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Chapter

Biosynthesis of Zinc Nanocomplex Employing for Plant Growth Promotion and Bio-Control of *Pythium ultimum*

Shaima M.N. Moustafa and Rania H. Taha

Abstract

Green Biosynthesis method was used for the preparation of Zn(II) nano complex from the reaction of the schiff base ligand 2,2′-((1E,1′E)-(1,2-phenylenebis (azanylylidene)) bis(methanylylidene))bis(4-bromophenol) and Zn(II)sulphate. The nano complex was characterized by different physicochemical methods. Zinc nanoparticles (ZnNP-T) will be studied as an antifungal agent. In this study, we will investigate the ability of the myogenic Zinc nanoparticles for plant Growth Promotion and Bio-control of *Pythium ultimum*.

Keywords: Zn-Nanocomplex, *Trichoderma harzianum*, *Pythium ultimum*, antifungal activity, *Trigonella foenum-graecum*

1. Introduction

The rising interest of consumers for food integrity and for the social and environmental sustainability of agriculture systems has a special impact on the crop production fields. This circumstance forces us to look for new tools for crop protection that are not based on chemical control.

Since the rising demand production of organic food, using of biofertilizers and biopesticides were the best way to alternative eco-friendly production. In fact, *Trichoderma* spp. have the greatest resistant to pesticides, which categorizes as integrated control and good bio-control agents [1, 2] because of its ability to produce many enzymes, such as chitinases, glucanases and proteases. In addition to bio-control, *Trichoderma* spp. have been recognized as plant growth promoter and promoters of different plant defense mechanisms [1, 3].

Nanoparticles have very important role in the biotechnology industries [4], which considered one of the fastest growing fields because of their biological, chemical and physical characterization. The biosynthesis of nanoparticles complex from fungi are a significant branch, due to the fungi has the greatest tolerance and metal bioaccumulation capability. Biogenic methods for nanoparticles forming by plants [5, 6], fungi [7, 8], and bacteria [9, 10] have formerly been described.

A great promise in this area is the Schiff bases. A Schiff base can be defined as the nitrogen analogue of aldehyde in which the carbonyl group is replaced by the

azomethen group [11]. The transition metal complexes having O and N donor of Schiff bases ligands possess unusual configuration, structural liability, and are sensitive to the molecular environment [12].

Fenugreek (*Trigonella foenum-graecum* L) is an annual herb grown in North Africa, Egypt, India, Afghanistan, Bangladesh, Pakistan, Nepal, Iran, Turkey, Morocco, Spain, southern China and southern Europe. it is one of the commonly used herbs since ancient times. Medical Papyri of ancient Egyptian mentioned that it is used as food and antipyretic and in compounds used as incense for embalming and fumigation [13]. Several Arab writers described the plant and seeds as aperient, suppurative, emmenagogue, diuretic, useful in enlargements of spleen and liver, dropsy, and chronic cough. Fenugreek seeds contain 45.4% dietary fiber that blunts glucose absorption after a meal and regulate the production of cholesterol in liver [14, 15]. Seeds contain trypsine, alkaloids and chymotrypsine inhibitors [16, 17], anti-inflammatory steroidal saponin glycosides, flavonoids, furostanol steroidal saponins, flavone C-glycosides, spirostanol saponins and 17 amino acids, 7 of them being essential amino acids. Seeds considered as one of the most effective antidiabetic plants, used by traditional healers of northern Europe to treat diabetes, as stimulant and carminative, and in renal disorders. In Danish popular medicine, it is used to treat anxiety and depression. The seeds are used for wound dressing, rheumatism, stomachache, and leprosy, and have observed uterine stimulant activity [18].

However, its production may be affected by several exogenous stresses, including diseases caused by soil born pathogen microorganism. *Pythium* species affecting the different plants in younger stages and causing damping-off disease, this pathogenic fungi can caused root rot in later stages of plant growth [19, 20]. When the plant is in the seedling stage of its life cycle, infection by pathogenic *Pythium* species causes pre and post-emergence damping-off disease, which decaying the seeds and seedlings before and after the emergence of the plant from the soil surface, respectively. However, infected mature plants have also been found to show root rot symptoms.

Pythium spp. affect the young tissues, which have not developed to secondary thickenings; thus, the infection is limited to seedlings and youngest roots. The post-emergence damping-off disease of seedlings is associated with symptoms like water soaking, reduced growth, black or brown discoloration, wilting and root rot [21, 22]. In mature plants, water-soaked roots and lesions of stem at the soil line, stunted growth, and brown discoloration of roots are prevalent [23]. Due to their capability to disperse through different routes, their detection and Managements have become crucial. Therefore, the use of an integrated approach with bio and myogenic techniques for controlling of pathogenic *Pythium* spp. can be the most and best sustainable alternative to the traditionally used and dangerous chemical approach.

This study will focus on the management strategies of *Pythium ultimum* causing damping-off and root rot in Fenugreek (*Trigonella foenum-graecum* L) plants by preparation of Nano Schiff base complex, its characterization and its hopeful applications in different fields by using *Trichoderma harzianum*, which is a nonpathogenic, fast growing and environmentally friendly fungus. Therefore, the goals of the present study were: (a) to evaluate the pathogenicity capacity of nano metal complex against seedling. (b) to evaluate the tolerance of different concentrations of nano metal complex on the germination of seeds and possibility for acceleration of the growth and finally, (c) to evaluate the biosynthesis of nanoparticles complex for control of the disease caused by *P. ultimum* in seedlings.

2. Methodology

2.1 Materials

In this study, all chemicals used were of highest purity. Organic solvents used included C_2H_5OH and (DMF) were spectroscopic pure from British Drug House (BDH). Distilled water collected from glass equipment. The other materials such as, 5-bromo-salicylaldehyde (Sigma), *o*-phenelendiamine (Aldrich), $Zn(NO_3)_2$ (Merck) were also used.

2.2 Instrumentation

Microanalytical determinations were carried out in the Cairo University (Microanalytical center). The IR spectra were recorded on a Perkinelmer spectrophotometer (400 – 4000 cm^{-1}) (KBr technique). proton-NMR spectra (DMSO-*d*6) were measured at a Pruker spectrophotometer, using TMS as an internal standard. The uv–vis spectra were recorded on a Perkin-Elmer spectrophotometer. Mass spectra were recorded with the aid of a Shimadzu using a direct insertion probe (DIP) at temperature range 50–800°C. The nano-sized complex was characterized with (SEM) a scanning electron microscope.

2.3 Synthesis of the Schiff base ligand

A solution of 5-bromo-salicylaldehyde (4.02 g, 20 mmol.) in absolute ethanol (50 ml) was added to a hot stirred solution of *o*-phenylenediamine (1.08 g, 10 mmol.). This mixture was refluxed for 3 hr. in water bath and cooling, then, the products formed were collected by filtration. Yield (87.4%, 90.2%); M. P; 240 and 200°C; respectively (the ligand was synthesized in our previous work [24].

2.4 Studied micro-organisms

Isolates of *Pythium ultimum* and *Trichderma harzianum* were obtained from biology department –Jouf University, KSA. Precultures of *Trichderma harzianum* (MW459195) and *Pythium ultimum* (MW830915) were made by grown on potato dextrose agar (PDA) for 7 days at 26°C in dark.

2.5 Preparation of biomass of *Trichderma harzianum* (MW459195)

Two discs (5 mm in diameter) of *T. harzianum* (MW459195) was inoculated onto 100 ml PD broth in a 250 Erlenmeyer flask and incubated at 26°C for 7 days in the dark. Mycelium mats were collected by using filter paper (Whatman No. 1) and washed three times with sterilized distilled water to eliminate any adhering media may present. Mycelium mats (3 g) were dried for bio-synthesis of nanoparticles.

2.6 Pathogenicity tests of nano metal complex against seedling and germination of seeds

In order to use Nano metal complex as bio-control agent, it must not produce metabolic toxins that effect on seed germination and plant growth. Seeds of Fenugreek were soaked in the solution of sodium hypochlorite (2% w/v) for 3 min

and washed with sterile distilled H_2O several times. Ten ml of different concentration of nano metal complex (10, 20, 30 and 40 ppm) were prepared and mixed with 0.6 g of carboxymethyl cellulose (CMC). Fifty of Fenugreek seeds were added to the mixture and stirred thoroughly and allowed to dry overnight in a laminar flow cabinet at room temperature, and incubated at 20°C until seeds germinated. Ten ml of sterile distilled H_2O were used as control. After Fenugreek seeds were germinated and beginning to form radicles and plumules, the time of seed germination were determined. Choose the vital seeds and 3 seeds were planted in Erlenmeyer flasks, which containing sterilized 100 ml of WA (3%) was decanted in conical flasks with 10 ml of different concentration of nano-metal complex (10, 20, 30 and 40 ppm). All flasks were incubated in growth chamber at 25°C with 12 h photoperiod (91 μmol m^{-2} s^{-1}) for 4 week. All seeds were then used in study the pathogenicity of nano metal complex on Fenugreek seed germination and seedling elongation.

2.7 Biosynthesis of the nano metal complex

The Zn nano complex was prepared by mixing the Schiff base ligand (1 mmol) and 1 mmol of Zn (II) nitrate in the presence of *T. harzianum* powder as a reducing agent as a novel method for the preparation of Zn-nanocomplex. The mixture was ground with a pestle in an open mortar at room temperature. The melted mixture was then allowed to solidify. The solid was filtered off and crystallized twice-using C_2H_5OH to give Zn-nanocomplex as a pale brown crystal [25].

2.8 Bioassay of nano metal complex against *Pythium ultimum*

Sterilized warm Potato Dextrose Agar (PDA) medium was prepared, and poured in sterile Petri dish supplemented with 100 μl of different concentration of nano metal complex (10, 20, 30 and 40 ppm). Agar discs (10 mm) were taken from the margins colony grown on water agar, and then placed in the center of each Petri dish. All petri-dishes were incubated in the dark at 26°C. Radial fungal mycelial growth was measured by determined colony diameters in each dishes, after 6 days of incubation. The percentage inhibition of fungal growth was calculated using the formula:

$$\text{Inhibition}(\%) = \left((D - M) \div D\right) \times 100 \quad (1)$$

where D is the growth diameter of *P. ultimum* in the control (mm) and M is the growth diameter of *P. ultimum* in presence of Nano-metal complex (ppm) [26].

2.9 Microscopic analysis of the effect of nano metal complex on the mycelium growth

The microscopic images were taken at the faculty of Agriculture, Cairo University, using a scanning electron microscope (JOEL brand model JSM6400) at 1000 and 3000 x. Samples were taken from mycelium treatment with tested nano metal complex at 30 ppm, then the mycelium was placed on a glass slide and using a metal coating equipment of samples (EDWARDS E306A), a copper bath was applied to the samples for 20 min for subsequent visualization. Finally, the general morphological and structural characteristics of the *P. ultimum* mycelium were evaluated.

2.10 Evaluation of nano metal complex as antifungal activity and plant growth promotion in pot experiment

The ability of nano metal complex in different concentrations (10, 20, 30 and 40 ppm), its antifungal and growth enhancement effects of Fenugreek seedlings in soil infested with *P. ultimum* were tested. For preparation *P. ultimum* infested soil, the propagule suspension of *P. ultimum* was added at thirty-propagules/g soil to the autoclaved soil. Thirty seeds were used for each treatment. The surface of the seeds were sterilized with 2% sodium hypochlorite for 3 min, and washed by sterile distilled H_2O for 5 min and dried using sterile filter paper. Pre germination test was done to select viable seeds. The experiment was classified into five groups. Frist group: free *Pythium* soil were irrigated with sterile distilled water, while second groups: soil infested with *Pythium* and irrigated sterile distilled water. The remaining three groups: the soil infested with *Pythium* in each group and soil were irrigated with nano metal complex -solution with different concentrations (10, 20, 30 and 40 ppm). Pots were kept in a growth-illuminated cabinet (Precision, United States) at 25°C with 12 h photoperiod (91 μmol/ m^2 s^1) under humid conditions. Soil moisture content was preserved at 35%. Experiments were performed with ten replicate pots per treatment. Germination % was calculated after 48 and 72 hour by following formula:

$$\text{Germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100 \qquad (2)$$

After 10 days of germination, seedlings growth were determined in terms of shoot and root length (mm).

3. Results and discussion

In the present study, the analytical data of the previously prepared Schiff base ligand and its Zn nano metal complex suggest the structures as in **Figure 1**. The Schiff base ligand and its Zn-nano complex were subjected to elemental analyses (C, H, N and O). The results of elemental analyses with molecular formula and the M.P. were in **Table 1**. The results obtained were in well agreement with those of

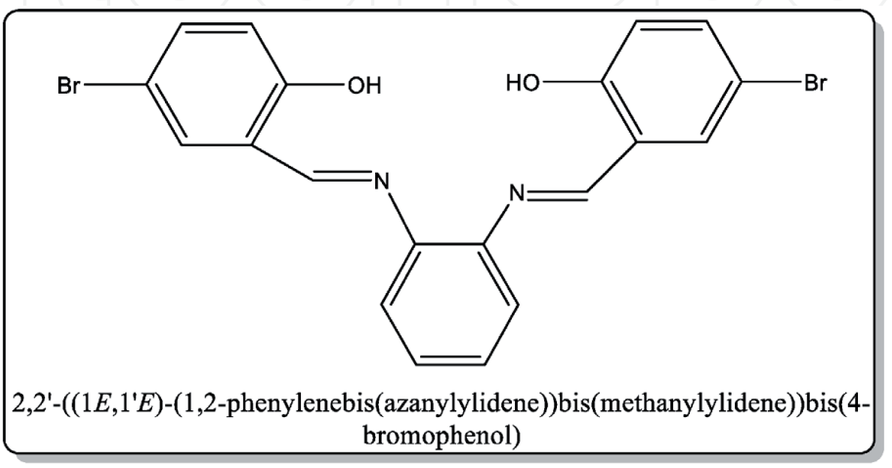

Figure 1.
Schematic route for preparation of the free ligand.

Compd. no. emprical formula	M.P. (°c)	Color (yield %)	(%) found (calcd.) Λm^*			
			C	H	N	
Free ligand(L), $C_{20}H_{14}Br_2N_2O_2$	200	Dark yellow (90.20)	36.89 (50.64)	2.93 (2.98)	12.01 (5.91)	—
(5)[(Zn)(L)]$(NO_3)_2$, $C_{20}H_{14}Br_2N_4O_8Zn$ nano complex	<350	Dark yellow (85.79)	33.59 (33.90)	1.95 (2.00)	7.86 (7.91)	11.25

*$^*ohm^{-1}cm^2mol^{-1}$.

Table 1.
Elemental analysis and some physical measurements of the free ligand and its metal nano complex.

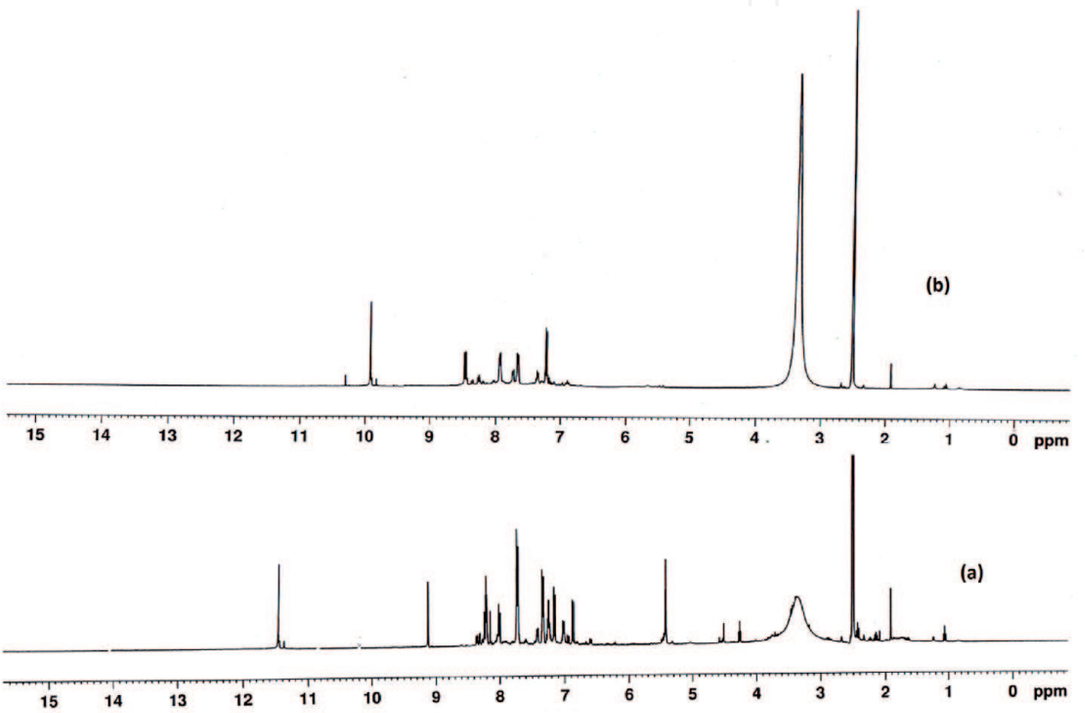

Figure 2.
^1H NMR data for (a) free ligand and (b) its Zn(II) nano complex.

Compd. no.	δ_{ph}	$\delta_{CH=N}$	δ_{OH}
L	6.465–8.175	9.335	11.247
Zn nano complex	6.451–7.684	8.252	10.210

Table 2.
^1H NMR data for the Schiff base ligand and its nano complex.

the suggested formulae. The M.P. were sharp, indicating the purity of the prepared compounds. The results of the elemental analyses of the isolated complex are in agreement with those required by the proposed formula of the complex.

The NMR spectrum of the Schiff base ligand (**Figure 2**) (**Table 2**) showed a singlet peaks at 9.335 ppm and at 11.247 ppm due to the azomethine and phenolic protons, respectively. In addition to multiplet signals at 6.465–8.175 ppm attributed to the aromatic protons [27, 28]. The comparison of the NMR data of the ligand and its Zn (II)metal complex confirms the mode of coordination between the ligand and its metal ion. By complexation, the spectrum of the nano-Zn complex display

Compd. no.	υ_{O-H} (phenolic)	$\upsilon_{CH=N}$ (azomethine)	υ_{C-O} (phenolic)	υ_{M-O}	υ_{M-N}	Additional bands
L	3456	1620	1290	—	—	—
Zn-nanocomplex	3401	1532	1240	550	515	1401,1372,1025 (coord.NO_3)

Table 3.
Infra-red spectral data of the free ligand and its metal complex.

a significant shift of the signals due to C=N group and OH protons indicating the involvement of both OH as well as C=N groups in coordination to the metal ions without their deprotonation suggesting that the ligand act as neutral tetradentate ligand, O_2N_2 coordination sphere.

The IR spectrum of the ligand was compared with those of its metal complex, in order to confirm the mode of bonding, **Table 3**.

The infrared spectrum of the schiff base ligand has a broad absorption band at 3456 cm^{-1} which were due to the phenolic group [29, 30]. The spectrum displays also medium band at 1290 cm^{-1} due to υ_{C-O} (phenolic group) [31]. Additionally, a strong band at1620 cm^{-1} was observed due to $\upsilon_{C=N}$ of azomethine group [32]. By careful comparison of the spectrum of the nano-metal complex with those of the Schiff base ligand it was found that: the band at 3456 cm^{-1} shifted to lower frequency region at 3401 cm^{-1} in the metal nano complex suggesting the involvement of OH group in complexation without its deprotonation. The strong bands at 1620 and 1290 cm^{-1} due to $\upsilon_{C=N}$ and υ_{C-O} (C=N and OH groups) are shifted to lower wave number (1532 cm^{-1}) and (1240 cm^{-1}), respectively in the metal complex indicating the coordination of nitrogen and oxygen to the metal ion. Also, the spectrum of the complex shows three bands at 1401 cm^{-1}, 1372 cm^{-1} and 1025 cm^{-1} corresponding to unidentate coordination mode of NO_3 group. All these results are consistent with the conductance data. Conclusive evidence of the bonding is also shown by observing new bands in the infrared spectrum of metal nano complex in low frequency region at 550 cm^{-1} and 515 cm^{-1} may be attributed to υ_{M-O} and υ_{M-N}, respectively that are not observed in the spectrum of the Schiff base ligand [33, 34].

The mass spectrum of the free ligand shows the parent peak at m/e = 474.36 (48.78%) that agree with the molecular formula $C_{20}H_{14}Br_2N_2O_2$. Also, the spectrum shows numerous peaks corresponding to various fragments, their intensity indicates the stability of the fragments. **Figure 3** represent the proposed pathway for the decomposition steps for the ligand.

The conductivity Λ_m value of the Zn-nanocomplex can be calculated using the following relation $\Lambda_m = K/C$, where C is the molar concentration of the nano metal complex solution and K is the specific conductance. The complex was dissolved in (10^{-3} M) DMF and the molar conductivity of the solution at 25 ± 2°C were measured **Table 1**. It is concluded from the results that; the complex is found to has molar conductance value of 11.25 $ohm^{-1}mol^{-1}cm^2$ indicating that this complex is non-electrolytic in nature. Also, the values indicate the bonding of the nitrate ions to metal cations [33, 35].

UV–vis spectra of the ligand and its metal complex was performed in DMF at room temperature at wavelength range 200–800 nm. The significant electronic absorption bands of the ligand, its nano complex and the magnetic moments of the complex are given in **Table 4**. 2 absorption peaks were observed in the spectrum of the ligand at 265 and 339 nm due to π-π* and n-π* transitions, respectively attributed to benzene and the azomethine groups [34, 36]. In the spectrum of metal complex, the absorption bands attributed to π-π* and n-π* transition were

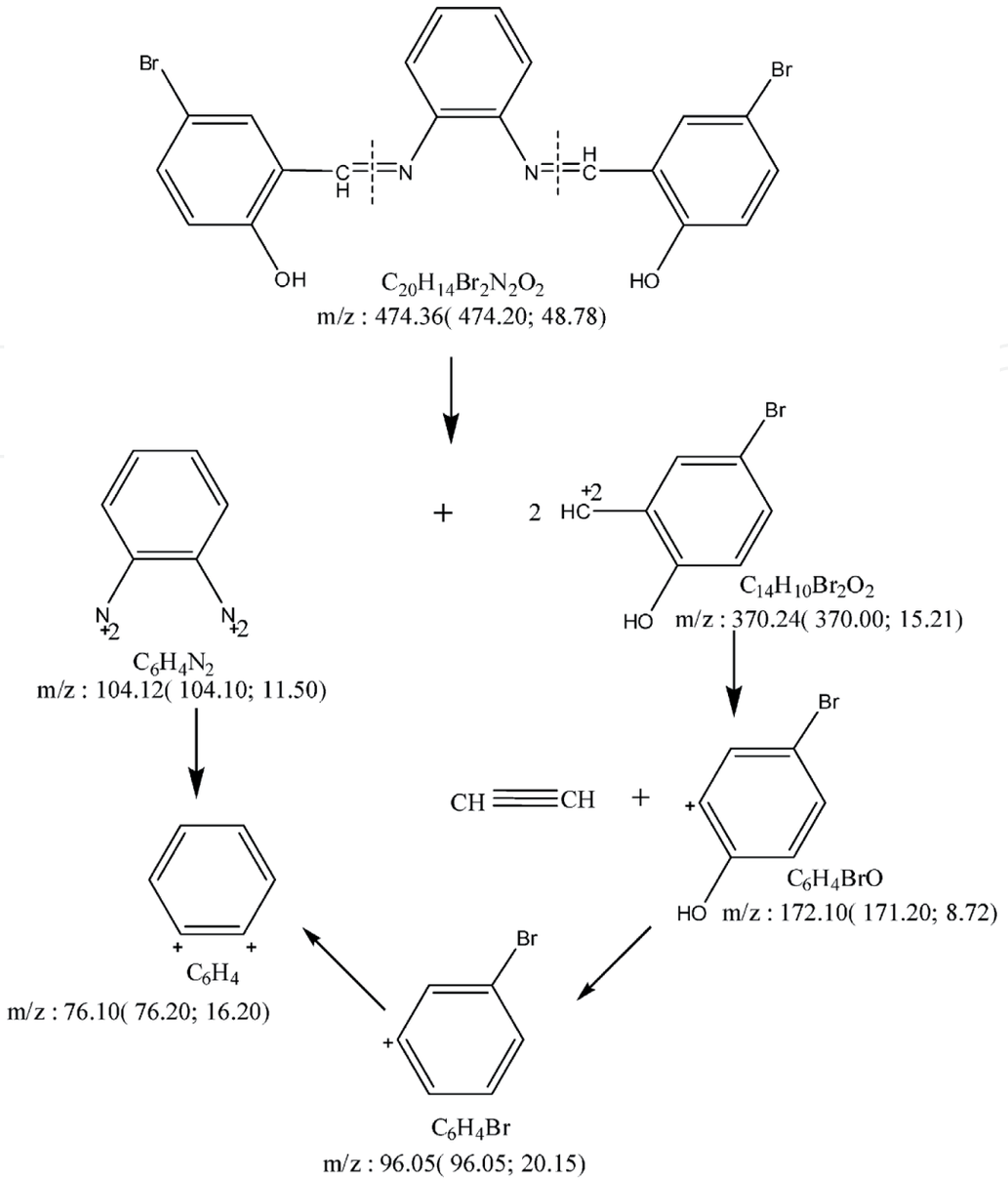

Figure 3.
Mass fragmentation pattern for the free ligand

Compd. no.	μ_{eff} (B.M.)	Absorption bands (nm)		
		π-π*	n-π*	d-d transition
L	—	265	339	—
Zn-nano complex	Diamagnetic	276	323	—

Table 4.
Magnetic moment and electronic spectral data of the ligand and its Zn-nano complex.

shifted to lower or higher frequency due to the coordination of the Schiff base ligand with the metal ions. Zn (II) complex is diamagnetic in nature with no *d-d* transition. So, according to all of this obtained data, the octahedral structure may be suggested.

The size and the morphology of the nano complex were studied by SEM (**Figure 4**). It was found that the particles are semispherical in nature with some agglomerations. The SEM images also revealed the stabilization of Zn(II) nanoparticles due to interaction with the Schiff base ligand [37].

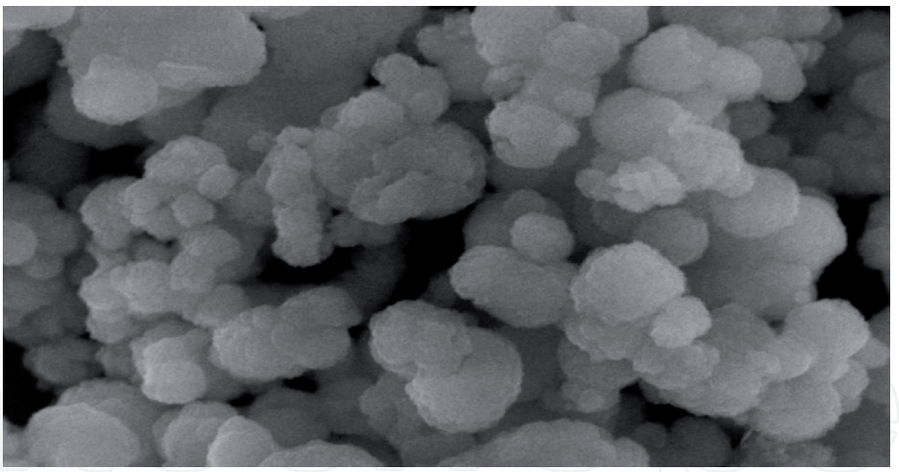

Figure 4.
SEM image of nanoparticle complex.

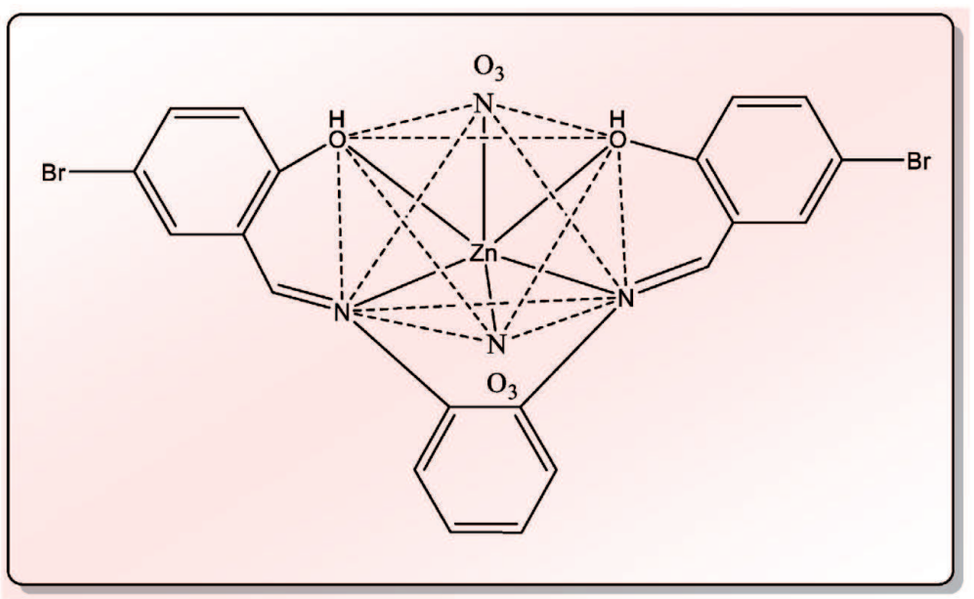

Figure 5.
Suggested structure of Zn nano complex.

Correlation of all this results, the structure of the complex can be suggested to be as in **Figure 5**.

Fungi are the most efficient microorganisms when it comes to the biological production of Mycogenic Metal Nanoparticles synthesis [38]. This is due to the fungi having enhanced processes, accumulating metals, have capacity for producing a high number of bioactive metabolites [39, 40], fungi do not require complex nutrients, these are easy to grow, easy to manipulate, also have high metabolites and production of biomass, and has high metal uptake and high wall-binding capability [41–43]. Our Experiment revealed that nano-metal complex had no obvious effect on the time required for Fenugreek seed germination, (**Figures 6** and 7). Nano-metal complex activated seeds germination, since seeds germinated 24 h prior to the germination of control. Results in **Figure 7** revealed that seedling elongation was retarded with different degrees in case of treated seeds compared with the controls. Nano-metal complex showed a slight retarding effect on Fenugreek seedling elongation compared with the control, especially after 7 days from seed germination.

Table 5 shows the antifungal influence of different concentration of nano metal complex (10, 20, 30 and 40 ppm) on *P. ultimum* mycelial growth on PDA

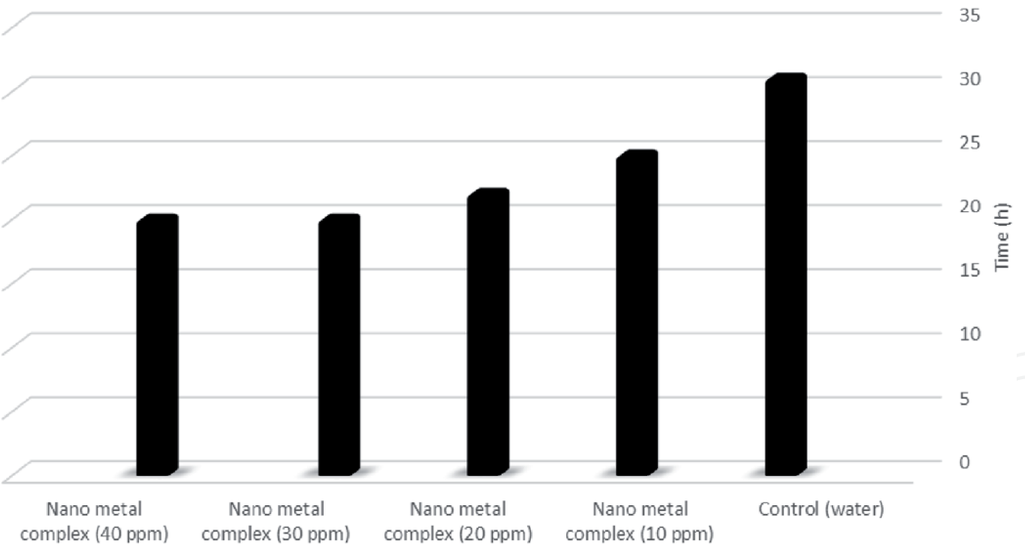

Figure 6.
Effect of nano-metal complex with different concentration on the time of seed germination.

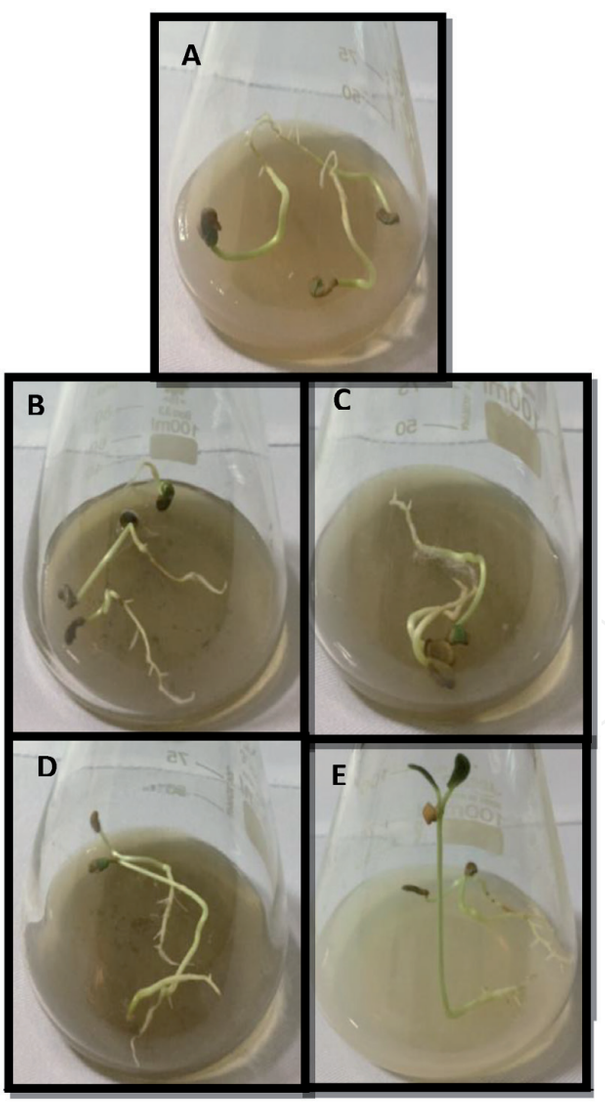

Figure 7.
Pathogenicity tests of nano metal complex against Trigonella foenum-graecum germinating seeds grown in Erlenmeyer flasks containing 2% water agar. (A) Control, (B) treated seeds with nano-metal complex (10ppm), (C) treated seeds with nano-metal complex (20 ppm), (D) treated seeds with nano-metal complex (30 ppm), (E) treated seeds with nano-metal complex (40 ppm) after 7 days at 25°C.

***Pythium* spp.**	**Mycelium growth (mm)**				
	control (metalaxyl)	**Nano metal complex (10 ppm)**	**Nano metal complex (20 ppm)**	**Nano metal complex (30 ppm)**	**Nano metal complex (40 ppm)**
P. ultimum	34** ±0.16*	65 ± 0.3	60 ± 0.13	52 ± 0.21	0

**Mycelium growth (mm).*
***Standard error of three replicates.*

Table 5.
Antifungal activity of nano metal complex against P. ultimum *on PDA medium at 26°C for 6 days in the dark.*

medium after 6 days of incubation at 26°C. Results reveled that significantly reduced of mycelial growth of *P. ultimum* at different concentrations (10, 20, 30 and 40 ppm), especially in 30 and 40 ppm compared to control ($p \leq 0.001$). As shown in **Figures 8** and **9** efficacy of nano metal complex at concentration 40 ppm, was the greatest one of all concentration tested *P. ultimum* growth inhibition, that observed no mycelium growth appear, follow by nano metal complex at concentration 30 ppm which mycelium growth were (32 mm). Percentage of inhibition of nano metal complex at different concentrations (10, 20, 30 and 40 ppm) were (23.52%, 29.4%, 62.4% and 100%) respectively. Biological control of damping-off diseases has been successfully. Applied using *T. harzianum* [44, 45]. El-Sayed [46] reported that *Trichoderma* spp. are known to control all pathogens either directly by inhibition of sporulation and growth of the pathogen mechanisms such as enzyme production and mycoparasitism, or indirectly by competing for nutrients and space, modifying the environmental conditions, or by promoting plant growth and enhancing plant defensive mechanisms and antibiosis. Microscopic examination of the mycelium showed coagulation of the protoplasm within the mycelium and lysis of cell wall of mycelium compared with the control (**Figure 10**).

Kamala and Indira [47], Evaluated the activity of three *Trichoderma* isolates (T73, T80 and T105) for their bio-control activity of *P. ultimum*. Also assayed the different bio control mechanisms such as protease, chitinase, β-1,3-glucanase

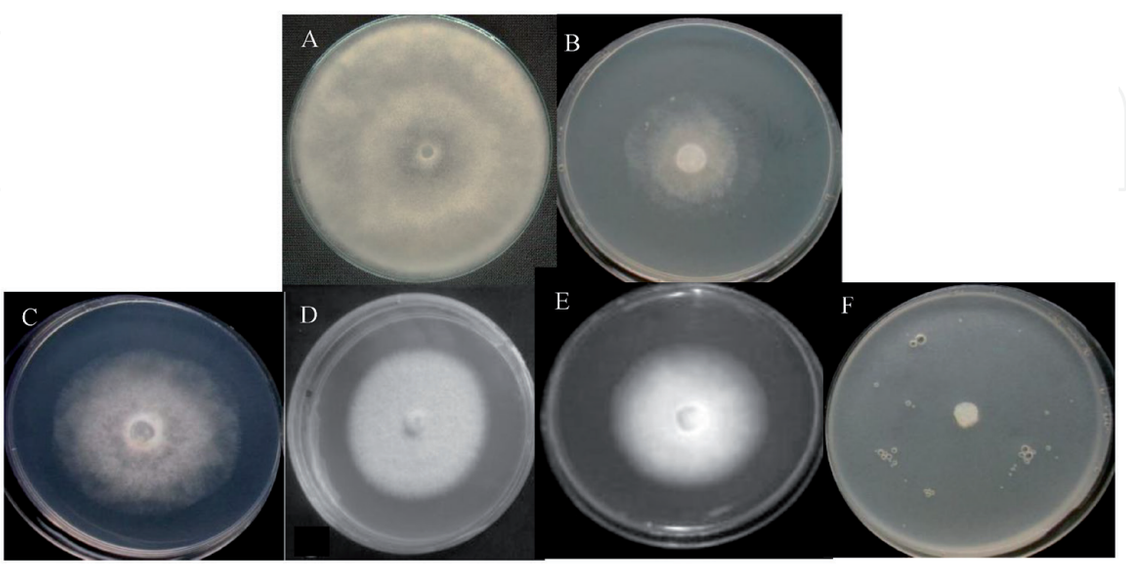

Figure 8.
Inhibition of mycelial growth of P. ultimum. (A) Control, (B) Control containing Metalaxyl, (C) Treated containing Nano-metal complex (10 ppm), (D) Treated containing Nano-metal complex (20ppm), (E) Treated containing Nano-metal complex (30ppm) and F) Treated containing Nano-metal complex (40ppm) on PDA at 26°C for 6 days at the dark.

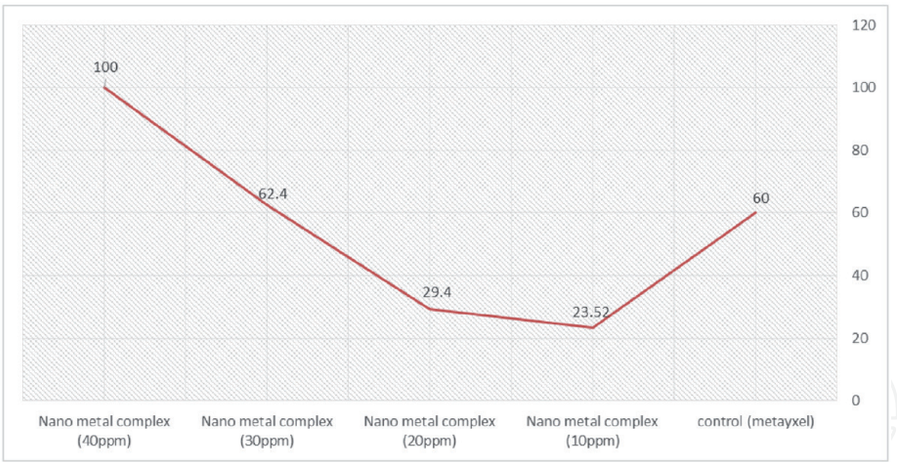

Figure 9.
Inhibition % of P. ultimum exposed to different concentration of nano metal complex, on PDA at 27°C for 6 days at the dark.

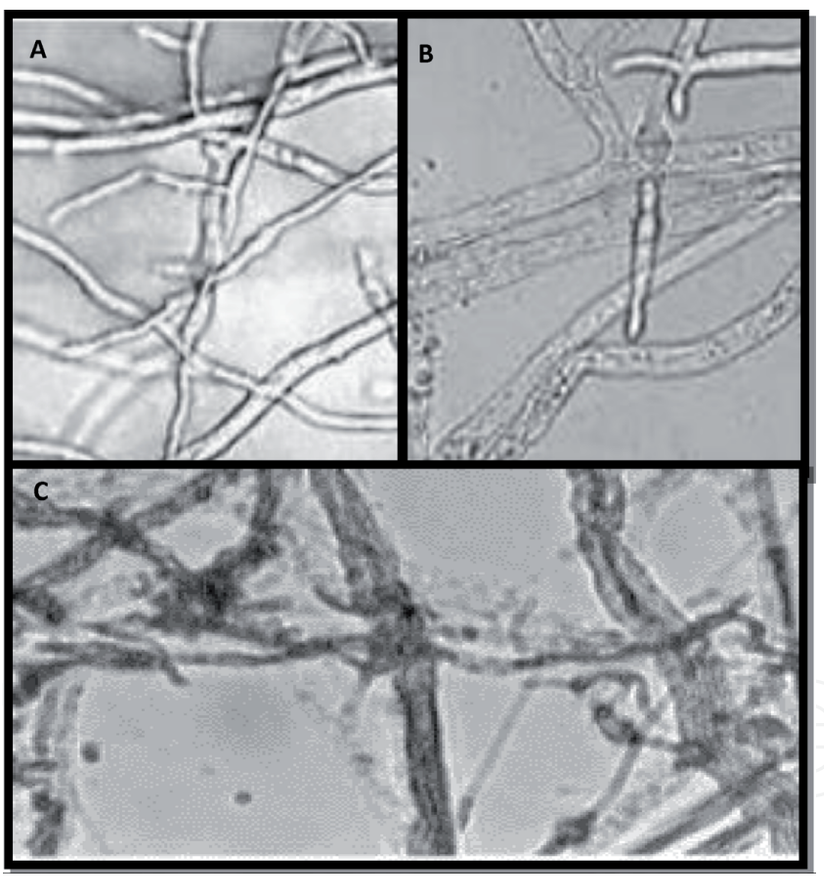

Figure 10.
Scanning electron micrograph showing the antagonistic effect of nano metal complex (30 ppm) against P. ultimum. (A and B) healthy mycelia of P. ultimum. (C) Abnormal structure, lysis and destruction of P. ultimum.

activity, cellulase and production of volatile and non-volatile compounds. Their results show that, *Trichoderma* (T-105) reduced the.

Pre and post-emergence damping-off disease or Root rot disease were incidence in infested soil with *P. ultimum* and showed the highest disease control percentage under in vitro as well as the pot experiment [48–50]. In our study, we evaluated the biogenic Nano-metal complex in pot experiments, which reveal that data in **Table 6** have differences in the germination%, that It observed the beginning of germination

Treatment	Germination % after 20 h	Germination % after 24 h	Germination % after 48 h	Length (mm)	
				Shoot	Root
Control	33.3 ± 0.2	73.33 ± 0.16	100	130	69.5
Nano-metal complex "10 ppm"	60 ± 0.05	93.33 ± 0.13	100	150	81.8
Nano-metal complex "20 ppm"	66.6 ± 0.1	96.67 ± 0.2	100	180	95.2
Nano-metal complex "30 ppm"	66.6 ± 0.12	96.67 ± 0.12	100	180	96.5
Nano-metal complex "40 ppm"	66.6 ± 0.2	96.67 ± 0.22	100	180	96.5

Table 6.
Effect of nano-metal complex treatments on germination (%) and root and shoot lengths (mm) of fenugreek.

of seeds after 20 hour in Nano-metal complex 20, 30 and 40 ppm about 60%,compared with control 33.3%. gradullary, the germination % of seeds increase with increasing time until 48 h, all seeds were germinate 100%.

With observance to root and shoot lengths, results in **Table 6** show that Nano-metal complex had effects in both concentration 20–40 ppm for shoot length about (180 mm), the Nano-metal complex (10 ppm) treatment recorded the lowest value for root length about 150 mm. In addition in the same concentration for root length about (95.2 mm, 96.5 mm and 96.5 mm), respectively. The Nano-metal complex (10 ppm) treatment recorded the lowest value for root length about 81.8 mm, **Figures 11** and **12**. From previous results, it can be concluded that Nano-metal complex (40 ppm) had the best record in both germination as well as shoot and root length.

The increased applications of Metal Nano Particles in several area required new biocompatible, safe, and active nanostructures with less dangerous by products of synthesis reactions [51]. Mycogenic Nano-Particles are observed as biocompatible,

Figure 11.
The effect of bio-control agent and plant growth promotion of nano metal complex on Trigonella foenum-graecum seeds germination in pots containing sandy loam soil cultivated with P. ultimum. (1) Control seeds sown in free Pythium soil, (2–5) seeds sown in soil infested with P. ultimum and irrigated with nano-metal complex (40,30,20, 10 ppm), respectively after 4 weeks.

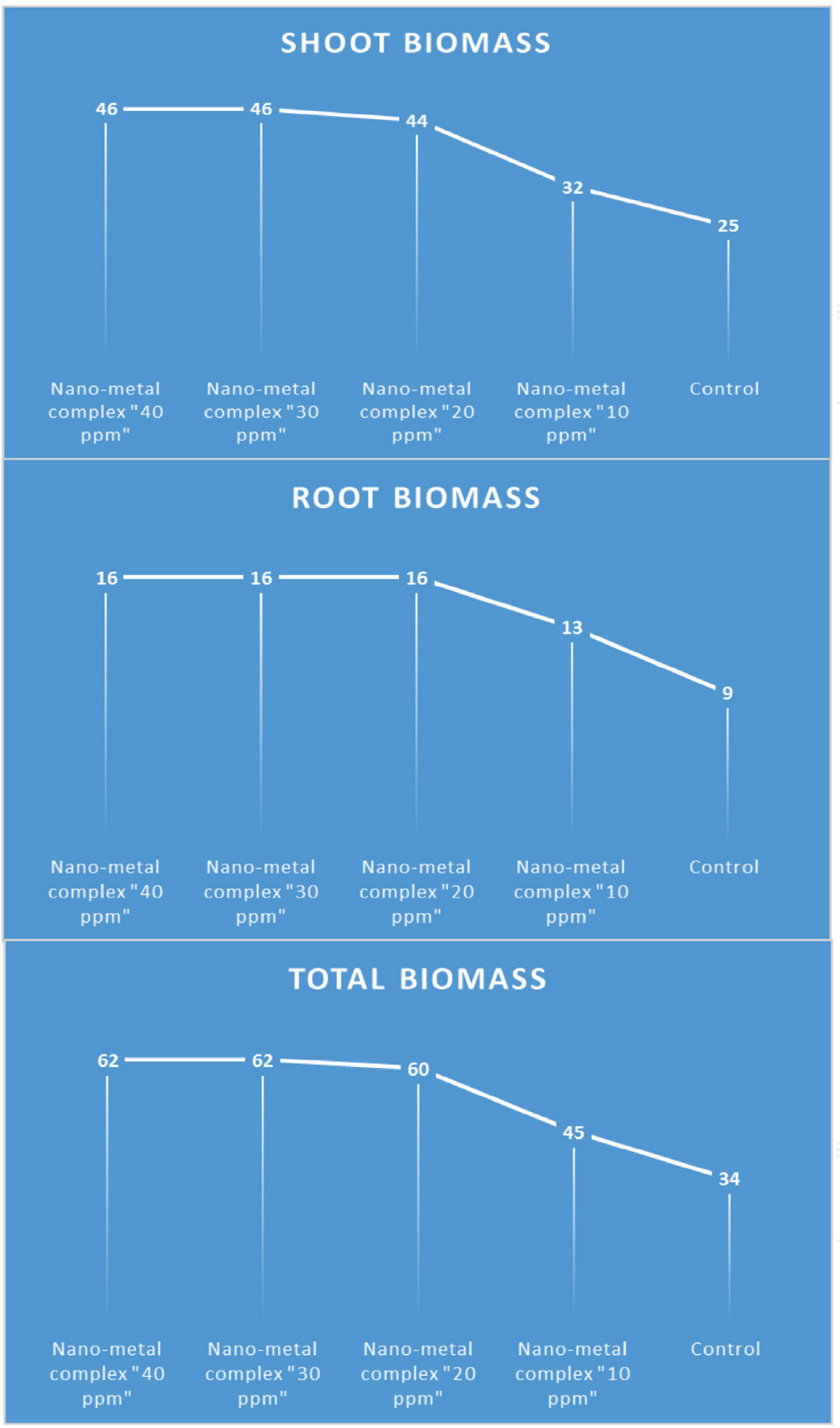

Figure 12.
Effect of nano-metal complex on Trigonella foenum-graecum plant biomass after 4 weeks of planting. (1) Shoot biomass. (2) Root biomass. (3) Total biomass.

eco-friendly, less toxic, safe, and cheapest alternatives, with lowest consumption of energy and highest yields when compared with other physical or chemical synthesis [26, 52]. While well-known advantages of Metal Nano-Particles as the best alternatives against antimicrobial species of pathogenic fungi and other microflora, many

challenges and opportunities are ahead of us. Given that the effects of NPs result from a combination of multiple, the potential development of resistance against them is more difficulty and less likely [53]. In future should be focus on testing new isolates, discovering new Metal Nano-Particles, and clarifying their structures and mode of its action as antimicrobial agents. Novel discovery of mycogenic MNPs include the study of extremophilic and endophytic isolates. While the endophytic has increased its relevance during the last years, the extremophilic is still limited in studies and focused on other purpose rather than their use against antimicrobial species [54].

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The data presented in this study are available upon request from the corresponding author.

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