The Impact of Cu Ion, Two Novel Schiff Base Ligands and their Copper (II) Complexes on the Biological Activity of the Entomopathogenic Nematodes

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Abstract

Background. Many biotic and abiotic soil components affect entomopathogenic nematode (EPN's) activity, infectivity, host finding ability and the rate of reproduction. Since, copper being an essential element, could be toxic at its elevated concentrations in soils, the study was aimed therefore to evaluate the biological effect of Cu ion and safer alternatives, i.e. Schiff base ligands and their copper complexes on EPN's juveniles. ***Methods.*** Two novel Schiff base ligands of 2-amino 3- cyano 1, 5 diphenylpyrrole and salicylaldehyde (HL^1) or 2- hydroxy11-naphthylaldehyde (HL^2) and their copper (II) complexes were synthesized and characterized. Their effect on the infectivity and reproduction potential of the Egyptian entomopathogenic nematodes (EPN's) *Heterorhabditis bacteriophora* and the imported *Steinernema carpocapsae* were tested at 1.5 mg/l and 11.0 mg/l. ***Results.*** The infectivity of Cu (II) ion treated *H. bacteriophora* and *S. carpocapsae* juveniles (at low and high concentrations) generally reduced, (33.30 % and 11.50%) and (88% and 75%) respectively, as compared with that of control. The infectivity of the ligands and complexes treated *H. bacteriophora* and *S. carpocapsae* juveniles at both concentrations matches that of the non-treated nematodes (100%). The reproduction of *H. bacteriophora* and *S. carpocapsae* decreased with increasing concentrations of copper (II), the ligands (HL^1, HL^2) and complexes (C1, C2) except in HL^1 for *H. bacteriophora* and C2 for *S. carpocapsae*. The difference in reproduction potentials of the tested EPN's due to the dose variations of the agents was observed to be insignificant. ***Conclusions.*** Although the pollution of soil with copper (II) ions affects nematode infectivity and reproduction potential, Schiff base ligands and their copper complexes were found to be less harmful and hence the latter could be used in combination with fertilizers to overcome one of the abiotic factors enhancing the field efficacy of EPN.

Significance | Schiff bases complex in retaining the activity of entomopathogenic nematodes.

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Abbreviations: HL^1, 2-amino 3- cyano 1, 5 diphenylpyrrole and salicylaldehyde; HL^2, 2- hydroxy11-naphthylaldehyde; EPN's, entomopathogenic nematodes; IJs, Infective juveniles; Hb, *Heterorhabditis bacteriophora*; Sc, *Steinernema carpocapsae*; C1, Copper complex 1 of ligand HL^1; C2: Copper complex 2 of ligand HL^2; DMSO: Dimethyl sulfoxide.

Introduction

Environmental concerns about chemical insecticides serve as a strong impetus for the development of biological control agents or biopesticides. Biopesticides can be safer, more biodegradable, and less expensive to develop. Among biopesticides, insect parasitic nematodes in the families Steinernematidae and Heterorhabditidae possess tremendous potential as alternative to chemicals. They have a unique association with symbiotic bacteria, *Xenorhabdus*

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and *Photorhabdus* (Enterobacteriaceae), respectively (Akhurst, 1995, Boemare et al; 1993, Hussein and Abd el Aty, 2012) which is essential for the usefulness of these nematodes in the control of insects (Nouh and Hussein, 2014; Hussein et al, 2015).

However, the field efficacy of these nematodes is limited because of their vulnerability to environmental conditions such as low humidity and solar radiation (Gaugler et al., 1992). Also, many biotic and abiotic soil components affect nematode activity, infectivity, host finding ability and the rate of reproduction. Copper, an essentially required element by all organisms, could be toxic at its elevated concentrations in soils and may result in a range of effects including reduced biological activity and the subsequent loss of fertility (Dumestre et al. 1999). Pesticide application is of particular interest among different sources of copper pollution such as mining, industrial discharge and fertilizers (Nor, 1987). Besides its use as fungicides and algicides, it is also used in animal nutrition and fertilizers. Again, copper is used to kill the slugs and snails in irrigation and municipal water treatment systems (Kamrin, 1997). Hence, significant uncertainty remains in the prediction of impacts of pesticide application on the agricultural ecosystem (Liess, 2004).

Contrarily, Schiff bases and their complexes have been studied extensively as they possess many interesting features including biological and pharmacological activities (Sinha et al 2008, Panneerselvam et al 2005 and Karthikeyan et al 2006), e.g., their complexing ability towards some toxic metals (Sawodny and Riederer, 1977). Also, the complexes of the Schiff base have a variety of applications in biological, clinical and pharmacological areas (Hitoshi et al, 1997).

The aim of the present study is, therefore, to evaluate the biological effect of Cu ion, two Schiff base ligands and two complexes of copper on *H. bacteriophora* and *S. carpocapsae* juveniles. This will include the determination of influence of these agents on the infectivity and the rate of reproduction of the nematodes.

Materials and methods

Nematode source

One endemic Egyptian species, *Heterorhabditis bacteriophora* and one exotic *Steinernema* species were used in the present study. The *Steinernema carpocapsae* (ALL) strain was obtained from Dr. Ralf Udo-Ehlers, Kiel University, Germany.

Preparation of the Schiff base ligands HL^1 and HL^2

The Schiff base ligands were prepared by the condensation of equimolecular amounts of 2-amino 3-cyano 1, 5-diphenyl pyrrole with salicylaldehyde (HL^1) or 2-hydroxy 1- naphthylaldehyde (HL^2) in 25 ml absolute ethanol and a trace amount of P_2O_5 was added. The resulting mixture was refluxed for 1- 4 h. The solids products obtained (yellow and orange for HL^1 and HL^2, respectively), were dried under vacuum and kept dry in a desiccator over anhydrous $CaCl_2/P_4O_{10}$.

Preparation of solid complexes (C1 and C2)

The solid complexes were prepared by mixing hot alcoholic solutions of copper salts (chloride, nitrate, acetate or bromide) with ligand under study HL^1 or HL^2 in the presence of an appropriate amount of sodium acetate, AcONa (1:1:1) molar ratio . The reaction mixture was stirred for 3-8 days. Petroleum ether (60-80%) was added to assist a good precipitation. The solid complexes were filtered off, washed several times with ethanol and kept in a vacum desiccator before analysis.

Preparation of the tested samples for biological activity

The copper salt ($CuCl_2.2H_2O$) was dissolved in distilled water. Distilled water was used for the control. Ligands and complexes were dissolved in distilled water and dimethyl sulfoxide (DMSO) in a ratio of (1000 ml: 5ml), respectively at the following concentrations (1.5 and 11 mg/L). Distilled water and DMSO were used for the control.

Nematode biological activity

Infectivity bioassay

The infectivity was checked for nematodes treated with Cu (II) ions, two ligands and two complexes under low and high concentrations (1.5 mg/L and 11 mg/L) according to Abdel Rahman and Hussein (2007) against the wax moth larvae, *Galleria mellonella*. Full grown larvae of *G. mellonella* kept in 1.5 ml Eppendorf tubes, lined with double layer filter paper (Whatman No. 1), were subjected to nematode infection at a dose level of 50 IJs/larva in 300 μl distilled water and kept at 25°C, in dark for 48 hrs. Three replicates, each with 10 larvae and a control treatment containing untreated nematodes were done.

Rate of reproduction

Larvae of *G. mellonella*, revealing the signs of nematode infection in the infectivity test, were washed twice with distilled water to remove any nematode juveniles, and used as the host insect for measuring the reproduction rate. The larvae were then transferred to White traps (White, 1927). The emerging juveniles from each larva were received in distilled water and stored at 15 °C until counted. After all infected larvae have ceased producing nematode juveniles, the number of IJs of each population was counted. Three replicates were done.

Data analysis

Data expressed in percentage were transformed to arcsine values to ensure normality. Statistical significance was determined by analysis of variance (Student's t-test and Duncan's multiple range tests) using the software package Costat (Cohort Inc., Berkeley, CA, USA). Results are recorded as mean ± standard deviation (SD) (Duncan, 1955).

Results and Discussion

Nematode Biological Activity

Infectivity bioassay

Data in table (1) showed a reduction in mortality of wax moth larvae, *G. mellonella* infected by Cu (II) ion treated *H. bacteriophora* (Hb) juveniles. Mortality was recorded as 33.30% and

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Table 1 | **Infectivity of *Heterorhabditis bacteriophora* juveniles to *Galleria mellonella* after treatment with two concentrations of Cu (II) ion, two ligands (HL^1, HL^2) and two complexes of copper (C1, C2).**

Metal ion, Legands, Complexes	Infectivity percentage of *G. mellonella* larvae caused by *H. bacteriophora* juveniles		
	Control	Concentration	
		1.50 mg/l	11.0 mg/l
Cu (II)	100	33.30	11.50
HL^1	100	100	100
HL^2	100	100	100
C1	100	100	100
C2	100	100	100

Table 2 | **Infectivity of *Steinernema carpocapsae* juveniles to *Galleria mellonella* after treatment with two concentrations of Cu (II) ion, two ligands (HL^1, HL^2) and two complexes of copper (C1, C2).**

Metal ion, Legands, Complexes	Infectivity percentage of *G. mellonella* larvae caused by *S.carpocapsae* juveniles		
	Control	Concentration	
		1.50 mg/l	11.0 mg/l
Cu (II)	100	88	75
HL^1	100	100	100
HL^2	100	100	100
C1	100	100	100
C2	100	100	100

Table 3 | **Rate of reproduction (RR) of *Heterorhabditis bacteriophora* juveniles to *Galleria mellonella* after treatment with different concentrations of Cu (II) ion, two ligands (HL^1, HL^2) and two complexes of copper (C1, C2).** * Significantly different than control (T-test, P<0. 05) ns: not significantly different

Cu (II) ions, Ligands, Complexes	Rate of reproduction (RR) of *G. mellonella* larvae caused by *H. bacteriophora* juveniles						
	Control	Concentration					
		1.50 mg/l			11.0 mg/l		
	RR	RR	F	LSD	RR	F	LSD
Cu (II)	98333.3^{a}	68855.56^{b}	15.87 **	17635.75	28805.41^{b}	52.16 ***	22078.25
HL^1	100292.4^{a}	29279.29^{b}	84.08 ***	17255.14	23437.8^{b}	159.33 ***	18566.38
HL^2	106935.6^{a}	35444.43^{b}	116.61 ***	14648.07	22175.72^{b}	346.25 ***	10149.29
C1	98001.67^{a}	19127.02^{b}	375.09 ***	9064.61	29461.53^{b}	236.64 ***	9927.56
C2	97295.95^{a}	59238.2^{b}	26.49 ***	16475.66	44740.17^{b}	126.97 ***	10392.14

Table 4 | **Rate of reproduction of *Steinernema carpocapase* juveniles to *Galleria mellonella* after treatment with different concentrations of Cu (II) ion, two ligands (HL^1, HL^2) and two complexes of copper (C1, C2).**

Cu (II) ions, Ligands, Complexes	Rate of reproduction of *G. mellonella* larvae caused by *S. carpocapase* juveniles						
	Control	Concentration					
		1.50 mg/l			11.0 mg/l		
	RR	RR	F	LSD	RR	F	LSD
Cu (II)	100222.2^{a}	93400.02^{a}	1.72 (ns)	25700.36	42181.41^{b}	92.76 ***	13426.97
HL^1	99314.73^{a}	59166.29^{b}	17.02 **	21083.20	50368.8^{b}	21.50 ***	23655.37
HL^2	97273.06^{a}	68759.43^{b}	92.24 ***	6926.51	44036.72^{b}	110.87 ***	11266.93
C1	97201.69^{a}	64444.02^{b}	40.09 *	11527.37	55620.53^{b}	84.19 ***	10097.07
C2	98026.08^{a}	92255.2^{b}	5.42 *	8840.46	92066.17^{b}	5.75 *	9851.49

11.50% for low and high concentrations, respectively as compared with that of control. Meanwhile, the infectivity of the Hb juveniles, treated with copper ligands and complexes at low and high concentrations, matched that of the non-treated nematodes (100%).

The juveniles of *S. carpocapsae* (Sc) tolerated Cu (II) ion treatment better than *H. bacteriophora* did. The mortality percentages (88% and 75%) of wax moth larvae, infected by Cu (II) ion treated Sc nematodes, were therefore higher at low and high concentrations, respectively (Table 2). In case of copper ligands and complexes treatment, the infectivity of the Sc juveniles was similar to the Hb nematodes, matching that of the non-treated nematodes, both at low and high concentrations (Table 2).

Thus, it was implied analyzing the data that, in terms of infectivity, H. bacteriophora and S. carpocapase exhibited a strong resistance to all the treatments used in this study. As assumed by Jaworska et al. (1994), the non permeability of biological membrane of the tested nematodes could be the major factor of such resistance. The ligands and complexes of copper reduced copper's toxicity and as a result, the infectivity of the nematodes increased up to that of the control. Our results are also in agreement with the findings of Keskioglu et al (2008) who reported that Schiff bases 1,4-bis [3-(2-hydroxy-1-naphthaldimine) propyl]piperazine have no activity against the fungi Candida albicans.

Rate of reproduction

It was observed that the reproduction potential of *H. bacteriophora* significantly decreased when the IJs were treated with low and high concentrations of Cu (II) ions, the two ligands and the two complexes of copper as compared with the control (Table 3). At low concentration, the highest reproduction potential (68855.56 IJs/larva) was observed for Cu (II) followed by C2, HL^2, HL^1 and C1. Again, at high concentrations, the scenario was different where copper complex 2 (C2) treated IJs were observed with the highest rate of reproduction (44740.17 IJs/larva) among other treatments and the rank was followed by C1, Cu (II) ions, HL^1 and HL^2.

The variation in reproduction potentials of the Hb juveniles due to low and high concentrations of all treatments were noticed significant except HL^1, for which the dose variation i.e. low and high concentrations, did not cause any significant difference on the reproduction potential (29279.29 IJs/larva and 23437.8 IJs /larva), respectively.

Likewise, the reproduction potential of *S. carpocapsae* was found to be decreased significantly when the IJs were subjected to the above mentioned similar treatments (Table 4). At low concentration, Cu (II) was found to be responsible for the highest reproduction potential (93400.02 IJs/larva) followed by C2, HL^2, C1 and HL^1. But at high concentration, the highest reproduction potential was observed with copper complex 2 (C2) followed by C1, HL^1, HL^2 and finally Cu (II), with lowest rate of reproduction (42181.41 IJs/larva). Except for C2, the dose variation i.e. the low and high concentrations of the treatments caused significant differences on the reproduction potential of Sc juveniles. Especially, Cu (II) ions caused a sharp decrease in the reproduction potential as shifted from low to high concentration (93400.02 IJs/larva and 42181.41 IJs/larva respectively) whereas no difference in that case was observed for copper complex 2 (C2) treated Sc juveniles with dose switching.

Observed effects of some metal ions including Cu (II) on the infectivity and reproduction potential of the IJs can be interpreted as either indirect effect as they affect the symbiotic bacteria associated with the nematodes (Akhurst and Boemare, 1990) or direct effect of the ions on those organisms. The observation of the offspring from Cu ion and its ligands or complexes treated generations of H. bacteriophora and S. carpocapsae revealed that they affect the reproduction potential reducing the number of offspring and the effect was more intense on the latter species. These results are consistent with those of Jaworska and Gorczyca, (2002) and Dumestre et al. (1999).

Conclusion

The present study mainly aimed to evaluate the biological effect of Cu ion, two Schiff base ligands and two complexes of copper on *H. bacteriophora* and *S. carpocapsae* juveniles which was established by determination of their influence on the infectivity and the rate of reproduction of the nematodes. The present study results indicate that the pollution of soil with copper affect nematode infectivity and reproduction potential. Meanwhile, the complexation of the Schiff base and their complexes with copper reduce this harmful effect which could be a better solution to use them combinedly with the fertilizers to overcome one of the abiotic factors which limits the infectivity of the EPN's and increase their field efficacy. This study considers the first record of studying the effect of Schiff bases complex on the viability of the entomopathogenic nematodes.

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Competing financial interests

The authors declare that they have no competing interests.

Author contributions

MAH and RMAE suggested the idea and designed the research. HAE, HKM and AEM prepared the Schiff base ligands and the solid complexes. MAH and RMAE conducted activity bioassays. MAH analyzed the data and wrote the manuscript. All authors contributed to the writing and approved the manuscript.

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