



Insecticidal activity of the schiff - base derived from anthranilic acid and acetoacetanilide and its copper complex on *Spodoptera litura* (Fab.)

N.Raman, J.Joseph, S. Muthu Kumar, S.Sujatha and K.Sahayaraj*

ABSTRACT

The schiff - base transition metal complexes are an important field in the coordination chemistry and find extensive application in different fields. The understanding of relationship between metals and various biological systems is a subject of great interest for researchers. Since nobody has utilized these compounds in agriculture sector particularly for insect pest management so far, this investigation deals with the impact of schiff-base derived from anthranilic acid and acetoacetanilide and its copper complex on fourth instar larvae of *Spodoptera litura* (Fab.) (Lepidoptera : Noctuidae) by leaf dip method. Results reveal that schiff-base has more impact ($LD_{50} = 0.01293\%$) than its copper complex ($LD_{50} = 0.03243\%$) at 96 h. In addition to insecticidal activity, these compounds also interfere with the physiology of *S. litura*. We concluded from these studies that both schiff - base and copper complex could be incorporated in the integrated management of *S. litura*.

INTRODUCTION

The common cutworm *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) is widely distributed throughout Japan except for Hokkaido and Northern areas of Honshu, Central and Southern China, other southern Asian countries, Ocean and islands fronting the Pacific Ocean. This pest is a major polyphagous pest, reported to attack a wide range of vegetables, cash and fiber crops. The field efficacy and sustainability of these group insecticides depend on the target pest. The level of susceptibility of field populations of *S. litura* to insecticides differs significantly and constitutes a service threat to crop production in many regions. With the development of high degree of resistance to pyrethroids in *S.litura* over past decade, there has been a shift in the use of different groups of insecticides other than pyrethroids for its management. Among those chemical insecticides constitute 70% and are widely used all over India to control this pest (Anonymus, 1997). Resistance to wide range of insecticides to *S. litura* (Armes *et.al.*, 1997; Kranthi *et al.*, 2001, 2002; Kodandarm and Swaran, 2007) has been reported elsewhere in India.

The literature reveals that schiff - base ligands are excellent coordinating ligands. This ligand system has both nitrogen and oxygen donor sites. During the past three decades, considerable attention has been paid to the chemistry of the complexes of the schiff - base containing nitrogen and other donors. This may be attributed to their stability, biological activity and potential applications in many fields such as oxidation catalysis

(Gaber *et.al* 1989; Thaker *et al.*, 2005), electrochemistry, etc; making the behaviour of schiff - base complexes with transition metals a worthy research topic. In recent years a considerable amount of work has been done on the coordination chemistry of copper(II) complexes with schiff - base ligands to model the physical and chemical behaviour of biological copper systems (Hussain *et al.*, 1999). A literature search revealed that no work had been done on the condensation product of acetoacetanilide and anthranilic acid. In this paper, we describe the synthesis, characterization and insecticidal activity of the schiff - base derived from anthranilic acid and acetoacetanilide and its copper complex on *S. litura* third instar larval mortality (LD_{50}) using leaves dip method.

MATERIALS AND METHODS

Collection and rearing of the pest

Life stages of *S. litura* were collected from the castor fields of Alangulam, Tirunelveli district, Tamil Nadu, India, and reared in the laboratory on castor leaves at $27 \pm 1.5^\circ\text{C}$ and $75 \pm 5\%$ RH. Laboratory emerged adults were maintained on 5% sugar solution. Eggs were maintained in the laboratory conditions. Laboratory emerged third instar *S. litura* (> 6 h) was used for this experiment.

Synthesis of the schiff - base ligand (HL)

An ethanolic solution (50 mL) of anthranilic acid (1.02 g, 5 mmol) and acetoacetanilide (2.03 g, 10 mmol) was boiled under reflux for *ca.* 5 h. The solution was reduced to one-third of its original volume on a water bath and the hot solution was poured into hot water to remove any

Table.1 Components of the probit analysis and LD₅₀ value (in Y.) for the tobacco caterpillar *S. litura* exposed to different concentrates of schiff - base ligand and copper complex.

Compounds	Time after Treatment (h)	Slope (+SE)	LD ₅₀	Fiducial limit		Significance χ^2
				Lower	Upper	
Schiff base ligand	96	0.0152	0.0129	0.0109	0.0142	9.93
	120	0.0201	0.0052	0.0036	0.0092	6.32
	144	0.0425	0.0024	0.0019	0.0038	2.9382
Copper complex	96	0.0678	0.0324	0.0310	0.0346	0.0347
	120	0.0145	0.0087	0.0072	0.0095	0.6876
	144	0.0281	0.0017	0.0013	0.0026	0.4378

unreacted anthranilic acid. The pasty mass obtained after decantation was crystallized from ethanol.

Synthesis of copper complex

The schiff - base (2.97 g, 10 mmol) dissolved in hot ethanol (50 ml) was added to a hot solution of copper (II) chloride (5 mmol) in ethanol (25 ml). The pH of the reaction mixture was adjusted to 6-7 by the addition of aqueous ammonia. The solid complex separated was filtered, washed with ethanol and dried *in vacuo*.

All reagents (Merck) were used as supplied. Microanalytical data, ¹H-NMR and FAB mass spectra of the compounds were provided by the Regional Sophisticated Instrumentation Center, Central Drug Research Institute (RSIC, CDRI), Lucknow. Microanalyses were done using a Carlo Erba 1108 CHN Elemental Analyser. Metal content was determined gravimetrically using the ammonium oxalate method. The ¹H-NMR spectrum of the ligand was recorded at 300 MHz in CDCl₃ using TMS as the internal standard on a Bruker Avance DRX 300 FT-NMR spectrometer. The FAB mass spectrum of the complex was recorded on a JEOL SX 102/DA-6000 mass spectrometer/data system using argon/xenon (6 kV, 10 mA) as the FAB gas. The accelerating voltage was 10 kV and the spectra were recorded at room temperature using *m*-nitrobenzyl alcohol (NBA) as the matrix. The IR spectra of the samples were recorded on a Perkin-Elmer 783 spectrophotometer over the 4000-200 cm⁻¹ range using KBr disks. The UV-Vis. spectra were recorded on a Shimadzu UV-1601 spectrophotometer. Magnetic susceptibility measurement of the complex was carried out by Guoy balance using copper sulphate as the calibrant. The molar conductance of the copper complex was measured using a Systronic conductivity bridge.

Different concentrations (0.002, 0.004, 0.008 and 0.016%) of Schiff base ligand and its copper complex were prepared using distilled water and a drop of Tween.20 was added as an adjuvant. Doses were sprayed on fresh castor leaves and allowed to dry for few minutes. In all experiments a negative control was carried out by leaf dip method of

solvent (water). Three replications for each concentration were maintained.

RESULTS AND DISCUSSION

The caterpillars were considered dead if they displayed no observable response to a mechanical stimulus *i.e.* short term pressure applied with a needle. Mortality rates were recorded for 96, 120 and 144 h after treatment. In this bioassay, a randomized design was performed with 30 larvae for all the concentration. Mortality rate was subjected to corrected mortality and subsequently to probit analysis (Abott's, 1925).

The new tridentate Schiff base ligand, derived from anthranilic acid and acetoacetanilide and its Cu(II) complex have been synthesized as described in the experiment. The syntheses were straightforward and provided good yields of the desired products in pure form. The complex was found to be air stable and insoluble in water, but soluble in DMF and DMSO. The thermal analytical data showed that there were no water molecules in the complex. The elemental analytical data were in good agreement with the molecular formulae arrived for the ligand and its complex. The formulation of the complex was based on IR data, molar conductivity values, elemental analyses, which were in close agreement with the values calculated for the assigned molecular formulae, and on FAB-mass spectra studies. The FAB mass spectrum of the ligand shows a molecular ion peak at *m/z* = 296. Its IR spectrum shows a peak at *ca.* 1604 cm⁻¹ indicating the presence of azomethine group. The peak at 1710 cm⁻¹ is assigned to the vibrational frequency of the acetoacetanilide carbonyl group and the intense peak at 3160 cm⁻¹ is a characteristic feature of an –NH stretching frequency. The sharp bands in the 750-790 and 1520-1540 cm⁻¹ regions are due to aromatic ν C-H and ν C=C, respectively. The band observed at 1165-1175 cm⁻¹ is due to ν C-N. The broad band in the 3000-2800 cm⁻¹ region is due to an OH group.

The ¹H-NMR spectrum of the Schiff base in CDCl₃ at room temperature reinforces the conclusions drawn from the IR spectra. The ligand shows signals at 10.3 (=C-COOH), 8.9 (PhNH), 7.2-7.6 (phenyl multiplet), 3.5 (-C-CH₂) and 1.4

ppm ($-C-CH_3$). The proposed structure of the ligand deduced from the above data is given below:

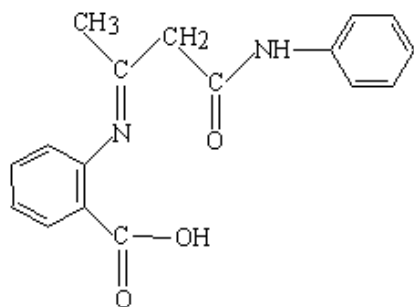


Figure 1. Structure of the Schiff base ligand

The FAB mass spectrum of the ligand shows a molecular ion peak at $m/z = 296$. The FAB mass spectrum of the copper complex shows molecular ion peak at $m/z = 653$ which is further evidence for the 1:2 nature of the complex. The molar conductance of the copper complex measured in DMSO reveals its non-electrolytic nature.

The IR spectrum of the free ligand was compared with the spectrum of the complex in order to study the binding mode of the Schiff base to the copper ion. The band appearing at 1710 cm^{-1} and assigned to the carbonyl group of the acetoacetanilide moiety $\nu(C=O)$ of the free ligand (Mathu *et al.*, 2000; Nakamoto *et al.*, 1977; Manimekalai, *et al.*, 2004), was shifted to lower frequency (1690 cm^{-1}) in the complex, suggesting the involvement of the carbonyl oxygen atom in coordination. Both the ligand and the complex show an intense peak at *ca.* 3160 cm^{-1} , which is a characteristic feature of the $-NH$ stretching frequency, indicating the existence of a free $-NH$ group (Mohapatra *et al.*, 1989; and Koksai *et al.*, 1996). The broad band at $2500\text{-}3000\text{ cm}^{-1}$ due to the carboxylic acid group νOH disappears in the complex and a new peak assigned to coordinated carboxylate appears at $1400\text{-}1600\text{ cm}^{-1}$. The proposed coordination mode is further supported by the presence of a new band at 410 cm^{-1} region, due to $M-O_{\text{carbo}}$ vibration (Raman *et al.*, 2004). The azomethine group, appearing at *ca.* 1604 cm^{-1} region in the free ligand, is shifted to lower frequency (1560 cm^{-1}) in the complex, indicating the involvement of azomethine nitrogen atom in coordination.

The electronic absorption spectra of the schiff - base and its copper complex were recorded in DMSO at 300 K. The absorptions in the ultraviolet region are attributed to transitions within the ligand orbital and those in the visible region are probably due to allowed metal-to-ligand charge transfer transitions. The schiff - base shows two absorptions, 28535 cm^{-1} and 34250 cm^{-1} , which are intra-ligand charge transfer transitions. The UV spectrum of the copper complex displays a broad band in the $12250\text{-}15500\text{ cm}^{-1}$ region, which is assigned as a ${}^2E_g \rightarrow {}^2T_{2g}$

transition, attributable to an octahedral geometry around the central metal ion (Raman *et al.*, 2003). The broadness of the band can be taken as an indication of Jahn-Teller distortion from the regular symmetry. The magnetic susceptibility value (1.9 BM) of the copper complex further indicates the octahedral arrangement of the ligand around the central atom. Based on the above spectral data, the structure of the copper complex was assigned as shown in Fig. 2.

Figure 2. Structure of the copper complex ($M = \text{Cu(II)}$)

The response of *S.litura* to the leaf dip method of Schiff base ligand and copper complex showed a positive relationship between dose and mortality. In a general way, LD_{30} , LD_{50} and LD_{90} values decreased when the time between application and evaluation increased. The data presented confirm that both schiff - base ligand and copper complex have potential insecticide activities. The newer chemicals produced 100% mortality when concentrations of $0.03\text{ }\mu\text{g/ml}$ and $0.032\text{ }\mu\text{g/ml}$ of schiff - base and copper complex were administered to *S. litura* larvae. Moreover anthranilic acid showed higher effectiveness against *S.litura*, as the LD_{50} values were lower than those obtained with copper complex, thus showing the highest susceptibility of the insect to the Schiff base ligand than copper complex. Almost immediately following the application of dose of both Schiff base ligand and copper complex, larval movement decreased and feeding drastically decreased. Also the results show the effectiveness of Schiff base derived from anthranilic acid and acetoacetanilide as insecticide against *S.litura*.

Typical intoxication symptoms such as spasmodic movements, regurgitation and faecal elimination, were observed. Marchini *et al.*, 1992; Navickiene *et al.*, 2007, thus confirming the acute toxicity of these compounds to *S. litura* caterpillar. This simple explanation for the observed insecticidal activity of schiff - base ligand and copper complex against *S.litura* is not the exact reason. However, many impacts took place on *S.litura* make it possible to conclude that Schiff base ligand and copper

complex may be good candidates for use in crop protection. According to Raman *et al.* (2007), the transition metal complexes of the Schiff base derivatives of anthranilic acid and acetoacetanilide bind to DNA in the Cu(II) form and enhances nuclease activity of Thymus DNA (CT-DNA). This character is useful to cause mortality in *S. litura*. The details on toxicity of Schiff - base ligand and copper complex in relation to different hours of exposure to *S. litura* are presented in Table 1. The LD₅₀ values of Schiff base ligand at 96 h are higher (0.0129) than at 120 and 144 h/h. Similar observation was also recorded for copper complex. Based on LD₅₀ values it was concluded that copper complex was more toxicity than Schiff base ligand. Additionally, further evaluation of these compounds on other stages of this pest should be carried out before recommending these as insecticides.

REFERENCES

- Abbots, W. S. 1925. A method of computing the effectiveness of insecticides. *Journal of Economic Entomology*, **18**: 265-267.
- Anonymous. 1997. Organophosphate insecticides –an Indian analysis. *The pesticide world*, **1**: 8-9.
- Armes, N. J., Wightmena, J. A., Jadhav, D. R., and Ranga Rao, G. V. 1997. Status of insecticide resistance *Spodoptera litura* in Andhra Pradesh India. *Pesticide Science*, **50**: 240-248.
- Gaber, M., Issa, R.M., Aly, F.A. and Ayad, M.I. 1989. Studies of Ti (IV) and Zr (IV) chelates with N₂O₂ Schiff bases of 2-hydroxy-1-naphthaldehyde with aromatic diamines. *Thermochim. Acta*, **155**: 309-316.
- Hussain Reddy, K., Sambasiva Reddy, P. and Ravindra Babu, P. 1999. Synthesis spectral studies and nuclease activity of mixed ligand copper(II) complexes of heteroaromatic semicarbazones/thiosemicarbazones and pyridine. *Journal of Inorganic Biochemistry*, **77**: 169-176.
- Kodandaram, M. H., and Swaran Dhingra. 2007. Variation in the toxicity of organophosphate insecticides to field populations of *Spodoptera litura*. *Indian Journal of Plant Protection*, **35**: 153-156.
- Koksai, K. R., Tumer, M. and Serin, S., 1996. Synthesis and characterization of binuclear Cu(II), Ni(II) and Co(II) chelates with tetradentate Schiff base ligands derived from 1,5, diamionaphthalene, *Synthetic Reaction Inorg. Met.-Org. Chemistry*, **26**: 1577-1588.
- Kranthi, K. R., Jadhav, D. R., Kranthi, S., Wanjari, R. R., Ali, S.S. and Russell, D. A. 2002. Insecticide resistance in five major insect pest of Cotton pests in India. *Crop Protection*, **21**: 449-460.
- Kranthi, K. R., Jadhav, D. R., Wanjari, R. R., Ali, S. S. and Russell, D. A. 2001. Carbamate and organophosphate resistance in cotton pests in India 1995 to 1999. *Bulletin of Entomological Research*, **91**: 37-46.
- Manimekalai, A., Sivakumar, B.S. 2004. Studies on benzoylhydrozone complexes derived from saturated six membered heterocyclic ketones. *Indian Journal of Chemistry*, **43A** : 2568-2572.
- Marchini, L.C., Alves, S.B. and Nakano, O. 1992. Sericultura in curso de entomologia aplicada a agricultura, FEALQ, *Piracicaba Brazil*, 70(5)-736.
- Mathu, N.T. and Radhakrishnan, P.K. 2000. Cobalt (II) complexes of (1, 2-diimino-4'-antipyrynyl) ethane and 4-N-(4'-antipyrylmethylidene) aminoantipyryne, *Transition Met. Chemistry*, **25**: 287-292.
- Mohapatra, M., Chakravorty, V. and Dash, K.C. 1989. Synthesis, spectral studies and nuclease activity of mixed ligand copper(II) complexes of heteroaromatic semicarbazones/thiosemicarbazones and pyridine. *Polyhedron*, **8**: 1509-1515.
- Nakamoto, K. 1977. Infrared and Raman Spectra of Inorganic and Coordination Compounds, Wiley Interscience, 3rd Edn, New York, 292 PP.
- Navickiene, H. M. D., Miranda, J.E., Bortolia, V.S., Kato, M. J., Bolzani, V.S. and Furlan, M. 2007. Toxicity of extracts and isobutyl amides free *Piper tuberculatum* potent compounds with potential for the control of the velvet bean caterpillar. *Anticarsia gemmatilis. Pest Management Science*, **63**: 399-403.
- Raman, N., Joseph, J. and Rajasekaran, K. 2007. Studies on the interaction of transition metal complexes of the Schiff base derived from anthranilic acid and acetoacetanilide with DNA. *Polish Journal of Chemistry*, **81**: 149-159.
- Raman, N., Kulandaisamy, A., Thangaraja, C. and Jeyasubramanian, K. 2003. Redox and antimicrobial studies of transition metal(II) tetradentate Schiff base complexes., *Transition Met. Chemistry*, **28**: 29-36.
- Raman, N., Kulandaisamy, A., Thangaraja, C., Manisankar, P., Viswanathan, S. and Vethi, C. 2004. Synthesis, structural characterization and electrochemical and antibacterial studies of Schiff base copper complexes. *Transition Met. Chemistry*, **29**: 129-135.
- Thaker, B.T., Tandal, P.K., Patel, A.S. Vyas, C.J., Jesani, M.S. and Patel, T.M. 2005. Synthesis and mesomorphic characterization of Cu(II), Ni(II) and Pd (II) complexes with azomethine and chalcone as bridging group. *Indian Journal of Chemistry*, **44A**: 265-270.

N.Raman¹, J. Joseph¹, S. Muthu Kumar², S. Sujatha² and K. Sahayaraj^{2*}

¹Department of Chemistry, VHNSN College, Virudhunagar-626 001, Tamil Nadu; ²Crop Protection Research Centre, Department of Advanced Zoology and Biotechnology, St. Xavier's College (Autonomous), Palayamkottai -627 002, Tamil Nadu, India, *Communication author, E-mail: ttn_ksraj@sancharnet.in.