EVALUATION OF MYCOBACILLIN AND VERSICOLIN AS AGRICULTURAL FUNGICIDES

III. GROWTH PATTERN AND ANTIBIOTIC PRODUCTION IN SOIL BY *ASPERGILLUS VERSICOLOR*

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Soil supports the growth of a jute pathogen *Colletotrichum gloeosporioides* but only to a limited extent that of its antagonist *Aspergillus versicolor*. The growth of the sensitive pathogen is considerably checked by the antagonist in mixed soil culture although versicolin production could not be demonstrated within the limits of assay. Both the sensitive and the antagonistic organisms grow well in soil-compost medium and versicolin production by the latter is also enhanced. The antagonistic effect of *Aspergillus versicolor* on *Colletotrichum gloeosporioides* is expectedly more marked in soil-compost medium than in soil medium.

Various attempts were made in the recent past to reduce the population of soil borne pathogens by applying micro-organisms instead of microbial products. Antagonistic effect of *Trichoderma viride* and *Penicillium nigricans* was reported by WRIGHT[1,2] who demonstrated the production of gliotoxin by *T. viride* and griseofulvin by *P. nigricans* in soil supplemented with organic manure. That a strain of *Bacillus subtilis* possesses some antagonistic effect on *Erwinia amylovora* was also reported.[3]

The production of several antibiotics in soil was studied by GOTTLIEB *et al*[4~6]. The survival pattern of some plant pathogens in presence of antagonists in soil was also demonstrated[7~10].

Versicolin, an antifungal antibiotic elaborated by *Aspergillus versicolor* has recently been reported to be highly active against rice and jute fungal pathogens[11]. The present work aims at exploring the possibility of using *Aspergillus versicolor*, a versicolin producer organism, to combat infection by a jute pathogen *Colletotrichum gloeosporioides*.

Materials and Methods

Organisms: *Aspergillus versicolor* N_5 which elaborates versicolin was used as the antagonist, *Colletotrichum gloeosporioides* a jute pathogen as the sensitive organism and *Trichophyton rubrum* as the test organism for bioassay of versicolin by the cup-plate method.

Media: The different media used were:

Medium A: 50 g soil (collected from paddy fields of 24-Parganas, West Bengal, India) per 100 ml of water (pH 6.1).

Medium B: 10 g of organic manure (collected from Agricultural Farm, Govt. of West Bengal) per 100 ml of water (pH 5.0).

Medium C: 10 g of organic manure and 50 g of soil per 100 ml of water (pH 5.8).

Medium D: 10 g of organic manure per 100 ml of SABOURAUD's medium (pH 5.1).

Medium E: 2 g of urea and 50 g of soil per 100 ml of water (pH 6.1).

Medium S: SABOURAUD's medium (pH 5.7).

Growth and fermentation experiments: Thirty ml of any one of the above media taken in a 100-ml

Erlenmeyer flask and sterilized for 45 minutes at 15 lbs pressure except Medium S which was autoclaved for 20 minutes were inoculated with 1 ml of aqueous spore suspension of the organisms prepared from 10 days' old slant cultures. In mixed culture experiments the inoculum consisted of 0.5 ml of aqueous spore suspension (10^5/ml) of each of the organisms *viz. A. versicolor* and *C. gloeosporioides*. Incubation was done at temperature (28~29°C) under stationary condition.

Determination of Growth: Growth was generally measured in terms of dry mycelial weight. In media containing insoluble matter *e.g.* compost medium or soil-compost medium, mycelial growth was separated by being drawn off with a pipette having a broken end when the growth was very feeble in the form of scattered bits of mycelia and in later stages when growth formed a thick mat, separation was conveniently effected with a forceps. In cases where growth was too scanty to be measured by mycelial weight, the medium containing the growth was uniformly dispersed and colony counts were determined by plate and dilution methods and these values were taken as a measure of growth. In mixed culture experiments where total mycelial weight could not give an index of individual fungal population, colony count was taken as a measure of growth. The growth medium containing the mixed cultures was thoroughly shaken and 1 ml of the suspension serially diluted and plated in SABOURAUD's agar media. The plates were incubated at 30°C for 2 days. The colonies developing on agar plates being distinguishable were then counted for each of the two cultures.

Antibiotic Assay: Versicolin was assayed microbiologically according to the method of DHAR and BOSE[12]. In experiments where the concentration of versicolin was very low, *e.g.* in soil-compost medium the antibiotic was first extracted with amyl acetate and then assayed microbiologically. To determine if soil or compost itself contains any antifungal activity towards the test organism *T. rubrum*, uninoculated sterile soil-compost medium was extracted with amyl acetate and the extract assayed. The soil or compost contained no antifungal activity.

Analysis of Organic Manure: Two samples of organic manure collected from an Agricultural Farm, Govt. of West Bengal, were analysed for their total nitrogen (nitrate and ammonia), free and protein amino acids according to the standard method[13].

Results

Growth Pattern of *Aspergillus versicolor* in Soil Media

The growth pattern of *A. versicolor* N_5 in different media is shown in Fig. 1 and Fig. 2. It appears that soil supported restricted growth of the organism (Fig. 1, Curve A). Addition of urea to the soil did not make any appreciable change in the amount of growth obtained (Fig. 1, Curve E). Enrichment of soil with organic manure caused a definite improvement in growth (Fig. 2, Curve C). The maximum growth supportable in this medium was only 76.7% of that obtainable in SABOURAUD's medium enriched with organic manure (Fig. 2, Curve D). Thus organic manure which contains free and protein amino acids (Table 1) plays an important role in the enhancement of growth of *A. versicolor* in soil.

Table 1. Analysis of organic manure

Name of sample	Total nitrogen %	Total ammonia and nitrate nitrogen %	Total free and protein amino acids %
W. B. Govt. Agricultural Farm	3.0	0.3	14
Agricultural Farm C.U.	2.34	0.21	12

Versicolin Production in Soil Media

The rate of versicolin production in different soil media is shown in Fig. 3. It shows that within the limit of assay by cup-plate method versicolin production does not occur in pure soil as well as soil-urea media (Fig. 3, Curves A, E), but it did occur when soil was enriched with 10% organic manure

Fig. 1. Growth pattern of *A. versicolor* N_5 in Medium A and Medium E.
Medium A: 50 g soil per 100 ml of water.
Medium E: 2 g of urea and 50 g soil per 100 ml of water.

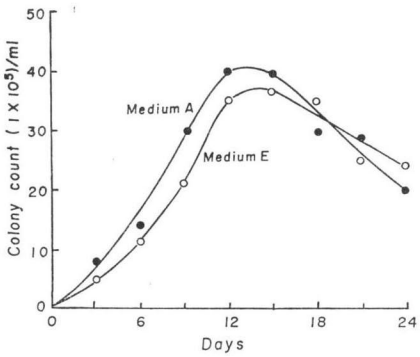


Fig. 2. Growth pattern of *A. versicolor* N_5 in different media.
Medium B: 10 g of organic manure per 100 ml water.
Medium C: 10 g of organic manure and 50 g soil per 100 ml of water.
Medium D: 10 g of organic manure per 100 ml of SABOURAUD's medium.
Medium S: SABOURAUD's medium.

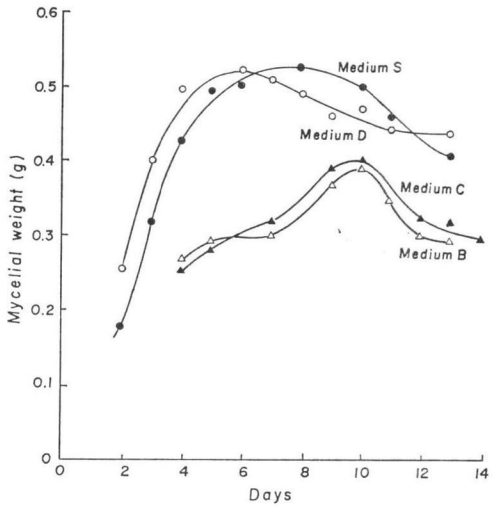
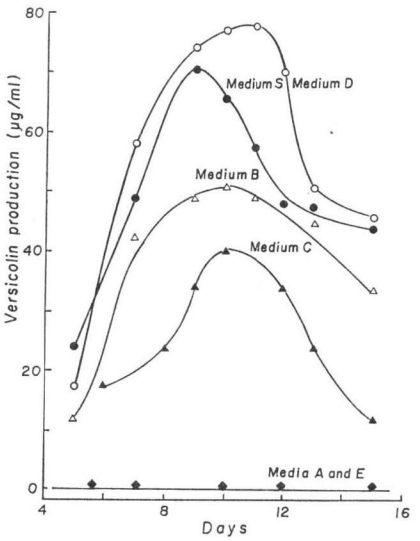


Fig. 3. Versicolin production by *A. versicolor* in different media.
Medium A: 50 g soil per 100 ml of water.
Medium B: 10 g of organic manure per 100 ml of water.
Medium C: 10 g of organic manure and 50 g soil per 100 ml of water.
Medium D: 10 g of organic manure per 100 ml of SABOURAUD's medium.
Medium E: 2 g of urea and 50 g soil per 100 ml of water.
Medium S: SABOURAUD's medium.



(Fig. 3, Curve C). However the yield was low being 56.3% of that obtainable in pure SABOURAUD's medium (Fig. 3, Curves C and S). It is of interest to note that the yield of the antibiotic which was fairly high in SABOURAUD's medium was further enhanced in presence of organic manure (Fig. 3, Curves S and D) which appears to have some additional stimulatory effect on versicolin production because of amino acid content, as previously observed[14].

Growth Pattern in Mixed Culture by Colony Count

The growth pattern of *A. versicolor* N_5 and *C. gloeosporioides*, studied in different media both in pure and mixed culture is shown in Figs. 4a, 4b and 4c. It appears that in pure culture *C. gloeosporioides* grows better than *A. versicolor* in soil medium (Fig. 4c) although in mixed culture in soil medium the growth of the former is considerably checked by the latter (Fig. 4c). Incorporation of organic manure into the soil medium which enhanced the growth of both the antagonist and plant pathogen, favoured relatively the former and thereby completely checked the growth of the latter (Fig. 4b). Thus the plant pathogen could not grow in mixed culture in soil medium not only in presence

Fig. 4a. The growth pattern of *A. versicolor* N_5 and *C. gloeosporioides* in pure and mixed cultures in Medium S.
Medium S: SABOURAUD's medium.

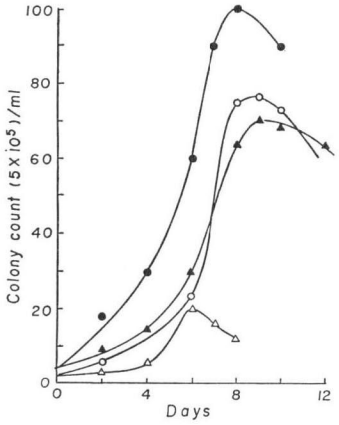


Fig. 4b. The growth pattern of *A. versicolor* N_5 and *C. gloeosporioides* in pure and mixed cultures in Medium C.
Medium C: 10 g of organic manure and 50 g soil per 100 ml of water.

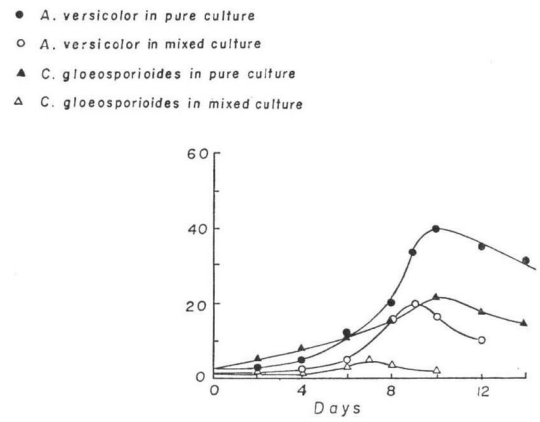


Fig. 4c. The growth pattern of *A. versicolor* N_5 and *C. gloeosporioides* in pure and mixed cultures in medium A.
Medium A: 50 g soil per 100 ml of water.

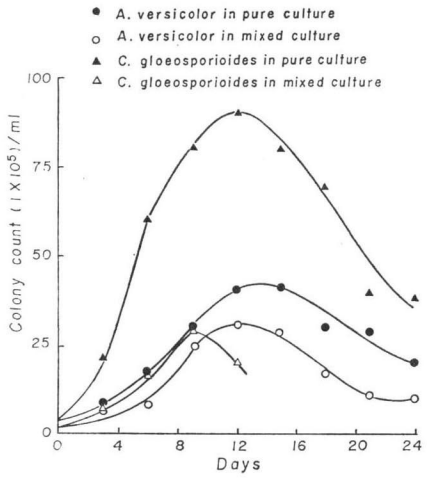
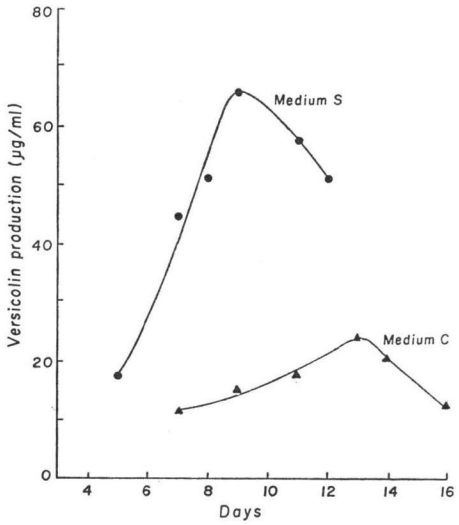


Fig. 5. Versicolin production by *A. versicolor* N_5 in mixed culture in Media C and S.
Medium C: 10 g of organic manure and 50 g soil per 100 ml of water.
Medium S: SABOURAUD's medium.



but also in absence of organic manure.

Versicolin Production in Mixed Culture

It appears that versicolin production occurred in mixed culture (Fig. 5, Curves S and C) although the yield of the antibiotic in pure culture showed some difference with that in mixed culture *e.g.* 71 μg/ml (Fig. 3, Curve S) as against 65 μg/ml (Fig. 5, Curve S) for Medium S or 40 μg/ml (Fig. 3, Curve C) as against 25 μg/ml (Fig. 5, Curve C) for Medium C.

Discussion

The study was undertaken to examine if it is possible to control the growth of plant pathogens by

the use of micro-organisms instead of microbial products. *A. versicolor* which produces versicolin was used as the antagonist and *C. gloeosporioides* as the sensitive plant pathogen. The growth pattern and antibiotic production of the antagonist was studied in soil media with or without enrichment by urea or organic manure. The growth of the antagonist in the soil medium was very restricted, but incorporation of organic manure greatly enhanced the growth rate. Concomitantly versicolin production which was nil within the limits of assay by the cup-plate method in pure soil and soil-urea medium was greatly favoured by incorporation of organic manure into the soil medium.

It is of interest to inquire if versicolin so produced *in situ* could inhibit the growth of the plant pathogen. It appears that *C. gloeosporioides* which grows in pure soil medium a little better than *A. versicolor*, was considerably inhibited by the antagonist whose versicolin production under identical condition could not be demonstrated by the cup-plate method of assay. This might be taken to mean that versicolin whose synthesis in pure soil medium was too small to be assayed microbiologically, was however sufficient to inhibit the growth of the plant pathogen. In mixed culture experiments using soil-compost media, the growth of the plant pathogen was completely inhibited by the antagonist whose versicolin production was also enhanced in soil-compost medium.

The possibility of control of plant pathogens by microbes instead of microbial products is therefore indicated.

References

1) WRIGHT, J. M.: The production of antibiotics in soil. II. Production of griseofulvin by *Penicillium nigricans*. Ann. Appl. Biol. 43: 288~296, 1955
2) WRIGHT, J. M.: The production of gliotoxin in soils. Nature 177: 896, 1956
3) DUN LEAVY, J. M.: Control of damping off of sugar beet by *B. subtilis*. Phytopathol. 42: 465, 1952
4) GOTTLIEB, D. & P. SIMINOFF: The production and role of antibiotics in the soil. II. Chloromycetin. Phytopathol. 42: 91~97, 1952
5) MARTIN, N. & D. GOTTLIEB: The production and role of antibiotics in the soil. III. Terramycin and aureomycin. Phytopathol. 42: 294~296, 1952
6) GOTTLIEB, D.; P. SIMINOFF & M. M. MARTIN: The production and role of antibiotics in soil. IV. Actidione and clavacin. Phytopathol. 42: 493~496, 1952.
7) BAKER, K. F.; N. T. FLENTJE, C. M. OLSEN & H. M. STRETTON: Effect of antagonists on growth and survival of *Rhizoctonia solani* in soil. Phytopathol. 57: 591~597, 1967
8) BAKER, R.: Mechanisms of biological control of soil borne pathogens. Ann. Rev. Phytopathol. 6: 263~294, 1968
9) SINGH, R. S.: Effect of *T. viridie* on the growth of *Pythium* sp. in sterilized and unsterilized soil. Naturwiss. 51: 173, 1964
10) RANGASWAMI, G. & M. RAMALINGAM: Survival of *Helminthosporium oryzae* in soil and its inhibition by *Bacillus mycoides*. Phytopathol. 52: 347~351, 1962
11) NANDI, J.; B. KUMAR DE & S. K. BOSE: Evaluation of mycobacillin and versicolin as agricultural fungicides. I. Antimicrobial spectrum and phyto-toxicity. J. Antibiotics 28: 988~992, 1975
12) DHAR, A. K. & S. K. BOSE: Mutagens for regeneration of an antibiotic-producing strain of *Aspergillus versicolor*. Appl. Microbiol. 16: 340~342, 1968
13) Soil Chemical Analysis. Edited by M. L. JACKSON: pp. 183~204, Prentice Hall of India Private Ltd. New Delhi, 1967
14) DHAR, A. K. & S. K. BOSE: Studies on versicolin, a new antifungal antibiotic from *Aspergillus versicolor*. II. Isolation and purification. Appl. Microbiol. 16: 749~752, 1968