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INTEGRATED MANAGEMENT OF *HETERODERA AVENAE* INFECTING WHEAT USING AM FUNGI, OIL CAKES AND NEEM PRODUCTS.

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In the present study, soil application of organic amendments has been explored as an alternative means for organic management of *Heterodera avenae*. The efficiency of various locally available organic amendments like neem cake, sesame cake and neem product like powdered neem leaves were tested under pot trials in combination with two major strains of VAM fungi isolated from local wheat fields, for the biocontrol of *Heterodera avenae* infecting wheat *Triticum aestivum,* L. var.wh-147. Two sets of experiments were framed using *Glomus fasciculatum* and *Glomus mosseae* respectively to see their individual potential in decreasing disease frequency. Results revealed that both species of VAM in combination with oil cakes and neem leaf extracts reduced nematode infection to varied degree.

Keywords : *Heterodera avenae,* Organic amendments, Vesicular arbuscular mycorrhiza, Wheat.

Arbuscular Mycorrhizal (AM) fungi are one of the most important component of rhizospheric microbial communities in agro ecosystems, forming symbiotic beneficial associations with more than 90% of terrestrial plants (Smith and Smith 1997). Mycorrhizal plants show resistance to all types of environmental stresses, predation by other soil organisms and tolerance to drought and salinity stresses. It also improves the activity of nitrogen-fixing organisms in the rhizosphere, nutrient uptake by plant and plant-water relations (Koske and Gemma 2005).

The cereal cyst nematode (CCN) *Heterodera avenae* Woll., has been reported as the most prevalent and damaging nematode to wheat and barley in different parts of the world (Jain *et al*. 2007). Eco-friendly management practices are used to check nematode population without adversely affecting environment. Plant parasitic nematodes and arbuscular mycorrhizal (AM) fungi occur together in the roots and rhizosphere of the same plant having a characteristic but opposite effect on plant vigor and growth. It has been reported by various workers that AM fungi have increased crop yield and enhanced plant vigor by reducing percentage nematode infection on various crops. Vesicular-arbuscular fungi do not colonize regions infected by endo-parasitic nematodes, and nematodes rarely infect regions colonized by AM fungi. The host-parasite relationship and interaction of nematode and AM fungi has been comprehensively reviewed (Arya and Saxena 2009). The plant roots profoundly colonized with mycorrhizal fungi can grow well even in presence of damaging levels of nematodes. In addition AM also enhances overall plant growth and vigor. Nematode-mycorrhizal interactions appear to be very specific and highly dependent on the association of specific plant cultivar, fungal and nematode species, the order of colonization by the symbionts and the soil nutrient level.

Cyst nematode, *Heterodera avenae* found in the wheat growing fields of Rajasthan state in India causing 'Molya' disease of wheat, causes heavy economic loss. In the local language 'Molya' means malformed as this nematode colonizes root system and causes its deformation. Survey done earlier, showed the association of AM with rhizosphere of wheat crop. Survey was followed by frequency distribution study of AM fungi, which indicated the predominance of two species viz. *G. mosseae* and *G. fasciculatum,* in most of the cultivated fields. Percent loss of the cereal crop in the surveyed areas ranged from 15 to 45 percent. Therefore, an experiment was framed to study the interaction of AM

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and *H.avenae* on wheat, integrated with organic amendments like neem products and oil cakes. Previous studies with AM fungi showed that it has the potential to reduce nematode population and at the same time it also promotes plant growth. In recent years, neem based pesticides and botanicals have also drawn attention for the management of nematodes and other pests. Beneficial properties of neem products like fewer side effects underline the outstanding suitability of neem products for use in Integrated Nematode management. Oil cakes have also shown same efficacy were applied against cereal cyst nematode.

MATERIALS AND METHODS

Soil samples were collected from fifteen local fields for isolation of AM species to be used in the test and were mass cultured on onion, wheat and buffelgrass. The identification was made by using standard key by Schenck and Pe'rez (1988). Spores were isolated from the soil by wet sieving and decanting technique (Gerdemann and Nicolson 1963) and cysts were collected from the soil and roots of wheat by simple sieving and floatation process. Mycorrhizal inoculums comprised of chlamydospores, azygospores, infective fungal hyphae and fungal roots, which were then placed just below the seeds during sowing. Plant growth period of 3-4 months gave good inoculum consisting of approximately 30-60spores/10g of soil. The earthen pots were filled with sterilized soil thoroughly amended with powdered neem leaves, neem and sesame oil cake and left for two weeks for proper decomposition of organics. After two weeks seeds were sown in these experimental pots. AM inoculum was applied at the time of seed sowing. Nematodes were applied to the one week old plants near the root zone @ 1000 second stage juveniles/pot.

In this trial combined impacts of oil cakes viz. Neem cake (NC) and Sesame cake (SC), neem products like Neem leaf (NL) and AM fungi were tested to reduce the disease incidence on wheat. The pots were arranged in randomized complete block design and experiment treatments were formulated in the two tests each with eight treatments using *G. fasciculatum* and *G. mosseae* respectively which are as follows:

Test I:

1. *Glomus fasciculatum* (4g) + Nematode.
2. *Glomus fasciculatum* (2g) + Neem cake (1g) + Neem leaf (1g) + Nematode.
3. *Glomus fasciculatum* (2g) + Sesame cake (1g) + Neem leaf (1g) + Nematode.
4. *Glomus fasciculatum* (2g) + Neem leaf (2g) + Nematode.
5. *Glomus fasciculatum* (2g) + Neem cake (1g) + Sesame cake (1g) + Nematode.
6. *Glomus fasciculatum* (1g) + Neem cake (1g) + Sesame cake (1g) + Neem leaf (1g) + Nematode.
7. Nematode alone inoculated plants (N).
8. Un-inoculated control plants (c).

Test II:

1. *Glomus mosseae* (4g) + Nematode.
2. *Glomus mosseae* (2g) + Neem cake (1g) + Neem leaf (1g) + Nematode.
3. *Glomus mosseae* (2g) + Sesame cake (1g) + Neem leaf (1g) + Nematode.
4. *Glomus mosseae* (2g) + Neem leaf (2g) + Nematode.
5. *Glomus mosseae* (2g) + Neem cake (1g) + Sesame cake (1g) + Nematode.
6. *Glomus mosseae* (1g) + Neem cake (1g) + Sesame cake (1g) + Neem leaf (1g) + Nematode.
7. Nematode alone inoculated plants (N).
8. Un-inoculated control plants (c).

All the above mentioned treatments were replicated four times. The experiment was completed after 90 days with the uprooting of plants and various growth parameters like shoot-root length, fresh and dry weight of plants, ear length and its fresh and dry weight, total cyst per plant, total spores per gram of soil, percent AM colonization were measured. The data was statistically analyzed using

Table 1: An integrated approach for nematode management with *Glomus fasciculatum*, oil cakes and neem products against *Heterodera avenae* infecting wheat.

S.No.	Treatments	Fresh weight (g)		Dry weight (g)		Ear length (cm)	Ear weight (g)		Total cysts /plant	Total eggs/cyst	% Root colonization
		Shoot	Root	Shoot	Root		Fresh	Dry			
1	2	3	4	5	6	7	8	9	10	11	12
1	GF + N	15.95	4.97	1.97	0.69	10.30	4.15	0.59	31.60 (5.69)	137.00 (11.74)	40.29 (6.41)
2	GF+NC+NL+N	17.33	5.84	2.83	0.94	12.80	5.00	0.78	10.66 (3.34)	109.68 (10.51)	69.70 (8.34)
3	GF+SC+NL+N	16.89	5.10	2.65	0.87	12.30	4.48	0.72	16.00 (4.07)	126.00 (11.26)	52.00 (7.21)
4	GF+NL+N	17.04	5.35	2.67	0.89	11.50	4.83	0.76	16.57 (4.16)	114.30 (10.73)	58.34 (7.68)
5	GF+SC+NC+N	16.42	5.33	2.12	0.77	11.36	4.36	0.63	27.00 (5.27)	130.00 (11.43)	63.30 (8.00)
6	GF+SC+NC+NL +N	19.55	6.93	2.84	1.02	14.10	5.26	0.86	9.30 (3.19)	113.66 (10.70)	43.00 (6.57)
7	N alone	11.10	1.67	1.12	0.28	7.20	0.64	0.19	268.64 (16.42)	229.60 (15.17)	0.00 (1.00)
8	Control	17.87	6.42	2.91	0.95	1.80	5.95	0.91	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
	SEM±	0.76	0.42	0.16	0.04	0.18	0.19	0.03	0.45	0.24	0.49
	CD at 5%	1.62	0.89	0.35	0.09	0.38	0.41	0.07	0.95	0.52	1.04
	CD at 1%	2.23	1.23	0.48	0.13	0.53	0.57	0.10	1.31	0.72	1.43

Values are mean of four replications. Figures in parenthesis are 1+*n* transformed values. N=Nematode, GF=Glomus fasciculatum, NC=Neem cake, SC=Sesame cake, NL=Neem leaf.

Analysis of variance. Critical difference at 5% and 1% level of significance were calculated. Root infection was assessed by using staining technique and slide method (Giovanneti and Mosse 1980).

RESULTS AND DISCUSSION

The results presented in the table-1 revealed that AM in combination with oil cakes and neem leaves significantly reduced the nematode infection along with disease incidence and promoted plant growth.

In the first set of experiment, AM treated plants had better growth where as 'N' alone inoculated plants were stunted with lesser, yellow pale leaves. Data showed that the fresh (19.55g) and dry (2.84g) shoot weight was found maximum in the treatments where GF was applied concomitantly with oil cakes and neem leaf powder. There was a gradual decrease in the fresh weight of shoot from GF+NC+NL (17.33g), GF+NL (17.04g), GF+SC+NL (16.89g), GF+SC+NC (16.92g), GF aloneapplied (15.95g) to N alone treated plants (11.10g) as mentioned in table-1. The present findings are comparable with those of Meena *et al.* (2009) on biopesticidal potential of different organic oil cakes which were found to be highly effective in promotion of the plant growth and reduction in the nematode population. As a result of application of neem cake amendments, diterpenoid, flavonoids and triterpene compounds are released which provides more

Table 2 : An integrated approach for nematode management with *Glomus mosseae*, oil cakes and neem products against *Heterodera avenae* infecting wheat

S.No.	Treatments	Fresh weight (g) Shoot	Fresh weight (g) Root	Dry weight (g) Shoot	Dry weight (g) Root	Ear length (cm)	Ear weight (g) Fresh	Ear weight (g) Dry	Total cysts /plant	Total eggs/cyst	% Root coloniz-ation
1	2	3	4	5	6	7	8	9	10	11	12
1	GM + N	15.88	4.81	2.15	0.73	11.03	4.46	0.60	33.0 (5.81)	200 (14.15)	44.62 (6.71)
2	GM+NC+NL +N	20.50	5.79	2.84	0.95	13.70	5.08	0.75	14.40 (3.88)	134.34 (11.62)	53.30 (7.36)
3	GM+SC+NL +N	20.84	5.55	2.62	0.87	13.70	5.00	0.68	17.33 (4.26)	154.62 (12.39)	72.67 (8.54)
4	GM+NL+N	18.10	5.35	2.45	0.79	11.90	4.70	0.64	29.31 (5.50)	165.68 (12.89)	61.67 (7.68)
5	GM+SC+NC +N	17.09	4.85	2.31	0.74	12.00	4.56	0.64	33.70 (9.19)	178.00 (13.33)	66.34 (8.13)
6	GM+SC+NC +NL+N	21.80	6.64	3.02	1.04	14.40	5.25	0.79	11.00 (3.38)	133.70 (11.50)	50.00 (7.01)
7	N alone	12.20	2.28	1.42	0.25	8.13	0.87	0.17	367.65 (19.20)	219.00 (14.82)	0.00 (1.00)
8	Control	23.40	6.35	3.12	0.96	14.30	5.81	0.87	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
	SEM ±	1.03	0.34	0.13	0.07	0.42	0.12	0.04	0.25	0.86	0.89
	CD at 5%	2.19	0.72	0.29	0.16	0.90	0.27	0.09	0.54	1.82	1.89
	CD at 1%	3.02	1.00	0.39	0.22	1.25	0.37	0.13	0.75	2.51	2.61

Values are mean of four replications. Figures in parenthesis are 1+n transformed values. N=Nematode, GF=Glomus mosseae, NC=Neem cake, SC=Sesame cake, NL=Neem leaf.

available nitrogen to plants and accelerates overall plant growth, thereby helping the plants to escape nematode attack. Likewise fresh and dry root weight showed the similar sequences with highest in 6.93 and 1.02g in GF+SC+NC+NL dose and then in descending order in control (6.42g and 0.95g), GF+NC+NL (5.84 and 0.94g), GF+NL (5.35g and 0.89g), GF+NC+SC (5.33g and 0.77g) and GF alone (4.97g and 0.69g) treated plants. In context to ear length, it was found to be maximum (14.1cm) in GF+NL+SC+NC and minimum in 'N' alone treated plants (7.2cm) which had small shriveled and less grains. It was 10.3cm in GF treated plant and addition of oil cakes and neem leaves enhanced it as seen in GF+NC+NL (12.8cm) and GF+SC+NL (12.3cm) treatments. In terms of fresh and dry weight of ear, maximum of 5.95g and 0.91g was recorded in control, followed by 5.76g and 0.86g in GF+SC+NC+NL, 5.00g and 0.78g in GF+NC+NL, 4.83g and 0.76g in GF+NL, 4.48g and 0.72g in GF+SC+NL and 0.64g and 0.19g in nematode alone treated plants. Significantly better growth was observed in ear length parameters with AM, oil cakes and neem leaf integrated treatments. In the second set of experiment, similar trend was observed with all different parameters of wheat growth treated with all tabulated treatments of *Glomus mosseae* (Table-2).

GF treatment reduced the cyst number which was further lowered by the addition of powdered neem leaves and oil cake. Counting done on the number of cyst per plant unveiled the maximum cyst production in 'N' treated plants (67.65). The number of cyst were less in GM+SC+NL+NC (11) and GM alone (33) treated plants. Javed *et al.* (2007) revealed that Neem cake was effective in preventing development of juvenile stage of nematodes by inducing defense mechanism in plant which subsequently delayed the development of nematode. Decrease in the nematode population and an increase in plant vigour, grown in soil amended with neem cake was reported by Mishra and Prasad (1974) in mung bean and wheat. It has been found by many workers (Srivastava 2002) that ammonia, glucocynate and allyl isothiocynate are the factors involved in nematode control when organic amendments are applied.

Neem cake is a non edible, cheap and easily available oil cake. Besides being non-toxic and non hazardous, it leaves no as such residual effects on plant. Substances released as a result of decomposition of organic amendments do possess nematicidal properties. The neem cake has been noticed to be highly suppressive for nematode soil population. Our results have been in accordance with the findings of others (Rizvi *et al.* 2012, Khan and Rathi 2001). So far as fungal type is concerned, an increase was found in both biomass and growth parameters. Both AM species were effective enough in managing *H.avenae* alone and in combination with different oil cakes, although results were better with GF and neem oil cake treatments. Thus in natural ecosystems, AM may have significant increase impact on plant growth and nematode reproduction dynamics. By and large collectively, AM should be subjugated for the bio-control of *H. avenae* as it is eco-friendly and gives very accomplishing results.

CONCLUSIONS

The present study showed the reduction in final *H.avenae* population on wheat plant, brought by an integrated approach with *G.fasciculatum* and *G. mosseae* in combination with sesame, neem oil cakes and neem leaves. The nematode inoculated plants without AM treatment showed reduced morphological parameters. In the first set of experiment, GF was applied alone and in combination with sesame oil cake and neem products. The treated plants exhibited increase in plant growth and vigour. In the second set of experiment, in place of GF, GM was used. Both AM fungi had antagonistic effect on *H.avenae,* though the extent varied with the applied AM species and formulated dose. The presence of mycorrhizae in plant roots caused alteration in root exudate, which in turn reduced the root attractiveness to plant parasitic nematodes, thus resulting in lesser nematode penetration. The possible control of the *H.avenae* by oil seed cakes and neem leaves may be due to toxic products resulting from the decomposition of such added organic amendments. Organic additives also improve nutrient status, encourage natural enemies, improves soil structure and chemical properties which altogether help in reducing nematode population.

AM fungi improved plant growth and reduced cyst number but addition of oil cakes and neem leaves enhanced this effect. Neem leaves proved to be the best and neem oil cake was better than sesame oil cake. *G. fasciculatum* was better in giving results than GM and thus *G. fasciculatum* could be considered as an efficient and potent biocontrol agent for cereal cyst nematode.

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