi	Rhi	-	-
		Rho	Rho	Rho	Rho	Rho	Rho	Rho	Rho	Rho	-
3	VRM(Gg)2	Al	Al	Al	Al	Al	Al	Al	Al	Al	-
		As	As	As	As	As	As	-	-	-	-
		Cha	Cha	Cha	Cha	Cha	-	-	-	-	-
		Cla	Cla	Cla	Cla	Cla	-	-	-	-	-
		Dre	Dre	Dre	Dre	Dre	-	-	-	-	-
		Fu	Fu	Fu	Fu	Fu	Fu	Fu	Fu	-	-
		Pe	Pe	Pe	Pe	Pe	Pe	Pe	Pe	Pe	-
		Rhi	Rhi	Rhi	Rhi	Rhi	Rhi	Rhi	Rhi	-	-
		Rho	Rho	Rho	Rho	Rho	Rho	Rho	Rho	Rho	-
4	Co(Gg)7	As	As	As	As	As	As	-	-	-	-
		Cha	Cha	Cha	Cha	Cha	-	-	-	-	-
		Cla	Cla	Cla	Cla	Cla	-	-	-	-	-
		Co	Co	Co	-	-	-	-	-	-	-
		Cu	Cu	Cu	Cu	Cu	Cu	-	-	-	-
		Fu	Fu	Fu	Fu	Fu	Fu	Fu	Fu	-	-
		Pe	Pe	Pe	Pe	Pe	Pe	Pe	Pe	Pe	-
		Rhi	Rhi	Rhi	Rhi	Rhi	Rhi	Rhi	Rhi	-	-
		Rho	Rho	Rho	Rho	Rho	Rho	Rho	Rho	Rho	-
5	K1	Al	Al	Al	Al	Al	Al	Al	Al	Al	-
		As	As	As	As	As	As	-	-	-	-
		Cha	Cha	Cha	Cha	Cha	-	-	-	-	-
		Cu	Cu	Cu	Cu	Cu	Cu	-	-	-	-
		Dre	Dre	Dre	Dre	Dre	-	-	-	-	-
		Fu	Fu	Fu	Fu	Fu	Fu	Fu	Fu	-	-
		Pe	Pe	Pe	Pe	Pe	Pe	Pe	Pe	Pe	-
		Rhi	Rhi	Rhi	Rhi	Rhi	Rhi	Rhi	Rhi	-	-
		Rho	Rho	Rho	Rho	Rho	Rho	Rho	Rho	Rho	-

Al- *Alternaria* spp, As- *Aspergillus* spp, Cha - *Chaetomium* spp, Cla- *Cladosporium* spp, Co- *Colletotrichum* spp, Cu- *Curvularia* spp, Dre- *Drechslera* spp, Fu- *Fusarium* spp, Pe- *Penicillium* spp, Rhi- *Rhizopus* spp, Rho- *Rhizoctonia* spp

for 1.30 h. So, the seed treatment of plants extract will not create any problem of pollution and the chemicals of plants extract are easily degrade in the soil.

Leaf extracts of *A. alliaceum* showed a wide spectrum of fungicidal activity, which indicating that the active principle could possibly be an ester [7]. Results signify the potentiality of *A. alliaceum* as a source of antifungal therapies and hence further work is necessary to evaluate its active principal potentiality in *in vivo* studies on other pathogens as this biofungicidal botanics is environmentally safe and could replace the toxic and hazardous synthetic compounds. Simultaneously investigations are also needed to characterize, formulate and marketwise the active principles of the extract which may provide leads for the discovery of a novel antifungal compounds from *A. alliaceum*.

4) Conclusion

It can be concluded that former should be scientifically acquainted trained about seeds, symptoms in early stage and method of intercropping.

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