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Factors affecting colonisation of groundnut stem by *Gliocladium virens* Miller, Giddens and Foster in soil

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ABSTRACT : Ability of *Gliocladium virens* Miller, Giddens and Foster to colonise groundnut stem pieces used as organic bait in soil was studied under varying conditions like inoculum density, soil pH, soil moisture and temperature. High temperature (40°C) was lethal for the saprophytic activities of the antagonist as colonisation of the bait was completely inhibited. In air dry soil (ca. 6.0% mhc) colonisation of bait was low (ca. 5.0 - 8.0%) particularly at lower temperature (20°C) and low level of inoculum (20 mg/100 g soil). Acid soil (pH 5.6) at 50% soil moisture and 30°C temperature gave maximum colonisation of bait. Per cent colonisation of bait was less at either side of this temperature moisture optima.

KEY WORDS : Antagonist, competitive colonisation, *Gliocladium virens*, inoculum, organic bait

Gliocladium virens Miller, Giddens and Foster, a potential antagonist, utilises organic substrates saprophytically for its survival and as points of proliferation in soil. Ahmad and Baker (1987) observed that the amount of cellulase produced by different strains of antagonists was directly correlated with their competitive saprophytic ability and rhizosphere competence which was partially explained as the ability of the antagonist to utilise cellulose substrates associated with the roots. Different species of *Trichoderma* are known to exhibit variable degrees of antagonism among themselves with different degrees of survival ability and capacity to proliferate on spruce litter over a range of temperature regimes but without any marked effect of it on their antagonistic properties (Widden and Scattonlin, 1988). The present experiment was designed to

study the effect of different factors like variable inoculum densities; soil moistures; temperatures and soil pH on the ability of *G. virens* to colonise groundnut stem pieces in natural soil.

MATERIALS AND METHODS

A potential isolate (15 GV_1, B.No. 4359) of *G. virens* was used in this experiment as test organism. The antagonist was isolated from soil collected from a mixed vegetable plot in Dhapa, Calcutta on *Trichoderma* specific medium (TSM). The inoculum as chlamydospores was produced and multiplied in glucose tartarate broth (GTB) (Brian and Hemming, 1950) medium for a period of 21 days. The mycelial mats were harvested in folds of filter paper, washed in distilled water, air dried, powdered in Willey mill, passed through a 40 mesh sieve and finally stored in a screw cap test tube at 5°C for future use.

The soils used in this experiment were collected from three different condition locations in West Bengal *viz.* Purulia (pH 5.6), casuarina forest (pH 7.0) and submerged pond (pH 8.0) with differring physico-chemical properties.

The soil samples were collected and processed as described earlier (Saha and Pan, 1995). Air dried and well pulverised natural soils (passed through 40 mesh sieve) were mixed thoroughly with antagonist inocula @ 15, 25 and 50 mg/ 100g soil, respectively. In each level of inoculum-soil mixture, the colony forming units (cfu) of antagonist per g of soil were determined on TSM immediately after mixing. One hundred gram of each of these inocula - soil mixtures was poured into glass jars (8 x 6 cm) into which 50 groundnut stem pieces of one cm length were buried. The moisture content of the air dried soil in each glass jar was adjusted to air dry 25, 50, 75 and 100 per cent soil mositure level on the basis of their water holding capacity by adding required quantity of distilled water. The open mouth of the glass jars were covered with perforated aluminium foil. Each treatment was replicated thrice. The jars were incubated at different temperatures (20, 25, 30 and 40°C). The baits were recovered after incubation for 6 days, cleansed by jet washing, surface sterilised with NaOCl (0.5%) solution and finally placed on TSM to record degree of colonisation of bait.

RESULTS AND DISCUSSION

Initial population at three inoculum levels with the test isolate of *G. virens* were ca. 8.7×10^7, 22.5×10^7 and 7.75×10^8 cfu/g soil, a population identified as optimum for biocontrol purposes (Baker, 1990; Sen *et al.*, 1994). The results presented in Table 1 to 3 on the saprophytic colonisation of groundnut stem pieces (baits) by *G. virens* revealed that the overall pattern of colonisation as a function of inoculum density, soil pH, soil moisture and temperature was almost similar.

At higher temperature (40°C) the antagonist failed to colonize baits. Similarly, in air dry soil (ca. 6% soil moisture) and at low temperature (20°C)

Table 1. Competitive colonization of groundnut stems by isolate 15 GV_1 of *G. virens* in Purulia soil (pH 5.6)

	Mean per cent colonization of groundnut stems														
	Moisture														
	Airdry			25% mhc			50% mhc			75% mhc			100% mhc		
	Levels of inoculum														
Temp.	15	25	50	15	25	50	15	25	50	15	25	50	15	25	50
20°C	8.25*	8.97	10.38	41.25	94.17	94.33	50.71	97.41	100.00	47.63	67.93	76.58	38.84	49.04	61.35
	(16.69)	(17.43)	(18.79)	(39.96)	(76.03)	(76.22)	(45.41)	(81.30)	(90.00)	(43.64)	(55.51)	(49.98)	(38.55)	(44.45)	(51.56)
25°C	8.25	9.68	11.28	45.51	97.43	99.09	56.59	97.14	100.00	46.61	71.68	84.04	44.69	47.45	64.94
	(16.69)	(18.13)	(19.62)	(42.42)	(80.77)	(84.53)	(48.79)	(80.26)	(90.00)	(43.06)	(57.85)	(66.45)	(41.95)	(43.54)	(53.69)
30°C	15.00	27.23	35.72	55.33	99.14	100.00	93.37	100.00	100.00	62.57	100.00	100.00	52.76	87.87	96.50
	(22.79)	(31.45)	(36.70)	(48.06)	(84.68)	(90.00)	(75.08)	(90.00)	(90.00)	(52.28)	(90.00)	(90.00)	(46.58)	(69.62)	(69.22)
40°C	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)

	SEM (±)	CD (P=0.05)
temperature	1.14	3.16
moisture	1.27	3.52
inoculum density	0.98	2.72

	SEM (±)	CD (P=0.05)
temperature x moisture	2.54	7.04
temperature x inoculum density	1.97	5.46
moisture x inoculum density	2.20	6.10
temperature x moisture x inoculum density	4.41	12.22

* Figures in parenthesis are angular transformed value

Table 2. Competitive colonization of groundnut stems by isolate 15 GV_1 of *G. virens* in Casuarina forest soil (pH 7.0)

	Mean per cent colonization of groundnut stems														
	Moisture														
	Airdry			25% mhc			50% mhc			75% mhc			100% mhc		
	Levels of inoculum														
Temp.	15	25	50	15	25	50	15	25	50	15	25	50	15	25	50
20°C	7.39[a] (15.77)	7.89 (16.31)	9.54 (17.99)	9.54 (17.99)	87.91 (69.65)	90.21 (71.77)	37.72 (37.89)	88.88 (70.52)	96.57 (79.32)	34.90 (36.21)	53.57 (47.04)	64.98 (53.72)	33.07 (35.10)	39.27 (38.80)	45.95 (42.58)
25°C	8.79 (5.04)	11.51 (19.83)	14.65 (22.50)	40.58 (39.57)	86.31 (68.28)	95.64 (77.95)	47.93 (43.81)	98.24 (82.38)	99.16 (84.74)	33.51 (35.37)	62.10 (52.00)	72.21 (58.19)	33.74 (35.51)	44.25 (41.70)	54.96 (47.85)
30°C	13.23 (21.33)	22.29 (28.17)	33.13 (19.35)	51.26 (45.72)	95.81 (73.35)	100.00 (90.00)	77.86 (61.93)	100.00 (90.00)	100.00 (44.67)	49.53 (44.67)	100.00 (90.00)	100.00 (90.00)	46.60 (43.05)	78.50 (62.37)	95.81 (78.19)
40°C	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)

	SEM (±)	CD (P=0.05)
temperature	0.73	2.02
moisture	0.81	2.24
inoculum density	0.63	1.75

	SEM (±)	CD (P=0.05)
temperature x moisture	1.63	4.52
temperature x inoculum density	1.26	3.49
moisture x inoculum density	1.41	3.91
temperature x moisture x inoculum density	2.82	7.79

[a] Figures in parenthesis are angular transformed value

Table 3. Competitive colonization of groundnut stems by isolate 15 GV_1 of *G. virens* in submerged pond soil (pH 8.0)

	Mean per cent colonization of groundnut stems														
	Moisture														
	Airdry			25% mhc			50% mhc			75% mhc			100% mhc		
	Levels of inoculum														
Temp.	15	25	50	15	25	50	15	25	50	15	25	50	15	25	50
20°C	5.92[a] (14.08)	6.59 (14.87)	7.94 (16.37)	37.01 (37.74)	91.25 (72.79)	95.93 (78.36)	40.85 (39.73)	93.72 (75.49)	97.40 (80.72)	94.70 (80.72)	37.00 (37.46)	89.36 (70.96)	35.04 (35.69)	38.58 (38.40)	50.73 (45.42)
25°C	8.93 (17.39)	11.68 (19.98)	12.67 (20.85)	42.85 (40.88)	94.26 (76.14)	99.14 (84.68)	51.14 (43.81)	97.52 (82.38)	100.00 (84.74)	100.00 (84.74)	44.70 (35.37)	72.44 (52.00)	44.10 (35.51)	48.41 (41.70)	60.45 (47.85)
30°C	12.32 (20.54)	22.10 (28.04)	33.80 (35.55)	50.46 (45.26)	95.78 (78.14)	100.00 (90.00)	76.21 (60.81)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	47.43 (43.53)	100.00 (90.00)	46.73 (43.12)	78.24 (62.19)	90.90 (72.44)
40°C	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)

	SEM (±)	CD (P=0.05)
temperature	0.73	2.02
moisture	0.81	2.24
inoculum density	0.63	1.75

	SEM (±)	CD (P=0.05)
temperature x moisture	1.63	4.52
temperature x inoculum density	1.26	3.49
moisture x inoculum density	1.41	3.91
temperature x moisture x inoculum density	2.82	7.79

[a] Figures in parenthesis are angular transformed value

colonisation of baits was low (ca. 15.0 - 8.0%) particularly at low inoculum level (15 mg/100g soil). The degree of colonisation of baits increased sharply with rise in incubation temperature up to 30°C and soil moisture to 50 per cent mhc. This combination appeared to be optimum for colonisation of baits at any soil pH. The level of colonisation increased with increase in inoculum density.

Increase in moisture content of soil from 50 to 75 per cent reduced the degree of colonisation of baits over 50 per cent mhc but it was still higher than colonisation at 25 per cent mhc. At saturation moisture, the general pattern of colonisation remained same, although colonisation level was slightly lower than that at 25 per cent mhc. It appeared from this experiment that there was no effect of soil pH on the pattern and degree of colonisation of the baits.

The germination of different spore forms of *G. virens* is known to behave differentially in response to soil pH (Saha, 1995). However, the addition of nutrients could greatly reduce the stasis effects of the soil (Lockwood, 1977) helping the spores to germinate, proliferate and subsequently colonise organic substrates in soil. These nutrients (sugars and amino acids) counteracted and nullified the inhibitory effect of pH visualised in the present experiments. The controversies over high (Dwivedi, 1993) and low (Ahmad and Baker,1987; Adams,1990) competitive saprophytic ability of *Trichoderma* does not apply here as *Trichoderma* always requires a nutrient source for becoming prolific in the soil (Kelley, 1976).

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