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Evaluation and quantitative structure-activity relationship of S-alkyl S,S-diaryl phosphorotrithioates for the nematicidal activity against root knot nematode (*Meloidogyne incognita*) (1)

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Summary – Twelve new S-alkyl S,S-diaryl phosphorotrithioates have been tested *in vitro* for nematicidal activity against the root knot nematode (*Meloidogyne incognita*) and their structure-activity relationships analysed by means of multiple regression analysis using physico-chemical substituent parameters for hydrophobic, electronic and steric properties. The major contribution to the nematicidal activity of the phosphorotrithioates in this series was due to the steric effect expressed in terms of STERIMOL parameter B4 of the alkyl groups attached to sulphur, the electronic nature (σ) and the lipophilicity (π) of the benzene ring substituents were also influencing the activity. Further, the nematicidal activity increased by attaching smaller alkyl groups to sulphur and by the presence of less lipophilic and more electron withdrawing groups in the benzene ring. The five compounds which showed good nematicidal activity *in vitro* were further evaluated under glass house conditions on soybean. These have significantly reduced the number of galls with maximum reduction in case of S-methyl S,S-di(4-chlorophenyl) phosphorotrithioate. Its nematicidal activity was found comparable to Nemacur*. Further evaluation of this compound showed no adverse effect on germination of soybean but significantly decreased the penetration of *M. incognita* juveniles when sprayed on the foliage, thus establishing its basipetal systemic character.

Résumé – ***Évaluation et relations quantitatives entre la structure et l'activité de S-alkyl S,S-diaryl phosphorotrithioates comme nématicides envers* Meloidogyne incognita** – Douze nouveaux S-alkyl S,S diaryl phosphotrithioates ont été testés pour leur activité nématicide envers *Meloidogyne incognita*; la relation structure/activité a été analysée par régression multiple en utilisant des paramètres physico-chimiques de substitution pour les propriétés hydrophobes, électroniques et stériques. Dans la série des phosphorotrithioates étudiée, la part prépondérante de l'activité nématicide est due à l'effet stérique – exprimé en termes de paramètres B4 du STERIMOL – des groupes alkyles liés au soufre; le caractère électronique (σ) et la lipophilie (π) des substituts aux anneaux benzéniques influencent également cette activité. De plus, l'activité nématicide est accrue par la liaison de petits groupes alkyles au soufre et par la présence sur les anneaux benzéniques de groupes moins lipophiles et provoquant des pertes d'électrons. Les cinq composés ayant montré une bonne activité nématicide *in vitro* ont été ensuite testés en serre sur soja. Ils ont diminué significativement le nombre de galles, le meilleur résultat étant dû au S-méthyle S,S-di(4-chlorophényle) phosphorotrithioate, lequel a une activité nématicide comparable à celle du Nemacur*. Des tests complémentaires ont montré que les composés n'ont aucun effet contraire sur la germination et qu'ils diminuent significativement la pénétration des larves de *M. incognita* lorsqu'ils sont appliqués sur le feuillage; il s'agit donc là de produits systémiques, agissant basipétalement.

Key-words : Nematodes, *Meloidogyne*, nematicides.

Among the organophosphorus compounds, phosphorothioates and dithioates are important groups exhibiting nematicidal activity and majority of the commercially available organophosphorus nematicides belong to these groups (Gupta & Roy, 1987). Further, a large number of phosphorothioates have been screened in the past (Gupta *et al.*, 1984, 1986, 1989) for their nematicidal activity. Recently the fungicidal activity of a few phosphorotrithioates, a newer subclass in this group has been reported from this laboratory (Gupta & Roy, 1990, 1991) but there is no report so far on the nematicidal activity in this class. This paper reports the nematicidal activity against *Meloidogyne incognita* and quantitative structure-activity relationships (QSAR) of a series of phosphorotrithioates viz. S-alkyl S,S-diaryl phosphorotrithioates. Some developmental studies on a lead compound are also described.

Materials and methods

COMPOUNDS

The compounds, S-alkyl S, S-diaryl phosphorotrithioates evaluated for nematicidal activity are listed in

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Table 1. Physico-chemical data and observed *versus* predicted nematicidal activity of S-alkyl S,S-diaryl phosphorotrithioates against *Meloidogyne incognita*.

Compd. No.	R (Fig. 1)	Z	M.P. (°C)	LC_{50} (ppm)	B4 (R)	π (Z)	σ (Z)	- *log* LC_{50} *(M)*	
								Obs.	Calc.
1	CH_3	H	a	35	2.04	0.00	0.00	3.95	4.30
2	CH_3	CH_3	a	51	2.04	0.56	−0.17	3.82	3.39
3	CH_3	Cl	79-80	36	2.04	0.71	0.23	4.02	4.04
4	CH_3	Br	106-107	70	2.04	0.86	0.23	3.83	3.89
5	C_2H_5	H	a	138	2.97	0.00	0.00	3.37	3.03
6	C_2H_5	CH_3	a	10 000	2.97	0.56	−0.17	1.55	2.11
7	C_2H_5	Cl	a	653	2.97	0.71	0.23	2.78	2.77
8	C_2H_5	Br	a	676	2.97	0.86	0.23	2.85	2.61
9	i-C_3H_7	H	82-83	616	3.16	0.00	0.00	2.74	2.77
10	i-C_3H_7	CH_3	59-60	3 589	3.16	0.56	−0.17	2.01	1.85
11	i-C_3H_7	Cl	52-64	776	3.16	0.71	0.23	2.72	2.51
12	i-C_3H_7	Br	75-76	5 129	3.16	0.86	0.23	1.99	2.35

a = column purified liquid.
R = aliphatic substituent.
Z = aromatic substituent.
M.P. = melting point.
LC_{50} = lethal concentration for 50 % mortality of nematode juveniles.
B4 = STERIMOL parameter for maximum width of the substituents.
π = Hansch Pi value, measure of hydrophobicity of the substituents.
σ = Hammett constant, an electronic parameter for the aromatic substituents.
Obs. = experimentally determined.
Calc. = calculated from Equation 3 (see Table 3).

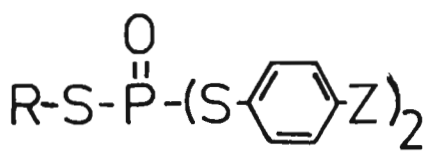

Fig. 1. S-Alkyl S,S-diaryl phosphorotrithioates.

Table 1. Methods for their preparation have been reported earlier (Gupta & Roy, 1991). The purity of the compounds was checked by TCL on silica gel coated plates using a mixture of benzene-acetone (9:1) for compounds 1-4 and a mixture of benzene-n-hexane (1:3) for compounds 5-12 as developing solvent and iodine vapours as visualising agent.

Nematicidal testing against *Meloidogyne incognita*

M. incognita juveniles used for the nematicidal testing were obtained by keeping the egg masses (collected from a stock culture maintained on tomato under glass house conditions) on a wire gauge support having two layers of tissue paper in a Petri dish containing water at 30 °C ± 1°.

In vitro *screening*

An emulsifiable concentrate (EC) of each compound was initially prepared by dissolving 50 mg of the compound in 2 ml acetone and 2-3 drops of Tween-80 as emulsifier. This was then made up to 25 ml with distilled water to obtain a stock solution of 2000 ppm. From the stock solution further concentrations were prepared by dilution with distilled water. One ml of nematode suspension containing about 100 juveniles of *M. incognita* was taken in vials (12 ml capacity) and one ml solution of double concentration of the test compounds was added to the vials to obtain the desired concentrations. The loosely capped vials were kept at 30 °C ± 1° for 72 h. A control of acetone containing Tween-80 was similarly prepared and stored along with the compounds. After the said period of exposure, the number of alive and dead nematodes were counted under stereoscopic binocular (Sethi & Prasad, 1982). The percent mortality at each concentration was worked out from the average of three replications according to Abbot's (1925) formula. The LC_{50} (lethal concentration for 50 % mortality of nematode juveniles) values (ppm) were determined from this data for five concentrations on a log probit scale and are presented in Table 1. The highest concentration used was 1000 ppm, the LC_{50} values greater than 1000 ppm have been obtained by extrapolation.

For QSAR study, LC_{50} (ppm) values were converted to molar LC_{50} values and their negative logarithm calcu-

lated. The – *log* LC_{50} *(M)* values are also given in Table 1.

Glass house testing

Of the twelve compounds tested *in vitro*, five which showed good activity were further evaluated against *M. incognita* under glass house conditions on soybean (*Glycine max* L.) cv. PK-327 as test plants.

Earthen pots of 10 cm diameter were each filled with 500 g steam sterilised mixture of soil and sand (3:1). One seed of soybean was sown in each pot. After 7 days of germination, 2000 freshly hatched *M. incognita* juveniles were inoculated around the root zone of growing plant in each pot. After 6 hours, 100 ml of 125 and 250 ppm concentration of each test compound was drenched into the pots. A control of acetone and a standard fenamiphos (Nemacur®) was similarly used. Each treatment including the control was replicated thrice. At 45 days after inoculation, the observations on shoot length, shoot weight, root length, root weight, number of pods, number of nodules and number of galls were recorded. The data were statistically analysed by analysis of variance technique and the results are presented in Table 2.

PHYSICO-CHEMICAL PARAMETERS AND CORRELATION ANALYSIS

Values for the various physico-chemical parameters of aliphatic (R) and aromatic substituents (Z) were taken from literature (Hansh & Leo, 1979). Hansh π values were used as a measure of hydrophobicity, Taft electronic parameter (σ^*) for aliphatic substituents and Hammet constant (σ) for aromatic substituents were used as electronic parameters. Steric parameters used were, Taft steric parameter (Es), molar refractivity (MR) and STERIMOL parameters L, B1 and B4 rep-

Table 2. Effect of S-alkyl S, S-diaryl phosphorotrithioates on the plant growth characters of soybean infected by *Meloidogyne incognita*.

Compd. No.	R (Fig. 1)	Z	Conc. (ppm)	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	No. of pods	No. of nodules	No. of galls
1	CH_3	H	125	51.67	10.77	23.93	3.73	11.67	13.67	17.33
			250	45.00	11.77	26.67	4.30	11.67	9.33	11.33
			CD at 5 %	7.71	4.49	11.06	1.44	7.62	8.24	8.21
			S.E (m)	3.86	2.25	5.53	0.72	3.81	4.12	4.11
2	CH_3	CH_3	125	49.00	13.67	28.67	4.33	13.33	15.33	21.00
			250	51,33	16.60	34.00	4.77	11.00	8.00	10.00
			CD at 5 %	11.51	7.26	4.56	1.95	6.14	11.28	6.92
			S.E. (m)	5.76	3.63	2.28	0.98	3.07	5.65	3.46
3	CH_3	Cl	125	58.00	19.33	44.33	3.63	15.33	9.33	13.67
			250	51.00	17.17	32.67	3.90	13.00	14.67	5.00
			CD at 5 %	9.32	8.47	6.63	1.57	8.34	7.02	7.68
			S.E. (m)	4.67	4.24	3.32	0.78	4.18	3.51	3.84
4	CH_3	Br	125	51.67	17.60	27.00	3.93	14.67	10.33	7.67
			250	51.67	22.73	33.00	4.63	20.33	10.33	5.33
			CD at 5 %	5.65	6.62	4.37	1.35	3.94	10.25	8.21
			S.E. (m)	2.83	3.31	2.18	0.67	1.97	5.13	4.11
5	C_2H_5	H	125	52.33	16.33	32.00	3.93	15.67	7.67	12.33
			250	54.33	16.67	33.67	4.40	15.00	15.67	11.67
			CD at 5 %	14.36	9.06	3.77	1.80	13.66	12.26	6.07
			S.E. (m)	7.19	4.53	1.88	0.90	6.84	6.14	3.04
Nemacur* (Standard)			125	60.67	26.50	32.00	7.40	22.00	27.33	4.00
			250	54.33	17.20	26.33	5.03	11.33	14.00	2.67
			CD at 5 %	16.23	6.95	13.78	4.08	4.52	10.74	4.52
			S.E. (m)	8.12	3.48	6.90	2.04	2.26	5.37	2.26
Control			–	28.67	5.63	18.33	4.67	0.00	4.67	25.00

R = aliphatic substituent.
Z = aromatic substituent.
CD = critical difference, indicates significant increase/decrease over control.
S.E. (m) = standard error of mean.

Table 3 . Correlations for the nematical activity of S-alkyl S, S-diaryl phosphorotrithioates.

Eq. No.	Regression equation $-\log LC_{50}(M) =$	n	s	r	$F_{\nu_1}{}^{\nu_2}$
1	6.703 (± 0.311) - 1.371 (± 0.637) B4 (R)	12	0.484	0.835	$F_{1,10} = 23.03$
2	6.965 (± 0.311) - 1.371 (± 0.635) B4 (R) - 0.492 (± 0.956) π Z)	12	0.476	0.858	$F_{2,9} = 12.60$
3	7.102 (± 0.236) - 1.371 (± 0.483) B4 (R) - 1.024 (± 0.844) π (Z) + 2.019 (± 1.628) σ (Z)	12	0.355	0.933	$F_{3,8} = 17.83$

R = aliphatic substituent.
Z = aromatic subsituent.
LC_{50} = lethal concentration for 50 % mortality of nematode juveniles.
B4 = STERIMOL parameter for maximum width of the substituents.
π = Hansch Pi value, measure of hydrophobicity of the substituents.
σ = Hammett constant, an electronic parameter for the aromatic substituents.
Figures in brackets = 95 % confidence intervals.
n = No of compounds included in the correlation study.
s = standard deviation.
r = correlation coefficient.
F_{ν_1}, F_{ν_2} = F ratio of the correlation.

resenting respectively length, minimum and maximum width of the substituents. The values of B4 (R), π (Z) and σ (Z) used in the regression equations (Table 3) are given in Table 1.

The structure-activity correlations were analysed by the multiple regression analysis technique (Hansch & Fujita, 1964) using measured – *log LC_{50} (M)* values for nematicidal activity against *M. incognita* as the dependent variable and physico-chemical parameters for hydrophobic, electronic and steric properties of each member of the series as independent variables. The levels of significance of correlations and terms were examined by F and t tests.

Table 4. Correlation matrix for the parameters used in regression equations.

	B4 (R)	π (Z)	σ (Z)
B4 (R)	1.00	0.00	0.00
π (Z)		1.00	0.51
σ (Z)			1.00

R = aliphatic substituent.
Z = aromatic substituent.
B4 = STERIMOL parameter for maximum width of the substituents.
π = Hansch Pi value, measure of hydrophobicity of the substituents.
σ = Hammett constant, an electronic parameter for the aromatic substituents.

Table 5. Effect on germination and penetration of *Meloidogyne incognita* juvenile on soybean of S-methyl S,S-di(4-chlorophenyl) phosphorotrithioate.

Conc. (ppm)	% Germination after			Number of *M. incognita* juvenile penetrated after		
	2 days	4 days	6 days	1 week	2 weeks	3 weeks
125	70.00	83.33	83.33	12.00	11.67	23.33
250	70.00	90.00	93.33	15.67	10.00	16.67
Control	80.00	96.67	93.33	20.00	27 33	33.00
CD at 5 %	23.07	14.89	16.31	4.04	5.95	8.29
S.E. (m)	11.54	7.45	8.16	2.02	2.98	4.15

C.D. = critical difference, indicates significant increase/decrease over control.
S.E. = standard error of mean.

Developmental studies on the lead compound

Further developmental studies were carried out on the most active compound, S-methyl S, S-di(4-chlorophenyl) phosphorotrithioate.

Effect on germination of soybean seeds

Petri dishes (100 mm diameter) were fitted with double layer of tissue paper, soaked with 10 ml of 125 and 250 ppm concentration of S-methyl S, S-di(4-chlorophenyl) phosphorotrithioate, replicated three times. Ten seeds of soybean (cv. PK-327) were sown in each Petri dish. A control of acetone containing Tween-80 similarly prepared as the concentrations of the chemical was also used. Observations on germination were recorded after 2, 4 and 6 days of sowing. The percent germination are given in Table 5.

Effect of penetration of M. incognita *juveniles into the roots of soybean*

Wax coated ice cream cups (100 ml capacity) were filled with 100 g steam sterilized mixture of soil and sand (3:1). One seed of soybean, cv. PK-327, was sown in each cup. After 7 days of germination 200 freshly hatched juveniles of *M. incognita* were inoculated around the growing seedlings. After 1 hour, 5 ml each of 125 and 250 ppm concentration of S-methyl S, S-di(4-chlorophenyl) phosphorotrithioate were sprayed on the foliage of the plant. A control of acetone containing Tween-80 was also used and each treatmemt replicated thrice. After 1, 2 and 3 weeks of spray, juveniles penetrated into roots were stained and counted under stereoscopic binocular (Byrd *et al.*, 1983). The results are presented in Table 5.

Results and discussion

The nematicidal activity results (Table 1) show that the activity of S-alkyl S, S-diaryl phosphorotrithioates

against *M. incognita* was dependent on the nature of the alkyl group attached to sulphur. The order of activity was found to be CH_3S- > C_2H_5S- > i - C_4H_7S- except when methyl was present in the benzene ring at para position in which case the ethyl homologue (6) exhibited the least activity. Further, the LC_{50} values of the compounds in this series do not indicate any definite trend for the variation in nematicidal activity based on the electronic nature of the benzene ring substituents. However, some generalisations can be made for the nematicidal activity of the compounds of this series based on benzene ring substituents irrespective of the alkyl group attached to sulphur. The phenyl derivatives (1, 5, 9) have exhibited the highest nematicidal activity. Substitution by chlorine atom at the para position (3, 7, 11) resulted in diminished activity as compared to the corresponding phenyl derivatives, but have shown higher activity than the corresponding 4-methyl (2, 6, 10) and 4-bromo (4, 8, 12) analogues. Introduction of bromine atom at the para position resulted in the least activity of the compounds (4, 12) except for the 4-bromo analogue (8) of S-ethyl S, S-diaryl phosphorotrithioates in which case the 4-methyl analogue (6) showed the least activity. S-methyl S,S-diphenyl phosphorotrithioate and S-methyl S,S-di(4-chlorophenyl) phosphorotrithioate have shown the highest activity (LC_{50} = 35 and 36 ppm respectively) *in vitro* in this series.

In order to clarify the effect of alkyl and aryl substituents on nematicidal activity precisely, quantitative structure-activity relationships using measured LC_{50} values and various substituent parameters for hydrophobic, electronic and steric properties were analysed. Several regression equations were obtained, a few relevant (Table 3) have been selected and are discussed here.

In these equations n is the number of compounds included in the correlation, s is the standard deviation, r is the correlation coefficient and F_{v_1, v_2} is the F ratio of the correlation where $v_1 = m$ and $v_2 = n\text{-}m\text{-}1$; m is number of independant variables used in the correlation. The figures in parenthesis are the 95 % confidence intervals for the respective constants.

Equation (1) shows that 69.72 % (r = 0.835) of the variation in the nematicidal activity of S-alkyl S,S-diaryl phosphorotrithioates against *M. incognita* is due to variation in B4 (R), the STERIMOL parameter for maximum width of the alkyl group attached to sulphur. Eq. (1) and the B4 (R) term in it is significant at 99 % level. Introduction of a π (Z) term to Eq. (1) slightly improved the correlation (r = 0.858). Addition of one more term σ (Z) to Eq. (2) further improved the correlation (r = 0.933) in Eq. (3) which accounts for 87.05 % of the variation in the nematicidal activity. Further addition of any other term to Eq. 3 did not improve the correlation. Eq. 3 is thus, the statistically best fit for determining the nematicidal activity of S-alkyl S,S-diaryl phosphorotrithioates against *M. incognita*. The Eq. (3) is significant at 99 % level, the B4 (R) term in it is justified at 99 % level and the π (Z) and σ (Z) term at 95 % level. The $-\ log\ LC_{50}$ values calculated from Eq. (3) (Table 1) are comparable with the observed values. A correlation matrix for the parameters used in this regression equation is given in Table 4. Further in these equations – ve sign with B4 (R) and π (Z) terms indicate that smaller the B4 (R) value of the alkyl group attached to sulphur and less lipophilic is the benzene ring substituent, more will be the activity of the compound. The +ve coefficient with σ (Z) term means that the presence of electron withdrawing groups in the benzene ring will enhance the nematicidal activity. The stronger the electron withdrawing substituent in the benzene ring, the greater will be the nematicidal activity of the compound.

The QSAR study thus clearly states that although the major contribution is from the alkyl groups attached to sulphur, the benzene ring substituents are also significantly influencing the nematicidal activity of the compounds in this series against *M. incognita*.

The results of the glass house tests (Table 2) indicate that all the five test chemicals have significantly reduced the number of galls on the roots of soybean and did not show any significant adverse effect on the plant growth characters. S-methyl S,S-di(4-chlorophenyl) phosphorotrithioate has shown the maximum reduction in the number of galls and also showed a significant increase in the plant growth characters such as shoot length, shoot weight, root length, number of pods and number of nodules. Its activity is at par with that of Nemacur®.

The results of further developmental studies (Table 5) carried out on the most active compound, S-methyl, S,S-di(4-chlorophenyl) phosphorotrithioate reveal that it does not have any significant detrimental effect on the germination of soybean but have significantly decreased the penetration of *M. incognita* juvenile into the roots. This indicates that spraying in foliage of soybean by the chemical had moved downward to protect the roots from invasion by nematode juveniles thus establishing the basipetal systemic character of this potential nematicide.

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