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Assessment of new chemistry insecticides for their nematicidal potential against root knot nematode, *Meloidogyne incognita*

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Abstract

The present research work was conducted to check the nematicidal potential of new chemistry insecticides against root knot nematode, *Meloidogyne incognita* (Kofoid and White) due to the current unavailability of nematicides in local market. Assessment of twenty new chemistry insecticides available in the market of both bio and synthetic origin was done against *M. incognita*. Effect on egg hatching and juvenile's mortality of *M. incognita* was assessed under *in-vitro* conditions. Three concentrations of each new chemistry insecticide were prepared *viz.*, S, S/2, S/4 according to recommended dose of each insecticide. Data on egg hatching was recorded after 2, 4 and 6 days and on mortality after 24, 48 and 72 hours. Maximum hatching inhibition and mortality percentage was recorded in synthetic insecticides; Cartap and priority and bio; Nova Star and Proclaim. After 24 hours of incubation Cartap and priority at S, S/2 and S/4 caused maximum (85.67, 71.67, 63.00) and (76.33, 64.00, 53.00) mortality, respectively. After 2 days of incubation, Cartap and priority at S, S/2 and S/4 caused (90.00, 84.00, 72.00) and (66.33, 60.67, 50.33) % egg hatching inhibition, respectively. These four insecticides were selected and evaluated further against mobility of juveniles. Minimum number of J2's were recovered in Cartap (93.68) followed by other chemicals while maximum were recovered in control (250.4). The findings of present investigation suggested suitable insecticides available in the market for growers having nematode problem in field to plan better management strategies for better crop yield.

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Introduction

Root-knot nematode was reported first time by Berkeley in 1855 on cucumber. The type specie of the genus *Meloidogyne exigua* was described by Goeldi in 1887 from where Chitwood found the name *Meloidogyne* which is currently used for root-knot nematodes. It is of Greek origin which means "apple-shaped female". In Four new species were described within the genus *Meloidogyne* by Chitwood in 1949. Most of the species of *Meloidogyne* belong to tropical to sub-tropical climates however *M. hapla* and *M. chitwoodi* are well adjusted to temperate climates (Brodie *et al*., 1993).

Severe infections of root-knot nematode results in reduced yields on various crops and also affects consumer's acceptance. Mainly three factors affect root galling including population density, *Meloidogyne* specie, race, and host plant specie. As the population density of nematodes increases in particular location, number of galls will increase (Barker *et al*., 1998).

Root-knot nematodes cause severe losses in vegetables throughout the world. Yield losses upto 24% due to *M. incognita* and *M. javanica* were reported (Kathy, 2000). Disease infestation and prevalence was 32% and 60% respectively due to *M. incognita* in Pakistan (Javed *et al*., 2010; Kamran *et al*., 2010). In Pakistan yield losses due to *M. incognita* and *M. javanica* were 40%(Anwar and Mckenry, 2012). A range of strategies employed for the management of root-knot nematodes including cultural practices, biological control, sanitation, soil amendments and host plant resistance. But unfortunately all these practices are unable to protect the crops under field conditions because these are not cost-effective and require extra labour (Kerry, 1990). So, the most practical alternative like chemical control should be used to protect the plants under field conditions. Chemical control through nematicides is the quickest way to reduce the root-knot nematode population under field conditions in a short period of time. Though, the use of some nematicides and fumigants has been restricted due to concerns about the health hazards to humans and environment safety (Rich *et al*., 2004). However, chemical control still endures to be the main approach for the management of nematodes. The chemicals preferably used should possess a high rate of nematode suppression in a short time and have no phytotoxic. Information about the level of nematode infestation in the soil is a prerequisite to avoid the needless use of nematicides (Dubey and Trivedi, 2011). Lamberti *et al.* (2000) reported that non-fumigant nematicides can be easily and safely applied as compared to fumigants, which are most widely used such as carbofuran, aldicarb, fenamiphos, fosthiazate cadusafos, oxamyl, ethoprop and organophosphate based nematicides.

Rehman *et al*. (2009) incorporated various bio-products into the soil to lessen the population of *M. incognita*. Abamactin proved the best for reducing the invasion and development of *M. incognita* followed by emamectin whereas azadirachtin reduced the number of eggs per egg mass and proved to have nematostatic properties. Therefore, this research work was conducted to check the nematicidal potential of new chemistry insecticides against root knot nematode, *M. incognita*.

Materials and methods

Collection of diseased plants

Eggplant roots and soil samples infested with root-knot nematodes were collected from the vegetable production areas of University of Agriculture Faisalabad. 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.

Mass culturing of root knot nematodes

Perineal patterns of mature females were prepared for different root-knot nematode species (Jepson, 1987). The sterilization of sandy loam soil was done in

oven at 120°C for 20 minutes (Talavera and Mizukubo, 2003) and then it was stored for two weeks at 25°C before using them for experimental purpose. Seeds of tomato (var. Moneymaker) were collected from Ayub Agriculture Research Institute, Faisalabad. Seeds were planted in seedling trays containing sterilized soil. Three weeks old seedlings were transplanted in earthen pots (20-cm dia.). In order to make pure culture of field population, single egg mass inoculation of *M. incognita* was done. Single mature egg mass was 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 further studies.

Assessment of nematicidal effect of new chemistry insecticides on egg hatching of M. incognita

Four concentrations (2S=Double dose, S=Recommended dose, S/2= Half dose, S/4= Quarter dose) of each chemical were prepared according to recommended dose by adding requisite amount of distilled water. For hatching test, population of *M. incognita* maintained on the roots of egg plant from single egg mass culture was used, eggs of *M. incognita* were isolated by the method of Hussey and Barker (1973). Single egg mass of uniform size containing about 250 eggs was placed in each Petri dish.Four concentrations of each chemical were added in Petri dishes. Five replications were done for each chemical and incubated at 28°C ± 2 in a completely randomized design. Data was recorded after 2, 4 and 6 days of incubation.

Percent egg hatching was calculated by following formula:

$$\frac{\text{No. of hatched eggs}}{\text{Total no. of eggs}} X\ 100 = \%\ \text{egg hatching}$$

After each count the egg masses were washed with 1 mL of distilled water in their respective plates and transferred to fresh concentrates of chemicals.

Assessment of nematicidal effect of new chemistry insecticides on mortality of M. incognita

For mortality test, all the experimental protocol and conditions were similar as in experiment No. 1 except freshly hatched second stage juveniles of *M. incognita* were used. Juveniles of *M. incognita* were extracted from the eggs and 1 ml of the suspension containing 80 juveniles was placed in each Petri dish.

Juveniles mortality was calculated by following formula:

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$$\frac{\text{No. of juveniles killed}}{\text{Total no. of juveniles}} X\ 100 = \%\ \text{juveniles mortality}$$

Juveniles were considered dead if they did not move when probed with a fine needle (Abbasi *et al.*, 2008) and were considered alive if they moved or appeared as a winding shape (El-Rokiek and El-Nagdi, 2011).

Mobility of juveniles of root knot nematode in soil amended with new chemistry insecticides

Among the Bio and synthetic chemicals which showed significant results under *in vitro* conditions were selected and their efficacy was evaluated for nematode mobility in soil. One thousand J2s of *M. incognita* were inoculated in each plastic pot (8.5 cm top dia.; 7.5 cm bottom dia.; 4.5 cm depth) of sterilized and normal soil. There were ten replication for each treatment and was placed under completely randomized design (CRD). Data were recorded after 24, 48 and 72 hours by following formula:

% decrease over control=C-T/C×100.

The data were subjected to analysis of variance (ANOVA) by Mstat version 1.3

Results

Assessment of nematicidal effect of new chemistry insecticideson mortality of M. incognita

Mortality of juveniles was increased as concentration of chemicals increased and this trend almost increased with time interval. All the thirteen chemicals of synthetic nature were statistically significant at different doses and time intervals. After 24 hours of incubation Cartap and priority at S, S/2 and S/4 caused maximum (85.67, 71.67, 63.00) and (76.33, 64.00, 53.00) mortality, respectively, and the

same concentration of Ulala and Momentum caused minimum (16.67, 13.33, 9.00) and (19.00, 14.00, 9.67) mortality respectively. All the treatments significantly varied from the control one at different time intervals (24, 48 and 72 hrs.) and doses. There were 7 bio chemicals were used, After 24 hours of incubation Nova Star and Proclaim at S, S/2 and S/4 caused (64.67, 60.67, 56.00) and (59.00, 53.00, 47.00) mortality, respectively, and the same concentration of Astra and Neemax caused (20.67, 20.00, 15.67) and (28.00, 25.33, 20.67) mortality respectively (Table 1,2). All the treatments significantly varied from each other at different time intervals (24, 48 and 72 hrs.) and doses.

Table 1. Assessment of nematicidal effect of new chemistry insecticides (synthetic) on mortality of *M. incognita*.

Time interval	24hrs			48hrs			72hrs		
Treatments	S	S/2	S/4	S	S/2	S/4	S	S/2	S/4
Rugby	94.67b	82.33gh	66.67opq	99.67 a	88.00de	74.33kl	99.67a	93.00bc	81.00ghi
Cartap	85.67ef	71.67lmn	63.00r	90.00cd	80.67ghi	69.00no	95.67b	78.67ij	65.33pqr
Priority	76.33jk	64.00pqr	53.00tu	83.00fg	70.67mn	65.00pqr	89.00d	79.67hi	6367qr
Actara	63.67qr	53.00tu	45.33zabc	73.00lm	64.67pqr	56.00st	79.67hi	67.000p	52.33uv
Minto	57.33s	48.00wxyz	38.00ghi	65.33pqr	53.67tu	46.33yzab	71.00mn	63.00r	50.67uvw
Kalex	43.67abcde	30.67nopq	28.67opqrs	53.33tu	44.00abcd	35.33ijk	64.67pqr	49.00wxy	37.67ghi
Drift	35.67ijk	28.00pqrst	20.67yzabc	46.67xyza	33.67klmn	27.00rstu	58.00s	49.67vwx	41.67def
Confidar	31.33lmno	26.00stuv	20.33zabc	43.00cde	37.00hij	30.67nopq	53.00tu	43.33bcde	35.00ijk
Pirate	27.67 qrst	22.00 xyzab	17.67 cdef	39.00 fgh	30.000pqr	23.67vwxy	49.00wxy	37.67ghi	31.00mnop
Barood	23.00 vwxyz	17.00 defg	13.33 ij	34.00 jklm	27.00 rstu	20.67 yzabc	40.67 efg	35.000 ijk	25.67 stuvw
Belt	21.00 yzab	16.33 efghi	11.00 jk	25.00 tuvwx	19.00 bcde	15.00 fghi	34.33 jkl	25.67 stuvw	19.33 bcde
Momentum	19.00 bcde	14.00 ghij	9.67 k	23.67 vwxy	19.67 abcd	17.00 defg	27.67 qrst	24.33 uvwx	20.33 zabc
Ulala	16.67 defgh	13.33 ij	9.00 k	19.00 bcde	13.67 hij	11.00 jk	22.67 wxyza	19.00 bcde	15.00 fghi

Table 2. Assessment of nematicidal effect of new chemistry insecticides (Bio) on mortality of *M. incognita*.

Time interval	24hrs			48hrs			72hrs		
Treatments	S	S/2	S/4	S	S/2	S/4	S	S/2	S/4
Nova Star	64.67 de	60.67 fgh	56.00 ijk	71.67 c	64.67 de	59.00 dhi	83.33 a	76.00 b	66.00 d
Proclaim	59.00 dhi	53.00 klm	47.00 nopq	66.33 d	61.00 efg	54.00 jkl	77.00 b	70.33 c	59.00 ghi
Match	53.67 jklm	50.00 mn	45.00 pqr	61.67 efg	61.33 efg	57.00 hij	70.67 c	64.33 def	56.67 ijk
Timer	47.33 nopq	41.67 rst	38.00 tuv	54.67 jk	50.33 lmn	49.00 no	59.00 ghi	53.00 klm	47.67 nop
Neemax	45.00 pqr	38.67 tuv	33.00 wx	47.00 nopq	43.00 rs	41.00 st	50.67 lmn	45.33 opqr	41.00 st
Astra	28.00 yz	25.33 z	20.67 a	36.33 uvw	33.67 wx	30.67 xy	43.67 qrs	40.67 st	39.00 tu

Assessment of nematicidal effect of new chemistry insecticides on egg hatching inhibition of M. incognita

Percent egg hatching inhibition was increased as concentration of chemicals increased and this trend almost increased with time interval. There were 20 new chemistry insecticides, which were evaluated at different concentrations and time intervals. All the new chemistry insecticides were statistically significant at different doses and time intervals. After 2 days of incubation, Cartap and priority at S, S/2 and S/4 caused (90.00, 84.00, 72.00) and (66.33, 60.67, 50.33) % egg hatching inhibition, respectively, and the same concentration of Ulala and Momentum caused (15.00, 11.00, 9.00)and (22.00, 24.67, 30.67) % egg hatching inhibition respectively. All the treatments significantly varied from the control at different time intervals (24, 48 and 72 hrs.) and doses.

There were 7 bio (new chemistry) chemicals were used, After 2 days of incubation Nova Star and Proclaim at S, S/2 and S/4 caused (57.67, 51, 46.67) and (49, 42.33, 35.67)% egg hatching inhibition, respectively (Table 3,4). All the treatments significantly varied from the control at different time

intervals (2, 4 and 6 days.) and doses.

Mobility of juveniles of root knot nematode in soil amended with new chemistry insecticides

There were 4 new chemistry insecticides evaluated against mobility of *M. incognita*, five replications were made for each insecticide, two bio and two synthetic new chemistry insecticides and last one was controlled. New chemistry insecticides which showed significant results in mortality and hatching experiments were evaluated against mobility of *M. incognita* on eggplant. Effect of New chemistry insecticides on mobility of juveniles (J2s) of *M. incognita* was observed after three days on number of J2s recovered, recovery percentage and % reduction over control. Reaction of all the treatments varied significantly on recovery of J2s after three days (Table 5). Percentage of reduction over control was observed in Cartap (19.28) and Nova Star (29.31).

Table 3. Assessment of nematicidal effect of new chemistry insecticides (Bio) on egg hatching inhibition of *M. incognita*.

Time interval	2 days			4 days			6 days		
Treatments	S	S/2	S/4	S	S/2	S/4	S	S/2	S/4
Nova Star	57.67 e	51 hi	46.67 jk	75.33 b	63.33 d	54 fg	86.33 a	74 b	62 d
Proclaim	49 ij	42.33 lm	35.67 pq	61 d	55.67 ef	46 k	74.67 b	67 c	57.33 e
Match	40.33 mn	33.33 qrs	27.33 uv	53.33 fgh	44.67 kl	35.67 pq	68.33 c	60.67 d	53 fgh
Timer	27.33	22.33 za	16 cd	36.33 op	28.67 tu	20.67 ab	54.33 fg	46 k	39 no
Neemax	32.33 rs	27 uvw	24.33 wxyz	44.33 kl	32.33 rs	24.67 vwxyz	51.67 ghi	43 lm	34 pqr
Astra	23.33 xyza	19.33 b	15.67 cd	36.67 op	32.33 rs	25 vwxyz	33 qrs	25.33 vwxy	18.33 bc

Discussion

In recent studies, twenty new chemistry insecticides were evaluated for their nematicidal effects against *M. incognita* on eggplants. All new chemistry insecticides showed significant results under *in-vitro* condition against *M. incognita* Juveniles egg mortality and % egg hatching at different doses and time intervals.

Table 4. Assessment of nematicidal effect of new chemistry insecticides (synthetic) on egg hatching inhibition of *M. incognita*.

Time interval	2 days			4 days			6 days		
Treatments	S	S/2	S/4	S	S/2	S/4	S	S/2	S/4
Rugby	99.67 a	98.00 ab	89.33 d	99.67 a	95.67 bc	87.67 de	99.67 a	98.67 ab	96.33 abc
Cartap	90.00 d	84.00 f	72.00 hi	94.00 c	90.33 d	84.67 ef	99.33 a	95.33 bc	89.67 d
Priority	66.33 klm	60.67 pq	50.33 vwxy	79.00 g	70.67 hij	63.00 mnop	87.33 def	80.33 g	72.67 h
Actara	57.00 rs	50.33 vwxy	45.00 abcd	69.00 ijk	61.33 nopq	54.00 stu	74.00 h	67.33 jkl	61.00 opq
Minto	53.67 stuv	46.33 zabc	37.67 hijkl	61.33 nopq	54.33 st	47.67 xyzab	69.00 ijk	60.67 pq	53.67 stuv
Kalex	48.67 wxyz	40.67 fghi	35.00 lm	56.33 rs	50.67 uvwx	45.67 zabc	64.67 lmn	57.00 rs	50.00 wxy
Drift	44.00 cdef	35.33 klm	27.00 rst	56.67 rs	52.00 tuvw	46.33 zabc	64.33 lmno	56.00 rs	48.67 wxyz
Confidar	41.33 efg	35.00 lm	27.33 qrs	54.67 st	48.33 xyza	41.67 defg	59.33 qr	49.00 wxyz	41.00 fgh
Pirate	35.33 klm	31.00 nop	23.67 tuvw	50.67 uvwx	44.67 bcde	36.67 jklm	56.67 rs	48.67 wxyz	37.33 ijkl
Barood	31.00 nop	25.00 stuv	19.00 yz	40.00 ghij	34.33 lmn	25.00 stuv	47.00 yzabc	37.67 hijkl	33.33 mno
Belt	26.33 rstu	19.00 yz	14.33 abc	38.67 ghijk	31.00 nop	24.67 stuvw	44.67 bcde	38.67 ghijk	27.67 pqrs
Momentum	22.00 vwxy	24.67 stuvw	30.67 opq	29.00 pqr	21.33 wxy	14.00 bc	37.33 ijkl	30.67 opq	23.67 tuvw
Ulala	15.00 ab	11.00 cd	9.00 d	23.33 uvw	19.33 xyz	14.00 bc	29.33 pqr	22.67 vwx	17.67 za

There were several researches revealed the nematicidal effects of different chemicals against the mortality, % egg hatching and egg hatching of root knot nematode. Nematicidal activity of some chemicals attributed due to their specific mechanism (Cayrol *et al.*, 1993; Safdar *et al.*, 2012) these findings are inline with our results. Rugby and Cartap caused maximum reduction in nematode population under *in*

vitro condition as they belong to organophosphate and carbamate group respectively. Nematodes locomotion depends upon motor neurons and interneuron's that use a neurotransmitter acetylcholine. Acetylcholinesterase a critical enzyme in nervous system of nematodes, these chemicals caused inactivation of this enzyme so the locomotion of nematode retarded by this mechanism (Johnson and Stretton, 1987). Our results are in accordance with the findings of (Abbas el at., 2015), that both the synthetic and bio nature of new chemistry insecticides have potential on mortality and hatching inhibition of *M. incognita*.

Another synthetic chemical priorityactive ingredient pyriproxafen, as belongs to juvenile hormone mimics group which caused significant inhibition in hatching, % egg hatching and mortality of *M. incognita* and applied in pre-metamorphic instar, these compounds disrupt and prevent metamorphosis.

Table 5. Mobility of juveniles of root knot nematode in soil amended with new chemistry insecticides.

Treatments	No. of J2 recovered (After 3 days)	Recovery (%)
Cartap	93.68	19.28
Priority	110.62	22.12
Nova Star	146.54	29.31
Proclaim	156.56	31.26
Control	250.44	50.098

This group of chemicals caused hormonal imbalance in nematodes at juvenile stage. Target protein responsible for biological activity is unknown or uncharacterized (IRAC). Bio chemicals also reduced nematode population by increasing mortality and inhibition percentages. Nova Star contains Abamectin and Bifenthrin as active ingredient, it was the most successful chemical in reducing nematode population. Its nematicidal potential was due to the blockage of electrical activity in nerve and muscle cells. As it belongs to avermectins that also have a role in human health and crop protection. As abamectin belongs to Glutamate-gated chloride channel (GluCI) allosteric modulators which affects the nerve and muscle tissues and in severe cases caused paralysis. Glutamate is an important inhibitory neurotransmitter in insects. Bifenthrin belongs to pyrethroids group, member of this group act as sodium channel modulators. Members of this group keep sodium channel open, causing hyper excitation and, in some cases, nerve block. Sodium channel are involved in the propagation of action potentials along nerve axons. Another bio chemical proclaim caused maximum increase in mortality and decrease in hatching of *M. incognita* as compared to other chemicals (Dybas*et al.*, 1989).After treating the juveniles with standard concentrations of new chemistry insecticides, % egg hatching of juveniles was observed.

It is possible that if the static (paralyzed) juveniles were not returned to water they might be mistaken as dead or the time of the observation for their revival was not long enough. Another explanation for the high mortality might be that juveniles died from lack of oxygen if the levels of oxygen were low in the amended dishes (Khurma and Singh, 1997). However, this should have been avoided by the use of small volumes of extract in containers with large surface to air contact.

It may be summarized from present findings that new chemistry insecticides have nematicidal potential against *M. incognita*.

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