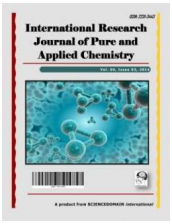

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Synthesis of Novel 1,2,4-triazole-DTC Based Metallo-phosphorous Nanoformulations as Larvicide against *Aedes aegypti*

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Authors' contributions

This work was carried out in collaboration between all authors. Author KG designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AS and DKK managed the analyses of the study. Author DKK helped in evaluation of the data for larvicidal potential. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The present study involved the synthesis of heteroleptic metal complexes and their nanoformulations as potential larvicidal agent.
Study Design: Metal complexes nanoformulations.
Place and Duration of Study: Department of Chemistry, Punjab agricultural University, Ludhiana, Punjab, India between January 2016 and February 2017.
Methodology: Heteroleptic metal complexes of Cu(II), Co(II) and Fe(III), using 1,2,4-triazole-dithiocarbamate, isothiocyanate and triphenyl phosphine ligands were prepared and converted to water dispersible nano-formulations using PVP (Polyvinyl pyrollidine) as capping as well as stabilizing agent and sodium dodecyl sulfate (SDS) as surfactant. The structures of metal complexes and their nanoformulations were characterized on the basis of elemental analysis, spectral techniques, and TEM analysis. The anti-larval evaluation of the nano-formulations against

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Aedes aegypti was made on the basis by the standard guidelines of World Health Organization (2005).
Results: The anti-larval evaluation of the nano-formulations against *Aedes aegypti* revealed that the formulations were found to be highly active against the test specie at concentrations as low as 10 ppm, with copper analogues being the most active among series.
Conclusion: The less toxic behaviour of these metal complexes as aqua nano formulations endorsed their use in place of earlier used phospho-analogues.

Keywords: One-pot synthesis; dithiocarbamate; triphenyl phosphine; heteroleptic; metal complexes; nanoformulation; anti-larval.

1. INTRODUCTION

Aedes aegypti is considered as primary vector of viral diseases like dengue fever, chikungunya, yellow fever etc. in tropical and sub-tropical areas of the world [1]. No vaccination has been developed against the diseases so far. Management of mosquitoes is the only way to protection. Out of the various control methodologies, the eradication of the larvae at breeding ground is the most efficient way, as it is the most concentrated and localised stage of the mosquitoes [2]. Various reported chemical control measures preferably affects adult stages keeping the larval stages unaffected [3]. Moreover, the chemical control strategies have led to the development of resistance in many mosquito strains [4] and their non-specific action on other species is causing ecological imbalance [5,6]. Thus, the search for novel effective molecules, which could eradicate the larvae at breeding ground, highlighted the need for new strategies for mosquito control.

Organophosphorous, dithiocarbamates and 1,2,4-triazoles are bioactive moieties used worldwide, in agrochemicals, pharmaceuticals and other industrial fields [7-9]. Dithiocarbamates and 1,2,4-triazoles are the heterorganic moieties with moderate toxicity [10,11]. Organophosphorus also forms a widely used class of pesticides which are still needed as insecticides at the time of sudden and severe outbreak of diseases [12-14]. But their toxicity and tendency to develop resistance, demands their redesigning, so as to use them as resistance measure as and when required [15,16]. On the other hand, complexation of heterorganic ligands with bioactive metals has an immense potential to portray an augmented bioactivities to them, which is further influenced by the design and bioactivities of the ligands along with the type of the metal ions used [17].

Most of the organic and inorganic molecules are not able to act at breeding ground of mosquitoes because of their water insolubility/dispersibility. Solubility/dispersibility is the most limiting factor for any substance to act as larvicide in water [18], so that larvae could be able to take the substance into its gut. The essence of nanotechnology, i.e. preparing the nano-formulations of the bioactive molecules seems to provide solution to the problem of water insolubility. The nano-formulations also impart diversified topological parameters which are responsible for improved bioactivity than the molecules in bulk providing additional benefits with greater effectiveness at low doses [19].

In the multi-component regime for the synthesis of bioactive molecules, we endeavoured to design complexes of Cu (II), Co (II) and Fe (III) with heteroleptic ligands *viz.* 1,2,4-triazole-dithiocarbamate, triphenyl phosphine and isothiocyanate, in variable ratios. The synthesized complexes were converted to nano aqua formulations for evaluation of their larvicidal potential against *Aedes aegypti,* using Temephos [(*O,O'*-(thiodi-4,1-phenylene)bis(*O,O*-dimethylphosphorothiolate)] as positive control. The *in silico* toxicity analysis of the prepared complexes has been done for rationalization of the results.

2. MATERIALS AND METHODS

All the reagents and solvents were commercially available, analytical grade materials and were used as supplied, without further purification. Deionized water was used for preparation of all the aqua formulations. Molar conductance of all the complex derivatives was recorded on digital conductivity meter at room temperature. IR spectra were recorded on Perkin Elmer FT-IR spectrometer using KBr disc (range 4000–400 cm^{-1}). UV-Vis spectra were recorded on Shimadzu Europa GmbH spectrophotometer in the range of 200-800 nm. CHNS Analysis was

made on Thermo Finnigan instrument. Transmission electron microscopic (TEM) studies were made on Hitachi TEM system operated at 100 kV by dispersing the sample onto a Cu grid with holey carbon supporting films. The Larvicidal activity of the nano-dispersions was tested by WHO method against the *Aedes aegypti.*

2.1 Synthesis of Heteroleptic Metal Complexes

2.1.1 Synthesis of [(1,2,4-triazole-1-carbodithioate)(PPh_3)(SCN)] cobalt (II) complex (1)

1,2,4-Triazole (1.04 g, 0.015 moles) was dissolved in 10 ml of methanol and potassium carbonate (1.5 g) was added to it. The reaction mixture was cooled by keeping in ice bath and carbon disulphide (1.5 ml, 0.02 moles) was added to the stirring mixture. The mixture was stirred till the appearance of reddish yellow coloration. Triphenyl phosphine (2.62 g, 0.01 moles) dissolved in small amount of chloroform and sodium thiocyanate (0.81 g, 0.01 moles) dissolved in distilled water was dropwise added to the same flask under vigorous stirring followed by the addition of aqueous solution of cobaltous chloride (2.37 g, 0.01 moles) in the same reaction mixture with formation of reddish brown precipitates. The reaction mixture was stirred for additional 1 h and the precipitates so formed were separated and washed with distilled water followed by chloroform to obtain the pure solid, which was dried under vacuum and stored for further use.

Yield: 72%. Mp: > 300℃. FTIR (KBr, cm^{-1}): 3110 (υ_{C-H}), 2077 (NCS), 1537 (υ_{N-CSS}), 996 (υ_{C-SS}), 543 (υ_{M-S}). UV-Vis (DMF, λ_{max}/nm): 234 & 265 (π-π^*), 375 (LMCT). Anal. Calcd. for $C_{22}H_{17}CoN_4PS_3$ (%):C, 50.47; H, 3.27; N, 10.70; S, 18.38; Co, 11.26. Found (%):C, 50.43; H, 3.24; N, 10.74; S, 18.34; Co, 11.23.

2.1.2 Synthesis of [(1,2,4-triazole-1-carbodithioate)(PPh_3)(SCN)] copper (II) complex (2)

The similar methodology as explained for complex 1 was employed for the synthesis of complex 2, with the use of aqueous cupric chloride (1.70 g, 0.01 moles) to obtain the bluish green solid precipitates.

Yield: 76%. Mp: > 300℃. FTIR (KBr, cm^{-1}): 3138 (υ_{C-H}), 2075 (NCS), 1518 (υ_{C-NSS}), 997 (υ_{C-SS}), 528 (υ_{M-S}). UV-Vis (DMF, λ_{max}/nm): 233 & 273 (π-π^*), 332 (LMCT). Anal. Calcd. for $C_{22}H_{17}CuN_4PS_3$ (%): C, 50.03; H, 3.64; N, 10.61; S, 18.21; Cu, 12.03. Found (%):C, 50.01; H, 3.28; N, 10.67; S, 18.25; Cu, 12.13.

2.1.3 Synthesis of [(1,2,4-triazole-1-carbodithioate)(PPh_3)(SCN)] iron (III) complex (3)

Complex 3 was prepared by the same reaction procedure as explained above involving the use of aqueous ferric chloride (1.62 g, 0.01 moles) to obtain the brownish black solid precipitates.

Yield: 55%. Mp: 259℃. FTIR (KBr, cm^{-1}): 3325 (υ_{O-H}), 3124 (υ_{C-H}), 2097 (NCS), 1535 (υ_{C-NSS}), 996 (υ_{C-SS}), 458 (υ_{M-S}). UV-Vis (DMF, λ_{max}/nm): 231 & 273 (π-π^*), 400 (LMCT). Anal. Calcd. for $C_{22}H_{21}ClFeN_4O_2PS_3$ (%):C, 44.64; H, 3.58; N, 9.47; S, 16.25; Fe, 9.43. Found (%):C, 44.59; H, 3.54; N, 9.42; S, 16.21; Fe, 9.48.

2.1.4 Synthesis of [(1,2,4-triazole-1-carbodithioate)(PPh_3)$_2$] cobalt (II) complex (4)

1,2,4-Triazole (1.04 g, 0.015 moles) was dissolved in 10 ml of methanol and potassium carbonate (1.5 g) was added to it. The reaction mixture was cooled by keeping in ice bath and carbon disulphide (1.5 ml, 0.02 moles) was added to the stirring mixture. The mixture was stirred till the appearance of reddish yellow coloration. Triphenyl phosphine (5.34 g, 0.02 moles) dissolved in small amount of chloroform was added to the same flask under vigorous stirring. The aqueous solution of cobaltous chloride (2.37 g, 0.01 moles) was drop-wise added to the same mixture with appearance of brown precipitates. The reaction mixture was stirred for additional 1 hr and the precipitates so formed were separated and washed with distilled water followed by chloroform to obtain the pure solid.

Yield: 75%. Mp: > 300℃. FTIR (KBr, cm^{-1}): 1495 (υ_{C-NSS}), 963 (υ_{C-SS}), 512 (υ_{M-S}). UV-Vis (DMF, λ_{max}/nm): 230 & 275 (π-π^*), 395 (LMCT). Anal. Calcd. for $C_{39}H_{32}ClCoN_3P_2S_2$ (%):C, 61.38; H, 4.23; N, 5.51; S, 8.40; Co, 7.72. Found (%): C, 61.32; H, 4.26; N, 5.48; S, 8.43; Co, 7.76.

2.1.5 Synthesis of [(1,2,4-triazole-1-carbodithioate)(PPh_3)$_2$] copper (II) complex (5)

Complex 5 was prepared by the same reaction procedure as explained above for complex 4,

involving the use of aqueous cupric chloride (1.70 g, 0.01 moles) to obtain the bluish green solid precipitates.

Yield: 72%. Mp: > 300℃. FTIR (KBr, cm^{-1}): 3125 (υ_{C-H}), 1539 (υ_{C-NSS}), 996 (υ_{C-SS}), 532 (υ_{M-S}). UV-Vis (DMF, λ_{max}/nm): 226 & 272 (π-π^*), 400 (LMCT). Anal. Calcd. for $C_{39}H_{32}ClCuN_3P_2S_2$ (%):C, 61.01; H, 4.20; N, 5.47; S, 8.35; Cu, 8.28. Found (%): C, 61.09; H, 4.28; N, 5.43; S, 8.39; Co, 8.33.

2.1.6 Synthesis of [(1,2,4-triazole-1-carbodithioate)(PPh_3)$_2$] iron (III) complex (6)

Aqueous ferric chloride (1.62, 0.01 moles) was used for preparation of complex 8 by the same reaction procedure as explained above, to obtain the brownish black solid precipitates.

Yield: 80%. Mp: 280℃. FTIR (KBr, cm^{-1}): 3318 (υ_{O-H}), 3095 (υ_{C-H}), 1565 (υ_{C-N}), 992 (υ_{C-S}), 492 (υ_{M-S}). UV-Vis (DMF, λ_{max}/nm): 233 & 264 (π-π^*), 459 (LMCT). Anal. Calcd. for $C_{39}H_{36}Cl_2FeN_3O_2P_2S_2$ (%):C, 56.33; H, 4.36; N, 5.05; S, 7.71, Fe, 6.72. Found (%):C, 56.35; H, 4.39; N, 5.08; S, 7.76; Fe, 6.78.

2.1.7 Synthesis of [(1,2,4-triazol-4ylcarbamodithioate)(PPh_3)(SCN)] obalt (II) complex (7)

4-Amino-1,2,4-triazole (1.26 g, 0.015 moles) was dissolved in 10 ml of methanol and potassium carbonate (1.5 g) was added to it. The reaction mixture was cooled by keeping in ice bath and carbon disulphide (1.5 ml, 0.02 moles) was added to the stirring mixture. The mixture was stirred till the appearance of yellow coloration. Triphenyl phosphine (2.67 g, 0.01 moles) dissolved in small amount of chloroform and sodium thiocyanate (0.81 g, 0.01 moles) dissolved in distilled water was added to the same flask under vigorous stirring. The aqueous solution of cobaltous chloride (2.37 g, 0.01 moles) was drop-wise added to the same mixture with appearance of reddish brown precipitates. The reaction mixture was stirred for additional 1 hr and the precipitates so formed were separated and washed with distilled water followed by chloroform to obtain the pure complex 7. The solid was dried under vacuum and stored for further use.

Yield: 75%. Mp: > 300℃. FTIR (KBr, cm^{-1}): 3289 (υ_{N-H}), 3116 (υ_{C-H}), 2069 (NCS), 1538 (υ_{C-N}), 996 (υ_{C-S}), 516 (υ_{M-S}). UV-Vis (DMF, λ_{max}/nm): 233 & 272 (π-π^*), 382 (LMCT). Anal. Calcd. for $C_{22}H_{18}CoN_5PS_3$ (%): C, 49.07; H, 3.37; N, 13.00; S, 17.86; Co, 10.94. Found (%): 49.12; H, 3.38; N, 13.01; S, 17.89; Co, 10.91.

2.1.8 Synthesis of [(1,2,4-triazol-4ylcarbamodithioate)(PPh_3)(SCN)] copper (II) complex (8)

The similar methodology as explained for complex 7 was employed for the synthesis of copper complex 8 with the use of aqueous cupric chloride (1.70 g, 0.01 moles) to obtain the yellowish green solid precipitates.

Yield: 74%. Mp: 289℃. FTIR (KBr, cm^{-1}): 3310 (υ_{N-H}), 3112 (υ_{C-H}), 2074 (NCS), 1534 (υ_{C-NSS}), 993 (υ_{C-SS}), 518 (υ_{M-S}). UV-Vis (DMF, λ_{max}/nm): 230 & 276 (π-π^*), 380 (LMCT). Anal. Calcd. for $C_{22}H_{18}CuN_5PS_3$ (%):C, 48.65; H, 3.34; N, 12.89; S, 17.71; Cu, 11.70. Found (%): C, 48.69; H, 3.38; N, 12.92; S, 17.76; Cu, 11.73.

2.1.9 Synthesis of [(1,2,4-triazol-4ylcarbamodithioate)(PPh_3)(SCN)] iron (III) complex (9)

Complex 9 was prepared by the same reaction procedure as explained above for complex 7, involving the use of aqueous ferric chloride (1.62, 0.01 moles) to obtain the brownish black solid precipitates.

Yield: 45%. Mp: > 300℃. FTIR (KBr, cm^{-1}): 3363 (υ_{N-H}), 3054 (υ_{C-H}), 2097 (NCS), 1508 (υ_{C-N}), 998 (υ_{C-S}), 515 (υ_{M-S}). UV-Vis (DMF, λ_{max}/nm): 237 & 264 (π-π^*), 365 (LMCT). Anal. Calcd. for $C_{22}H_{18}ClFeN_5PS_3$ (%):C, 44.49; H, 3.90; N, 11.28; S, 15.49; Fe, 8.99. Found (%):C, 44.44; H, 3.92; N, 11.23; S, 15.45; Fe, 8.93.

2.1.10 Synthesis of [(1,2,4-triazol-4ylcarbamodithioate)(PPh_3)$_2$] cobalt (II) complex (10)

4-Amino-1,2,4-triazole (1.04 g, 0.015 moles) was dissolved in 10 ml of methanol and potassium carbonate (1.5 g) was added to it. The reaction mixture was cooled by keeping in ice bath and carbon disulphide (1.5 ml, 0.02 moles) was added to the stirring mixture. The mixture was stirred till the appearance of yellow coloration. Triphenyl phosphine (5.34 g, 0.02 moles) dissolved in small amount of chloroform was added to the same flask under vigorous stirring. The aqueous solution of cobaltous chloride (2.42

g, 0.01 moles) was drop-wise added to the same mixture with appearance of brown precipitates. The reaction mixture was stirred for additional 1 hr and the precipitates so formed were separated and washed with distilled water followed by chloroform to obtain the pure complex 10. The product was vacuum dried and stored for further use.

Yield: 85%. Mp: > 300℃. FTIR (KBr, cm^{-1}): 3322 (υ_{N-H}), 3056 (υ_{C-H}) 1586 (υ_{C-NSS}), 997 (υ_{C-SS}), 514 (υ_{M-S}). UV-Vis (DMF, λ_{max}/nm): 234 & 269 (π-π^*), 420 (LMCT). Anal. Calcd. for $C_{39}H_{33}ClCoN_4P_2S_2$ (%):C, 60.19; H, 4.27; N, 7.20; S, 8.24; Co, 7.57. Found (%): C, 60.22; H, 4.26; N, 7.25; S, 8.89; Co, 7.53.

2.1.11 Synthesis of [(1,2,4-triazol-4-ylcarbamodithioate)(PPh_3)$_2$] copper (II) complex (11)

Complex 11 was prepared by the same reaction procedure as explained for complex 10, involving the use of aqueous cupric chloride (1.70 g, 0.01 moles) to obtain the bluish green solid precipitates.

Yield: 80%. Mp: > 300℃. FTIR (KBr, cm^{-1}): 3345 (υ_{N-H}), 3116 (υ_{C-H}), 1545 (υ_{C-NSS}), 989 (υ_{C-SS}), 549 (υ_{M-S}). UV-Vis (DMF, λ_{max}/nm): 228 & 272 (π-π^*), 410 (LMCT). Anal. Calcd. for $C_{39}H_{33}ClCuN_4P_2S_2$ (%): C, 59.87; H, 4.28; N, 7.11; S, 8.16; Cu, 8.20. Found (%): C, 59.84; H, 4.25; N, 7.16; S, 8.19, Cu, 8.12.

2.1.12 Synthesis of [(1,2,4-triazol-4ylcarbamodithioate)(PPh_3)$_2$] iron (III) complex (12)

Ferric chloride (1.62, 0.01 moles) was used to obtain dark brown precipitates of complex 12 by the same reaction procedure as explained above for complex 10.

Yield: 80%. Mp: > 300℃. FTIR (KBr, cm^{-1}): 3347 (υ_{N-H}), 3164 (υ_{N-H}), 1565 (υ_{C-NSS}), 992 (υ_{C-SS}), 492 (υ_{M-S}). UV-Vis (DMF, λ_{max}/nm): 233 & 264 (π-π^*), 459 (LMCT). Anal. Calcd. for $C_{39}H_{33}Cl_2FeN_4P_2S_2$ (%):C, 55.33; H, 4.41; N, 6.62; S, 7.58, Fe, 6.60. Found (%):C, 55.35; H, 4.42; N, 6.65; S, 7.54; Fe, 6.63.

2.2 Standardization and Synthesis of Nano-formulations of Metal Complexes

The synthesized metal complexes (1-12) were finely grounded using a pestle mortar. Different amounts *viz.* 2.0, 1.0, 0.5, 0.2 and 0.1 g of the metal complexes were mixed with 0.2 g of PVP and the mixture was slowly dispersed in 100 ml of distilled water containing 0.2 g of SDS, while ultrasonication. The sonication was continued for 20 minutes and the nano-dispersion was allowed to age for 1 hour. The formulation with clear appearance containing maximum amount of solid complex by weight were analysed by TEM micrographs to get the optimum amount required to prepare 100 ml of nano-formulation. All the other nano-formulations were prepared by the same standard method and were diluted to 500 ppm of active ingredient, which was stored as stock solution for larvicidal evaluation.

2.3 Larvicidal Evaluation

2.3.1 Collection and culture of mosquito larvae

Water samples from different types of standing water bodies like ponds, paddy fields, road side ditches, gardens, nurseries etc. were collected with the help of plastic dippers. *Aedes* larvae were taken from the collected water samples by identifying them on the basis of their morphological features following the standard keys [20].

2.3.2 Dose response larvicidal bioassay

The larvicidal activity was assessed by the standard guidelines of World Health Organization (2005), with some modifications [21].

Stock solutions of 500 µg/mL of water dispersible nanoparticles of metal complexes were used as such prepared in section 2.2 by standardized procedure. The serial dilutions of the stock solution were made to get the required concentrations of 250, 100, 50, 25, 10 and 5 µg/mL. Twenty, 4^{th} instar larvae were individually added to 100 ml of different concentrations of nano-formulations in a Petri plates. A control set having 100 ml of de-chlorinated water, SDS and PVP along with the larva (n=20), was also run simultaneously along with each set. The experimental media, in which 100% mortality rate of larvae occurred were selected for a dose response bioassay. Based on the screening results, the numbers of dead larvae were counted 24 hr post treatment, and the percent mortality was recorded from the average of three replicates. The mortality data was subjected to probit analysis for calculating median lethal concentration (LC_{50}) and statistics at 95% upper

and lower fiducial limits [22]. Prior to analysis, mortality in treatments was corrected for that in control using Abbott formula [23].

2.4 *In silico* Toxicity Analysis

Toxtree v2.6.6 is an open-source software application that places chemicals into categories and predicts various kinds of toxic effect by applying different decision tree approaches [24]. Toxtree was developed by Idea Consult Ltd. (Sofia, Bulgaria) under the terms of an ECB contract. The software is made freely available by ECB as a service to scientific researchers and anyone with an interest in the application of computer-based estimation methods in the assessment of chemical toxicity. The new module with the revised list of SAS includes also structure–activity relationships (SAR) models that enable the toxicity evaluations for a number of chemical classes to be fine-tuned.

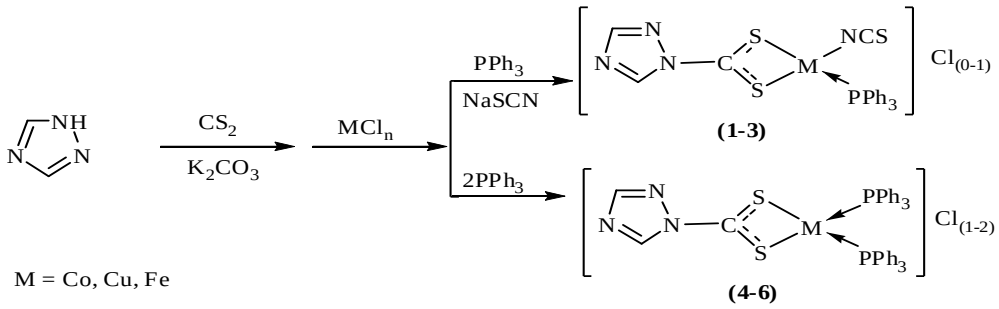
In order to find out the toxic hazards of all the synthesized compounds, two dimensional models of the compounds were first converted into its simplified molecular-input line-entry system *(SMILES format)* using an online SMILES translator. Then simply putting the SMILES code into the Chemical identifier row available in the Toxtree software we can easily get the toxic characters.

3. RESULTS AND DISCUSSION

3.1 Chemistry

The heteroleptic complexes were prepared according to the synthetic procedure shown in Schemes 1 and 2. Dithiocarbamate ligands were first prepared by reaction of 1,2,4-triazole and 4-amino-1,2,4-triazole with carbon disulphide in basic medium followed by addition of metal salt and triphenyl phosphine along with the addition of sodium thiocyanate in two different molar ratios to yield heteroleptic metal complexes (1-12). The structures of the target molecules were elucidated using UV, IR, and elemental analysis.

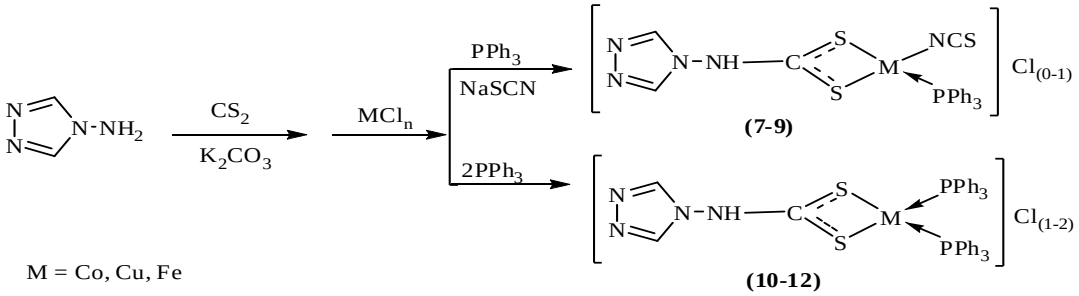
The complexes formed were stable but insoluble in most of the solvents leading to its poor applicability as larvicidal agent, against mosquitoes in water. Thus, the essence of nanotechnology was added to make the biologically active molecules water dispersible with help of surfactant SDS and stabilizing them by coating with PVP. The synthesized nano-aqua formulations at different concentrations were prepared, characterized and evaluated for their larvicidal potential. The transperancies of the nano-qua formulations so formed had been observed over a dark background. The result in terms of clarity is shown in Fig. 1.



(1-3)

(4-6)

Scheme 1: Synthesis of metal complexes of 1,2,4-Triazole



(7-9)

(10-12)

Scheme 2: Synthesis of metal complexes of 4-Amino-1,2,4-triazole

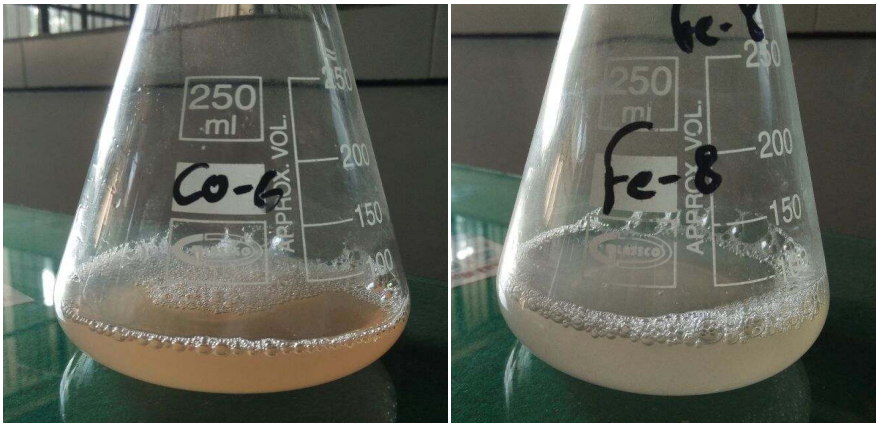


Fig. 1. Nano aqua formulations of heteroleptic cobalt complex (a), and heteroleptic iron complex (b)

3.2 Characterization

3.2.1 Molar conductance

The molar conductance of 10^{-3} M (DMSO) transition metal complexes were recorded at room temperature using Digital conductivity meter. The cell constant of the conductivity cell used was 1 cm^{-1}. All the complexes are coloured, stable in air and insoluble in most of the organic solvents except DMSO and DMF. The molar conductivity of various adducts in 10^{-3} M solution are found to be in the range of 6-14 $ohm^{-1}cm^2mol^{-1}$ (Table 1). These values observed for all these adduct are lower than the values which can be expected for uni-valent or di-valent electrolytes in these solvents suggesting that these complexes are non-electrolytic in nature.

3.2.2 IR Spectra

The characteristic bands in the infrared spectra are direct significance of the formation of complexes. It involved the single bands in the region, 950-1050 cm^{-1} which exhibited the bidentate nature of coordination, on the basis of Bonati and Ugo criterion [25]. The other band lies between 1450 and 1600 cm^{-1} which referred to the thioureide band, it is an intermediate between single and double bonded C-N and its position indicate the shift of electron density towards the coordinating metal ion. The Infrared active frequency was sensitive to both chain length and the steric bulk of the substituent. The additional sharp bands in the region of 3100-3200 cm^{-1} referred to the N-H stretch of 4-amino-1,2,4-traizole as appeared only in complex (6-12) and a broad band ahead referred to the presence of water molecules coordinated to the cobalt complexes (1, 4, 7 and 12). A strong band of metal-sulfur linkage is seen in the lower region confirming the coordination of dtc ligand to the metal. The observed stretching frequencies in the region of 2050-2100 cm^{-1} are attributed to the 'N'-coordinated isothiocyanate anion. Fig. 2 and 3 showed the IR-spectra of complex 4 and 9.

3.2.3 Electronic spectra

Dithiocarbamate generally show three bands in the UV region that are ascribed to intramolecular-intraligand transitions corresponding to π-π^* transitions of N-C=S and S-C=S groups and n-π^* transition due to lone pair of electrons on sulphur atom [26]. In the complexes, bands below 300 nm are attributed to the intraligand transitions. A moderate band due to ligand metal charge transfer (LMCT) appeared above 350 nm in most of the cases whereas a weak band in higher visible region due to d-d transition is less pronounced [27]. Figs. 4 and 5 showed UV-Visible spectra of complex 1 and 6.

3.2.4 Elemental Analysis

The elemental analysis of the complexes helped in documenting the percentage of carbon, hydrogen, nitrogen and sulphur, which corroborated the structures of the metal complexes in coordination with the results of Infrared and UV-Visible spectroscopy and the high carbon and hydrogen percentage also confirmed the presence of triphenyl phosphine moiety in the molecule. The percentages of metal in the complexes were estimated by titrametric method [28].

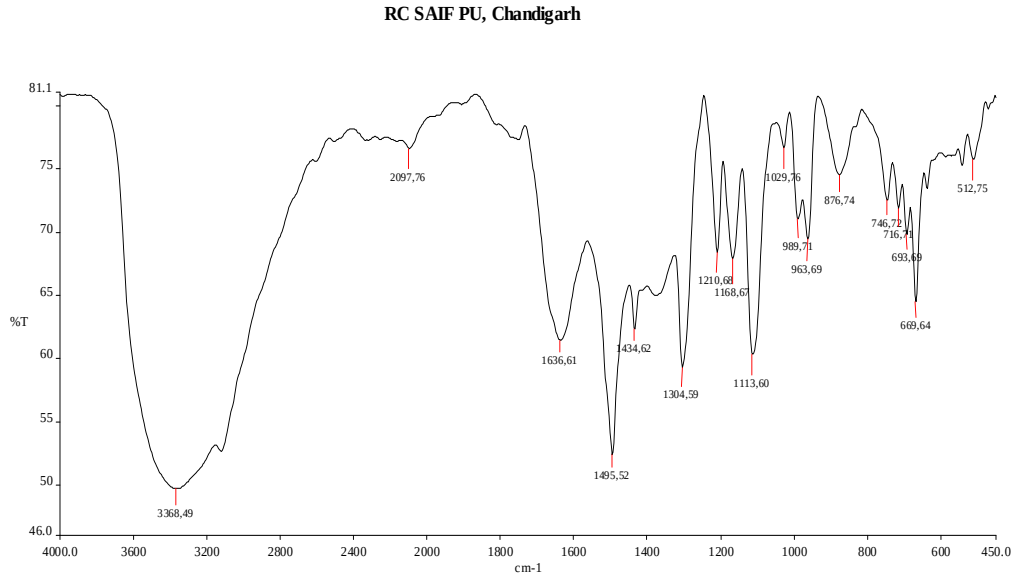


Fig. 2. FTIR spectra of complex 4

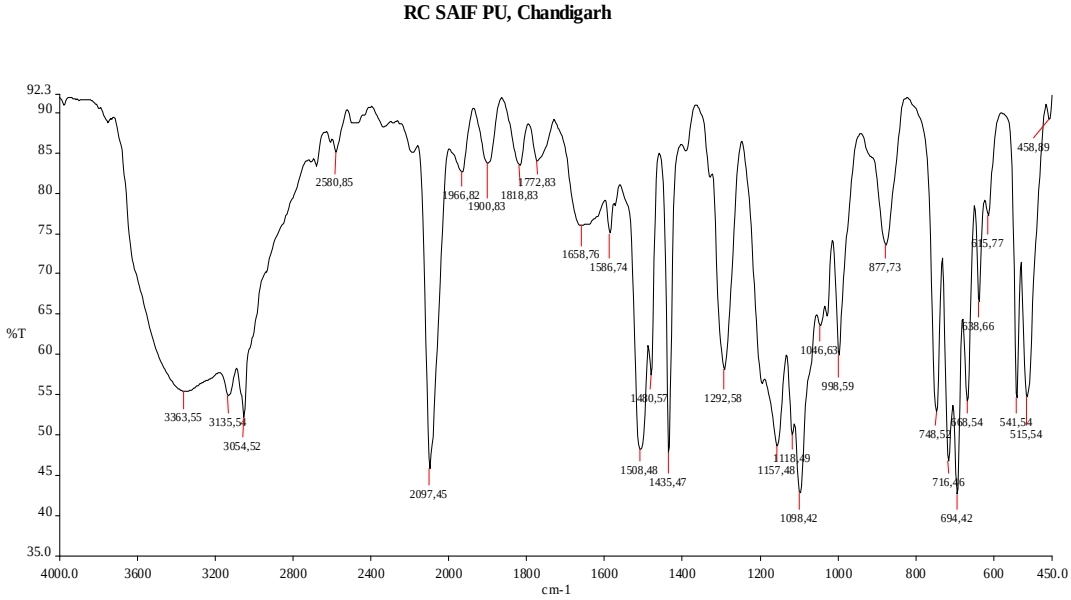


Fig. 3. FTIR spectra of complex 9

3.2.5 TEM Analysis

The TEM analysis made to find the size of the nanoparticles appeared as clusters of irregular particles surrounded by bright hyaline coating (Fig. 6). The particle size measured provides point-to-point resolution. The picture showed the clusters of irregular particles with size ranged from 15 to 30 nm.

3.3 Larvicidal Evaluation of Nano-formulation under Laboratory Conditions

Mortality of the larvae after 24 hours of treatment with different concentrations of metal complex nanoformulations were recorded along with control sets. Preliminary screening of the compound was done by calculating the highest

mortality of larvae within the time duration of testing. The marked efficacy of different concentrations of metal complexes nanoformulations has been enumerated in Table 1.

All the synthesized metal complexes showed moderate to significant toxic effect against *A. aegypti* at different test concentrations. Amongst them, two complexes 2 and 8 exhibited most promising larvicidal potential (LC_{50} values, 9.907 and 7.534 µg/mL, respectively) followed by complexes 5, 7 and 11 (LC_{50} values, 10.422, 13.635 and 13.405 µg/mL, respectively). The results are in agreement with the results of Sundaramurthy and Kannappan, who reported the best potential of Cu complexes against *A. ageypti,* in comparison to other bioactive metals [29]. The mode of action of the prepared organophosphorous appears to be different, as there is no P=O linkage in the complex, which makes it acetylcholinesterase (AChE) inhibitor and thus has lower side effects on humans [30]. The presence of bis-triphenyl phosphine in the complexes resulted in the decreased larvicidal activity of the complexes which is probably due to the steric hindrance by bulky PPh_3 groups. The presence of –NCS ligands has been found to have endorsing effect in the series of complexes as the complexes 4, 5, 6, 10, 11 and 12 were found to be less effective against the *Aedes* larvae.

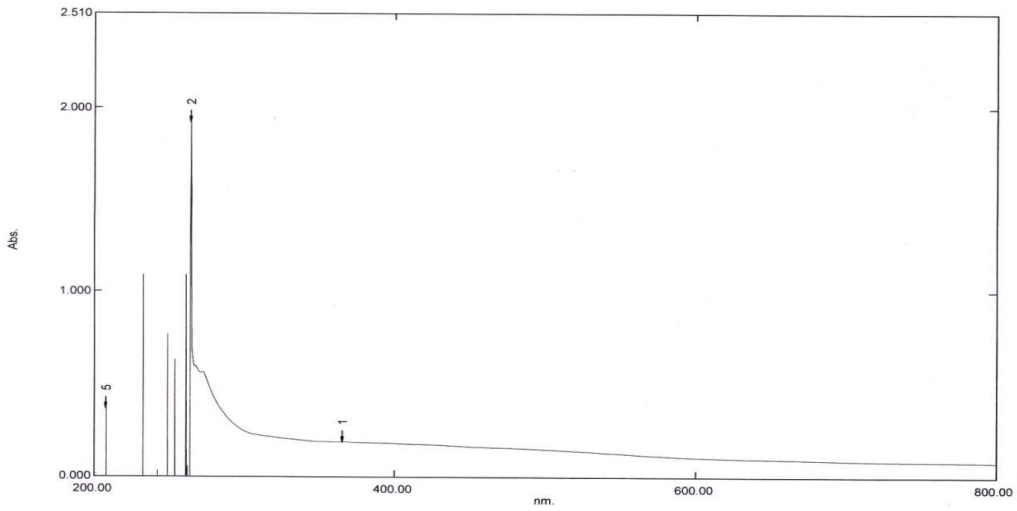


Fig. 4. UV-Visible spectrum of complex 3

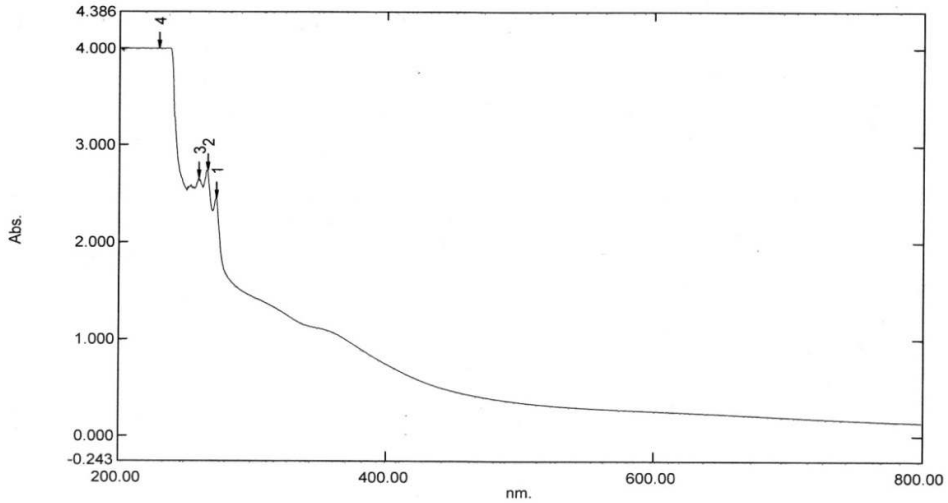


Fig. 5. UV-Visible spectrum of complex 8

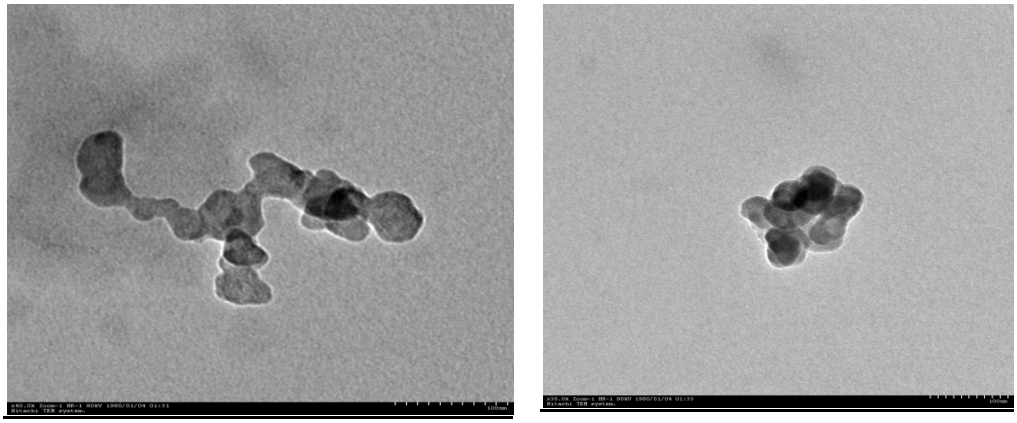


Fig. 6. TEM images of complex 4 (250 ppm), and complex 10 (100 ppm)

Table 1. Larvicidal activity of nano-formulations of metal complexes against *Aedes aegypti*

Complex concentration (µg/mL)	Larval mortality (%)						LC_{50} (µg/mL)
	250	100	50	25	10	5	
1	100	95	80	63.33	40	15	15.521 (4.664-32.172)
2	100	100	80	71.66	55	31.6	9.907 (4.817-15.591)
3	100	100	78.33	63.33	36.66	8.33	17.108 (11.732-23.944)
4	100	86.66	73.66	60	33.33	8.33	20.547 (14.841-27.575)
5	100	96.66	88.33	71.66	51.66	28.33	10.422 (8.551-12.372)
6	100	95	75	55	33.33	11.66	19.16 (16.511-22.103)
7	100	88.33	85	65	45	21.66	13.635 (11.238-16.201)
8	100	98.33	88.33	73.33	61.66	40	7.534 (5.781-9.319)
9	100	85	70	60	40	18.33	22.722 (13.275-36.845)
10	100	78.33	68.33	56.66	28.33	6.66	24.848 (15.818-37.487)
11	100	93.33	83.33	63.33	46.66	21.66	13.405 (11.142-15.823)
12	100	85	70	61.66	50	20	15.288 (7.8-24.731)
Control	0	0	0	0	0	0	-
Temephos	100	100	100	100	100	100	4.125 (3.245-4.652)

No. of replicates 3@ 20 larvae/replicate

Chemical analysis of the complexes clearly indicated the safer mode of their application as these are not soluble in water and the prepared nano-formulations, inflicted less direct bioavailability to living tissue. The designed complexes have triazole and DTC moieties, which are permissible moieties for use (US EPA) along with NCS and PPh_3 group that form strong bonds with the heavy transition metals *via* soft acid-soft base interaction. Overall, the complexes are expected to have topical mode of action on the larval gut. They are expectedly less toxic to mammals and plant kingdom. Moreover, copper in most potent compounds is present in bound form which inflicts lower toxicity than free copper ions [31]. Further research in this field, advocates the use of Cu and NCS ions as metal and ligands for designing of bioactive molecules of larvicidal behaviour against *A. ageypti* larvae.

Comparison of the results with reference standard indicated a very high larvicidal potential of Temephos (LC_{50}, 4.125 µg/mL, 24 hours) as compared to the tested nano-formulations. Moreover, nano-formulating the metal complexes with water as base provide eco-friendly alternate by eradicating the use of organic solvents [32]. So, the present larvicidal results of water based nano-formulations are a significant outcome in favour of less toxic metal complexes as larvicidal agents.

3.4 *In silico* Toxicity Analysis

The results of toxicity showed that all the compounds belong to class III level of toxicity

which referred to high toxicity, and it was same as that of the already used commercial standards. Carcinogenicity studies indicated that the iron complex showed negative results for both genotoxic and non-genotoxic kind of carcinogenicity whereas, complexes of cobalt and copper were considered to have some non-genotoxic carcinogenic nature. The results of mutagenicity on the basis of their activity on *S. typhimurium* TA 100, was also nil for all the complexes. But the standard Temephos, an organophosphorous has greater limitations in terms of its toxic effect on non-target organisms [33].

4. CONCLUSION

The present investigation has important implications in the practical control of mosquito larvae in the polluted aquatic ecosystem. In comparison to Temephos and chlorinated agrochemicals (DDT), these metal complexes nano-formulations can be considered as a better option due to lower toxicity and water dispersibility. Further tailoring and alteration of the effective moieties is recommended to get more effective and eco-friendly solutions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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