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Exploitation of the Nematicidal Potential of Bio- and Synthetic Chemicals Against *Meloidogyne incognita* and Their Impact on Phytotoxicity and Nematode Reproduction

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Abstract.- Present investigation was conducted to exploit the nematicidal potential of bio and synthetic chemicals against *Meloidogyne incognita* (Kofoid and White) on tomato. Effect of twenty chemicals currently available in market was evaluated against *M. incognita.* Hatching inhibition and juvenile's mortality of *M. incognita* was assessed under *in vitro* conditions. Four concentrations of each chemical were prepared viz., 2S, S, S/2, S/4 according to recommended dose of each chemical. Data on hatching inhibition was recorded after 2, 4 and 6 days and on mortality after 12, 24, 48 and 72 h. Maximum hatching inhibition and mortality percentage was recorded in synthetic [Cartap (Thiocarbamate), Virtako (Thiamethoxam + chlorantraniliprole)] and bio [Cure (Abamectin), Azadirachtin (Aza)] chemicals. These four chemicals were selected and evaluated further against mobility of juveniles and for their phytotoxic effect on tomato. Minimum number of J2's were recovered in Cartap (95.67) followed by other chemicals while maximum were recovered in control (238.10). Tomato plants were examined for following symptoms yellowing or browing, wilting, necrosis and plant mortality after two months, none of the chemical was found to be phytotoxic. Efficiency of selected chemicals was evaluated at different time intervals (7, 14 and 28 days) against *M. incognita* on nematode reproduction parameters. A gradual decline was noted in the effectiveness of chemicals with the increase in time interval. Galling index was increased in all the chemicals after 28 days interval as compared to 7 and 14 days. The results of present investigation suggest suitable chemicals for grower having nematode problem in field to incorporate it in management strategies.

Key words: Nematicidal, hatching, mortality, phytotoxic, management.

INTRODUCTION

Management of *Meloidogyne incognita* is difficult due to its wide host range including more than 3,000 plant species (Abad *et al.*, 2003). Root-knot nematodes cause severe losses in vegetables throughout the world. Yield losses upto 24% due to *M. incognita* and *M. javanica* (Treub) 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). Population density of *M. incognita* was reported at higher level on tomato (Kamran *et al*., 2013). 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

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widely used such as carbofuran, aldicarb, fenamiphos, fosthiazate cadusafos, oxamyl, ethoprop and organophosphate based nematicides.

The effectiveness of various chemicals for the management of root-knot nematodes was evaluated on sunflower cultivars. The results revealed that out of five nematicides, Rugby-10 G (cadusaphos) was most effective followed by Unihypo-3.6 G and Furadan-3 G (carbofuran) (Rehman *et al.*, 2006). Bhosle *et al.* (2012) revealed that application of carbofuran and phorates in granular form were relatively effective in minimizing the root-knot nematode population and in increasing the yield of okra. Nematicides based on micro-organisms are referred as biopesticides; they have the potential to reduce the nematode population in the soil (Arora *et al.*, 2000) and can be successfully used in integrated disease management. The role of abamectin for the management of root-knot nematode on cotton as a seed treatment was studied. The findings revealed that final nematode population density was reduced due to the treatment of abamectin (Monfort *et al.* 2006). 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. In Pakistan, true nematicides are not available in public domain; therefore present study was conducted to exploit nematicidal potential of available bio and synthetic chemicals against *M. incognita*.

MATERIALS AND METHODS

Collection of diseased plants

Tomato 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.

Identification

Perineal patterns of mature females were prepared for different root-knot nematode species (Jepson, 1987). At least 10 perineal patterns were examined to make the identification.

Mass culturing of root knot nematodes

The sterilization of sandy loam soil was done in oven at 120°C for 20 min (Talavera and Mizukubo, 2003) and then it was stored for two weeks at 25°C before using them for experimental purpose. Seeds of tomato (*Lycopersicon esculentum)* '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 diam.). 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.

Evaluation of inhibitory effects of bio and synthetic chemicals on 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±2°C in a completely randomized design. Data was recorded after 2, 4 and 6 days of incubation.

Percent egg hatching was calculated and corrected by Abbott's formula (Abbott, 1925):

$$\frac{t-c}{100-c} X\ 100 = \textbf{Hatching inhibiton (\%)}$$

where t, percent hatching inhibition in the chemical

(bio/synthetic); c, percent hatching inhibition in the control.

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.

Evaluation of nemastatic/nematicidal effects of bio- and synthetic chemicals 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 and corrected by Abbott's formula (Abbott, 1925):

$$\frac{t-c}{100-c} X\ 100 = \textbf{Mortality}\ (\%)$$

where t, percent mortality in the chemical (bio/synthetic); c, percent mortality in the control.

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).

Effect of bio and synthetic chemicals on mobility of juveniles of M. incognita *and their impact on phytotoxicity*

Among the bio- and synthetic chemicals which showed significant results in hatching and mortality experiments were selected and their efficacy was evaluated against nematode mobility in soil. Five hundred J_2's of *M. incognita* were inoculated in each plastic pot (8.5 cm top diam.; 7.5 cm bottom diam.; 4.5 cm depth) of sterilized soil. Each treatment was replicated fifteen times and placed under completely randomized design (CRD). Data was recorded after three days on the basis of number of juveniles recovered, recovery percentage and % reduction over control.

For phytotoxic effect three week old seedlings of tomato cv. Moneymaker were dipped in selected chemicals for 20-30 min while the seedlings dipped in water served as control. Then the seedlings were transplanted in earthen pots (10 cm diam.). Data on phytotoxicity was recorded on the basis of yellowing or browning, wilting, necrosis, burning and plant mortality after two months.

Efficiency of bio and synthetic chemicals against M. incognita *at different time intervals*

Efficiency of selected bio and synthetic chemicals was evaluated at different time intervals against *M. incognita*. Three weeks old seedlings of tomato cv. Moneymaker were transplanted in earthen pots of (10 cm dia.) containing amended soil with bio and synthetic chemicals. At different time intervals of 7, 14 and 28 days 750 J_2's of *M. incognita* were inoculated in each pot. Three sets of treatments with fifteen replications were placed under CRD. Data was recorded after 35 days on visual estimation of root knot nematode galling index on root system of tomato by using galling index 0-10 scale (Bridge and Page, 1980), number of egg masses were counted by staining them with phloxine B (Holbrook *et al.*, 1983), number of females/root system were recorded by staining in boiling 0.1% acid fuchsin solution (McBeth *et al.*, 1941) for 1 min.

RESULTS

Inhibitory effects of bio- and synthetic chemicals on hatching

Inhibitory effect of twenty bio and synthetic chemicals was evaluated against percent hatching inhibition of *M. incognita.* The results revealed that all the treatments varied significantly in their potential towards *M. incognita*. Hatching of *M. incognita* was significantly varied in synthetic chemicals (Table I). Among the twelve chemicals Rugby caused maximum percent hatching inhibition followed by Cartap and Virtako as compared to other chemicals after 2 days. Percent (%) hatching inhibition in each chemical was affected by concentrations. Maximum inhibition was observed in 2S and S concentrations of all the chemicals while minimum was recorded in S/4 concentration. After 4 days% hatching inhibition was higher in Rugby, Cartap and Virtako while lower in Silk and

Table I.- Evaluation of synthetic chemicals on % hatching inhibition of *Meloidogyne incognita* after 2, 4 and 6 days.

Treatments	% Hatching inhibition at different concentrations [a]				Mean [b]
	2S	S	S/2	S/4	
After 2 days					
Rugby	100.00 ± 0.00a[d]	100.00 ± 0.00a	96.20 ± 0.58b	85.00 ± 0.55d	95.30 ± 1.42A
Regent	30.40 ± 0.51opq	27.40 ± 0.40rs	20.80 ± 0.37tu	17.20 ± 0.37wx	23.95 ± 1.21G
Movento	31.00 ± 0.32op	28.00 ± 0.32qr	18.80 ± 0.37uvw	16.00 ± 0.32xy	23.45 ± 1.44G
Alance	37.00 ± 0.71m	34.20 ± 0.37n	25.20 ± 0.49s	20.40 ± 0.51tuv	29.20 ± 1.56F
Coredor	46.60 ± 0.51k	41.00 ± 0.45l	32.80 ± 0.37no	28.40 ± 0.68qr	37.20 ± 1.64E
Cartap	100.00 ± 0.00a	93.00 ± 0.32c	80.20 ± 0.58e	74.20 ± 0.37f	86.85 ± 2.34B
Arrivo	58.00 ± 0.45i	51.40 ± 0.51j	43.40 ± 0.51l	38.20 ± 0.37m	47.75 ± 1.75D
Virtako	70.60 ± 0.60g	60.80 ± 0.37h	48.20 ± 0.37k	42.00 ± 0.45l	55.40 ± 2.55C
Steward	30.00 ± 0.32pq	27.20 ± 0.37rs	20.40 ± 0.51tuv	17.20 ± 0.37wx	23.70 ± 1.19G
Silk	14.40 ± 0.51y	10.60 ± 0.40zz1	5.00 ± 0.32z2	3.00 ± 0.32z2	8.25 ± 1.05I
Vimax	22.20 ± 0.37t	18.00 ± 0.32vwx	11.20 ± 0.37z	8.20 ± 0.37z1	14.90 ± 1.28H
Actara	59.00 ± 0.32hi	52.40 ± 0.51j	41.60 ± 0.40l	37.40 ± 0.51m	47.60 ± 1.97D
Mean [c]	49.93 ± 3.55A	45.33 ± 3.49B	36.98 ± 3.42C	32.27 ± 3.15D	
After 4 days					
Rugby	100.00 ± 0.00a[d]	100.00 ± 0.00a	100.00 ± 0.00a	90.60 ± 0.68b	97.65 ± 0.95A
Regent	44.20 ± 0.37n	39.20 ± 0.37opq	35.00 ± 0.32rs	27.80 ± 0.58wx	36.55 ± 1.39I
Movento	46.40 ± 0.51mn	41.20 ± 0.37o	34.20 ± 0.37st	29.00 ± 0.32vw	37.70 ± 1.53H
Alance	54.80 ± 0.66hi	49.40 ± 0.51kl	40.40 ± 0.24op	37.00 ± 0.32qr	45.40 ± 1.64G
Coredor	62.00 ± 0.32fg	57.40 ± 0.51h	46.20 ± 0.58mn	38.40 ± 0.51pq	51.00 ± 2.14F
Cartap	100.00 ± 0.00a	97.80 ± 0.37a	89.00 ± 0.71b	81.40 ± 0.51c	92.05 ± 1.71B
Arrivo	70.40 ± 0.51e	64.00 ± 0.45f	56.00 ± 0.55h	49.20 ± 0.37l	59.90 ± 1.85D
Virtako	81.00 ± 0.45c	74.80 ± 0.58d	60.40 ± 0.51g	52.40 ± 0.51ij	67.15 ± 2.61C
Steward	44.80 ± 0.58mn	37.20 ± 0.37qr	33.00 ± 0.45stu	25.40 ± 0.51xy	35.10 ± 1.63J
Silk	30.60 ± 0.40uv	24.80 ± 0.37y	19.00 ± 0.32zz1	14.20 ± 0.37z2	22.15 ± 1.42L
Vimax	37.00 ± 0.45qr	32.20 ± 0.37tu	21.40 ± 0.51z	18.20 ± 0.37z1	27.20 ± 1.77K
Actara	71.20 ± 0.58e	63.00 ± 0.71fg	52.00 ± 0.71jk	47.40 ± 0.51lm	58.40 ± 2.16E
Mean [c]	61.87 ± 2.89A	56.75 ± 3.05B	48.88 ± 3.10C	42.58 ± 2.94D	
After 6 days					
Rugby	100.00 ± 0.00a[d]	100.00 ± 0.00a	100.00 ± 0.00a	95.40 ± 0.51b	98.85 ± 0.47A
Regent	48.60 ± 0.40lm	42.40 ± 0.51pqr	38.00 ± 0.32tu	30.20 ± 0.58w	39.80 ± 1.55I
Movento	50.60 ± 0.51kl	44.40 ± 0.51op	39.00 ± 0.45st	33.20 ± 0.37v	41.80 ± 1.49H
Alance	60.40 ± 0.51h	56.20 ± 0.37i	43.40 ± 0.51opq	39.20 ± 0.37st	49.80 ± 2.02G
Coredor	68.40 ± 0.51f	61.20 ± 0.37h	52.40 ± 0.51jk	45.20 ± 0.37no	56.80 ± 2.02F
Cartap	100.00 ± 0.00a	100.00 ± 0.00a	93.40 ± 0.51b	87.00 ± 0.55c	95.10 ± 1.25B
Arrivo	76.40 ± 0.51d	67.20 ± 0.66f	59.40 ± 0.68h	52.60 ± 0.81jk	63.90 ± 2.06E
Virtako	95.20 ± 0.37b	88.00 ± 0.71c	71.40 ± 0.51e	64.00 ± 0.55g	79.65 ± 2.88C
Steward	47.40 ± 0.51mn	41.00 ± 0.55qrs	35.80 ± 0.37uv	29.00 ± 0.32w	38.30 ± 1.56J
Silk	35.40 ± 0.51uv	28.00 ± 0.32w	25.00 ± 0.32x	20.40 ± 0.51y	27.20 ± 1.27L
Vimax	40.40 ± 0.51rst	36.00 ± 0.55u	29.40 ± 0.51w	23.20 ± 0.58x	32.25 ± 1.52K
Actara	78.80 ± 0.37d	69.20 ± 0.37ef	60.40 ± 0.51h	54.60 ± 0.51ij	65.75 ± 2.11D
Mean [c]	66.80 ± 2.90A	61.13 ± 3.06B	53.97 ± 3.01C	47.83 ± 3.02D	

Values (± SE) are mean of five replicates.

[a] Individual mean % hatching inhibition at different concentrations.

[b] Overall mean % hatching inhibition after 4 days.

[c] Overall mean % hatching inhibition at different concentrations.

[d] Means sharing similar letter in a row or in a column are statistically non-significant (*P>0.05*) according to Tukey Test. Small letters represent comparison among interaction means and capital letters are used for overall mean.

Table II.- Evaluation of biochemicals on % hatching inhibition of *Meloidogyne incognita* after 2, 4 and 6 days.

Treatments	% Hatching inhibition at different concentrations [a]				Mean [b]
	2S	S	S/2	S/4	
After 2 days					
Azadirachtin	53.20 ± 0.66b[d]	45.80 ± 0.66c	35.40 ± 0.51fg	31.00 ± 0.71hi	41.35 ± 2.02B
Neemix	26.20 ± 0.49k	22.40 ± 0.51mn	18.40 ± 0.51p	15.20 ± 0.49q	20.55 ± 0.98E
Neemakill	42.00 ± 0.32d	37.80 ± 0.49ef	30.60 ± 0.40hi	27.00 ± 0.71jk	34.35 ± 1.37C
Astra	14.20 ± 0.37q	10.80 ± 0.37r	5.20 ± 0.37s	3.40 ± 0.51s	8.40 ± 1.01F
Cure	62.40 ± 0.93a	55.00 ± 0.55b	42.20 ± 0.37d	36.60 ± 0.51ef	49.05 ± 2.36A
Spintor	25.00 ± 0.32klm	23.20 ± 0.37lmn	19.40 ± 0.51op	14.60 ± 0.68q	20.55 ± 0.94E
Radiant	33.20 ± 0.49gh	29.20 ± 0.37ij	21.60 ± 0.40no	18.20 ± 0.66p	25.55 ± 1.38D
Timer	43.00 ± 0.55cd	39.00 ± 0.55e	30.40 ± 0.51hi	25.80 ± 0.66kl	34.55 ± 1.58C
Mean [c]	37.40 ± 2.38A	32.90 ± 2.14B	25.40 ± 1.74C	21.48 ± 1.61D	
After 4 days					
Azadirachtin	70.60 ± 0.51b[d]	64.40 ± 0.51c	52.60 ± 0.68ef	45.60 ± 0.40g	58.30 ± 2.26B
Neemix	43.40 ± 0.51gh	36.00 ± 0.55i	27.20 ± 0.49jkl	21.80 ± 0.58m	32.10 ± 1.91E
Neemakill	60.40 ± 0.51d	53.00 ± 0.71ef	43.20 ± 0.58gh	36.40 ± 0.51i	48.25 ± 2.12C
Astra	29.20 ± 0.58jk	24.40 ± 0.51lm	15.40 ± 0.51n	11.80 ± 0.37o	20.20 ± 1.61F
Cure	80.20 ± 0.86a	73.40 ± 0.51b	61.20 ± 0.58d	55.40 ± 0.51e	67.55 ± 2.26A
Spintor	42.20 ± 0.58h	36.80 ± 0.80i	26.40 ± 0.51kl	23.00 ± 0.55m	32.10 ± 1.80E
Radiant	52.20 ± 0.66f	45.40 ± 0.51g	34.00 ± 0.71i	29.60 ± 0.51j	40.30 ± 2.08D
Timer	61.40 ± 0.93cd	55.20 ± 0.37ef	43.40 ± 0.51gh	37.00 ± 0.71i	49.25 ± 2.22C
Mean [c]	54.95 ± 2.49A	48.58 ± 2.42B	37.93 ± 2.26C	32.58 ± 2.10D	
After 6 days					
Azadirachtin	84.40 ± 0.75b[d]	77.80 ± 0.86c	66.00 ± 0.71e	58.00 ± 0.55gh	71.55 ± 2.37B
Neemix	60.40 ± 0.68fg	54.60 ± 0.51ij	44.80 ± 0.66l	39.40 ± 0.51m	49.80 ± 1.90F
Neemakill	74.20 ± 0.66d	68.20 ± 0.49e	55.80 ± 0.37hi	50.40 ± 0.51k	62.15 ± 2.19D
Astra	33.00 ± 0.71n	28.60 ± 0.60o	21.40 ± 0.51p	19.40 ± 0.51p	25.60 ± 1.28G
Cure	90.00 ± 0.63a	83.40 ± 0.51b	74.20 ± 0.66d	68.40 ± 0.51e	79.00 ± 1.92A
Spintor	59.20 ± 0.58fg	54.20 ± 0.66ij	45.40 ± 0.51l	38.00 ± 0.71m	49.20 ± 1.89F
Radiant	69.00 ± 0.71e	62.20 ± 0.66f	50.80 ± 0.37k	46.00 ± 0.55l	57.00 ± 2.10E
Timer	75.20 ± 0.66cd	68.60 ± 0.51e	59.20 ± 0.66fg	52.20 ± 0.66jk	63.80 ± 2.04C
Mean [c]	68.18 ± 2.67A	62.20 ± 2.55B	52.20 ± 2.40C	46.48 ± 2.21D	

Values (± SE) are mean of five replicates.
[a] Individual mean % hatching inhibition at different concentrations.
[b] Overall mean % hatching inhibition after 6 days.
[c] Overall mean % hatching inhibition at different concentrations.
[d] Means sharing similar letter in a row or in a column are statistically non-significant (*P>0.05*) according to Tukey Test. Small letters represent comparison among interaction means and capital letters are used for overall mean.

Vimax (Table I). Rugby at its all concentration caused (98.85) % hatching inhibition while Cartap and Virtako caused (95.10, 79.65) respectively after 6 days of incubation (Table I). Regression analysis showed linear relationship between % hatching inhibition and concentrations of synthetic chemicals. The relationship showed as the concentration lowered from 2S to S/4, a significant decreased in % hatching inhibition was observed. Time duration also affected hatching inhibition percentage, as the time duration increases, a significant increase in % hatching inhibition was recorded. The relationship between Cartap, Virtako and % hatching inhibition and was observed through regression analysis (Fig.1) respectively. Effect of biochemicals on % hatching inhibition of *M. incognita* was also evaluated. Results revealed that % hatching inhibition was significantly varied in all the treatments (Table II). Cure and Azadirachtin caused higher % hatching inhibition as compared to other bio chemicals after 2 days. After 4 days of incubation Cure and Azadirachtin caused (67.55%,

Table III.- Evaluation of synthetic chemicals on % mortality of *Meloidogyne incognita* juveniles after 24, 48 and 72 h.

Treatments	% Juvenile mortality at different concentrations [a]				Mean [b]
	2S	S	S/2	S/4	
After 24 h					
Rugby	100.00 ± 0.00a[d]	100.00 ± 0.00a	72.26 ± 0.55e	57.58 ± 0.78i	82.46 ± 4.20A
Regent	30.85 ± 0.37pqr	30.65 ± 0.58qr	26.83 ± 0.37st	20.80 ± 0.74vwx	27.28 ± 0.97G
Movento	33.07 ± 0.45nopq	30.04 ± 0.69qr	23.41 ± 0.67uv	17.78 ± 0.50xyz	26.08 ± 1.39G
Alance	44.12 ± 0.53k	37.69 ± 0.49lm	32.46 ± 0.51opqr	23.82 ± 0.49tuv	34.52 ± 1.72F
Coredor	52.76 ± 0.43j	46.53 ± 0.58k	33.87 ± 0.36nop	26.83 ± 0.48st	40.00 ± 2.35E
Cartap	90.15 ± 0.68b	81.50 ± 0.54c	63.41 ± 0.53h	50.15 ± 0.58j	71.30 ± 3.58B
Arrivo	65.23 ± 0.51gh	56.58 ± 0.44i	43.92 ± 0.63k	32.06 ± 0.70opqr	49.45 ± 2.90D
Virtako	75.87 ± 0.78d	70.46 ± 0.32ef	45.53 ± 0.74k	34.47 ± 0.26no	56.58 ± 3.94C
Steward	29.65 ± 0.75rs	22.62 ± 0.25uvw	16.38 ± 0.61z	11.76 ± 0.75z1	20.10 ± 1.57H
Silk	22.21 ± 0.49uvw	19.79 ± 0.50wxy	6.12 ± 0.70z2z3	3.71 ± 0.53z3	12.96 ± 1.88J
Vimax	24.82 ± 0.21tu	16.78 ± 0.50yz	11.36 ± 0.51z1	7.74 ± 0.39z2	15.18 ± 1.49I
Actara	67.84 ± 0.53fg	56.78 ± 0.61i	40.70 ± 0.40l	35.88 ± 0.58mn	50.30 ± 2.93D
Mean [c]	53.05 ± 3.31A	47.45 ± 3.28B	34.69 ± 2.49C	26.88 ± 2.03D	
After 48 h					
Rugby	100.00 ± 0.00a[d]	100.00 ± 0.00a	77.72 ± 0.38e	65.36 ± 0.76gh	85.77 ± 3.42A
Regent	35.79 ± 0.71q	32.34 ± 0.63r	27.28 ± 0.65st	24.05 ± 0.44uv	29.87 ± 1.07G
Movento	36.40 ± 0.54q	31.54 ± 0.47r	25.87 ± 0.20tu	17.56 ± 0.41w	27.84 ± 1.62H
Alance	56.87 ± 0.36k	46.53 ± 0.51mn	40.66 ± 0.67p	24.86 ± 0.37tuv	42.23 ± 2.67F
Coredor	65.97 ± 0.43gh	54.83 ± 0.44k	40.45 ± 0.35p	29.92 ± 0.55rs	47.79 ± 3.16E
Cartap	95.75 ± 0.37b	87.04 ± 0.36c	75.49 ± 0.39e	66.78 ± 0.45g	81.26 ± 2.54B
Arrivo	77.31 ± 0.60e	61.52 ± 0.66ij	48.96 ± 0.55lm	40.85 ± 0.69p	57.16 ± 3.17D
Virtako	83.18 ± 0.56d	75.89 ± 0.46e	60.70 ± 0.47j	42.48 ± 0.35op	65.57 ± 3.59C
Steward	40.86 ± 0.34p	25.26 ± 0.58tuv	16.14 ± 1.00w	10.88 ± 0.48xy	23.28 ± 2.63I
Silk	30.13 ± 0.32rs	22.43 ± 0.51v	10.47 ± 0.63xy	8.05 ± 0.52y	17.77 ± 2.07K
Vimax	30.33 ± 0.21r	26.47 ± 0.86tu	16.76 ± 0.20w	13.11 ± 0.59x	21.67 ± 1.62J
Actara	71.65 ± 0.28f	63.74 ± 0.68hi	49.56 ± 0.50l	45.31 ± 0.63no	57.56 ± 2.45D
Mean [c]	60.35 ± 3.19A	52.30 ± 3.24B	40.84 ± 2.82C	32.43 ± 2.49D	
After 72 h					
Rugby	100.00 ± 0.00a[d]	100.00 ± 0.00a	92.17 ± 0.76b	79.59 ± 0.37c	92.94 ± 1.92A
Regent	38.14 ± 0.58op	33.20 ± 0.37q	28.66 ± 0.51s	22.68 ± 0.52t	30.67 ± 1.33I
Movento	44.12 ± 0.47n	37.10 ± 0.65p	29.49 ± 0.40rs	18.56 ± 0.41uv	32.32 ± 2.19H
Alance	67.42 ± 0.51fg	49.48 ± 0.49kl	45.15 ± 0.47mn	30.72 ± 0.68qrs	48.19 ± 3.01G
Coredor	69.49 ± 0.49ef	61.24 ± 0.35i	47.62 ± 0.45lm	30.72 ± 0.40qrs	52.27 ± 3.38F
Cartap	100.00 ± 0.00a	92.58 ± 0.38b	80.62 ± 0.33c	75.87 ± 0.46d	87.27 ± 2.20B
Arrivo	80.62 ± 0.37c	70.52 ± 0.36e	63.10 ± 0.29hi	46.19 ± 0.28mn	65.11 ± 2.89D
Virtako	90.11 ± 0.51b	80.62 ± 0.59c	69.48 ± 0.26ef	56.90 ± 0.50j	74.28 ± 2.85C
Steward	45.36 ± 0.49mn	30.72 ± 0.40qrs	22.68 ± 0.41t	16.08 ± 0.21vw	28.71 ± 2.51J
Silk	31.74 ± 0.74qr	23.09 ± 0.72t	15.26 ± 0.25w	7.63 ± 0.04y	19.43 ± 2.07L
Vimax	40.20 ± 0.50o	32.36 ± 0.84q	19.18 ± 0.38u	11.95 ± 0.78x	25.92 ± 2.55K
Actara	76.49 ± 0.37d	65.57 ± 0.64gh	56.90 ± 0.44j	51.96 ± 0.70k	62.73 ± 2.15E
Mean [c]	65.31 ± 3.09A	56.37 ± 3.24B	47.53 ± 3.16C	37.40 ± 3.05D	

Values (± SE) are mean of five replicates.

[a] Individual mean mortality at different concentrations.

[b] Overall mean mortality after 72 h.

[c] Overall mean mortality at different concentrations.

[d] Means sharing similar letter in a row or in a column are statistically non-significant (*P>0.05*) according to Tukey Test. Small letters represent comparison among interaction means and capital letters are used for overall mean.

58.30%) hatching inhibition respectively at their all concentration (Table II). Maximum inhibition percentage was observed in Cure and Azadirachtin at their all concentration from other chemicals after 6 days of incubation (Table II). Regression analysis showed linear relationship between concentrations of bio chemicals and % hatching inhibition. Time duration also affected hatching inhibition percentage, as its higher value was reached after 6 days of incubation. Regression curves were also drawn between Cure, Azadirachtin and % hatching inhibition (Fig. 1).

Nemastatic/nematicidal effects of bio- and synthetic chemicals on mortality

Effect of all the chemicals varied significantly on % juveniles mortality of *M. incognita* (Table III). After 12 h of incubation Rugby caused maximum % juveniles mortality (71.00) at its 2S concentration followed by S, S/2 and S/4 concentrations. Juveniles mortality percentage was maximum in Rugby, Cartap and Virtako at their all concentrations after 24 h (Table III). In Rugby, Cartap and Virtako % mortality was (100.00, 95.75, 83.18) in 2S concentration while at S/4 concentration % mortality was (65.36, 66.78, 42.48) respectively (Table III) after 48 h. Mean mortality percentage was significantly higher in Rubgy (92.94) followed by Cartap (87.27) and Virtako (74.28) while lower in Silk (19.43) at their all concentrations after 72 h of incubation (Table III). Regression analysis between % juveniles mortality and concentrations of synthetic chemicals was showed in Figure 2. A linear relationship was observed in mortality and concentrations of chemicals after all time duration. Regression equations were also drawn for the chemicals which caused maximum mortality at their all concentrations after all time intervals. Mortality of *M. incognita* was significantly affected by bio chemicals (Table IV). Among the bio chemicals Cure caused maximum % juveniles mortality after 12 h of incubation followed by Azadirachtin. After 24 h of incubation concentration effect remained significant, as Cure caused 76.62% mortality at 2S followed by S (70.57), S/2 (57.89) and S/4 (48.41) concentration, respectively (Table IV). In Cure and Azadirachtin % mortality was increased after 48 h of incubation at their all concentrations (Table IV). After 72 h Cure and Azadirachtin caused maximum mean mortality (75.44, 68.91), respectively, from all other chemicals at their all concentration (Table IV). The relationship between % mortality and biochemicals was observed through regression analysis. Regression equations between Cure, Azadirachtin and % mortality showed as the concentration of chemicals increased, % mortality increased significantly with the increase in time duration. In regression analysis a progressive increase was noted in mortality with the increase in concentration (Fig. 2).

Effect of bio- and synthetic chemicals on mobility of juveniles and their impact on Phytotoxicity

Bio- and synthetic chemicals which showed significant results in mortality and hatching experiments were evaluated against mobility of *M. incognita* and for phytotoxic effect on tomato. Effect of bio- and synthetic chemicals 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 V). Minimum number of J2s were recovered in Cartap (95.67) followed by other chemicals while maximum were recovered in control (238.1). Maximum percentage of reduction over control was observed in Cartap (60) followed by Virtako (55), Cure (39) and Azadirachtin (34). To check phytotoxic effect, plants were examined for following symptoms yellowing or browing, wilting, necrosis, burning and plant mortality after two months. None of the chemical was found to be phytotoxic.

Efficiency of bio- and synthetic chemicals at different time intervals

Efficiency of selected bio- and synthetic chemicals was evaluated at different time intervals; 7, 14 and 28 days against *M. incognita*. Data on galling index, number of egg masses and number of females/root system was recorded after 35 days of inoculation. Among synthetic chemicals (Cartap, Virtako) galling index varied significantly while in bio chemicals (Cure and Azadirachtin) results were statistically non significant (Table VI). All the

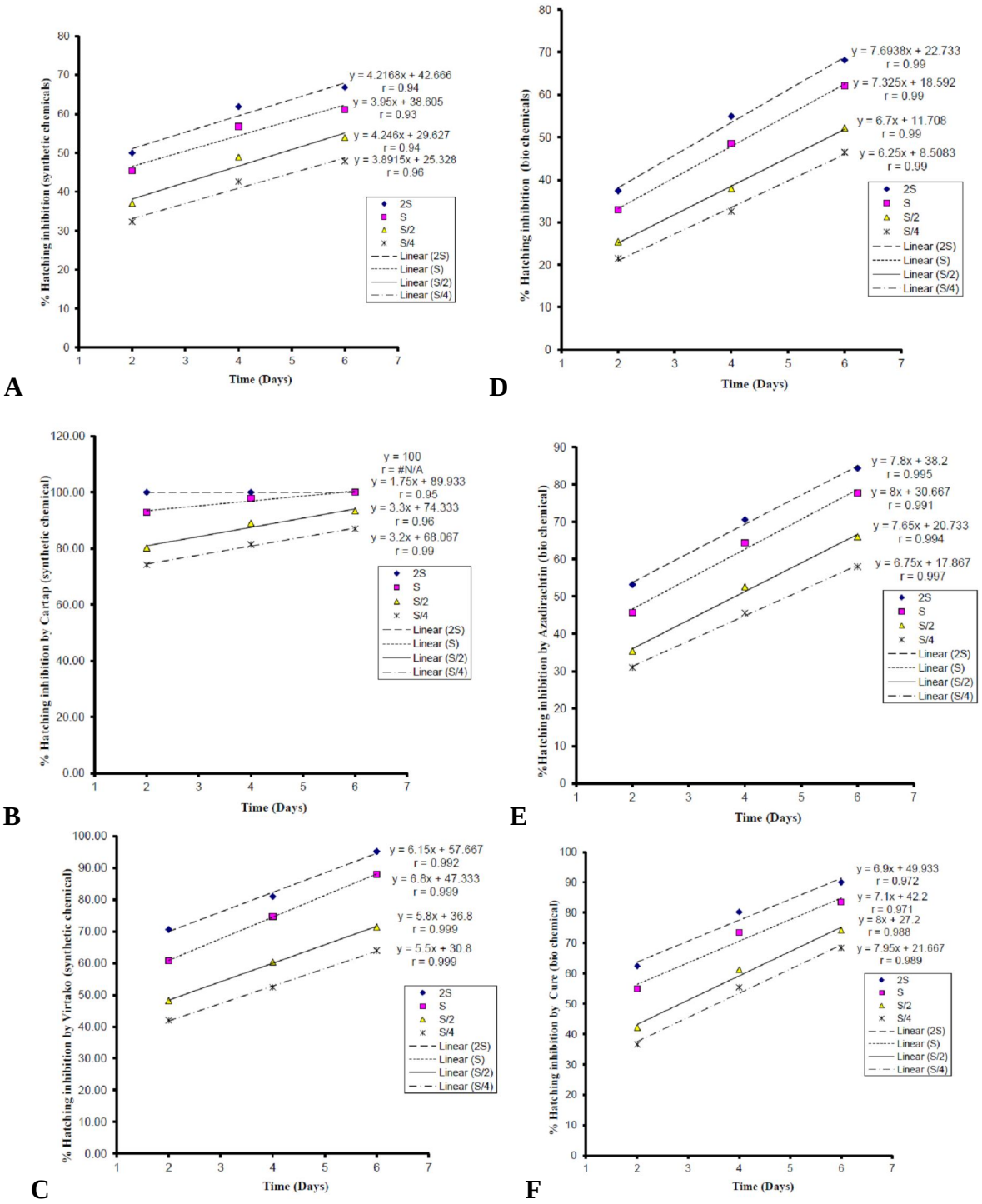

Fig. 1. Relationship between % hatching inhibition and time (days) by synthetic chemicals (A), Cartap (B), Virtako (C), biochemicals (D), Azadirachtin (E), and Cure (F), at four level of their concentrations.

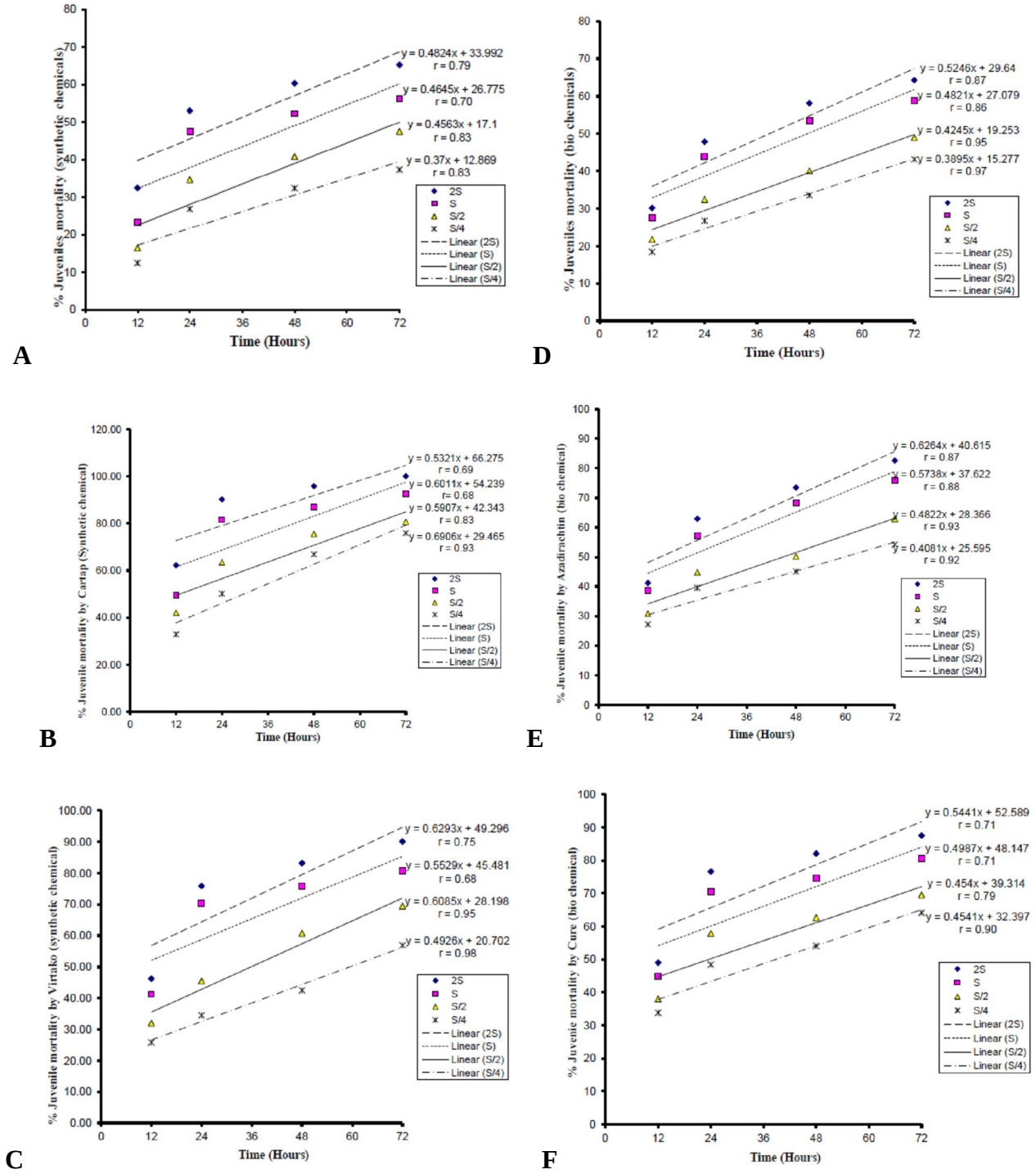

Fig. 2. Relationship between % juveniles mortality and time (hours) by synthetic chemicals (A), Cartap (B), Virtako (C), biochemicals (D), Azadirachtin (E) and Cure (F), at four level of their concentrations.

Table IV.- Evaluation of Bio chemicals on % mortality of *Meloidogyne incognita* juveniles after 12, 24, 48 and 72 h.

Treatments	% Juvenile mortality at different concentrations [a]				Mean [b]
	2S	S	S/2	S/4	
After 12 h					
Azadirachtin	41.20 ± 0.37c[d]	38.60 ± 0.24d	30.80 ± 0.37f	27.20 ± 0.37gh	34.45 ± 1.31B
Neemix	22.40 ± 0.24ijk	20.80 ± 0.58kl	15.80 ± 0.37m	12.20 ± 0.37n	17.80 ± 0.95E
Neemakill	33.80 ± 0.37e	31.40 ± 0.24f	26.40 ± 0.24h	23.20 ± 0.37ij	28.70 ± 0.96C
Astra	9.20 ± 0.37o	6.00 ± 0.45p	1.00 ± 0.32q	0.60 ± 0.24q	4.20 ± 0.84F
Cure	49.00 ± 0.32a	44.80 ± 0.37b	38.00 ± 0.32d	33.80 ± 0.37e	41.40 ± 1.36A
Spintor	21.60 ± 0.40jkl	20.40 ± 0.24l	15.60 ± 0.40m	10.80 ± 0.37no	17.10 ± 0.99E
Radiant	28.80 ± 0.37g	26.00 ± 0.32h	20.40 ± 0.51l	15.80 ± 0.37m	22.75 ± 1.17D
Timer	35.00 ± 0.32e	31.80 ± 0.37f	26.20 ± 0.37h	23.80 ± 0.37i	29.20 ± 1.03C
Mean [c]	30.13 ± 1.87A	27.48 ± 1.81B	21.78 ± 1.70C	18.43 ± 1.59D	
After 24 h					
Azadirachtin	62.93 ± 0.59c[d]	57.28 ± 0.57d	44.79 ± 0.38h	39.54 ± 0.37ij	51.13 ± 2.16B
Neemix	36.92 ± 0.57kl	32.49 ± 0.58mn	20.60 ± 0.40pq	17.17 ± 0.50rs	26.80 ± 1.89F
Neemakill	51.44 ± 0.36ef	48.41 ± 0.44g	37.73 ± 0.48jkl	30.88 ± 0.19mn	42.11 ± 1.90D
Astra	19.39 ± 0.51pqr	16.57 ± 0.41s	9.72 ± 0.61t	7.30 ± 0.43t	13.25 ± 1.15G
Cure	76.62 ± 0.40a	70.57 ± 0.45b	57.89 ± 0.29d	48.41 ± 0.36g	63.37 ± 2.52A
Spintor	35.92 ± 0.57l	33.30 ± 0.47m	21.82 ± 0.38p	18.58 ± 0.52qrs	27.40 ± 1.70F
Radiant	45.79 ± 0.46h	40.76 ± 0.27i	28.26 ± 0.56o	21.82 ± 0.38p	34.16 ± 2.20E
Timer	53.65 ± 0.25e	51.03 ± 0.55f	38.74 ± 0.47ijk	30.27 ± 0.48no	43.42 ± 2.18C
Mean [c]	47.83 ± 2.65A	43.80 ± 2.51B	32.44 ± 2.31C	26.75 ± 1.98D	
After 48 h					
Azadirachtin	73.46 ± 0.62b[d]	68.16 ± 0.36c	50.20 ± 0.38h	45.10 ± 0.38ijk	59.23 ± 2.73B
Neemix	46.94 ± 0.25i	42.85 ± 0.62k	30.81 ± 0.58o	25.30 ± 0.49p	36.48 ± 2.02F
Neemakill	65.10 ± 0.36de	60.40 ± 0.67f	43.68 ± 0.25jk	35.10 ± 0.57mn	51.07 ± 2.80D
Astra	24.69 ± 0.40p	20.81 ± 0.59q	16.52 ± 0.62r	13.06 ± 0.51s	18.77 ± 1.04G
Cure	82.04 ± 0.66a	74.49 ± 0.33b	62.66 ± 0.44ef	54.07 ± 0.67g	68.32 ± 2.48A
Spintor	46.12 ± 0.53ij	44.08 ± 0.38jk	30.41 ± 0.48o	23.67 ± 0.60p	36.07 ± 2.16F
Radiant	60.41 ± 0.59f	54.89 ± 0.59g	38.98 ± 0.39l	34.49 ± 0.23n	47.19 ± 2.48E
Timer	66.32 ± 0.64cd	61.42 ± 0.49f	47.14 ± 0.46i	37.55 ± 0.37lm	53.11 ± 2.63C
Mean [c]	58.13 ± 2.73A	53.39 ± 2.56B	40.05 ± 2.13C	33.54 ± 1.93D	
After 72 h					
Azadirachtin	82.60 ± 0.33b[d]	75.96 ± 0.37c	62.90 ± 0.35f	54.19 ± 0.39i	68.91 ± 2.54B
Neemix	57.51 ± 0.39gh	51.09 ± 0.30j	42.17 ± 0.54l	35.53 ± 0.60m	46.57 ± 1.94F
Neemakill	68.08 ± 0.46d	63.73 ± 0.37ef	56.26 ± 0.51hi	50.67 ± 0.50j	59.69 ± 1.55D
Astra	28.70 ± 0.52n	24.55 ± 0.60o	17.92 ± 0.33p	14.40 ± 0.42q	21.39 ± 1.30G
Cure	87.56 ± 0.47a	80.51 ± 0.42b	69.53 ± 0.36d	64.14 ± 0.62ef	75.44 ± 2.11A
Spintor	56.26 ± 0.51hi	50.68 ± 0.42j	41.76 ± 0.41l	34.50 ± 0.42m	45.80 ± 1.92F
Radiant	64.14 ± 0.62ef	58.75 ± 0.35g	45.07 ± 0.42k	40.30 ± 0.44l	52.07 ± 2.24E
Timer	69.53 ± 0.41d	65.60 ± 0.38e	56.06 ± 0.54hi	51.50 ± 0.44j	60.67 ± 1.67C
Mean [c]	64.30 ± 2.72A	58.86 ± 2.62B	48.96 ± 2.40C	43.16 ± 2.31D	

Values (± SE) are mean of five replicates.
[a] Individual mean mortality at different concentrations.
[b] Overall mean mortality after 72 h.
[c] Overall mean mortality at different concentrations.
[d] Means sharing similar letter in a row or in a column are statistically non-significant (*P>0.05*) according to Tukey Test. Small letters represent comparison among interaction means and capital letters are used for overall mean.

chemicals varied significantly (P=0.05) in their response towards number of egg masses on root system of tomato. Maximum number of egg masses were observed in control (222.5) treatment while minimum were recorded in Cartap (65.8) followed by other chemicals. A declining trend was observed

Table V.- Mobility of *Meloidogyne incognita* in soil amended with bio- and synthetic chemicals and their impact on phytotoxicity.

Treatments	No. of J2 recovered (after 3 days)	Recovery (%)	% reduction over control[2]	Phytotoxicity[3]
Cartap	95.67[1] e	19.13 e	60	Nil
Virtako	112.3 d	22.47 d	55	Nil
Abamectin	145.3 c	29.05 c	39	Nil
Azadirachtin	158.3 b	31.65 b	34	Nil
Control	238.1 a	47.63 a	-	Nil

[1] Means with in a column sharing the same letter are not significantly different from each other at *P = 0.05* according to Bartlett's test
[2] % decrease over control=C-T/Cx100
[3] recorded on the basis of yellowing or browning, wilting, necrosis, burning and plant mortality

Table VI.- Efficiency of bio- and synthetic chemicals in amended soil at different time intervals.

Treatments	After 7 days			After 14 days			After 28 days		
	Galling index	No. of egg masses	No. of females/root system	Galling index	No. of egg masses	No. of females/root system	Galling index	No. of egg masses	No. of females/root system
Cartap	2.2[1] d	65.8 e	77.4 e	2.80 d	87.4 e	105.4 e	4.00 d	132.5 e	163.5 e
Virtako	2.7 c	80.6 d	98.6 d	3.46 c	110.5 d	139.1 d	4.20 cd	158.5 d	184.5 d
Abamectin	3.2 b	92.5 c	107.5 c	3.73 c	132.2 c	160.7 c	4.40 bc	175.3 c	196.6 c
Azadirachtin	3.5 b	105.6 b	128.3 b	4.13 b	155.4 b	176.8 b	4.60 b	192.4 b	220.7 b
Control	5.4 a	222.5 a	237.6 a	5.20 a	216.5 a	235.5 a	5.53 a	225.6 a	243.8 a
LSD	0.36	0.42	0.36	0.31	0.35	0.44	0.31	0.36	0.39

[1]Means with in a column sharing the same letter are not significantly different from each other at *P = 0.05* according to Bartlett's test

in the efficiency of bio and synthetic chemicals in terms of galling index, number of egg masses and number of females/root system after 14 days. In case of Cartap and Virtako minimum number of females (105.4, 139.1) were recorded as compared to Azadirachtin and Cure (176.8, 160.7) respectively. Galling index was increased in all the chemicals after 28 days interval as compared to 7 and 14 days. In Cartap number of egg masses was observed (132.5) after 28 days while these were (65.8, 87.4) after 7 and 14 days respectively, similar behavior was observed in all other treatments containing chemicals (Table VI).

DISCUSSION

In present investigation, nematicidal potential of various bio- and synthetic chemicals was evaluated against *M. incognita*. All the chemicals under *in vitro* studies showed different levels of hatching inhibition percentage and J2s mortality after different time duration. Several researchers reported about nematicidal potential of chemicals (Cayrol *et al*., 1993; Safdar *et al*., 2012) on hatching inhibition and J_2 mortality. Nematicidal activity of different chemicals was attributed due to different mechanisms. Rugby and Cartap as belongs to organophosphate and carbamate group respectively both caused maximum reduction in nematode population under *in vitro* studies. Their nematicidal activity was due to the inactivation of acetylcholinesterase which is critical enzyme in nervous system of nematodes as nematode locomotion depends upon motor neurons and interneurons that use a neurotransmitter acetylcholine whose activity is stopped acetylcholinesterase (Johnson and Stretton, 1980, 1987). Another synthetic chemical Virtako caused significant inhibition in hatching of *M. incognita*, its nematicidal activity attributed to thiamethoxam and chlorantraniliprole. Thiamethoxam caused reduction due to contact and by binding to acetylcholine

receptor site, damaging the nervous system, ultimately paralysis and death (Yamamoto, 1999). Chlorantraniliprole belongs to anthranilic diamides having a different mode of action by liberating and exhaustion of calcium from muscle cells which results in impaired muscle cells, paralysis and death (Cordova *et al*., 2006). Due to its diversified activity reduction of nematode was higher in Virtako. Previously its toxicity was evaluated against insects (Cordova *et al*., 2006; Dinter *et al*., 2008). Bio chemicals also reduced nematode population by increasing mortality and inhibition percentages. Cure 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 (Dybas *et al*., 1989). It also binds with gamma-aminobutyric acid that leads to the condition of hyperpolarisation and paralysis (Bloomquist, 1996). Another bio chemical Azadirachtin also caused maximum increase in mortality and decrease in hatching of *M. incognita* as compared to other chemicals. As a chemical compound, azadirachtin belongs to limonoid group that is secondary metabolite present in neem (Kosma *et al*., 2011). Its nematicidal activity was also due to presence of alkaloids, quercetin, kaemferol and limnoids (Khan *et al*., 1974; Alam, 1993).

Bio- and synthetic chemicals which caused significant mortality and hatching inhibition were evaluated against mobility of *M. incognita* and for phytotoxic effect on tomato. Cartap, Virtako, Cure and Azadirachtin were selected from bio and synthetic chemicals and tested further. Rugby was not selected due to its phytotoxic effects recorded on *Poa annua* (McClure and Schmitt, 2012). This was also included in the list of those chemicals whose utilization was banned from February 2008 by French agriculture ministry. Due to its promising nematicidal activity it was evaluated as a standard to check the potential of other chemicals. Phytotoxicity is actually the assessment of temporary or long lasting damage to the plant caused by a chemical compound or pesticide (Short, 1981). In the present study none of the chemical was found to be phytotoxic on their recommended doses after two months. García-Hernández *et al.* (2001) reported the phytotoxic effects of chemicals above recommended doses but not at recommended. Phytotoxic effects of different chemicals were evaluated on different crops in different studies (Raymond *et al*., 2002; Fanigliulo and Sacchetti, 2008). Recovery of juveniles was assessed after three days from soil in Cartap, Virtako, Cure and Azadirachtin. As first two days of contact with the host are crucial for the penetration of nematodes (Nwauzor and Fawole, 1992), so minimum recovery of juveniles from the chemicals indicates a population reduction at a critical period. In our results recovery percentage was decreased in all the chemicals tested as similar with the findings of others (Lei *et al*., 2010; Saad *et al*., 2011; Moosavi, 2012).

Cartap, Virtako Cure and Azadirachtin showed a decreasing trend in efficacy with the increase in time interval. Maximum population of nematodes was observed after 28 days interval in all chemicals. Chemicals were applied in soil as a single dose before transplantation of tomato plants. A direct relation was observed between efficacy of chemicals and time by (Deliopoulos *et al*., 2010), as with the passage of time, the efficacy of chemicals decreased with the increase in nematode population. Degradation of chemicals from the soil was attributed in three ways *viz.*, leaching, chemical and biological degradation (Dunn and Noling, 2003). Susceptibility of chemicals varied towards degradation, organophosphate and carbamates were found to be more susceptible to biological degradation (Laveglia and Dahm, 1977). Biological include microbial degradation caused by bacteria (Cain and Head, 1991), fungi (Jones, 1976) and algae (Zuckerman *et al*., 1970). Galling index, number of females and number of egg masses were higher at third time interval due to decrease in efficacy of chemicals. So efficiency and time are negatively correlated. It may be concluded from these findings that bio and synthetic chemicals have nematiticidal potential against *M. incognita* through diversified mechanisms.

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