



**Research Paper**

**SOIL BORNE FUNGI PROMOTE SEED GERMINATION *In Vitro* ON *Vigna* SPECIES**

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**Abstract**

Soil provides unique habitat for a large number of microorganisms and these microorganisms interact with plants and bring about various effects. Not only bacteria but fungi are also able to stimulate root growth, secondary root initiation and seed germination. Therefore, the present study was focused on the effects of six different soil borne fungi *Fusarium* spp., *Trichoderma* spp., *Aspergillus* spp., *Alternaria* spp., *Colletotrichum* spp. and *Penicillium* spp. on the seed germination of *Vigna mungo* (Black gram), *Vigna radiate* (Green gram) and *Vigna unguiculata* (Cowpea). Twenty five surface sterilized seeds were soaked in 50 ml of fungal spore suspension ( $10^8$  spores/ml) for 24 hours separately. After soaking, seeds were transferred into sterile petridishes moistened with sterile filter paper. Plates were kept in dark at room temperature for one to four days and the development of germ tube was measured. Results were subjected to analysis of variance (ANOVA) ( $P < 0.05$ ) followed by Tukey test. Particular fungal genera showed different degree of germination effect on different plant seeds. Likewise different fungal genera exhibited different effects on a particular plant seed variety. After 24 hours of incubation for germination, highest growth rate was observed in cowpea which was produced by *Trichoderma* spp., *Alternaria* spp. and *Colletotrichum* spp. However, *Fusarium* spp. and *Penicillium* spp. suppressed the growth of germ tube of all three plants tested. But after 48 hours of incubation period, *Alternaria* spp. promoted the highest growth of germ tubes on black gram and green gram seeds. Almost all the tested fungi enhanced the length of germ tube in all treated seeds compared with control except *Penicillium* spp. which inhibited seed germination on green gram and cow pea.

Key words: Seed germination, soil borne fungi, *Vigna mungo*, *Vigna radiate*, *Vigna unguiculata*.

**INTRODUCTION**

Soil provides unique habitat for a large number of microorganisms such as fungi, bacteria, cyanobacteria, algae, nematodes and viruses belonging to innumerable genera and species. Soil microorganisms interact with plants and bring about various effects such as beneficial, harmful, neutral and variable(1). The rhizosphere is particularly suitable for the growth of soil microorganisms. Not only bacteria but fungi are also able

to stimulate root growth, secondary root initiation and seed germination. Beneficial mycorrhizal associations are to be found in many of our edible and flowering crops. These associations include at least 80% of the *Brassica* and *Solanum* families, as well as the majority of tree species, especially in forest and woodlands(2). Fungal metabolites such as amino acids, cyclicpeptides, aromatic phenols and plant growth regulators are released to the rhizosphere environment(3). Enzymes of fungi participated in the breakdown of cell wall and maceration of plant tissues, which leads to invasion of plant by pathogens (4). Some fungal genera of, *Aspergillus*, *Fusarium*, *Penicillium* and *Rhizoctonia* commonly produce toxic substances (5). Nevertheless, some of the interesting results were produced by some soil borne fungi by secreting plant hormones themselves which induce the germination of seeds. *Vigna mungo* (Black gram ) , *Vigna radiate* ( Green gram) and *Vigna unguiculata* ( Cowpea) are the most extensively used pulse crop in Sri Lanka. Therefore present study was conducted to screen the effects of six soil borne fungi on the germination of black gram, green gram and cowpea seeds.

## MATERIALS AND METHODS

### Collection of plant seeds and fungal culture

Plants seeds, *Vigna mungo* (Black gram), *Vigna radiate* ( Green gram) and *Vigna unguiculata* ( cowpea) were collected from Chemical institute in Colombo [ CIC (Pvt Ltd)] Seeds Agro Business outlet in Jaffna. Six genera of soil borne fungi, *Fusarium* spp., *Trichoderma* spp., *Aspergillus* spp., *Alternaria* spp. , *Collettrorichum* spp and *Penicillium* spp were obtained from laboratory culture collection of Department of Botany, University of Jaffna and these were sub cultured on Potato dextrose agar medium and incubated at room temperature for 3-5 days.

### Soaking of seeds in fungal spore suspensions

Fungal spores suspensions were prepared separately by using the ringer's solution and the number of spores were counted by the Haemocytometer. Fungal spore concentration was maintained as  $10^8$  spores/ml for each fungal genera by the dilution technique. After that 25 surface sterilized healthy seeds were soaked in 50 ml of the particular fungal spore suspension for 24 hours. Above procedure was repeated to other fungi as well as other plant varieties. Seeds were soaked in 50 ml of sterile ringer's solution used as control(6,7).

### Seed germination study

After 24 hours of soaking, seeds were transferred into sterile petridishes moistened with sterile filter paper. Plates were kept in dark at room temperature for one to four days and the development of germ tube was measured after 24 and 48 hours of incubation(8).

### Statistical Analysis

The results were subjected for analysis of variance, Tukey test ( $P=0.05$ ) using statistical software SPSS windows version 13.0.

## RESULTS AND DISCUSSION

Seed germination depends on the types of fungal species, variety of plant seeds, concentration of inoculum, rate of colonization, internal physical factors of seed and the amount of growth promoting substances produced(7). Particular fungal genera showed different degree of germination effect on different plant seeds. Likewise different fungal

genera exhibited different effects on a particular plant seed variety. After 24 hours of incubation for germination, highest growth rate was observed in cowpea which was produced by *Trichoderma* spp. (10.30 mm), *Alternaria* spp.(10.20 mm) and *Colletotrichum* spp.(9.83 mm) and no significant difference was observed in their activity on seed germination. In black gram, length of germ tube was highly induced by *Alternaria* spp.(7.2mm) and *Colletotrichum* spp.(7.08mm), which was followed by *Aspergillus* spp.(6.58mm) and *Trichoderma* spp.(6.58mm) when compared with control. Green gram seed germination was highly induced by *Colletotrichum* spp. (8.25mm). However, *Fusarium* spp. and *Penicillium* spp. suppressed the growth of germ tube of all three plants tested. But the suppressive effect was more prominent by the *Penicillium* spp.(4.12, 5.11,6.12mm). These inhibitory effect might be due to the secondary metabolites of fungi which able to degrade seed quality and reduce the seed viability(9).(Table1).

**Table 1: Effects of soil borne fungi on seed germination after 24 hours of incubation**

	<b>Black gram</b>	<b>Green gram</b>	<b>Cow pea</b>
Control	6.18 ± 0.0288 <sup>c</sup>	7.62 ± 0.0251 <sup>bc</sup>	8.85 ± 0.05 <sup>b</sup>
<i>Aspergillus</i> spp	6.58 ± 0.0288 <sup>b</sup>	7.98 ± 0.0288 <sup>ab</sup>	7.52 ± 0.0251 <sup>c</sup>
<i>Colletotrichum</i> spp	7.08 ± 0.0763 <sup>a</sup>	8.25 ± 0.2179 <sup>a</sup>	9.83 ± 0.1154 <sup>a</sup>
<i>Fusarium</i> spp	5.65 ± 0.4932 <sup>d</sup>	7.50 ± 0.05 <sup>d</sup>	7.36 ± 0.3883 <sup>c</sup>
<i>Trichoderma</i> spp	6.58 ± 0.3362 <sup>b</sup>	6.08 ± 0.7637 <sup>e</sup>	10.30 ± 0.2645 <sup>a</sup>
<i>Alternaria</i> spp	7.2 ± 0.1 <sup>a</sup>	7.80 ± 0.7637 <sup>b</sup>	10.20 ± 0.00 <sup>a</sup>
<i>Penicillium</i> spp	4.12± 0.015 <sup>e</sup>	5.11± 0.0251 <sup>f</sup>	6.12± 0.115 <sup>e</sup>

**Values are mean ± Standard deviation (SD), values with different superscripts on the same column show significant ( p<0.05) difference.**

Nevertheless, after 48 hours of incubation period, *Alternaria* spp. exhibited the highest growth of germ tubes on black gram(34.53mm) and green gram(29.82mm) seeds. But unfortunately the previous study stated that the reduction in percentage of seed germination on soy bean which was soaked in culture filtrates of *Alternaria tenuis*(10). Cow pea seed germination was highly induced by *Trichoderma* spp(32.21mm) and *Aspergillus* spp.(32.21mm). It was reported that the secondary metabolites of *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus nidulans* inhibited the seed germination and root-shoot length of all the selected cereals and pulses. Wheat, green gram and black gram were more susceptible to the metabolites of *Aspergillus niger*(11). Similar results were also reported by Pangrikar et al.(12). The production of secondary metabolites by fungi is known to degrade seed quality and reduce the seed viability(11). On the other hand *Fusarium* spp and *Penicillium* spp were found to promote seed germination and seedling growth of *Xyris complanata*(13). Most interestingly this study showed that almost all the tested fungi enhanced the length of germ tube in all treated seeds compared with control expect *Penicillium* spp. which inhibited seed germination on green gram and cow pea. (Table2).

**Table 2: Effects of soil borne fungi on seeds germination after 48 hours of incubation**

	Black gram	Green gram	Cowpea
Control	21.25 ± 0.05 <sup>f</sup>	16.51 ± 0.0288 <sup>e</sup>	22.20 ± 0.1802 <sup>e</sup>
<i>Aspergillus</i> spp	29.75 ± 0.0579 <sup>d</sup>	21.15 ± 0.1322 <sup>d</sup>	32.21 ± 0.0288 <sup>a</sup>
<i>Colletotrichum</i> spp	33.68 ± 0.0288 <sup>b</sup>	28.81 ± 0.0288 <sup>b</sup>	31.25 ± 0.05 <sup>b</sup>
<i>Fusarium</i> spp	31.11 ± 0.0288 <sup>c</sup>	28.50 ± 0.00 <sup>c</sup>	26.76 ± 0.0763 <sup>c</sup>
<i>Trichoderma</i> spp	24.06 ± 0.1154 <sup>e</sup>	16.40 ± 0.05 <sup>e</sup>	32.21 ± 0.1892 <sup>a</sup>
<i>Alternaria</i> spp	34.53 ± 0.0577 <sup>a</sup>	29.82 ± 0.0288 <sup>a</sup>	25.26 ± 0.0230 <sup>d</sup>
<i>Penicillium</i> spp	23.13 ± 0.015 <sup>e</sup>	14.11 ± 0.0151 <sup>f</sup>	21.12 ± 0.215 <sup>f</sup>

**Values are mean ± Standard deviation (SD), values with different superscripts on the same column show significant (  $p < 0.05$  ) difference.**

## CONCLUSION

Particular fungal genera showed different degree of germination effect on different plant seeds. Likewise different fungal genera exhibited different effects on a particular plant seed variety. After 24 hours of incubation for germination, highest growth rate was observed in cowpea which was produced by *Trichoderma* spp., *Alternaria* spp. and *Colletotrichum* spp. However, *Fusarium* spp. and *Penicillium* spp. suppressed the growth of germ tube of all three plants tested. But after 48 hours of incubation period, *Alternaria* spp. promoted the highest growth of germ tubes on black gram and green gram seeds. Almost all the tested fungi enhanced the length of germ tube in all treated seeds compared with control except *Penicillium* spp. which inhibited seed germination on green gram and cow pea. Field studies could be developed in future to monitor the effect of these beneficial soil borne fungi.

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