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COMPARATIVE NEMATICIDAL ACTIVITIES OF NITROGEN-NITROGEN DONOR IMINES AND THEIR MANGANESE AND TIN COMPLEXES

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Nematicidal activity of manganese (II) and organation (IV) complexes with imines having nitrogen-nitrogen donor system have been studied. The very little amount (ppm solution) of complexes retards the hatching percentage of eggmasses the spp. used in *Meloidogyne incognita.* All complexes are tested with different concetration of solutions against the hatching of eggs. Manganese (II) and tin (IV) complexes were prepared from column method in different unimolar and bimolar ratios. Chemical treatment was given to brinjal roots from step by step procedure. All results are compared with the control. The results revealed that activity increased with increasing complexation, i.e. the newly synthesized complexes were found to be more toxic in inhibiting the growth of nematode than the parent ligands themselves.

Keywords : Organotin (IV) complexes, Manganese (II) complexes, nematicidal and sulphonamide-imine.

Introduction

The plant parasitic nematodes infect almost every plant on this earth causing heavy economic losses. The plant parasitic nematodes are soil-inhabiting microscopic roundworms tht feed on plant roots and are responsible for heavy yield losses. The assessment of the Society of Nemtologist Committee on crop losses indicates annual losses in the United States due to plant parasitic nemtodes to the tune of $ 1,038,374, 300 in field crops, 225, 145, 900 in fruit and nut crops, $ 266, 989, 100 in vegetable crops and $ 59, 817, 634 in ornamental crops[1].

In India plant parasitic nematodes *Meloidogyne incognita* and *Meloidogyne javanica* are the most abundant in the plains[2]. Yadav and Naik[3] has earlier reported nematode *Meloidogyne* as the most abundant followed by other phytonematodes in Rajasthan. The use of methyl bromide for nematode control as a soil fumigant was suggested long back[4,5,6]. With the use of methyl bromide in field as well as under green house conditions, Taylor[7] found good control of roo-knot nematodes.

Kochansky and Feldmesser[8] revealed the structure-activity studies on nematicidal activity of dialkyl carbamates and thiocarbamates. The effect of carbamate, organophosphate and avermectin nematicides on oxygen consumption by three *Meloidogyne* spp. was studied by Nordmeyer and Dickson[9].

In the present studies biological activity of tin and manganese complexes are tested to control nematode *Meloidogyyne incognita* in *in vitro* conditions.

Materials and Methods

Part-I (Chemical)

Synthesis of Ligand : All the chemicals were dried and purified before use and the reaction were carried out with a distillation assembly, fitted with condenser and protected from moisture using $CaCl_2$ drying tubes.

Sulphonamide imine ligands were prepared by condensation of reactants with different sulpha drugs in 1:1 molar ratio in alcoholic medium. The contents were refluxed for 5-6 hours, on water bath. After cooling (overnight) fine crystals of ligand were obtained, recrystallised from the same solvent and dried under reduced pressure.

Synthesis of Manganese (II) Complexes[10,11]: Exact amount of the manganese chloride or acetate (hydrous) was mixed with the imines in uni or bimoloar ratios. Contents were refluxed for 15-16 hours by column method. After reaction the solvent of R. B. flask continuously removed by ration-head. Product was dried *in vacuo* after being repeatedly washed with dry cyclohexane. At last the compound were recrystallized in methanol.

$$[Mn(X)_2 . 4H_2O]+SBH \xrightarrow{1:1} [Mn(X)(SB) H_2O]+ XH+3H_2O$$

$$[Mn(X)_2 . 4H_2O]+2SBH \xrightarrow{1:2} [Mn(SB)_2] +2XH+4H_2O$$

X=Cl or CH_3COO and SB = anion of the ligand molecule HSB.

Synthesis of Organotin (IV) Complexes : A calculated amount of sodium salt of the ligand in dry methanol was added to the organotinhalide in 1:1 and 1:2 molar ratios. The mixture was refluxed over a ratio-head for 15-16 hours and the white precipitate of sodium chloride obtained, was removed under suction.

Compounds were dried under reduced pressure for 3-4 hours. These were purified by repeated washing with n-hexane and ether. All the compounds were isolated as powdered solids

$$[R_2SnCl_2]+SBNa \xrightarrow{1:1} [R_2SnCl(SB)] + NaCl$$

$$[R_2SnCl_2]+2SBNa \xrightarrow{1:2} [R_2Sn(SB)_2] + 2NaCl$$

R= CH_3 or C_6H_5

$$[Ph_3SnCl]+SBNa \xrightarrow{1:1} [Ph_3Sn(SB)] + NaCl$$

Part-II (Botanical)

Preparation of inoculum : The pure culture of nematode *Meloidogyne incognita* chitwood was maintained and multiplied on brinjal plants. For this soil was autoclaved, filled in earthen pots and seedsowing of brinjal plants was done. Fertilizer used was Hoagland's complete nutrient solution, 25 ml/pot once a week. Egg masses were isolated in sterile water and the eggs were allowed to incubate in a Baermann funnel for 48-72 hours. As the juveniles hatched out of eggs, they passed through the double layers of tissue paper and collected in a tube below. The suspension was diluted with sterile water, stirred with a magnetic stirrer for obtaining a homogenous suspension, 5ml of it contained the desired number of juveniles. Nematode inoculation was done when brinjal seedlings were 2 weeks old, by pipetting and pouring 5ml of juvenile suspension in three holes made around the base of seedlings, afterwards the holes were plugged with soil.

Isolation of Eggmasses : After 60 days brinjal plants were uprooted, galls with eggmasses were washed with sterile water. Eggmasses were hand picked out and put in the watchglasses, having distill water. In each eggmass, eggs were counted with the help of nematode counting dish. These eggs were further treated with different ppm of tin alongwith untreated control.

Treatment : Organotin (IV) complexes with 25, 50 and 100 ppm concentration and manganese (II) complexes with only 100 ppm concentration were taken. Solvent used is methanol and since methanol is a evaporating solvent, some water was mixed in solution of complexes and these two also are non-toxic for plants. Each treatment was replicated four times and mean of four such readings was taken. The experiment was conducted at room temperature 30±2°C. Two hundred eggs of *M. incognita* were used per replicate sample.

The method followed for obtaining quantities of clean *Meloidogyne incognita* eggs was that of Mc. clure *et. al.*[12] and the step by step procedure was as follows :

1. Brinjal plants infected with *M. incognita* were harvested from infested pots. The roots were washed thoroughly and cut into small 1-2 cm pieces.
2. The chopped pieces were placed in a beaker in 100ml of tap water, 500ml of 1% NaOCl added and the suspension was vigorously shaken for 5 minutes.
3. After a vigorous shake, the suspension was poured quickly through nested 150 and 400 mesh sieves. The egss which were retained on the 400 mesh sieve were washed with sufficient quantity of distilled water.
4. Eggs which passed through the 400 mesh sieve (pore size 37 μm) were recovered by repeated sieving and rinsing.
5. Eggs were eluted from the sieves and transferred to 40 ml of water.
6. A centrifuge tube was two third filled with 20% sucrose solution and the egg-water suspension was centrifuged at 500g for 5 minutes.

7. A silver layer containing the suspended eggs at the junction of sugar solution and egg suspension was removed with the help of a pipette and quickly poured on to a 400 mesh sieve.
8. The eggs retained on the sieve were washed three times with distilled water thoroughly and collected in a beaker.
9. The eggs obtained by this method were free from debris and therfore easy to count.

In each nematode hatching dish 200 eggs were taken and treated with the treatment. The number of juvenils were counted after24, 48 and 72 hours. After 72 hours, sieves containing the unhatched eggs were removed from the test solution, washed thoroughly with distill water and left in distill water for 24 hours to record further hatching if any.

Results and Discussion

All results are shown in Tables 1 to 5. The results revealed that:

Table 1 : Nematicidal screening data of metal complexes with different ligands, hatching parentage after 24 hours, (conc. 100 ppm)

Ligand	Unimolar Manganese Complexes		Bimolar Manganese Complexes
HSB_1 15.0	$[Mn(CH_3COO)(SB_1)H_2O]$ 13.8	$[MnCl\ (SB_1)\ H_2O]$ 14.0	$[Mn(SB_1)_2]$ 12.0
HSB_2 16.3	$[Mn(CH_3COO)(SB_2)H_2O]$ 15.2	$[MnCl\ (SB_2)\ H_2O]$ 15.1	$[Mn(SB_2)_2]$ 12.4
HSB_3 15.9	$[Mn(CH_3COO)(SB_3)H_2O]$ 15.0	$[MnCl\ (SB_3)\ H_2O]$ 15.5	$[Mn(SB_3)_2]$ 12.0
HSB_4 13.1	$[Mn(CH_3COO)(SB_4)H_2O]$ 12.0	$[MnCl\ (SB_4)\ H_2O]$ 12.5	$[Mn(SB_4)_2]$ 10.5
HSB_5 14.2	$[Mn(CH_3COO)(SB_5)H_2O]$ 13.8	$[MnCl\ (SB_5)\ H_2O]$ 13.9	$[Mn(SB_5)_2]$ 13.0
HSB_6 15.0	$[Mn(CH_3COO)(SB_6)H_2O]$ 14.4	$[MnCl\ (SB_6)\ H_2O]$ 14.7	$[Mn(SB_6)_2]$ 13.4

Table 2 : Nematicidal screening data of metal complexes with different ligands, hatching parentage after 24 hours, (conc. 25 ppm)

Ligand	Unimolar Tin Complexes		
HSB_1 22.1	$[Me_2SnCl\ (SB_1)]$ 18.4	$[Ph_2SnCl\ (SB_1)]$ 16.9	$[Ph_3Sn\ (SB_1)]$ 14.2
HSB_2 23.7	$[Me_2SnCl\ (SB_2)]$ 20.3	$[Ph_2SnCl\ (SB_2)]$ 18.2	$[Ph_3Sn\ (SB_2)]$ 16.1
HSB_3 24.3	$[Me_2SnCl\ (SB_3)]$ 20.5	$[Ph_2SnCl\ (SB_3)]$ 19.1	$[Ph_3Sn\ (SB_3)]$ 16.4

Table 3 : Nematicidal screening data of metal complexes with different ligands, hatching parentage after 24 hours, (conc. 50 ppm)

Ligand	Unimolar Tin Complexes		
HSB_1 17.5	$[Me_2SnCl\ (SB_1)]$ 14.0	$[Ph_2SnCl\ (SB_1)]$ 13.9	$[Ph_3Sn\ (SB_1)]$ 12.6
HSB_2 19.4	$[Me_2SnCl\ (SB_2)]$ 15.9	$[Ph_2SnCl\ (SB_2)]$ 15.7	$[Ph_3Sn\ (SB_2)]$ 11.3
HSB_3 20.0	$[Me_2SnCl\ (SB_3)]$ 16.6	$[Ph_2SnCl\ (SB_3)]$ 15.9	$[Ph_3Sn\ (SB_3)]$ 11.8

Table 4 : Nematicidal screening data of metal complexes with different ligands, hatching parentage after 24 hours, (conc. 25 ppm)

Ligand	Bimolar Tin Complexes	
HSB_1 22.2	$[Me_2Sn(SB_1)_2]$ 15.5	$[Ph_2Sn(SB_1)_2]$ 12.1
HSB_2 23.8	$[Me_2Sn(SB_2)_2]$ 17.2	$[Ph_2Sn(SB_2)_2]$ 13.7
HSB_3 24.2	$[Me_2Sn(SB_3)_2]$ 18.0	$[Ph_2Sn(SB_3)_2]$ 14.5

Table 5 : Nematicidal screening data of metal complexes with different ligands, hatching parentage after 24 hours, (conc. 50 ppm)

Ligand	Bimolar Tin Complexes	
HSB_1 17.4	$[Me_2Sn(SB_1)_2]$ 11.9	$[Ph_2Sn(SB_1)_2]$ 9.5
HSB_2 19.3	$[Me_2Sn(SB_2)_2]$ 13.2	$[Ph_2Sn(SB_2)_2]$ 10.7
HSB_3 20.1	$[Me_2Sn(SB_3)_2]$ 13.5	$[Ph_2Sn(SB_3)_2]$ 10.5

(1) The metal chelates are more active than their parent ligands.

(2) Tin complexes are more toxic than the manganese complexes.

(3) By increasing the concentration of solution, activity increase.

(4) Bimolar complexes are more active than the unimolar complexes.

(5) $Ph_3Sn(SB)$ complexes are more active in all unimolar tin complexes.

(6) Comparatively $Ph_2Sn(SB)_2$ complexes are more toxic than $Me_2Sn(SB)_2$ in bimolar tin complexes.

(7) $[Mn(CH_3COO)(SB)H_2O]$ complexes are more active than $[MnCl(SB)H_2O]$ in unimolar manganese complex.

Theory of action : These chemicals act by impairing nematode neuro-muscular activity, thereby reducing their movement, invasion, feeding and consequentially the rate of development and reproduction[13,14]. However at substantial rates the chemicals acted against the root-knot nematode by inhibiting egg hatching, their movement and host invasion by infective juveniles and checked further development of second stage juveniles.

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