

NEMATICIDAL EFFICACY AND PERSISTENCE OF CARBOSULFAN, FENAMIPHOS AND TRIAZOPHOS IN CHICKPEA FOLLOWING SEED TREATMENT

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Summary. Seed treatment has proved to be a low cost and effective nematode management option with minimal environmental contamination. Absorption of carbosulfan, fenamiphos and triazophos by seeds of chickpea, *Cicer arietinum* L., (cv. Pusa 362), following soaking in 0.1% acetonic solution of the nematicides, increased by 55, 36 and 32% of fenamiphos, carbosulfan and triazophos, respectively, compared to that in aqueous solutions. The amount of toxicant incorporated into seeds influenced the concentration of its residues in both roots and shoots. This affected the control of the root-knot nematode, *Meloidogyne incognita*, as well as of reniform nematode, *Rotylenchulus reniformis*, in the field. The residues in roots and shoots of chickpea persisted beyond 90 days but residues only occurred in green seeds at trace to non-detectable levels. The reduction in root-knot nematode population in the soil was correlated with the decrease in root galling of chickpea ($r = 0.70$, significant at $P = 0.05$). Also, root galling was negatively correlated with the nematicide concentration in the roots ($r = 0.65$, significant at $P = 0.05$). A seed concentration of 53.6 μg triazophos/g was sufficient to reduce the soil population as well as root infection. Seed treatment with triazophos at 100 $\mu\text{g}/\text{ml}$ of 0.1% acetonic solution can be safely recommended for managing nematodes in chickpea.

Key words: Control, *Cicer arietinum*, *Meloidogyne incognita*, nematicides, *Rotylenchulus reniformis*.

Targeting seeds for nematicidal treatment is low cost and one of the easiest methods of applying chemicals for control of nematode problems in crops. It requires only a limited amount of active ingredient of the toxicant, generally 1-2% by weight of seed, using binding agents such as gum arabic or starch. The method is both recommendable and effective against nematodes, while minimizing environment contamination, in a variety of crops, including cereals, pulses, and vegetables (Uebayashi *et al.*, 1971; Sivakumar *et al.*, 1974; Kaushal and Seshadri, 1989; McGarvey *et al.*, 1990; Chiba *et al.*, 1993). And at the much lower dose of 125 to 500 $\mu\text{g}/\text{ml}$ seed absorption of the toxicant from aqueous solution was still found effective against *Meloidogyne incognita* (Kofoid *et al.* White) Chitw. on cowpea and French bean (Hong and Sethi, 1988; Kumar, 1996). In most of these studies the actual concentrations of the nematicides in seeds, plants or fruits were not quantified and were considered environmentally safe. The present study was, therefore, undertaken to evaluate toxicant impregnation in chickpea seeds in aqueous and acetonic solutions of two organophosphate nematicides, fenamiphos and triazophos, and a procarbamate nematicide, carbosulfan, and to determine their levels in roots and shoots and assess their nematicidal efficacy.

MATERIALS AND METHODS

Technical grade nematicides, reagents and solvents

Technical grade carbofuran (the major metabolite of carbosulfan) of 99% purity and carbosulfan of 98% purity were obtained from FMC Corporation, USA; fenamiphos (91.9% purity) was obtained from Bayer, Leverkusen and triazophos (97.6% purity) from Hoestch, West Germany. Analytical fenamiphos sulfoxide and fenamiphos sulfone of purity >90% were prepared as per the method available in the literature (Khazanichi *et al.*, 1992). Emulsifiable concentrates of the nematicides were procured from Bayer India Ltd., Rallis India Ltd., and Hoestch India Ltd. All reagents used were of analytical grade and solvents for liquid chromatography were HPLC grade. Solvents for liquid chromatography (LC) were degassed prior to use. All other solvents were glass-distilled before use.

Experiments in vitro and in vivo

Experiments *in vitro* and *in vivo* were conducted according to a factorial design, with three nematicides each at three concentrations and with three replicates.

Seed treatment. Two hundred g of chickpea cv. Pusa 362 seeds were soaked in 800 ml of aqueous or 0.1% acetonic solutions (100, 200 and 400 $\mu\text{g}/\text{ml}$) of carbo-sulfan (Marshal, 25% emulsifiable concentrate (EC)), fenamiphos (Nemacur, 40% EC) and triazophos (Hostathion, 40% EC) in glass beakers for 12 h at 18 ± 2 °C. The treated seeds were washed with tap water and

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dried on blotting paper before analysis of pesticide concentrations.

Seed germination. The germination of the treated seeds was tested *in vitro*. For this, twenty treated seeds per replicate were placed on moist tissue paper in 10-cm-diameter Petri dishes and kept at $25 \pm 2^\circ\text{C}$ for 24 h in an incubator.

Field experiment. Experiments were conducted at the Indian Agriculture Research Institute, New Delhi in microplots naturally infested with 2.1-2.6 second stage juveniles (J2)/g soil of the root-knot nematode, *Meloidogyne incognita*, and 1.9-2.4 juveniles and vermiform females/g soil of the reniform nematode, *Rotylenchulus reniformis* Linford *et* Oliveira. Seeds were sown at inter- and intra-row spacings of 30 and 15 cm, respectively, in plots of 4 m² in November. The soil was alluvial with pH 8.2, cation exchange capacity of 2.10 me per 110 g, 73.2% sand, 7.6% silt, 19.0% clay and 0.91% organic

carbon. A single dose of the recommended fertilizer (25 kg nitrogen, 50 kg phosphorus and 25 kg potassium/ha) was applied one day before sowing. The plants were irrigated at intervals of 30 days. The plots were kept free of weeds by hand-hoeing. Samples of roots and shoots were taken after 45 and 90 days and of green pods at 100, 110 and 120 days after sowing for analysis of nematicide residues.

Nematicidal efficacy. The nematode population in soil was estimated on days 0, 45 and 90 after sowing by processing 200 cm³ soils by Cobb's modified decanting and sieving and Baermann funnel techniques. Root knot index was estimated on day 90 after sowing according to Taylor and Sasser (1978). The levels of toxicant impregnated in the seeds and their concentrations in roots were correlated with the decline in soil population levels of nematodes and reduction in root galling of chickpea plants.

Table I. Absorption of nematicides by chickpea seeds after soaking in aqueous and acetic solutions of nematicides and the effects on seed germination.

Treatment	Dose ($\mu\text{g/ml}$)	Average toxicant impregnation* ($\mu\text{g/g}$)		% increase in acetic solution	% seed germination	
		Aqueous solution	0.1 % acetic solution		Aqueous solution	0.1% acetic solution
Carbosulfan	100	132.5	180.4	36.2	98.4	98.4
	200	216.7	290.1	33.8	100.0	100.0
	400	386.7	472.6	22.2	100.0	98.4
Fenamiphos	100	280.5	434.9	55.2	100.0	100.0
	200	460.4	715.2	55.4	98.4	96.7
	400	847.2	1300.8	53.4	96.7	96.7
Triazophos	100	053.6	070.8	32.2	100.0	100.0
	200	120.4	146.3	21.5	96.7	100.0
	400	207.5	240.8	16.1	100.0	98.4
Check	-	-	-	-	100.0	98.4

		Statistical parameters			
		Average			CD (P = 0.5)
		1	2	3	
Chemicals (3)		16.35	25.16	11.45	2.98
	Dose (3)	13.05	17.17	22.75	
Chemical \times Dose:	1	Cabosulfan	Fenamiphos	Triazophos	5.16
	2	12.47	15.88	20.70	
	3	18.80	24.10	32.59	
Application method \times Chemical:		Aqueous solution	Acetic solution		2.06
	1	15.30	17.40		
	2	22.44	27.89		
	3	10.90	12.01		

*Average of 3 replicates. Chemical 1 = carbosulfan, 2 = fenamiphos, 3 = triazophos; Dose 1 = 100, 2 = 200, 3 = 400 $\mu\text{g/ml}$. For statistical analysis, data were square root transformed.

Residue analysis. Ten g of treated chickpea seeds per replicate were processed to determine the concentration of the nematicides incorporated into the seeds; the samples were analysed 12 h after treatment. Plant samples (25 g per replicate) from the field experiment were analyzed for residues in roots, shoots and fruit. Chemical residues were extracted three times from seed and plant samples with 150, 100 and 75 ml portions of acetone in a Waring blender. The combined extract was evaporated at 40 °C using a Buchi type rotary vacuum evaporator. To the residue, 5 ml of saturated sodium chloride solution and then 20 ml of water were added. The resulting solution was transferred to a separating funnel and partitioned with 100, 75, and 50 ml portions of chloroform. The chloroform extract was passed through a bed of anhydrous sodium sulphate over a plug of cotton. The clean-up procedure for analysis of residues of fenamiphos was a coagulation technique (Meher *et al.*, 1987). In brief, the chloroform extract was evaporated to dryness and thereafter 2 ml of acetone, 100 ml coagulating solution (1 g ammonium chloride, 2 ml of 75% phosphoric acid and 200 ml of water) and 0.5 g Hyflo Supercel were added. The mixture was shaken, allowed to stand for 1 h, filtered through a 0.5 cm layer of Hyflo Super Cell and washed with an additional 20 ml of coagulating solution. The filtrate was partitioned with 125, 75 and 50 ml portions of chloroform and dried over anhydrous sodium sulphate. The chloroform extract was then evaporated to dryness and the volume made up to 2 ml with acetone for GC analysis. To clean samples for carbosulfan and triazophos analysis, the chloroform extract was passed through a charcoal-florisil (1:3) column (32 × 2 cm) over a layer of sodium sulphate, dried at 40 °C and dissolved in 3 ml of acetonitrile for liquid chromatography. The residues of the test nematicides were reported as total residues comprising parent material and main toxic metabolites: the residues of fenamiphos were analysed as fenamiphos, its sulfoxide and sulfone; triazophos was determined as parent compound; carbo-sulfan residues were analysed as the parent compound carbosulfan and its degradation product carbofuran.

A Hewlett Packard 6890 gas chromatograph equipped with flame photometric detector and 15 m Hp5 capillary column (15 m, id 0.53 mm and film thickness 5 µm) and a chemstation were used for analysis of residues of fenamiphos and triazophos. The temperature (°C) of the injection port was 260, column 180 and detector 260. Nitrogen, hydrogen and air flow rates were 60, 150 and 120 ml/min, respectively.

A Hewlett Packard binary gradient high pressure liquid chromatograph series 1100, with Rheodyne manual injector 20 µl, UV-visible variable wavelength detector set at λ max 210 nm and a 4.6 × 200 mm Lichrosorb 5 µm C-18 reverse phase column, was used for analysis of the residues of carbosulfan. The mobile phase comprised acetonitrile and water (90:10) at a flow rate of 0.7 ml/min.

Statistical analysis.

Multivariate analysis of variance (MANOVA) and multiple correlation regression analysis were performed through software version 3.0 of Statistical Package for Social Scientists (SPSS) for data analysis and interpretation.

RESULTS AND DISCUSSION

Analytical technique. The retention times of triazophos, fenamiphos, fenamiphos sulfoxide and fenamiphos sulfone under the given conditions of gas chromatography were 4.0, 7.2, 13.7 and 17.8 min, respectively. The analysis time was 20 min. The peak areas were found to be linear in the test concentrations range of 0.1-1.0 µg/ml. The limit of detection (LOD) of the test nematicides was 0.001 µg/ml and the limit of quantification (LOQ) 0.003 µg/g plant tissues. Liquid chromatography was used for quantification of carbosulfan and its main degradation product carbofuran. Carbofuran eluted at a retention time of 4.8 min. Carbosulfan appeared at 10.3 min. The analytical method could detect 0.01 µg/ml of the test nematicides and had a LOQ of 0.04 µg/g plant matrices.

The average recoveries of triazophos and fenamiphos and its toxic metabolites ranged from 85 to 96% and those of carbosulfan 82 to 87% following fortification at 0.1 and 1.0 µg/g plant matrices. Similar recoveries of >85% for the test nematicides and their main toxic metabolites were reported earlier (Leppert *et al.*, 1983; Meher *et al.*, 1987; Tejada *et al.*, 1995; Wong *et al.*, 2004; Eswara Reddy *et al.*, 2007; Soler *et al.*, 2007). The recoveries being satisfactory, no correction factor was used in the final calculations of amounts of residues. The residues have been reported as the total toxic residues.

Germination and phytotoxicity. Soaking chickpea seeds in 100-400 µg/ml acetonic (0.1%) or aqueous solutions of carbosulfan, fenamiphos or triazophos for 12 h did not affect seed germination (Table I). No symptoms of phytotoxicity were discernible during crop growth.

Absorption by seed. Major metabolites observed in seeds 12 h after treatments were carbosulfan and carbofuran (approximately in the ratio of 2:3), fenamiphos and triazophos following soaking in aqueous solutions of the nematicides at 18 ± 2 °C (Table I). Absorption more than doubled with increase of dosage rates from 100 to 400 µg/ml. The absorption of toxicant by seed was greatest (281-847 µg/g) for fenamiphos, followed by carbosulfan (133-387 µg/g) and triazophos (54-208 µg/g) in aqueous solution. Toxicant incorporation was greater in acetonic than aqueous solutions. Absorption of fenamiphos by the seeds increased by 55% in acetonic solution compared to that in aqueous solution, and the increase was not affected by the rate of application.

However, with increasing rates of application of carbosulfan and triazophos, the percentage increase of toxicant absorption from acetonic versus aqueous solutions decreased. This suggests that the mechanisms of absorption by the seeds differed between chemicals. Fenamiphos absorption was greatest, followed by carbosulfan and triazophos. Absorption increased differentially with dosage and application method. This could be due to inherent characteristics of the compounds, water solubility and enhanced polarity in acetonic solution (Khan, 1980; Andergy and Lichtenstein, 1981). Water solubility of fenamiphos and carbofuran (the major metabolite of carbosulfan) is 700 µg/ml and that of triazophos 30-40 µg/ml. Non-polar compounds tend to be adsorbed on plant surfaces whilst polar compounds are readily absorbed and translocated in solution, fur-

ther affecting the plant concentration of pesticides (Khan, 1980).

Translocation and persistence in the plant. In plant parts carbofuran, fenamiphos, fenamiphos sulfoxide and triazophos were the predominant metabolites, accounting for >90% of the total residues. Rapid degradation of carbosulfan (Iwata *et al.*, 1983; Nigg *et al.*, 1984; Gupta *et al.*, 2007) and fenamiphos (Waggoner and Khasawinah, 1974) in plants is on record. However, the seed concentration of the nematicides exhibited good correlation with the toxicant level in roots 45 ($r = 0.85$, significant at $P = 0.05$) and 90 ($r = 0.71$, significant at $P = 0.05$) days after sowing. The root residue levels at 45 and 90 days were also well correlated ($r = 0.92$, significant at $P = 0.01$). The total nematicide residues in roots

Table II. Total toxic residues in roots and shoots of a chickpea crop grown after seed treatment with aqueous and acetonic solutions of nematicides.

Treatment	Dose (µg/ml)	Average concentration of residues (µg/g) in plant tissues*							
		Days							
		45				90			
		Root		Shoot		Root		Shoot	
		1	2	1	2	1	2	1	2
Carbosulfan	100	0.543	1.208	0.113	0.320	0.008	0.261	0.006	0.011
	200	1.201	2.541	0.259	0.603	0.019	0.590	0.017	0.024
	400	2.086	4.293	0.484	1.308	0.042	1.003	0.031	0.038
Fenamiphos	100	0.682	1.376	0.156	0.366	0.096	0.301	0.014	0.021
	200	1.025	2.674	0.267	0.705	0.152	0.687	0.029	0.055
	400	2.687	5.217	0.403	1.397	0.278	0.931	0.050	0.121
Triazophos	100	0.180	0.210	0.021	0.033	0.006	0.009	0.002	0.006
	200	0.351	0.362	0.064	0.078	0.010	0.016	0.003	0.009
	400	0.673	0.709	0.150	0.175	0.024	0.039	0.006	0.017
		Statistical parameters							
		Roots				Shoots			
		Average			CD (P = 0.05)	Average			CD (P = 0.05)
		1	2	3		1	2	3	
Chemicals (3)		1.15	1.34	0.22	0.61	0.27	0.30	0.05	0.20
Dose (3)		0.41	0.80	1.50	0.61	0.09	0.18	0.35	0.20
Application methods (2)		0.56	1.25		0.64	0.12	0.29		0.16

*Average of 3 replicates. Application method: 1 = aqueous solution; 2 = 0.1% acetonic solution. Chemical: 1 = carbosulfan, 2 = fenamiphos, 3 = triazophos. Dose: 1 = 100, 2 = 200; 3 = 400 µg/ml.

Table III. Total toxic residues in green seeds of a chickpea crop grown after seed treatment with aqueous and acetic solutions of nematicides.

Treatment	Dose ($\mu\text{g/ml}$)	Average residues ($\mu\text{g/g}$) in fruits*					
		Days after sowing					
		100		110		120	
		1	2	1	2	1	2
Carbosulfan	100	TR	TR	ND	ND	ND	ND
	200	TR	TR	TR	TR	ND	ND
	400	TR	0.012	TR	TR	ND	ND
Fenamiphos	100	0.003	0.004	ND	ND	ND	ND
	200	0.005	0.008	ND	0.003	ND	ND
	400	0.007	0.012	0.003	0.005	ND	ND
Triazophos	100	0.004	0.003	ND	ND	ND	ND
	200	0.005	0.004	ND	ND	ND	ND
	400	0.004	0.007	0.003	0.005	ND	ND

*Average of 3 replicates. 1 = Aqueous solution, 2 = 0.1% Acetic solution, ND = Non-detectable, TR = Traces (<0.001)

and shoots of chickpea, measured at mid-season of the chickpea crop (45 days after sowing treated seeds in field microplots) were highest for fenamiphos (0.68-2.68 $\mu\text{g/g}$ root; 0.16-0.40 $\mu\text{g/g}$ shoot), followed by carbosulfan (0.54-2.09 $\mu\text{g/g}$ root; 0.11-0.48 $\mu\text{g/g}$ shoot) and triazophos (0.18-0.67 $\mu\text{g/g}$ root; 0.02-0.15 $\mu\text{g/g}$ shoot), in the same pattern as the levels of residues in seeds (Table II). The levels increased with the dosage rates and more so with treatment in acetic than aqueous solution. The increase in residue levels in both roots and shoots were more pronounced for fenamiphos and carbosulfan than for triazophos. However, the residue levels in both roots and shoots declined by 90 days, although residues were still detectable at this time and therefore persisted beyond 90 days. The persistence of carbofuran in pea (Meher *et al.*, 1985), fenamiphos in cowpea, pea and okra (Meher and Sethi, 1992) in India and triazophos in wheat in China (Li *et al.*, 2008) has been recorded beyond 90 days from application.

Residues in green seeds. The residues in green seeds on three different picking occasions decreased, leaving behind only trace (0.003-0.009 $\mu\text{g/g}$) to non-detectable levels in green seeds by 120 days, but with up to 0.012 $\mu\text{g/g}$ after 100 days from the high rate (400 $\mu\text{g/ml}$) of treatment of carbosulfan and fenamiphos in acetic solutions (Table III). In fruits, the residues of triazophos have been reported to persist beyond 10 days in brinjal and okra (Raja *et al.*, 1999; Eswara Reddy *et al.*, 2007). Half-lives for dissipation of carbosulfan were 2 days in okra fruit (Gupta *et al.*, 2007) and 4 days in citrus foliage (Iwata *et al.*, 1983), and 8 days for carbofuran in citrus foliage (Iwata *et al.*, 1983). However, the residues of the nematicides in the green seeds of chickpea were far below the Codex maximum residue limit of 0.2 $\mu\text{g/g}$ of fenamiphos allowed in potato (Smart, 1987), the recommended 0.02 $\mu\text{g/g}$ for triazophos in broad beans (Anonymous, 2007) and the 0.1 $\mu\text{g/g}$ for carbofuran in

fruits and vegetables (Dureja and Gajbhiye, 2004) or the German MRL of 0.02 $\mu\text{g/g}$ carbofuran and fenamiphos and 0.01 $\mu\text{g/g}$ triazophos for plant products (Anonymous, 2006); hence, they may be considered as safe.

Nematicidal activity. The pre-treatment plant parasitic nematode populations of the soil comprised mainly *M. incognita* and *R. reniformis* (Table IV). At sowing, the *M. incognita* population was 2.1-2.6 J2/g soil and that of *R. reniformis* 1.9-2.4 juveniles and vermiform females/g soil. At mid-season, about 45 days after sowing, the population *M. incognita* in the soil had declined by 55-85, 38-70 and 45-76% in treatments with aqueous solutions of fenamiphos, carbosulfan and triazophos, respectively. In the check, there was a 4.3% increase in population. The reductions were greater (61-84, 47-79, 48-79%) in treatments with 0.1% acetic water solutions of fenamiphos, carbosulfan and triazophos, respectively. By day 90, the population reduction of *M. incognita* was 80-90% in the various treatments as against a 12% population increase in the check. The relatively small increase in nematode population in the check was due to the low temperatures prevailing in winter.

The effects of the treatments on *R. reniformis* in soil were similar but less pronounced, with mid-season population reductions of 54-77, 50-75 and 28-63% following treatment in aqueous solutions, and higher reductions of 60-81, 59-81 and 32-66% following treatment in acetic solutions, of triazophos, fenamiphos and carbosulfan, respectively. Final population reductions of 55-86% were achieved by day 90 compared to an increase of about 16% in the check.

In general, the treatments were more effective against *M. incognita* compared to *R. reniformis*. Multivariate Analysis of Variance revealed that all chemicals were equally effective against *M. incognita*. However, their efficacy differed against *R. reniformis*, with triazophos being the most effective followed by fenamiphos and car-

bosulfan, which were at par with each other. The nematocidal action increased with dosage and application method and continued up to 90 days.

Estimation of root galling on a 0-5 scale (Taylor and Sasser, 1978) showed root gall indices of 1.0-1.3 in triazophos, 1.0-1.4 in fenamiphos and 1.0-1.6 in carbosulfan treatments. In the check the root-knot index was

3.2. The effect on root knot index did not appear to differ across treatments (Table IV).

The population reduction of *M. incognita*, unlike that of *R. reniformis*, in soil and concentration of nematocides in root and root galling were well correlated ($r = 0.65$ and 0.70 , significant at $P = 0.05$). The decline in the *M. incognita* population and the resultant reduction

Table IV. Effect of treatment of seeds of chickpea on root-knot and reniform nematodes in soil and root galling, in a winter chickpea crop.

Treatment	Dose ($\mu\text{g/ml}$)	Application method	Nematode population/ cm^3 soil*						Root- knot index
			<i>Meloidogyne incognita</i> (J2)			<i>Rotylenchulus reniformis</i> (juveniles and females)			
			1	2	3	1	2	3	
Carbosulfan	100	1	2.15	1.33	0.43	2.00	1.44	0.89	1.6
		2	2.15	1.14	0.39	2.00	1.36	0.85	1.4
	200	1	2.44	0.98	0.29	1.98	1.17	0.77	1.2
		2	2.44	0.76	0.25	1.98	1.04	0.69	1.1
	400	1	2.21	0.67	0.18	2.31	0.86	0.56	1.1
		2	2.21	0.46	0.16	2.31	0.78	0.48	1.0
Fenamiphos	100	1	2.56	1.14	0.37	2.44	1.21	0.77	1.4
		2	2.56	1.00	0.33	2.44	1.00	0.68	1.2
	200	1	2.38	0.84	0.27	2.53	0.92	0.68	1.1
		2	2.38	0.69	0.20	2.53	0.74	0.52	1.0
	400	1	2.46	0.48	0.18	2.37	0.58	0.43	1.0
		2	2.46	0.39	0.16	2.37	0.44	0.39	1.0
Triazophos	100	1	2.21	1.21	0.32	2.18	1.01	0.68	1.5
		2	2.21	1.15	0.28	2.18	0.87	0.57	1.2
	200	1	2.58	0.82	0.24	1.94	0.68	0.54	1.3
		2	2.58	0.76	0.19	1.94	0.61	0.41	1.1
	400	1	2.33	0.55	0.19	2.00	0.46	0.31	1.2
		2	2.33	0.48	0.16	2.00	0.38	0.27	1.0
Check	-	-	2.57	2.68	2.89	2.36	2.77	2.74	3.2

Statistical parameters

	Averages						CD (P = 0.05)
	Root-knot			Reniform			
	1	2	3	1	2	3	
Chemicals	1.54	1.55	1.53	1.63	1.62	1.45	0.010
Dose	2.71	1.27	1.17	2.62	1.36	1.20	0.038
Nematode population	2.42	1.29	0.91	2.24	1.34	1.12	0.011
Application method	1.56	1.52		1.59	1.54		0.023

* Average of 3 replicates. Chemical: 1 = carbosulfan, 2 = fenamiphos, 3 = triazophos. Dose: 1 = 100, 2 = 200; 3 = 400 $\mu\text{g/ml}$. Nematode population: 1 = initial, 2 = mid season, 3 = final population. Application method: 1 = aqueous; 2 = 0.1% acetic solutions.

in root knot indices could be attributed mainly to absorption of nematicides by the seeds. Vadhera *et al.* (1998) reported 65% reduction in nematode population and 40% yield increase of chickpea after soaking seeds with carbosulfan (0.01%). Reductions in invasion, root galling and infestation following seed treatments with carbosulfan, fenamiphos and triazophos have improved the growth and yield of various crops (Siddiqui *et al.*, 1993; Sirohi and Siyanand, 1994, Kumar, 1996; Joshi and Patel, 1997; Verma and Gupta, 1997). Seed treatment has also been reported to prevent invasion or completion of the life cycle of *Heterodera avenae* on wheat (Kaushal and Seshadri, 1989). The reductions in nematode populations and infection levels could be due to adverse influences on orientation towards roots, invasion, development, reproduction and fecundity coupled with a direct poisoning effect (Di Sanzo, 1973; McLeod and Khair, 1975; Greco and Thomason, 1980; Marban-Mendoza and Viglierchio, 1980a, b, c). The better nematicidal action against root-knot compared to reniform nematodes was probably due to a smaller rate of degradation of the nematicides and a greater inhibition of acetylcholine esterase in root-knot than in reniform nematode (Nordmeyer and Dickson, 1990; Zhu and Clark, 1995). Low concentrations of non-fumigant nematicides have been reported to influence nematode behaviour and are considered important for nematode management (McLeod and Khair, 1975; Marban-Mendoza and Viglierchio, 1980a, b, c; Wright and Womack, 1981; Bostian *et al.*, 1984). A very low concentration of fenamiphos (0.003 µg/ml) has been reported to inhibit attraction of *Pratylenchus vulnus* Allen *et* Jensen by roots of bean (Marban-Mendoza and Viglierchio, 1980b). Significant reductions in invasion of roots of cowpea by *M. incognita* were observed when J2 were exposed to the still lower concentrations of 0.0005 to 0.0200 µg/ml fenamiphos for 24 to 48 h (Meher and Sethi, 1988). A root concentration of 1.9 µg fenamiphos/g, achieved 7 days after a foliar spray at 100 µg/ml, was enough to prevent the entry of *M. incognita* juveniles into roots of cowpea (Meher and Sethi, 1991).

It may be inferred from our results that seed treatment with triazophos at 100 µg/ml in aqueous solution containing 0.1% acetone is effective in reducing the soil population of *M. incognita* and *R. reniformis* and root galling by *M. incognita*. Besides, the residues of this chemical in green seeds were far below the maximum limit admitted. Therefore, triazophos is the most recommended chemical for the control of these nematodes in chickpea.

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