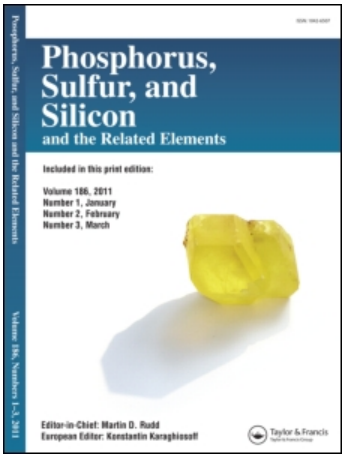

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NEMATICIDAL, INSECTICIDAL, ANTIFERTILITY, ANTIFUNGAL AND ANTIBACTERIAL ACTIVITIES OF SALICYLANILIDE SULPHATHIAZOLE AND ITS MANGANESE, SILICON AND TIN COMPLEXES

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Mukta Jain,[a] *Shweta Gaur,*[a] *S. C. Diwedi,*[b] *S. C. Joshi,*[c] *R. V. Singh,*[a] *and Anil Bansal*[d]
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*A facile synthesis and studies on the stereochemistry and biochemical aspects of some organosilicon(IV), organotin(IV), and manganese(II) complexes derived from imine having N∩N∩O donor system is reported. The imine was prepared by the condensation of salicylanilide with sulphathiazole. This imine reacts with organosilicon(IV)chloride, organotin(IV)chloride, and hydrated manganese(II) chloride to yield compounds having M—O and M←N bonds. The structures of the compounds have been elucidated by physicochemical and spectral (IR, ^{1}H NMR, ^{13}C NMR, ^{29}Si NMR, ^{119}Sn NMR, and ESR) studies, which clearly point to a trigonal bipyramidal geometry around silicon(IV) and tin(IV), and tetrahedral geometry around manganese(II), as the active lone pair of the nitrogen is also included in the coordination sphere. In the search for better fungicides and bactericides, studies were conducted to assess the growth-inhibiting potential of the synthesized complexes against various pathogenic fungal and bacterial strains. These complexes are highly active against nematode (*Meloidogyne incognita*) and insect (*Trogoderma granarium*). The activity will be increased with increasing concentration. These studies demonstrate that the concentrations reached levels that are sufficient to inhibit and kill the pathogens. All compounds have also been found to act as sterilizing agents by reducing the production of sperm in male mice.*

Keywords: Insecticides; manganese complexes; nematodes; pesticides; silicon complexes; tin complexes

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INTRODUCTION

Imines bonding through nitrogen[1] and oxygen atoms of biologically active ligands provide models for metal ligand-binding sites in several enzymes. Considerable importance has been given to the metal complexes of these ligands on account of their biological properties. They are known to function as antimicrobial, antifertility, antimalarial, and antileukemic agents.[1] The sulphonamides have long been known to possess conspicuous biocidal activity, and the ligands derived from them also inherit these characteristics. The heterocyclic compounds with both sulphur and nitrogen atoms in the ring system have also been used in the synthesis of biologically active complexes.

The rapid rise in the industrial, agricultural, biological, and medical applications of organosilicon(IV),[2] organotin(IV),[3] and manganese(II)[4] compounds during the last few decades have led to their accumulation in the environment and in biological systems. Hydrated manganese(II), organotin(IV), and organosilicon(IV) complexes with sulphonamide imines having N$^{\cap}$N donor system have been described in the literature[5–12] for their high activity/toxicity against the root-knot nematode *Meloidogyne incognita*, pathogenic fungi, viz. *Aspergillus niger*, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Alternaria Alternata*, and bacteria, viz. *Escherichia coli*, *Klebsiella aerogenous*, *Pseudomonas cepacicola*, and *Staphylococcus aureus*. The biochemistry of synthetic organometallics has been a subject of active research, and its importance may be judged by the large number of articles in the literature[13] relating to their biochemical significance. However, it is noteworthy that the biological activity gets enhanced on undergoing complexation with metal ions.[14] Stereochemical and biochemical aspects of some of the organosilicon(IV) and organotin(IV) complexes of salicylanilide and its thiosemicarbazone have been described.[15]

EXPERIMENTAL

Adequate care was taken to keep the organosilicon(IV), organotin(IV), and manganese(II) complexes, chemicals, and glass apparatus free from moisture. Clean and well-dried glass apparatus fitted with quickfit interchangeable standard ground joints was used throughout the experimental work. All the chemicals and solvents used were dried and purified by standard methods.

Preparation of the Ligand

The synthesis of this ligand was carried out by dissolving salicylanilide in water and sulphathiazole in alcohol. The solutions were then mixed

together and refluxed for five/six hours. After cooling, the precipitate of sulphonamide imine was purified by recrystallization from the same solvent. The analysis and physical properties of this ligand are given in Table I.

Synthesis of the Organometal(IV) Complexes

For the preparation of these complexes, a calculated amount of Me_2SiCl_2, Ph_2SiCl_2, Ph_3SiCl, Me_2SnCl_2, Ph_2SnCl_2, and Ph_3SnCl in about 30 ml of methanol in a round bottom flask were taken, and a requisite amount of sodium salt of the ligand in the same solvent was added to this solution. The contents were refluxed over a fractionating column for 12–14 h. After the completion of the reaction, the excess of the solvent was removed and NaCl was precipitated out and removed by filtration. The complexes were subsequently dried for 3–4 h and then repeatedly washed with methanol and n-hexane so as to ensure their purity and then dried a second time under reduced pressure. The synthetic details and elemental analyses of the resulting silicon complexes are listed in Table I.

Synthesis of the Manganese(II) Complexes

Hydrated manganese dichloride $MnCl_2 \cdot 4H_2O$ was dissolved in methanol, and the methanolic solution of the ligand was added to it in 1:1 molar ratio. The mixture was heated under reflux for 10–12 h. The solvent was removed and the products obtained were washed with n-hexane and dried in vacuo. The details of these reactions and the analyses of the resulting products are recorded in Table I.

Based on the coordination sites available in the ligand system, this behaves as bibasic tridentate, as shown in Scheme 1.

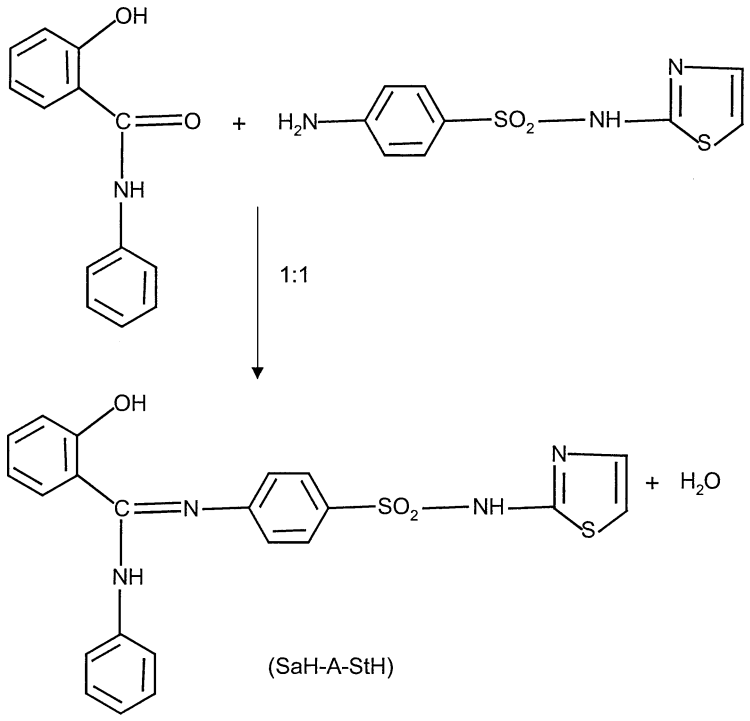
Analytical Methods and Physical Measurements

Nitrogen and sulfur were estimated by the Kjeldahl's and Messenger's methods, respectively. Silicon and tin were determined gravimetrically as SiO_2 and SnO_2. The conductance was measured with a conductivity bridge type 304 Systronics model, and the molecular weights were determined by the Rast Camphor method. Magnetic measurements were carried out at room temperature by the Gouy's method using $NiCl_2$ solution as the calibrant.

Infrared (IR) spectra were recorded on a Perkin-Elmer 577 Grating Spectrophotometer in the range 4000-200 cm^{-1}, as Nujol mulls using CsI Cell. ^1H NMR spectra were recorded in DMSO-d_6, ^{13}C and $^{29}Si/^{119}Sn$

TABLE I Physical Properties and Analytical Data of Salicylanilidesulphathiazole and its Manganese, Silicon and Tin Complexes

Reagent (g)			Formula	M.P. (°C)	Yield (%)	Analysis % found (Calcd.)					Mol. wt. found (calcd.)
						C	H	N	S	M	
(SaH-A-StH)	—	—	$C_{22}H_{18}N_4O_3S_2$	109–111	80	58.39	3.85	12.19	14.06	—	432
			White solid			(58.65)	(4.02)	(12.43)	(14.23)		(450.52)
Ph_3SiCl	(SaH-A-StH)	Na	$C_{40}H_{32}N_4O_3S_2Si$	102–104	75	67.50	4.29	7.48	8.78	3.64	684
0.60	0.92	0.05	Peach solid			(67.77)	(4.54)	(7.90)	(9.04)	(3.96)	(708.91)
Me_2SiCl_2	(SaH-A-StH)	Na	$C_{24}H_{22}N_4O_3S_2Si$	105–107	74	56.60	4.14	10.87	12.41	5.28	486
0.29	1.02	0.05	Cream solid			(56.89)	(4.37)	(11.05)	(12.65)	(5.54)	(506.66)
Ph_2SiCl_2	(SaH-A-StH)	Na	$C_{34}H_{26}N_4O_3S_2Si$	130–132	76	64.51	3.90	8.49	9.84	4.14	612
0.51	0.91	0.05	White solid			(64.73)	(4.15)	(8.88)	(10.16)	(4.45)	(630.80)
Ph_3SnCl	(SaH-A-StH)	Na	$C_{40}H_{32}N_4O_3S_2Si$	103–105	74	59.82	3.88	6.75	7.79	14.61	776
0.81	0.95	0.05	Cream solid			(60.09)	(4.03)	(7.00)	(8.02)	(14.84)	(799.52)
Me_2SnCl_2	(SaH-A-StH)	Na	$C_{24}H_{22}N_4O_3S_2Sn$	85–87	76	48.02	3.60	9.12	10.52	19.59	585
0.43	0.87	0.04	Cream solid			(48.26)	(3.71)	(9.38)	(10.73)	(19.87)	(597.27)
Ph_2SnCl_2	(SaH-A-StH)	Na	$C_{34}H_{26}N_4O_3S_2Sn$	145–147	72	56.48	3.33	7.49	8.70	16.19	703
0.54	0.71	0.04	White solid			(56.60)	(3.63)	(7.76)	(8.88)	(16.45)	(721.41)
$MnCl_2 \cdot 4H_2O$	(SaH-A-StH)	—	$C_{22}H_{18}N_4O_4S_2Mn$	125–127	79	50.23	3.16	10.32	11.86	10.20	494
0.43	0.97		Light yellow solid			(50.67)	(3.47)	(10.74)	(12.29)	(10.53)	(521.45)



SCHEME 1 Ligand synthesis.

NMR spectra were recorded in methanol, using TMS as the internal/external standard for ^{1}H, ^{13}C, ^{29}Si NMR, and ^{119}Sn NMR spectra.

RESULTS AND DISCUSSION

The reactions of triphenylchlorosilane, diphenyldichlorosilane, dimethyldichlorosilane, triphenyltinchloride, diphenyltindichloride, and dimethyltindichloride with the sodium salt of salicylanilidesulphathiazole in 1:1 molar ratio in perfectly dry methanol give products of the types $R_2M(N^{\cap}N^{\cap}O)$ or $R_3M(HN^{\cap}N^{\cap}O)$. The reactions proceed smoothly with the precipitation of NaCl. These reactions can be represented by the following equations:

$$R_2MCl_2 + HN^{\cap}N^{\cap}OH \xrightarrow[MeOH]{Na} R_2M(N^{\cap}N^{\cap}O) + 2NaCl$$

$$Ph_3MCl + HN^{\cap}N^{\cap}OH \xrightarrow[MeOH]{Na} Ph_3M(NH^{\cap}N^{\cap}O) + NaCl$$

where HN∩N∩OH is the donor system of the ligand molecule, M = Si or Sn, and R = Me or Ph. The resulting complexes have been obtained as colored solids soluble in most of the common organic solvents. Complexes were found to be monomeric, as evidenced by their molecular weight determinations. The low value of molar conductivity (17–27 ohm^{-1} cm^2 mol^{-1}) of the resulting metal complexes in anhydrous DMF shows them to be nonelectrolytes in nature. The mode of bonding in these metal chelates has been proposed on the basis of infrared and NMR spectral evidences.

IR Spectra

The IR spectrum of the ligand exhibits a strong band due to ν(C═N) at 1615 cm^{-1}, which shifts to lower frequency side, showing the coordination through the azomethine nitrogen. Two broad bands at 3100–3410 cm^{-1} due to ν(OH/NH) arise in the spectrum of the ligand. The band due to ν(OH) is absent in the metal complexes, while that due to ν(NH) remains at the same position in case of triphenyl complexes, showing the bonding through the oxygen atom[8] and noninvolvement of NH group in bonding, respectively. In the IR spectra of dimethyl and diphenyl metal complexes the bands due to ν(NH/OH) remain absent, showing the bonding of phenolic oxygen and (NH) nitrogen to the metal atom after the deprotonation of the functional groups. New bands in the regions 418–423 cm^{-1}, 527–532 cm^{-1}, 580–585 cm^{-1}, and 620–623 cm^{-1} are due to ν(Sn←N), ν(Sn—O), ν(Si←N), and ν(Si—O) modes, respectively, which further support the coordination through azomethine nitrogen to the metal atom and bonding of oxygen with the metal atom.[8–10]

Reaction of hydrated Manganese(II) chloride with bibasic tridentate sulphonamide imine in unimolar ratio in methanol proceeds as depicted below.

$$MnCl_2 \cdot 4H_2O + HN{\cap}N{\cap}OH \xrightarrow[MeOH]{1:1} [Mn(H_2O)(N{\cap}N{\cap}O)] + 3H_2O + 2HCl$$

This reaction is quite facile and the yield is almost quantitative. The resulting complex is colored solid, and its composition is supported by the elemental analyses. The nonelectrolytic nature of the complex has been proposed on the basis of molar conductance value, which is 15 ohm^{-1} cm^2 mol^{-1}. The molecular weight determinations show the monomeric nature of the complex. This is soluble in methanol, DMF, and DMSO.

On complexation the bands due to ν(OH) and ν(NH) disappear, indicating chelation through phenolic oxygen and nitrogen of (—NH—). New bands at 465 cm^{-1} and 372 cm^{-1} due to ν(Mn—O) and ν(Mn←N)

appear in the spectrum of the complex, also indicating the pattern of complexation.[6] A new band also appears at *ca* 700 cm^{-1}, which is due to the coordinated water molecule.

^{1}H NMR Spectra

The proton magnetic resonance spectral data of the ligand and its corresponding metal complexes have been recorded in DMSO-d_6. The ^{1}H NMR spectrum of the ligand shows a signal at δ 10.28 ppm due to NH proton, which remains in the case of triphenyl metal complexes at almost the same position, showing noninvolvement of NH group in bonding. A signal at δ 12.10 ppm due to OH proton disappears in the spectra of the complexes, indicating the bonding through oxygen to the metal atom. However, in case of diorganometal complexes, disappearance of NH and OH signals in the organometallic derivatives indicates the coordination of the azomethine nitrogen as well as covalent bond formation between metal and oxygen and nitrogen due to the deprotonation. Further, in the spectra of the metal complexes a downfield shift (δ 8.30–7.10 ppm) in the position of the aromatic protons also indicates the coordination of azomethine nitrogen to the metal atom.

^{13}C NMR Spectra

The ^{13}C NMR spectral data for the ligand and its metal complexes in MeOH support the coordination of salicylanilidesulphathiazole through the azomethine nitrogen.[11,12] The shifts in the positions of carbon atoms adjacent to atoms involved in complex formation indicate the bonding pattern of the complexes. The downfield shift of signals attached to azomethine nitrogen from δ 168.24–162.50 ppm in the spectra of the complexes further supports the involvement of the azomethine group in complexation.

^{29}Si and ^{119}Sn NMR Spectra

In order to confirm the geometry of the complexes, ^{29}Si NMR and ^{119}Sn NMR spectra were recorded in MeOH. Sharp signals at δ −92, −98, and −95 ppm are assigned to $Ph_3Si(HN^{\cap}N^{\cap}O)$, $Me_2Si(N^{\cap}N^{\cap}O)$, and $Ph_2Si(N^{\cap}N^{\cap}O)$ complexes, respectively, which have penta-coordinated states around the silicon atom. Similarly, in case of tin complexes[5–9] sharp signals at δ −145, −120, and −135 ppm are indicating the penta-coordinated state around the tin atom for $Ph_3Sn(HN^{\cap}N^{\cap}O)$, $Me_2Sn(N^{\cap}N^{\cap}O)$, and $Ph_2Sn(N^{\cap}N^{\cap}O)$ type of complexes, respectively.

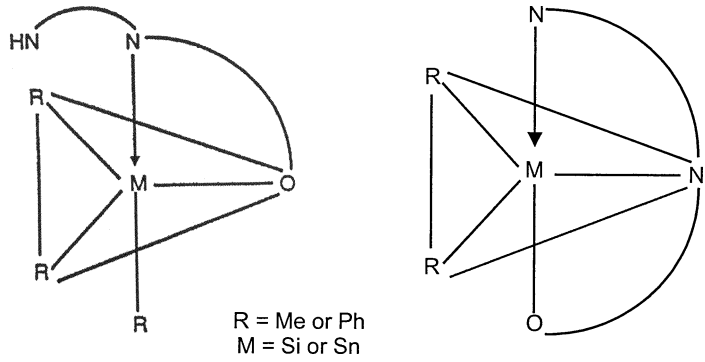


FIGURE 1 Structures of the complexes.

On the basis of the results so far discussed, including the analytical as well as spectral data, suitable penta-coordinated trigonal bipyramidal geometrics have been suggested for the metal complexes (Figure 1).[7–13]

ESR Spectra

The electron spin resonance studies of the Mn(II) complex of salicylanilide sulphathiazole at the room temperature show only one isotropic signal centered at 2.130 g, indicating a tetra-coordinated structure to this complex.[6]

Magnetic Susceptibility Measurements

The magnetic moment value of this complex at the room temperature is 5.8, which suggests a high spin state for this derivatives with central manganese atom being surrounded by tetra-coordinated environment (Figure 2).

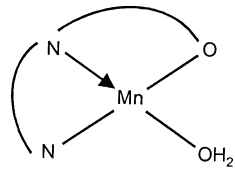


FIGURE 2 Structure of the manganese complex.

TABLE II Antifungal Screening Data of the Ligand and Its Metal Complexes Inhibition (%) After 96 h (Conc. 25, 50, and 100 ppm)

	Aspergillus niger			*Macrophomina phaseolina*			*Fusarium oxysporum*			*Alternaria alternata*		
Compound	25	50	100	25	50	100	25	50	100	25	50	100
(SaH-A-StH)	40	51	70	39	50	67	45	58	65	46	59	67
Me_2Si(Sa-A-St)	45	55	75	44	55	73	49	60	69	49	61	70
Ph_2Si(Sa-A-St)	49	58	78	50	59	76	51	63	75	52	64	74
Ph_3Si(Sa-A-StH)	52	62	81	51	61	79	53	65	77	54	66	77
Me_2Sn(Sa-A-St)	46	57	77	45	57	74	50	62	71	51	61	72
Ph_2Sn(Sa-A-St)	50	59	79	52	61	77	53	64	75	53	65	75
Ph_3Sn(Sa-A-StH)	53	63	82	54	64	81	55	66	78	55	68	79
Mn(Sa-A-St)H_2O	42	54	73	—	—	—	—	—	—	—	—	—
Bavistin	69	86	98	72	82	96	70	91	100	71	86	100

Biocidal Activity

Fungicidal and bactericidal activities of the imine and its metal complexes against pathogenic fungi and bacteria are recorded in Tables II and III.

EXPERIMENTAL

Radial Growth Method for Antifungal Activity[16]

Potato dextrose agar medium (glucose, 20 g; starch, 20 g; agar-agar, 20 g; and 1000 ml of H_2O) (1:1:1:5) was prepared in flask and sterilized. To this medium was added requisite amount of the compound

TABLE III Antibacterial Screening Data of the Ligand and Its Metal Complexes Inhibition (mm) After 24 h (Conc. 500 and 1000 ppm)

	E. coli (–)		*Klebsiella aerogenous* (–)		*Pseudomonas cepacicola* (–)		*Staphylococcus aureus* (+)	
Compound	500	1000	500	1000	500	1000	500	1000
(SaH-A-StH)	8	11	8	12	12	14	12	15
Me_2Si (Sa-A-St)	10	15	9	15	14	15	15	17
Ph_2Si(Sa-A-St)	14	17	12	17	16	17	16	18
Ph_3Si(Sa-A-StH)	15	18	14	18	18	18	17	19
Me_2Sn(Sa-A-St)	12	16	10	17	15	16	15	17
Ph_2Sn(Sa-A-St)	16	18	13	18	17	17	16	19
Ph_3Sn(Sa-A-StH)	17	19	16	19	18	19	18	19
Mn(Sa-A-St)H_2O	9	10	10	11	13	15	14	16
Streptomycin	1	2	3	5	2	5	15	17

after being dissolved in methanol so as to get a certain final concentration. A series of concentrations were prepared. The medium was than poured into the Petri plates and a small disc (0.7 cm) of the fungus culture was cut with a sterile cork borer and transferred aseptically to the center of a Petri dish containing the medium with a certain amount of the compound. Suitable checks were kept so that the culture discs were grown under the same conditions on potato dextrose media (PDA) without the compound. These Petri dishes were wrapped in polythene bags containing a few drops of alcohol and were placed in an incubator at 25 ± 2°C. Three replicates were used in each case. After 96 h the colony diameter compared with check was taken as a measure of fungitoxicity. The amount of growth inhibition was calculated by the equation

$$\% \text{ inhibition} = (dc - dt) \times 100 \times dc^{-1}$$

where dc is the diameter of the fungal colony in control or check plate and dt is the diameter of the fungal colony in test plate.

Paper Disc Plate Method for Antibacterial Activity[17]

Flat-bottomed 90 mm pyrex petridishes were used. Fifteen milliliters of agar medium (peptone, 5 g; beef extract, 5 g; NaCl, 5 g; agar–agar, 20 g; and 1000 ml of distilled water) was pipetted into the petridish. After the agar solidified, 5 ml of warm seeded agar was applied. The seeded agar was prepared by cooling the molten agar to 40°C and then adding the amount of bacterial suspension. The plate was tilted to ensure even coverage before the agar solidifies. These dishes with tops in place are stacked in refrigerator upside down to prevent condensation of moisture. The compounds were dissolved in methanol in 500 and 1000 ppm concentrations.

Paper discs of Whatman No. 1 with a diameter of 5 mm were soaked in these solutions of varied concentrations. The discs were dried and placed on the medium previously seeded with organisms in petri dishes at suitable distances. The petri plates were stored in an incubator at 28 ± 2°C for 24 h. The zone of inhibition that formed around each disc containing the test compound was measured accurately in mm.

Mode of Action[18]

PDA rich in carbohydrates is utilized as the major nutrient source by the microbes with the help of various enzymes (viz., amylase, cellulase, and pectinase). Metal-based fungicides inhibit a wide range of enzymes involved in various metabolic pathways and ultimately cause

cell death. Early work on the mode of action of fungicides showed that these compounds inhibit cell division. It was later shown that specific site of action is β-tubuline, a polymeric protein found in microtubules—essential component of the cytoskeleton. Phenyl and amine groups in complexes affected nucleic acid synthesis and mitochondrial electron transport.

1. *Carbon catabolic regulation*. During the periods of rapid utilization of the carbon source, particularly of glucose or sucrose, either the formations of enzymes in the secondary metabolic pathways leading to toxins would be repressed or the activity of these pathways would be inhibited.
2. *Nitrogen catabolic repression*. Excessive levels of rapidly assimilated forms of nitrogen (e.g., ammonium ion) could repress the formation of enzymes concerned with nitrogen transformation of toxins intermediates.
3. *Feedback regulation*. As toxins accumulate, they would in some instances limit their own biosynthesis by inhibiting the activity of one or more enzymes earlier in their synthetic pathways.
4. *Feed back regulation by primary precursors*. Primary metabolites that are precursors of toxins could act similarly by inhibiting enzymes in primary pathways.
5. *Energy charge regulation*. High phosphate levels could reduce the availability of high-energy phosphate (i.e., ATP and ADP). This would effectively inhibit a number of key reactions in primary metabolism, which in turn would cause a reduction in the activity of secondary pathways linked to the toxin production.
6. *Induction*. The addition of certain primary metabolites (termed effectors) could induce the formation of enzymes in secondary pathways leading to the toxin production.

DISCUSSION

All the metal compounds are more active than the parent ligand against the same microorganism. The increased biocidal activity of the complexes can also be explained by considering the chelation theory.[19] The chelation reduces the polarity of the central ion mainly because of partial sharing of its positive charge with the donor groups and possible π-electron delocalization over the whole chelate ring. Such chelation increases the lipophilic character of the central atom, which subsequently favors its permeation through the lipoid layer of the membrane. It is seen that lower concentration of compounds can check the sporulation

in fungi, and a higher concentration inhibits the growth of organisms almost completely.

The effect of resonating rings on toxicity may be appraised in the light of modern electronic theory. According to Gilman, resonance energy is the energy in excess of the sum of the energy of the separate bonds making up the molecule. The Arrhenius activation theory states that the excess of the molecular energy seems to activate molecules and produce a more rapid rate of chemical reaction. Resonating structures such as benzene rings (in present case) may serve as powerhouse to activate potentially reactive groupings. If toxicity is dependent on one or more chemical reactions, then any molecule that would increase the rate of chemical reactions must enhance toxicity.[20]

Hydrolysis plays a very vital role in this subject. Fungal and bacterial cells accumulate the water-soluble metal/metalloid complexes, which later dissociate to give free central atom or its complex ion. These ions denature the proteins. Enzymes are proteins, and it is expected that the central atoms inactivate these catalysts. However, not all enzymes are equally inactivated by low concentrations of these complexes; therefore, low concentration seems to be less effective against growth. Complexes that have amido groups or reactive halogen atom tend to hydrolyse to form compounds that have modified activity spectrum. The active halogen atoms of the complexes get hydrolysed in aqueous suspensions. The halogen is replaced by hydroxyl ion, and as a result of slight alkaline pH an increase in activity was observed.

The introduction of a lipophilic substituent,[21] either aryl or alkyl, often conferred toxicity, as did the substitution of polar groups such as, $-NH_2$, $-NO$, $-OH$, and $-SH$. A combination of lipophilic and lipophobic groups was occasionally more active than either alone.

It is interesting to see that among all metal complexes the triphenyltin(IV) derivatives with the present ligand are the most active ones in inhibiting the growth of microorganisms, and manganese(II) derivative is the list active. The results of biological activity have been compared with the conventional fungicidal *Bavistin* and conventional bactericide *Streptomycin*, taken as standards in either cases.

Nematicidal Activity

In India plant parasitic nematodes *Meloidogyne incognita* and *M. javanica* are the most abundant in the plants. *M. incognita* produces galls on the roots of many host plants and is responsible for 44.87% yield loss in brinjal. The root-knot nematode (**M. Spp.**) produces galls on the root of many vegetables crops, pulses, some of the fruit crops, tobacco, ornamental crops, and causes severe losses. The avoidable yield

losses due to *M. incognita* were estimated to be 28.08, 33.68, 43.48, and 28.60% in Okra, brinjal, french been, and cowpea, respectively.

Nematodes cause heavy economic losses to plants. Estimated overall average yield loss on the world's major crop due to damage by plant parasitic nematodes is 12.3%.[22–25]

The method followed by obtaining quantities of clean *M. incognita* eggs was that of Jain et al.,[26] and the step-by-step procedure is as follows:

1. Brinjal plants infected with *M. incognita* were harvested from infected pots. The roots were washed thoroughly and cut into small 1–2 cm pieces.
2. The chopped pieces were placed in a beaker in 100 ml of tap water, 500 ml of 1% NaOCl was added, and the suspension was vigorously shaken for 5 min.
3. After a vigorous shake, the suspension was poured quickly through nested 150 and 400 mesh sieves. The eggs that were retained on the 400 mesh sieve were washed with a sufficient quantity of distilled water.
4. Eggs that 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-thirds filled with 20% sucrose solution, and the egg–water suspension was centrifuged at 500 g for 5 min.
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 onto 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 therefore easy to count.

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

Water and methanol solutions were used for this experiment. Each treatment was replicated four times and the mean of four such readings was taken. The experiment was conducted at room temperature, 30 $\pm$ 2°C. Two hundred eggs of *M. incognita* were used per replicate sample. The indirect nematostatic effects of nonfumigant nematicides resulting

from impairment of neuromuscular activity interfere with movement, feeding, invasion, development, reproduction, fecundity, and hatching of nematodes is considered more important than their direct killing action, and hence much smaller amounts of nonfumigant than fumigant nematicides are needed in plant protection against nematodes.[27]

Antifertility Activity

Adult colony-bred healthy Wistar rats of proven fertility weighing 200–250 g were used for this study. The animals were maintained under controlled temperature (22 ± 3°C) and constant photoperiodic conditions (12 h light:12 h dark cycle). Commercial rat food pellet (Lipton, India Ltd.) and water were available ad libitum. The rats were divided randomly into 9 groups containing 6 animals each. Group A served as vehicle (olive oil)-treated controls. In group B the ligand salicylanilidesulphathiazole 50 mg/kg b. wt. suspended in olive oil was given orally for a period of 60 days. Animals of groups C, D, E, F, G, H, and I received similar doses of salicyalanilide–sulphathiazole metal complexes for 60 days.

The animals were screened for fertility test and were weighted and autopsied for detailed biochemical studies on the last day after final dosing. Testes and other reproductive organs were dissected out and weighted. Sperm motility, sperm density, sialic acid, total protein, cholesterol, fructose, and acid and alkaline phosphatase were estimated by standard laboratory techniques.

Male rats exposed to the ligand and its magnesium, tin, and silicon complexes showed altered reproductive activity.

Body and Organ Weight

There were no significant differences in the body weight of the treated groups as compared with control at the end of the experimental period. However, the weights of testes, epididymis, seminal vesicle, and ventral prostate were decreased significantly in the ligand ($P \leq 0.01$) and its various complexes ($P \leq 0.01$ to 0.001).

Sperm Dynamics and Fertility

The sperm motility in cauda epididymis was decreased significantly in ligand ($P \leq 0.01$) and its complexes treated animals ($P \leq 0.001$), as shown in Table IV. Also, a significant decrease in sperm density in testes and cauda epididymis ($P \leq 0.01$ to 0.001) were observed in ligand and its various complexes treated rats.

TABLE IV Altered Sperm Dynamics and Fertility After Treatment with the Ligand and Its Manganese, Silicon, and Tin Complexes

Compound	Sperm motility caudal epididymis	Sperm density (million/ml)		Fertility Test (%)
		Testes	Epididymis	
A Control	70.0 ± 6.10	1.90 ± 0.20	51.0 ± 1.85	98 + ve
B (SaH-A-StH)	60.0 ± 3.0^a	1.08 ± 0.10^a	42.0 ± 1.70^a	65 − ve
C $Mn(Sa\text{-}A\text{-}St)H_2O$	45.0 ± 5.0^b	0.81 ± 0.11^a	30.0 ± 1.70^b	75 − ve
D $Me_2Si(Sa\text{-}A\text{-}St)$	46.0 ± 6.0^b	0.82 ± 0.11^a	31.0 ± 1.80^b	81 − ve
E $Ph_2Si(Sa\text{-}A\text{-}St)$	40.0 ± 7.0^b	0.72 ± 0.10^b	30.0 ± 1.90^b	90 − ve
F $Ph_3Si(Sa\text{-}A\text{-}StH)$	41.0 ± 8.0^b	0.70 ± 0.20^b	28.0 ± 1.60^b	96 − ve
G $Me_2Sn(Sa\text{-}A\text{-}St)$	38.0 ± 6.0^b	0.61 ± 0.18^b	25.0 ± 1.80^b	95 − ve
H $Ph_2Sn(Sa\text{-}A\text{-}St)$	35.0 ± 7.0^b	0.51 ± 0.20^b	22.0 ± 1.30^b	98 − ve
I $Ph_3Sn(Sa\text{-}A\text{-}StH)$	30.0 ± 7.1^b	0.35 ± 0.10^b	20.5 ± 1.60^b	100 − ve

Values are mean ± SEM six determinations.

a = $P \leq 0.01$; b = $P \leq 0.001$.

Group A compared with Group B. Group C compared with Group B. Groups D, E, F, G, H, and I compared with Group B.

Biochemical Changes

Marked reductions ($P \leq 0.01$ to 0.001) in sialic acid and protein contents of testes, epididymis, ventral prostate, and seminal vesicle were observed in the ligand and its complexes treated animals when compared with control (Table V). However, a sharp increase in testicular cholesterol, and acid and alkaline phosphatase contents were observed in various treated groups. Seminal vesicular fructose contents were decreased significantly (Table V).

In the present study, ligand and its complexes were administered to rats at the dose levels 9–50 mg/kg/day for 60 days which brought about marked alterations in the weights of testes, epididymis, seminal vesicle, and ventral prostate. Significant decline in the testes weight may be due to the decrease in number of spermatogenic elements and spermatozoa.[28] Reduction in the weights of sex accessory organs directly support the reduced availability of androgens.[29] Suppression of gonadotropins might have caused decrease in sperm density in testes.[30] Low caudal epididymal sperm density may be due to alterations in androgen metabolism,[31] and 65–100% negative fertility may be attributed to lack of forward progression and reduction in the density of spermatozoa and altered biochemical milieu of cauda epididymis. Decline in total protein concentration in testes and other accessory reproductive organs indicated suppressed androgen activity.[32] Furthermore, reduced contents of sialic acid in various reproductive organs reported herein suggest adverse effects on the metamorphosis and maturational

TABLE V Effects of the Ligand and its Manganese, Silicon, and Tin Complexes on Tissue Biochemistry

Compound	Total protein (mg/g)				Sialic acid (mg/g)				Cholesterol (mg/g) testes	Fructose (mg/g) seminal vesicle	Phosphatases (mg/ip liberated/hr/mg tissue)	
	Testes	Epididymis	Seminal vesicle	Ventral prostate	Testes	Epididymis	Seminal vesicle	Ventral prostate			Acid	Alkaline
A Control	25.0 ± 17.0	230.0 ± 20.0	240.0 ± 18.0	220.0 ± 15.0	7.6 ± 0.7	6.7 ± 0.5	6.9 ± 0.4	6.9 ± 0.6	7.7 ± 0.2	460.0 ± 30.0	2.9 ± 0.18	10.5 ± 0.65
B (SaH-A-StH)	210.0 ± 15.0[a]	180.0 ± 10.0[a]	190.0 ± 10.0[a]	170.0 ± 10.0[a]	6.6 ± 6.3[a]	5.1 ± 0.3[a]	5.2 ± 0.3[a]	5.1 ± 0.5[a]	8.5 ± 0.1[a]	400.0 ± 10.0[a]	3.5 ± 0.10[a]	13.0 ± 0.50[a]
C Mn(Sa-A-St)H_2O	150.0 ± 15.0[b]	135.0 ± 10.0[b]	128.0 ± 10.0[b]	115.0 ± 12.0[b]	4.1 ± 0.2[b]	4.0 ± 0.2[b]	4.2 ± 0.3[b]	3.9 ± 0.2[b]	11.0 ± 0.2[b]	300.0 ± 10.6[b]	4.5 ± 0.20[b]	16.2 ± 0.60[b]
D Me_2Si(Sa-A-St)	152.0 ± 14.0[b]	130.0 ± 15.0[b]	130.0 ± 12.0[b]	110.0 ± 11.0	4.2 ± 0.1[b]	4.1 ± 0.3[b]	4.0 ± 0.1[b]	3.7 ± 0.1[b]	11.5 ± 0.3[b]	280 ± 15.3[b]	4.4 ± 0.18[b]	16.8 ± 0.65[b]
E Ph_2Si(Sa-A-St)	140.0 ± 10.8[b]	127.0 ± 13.0[b]	131.0 ± 12.0[b]	105.0 ± 10.0[b]	4.0 ± 0.2[b]	4.2 ± 0.2[b]	3.9 ± 0.1[b]	3.8 ± 0.2[b]	11.8 ± 0.4[b]	260.0 ± 12.0[b]	4.5 ± 0.11[b]	16.1 ± 0.70[b]
F Ph_3Si(Sa-A-StH)	145.0 ± 10.9[b]	125.0 ± 14.0[b]	118.0 ± 15.0	112.0 ± 13.0	3.8 ± 0.1[b]	3.7 ± 0.1[b]	3.6 ± 0.1[b]	3.3 ± 0.1[b]	11.7 ± 0.3[b]	220.0 ± 15.0[b]	4.3 ± 0.10[b]	16.7 ± 0.50[b]
G Me_2Sn(Sa-A-St)	130.0 ± 9.5[b]	124.0 ± 13.0[b]	120.0 ± 16.0[b]	110.0 ± 14.0[b]	3.9 ± 0.2[b]	3.5 ± 0.1[b]	3.8 ± 0.2[b]	3.1 ± 0.3[b]	11.9 ± 0.2[b]	224.0 ± 17.8[b]	4.8 ± 0.11[b]	17.0 ± 0.60[b]
H Ph_2Sn(Sa-A-St)	125.0 ± 9.7[b]	120.0 ± 14.0[b]	117.0 ± 17.0	108.0 ± 18.0[b]	3.5 ± 0.1[b]	3.4 ± 0.2[b]	3.5 ± 0.3[b]	3.0 ± 0.3[b]	12.1 ± 0.3[b]	200.0 ± 13.0[b]	4.7 ± 0.12[b]	17.1 ± 0.65[b]
I Ph_3Sn(Sa-A-StH)	110.0 ± 5.0	107.0 ± 6.0	108.0 ± 7.0[b]	100.0 ± 17.0[b]	3.0 ± 0.5[b]	3.1 ± 0.6[b]	3.8 ± 0.5[b]	3.2 ± 0.3[b]	12.5 ± 0.8[b]	180.0 ± 15.0[b]	5.0 ± 0.90[b]	18.0 ± 0.90[b]

Values are mean ± SEM six determinations.

a = $P \leq 0.01$; b = $P \leq 0.001$.

Group A compared with Group B. Group C compared with Group B. Groups D, E, F, G, H, and I compared with Group B.

stages of spermatid.[33] The rise in the testicular cholesterol contents due to various compounds treatment suggests suppressed androgen biosynthesis.[34] An increase in testicular acid and alkaline phosphatase activities indicates metabolic disturbance and impairment of the functional integrity of the testes.[35]

Thus the results of present study reveal that the ligand and its manganese, silicon, and tin complexes altered the reproductive function of male rats, and the complexes of tin and silicon are more active than the manganese complex in inhibiting fertility in male rats.

Insecticidal Activity

Many insects cause damage to the stored products and other commodities such as food, grain, paper, books, furniture, timber, and clothing. The greatest importance of insects lies in their being pests of crops and animals. A pest is an animal whose population buildup increases above certain level of economic injury, and its existence conflicts with human welfare, convenience, and profit.[36]

The stock culture was established in the laboratory on the grains of wheat in large glass jars. Inside the jars, 100 pairs of fresh insects were released on disinfested wheat grains. Healthy conditions of the stock culture were maintained by frequent replacement of the state grains with fresh ones. The jars were kept at $35 \pm 2^{\circ}C$ temperature and $60 \pm 10\%$ relative humidity. After the stock culture bloomed to its youth, subsequent cultures were also established by releasing few parts of freshly emerged adult beetles on disinfested wheat grains in smaller glass jars. After allowing 7–8 days for oviposition, beetles were removed. A continuous supply for experimentation was thus maintained by repeating the process every week. Fresh wheat was used for subculture to prevent the food effects. To rule out the possibilities of infection, all the jars and grains were sterilized before use. The insects were transferred with the help of forceps and hairbrushes.

Assessment of the Toxicity of the Chemicals

Ovicidal Action

Eggs were treated by contact method to determine the efficacy of chemicals as ovicide. A thin film of desired concentration was prepared by spreading 1 ml of chemical solutions on Petri dishes (5.0 cm diameter). The solvent was allowed to evaporate. Twenty eggs of 0–24 h were kept in contact with the insecticidal film throughout their incubation period. A control was also run with each set of experiments in which the eggs were kept in 1 ml of solvent. Percent egg mortality and percent

corrected egg mortality was calculated by Abbott's formula.[37]

$$\text{Abbott's Corrected Mortality} = \frac{\%\ \text{Kill in treated} - \%\ \text{Kill in control}}{100 - \%\ \text{Kill in control}} \times 100$$

Larvicidal Action

Larvicidal efficacy of chemicals was assessed by feeding method. First instar larvae separated from subculture were kept in vials containing 5 g of topically treated wheat grains with 1 ml of chemicals. Larvae were allowed to continue their development on this diet until the pupae formation. Each dose was replicated thrice. In the control the food was treated with solvent only. Percent larvae mortality and percent corrected mortality was calculated by Abbott's formula.

Pupicidal Action

Last larval instars were stored out from the subculture and were kept in separate containers on the same rearing media. Pupae of known age (0–12 h) were taken out and were dipped in the desired concentration of chemicals. Three replicates were set for each dose along with a control, and after 96 h, total emergence and pupal mortality was recorded. Percent pupal mortality and pupal corrected mortality was calculated by Abbott's formula.

Adulticidal Action

The adulticidal action was assessed by contact method. Five grams of wheat grains were treated with 1 ml of respective doses. The solvent was allowed to evaporate completely. Experiment was replicated thrice along with a control. Newly emerged adults were taken from the subculture and were released in the plastic vials containing treated food. Observations were taken after 48 h, and percent corrected mortality was calculated by Abbott's formula.

Mode of Action[38,39]

Insecticides act on target organisms in different ways depending on their physical and chemical makeup. Some insecticides are physical poisons causing asphyxiation, some are protoplasmic poisons, and a few are respiratory poisons, but the majority of them are nerve poisons. The action of insecticides upsets the normal behaviour and actions of the target organisms, and the surest and quickest way to achieve this is to poison the nervous system.

Ovicidal Action

It was observed that few egg shells split and undeveloped larvae failed to come out of the eggs. It was also observed that some other treated eggs disfigured and stuck to the surface as dried yellow mass without showing shell splitting. It can be suggested that probably the toxic substances of the chemicals interfere with normal embryonic development, which in turn may result in certain disturbances during process of cell division and blastokinesis, thus exhibiting impressive ovicidal properties against *Trogoderma granarium* (Table VI).

Larvicidal Action

High larvicidal activities were performed by chemicals. It was observed that earlier larval instars were more sensitive than the later instars. This suggests that chemicals penetrate into the insect cuticle. The chemical-treated grains were slowly or much less fed to the larvae of *Trogoderma granarium*, which leads to starvation in the developing larvae.

Pupicidal Action

The pupicidal action of chemicals may be due to the chemical that enters into the puparium and disrupts the normal metabolic activities of the developing insect.

TABLE VI Ovicidal Data of the Ligand and Its Metal Complexes

Compound	Dose level ppm	Average no. of eggs hatched	Average no. of eggs unhatched	% eggs hatched	% eggs unhatched	% corrected mortality
(SaH-A-StH)	100	14	6	70	30	26.31
(SaH-A-StH)	200	12	8	60	40	36.84
[Mn(Sa-A-St)H_2O]	100	14	6	70	30	26.31
[Mn(Sa-A-St)H_2O]	200	11	9	55	45	42.10
Me_2Si(Sa-A-St)	100	12	8	60	40	36.84
Me_2Si(Sa-A-St)	200	8	12	40	60	57.89
Ph_2Si(Sa-A-St)	100	10	10	50	50	47.36
Ph_2Si(Sa-A-St)	200	6	14	30	70	68.42
Ph_3Si(Sa-A-StH)	100	9	11	45	55	52.63
Ph_3Si(Sa-A-StH)	200	6	14	30	70	68.42
Me_2Sn(Sa-A-St)	100	11	9	55	45	42.10
Me_2Sn(Sa-A-St)	200	7	13	35	65	63.15
Ph_2Sn(Sa-A-St)	100	10	10	50	50	47.36
Ph_2Sn(Sa-A-St)	200	5	15	25	75	73.68
Ph_3Sn(Sa-A-StH)	100	8	12	40	60	57.89
Ph_3Sn(Sa-A-StH)	200	4	16	20	80	78.94

TABLE VII Adulticidal Data of the Ligand and Its Metal Complexes

Compound	Dose level ppm	No. of adults in each vial	Average mortality after 48 h	% adult motrality	% corrected mortality
(SaH-A-StH)	100	20	5	25	21.05
(SaH-A-StH)	200	20	7	35	31.57
[Mn(Sa-A-St)H_2O]	100	20	6	30	26.31
[Mn(Sa-A-St)H_2O]	200	20	9	45	42.10
Me_2Si(Sa-A-St)	100	20	8	40	36.84
Me_2Si(Sa-A-St)	200	20	12	60	57.89
Ph_2Si(Sa-A-St)	100	20	10	50	47.36
Ph_2Si(Sa-A-St)	200	20	13	65	63.15
Ph_3Si(Sa-A-StH)	100	20	11	55	52.63
Ph_3Si(Sa-A-StH)	200	20	15	75	73.68
Me_2Sn(Sa-A-St)	100	20	9	45	42.10
Me_2Sn(Sa-A-St)	200	20	13	65	63.15
Ph_2Sn(Sa-A-St)	100	20	11	55	52.63
Ph_2Sn(Sa-A-St)	200	20	14	70	68.42
Ph_3Sn(Sa-A-StH)	100	20	12	60	57.89
Ph_3Sn(Sa-A-StH)	200	20	14	70	68.42

Adulticidal Action

The chemicals that are applied as contact as well as stomach poisons seemed to be the most hazardous to the beetles. When mixed with their food, these chemicals penetrate rapidly through the body wall of insects and thereby obstruct the normal respiratory activities of adults by adversely affecting the spiracles (Table VII). Toxicity in metal complexes increase in this order; manganese < silicon < tin. Dimethyltin and dimethylsilicon complexes are less active than their triphenyltin and triphenyl silicon complexes.

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