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Biological Investigation of Nano-Organometallic Agents against Bacteria and *Chilo polychrysus*

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Abstract

Nanotechnology is the new hope, and is hailed as having the potential to increase the efficiency of energy consumption, help clean the environment, and solve major health problems. This research aims to increase the biological activities of natural syringaldehyde. The synthesis of syringaldehyde derivatives and controls the size of the material particles in the nanometer ranges. Nano-organic compounds (**L1** - **L2**) and nano-organometallic compounds (**C1** - **C4**) are used for the study of physicochemical characterization and biological activities. Antioxidant capacities were the DPPH and FRAP techniques, and bacterial abilities against *Escherichia coli*, *Salmonella* spp. *and Staphylococcus aureus*. The syringaldehyde salicylic hydrazone (**L2**) showed a high scavenging ability to DPPH. Free radical exhibited with IC_{50} values as low as 51.109 ppm. The ability of antioxidants by FRAP showed that substances are capable of reducing Fe^{3+} and most of **C3**, the ability to inhibit the growth of bacteria with the lowest MIC and MBC values and the ability to *Chilo polychrysus* (Meyrick) found that **C1** and **C2** showed LT_{50} at 24 h and 48 h (19.00 and 19.33). These particles should develop as biological agents to reduce the use of chemicals that are harmful to humans and the environment.

Keywords

Nanotechnology, Natural Products, Biological Activity, Schiff Base, *Chilo polychrysus*

1. Introduction

The growth of the agricultural economy is influenced by many factors, both within and outside the country, as well as natural factors, which are variable and difficult to predict. Thai economy in the third quarter, overall, agriculture continues to grow 2.2 percent as a result of the most important agricultural products and agricultural prices are good. For GDP, the agricultural sector of the year 2021, can expand in the direction that increases in 2020, which the agricultural sector can expand by 3.3 percent [1], although it has been affected by natural disasters, due to the price level of the product and the amount that is likely to increase for the year 2021, the agricultural sector has expanded by 3.6 percent compared to the previous year because the major producers have been affected by the fluctuation of weather. Risk factors that may result in agriculture sectors, such as oil price levels are still at a high level, the situation of the uncertainty of the global economic recovery [2]. The Thai agricultural economy from the beginning of 2021 to 2022 is still likely to grow continuously. There are supporting factors both from agricultural prices that are likely to increase and demand for agricultural products from Thailand in foreign countries. Due to the fluctuating weather, the spread of diseases and pests is more violent. Agricultural products are damaged, causing many counties to be concerned about food sufficiency [3]. The result of the demand for agricultural and agricultural industries in the world market is a very high recording of the positive factors of demand in foreign markets. Therefore, the control of plant diseases by biocontrol, which reduces the population and reduces the activity of plant germs that will cause disease to a level that does not cause economic damage to plants by living organisms in nature [4] [5] [6]. There are many living things or microbes that have the ability to be an opposition to the causes of plant diseases and each type has different treasures in the hostility. Current, new plant disease control methods are being researched to reduce the dangers of using agricultural chemicals and encourage farmers to use biological control of plant diseases. It is considered an accepted method that works well. Disease control mechanisms and biological control systems have been studied. Bring knowledge in science and technology, nanotechnology, and biotechnology to help solve problems or create value for agricultural products to have quality and safety for consumers. From the above reasons and necessity, the research team is interested in combining natural products with knowledge of nanoscience and nanotechnology to be used to control bacteria that cause human disease and *Chilo polychrysus* of rice. Because rice is an economic crop that brings income into the country as the top of all the economic crops. Therefore, solving the problem of rice enemies is urgent.

2. Materials and Methods

2.1. Chemicals and Instruments

All of the chemicals were purchased from Sigma Aldrich Chemicals (USA). Analytical grade solvents were employed during all syntheses. The elemental analyses were recorded on CHNS Analyzer, Perkin Elmer PE2400 Series II at Scientific and Technological Research Equipment Center Chulalongkorn University, Bangkok, Thailand. The morphology of particles was carried out by using Scanning Electron Microscope (Model JCM-6010 LV) and the infrared spectra were recorded on a Perkin Elmer 283-B infrared spectrophotometer.

2.2. Synthesis of Hydrazone Schiff Base Ligands

All ligands were prepared by condensation of syringaldehyde with one of the five hydrazides indicated (Figure 1) in the molar ratio 1:1 in MeOH (5 ml) with constant stirring at 40˚C for 6 hrs and concentrating the reaction mixture before allowing it to cool at room temperature. The products were obtained with high percentages of the yield as shown in Table 1.

2.3. Synthesis of Copper Complexes

Synthesis of nanoparticles by using bottom-up technique between certain metal (II) chloride dihydrate or metal nitrate was added to the methanolic solution of syringaldehyde hydrazones in the molar ratio of 1:1 over a period of 3 hrs with constant stirring in dark. The products were separated by centrifugation and dried in vacuum.

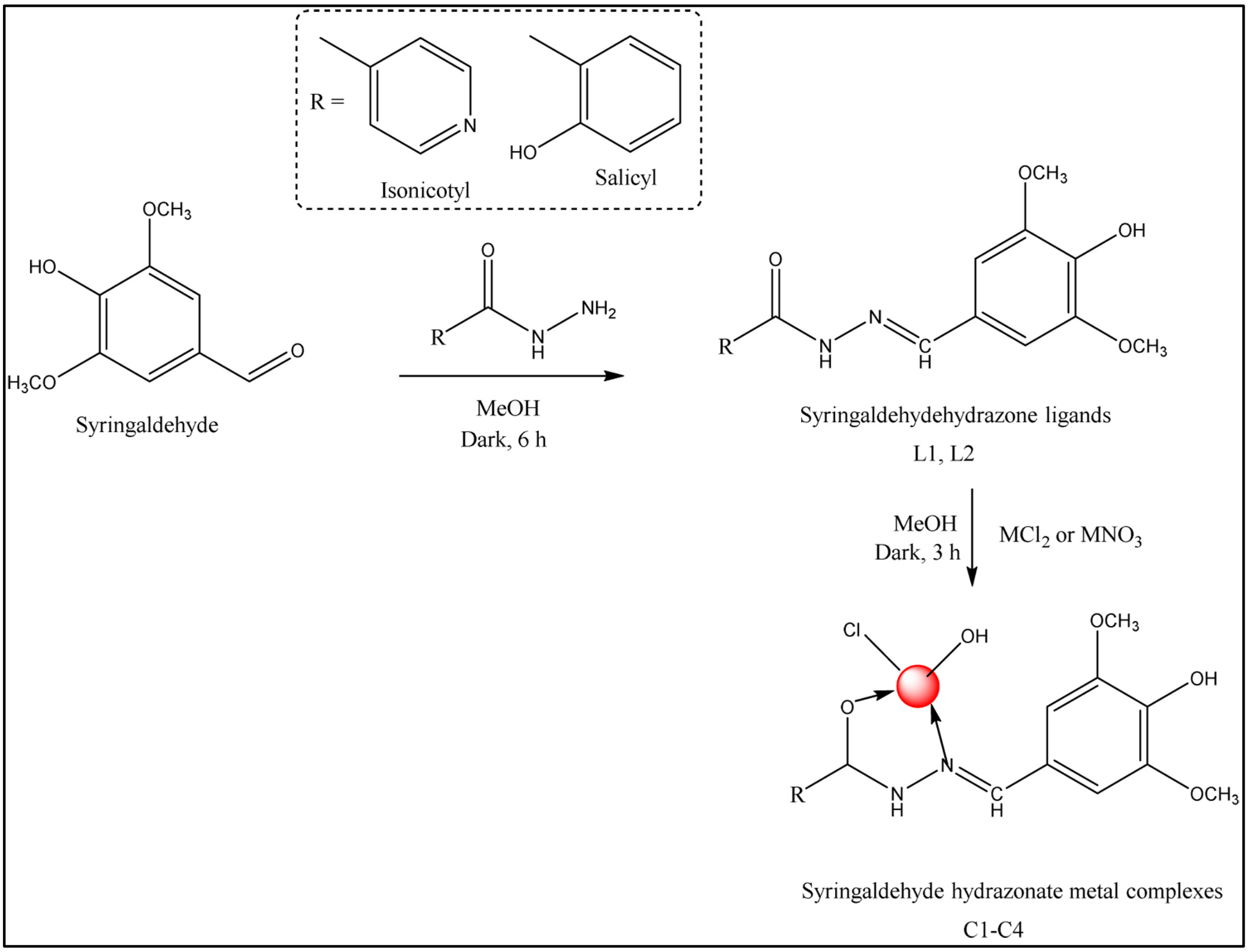

Figure 1. General synthesis scheme for preparation of ligands and metal particles.

Table 1. Physicochemical properties of samples.

Trivial name	Code	Formula	Meting point (˚C)	Color of samples	Particle size (nm)
SRA-SAL	L1	$C_{16}H_{16}N_2O_5$	234.6	yellow	139.86
SRA-SAL-Cu	C1	$[C_{16}H_{16}N_2O_5CuCl(H_2O)]$	288.9	green	74.16
SRA-SAL-Ag	C2	$[C_{16}H_{16}N_2O_5AgNO_3(H_2O)]$	227.2	brown	85.24
SRA-INH	L2	$C_{15}H_{15}N_3O_4$	229.2	yellow	150.86
SRA-INH-Cu	C3	$[C_{15}H_{15}N_3O_4CuCl(H_2O)]$	195.7	green	56.38
SRA-INH-Ag	C4	$[C_{15}H_{15}N_3O_4AgNO_3(H_2O)]$	213.3	yellow	70.86

2.4. Antioxidant Activity by DPPH Method

The assay was performed as described Sharma *et al.* [7], the DPPH radical scavenging activity of the compounds was measured. Briefly, 0.2 mM, 2-Diphynyl-1-picrylhydrazyl (DPPH) solution was prepared with methanol. 100 µL sample was added to 3.9 cm^3 DPPH solution and was incubated in dark for 30 min at a temperature of 37˚C then the absorbance was measured at λ_{max} = 517 nm, calculate the percentage of anti-free radical activities.

2.5. Antioxidant Activity by FRAP Method

Prepare FRAP (ferric reducing/antioxidant power) by preparing acetate buffer pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridly-s-trizine) in solution 40 mM of HCL, and 20 mM of ferric chloride and then mix at the proportion of 10:1:1 (v/v). This will get a FRAP solution, then prepare a sample solution in purified water with the required concentrations [7].

2.6. Antibacterial Activities

All the newly synthesized compounds were screened for antibacterial activity against both gram-negative (*Escherichia coli* and *Salmonella* spp.) and gram- positive bacteria (*Staphylococcus aureus*) by determining minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) using the broth dilution method. Bacterial colonies were taken from fresh cultures and incubated in the tubes containing 5 mL of trypticase soy broth at 37˚C for 6 hours until achieving visible growth. Turbidity was adjusted to 0.5 McFarland standard by adding 0.05 mL of 1% w/v $BaCl_2{\cdot}2H_2O$ in phosphate buffered saline (PBS) to 9.95 mL of 1% v/v H_2SO_4 in PBS. Finally, 10 mg of each compound was dissolved in 10 mL of dimethyl sulfoxide (DMSO) to obtain concentration of 1 mg/mL [8].

2.7. Experimental Design: Anti-*Chilo polychrysus* (Meyrick)

The research team conducted a preliminary study and selected the best specific compounds against the larveae of the rice dark-head stem borer in the 1.5 × 1.0 × 1.0 m^3 plots, each plot containing 40 borer larveae (F1). The experimental plan was completely randomized design (CRD), there were a total of 5 treatments, 3 replications, 20 tillers per plot of rice. The sample was prepared to be concentrated with 10.0 ppm mixed with surfactant and sprayed in the planting rice aged 45 days [9].

2.8. Statistic

All experiments were carried out in triplicates. Data obtained were analyzed by using one-way analysis of variance (ANOVA) and Pearson's correlation coefficient was performed. Significant differences between the means at 95% ($p < 0.05$) level were considered statistically significant. Data was recorded as mean ± standard deviation.

3. Results

3.1. Physicochemical Characterization of Samples

Physical chemistry feature of compound from synthesis, both ligands; Syringaldehyde salicylic hydrazide (**L1**) Syringaldehyde isonicotinic acid hydrazone (**L2**), and complexes between ligands and metal ions (Cu^{2+} and Ag^{+}) are C1, C2, C3 and C4 as in **Figure 1**.

The synthesized substances will have colors, molecular formula, melting point, percentage of elemental substances, and percentage of products in different figures as in **Table 1** and **Table 2**, respectively.

All synthesized substances are polar compounds but polarity is high in ligand L1 and L2. These ligands are reacted with Lewis acid (copper and silver) to form C1, C2, C3 and C4 complexes with reduced polarity due to reduced charge. This is caused by the neutralization of negatively charged ligands with positively charged metal ions of copper and silver.

Table 2. Percent yield, molecular weight and elemental analysis of samples.

Samples	% yield	MW	% C	% H	% N	% O
L1	86.91	316.32	60.69* (60.55)#	5.05 (4.95)	8.85 (8.90)	25.29 (25.25)
L2	97.14	301.31	59.73 (60.01)	4.83 (4.75)	13.93 (13.85)	20.62 (20.55)
C1	77.07	450.80	42.59 (41.89)	3.54 (3.62)	6.21 (6.15)	17.74 (17.80)
C2	80.23	486.19	39.49 (40.13)	3.29 (3.35)	5.75 (5.80)	16.45 (16.50)
C3	66.14	435.75	41.30 (41.23)	0.26 (0.30)	9.63 (9.56)	14.68 (14.59)
C4	86.54	471.18	38.20 (38.15)	3.18 (3.20)	0.91 (1.02)	13.58 (13.61)

Note: *Cal, #Found.

Both ligands and complexes synthesized in this research showed high percentage yields (66.14% - 79.14%) by condensation reaction between syringaldehyde and hydrazide in a 1:1 mass stoichiometric ratio. The complex is green in color, ligands conjugate with metal salts. From the study of chemical composition, it was found that complexes have a general formular as [M(ligand)(H_2O)NO_3/Cl], corresponding to our previous studies [10] [11] and others [12] [13].

3.2. IR Spectra

The significant peaks in ir spectra of parent compounds and their probable assignments useful for determining the coordination mode of the coordination mode of the present ligands are summarized in **Table 3**.

The parent compound exhibits the carbonyl stretching frequency band at 1671 cm^{-1}, when this compound reacts with an amine group, a new Schiff base is formed, this functional group was replaced by the imino group, which showed additional vibration band at 963 - 976 cm^{-1}. There are also stretching frequency bands at 1600 - 1615 cm^{-1} due to the formation of new hydrazinic N-N and C=O bands. The presence of ν(N-H) and ν(C=O) bands in the ligands indicate that these compounds are stable in the keto form in nature [14], but upon interaction of the hydrazinic carbonyl group with metal, they migrate to lower wavenumber (1323 - 1372 cm^{-1}) indicating enolization and metal interaction with the phenolate group, while the presence of a broader band at 1600 - 1615 cm^{-1} due to the vibration of the azomethine group (>C=N-N=C<) and when complexation with metal the ν(N-N) band moves to the higher wavenumber side (as in **Table 3**). The appearance of new bans around 511 - 570 and 480 - 485 cm^{-1} were bands between ν(Cu-O) and ν(Cu-N) [15]. In addition, the vibration attributed to the nitrate group $\nu(NO_3)$ was observed at 1385 cm^{-1} and the ν(Ag-O) vibration mode was found at 510 - 515 cm^{-1} in the spectra of complexes C2 and C4, respectively [16] [17].

3.3. Antioxidant Activities

The ability of anti-free radical activities by using the DPPH and FRAP methods.

Table 3. IR spectra of ligands (L1 - L2) and their metal complexes (C1 - C4).

Compound	ν (N-H)	ν (C=O)	ν (N-N)	ν (C=N)	ν (C=N-N=C)	ν (C-O)	Cu-O/ Ag-O	Cu-N/ Ag-N
Precursor	3282	1671 (vs)	-	-	-	-	-	-
L1	3291	1642	963 (s)	1587	-	-	-	-
C1	-	-	974	1564	1612 (sh)	1357 (s)	511	480
C2	-	-	985	1537	1600 (sh)	1365 (s)	510	715
L2	3225	1653	964	1587	-	-	-	-
C3	-	-	980	1509	1615 (sh)	1323 (s)	570	485
C4	-	-	986	1553	1610 (sh)	1360 (s)	515	714

Note: vs = very strong; s = strong; sh = shoulder.

The calculation of inhibitory concentration 50% is illustrated in Table 4, Figure 2 and Figure 3.

It is found that the novel Schiff base ligands anti-free radical DPPH at a high percentage. All compounds (particles) showed anti-free radical DPPH and have a concentration dependence on both the ligands and metal complexes but hydrazonate metal complexes have low proficiency than independent ligands because of the bigger structure resulting in the steric effect that makes it difficult to provide hydrogen free radical. For anti-free radical activity by reducing Fe^{3+} to be Fe^{2+} (FRAP) it is found that ligands and their complexes showed low ability to reduce iron because the molecule has difficulty in providing single electron transfer. All particles showed higher activity in both techniques than the standard ascorbic acid, especially C1 particles showed the best potency because of small particles. Antioxidants have a differing capacity to stop the propagation of free radicals, influencing this both the structure of the antioxidant and the structure of the compounds to be oxidized [18] [19].

Table 4. Value of IC_{50} of samples from anti-free radical DPPH.

Samples	IC_{50} (inhibitory concentration 50%)	
	DPPH	FRAP
L1	60.06[e]	139.31[d]
L2	59.95[e]	160.66[b]
C1	55.76[e]	119.13[f]
C2	87.98[d]	154.8[c]
C3	113.85[c]	126.10[e]
C4	134.47[b]	153.20[c]
Ascorbic acid	241.21[a]	261.01[a]
C.V. (%)	6.09	1.09

Note: The different of values: a > b > c > d > e > f.

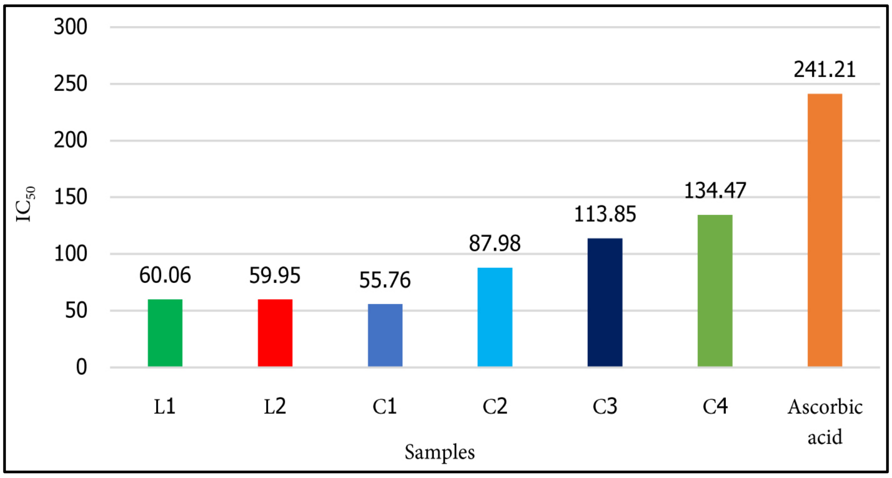

Figure 2. IC_{50} of the compounds compare with vitamin C standard in DPPH method.

3.4. Antibacterial Activities

Prepare solution of samples in different concentrations and the screening of the *in vitro* antibacterial activity against both Gram-negative bacteria (*Escherichia coli*, *Salmonella* spp.) and Gram-positive bacteria (*Staphylococcus aureus*) for 24 hours. Then measure MIC and MBC which find the results as in Table 5, Figure 4 and Figure 5, respectively.

All the green synthesized nanoparticles showed good MIC and MBC antibacterial activity, especially copper nanoparticles (C3) with small particle sizes (56.38 nm) showed the best efficiency to inhibit cell growth. The mechanism of action for antibacterial activity starting from metal ions is released from metal nanoparticles (NPs) during the redox reaction by exchanging electron and ROS free radicals to react with DNA, protein enzymes and mitochondria [20] [21].

3.5. Biological Activities to *Chilo polychrysus* (Meyrick)

Test results for the ability of nanoparticles against larvae (F1) as in Table 6.

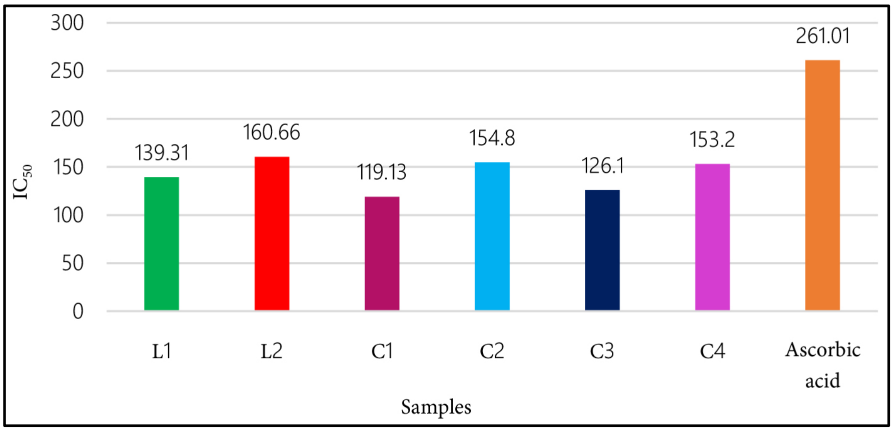

Figure 3. IC_{50} the compounds compare with vitamin C standard in FRAP method.

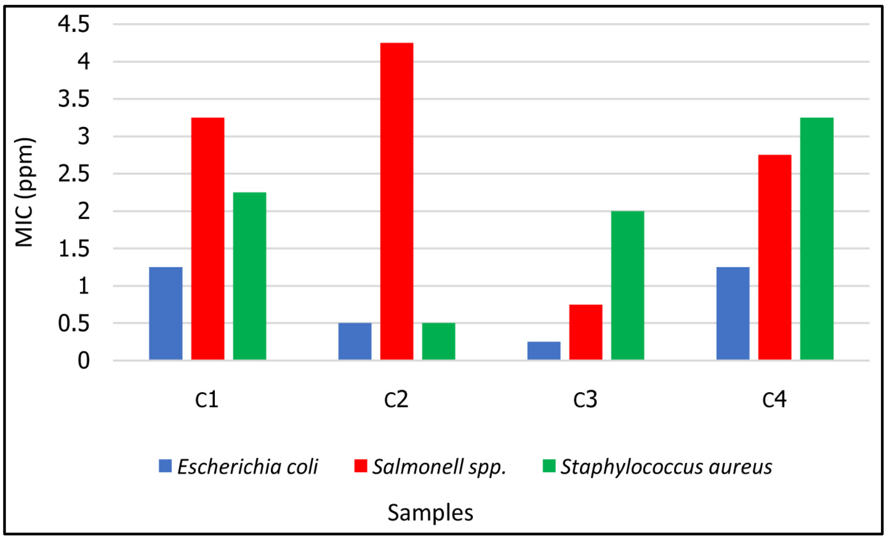

Figure 4. The MIC values (ppm) of copper particles (C1, C3) and silver particles (C2, C4).

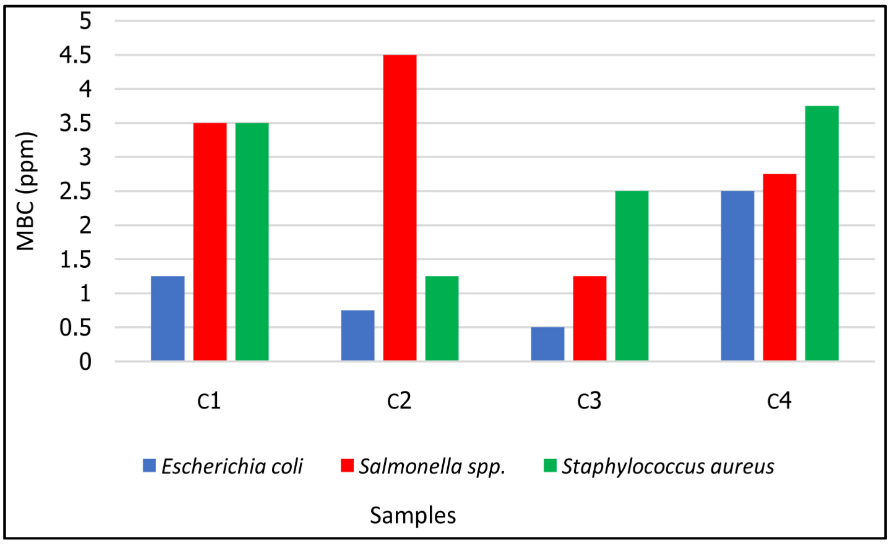

Figure 5. The MBC values (ppm) of copper particles (C1, C3) and silver particles (C2, C4).

Table 5. The MIC and MBC of nano-organometallic particles against the tested bacteria.

Bacteria	MIC and MBC (ppm)	Compounds			
		C1	C2	C3	C4
Escherichia coli	MIC	1.25 ± 0.71	0.50 ± 0.71	0.25 ± 0.00	1.25 ± 0.00
	MBC	1.250.71	0.75 ± 0.00	0.50 ± 0.71	2.50 ± 0.71
Staphylococcus aureus	MIC	2.50 ± 0.00	0.50 ± 0.71	2.00 ± 0.00	3.25 ± 0.71
	MBC	3.50 ± 0.71	1.25 ± 0.00	2.50 ± 0.71	3.75 ± 0.00
Salmonella spp.	MIC	3.25 ± 0.00	4.25 ± 0.00	0.75 ± 0.71	2.75 ± 0.00
	MBC	3.50 ± 0.71	4.50 ± 0.71	1.25 ± 0.00	2.75 ± 0.00

Note: Values are expressed as Mean ± S.D (n = 3).

Table 6. The survival rate of *Chilo polychrysus* (Meyrick) after contact to sample concentration at 10 ppm at 1, 12, 24 and 48 hours with LT_{50} values.

Samples	Survival rate (%) after treatment				LT_{50} (hours)
	1 hr	12 hrs	24 hrs	48 hrs	
C1	37.00^{b}	29.00^{c}	19.00^{c}	14.00^{d}	24 hr
C2	37.66^{b}	34.33^{b}	24.00^{b}	19.33^{b}	48 hr
C3	39.00^{a}	35.66^{b}	25.00^{b}	25.00^{b}	na
C4	39.00^{a}	37.33^{a}	25.33^{b}	23.66^{b}	na
Control; DMSO (5%)	39.33^{a}	38.00^{a}	34.66^{a}	33.00^{a}	na
C.V. (%)	2.51	2.22	3.76	4.19	

Note: na = not available. The different of values: a > b > c > d.

It was found that after larvae were exposed to nanoparticles, they move violently at first. Then, the movement became less and less and death began to occur towards the end of the hour. After 24 hours, the mortality rate increased, especially C1 (LT_{50} 19.00), and by 48 hours, the larvae exposed to C2 particles had a lower survival rate (LT_{50} 19.33). Nanoparticles have a significant impact on insect antioxidants and detoxifying enzymes leading to oxidative stress and cell death [22] [23].

4. Conclusion

The synthesis of nanoparticles with the bottom-up technique by using natural syringaldehyde hydrazone is a reducing agent and controls the size of the material particle in the nanometer ranges. All particles showed antioxidant capacity with IC_{50} lower than the ascorbic acid standard. The C3 particle inhibits the growth of bacteria with the lowest MIC and MBC values. Besides that, C1 and C2 particles affect the survival rate of *Chilo polychrysus* (Meyrick) with LT_{50} values at 19.00 and 19.33, respectively. These particles should develop as biological agents to reduce the dangers of using agricultural chemicals and encourage farmers to use biological control of plant diseases.

Acknowledgements

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Conflicts of Interest

The authors declare on conflicts of interest regarding the publication of this paper.

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