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Soil Fungistasis against *Fusarium oxysporum* f. sp. *ciceri*, causing Wilt of Chickpea

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A marked inhibition of the macroconidial germination of *Fusarium oxysporum* f. sp. *ciceri* due to soil fungistasis by two soil types was observed. Fungistasis was more pronounced in top soils than in sub-soils. The fungistatic factor(s) was found to be seasonally variable, with higher levels of inhibition during summer and reduced inhibition during the other seasons. The seasonal variation in soil fungistasis was correlated with the atmospheric temperature, while rainfall and soil *p*H failed to show any relationship. Increase in soil depth was inversely related to soil fungistasis. Soil amendments with various forms of nitrogen and chitin enhanced the inhibition while cellulose and oatmeal amendments did not affect the fungistatic activity.

Key Words: Soil fungistasis, *Fusarium*, Soil-borne disease, Chickpea, Soil amendments

Introduction

As most of the soil-borne plant pathogenic fungi have to survive inter-substrate periods, the spontaneous germination of propagules or continuous hyphal growth would be highly disadvantageous to survival, as such, the 'exogenous dormancy' induced by soil fungistasis (Sussman 1965) provides the pathogen an added advantage for a long time survival. The degree of fungistasis in natural soils has been found to vary with soil depth (Griffiths 1966) and from season to season (Dobbs & Carter 1962, Dutta & Isaac 1979). Lockwood (1964, 1977) suggested the involvement of biological factors in soil fungistasis. Watson and Ford (1972) reviewed various hypotheses on soil fungistasis and proposed the concept of 'inhibitor-stimulator' balance, which controls the induction, maintenance and release of fungistasis. An attempt has been made in the present study to understand the soil fungistasis of two soil types in relation to seasonal changes, soil depth and soil amendments against the soil-borne chickpea wilt pathogen, *Fusarium oxysporum* f. sp. *ciceri*.

Materials and Methods

In the present study, red sandy loam soil from the Osmania University research fields (sand 74.8%; silt 17.2%; clay 8%; *p*H 8.17) and black soil from the fields near Patancheru, Hyderabad (sand 25.11%; silt 35.16%; clay 39.53%; *p*H 7.93) were employed. The culture of *F. oxysporum* f. sp. *ciceri* (Padw.) Snyd. & Hans. was obtained from the ICRISAT, Patancheru, Hyderabad and maintained on potato-sucrose-agar medium. Seasonal variation of fungistasis was studied in top and sub-soils for a period of one year employing the cellophane method (Dobbs & Hinson 1953) and agar slide method (Chinn 1953). However, only agar slide method was used for studying the vertical distribution. Soils were amended separately with cellulose, oatmeal and chitin at 2% level and also with different sources of nitrogen (0.5%N). In addition to the agar slide method, a modified soil emanation agar method (Hora & Baker 1972) was also employed for studying the effect of soil amendments.

Cellophane Method

A thin cellophane paper was cut into two inch squares, boiled to remove the surface dressing and autoclaved at 10 lbs. pressure for 5 min along with water. Tween-80 was applied to act as an adhesive and then the spore suspension was applied. The squares were later folded with the spores inside, partially buried in moist soil taken in porcelain jars and pressed firmly so as to have an intimate contact with the soil. The cellophane was removed after 24 hr of incubation and observed for fungistatic activity.

Agar Slide Method

The slides were dipped in the spore suspension made in 1% water agar. The slides carrying the spore suspension were inserted in the porcelain jars containing soil and were completely covered with the soil. After 24 hr of incubation, slides were removed carefully and rinsed away the adhering soil. Agar from one side of the slide was removed completely and observations were made from the agar remaining on the other side.

In the first series of experiments soils were autoclaved and in the second the soils were amended with 1% glucose solution.

Soil Emanation Agar Method

Spore-seeded agar discs (5mm diam.) were exposed for 24 hr above 100g soil taken in sterile Petriplates. Agar discs exposed above sterile distilled water served as control. After the incubation period the discs were observed for the fungistatic activity.

Results

Two levels of fungistasis were noted against the macroconidia of *F. oxysporum* f. sp. *ciceri* with the cellophane method in red sandy loam soil. The inhibition was maximum during summer (February-May) while reduced inhibition was noted during rest of the year. Similar pattern of inhibition was observed in the sub-soils of red sandy loam soil (figure 1). Cellophane method recorded two levels of fungistasis in top black soil also, but the inhibition during winter was not consistent though it was low. Black sub-soil supported high percentage germination of conidia showing no clear-cut demarcation between different seasons. The agar slide method recorded

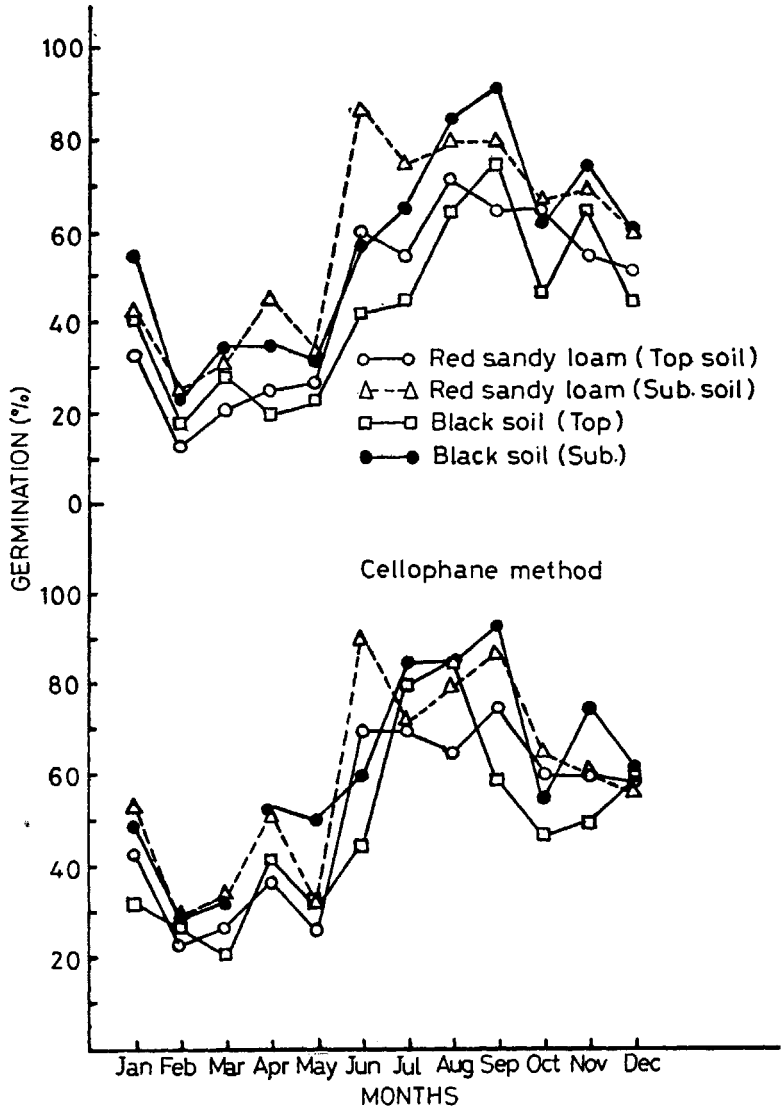

Figure 1 Percentage germination of *F. oxysporum* f. sp. *ciceri* in red sandy loam and black soils

low percentages of germination during summer and late winter (November and December) in red sandy loam soil while minimum inhibition was noticed during rainy season (June–October). Sub-soil clearly exhibited two levels of inhibition with minimum during rainy season and maximum during summer months. Black soil also exhibited the same pattern as the red sandy loam soil. Sub-soils exhibited comparatively less inhibition than top soils. Soil treatments like autoclaving and addition of 1% glucose annuled the fungistasis to a greater

extent. No relation was apparent between the pattern of rainfall during the year and soil *p*H but there appears to be a correlation between the temperature and fungistatic activity (figure 2).

In red sandy loam soil, an initial increase in spore germination percentage was recorded up to the depth of 90 cm but it declined gradually to 45% at 180 cm depth. In black soil, a gradual increase in germination occurred as the soil depth increased (figure 3). In general, the vertical distribution of fungistasis was similar in both the soils. Autoclaving and glucose amendment (1%) greatly reduced the inhibition of spore germination.

A positive response to various amendments was observed in red sandy loam soil and black soil (figure 4). Of all the amendments to red sandy loam soil, maximum inhibition was noticed in soils amended with nitrate + ammonium and urea followed by sodium nitrate, ammonium chloride, chitin and cowdung. No inhibition was noticed in oatmeal-amended soils, while cellulose-amended soils supported 87% germination. In black soil, nitrate-amendment produced the highest inhibition with agar slide method

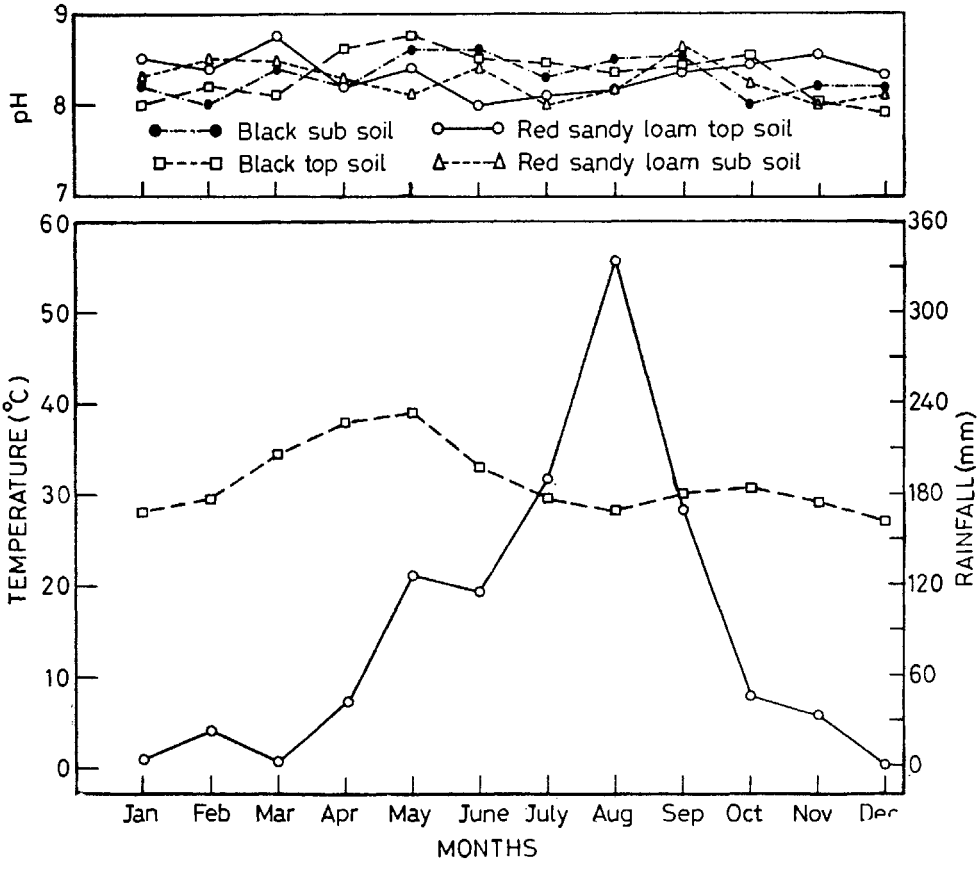

Figure 2 Distribution of soil *p*H, temperature and rainfall during the year 1979

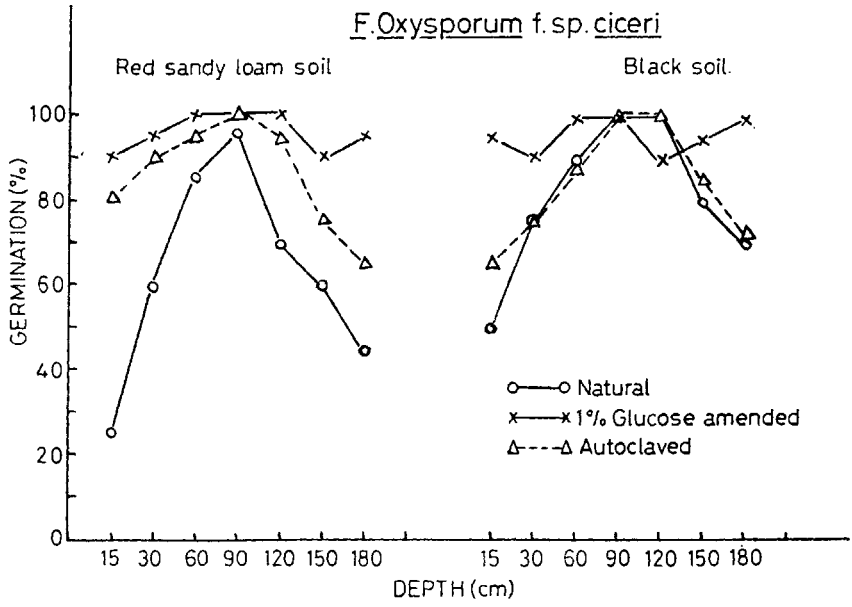

Figure 3 Vertical distribution of soil fungistasis in red sandy loam and black soils against *F. oxysporum* f. sp. *ciceri*

followed by ammonium chloride, nitrate + ammonium, urea and cowdung. Different levels of fungistasis were recorded with the soil emanation agar method under various amendments. Chitin- and ammonium-amended soils produced considerable inhibition whereas other amendments produced little inhibition. Only chitin-amendment proved inhibitory in black soil with the soil emanation method.

Discussion

In the present study, the inhibition is confined mostly to the surface layers. Decrease in fungistatic activity with increasing soil depth is obviously due to the reduction of microbial activity which in turn offers less competition for nutrients; and the reocurrence of inhibition at greater depths indicates the influence of inhibitors of abiotic origin (Dobbs et al. 1960, Griffiths 1966).

Soil amendments with nitrogen, irrespective of the form of nitrogen, suppressed the germination of conidia of *F. oxysporum* f. sp. *ciceri* in both the soils, while oatmeal and cellulose-amendments enhanced the germination. The availability of carbon might have been affected due to nitrogen amendments. Cellulose and oatstraw with and without nitrogen did not affect the conidial germination of *F. solani* f. sp. *phaseoli* (Adams et al. 1968). Chitin-amendment would selectively enhance the development of populations which would produce toxins harmful to fungi (Alexander 1978) and the volatile ammonia from the chitin-amended soils influences the spore germination as a volatile fungistatic factor (Schippers & Palm 1973). Higher levels of fungistatis were noticed in amended soils than the non-amended soils (Kanaujia 1976, Mishra & Pandey 1978). The

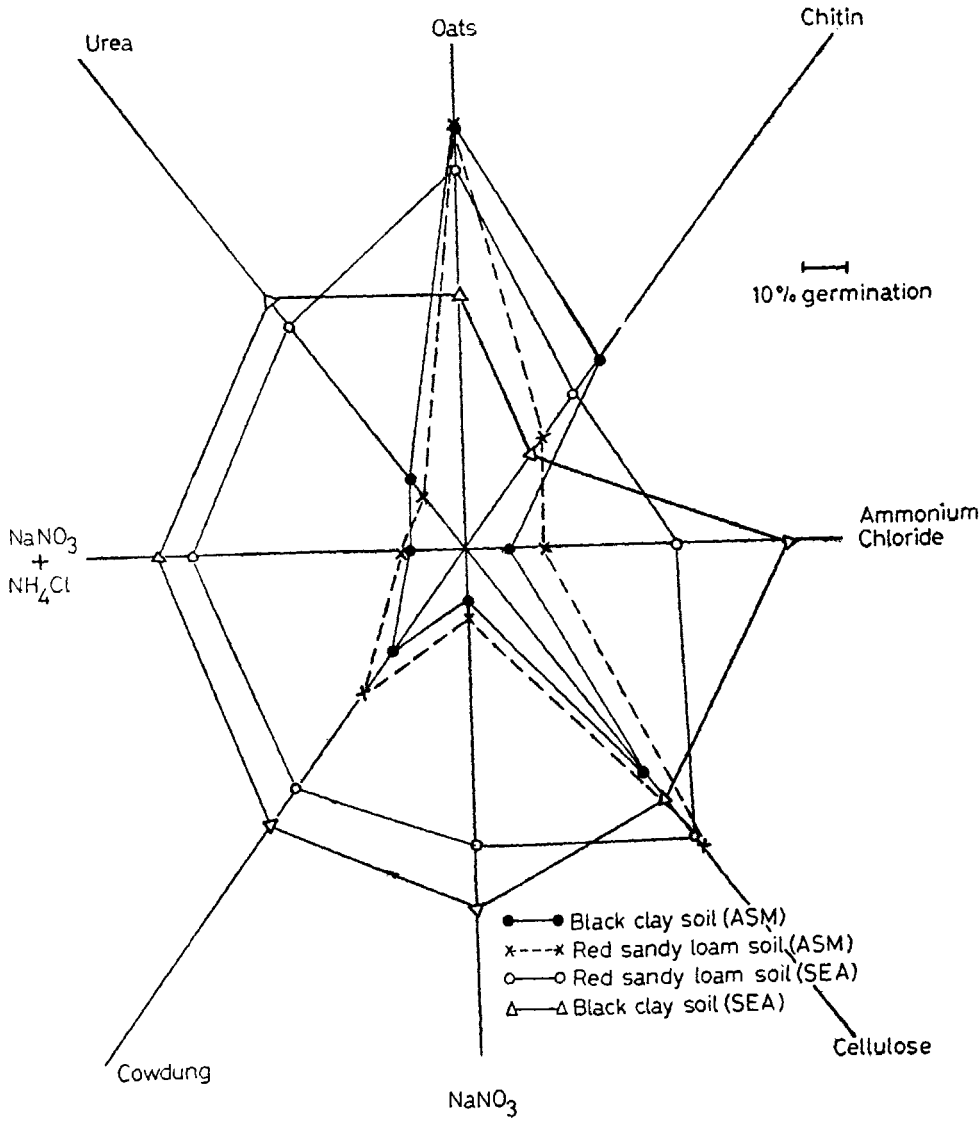

Figure 4 Influence of various amendments on soil fungistasis against *F. oxysporum* f. sp. *ciceri* in agar slide method (ASM) and soil emanation agar method (SEA)

fungistasis was more pronounced in amended soils and the soil emanation agar method was more sensitive in estimating the volatile fungistatic factor than the agar slide method. These results suggest the involvement of a volatile fungistatic factor, probably ammonia, in soils amended with chitin and ammonium chloride.

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