

Prof. T. Pullaiah,

Professor of Botany,

Sri Krishnadevaraya University,

Anantapur 515003, A.P.

The Journal of the Indian Botanical Society

Vol. XXXII]

1953

[Nos. 1 & 2

DAMPING-OFF OF TOMATO SEEDLINGS

BY BABU SINGH AND H. C. SRIVASTAVA

Plant Pathological Laboratory, Govt. Agricultural College, Kanpur, U.P.

(Received for publication on December 8, 1952)

THE tomato crop suffers heavily all over the Uttar Pradesh in the seedling stage by 'damping-off'. The disease has been found to be of cosmopolitan occurrence and was investigated in foreign countries by Alexander, Young and Kiger (1931-32), and Brown (1944), but so far no comprehensive work has been done in India, on this problem. The disease has been noticed in various nurseries at Kanpur and its neighbouring villages for several years, both in the rainy and winter seasons, but the damage is especially severe during rains. The nursery owners usually raise seedlings in the same seedbeds, year after year, and thus suffer tragic losses due to this disease. The percentage of damage was estimated by throwing one foot hollow square in seedbeds at random, and then counting the affected and healthy seedlings inside the square. During the rainy season of 1947-48, the disease was surveyed in various nurseries of the State, and the average percentage of damage, is given below. Almost every seedbed in the locality mentioned below was found affected.

Kanpur ..	60-80%	Allahabad ..	60-70%
Unao ..	58-78%	Banares ..	62-64%
Kannauj ..	60-80%	Meerut ..	48-52%
Bulandshahr ..	50-60%	Agra ..	65-74%

Considering the economic importance of the crop and the losses caused by the disease, the present investigation was undertaken.

Symptoms

The disease is characterised by sudden collapsing of the seedlings in the seedbeds. The seedling begins to rot from the base resulting in its toppling over abruptly and then drying slowly from base to the top. In a few cases the disease started from the root hairs and killed the seedlings in the same way (Fig. 1 A). Some of the seedlings, even at an early age, become twisted at their base and brown spots appear on their roots. Top infection is also common, whereby the upper portion of the seedling rots first, while the lower portion remains normal (Fig. 1 B). Such seedlings are often associated with the disease at their base as well, which ultimately hastens their mortality. Brown

and dark brown lesions are formed on some of the seedlings, encircling any part of their stout stem, hence forming a 'Sore-Shin'. Constricted lesions, shining milky white in colour, are formed at the base of the stem and young white sclerotia, which at later stage turn brown are attached to it. Local lesions, brown to dark tan in colour, with margins showing violet tinge, are found on the affected portion of the seedlings. Black pycnidia are seen on the surface of the lesions. When a seedling after infection topples over suddenly and touches the ground by its middle portion, secondary roots are given out at the point of contact with the soil, the upper part of the seedling remains normal.

"Pre-emergence" damping-off, *i.e.*, rotting of the seeds and the seedlings before actual emergence from the soil is also very common, and as a result of this, scattered small blank patches are usually found in the seedbeds. "Post-emergence" damping-off is severe when the seedlings are in cotyledonous stage, and this is the "critical period" for them. The disease is also common even after the formation of the secondary leaves.

Isolation of Causal Organisms

The diseased seedlings of tomato were collected from various places, and large-scale isolations from them revealed the presence of the following fungi. The percentage occurrence of each fungus in the isolations is given below:—

1. <i>Pythium sp.</i> No. 1..	..	90%
2. <i>Pythium sp.</i> No. 2..	..	55%
3. <i>Phytophthora sp.</i>	60%
4. <i>Rhizoctonia sp.</i>	82%
5. <i>Sclerotium rolfsii</i>	25%
6. <i>Phoma sp.</i>	20%
7. <i>Fusarium sp.</i>	15%
8. <i>Acrothecium sp.</i>	5%
9. <i>Alternaria sp.</i>	4%
10. <i>Helminthosporium sp.</i>	2%

Pythium sp. No. 1 was invariably found associated with *Phytophthora sp.*, but it was obtained in the pure form by inoculating the mixture of the two fungi on Bhindi buds (*Hibiscus esculentus* L.). On the other hand *Phytophthora sp.* was recovered in the culture free from *Pythium sp.*, by inoculation on Castor leaves (*Ricinus communis* L.) and re-isolating it from inoculated leaves. The parasitic fungi (Table II) were studied in detail.

Morphology and Identity of the Fungi

Pythium sp. No. 1.—Hyphæ 4–12 μ in diameter; appressoria 10–30 μ , sporangia (prosporangia) had a linear structure, 80–250 μ in length and an evacuation tube was given out from it, which was broader than hypha and longer than sporangium. A vesicle was formed at the tip of evacuation tube in which zoospores were formed, they measured 12–15 μ while swimming and 10 μ when encysted. They germinate

by producing 1-3 germ tubes. Terminal oogonium measured $15-20\ \mu$ and had a tendency to bend towards antheridium. The antheridia were monoclinal, oospores aplerotic, thickwalled and measured $10-20\ \mu$, with an average of $15.5\ \mu$, oospores were formed in clusters at one place (Fig. 2). The species agreed with the measurements and

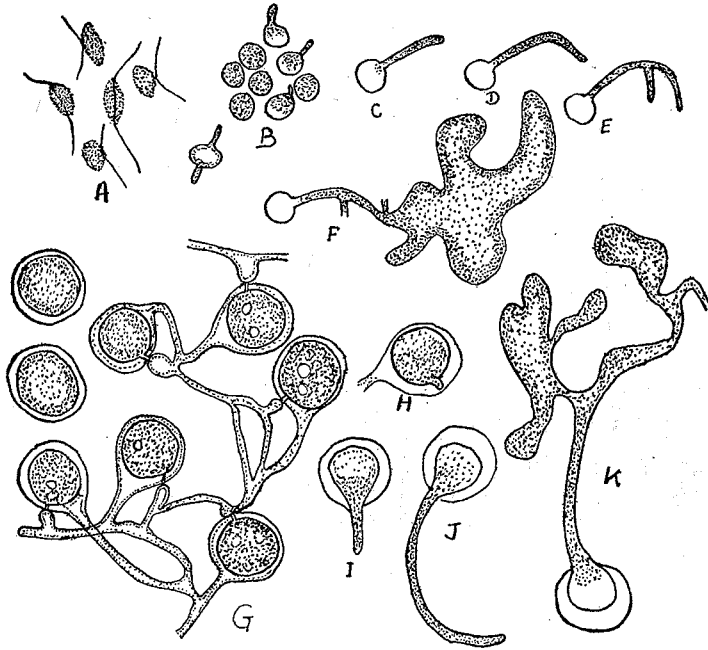


FIG. 2. A. Biciliated zoospores as they swim, $\times 1,100$. B. Encysted zoospores germinating *in situ*, $\times 1,100$. C, D, E & F. Various stages of germination of zoospores, $\times 1,100$. G. Oospores formed in cluster from a strand of hypha, $\times 600$. H, I, J & K. Various stages of germination of oospores, $\times 600$.

characters of *Pythium indicum* Balakrishnan, reported by Balakrishnan (1948) from Madras (South India) causing the fruit rot of Bhindi (*Hibiscus esculentus* L.).

Pythium sp. No. 2.—The hyphae unseptate, measuring $3-7\ \mu$ but septa were present in older ones. The zoospores were $8-12 \times 6-8\ \mu$ while moving and $8-9\ \mu$ at rest. Oogonia were $20-26\ \mu$ in diameter and formed generally on short lateral stalks. Oospores round, smooth and measured $14-22\ \mu$ in diameter. The species was identified as *Pythium aphanidermatum* (Edson) Fitz.

Phytophthora sp.—Hyphae $5-6\ \mu$ in width, sporangia borne on simple stalk, varying in size from $25-50 \times 20-40\ \mu$. Zoospores $8-12\ \mu$ while moving and $7-11\ \mu$ during rest. Chlamydospores $20-60\ \mu$ in diameter, oogonium $15-27\ \mu$ with an average of $23.8\ \mu$ in diameter. Oospores ranged from $13-27\ \mu$ but generally $15-20\ \mu$ in diameter. The species was identified as *Phytophthora parasitica* Dastur.

Rhizoctonia sp.—Mycelium 7–9 μ in diameter and the cells were 40–130 μ long. Two different sizes of sclerotia were found in nature as well as in culture medium named as type A and B. Their measurements are tabulated below:—

Type	Source	Range		Average of 200 sclerotia	
		mm.	μ	mm.	μ
A	1 Tomato seedlings ..	0.9–1.5	900–1500	1.2	1200
	2 Potato dextrose agar medium	0.9–1.8	900–1800	1.35	1350
B	1 Tomato seedlings ..	2.5–3.0	2500–3000	2.7	2700
	2 Potato dextrose agar medium	2.8–3.5	2800–3500	3.1	3100

Generally the sclerotia were found to be larger in culture medium as compared with those found in nature. The species was identified as *Rhizoctonia solani* Kuhn. The basidial stage of the fungus was found on the damped-off seedlings and the measurements were identical with *Corticium solani* (Prill. and Declr.) Bourd. and Galz. The basidia and basidiospores were found in abundance in nature but when transferred to potato dextrose agar medium, they produced sclerotia only. On onion agar medium, few basidia were found, in old cultures.

Sclerotium rolfsii Sacc.—Mycelium broad, sclerotia white in the beginning, turning brown afterwards. The variation in the size of sclerotia is as follows:—

Serial No.	Source	Range		Average	
		mm.	μ	mm.	μ
1	Tomato tissues ..	0.4–1.5	400–1500	0.95	950
2	Potato dextrose agar medium ..	1.0–2.0	1000–2000	1.5	1500
3	Maize meal agar ..	0.9–2.5	900–2500	1.7	1700

Phoma sp.—Mycelium first hyaline but later on turned light brown in colour, and was generally inter-cellular. Pycnidia light brown to dark in colour, and found below the epidermis. There was much variation in their size, e.g., from 130 μ to 420 μ but the largest number fell between 180–350 μ . The pycnosporos also vary in size, and the largest number fell between 3.5–7.0 μ in length and 2.5–3.9 μ in breadth out of 200 measurements. The species was identified as *Phoma beta* (Oudem) Frank.

Fusarium sp.—Mycelium aseptate, conidia formed on short conidiophores were hyaline, falcate and acute. Both micro and macro-conidia were abundant in cultures. Macro-conidia were 3–5 septate.

Germination Studies of *Pythium indicum* Balakrishnan

The germination studies of *Pythium indicum* were undertaken because no account has so far been published from India. Other pathogenic fungi isolated have already been studied by various authors.

Germination of zoospores in distilled, sterilised, tap water and in 0.1 to 4.0% concentrations of glucose solution was done but no difference was observed in the treatments. Oogonium gave germ tube in water from 24–48 hours and oospores from 4–8 days when kept in water, which was changed twice daily.

Sporangia were formed by the mycelium given by zoospores within 24 hours.

In darkness the germination of zoospores was only 1–3.5%, and no sporangia were formed from the mycelium given out by them. When sporangia were kept in darkness, they failed to give the evacuation tubes, but as soon as they were exposed to light, evacuation tubes were formed. Oospores did not germinate in darkness.

The germination of zoospores and formation of sporangia was very much enhanced, when some food material was placed in water, e.g., agar pieces, dead ants or pieces of tomato seedlings.

Viability of Oospores of *Pythium indicum* Balakrishnan

Two methods were adopted to test the viability of the oospores:—

(i) The oospores were mixed with dry sterilised soil in a conical flask and kept at room temperature, in the laboratory. The range of temperature was 18–26° C. Suspension of oospores along with sterilised soil was prepared in sterilised water. Drops from it were kept on sterilised slides and the percentage of germinated oospores was noted. This was repeated every fortnightly, to determine the viability of oospores of different ages. (ii) Drops of the suspension of oospores in sterilised water were kept on sterilised slides and were allowed to dry inside a sterilised Petri dish. Such dried oospores were again moistened with sterilised water, after definite intervals and the percentage of germination was noted and the results are tabulated in Table I.

It is evident from Table I, that the percentage of germination of oospores is adversely affected by age and exposure to dry conditions. The oospores kept in soil remained viable for more than 150 days whereas when they were dried on slides, they germinated only upto 105 days. There was less germination with the increase in age of oospores.

TABLE I
Showing the viability of oospores of *Pythium indicum*

Age of culture days	% germination of oospores after													
	4 days		5 days		6 days		7 days		8 days		9 days		10 days	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
15	78.0	65.3	85.0	68.4	89.2	69.0	92.0	72.0	95.6	78.0	97.2	82.0	100.0	89.0
30	62.0	62.0	70.5	62.0	85.0	65.3	88.0	68.3	92.0	70.2	97.6	75.0	100.0	79.3
45	60.2	57.3	60.7	59.4	71.8	62.0	82.0	64.3	89.7	69.3	94.3	72.0	98.8	75.6
60	55.8	52.4	59.2	54.2	63.8	57.2	69.0	60.1	75.0	63.0	82.3	65.0	92.0	70.3
75	52.8	47.0	56.2	48.0	60.2	52.0	65.0	53.0	69.3	55.0	72.0	57.0	85.0	65.0
90	46.7	30.4	49.2	36.2	55.2	40.3	59.2	43.0	63.0	45.0	69.5	48.0	77.2	56.0
105	42.8	15.4	47.2	20.0	52.3	23.0	56.4	24.0	62.0	25.0	67.0	26.7	73.2	27.0
120	39.1	..	43.3	..	45.7	..	50.3	..	52.4	..	59.2	..	65.0	..
135	32.3	..	39.2	..	42.0	..	44.5	..	51.2	..	48.0	..	50.0	..
150	25.2	..	28.2	..	30.2	..	38.4	..	40.2	..	43.2	..	45.2	..

A = Germination percentage of oospores of *Pythium indicum* from sterilised soil.

B =

.. denotes nil.

.. denotes nil.

.. denotes nil.

Pathogenicity

A number of inoculation experiments to test the pathogenicity of different fungi isolated were carried out in the laboratory. In one set of pots, sterilised soil was filled and thoroughly mixed with the fungus culture grown on corn meal sand, and the seeds were sown. In the second set of pots, seeds were sown in sterilised soil and then the soil was watered with spore suspension. In the third set of pots,

TABLE II

Summary of the inoculation experiments carried out with different fungi isolated from Tomato seedlings

Name of the fungus	I Set		II Set		III Set		IV Set	
	% E	% D	% E	% D	% E	% D	% E	% D
<i>P. indicum</i>	69.0	83.4	73.0	63.1	55.0	90.2	92.0	..
<i>P. aphanidermatum</i>	58.0	80.5	79.0	58.0	65.0	82.2	92.5	..
<i>R. solani</i>	74.0	82.4	79.0	60.1	60.0	80.6	93.0	..
<i>Phytophthora parasitica</i>	78.0	55.6	88.0	43.9	71.0	58.1	92.0	..
<i>Sclerotium rolfsii</i>	14.0	85.1	75.0	30.2	17.0	82.0	94.0	..
<i>Phoma beta</i>	88.0	32.8	92.0	10.0	84.0	36.7	92.0	..
<i>Fusarium sp.</i>	85.0	19.4	88.0	8.7	83.0	29.2	93.0	..

E = emergence.

D = damage.

.. denotes nil.

the seeds were sown in sterilised soil mixed with fungus culture and then the latter was watered with spore suspension. In the fourth set, the seeds were sown in sterilised soil in pots to serve as control. The emergence and damage percentage as observed in each case is given in Table II.

The above table shows that *Pythium indicum* Balakrishnan, *Pythium aphanidermatum* (Edson) Fitz., *Rhizoctonia solani* Kuhn., *Phytophthora parasitica* Dastur, *Phoma beta* (Oudem) Fr., *Sclerotium rolfsii* Sacc., and *Fusarium sp.* are pathogenic while *Alternaria sp.*, *Helminthosporium sp.*, and *Acrothecium sp.* are non-pathogenic. *Pythium indicum* proved to be the most destructive organism.

Inoculation of seedlings of different ages.—Experiments were set in which 3 days to 5 weeks-old seedlings were inoculated with different fungi in order to see their virulence in relation to the age of the host. The fungus was inoculated by several methods, i.e., by keeping the fungus culture by the side of hypocotyl of the seedlings, by spraying spore suspension on the seedlings, and by spraying spore or sclerotial

suspension on the soil. Twelve seedlings were inoculated in each set, and the number of damped-off seedlings were counted and tabulated in Table III. The seedlings were sprayed with distilled water for control.

TABLE III

Showing the number of damped-off seedlings of different ages on inoculation by different methods

Name of fungus	Method of inoculation	Age of the seedlings					
		3 Days	1 Week	2 Weeks	3 Weeks	4 Weeks	5 Weeks
<i>Pythium indicum</i>	a. Culture on hypocotyl ..	12	10	9	6	5	1
	b. Zoospore suspension on seedlings ..	10	10	8	7	7	4
	c. Zoospore, oospore suspension in soil ..	12	8	6	5
<i>Pythium aphanidermatum</i>	a. Culture on hypocotyl ..	12	10	10	5	6	4
	b. Zoospore suspension on seedlings ..	10	8	7	5	3	..
	c. Zoospore, oospore suspension in soil ..	11	7	4	3
<i>Phytophthora parasitica</i>	a. Culture on hypocotyl ..	10	11	9	6	5	..
	b. Zoospore suspension on seedlings ..	8	7	6	5
	c. Zoospore, oospore suspension in soil ..	10	8	7	4	2	..
<i>Rhizoctonia solani</i>	a. Culture on hypocotyl ..	10	11	10	9	8	8
	b. Sclerotial suspension in soil ..	8	7	9	8	6	4
<i>Phoma betæ</i>	a. Culture on hypocotyl ..	8	7	8	6	7	7
	b. Spore suspension in soil ..	6	7	7	6	6	6
<i>Fusarium sp.</i>	a. Culture on hypocotyl ..	4	3	2	4	2	..
	b. Spore suspension in soil ..	3	2	1
<i>Sclerotium rolfsii</i>	a. Culture on hypocotyl ..	2	1	2	3	1	2
	b. Sclerotial suspension in soil ..	5	2
Sterilised soil (control)	Distilled water sprayed

.. denotes nil.

It is evident from Table III that *Pythium* species infect seedlings of all ages tested in the nurseries, but the older seedlings were less susceptible to *Phytophthora parasitica* Dastur. *Phoma betæ* (Oudem) Fr. and *Rhizoctonia solani* were found to infect old seedlings. There was no disease in control.

Inoculation experiments with different fungi in combination.—In order to see the effect on incidence of the disease, when more than one pathogenic fungus were present in the soil, an experiment was laid out

in which different combinations of the fungi were mixed with sterilised soil and 100 seeds were sown in each pot (size: Diameter 2 ft., height 6 inches). The percentage damage is tabulated in Table IV. Seven flasks of the fungus culture, grown on corn meal sand, were thoroughly mixed in each pot used in the experiment and where more than one fungus was used, equal proportions of the inoculum from different fungi were kept. The data from inoculation of individual fungus is given in Table II, in I set of pots and the damping-off percentage is the total of pre- and post-emergence loss; and has not been repeated in Table IV.

TABLE IV

Summary of the inoculation experiments carried out with combinations of pathogenic fungi

Different combinations of fungi	% Emergence	Damping-off %		
		Pre-	Post-	Total
Sterilised soil + seeds	88
<i>P. indicum</i> + <i>P. aphanidermatum</i>	38	50	38	88
" + <i>R. solani</i>	36	52	39	91
" + <i>Phoma betæ</i>	60	28	46	74
" + <i>Fusarium</i> sp.	74	20	47	67
" + <i>Phytophthora parasitica</i>	59	29	47	76
" + <i>Sclerotium rolfsii</i>	50	38	6	44
<i>R. solani</i> + <i>Phytophthora parasitica</i>	62	30	44	74
" + <i>Phoma betæ</i>	62	16	21	37
" + <i>Fusarium</i> sp.	60	20	18	38
" + <i>S. rolfsii</i>	60	28	5	33
" + <i>P. aphanidermatum</i>	34	54	32	86
<i>Phytophthora</i> sp. + <i>Phoma betæ</i>	76	12	26	38
" + <i>Fusarium</i> sp.	78	10	22	32
" + <i>S. rolfsii</i>	53	36	8	44
<i>Phoma betæ</i> + <i>Fusarium</i> sp.	82	6	8	14
" + <i>S. rolfsii</i>	30	58	2	60
<i>Fusarium</i> sp. + <i>S. rolfsii</i>	32	56	2	58
<i>Pythium</i> spp. + <i>R. solani</i> + <i>S. rolfsii</i>	38	50	35	85
" + " + <i>Phytophthora</i> sp.	36	54	42	96
" + " + <i>Fusarium</i> sp.	51	37	20	57
" + " + <i>Phoma betæ</i>	56	32	32	64
<i>R. solani</i> + <i>S. rolfsii</i> + <i>Phytophthora</i> sp.	58	30	40	70
" + " + <i>Fusarium</i> sp.	65	23	12	35
" + " + <i>Phoma betæ</i>	64	24	25	49
<i>S. rolfsii</i> + <i>Phytophthora</i> sp. + <i>Fusarium</i> sp.	62	26	10	36
" + " + <i>Phoma betæ</i>	60	28	5	33
<i>Phoma betæ</i> + <i>Fusarium</i> sp. + <i>Phytophthora</i> sp.	75	13	8	21
<i>Pythium</i> spp. + <i>R. solani</i> + <i>S. rolfsii</i> + <i>Phytophthora</i> sp.	38	50	40	90
" + " + " + <i>Fusarium</i> sp.	48	40	28	68
" + " + " + <i>Phoma betæ</i>	50	38	25	63
<i>Phytophthora</i> sp. + " + " + "	49	39	25	64
" + " + " + <i>Fusarium</i> sp.	46	42	22	64
" + <i>Phoma betæ</i> + " + "	45	43	19	62
All pathogenic fungi mixed	36	52	47	99

The data show that *Pythium* singly (refer Table II, Set I) as well as in combination is the most virulent parasite, while *S. rolfsii* loses its virulence in combination. The damage becomes maximum when *Rhizoctonia* sp., *Pythium* spp., and *Phytophthora* sp. were mixed and introduced into the soil.

Cross-Inoculation

The fungi isolated from damped-off tomato seedlings were introduced in the sterilised soil and the seeds of different vegetables and ornamentals were sown in it. Similarly the fungi isolated independently from different damped-off vegetable and ornamental seedlings were inoculated in the soil and tomato seeds were sown. The results of the experiment show that the fungi responsible for damping-off in tomato seedlings can also cause the disease in cabbage (*Brassica oleracea* var. *caulorapa*), cauliflower (*Brassica oleracea* var. *botrytis*), tobacco (*Nicotiana glauca*) and balsamina (*Impatiens balsamina*) seedlings and vice versa.

Inoculation experiments on different varieties of tomato.—The virulence of these fungi was tested on different varieties of tomato. Three varieties were selected, in which Marglobe was an improved one, having fast growth and yielding big fruits. The second one was Ponderosa, a medium and the third one Oxheart, a slow growing, short statured and yielding small fruits. The percentage of pre- and post-emergence damping-off is tabulated in Table V.

TABLE V

Showing the inoculation of different fungi on different varieties of tomato

Name of the fungus	Marglobe % damage		Ponderosa % damage		Oxheart % damage	
	Pre-	Post-	Pre-	Post-	Pre-	Post-
<i>P. indicum</i>	..	25 60	15 50	9 30
<i>P. aphanidermatum</i>	..	24 55	12 40	4 20
<i>Phytophthora parasitica</i>	..	20 40	12 32	.. 12
<i>Rhizoctonia solani</i>	..	15 58	10 42	2 14
<i>Phoma beta</i>	..	8 28	4 20	.. 4
<i>Fusarium</i> sp.	..	6 15	2 8
<i>Sclerotium rolfsii</i>	..	75 8	70 ..	52

'Pre-' stands for pre-emergence loss.

'Post-' stands for post-emergence loss.

From the above table it is quite clear that Marglobe is easily susceptible, while Oxheart is less. Marglobe is a prominent variety but is heavily attacked by the disease.

Control Measures

The study of the problem reveals that the disease generally starts from the soil, therefore, all attempts were made to destroy the fungi in the soil or to protect the seeds through seed treatments from their invasion. A number of the fungicides were compared to see their efficacy in controlling the disease, in statistically laid out plots and their results were analysed and interpreted.

A. Seed treatments.—Nine fungicides were used for seed treatment, viz., Agrosan G, Ceresan, Spergon, Phygon, Zinc oxide, Copper carbonate, Copper sulphate, Formalin and Mercuric chloride. To ascertain the best strength of Copper sulphate, Formalin and Mercuric chloride, to be used as seed treatment, an experiment was laid out in which the seeds were soaked in different percentage of the above fungicides and sown in the soil, which was already infested with the pathogenic fungi. The emergence and damping-off percentage was noted and the analysis of the results revealed that 3% Formalin (considering the commercial formalin as cent. per cent.); 3% Copper sulphate; 0.15% Mercuric chloride are the best strength which can be used safely. The rest of the fungicides were dusts, and the seeds were dusted with them. The excess of the dusts was shaken off.

In comparing the efficacy of all the above fungicides, the treated seeds were sown in soil inoculated with the causal organisms. For check (control), untreated seeds were sown in inoculated soil. To see the effects of various chemicals and fungicides on seed germination, treated seeds were also sown in sterilized soil. Pre- and Post-emergence damping-off were noted and the data was statistically analysed.

TABLE VI

Analysis of variance of Pre-emergence damping-off

Due to	Degrees of freedom	Sum of squares	Mean square
Blocks ..	3	7.2	2.4
Treatments ..	9	10259.3	1139.9
Fungi ..	4	1455.7	363.9
Treatments × Fungi ..	36	1124.5	31.2
Error ..	147	967.4	6.6
Total ..	199	13814.1	..

The above table denotes that the variation due to treatments is significant at 1% level and the symbolical representation of the average percentage of damped-off seedlings placed in order is as follows:—

Ceresan	Agrosan	Phygon	Zinc oxide	Spergon	Copper carbonate	Copper sulphate	Formalin	Mercuric chloride	Control
8.3	9.6	10.2	10.7	11.5	12.4	13.3	14.4	15.0	36.6

The above representation shows that Ceresan is the best fungicide for controlling Pre-emergence damping-off. Then comes Agrosan G, which is less efficacious in controlling the disease than Ceresan and is closely followed by Phygon. Though the percentage of damping-off in Phygon is slightly greater than that in Agrosan G, yet this difference is insignificant and can be attributed to random causes. Each and every fungicide is effective for reducing the disease percentage as obtained from Control.

TABLE VII

Analysis of variance of Post-emergence damping-off

Due to		Degrees of freedom	Sum of squares	Mean squares
Blocks	..	3	48.6	16.2
Treatments	..	9	19403.8	21559.9
Fungi	..	4	5206.5	1310.6
Treatments × Fungi	..	36	1800.7	500.2
Error	..			
Total	..	199	27416.8	..

The above table shows that the variation due to blocks, treatments, fungi, and interaction between different fungi and treatments are highly significant. The symbolical representation of the fungicides in controlling the post-emergence damping-off, in order of their fungistatic action is as follows:—

It is evident from the above representation that Copper sulphate and Formalin lessend the post-emergence damping-off, against Agrosan G and Ceresan, which proved better, in controlling pre-emergence

Copper sulphate	Formalin	Agrosan	Ceresan	Mercuric chloride	Copper carbonate	Zinc oxide	Control	Spergon	Phygon
22.6	27.5	35.9	41.1	45.0	45.6	49.1	51.1	51.7	51.9

damping-off. Copper carbonate and Mercuric chloride do not differ significantly among themselves. Spergon, Phygon and Control come under one line, it means that these two fungicides have no effect in controlling post-emergence damping-off. Since Ceresan and Agrosan G are better in controlling pre- and post-emergence losses as compared with other fungicides, therefore these are considered to be the best seed treatments.

B. Soil treatment (before sowing).—Formaldehyde dust was mixed with the soil before sowing the seeds. A number of experiments were laid out statistically to find out the proper absorbant and correct weight of the fungicide to be used. The formula is given below:—

	Commercial formalin	Absorbant	15 parts
(a)	Wood charcoal	..	85 parts
(b)	Charcoal ash	..	85 parts
(c)	Soil (finely dusted)	..	85 parts

The dust was mixed to the upper 3 or 4 inches of the soil, and then the seedbed was covered with wax paper. The seeds were sown after several days of treatment. Six different weights were used, viz., 8 gm., 16 gm., 24 gm., 32 gm., 40 gm. and 60 gm., per square foot. The data obtained was statistically analysed and interpreted as follows:

TABLE VIII
Analysis of variance

Due to	Degrees of freedom	Sum of squares	Mean squares
Weights ..	5	11151.0	2230.2
Fungi ..	4	590.7	147.7
Weight × Fungi ..	10	1591.1	79.6
Methods ..	2	75.4	37.7
Weights × Methods ..	10	107.4	10.7
Fungi × Methods ..	8	20.1	2.5
Fungi × Weight × Methods ..	40	75.0	1.9

The interaction between weights and methods, fungi and methods, fungi, weights and methods, were separately analysed and the symbolical representation for the different weights runs as follows:—

40 gm.	32 gm.	24 gm.	16 gm.	8 gm.	Check
10.7	12.5	14.1	18.7	22.3	43.6

Since 60 gm. per square foot delayed the seed germination and often caused injury to the seeds, therefore its data was not considered in the comparison. The representation shows that the use of 40 gm., 32 gm. and 24 gm., do not differ significantly, *i.e.*, the use of any of these weights will check the disease equally well, therefore to avoid the risks, 32 gm. per square foot, is considered to be the best treatment. The fungi have got definite effect in producing the disease and regarding, different methods of absorbants, the following is their symbolical representation:—

Charcoal ash	Charcoal powder	Soil
19.6	19.8	21.6

It denotes that the Charcoal ash is the best absorbant. Agrosan G and Ceresan had already been considered to be the best seed treatments and formaldehyde dust at the rate of 32 gm. per square foot, as best soil treatment. An experiment was therefore laid out in which the treated seeds with both the dusts were sown in beds, the soil of which was treated with formaldehyde dust. It was seen that the disease was absent from such beds (Figs. 1 C & E; D & F).

C. Controlling the disease after sowing.—Cheshunt compound and 2.5% formalin (commercial) were used as spray in the seedbeds to control the disease after sowing. An experiment was statistically laid out, in order to compare the above two fungicides. The seedbeds selected for the experiment were inoculated with the pathogenic fungi, as usual, leaving check beds to see the effect of the fungicide on the seedlings. The seeds were sown and the next day all the beds were sprayed with the above fungicides. The beds were then divided in three groups, in the first group, no subsequent spraying was done; in second, spraying was done every seventh day; while in the third, spraying was done every third day, and all this was maintained upto 21 days. The critical statistical study of the data revealed that spraying at every third day was better in the case of both the fungicides. Both of them stand on equal footing but cheshunt compound was slightly better, as formalin often kills the seedlings due to its toxic nature.

The results obtained from the experiments on controlling the damping-off in tomato, denote that, Ceresan is the best seed treatment and

Formaldehyde dust as the best soil treatment, at the rate of 32 gm. per square foot, applied to upper 3 or 4 inches layer of soil, a fortnight before sowing, while when they both were applied, the disease was found almost controlled.

Summary

Damping-off of tomato seedlings is a serious problem of nurseries. of Uttar Pradesh, and ten fungi were isolated from the diseased specimens, brought from several places. Out of them only seven could be proved pathogenic. These have been identified as *Pythium indicum* Balakrishnan, *Pythium aphanidermatum* (Edson) Fitz., *Phytophthora parasitica* Dastur, *Rhizoctonia solani* Kühn., *Sclerotium rolfsii* Sacc., *Phoma betæ* (Oudem.) Fr., and *Fusarium* sp.

A method of separating mixed cultures of *Pythium indicum* and *Phytophthora parasitica* was found out by inoculating them on *Hibiscus esculentus* and *Ricinus communis* respectively. The oospores of *Pythium indicum* were found to be viable for more than 150 days.

The pathogenic fungi infected the seedlings upto the age of 5 weeks. Varietal tests show that Marglobe is the most susceptible and Oxheart the least susceptible variety.

Various wet and dry seed treatments were carried out to control the disease, and statistical analysis of the data obtained shows that Ceresan is the best seed treatment in controlling pre-emergence damping-off.

Formaldehyde dust was used for soil treatment, and three different absorbants were taken, out of which Charcoal ash proved slightly better than others. Its application at the rate of 24-40 gm. per square foot of upper 3 inches of the soil proved to check the disease. A combined application of Ceresan as seed treatment, and formaldehyde dust at the rate of 24-40 gm. ($\frac{1}{2}$ — $\frac{3}{4}$ chattaks) per square foot, as soil treatment controlled the disease.

After sowing, spraying with 2.5% formaline and cheshunt compound checked the disease.

ACKNOWLEDGEMENT

The authors are greatly indebted to Dr. B. L. Sethi for encouragement and keen interest during the course of investigation and to the U.P. Scientific Research Committee for partly financing the work.

The writers are also thankful to Sri. N. P. Srivastava for the help given in analysing the data statistically.

LITERATURE CITED

1. ALEXANDER, L. J., YOUNG, H. C. AND KIGER, C. M. 1931. Causes and control of damping-off of tomato. Ohio. Agri. Expt. Bull. 496.
2. BALAKRISHNAN, M. S. 1948. South Indian Phycomycetes. I. *Pythium indicum* sp. nov. causing fruit rot of *Hibiscus esculentus* L. Proc. Ind. Acad. Sci., B, 27.

3. BEWEY, W. F. 1921. Control of damping-off and foot rot of tomatoes. Jour. Minn. Agr. (Gr. Brit.). 28: 653-54.
4. ————. 1920. Damping-off and foot rot of tomato seedlings. Ann. Appl. Biol. 7: 156-72.
5. BRIEN, R. M. AND CHAMBERLAIN, E. E. 1936. Tomato seedlings damping-off. I. Control by seed treatments. New Zealand Jour. Agr. 52: 257-67.
6. BROWN, W. 1941. Damping-off disease of tomato. Gardener's Chronicle Ser. 3, 109: 55.
7. BUTLER, E. J. 1907. Studies in the genus *Pythium*. Mem. Dept. Agr. India Bot. Series, Vol. I.
8. CUNNINGHAM, H. S. AND SHARVELLE, E. G. 1940. Organic seed treatments for lima beans. Abs. Phytopath. 30.
9. DASTUR, J. F. 1912. *Phytophthora parasitica*—a new disease of the castor oil plant. Mem. Dept. Agr. India Bot. Series. Vol. 5.
10. GODBOUT, F. L. 1930. Some studies of seed treatments. Canada Phyto. Sec. Prec. 47-54.
11. GUTTERMAN, C. F. AND MASSEY, L. M. 1935. A liquid formaldehyde treatment to control damping-off of flower seedlings. Abs. Phytopath. 25.
12. HAENSLER, C. M. 1935. Formaldehyde does well in controlling damping-off N.J. Agr. 17: 1.
13. HIGGINS, B. B. 1927. Physiology and parasitism of *Sclerotium rolfsii*. Phytopath. 17.
14. HORSEFALL, J. G. 1930. Combating damping-off of tomato by seed treatment. N.Y. (Geneva) Agr. Expt. Sta. Bull. 585.
15. ————. 1932. Dusting tomato seeds with copper sulphate. N.Y. (Geneva) Agr. Expt. Sta. Tech. Bull. 198.
16. ————. 1934. Zinc oxide as seed and soil treatment. N.Y. (Geneva) Agr. Expt. Sta. Bull. 650.
17. ————. 1934. Dusting with red copper oxide. N.Y. (Geneva) Agr. Expt. Sta. Bull. 643.
18. ————. 1935. Red oxide of copper as dust fungicide for combating damping-off by seed treatments. N.Y. (Geneva) Agr. Expt. Sta. Bull. 615.
19. MONTIETH, J. AND DAHL, A. S. 1928. Comparison of some strains of *Rhizoctonia* in cultures. J. Ag. Res. 36 : 897-903.
20. SHAW, F. J. F. 1912. Morphology and parasitism of *Rhizoctonia* sp. Mem. Dept. Agr. India Bot. Series. Vol. 5.
21. SMALL, T. 1928. *Rhizoctonia* foot rot of tomato. Ann. Appl. Biol. 14: 290-95.
22. SRIVASTAVA, H. C. 1949. Unpublished thesis on damping-off of tomato seedlings submitted to Agra University.
23. SUBRAMANIAM, L. S. 1919. A *Pythium* disease of ginger, tobacco and papaya Mem. Dept. Agr. India Bot. Series. Vol. 10.
24. TINT HOWARD. 1945. Studies in *Fusarium* damping-off of conifers. I. The comparative virulence of certain fusaria. Phytopath. 35: 421-39.
25. VAN HALTERN, F. 1935. Control of tomato seedbed diseases. Ga. Agr. Expt. Bull. 187.
26. WILSON, J. D. 1932. The use of formaldehyde dust in growing celery seedlings. Ohio Agr. Expt. Sta. Bimonth. Bull. 159.
27. ———— AND TILFORD, P. E. 1933. The use of formaldehyde dust in growing seedling. Ohio Agr. Expt. Sta. Bull. 520.
28. WRIGHT, E. 1945. Relation of micro-fungi and micro-organism of soil to damping-off of broad-leaf nurseries. J. Ag. Res. 70.

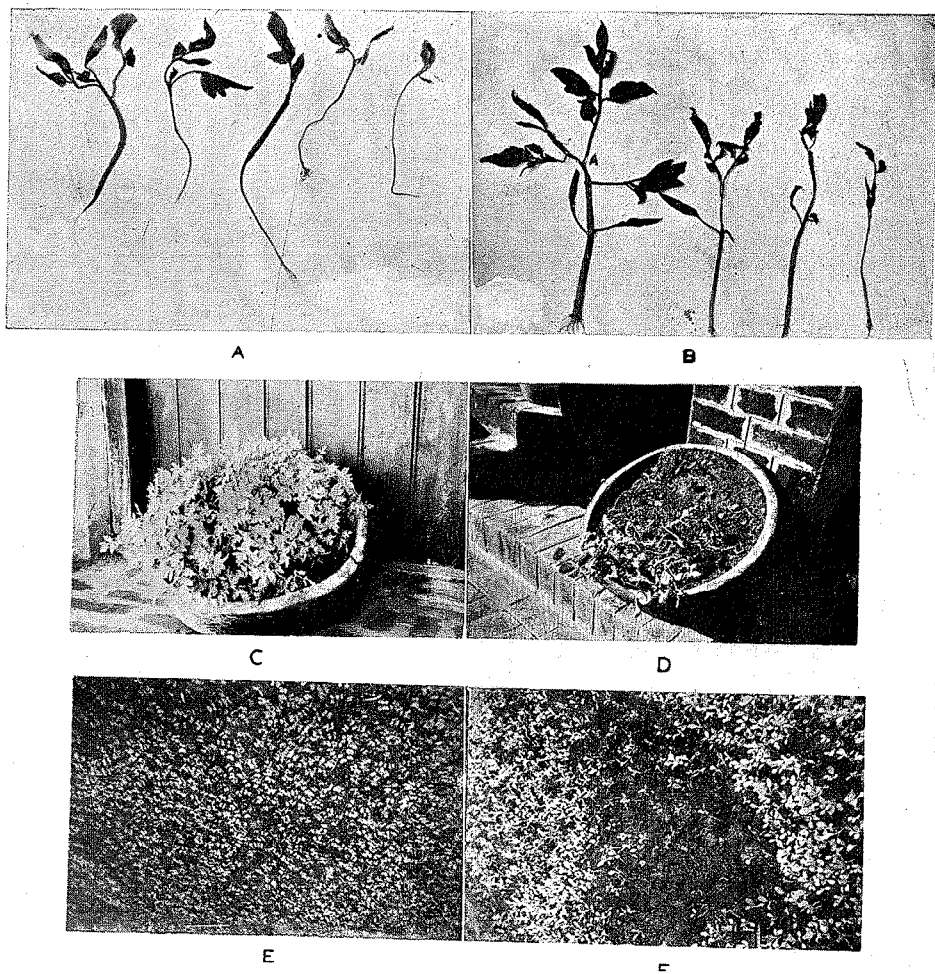


FIG. 1. *A.* Damped-off tomato seedlings, exhibiting base infection. *B.* Seedlings showing 'Top infection' and the advancement of the disease is downwards. *C & E.* Pot and bed showing healthy seedlings growing, where seeds were treated with Ceresan are sown in the soil treated with Formaldehyde dust. *D & F.* Pot and bed (check) where untreated seeds are sown in untreated soil.

Babu Singh and H. C. Srivastava