Note from TLC Products: Chitatrol / NemaFix and ACF-SRP are identical products. ACF-SRP is the correct name, and Chitatrol / NemaFix were earlier names used.

Report

Evaluation of the efficacy of different concentrations and application intervals of a biological-based product Chitatrol / NemaFix against a mixed *Meloidogyne incognita* and *Meloidogyne javanica* population

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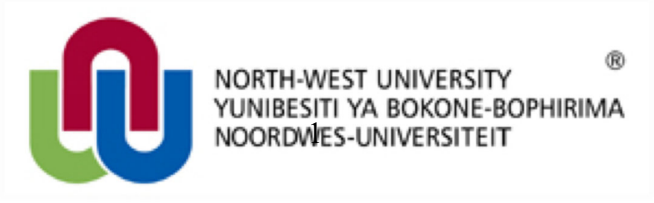

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1 Objective

To evaluate the effect of different concentrations of a biologically-based product, applied at various time intervals, on the population densities of a mixed *Meloidogyne incognita* and *Meloidogyne javanica* population in a pot experiment under prevailing temperature conditions.

2 Materials and methods

2.1 *Meloidogyne* population used

Eggs and second-stage juveniles of a mixed, *in vivo* reared *M. incognita* and *M. javanica* population (70:30 ratio) were extracted from roots of infected tomato plants (cv. Floradade) using Riekert's (1995) modified NaOCl-method. To hatch J2, eggs that were extracted were placed on a 25-µm-mesh sieve which was submerged into a container filled with tap water (approximately 5 cm deep). This container with eggs was incubated in a temperature-regulated chamber at 26 °C for 48 h.

2.2 Experimental setup and *Meloidogyne* spp. inoculation

Ten-liter capacity plastic pots were filled with Telone II fumigated soil (active substance 1.3 dichloropropene, Telone® II is a preplant soil fumigant for control of all major species of nematodes, including root knot, lesion, stubby root, dagger, ring, and cyst nematodes.) at a dosage rate of 150 l/ha (about 15 gallons per acre). The soil used was sandy loam soil (5.3 % clay, 93.6 % sand, 1.1 % silt and 0.47 % organic matter content) with pH (H_2O) of 6.8. The mean temperature range during the duration of the experiment was 21.6 to 29.4 degrees Celsius. The experimental layout was a randomised complete block design, with six replicates for each treatment. The treatments applied at the various time intervals are listed in Table 1. Preparation of the product concentrations as well as application of the treatments were done by Ms Isabelle Barnard.

One day before planting, on 25 January 2017, ±10 000 eggs and J2 (50:50 ratio) were inoculated and incorporated in the top 5-10 cm of the soil in each pot. The J2 used were obtained as described in Paragraph 2.1. One seed of maize cultivar DKC 78-79 BR was planted (approximately 4cm deep) per pot and the first product application done. The plants were watered three times per week with tap water and particularly after each product application when 500 ml water was applied to ensure that the

product was washed into the soil. Nutrifeed was applied as liquid fertilizer directly after seedling emergence as recommended by the supplier of the product and again at 35 days after seedling emergence.

The trial were terminated on 27 March 2017 (60 days after planting and inoculation) and the root system of each plant excised from the aerial parts, which were weighed and the data recorded. The root system of each plant were then removed from each pot together with approximately 200g rhizosphere soil. After weighing of the root system of each plant and recording the data, *Meloidogyne* spp. eggs and J2 were extracted using the adapted NaOCl method of Riekert (1995). The J2 were extracted from the soil obtained from each pot using the decanting- and sieving-, followed by the sugar-flotation method (Hooper *et al*., 2005). Nematode eggs and J2 were counted in a De Grisse (1963) counting dish using a stereomicroscope (60x magnification) and data recorded.

Nematode and plant (aerial mass) data were subjected to Analyses of Variance, with means separated by Tukey's HSD Test ($P \leq 0.05$). Nematode and plant data were subjected to the statistical programme Statistica for Windows, Version 13.2 (http:///www.statsoft.com).

2.3 *In vivo* tunnel experiment

Treatments and time intervals when the treatments were applied are listed in Table 1.

Table 1. The different treatment concentrations of a biologically-based product used at different time intervals to evaluate its effect on population densities of a mixed *Meloidogyne incognita* and *Meloidogyne javanica* population.

							Total application rate/ha		Dates applied								
Treatment	NemaFix powder kg/ha/application	Nemafix brew* L/ha/application	Brew Time	Dosage ml Brew/pot	Application Frequency	Nr Applications/ 9 weeks	NemaFix powder Total kg/ha	NemaFix Brew Total Litre/ha									
1	1	200	72 hours	1	Monthly	3	3	600	26 Jan				23 Feb				24 Mrt
2	1	200	72 hours	1	Weekly	9	9	1800	26 Jan	2 Feb	9 Feb	16 Feb	23 Feb	2 Mrt	9 Mrt	17 Mrt	24 Mrt
3	5	1000	72 hours	5	Monthly	3	15	3000	26 Jan				23 Feb				24 Mrt
4	5	1000	72 hours	5	Weekly	9	45	9000	26 Jan	2 Feb	9 Feb	16 Feb	23 Feb	2 Mrt	9 Mrt	17 Mrt	24 Mrt
5	10	2000	72 hours	10	Monthly	3	30	6000	26 Jan				23 Feb				24 Mrt
6	10	2000	72 hours	10	Weekly	9	90	18000	26 Jan	2 Feb	9 Feb	16 Feb	23 Feb	2 Mrt	9 Mrt	17 Mrt	24 Mrt
7	Control																

* 1 kg Nemafix/200 Litre water

Application rate per pot calculations:

25.5	cm deursnee
12.75	cm radius
0.1275	radius
3.1416	pi
0.05	m^2 oppervlak
0.0005	% van ha

Treatment 1 and 2		Treatment 3 and 4		Treatment 5 and 6	
200	L/ha	1000	L/ha	2000	L/ha
200000	ml/ha	1000000	ml/ha	2000000	ml/ha
1.0	ml/pot	5	ml/pot	10	ml/pot

Previous layout:

Treatment	NemaFix kg/ha	Brew Volume L/ha	Brew Time	Nr Applications/ 12 weeks	kg/ha/ 12 weeks
1	1	200 liter	48 hours	3	3
2	1	200 liter	48 hours	6	6
3	1	200 liter	48 hours	12	12
4	2	200 liter	48 hours	3	6
5	2	200 liter	48 hours	6	12
6	2	200 liter	48 hours	12	24

3 Results and discussion

3.1 Nematode and plant data

Table 1. *Meloidogyne incognita* and *Meloidogyne javanica* egg and second-stage juvenile (J2) data per root system of maize plants (cultivar DKC 78-79 BR) and J2 numbers per 200g soil, as well as the root and aerial masses of maize plants for different treatment concentrations of a biologically-based product applied at different time intervals to evaluate their effect on nematode population densities.

Treatment	*Meloidogyne* spp. egg and J2 numbers per root system	% decrease in *Meloidogyne* spp. population densities	*Meloidogyne* spp. J2 numbers per 200g soil	Root mass of maize plants (g)	Aerial mass of maize plants (g)
1	10 547*ab	46	73** a	78.5 a	134.9 a
2	10 692 ab	46	153 a	75.5 a	177.3 a
3	10 130 ab	49	218 a	75.0 a	166.1 a
4	4 073 a	79	63 a	60.9 a	168.8 a
5	8 050 ab	59	283 a	71.3 a	164.5 a
6	13 225 ab	33	193 a	68.4 a	185.9 a
7 (Untreated control)	19 700 b	-	323 a	63.4 a	154.3 a
P value	***0.019***	-	*0.79*	*0.846*	*0.644*
F ratio	*3.056*	-	*2.140*	*0.441*	*0.710*

*Real means; Treatments with the same letters per column do not differ significantly from each other according to Tukey's Test where P = 0.05

Treatment 4 differed significantly ($P \leq 0.05$) from the Untreated Control (Treatment 7) and from Treatment 1, 2, 3, 5, and 6 (Table 1). Hence, Treatment 4 contained the least number of eggs and J2 in maize roots followed by Treatments 5, 3, 1, 2 and 6. The percentage reduction in *Meloidogyne* spp. population densities ranged from 33% (Treatment 6) to 79% (Treatment 4).

Second-stage juvenile numbers did not differ significantly at the P = 0.05 signficance level among the treatments and ranged from 63 (Treatment 4) to 323 (Treatment 7) (Table 1). However, pot experiments with nematodes are well known to vary considerably from plot to plot. To attain statistical significance, a greater number of pots per version would most likely have been required to overcome the natural variance from plot to plot.

Again, the natural variance from pot to pot within a treatment group made difference at P = 0.05 problematic with just 6 pots, but a qualitative trend of greater root mass and aerial plant mass for treated pots was evident though not significant at p = 0.05 due to pot to pot variability (Table 2).

Table 2. Root and aerial masses of maize plants for different treatment concentrations of a biologically-based product applied at different time intervals to evaluate their effect on nematode population densities.

Treatment	Root mass of maize plants (g)	Aerial mass of maize plants (g)	Combined Root Plus Arial Mass (g)
1	78.5	134.9	213.4
2	75.5	177.3	252.8
3	75	166.1	241.1
4	60.9	168.8	229.7
5	71.3	164.5	235.8
6	68.4	185.9	254.3
7 (Untreated control)	63.4	154.3	217.7

4 Conclusions

According to results obtained from this experiment, application of Treatment 4 in particular proved to reduce *Meloidogyne* spp. numbers significantly (79%) compared to that recorded for the Untreated Control. Although nematode egg and J2 numbers were not significantly reduced after application of Treatment 5, this treatment also resulted in a substantial reduction in population levels of the mixed *Meloidogyne* spp. used when compared to the Untreated Control. Important to bear in mind, however, is the variation in nematode data obtained among the six replicates (although within valid statistical limits) is proposed to be the reason for this phenomenon.

It is recommended that the product be evaluated during the next summer-growing season in soils infested with *M. incognita* and *M. javanica* in micro-plots and/or small field plots under prevailing climate conditions, preferably in two different climatic zones. This way the efficacy of the product can be validated before full-scale field experiments are done for registration purposes. The tunnel experiment done and reported on in this report as well as micro-plot and/or small-field experiments will also be valuable as supportive data together with full-scale field experiments when the product is filed for registration.

References

De Grisse, A. (1963) A counting dish for nematodes excluding border effect. *Nematologica* 9:162.

Hooper, J.H., Hallmann, J. & Subbotin, S. (2005) Methods for extraction, processing and detection of plant and soil nematodes. In Luc, M., Sikora, R.A. & Bridge, J. (*eds*) Plant parasitic nematodes in subtropical and tropical agriculture. 2nd edition. Wallingford, CABI Publishing. pp. 53-86.

Riekert HF. (1995) An adapted method for extraction of root-knot nematode eggs from maize root samples. *African Plant Protection* 1:41-43.