EVALUATION OF SUPPRESSIVE EFFECT OF TRAP CROPS ON *HETERODERA SCHACHTII* AND *MELOIDOGYNE CHITWOODI* UNDER GREENHOUSE CONDITIONS

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Summary. Two experiments were conducted in pots in a greenhouse to study the effect of green manure cultivars on the suppression of *Heterodera schachtii* and *Meloidogyne chitwoodi*. Oilradish cv. Comet showed maximum fresh shoot weight and dry root weight after eight weeks of growth. Six cultivars (Colonel, Defender, Comet, Luna, Adagio and Metex), out of seven tested, reduced cyst, egg and larval population of *H. schachtii* compared to fallow. Among all oil radish cultivars, the greatest reduction of *M. chitwoodi* population in the root was observed in Comet (98.7%) followed by Defender (94.9%).

Keywords: Columbia root-knot nematode, control, trap crops, *Raphanus sativus*, *Sinapis alba*, sugar beet cyst nematode.

The sugar beet cyst nematode, *Heterodera schachtii* Schmidt, is a major root parasite of sugar beet in Idaho, USA. It causes serious stand and yield reductions wherever sugarbeet is produced, particularly in fields located where sugarbeets have been grown consistently for many years (Held *et al.*, 2000). Several management tactics have been used effectively to control *H. schachtii* on sugar beet. One approach is planting of trap crops, such as nematode-resistant varieties of oil radish and mustard, to stimulate nematode egg hatch (Hafez and Sundararaj, 1999). Among management practices, trap crops are promising alternatives to nematicides for control of the sugarbeet cyst nematode (Krall *et al.*, 2000). Initially, the nematode resistant varieties of oil radish and white and yellow mustard were developed in Germany, and adapted for many sugarbeet growing regions in the United States (Wilson *et al.*, 1993). Studies were conducted on the use of oil radish as a second crop to reduce soil populations of *H. schachtii* and, therefore, reduce nematicide use (Koch *et al.*, 1998). In pot experiments it was found that egg numbers of *H. schachtii* were reduced much more rapidly under radish than under spinach or in pots left unplanted (Brzeski and Baksik, 1983).

Earlier studies indicated that trap crops reduced the *H. schachtii* population significantly while increasing sugar beet yield. Percent reduction of nematode population was more with oilradish than with white mustard (Hafez and Sundararaj, 2000b). Green manure crops are also used for the management of root-knot nematode *Meloidogyne hapla* Chitw. (Melakeberhan, *et al.*, 2008), *Meloidogyne incognita* (Kofoid *et* White) Chitw. (Kratochvil *et al.*, 2004), *Meloidogyne* sp. (Curto *et al.*, 2007) and *Meloidogyne javanica* (Treub) Chitw. (Sharma and Scolari, 1984; Vawdrey and Stirling, 1997). The Columbia root-knot nematode, *Meloidogyne chitwoodi* Golden, O'Bannon, Santo, *et* Finley has been recognized as a major nematode pest on potato in Idaho and found in abundance especially in sandy soils (Hafez and Sundararaj, 2000a). Females feeding in the tubers and the development of juvenile stages live young specimens cause enlargement or bumps in the outer layers of the tubers, rendering them useless for either fresh packing or processing (Anon., 1986). Root-knot nematodes have wide host ranges (Al-Rehiayani *et al.*, 1999) leading to population increases when other susceptible crops are grown in rotation with potato (Pinkerton *et al.*, 1991). The present study was undertaken to evaluate the efficacy of newly available oil radish (*Raphanus sativus* L.) and mustard (*Sinapis alba* L.) cultivars on the multiplication of *H. schachtii* and *M. chitwoodi* in pots under greenhouse conditions.

The first experiment was conducted to compare the reproduction rate of *H. schachtii* on oil radish (cvs Colonel, Comet, Defender) and mustard (cvs Luna, Mustard blend) (Table I). The experiment was arranged according to a completely randomized design with five replicates. Ten seeds of each cultivar were planted per square pot (12.5 cm × 12.5 cm top section, 9 cm × 9 cm bottom section, 13 cm deep, 1500 cm^3 soil volume) filled with a field soil naturally infested with *H. schachtii* (8 eggs and juveniles/cm^3 soil) and thinned to five plants per pot 14 days after sowing. Plants were held at 27 °C during the day and night with ambient natural light. Pots were irrigated as needed and fertilized weekly with a 0.01 % 20-20-20 N-P-K fertilizer solution.

Plants were allowed to grow for 70 days and then harvested. At harvest, roots were removed, gently rinsed with tap water and observed for female nematodes using a 10× illuminated magnifying lens. The soil was moved from each pot into a tray, thoroughly mixed and a subsample of 500 cm^3 taken for nematode extraction. Therefore, the soil sub-sample was washed through a 70 mesh sieve into a basin. Soil debris and cysts remaining on the 70 mesh sieve were subjected to the centrifugation extraction technique (Jenkins, 1964) to extract the cysts.

Table I. Mean numbers of cysts, eggs and second stage juveniles in the pots containing soil infested with *Heterodera schachtii* and planted with green manure cultivars, 70 days after sowing.

Green manure cultivar	Cysts			Eggs and juveniles at harvest		
	Per pot	% Reduction from fallow	Per 500 cc soil	Per cyst	Per pot at harvest	% reduction per pot compared to fallow
Colonel	3.8 b	69.4	16.0 c	30.2 d	138.6 b	86.3 a
Defender	4.4 b	64.5	4.0 c	42.2 bcd	197.6 b	80.4 a
Comet	2.2 b	82.3	0.0 c	34.8 cd	57.8 b	94.2 a
Luna	8.2 b	33.9	8.0 c	70.4 bc	460.0 b	54.4 a
Mustard blend	92.2 a	-643.5	88.0 a	139.0 a	13270.0a	-1215 b
Adagio	7.0 b	43.5	4.0 c	6.0 bcd	453.2 b	55.1 a
Metex	10.0 b	19.4	14.0 c	71.8 b	805.4 b	20.2 a
Fallow	12.4 b	0.0	50.0 b	65.6 bcd	1009.0 b	

Cysts were collected in a 25-ml beaker and then transferred on a counting dish and counted under a stereo zoom microscope. Each cyst was crushed with a needle and eggs and second stage juveniles were counted. The soil and water that had passed through the sieve into the basin were used to extract eggs and larvae by sieving on a 400 mesh sieve. The nematodes were separated from soil remaining on the 400 mesh sieve by centrifugal flotation (Jenkins, 1964) and eggs, juveniles and males were collected in a 25-µm aperture sieve. The nematodes were counted using a compound microscope at 100× magnification. The final *H. schachtii* populations recovered from each pot were estimated by extrapolation.

The second experiment was conducted to assess the reproduction of *M. chitwoodi* on oil radish and mustard cultivars in comparison with that on susceptible tomato cv. Payett, which served as a control (Table II). Experimental design, pot size environmental conditions and maintenance were as described in the first experiment. However, the pots were filled with 1500 cm^3 of a sterilized (1:1, v/v) sand and soil mixture (50% sand, 25% silt, 25% clay; pH 7.5).

The inoculum of *M. chitwoodi* was extracted from infected Russet Burbank potato tubers by chopping the tubers into pieces, placing them in a mist chamber for one week and collecting juveniles at two-day intervals. A total of 2000 second-stage juveniles (J2) of *M. chitwoodi* were inoculated into each pot by pipetting aliquots of the nematode water suspension into 3-cm deep holes in the soil around the base of the plant stem. Plants were grown for 56 days to allow for nematode reproduction. At harvest, plant shoots were cut at 1 cm above the soil level. Soil with roots was stored in bags in a refrigerator at 3 °C and nematodes were extracted within a week. Roots were removed from the bag and the attached soil was collected by shaking into a plastic tray before gently rinsing the roots with tap water. This water was saved and used during the extraction of the nematodes from the soil. Roots were blotted dry using a paper towel to absorb excess water and their fresh weight was recorded. Then they were finely chopped and enclosed in muslin cloth, placed on a funnel and kept in a mist chamber for ten days to extract nematodes. Thereafter, root samples were taken out of the mist chamber, put individually in paper covers, kept in a drying oven at 37 °C for four days and their dry weights recorded.

The soil was moved from each pot into a tray, thoroughly mixed, and a sample of 500 cm^3 taken for nematode extraction. Each sample was pre-soaked with rinse water from the corresponding roots for 30 minutes and

Table II. Mean nematode population density in the green manure cultivars inoculated with *Meloidogyne chitwoodi* and harvested 8 weeks after sowing.

Green manure cultivar	Shoot weight (g)		Root weight (g)		*M. chitwoodi* population		% reduction of root population
	Fresh	Dry	Fresh	Dry	In soil (pot)	Per g root	
Colonel	29.2 b	5.7 b	12.0 c	1.3 b	960 b	1885 b	51.7
Defender	33.6 ab	6.5 a	15.2 a	2.6 ab	118 d	200 b	94.9
Comet	36.4 a	6.8 a	16.1 a	3.4 a	40 d	52 b	98.7
Luna	15.6 c	4.1 c	13.4 b	2.8 ab	1112 a	1415 b	63.7
Mustard blend	14.0 c	3.8 c	10.3 c	1.6 b	552 c	1838 b	52.9
Tomato	-	-	15.0 a	3.0 a	1088 a	3903 a	

then passed through a 70 mesh sieve into a 2-litre bowl. The sieved soil and water in the bowl were then passed through a 400 mesh sieve. The residue on the 400 mesh sieve was collected in a 200 ml beaker and allowed to settle at 3 °C for 3 hours. The excess water on top of the beaker was siphoned off, leaving the residue containing nematodes on the bottom of the beaker undisturbed. Nematodes were separated from the residue by the centrifugal flotation technique (Jenkins, 1964), collected on a 500 mesh screen and counted using a compound microscope at 100× magnification. The final *M. chitwoodi* populations recovered from each pot were estimated by extrapolation. Nematode population per g dry root was obtained by dividing the total nematode population in the root of each treatment by the total root dry weight.

There was a significant reduction in the cyst, egg and juvenile populations of *H. schachtii* by all green manure cultivars, compared to fallow (Table I), except with Mustard blend, which instead increased significantly both cyst (7.4-fold) and eggs and juveniles (13.1-fold) per pot. The greatest reduction in cyst numbers was observed in Comet (82.3%) followed by Colonel (69.4%) and Defender (64.5%). The results agree with Nowakowski and Szymczak-Nowak (2006), who found that the greatest reduction of the population of the sugar beet cyst nematode in the soil was given by Colonel, although these authors found that the largest fresh and dry matter production of aboveground and root biomass were in Remonta and Adagio. However, Dobosz *et al*. (2005) reported that Colonel did not significantly decrease the sugar beet cyst nematode population density compared with the fallow treatment.

More variability was observed in the numbers of eggs and juveniles per cyst (Table I). The least eggs and juveniles per cyst was in Colonel (30.2) followed by Metex (71.8). Similarly, Smith *et al*. (2004) reported that cultivars of oil radish were superior to most of the mustard cultivars in reducing nematode populations and, in a field study, Colonel reduced nematode populations more than Adagio and Rimbo when Pi was lower than 2.5 *H. schachtii* eggs and J2/cm^3 soil. The greatest reduction in nematode populations was attributed to the production of the greatest amount of biomass, whose roots in turn release more nematode suppressive factors. This concept was proved by Nowakowski and Szymczak-Nowak (2003) and Szymczak-Nowak and Nowakowski (2002), who demonstrated that the highest fresh and dry matter yields of roots and shoots were obtained with Colonel and Metex coupled with the greatest reduction in nematode population.

There was a significant reduction in *M. chitwoodi* root populations by the green manure cultivars compared with the roots of the control tomato plants. Among the three oil radish cultivars tested, the reduction in root population was greatest in Defender (94.9%) followed by Colonel (51.7%). Earlier studies indicated that addition of the bacterium *Bacillus megaterium* (Al-Rehiayani *et al*., 1999) along with green manure significantly decreased infection of potato tubers by *M. chitwoodi* and increased the tuber yield. Similarly, Mojtahedi *et al*. (1993) found that planting a green manure crop in the fall and incorporating it in the spring limited *M. chitwoodi* damage. Among all of the mustard cultivars, the greatest nematode reduction in roots was in the pots planted with Comet (98.7%) followed by Luna and Mustard blend. However, there was no significant difference in nematode soil population among the cultivars of green manure tested.

The largest populations of *M. chitwoodi* in the soil and in the roots were observed in the pots planted to tomato. All cultivars, except Luna, reduced significantly the population of *M. chitwoodi* in the soil compared to tomato, with cvs Defender and Comet giving the greatest reduction, which was also significantly more than that of the other cultivars. In the roots, the nematode populations were reduced significantly by all green manure cultivars. However, although Defender and Comet gave again the greatest reduction (94.9% and 98.7%, respectively), the observed differences among cultivars were not significant probably because of the large variability among the replicates.

There were significant differences in dry shoot weight and dry root weight among the green manure cultivars, but the differences observed in fresh weight were not significant. The greatest shoot dry weights were observed in Defender (6.5 g) and Comet (6.8 g) followed by Colonel (5.7 g). The lowest dry shoot weights were observed in Luna and Mustard blend. The greatest dry root weight was observed in Comet (3.4 g). There were no significant differences in dry root weight among the cultivars Defender, Comet and Luna. Dry root weight in Colonel and Mustard blend was higher than Comet, however, and statistically on par with Defender and Luna.

Earlier studies by Hafez and Sundararaj (1998) indicated that there was a difference in biomass production among the green manure cultivars and planting date (Hafez and Sundararaj, 1999). Such an increase in biomass production is also one of the factors that enhances the nitrogen level in the soil. The current studies indicated that six cultivars, out of seven tested, are promising green manure crops to be considered for sugar beet cyst nematode management in Idaho. Similarly the cultivars Defender and Comet have potential to be incorporated into the soil as a management strategy for the Columbia root knot nematode on potato.

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