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# Fungal Succession in Composite Soil on Staled Agar Disc at different Staling Periods

**S.K. Dwivedi and Sangeeta***

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

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## ABSTRACT

In the present study the fungal colony interaction of composite soil was assessed under *in vitro* condition in virgin and staled agar discs after different staling periods i.e. 24, 48, 72, 96 and 120 hours by using Warcup method to examine the tolerance potential of soil mycoflora present in the composite soil against the growth substances produced by the precolonized fungal colonies. It was observed that fungal growth pattern was different in each agar disc plate. It was also noticed that there was a successive decrease in the number of fungi colonizing on the reverse side of the staled agar discs. Only the most resistant microfungi i.e., *Aspergillus flavus, A. niger, A. luchuensis, Aspergillus sulphureus ,Penicillium citrinum, Penicillium chrysogenum* and *Trichoderma viride* were able to persist on staled agar disc after 96 and 120 hours of long staling periods.

## 1) INTRODUCTION

Fungal growth substances released from the early established fungal colonies play a very significant role in the growth and development of the mycoflora present in the composite soil inocula. The growth and development of the soil inhabiting microfungi totally depend upon the quantity and quality of the antibiotic substances secreted by the microfungi. Antibiotics released from the early established microfungi can slow down or inhibit the growth of the soil inhabiting microfungi. In the present study the composite soil inocula was evaluated for its potentiality to get established on staled agar disc after 24, 48, 72, 96 and 120 hrs of staling periods.

## 2) MATERIALS AND METHODS

### a. Sampling

The composite soil was collected from sites in the vicinity of Sitapur District, Uttar Pradesh, India.

### b. Study of the effect of fungal staling growth substances on establishment of composite soil mycoflora by using Warcup method

Sampled soil was crushed and sieved under aseptic conditions. About 15 ml of sterilized Czapek-Dox Agar medium was poured in petri plates. After solidification of agar in petri plates, soil impression was given over the whole agar disc by using a flat bottomed glass beaker, 5 series of plates each with 3 replicates were incubated at 25±2$^0$C for 24, 48, 72, 96 and 120 hrs of staling and thereafter the whole agar disc was placed upside down and the same composite soil inocula was pressed over the reverse agar discs and the fungi were observed and recorded after the full growth of fungal colonies. The percent of colonization was calculated by using the following formula.

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

No. of species present in soil is referred as species diversity. Population density was measured in terms of colony forming unit (CFU).

## 3) RESULTS

The result of the present papers has totally focused on the fact that the growth and colonization potential of the fungal community present in the composite soil inocula completely depends upon the antagonistic activity and fast growth rate. The result revealed that there was a successive decrease in the number five different staling periods i.e. 24,48,72,96 and 120 hours respectively.

### *3.1 Grouping of fungal species on the basis of tolerance potential*

On the basis of the tolerance potentiality of the composite soil

* Corresponding Author: Ms. Sangeeta
*Email address :* sangibhushan7184@gmail.com

mycoflora against the fungal staling growth substances produced by the earlier established mycoflora the fungal species were categorized into VI groups (Table No. 1).The fungal species which loomed in virgin (control) agar plate disappeared in the rest of the Petri plates after 24, 48, 72, 96 and 120 hours of staling periods, were placed in group I whereas the fungal sps. which were present after 24 hours of staling period were placed in group II Similarly, the fungal sp. second in order of tolerance potential because they were present on the reverse side of the agar disc plate after 96 hours of staling but were absent in agar plate after 96 hours of staling. Similarly *T. koningii, F. oxysporum* and sterile mycelium ranked third followed by *P. italicum, P. oxalicum, F. longipes, Helminthosporium, Rhizopus* sp. and *Chaetomium globosum* respectively.

***Fungal population***

**Table 1:** Colonization pattern of composite soil mycoflora in the presence of staled agar after different staling periods i.e. 24, 48, 72, 96 and 120 hours

| **Names of the fungal species** | **Different staling periods (h)** | | | | | |
|---|---|---|---|---|---|---|
| **Group I** | **0** | **24** | **48** | **72** | **96** | **120** |
| *Alternaria alternata* | + | - | - | - | - | - |
| *Humicola sp.* | + | - | - | - | - | - |
| **Group II** | | | | | | |
| *Helminthosporium* | + | + | - | - | - | - |
| *Rhizopus sp.* | + | + | - | - | - | - |
| *Chaetomium globosum* | + | + | - | - | - | - |
| **Group III** | | | | | | |
| *Penicillium italicum* | + | + | + | - | - | - |
| *Penicillium oxalicum* | + | + | + | - | - | - |
| *Fusarium longipes* | + | - | + | - | - | - |
| **Group IV** | | | | | | |
| *Trichoderma koningii* | + | + | + | + | - | - |
| *Fusarium oxysporum* | + | + | + | + | - | - |
| Sterile mycelium | + | - | + | + | - | - |
| **Group V** | | | | | | |
| *Penicillium chrysogenum* | + | + | - | + | + | - |
| *Aspergillus sulphureus* | + | - | + | + | + | - |
| *Trichoderma viride* | + | + | + | + | + | - |
| **Group VI** | | | | | | |
| *Penicillium citrinum* | + | + | + | + | + | + |
| *Aspergillus luchuensis* | + | + | - | - | + | + |
| *Aspergillus niger* | + | + | + | + | - | + |
| *Aspergillus flavus* | + | - | + | + | + | + |
| **Total species** | **18** | **12** | **11** | **9** | **6** | **4** |

+ = Presence, - = Absence

which do not get disappeared after 48 hours of staling period were placed in group III and so on . This means that the fungal species which were present after120 hours of the long staling period were placed in group VI and they were highly antagonistic and have a maximum tolerance potential as compared to the fungal species of other 4 groups. The result (Table No. 1) revealed that after 120 hours of staling only *P. citrinum, A. luchuensis, A. niger* and *A. flavus* unexpectedly loomed on the reverse side of the agar disc showed that they have high tolerance potential against the fungal staling growth substances released by the early established fungal colonies whereas *P. chrysoginum , A. sulfureus* and *T. viride* ranked

The microbial population of the highly resistant microfungi has been presented in Table 2.*Aspergillus flavus, P. citrinum, A. niger* and *A. luchuensis* ranged between 4.33 to 0.67 cfu/g soil. The microbial population of *A. flavus* (4.33 cfu/g soil), *P. citrinum* (4), *A. niger* (14) and *A. luchuensis* (5.38) were highest in the virgin agar plate in comparison to microorganisms present in other groups i.e. from group I to group V. It was noticed that the fungal diversity and fungal population decreases gradually with the increase in the fungal staling periods. In the virgin agar disc plate 18 fungal species were present but as the staling period increased the number of fungal species decreased. Only 16 fungal species were present

after 24 hours of staling periods whereas 13, 10 and 7 fungal species were present after 48, 72 and 96 hours of staling period. After 120 hours of staling only 4 fungal species were

In virgin agar disc plate the colonization percent of dominant sps. Were *P. citrinum* (9.63%), *A. luchuensis* (8.89%), *A. niger* (8.89%) and *A. flavus* (11.85%) followed by *P.*

**Table 2:** Fungal population in composite soil inocula on staled agar discs after 24, 48, 72, 96 and 120 hours of staling periods (Warcup method)

| **Fungal population (cfu/g soil )** | | | | | | |
|---|---|---|---|---|---|---|
| **Names of the fungal species** | | **Different staling periods (h)** | | | | |
| **Group I** | **0** | **24** | **48** | **72** | **96** | **120** |
| *Alternaria alternata* | 1.33 ± 0.58 | | | | | |
| *Humicola* | 1.00 ± 0.00 | | | | | |
| **Group II** | | | | | | |
| *Helminthosporium sp.* | 1.33 ± 0.58 | 1.33 ± 0.58 | | | | |
| *Rhizopus sp.* | 1.33 ± 0.58 | 0.67 ± 0.58 | | | | |
| *Chaetomium globosum* | 1.00 ± 0.58 | 0.67 ± 0.58 | | | | |
| **Group III** | | | | | | |
| *Penicillium italicum* | 1.67 ± 0.58 | 1.00 ± 0.00 | 1.00 ± 1.00 | | | |
| *Penicillium oxalicum* | 2.00 ± 0.00 | 1.67 ± 0.58 | 0.67 ± 0.58 | | | |
| *Fusarium longipes* | 2.33 ± 0.58 | 0.00 ± 0.00 | 0.67 ± 0.58 | | | |
| **Group IV** | | | | | | |
| *Trichoderma koningii* | 2.67 ± 0.58 | 2.00 ± 0.00 | 1.67 ± 0.58 | 1.33 ± 0.58 | | |
| *Fusarium oxysporum* | 2.33 ± 0.58 | 1.33 ± 0.58 | 1.33 ± 0.58 | 0.67 ± 0.58 | | |
| White Sterile mycelium | 2.33 ± 0.58 | 0.00 ± 0.00 | 0.67 ± 0.58 | 1.67 ± 0.58 | | |
| **Group V** | | | | | | |
| *Penicillium chrysogenum* | 2.67 ± 0.58 | 2.67 ± 0.58 | 0.00 ± 0.00 | 1.67 ± 0.58 | 0.67 ± 0.58 | |
| *Aspergillus sulphureus* | 3.00 ± 0.00 | 0.00 ± 0.00 | 1.67 ± 0.58 | 1.33 ± 0.58 | 0.67 ± 0.58 | |
| *Trichoderma viride* | 2.33 ± 0.58 | 2.00 ± 0.00 | 1.67 ± 0.58 | 1.00 ± 0.00 | 0.67 ± 0.58 | |
| **Group VI** | | | | | | |
| *Penicillium citrinum* | 4.00 ± 1.00 | 3.33 ± 0.58 | 0.00 ± 0.00 | 0.00 ± 0.00 | 2.00 ± 0.00 | 1.00 ± 1.00 |
| *Aspergillus luchuensis* | 5.33 ± 0.58 | 0.00 ± 0.00 | 3.67 ± 0.58 | 2.67 ± 0.58 | 2.33 ± 0.58 | 1.33 ± 0.58 |
| *Aspergillus niger* | 4.00 ± 1.00 | 3.33 ± 0.58 | 3.00 ± 0.00 | 2.33 ± 0.58 | 0.00 ± 0.00 | 0.67 ± 0.58 |
| *Aspergillus flavus* | 4.33 ± 0.58 | 3.00 ± 0.00 | 3.00 ± 1.00 | 2.33 ± 0.58 | 1.67 ± 0.58 | 1.00 ± 0.00 |
| ****=significant at 1%** | ****** | ****** | ****** | ****** | ****** | ****** |
| **CV%** | **23.09** | **30.12** | **45.83** | **46.18** | **68.47** | **136.95** |

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

able to exist. It was also observed that the number of fungal count also decreases with the increase in staling periods.

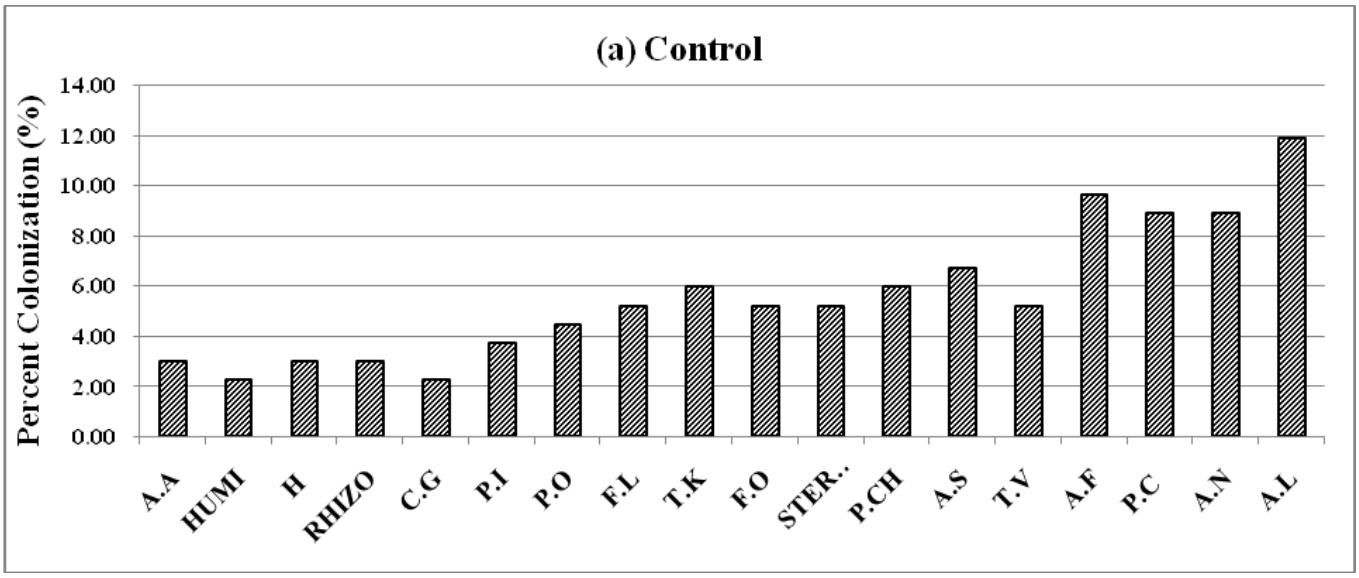
### *3.2 Colonization percentage*

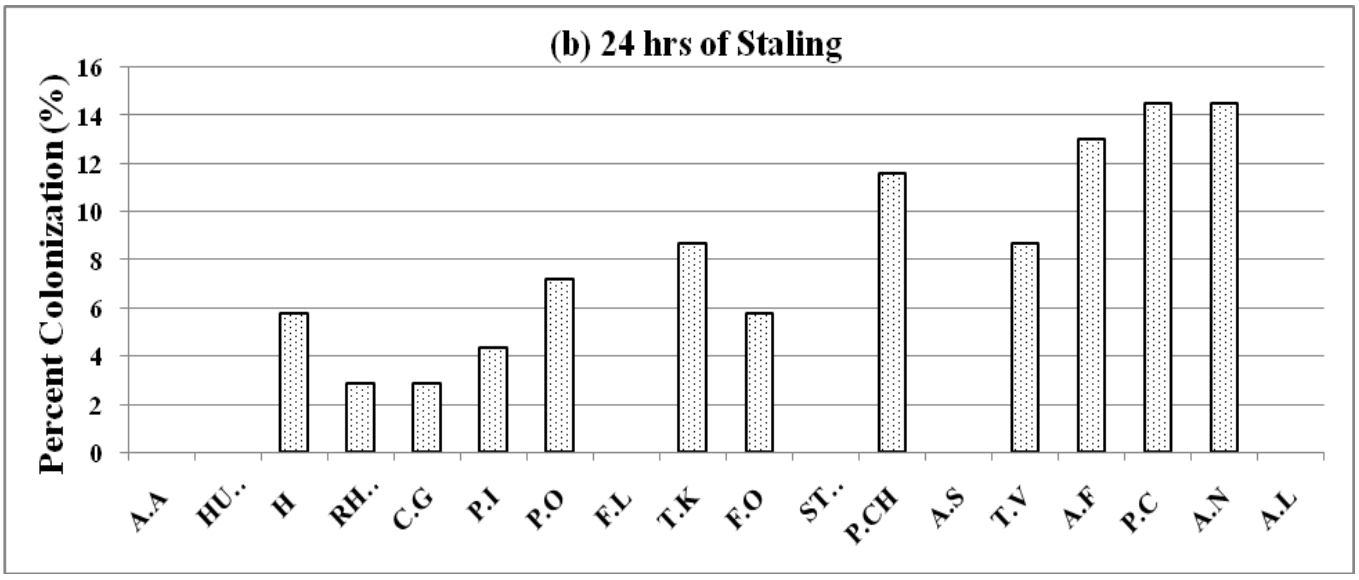
The result of the percent of colonization (Fig. 1) revealed that the most tolerant microfungal communities have highest percent colonization. It was observed that percent colonization of *P. citrinum* (25%), *A. luchuensis* (25%), *A. niger* (16.67%) and *A. flavus* (33.33%) in staled agar disc after 120 hours of staling whereas colonization percent after 96 hours of staling were *P. citrinum* (20.83%), *A. luchuensis* (25%) and *A. flavus* (29.17%) followed by *P. chrysogenum*, *A. sulphurous* and *T. viride* having 8.33% colonization percent and were placed in group V.

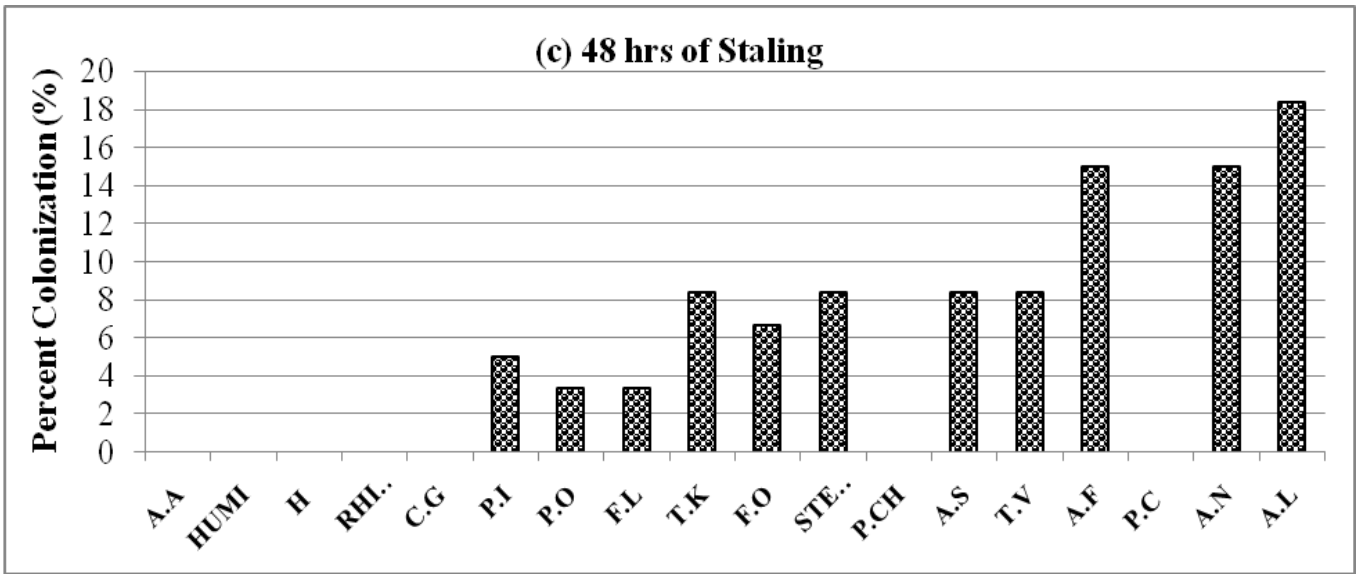
*chrysogenum* (5.93%), *A. sulphureus* (6.67%), *Trichoderma viride* (5.19%) and so on.

## 4) DISCISSION

It has been reported that most soils inhibit the germination and growth of fungi to a certain extent and the phenomenon is known as soil fungistasis [1] and was described by Dobbs and Hinson [2]. The fungistasis intensity depends on the physical and chemical property as well as on the soil microbial activity [3,4,5]. Fungi differ in sensitivity to fungistasis. It was reported that phytopathogenic fungi are more sensitive to fungistasis than saprophytic fungi [6].


(a) Control
Percent Colonization (%)
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
A.A
HUMI
H
RHIZO
C.G
P.I
P.O
F.L
T.K
F.O
STER..
P.CH
A.S
T.V
A.F
P.C
A.N
A.L


(b) 24 hrs of Staling
Percent Colonization (%)
16
14
12
10
8
6
4
2
0
A.A
HU..
H
RH..
C.G
P.I
P.O
F.L
T.K
F.O
ST..
P.CH
A.S
T.V
A.F
P.C
A.N
A.L


(c) 48 hrs of Staling
Percent Colonization (%)
20
18
16
14
12
10
8
6
4
2
0
A.A
HUMI
H
RHI..
C.G
P.I
P.O
F.L
T.K
F.O
STE..
P.CH
A.S
T.V
A.F
P.C
A.N
A.L

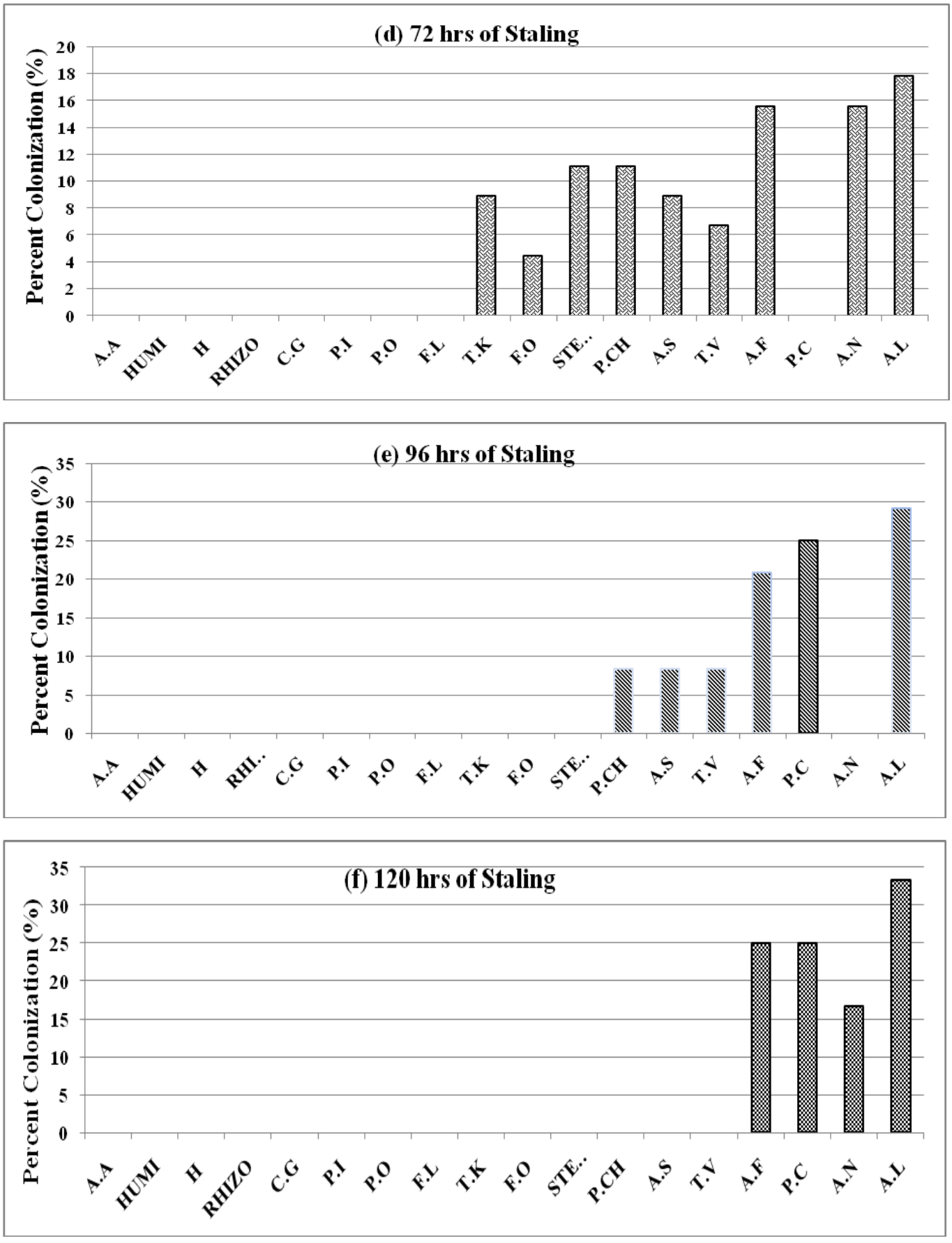



**Fig. 1:** Percent colonization of composite soil mycoflora (1:1000 dilution) on nutrient virgin agar and staled agar disc after different periods of staling (a) Control, (b) After 24 Hrs of staling, (c) After 48 Hrs of staling, (d) After 72 Hrs of staling, (e) After 96 Hrs of staling and (f) After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

The fungus culture becomes staled after a period of growth. The phenomenon of staling is a complex process and is generally detected by reduction in growth rate. Many workers have focused on the metabolic products as growth inhibitor, in relation to the process of staling [7]. In the past years much emphasis has been paid on effect on fungal staling growth product in microbial interaction [8]. Most of such studies were conducted on agar plate which gives a presumptive result of antagonism [9]. However, the result obtained gave the necessary information about microbial interaction [10]. In the present study the effect of the fungal staling growth substances of precolonized fungal colonies on the growth of the mycoflora of the composite soil mycoflora have received little attention. It was noticed that there was a change in the growth pattern of the fungal colonies after different staling periods this may be due to the diffused growth substances produced from the earlier established fungal colonies [11]. Antibiotics beside pH play a significant role in the mycostatic phenomena leading to colonization [12].

There was a retrogressive decrease in the number of fungal colonies on the reverse side of the agar disc after different staling period. This successive decrease in the fungal population was may be due to the diffusion of antibiotics produced from the early established fungal colonies [13,14]. The colonization percentage washighest for the fungal species whose microbial population was high. This was due to the fact that the colonization percentage of a particular fungus depends upon its population level in the soil [15]. The growth rate and colonization percent depend upon fungal population and its tolerance potential.

## 5) CONCLUSION

From the present study it may be concluded that interfungal competition and growth of fungal colonies on staled agar disc plate is a very complicated process. The growth, establishment and percent colonization of a fungal species on staled agar disc depends upon its population level in soil and tolerance potential against the fungal growth substances released by the early established fungal communities of composite soil inocula.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