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CONCOMITANT EFFECT OF VAM AND NEEM PRODUCTS ON *HETERODERA AVENAE* INFECTED WHEAT

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The cereal cyst nematode *Heterodera avenae* is a serious pest causing 'Molya' disease of wheat. A systemic trial was undertaken to study the role of VAM as a natural, ubiquitous, suppressive agent on *H.avenae*. Influence of VAM as an efficient biocontrol agent in conjunction with various pure and crude neem products viz. Neem leaf, Neem cake, Nimin, Achook, and Neemax on *Heterodera avenae* Population dynamics and its beneficial effects on *Triticum aestivum*. L var. wh-147 growth was investigated in twinsets of experiment under pot trials. Establishment of *Glomus fasciculatum* and *Glomus mosseae* in the root system of host, resulted in decrease in the final nematode population and significant plant growth enhancement. The plants treated with various neem products were at par with VAM treated plants. Application of VAM integrated with Nimin, Achook, Neemax, Neem leaf and Neem cake outbreaked in increase in length, fresh and dry weight of root,shoot and ears as compared to their individual incoculation and proved to be novel strategy for controlling nematode pest.

Key words: VAM, *Glomus fasciculatum, Glomus mosseae,* Neem products, *Triticum aestivum L., Heterodera avenae,*cereal cyst nematode.

VAM fungi are ubiquitous, living, naturally existing component of dynamic microbial communities which are associated with 95% of plants. The role of VAM fungi is well known is phosphorus uptake and plant growth (Hall, 1984 ; Koide, 1991) which aims to the study of transport of phosporus and carbon in VAM fungi (Jackobson, 1995). The cereal cyst nematode *Heterodera avenae* is responsible for the serious "molya" disease of wheat crop in North western region of the country comprising chiefly of Rajasthan, Haryana, Delhi, Punjab, Himachal pradesh and some regions of U.P and J.K. In general, a yield reduction of about 50% or even a total loss has been recorded (Mathur, 1969). VAM have been demonstrated to contribute to reduction in nematode population variously (Baltruschat *et al.*, 1973; Reddy *et al.*, 1997; Rao *et al.*, 1996 Nagesh and Reddy, 1999). VAM fungi are symbiotically present in the roots of most of the angiospermic and crop plants (Tester *et al.*, 1997).These two groups of micro organisms exert a characteristic but opposite effect on. plant health, The mycorrhiza make the plants resistant to diseases, stress and other besides helping in phosphorus uptake, enhance plant growth, biomass and yield.The potential role of mycorrhizal fungi as a biocontrol agent for nematode infection has received considerable attention (Jain and Hasan, 1994; Hasan and Jain, 1992). Keeping in view the economic importance of "Molya" disease, the present study was undertaken to test relative performance of VAM and various neem products on population dynamics of *Heterodera avenae* on wheat. Comparative efficacy of VAM fungi in combination with neem products against nematode was studied by various workers (Nagesh *et al.*, 1999; P; Trivedi *et al.*, 1978; Mojumdar and Mishra, 1991,94)

MATERIALS AND METHODS

Surface sterilized seeds of wheat, *Triticum aestivum* (L.) variety WH-147, with 0.1% $HgCl_2$ solution were sown in 15cm diameter earthen pots each containing autoclaved soil. Randomized complete block design experiment was set up under pot trials.The two VAM spp., *Glomus mosseae* and *Glomus fasciculatum* were multiplied individually on *Cenchrus ciliaris,* onion and wheat for three months and were added to pots during seed sowing. Roots chopped into small bits and mixed with soil in which they were grown constitued mycorrizal inoculum. Inoculum (3g/kg) consisted of chlamydospores (255/3g and 210/3g *Glomus mosseae and Glomus fasciculatum* respectively) along with hyphae and infected roots and was placed just below the seed in pots. Cysts of *H.avenae* were extracted from wheat plant roots and the native soil of the local wheat fields by simple floatation and screening process. For inoculation the second stage juveniles (L2S) were obtained by crushing healthy cysts in distilled water., The resulting suspension containing eggs was incubated in BOD at optimum temperature of 18±1 degree Celcius for *H.avenae* cysts upto 48 hours before inoculation. One week old seedlings were inoculated to

the plants by pouring larval suspension @ 1000 larvae/ pot in 3 holes made equidistant around the seedlings and then plugged with sterilized soil. 5ml. of complete Hoagland's nutrient solution was added weekly to each pot to avoid mineral deficiency.

Each treatment was replicated four times. Data on biometrics, nematode multiplication, spore counts and per cent mycorrhizal root infection were recorded after 90 days at the experiment termination.

VAM root infection level were assessed from randomly selected root material after cutting the entire root system into 1 cm pieces. Roots were cleared in KOH and stained in Trypan blue (Phillips and Hayman, 1970). Per cent root colonization was determined as suggested by Giovannette and Mosseae (1980). Entire data was statistically analysed. Pure and crude neem products viz neem cake, neem leaf, Neemax, Nimin and Achook were used in the experiment which were available in the market. Neem leaf powder was made in the laboratory by drying and powdering neem leaf. Finely powdered neem cake (*Azadirachta indica* A. Juss) at the rate of 3g/kg was incorporated into potted. soil. Mycorrhizal incoulation and neem products were given at half the dose each in combined treatments. Nimin, Achook and Neemax were applied at the time of nematode inoculation. Neemax is a product of Ecomax agrosystems, it is a neem seed powder. Nimin and Achook are the neem based pesticides produced by Godrej /Agrovet Ltd.

RESULTS AND DISCUSSION

Effect of VAM and neem based products on host response to nematode parasitism and on nematode infection, development and reproduction are summarized in table:1 and 2. In first set of experiment better growth of aerial parts was observed in the GF+Nm+Nc+Ac+Nx+NL treatment followed by GF+NL, Gf+Nm, GF+Ac and GF+Nx. GF solitary reduced the nematode multiplication but with least efforts as compared to above mentioned treatments. The fresh and dry weight of shoot and root was more in the integrated treatment and GF+NL treatment in contrast to GF+NC treated Plants. Gm+N gave results in between, same results were a btained in the second set of experiment in thethe case of *Glomus mosseae* in which integrated treatment gave best result with maximum length, fresh and dry weight of shoot and root followed by Gm+NL and GM+Nematode. Establisment of *Glomus mosseae* and *Glomus fasciculatum* along with Neem products in the roots of host plant not only resulted in decrease in the population but also increase in the fresh and dry weight of root, shoot and fruit as compared to nematode alone inoculated plants. Thus VAM fungi improves plant nutrition and aid the host in compensation for the damage caused by parasitic nematode, thereby increasing the host tolerance to test pests. Similarly observations have shown that prior application of mycorrhiza to nematode reduced nematode infestation more than simultaneous or nematode preceding mycorrhiza (Suresh and Bagyaraj, 1984 ; Sharma and Trivedi, 1994; Nagesh *et al.*, 1999). VAM fungi may increase root growth, expand the absorptive capacity of the root system for nutrient or water and enhance cellular processess in roots (Hayman,1982).

Nematode multiplication rate was least in integrated treatment in first as well as in the second experiment where VAM was applied with NL, NC, Nm, Ac and Nx respectively as compared to their solo application. Oil cakes and Nimin when combined with VAM gave better result than VAM combined with neem based pesticides. Saleh and Sikora (1988) and Menge (1982) reported that fungicide used for the control of soil borne pathogen adversely affect VAM symbiosis. Similar Observation was recorded by several workers on different crops. Inoculation of endomycorrhiza with neem cake amended on tuberose and brinjal resulted in effective management of root-knot and reniform nematode management (Khan and Parvatha Reddy 1992; Rao *et al., 1993,1994*). The final population of cyst nematode was found to be significantly affected by the presence of GF,GM, GF+NL and GF+Nm. It was maximum in plants treated with GF and GM alone. Addition of neem leaf or neem cake accelerates VAM multiplication. *Glomus mosseae* was shown to grown saprophytically in soil, its growth was enhanced and infectivity to clover was increased when the soil was amended with organic matter (Hepper and Warner 1983; Nagesh *et al.*, 1999), Jain and Hasan 1995, observed that *Glomus fasciculatum* along with Achook as soil treatment @ 2kg/ha was found to be most effective both in managing the nematode incidence to a safer level and increased the forage biomass.

Table 1: Concomitant influence of crude and purified neem products in combination with *Glomus fasciculatum* on *Heterodera avenae* infecting wheat.

S.No.	Treatments	Length (cm) Shoot	Root	Fresh weight (g) Shoot	Root	Fresh weight (g) Shoot	Root	Ear length (cm)	Ear wt. (g) Fresh	Dry	Total cyst/ plant	Total eggs/ cyst	% VAM Colonization	Spores/50g of soil
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	GF+NL	74.06	29.63	19.20	6.02	2.13	0.65	15.70	4.08	0.79	11.00 (3.42)	155.00 (12.46)	47.00 (6.89)	40.00 (6.39)
2	GF+Nm	73.26	28.96	18.56	5.83	2.09	0.67	15.40	4.06	0.78	17.00 (4.22)	188.65 (13.76)	29.32 (5.49)	52.00 (7.26)
3	GF+NC	72.73	28.73	17.86	5.79	2.04	0.64	15.45	4.03	0.75	24.00 (4.96)	214.32 (14.67)	36.65 (6.13)	37.00 (6.14)
4	GF+Ac	71.36	27.93	17.06	5.23	2.01	0.61	14.90	3.87	0.71	25.66 (5.15)	216.64 (14.61)	29.67 (5.53)	49.32 (7.06)
5	GF+Nx	70.70	26.90	16.60	5.14	1.92	0.54	14.00	3.82	0.66	34.00 (5.90)	233.34 (15.26)	31.35 (5.66)	50.64 (7.19)
6	GF+N	69.60	26.30	15.86	5.13	1.91	0.46	13.73	3.55	0.64	47.00 (6.92)	245.61 (15.70)	24.00 (4.86)	40.65 (6.40)
7	GF+NL+Nm +NC+Ac+Nx	75.65	30.80	15.60	6.35	2.23	0.68	15.76	4.17	0.80	12.64 (3.64)	145.36 (12.07)	35.00 (5.96)	43.00 (6.62)
8	N alone	48.83	25.03	12.76	2.42	1.43	0.35	7.53	0.83	0.15	55.34 (7.49)	219.18 (14.84)	0.00 (1.00)	0.00 (1.00)
9	Control	74.23	30.73	19.76	6.67	2.21	0.72	16.26	4.40	0.81	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
	SEM ±	0.50	0.21	0.54	0.04	0.08	0.03	0.34	0.92	0.03	0.40	0.54	0.39	0.41
	CD at 5%	1.06	0.45	1.14	0.09	0.18	0.06	0.72	1.93	0.06	0.84	1.13	0.83	0.86
	CD at 1%	1.46	0.62	1.57	0.13	0.25	0.09	0.99	2.64	0.09	1.15	1.56	1.14	1.18

Values are means of four replications. Figures in parenthesis are $\sqrt{n+1}$ transformed values.

GF=*Glomus fasciculatum*, NL=Neem Leaf, Nm=Nimin, NC=Neem Cake, Ac=Achook, Nx=Neemax, N=Nematode.

Table 2: Concomitant influence of crude and purified neem products in combination with *Glomus mosseae* on *Heterodera avenae* infecting wheat.

S.No.	Treatments	Length (cm) Shoot	Root	Fresh weight (g) Shoot	Root	Fresh weight (g) Shoot	Root	Ear length (cm)	Ear wt. (g) Fresh	Dry	Total cyst/ plant	Total eggs/ cyst	% VAM Colonization	Spores/50g of soil
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	GM+NL	73.6	30.10	18.88	6.11	2.13	0.64	15.46	4.07	0.78	14.33 (3.83)	165.32 (12.91)	45.00 (6.77)	47.32 (6.94)
2	GM+Nm	73.13	29.50	18.30	6.00	2.05	0.62	15.10	4.02	0.72	20.65 (4.63)	170.62 (13.09)	44.65 (6.73)	28.35 (5.39)
3	GM+NC	72.56	29.10	17.46	5.76	1.99	0.58	14.76	3.97	0.67	31.00 (5.64)	183.66 (13.58)	34.64 (5.95)	41.28 (6.47)
4	GM+Ac	72.3	28.10	16.73	5.55	1.89	0.61	14.43	3.85	0.61	30.31 (5.58)	195.35 (13.99)	45.00 (6.77)	47.65 (6.96)
5	GM+Nx	71.76	27.50	16.26	5.18	1.92	0.54	13.96	3.69	0.55	33.67 (5.88)	198.33 (14.11)	38.30 (6.22)	47.65 (6.96)
6	GM+N	69.96	27.80	15.90	5.04	1.91	0.46	12.96	3.54	0.51	40.00 (6.39)	218.00 (14.80)	44.00 (6.69)	27.00 (5.26)
7	GM+NL+Nm +NC+Ac+Nx	75.33	31.36	19.70	6.66	2.23	0.68	15.93	4.24	0.83	11.34 (3.42)	145.67 (12.09)	47.39 (6.93)	22.00 (4.76)
8	N alone	47.76	22.20	12.23	2.13	1.43	0.35	7.46	0.77	0.15	64.60 (8.08)	264.56 (16.21)	0.00 (1.00)	34.68 (5.89)
9	Control	73.9	30.74	19.60	6.43	2.21	0.72	16.30	4.43	0.85	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
	SEM ±	0.23	0.40	0.30	0.12	0.08	0.03	0.18	0.07	0.03	0.49	0.55	0.34	0.39
	CD at 5%	0.49	0.85	0.64	0.25	0.25	0.09	0.38	0.16	0.06	1.04	0.97	0.71	0.82
	CD at 1%	0.68	1.17	0.88	0.35	0.18	0.06	0.52	0.22	0.09	1.42	1.50	0.09	1.13

Values are means of four replications. Figures in parenthesis are $\sqrt{\ }$ transformed values.

GM=*Glomus mosseae*, NL=Neem Leaf, Nm=Nimin, NC=Neem Cake, Ac=Achook, Nx=Neemax, N=Nematode.

Data on percentage of mycorrhizal infection which varied from 24% to 47% in the first test and 23% to 47.32% in the second test and spore count showed that overall percentage of root infection by *Glomus mosseae* was almost at par with *Glomus fasciculatum*. The introduction of *H. avenae* affected the root adversely and it was observed that per cent mycorrhizal infection decreased with nematode infection. Nagesh *et al.*, (1999) mentioned that *Glomus fasciculatum* singly or in combination with neem cake colonized better than *Glomus mosseae*. Pandey (1995) also tested performances of oil seed cakes, pesticides and dry leaf powders on reproduction potential of nematodes.

An eco-friendly approach was applied in the present investigation with the combination of part and product of botanicals. Maximum reduction in multiplication was found in the integrated combination of *G. fasciculatum* and *G. mosseae* both with neem leaf neem cake, a chook. Nimin and Neemax as compared to their alone application. It can be inferred from the results that various organics and nematicides have different mode of action and may depend accordingly the plant part used. The leaf powders have nonpersistance characteristics, however oil cakes have residual effect on subsequent generation of nematodes and their requirement in large quantities at field level have drawn attention to neem based pesticides which in turn are expensive. To overcome all these difficulties simultaneous application of both with VAM develops a cheaper and effective nematode inhibition device, use of which becomes economically feasible.

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REFERENCES

Baltruschat H Sikora RA & Schonbeck F 1973 Effect of VA mycorrhiza (*Endogone mosseae*) on the establishment of *Thielaviopsis basicola* and *Meloidogyne incognita* in tobacco *Int Cong Plant Path Minnesota* NY 0661.

Bolan N S 1991 A critical review of the role of mycorrhizal fungi in the uptake of phosphorus by plants. *Plant soil* **134** 189-207.

Giovannetti M & Mosse B 1980 An evaluation of technique for measuring Vesicular-arbuscular mycorrhizal infection in roots *New Phytol* **84** 489-500.

Hall IR 1984 Field trials assessing the effect of inoculating agricultural soils with endomycorrhizal fungi. *J Agric Sci* **102** 725-731.

Hasan N & R K Jain 1992 In Dwivedi *BK* (eds) *Recent advances in Nematology* Bioved Research Society Allahabad 219.

Hayman *DS* 1982 Practical aspects of Vesicular-arbuscular mycorrhiza *In: Advances in Agricultural Microbiology* (eds NS SubbaRao) Oxford and IBH publishing Co. Oxonion press Ltd Faridabad pp. 325-373.

Hepper *CM* & Warner A 1983 Role of organic matter in growth of VAM in soil *Trans Brit Mycol Soc* **81** 155.

Jackobson I 1995 Transport of phosphorus and carbon in VA mycorrhizaes In: Mycorrhiza (eds Varma and B Hock) Springle and Warlock pp 297-324.

Jain *R K* & N Hasan 1994 In: (Singh T and PC Trivedi-eds) Vistas in seed biology Printwell Publisher, Jaipur **49**.

Jain *RK* & Hasan N 1995 Vesicular-arbuscular mycorrhizal (VAM) fungi a potential biocontrol agent for nematodes In *Nematode Pest management an appraisal of Eco friendly* Approaches (Eds. Gopal Swarup *et al.*) pp217-227. Nematological society of India IARI New Delhi.

Khan *RM* & Parvatha Reddy P 1992 Nematode pests of ornamental crops. In *Nematode pest of crops* (D.S. Bhatt and R.K. Wallia) pp 250-257. CBS Publisher and Distribution, Delhi pp 381.

Koide R 1991 Nutient supply nutrient demand and plant responses to mycorrhizal infection, New Phytol **117** 365-286.

Mathur *BN* 1969 Studies on cereal root eelo wrm (*Heterodera avenae* Woll) with special reference to 'molya' disease of wheat and barley in Rajhasthan. *Ph.D Thesis*, University of Rahasthan, 233pp.

Menge *JA* 1982 Effect of soil fumigants and fungicides on Vesicular arbuscular fungi. Phytopathology **11** 171-196.

Majumdar V & Mishra *SD* 1991 Nematicidal efficacy of some plant products and management of *Meloidogyne*

incognita in pulse crop by soaking seeds in their aqueous extracts. *Current Nematology* **2** 27-32.

Mojumdar V & Mishra *SD* 1994 Comparative efficacy of neem products and Marshall as seed treatment against *Meloidogyne incognita* infecting chickpea *National symposium on pests of Agricultural importance and their management, Bioved Research Soc*, Allahabad Feb 19-21 (Abstr) 36.

Nagesh M & Parvatha Reddy P 1999. Studies on correlation between *Glomus fasciculatum* spore density, root colonisation and *Meloidogyne incognita* infection on *Lycopersicon esculentum. Ziet shrift fur Pflanzen krankheiten wnd Pflanzenschutz* 106 (1) 82.

Nagesh M, Parvatha Reddy & *MS* Rao 1999 Comparative efficacy of VAM fungi in combination with neem cake against *Meloidogyne incognita* on *Grossandra undulaefolia Mycorrhiza* News **11** 11.

Pandey Rakesh 1997 Performance of oil seed cake pesticides and dry leaf matter on the reproduction potential of *Meloidogyne incognita* and yield of japanese mint H-77. *Ninth Annual Meet of Nematological Society of India* IARI. New Delhi 26-28th March.

Parvatha Reddy P, Nagesh M & *MS* Rao 1997. Integrated management of burrowing nematode *Radopholus similis* using endomycorrhiza *Glomus mosseae* and oil cakes. *Pest management Horticultural Ecosystmens* **3**(1) 25.

Phillips JM & Hayman DS 1970 Improved procedures for clearing roots and obtaining parasitic and Vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection *Trans Brit Mycol Soc* **55** 158-161.

Rao MS, Mohandas S & Parvatha Reddy P 1993 Management of reniform nematode on egg plants by integrating botanicals (Castor cake) mycorrhiza *(Glomus fasciculatum)* and bioagent *(Paecilomyces lilacinus). Golden jubilee Symposium on Horticulture Research changing Scenario, Bangalore* pp 259-260.

Rao MS, Parvatha Reddy P & Mohandas S 1996. Effect of integration of *Calotropis procera* leaf and *Glomus fasciculatum* on the management of *Meloidogyne incognita* infesting tomato. *Nematologica Mediterranea* **24** (1) 59.

Rao MS, Parvatha Reddy P & Nagesh M 1994 Use of biotic agents *Paecilomyces lilacinus* along with seed treatment of okra with neem cake suspension for the management of root knot nematode under field condition. *National symposium on Emerging trends in Pest management* Nauni.

Saleh HM & RA Sikora 1988 Effect of oientozen benomyl and carbendezim on interaction between the endomycorrhizal fungus *Glomus fasciculum* and the root-knot nematode on *Meloidogyne incognita* on cotton Nematologica **34** 432-442.

Suresh CK & Bagyaraj DJ 1984 Interaction between a Vesicular-arbuscular mycorrhiza and a root knot nematode and its effect on growth and chemical composition of tomato. *Nematologica medit* **12** 32-39.

Tester M Smith SE & Smith FA 1987 The phenomenon of "Non mycorrhizal" plants. *Can J Bot* **65** 419-431.

Trivedi PC, Bhatnagar A & Tiagi B 1978 Control of root knot nematode on *Capsicum annum* by application of oil cake. *Indian Phytopathology* **31** 75-76.