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Research Article

Can VAM Occurring In The Rhizosphere Of Cowpea Be A Source Of Natural Antagonist To *Heterodera Cajani* Population?

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ABSTRACT
Two VAM species viz. *Glomus mosseae* and *Glomus fasciculatum* were tested against *Heterodera cajani* population infecting cowpea. In the first set of experiment mixed inoculation of VAM was found more effective in managing *H. cajani* population whereas in single VAM spp. inoculation *G. fascicula*tum proved more beneficial. In second set of experiment degree of mitigative effect of VAM spp. on *Heterodera cajani* depended on the time of VAM and nematode inoculation. Two weeks prior inoculation of *Glomus fasciculatum* resulted in greater reduction in nematode population.

Key Words: VAM, *Glomus mosseae*, *Glomus fasciculatum*, Nematode, *Heterodera cajani*.

INTRODUCTION
Plant parasitic nematodes along with VAM fungi share the same ecological niche of most agro-ecosystems while VAM act as obligate symbiont and enhance plant growth whereas nematode act as obligate parasite and reduce the plant growth. Recent research has indicated that VAM fungi has the potential as 'biocontrol" agent when both group of organisms occur simultaneously in the rhizosphere of the same plant. Cowpea commonly called as *lobia* is grown as dry land *kharif* crop, is mainly valued as feed for livestock and also valued as cover or green manure crop. Along with food and feed value it also helps in improving soil fertility by fixing atmospheric nitrogen in the soil with the help of root nodules inhabited by nitrogen fixing *Rhizobium* spp. (Rachie, l985). A number of plant parasitic nematodes have been recorded on cowpea (*Vigna unguiculata*. L.walp.). *Heterodera cajani* infection on cowpea has been reported from the state of UP, Delhi, Rajasthan, Haryana and AP (Sharma and Sethi, 1975; Sharma and Sethi, l976; Koshy, 1967).

Mycorrhizal infection has particular value for legume because nodulation and symbiotic nitrogen fixation by rhizobia requires an adequate phosphorus supply and restricted root system of legume leads to poor competition for soil phosphorus (Carling *et a1*., 1978). Several legumes grow poorly and fail to nodulate in autoclaved soil unless they were mycorrhizal. This is probably due to phosphorus deficiency, since adequate phosphorus is important for satisfactory nodulation and nitrogen fixation (Hayman, 1986). Ray and Dalei (1998) revealed the *G. fasciculatum* alone as well as in combination with legume bacterium increases various growth parameters of green gram.

Baltruschat *et al.*, (1973) were the first to show that plants preinoculated with VAM fungi *G. mosseae* were less susceptible to root-knot infection. Many workers have demonstrated a reduction in nematode population densities (Cooper and Grandison, 1986; Grandison and Cooper, 1986; Jain and Hasan, 1988). Odeyemi *et al.*, (2010) also reported a significant lower root galling in four improved cowpea varieties treated with *G. mosseae*. They reported *G. mosseae* as a potential bio control agent for *M. incognita* on several cowpea varieties.

VAM beneficial symbiotic association with root increases the plant ability to absorb phosphorus, minor elements and water uptake (Gerdemann, l986; Hayman, 1982). Prior application of VAM to the nematode reduced the nematode population and nematode multiplication on various plants (Suresh and Bagyaraj, 1984; Sharma and Trivedi, 1994; Jain *et al.*, 1998) and also enhances the total biomass and yield of plants (Sanni, 1976; Lingaraju and Goswami, 1993; Jothi and Sunderababu, l998).

Thus the objective of present investigation was to study the relative effect of two species of VAM fungi (*Glomus fasciculatum* and *G. mosseae*) on *Heterodera cajani* infecting cowpea and also to study the effect of inoculation time of *Heterodera cajani* and *Glomus* spp. (*G. fasciculatum* and *G. mosseae*) singly and in combination on cow pea plant.

MATERIALS AND METHODS
Two different sets of experiments were performed under the present study.

Experiment I: Effect of single or mixed inoculums of *Glomus* sps. on *Heterodera* population

Experiments using *Glomus fasciculatum* (G.F.) and *G. mossea*e (G.M.) singly or in combination with *Heterodera cajani* comprised of eight treatments with five replications were performed. The treatments were as follows:

(1) G.F (*Glomus fasciculatum*).

(2) G.M (*Glomus mosseae*).

(3) G.F+G.M (*G. fasciculatum* + *G. mosseae*)

(4) G.F+N (*G. fasciculatum* + Nematode).

(5) G.M+N (*G.m*osseae + Nematode).

(6) G.M+G.F+N (*G. mosseae* + *G.fasciculatum* + Nematode).

(7) N (Nematode).

(8) C (un-inoculated control).

Experiment II: Effect of inoculation time of *Glomus* sps and *Heterodera cajani*

Here effect of inoculation time of *H. cajani* and *Glomus* spp., singly or in combination, on cowpea consisted of thirteen treatments with five replications was studied. The treatments were as follows:

(1) G.M (*Glomus mosseae*)

(2) G.M+N (*Glomus mosseae* + Nematode)

(3) $G.M_7$+N

(4) $G.M_{15}$+N

(s) N_7+G.M

(6) N_{15}+G.M

(7) G.F (*Glomus fasciculatum*)

(8) G.F+N (*Glomus fasciculatum* + Nematode)

(9) $G.F_7$+N

(10) $G.F_{15}$+N

(11) N_7+G.F

(12) N_{15}+G.F

(13) N alone.

*Numeric subscripts represent days (i.e., 7 or 15 days) prior to inoculation of nematode (N)/VAM fungi as given

Mycorrhizal fungi inoculum of *G. mosseae* and *G. fasciculatum* was cultured and maintained on onion roots and *Cenchrus* spp. for three months. Roots chopped into small bits and mixed with the soil, in which they were grown, constituted the mycorrhizal inoculum. Inoculum consisted of l40 chlamydospores/l0g (in case of G.M) and 158 chlamydospores/10g (in case of G.F.) were subjected to experimental pots approximately one week and two weeks prior to seed sowing. Surface sterilized seeds of highly susceptible variety of cowpea were sown in 15cm diameter pots containing sterilized soil. One thousand freshly hatched larvae of *Heterodera cajani* were inoculated in the form of suspension in distilled water by making holes around the roots of one week old seedlings.

Observations were taken after sixty days of inoculation. Data on plant growth characters, nematode multiplication and spore count with per-cent mycorrhizal infection were recorded after sixty days depending upon the time of nematode inoculation. VAM root infection levels were assessed from randomly selected root material after cutting the entire root system into 1 cm pieces. Roots were boiled in10%KOH for 5min. rinsed several times in tap water and stained in 0.1% trypan blue in lactoglycerol (lactic acid : glycerol : distilled water l:2:l VA/A/) and were transferred to lactoglycerol for storage. Per-cent VAM infection in root was estimated by the method given by Phillips and Hayman (1970). Data were recorded at frequency distributions from samples containing 25, 50, 100 root segments and the percentage mycorrhizial colonization was calculated from the frequency distribution by using the formula of Goivannetti and Mosse (1980).

$$\% \text{ Colonization} = \frac{\text{Number of VAM positive segments}}{\text{Total number of segments scored}} \text{ X } 100$$

Spore count in soil was determined using wet sieving and decanting technique followed by Gerdemann and Nicolson (1963). All the data's were subjected to statistical analysis.

RESULTS AND DISCUSSION

It is evident from the Tables 1 and 2, and results obtained from our investigation that both the *Glomus* spp. (*Glomus fasciculatum* and *G. mosseae*) proved beneficial to the plants by enhancing vigour of plant, plant growth parameters viz. fresh and dry weight, nodulation etc. A remarkable reduction in cyst, egg population and final nematode population was observed.

The results presented in Table l showed that the addition of mycorrhizal fungi tended to mitigate the adverse effect of nematode to a varied extent and plant growth characters were improved. Maximum growth parameters was observed in plants receiving mixed inoculum of both the species of *G. fasciculatum and G. mosseae* respectively. This enhancement in plant growth parameters, mycorrhizal colonization and biomass production emphasis the findings of Powell and Daniel (1978) who reported an increase in plant growth when *Glomus tenue* was added to host pot cultures already colonized by other VAM fungi. Ross and Ruttencutter (1977) also found less colonization in soyabean and peanut roots receiving single inoculum of *Gigaspora macrocarpum* rather than inoculated with *G.macrocarpum* + *G.gigantea*. The effect of nematode was minimized in the plants treated with combination of G.F+G.M+N here the cyst number /root system was noted to be minimum as compared to N alone treated plant.

It was also observed that mycorrhizal symbiont caused considerable reduction in eggs and larvae of *H. cajani* in VAM infected cysts (Fig:1) Some of the cyst showed the presence of chlamydospores of VAM and hyphal attachment of the fungus. These observation are in accordance with the work of Rozypal (1934) who reported the presence of mycorrhizal fungus in empty cysts.

Table 1. Effect of *Glomus* spp. singly or in combination with *H. cajani* on cowpea*

Treatment	Fresh Wt. (g)		Dry Wt. (g)		Number of Nodules	Number of Cyst	Number of Egg/Cyst	% VAM Colonization	Spore Count	Final Cyst Population
	Shoot	Root	Shoot	Root						
1. G. F.	29.70	6.08	6.36	1.40	28.3	0 (1)	0 (1)	72.1 (8.54)	1592.4 (39.91)	0 (1)
2. G. M.	26.50	5.57	5.93	1.26	25.6	0 (1)	0 (1)	69.7 (8.41)	1565.1 (39.57)	0 (1)
3. G.M.+G.F.	30.70	6.41	6.84	1.62	32	0 (1)	0 (1)	76.1 (8.78)	1725.1 (41.54)	0 (1)
4. G.M. + N	20.83	4.56	2.21	1.00	20	566.3 (23.79)	23.6 (4.92)	62.5 (7.96)	1313.1 (36.24)	4075.5
5. G.G. + N	22.99	4.82	3.96	1.14	23	510.1 (22.60)	21 (4.69)	66.1 (8.19)	1331.6 (36.50)	4050
6. G.M. + G.F. + N	25.19	5.00	4.83	1.17	25	391.4 (19.80)	16.3 (4.15)	68.6 (8.34)	1482.0 (38.50)	3910.1
7. ‘N’ alone	11.94	3.94	2.10	0.66	10	691 (26.3)	72.6 (8.57)	0 (1)	0 (1)	4160
8. ‘C’	28.32	5.91	6.09	1.30	30	0 (1)	0 (1)	0 (1)	0 (1)	0 (1)
SEM	3.99	0.44	0.31	0.14	1.86	1.87 (0.14)	1.23 (0.06)	20.93 (0.16)	12.58 (0.16)	-
CD at 5%	9.20	1.03	0.71	0.33	4.29	4.33 (0.34)	2.83 (0.15)	48.26 (0.37)	29.01 (0.38)	-
CD at 1%	13.38	1.50	1.04	0.49	6.25	6.30 (0.49)	4.13 (0.22)	70.22 (0.53)	42.20 (0.56)	-

*Observations are means of five replicates. Figures in parenthesis are $\sqrt{n+1}$ transformed values.

G.F. - *Glomus fasciculatum*, GM - *Glomus* mosseae, N-Nematode, C-Control

Table 2. Effect of inoculation time of *H. cajani* and *Glomus* spp. singly or in combination on cowpea*

Treatment	Fresh wt. (g)		Dry Wt. (g)		Number of Nodules	Number of Cyst	Number of Egg/Cyst	Percentage Infection	Spore Count	Final Cyst Population
	Shoot	Root	Shoot	Root						
1. G.M.$_{15}$+N	23.34	4.46	4.64	0.92	25	456 (21.3)	50 (7.14)	65 (8.12)	1070 (32.72)	3700.5
2. M.M.$_{7}$+N	21.92	4.20	4.44	0.84	23	473 (21.7)	51.2 (7.21)	63.1 (8.0)	1056.2 (32.51)	4470.0
3. G.M + N	20.86	4.00	3.21	0.71	21	684.5 (26.1)	61.3 (7.89)	57 (7.61)	1142 (33.80)	4476
4. G.M.	27.43	6.22	5.90	1.40	26	-	-	69 (8.36)	1265 (35.58)	-
5. N$_{15}$+G.M.	17.32	2.82	3.20	0.49	19	574 (23.9)	60 (7.81)	47 (6.92)	794 (28.19)	4472.3
6. N$_{7}$+G.M.	19.16	3.30	3.54	0.52	17.6	561 (23.7)	58 (7.68)	49 (7.07)	898.1 (29.98)	4410
7. G.F$_{15}$+N	25.58	5.70	5.78	1.26	29	435.4 (20.88)	44 (6.70)	68 (8.30)	1244 (35.28)	2894.2
8. G.F$_{7}$+N	24.22	5.54	4.88	1.18	28	444.3 (21.09)	46 (6.85)	65 (8.12)	1067.5 (32.68)	3658.6
9. G.F. + N	22.9	4.82	3.99	1.14	23	675.4 (25.9)	60.03 (7.81)	58 (7.68)	1154 (33.98)	4418.7
10. G.F.	29.72	6.46	6.36	1.98	29	-	-	71.3 (8.50)	1278 (35.76)	-
11. N15+G.F	18.7	3.46	3.43	0.55	19	566 (23.8)	57.8 (7.66)	50 (7.14)	802.4 (28.34)	4456.3
12. N7+G.F	19.74	3.92	3.96	0.60	20	548 (23.4)	56.1 (7.55)	52 (7.28)	907.3 (30.13)	4409
13. 'N' alone	10.9	2.50	2.02	0.37	9	695 (26.38)	63.1 (8.00)	0 (1)	0 (1)	4500
SEM	0.59	0.23	1.40	0.23	1.38	3.68 (1.56)	2.02 (0.1)	16.58 (0.01)	6.50 (0.35)	-
CD at 5%	1.78	0.70	4.23	0.71	4.16	11.08 (4.71)	6.10 (030)	49.95 (0.04)	19.57 (1.05)	-
CD at 1%	1.27	0.50	3.03	0.51	2.98	7.95 (3.37)	4.37 (0.21)	35.82 (0.03)	14.04 (0.75)	-

**Observations are means of five replicates. Figures in parenthesis are* $\sqrt{n+1}$ *transformed values.*

***Abbrv.** G.F.- Glomus fasciculatum, GM - Glomus mosseae, N- Nemato*

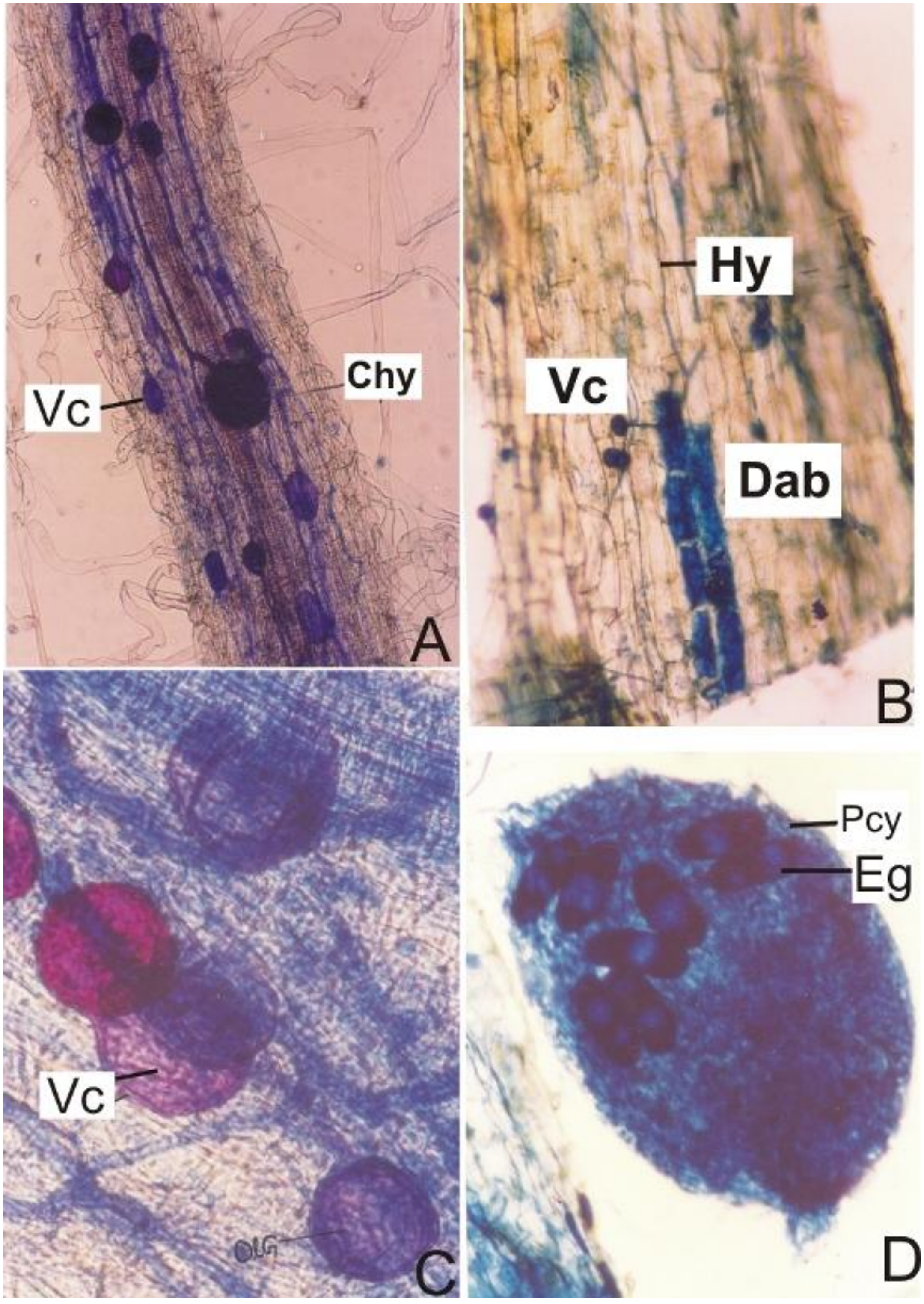

Figure 1. Interaction of VAM fungi (*G. fasciculatum*) on *Heterodera cajani* cyst and cowpea roots. A Intramatrical mycelium showing dichotomous branching. B Degenerating arbuscles with granular appearance with intercalary vesicles. C Magnified view of root showing spores with vesicles and oil globules. D Mature *H. cajani* cyst filled with eggs parasitized with VAMF hyphae outside the host root. (***Abbrv***. Vc-vesicle, Chy-chalamydospore, Hy-hyphae, Dab-degenerating arbuscules, Olg-oilglobules, Eg-egg, Pcy-parasitized cyst).

Presence of VAM chlamydospores especially of *Glomus* spp. were recorded in different *Heterodera* spp. by several workers (Gerdeman and Nicolson, 1963; Willcox and Tribe, 1974; Graham and Stone, 1975; Tribe, 1977). In treatments where single *Glomus* spp. was used *Glomus fasciculatum* was proved best as compared to the *G. mos*seae. These findings are in accordance with Manjunath and Bagyaraj 1984; Jain and Sethi, 1988; Kassab and Taha, 1990; and Jain and Hasan, 1994.

In the second experiment out of all the thirteen treatments the result presented in Table 2 indicated that either of the two fungi alone enhanced the various growth parameters of plant as compared to plants receiving the *H. cajani* treatments along with two VAM spp.

Degree of mitigative effect of VAM spp. on *H. cajani* depended on the time of VAM inoculation. Two weeks prior introduction of VAM spp. to the nematodes resulted in greater reduction of nematode population. (Table 2).Maximum reduction in cyst population was reported in G.F_{15}□N which is in the accordance with the work of earlier workers (Jain and Sethi, 1988b; Suresh and Bagyaraj, 1984; Sharma *et al.*, 1994; Jain *et a1.*,1998). This VAM fungi in host plant roots may change its root exudation pattern and affect the attractiveness of root to plant parasitic nematodes and ultimately fewer nematode penetrate the roots. This altered chemotactic attractions of nematode to the roots and affect exclusion in those nematodes spp. that requires hatching stimulus (Baker and Cook, 1982; Garrett, 1970 and Wallace, 1983).

The other reason for reduction in nematode population might be due to the direct competetion of VAM fungi with the nematode for the space in root tissues (Davis and Menge, 1980).

In the present findings reduction in nodulation of cowpea roots were observed in nematode infested plant and addition of VAM spp. tended to reduce the adverse effect of *H. cajani* on cowpea roots which was favoured by

Sivaprasad *et al.*, (1999). Selveira *etal.*, (1995) also showed that inoculation of beans with both, *Glomus etunicatum* and plant growth promoting rhizobacteria *Pseudomonas putida* resulted in increase in root growth, nodulation, uptake and use efficiency of nitrogen and phosphorus.

Mycorrhizal association is reported to alter the root physiology by increasing amino acids and reducing sugar contents (Nemac and Meredith 1981) in the roots. Thus loss of these compounds due to nematode infection was compensated to some extent due to VAM and develop host tolerance towards cyst infection. Lignin and phenols were found significantly more in mycorrhizal roots which are known for their role in host resistance (Bhatia et *a1.*, 1972; Krishna and Bagyaraj, 1984) especially in influencing penetration of roots by the nematode.

Thus the results achieved from this study indicate a strong potentiality of using *Glomus fasciculatum* in Rajasthan state as one of the biocide for *Heterodera cajani* on cowpea.

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