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# STUDIES ON SOIL FUNGISTASIS: EFFECT OF CERTAIN PHYSICAL AND BIOLOGICAL FACTORS

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SUMMARY

The effect of different physical and biological factors like soil sterilization, incubation period of soil, spore age, amendment of certain fungal species and their metabolites on soil fungistasis has been investigated.

Different degree of sterilization affected the fugistasis differently. Soil heating above 80°C completely annulled the fungistasis. No fungistasis was recorded in soil samples steamed for 15 mts in an autoclave.

Incubation of soil samples to longer duration resulted in increased fungistasis. Maximum fungistatic value was noted in samples incubated for 15 days at 25 $\pm$ 1°C.

Spore age also played important role in fungistasis. A positive relation was noted in the spore age and fungistasis upto 30 days of age and thereafter the increase in fungistasis was not well marked.

Varying inhibitory effect was noted on the spore germination of the test fungi in relation to amendment of certain fungi individually and in different combinations to the soil. *Aspergillus flavus* alone and in combination of *Aspergillus niger* proved most inhibitory. The filtrate of the different fungi also induced fungistasis in soil. In this case also *A. flavus* was most effective.

## INTRODUCTION

The origin of soil fungistasis is considered to be biological but which constituent (s) of soil micro-population is (are) particularly responsible for this phenomenon is not yet clear. The origin of fungistasis in soil by microbial activities is evident from the fact that it can be completely removed from soil by sterilization, fumi-

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gation or by long air drying and that it is lessened when microbial activities are reduced and can be restored in sterilized soil inoculated with natural soil, specific organisms and/or plant materials[8 9].

The phenomenon of fungistasis, however, is not yet clearly understood in relation to different physical and biological factors like different degree of sterilization, incubation period, spore age, addition of fungal species and their metabolites to the soil. In this paper an effort has been made to investigate the role of the soil parameters on the soil fungistasis of two cultivated fields.

## MATERIALS AND METHODS

Soil samples for the study of soil fungistasis were collected from two cultivated fields cropped with *Cajanus cajan* and *Lathyrus sativus*. The soil samples collected at different stages of the plants growth were air dried, finely powdered and then used for the experiments detailed below.

Soil samples collected were kept separately in an electric oven at 40, 50, 60, 70, 80, 100 and 120°C for 6 hrs and the sterilization of soil samples was done in autoclave for different time intervals *i.e.* 5, 10, 15 and 20 mts at 15 lb/sq. inch pressure. The soil samples thus treated were tested for fungistasis by method of Jackson[5].

Mycoflora of the above soil samples was also estimated by dilution plate method using Martin's medium.

In another set of the experiment the soil samples collected from the fields of the two crops after powdering and sieving were packed in the petriplates. The plates were incubated at 25 $\pm$ 1°C for 1, 4, 7 and 15 days and thereafter fungistasis was assessed by a method suggested by Jackson[5]. Throughout the course of study the moisture status of the soil samples was maintained at 25 percent, by adding sterilized distilled water.

This part of the experiment consisted of the evaluation of fungistasis in relation to spore age The spores of *Rhizopus nigricans, Aspergillus flavus, Penicillium oxalicum, Verticillium albo-atrum, Curvularia lunata, Helminthosporium sativum* and *Fusarium oxysporum* harvested from 7, 10, 15, 20, 25, 30, 45, 60 and 90 days-old culture medium (PDA) were used for the study of fungistasis in relation to spore age. The test fungi were maintained in triplicate for each type of spore age. After the desired age the spores were removed and spore suspension was prepared in sterilized double distilled water, centrifuged at 5000 rpm, diluted with sterilized double distilled water so that each microscopic field contained about 50 spores. After preincubation (6–8 h) one drop of the above suspension was pipetted out separately on agar blocks with a sterilized pipette. Inoculated plates were again incubated at 25 $\pm$ 2°C for 24 h and thereafter the agar blocks were gently removed from the surface of the soil. The surface of the block which was in contact

with the soil was then sliced off with the help of cover glass and kept on slide. All the blocks removed at a time were killed and stained with lactophenol cotton blue and were examined under microscope for the germination of fungal spores. The percentage inhibition was calculated on the basis of 1000 spore number counted.

In this part of the investigation the effect of soil amendment with seven days old cultures of *Rhizopus nigricans, Mucor hiemalis, Aspergillus niger, A. flavus, A. sydowi, A. terreus, Penicillium oxalicum, Curvularia lunata, Acrophialophora fusispora, Alternaria lumicola, Helminthosporium sativum* and *Fusarium oxysporum* on fugistasis was studied. Ten circular agar blocks of uniform size (1 cm) of the above fungi grown on PDA medium were mixed separately to 50 g of sterilized soil. The plates were then incubated at 25 ± 1°C for 15 days and thereafter, fungistasis was determined. For an other set of this experiments the fungi grown similarly were mixed together in different combinations, *viz A. niger + A. flavus, A. flavus + A. tenuis, A. niger + H. sativum, A. tenuis + F. oxysporum* and *A. tenuis + H. sativum*. In this case five blocks of each constituent measuring 1 cm in diameter were mixed and incubated as described above and the fungistasis was assessed. The fungistasis of sterilized soil was also determined for reference.

The effect of culture filtrate of *M. hiemalis, A. niger, A. flavus, A. sydowi, P. oxalicum, A. tenuis, H. sativum* and *F. oxysporum* on the fungistasis was studied in this experiment. The fungi were grown separately in 250 ml flasks containing 100 ml sterilized liquid Czapek's medium in an incubator (25 ± 1°C) for 15 days. The flasks were intermittently shaken during the incubation period and thereafter the medium was filtered through Whatman filter paper no. 1 and finally through millipore filter (0.22 μ). The clear solution thus obtained was designated as culture filtrate. The culture filtrate of above fungi was mixed with sterilized soil at the rate of 1 ml per 10 g of soil and the fungistasis was assessed.

In all the experiments the fungistasis was assessed by the method suggested by Jackson[5] and the test organisms used were *Rhizopus nigricans, Aspergillus flavus, Penicillium oxalicum, Verticillium albo-atrum, Curvularia lunata, Helminthosporium sativum* and *Fusarium oxysporum*. The fungistasis has been expressed in terms of percentage inhibition which has been calculated on the basis of 1000 spore number counted.

## RESULTS

### *Effect of sterilization on fungistasis*

(i) Oven sterilization. A perusal of the table 1 indicates that as the temperature increases the fungistatic value decreased. The fungistasis was very much reduced at 80°C. At 100°C and above the fungistasis was lost.

The soil samples of *L. sativus* field exhibited higher fungistatic value than the samples from *C. cajan* field. The organisms tested

TABLE 1

Effect of soil sterilization (in hot air oven) on fungistasis

| Temp. (°C) | Test fungi | | | | | | | Mycopopulation (cols./plate) |
|---|---|---|---|---|---|---|---|---|
| | RN | AF | PO | VA | CL | HS | FO | |
| *Cajanus cajan* | | | | | | | | |
| 40 | 74 | 83 | 82 | 54 | 32 | 33 | 52 | 57 |
| 50 | 66 | 72 | 71 | 38 | 26 | 28 | 28 | 39 |
| 60 | 58 | 59 | 61 | 23 | 12 | 13 | 12 | 27 |
| 70 | 43 | 48 | 50 | 16 | 7 | 1 | 4 | 12 |
| 80 | 33 | 36 | 41 | 0 | 0 | 0 | 0 | 2 |
| 100 | 24 | 35 | 28 | 0 | 0 | 0 | 0 | 0 |
| 120 | 26 | 30 | 27 | 0 | 0 | 0 | 0 | 0 |
| *Lathyrus sativus* | | | | | | | | |
| 40 | 77 | 86 | 82 | 67 | 38 | 35 | 54 | 104 |
| 50 | 70 | 79 | 76 | 43 | 26 | 29 | 36 | 62 |
| 60 | 59 | 62 | 66 | 26 | 9 | 11 | 13 | 36 |
| 70 | 37 | 45 | 57 | 10 | 2 | 2 | 6 | 11 |
| 80 | 26 | 37 | 44 | 0 | 0 | 0 | 0 | 3 |
| 100 | 25 | 33 | 28 | 0 | 0 | 0 | 0 | 0 |
| 120 | 23 | 32 | 28 | 0 | 0 | 0 | 0 | 0 |
| *Control* | | | | | | | | |
| | 26 | 31 | 28 | 0 | 0 | 0 | 0 | — |

RN = *Rhizopus nigricans*, AF = *Aspergillus flavus*, PO = *Penicillium oxalicum*, VA = *Verticillium albo-atrum*, CL = *Curvularia lunata*, HS = *Helminthosporium sativum*, FO = *Fusarium oxysporum*.

TABLE 2

Effects of steam sterilization of soil on fungistasis

| Time of sterilization in mts | Test fungi | | | | | | | Mycopopulation (cols./plate) |
|---|---|---|---|---|---|---|---|---|
| | RN* | AF | PO | VA | CL | HS | FO | |
| *Cajanus cajan* | | | | | | | | |
| 5 | 64 | 69 | 62 | 45 | 31 | 36 | 37 | 45 |
| 10 | 39 | 46 | 41 | 13 | 5 | 3 | 9 | 17 |
| 15 | 23 | 30 | 29 | 0 | 0 | 0 | 0 | 0 |
| 20 | 24 | 26 | 31 | 0 | 0 | 0 | 0 | 0 |
| *Lathyrus sativus* | | | | | | | | |
| 5 | 69 | 78 | 71 | 55 | 31 | 38 | 48 | 59 |
| 10 | 42 | 44 | 40 | 34 | 3 | 6 | 13 | 34 |
| 15 | 24 | 32 | 31 | 0 | 0 | 0 | 0 | 0 |
| 20 | 13 | 31 | 29 | 0 | 0 | 0 | 0 | 0 |
| *Control* | | | | | | | | |
| — | 25 | 30 | 28 | 0 | 0 | 0 | 0 | 0 |

* see Table 1

showed following trend of variation: *A. flavus* > *P. oxalicum* > *R. nigricans* > *V. albo-atrum* > *F. oxysporum* > *H. sativum* > *C. lunata* in case of both the sets of soil. Decrease in mycoflora was noted when the temperature increased and above 80°C no fungi were recorded (Table 1).

(ii) Steam sterilization. The trend of variation in fungistatic values of the different fungi was nearly the same as in case of oven sterilization. No fungistasis was noted in the soil samples steamed for 15 mts and above (Table 2).

The mycopopulation also decreased with increase in steaming period. After 15 mts of steaming no fungal species could be isolated from the soil samples (Table 2).

*Evaluation of fungistasis in relation to incubation period*

Increase in fungistasis was marked with increase in incubation period. The soil samples incubated for 15 days exhibited maximum fungistatic value. Generally the fungistasis of *C. cajan* soil was higher than that of *L. sativus*. The sensitivity of different test organisms to fungistasis varied greatly and was generally maximum in the case of *A. flavus*. Other fungal species exhibited the following pattern; *R. nigricans* > *V. albo-atrum* > *C. lunata* > *F. oxysporum* > *H. sativum* (*Cajanus cajan*) and *R. nigricans* > *V. albo-atrum* > *F. oxysporum* > *H. sativum* > *C. lunata* (*Lathyrus sativus*) (Figs. 1 and 2).

*Effect of spore age on soil fungistasis*

With increase in spore age, increase in soil fungistasis was recorded for all the test fungi. Soil samples exhibited marked increase in fungistasis upto 30 days of spore age and thereafter the figures obtained for fungistasis did not vary much (Fig. 3).

Amongst the various soil samples amended with individual fungal species maximum fungistasis was recorded in soil amended with *A. flavus*. The remaining fungal species exhibited following trend of variation in fungistasis: *A. niger* > *H. sativum* > *P. oxalicum* > *A. terreus* > *A. sydowi* > *F. oxysporum* > *A. tenuis* > *C. lunata* > *R. nigricans* > *A. fusispora* > *H. hiemalis* in case of *Cajanus cajan*; *A. niger* > *A. sydowi* > *P. oxalicum* > *A. terreus* > *H. sativum* > *F. oxysporum* > *A. tenuis* > *C. lunata* > *A. fusispora* > *R. nigricans* > *M. hiemalis* in case of *Lathyrus sativus* (Table 3).

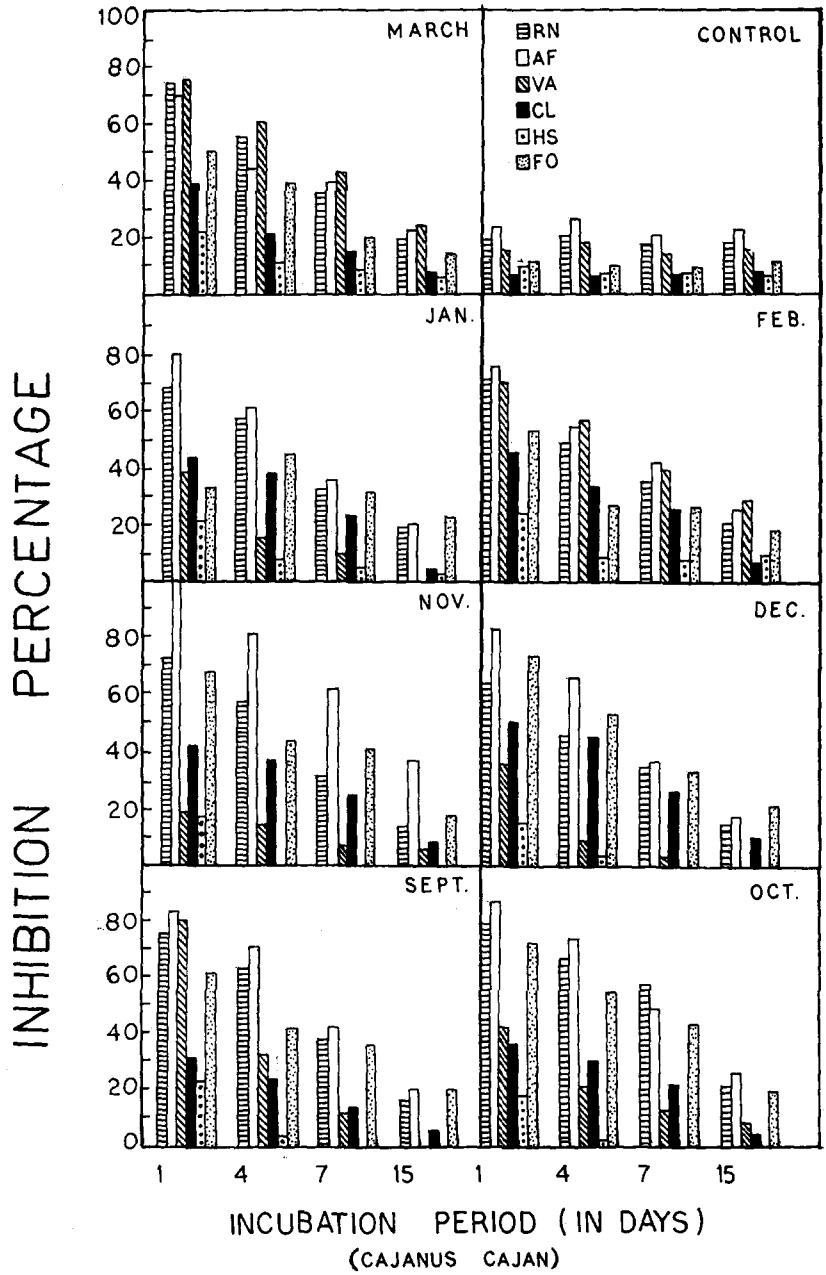


Fig. 1. Effect of length of incubation period on rate of fungistasis (percentage inhibition).

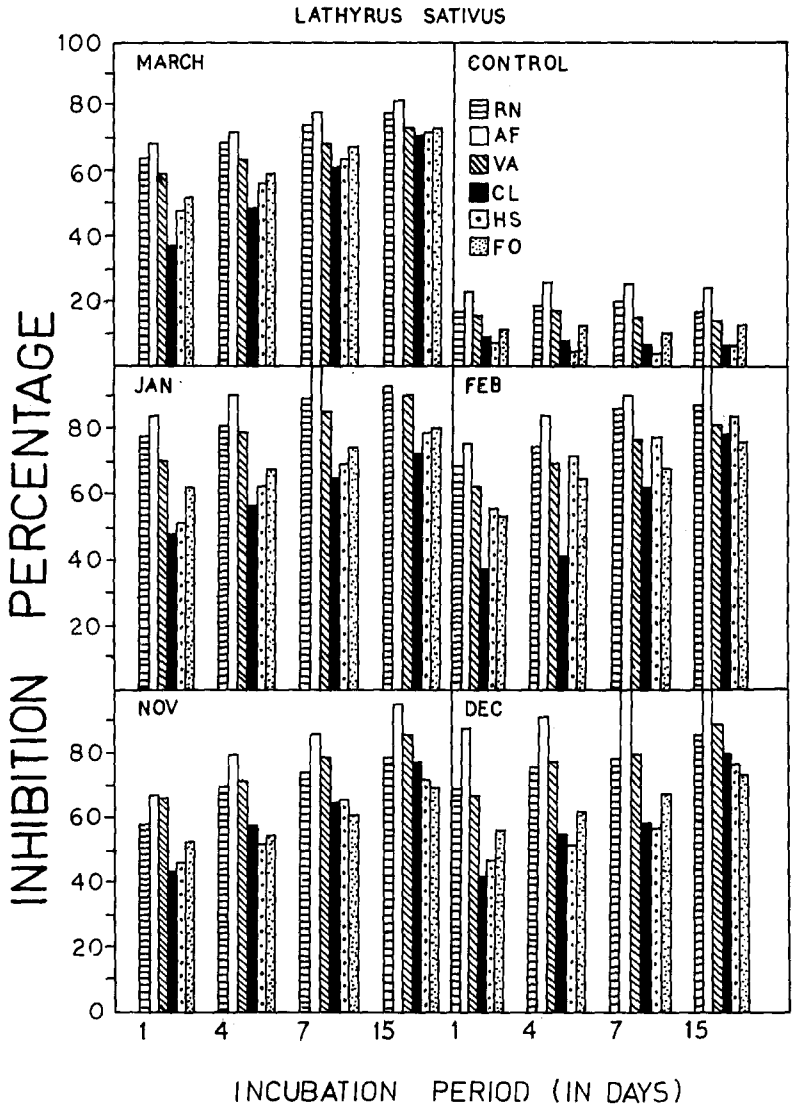


Fig. 2. Effect of length of incubation period on rate of fungistasis (percentage inhibition).

Maximum fungistasis was observed in the soil samples amended with the mixture of *A. flavus* and *A. niger*. In other sets the value obtained was comparatively low (Table 3).

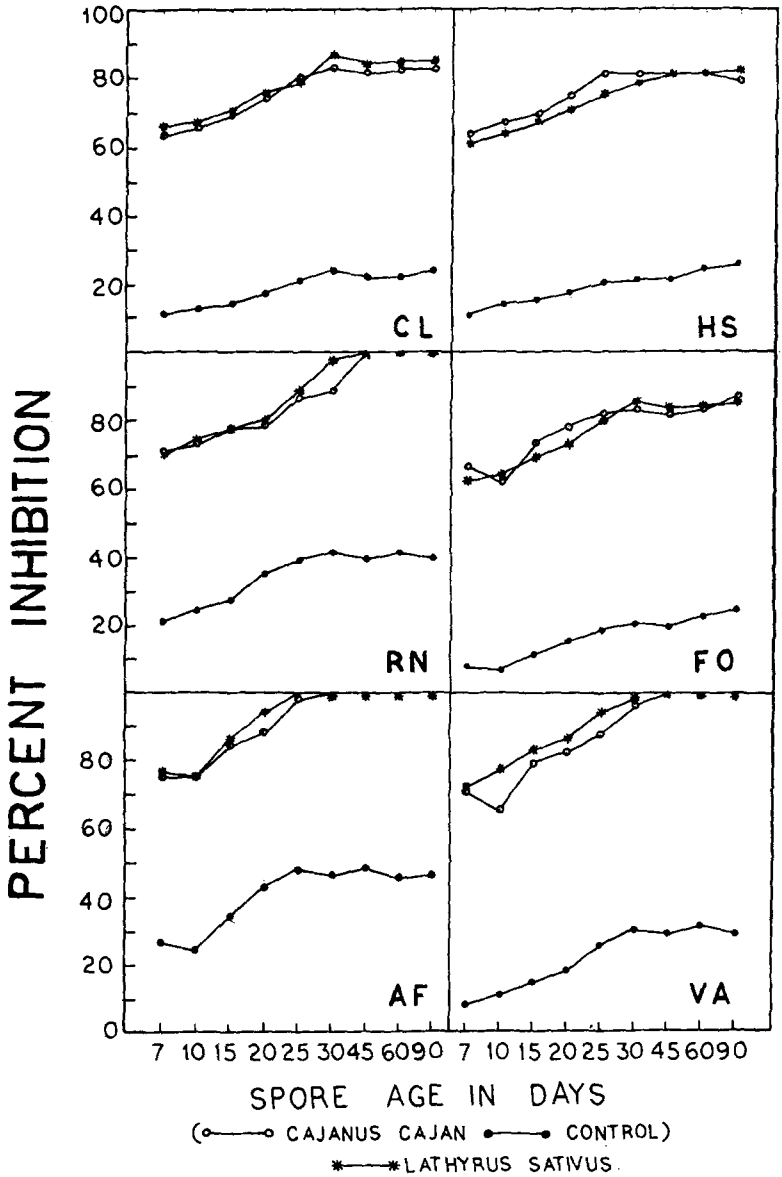


Fig. 3. Effect of Spore age on soil fungistasis.

TABLE 3

Variation in fungistasis in soil samples of *Cajanus cajan* and *Lathyrus sativus* amended with certain fungal species

| Fungi amended | Test fungi | | | | | | |
|---|---|---|---|---|---|---|---|
| | RN* | AF | PO | VA | CL | HS | FO |
| *Rhizopus nigricans* | 27(29)** | 42(33) | 31(30) | 25(32) | 12(19) | 11(15) | 22(23) |
| *Mucor hiemalis* | 24(28) | 37(35) | 28(25) | 27(29) | 11(14) | 9(13) | 19(21) |
| *Aspergillus niger* | 64(71) | 94(97) | 81(86) | 89(100) | 58(61) | 69(64) | 77(81) |
| *A. flavus* | 69(77) | 100(100) | 85(90) | 97(100) | 67(67) | 72(69) | 81(87) |
| *A. sydowi* | 59(67) | 91(91) | 77(81) | 81(91) | 51(58) | 52(61) | 64(80) |
| *A. terreus* | 61(65) | 93(93) | 79(84) | 83(93) | 53(54) | 54(63) | 66(76) |
| *Penicillium oxalicum* | 63(66) | 95(96) | 69(95) | 87(94) | 55(56) | 57(65) | 68(77) |
| *Acrophialophora fusipora* | 26(32) | 40(31) | 34(27) | 30(36) | 15(21) | 8(16) | 26(27) |
| *Curvularia lunata* | 31(37) | 48(38) | 35(31) | 37(44) | 19(25) | 13(18) | 33(33) |
| *Helminthosporium sativum* | 66(63) | 96(95) | 81(77) | 86(87) | 58(55) | 61(56) | 74(69) |
| *Alternaria tenuis* | 37(46) | 49(54) | 42(39) | 44(51) | 27(29) | 29(33) | 41(41) |
| *Fusarium oxysporum* | 58(61) | 89(87) | 73(74) | 81(79) | 53(53) | 56(57) | 68(70) |
| *A. niger* + *A. flavus* | 72(82) | 100(100) | 84(94) | 100(100) | 87(71) | 94(78) | 89(84) |
| *A. flavus* + *A. tenuis* | 70(85) | 100(100) | 81(91) | 100(100) | 84(69) | 91(75) | 92(79) |
| *A. niger* + *H. sativum* | 69(81) | 100(100) | 80(89) | 100(100) | 81(67) | 89(73) | 90(83) |
| *A. tenuis* + *F. oxysporum* | 66(74) | 94(100) | 82(86) | 94(100) | 80(64) | 87(71) | 89(81) |
| *A. tenuis* + *H. sativum* | 63(83) | 96(100) | 83(90) | 91(100) | 82(63) | 83(77) | 94(85) |
| Sterilized soil | 22(25) | 29(28) | 27(23) | 21(24) | 9(11) | 6(12) | 13(17) |
| Control | 17(19) | 25(26) | 21(19) | 16(17) | 7(9) | 6(6) | 14(12) |

* See Table 1; ** Figures in the brackets stand for *L. sativus*

*Effect of fungal metabolites*

The soil samples amended with fungal metabolites exhibited much variation in fungistatic values. Maximum effect was observed in the samples amended with metabolites of *A. flavus* followed by *A. niger, H. sativum, A. tenuis, P. oxalicum, A. sydowi, F. oxysporum, M. hiemalis* in *Cajanus cajan* soil, and *A. niger, H. sativum, P. oxalicum, A. sydowi, A. tenuis F. oxysporum, M. hiemalis* in *Lathyrus sativus* soil (Table 4).

Significant variation was observed in the fungistatic value of the test fungi amended with fungal filtrate of the different fungi ($P = 0.05$).

## DISCUSSION

It is evident from table 1 and 2 that sterilization of the soil samples removed the fungistatic effect. Different degree of steriliza-

TABLE 4

Effects of cultural filtrate of certain fungi on fungistasis of *Cajanus cajan* and *Lathyrus sativus* field soi

| Fungi used | Test fungi | | | | | | |
|---|---|---|---|---|---|---|---|
| | RN* | AF | PO | VA | CL | HS | FO |
| *Mucor hiemalis* | 22(25)** | 31(37) | 23(30) | 27(29) | 15(11) | 14(13) | 20(17) |
| *Aspergillus niger* | 83(92) | 100(97) | 98(94) | 95(58) | 75(77) | 73(79) | 87(91) |
| *A. flavus* | 84(100) | 100(100) | 99(100) | 96(100) | 77(81) | 76(84) | 89(100) |
| *A. sydowi* | 71(77) | 83(89) | 79(83) | 87(84) | 65(68) | 68(69) | 78(74) |
| *Penicillium oxalicum* | 75(79) | 84(91) | 82(85) | 86(78) | 57(61) | 59(63) | 80(68) |
| *Helminthosporium sativum* | 79(83) | 89(98) | 82(89) | 82(85) | 65(61) | 64(56) | 71(58) |
| *Alternaria tenuis* | 77(79) | 85(89) | 81(84) | 79(73) | 62(61) | 61(58) | 67(68) |
| *Fusarium oxysporum* | 65(59) | 70(67) | 77(63) | 54(62) | 35(38) | 46(41) | 45(49) |
| Czapek's solution | 6(9) | 14(11) | 8(6) | 0(0) | 0(0) | 0(0) | 0(0) |
| Water | 17(15) | 23(26) | 19(21) | 17(19) | 9(10) | 10(7) | 16(13) |
| Control | 16(15) | 25(23) | 20(19) | 17(16) | 8(8) | 7(7) | 12(11) |

* See Table 1 ** Figures in the brackets stand for *L. sativus*

tion affected the fungistasis differently. The minimum fungistasis was recorded in the soil which was fully sterilized. Dobbs and Hinson[1] have concluded that autoclaving destroys the toxic microorganisms present in soil during sterilization. Increase in fungistasis in sterilized soil reinoculated with *Streptomyces* spp., bacteria and fungi has been reported by various workers[2 7 8 11]. Griffiths and Dobbs[4] demonstrated that autoclaved soil released a high concentration of reducing sugars into the soil which annulled the fungistasis of soil.

A continuous increase in fungistasis with the increase in incubation period was clearly marked (Figs 1–2). Incubation of soil samples under adequate moisture condition and suitable temperature to longer duration probably encouraged the multiplication of soil microbes which ultimately induced higher degree of fungistasis. Besides this, the longer duration of incubation probably resulted in accumulation of higher amount of fungistatic factor which ultimately induced high fungistasis.

From the findings presented in Fig. 3 it is evident that as the age of the spores of the test fungi increases, the inhibition percentage is also enhanced. This variation in spore germination was not much marked in the spore aged 25 days or more. With the advancement of spore age the viability of the fungus spores decreases which possibly resulted in increase in fungistasis.

The different fungal species isolated from the rhizosphere of the two plants when inoculated in sterilized soil samples individually and in different combinations inhibited the fungal spore germination of the test fungi (Table 3). *A. flavus* alone and in combination with *A. niger* proved highly fungistatic for the fungi tested. Both the fungi mentioned are well known for production of organic acids which probably affected the spore germination of the fungi under test. Fast growing habit and heavy sporulating nature of the two fungi may also affect the test fungi by nutrient deprivation in soil. Kiem and Webster[6] indicated that microbial activity was responsible for the inhibition of germination of sclerotia of *S. oryzae*. Inhibition was observed when the organisms were tested individually or in different groups. They suggested that inhibition of the sclerotia was probably due to formation of certain inhibitory substances by the organisms used. Similar results were also obtained by the authors in the present investigation. The metabolites of different fungi when amended induced fungistasis (Table 4). Park[10] remarked that the growth of a fungus may be inhibited by the accumulation of autotoxic metabolic by products or staling substances and not by the depletion of nutrients or of space. Griffin[3] also suggested that the fungistatic effect of natural soil may result in part from the general saprophytic activities of the soil microflora, and toxic metabolites other than specific antibiotic substances. Lockwood and Lingappa[8] noted that sterilized soil inoculated with various fungi, actinomycetes and bacteria produced some inhibitory effect on fungal spore germination while others did not show any such effect when tested against *Glomerella cingulata*.

In conclusion it may be said that the fungistasis in soil is a consequence of microbes inhabiting the environment. The mode of their action, however, is still not clear from the results of the present investigation. Whether they affect the spore germinations directly or through their metabolites is controversial because when tested either way the effect is inhibitory.

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