

**International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)**

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ISOLATION OF MYCOFLORA PRESENT IN THE RHIZOSPHERE OF COWPEA GROWN FROM STORED SEEDS

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Received 4th Oct. 2020; Revised 5th Nov. 2020; Accepted 7th Dec. 2020; Available online 1st Sept. 2021

<https://doi.org/10.31032/IJBPAS/2021/10.9.5602>

ABSTRACT

A field study was undertaken to isolate the fungi present in the rhizosphere of cowpea plant grown from stored cowpea seeds. Periodically stored cowpea (3, 6, 9 and 12 months) seeds were sown in the field and rhizospheric soil was collected at five different growth stages (15, 30, 45, 60, 75 days after sowing) of the plants for the isolation of fungal flora. Result revealed that, fungal population was less at 15 days after sowing (DAS) which was gradually increased upto 45 DAS and decreased gradually from 60 DAS. The rhizosphere soil was associated with *Aspergillus* sp., *Alternaria* sp. *Penicillium* sp. *Chaetomium globosum*, *Curvularia lunata*, *Fusarium solani*, *Helminthosporium solani*, *Monilia* sp., *Rhizopus* sp. and *Verticillium* sp. in all the growth stages of plant. Therefore, present study confirmed that there is gradual increase in the fungal flora from seedling stage to maximum vegetative stage of the plant and decrease towards senescence stage.

Keywords: Cowpea, Mycoflora, Rhizosphere, Root exudation

INTRODUCTION

The rhizosphere is the zone of soil surrounding a plant root where the biology and chemistry of the soil are influenced by the root. This zone is about 1 mm wide, but has no distinct edge. Microbiological activity in the rhizosphere is much greater

than in soil away from plant roots [1]. Some microorganisms also provide nutrients for the plants. All these activity makes the rhizosphere the most dynamic environment in the soil and it is an area of intense biological and chemical activity influenced

by compounds exuded by the root, and the compounds are used by microorganisms as food. As plant roots grow through soil they release water soluble compounds such as amino acids, sugars and organic acids that supply food for the microorganisms [2]. This is the reason, the rhizosphere has been called the last frontier in agricultural science and possibly rhizosphere interaction is mediated by root exudates. Root exudation can increase or decrease soil nutrients availability by altering soil chemistry and soil biological processes [2]. Roots release a wide range of compounds. These compounds include sugars, polysaccharides, amino acids, aliphatic acids, aromatic acids, fatty acids, sterols, phenolics, enzymes, vitamins, plant growth regulators and other secondary metabolites [3, 4]. The influence of root exudates on rhizosphere microorganisms varies with plant age as well as plant type [5]. Rhizodeposition of nutrients by plant roots supports increased microbial growth in comparison with that of the bulk soil communities. During the developmental stage of plant i.e. the change from young seedling, flowering to senescence may influence the microbial community structure [6]. The composition of the microbial community in the rhizosphere also changes with time in response to the changing root exudation patterns that vary during the life cycle and

seasonal response of plants [7]. In cognizance with the above, a field study was undertaken to isolate the fungal flora present in the rhizosphere of cowpea grown from the periodically stored cowpea seeds.

MATERIALS AND METHODS

For isolation the fungi present in rhizosphere, cowpea seeds were sown after the end of each storage period i.e. three, six, nine and twelve months. The seeds were sown in plots having 24 sq. m size with three replicates. Further fungi associated with rhizosphere were isolated from the sample plants at five different growth stages of the plant life i.e. 15, 30, 45, 60 and 75 days after sowing. All the plants were removed with its root system intact with the loosely adhering soil from each sampling site and were transferred to the laboratory in sterile containers. The fungal species present in rhizosphere were isolated by serial dilution method. In this method, 1g of soil was added to 100 ml of distilled water to make microbial suspension. This suspension was used to make serial dilution of 10^{-1} - 10^{-5} . 1ml of 10^{-5} dilution was added to sterile Petri dishes containing sterilized Potato Dextrose Agar (PDA), incubation for seven days at $25\pm 2^{\circ}\text{C}$. After incubation, Petri dishes were observed and fungal colonies developed in the cultures were counted and identified. The number of colonies produced by fungi was multiplied

by the reciprocal of dilution factor to obtain the total fungal population per gram of soil.

Identification of isolated fungi:

Isolated fungi were identified both macroscopically and microscopically. The fungal colonies were counted and identified on the basis of colony characters, morphology and reproductive characteristics. The percentage incidence of occurrence of each fungus was also calculated.

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.

RESULTS

The result presented in **Table 1 to 4** represents fungi associated with the rhizosphere of cowpea seeds stored for different periods (3, 6, 9 and 12 months). Altogether, sixteen fungal species were isolated from the rhizosphere of cowpea. These were *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus ochraceus*, *Aspergillus terreus*, *Aspergillus fumigatus*, *Alternaria alternata*, *Alternaria triticina*, *Curvularia lunata*, *Chaetomium globosum*, *Fusarium solani*, *Helminthosporium solani*, *Monilia* sp., *Penicillium chrysogenum*, *Penicillium citrinum*, *Rhizopus* sp. and *Verticillium* sp. Out of these, *Aspergillus flavus*, *Aspergillus*

ochraceus, *Aspergillus terreus*, *Aspergillus fumigatus*, *Chaetomium globosum*, *Fusarium solani*, *Penicillium chrysogenum* and *Penicillium citrinum* were isolated under stored as well as field condition.

Rhizosphere study of plants grown from three months stored cowpea seeds at the stage of 15 days after sowing (DAS) revealed that, incidence of, *Aspergillus niger* (16.8%) and *Aspergillus flavus* (15.7%) was highest followed by *Alternaria alternata* (9.6%), *Curvularia lunata* (8.9%), *Fusarium solani* (8.4 %), *Aspergillus ochraceus* (7.1%), *Helminthosporium solani* (5.9%), *Penicillium chrysogenum* (4.9%), *Aspergillus terreus* (4.7%), *Alternaria triticina* (3.5 %), *Aspergillus fumigatus* (3.4 %), *Monilia* sp. (3.3 %), *Penicillium citrinum* (3.1 %), *Chaetomium globosum* (2.6 %), *Rhizopus* sp. (1.3 %) and *Verticillium* sp. (0.8 %). Similarly, at 30 DAS, incidence of *Aspergillus niger* was highest (15.7 %) and remaining fungal species found were in the range of 0.7 -12.2 %. It was observed that 45 DAS, the incidence of *Aspergillus niger* (11.9 %) was highest followed by *Aspergillus flavus* (11.6 %) and the remaining fungal species were in the range of 2.7- 8.2 %. On the other hand, at 60 DAS, the incidence of *Helminthosporium solani* was highest (10.4 %) followed by *Aspergillus flavus* (9.4 %) and the remaining fungal species were in

the range of 2.2 -9.0%. The incidence of *Helminthosporium solani* at 75 DAS was highest (14.8 %) followed by *Alternaria alternata* (10.1 %), *Penicillium chrysogenum* (10.1 %) and the remaining fungal species found were in the range of 1.1- 9.1% (Table 1, Figure 1A).

Similarly, rhizosphere study of plant grown from six months stored cowpea seeds 15 DAS revealed that, incidence of *Aspergillus flavus* (13.9 %) followed by *Aspergillus niger* (12.1 %), *Curvularia lunata* (9.8%), *Alternaria alternata* (9.1 %), *Fusarium solani* (8.8 %), *Helminthosporium solani* (7.6 %), *Aspergillus ochraceus* (7.4 %), *Penicillium chrysogenum* (6.2 %), *Aspergillus terreus* (4.8 %), *Aspergillus fumigatus* (4.3 %), *Alternaria triticina* (4.2 %), *Penicillium citrinum* (4.0 %), *Rhizopus* sp. (3.6 %), *Chaetomium globosum* (3.3 %) and *Monilia* sp. (1.0 %). Similarly, at 30 DAS incidence of *Aspergillus flavus* was highest (16.1 %) followed by *Aspergillus niger* (12.3 %) and remaining fungal species were found in the range of 0.9- 9.8%. At 45 DAS, the incidence of *Penicillium chrysogenum* was highest (11.0 %) followed by *Fusarium solani* (9.9 %) and the remaining fungal species were in the range of 2.3- 9.8%. On the other hand, at 60 DAS, the incidence of *Alternaria alternata* (12.0 %) and *Penicillium chrysogenum* (12.0 %) was highest followed by *Helminthosporium*

solani (10.2 %) and the remaining fungal species were in the range of 1.6-9.1%. The incidence of *Helminthosporium solani* at 75 DAS was highest (15.6 %) followed by *Penicillium chrysogenum* (13.2 %) and the remaining fungal species were found in the range of 1.1- 10.8% (Table 2, Figure 1B).

Rhizosphere study of plants grown from nine months stored cowpea seeds at 15 DAS revealed that, incidence of *Alternaria alternata* (15.3 %) was highest followed by *Aspergillus niger* (14.0 %), *Penicillium chrysogenum* (12.9 %), *Fusarium solani* (10.5 %), *Helminthosporium solani* (9.7 %), *Curvularia lunata* (9.2 %), *Aspergillus ochraceus* (5.6 %), *Aspergillus terreus* (4.8 %), *Aspergillus flavus* (4.0 %), *Aspergillus fumigatus* (3.4 %), *Chaetomium globosum* (3.3 %), *Alternaria triticina* (2.1 %), *Monilia* sp. (2.1 %), *Rhizopus* sp. (1.7 %) and *Verticillium* sp. (1.5 %). Similarly, at 30 DAS, incidence of *Alternaria alternata* (14.7 %) was highest followed by *Helminthosporium solani* (12.8 %) and remaining fungal species were found in the range of 0.9- 10.5%. At 45 DAS, the incidence of *Penicillium chrysogenum* was highest (13.8 %) followed by *Alternaria alternata* (12.5 %) and the remaining fungal species were in the range of 2.4- 10.9%. Similarly, at 60 DAS, the incidence of *Penicillium chrysogenum* was highest (14.9 %) followed by *Alternaria alternata* (14.1

%) and the remaining fungal species were in the range of 0.8- 12.1%. The incidence of *Helminthosporium solani* at 75 DAS was highest (15.4 %) followed by *Penicillium chrysogenum* (15.1 %), *Curvularia lunata* (12.7 %) and the remaining fungal species were in the range of 1.2- 9.9% (**Table 3, Figure 1C**).

Study of fungal species isolated from rhizosphere grown from the cowpea seeds stored for twelve months, revealed that incidence of *Alternaria alternata* (17.5 %) was highest followed by *Helminthosporium solani* (13.2 %), *Aspergillus niger* (11.9 %), *Penicillium chrysogenum* (11.0 %), *Fusarium solani* (10.6 %), *Curvularia lunata* (8.7 %), *Aspergillus flavus* (6.7 %), *Aspergillus ochraceus* (5.6 %), *Aspergillus terreus* (4.2 %), *Aspergillus fumigatus* (4.1 %), *Chaetomium globosum* (2.3 %), *Rhizopus* sp. (2.2 %), *Verticillium* sp. (1.1 %) and *Alternaria triticina* (1.0 %) at 15 DAS. Similarly, at 30 DAS incidence of *Helminthosporium solani* was highest (14.1 %) followed by *Aspergillus niger* (12.3 %) and remaining fungal species were found in the range of 0.9- 11.6%. At 45 DAS, the incidence of *Penicillium chrysogenum* was highest (12.9 %) followed by *Helminthosporium solani* (10.9 ± 1.1%) and the remaining fungal species were in the range of 1.8-10.4%. Similarly, at 60 DAS,

the incidence of *Penicillium chrysogenum* was highest (16.2%) followed by *Helminthosporium solani* (13.8 %) and the remaining fungal species were in the range of 1.0-11.2%. The incidence of *Penicillium chrysogenum* at 75 DAS was found highest (16.9 %) followed by *Alternaria alternata* (15.5 %) and the remaining fungal species found were in the range of 1.5-15.2% (**Table 4, Figure 1D**).

It was observed that number of fungi in rhizosphere differed at successive growth stages in the life of the cowpea plant (**Figure 2**). Prevalence of fungi was less at 15 DAS which gradually increased upto 45 DAS. Plants attained maximum vegetative growth during this period. It was followed by gradual decline at senescence stage (75 DAS).

Table 1: Prevalence of fungi (in percentage) associated with rhizosphere of cowpea at different growth stages (three months stored seeds)

Sl. No.	Fungi isolated	Growth periods (days after sowing)				
		15	30	45	60	75
1	<i>Aspergillus flavus</i>	15.7 ^a ± 2.7	12.2 ^{ab} ± 2.4	11.6 ^{ab} ± 2.3	9.4 ^{ab} ± 1.8	3.1 ^{def} ± 2.0
2	<i>Aspergillus fumigatus</i>	3.4 ^{bc} ± 1.5	4.6 ^{cde} ± 1.3	4.3 ^{cde} ± 0.6	2.2 ^f ± 0.9	2.3 ^{ef} ± 1.4
3	<i>Aspergillus niger</i>	16.8 ^a ± 5.4	15.7 ^a ± 4.1	11.9 ^a ± 2.6	7.5 ^{abcde} ± 1.1	6.8 ^{bcdef} ± 0.9
4	<i>Aspergillus ochraceus</i>	7.1 ^{bc} ± 0.9	10.2 ^{bc} ± 1.8	7.2 ^{bcde} ± 1.5	9.0 ^{abc} ± 1.6	6.0 ^{bcdef} ± 2.8
5	<i>Aspergillus terreus</i>	4.7 ^{bc} ± 1.4	7.6 ^{bcd} ± 0.6	6.4 ^{cde} ± 0.5	5.2 ^{bcdef} ± 0.7	1.1 ^f ± 1.1
6	<i>Alternaria alternata</i>	9.6 ^b ± 1.3	6.9 ^{bcd} ± 1.6	7.0 ^{cde} ± 1.2	7.4 ^{abcde} ± 1.0	10.1 ^{ab} ± 0.9
7	<i>Alternaria triticina</i>	3.5 ^{bc} ± 1.6	3.6 ^{de} ± 1.6	4.7 ^{cde} ± 1.4	4.1 ^{def} ± 1.5	2.3 ^{ef} ± 1.4
8	<i>Chaetomium globosum</i>	2.6 ^{bc} ± 1.1	3.6 ^{de} ± 1.6	3.9 ^{cde} ± 1.1	4.6 ^{cdef} ± 1.4	3.2 ^{def} ± 2.1
9	<i>Curvularia lunata</i>	8.9 ^b ± 1.6	8.0 ^{bcd} ± 1.2	8.2 ^{abc} ± 1.1	8.0 ^{abcd} ± 1.8	8.9 ^{bcd} ± 0.9
10	<i>Fusarium solani</i>	8.4 ^b ± 2.3	6.2 ^{cde} ± 1.7	7.5 ^{abcd} ± 1.3	7.2 ^{abcde} ± 1.6	9.1 ^{bc} ± 1.3
11	<i>Helminthosporium solani</i>	5.9 ^{bc} ± 1.7	6.6 ^{bcd} ± 1.3	8.1 ^{abc} ± 0.9	10.4 ^a ± 1.5	14.8 ^a ± 2.0
12	<i>Monilia</i> sp.	3.3 ^{bc} ± 2.4	3.5 ^{de} ± 1.8	4.8 ^{cde} ± 1.5	7.1 ^{abcde} ± 1.9	8.6 ^{bcd} ± 3.6
13	<i>Penicillium chrysogenum</i>	4.9 ^b ± 1.4	4.9 ^{cde} ± 1.4	5.9 ^{cde} ± 1.9	6.7 ^{abcdef} ± 0.5	10.1 ^{ab} ± 0.7
14	<i>Penicillium citrinum</i>	3.1 ^{bc} ± 1.3	3.2 ^{de} ± 1.4	3.3 ^{de} ± 1.0	4.0 ^{def} ± 1.3	7.8 ^{bcd} ± 1.0
15	<i>Rhizopus</i> sp.	1.3 ^c ± 1.3	2.5 ^{de} ± 1.6	2.7 ^{de} ± 1.2	4.0 ^{def} ± 1.7	2.1 ^{ef} ± 2.1
16	<i>Verticillium</i> sp.	0.8 ^c ± 0.8	0.7 ^c ± 0.7	2.7 ^c ± 0.7	3.2 ^{ef} ± 0.8	3.7 ^{cdef} ± 1.6

Table 2: Prevalence of fungi (in percentage) associated with rhizosphere of cowpea at different growth stages (six months stored seeds)

Sl. No.	Fungi isolated	Growth periods (days after sowing)				
		15	30	45	60	75
1	<i>Aspergillus flavus</i>	13.9 ^a ± 2.6	16.1 ^a ± 3.1	9.8 ^{ab} ± 3.3	6.5 ^{bcd} ± 1.9	6.2 ^{bcd} ± 1.8
2	<i>Aspergillus fumigatus</i>	4.3 ^{bcd} ± 2.0	2.7 ^{def} ± 1.8	5.2 ^{bcd} ± 0.8	2.3 ^c ± 0.9	2.5 ^d ± 1.5
3	<i>Aspergillus niger</i>	12.1 ^{ab} ± 4.6	12.3 ^{ab} ± 4.4	8.1 ^{abcd} ± 2.7	3.9 ^{cde} ± 1.1	6.2 ^{bcd} ± 1.8
4	<i>Aspergillus ochraceus</i>	7.4 ^{abcde} ± 2.2	8.9 ^{bcd} ± 2.5	4.7 ^{bcd} ± 1.9	8.7 ^{abcd} ± 2.3	5.2 ^{bcd} ± 2.6
5	<i>Aspergillus terreus</i>	4.8 ^{bcd} ± 2.1	3.9 ^{cdef} ± 1.7	3.7 ^{cde} ± 1.8	4.0 ^{cde} ± 1.1	1.1 ^d ± 1.1
6	<i>Alternaria alternata</i>	9.1 ^{abc} ± 0.7	9.2 ^{bcd} ± 1.6	8.4 ^{abcd} ± 1.5	12.0 ^a ± 2.6	10.5 ^{abc} ± 1.7
7	<i>Alternaria triticina</i>	4.2 ^{cde} ± 1.9	0.9 ^{ef} ± 0.9	4.1 ^{cde} ± 1.5	2.3 ^c ± 0.9	2.2 ^d ± 1.4
8	<i>Chaetomium globosum</i>	3.3 ^{cde} ± 1.4	4.4 ^{cdef} ± 1.9	3.3 ^{de} ± 1.5	3.5 ^{de} ± 1.6	2.1 ^d ± 2.1
9	<i>Curvularia lunata</i>	9.8 ^{abc} ± 2.9	9.8 ^{bc} ± 1.5	9.2 ^{abc} ± 1.1	9.1 ^{abc} ± 1.4	10.0 ^{abc} ± 1.9
10	<i>Fusarium solani</i>	8.8 ^{abcd} ± 2.9	7.4 ^{abcde} ± 2.1	9.9 ^{ab} ± 1.7	9.1 ^{abc} ± 2.2	10.8 ^{ab} ± 1.0
11	<i>Helminthosporium solani</i>	7.6 ^{abcde} ± 2.3	8.0 ^{bcd} ± 1.5	9.7 ^{ab} ± 1.1	10.2 ^{ab} ± 1.1	15.6 ^a ± 2.0
12	<i>Monilia</i> sp.	1.0 ^{de} ± 1.0	3.0 ^{cdef} ± 1.3	4.9 ^{bcd} ± 1.5	6.3 ^{bcd} ± 2.8	4.8 ^{cd} ± 2.2
13	<i>Penicillium chrysogenum</i>	6.2 ^{abcde} ± 1.8	6.7 ^{bcdef} ± 2.2	11.0 ^a ± 1.0	12.0 ^a ± 1.2	13.2 ^a ± 2.2
14	<i>Penicillium citrinum</i>	4.0 ^{cde} ± 1.7	3.9 ^{cdef} ± 1.7	3.2 ^d ± 0.8	4.2 ^{cde} ± 1.3	5.0 ^{bcd} ± 1.3
15	<i>Rhizopus</i> sp.	3.6 ^{cde} ± 3.6	3.0 ^f ± 1.9	2.4 ^e ± 1.6	4.3 ^{cde} ± 1.8	2.1 ^d ± 2.1
16	<i>Verticillium</i> sp.	0.0 ^e ± 0.0	0.0 ^f ± 0.0	2.3 ^c ± 0.9	1.6 ^e ± 0.9	2.6 ^d ± 1.6

Table 3: Prevalence of fungi (in percentage) associated with rhizosphere of cowpea at different growth stages (nine months stored seeds)

Sl. No.	Fungi isolated	Growth periods (days after sowing)				
		15	30	45	60	75
1	<i>Aspergillus flavus</i>	4.0 ^{cdef} ± 1.7	9.4 ^{abc} ± 3.2	4.5 ^{cdef} ± 2.2	6.2 ^{bc} ± 1.7	6.3 ^{cdef} ± 1.8
2	<i>Aspergillus fumigatus</i>	3.4 ^{cdef} ± 2.3	2.7 ^{cd} ± 1.8	4.4 ^{ab} ± 0.3	1.7 ^{cd} ± 1.1	2.6 ^{fg} ± 1.6
3	<i>Aspergillus niger</i>	14.0 ^a ± 4.7	8.1 ^{abc} ± 1.2	7.8 ^{bcd} ± 1.5	5.2 ^{bcd} ± 0.7	9.2 ^{bcd} ± 2.8
4	<i>Aspergillus ochraceus</i>	5.6 ^{bcd} ± 2.6	8.6 ^{abc} ± 2.2	4.3 ^{def} ± 1.3	6.0 ^{bc} ± 1.9	4.9 ^{cdefg} ± 2.2
5	<i>Aspergillus terreus</i>	4.8 ^{cdef} ± 2.1	1.2 ^d ± 1.2	2.8 ^{def} ± 1.2	2.8 ^{cd} ± 1.2	1.2 ^{fg} ± 1.2
6	<i>Alternaria alternata</i>	15.3 ^a ± 2.9	14.7 ^a ± 2.8	12.5 ^{def} ± 2.5	14.1 ^a ± 3.1	9.9 ^{abc} ± 1.2
7	<i>Alternaria triticina</i>	2.1 ^{def} ± 1.3	0.9 ^d ± 0.9	3.5 ^{abc} ± 1.7	1.7 ^{cd} ± 1.1	1.2 ^{fg} ± 1.2
8	<i>Chaetomium globosum</i>	3.3 ^{cdef} ± 1.3	4.5 ^{bcd} ± 1.9	2.5 ^{abc} ± 1.0	0.0 ^d ± 0.0	0.0 ^g ± 0.0
9	<i>Curvularia lunata</i>	9.2 ^{abcde} ± 2.7	10.5 ^{ab} ± 2.2	10.3 ^{ef} ± 1.5	10.1 ^{ab} ± 1.7	12.7 ^{ab} ± 1.9
10	<i>Fusarium solani</i>	10.5 ^{abc} ± 3.3	8.1 ^{abc} ± 2.3	10.9 ^{abc} ± 1.9	11.1 ^a ± 2.3	8.8 ^{bcd} ± 1.3
11	<i>Helminthosporium solani</i>	9.7 ^{abcd} ± 2.6	12.8 ^a ± 2.7	9.6 ^{cde} ± 0.9	12.1 ^a ± 1.2	15.4 ^a ± 1.8
12	<i>Monilia sp.</i>	2.1 ^{def} ± 1.3	4.9 ^{bcd} ± 2.6	6.7 ^a ± 2.3	5.9 ^{bc} ± 2.8	5.0 ^{cdefg} ± 2.2
13	<i>Penicillium chrysogenum</i>	12.9 ^{ab} ± 2.1	8.8 ^{abc} ± 2.3	13.8 ^{def} ± 2.2	14.9 ^a ± 0.9	15.1 ^a ± 3.3
14	<i>Penicillium citrinum</i>	0.0 ^f ± 0.0	3.9 ^{bcd} ± 1.7	4.0 ^f ± 1.2	2.8 ^{cd} ± 1.2	3.6 ^{defg} ± 2.4
15	<i>Rhizopus sp.</i>	1.7 ^{ef} ± 1.7	0.9 ^d ± 0.9	0.0 ^{ef} ± 0.0	4.6 ^{cd} ± 1.9	1.2 ^{fg} ± 1.2
16	<i>Verticillium sp.</i>	1.5 ^{ef} ± 1.5	0.0 ^d ± 0.0	2.4 ^{ef} ± 0.9	0.8 ^{cd} ± 0.8	2.8 ^{efg} ± 1.7

Table 4: Prevalence of fungi (in percentage) associated with rhizosphere of cowpea at different growth stages (twelve months stored seeds)

Sl. No.	Fungi isolated	Growth periods (days after sowing)				
		15	30	45	60	75
1	<i>Aspergillus flavus</i>	6.7 ^{cdef} ± 2.0	7.0 ^{cde} ± 1.7	4.6 ^{fg} ± 0.9	3.0 ^{de} ± 1.2	1.5 ^{de} ± 1.5
2	<i>Aspergillus fumigatus</i>	4.1 ^{efg} ± 2.5	4.3 ^{def} ± 1.3	3.3 ^{gh} ± 0.2	1.9 ^e ± 1.1	3.4 ^{de} ± 2.1
3	<i>Aspergillus niger</i>	11.9 ^{abc} ± 3.2	12.3 ^{ab} ± 1.7	9.8 ^{bcd} ± 0.6	8.1 ^{cd} ± 0.7	8.5 ^{bcd} ± 2.6
4	<i>Aspergillus ochraceus</i>	5.6 ^{defg} ± 1.9	7.2 ^{cde} ± 1.8	6.4 ^{ef} ± 0.9	3.9 ^{de} ± 1.8	6.8 ^{cde} ± 3.1
5	<i>Aspergillus terreus</i>	4.2 ^{efg} ± 1.8	0.9 ^f ± 0.9	2.6 ^{gh} ± 0.7	3.9 ^{de} ± 1.0	0.0 ^e ± 0.0
6	<i>Alternaria alternata</i>	17.5 ^a ± 2.8	11.6 ^{abc} ± 2.1	10.4 ^{bc} ± 0.8	11.2 ^{bc} ± 1.5	15.5 ^{ab} ± 3.5
7	<i>Alternaria triticina</i>	1.0 ^{fg} ± 0.9	1.6 ^f ± 0.9	3.9 ^{gh} ± 0.7	2.8 ^e ± 1.1	1.7 ^{de} ± 1.7
8	<i>Chaetomium globosum</i>	2.3 ^{fg} ± 1.4	3.3 ^{def} ± 1.6	1.8 ^h ± 0.8	1.0 ^e ± 1.0	1.5 ^{de} ± 1.5
9	<i>Curvularia lunata</i>	8.7 ^{bcd} ± 1.3	8.4 ^{bcd} ± 1.9	7.7 ^{de} ± 0.5	10.6 ^{bc} ± 0.9	8.5 ^{bcd} ± 3.8
10	<i>Fusarium solani</i>	10.6 ^{bcd} ± 3.1	6.9 ^{cde} ± 2.0	8.2 ^{cde} ± 1.1	10.9 ^{bc} ± 2.5	12.0 ^{abc} ± 2.2
11	<i>Helminthosporium solani</i>	13.2 ^{ab} ± 1.5	14.1 ^a ± 2.4	10.9 ^{ab} ± 1.1	13.8 ^{ab} ± 1.8	15.2 ^{ab} ± 1.6
12	<i>Monilia sp.</i>	0.0 ^g ± 0.0	2.6 ^{ef} ± 1.1	4.4 ^{gh} ± 1.3	4.2 ^{de} ± 2.2	1.8 ^{de} ± 1.8
13	<i>Penicillium chrysogenum</i>	11.0 ^{bcd} ± 1.9	11.3 ^{abc} ± 0.9	12.9 ^a ± 0.7	16.2 ^a ± 2.9	16.9 ^a ± 2.6
14	<i>Penicillium citrinum</i>	0.0 ^g ± 0.0	3.4 ^{def} ± 1.5	3.8 ^{gh} ± 0.5	3.9 ^{de} ± 1.0	1.8 ^{de} ± 1.8
15	<i>Rhizopus sp.</i>	2.2 ^{fg} ± 1.4	1.7 ^f ± 1.1	5.1 ^{fg} ± 0.6	2.8 ^e ± 1.9	3.2 ^{de} ± 1.9
16	<i>Verticillium sp.</i>	1.1 ^{fg} ± 1.1	3.5 ^{def} ± 0.9	3.9 ^{gh} ± 0.7	1.8 ^e ± 1.1	1.7 ^{de} ± 1.7



Figure 1A: Cowpea plants grown from three months stored seeds (at 30 DAS)



Figure 1B: Cowpea plants grown from six months stored seeds (at 30 DAS)



Figure 1C: Cowpea plants grown from nine months stored seeds (at 30 DAS)



Figure 1D: Cowpea plants grown from twelve months stored seeds (at 30 DAS)

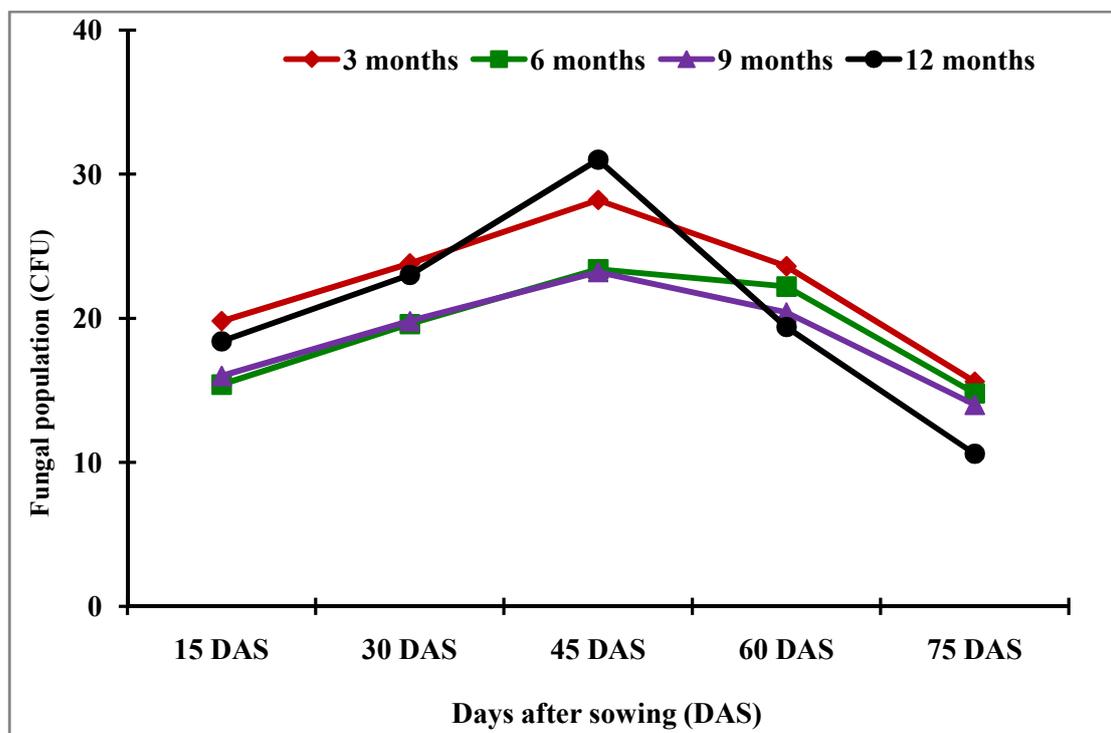


Figure 2: Fungal population in rhizosphere of cowpea) at different growth stages

DISCUSSION

During present study, quantitative estimation of fungi present in rhizosphere of cowpea throughout the growth period revealed that, fungal population showed an increasing trend till the plant attained maximum vegetative growth and gradually decreased towards the senescence of the plant. Abdel-Rahim *et. al.* [5] found relatively smaller number of microorganisms in the early stages of plant growth, which increased in number after the plant had reached considerable size and decreased after senescence of plant. Greatest number of fungi was observed when the plant attained maximum vegetative growth and fruiting. Lakshmi and Sreeramulu [8] also reported that, fungal population in rhizosphere increased with the growth of the plant and decreased after senescence of sesame. In the present studies fungal population of rhizosphere also decreased with the senescence of plant after maturation of fruit. Variation in the fungal population with the ageing of the crop may be ascribed to physiological stimulation of the microorganisms, the excretion of root exudates during the life cycle of the plant [9]. Progressive interaction between the roots and the microorganisms is accompanied by continuous availability of nutrients for the growth of the microorganisms [10]. Decline of fungi in

the rhizosphere soil at senescence could be due to reduction in the growth of the plants which in turn results in the reduction of the rate of root exudation [11].

CONCLUSION

The present study revealed that fungal population in the rhizosphere of cowpea is highest at the maximum vegetative stage of the plant and gradually decline towards senescence stage. Moreover, *Aspergillus* is the dominate species during all the growth stages of plant.

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