The Pharma Innovation Journal 2023; 12(4): 2709-2712

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(4): 2709-2712

www.thepharmajournal.com
Received: 02-01-2023
Accepted: 12-03-2023

Bhagyashali V Hudge
College of Horticulture, Mojerla, Wanaparthy, Sri Konda Laxman Telangana State Horticultural University, Hyderabad, Telangana, India

T Navya Swetha
College of Horticulture, Mojerla, Wanaparthy, Sri Konda Laxman Telangana State Horticultural University, Hyderabad, Telangana, India

Bontha Rajasekar
College of Horticulture, Mojerla, Wanaparthy, Sri Konda Laxman Telangana State Horticultural University, Hyderabad, Telangana, India

Dr. Purnima Mishra
College of Horticulture, Mojerla, Wanaparthy, Sri Konda Laxman Telangana State Horticultural University, Hyderabad, Telangana, India

Corresponding Author:
Bhagyashali V Hudge
College of Horticulture, Mojerla, Wanaparthy, Sri Konda Laxman Telangana State Horticultural University, Hyderabad, Telangana, India

Effect of environmental factors on the growth of *Pythium ultimum* Trow. Which causes Soybean damping-off

Bhagyashali V Hudge, T Navya Swetha, Bontha Rajasekar and Dr. Purnima Mishra

Abstract
Environmental studies on different soil types indicated that significantly highest pre-emergence damping-off with lowest germination was noted in clayey soil while sandy loam and sandy soil responded with significantly least seed rot. In respect of temperature, *Pythium ultimum* was found to grow in temperature range of 20 ºC to 35 ºC, with maximum growth in the temperature range of 25 ºC to 35 ºC. The maximum sporangial formation was found in temperature range of 25 ºC to 35 ºC. The oospore formation was maximum and was at par 30 ºC, 35 ºC and 25 ºC. In pH studies (*in-vitro*), the pathogen *Pythium ultimum* Trow. could grow in the pH range of 4 to 10, however, significantly highest growth of the pathogen and formation of sporangia and oospore was noted at pH 6.

Keywords: Damping-off, *Pythium ultimum*, soil types, temperature, pH, soybean

Introduction
With the growing importance of soybean [*Glycine max* (L.) Meril.] in world economy it has become necessary to stabilize the production and to increase productivity of soybean in India. Soybean is better known for poor germination and less final plant stand due to pre and post emergence damping-off disease. Disease severity, prevalence and agronomic losses are closely associated with environmental conditions, crop management practices and reaction of soybean cultivars to infection by the many plant pathogens. Soybean crop is attacked by more than dozens of diseases of fungal, bacterial and viral nature leading to great loss to the yields. Early planting, wet, poorly drained or compacted soils are especially prone to these diseases. *Pythium*, *Phytophthora*, *Rhizoctonia* and *Fusarium* are the most common soil borne fungi causes early-season soybean diseases (Hendrix and Campbell, 1973; Van der Plaats-Niterink, 1981) [4, 8]. Among these, *Pythium* species such as *Pythium ultimum*, *Pythium aphanidermatum*, *Pythium debaryanum*, *Pythium myriotylum* are associated with pre-emergence and post-emergence damping-off, are of worldwide distribution which always leads to poor stand in field and consequently reduces yield.
Soil moisture is a highly decisive factor in inducing losses by pre and post emergence damping-off. When soil moisture is increased from -1.8 to -0.018 bars matric potential at 20 ºC, reduction in emergence from 66 to 15 per cent has been reported by Schlub and Lockwood (1981) [6] due to seed and seedling root of soybean caused by *P. ultimum.* The severity of damping off and root rot by *P. ultimum* was enhanced by relatively high soil moisture levels in different crops and thus provided a suitable environment for the rapid diffusion of seed exudates through soil.
Soil type also affect the inoculum development as different soil properties (texture and structure) affect the water content at saturation, field capacity and permanent wilting point. Tripathi and Grover (1974) [7] observed least pre and post emergence damping off in sandy loam soils. They compared incidence of damping off in loamy, clayey loam and heavy clay loam soils and noted that pre and post emergence damping off was maximum in heavy clay loam soils. Ferriss and Baker (1990) [2] evaluated eleven soybean seed lots using standardized laboratory tests of seed quality and greenhouse tests of percentage and speed of emergence from different soils infested with *Pythium ultimum* and overall results indicated that the seed quality test which predicts soybean emergence can vary with seedbed conditions.
Different *Pythium* spp. differ in their temperature requirement for growth and sporulation. *Pythium aphanidermatum* has relatively higher temperature requirement ranging from 24º-30

ºC for infection on soybean. *Pythium ultimum* and *Pythium debaryanum* has relatively less temperature requirement for 15-20 ºC for infection on soybean. The moderate to cool temperature always favour disease development by *Pythium ultimum.* In case of pea overall damping off by *P. ultimum* was reduced at 18/25 ºC (night/day) as compared to 13/20 ºC. It has been also observed that the temperature requirement for pre and post emergence damping off was also varies. *Pythium butleri* caused more pre-emergence damping off at 20 ºC and post-emergence damping off at 35 ºC. Tripathi and Grover (1974) [7] in general noted the temperature range of 20-40 ºC ideal for the damping-off disease development.

Soil pH plays very important role by influencing growth of sporulation of pathogen. Griffin (1958) [3] noted high incidence of damping off at neutral or alkaline soil. He observed that conversion of thick to thin (dormant to germinable) oospores was maximum at pH 7, saturated with water at 25 ºC. Tripathi and Grover (1974) [7] however noted maximum damping off at pH 6. Dahiphale (2006) [1] also noted 6.5 pH as an optimum pH for growth and sporulation of *P. ultimum*.

With consideration of importance of environmental factors on soybean damping-off disease the present investigation was aimed to study the effect of soil types on germination of soybean seeds. Also, to study the effect of different temperature and pH levels on the growth and sporulation of *P. ultimum*, *in-vitro*.

Materials and Methods

a) Soil type

Four soil types i.e., heavy soil (black cotton), sandy soil, loamy soil (red), sandy loam soil was tested. The experiment was planned in split plot design. Inoculation (I_1) and control (I_0) served as main treatments and soil types served as sub treatments.

Sick soil was developed by inoculating these soil types with *Pythium* inoculum. Four replications with 4 pots per replications were maintained. Four seeds of soybean variety JS-335 were sown in each pot. Observations on germination were recorded after one week of sowing.

b) Temperature

Effect of temperature on the growth of the pathogen was studied, *in vitro*. Different levels of temperature were maintained in refrigerator, incubator and hot air oven at 0 ºC, 5 ºC, 10 ºC, 15 ºC, 20 ºC, 25 ºC, 30 ºC and 35 ºC as per the adjustability of the instrument. Experiment was conducted on PDA in Petri plates. Design of the experiment was RBD. For each treatment of temperature four replications of Petri plates were maintained. PDA was sterilized and poured in sterile Petri plates. These Petri plates were inoculated with 5mm inoculum disc, and then incubated at respective temperature level. One set of Petri plates was incubated at room temperature. Observations on growth, sporulation and other cultural characteristics were recorded after 4 days of incubation.

c) pH

Effect of different pH levels *viz*. 3, 4, 5, 6, 7, 8, 9 and 10 on the growth of pathogen were studied, *in vitro*. Autoclaved melted 8 PDA flasks (250 ml) were cooled down for some time and then by adding H_2SO_4 and KOH, pH of media was adjusted with the help of pH meter. For acidic media H_2SO_4 and for alkaline media KOH were added. All flasks containing adjusted pH of media were further autoclaved. Then autoclaved media was poured in sterilized Petri plates. Four Petri plates were maintained for each pH treatment. Inoculum disc (5mm) was plated in each Petri plates. These Petri plates were incubated at 25 ºC. After 3 days of incubation, observations on growth, sporulation and other cultural characteristics were recorded.

Results

Effect of soil types on pre-emergence damping-off

For assessing the role of soil types an experiment was planned in split plot design with sick soil (I_1) and sterile soil (I_0) as two main treatments. Soil types served as sub treatments. After collecting different soil types, they were added with sorghum flour 25g/kg of soil. The different soil types were sterilized in autoclave with 15-pound pressure/sq. inch. for 1 hr. After sterilization each soil type was inoculated with 5mm inoculum disc in each plastic container. After incubation of 4 days under stagnant, humid situation under polythene sheet. The seeds of JS-335 were sown in I_1 and in I_0. The observations on germination percentage were noted after 10 days of sowing in Table 1.

Table 1: Germination (%) as influenced by soil types

Sr. No.	Soil Type	Germination % (Arc sin values)			Main Tr.	
		I_0	I_1	Mean		
1.	Sandy	84.89	28.04	56.47		
2.	Loamy	79.80	24.04	51.92		
3.	Sandy Loam	61.50	31.09	46.30		
4.	Clayey soil	51.45	20.11	35.78	SE±	C.D.@0.05
	Mean	69.41	25.82	47.61	3.08	12.09*
Sub Tr.			SE±	1.86		
			C.D.@0.05	5.38*		
Interaction (I ×S)						
		SE±	C.D.@0.05			
		2.64	7.62*			

From this experiment it was observed that all soil types in general have significantly reduce germination when inoculum was added to them. The soil types also differed significantly in reducing the germination. Significantly lowest germination was observed in clayey soil. Sandy and loamy soil were at par for response to germination which gave significantly higher germination. The interaction I×S was also significant. The pathogen had significantly reduced germination in all the soil types, thereby indicating the major role of *Pythium ultimum* in causing seed rot. Significantly highest losses in germination and highest seed rot by the pathogen was noted in clayey soil. This may be due to highest water holding capacity of clayey soil which allowed maximum multiplication of the pathogen and conversion of thick-walled oospores into thin-walled oospores, thereby creating virulent effective inoculum potential.

The present findings are in confirmative with Schlub and Lockwood (1981) [6], Lumsden and Ayers (1975) [5]. Tripathi and Grover (1974) [7] who also noted highest incidence of pre- and post-emergence damping off in heavy clay loam soils.

Temperature studies

With *in vitro* experiment in RBD the optimum temperature requirement of the pathogen was decided. The experiment was planned with 9 temperature levels and 4 replications.

PDA was prepared, autoclaved and poured in sterile Petri plates. Inoculum disc (5mm) was plated at the center. For each temperature level 4 Petri plates were maintained. The different temperature levels were adjusted either in fridge, A.C., incubator or hot air oven as per the requirement of temperature level. After 3 days of incubation at given temperature the observations on colony diameter and cultural characteristics were noted which are given in Table 2.

From table 2. it can be inferred that pathogen could grow in the temperature range of 5 to 35 ºC. Significantly highest growth was recorded at 30 ºC. The temperature of 25 ºC and fluctuating room temperature (27-30 ºC) were also at par. Rest of the temperature levels were significantly inferior to 30, 25 ºC and room temperature (27-30 ºC).

Observations on cultural characters and sporulation of *Pythium ultimum* were also noted. Sporangial formation occurred in the temperature range of 10 to 35 ºC and was maximum from 25 to 30 ºC. Oospore formation occurred in the temperature range of 20 to 35 ºC and was maximum from 25 to 35 ºC.

pH in relation to growth and sporulation of *Pythium ultimum*: pH plays very important role in building up of inoculum and thereby development of epidemic. In order to know the optimum pH for growth and sporulation of *Pythium ultimum* infecting soybean the present investigation was undertaken in RBD with 8 levels of pH and 4 replications. PDA was prepared and autoclaved at 15 lbs pressure/sq. inch. Before pouring, acidic pH of media was adjusted by adding few drops of H_2SO_4. Higher levels of acidic pH required correspondingly more drops of H_2SO_4. The naturally prepared medium had pH of 6 and therefore for adjusting pH 7 and other alkaline pH levels, few drops of NaOH were added in increasing order. For each pH level 4 Petri plates were poured. After cooling, plates were inoculated with 5mm inoculum disc and incubated at room temperature (28 ± 4 ºC). The observations on colony diameter were taken horizontally and vertically, taking their mean as mean colony diameter in mm. The observations on margin, topography, colour and pigmentation were also noted. For sporulation 4 slides of each pH levels were prepared and their comparative abundance especially of sporangia and oospores were noted after 4 days of inoculation. The observations are given in Table 3.

From table 3, it can be concluded that pathogen could grow in the pH range of 4 to 10. It could not grow at pH 3. Significantly highest growth was induced at pH 6 which was superior over pH 4, 5, 7, 8, 9 and 10. On the basis of $(\sqrt{x}+1)^2$ transformation pH 7 and 8 were at par. Sporangial formation occurred in the pH range of 4-10 and was maximum at pH 6, 7, 8 and 9. Oospore formation occurred in the range of pH 5 to 10 and was maximum at pH 6 and 7.

Present findings are in agreement with Dahiphale (2006) [1]. The favorable pH allowed the conversion of thick, dormant oospores to thin germinable oospores in saturated soils at 25 ºC, thereby creating very effective inoculum for disease epidemics. Similar observations were also noted in the field experiments wherein frequent rains and soil saturation, favorable temperature caused maximum losses by pre-emergence damping -off led to poor germination in most of the varieties sown.

Table 2: Effect of temperature on mycelial growth, cultural characters and sporulation of *Pythium ultimum*

		Mean colony diameter			Cultural characters			Sporulation		
Sr. No	**Temperature (°C)**	**Original value (mm)**	**% Trans.**	**Arc sin value**	**Margin**	**Topography**	**Color**	**Pigmentation**	**Sporangia**	**Oospores**
1	0	5.00	5.55	3.18 ^{g}	-	-	-	-	-	-
2	5	15.50	17.21	9.91 ^{f}	Circular	Aerial	White	Faint Pinkish	-	-
3	10	45.50	50.55	30.37 ^{e}	—"—	—"—	—"—	—"—	+	-
4	15	61.50	68.32	43.10 ^{d}	—"—	—"—	—"—	—"—	++	+
5	20	86.00	95.55	73.07 ^{b}	—"—	Flat	—"—	—"—	+++	++
6	25	90.00	100	89.98 ^{a}	—"—	Flat	—"—	—"—	++++	+++
7	30	90.00	100	89.98 ^{a}	—"—	Flat	—"—	—"—	++++	+++
8	Room Temp. (27-30 ºC)	90.00	100	89.98 ^{a}	—"—	Flat	—"—	—"—	++++	+++
9	35	82.00	91.11	65.67 ^{c}	—"—	Flat	—"—	—"—	++++	+++
	SE±	0.48	0.53	0.67						
	C.D. at 5%	1.40*	1.56*	1.96*						
	C.V. (%)	1.53	1.53	2.44						

Note: -: Absent, +: Poor, ++: Fair, +++: Good, ++++: Excellent.
Figures with same letters are statistically at par.

Table 3: Effect of pH on mycelial growth and cultural characters of *Pythium ultimum*

		Mean colony diameter			Cultural characters				Sporulation	
Sr. No.	**pH Levels**	**Original value (mm)**	**% Trans.**	**Arc sin value**	**Margin**	**Topography**	**Color**	**Pigmentation**	**Sporangia**	**Oospores**
1.	pH3	5.00	6.85	3.92^{h}	-	-	-	-	-	-
2.	pH4	56.75	77.73	51.04^{f}	Circular	Aerial	White	Faint Pinkish	++	-
3.	pH5	66.50	91.09	65.68^{c}	—"—	Flat	—"—	—"—	+++	++
4.	pH6	72.00	98.63	81.88^{a}	—"—	Flat	—"—	—"—	++++	+++
5.	pH7	69.00	94.52	71.08^{b}	—"—	Flat	—"—	—"—	++++	+++
6.	pH8	64.25	88.01	61.70^{d}	—"—	Flat	—"—	—"—	++++	++
7.	pH9	61.50	84.24	57.47^{e}	—"—	Flat	—"—	—"—	++++	++
8.	pH10	52.75	72.25	46.27^{g}	—"—	Flat	—"—	—"—	+++	++
	SE±	0.57	0.78	1.32						
	C.D. at 5%	1.67*	2.29*	3.88*						
	C.V. (%)	2.04	2.04	4.82						

Note: -: Absent, +: Poor, ++: Fair, +++: Good, ++++: Excellent
Figures with same letters are statistically at par

Conclusion

A set of combination of temperature, moisture, pH and organic carbon levels and soil types influencing the damping off in soybean and other many pulse crops have been worked out. When all conditions were favorable to pathogen, no germination occurred. Temperature plays a very important role in developing inoculum of *Pythium ultimum* in soil. So as to create the artificial epiphytotic of *Pythium ultimum* it was necessary to optimize the temperature requirement of the pathogen. This was studied in depth by many Americans as well as Indian scientists. With reduction of temperature and moisture, there was drastic reduction in pre-emergence damping off. The author also observed similar trend in the interaction of soybean and *Pythium ultimum*.

References

1. Dahiphale SJ. Physiological investigations of *Pythium ultimum* Trow causing damping off in tomato. M.Sc. (Agri.) These. Marathwada Agricultural University, Parbhani (M.S.); c2006.
2. Ferriss RS, Baker JM. Relationships between soybean seed quality performance in soil. Seed Sci. Technol. 1990;18(1):51-73.
3. Griffin DM. Influence of pH on the incidence of damping off. Trans. Br. Mycol. Soc. 1958;41:483-490.
4. Hendrix FF, Campbell WA. *Pythiums* as plant pathogens. Annu. Rev. Phytopath. 1973;11:77-98.
5. Lumsden RD, Ayers WA. Influence of soil environment on the germinability of constitutively dormant oospores of *Pythium ultimum.* Phytopath. 1975;65:1101-1107.
6. Schlub RL, Lockwood JL. Etiology and epidemiology of seedling rot of soybean by *Pythium ultimum.* Phytopath. 1981;71:134-138.
7. Tripathi NN, Grover RK. Inoculum potential and soil factors affecting the pathogenesis of *Pythium butleri* in causing damping-off of tomato. The paper was presented at the Symposim on, Ecology of Soil Microorganisms held on 27-29 December, 1974, 41B(5).
8. Van der Plaats-Niterink AJ. Monograph of the genus *Pythium.* Studies in Mycology Centralburean Voor Schimmelcultures Baarn, The Netherlands. 1981;21:1-239.