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EFFECT OF DELAY IN PLANTING AFTER APPLICATION OF CHICKEN MANURE ON *MELOIDOGYNE JAVANICA* AND *PAECILOMYCES LILACINUS*¹

by

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Summary. A delay in planting tomato plants in soil treated with chicken manure decreased numbers of juveniles and galling due to *Meloidogyne javanica*. The greatest decrease was obtained with a six or eight week delay in combination with *Paecilomyces lilacinus*. Egg parasitism and tomato shoot dry weights significantly increased with delay in planting and were highest where planting was delayed for six or eight weeks.

Organic matter suppress the pathogenicity of root-knot nematodes on crops (Owino *et al.*, 1993; Oduor-Owino *et al.*, 1993). This has been attributed, in part, to the toxicity of some of the products of decomposition and/or to changes induced in the soil environment which favour the activity of nematode egg parasites or other soil-borne antagonists that destroy plant parasitic nematodes (Dackman *et al.*, 1972). It has also been suggested that sub-lethal doses of toxic compounds from specific amendments affect nematode susceptibility to fungal antagonists (Pandey and Sikora, unpublished). Since the concentration and levels of toxic products from decomposing organic matter vary with the decomposition period (Sayre *et al.*, 1965; Johnson, 1974), it is hypothesized that levels of control of root-knot nematodes and efficacy of fungal egg parasites against nematodes would also vary.

A greenhouse test was conducted to investigate effect of time of planting tomato (*Lycoper-*

sicon esculentum Mill), cv. Moneymaker in soil amended with chicken manure and infested with *Paecilomyces lilacinus* (Thom) Samson on fungal parasitism of *Meloidogyne javanica* (Treub) Chitw. eggs and on tomato growth.

Material and methods

Paecilomyces lilacinus was isolated from *M. javanica* eggs as previously described (Oduor-Owino *et al.*, 1993). The fungus was cultured on potato dextrose agar (PDA) medium (Jatala *et al.*, 1981) in 9 cm diam. Petri dishes for ten days at 20 °C. The PDA contained 0.1% chloramphenicol antibiotic (Sikora *et al.*, 1990). For pathogenicity tests, inoculum from source cultures was transferred to a new PDA in Petri dishes and incubated for 10 days at 18 °C. Pure cultures were used for the greenhouse test. Ten-day old chicken manure was incorporated into steam sterilized field soil contained in 13-

¹ Part of Ph. D thesis of first author.

cm diam. pots at the ratio of 1:8 (w/w) (chicken manure: soil). Soil without organic matter served as control. The soil was collected from a field that had been left fallow for three years. *P. lilacinus*, cultured on PDA, was immediately added into each pot at the rate of eight macerated PDA plugs (7 mm diam.) per pot and mixed. Soils without the fungus was also included as treatments. The soil was then inoculated with 4000±50 eggs and 200±50 juveniles of *M. javanica* suspended in 20 ml of distilled water and mixed thoroughly. Thirty-day old seedlings of tomato cv. Moneymaker were transplanted singly into each pot 0, 2, 4, 6 or 8 weeks after incorporation of chicken manure, nematode inoculation and fungal infestation. Fertilizer (N:P:K 20:10:10) was used at planting at the rate of 2 g/pot. All pots were arranged on a greenhouse bench in a randomized block design with six replications per treatment per planting period and were kept moist by watering twice per week. The experiment was terminated 50 days after planting and shoot dry weights and galling index (0-4 scale where 0 = no galls and 4 = 76-100% of the root system galled) assessed. The number of juveniles per 300 cm³ of soil was also determined (Southey, 1986). The percentage of parasitized eggs was assessed by extracting eggs from galled roots (Barker, 1985), and placing about 1000 eggs on water agar and counting the proportion of eggs

showing fungal growth (parasitized eggs) under the microscope after two days (Kerry and Crump, 1977). Analysis of variance was used to analyse the data. Least significance differences (LSD) were used to compare treatment means. Regression and/or correlation statistics were also used.

Results

Galling index and numbers of juveniles were low ($P = 0.05$) in soils in which chicken manure was left to decompose for 6-8 weeks before planting (Tables I and II). The least galling intensity was observed on tomatoes planted eight weeks after the chicken manure and *P. lilacinus* treatment. A combination of chicken manure and *P. lilacinus* was most effective in suppressing gall formation and nematode population in most cases. Planting tomato 0-2 weeks after incorporation of chicken manure had no significant effect on galling intensity or nematode population. Large ($P = 0.05$) galls were observed in soils without chicken manure (Table I). Egg parasitism (Table III) and tomato growth (Table IV) increased ($P = 0.05$) as planting was delayed and were greatest in soils where *P. lilacinus* and chicken manure had interacted for eight weeks before planting tomato. Planting six or eight weeks after inoculation and amending soil with

TABLE I - Effect of delay in planting on mean galling index due to *Meloidogyne javanica* 50 days after field soil was incorporated with chicken manure and *Paecilomyces lilacinus*.

| Treatments | Time of planting (weeks) | | | | |
|---|--------------------------|-----|-----|-----|------|
| | 0 | 2 | 4 | 6 | 8 |
| Chicken manure (CM) + <i>M. javanica</i> (MJ) | 3.8 | 3.6 | 3.0 | 1.6 | 1.6 |
| CM + <i>P. lilacinus</i> (PL) + MJ | 3.7 | 3.6 | 2.6 | 0.4 | 0.3 |
| PL + MJ | 4.0 | 3.9 | 3.8 | 3.8 | 3.9 |
| MJ alone (control) | 4.0 | 3.8 | 4.0 | 3.9 | 3.9 |
| L.S.D. (0.05) | NS | NS | 0.9 | 2.5 | 2.45 |

TABLE II - Effect of delay in planting on mean numbers of *M. javanica* juveniles per 300 cm³ soil 50 days after field soil was incorporated with chicken manure and infested with *P. lilacinus*.

| Treatments | Time of planting (weeks) | | | | |
|---|--------------------------|------|------|------|------|
| | 0 | 2 | 4 | 6 | 8 |
| Chicken manure (CM) + <i>M. javanica</i> (MJ) | 430 | 428 | 400 | 161 | 143 |
| CM + <i>P. lilacinus</i> (PL) + MJ | 425 | 426 | 401 | 150 | 140 |
| PL + MJ | 520 | 530 | 500 | 501 | 495 |
| MJ alone (control) | 530 | 508 | 509 | 520 | 545 |
| L.S.D. (0.05) | 89.4 | 85.4 | 80.3 | 18.5 | 45.2 |

TABLE III - Effect of delay in planting on fungal parasitism (%) of *M. javanica* by *P. lilacinus* 50 days after planting tomato in field soil incorporated with chicken manure and *P. lilacinus*.

| Treatments | Time of planting (weeks) | | | | |
|---|--------------------------|------|------|------|------|
| | 0 | 2 | 4 | 6 | 8 |
| Chicken manure (CM) + <i>M. javanica</i> (MJ) | 0.2 | 0.3 | 0.5 | 0.5 | 0.7 |
| CM + <i>P. lilacinus</i> (PL) + MJ | 16.4 | 20.4 | 25.0 | 29.6 | 30.6 |
| PL + MJ | 16.6 | 18.4 | 17.6 | 19.8 | 19.0 |
| MJ alone (control) | 0.1 | 0.2 | 0.2 | 0.3 | 0.4 |
| L.S.D. (0.05) | 2.9 | 1.3 | 6.5 | 8.3 | 9.1 |

chicken manure resulted into high ($P = 0.05$) levels of egg parasitism and tomato growth (Tables III and IV). In general, chicken manure had a stimulatory effect on shoot growth, whether used alone or in combination with *P. lilacinus*. Regression and correlation statistics (Table V) reveal that delay in time of planting from zero to 8 weeks resulted in significant reductions in the galling index for treatments with chicken manure alone ($r = -0.95$; $b = -0.3$) and chicken manure plus *P. lilacinus* ($r = -0.95$; $b = -0.05$). In contrast, delay in time of planting was associated with increases ($P = 0.05$) in egg parasitism ($r = 1$; $b = 1.9$) and dry shoot weights ($r = 0.85$; $b = 0.3$). Variations in time of planting had no significant effect on gall index, egg parasitism, number of juveniles and tomato growth in soils not amended with chicken manure.

Discussion

The low galling index and numbers of *M. javanica* juveniles from soils amended with chicken manure alone or in combination with *P. lilacinus* indicate that these treatments had an inhibitory effect on nematode development. However, it is unlikely that the low numbers of nematodes and galling intensity could be attributed to egg parasitism. The level of egg parasitism in all pots was too low to produce a significant decrease in nematode population or gall index. The increased egg parasitism and growth of the tomato plants with time of planting could be attributed to changes in the quality and quantity of decomposition products and/or nutrients that enhance nutrition (Sayre *et al.*, 1965). However, whether the chicken manure used in

TABLE IV - Mean dry shoot weights of tomato plants planted in field soil over an 8-week period after soil treatments with chicken manure and infestation with *M. javanica* and *P. lilacinus*.

| Treatments | Time of planting (weeks) | | | | |
|---|--------------------------|-----|-----|-----|------|
| | 0 | 2 | 4 | 6 | 8 |
| Chicken manure (CM) + <i>M. javanica</i> (MJ) | 3.0 | 3.3 | 3.8 | 4.7 | 5.2 |
| CM + <i>P. lilacinus</i> (PL) + MJ | 3.1 | 3.0 | 3.8 | 4.6 | 5.0 |
| PL + MJ | 1.3 | 1.7 | 1.8 | 1.7 | 1.5 |
| MJ alone (control) | 1.4 | 1.8 | 1.5 | 1.6 | 1.4 |
| L.S.D. (0.05) | 1.1 | 0.9 | 1.5 | 2.1 | 2.32 |

TABLE V - Regression coefficient for the relationships between time of planting tomato in soil inoculated with *P. lilacinus* and *M. javanica* alone or in combination with chicken manure and gall index (MGI), number of juveniles (NJ), egg parasitism (MEP) and dry shoot weight of tomato (MDSW).

| Treatments | MGI | | NJ | | MEP | | MDSW | |
|---|-------|-------|--------|-------|------|------|------|------|
| | b | r | b | r | b | r | b | r |
| Chicken manure (CM) + <i>M. javanica</i> (MJ) | -0.3* | -0.95 | -42.1* | -0.89 | 0.1 | 0.94 | 0.3 | 1.0 |
| CM + <i>P. lilacinus</i> (PL) + MJ | -0.5 | -0.95 | -42.3 | -0.89 | 1.9* | 1.0 | 0.3 | 0.85 |
| PL + MJ | 0.0 | 0.55 | -3.9 | -0.83 | 0.3 | 0.77 | 0.0 | 0.32 |
| MJ alone | 0.0 | 0.55 | +2.1 | +0.44 | 0.0 | 0.95 | 0.0 | 0.0 |

* Significant at P = 0.05.

this study stimulated egg parasitism by *P. lilacinus* or enhanced the activity of other microbes against *M. javanica* is not clear. Even if it did, the level of egg parasitism would be even lower if non-sterile field soil was used. Thus, it is unlikely that the chicken manure could significantly stimulate egg infection in the field.

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Literature cited

- BARKER K. R., 1985. Nematode extraction and Bioassay. In, *An Advanced Treatise on Meloidogyne* vol. II *Methodology*, pp. 19-38 (K. R. Barker, C. C. Carter and J. N. Sasser Eds) North Carolina State University graphics.
- DACKMAN C., JANSSON H. B. and NORDBRING-HERZ B., 1992. Nematophagous fungi and their activities in soil. In *Soil Biochemistry*, pp. 95-130 (G. Stotzy and J. M. Bollag Eds) Marcel Dekker, Inc.
- JATALA P., SALAS R., KALTENBACH R. and BOCANGEL M., 1981. Multiple application and long term effect of *Paecilomyces lilacinus* in controlling *Meloidogyne incognita* under field conditions *J. Nematol.*, 13: 445.
- JOHNSON L. F., 1974. Extraction of oat straw, flax and amended soil to detect substances toxic to root-knot nematodes. *Phytopathology*, 64: 1471-1473.
- KERRY B. R. and CRUMP D. H., 1977. Observations of fungal parasites of females and eggs of the cereal cyst nematode, *Heterodera avenae*. *Nematologica*, 23: 193-201.
- ODUOR-OWINO P., WAUDO S. W. and MAKHATSA W. L., 1993. Effect of organic amendments on fungal parasitism of

- Meloidogyne incognita* eggs and growth of tomato (*Lycopersicon esculentum* Mill) cv. Moneymaker. *Intern. J. Pest Manag.*, 39: 459-461.
- OWINO P. O., WAUDO S. W. and SIKORA R. A., 1993. Biological control of *Meloidogyne javanica* in Kenya: effect of plant residues, benomyl and decomposition products of mustard (*Brassica campestris*). *Nematologica*, 39: 127-134.
- SAYRE R. M., PATRICK Z. A. and THORPE H. J., 1965. Identification of a selective nematocidal component in extracts of plant residues decomposing in soil. *Nematologica*, 11: 263-268.
- SIKORA R. A., HEIMER M. and SCHUSTER R. P., 1990. Reflection on the complexity of fungal infection of nematode eggs and the importance of facultative perthophitic fungal pathogens in biological control of *Globodera pallida*. *M. ed. Fac. Landbouww Gent*, 55: 699-712.
- SOUTHEY J. F. M., 1986. *Laboratory methods for work with plant and soil nematodes (London)* Ministry of Agriculture, Fisheries and Food, HMSO, 200 pp.