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USE OF ORGANIC AMENDMENTS ON THE EFFICACY OF BIOCONTROL AGENTS IN THE CONTROL OF ROOT ROT AND ROOT KNOT DISEASE COMPLEX OF OKRA

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Summary. Use of *Verticillium chlamydosporium*, *Paecilomyces lilacinus*, *Rhizobium meliloti* or soil amendment with *Stoechospermum marginatum* seaweed controlled the infection of *Meloidogyne javanica* root knot nematode and *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium solani* infection of okra. Neem cake and datura powder were also effective against *M. javanica* and *M. phaseolina* infection. The combined use of *V. chlamydosporium* and neem cake completely controlled the *M. javanica* infection. The greatest height and fresh weight of shoot were produced when *R. meliloti* was used with seaweed followed by *V. chlamydosporium* used with neem cake.

Organic amendments that are generally used for increasing agricultural productivity have been shown to have a suppressive effect on plant parasitic nematodes (Alam, 1976). Neem cake (Alam, 1990), Stoechospermum marginatum (Abid et al., 1993) and datura powder (Shahwar et al., 1994) have shown promising results in the control of root knot nematodes. The use of biocontrol agents such as Verticillium chlamydosporium (de Leij, 1992), Paecilomyces lilacinus (Jatala, 1985) and rhizobia also provide significant control of root knot nematodes (Parveen et al., 1993) and root infecting fungi (Ehteshamul-Haque and Ghaffar, 1993) but if they are to be fully exploited as biocontrol agents they should be compatible with any organic amendments that are applied to the soil

An experiment was, therefore, carried out to ascertain the effect of organic amendments of neem cake, seaweed [Stoechospermum marginatum (C. Agardh) Kützing] and datura powder on the efficacy of *V. chlamydosporium* Goddard, *P. lilacinus* (Tham) Samson and *R. meliloti* Dangeard in the control of root rot and root knot disease complex of okra.

Materials and methods

Powdered neem cake, air dried leaves of *Datura fastuosa* L. or seaweed (*S. marginatum*) were mixed in sandy loam soil, and put into 8 cm diam. plastic pots, 250 g in each. The soil had a natural infestation of 3-11 sclerotia per g of soil of *Macrophomina phaseolina* (Tassi)

Goid., as determined by wet seiving and dilution technique (Sheikh and Ghaffar, 1975), 5-10% colonization of Rhizoctonia solani Kühn on sorghum seeds used as baits (Wilhelm, 1955) and 3500 cfu g-1 of mixed population of Fusarium solani (Mart) Appel et Wollenw. emend. Snyd. et Hans and F. oxysporum Schlecht. emend. Snyd. et Hans as assessed by soil dilution technique (Nash and Snyder, 1962). Pots were watered daily and kept at 50% W.H.C. (Keen and Raczkowski, 1921). Three weeks after the application of the soil amendment treatments an aqueous suspension of V. chlamydosporium, P. lilacinus (10⁷ cfu ml⁻¹) multiplied on Potato Dextrose Agar and R. meliloti (108 cfu ml⁻¹) multiplied on Yeast Extract Mannitol agar, were drenched in each pot at 25 ml/pot. Five seeds of okra [Abelmoschus esculentus (L.) Moench] were sown in each pot. In another set, egg masses of Meloidogyne javanica (Treub)

Chitw. of equal size obtained from brinjal plants (*Solanum melongena* L.) were inoculated near the roots of 5 day old seedlings at 10 egg masses per pot. Pots without soil amendment or biocontrol agents served as a control. The pots were randomized on a screen house bench with each treatment replicated three times.

Plants were uprooted after six weeks growth and root knot index recorded on a 0-5 scale (Taylor and Sasser, 1978). Data on height and fresh shoot weight were also recorded. The method used by Short *et al.*, (1980) was modified to determine the incidence of fungi in which roots were washed in running tap water, five 1 cm long root pieces were surface disinfected with 1% Ca(OCl)₂ and plated onto Potato Dextrose Agar plates containing penicillin (100,000 units/litre) and streptomycin (0.2 g/litre). The dishes were incubated for 5 days at 28 °C and the incidence of root infecting fungi re-

Table I - Effect of organic amendments and microbial antagonists in the control of root knot nematode on okra.

Treatments	Fresh weight of shoot (g)	Height of shoot (cm)	Root knot index
V. chlamydosporium (CV)	1.3	26.0	2.7
P. lilacinus (PL)	2.4	33.3	3.0
R. meliloti (RM)	1.3	26.4	3.3
S. marginatum (SM)	2.2	30.6	3.2
Neem cake (NC)	1.3	24.5	3.1
D. fastuosa (DF)	1.6	29.0	3.5
SM+VC	2.4	35.0	2.8
SM+PL	2.6	36.9	2.4
SM+RM	3.3	39.6	2.8
NC+VC	3.0	38.6	0.0
NC+PL	2.7	33.8	1.0
NC+RM	2.1	35.3	3.0
DF+VC	2.8	30.6	4.1
DF+PL	2.9	34.4	3.5
DF+RM	2.2	30.5	2.8
Control	1.2	22.8	4.4
LSD 0.05	1.2	6.9	1.1

Table II - Effect of organic amendments and microbial antagonists in the control of root infecting fungi on okra in soil artificially infested with root knot nematode.

Treatments	% Infection		
	M. phaseolina	R. solani	F. solani
V. chlamydosporium (CV)	72.0	0.0	0.0
P. lilacinus (PL)	44.3	0.0	11.0
R. meliloti (RM)	50.0	8.3	8.3
S. marginatum (SM)	66.6	0.0	0.0
Neem cake (NC)	33.3	66.3	22.0
D. fastuosa (DF)	61.0	33.0	11.0
SM+VC	50.0	33.3	0.0
SM+PL	44.3	0.0	27.6
SM+RM	66.6	0.0	0.0
NC+VC	83.3	100	16.6
NC+PL	27.6	66.6	11.0
NC+RM	33.3	100	0.0
DF+VC	50.0	0.0	0.0
DF+PL	72.0	11.0	22.0
DF+RM	38.6	33.3	22.0
Control	100	36.0	19.3
LSD 0.05 (Treatments) = 19.5			. 2
LSD 0.05 (Pathogens) = 9.7			

corded. Data were analyzed and subjected to Factorial ANOVA (FANOVA) according to Gomez and Gomez (1984).

Results and discussion

Neem cake significantly (P<0.05) controlled the infection of root knot nematode. *V. chlamy-dosporium* was found more effective than *P. lilacinus* or rhizobia. Gall formation was completely controlled when neem cake was used with *V. chlamydosporium*. Combined use of *S. marginatum* with *P. lilacinus* or rhizobia gave better results than when used separately for the control of root knot nematode. Datura powder effectively controlled the infection of *M. javanica* when used with rhizobia. Height and fresh weight of shoots of okra was greater when rhi-

zobia was used with seaweed, followed by *V. chlamydosporium* with neem cake (Table I).

Significant control of M. phaseolina infection was observed where P. lilacinus or R. meliloti were used alone or when soil was amended with seaweed, neem cake or datura powder. The greatest reduction in M. phaseolina infection occured when P. lilacinus was used with neem cake, and was followed by neem cake used alone, or neem cake with R. meliloti. Complete control of R. solani infection was obtained by V. chlamydosporium and P. lilacinus when used alone, or by soil amended with seaweed. The use of P. lilacinus or R. meliloti with seaweed or V. chlamydosporium with datura powder also completely controlled R. solani infection as did V. chlamydosporium or soil amendment with seaweed. The use of V. chlamydosporium with seaweed or datura powder and R. *meliloti* with seaweed or neem cake also completely controlled *F. solani* infection (Table II).

In the present study use of organic amendments with neem cake, datura powder and seaweed showed promising results in the control of root infecting fungi and root knot nematode.

Literature cited

- ABID M., ZAKI M. J. and MAQBOOL M. A., 1993. Use of brown algae, *Stoechospermum marginatum* for the control of root knot nematode in brinjal. *Marine Res.*, 2: 39-44.
- ALAM M. M., 1976. Organic amendments in relation to nematodes. Ph. D. Thesis, Aligarh Muslim University, Aligarh. pp. 150.
- ALAM M. M., 1990. Neem in nematode control. pp. 51-55. In: *Nematode biocontrol (Aspects and Prospects)*. M. S. Jairajpuri, M. M. Alam & I. Ahmad (Eds.). CBS Publishers & Distributors (Pvt.) Ltd. Delhi-110032, India.
- DE LEIJ F. A. A. M., 1992. The significance of ecology in the development of *Verticillium chlamydosporium* as a biological control agent against root-knot nematodes (*Meloidogyne* spp.). Ph. D. Thesis, Rothamsted Experimental Station, UK. pp. 140.
- EHTESHAMUL-HAQUE S. and GHAFFAR A., 1993. Use of rhizobia in the control of root rot diseases of sunflower, okra, soybean and mungbean. *J. Phytopathology*, *138*: 157-163.
- Gomez K. A. and Gomez A. A., 1984. *Statistical procedures* for agricultural research. 2nd ed. Wiley, New York, pp. 680.

- JATALA P., 1985. Biological control of nematodes. In: An advanced Treatise on Meloidogyne, Vol. I. Biology and control, (eds.) J. H. Sasser and C. C. Carter, Coop. Publ. Dept. Plant Pathology, North Carolina State University and the United States Agency for Int. Dev., Raleigh, NC. pp. 303-308.
- KEEN B. A. and RACZKOWSKI H., 1921. The relation between clay content and certain physical properties of soil. *J. Agric. Sci.*, 11: 441-449.
- NASH S. M. and SNYDER W. C., 1962. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. *Phytopathology*, *52*: 567-572.
- Parveen S., Ehteshamul-Haque S. and Ghaffar A., 1993. Biological control of *Meloidogyne javanica* on tomato and okra in soil infested with *Fusarium oxysporum*. *Pak. J. Nematol.*, 11: 151-156.
- Shahwar D., Abid M., Atta-ur-Rehman, Maqbool M. A. and Choudhry M. I., 1994. Nematicidal compounds from *Datura fastuosa*. In: Proceedings of *4th IUPAC Symposium on the chemistry of Natural products*. HEJ Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan (In press).
- Sheikh A. H. and Ghaffar A., 1975. Population study of sclerotia of *Macrophomina phaseolina* in cotton fields. *Pak. J. Bot.*, 7: 13-17.
- SHORT G. E., WYLLIE T. D. and Bristow P. R., 1980. Survival of *Macrophomina phaseolina* in soil and in residue of soybean. *Phytopathology*, 70: 13-17.
- Taylor A. L. and Sasser J. N., 1978. *Biology, identification and control of root-knot nematodes (Meloidogyne* species). North Carolina State University Graphics, Raleigh, USA, 111 pp.
- WILHELM S., 1955. Longevity of the *Verticillium* wilt fungus in the laboratory and field. *Phytopathology*, 45: 180-181.