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STUDIES ON RELATIONSHIP BETWEEN STORAGE FUNGI OF COWPEA AND THEIR COLONIZATION IN THE ROOTS

SARMA AK* AND DAS G

Assam Down Town University, Guwahati 781026, Assam, India

***Corresponding Author: Anjan Kumar Sarma: E Mail: aksbot82@gmail.com; Ph: 8638671212**

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ABSTRACT

A laboratory study was conducted to study the relationship between storage fungi of cowpea and their colonization of the roots. Freshly harvested cowpea seeds after drying were stored for a year to determine the fungi appeared during storage. Sampling was made after one year of storage and found that stored cowpea seeds were associated with twenty different fungal strains. Among the fungal species *Aspergillus flavus* and *Aspergillus ochraceus* was the most dominant species isolated from surface sterilized and non sterilized seeds respectively. To determine the relationship between fungi present during storage and their colonization of root, cowpea seeds were grown in three series of pots viz. surface sterilized seeds in natural soil, non sterilized seeds in sterilized soil and non sterilized seeds in natural soil. When surface sterilized seeds grown in natural soil, some fungal species already isolated from the surface sterilized seeds were absent in the soil of colonization. Fungi associated with rhizosphere of cowpea grown from non sterilized seeds in sterilized soil were almost similar to that of the fungi isolated from the sterilized and non sterilized seeds. All the fungi present in the rhizosphere of cowpea from surface sterilized seeds in natural soil as well as non sterilized seeds in sterilized soil were observed when non sterilized seeds were grown in natural soil. Therefore, present study confirmed that there is a movement of some storage fungi from seeds to their developing roots during the seed germination.

Keywords: Cowpea, Mycoflora, Storage, Rhizosphere

INTRODUCTION

Cowpea is one of the important annual food crop grown mainly to be used as pulse, vegetable and fodder. Cowpea is drought tolerant warm-weather crop well adapted to the drier regions of the tropics, where other food legumes do not perform well. It has a useful ability to fix atmospheric nitrogen through root nodules. Poor soil with more than 85% sand, less than 0.2% organic matter and low level of phosphorus is also favourable for the growth of cowpea **[1]**. Globally, current production levels of cowpea are estimated at 55 million metric tons annually, nearly 60% of this being grown in developing countries.

Storage fungi are the fungi which invade grains or seeds during storage. Storage fungi are usually not present to any serious extent before harvest. Small inoculum of spores of storage fungi may be present on grain going into storage or may be present on spilled grain present in harvest, handling and storage equipment or structures. Under improper storage conditions this small amount of inoculum can increase rapidly leading to significant problems. The development of storage fungi in stored grain is influenced by the moisture content and temperature of the stored grain, the condition of the grain to be stored, the duration for which grain is stored and the amount of insect and mite activity in the grain. The most harmful toxigenic species present in stored grains belong to the group of xerophilic species i.e. *Aspergillus* and *Penicillium* **[2]**. A wide variety of fungi (both pathogenic and saprophytic) are present both externally and internally in seeds during storage. These storage fungi may cause seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity, as well as seedling damage resulting in development of disease at later stages of plant growth by systemic or local infection **[3]**. This may reduce their quality and impair seed germination resulting in the production of abnormal seedling **[4]**. Borah *et. al.* **[5]** while studying diversity of seed mycoflora of flood prone low land rice genotype reported *Apergillus niger, A. flavus, A. terreus, A. candidus*, species of *Penicillium* and *Cladosporium* which contaminated the seeds as externally seed borne fungi while *Alternaria oryzae, Fusarium moniliforme* and *Sarocladium oryzae* appear to be internally seed borne being deeply seated inside the seed. Ali Khan *et. al.*, **[6]** isolated seven storage fungi from the four mustard varieties and out of which *Aspergillus flavus* was the most predominant. Park *et. al.* **[7]** found *Penicillum citrinum* and *Aspergillus candidus* were the most

prevalent species while *Fusarium proliferatum* was found as the dominant species on mycoflora of rice. Bidyapati *et. al.* **[8]** also reported that *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Colletotrichum capsici* and *Fusarium moniliforme* were most frequent while studying effect of seed borne fungi of chilli seeds during storage. In cognizance of the above a present study was undertaken to determine the relationship between storage fungi of cowpea and their colonization in the roots.

MATERIALS AND METHODS

The cowpea seeds stored for a year were examined and storage fungi were isolated by using standard agar plate method. The seeds were surface sterilized with 0.1% $HgCl_2$ solution before plating for the isolation of fungi.

To determine the relationship between fungi present during storage (one year stored cowpea seeds) and their colonization of root, cowpea plants were grown in three series of pots. The pots were watered regularly to maintain moisture level (60%) throughout the experimental period. The series were as follows:

Surface sterilized seeds in natural soil to determine the mycoflora originating from soil:

Seeds were surface sterilized with 0.1% $HgCl_2$ for 2 minutes and sown in five pots filled with natural soil. The mouth of the pots was covered with sterile cellophane paper and the growing seedlings were allowed to protrude through small slits to check air borne contamination.

Non-sterilized seeds in sterilized soil to determine the mycoflora originating from seed:

The seeds were sown in five pots containing sterile soil. Non-sterilized seeds were sown in each pot. The mouth of the pots was covered with sterile cellophane paper and the growing seedlings were allowed to protrude through small slits made on the cellophane paper to check air borne contamination.

Non-sterilized seeds in natural soil (control):

Non-sterilized seeds were sown in five pots filled with natural soil and used as control.

The rhizosphere was sampled at 5, 10, 15, 20 and 25 days of growth of plants. At each sampling, plants were removed from the series and the root system together with the loosely adhering soil was used for the study. The fungal species present in rhizosphere were isolated by standard serial dilution method. The isolated fungal colonies were counted and identified on the basis of colony characters, morphology and reproductive characteristics.

Statistical analysis:

To minimize experimental errors and attainment of proper degrees of freedom five replications were taken. Duncan Multiple Range Test (DMRT) was done using SPSS software.

RESULT

To study the colonization of storage fungi in the roots of surface sterilized and non sterilized cowpea seeds was done to observe the invasion of storage fungi in the rhizosphere. Twenty three fungal species belonging to twelve genera were isolated from surface sterilized and non sterilized cowpea seeds by agar plate method **(Figure 1)**. *Aspergillus ochraceus*, *Aspergillus flavus, Aspergillus niger*, *Trichoderma viride*, *Aspergillus terreus*, *Trichoderma harzianum*, *Penicillium citrinum* and *Penicillium chrysogenum* were most frequent isolates from the non sterilized cowpea seeds. The percentage occurrence of other fungi in non sterilized cowpea seeds were *Cladosporium herbarum*, *Mucor mucedo*, *Sporotrichum* sp., *Botrytis cinerea*, *Fusarium moniliforme* , *Penicillum frequentans*, *Penicillum oxalicum*, *Fusarium solani*, *Cephalosporium gramineum*, *Zygosporium* sp., *Penicillum notatum*, *Geotrichum candidum*, *Aspergillus fumigatus*, *Chaetomium globosum* and *Penicillum expansum*. However, *Aspergillus flavus* was the most dominant fungi isolated from sterilized seeds of cowpea followed by *Aspergillus ochraceus*, *Aspergillus terreus*, *Penicillium chrysogenum*, *Penicillium oxalicum*, *Chaetomium globosum*, *Sporotricchum* sp., *Trichoderma viride*, *Aspergillus fumigatus*, *Cephalosporium gramineum*, *Fusarium moniliforme*, *Trichoderma harzianum*, *Penicillium citrinum*, *Zygosporium* sp., *Aspergillus niger*, *Botrytis cinerea*, *Cladosporium herbarum*, *Fusarium solani*, *Penicillium notatum*, *Penicillium expansum*, *Penicillium frequentans*, *Geotrichum candidum* and *Mucor mucedo*.

Surface sterilized seeds in natural soil to determine the mycoflora originating from soil:

Fungi associated with the rhizosphere of young cowpea plant grown (5, 10, 15, 20, 25 DAS) from sterilized seeds in natural soil were *Alternaria alternata, Alternaria triticina, Aspergillus candidus*, *Curvularia lunata, Helminthosporium solani*, *Monilia* sp., *Phoma* sp., *Rhizopus* sp., *Trichoderma harzianum*, *Verticillium* sp. and sterile mycelia **(Table 1)**. The growing root showed internal seed borne as well as soil borne fungi. Some fungal species isolated from the surface sterilized seeds by agar plate method were absent in the soil of colonization **(Figure 2A)**.

Non sterilized seeds in sterilized soil to determine the mycoflora originating from seed:

Fungi associated with rhizosphere of cowpea grown (5, 10, 15, 20, 25 DAS) from non

sterilized seeds in sterilized soil were *Alternaria alternata, Aspergillus niger*, *Aspergillus flavus, Aspergillus ochraceus*, *Aspergillus terreus*, *Aspergillus fumigatus, Botrytis cinerea*, *Cephalosporium gramineum*, *Cladosporium herbarum*, *Chaetomium globosum*, *Curvularia lunata*, *Fusarium solani, Helminthosporium solani*, *Penicillium chrysogenum, Penicillium citrinum* and *Verticillium* sp. **(Table 2)**. Almost similar fungal species (external as well as seed borne) were isolated from the sterilized and non sterilized seeds of cowpea by agar plate method **(Figure 2B)**.

Non sterilized seeds in natural soil (Control): Fungi associated with rhizosphere of cowpea grown (5, 10, 15, 20, 25 DAS) from non sterilized seeds in natural soil (control) were *Alternaria alternata, Alternaria triticina*, *Aspergillus niger, Aspergillus flavus, Aspergillus ochraceus, Aspergillus terreus, Aspergillus fumigatus, Aspergillus candidus*, *Botrytis cinerea*, *Cephalosporium gramineum*, *Cladosporium herbarum*, *Chaetomium globosum, Curvularia lunata, Fusarium solani, Helminthosporium solani*, *Monilia* sp., *Penicillium chrysogenum, Penicillium citrinum*, *Rhizopus* sp. and *Verticillium* sp. **(Table 3)**. In the present study, all the external and internal seed borne and soil borne fungi were isolated from the growing root. All the fungi present in the rhizosphere of cowpea from surface sterilized seeds in natural soil as well as non sterilized seeds in sterilized soil were observed in control **(Figure 2C)**.

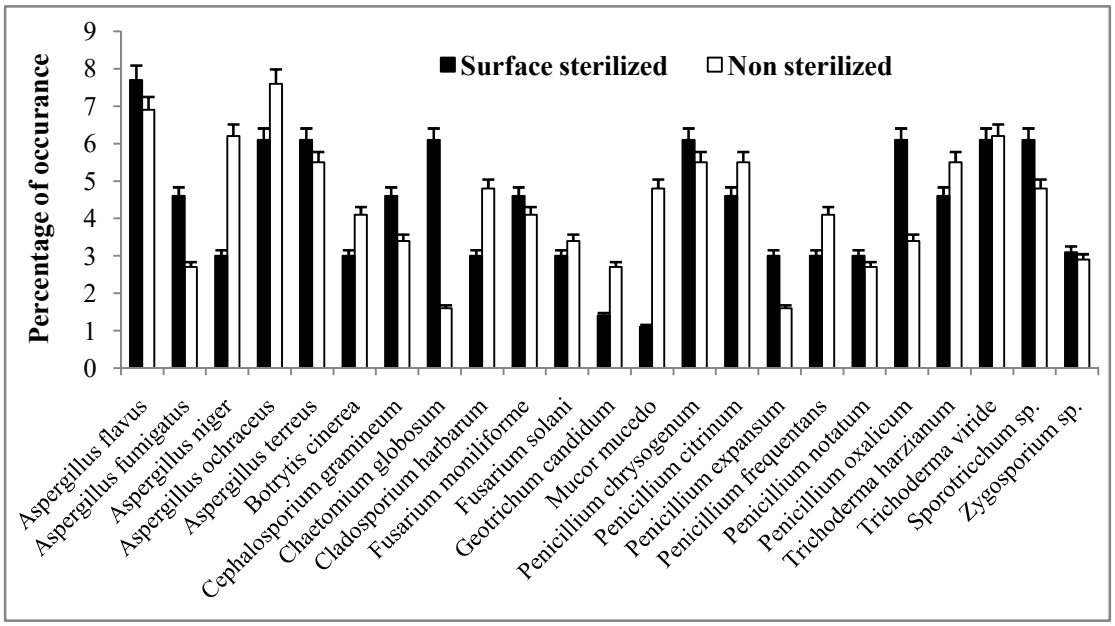

Figure 1: Occurrence of storage fungi of cowpea seeds (one year stored seed)

Table 1: Fungal incidence (percentage) in rhizosphere of young cowpea grown from surface sterilized seeds in natural soil

Sl. no	Type of fungi	Age of roots (Days after sowing)				
		5	10	15	20	25
1	*Alternaria alternata*	$11.1^{b} \pm 0.40$	$5.0^{d} \pm 0.58$	$2.8^{g} \pm 0.23$	$8.8^{d} \pm 0.92$	$7.4^{e} \pm 1.6$
2	*Alternaria triticina*	$11.1^{b} \pm 0.87$	$10.0^{c} \pm 1.2$	$5.4^{f} \pm 0.58$	$7.4^{de} \pm 0.81$	$7.4^{e} \pm 0.46$
3	*Aspergillus candidus*	$44.5^{a} \pm 4.3$	$35.0^{a} \pm 2.9$	$27.0^{a} \pm 3.5$	$23.5^{a} \pm 1.7$	$26.6^{a} \pm 2.2$
4	*Curvularia lunata*	$11.1^{b} \pm 0.75$	$10.0^{c} \pm 1.7$	$16.2^{b} \pm 1.3$	$13.2^{c} \pm 1.4$	$11.7^{c} \pm 1.2$
5	*Helminthosporium solani*	$11.1^{b} \pm 1.3$	$5.0^{d} \pm 1.2$	$8.1^{e} \pm 1.5$	$5.9^{ef} \pm 0.29$	$5.3^{f} \pm 0.40$
6	*Monilia* sp.	$0.00^{c} \pm 0.0$	$0.00^{e} \pm 0.0$	$0.00^{h} \pm 0.0$	$1.5^{g} \pm 0.29$	$2.2^{h} \pm 0.46$
7	*Phoma* sp.	$11.1^{b} \pm 1.2$	$5.0^{d} \pm 0.58$	$5.4^{f} \pm 0.46$	$5.9^{ef} \pm 0.17$	$4.3^{fgh} \pm 0.40$
8	*Rhizopus* sp.	$0.00^{c} \pm 0.0$	$10.0^{c} \pm 0.58$	$5.4^{f} \pm 0.35$	$4.4^{f} \pm 0.35$	$3.2^{g} \pm 0.35$
9	*Trichoderma harzianum*	$0.00^{c} \pm 0.0$	$0.00^{e} \pm 0.0$	$13.5^{c} \pm 1.9$	$7.4^{de} \pm 0.58$	$9.6^{d} \pm 0.58$
10	*Verticillium* sp.	$0.00^{c} \pm 0.0$	$0.00^{e} \pm 0.0$	$5.4^{f} \pm 0.58$	$4.4^{f} \pm 0.46$	$3.2^{gh} \pm 0.35$
11	Sterile mycellia	$0.00^{c} \pm 0.0$	$20.0^{b} \pm 1.7$	$10.8^{d} \pm 0.81$	$17.6^{b} \pm 1.6$	$19.1^{b} \pm 0.86$

Table 2: Fungal incidence (percentage) in rhizosphere of young cowpea grown from non sterilized seeds in sterilized soil

Sl. no.	Type of fungi	Age of roots (Days after sowing)				
		5	10	15	20	25
1	*Alternaria alternata*	$0.00^{c} \pm 0.0$	$0.00^{f} \pm 0.0$	$0.00^{f} \pm 0.0$	$2.3^{gh} \pm 0.17$	$3.2^{ef} \pm 0.46$
2	*Aspergillus flavus*	$13.3^{a} \pm 1.2$	$6.5^{d} \pm 0.52$	$11.6^{b} \pm 0.81$	$8.1^{e} \pm 1.1$	$7.1^{d} \pm 0.81$
3	*Aspergillus fumigatus*	$13.3^{a} \pm 1.6$	$9.8^{c} \pm 0.58$	$6.9^{c} \pm 0.75$	$9.3^{de} \pm 1.2$	$7.9^{d} \pm 0.52$
4	*Aspergillus niger*	$13.3^{a} \pm 1.7$	$9.8^{c} \pm 1.5$	$11.6^{b} \pm 1.0$	$18.6^{a} \pm 1.8$	$20.6^{a} \pm 1.8$
5	*Aspergillus ochraceus*	$6.6^{b} \pm 0.69$	$6.5^{d} \pm 0.86$	$4.7^{d} \pm 0.17$	$8.1^{e} \pm 1.1$	$10.3^{c} \pm 1.79$
6	*Aspergillus terreus*	$6.6^{b} \pm 0.80$	$9.8^{c} \pm 0.58$	$6.9^{c} \pm 1.9$	$5.8^{f} \pm 0.34$	$3.9^{e} \pm 0.87$
7	*Botrytis cinerea*	$0.00^{c} \pm 0.0$	$0.00^{f} \pm 0.0$	$4.6^{d} \pm 0.46$	$2.3^{gh} \pm 0.40$	$3.2^{ef} \pm 0.23$
8	*Cephalosporium gramineum*	$0.00^{c} \pm 0.0$	$0.00^{f} \pm 0.0$	$2.3^{e} \pm 0.06$	$3.5^{g} \pm 0.29$	$2.4^{ef} \pm 0.35$
9	*Chaetomium globosum*	$0.00^{c} \pm 0.0$	$3.2^{e} \pm 0.46$	$2.3^{e} \pm 0.40$	$3.5^{g} \pm 0.28$	$3.2^{ef} \pm 0.46$
10	*Cladosporium herbarum*	$6.6^{b} \pm 0.0$	$3.2^{e} \pm 0.46$	$6.9^{c} \pm 0.64$	$5.8^{f} \pm 1.7$	$3.9^{e} \pm 0.06$
11	*Curvularia lunata*	$13.1^{a} \pm 1.9$	$18.8^{b} \pm 1.9$	$14.4^{a} \pm 2.4$	$14.1^{b} \pm 2.4$	$14.5^{b} \pm 1.67$
12	*Fusarium solani*	$0.00^{c} \pm 0.0$	$9.8^{c} \pm 0.98$	$0.00^{f} \pm 0.0$	$5.8^{f} \pm 0.69$	$7.1^{d} \pm 0.52$
13	*Helminthosporium solani*	$0.00^{c} \pm 0.0$	$3.2^{e} \pm 0.81$	$2.3^{e} \pm 0.40$	$1.2^{hi} \pm 0.12$	$1.6^{fg} \pm 0.35$
14	*Penicillium chrysogenum*	$13.6^{a} \pm 1.6$	$0.00^{f} \pm 0.0$	$13.9^{a} \pm 2.4$	$11.6^{c} \pm 1.3$	$11.1^{c} \pm 2.1$
15	*Penicillium citrinum*	$13.6^{a} \pm 1.8$	$16.2^{b} \pm 2.3$	$11.6^{b} \pm 2.1$	$0.00^{i} \pm 0.0$	$0.00^{g} \pm 0.0$
16	*Verticillium* sp.	$0.00^{c} \pm 0.0$	$3.2^{e} \pm 0.46$	$0.00^{f} \pm 0.0$	$0.00^{i} \pm 0.0$	$0.00^{g} \pm 0.0$

Table 3: Fungal incidence (percentage) in rhizosphere of young cowpea grown from non sterilized seeds in non-sterilized soil

Sl. no.	Type of fungi	Age of roots (Days after sowing)				
		5	10	15	20	25
1	*Alternaria alternata*	$4.0^{e} \pm 1.2$	$5.8^{de} \pm 1.0$	$6.1^{bc} \pm 0.64$	$5.5^{def} \pm 0.87$	$4.9^{efg} \pm 0.52$
2	*Alternaria triticina*	$2.0^{f} \pm 0.58$	$2.9^{gh} \pm 0.06$	$3.7^{de} \pm 0.29$	$4.6^{ef} \pm 0.69$	$4.2^{fg} \pm 0.92$
3	*Aspergillus candidus*	$4.0^{e} \pm 1.2$	$3.9^{fg} \pm 0.75$	$4.3^{cd} \pm 0.58$	$3.7^{f} \pm 0.64$	$3.5^{g} \pm 0.29$
4	*Aspergillus flavus*	$8.0^{c} \pm 0.23$	$6.8^{cd} \pm 1.2$	$6.7^{b} \pm 0.98$	$7.9^{b} \pm 0.64$	$9.1^{bc} \pm 1.6$
5	*Aspergillus fumigatus*	$6.0^{d} \pm 1.2$	$5.8^{de} \pm 0.35$	$6.1^{bc} \pm 0.75$	$7.4^{bc} \pm 0.81$	$10.1^{ab} \pm 1.6$
6	*Aspergillus niger*	$12.0^{a} \pm 1.7$	$10.9^{a} \pm 1.6$	$10.8^{a} \pm 2.3$	$11.3^{a} \pm 1.7$	$11.4^{a} \pm 2.5$
7	*Aspergillus ochraceus*	$6.0^{d} \pm 1.2$	$5.8^{de} \pm 0.58$	$4.3^{cd} \pm 0.75$	$4.6^{ef} \pm 0.35$	$4.2^{fg} \pm 0.46$
8	*Aspergillus terreus*	$4.0^{e} \pm 1.2$	$3.9^{fg} \pm 0.81$	$4.9^{cd} \pm 0.51$	$3.7^{f} \pm 0.75$	$3.8^{g} \pm 0.23$
9	*Botrytis cinerea*	$2.0^{f} \pm 0.58$	$2.9^{gh} \pm 0.23$	$4.9^{bcd} \pm 0.29$	$3.7^{f} \pm 0.40$	$3.8^{g} \pm 0.46$
10	*Cephalosporium gramineum*	$6.0^{d} \pm 1.2$	$4.9^{ef} \pm 0.51$	$6.1^{b} \pm 0.98$	$6.9^{bcd} \pm 0.64$	$5.9^{ef} \pm 0.52$
11	*Chaetomium globosum*	$2.0^{f} \pm 0.58$	$4.9^{ef} \pm 0.40$	$3.7^{de} \pm 0.40$	$4.2^{ef} \pm 0.23$	$4.2^{fg} \pm 0.46$
12	*Cladosporium herbarum*	$4.0^{e} \pm 0.58$	$7.8^{bc} \pm 0.64$	$6.7^{bc} \pm 1.4$	$5.1^{ef} \pm 1.2$	$6.3^{de} \pm 0.98$
13	*Curvularia lunata*	$6.0^{d} \pm 1.2$	$8.7^{b} \pm 0.40$	$6.1^{bc} \pm 0.98$	$8.3^{b} \pm 0.64$	$7.7^{cd} \pm 1.1$
14	*Fusarium solani*	$4.0^{e} \pm 0.87$	$2.9^{gh} \pm 0.29$	$4.3^{cd} \pm 0.40$	$4.6^{ef} \pm 0.81$	$3.8^{g} \pm 0.35$
15	*Helminthosporium solani*	$4.0^{e} \pm 0.58$	$3.9^{fg} \pm 0.58$	$4.9^{bcd} \pm 0.75$	$3.7^{f} \pm 0.17$	$3.5^{g} \pm 0.29$
16	*Monilia* sp.	$4.0^{e} \pm 0.87$	$2.9^{gh} \pm 0.58$	$2.4^{ef} \pm 0.35$	$1.9^{g} \pm 0.17$	$1.4^{h} \pm 0.23$
17	*Penicillium chrysogenum*	$8.0^{c} \pm 0.69$	$6.8^{cd} \pm 1.2$	$6.7^{b} \pm 0.87$	$6.0^{cde} \pm 0.87$	$6.3^{de} \pm 0.98$
18	*Penicillium citrinum*	$10.0^{b} \pm 1.7$	$5.8^{de} \pm 0.69$	$4.9^{bcd} \pm 0.34$	$4.6^{ef} \pm 0.46$	$4.2^{fg} \pm 0.92$
19	*Rhizopus* sp	$2.0^{f} \pm 0.58$	$0.8^{i} \pm 0.28$	$0.6^{g} \pm 0.12$	$0.9^{g} \pm 0.06$	$0.7^{h} \pm 0.17$
20	*Verticillium* sp.	$2.0^{f} \pm 0.58$	$1.9^{hi} \pm 0.17$	$1.8^{fg} \pm 0.12$	$1.4^{g} \pm 0.23$	$1.0^{h} \pm 0.29$

A: surface sterilized seed in natural soil

B: Non sterilized seed in sterilized soil

C: Non-sterilized seed in natural soil (Control)

Figure 2: Pot culture of cowpea to determine the rhizosphere mycoflora originating from seed or soil

DISCUSSION

Present study has clearly indicated that, fungi present in the stored seeds (both external as well as internal) travel from seeds to the developing roots. This was confirmed when non sterilized cowpea seeds were sown in sterilized soil in pot experiments. The ability of various fungi to travel from inoculated seeds to the developing roots was studied by Chao *et. al* **[9]**. During the study they found that when *Trichoderma harzianum* inoculated pea seeds were grown in sterilized soil, the inoculated fungal species was detected in the rhizosphere in the upper half of the roots. Rangaswami **[10]** reported that when the seeds are sown in the soil, interaction between the seed borne mycoflora and soil microorganisms took place under the influence of the chemical exudates by the germinating seed. Such interaction varies with the type of seed and microflora present, the soil and the environmental conditions where they act together. This would, however, vary with the plant species, the nature and population of

microorganisms on the seed, the soil qualities, specifically the nature of its microflora and the environmental conditions of germination and growth of the seed in the soil. Oliver *et. al*. **[11]** studied movement of microorganisms present in the seeds to the rhizosphere and the mechanism of rhizosphere colonization which may involve active microbial movement or passive transport by percolating water or plant root. Further they reported that, nematodes can have an important role in rhizosphere colonization by microorganism in soil.

CONCLUSION

The present study revealed that there is a movement of some storage fungi from seeds to their developing roots during the germination of seeds. Hence, there is need to investigate the best way of storage of cowpea which could reduce fungal infection as there is a movement of movement of such pathogenic fungi to the developing roots.

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