Effect of Fungal Staling Growth Substances of Precolonized Microfungi on Colonization of Some Potential Microfungi of Composite Soil Inocula

S. K. Dwivedi and Sangeeta

Department of Environmental Science, Babasaheb Bhimrao Ambedkar (A Central) University, Raibareli Raod, Lucknow-226025, Uttar Pradesh, India.

Abstract- Antibiotics actively generated as staling growth product by the earlier established fungal colonies that inhibit the growth of soil inhabiting microfungi. In the present study, the composite soil mycoflora was assessed for its capability to get established on virgin and staled agar discs after 24, 48, 72, 96 and 120hrs under *in vitro* condition. It was experimentally monitored that the intricate pattern of fungal growth varied widely in each staled agar disc plate. The interim result from the experiment showed that the sum of fungi colonizing the staled agar disc reduces gradually with time from 24 to 120 hrs. Based on their racial low to high endurance capacity against the staled growth products or antibiotics actively generated by pre-colonized fungal colonies, they were sorted in VI groups. The dominating microfungi which could persist up to 96 to 120 hours were *Aspergillus flavus, A. luchuensis, A. niger, A. sulphureus, Penicillium citrinum, P. chrysogenum, P. italicum, Trichoderma viride* and *T. koningii*.

Index Terms- Antibiotics, Composite soil, Fungal antagonists, Soil mycoflora, Staled products.

I. Introduction

The competitive endurance and establishment of soil mycoflora depend upon its quantitative and qualitative antibiotic secretion and tolerance potential against other fungi. As yet, the study on the effect of fungal staling toxic growth substances released from pre-colonized microfungi, on colonization of some potential mycoflora of composite soil inocula has received a little attention. Rao (10) extensively studied the inter-specific fungal competition for substrate colonization. Later, Dwivedi and Garrett (4) reported that the tolerance potential of microfungi to mycostatic substances such as antibiotics or staling growth products play a very singificant role in their colonization on nutrient agar plates. Working on fungal competition in agar plate, they also reported that the species spectrum of fungi colonizing on nutrient agar plates changed successively with the increase of time i.e. degree of staling caused by pre-colonized fungal colonies. The present research paper deals with the study of competitive survival and colonization potential of some composite soil mycoflora on staled agar plates after different time intervals, i.e. 24, 48, 72, 96 and 120 hours.

II. Materials and Methods

Soil Sampling

The composite soil was collected by using standard techniques from different sites near Sitapur District, Uttar Pradesh, India during the winter season. The collected soil samples were brought into the laboratory for further studies.

Effect of fungal staling growth substance on the growth and colonization of microfungi of composite soil inocula

The soil samples were mixed thoroughly in the laboratory under aseptic conditions and sieved. The soil:water suspension of 1:1000, 1:10000 and 1:100000 dilution was prepared by using sterilized distilled water. About 15ml of the sterilized and cooled down Czapek-Dox agar medium was poured in Perti-plates. After solidification of agar, 1ml of each soil:water suspension of the above dilutions was poured in respective Petri-plates. Control was also maintained for each dilution separately. Inoculated plates were incubated at 25±2^{o}C for 24, 48, 72, 96 and 120 hours and there after the entire circle of agar in each Petri-plate was reversed i.e. placed upside down after the completion of respective hours, and one ml of corresponding soil:water suspension was poured over the reversed agar discs and the fungi grown were recorded and identified with the help of relevant literatures.

The percent colonization of the fungi on virgin and staled agar media was determined by the following formula

$$\% \text{ Colonization} = \frac{\text{Total no. of the respective fungal colonies}}{\text{Total no. of all fungal colonies}} \times 100$$

III. Data analysis

The data were expressed as Mean ± SD, n=3 and were analyzed statistically by using analysis of variance technique. A probability of 0.05 or less was considered as significant. Correlation test was applied to compare the percent colonization of composite soil fungal species after different staling periods (in Hrs.).

IV. Result

The result of the present study showed that the competition for survival among the microfungi in the soil is a highly complex natural process. The endurance and colonization ability of

microfungi entirely depends upon the potentiality of antibiotic production and fast growth rate. The observation recorded revealed that there was a significant decrease in the number of fungi colonizing on the reverse of agar discs with an increase in the staling time period from 24 hours to 120 hours respectively. This might be due to the diffusion of the staling growth substances released from pre-colonized microfungal colonies of composite soil inocula before the reversal of the agar discs. Based on the tolerance potential against the staling growth product of the earlier established microfungi, the composite soil mycoflora were classified into I to VI groups (Table 1-3). The microfungi having the best tolerance potential and colonizing abilities were placed in the group VI followed by the groups V, IV, III, II and I. The fungi of the group I have the minimum tolerance potential. It was noticed that the comparatively less numbers of fungi could colonize the staled agar discs after 96 and 120 hours of staling periods.

The result of 1:1000 dilution (Table No. 1) showed that after 120 hours of a long staling period, *Aspergillus flavus, A. luchuensis, A. niger* and *Penicillium citrinum* have maximum tolerance potential for antibiotics or staling growth product diffused by the pre-colonized fungi. Their total quantities (Table No. 4) were also high compared to other microfungi of the same composite soil inocula. Only three fungal species (*Helminthosporium sp., Fusarium longipes* and *Rhizopus*) appeared after 48 hours of staling period whereas four fungal species (*Chaetomium globosum, Alternaria solani, Humicola* and *Alternaria alternata*) were recorded after 24 hours of staling period. Similarly, the fungal species that were observed after 96 and 120 hours of staling period were minimum in the number. Those fungi that loomed on the nutrient virgin agar plate (*Chaetomium globosum, Alternaria solani, Humicola* and *Alternaria alternata*) (Table No. 1) but absent in staled agar disc plates revealed that they were extremely susceptible to the staled growth substances. Similarly, *Aspergillus flavus, A. luchuensis* and *A. niger* were found to be exceptionally dominating in 1: 10000 dilution (Table No. 2). Therefore, these were placed in the group VI followed by *Aspergillus sulphureus, Penicillium citrinum* and *P. italicum* respectively. In case of 1: 100000 dilution (Table No. 3), two dominant fungal species were observed i.e. *Aspergillus luchuensis* and *A. niger* followed by *Aspergillus flavus* and *Penicillium citrinum*. In all the three dilutions 1:1000, 1:10000 and 1:100000 (Table No. 1, 2 and 3), almost the same pattern of fungal colonization was monitored i.e. the number of fungal species and fungal colonies of composite soil inocula decreases successively with the gradual increase in the staling period. After 96 and 120 hours of staling period coparatively few, the highly resistant and tolerant species of fungi have survived and colonized on staled agar discs.

The observation (Table No. 4) revealed that the number of colonies of the most tolerant microfungi, i.e. *Aspergillus flavus* (5×10^3 cfug^{-1} soil), *Penicillium citrinum* (3.67×10^3 cfug^{-1} soil), *A. niger* (4.33×10^3 cfug^{-1} soil) and *A. luchuensis* (6×10^3 cfug^{-1} soil) were maximum as compared to microfungi of other given groups. The overall result of analysis mentioned in Table No. 4, 5 and 6 revealed that the number of microfungal colonies viewed on the staled agar disc plate, decreased gradually with the progressive increase in staling periods from 24 hours to 120 hours respectively.

The percent colonization of the respective fungal species revealed that the growth potential of the relatively dominant microfungi existing in composite soil inocula was highest comparatively to the less tolerant (against staling substances) microfungal communities. (Fig. 1, Fig. 2 and Fig. 3) It was observed that the *Aspergillus flavus* (8.93%), *Penicillium citrinum* (6.55%), *A. niger* (7.74%) and *A. luchuensis* (10.71%) (Fig. 1a) showed the maximum percent colonization followed by *Trichoderma viride* (5.95%), *A. sulphureus* (5.36%), *Fusarium oxysporum* (5.36%), *P. chrysogenum* (4.76%), *Rhizoctonia solani* (4.17%) and so on. *Chaetomium globosum* (1.19%) and *Humicola* (1.79%) were found to have remarkably low colonization ability. There were many fungal species which were present in the control plate but completely disappeared from staled agar disc plates such as *Drechslera* and *Alternaria solani* (Fig. 2). In the same way *Humicola, Fusarium longipes* and *Rhizoctonia solani* were present in staled agar disc after 24 hours of staling period but were lacking in 48, 72, 96 and 120 hours of staled agar disc plates. It was observed that after 120 hours of staling period only three fungal species were seemed to be established, i.e. *Aspergillus flavus, A. niger* and *A. luchuensis* having colonization percentage around 33%. Similar pattern of colonization was recorded in 1:100000 dilution (Fig. 3) where two fungal species i.e. *A. niger* (33.33%) and *A. luchuensis* (66.67%) survived.

The significant values of the simple linear correlation coefficients (r) indicate that they were significantly different from zero at the 5% and 1% probability level. The significant, high r value indicates that fungal colonization and staling period are highly associated with one another in a linear way.

V. Discussion

The study of inter-specific fungal competition in composite soil mycoflora in the presence of staling growth substances progressively released from the earlier established fungal colonies of composite soil inocula has received little attention. In the present study, it was observed that the number of fungi colonizing on the reverse agar disc decreases gradually with the increase in the staling period this may be due to the diffusion of the antibiotics or staling growth substances actively produced by the pre-colonized fungal colonies (1; 3). It was also observed that the fungal population (i.e. number of fungal colonies) of the extremely tolerant and dominating fungi were higher as compared to surprisingly less endure microfungi of the composite soil inocula. The staling phenomenon is a very perplex process and it is detected by the decrease in growth rate (11). The success of colonization of a particular fungus depends upon its population level in the soil (4). It was also noticed that the fungi which loomed on nutrient virgin agar disc disappeared from the staled agar disc, this failure in the colonization of fungi after different staling periods on staled agar disc might be due to the low growth rate and less tolerance potential against the staling growth substances. The similar result was reported by (4; 5). Upadhyay et al., (14) reported that besides pH, nutrient supply in staling growth product, antibiotics play a very significant role in the colonization. Arora et al., (2) studied the effect of fungal staling growth products some dominant rhizospheric fungi such as *Aspergillus candidus, A.fumigatus, Chaetomium globosum,*

Fusarium chlamydosporum, *F. cutmorum*, and *Penicitlium citrinum* against *Rhizoctonia solani* and found that *R. solani* possessed strong tolerance potential for staling growth substances and not a single antagonist could inhibit it growth successfully. The success of the competition depends upon the tolerance to staling product and antibiotic secretion ability (6). Leaf inhabiting microfungi may inhibit the pathogen by producing antibiotics which caused the mycostasis on the leaf surface (15; 7; 12; 13). The competitive fungal species utilizes the toxic metabolites on antibiotics for their establishments (9). Makut and Owolewa, (8) isolated the *Absidia corymbifera, Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, Cladosporium herbarum, Curvularia lunata, Penicillium* sp., *Rhizopus stolonifer* and *Trichoderma viride*, which are antibiotic producing fungi present in soil.

VI. Conclusion

Interspecific fungal competition for the survival in the soil is a very perplex process. The exponential growth and colonization ability of a composite soil mycoflora depend upon its growth rate and racial tolerance potential against the antibiotics or staling growth substances actively produced by the pre-colonized microfungi of the composite soil inocula.

Acknowledgements

The authors are thankful to the Head, Department of Environmental Science, B.B.A.U., Lucknow for providing the necessary facilities. One of us (Sangeeta) wishes to thank UGC, New Delhi for financial support by providing Rajiv Gandhi National Fellowship.

References

[1] Arora DK, Dwivedi RS (1975) Effect of fungal staling growth products on growth behavior of rhizosphere microfungi. Experientia. 32(7): 862-864.

[2] Arora DK, Gupta RC, Tandon RN (1977) Effect of fungal staling growth products on colony interaction among Rhizoctonia solani Kuhn and some potential rhizosphere fungi. Experientia 35(2): 200-201.

[3] Dwivedi SK (1988) Soil fungi of guava orchards with emphasis on antibiosis. Nat Acad sci Letters 11 (1): 7-9.

[4] Dwivedi RS and Garrett SD (1968) Fungal completion in agar plate colonization from soil inocula. Trans. Br. Mycol. Soc., 51: 95-101.

[5] Dwivedi RS, Arora DK (1975) Fungal colonization of staled agar discs from rhizosphere soil inocula of pigeon pea.

[6] Dwivedi SK, Dwivedi P (2004) Microbial Interactions and Biocides.In: Environmental Microbiology and Biotechnology (eds.)Singh, D.P. and Dwivedi, S.K. New Age International Publishers. 203-217.

[7] Fokkema NJ (1976) In: Microbiology of aerial plant surfaces. Academic Press, London p. 486.

[8] Makut MD, Owolewa OA (2011) Antibiotic-Producing Fungi Present In The Soil Environment Of Keffi Metropolis, Nasarawa State, Nigeria. Trakia Journal of Sciences,9(2) 33-39.

[9] Qureshi SA (2003) Studies On Antibiotics From Soil Fungi. Thesis, Studies On Antibiotics From Soil Fungi. University Of Karachi, Pakistan.

[10] Rao AS (1959). A comparative study of competitive saprophytic ability in twelve root- infecting fungi by agar plate method. Trans. Br. Mycol. Soc., 42: 97-111.

[11] Robinson PM (1968) Aspects of staling in liquid cultures of fungi. New Phytol 68, 351-357.

[12] Skidmore AM (1976) In: Microbiology of aerial plant surfaces, Academic Press, London p. 507.

[13] Upadhyay RK, Dwivedi RS (1977). Proc. Indian natl Sci. Acad. 43B, 33.

[14] Upadhyay RK, Arora DK, Dwivedi RS (1980) Staling growth products of phyllosphere fungi. Experientia. 36(1):66-67.

[15] Upadhyay RK, Arora DK (1979) Role of fungal staling growth products in inter-specific competition among phylloplane fungi. Experientia. 36: 185-186.

Authors

First Author – S. K. Dwivedi, Department of Environmental Science, Babasaheb Bhimrao Ambedkar (A Central) University, Raibareli Raod, Lucknow-226025, Uttar Pradesh, India.
Second Author – Sangeeta, Department of Environmental Science, Babasaheb Bhimrao Ambedkar (A Central) University, Raibareli Raod, Lucknow-226025, Uttar Pradesh, India.

Table No. 1: Grouping of composite soil mycoflora (1:1000 dilution) and their colonization pattern in presence of staled agar after different staling periods i.e. 24, 48, 72, 96 and 120 hours

Names of the fungal species	0 Hrs	24 Hrs	48 Hrs	72 Hrs	96 Hrs	120 Hrs
Group I						
Chaetomium globosum	+	-	-	-	-	-
Alternaria solani	+	-	-	-	-	-
Humicola	+	-	-	-	-	-
Alternaria alternata	+	-	-	-	-	-
Group II						
Helminthosporium sp.	+	+	-	-	-	-
Fusarium longipes	+	+	-	-	-	-
Rhizopus nigricans	+	+	-	-	-	-
Group III						
Drechslera	+	+	+	-	-	-
Penicillium oxalicum	+	+	+	-	-	-
Group IV						
Rhizoctonia solani	+	+	+	+	-	-
Sterile mycelium	+	+	+	+	-	-
Fusarium oxysporum	+	+	+	+	-	-
Group V						
Penicillium chrysogenum	+	+	+	+	+	-
Penicillium italicum	+	+	+	+	+	-
Aspergillus sulphureus	+	+	+	+	+	-
Trichoderma viride	+	+	+	+	+	-
Trichoderma koningii	+	+	+	+	+	-
Group VI						
Aspergillus flavus	+	+	+	-	+	+
Penicillium citrinum	+	+	+	+	+	+
Aspergillus niger	+	-	+	+	-	+
Aspergillus luchuensis	+	+	+	+	+	+
Total species	21	16	14	11	8	4

+ = Presence, - = Absence

Table No. 2: Grouping of composite soil mycoflora (1:10000 dilution) and their colonization pattern in presence of staled agar after different staling periods i.e. 24, 48, 72, 96 and 120 hours

Names of the fungal species	0 Hrs	24 Hrs	48 Hrs	72 Hrs	96 Hrs	120 Hrs
Group I						
Drechslera	+	-	-	-	-	-
Alternaria solani	+	-	-	-	-	-
Group II						
Humicola	+	+	-	-	-	-
Fusarium longipes	+	+	-	-	-	-
Rhizopus nigricans	+	+	-	-	-	-
Group III						
Trichoderma viride	+	+	+	-	-	-
Penicillium oxalicum	+	+	+	-	-	-
Group IV						
Penicillium chrysogenum	+	+	-	+	-	-
Sterile mycelium	+	+	+	+	-	-
Fusarium oxysporum	+	-	+	+	-	-
Group V						
Penicillium italicum	+	-	-	+	+	-
Aspergillus sulphureus	+	+	-	+	+	-
Penicillium citrinum	+	+	+	+	+	-
Group VI						

Aspergillus flavus	+	+	+	-	+	+
Aspergillus niger	+	-	+	+	-	+
Aspergillus luchuensis	+	+	+	+	+	+
Total species	16	11	8	8	5	3

+ = Presence, - = Absence

Table No. 3: Grouping of composite soil mycoflora (1:100000 dilution) and their colonization pattern in presence of staled agar after different staling periods i.e. 24, 48, 72, 96 and 120 hours

Names of the fungal species	0 Hrs	24 Hrs	48 Hrs	72 Hrs	96 Hrs	120 Hrs
Group I						
Alternaria solani	+	-	-	-	-	-
Group II						
Rhizopus nigricans	+	+	-	-	-	-
Penicillium oxalicum	+	+	-	-	-	-
Group III						
Drechslera	+	+	+	-	-	-
Fusarium oxysporum	+	+	+	-	-	-
Group IV						
Trichoderma koningii	+	+	+	+	-	-
Aspergillus sulphureus	+	+	-	+	-	-
Group V						
Aspergillus flavus	+	-	+	+	+	-
Penicillium citrinum	+	+	+	-	+	-
Group VI						
Aspergillus niger	+	+	+	-	-	+
Aspergillus luchuensis	+	+	+	-	+	+
Total species	11	9	7	3	3	2

+ = Presence, - = Absence

Table No. 4: Fungal population in composite soil inocula (1:1000 dilution) on staled agar discs after 24, 48, 72, 96 and 120 hours of staling periods

Names of the fungal species	Fungal population (cfu/g soil X 103) Different staling periods (Hours)					
	0 Hrs	24 Hrs	48 Hrs	72 Hrs	96 Hrs	120 Hrs
Group I						
Chaetomium globosum	0.67 ± 0.58					
Alternaria solani	1.33 ± 0.58					
Humicola	1.00 ± 1.00					
Alternaria alternata	2.00 ± 1.00					
Group II						
Helminthosporium sp.	2.00 ± 1.00	2.67 ± 0.58				
Fusarium longipes	2.33 ± 0.58	2.67 ± 0.58				
Rhizopus nigricans	1.67 ± 0.58	2.33 ± 0.58				
Group III						
Drechslera	2.33 ± 0.58	2.67 ± 0.58	2.00 ± 1.00			
Penicillium oxalicum	2.67 ± 0.58	2.33 ± 0.58	1.67 ± 0.58			
Group IV						
Rhizoctonia solani	2.33 ± 0.58	2.00 ± 1.00	1.67 ± 0.58	1.00 ± 1.00		
Sterile mycelium	2.33 ± 0.58	2.67 ± 0.58	1.67 ± 0.58	1.33 ± 0.58		
Fusarium oxysporum	3.00 ± 1.00	3.00 ± 1.00	2.33 ± 0.58	2.00 ± 1.00		
Group V						
Penicillium chrysogenum	2.67 ± 0.58	2.67 ± 0.58	1.67 ± 0.58	1.33 ± 0.58	1.00 ± 0.00	
Penicillium italicum	2.00 ± 0.00	2.00 ± 1.00	1.67 ± 0.58	1.33 ± 0.58	0.67 ± 0.58	
Aspergillus sulphureus	3.00 ± 1.00	2.67 ± 0.58	1.67 ± 0.58	1.33 ± 0.58	1.33 ± 0.58	

Trichoderma viride	3.33 ± 1.53	3.33 ± 1.15	2.67 ± 0.58	2.33 ± 0.58	1.67 ± 0.58	
Trichoderma koningii	2.33 ± 0.58	2.67 ± 0.58	2.33 ± 0.58	2.00 ± 1.00	1.67 ± 1.15	
Group VI						
Aspergillus flavus	5.00 ± 1.00	5.00 ± 1.00	4.33 ± 0.58	0.00 ± 0.00	1.67 ± 0.58	1.00 ± 0.00
Penicillium citrinum	3.67 ± 1.15	3.67 ± 0.58	3.33 ± 0.58	2.67 ± 0.58	2.33 ± 0.58	1.67 ± 0.58
Aspergillus niger	4.33 ± 1.53	0.00 ± 0.00	4.33 ± 0.58	3.67 ± 0.58	0.00 ± 0.00	0.67 ± 0.58
Aspergillus luchuensis	6.00 ± 1.00	5.67 ± 0.58	4.67 ± 0.58	4.00 ± 1.00	2.67 ± 0.58	1.67 ± 0.58
ns= Non significant						
**= Significant at 1%	**	**	**	**	**	ns
CV%	33 %	25.8 %	24 %	37.8 %	42.3 %	40 %

Mean ± SD, n=3, CV= Coefficient of variation

Table No. 5: Fungal population in composite soil inocula (1:10000 dilution) on staled agar discs after 24, 48, 72, 96 and 120 hours of staling periods

Names of the fungal species	Fungal population (cfu/g soil X 104)					
	Different staling periods (Hours)					
	0 Hrs	24 Hrs	48 Hrs	72 Hrs	96 Hrs	120 Hrs
Group I						
Drechslera	1.33 ± 0.58					
Alternaria solani	0.67 ± 0.58					
Group II						
Humicola	0.67 ± 0.58	0.67 ± 0.58				
Fusarium longipes	1.67 ± 0.58	1.33 ± 0.58				
Rhizopus nigricans	1.33 ± 0.58	1.33 ± 0.58				
Group III						
Trichoderma viride	1.67 ± 0.58	1.67 ± 0.58	0.67 ± 0.58			
Penicillium oxalicum	1.33 ± 0.58	0.67 ± 0.58	0.33 ± 0.58			
Group IV						
Penicillium chrysogenum	1.33 ± 0.58	1.00 ± 0.00	0.67 ± 0.58	0.33 ± 0.58		
White sterile mycelium	1.67 ± 0.58	1.33 ± 0.58	0.67 ± 0.58	0.33 ± 0.58		
Fusarium oxysporum	1.67 ± 0.58	1.33 ± 0.58	0.67 ± 0.58	0.67 ± 0.58		
Group V						
Penicillium italicum	1.67 ± 0.58	1.33 ± 0.58	0.67 ± 0.58	0.33 ± 0.58	0.33 ± 0.58	
Aspergillus sulphureus	2.33 ± 0.58	1.33 ± 0.58	0.33 ± 0.58	0.33 ± 0.58	0.33 ± 0.58	
Penicillium citrinum	2.33 ± 0.58	1.67 ± 0.58	1.00 ± 0.00	0.67 ± 0.58	0.67 ± 0.58	
Group VI						
Aspergillus flavus	2.67 ± 0.58	1.67 ± 0.58	0.67± 0.58	0.00 ± 0.00	0.67 ± 0.58	0.67 ± 0.58
Aspergillus niger	2.33 ± 0.58	0.00 ± 0.00	0.67 ± 0.58	0.67 ± 0.58	0.00 ± 0.00	0.67 ± 0.58
Aspergillus luchuensis	2.33 ± 0.58	1.67 ± 0.58	0.67 ± 0.58	0.67 ± 0.58	1.00 ± 0.00	0.67 ± 0.58
ns= Non significant,						
*= Significant at 5%,						
**= Significant at 1%	**	*	ns	ns	ns	ns
CV%	34.2 %	44.2 %	86 %	123.7 %	94.3 %	86.2 %

Mean ± SD, n=3, CV= Coefficient of variation

Table No. 6: Fungal population in composite soil inocula (1:100000 dilution) on staled agar discs after 24, 48, 72, 96 and 120hours of staling periods

Names of the fungal species	Fungal population (cfu/g soil X 105)					
	Different staling periods (Hours)					
	0 Hrs	24 Hrs	48 Hrs	72 Hrs	96 Hrs	120 Hrs
Group I						
Alternaria solani	0.67 ± 0.58					
Group II						
Rhizopus nigricans	0.67 ± 0.58	0.33 ± 0.58				
Penicillium oxalicum	0.67 ± 0.58	0.67 ± 0.58				
Group III						
Drechslera	0.33 ± 0.58	0.33 ± 0.58	0.33 ± 0.58			

Fusarium oxysporum	1.00 ± 0.00	0.67 ± 0.58	0.67 ± 0.58			
Group IV						
Trichoderma koningii	0.67 ± 0.58	0.67 ± 0.58	0.67 ± 0.58	0.67 ± 0.58		
Aspergillus sulphureus	0.67 ± 0.58	0.67 ± 0.58	0.00 ± 0.00	0.33 ± 0.58		
Group V						
Aspergillus flavus	1.33 ± 0.58	0.00 ± 0.00	0.67 ± 0.58	1.00 ± 0.00	0.33 ± 0.58	
Penicillium citrinum	1.00 ± 1.00	0.67 ± 0.58	0.33 ± 0.58	0.00 ± 0.00	0.33 ± 0.58	
Group VI						
Aspergillus niger	1.33 ± 0.58	0.67 ± 0.58	0.67 ± 0.58	0.00 ± 0.00	0.00 ± 0.00	0.33 ± 0.58
Aspergillus luchuensis	1.67 ± 0.58	1.33 ± 0.58	0.67 ± 0.58	0.00 ± 0.00	0.33 ± 0.58	0.67 ± 0.58
ns= Non significant,						
*= Significant at 5%	ns	ns	ns	*	ns	ns
CV%	66.3 %	91.3 %	108.0 %	101.0 %	200 %	115.5 %

Mean ± SD, n=3, CV= Coefficient of variation

Table No. 7: Computation of Linear correlation coefficient between the precent colonization of composite soil fungal species (1:1000 dilution) after different staling periods (in Hours)

	Control	24 Hrs	48 Hrs	72 Hrs	96 Hrs	120 Hrs
Control	1**					
24 Hrs	0.714**	1**				
48 Hrs	0.910**	0.606**	1**			
72 Hrs	0.687**	0.354	0.763**	1**		
96 Hrs	0.686**	0.704**	0.670**	0.603**	1**	
120 Hrs	0.777**	0.538*	0.722**	0.584**	0.715**	1**

*= Significance at 5%, **= Significance at 1%. Perfect positive correlation (r) = +1.0, Weak positive correlation (r) = +0.1 to +0.30, Medium positive correlation (r) = +0.30 to +0.50, Strong positive correlation (r) = +0.50 to +1.0, Perfect negative correlation (r) = -1.0, Weak negative correlation (r) = -0.30 to -0.1, Medium negative correlation (r) = -0.50 to -0.30, Strong negative correlation (r) = -1.0 to -0.50.

Table No. 8: Computation of Linear correlation coefficient between the precent colonization of composite soil fungal species (1:10000 dilution) after different staling periods (in Hours)

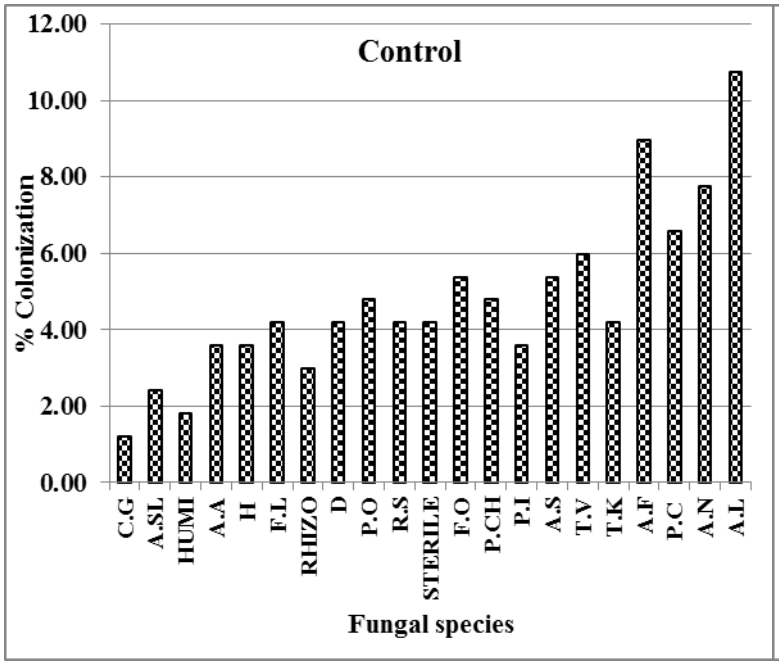
Control	24 Hrs	48 Hrs	72 Hrs	96 Hrs	120 Hrs
Control	1**					
24 Hrs	0.51*	1**				
48 Hrs	0.657**	0.504*	1**			
72 Hrs	0.539*	0.20166	0.710**	1**		
96 Hrs	0.682**	0.540*	0.490001	0.424005	1**	
120 Hrs	0.637**	0.039457	0.336227	0.337963	0.568*	1**

*= Significance at 5%, **= Significance at 1%. Perfect positive correlation (r) = +1.0, Weak positive correlation (r) = +0.1 to +0.30, Medium positive correlation (r) = +0.30 to +0.50, Strong positive correlation (r) = +0.50 to +1.0, Perfect negative correlation (r) = -1.0, Weak negative correlation (r) = -0.30 to -0.1, Medium negative correlation (r) = -0.50 to -0.30, Strong negative correlation (r) = -1.0 to -0.50.

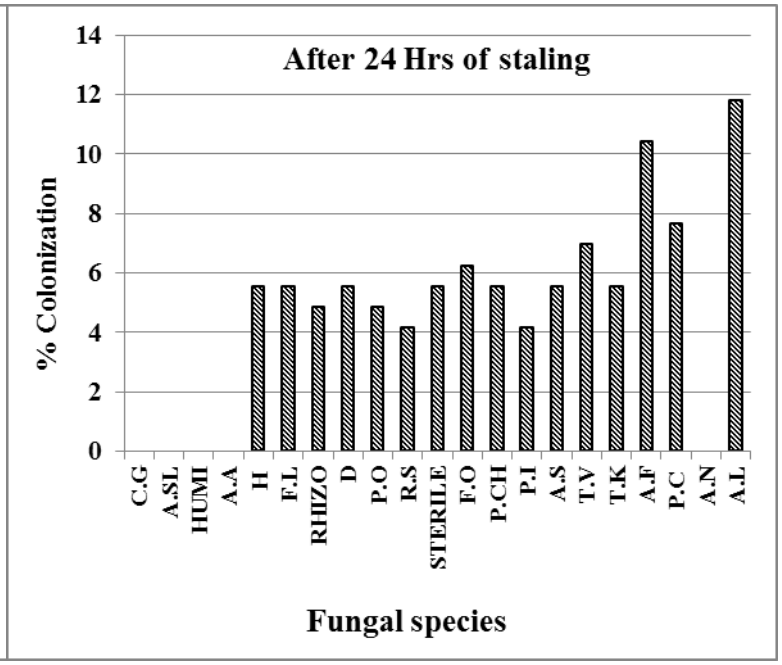
Table No. 9: Computation of Linear correlation coefficient between the precent colonization of composite soil fungal species (1:100000 dilution) after different staling periods (in Hours)

Control	24 Hrs	48 Hrs	72 Hrs	96 Hrs	120 Hrs
Control	1**					
24 Hrs	0.517	1**				
48 Hrs	0.686*	0.318	1**			
72 Hrs	0.161	-0.329	0.353	1**		
96 Hrs	0.683*	0.209	0.392	0.282	1**	
120 Hrs	0.736**	0.703*	0.447	-0.244	0.391	1**

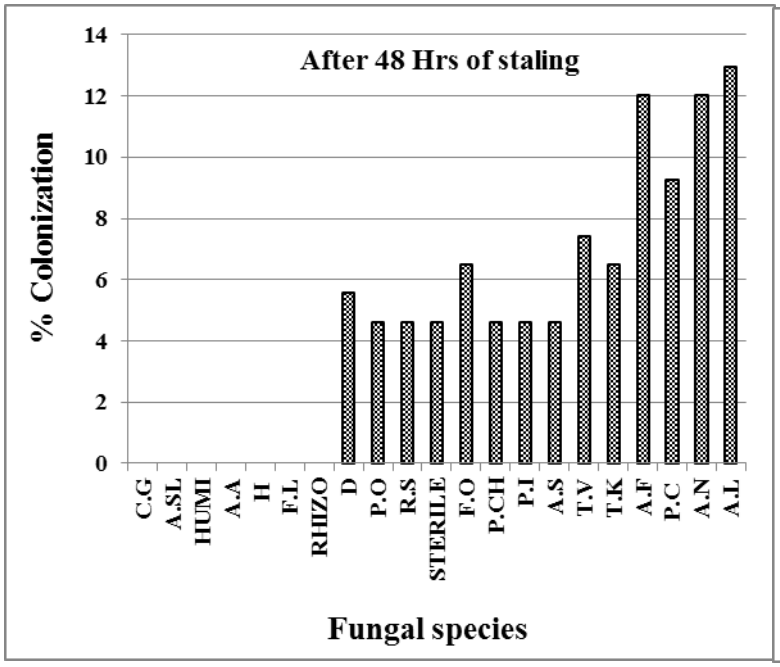
*= Significance at 5%, **= Significance at 1%. Perfect positive correlation (r) = +1.0, Weak positive correlation (r) = +0.1 to +0.30, Medium positive correlation (r) = +0.30 to +0.50, Strong positive correlation (r) = +0.50 to +1.0, Perfect negative correlation (r) = -1.0, Weak negative correlation (r) = -0.30 to -0.1, Medium negative correlation (r) = -0.50 to -0.30, Strong negative correlation (r) = -1.0 to -0.50.


Control
% Colorization
12.00
10.00
8.00
6.00
4.00
2.00
0.00
C.G
A.SL
HUMI
A.A
H
F.L
RHIZO
D
P.O
R.S
STERILE
F.O
P.CH
P.I
A.S
T.V
T.K
A.F
P.C
A.N
A.L
Fungal species

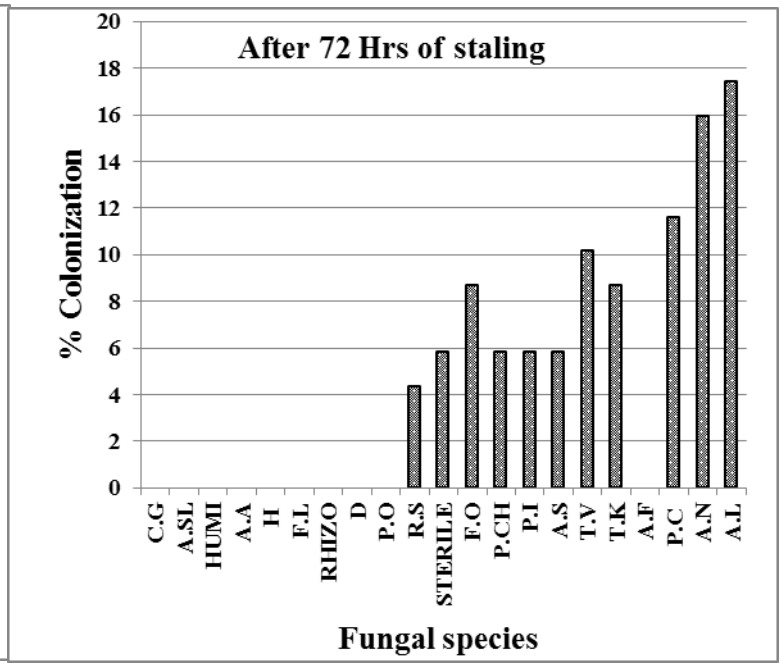
(a)


After 24 Hrs of staling
% Colonization
14
12
10
8
6
4
2
0
C.G
A.SL
HUMI
A.A
H
F.L
RHIZO
D
P.O
R.S
STERILE
F.O
P.CH
P.I
A.S
T.V
T.K
A.F
P.C
A.N
A.L
Fungal species

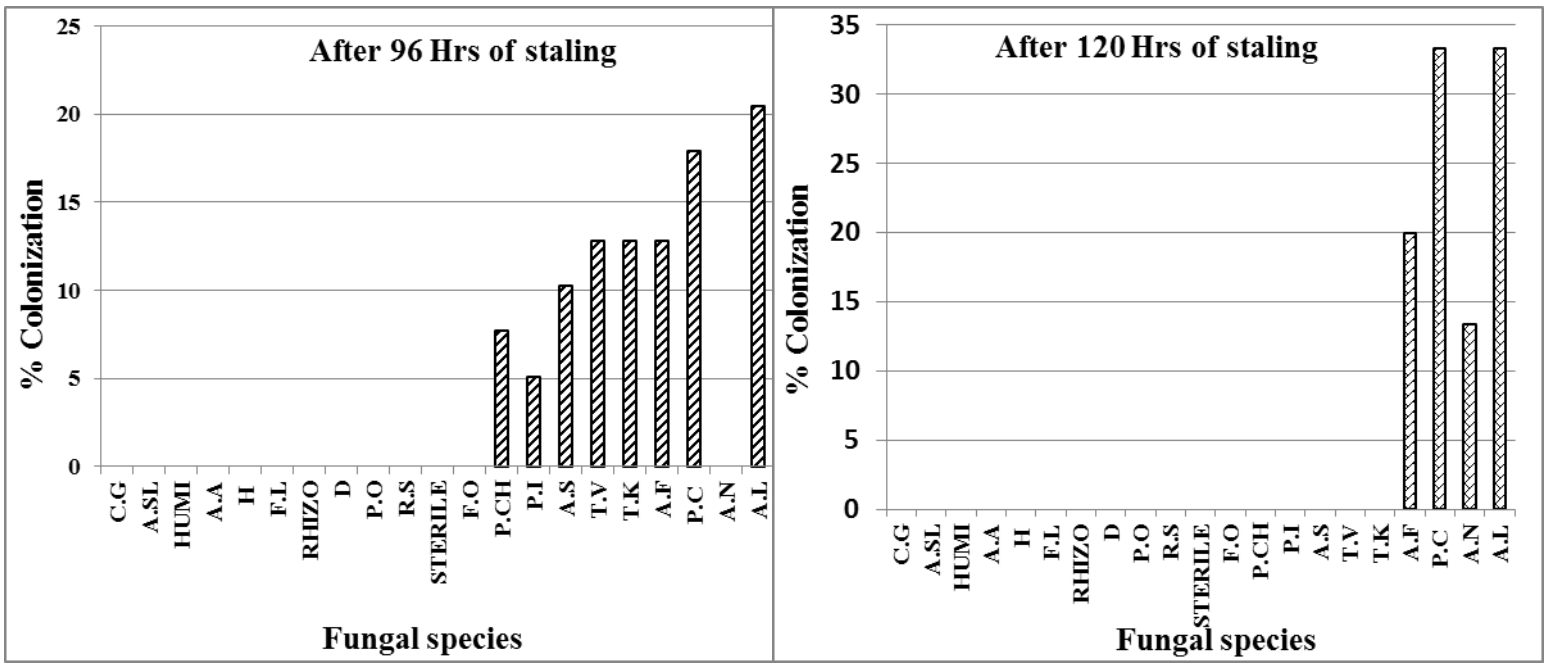
(b)


After 48 Hrs of staling
% Colonization
14
12
10
8
6
4
2
0
C.G
A.SL
HUMI
A.A
H
F.L
RHIZO
D
P.O
R.S
STERILE
F.O
P.CH
P.I
A.S
T.V
T.K
A.F
P.C
A.N
A.L
Fungal species

(c)

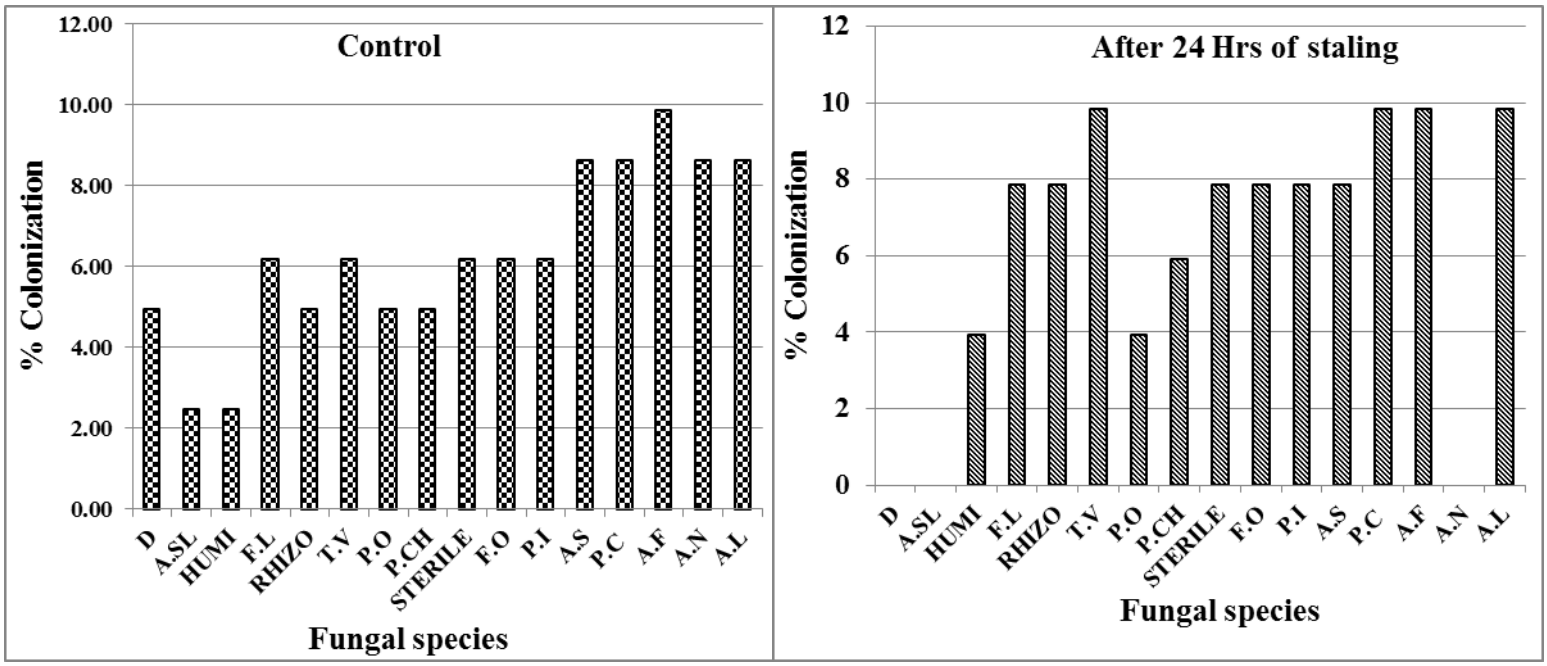

After 72 Hrs of staling
% Colonization
20
18
16
14
12
10
8
6
4
2
0
C.G
A.SL
HUMI
A.A
H
F.L
RHIZO
D
P.O
R.S
STERILE
F.O
P.CH
P.I
A.S
T.V
T.K
A.F
P.C
A.N
A.L
Fungal species

(d)

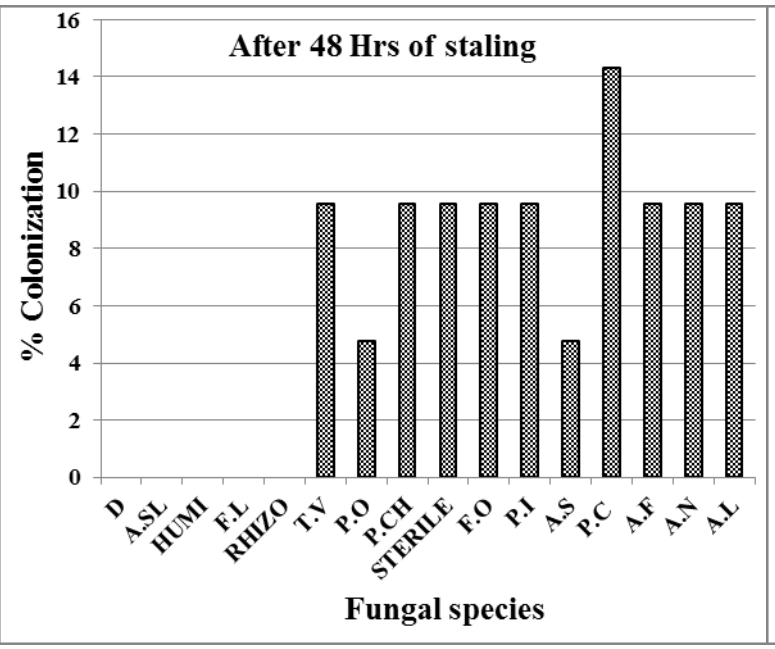


(e) (f)

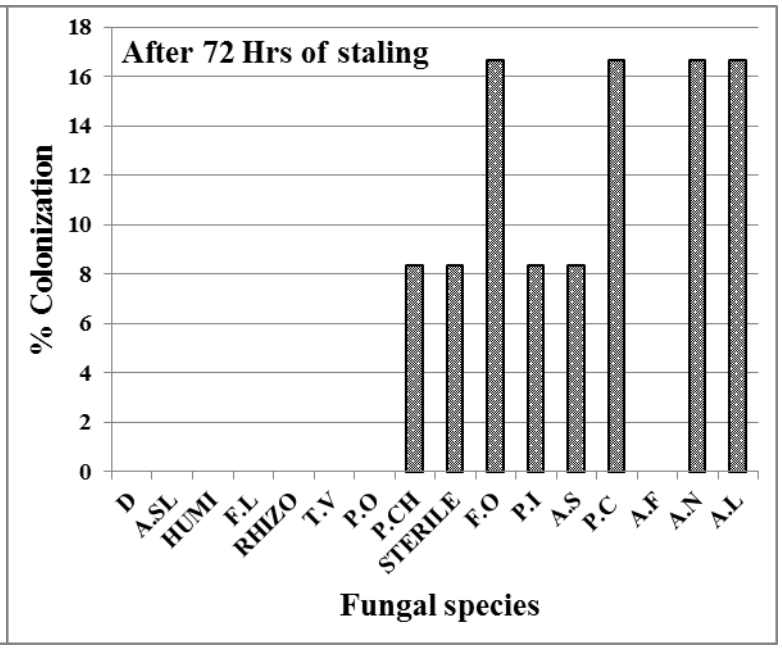
Fig. 1: Percent colonization of composite soil mycoflora (1:1000 dilution) on nutrient virgin agar and staled agar disc after different periods of staling (a) Control, (b) After 24 Hrs of staling, (c) After 48 Hrs of staling, (d) After 72 Hrs of staling, (e) After 96 Hrs of staling and (f) After 120 Hrs of staling. C.G - *Chaetomium globosum*, A.SL - *Alternaria solani*, HUMI – *Humicola*, A.A - *Alternaria alternata*, H – *Helminthosporium*, F.L - *Fusarium longipes*, RHIZO – *Rhizopus nigricans*, D – *Drechslera*, P.O - *Penicillium oxalicum*, R.S - *Rhizoctonia solani*, STERILE - *Sterile mycelium*, F.O - *Fusarium oxysporum*, P.CH - *Penicillium chrysogenum*, P.I - *Penicillium italicum*, A.S - *Aspergillus sulphureus*, T.V - *Trichoderma viride*, T.K - *Trichoderma koningii*, A.F - *Aspergillus flavus*, P.C - *Penicillium citrinum*, A.N - *Aspergillus niger*, A.L - *Aspergillus luchuensis*



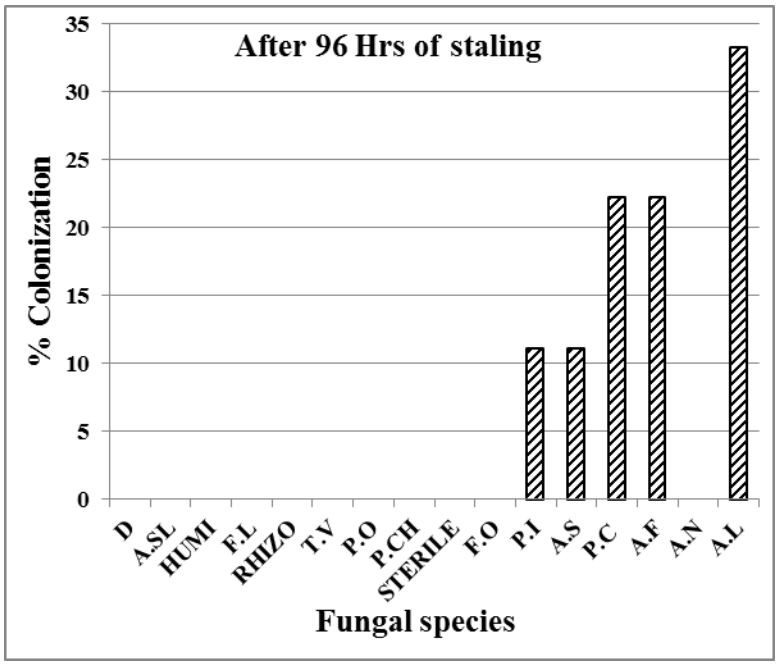
(a) (b)



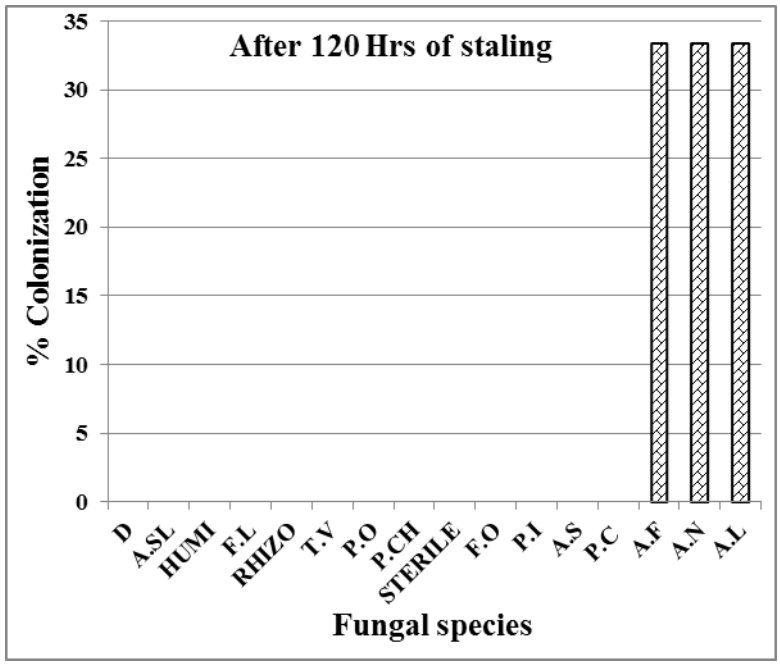
(c)



(d)



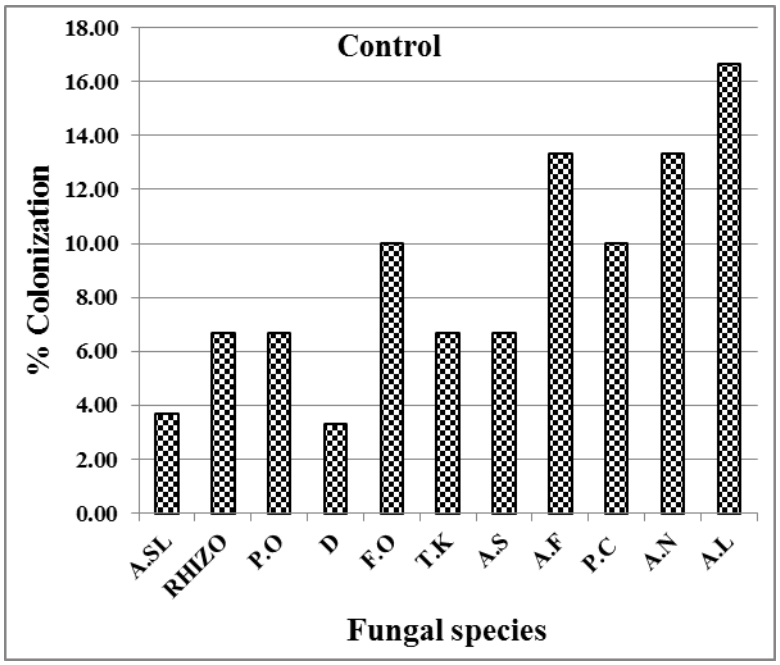
(e)



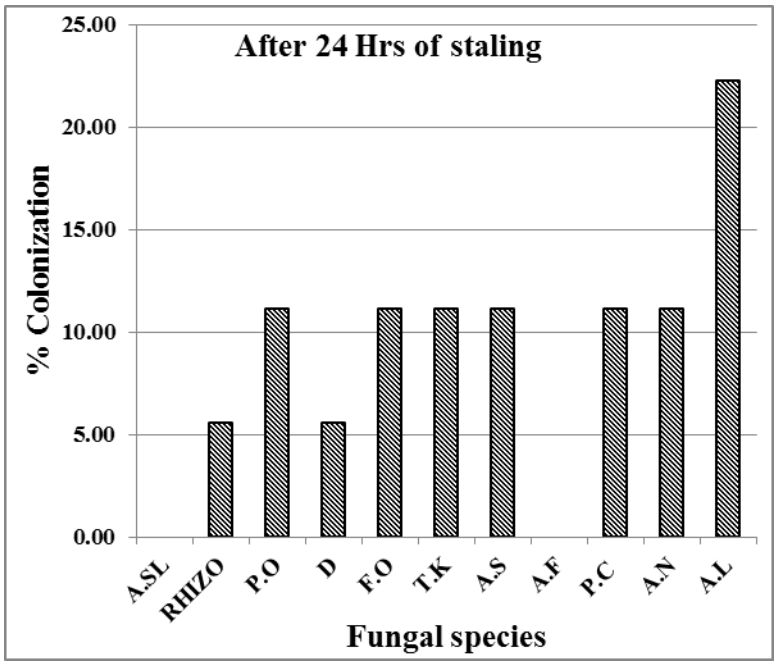
(f)

Fig. 2: Percent colonization of composite soil mycoflora (1:10000 dilution) on nutrient virgin agar and staled agar disc after different periods of staling (a) Control, (b) After 24 Hrs of staling, (c) After 48 Hrs of staling, (d) After 72 Hrs of staling, (e) After 96 Hrs of staling and (d) After 120 Hrs of staling. D – *Drechslera,* A.SL - *Alternaria solani,* HUMI – *Humicola,* F.L - *Fusarium longipes,* RHIZO – *Rhizopus nigricans,* T.V - *Trichoderma viride,* P.O - *Penicillium oxalicum,* P.CH - *Penicillium chrysogenum,* STERILE - *Sterile mycelium,* F.O - *Fusarium oxysporum,* P.I - *Penicillium italicum,* A.S - *Aspergillus sulphureus,* P.C - *Penicillium citrinum,* A.F - *Aspergillus flavus,* A.N - *Aspergillus niger,* A.L - *Aspergillus luchuensis.*

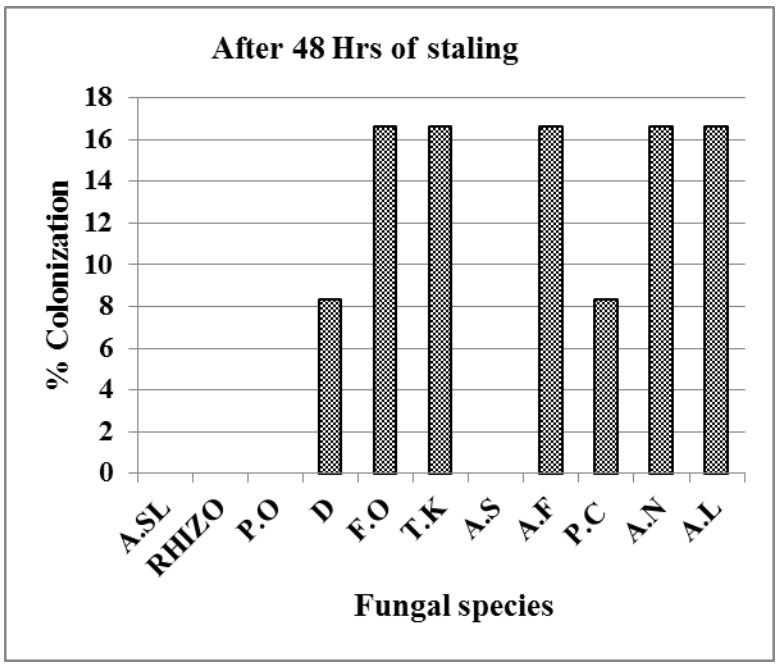
.


Control
% Colonization
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
A.SL
RHIZO
P.O
D
F.O
T.K
A.S
A.F
P.C
A.N
A.L
Fungal species

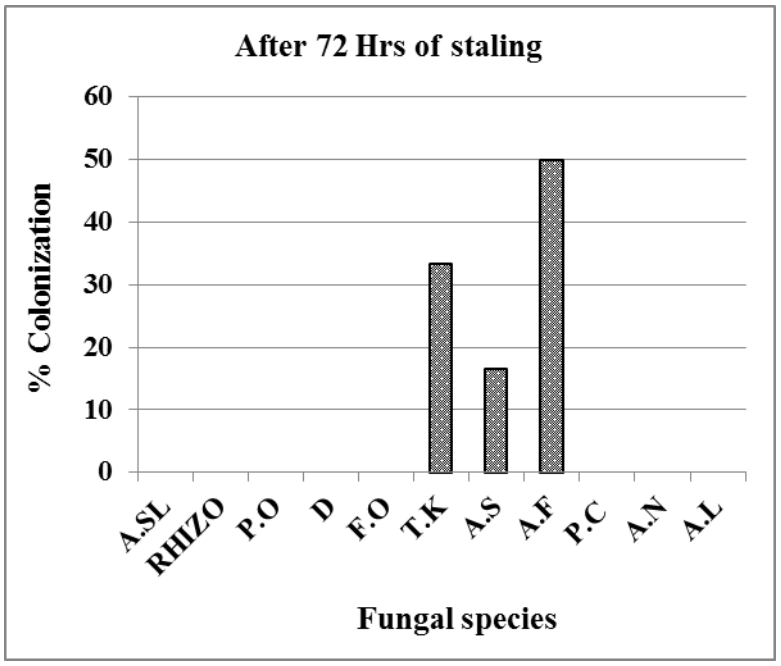
(a)


After 24 Hrs of staling
% Colonization
25.00
20.00
15.00
10.00
5.00
0.00
A.SL
RHIZO
P.O
D
F.O
T.K
A.S
A.F
P.C
A.N
A.L
Fungal species

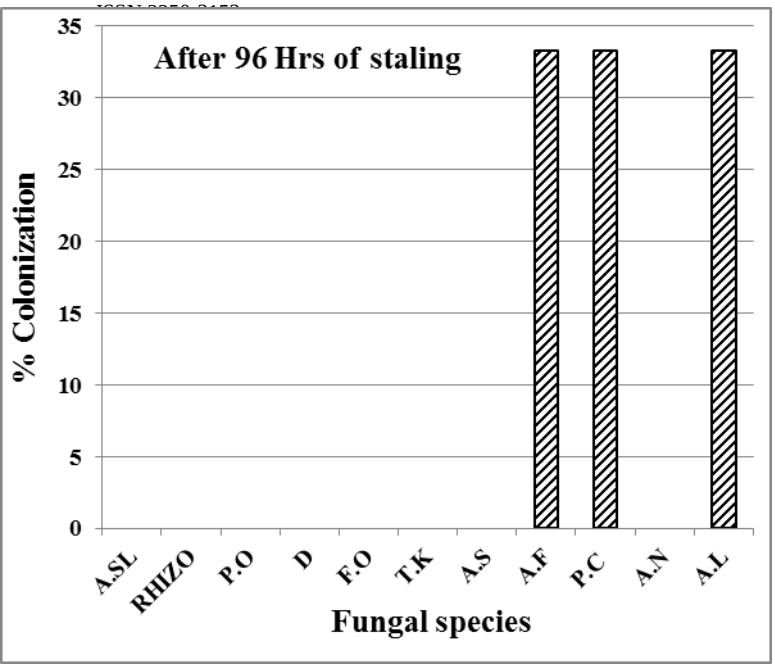
(b)


After 48 Hrs of staling
% Colonization
18
16
14
12
10
8
6
4
2
0
A.SL
RHIZO
P.O
D
F.O
T.K
A.S
A.F
P.C
A.N
A.L
Fungal species

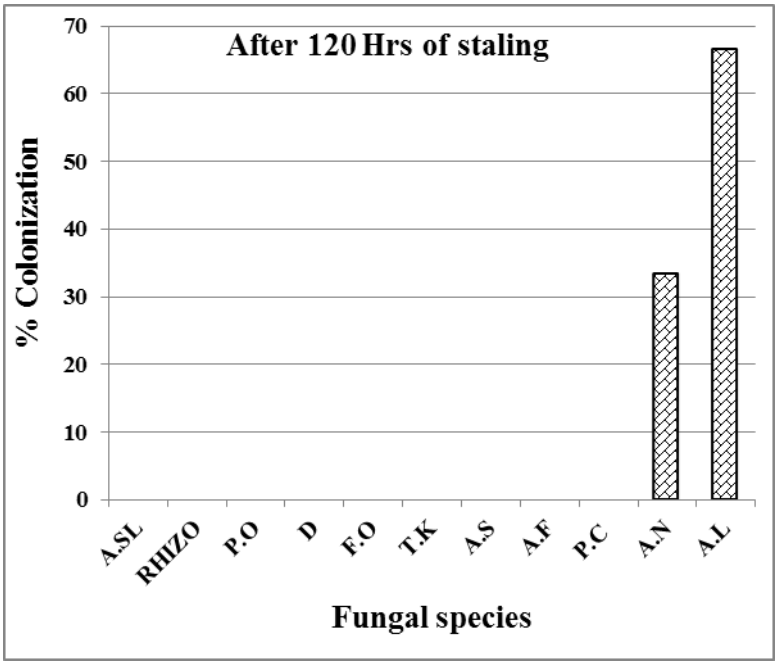
(c)


After 72 Hrs of staling
% Colonization
60
50
40
30
20
10
0
A.SL
RHIZO
P.O
D
F.O
T.K
A.S
A.F
P.C
A.N
A.L
Fungal species

(d)



(e)



(f)

Fig. 3: Percent colonization of composite soil mycoflora (1:100000 dilution) on nutrient virgin agar and staled agar disc after different periods of staling (a) Control, (b) After 24 Hrs of staling, (c) After 48 Hrs of staling, (d) After 72 Hrs of staling, (e) After 96 Hrs of staling and (d) After 120 Hrs of staling . A.SL - *Alternaria solani,* RHIZO – *Rhizopus nigricans,* P.O - *Penicillium oxalicum,* D – *Drechslera,* F.O - *Fusarium oxysporum,* T.K - *Trichoderma koningii,* A.S - *Aspergillus sulphureus,* A.F - *Aspergillus flavus,* P.C - *Penicillium citrinum,* A.N - *Aspergillus niger,* A.L - *Aspergillus luchuensis.*