

STIMULATION OF *RHIZOBIUM TRIFOLII* BY *STACHYBOTRYS ATRA*

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During studies on the interaction of *Stachybotrys atra* with soil microorganisms, *S. atra* was found to inhibit a range of fungi, actinomycetes and bacteria in agar culture (Butt & Ghaffar, 1972). *S. atra* did not inhibit *Rhizobium trifolii*, the root nodule bacterium of clover (*Trifolium alexandrinum*). *R. trifolii*, however was found to be stimulated by the metabolites secreted by *S. atra*. This is reported below:-

The culture of *S. atra* previously found colonizing roots of cotton (K.U. Bot. Dept. acc. No. 88) and *R. trifolii* isolated from nodules of clover (K.U. Bot. Dept. acc. No. 141) were used in this study.

Stimulation in agar culture:

Petri dishes containing about 15 ml of Czapek Dox agar, pH 7.0 were inoculated with *S. atra* by a making a streak on one side of the dish. The dishes were incubated for 4 days at 30°C after which a loopful of the *Rhizobium* was streaked at right angles to the *S. atra* culture. After 2 days, the gradual spreading in the medium containing secretions from *S. atra* cultures gave an indication of the stimulatory effect (Fig. 1) as compared to no stimulation in the control plates.

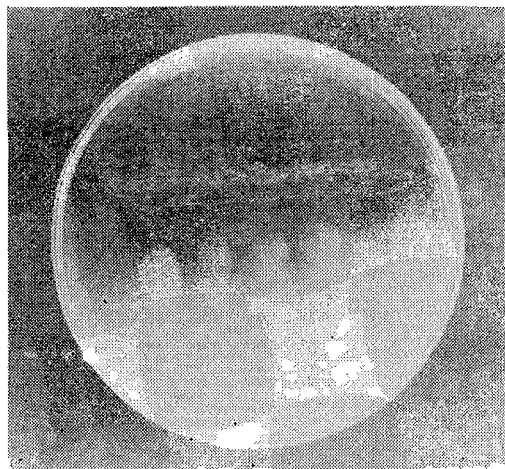


Fig. 1. Stimulation of *Rhizobium trifolii* by *Stachybotrys atra* on Czapek Dox agar at 30°C. *S. atra* on top of the dishes, *R. trifolii* streaked at right angles to *S. atra* culture; 2 streaks on the left made towards *S. atra* culture whereas 2 on the right streaked away from *S. atra*.

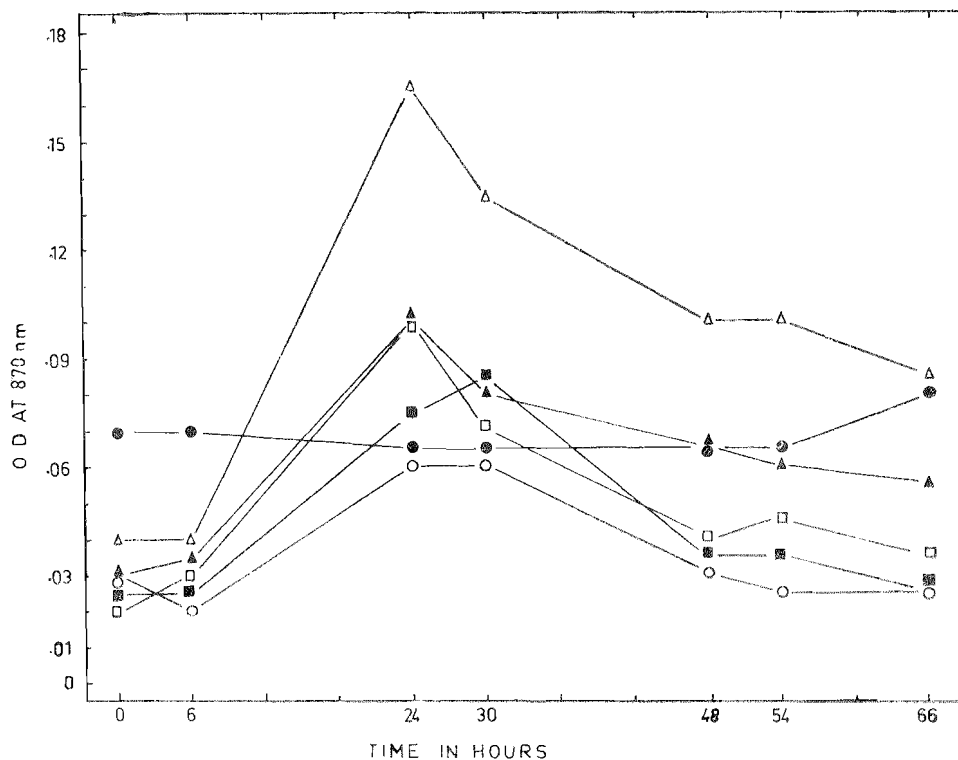


Fig. 2. Rate of growth of *Rhizobium trifolii* in Czapek Dox broth at 30°C containing different concentrations of *Stachybotrys atra* culture filtrate.

○ = 0:1, ● = 1:10, △ = 1:20, ▲ = 1:30, □ = 1:50, and ■ = 1:100 ratio of filtrate:broth (v/v).

Stimulation in broth culture:

S. atra was grown as still cultures on Czapek Dox broth at 30°C for 10 days from which culture filtrate of T.V. units 30 was obtained i.e., the number of times the original culture filtrate can be diluted and still prevent the germination of *Trichoderma viride* conidia (Ghaffar & Kaniz, 1970). The filtrate was mixed to Czapek Dox broth, pH 6.5, in 0:1, 1:10, 1:20, 1:30, 1:50 and 1:100 ratio of filtrate:broth (v/v). Five ml of these were inoculated with 0.1 ml of rhizobial cell suspension obtained from 2 day old *R. trifolii* slants and incubated at 30°C. Each treatment was replicated three times and the mean calculated. The rate of multiplication of rhizobial cells was observed by turbidity method and is expressed as Optical Density (O.D.) at wave length 870 nm (Fig. 2). At 1:10 dilution there was a lag of 54 hours after which a slight stimulation of *R. trifolii* was

noticed. In rest of the dilutions however, a marked stimulation of *R. trifolii* was observed after 24 hours as compared to control. After this stimulatory effect there appeared to be a decline in the rhizobial cells in all the treatments, including the control series due to depletion of nutrients or autolysis.

Conclusions

Preliminary results obtained during this investigation gave an indication of stimulation of *R. trifolii* by *S. atra*. The use of organisms stimulating *Rhizobia* is of practical importance keeping in view the increased yields of clover after treatment with mixed inocula of *R. trifolii*, *Pseudomonas* sp. and *Achromobacter* sp. (Allen & Allen, 1950). Similarly, two *Actinomyces* spp. have also been found to stimulate nodulation of red clover in sterilized soils (Robinson, 1945). Studies on the stimulatory effects of *S. atra* on *R. trifolii* in soil affecting nodulation and increased yield of clover, therefore, needs investigation. Considering the antagonistic effects of soil micro-organisms isolated from the root region of clover on *R. trifolii* (Hattingh & Louw, 1966; 1969), the use of *S. atra* will have added significance because of its wide antimicrobial spectrum (Butt & Ghaffar, 1972) and at the same time showing stimulatory effects on *R. trifolii* without any phytotoxic effects on clover, (*T. alexandrinum*).

References

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