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Larvicidal activity of copper(II) complexes with 1,10 phenathroline and 2,2'-bipyridyl against *Culex* and *Anopheles* mosquito larvae

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Abstract: In the present study five ligands such as alanine(H_2SAla)**,** phenylalanine **(**H_2SPhe), leucine (H_2SLeu), isoleucine(H_2SIle), tryptophan (H_2STrp) were prepared by the addition of respective aminoacids with salicylaldehyde. Totally 10 Cu(II) complexes were synthesized by the addition of Cu(II) acetate monohydrate to ligand in aqueous solution with 1,10-phenanthroline and 2,2'-bipyridyl which led to the precipitation of complexes. Totally ten Cu(II) complexes were studied for mosquito larvicidal and Cu(II) complexes were investigated at a concentration of 0.5% of each compound against *Culex* early instar larvae. Thus the larvicidal effect may make these complexes to serve as potential insecticidal agents in mosquito control strategy. The Cu(II) complexes [Cu(SAla)phen(H_2O)].H_2O, [Cu(SAla)bpy(H_2O)].H_2O were found to cause mortality of the larvae after 18 hours. The mortality of larvae was seen after 24 hour itself for all the other metal complexes. Hence the above two complexes were chosen for LC_{50}, LC_{90} analysis in *Culex* and *Anopheles* mosquito larvae.

Introduction

The complexes of transition metals with amino acids in proteins and peptides are utilized in numerous biological processes, such as oxygen conveyer, electron transfer and oxidation. In these processes, the enzymatic active site, which is very specific, forms complexes with divalent metal ions (1). 1,10-phenanthroline (1,10-phen), 2,2'-bipyridine (2,2'-bipy) and their substituted derivatives, both in the metal-free state and as ligands coordinated to transition metals, disturb the functioning of a wide variety of biological systems. When the metal-free N, N-chelating bases are found to be bioactive it is usually assumed that the sequestering of trace metals is involved, and that the resulting metal complexes are the actual active species.

Mosquitoes which transmit a number of diseases such as malaria (*Anopheles)*, filariasis (*Culex, Mansonia*) and dengue (*Aedes aegypti*) etc., and cause millions of deaths every year, are the most important group of insects in term of public health (2). *Anopheles stephensi* is a major malarial vector in India. Currently, about 40% of the world's total population lives in areas where malaria is endemic (3). *Culex quinquesfasciatus* is one of the potential vectors of *Wuchereria bancrofti*, the causative agent of human lymphatic filariasis infecting over 120 million people all over the world (4).

To prevent mosquito borne diseases and improve public health it is necessary to control them. The approach to combat these diseases largely relied on interruption of the disease transmission cycle by either targeting the mosquito larvae through spraying of stagnant water breeding sites or by killing the adult mosquitoes using insecticides. But in recent years, mosquito control programmes have been suffering from failures because of increased insecticide resistance (5). Besides insecticidal resistance repeated use of synthetic

insecticides for mosquito control has disrupted natural biological systems. It has also resulted in undesirable effects on non-target organisms. For this reasons, it becomes necessary to search for alternative vector-control methods, which could be environmentally safer and specific in their action (6).

Schiff bases form an interesting class of chelating ligands that has enjoyed popular use in the coordination chemistry of transition and inner transition metals which show various Industrial, biological and catalytic applications (7). Metal complexes of Schiff bases derived from heterocyclic systems particularly those containing thiazole ring system find extensive applications in various fields (8). The manganese(II), cobalt(II), nickel(II), copper(II) and zinc(II)complexes with the title Ligand were synthesized and screened for antibacterial**,** antifungal and larvicidal activities of the ligand and complexes by Mohanan et al. (7)

The insecticidal activity has been reported for esters of 2,2,3,3-tetramethylcyclopropane carboxylic acid (9), amimic of the acid part of a pyrethroid, a series of triorganotin 2,2,3,3-tetramethylcyclopropane carboxylates were synthesized and screened against the *Aedes aegypti* (*Ae*. *aeypti*), *Anopheles stephensi* (*An*. *stephensi*) and *Culex pipiens quinquefasciatus* (*Cx*. *p*. *quinquefasciatus*) larvae. Song et al (10) reported the synthesis, larvicidal, QSAR and structural studies of some triorganotin 2,2,3,3-tetramethylcyclopropanecar boxylates.

In the literature, it has also been reported that copper complexes with drugs are much more active in the presence of a nitrogen donor heterocyclic ligand, such as pyridine (=py) and 2,2'-bipyridine (=bipy) and 1,10-phenanthroline (=phen) (11; 12). In present study report on the synthesis of Cu(II) complexes of aminoacid derived Schiff base containing 2,2’ bipyridine (=bipy) and 1,10-phenanthroline (=phen) and larvicidal and hemolytic assay of the Cu(II) complexes. The literature survey reveals that the mechanism of biological activity and toxicological effect of copper(II) 1,10-phenanthroline and copper(II) 2,2'-bipyridyl complexes are very minimum. In this view the present research is planned with the following objectives to synthesize the copper(II) complexes with five different aminoacid derived schiff bases using 2,2'-bipyridyl and 1,10-phenanthroline and to evaluate the mosquito larvicidal activity of the Cu(II) complexes

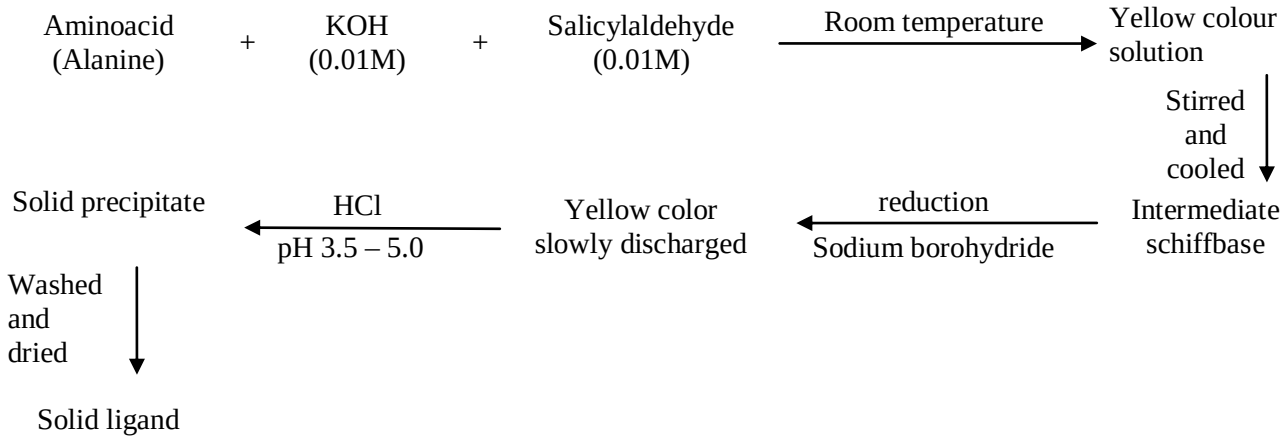
M**aterials and methods**

Materials

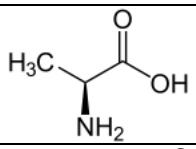
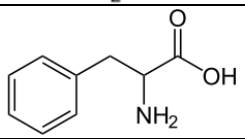
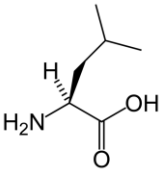
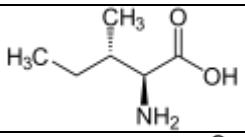
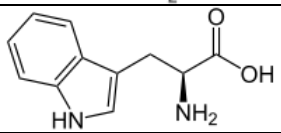
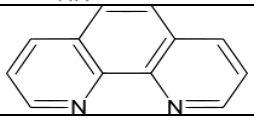
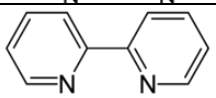
All chemicals used in the synthesis of the copper complex were analytical grade while L-alanine, L-phenylalanine, L-leucine, L-isoleucine, L-tryptophan, salicylaldehyde, copper(II) acetate monohydrate, 1, 10-phenanthroline and 2, 2'- bipyridyl were obtained from Merck Specialities Private Limited.

Preparation of Ligands

All the ligands and complexes were synthesized by the method of Koh et al., (13) Ligand Preparation



Ligands used in the present study

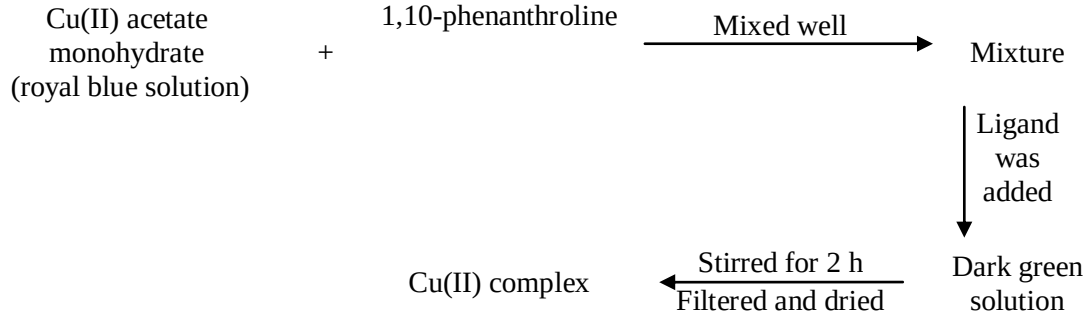
Name of the Ligand	Molecular formula	Structure
Alanine	$C_3H_7NO_2$	
Phenylalanine	$C_9H_{11}NO_2$	
Leucine	$C_6H_{13}NO_2$	
Isoleucine	$C_6H_{13}NO_2$	
Tryptophan	$C_{11}H_{12}N_2O_2$	
1,10-phenanthroline	$C_{12}H_8N_2$	
2,2'-bipyridyl	$C_{10}H_8N_2$	

Synthesis of copper(II) complexes

Synthesis of copper(II) complexes using 1,10-phenanthroline

In a clean beaker 0.2 g of copper(II) acetate monohydrate (1 mM) was taken and dissolved in 15 mL of ethanol. It was stirred for 10-15 minutes. To the royal blue solution formed 0.18 g of 1,10-phenanthroline (1 mM) in 10 mL ethanol was added and mixed well. To this 0.2 g of the corresponding ligand (1mM) in 10 mL double distilled water with KOH (1 mL, 1 M) was added. The resulting dark green solution was stirred for 2 hours at room temperature and then filtered and left for several days. The resulting solid precipitate was dried in a dessicator for few more days to afford the desired product.

Synthesis of Cu(II) complex with 1,10- phenanthroline



Synthesis of copper(II) complexes using 2,2'-bipyridyl

In a clean beaker 0.2 g of copper(II) acetate monohydrate (1 mM) was taken and dissolved in 15 mL ethanol. It was stirred for 10-15 minutes. To the royal blue solution formed 0.1564 g of 2,2'-bipyridyl (1 mM) in 10 mL ethanol was added and mixed well. To this 0.2 g of the corresponding ligand (1 mM) in 10 mL double distilled water with KOH (1 mL, 1 M) was added. The resulting dark green solution was stirred for 2 hours at room temperature and then filtered and left for several days. The resulting solid precipitate was dried in a dessicator for few more days to afford the desired product.

The similar methodology was followed to synthesize copper(II) complexes using five ligands with 1,10-phenanthroline and 2,2'-bipyridyl. They include copper-salicylaldehyde–alanine-1,10-phenanthroline, copper–salicylaldehyde-alanine-2,2'-bipyridyl, copper-salicylaldehyde–phenylalanine-1,10-Phenanthroline, copper-salicylaldehyde–phenylalanine-2,2'-bipyridyl, copper-salicylaldehyde–leucine-1,10-phenanthroline, copper-salicylaldehyde-leucine-2,2'-bipyridyl, copper-salicylaldehyde–isoleucine-1,10-phenanthroline, copper-salicylaldehyde-isoleucine- 2,2'-bipyridyl, copper-salicylaldehyde-tryptophan-1,10-phenanthroline, copper-salicylaldehyde-tryptophan-2,2'-bipyridyl.

***In vitro* evaluation of Larvicidal activity**

The mosquito larvae were collected from water habitats of Thanjavur and Tiruchirappalli district in wide mouth container. The samples were brought to the laboratory, morphologically identified suing standard manual and further used for larvicidal assay**.**

Cleaned sterile test tubes were taken and 5 early instar larvae of *Culex* and *Anopheles* sp. were taken in 1 mL of distilled water. To that 0.5% concentration of synthesized complexes in aqueous and solvent extracts were added. 5 larvae taken in distilled water along with the respective solvent served as control. The tubes were kept for 24 hours and observed for mortality of the larvae. The LC_{50} and LC_{90} of Cu(II) complex were determined in mosquito larvae of *Culex* and *Anopheles* sp(14). The results were statistically analyzed by the method of standard error.

Results and Discussion

To overcome the general problems of ligand instability for schiff bases of aminoacids and to introduce greater ligand flexibility and model the proton shifted intermediate of transamination reactions, an *in vitro* reduction was carried out on salicylaldehyde and aminoacid mixture. Optically active Schiff bases for example, salicylaldehyde and alanine, valine, phenylalanine and histidine have been reported (15) by refluxing the reagents for 2 h. A survey of such ligand preparation does not show any apparent pattern when racemeization occurs, although the presence of a metal may favour the retention of chirality by acting as a template. Copper(II) complexes were synthesised and larvicidal effects were analysed as follows.

Synthesis of copper(II) complexes with aminoacid derived schiff base using 2,2'-bipyridyl and 1,10 phenanthroline

In the present study five ligands such as alanine(H_2SAla)**,** phenylalanine (H_2SPhe), leucine(H_2SLeu), isoleucine(H_2SIle), tryptophan (H_2STrp) were prepared by the addition of respective aminoacids with salicylaldehyde . Totally 10 Cu(II) complexes were synthesized by the addition of Cu(II) acetate monohydrate to ligand in aqueous solution with 1,10-phenanthroline and 2,2'-bipyridyl which led to the precipitation of complexes. The complexes were named as copper-salicylaldehyde-alanine-1,10-phenanthroline [Cu(SAla) phen(H_2O)].H_2O, copper-salicylaldehyde-alanine-2,2'-bipyridyl [Cu(SAla)bpy(H_2O)].H_2O, copper-salicyl aldehyde-phenylalanine-1,10-phenanthroline [Cu(SPhe)phen(H_2O)].H_2O, copper-salicylaldehyde-phenyl alanine-2,2'-bipyridyl [Cu(SPhe)bpy(H_2O)].H_2O, copper-salicylaldehyde-leucine-1,10-phenanthroline [Cu (SLeu)phen(H_2O)].H_2O, copper-salicylaldehyde-leucine-2,2'- bipyridyl [Cu(SLeu)bpy(H_2O)].H_2O, copper-salicylaldehyde-isoleucine-1,10 phenanthroline [Cu(SIle)phen(H_2O)].H_2O, copper-salicylaldehyde-isoleucine 2,2'-bipyridyl [Cu(SIle)bpy(H_2O)].H_2O, copper-salicylaldehyde-tryptophan-1,10-phenanthroline [Cu(STrp) phen(H_2O)].H_2O, copper-salicylaldehyde-tryptophan-2,2'-bipyridyl [Cu(STrp)bpy(H_2O)].H_2O. All the complexes were found to be stable at room temperature.

Larvicidal activity of copper(II) complexes with 1,10-phenanthroline and 2,2'-bipyridine

In the present study, Cu(II) complexes of aminoacid derived schiff base containing 2,2'-bipyridine (bpy) and 1,10-phenanthroline (phen) were prepared and their larvicidal activity were evaluated.

Despite advances in medical science, mosquito-borne diseases including malaria, filariasis, dengue and the viral encephalitis remain the most important diseases of humans. Thus, there is an urgent need for new agents to control mosquito vectors of disease. In the present study the Larvicidal effect of Cu(II) complexes were investigated at a concentration of 0.5% of each complexes against *Culex* early instar larvae and the results were described. The metal complexes [Cu(SAla)phen(H_2O)].H_2O, [Cu(SAla)bpy(H_2O)].H_2O were found to cause mortality of the larvae after 18 hours. The mortality of larvae was seen after 24 hour itself for all the other 8 Cu(II) complexes. The aqueous extract of all the Cu(II) complexes showed 100% mortality. The Cu(II) complex effect on *Culex* and *Anopheles* mosquito larva was studied and their LC_{50}, LC_{90} values were calculated (Fig. 1, 2). The present findings were similar to the report of Chandraleka et al (16).

Fig.1 Screening of Mosquito larvicidal activity of Cu(II) complexes (LC_{50}values)

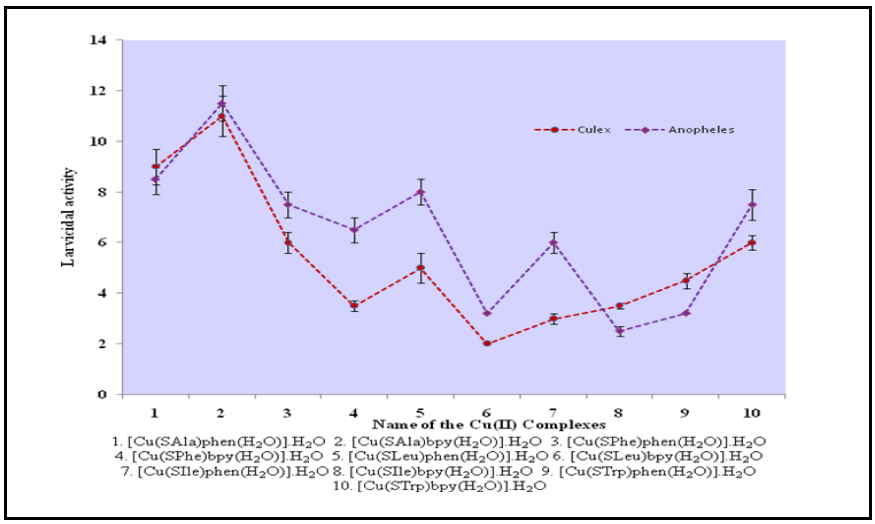
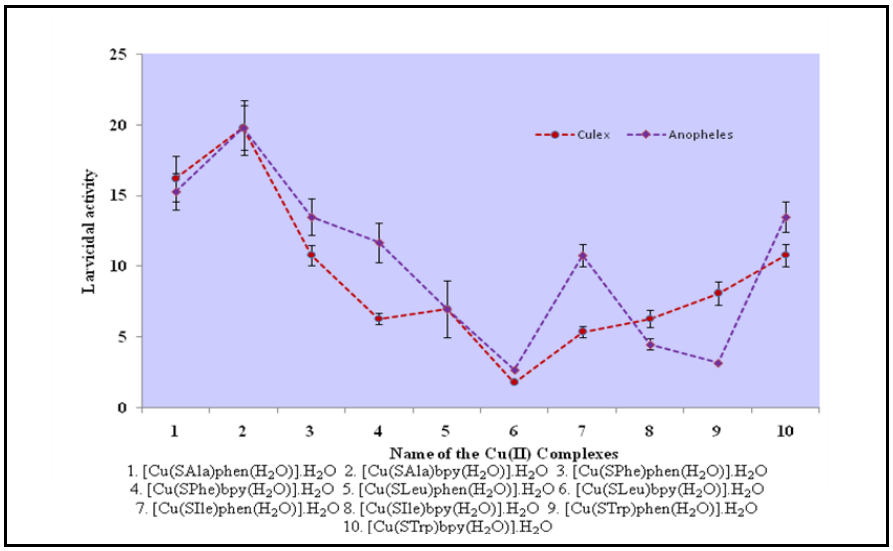


Fig.2 Screening of Mosquito larvicidal activity of Cu(II) complexes (LC_{90}values)



The *Culex* mosquito larva mortality was evaluated of different concentration of Cu(II) complexes [Cu(SAla)phen(H_2O)].H_2O, [Cu(SAla)bpy(H_2O)].H_2O in the range of 10 to 100 ppm. Among the concentration, larva mortality was not recorded from 10 to 50 ppm concentration for the period of 48 hrs, whereas the percentage of mortality was significantly increased from the 60 to 100 ppm. The minimum time required for larvicidal activity of *Culex* mosquito is 3 hrs at 100 ppm. The Cu(SAla)bpy(H_2O)].H_2O was highly significant in larvicidal activity (Table 1; Fig. 3; 4). Thus the larvicidal effect of these synthesized Cu(II) complexes may make these complexes to serve as potential insecticidal agents in mosquito control plans. The mosquito larvicidal order of Cu(II) complexes as follows

Table 1. Mortality rate of Mosquito larvae treated with Cu(II) complexes

S. No.	Concentration of the Cu(II) complex (ppm)	Time (Hrs)	[Cu(SAla)phen(H_2O)].H_2O		[Cu(SAla)bpy(H_2O)].H_2O	
			Number of larva death	% Mortality ± SD	Number of larva death	% Mortality ± SD
1.	100 ppm	3	-	-	-	-
		6	-	-	-	-
		9	-	-	6	40 ± 3.0
		12	1	6.7 ± 0.00	10	66.7 ± 4.583
		15	2	13.3 ± 0.00	11	73.3 ±3.464
		18	2	13.3 ± 0.00	13	86.7 ± 1.732
		21	4	26.7 ± 0.00	15	100 ± 0.00
		24	4	26.7 ± 0.00	15	100 ± 0.00
2.	90 ppm	3	-	-	-	-
		6	-	-	-	-
		9	-	-	3	20 ± 2.121
		12	-	-	6	40 ± 3.00
		15	-	-	8	53.3 ± 3.464
		18	2	13.3 ± 0.00	10	66.7 ± 1.732
		21	3	20 ± 0.00	13	86.7 ± 1.732
		24	3	20 ± 0.00	15	100 ± 0.00
3.	80 ppm	3	-	-	-	-
		6	-	-	-	-
		9	-	-	3	20 ± 2.121
		12	-	-	4	26.7 ± 4.243
		15	-	-	9	60 ± 3.00
		18	-	-	11	73.3 ± 1.732
		21	2	13.3 ± 0.00	14	93.3 ± 1.732
		24	2	13.3 ± 0.00	15	100 ± 0.00
4.	70 ppm	3	-	-	-	-
		6	-	-	-	-
		9	-	-	3	20 ± 2.121
		12	-	-	4	26.7 ± 0.00
		15	-	-	6	40 ± 3.00
		18	-	-	8	53.3 ± 1.732
		21	1	6.7 ± 0.00	10	66.7 ± 1.732
		24	1	6.7 ± 0.00	15	100 ± 0.00
5.	60 ppm	3	-	-	-	-
		6	-	-	-	-
		9	-	-	2	13.3 ± 0.00
		12	-	-	3	20 ± 2.121
		15	-	-	6	40 ± 3.00
		18	-	-	8	53.3 ± 1.732
		21	-	-	11	73.3 ± 1.732
		24	1	6.7 ± 0.00	15	100 ± 0.00
6.	50 ppm	3	-	-	-	-
		6	-	-	-	-
		9	-	-	-	-
		12	-	-	3	20 ± 2.121
		15	-	-	4	26.7 ± 4.243
		18	-	-	6	40 ± 3.00
		21	-	-	6	40 ± 3.00
		24	1	6.7 ± 0.00	9	60 ± 6.00

Values in each % mortality column is the mean of three replicates ± S.E

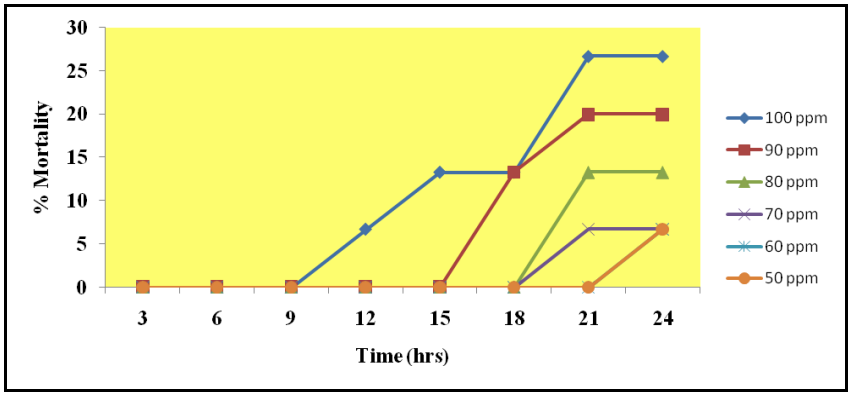


Fig. 3. Mortality rate of Mosquito larvae treated with [Cu(SAla)phen(H_2O)].H_2O

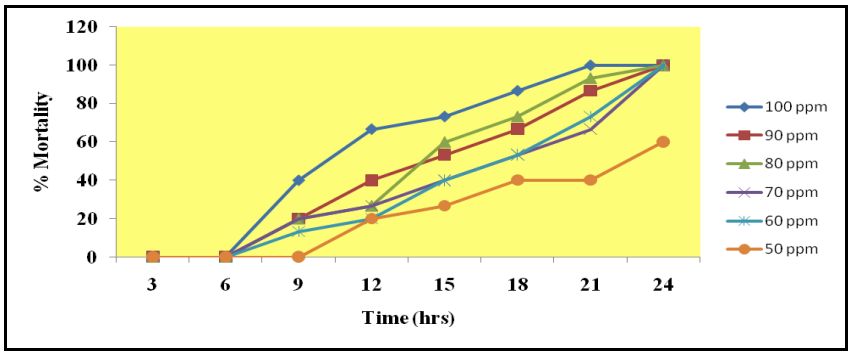


Fig. 4. Mortality rate of Mosquito larvae treated with [Cu(SAla)bpy(H_2O)].H_2O

a) *Culex* mosquito larvae > [Cu(SAla)bpy(H_2O)].H_2O > [Cu(STrp)bpy(H_2O)].H_2O > Cu(SIle)bpy(H_2O)].H_2O > [Cu(SPhe)bpy(H_2O)].H_2O > Cu(SLeu)bpy(H_2O)].H_2O > [Cu(SAla)phen(H_2O)].H_2O > [Cu(SPhe)phen(H_2O)].H_2O > [Cu(STrp)phen(H_2O)].H_2O > [Cu(SLeu)phen(H_2O)]. H_2O > [Cu(SIle)phen(H_2O)]. H_2O

b) *Anopheles* mosquito larvae > [Cu(SAla)phen(H_2O)].H_2O > [Cu(SPhe)phen(H_2O)].H_2O > Cu(SIle)phen(H_2O)].H_2O > [Cu(SLeu)phen(H_2O)].H_2O > [Cu(STrp)phen(H_2O)].H_2O > [Cu(SAla)bpy(H_2O)].H_2O > [Cu(STrp)bpy(H_2O)].H_2O > [Cu(SPhe)bpy(H_2O)].H_2O > [Cu(SIle)bpy(H_2O)].H_2O > [Cu(SLeu)bpy(H_2O)].H_2O

The Cu(II) complexes with 2,2'-bipyridine and 1,10-phenanthroline is possess the antimicrobial activity against the bacterial and fungal pathogens (17,18). In addition to timicrobial activity, Cu(II) complexes with 2,2'-bipyridine (bpy) and 1,10-phenanthroline is showed the significant mosquito larvicidal activity is helpful to minimize the breeding of *Culex* and *Anopheles* mosquito larvae in extending with field and other experimental trials.

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