



## **INTERACTION STUDIES BETWEEN CEREAL CYST-NEMATODE AND VESICULAR-ARBUSCULAR MYCORRHIZAL (VAM) FUNGI ON BARLEY IN JAIPUR, DISTRICT, RAJASTHAN**

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Cereal-cyst nematode (*Heterodera avenae*) is one of the most serious pathogen of wheat and barley crops in the state of Rajasthan, limiting agricultural productivity. In the present interaction study the potential of VAM species viz. *Glomus fasciculatum* and *Glomus aggregatum* singly and in combination was evaluated against *H. avenae* infestation on barley roots. Analysis of covariance of yield and plant growth data indicated better growth and yield of barley when its roots were associated with VAM species. A significant reduction in *H. avenae* cyst population and egg content of the cysts to minimum level was evident in VAM associated plants. Host's roots and VAM species interaction was found to be beneficial symbiotic association which resulted in improved plant growth and yield of the host plant.

**Keywords:** Barley; *Heterodera avenae*; *Glomus fasciculatum* and *Glomus aggregatum*; Symbiotic association.

### **Introduction**

In India, *Heterodera avenae* was first reported from Sikar district of Rajasthan state<sup>1,2</sup>. The nematode was found to be a serious pathogen of wheat and barley causing "Molya disease" characterized by deformed root system. Survey studies revealed that due to congenial climatic conditions and sandy soil, the nematode established and distributed in different areas of Rajasthan including Jaipur district<sup>3</sup>. Barley is an important food and forage crop, extensively grown in the state of Rajasthan. It was found to be severely infested with the nema-pathogen that results in an atrocious state of the host and causes great loss to the crop production every year. Amongst the various kinds of organisms engaged in the

biocontrol of nematodes, VAM fungi are the most promising group. The role of VAM as biocontrol agents in limiting yield losses due to nematodes infestations was investigated by different workers<sup>4,5</sup>. Their beneficial symbiotic association with host roots increases the plants ability to absorb Phosphorus (P), minor elements and water<sup>6</sup>. During survey of Jaipur district areas, plant samples collected from *H.avenae* infested local fields, when observed showed intimate association of *Glomus* species with the roots of barley and the cysts of *H. avenae*. Therefore, it was logical to further explore the nature of interaction between the VAM fungi, Cereal-cyst nematode and the host plant barley. In the present study interaction between the nematode and *Glomus* species

viz. *Glomus fasciculatum* and *Glomus aggregatum* individually and their combined effect on the growth and yield of barley were evaluated.

### Material and Methods

*Isolation, Identification and Culture of VAM fungi:* Spores of VAM spp. were isolated from the soil samples collected from different localities by using standard wet sieving and decanting technique given by Gerdemann and Nicolson<sup>7</sup>. For identification of VAM spp. the root pieces retained on 710 µm sieve were examined under a dissecting microscope to assure the presence of hyphae with spores and sporocarps of VAM fungi. The organic matter retained on 250 µm sieve was examined under stereomicroscope for the presence of large spores and sporocarps. Similarly matter retained on 106 µm and 53 µm sieves were also observed for the presence of clusters of small spores and detached spores respectively. For counting and identification of spores Doncaster nematode counting dish was used.

For examining VAM hyphae and spores associated with host's roots, roots pieces were boiled in 10% KOH solution for 5 minutes, washed in tap water stained in 0.1% Trypan blue in lactoglycerol for 12 hrs and then transferred to lactoglycerol.

From the VAM infested cysts spores and hyphae were isolated simply by crushing the cysts in watch glasses containing D.W. and were examined for the identification of VAM species under stereomicroscope. For identification purpose standard key given by Trappe<sup>8</sup> was used. Two *Glomus* species viz., *G. fasciculatum* and *G. aggregatum* were identified.

Above said identified two species were maintained separately on wheat for getting pure inoculums. For pure cultures, sterilized wheat grain were sown in pots

containing steam sterilized soil + sand (3:1) mixture. The mycorrhizal inoculum consisted of chlamyospores of each *Glomus* species obtained from the local field soil and infested *H. avenae* cysts were placed just near the roots of 1 week old wheat seedling. After 90 days plants were uprooted and roots were washed, processed and examined for the presence of each *Glomus* species.

*Experimental study:* For the interaction study pot trials were set. Pure inoculum of each *Glomus* species was added separately as well as in combination to the 15 cm diameter pots containing autoclaved soil + sand (3:1) mixture and mixed thoroughly. Inoculum of both the species consisted on an average 280 chlamyospores per 10g hyphae plus infected roots. Surface sterilized seeds of barley variety RD103 were sown in VAM inoculated pots. One week old seedlings were nematized with one thousand freshly hatched active juveniles of *H.avenae* around the roots for infestation. Following treatments were comprised for experimental set up:

1. *G. fasciculatum* [G.F.(10g) + *G. aggregatum* [G.A.(10gm)]
2. G.F.(10g)
3. G.A. (10g)
4. G.F. (10g)+G.A. (10g)+Nematode (N)
5. G.F. (20g) + G.A. (20g) + N
6. G.F. (10g) + N
7. G.F. (20g) + N
8. G.A. (10g) + N
9. G.A. (20g) + N
10. N

All the treatments were replicated four times. Data on plant growth characters, nematode reproduction and spore count with percent mycorrhizal root infection were recorded after 90 days of nematization. Number of cysts per root system plus per

100g soil and average cyst content (number of eggs) were also recorded. VAM root association levels were assessed from randomly collected root material after cutting the entire root system into 1 cm pieces. Roots were stained and preserved as described before. The percent VAM colonization was calculated from the frequency distribution using following formula given by Giovannetti & Mosse<sup>9</sup>.

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

Spores count in soil was also determined by the technique described earlier.

### Observations and Results

Analysis of soil samples collected from *H. avenae* infested fields showed presence of spores, sporocarps and hyphae of VAM fungi. Barley roots examined revealed presence of extra as well as intrametrical hyphae bearing spherical vesicles at their tips and in intercalary positions containing oil globules. Arbuscules developed from intracellular hyphae were also evident in host cells. Hyphae were abundantly observed in the cortical region of roots, but they were absent in endodermal and stellar region. Young roots were found to be containing plenty of arbuscules, but in older roots vesicles with oil globules, chlamydospores and sporocarps were abundant.

Similarly crushed cysts also showed presence of both *Glomus fasciculatum* and *Glomus aggregatum* species. In some cysts, cyst content (eggs) was totally replaced by VAM hyphae and spores. (Fig.1, 2). The most commonly identified species; *G. fasciculatum* and *G. aggregatum* were characterized as follows:

*G. fasciculatum*: Spores globose to subglobose, ovate, ellipsoid or irregular; dimensions at maturity 50-125  $\mu\text{m}$ ; surface smooth to roughened; double

walled; yellow sporogenous hyphae thickened at the point of attachment with a diameter 5 to 20  $\mu\text{m}$  (Fig-1a)

*G. aggregatum*: spores subglobose, ovate, ellipsoid or irregular; dimensions at maturity 225-250  $\mu\text{m}$ ; surface coarse-roughened, double walled; sporogenous hyphae white to hyaline, non perceptible, cylindrical or flared towards point of attachment, pore closed by septum or inner wall (Fig-1b).

The mycorrhizal culture multiplied on wheat for experimental purpose also consisted of chlamydospores, along with hyphae and infected roots.

*Experimental Study*: Data depicted in Table-1, showed effect of *G. fasciculatum* and *G. aggregatum* singly and in combination on plant growth and yield of barley and multiplication of *H. avenae*. A significant reduction in disease incidence, better plant growth and yield in terms of seed weight were obtained by the treatment of *Glomus* species as compared to untreated control and only *H. avenae* infested plants.

Regarding plant growth parameters, like root-shoot lengths were recorded to be maximum in G.F. (10g) + G.A. (10g) treatment and were minimum in 'N' treated plants. Intermediate shoot and root lengths were evident in the treatments, where single dose (10g) of each species and higher dose in combinations were used.

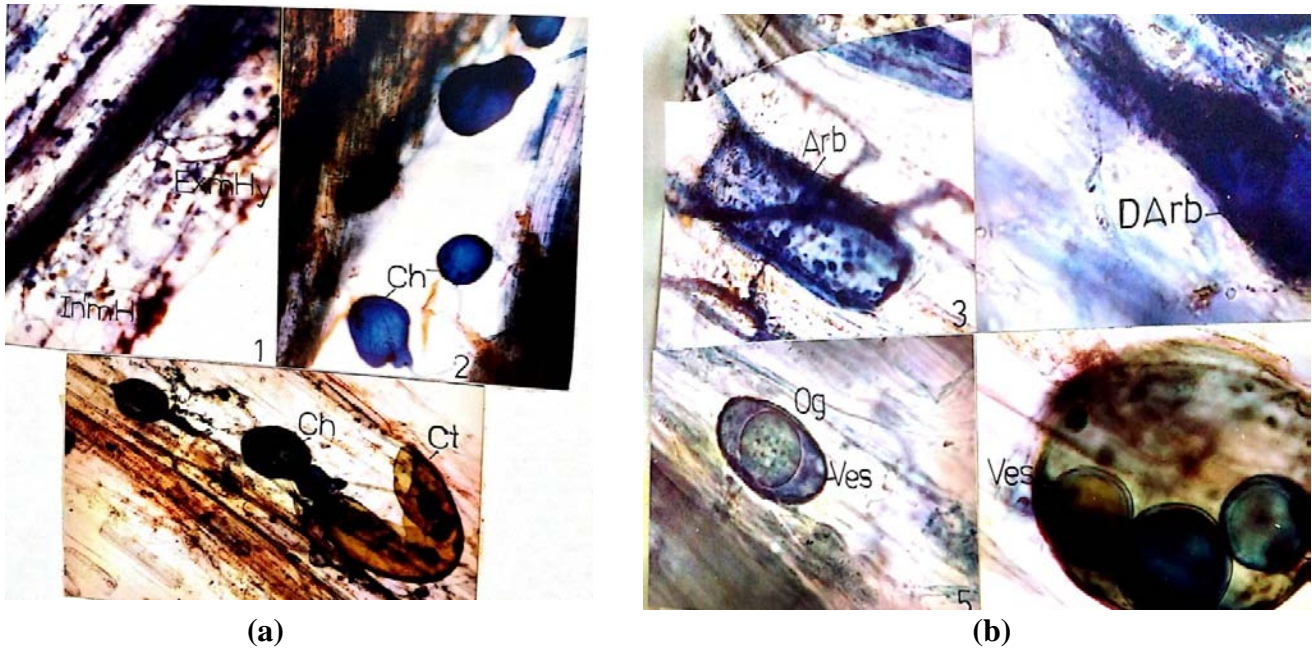
Generally, both fresh and dry weights of shoot were higher in VAM treated plants as compared to 'N' alone treated plants. Shoot fresh weight ranged between 2.852g to 14.898 g, being maximum in G.F. (10g) + G.A. (10g) treatment and minimum in 'N' treated plant. Similar trend was evident regarding shoot dry weights and seed weight per plant.

In the case of roots also fresh and dry weights increased with increase in VAM

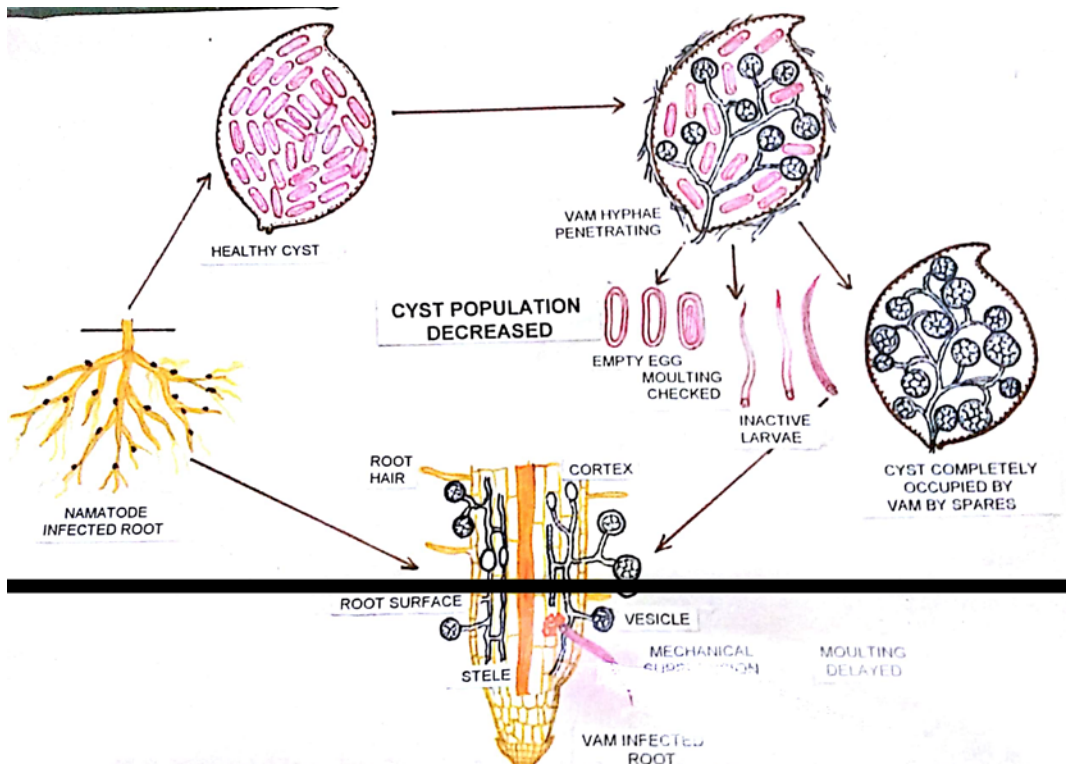
**Table: 1.** Interaction of VAM fungi with *H. avenae* infecting Barley (Mean of 4 replicates)

S. No.	Treatments	Length (CM)		Fresh Wt. (g)		Dry Wt. (g)		Seed Wt. (g)	Cysts per root system + per 100 g soil	No. of Egg/ Cyst	% Mycorrhizal infection	VAM Spore Count
		Shoot	Root	Shoot	Root	Shoot	Root					
1	2	3	4	5	6	7	8	9	10	11	12	13
1	GF (10g) +GA (10g)	74.5	40.00	14.898	4.875	1.655	0.609	1.325	0 (1)	0 (1)	99.16	3334.3
2	GF (10g)	67.6	38.8	13.754	3.495	13.84	0.439	1.221	0	0	85.30	1991.0
3	GA (10g)	61.4	37.1	6.735	1.948	0.846	0.256	1.002	0	0	80.3	2422.0
4	GF (10g)+GA(10g) + Nematode	54.1	31.4	4.751	0.753	0.590	0.115	0.500	67.8	260.60	91.3	2578.0
5	GF (20g) + GA (20g) + Nematode	61.1	34.6	6.252	1.748	0.782	0.246	0.630	26.3	211.00	100.0	2825.0
6	GF (10g) + N	46.3	20.8	4.032	0.602	0.502	0.075	0.227	100.0	231.3	64.0	4387.0
7	GF (20g)+N	48.7	30.8	4.195	0.602	0.533	0.094	0.481	72.6	165.3	60.1	2447.0
8	GA(10g)+N	45.2	12.6	3.201	0.153	0.413	0.057	0.075	126.3	300.6	87.4	4911.00
9	GA (20g)+N	45.9	20.3	3.555	0.500	0.444	0.073	0.125	101.0	245.0	98.9	1620.0
10	Nematode	36.4	9.2	2.852	0.152	0.353	0.017	0.027	162.6	338.3	0 (1)	0 O(1)
11	Control	60.2	34.4	5.224	0.904	0.654	0.217	0.601	0 (1)	0 (1)	0 (1)	0 (1)
	SEM±	0.293	0.388	0.029	0.015	0.008	0.011	0.008	0.512	0.516	0.308	0.171
	CD at 5%	0.611	0.810	0.061	0.032	0.018	0.024	0.017	1.070	1.079	0.644	0.357
	CD at 1%	0.833	1.103	0.081	0.042	0.024	0.130	0.022	1.456	1.467	0.875	0.485

GF = *Glomus fasciculatum*; GA = *Glomus aggregatum*; N= Nematode



**Fig. 1** (a) *G. fasciculatum* (Hyphae, arbuscules, vesicles, chlamydospores and sporocarps), (b) *G. aggregatum* (Hyphae, arbuscules, vesicles, chlamydospores)



**Fig. 2** Control of *Heterodera avenae* by VAM Fungi

inoculations. Root fresh weight (40g) was maximum in the G.F. (10g) + G.A.(10g) treatment, whereas it was minimum (9.2g) in nematode('N') treatment. Root dry weights also exhibited similar trend.

A marked decrease in disease manifestation was evident by the application of *Glomus* species. Maximum cyst number (162.6 per root system + 100g soil) was recorded in the 'N' treated plants. It reduced to minimum (26.3 per root system + 100 g soil) in the treatment, where 20g dosages of both the species were used. As compared to *G. aggregatum*, *G. fasciculatum* was more efficient in reducing cyst number.

The Cyst content (Eggs + larvae) did not show any definite trend. In the case of concomitant application of *Glomus* species most of the cysts were found to be empty containing hyphae with spores (Fig-2). Data revealed a negative correlation between the nematode and mycorrhizal association with the host plant.

Percent mycorrhizal infection was more in G.F. (10g) + G.A. (10g) treatment, which gradually increased with higher dosages (20g) of VAM, however it was recorded to be minimum in the G.A. (10g)+N treatment. Spore counts also showed similar trend. Data were found to be statistically significant.

### Discussion

Survey of study area revealed that *H. avenae* and *Glomus* species are indigenous to local fields of barley. Results of present investigation indicated better establishment of *Glomus* species on the root of barley naturally as well as in pot trial. Their association was found to be mutually beneficial symbiosis that caused better growth and yield of barley as compared to non mycorrhizal plants. Barley was proved to be the good host for establishment of VAM species. Hayman also reported a

considerable infection on barley host<sup>10</sup>. The genus *Chloris gayana* with 98.5% root colonization by *G. fasciculatum* was considered to be the best host by Srinivasan and Bagyaraj<sup>11</sup>. In the present investigation on an average percent root colonization by VAM ranged between 80.3-85.3 percent, so it can also serve as an efficient host.

The results obtained during pot trial indicated that barley and VAM fungi interaction mitigated the deleterious effect of the nematode on barley variety RD103 and increased host tolerance level against the pathogen.

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