Pakistan J. Zool., pp 1-8, 2020. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20190712040728

Effect of Different Application Methods on the Nematicidal Activity of Bio and Synthetic Chemicals against Root Knot Nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood

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Article Information
Received 12 July 2019
Revised 03 September 2019
Accepted 20 September 2019
Available online 01 June 2020

Authors' Contribution
HA, NJ and MK, SAK planned, designed and executed experimental work, MN, HA and IU formatted the manuscript. EH and AJ conducted data analyses.

Key words
Systemic, Root dip, Drench, Chemical, Nematode

ABSTRACT

Meloidogyne incognita (Kofoid and *White*) *Chitwood* is the most widespread and destructive pathogen of tomato, difficult to control due to its wide host range. In present investigation effect of bio (Cure, Azadirachtin) and synthetic (Cartap, Virtako) chemicals was evaluated on the induction of systemic activity by using modified split root technique. Efficacy of different application methods; soil drench and root dip was also tested. Results revealed that all the chemicals have more or less systemic effect against *M. incognita.* Cartap was found to be more effective in reduction of egg masses followed by Virtako in both treated and untreated root portion. Number of egg masses was reduced significantly in Cure and Azadirachtin in treated (45.7, 58.2) as well untreated root portion (93.7, 80.7) respectively. In soil drench treatment, a significant higher reduction in the nematode reproduction parameters was observed in all the chemicals as compared to root dip method. Efficacy of all the chemicals was also tested in microplots already infested with nematode. Significant suppression in nematode population was observed in all the tested chemicals when applied in infested microplots. The results of the present investigation suggest suitable management option for the growers having nematode problem in their field.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is an economically important vegetable, widely grown throughout the world including Pakistan. Its consumption as food was originated in Mexico in diverse ways such as sauses, drinks, salads and raw product. It contains vitamin A, C, E and lycopene which are beneficial for human health (Hobson and Davies, 1971). It contains antioxidants, good amounts of manganese and phytonutrients including flavonones, carotenoids, glycosides and fatty acid derivatives etc. average harvested yield of tomato is low because of a number of reasons including poor agronomic practices, non-availability of certified seed and by various diseases caused by fungi, viruses, nematode and bacteria. Root-knot nematode, *Meloidogyne incognita* L. is the most widespread and destructive pathogen of tomato (Fourie and McDonald, 2000).

In solanaceous crops 4-8% losses were recorded due to root-knot nematode (Koenning *et al*., 1999). Shahid *et al*. (2007) reported 75-100 % disease incidence on tomato while disease infestation and prevalence was reported upto 32% and 53%, respectively due to *M. incognita* (Kofoid and White) Chitwood in Pakistan (Kamran *et al*., 2010). In Pakistan yield losses due to *M. incognita* and *M. javanica* were recorded at 32-40% (Anwar and Mckenry, 2012). All the inoculums densities of *M. javanica* resulted in significant reductions in growth variables of both the cultivars over their controls (Mukhtar and Hussain, 2019).

Since 1950, fumigant and non-fumigant nematicides have been used extensively for the control of root-knot nematode in vegetables and nursery crops (Noling and Becker, 1994). Another category of chemicals having toxicity to nematodes was reported during 1960s with two general classes *i.e.* organophosphates and carbamates were recognized for the control of nematodes as well as insects

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0030-9923/2020/0004-0001 $ 9.00/0

(Weiden *et al*., 1965).

Fortnum *et al*. (2001) used nematicides to manage mixed populations of root-knot nematode on tobacco. Their findings revealed that 1,3-dichloropropene was more effective in reducing the mixed populations of *M. javanica*, *M. incognita* race 3 and *M. arenaria* race 2 as compared to ethoprop, and fenamiphos. Development of root-knot nematode was more affected at 1% concentration of Cadusafos (Safdar *et al*., 2012).

Various chemicals with systemic mode of action have been reported against plant parasitic nematodes (Javed *et al*., 2007; Oka *et al*., 2012). Cloyd (2002) reported that the active ingredient of systemic chemicals is absorbed by the roots and transported all through the plant (through the vascular tissues either of xylem or phloem). Previously different application methods have been tested to check the efficacy of chemicals against nematodes i.e. root dip (Harlan and Jenkins, 1967; Jayakumar *et al*., 2001), soil drench (Alam, 1993; Javed *et al*., 2008; Das *et al*., 2014), foliar spray (Johnson *et al*., 1995; Oka *et al*., 2012) and seed treatment (Cabrera *et al*., 2009).

The present investigation was conducted to evaluate the systemic activity of bio and synthetic chemicals {chemicals selected from previously conducted laboratory experiments (Abbas *et al*., 2015)} against *M. incognita* and their efficacy through different application methods.

MATERIALS AND METHODS

Nematicidal potential of bio and synthetic chemicals available in the market was evaluated against *M. incognita* in different concentrations and time intervals (Abbas *et al*., 2015). On the basis of the results, four chemicals were selected and evaluated in the present study.

Mass culturing of root knot nematode

Tomato roots and soil samples infested with root-knot nematode were collected from the vegetable production areas of University of Agriculture Faisalabad, Pakistan. Root and soil samples were processed separately to assess root-knot nematode population. The roots were separated from the soil, washed and weighed. The entire root system was chopped and incubated in a mist chamber for 5 days to hatch the eggs. Soil samples were thoroughly mixed and processed by Baermann funnel techniques for 3 days to collect nematodes. Perineal patterns of mature females were prepared for different root-knot nematode species. At least 10 perineal patterns of each specie were examined to identify the species. The sterilization of sandy loam soil was done in an oven at 120°C for 20 min (Talavera and Mizukubo, 2003) after which it was stored for two weeks at 25°C before using them for experimental purposes. Seeds of tomato (*Lycopersicon esculentum)* 'Moneymaker' were collected from Ayub Agriculture Research Institute, Faisalabad, Pakistan. Seeds were planted in seedling trays containing sterilized soil. Three weeks old seedlings were transplanted in earthen pots (20-cm diam.). In order to make a pure culture of field populations, single egg mass inoculation of *M. incognita* was done. Single mature egg masses were inoculated in pots around the root of young tomato seedlings. Mass culturing was done by inoculating new tomato seedlings with at least 15 egg masses, each obtained from pure culture in order to maintain sufficient inoculum for the present study.

Evaluation of systemic effect of chemicals

Split root technique

Sterilized soil was amended with Cartap, Virtako, Cure and Azadirachtin at their R concentration (Recommended dose) in the field , [four concentrations, R=Recommended dose, 2R=Double dose, R/2= Half dose, R/4=Quarter dose, of each chemical were prepared by adding requisite amount of distilled water for mortality experiment, R concentration was selected for the present study as maximum mortality was recorded in R concentration (Abbas *et al*., 2015)] subsequently pots were filled with this treated soil while the other half of the pots was filled with untreated sterilized soil. Three weeks old tomato cv. Moneymaker seedlings were transplanted with splitted roots into two equal halves; each half of the root system was transplanted into a separate pot, one half in treated soil (bio and synthetic chemicals) and another in untreated soil. A 2.5 cm ring of paper tube filled with soil was placed between the twin pots to provide support to the plant. After seven days these plants were inoculated with 500 freshly hatched J_{2s} of *M. incognita.* Cross contamination by soil or irrigation water was avoided.

Pots were collected after 35 days. Data recorded consisted of number of egg masses, number of females and eggs/egg mass for each half. Number of egg masses and females were counted. Eggs per egg mass were estimated by taking 10 uniformly sized egg masses from each root system, dispersed them with bleach (McClure *et al*., 1973) and five 0.5 ml aliquots of the resulting egg suspension were counted under a dissecting microscope at 40 X magnification.

Root dip treatment

Sterilized soil was filled in earthen pots of (15 cm diameter). Three weeks old seedlings of tomato cv. moneymaker were dipped in Cartap, Virtako, Cure and Azadirachtin at their R concentration for 30-35 minutes. Plants dipped in distilled water for the same time period served as control. Thereafter these seedlings were

transplanted in the earthen pots. One week after planting, plants were inoculated with 2000 freshly hatched J_2s of *M. incognita.* The experiment consisted of 10 replications under a completely randomized design.

Data on number of galls, number of females, number of egg masses, J_{2s}/100 cm^3 of soil and reproduction factor were recorded at 35 days after inoculation.

Soil drench treatment

All the experimental protocol was duplicated from previous study (root dip study) except that seedlings were transplanted in sterilized soil amended with bio and synthetic chemicals. Inoculation of 2000 freshly hatched J_{2s} of *M. incognita* was done one week after transplantation. The experiment consisted of 10 replications under a completely randomized design. Data on number of galls, number of females, number of egg masses, J_{2s}/100 cm^3 of soil and reproduction factor were recorded at 35 days after inoculation.

Evaluation of chemicals against M. incognita *in microplots*

Three weeks old tomato seedlings were transplanted in microplots (Department of Plant Pathology, University of Agriculture Faisalabad, Pakistan) in two rows. The microplots (7 x 3x 2 ft) were already infested with *M. incognita* and the infestation level was 108 J_{2s} per 100 cm^3 soil. Infestation level was assessed by taking five soil cores from each microplot. Soil cores were mixed thoroughly and a 100-cm^3 sub sample was used for nematode extraction on sieving-cum-modified Baermann funnel technique (Thistlethwayte, 1970). Plant to plant and row to row distance was 1 and 1.5 feet, respectively. One week after transplantation Cartap, Virtako, Cure and Azadirachtin were applied in the soil at the R concentration. All the treatments were replicated in Randomized complete block design. Three months after transplantation the following data was recorded. Plant growth responses viz., foliage weight (g), shoot length (cm) and yield and nematode reproduction parameters viz., number of galls, number of females, number of egg masses, J_{2s} /100 cm^3 of soil and reproduction factor were recorded.

All the experiments were repeated; the average of two year data was statistically analyzed and presented in the tables.

RESULTS

Evaluation through split root technique

Effect of bio and synthetic chemicals was evaluated on the induction of systemic activity by using a split root technique. Results revealed that all the chemicals have a systemic effect against *M. incognita* (Table I). Systemic effect of all the chemicals on number of females varied significantly (P=0.05) in all the chemicals. Maximum reduction in the number of females was observed in Cartap in treated (33.5) as well as in untreated roots portion (61.2). Virtako also showed systemic activity against *M. incognita* as it lowered number of females not only in treated portion but also in untreated one (Table I). Number of females was also decreased in Cure and Azadirachtin (103.7, 92.1) in untreated portion as compared to control (205.6). All the tested chemicals showed systemic activity in reducing egg masses of *M. incognita*. Cartap was found to be more effective in reduction of egg masses followed by Virtako in both treated and untreated root portion of tomato. Number of egg masses was reduced significantly in Cure and Azadirachtin in treated (45.7, 58.2) as well untreated root portion (93.7, 80.7) respectively. Eggs/egg mass were decreased significantly in all the chemicals on untreated root portion. Systemic activity was observed in all the chemicals. Maximum reduction in number of eggs/egg mass was observed in Cartap in both treated and untreated root portion (114.4, 148.2) respectively. Other treatments; Virtako, Cure and Azadirachtin also caused significant reduction in eggs/egg mass in untreated (162.5, 192.5, 184.1) root portion as compared to control treatment (248.8) respectively (Table I). All the chemicals showed significant variation in the development of *M. incognita* by testing through root dip and soil drench treatment. Effect of bio and synthetic chemicals was evaluated on reproductive parameters.

Assessment through root dip treatment

The effect of the chemicals varied significantly (P=0.05) from the untreated control on the development of *M. incognita* by using root dip technique (Table II). All the chemicals caused significant reduction in number of galls. Where the maximum reduction was observed in Cartap (122.2) followed by Virtako (138.4) and Cure (162.3). Significant lower number of galls was observed in Azadirachtin (182.4) as compared to control treatment (380.7). Number of females decreased significantly in root dip treatment of all the chemicals (Table II). Minimum number of females was recorded in Cartap (134.1) in comparison to control (395.8). A significant reduction in number of females was observed in Virtako, Cure and Azadirachtin (145.3, 180.5, 192.8) respectively. Higher number of egg masses was observed in control due to maximum infection of nematode. Minimum egg masses were recorded in all the chemicals (Table II). Cartap caused significant reduction in production of egg masses (110.2) followed by Virtako and Cure (117.5, 135.2) as compared to control (372.6) respectively. Bio and synthetic chemicals varied significantly in the recovery of J_{2s} from soil. Maximum number of were recovered in control (1259.0) while their number was decreased in all

Table I. Evaluation of systemic effect of bio and synthetic chemicals through split root technique.

Treatments	Treated root portion			Untreated root portion		
	No. of females	No. of egg masses	Eggs/egg mass	No. of females	No. of egg masses	Eggs/egg mass
Cartap	33.5[1] e	27.1 e	114.4 e	61.2 e	54.1 e	148.2 e
Virtako	42.7 d	34.3 d	128.7 d	74.6 d	63.5 d	162.5 d
Abamectin	60.2 c	45.7 c	146.2 c	103.7 b	93.7 b	192.5 b
Azadirachtin	74.4 b	58.2 b	158.1 b	92.1 c	80.7 c	184.1 c
Control	202.8 a	186.7 a	244.3 a	205.6 a	190.3 a	248.8 a
LSD	0.66	0.74	0.67	0.75	0.73	0.72

[1]Means within a column sharing the same letter are not significantly different from each other at $P = 0.05$.

Table II. Assessment of bio and synthetic chemicals against *M. incognita* through root dip treatment.

Treatments	No. of galls	No. of females	No. of egg masses	J_{2s}/100cm^3 of soil	RF[2]
Cartap	122.2 e	134.1 e	110.2 e	282.0 e	0.84 e
Virtako	138.4 d	145.3 d	117.5 d	298.2 d	0.89 d
Abamectin	162.3 c	180.5 c	135.2 c	336.4 c	1.01 c
Azadirachtin	182.4 b	192.8 b	148.1 b	354.5 b	1.06 b
Control	380.7 a	395.8 a	372.6 a	1259. a	3.77 a
LSD	0.718	0.693	0.643	0.816	0.004

[1]Means with in a column sharing the same letter are not significantly different from each other at $P = 0.05$.
[2]Reproduction factor (RF) = final population/initial population.

the chemicals; Cartap, Virtako, Cure and Azadirachtin (282.0, 298.2, 336.4, 354.5) respectively. Bio and synthetic chemicals caused a significant decline in the reproduction factor of *M. incognita* (Table II). Maximum reproduction was observed in control plants (3.77) while minimum reproduction was recorded in Cartap (0.84).

Assessment through soil drench treatment

Potential of bio and synthetic chemicals was also assessed through soil drench treatment against *M. incognita* (Table III). All the chemicals varied significantly (P=0.05) on the development of nematode. In soil drench treatment of a significant higher reduction in the number of galls was observed in all the chemicals as compared to root dip. Maximum number of galls was recorded in control (384.8) while a lower number was observed in Cartap (92.0) followed by Virtako, Cure and Azadirachtin (103.3, 115.1, 132.3) respectively (Table III). A significant reduction was observed in number of females through soil drench application of chemicals. Cartap proved to be more effective from other chemicals in reducing number of females. Virtako caused higher reduction in number of females as compared to Cure. In Azadirachtin (153.7) minimum number of females was observed as compared to control (398.2). Maximum reduction in number of egg masses was recorded in Cartap through soil drench (88.0) treatment as compared to root dip (110.2). Statistically significant (P=0.05) reduction in egg masses was observed in Virtako, Cure and Azadirachtin (98.2, 114.5, 122.7) respectively.

Number of J_{2s} recovered through soil drench treatment was lowered as compared to root dip treatment in all the chemicals. Maximum recovery was noted in control (1245.0) while minimum was observed in Cartap (202.1). A significant reduction was observed in other chemicals; Virtako, Cure and Azadirachtin (222.1, 245.4, 266.5) respectively (Table III). Reproduction factor of *M. incognita* showed a decline in soil drench treatment of chemicals in comparison to root dip treatment. Minimum reproduction rate was recorded in Cartap followed by other chemicals while maximum was in control.

Evaluation in microplots

Effect of bio and synthetic chemicals was significantly varied on tomato growth and nematode reproduction in microplots. A significant (P=0.05) increase in plant growth and decrease in nematode reproduction was recorded (Table IV). All the treatments significantly differ in their reaction towards foliage weight. Maximum foliage weight was recorded in healthy control plants.

Table III. Assessment of bio and synthetic chemicals against *M. incognita* through soil drench treatment.

Treatments	No. of galls	No. of females	No. of egg masses	J_{2s} /100cm^3 of soil	RF[2]
Cartap	92.0 e	98.1 e	88.0 e	202.1 e	0.60 e
Virtako	103.3 d	121.3 d	98.2 d	222.1 d	0.66 d
Abamectin	115.1 c	142.2 c	114.5c	245.4 c	0.73 c
Azadirachtin	132.3 b	153.7 b	122.7b	266.5 b	0.80 b
Control	384.8 a	398.2 a	376.8a	1245. a	3.73a
LSD	0.790	0.661	0.655	0.801	0.004

[1]Means with in a column sharing the same letter are not significantly different from each other at $P = 0.05$.
[2]Reproduction factor (RF) = final population/initial population

Table IV. Evaluation of bio and synthetic chemicals against *M. incognita* in microplots.

Treatments	Foliage weight (g)	Shoot length (cm)	Yield (g)	No. of galls	No. of females	No. of egg masses	J_{2s}/100cm^3 of soil	RF[2]
Cartap	176.6[1] b	54.2 b	894.7 b	224.3 e	242.2 e	196.4 e	294.3 e	2.72 e
Virtako	159.4 c	51.6 c	810.2 c	248.1 d	268.1 d	216.2 d	326.4 d	3.02 d
Abamectin	147.7 d	48.4 d	750.4 d	294.1 c	314.5 c	265.7 c	370.6 c	3.43 c
Azadirachtin	141.2 e	45.9 e	688.6 e	317.4 b	330.1 b	288.4 b	392.0 b	3.63 b
Control (N)	94.4 f	38.4 f	482.4 f	580.9 a	612.7 a	496.0 a	740.5 a	6.85 a
Control (H)	198.3 a	57.7 a	970.7 a	0.00 f	0.00 f	0.00 f	0.00 f	0.00 f
LSD	0.018	0.063	0.069	0.635	0.711	0.604	0.564	0.005

[1]Means with in a column sharing the same letter are not significantly different from each other at $P = 0.05$
[2]Reproduction factor (RF) = final population/initial population.

An increase in foliage weight was observed in Cartap (176.6) followed by Virtako and Cure (159.4, 147.7) respectively. A considerable increase in shoot length was observed in Cartap and Virtako over nematode control. Maximum shoot length was recorded in healthy control (57.7) while minimum was in nematode control (38.4). Maximum reduction in yield of tomato plants was recorded in control having nematodes only (482.4). Yield was maximum in healthy control (970.7) followed by Cartap (894.7), Virtako (810.2), Cure (750.4) and Azadirachtin (688.6) respectively. Galls number was decreased significantly in Cartap (224.3) as compared to control (580.9). A significant (P=0.05) decline in number of females was recorded in all the chemicals. Minimum number of females was observed in Cartap (242.2). Cartap caused a higher reduction in egg masses production (196.4) as compared to Virtako (216.2) which was more effective than Cure (265.7). Cartap caused significant reduction in J_{2s} recovery from soil (294.3) followed by Virtako (326.4), Cure (370.6) and Azadirachtin (392.0) respectively. Maximum reproduction factor was observed in control with only nematodes (6.85) while minimum was observed in Cartap (2.72). Virtako and Cure decreased reproduction factor significantly (Table IV).

DISCUSSION

Systemic activity of selected bio and synthetic chemicals was evaluated against *M. incognita* by using split root technique. Systemic effect of all the chemicals varied on number of females, number of egg masses and eggs/egg mass. Maximum systemic activity was observed in Cartap on untreated root portion. Split root technique was used to study the systemic effect of chemicals. Previously this technique was used by other workers to check systemic effect of different chemicals (Kheir *et al*., 1983; Javed *et al*., 2007).

Chemicals having systemic activity are absorbed by the root system preventing penetration and reproduction of nematodes (Sivakumar *et al*., 1976; Wright, 1981). Results of the present study revealed that reproduction of *M. incognita* was affected in untreated root portion, since lower numbers of females, egg masses and eggs/egg mass were observed in all the chemical treatments as compared to the untreated control. These results are similar with (Javed *et al*., 2007) who recorded reduction in egg masses and eggs/egg mass in untreated root portion with bioproducts as compared to the control. Cartap having thiocarbamate was found to be most effective.

Systemic effect of carbamates were reported by (Nelmes *et al*., 1973; Dubey and Trivedi, 2011). Systemic action of Virtako might be attributed to its active ingredients (thiamethoxam + chlorantraniliprole) as suppression in reproduction parameters was observed in untreated root portion, this action was also recorded by other researchers (Lanka *et al*., 2013). In bio chemicals, Azadirachtin showed more systemic activity as compared to Cure. Several investigations confirmed systemic activity of Azadirachtin (Larew, 1988; Osman and Port, 1990; Nisbet *et al*., 1993). Cure was less effective in reducing nematode density in untreated root portion than other chemicals that was suggested due to limited systemic activity of abamectin (Chukwudebe *et al*., 1996). Potential of chemicals might be attributed to inactivation of enzymes, damaged nervous system, paralysis, blockage of electrical activity, thus reduced feeding, penetration and development (Evans and Wrigh, 1982; Yamamoto, 1999) of nematodes. It could be concluded from these findings that systemic chemicals are more effective in suppressing nematode population as penetration and development of nematodes reduced in untreated root portions. Effectiveness of bio and synthetic chemicals was checked through root dip and soil drench treatment against *M. incognita*. Results revealed that soil drenching of chemicals was more successful in reducing nematode population as compared to root dip treatment. Lowest number of galls, J_2/100 cm^3 of soil and egg masses was recorded in soil drench application of Cartap as compared to root dip. These findings are in line with (Niles *et al*., 1984; Javed *et al*., 2008) who observed suppression in nematode population in soil drench treatment of various chemicals and this method was termed as most practical for the management of nematodes (Alam, 1993) having no negative effect on plant growth (Rosenberger and Meyer, 1988). Khan *et al*. (2012) evaluated root dip and soil application methods for nematicides both in individual and combined application against *M. graminicola*. Results showed that combined application of both methods was most successful in nematode reduction. Efficacy of different chemicals was evaluated against plant parasitic nematodes with either granular or liquid formulations as root dipping and soil drench application against several genus of nematodes like *M. incognita, M. arenaria, M. javanica, Tylenchulus semipenetrans, Rotylenchulus reniformis Radopholus similis* and *Ditylenchus dipsaci* (Jansson and Rabatin, 1997; Sivakumar and Gunasekaran, 2011).

Other application methods; injection methods, foliar spray and root dip of avermectins were tested against plant parasitic nematodes (Jansson and Rabatin, 1998) on different crops. Application of chemicals closer to plant parasitic nematodes was more effective in reducing infection (Cayrol *et al*., 1993). Effect of bio and synthetic chemicals was significantly varied in their reaction towards tomato growth and nematode reproduction in microplots. A significant increase in foliage weight, shoot length and yield of tomato plants was observed in all the chemical treatments. Reduction in reproductive parameters i.e., number of galls, number of females, number of egg masses, J_2/100 cm^3 was variable in all the chemicals. These findings are in agreement with (Khan *et al*., 2011; Pankaj *et al*., 2012) who reported increased plant growth and reduced nematode population under the of influence different chemicals. Reduction in nematode penetration due to the absorption of chemicals in roots (Hague and Gowen, 1987; Javed *et al*., 2007) act as factor in improving plant growth. Synthetic chemicals were found to be more effective in reducing nematode population and improving plant growth as compared to the bio chemicals. Cartap and Virtako caused maximum reduction in reproductive parameters. These results are in conformity with (Raddy *et al*., 2013) that tested the effectiveness of nematicides and bio products against *M. incognita* on tomato.

CONCLUSION

It could be summarized that bio and synthetic have the potential of reducing nematode population in the field conditions. They should be used at their recommended doses to avoid phytotoxic effects. Chemicals may also improve plant growth by reducing the damage to the root system.

Statement of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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