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Research Article

Influence of soil pH and moisture on the biocontrol potential of *Trichoderma harzianum* on *Phytophthora capsici*–black pepper system

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ABSTRACT: The role of abiotic factors in maintaining the population and proliferation of *Trichoderma harzianum* (Rifai) with respect to the survival and multiplication of *Phytophthora capsici* in black pepper system was studied in conducive soil under pot culture conditions. *Trichoderma harzianum* could grow profusely at a pH range of 4.0–5.0, while *P. capsici* could grow better at *p*H 5.5–6.0. *Trichoderma harzianum* survived and proliferated in soil at a pH range of 4.5–5.5 with 10–15% moisture level. *P. capsici* survived at a pH range of 4.5–7.0, but its multiplication was at higher pH levels (6.6–7.0) as indicated by the disease potential index and it was also found capable of surviving at all moisture levels tested. When *Phytophthora* infested soil was supplemented with *Trichoderma,* a disease reduction of 27.5–63.75% was noticed in 20 days application compared to soil without *Trichoderma* supplement. Hence for the biological control of *P. capsici* in black pepper system, the pH of the soil has to be maintained after at 4.5–6.0 in order to facilitate the growth and proliferation of *Trichoderma* which in turn will reduce the population of *Phytophthora.*

KEY WORDS: Biological control, black pepper, foot rot, *Trichoderma harzianum, Phytophthora capsici*, survival

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INTRODUCTION

Black pepper (*Piper nigrum* L.) is grown in soil that is moist, well-drained not too dry or susceptible to flooding, and rich in organic matter. This condition is often congenial for the growth and development of *Phytophthora capsici*, the cause of foot rot disease, the major threat for black pepper that causes severe economic losses (Anandaraj, 2004). Infected soil and plant debris form the primary source of inoculum for the initiation of the disease. The detectability of the pathogen was maximum during wet period (Nambiar *et al.,* 1979). Among the fungal biocontrol agents, *T. harzianum* and *T. viride* have been established as potential candidates against this disease (Sarma *et al.,* 1997, Rajan *et al.,* 2002). But for the use of *Trichoderma* as a biocontrol agent against *P.capsici*, an understanding of the role of abiotic factors such as soil moisture, temperature, pH and nutrient availability favoring the growth and establishment of the pathogen as well as biocontrol agents in *Trichoderma–P. capsici* black pepper system is required. Moreover, the potential of the bioagents can be better exploited only if the conditions are favorable for its growth, colonization and proliferation in the environment. This is possible only by understanding the factors favoring its growth and colonization. Hence in this experiment, an attempt was made to study the influence of soil pH and moisture on the survival of *P. capsici* as well as the growth and proliferation of *Trichoderma* and its suppressive effect on *P. capsici* in a conducive environment.

MATERIAL AND METHODS

The experiment was conducted at Indian Institute of Spices Research, Calicut, during 2007-08. Initially the effect of pH on the growth of *T. harzianum* and *P. capsici* was studied *in vitro*. Further, conducive soil was adjusted to different pH and moisture levels and supplemented with the biocontrol agent and planted with rooted cuttings of black pepper to study the effect *in vivo*. Conducive soil was collected from IISR experimental farm, Peruvannamuzhi. Rooted cuttings of black pepper variety Panchami having 3-4 leaf stage raised in potting mixture of soil, sand and FYM (1:1:1) in polythene bags were used for the experiment. These plants were transplanted in 12" x 12" size earthenware pots as per the experimental requirement.

Preparation of Trichoderma harzianum

Trichoderma harzianum (MTCC 5179), being used for the biocontrol of foot rot disease of black pepper and maintained in the biocontrol repository at Indian Institute

of Spices Research, Calicut, Kerala, was used. The isolate was grown on 100ml of potato dextrose agar (PDA) in 250 ml conical flask for 7 days. The spores formed on the surface of the medium were made into a suspension by adding 300 ml of sterile distilled water and filtered through three layers of sterile muslin cloth. After filtration, the spore load ml^{-1} was calculated using Haemocytometer. Spore suspension @ 5ml containing 10^7 CFUs ml^{-1} was injected to 1 kg sterilized coir compost at 25% moisture and packed in polypropylene bags and mixed thoroughly. The polybags were incubated at room temperature for 10 days and the CFU g^{-1} of the coir compost was determined by serial dilution plate method using *Trichoderma* selective medium (TSM). This multiplied media containing 10^8 CFUs g^{-1} was used as *Trichoderma* inoculum and added @ 100 g pot^{-1}.

Effect of pH on growth of T. harzianum *and* P. capsici in vitro

The effect of pH on the growth *T. harzianum* (MTCC 5179) was studied on PDA adjusted to pH ranging from 4.0 to 7.0 using 1N NaOH and 1N HCl (Sid Ahmed *et al*., 1999). The media was autoclaved at 121°C at 15 lbs for 20 minutes. Fifteen ml of the media was poured into 90 mm Petri dishes and inoculated with 5mm mycelial plugs of *T. harzianum* cut from the edge of 72h old-actively growing culture and incubated at ± 25°C. The diameter of the colony was measured at 48h. Similarly five mm mycelial plugs of *P. capsici* from 72h old-actively growing culture was inoculated at the centre of 90 mm Petri dish containing carrot agar adjusted to different pH and incubated at ± 25°C under ambient conditions. The diameter of the colony was measured at 72 h.

Effect of pH and moisture on the growth and proliferation of T. harzianum *and* P. capsici in vivo

The experiment was conducted in pot culture and designed in CRD in a split-split plot design. The main treatment consisted of five pH levels 4.5–5.0, 5.1–5.5, 5.6–6.0, 6.1–6.5 and 6.6–7.0. The sub-treatments were four moisture levels 35–40%, 30–35%, 20–25% and 10–15%. The sub-sub treatments consist of two treatments namely, conducive soil supplemented with *T. harzianum* and conducive soil without *Trichoderma* (control).

The initial pH of the conducive soil was measured, and then adjusted to different pH levels by the addition of lime. To standardize the amount of lime required to get the desired pH, different quantities of lime (Table 1) were mixed thoroughly with 12 kg soil in 12" x 12" earthenware pots in triplicate and saturated with water. The soil was incubated for 3 days and then the pH of the soil was measured using a pH meter. The quantity of lime that gave the desired pH was selected for adjusting the soil pH for the experiment.

Table 1. Standardization of pH using lime

Quantity of lime added to 12 kg soil	*p*H range
Initial soil	4.5 – 5.0 (4.7)
1gm	5.1 – 5.5
5gm	5.6 – 6.0
10gm	6.1 – 6.5
25gm	6.6 – 7.0

Soil of about 12 kg each was filled in earthenware pots of 12" x 12" and adjusted to five different pH ranging from 4.5–7.0 *viz.* 4.5–5.0 (control), 5.1–5.5, 5.6–6.0. 6.1–6.5 and 6.6–7.0. The soil moisture was adjusted by adding one liter of water at regular intervals to maintain a moisture level of 35–40% by irrigating the pots (twice daily), 30–35% (once daily), 20–25% (once in two days) and 10–15%, (once in 7 days), respectively. The pots were supplemented with *Trichoderma* enriched coir-compost @ 100 g pot^{-1} in the respective treatment.

Enumeration of *T. harzianum* from the treated soil was done by soil dilution plating by collecting soil samples from respective treatments at weekly intervals. The soil was air dried, suspended in 90 ml sterile distilled water taken in a 250 ml Erlenmeyer flask and stirred well for 20 min. From this serial dilutions were made up to 10^{-5} and 1ml of this was plated using *Trichoderma* selective medium (TSM). The plates were incubated at ± 25°C for five days for enumerating the colony forming units (CFUs).

Quantification of *P. capsici* (disease potential index) from the inoculated soil was done by soil dilution baiting technique using leaflets of *Albizzia falcataria* (Anandaraj and Sarma, 1990). Soil was diluted up to 10^{-10} dilution and the DPI was recorded. The data were analyzed statistically using Windowstat package.

RESULTS AND DISCUSSION

Effect of pH on the growth of T. harzianum *and* P. capsici in vitro

Significant difference in growth of *T. harzianum* was observed at pH f4.0–7.0. Growth was maximum at pH 4.0 (90.00 mm) followed by 4.5 (86.87 mm) and minimum growth (73.67mm) was observed at pH 6.0. However, in general, *Trichoderma* is found growing at all pH regimes, though there is a slight reduction in growth at pH 5.5–6.5. The growth of *P. capsici* was most supported by pH 5.5 (72.33mm) followed by pH 6.0 (71.00 mm). Comparatively lower growth was observed at pH 4.0 (56.00mm)

Effect of pH and moisture on the growth and proliferation of T. harzianum *and* P. capsici *in vivo*

The initial DPI of the conducive soil was observed as 1024 (reciprocal of the highest dilution). Rooted cuttings planted in this soil before *Trichoderma* application died in 4-7 days after planting due to the heavy inoculum load of *P. capsici*. In soil, at different pH and moisture levels, *Trichoderma* showed variation in population level. A pH of 4.5–5.5 was found highly suitable for the growth and establishment of *T. harzianum* (5.82–5.98 log cfu g^{-1}) at all the tested moisture levels (10–40%) (Table 2 and 3). But there was a decline in the population above pH 5.5 at all the moisture levels (3.48–4.81 log CFUs g^{-1}) and the trend continued throughout. This clearly indicated that pH above 5.5 is not suitable for the growth and multiplication of *T. harzianum* in the soil**.** Hence, *T. harzianum* prefers an acidic pH and it can survive and proliferate in soil having a pH range of 4.5–5.5 at moisture levels of 10–40%.

When *Phytophthora* infested soil was adjusted to different pH levels from 4.5 – 7.0, the maximum DPI was obtained at pH 7.0 (Table 4), but moisture level did not show any significant difference. This showed that *Phytophthora* is capable of surviving at all moisture levels, but pH is greatly influencing the population buildup (Tables 4 and 5).

When conducive soil was supplemented with *T. harzianum*, there was considerable reduction in DPI which ranged from 13.33 to 47.71% in 10 days and 27–64% in 20 days of application. Reduction was maximum at pH 4.5–6.0 (56.74–63.75%) and minimum at pH 6.6–7.0 (27.5%) indicating the maximum suppression by *Trichoderma* at acidic pH. Similarly maximum reduction (49.66%) was obtained at 10–15% moisture when compared to 26.76% at 35–40% (Table 6 and 7). This again confirmed the fact that pH 4.5–6.0 at 10–15% moisture level is suitable for exploiting the biocontrol potential of *T. harzianum* against *P. capsici.*

Table 2. Effect of pH on *T. harzianum* population (Log_4 $CFUg^{-1}$)

pH	35–40	30–35	20–25	>15%	Mean
4.5–5.0	5.53A	5.27B	5.600B	5.87A	5.53
5.1–5.5	5.27B	5.446A	5.74A	5.90A	5.65
5.6–6.0	4.94C	3.75C	4.26C	4.69B	4.60
6.1–6.5	4.58D	3.79C	4.14C	4.51B	4.36
6.6–7.0	4.63D	3.73C	4.12C	4.68B	4.43
CD (*P* = 0.5)	4.61	4.68	4.94	4.94	

Table 3. Effect of moisture on *T.harzianum* population (Log_4 $CFUg^{-1}$)

Moisture	pH 4.5–5.0	pH 5.1–5.5	pH.6–6.0	pH 6.1–6.5	pH 6.6–7.0
35–40	5.23a	4.89b	4.97b	5.48bc	4.20
30–35	5.15a	4.936b	4.97b	5.57ab	5.23
20–25	4.93b	4.96ab	5.43a	5.40c	5.24
>15%	5.19a	5.10a	5.53a	5.60a	5.41
CD (P = 0.5)	4.52	4.54	4.87	4.86	

Table 4. Effect of pH on the survival of *P.capsici* (DPI)

pH	10 days	20 days	Mean
4.5–5.0	304.00d	376.00d	340.00
5.1–5.5	384.00c	450.67bc	417.34
5.6–6.0	421.33c	490.67b	456.00
6.1–6.5	517.33b	405.33cd	461.33
6.6–7.0	853.33a	736.00a	794.67

Table 5. Effect of moisture on the survival of *P. capsici* (DPI)

Moisture	10 days	20 days	Mean
35–40	512.00	537.60	524.00
30–35	469.00	482.13	475.57
20–25	507.00	486.40	496.70
10–15	494.00	460.80	477.40
	NS	NS	

Table 6. Interaction of pH and *T. harzianum* on Disease Potential Index

*p*H	Conducive soil+ *T. harzianum*	Conducive soil	Conducive soil+ *T. harzianum*	Conducive soil	Conducive soil + *T. harzianum* (mean)	Conducive soil (mean)	% reduction
	10 days		20 days				
4.5–5.0	224.00	384.00	218.67	533.33	221.34	458.67	51.74
5.1–5.5	256.00	512.00	240.00	661.33	248.00	586.67	57.73
5.6–6.0	288.00	554.67	288.00	693.33	288.00	624.00	53.85
6.1–6.5	394.67	640.00	320.00	490.67	357.34	565.34	36.79
6.6–7.0	810.67	896.00	618.67	853.33	714.67	874.67	18.29

CD (P = 0.05) = 73.64 for DPI in 10 days and 64.43 for DPI in 20 days

Table 6. Interaction of pH and *T. harzianum* on Disease Potential Index

*p*H	Conducive soil+ *T. harzianum*	Conducive soil	Conducive soil+ *T. harzianum*	Conducive soil	Conducive soil + *T. harzianum* (mean)	Conducive soil (mean)	% reduction
	DPI in 10 days		DPI in 20 days				
35–40	477.87	546.13	409.60	665.60	443.74	605.87	26.76
30–35	375.47	563.20	315.73	648.53	345.60	605.87	42.96
20–25	366.93	648.53	341.33	631.47	354.13	640.00	44.67
10–15	358.40	631.47	281.60	640.00	320.00	635.74	49.66

CD (P = 0.05) = 73.64 for DPI in 10 days and 64.43 for DPI in 20 days

The present study very clearly indicated the role of abiotic factors like pH and moisture on the growth and proliferation of the biocontrol agent and the pathogen. A pH 4.0 was highly congenial for *in vitro* growth of *T. harzianum* (90.00 mm) whereas it was 5.5–6.0 in the case of *P. capsici*. A similar trend was noticed *in vivo* also. A pH above 5.5 was found not suitable for the proliferation of *Trichoderma* in the soil.

Similar studies were conducted by Meena and Paul (2008) and they observed that the population of *Trichoderma* was almost zero at alkaline pH. *Trichoderma* species favouring by acidic environment was also reported by Dewan and Sivasitamparam (1988) and Harman *et al.* (1991). Marshall (1982) found a significant reduction in damping off of snap bean when seeds were inoculated with *T. harzianum* in soil with a pH of 3.5. Chet and Baker (1980) reported that acidic pH levels enhanced *in vitro* growth of *T. harzianum* and stimulated its conidiophore formation and enhanced germination. The results of the present study are supported by the work of Chet and Baker (1981) that acidification of soil could induce suppressiveness for *Phytophthora* isolates.

This property of *T. harzianum* and *P. capsici* with respect to their reaction towards varying pH levels can be exploited for the successful utilization of *T. harzianum* for biological control. Moreover, the study also indicated that *Trichoderma* can survive at all moisture levels tested, however, maximum growth was at low moisture levels (>15%) as against *P. capsici* which favors a high moisture profile for multiplication *in vivo*. But Saju (2005) reported that porpagules of *T. harzianum* survived best in moisture levels of 30–60% whereas *T. viride* survived best at 20–60% and none of the moisture levels caused a complete disappearance of the propagules. They also observed that higher levels of moisture caused decrease in population but it maintained a population of 10^2–10^3 CFUs g^{-1}.

Our study is supported by similar studies conducted in South Africa with lettuce plants (Neumann and Laing, 2002). In lettuce *Pythium* control is best achieved through the integration of *Trichoderma* and optimum soil moisture. *Trichoderma* serves to minimize the negative effects of *Pythium* infection, providing a buffering capacity against the effects of poor soil moisture management. In black pepper also, when the pH of the soil was adjusted to varying levels, there was a drastic reduction in DPI of

P. capsici at pH 4.5 (1024 to 597). When *Trichoderma* was incorporated to infested soil, there was almost 51% reduction in DPI when compared to untreated control at the same pH. It clearly indicated that pH has got a definite role in enhancing the biocontrol potential of *Trichoderma*.

The ambient temperature was 28-34°C and there was no rain during the period which can interfere with the conditions under which the experiment was done. But the population build up was found inversely proportional to moisture. In contrast to this, Eastburn and Butler (1991) reported a positive correlation of *Trichoderma* population densities with moisture content. They observed a reduction in population of *Trichoderma* with decrease in moisture levels which is not in agreement with the findings of the present study.

Hence, based on the result of the present experiment on the ecology of *Trichoderma* and *Phytophthora* with respect to black pepper system, it is advisable that, for achieving better biocontrol potential of *Trichoderma*, the soil pH should be maintained at 4.5–6.0 and in case of nursery for planting material production, the moisture level should also be maintained at 10–15% for reducing the disease incidence due to *P. capsici* without affecting the plant growth.

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