INOCULUM POTENTIAL AND SOIL FACTORS AFFECTING THE PATHOGENESIS OF *PYTHIUM BUTLERI* IN CAUSING DAMPING-OFF OF TOMATO*

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Pythium butleri inoculum consisting of zoospore suspension, raised on oatmeal: sand or homogenized mycelium were almost equally effective in causing damping-off of seedlings of tomato variety S-12. With the increase in zoospore concentrations the post-emergence damping-off increased while in case of oatmeal: sand inoculum, the pre-emergence damping-off increased with increase in inoculum concentration and led to decrease in post-emergence damping off. The disease was always more in sterilized than unsterilized soil. The interaction between 5 days old inoculum and 10 days old tomato seedlings led to maximum damping-off, while any deviation in the age of inoculum or the host resulted in reduced damping-off. Among soil factors, high soil moisture, soil *p*H 6.0 and clay soil were found most conducive for disease development. The disease developed in the range of 20–40°C, yet pre-emergence damping-off was maximum at 20–25°C and post-emergence damping-off was maximum at 30–40°C, and over all damping off was maximum at 35°C.

INTRODUCTION

In recent years *Pythium butleri* Subramaniam has been found to cause serious damping-off of tomato (*Lycopersicon esculentum* Mill.) and other crops in nursery beds in different parts of North India. Although some information is available with regard to factors affecting disease development in case of *P. aphanidermatum* (Trujillo and Marclay 1967; Kraft and Erwin 1968; Thompson *et al.* 1971), *P. debaryanum* (Thompson *et al.* 1971), P. *ultimum* (Beach 1949; Kerr 1964; Laviollette and Athew 1971) and *P. myriotylum* (Frank 1967, 1974), yet no such information is available in case of *P. butleri.* The present studies were, therefore, initiated keeping in view the diversity of agroclimatic conditions of the soil that exist in a tropical country like India and seriousness of *P. butleri* in nursery beds causing damping-off of various vegetables.

MATERIALS AND METHODS

Pythium butleri (isolate Pb. 3) was obtained from damped-off seedlings of tomato variety S-12. For all tests tomato variety S-12 was used as test plant.

Inoculum potential—Three types of inocula of *P. butleri* were used in different proportions to determine the inoculum potential. Various types of inocula and methods of their preparations are given below :

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(i) *Oatmeal: sand inoculum*—It was prepared by the method described by Grover and Dutt (1973) with some modifications. The mixture of oatmeal: sand (1:9 and moistened with water) was sterilized for 1.30 hr at 15 p.s.i. and subsequently inoculated with three one-week-old mycelial discs (0.5 mm diameter) per 250 ml Erlenmeyer flask containing 100 g of the sand: oatmeal mixture. After 10 days of incubation at 30±1°C, this inoculum was mixed with sterilized or unsterilized soil, as the case may be, in the ratio of 1.5 : 8.5 and its variables. This mixture was put in 15 cm diameter earthen pots and allowed to stabilize for 5 days before use. Each pot contained approximately 1 kg soil.

(ii) *Homogenized mycelial inoculum*—The organism was grown on Czapek's nutrient solution (*p*H 6.5) for one week at 30±1°C. The mycelial mats were filtered, washed repeatedly and homogenized in a waring blender for 1.30 min in sterilized water. The inoculum density of mycelial propagules was adjusted turbidimetrically using Spectronic-20 Colorimeter at 500 nm wave length. At O.D. 2.00 there were approximately 500 viable mycelial propagules/ml. This inoculum and its variable concentrations were used at the rate of 30 ml/pot of 15 cm diameter containing 1 kg soil. The inoculum was added 4 days after sowing the seeds.

(iii) *Zoospore suspension*—Zoospores were produced by Kraft and Erwin's (1968) modified method. *P. butleri* was grown on french bean (*Phaseolus vulgaris* L.) agar medium (5 per cent bean decoction mixed with 2 per cent agar) for 48 hr at 30±1°C in Petri plates. Mycelial discs of 5 mm size were cut from peripheral region and suspended in sterilized water at the rate of 10 ml/10 mycelial discs in each Petri plate kept at 30±1°C. The water in plates was renewed twice, once after 48 hr, and then after 24 hr, after which they were placed at 25±1°C for next 24 hr. This led to the formation of vesicles and differentiation of zoospores. Petri plates were once again brought to 30 to 35±1°C. After two hours vesicles ruptured and abundant zoospores were liberated. The number of zoospores in the inoculum were adjusted microscopically with sterile water. One ml of different zoospore concentration was put on 5 days old seedlings of tomato grown on 2 per cent water agar medium in Petri plates. Each Petri plate contained 10 seedlings. Observation on mortality of seedlings was recorded after 5 days. Addition of zoospore suspension to soil or sand gave erratic seedling mortality, hence this method was not tried.

Inoculation tests were conducted mostly in pots (unless otherwise stated) at 28–30°C using 30–50 seeds per pot and 6 to 7 replicate pots for each test. Observation on pre- and post-emergence damping-off were recorded 5 and 10 days after inoculations respectively.

Soil factors

(i) *Soil type*—Four different types of soils viz. sandy loam, loam, clay loam and heavy clay loam obtained from different localities were used. These soils are used after sterilization at 15 lb. p.s.i. for 2 hr. Homogenized mycelial suspension was used to inoculate pots.

(ii) *Soil moisture*—Different moisture levels were adjusted in loamy soil by the method described by Dickson (1923). Bottom sealed plastic pots (10 cm diameter containing 500 g of soil) were fitted each with a glass tube (1 cm diameter) till mid way in the pot, through which desired amount of water was added. Difference

between wilting capacity and field capacity was calculated so as to obtain different moisture levels. Required amount of water was then added to the pots through the central tube with the help of a pipette after every 8 hours. The amount of water required was added on the total weight basis of a pot which was weighed everytime. Seeds were sown immediately after initial adjustment of moisture gradient. After 4 days of sowing, pots were inoculated with 20 ml homogenized mycelial suspension per pot.

(iii) *H-ion concentration*—River sand was neutralized by soaking in 5 N HCl for 24 hr followed by repeated washing with deionized water till it gave neutral reaction. After sun drying it was saturated with citric acid (0.1 M) disodium hydrogen phosphate (0.2 M) buffer at different *p*H for 7 days. Seed were sown and after 4 days these were inoculated with 20 ml homogenized mycelial suspension per pot. After every 4 days buffers were added to saturate these pots.

(iv) *Soil temperature*—The method of Thompson *et al.* (1971) was used to study the effect of temperature on damping-off of tomato. In this case also 20 ml of homogenized mycelial suspension was added to inoculate the pots.

Results

(i) *Inoculum potential*—When either oatmeal : sand or homogenized mycelial suspension was used as inoculum, it was found that damping-off increased with the increased concentration of inoculum. Zoospore suspension inoculum, however, produced cent per cent damping-off of seedlings when there were 10–15 zoospores per microscopic field in the suspension (Fig. 1). Inoculum grown in oatmeal : sand also produced cent per cent mortality when it was used at the rate of 45 per cent w/w in sterilized soil (Fig. 2). The homogenized mycelial suspension produced about 90 per cent mortality at the maximum inoculum concentration i.e. 500 propagules/ml (Fig. 1). Inoculation in unsterilized soil always led to reduced seedling mortality.

The mortality due to pre- and post- emergence damping-off, however, varied when different inocula were used. There was a linear increase in the pre-emergence damping-off when either oatmeal : sand medium or homogenized mycelial suspension was used. Post-emergence damping-off, however, first increased with the concentration of inoculum but subsequently decreased with further increase in inoculum. The maximum post-emergence damping-off in case of soil inoculum was observed in sterilized soil (30 per cent mortality) when its concentration was 10 per cent w/w in unsterilized soil. There was no pre-emergence damping-off when inoculum reached the concentration of 40 per cent w/w in case of sterilized soil and 50 per cent in case of unsterilized soil (Fig. 2).

(ii) *Effect of age of inoculum and host*—It was found that 5-day-old inoculum caused maximum (92.8 per cent) damping-off of 10-day-old seedlings (Table I). Any further increase in the age of inoculum or the seedlings led to decrease in damping-off so much so that 25-day-old inoculum did not infect 25-day-old seedlings.

(iii) *Soil factors: (a) Soil type*—Among the 4 types of soils taken, pre- and post-emergence damping-off were least in sandy loam soil, and maximum in heavy clay loam soil (Table II). The differences in mortality due to pre-emergence damping-off between other three soils were not marked.

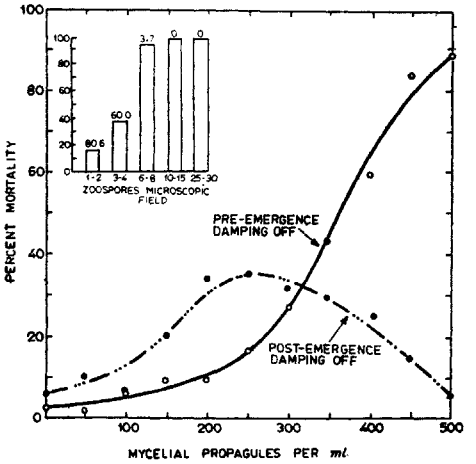

FIG. 1. Effect of concentrations of zoospore and mycelial propagules of *P. butleri* on damping-off of tomato seedlings.

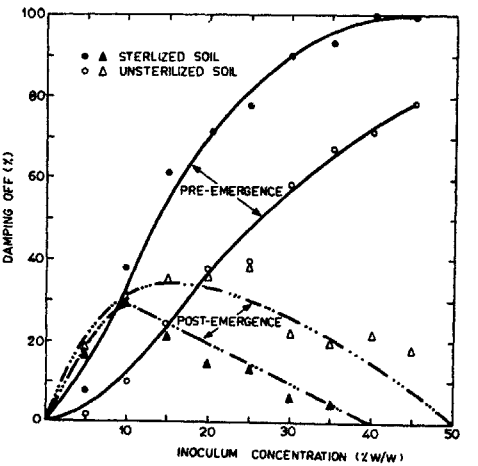

FIG. 2. Effect of concentrations of inoculum in sterilized and unsterilized soil on damping-off of tomato seedlings caused by *P. butleri*.

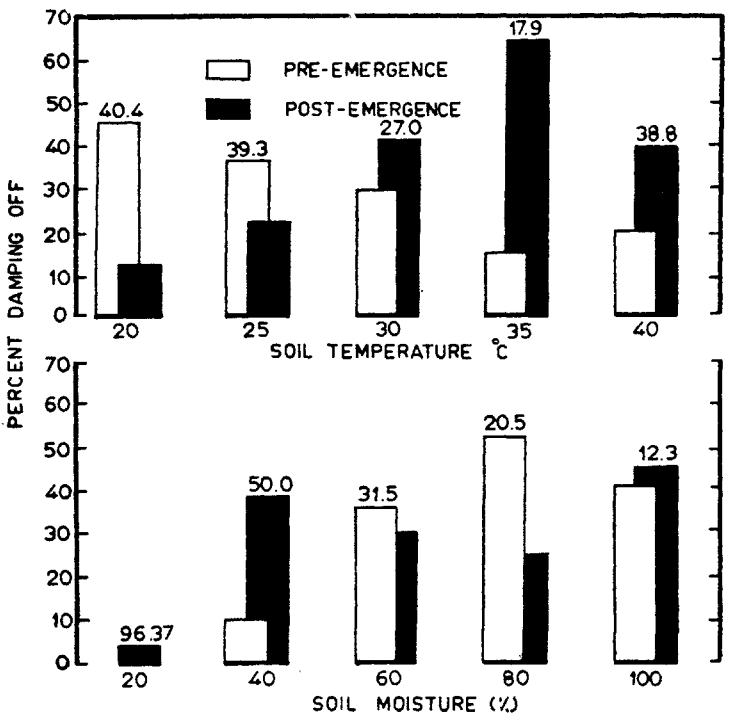

FIG. 3. Effect of soil temperature and moisture on damping-off of tomato seedlings caused by *P. butleri*.

N.B. Figures given on top of the bars indicate per cent final stand.

TABLE I

Interaction between ages of tomato seedlings and Pythium butleri *on damping-off*

Age of inoculum (days)	Per cent final stand of seedlings when inoculated at the age of* (days)				
	10	15	20	25	30
5	92.8	50.5	30.0	20.8	9.6
	(74.44)	(45.29)	(33.21)	(27.13)	(18.05)
10	57.0	36.6	21.3	14.7	6.8
	(49.02)	(37.23)	(27.49)	(22.55)	(15.12)
15	27.9	21.4	14.1	6.7	2.5
	(31.88)	(27.56)	(22.06)	(15.00)	(9.10)
20	8.6	6.9	5.4	2.3	1.4
	(17.05)	(15.23)	(13.44)	(8.72)	(6.80)
25	3.5	2.6	1.3	0.0	0.0
	(10.78)	(9.28)	(6.55)	(0)	(0)

M.E.S. = (±4.17); Plant age : L.S.D. (P=0.05)=(8.42); Inoculum age: M.E.S. = (±2.84), L.S.D. (P=0.05) = (5.73)

*Average of three replications.

Note : Figures in parentheses are transformed values.

TABLE II

Effect of soil types on damping-off of tomato caused by Pythium butleri

Soil type	Per cent mortality		Per cent final stand
	Pre-emergence	Post-emergence	
Heavy clay loam	35.2	36.8	28.0
Clay loam	18.5	28.5	53.0
Loam	17.4	27.0	55.6
Sandy loam	14.3	17.1	68.6

M.E.S. ± 3.7; L.S.D. 7.25

(*b*) *Soil moisture*—Loam soil was adjusted to different moisture levels ranging between wilting and field capacity of soil in 10 cm diameter bottom sealed plastic pots. Seven replicated pots were used in each treatment. It was observed that normal seed germination started in soil having 40 per cent moisture level or more.

TABLE III

Effect of sand pH on damping-off of tomato caused by Pythium butleri

Sand *p*H	Per cent mortality*		Per cent final stand
	Pre-emergence	Post-emergence	
5.0	0P	0P	—
6.0	34.6	36.2	29.2
7.0	31.4	20.4	48.2
7.5	34.8	25.2	40.0
8.0	30.8	32.0	37.2
M. E. S.			± 3.10
L.S.D. (P=0.05)			= 7.016

P = Phytotoxic; * = Average of three replications

The total damping-off increased with increase in moisture level in the soil. There was 50 per cent increase in total damping-off when the soil had 40–60 per cent moisture level as compared to when soil had 20 per cent moisture. Further increase in moisture level increased damping-off 4 to 5 times (Fig. 3).

Pre-emergence damping-off was absent at 20 per cent soil moisture but gradually increased to its maximum when soil had 80 per cent moisture. Post-emergence damping-off was considerably reduced when pre-emergence damping-off was maximum.

(*iii*) *H-ion concentration*—It was found that at *p*H 4 no seedlings emerged while at *p*H 5 some seedlings emerged which collapsed within 24 hr. Therefore, observations were recorded in a range of *p*H 6 to 8. Maximum damping-off was noted at *p*H 6 while minimum at *p*H 7. At *p*H 8.0 there was decrease in damping-off (Table III).

(*iv*) *Soil temperature*—Inoculated pots containing tomato seeds were put at different temperatures in incubators (6 replicated pots at different temperatures). It was seen that pre-emergence damping-off was maximum at 20°C while post-emergence damping-off was maximum at 35°C. Any further increase or decrease in temperature led to decrease in damping-off (Fig. 3).

DISCUSSION

It is well known that the inoculum potential of the pathogens determines the interaction between the host and the pathogen (Garrett 1960; Dimond and Horsfall 1965; Strobel and Mathre 1971). In case of *Pythium* sp. different workers used different kinds of inocula, such as mycelial (Klisiewicz 1968) or zoospore suspension (Kraft and Erwin 1968) and established the optimum inoculum density required to obtain maximum disease. In the present study using three different types of inocula viz. oatmeal : sand, mycelial suspension and zoospore suspension,

it was observed that all the three types of inocula were able to produce abundant damping-off on tomato and there was little difference between these inocula. However, when inoculum was used in sterilized or unsterilized soils it was found that there was more disease in sterilized soil than in unsterilized soil, irrespective of the type of inoculum for obvious reason of greater microbial competition in unsterilized soil. Increased inoculum concentration continued to increase the disease in all cases. There was, however, a marked effect in the proportion of pre- and post-emergence damping-off of tomato seedlings at different inoculum concentrations. The post-emergence damping-off was maximum to the tune of 30 to 40 per cent and this was reached at low inoculum densities Further increase in inoculum concentrations led to decrease in the post-emergence damping-off, so much so that there was no post-emergence damping-off at higher inoculum concentrations. On the other hand, there was a continuous linear increase in the pre-emergence damping-off of the seedlings with increased concentrations of the inoculum. The reason for low post-emergence mortality at higher inoculum concentration is that with the increase in inoculum density there is a corresponding increase in pre-emergence damping-off resulting in poor seedling stand for post-emergence damping-off.

The fact that *Pythium* spp. cause disease only on the young seedlings of different crops has been found to hold good in this case also. Five-day-old inoculum of *P. butleri* and 10-day-old tomato seedling resulted in maximum damping-off and any further increase in age of host or inoculum resulted in reduced disease severity. Changes in the physiology and anatomy of the roots of the vegetable crops have been considered by Leach (1947) as an important factor in increasing resistance in plants against such organisms. Loss of virulence in the pathogen *P. ultimum,* has been attributed by Mellano *et al.* (1970) to the change of vegetative phase to the reproductive phase of the organism. In *P. butleri* reproductive phase is initiated after 10 days of growth in culture and this may account for loss in its virulence at older ages. What happens in tomato roots with age is yet to be determined.

Various soil factors studied were critical in influencing disease development individually. Among the four types of soil tested the one having more clay content encouraged more disease. Kerr (1964) working with *P. ultimum* pointed out that increased disease in heavy and dry soil could be correlated with poor production of root exudates by pea.

Several workers have reported that high moisture level in the soil increased diseases due to *Pythium* spp. (Flor 1930; Harter and Zaumeyer 1931*a* and 1931*b*; Beach 1949). Frank (1974) observed that although *P. myriotylum* caused higher incidence of groundnut (*Arachis hypogaea* L.) pod rot in wet soils, yet some disease developed even in soil drier than the wilting range for mesophytes. In case of tomato also some disease was observed even at 20 per cent soil moisture level above the wilting capacity of soil.

When seedlings were raised on sand saturated with buffers of different *p*H it was noted that maximum disease was observed at *p*H 6 and with further increase in *p*H, disease intensity was reduced correspondingly.

P. butleri causes damping-off in the temperature range of 20–40°C and maximum disease was at 30–35°C. The pre- and post-emergence damping-off, however, had different optima i.e. 20–25°C and 30–40°C, respectively. Harter and

Zaumeyer (1931*a* and 1931*b*) and Monteith (1933) found maximum disease on bean (*Phaseolus vulgaris*) and turf grass (*Poa pretensis*) at 32.5°C and 35°C respectively. Thompson *et al.* (1971) observed that maximum disease in soybean (*Glycine max*) due to *P. aphanidermatum* was at 29–30°C while due to *P. debaryanum* and *P. ultimum* it was at 15–20°C. Generally, at lower temperatures seed germination is delayed which leads to greater accummulation of exudates and if the pathogen is aggressive at that temperature, there is likelihood of greater pre-emergence of damping-off (Leach 1947). But this may not explain as to why there is maximum mortality of seedlings at higher temperatures. The fact, however, remains that soil temperature greatly influenced both pre- and post-emergence damping-off caused by various *Pythium* spp.

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