

## Quantitative and qualitative effects of different silver nanoparticles concentrations on the pathogenicity and reproductive of root-knot nematode, *M. incognita* infecting susceptible sugarbeet variety

Gohar I.M.A.<sup>1</sup>, Elshaimaa A.E. Mohamed<sup>1</sup>, Nader R. Abdelsalam<sup>2</sup> and Amara F. Zaitoun<sup>2</sup>

<sup>1</sup>Sugar Crops Research Institute, Department of Sugar Crops Disease and Pests Research, Agricultural Research Center, 12619, Giza, Egypt.

<sup>2</sup>Agricultural Botany Department, Faculty of Agriculture (Saba Basha), Alexandria University, 21531 Alexandria, Egypt.

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### ABSTRACT

Sugarbeet (*Beta vulgaris* subsp. *vulgaris*) is the most economically valuable crop species in the order Caryophyllales. Root knot nematodes interrupt the physiology of the plant and able to cause economic importance great losses in production and quality of sugarbeet crop. Chemical nematicides are usually preferred for their effective control; the problems associated with nematicides application turned the workers vision to focus new alternative agents for nematode management programs. In this study, High throughput microcrystalline cellulose decorated silver nanoparticles (Ag-NPs) by different concentrations were evaluated as a nematicidal substance in outdoors pots experiment. The chosen tested concentrations were 20, 30, 40, 50, and 60 ppm/ml of Ag-NPs with four replicates/ each concentration along with two methods of application [one time application (AT1) and application two times (AT2)], applied to sugarbeet pots infested with *Meloidogyne incognita*. Applying Ag-NPs directly to infested sugarbeet pots achieved significant suppression at  $p \leq 0.05$  of root-knot nematode, *M. incognita* in terms of reducing numbers in soil, reproductive factor, and knot disease severity%. Efficacy % that related to untreated pots and relative efficacy% that proportionated to pots treated with Ethoprop 10% G get higher potential as of Ag-NPs of concentrations get higher. Time of application AT2 enhanced the efficacy of Ag-NPs at low concentration (under 50 ppm/ml) and occasionally above 50 ppm/ml. Effects of different Ag-NPs concentrations and times of application on yield components i.e. Root yield plant<sup>-1</sup>(g), Top yield plant<sup>-1</sup>(g) and sugar yield plant<sup>-1</sup>(g) of infested sugarbeet plant with root-knot nematode, *M. incognita*, were related to degree of Ag-NPs concentration to suppress nematodes activity. Different concentrations of Ag-NPs increased yield components i.e. Root yield plant<sup>-1</sup>(g), Top yield plant<sup>-1</sup>(g) and sugar yield plant<sup>-1</sup>(g) even at low concentration (20 ppm/ml) in comparison with control treatment (0.0 ppm/ml). The same trend for quality as sucrose, total soluble solids (T.S.S) and purity percentages of sugarbeet infested with root-knot nematode, *Meloidogyne incognita* in pots experiment. Avoidable loss percentage in roots and sugar yields plant<sup>-1</sup>(g) as an economic expression responded positively to different levels of Ag-NPs concentrations and to time of application AT2 in low concentration <50 ppm/ml. This study has demonstrated a potential environmentally friendly alternative for the management of the root-knot nematodes.

**Keywords:** Sugarbeet, *M. incognita*, silver nanoparticles, concentrations, efficacy %.

### Introduction

Sugarbeet (*Beta vulgaris* subsp. *vulgaris*) is deliberated as the major sugar crop in Egypt cultivated in 492.708 feddans contributing 57.7% of sugar production with an average production of 21.06 tons per feddan (Annual Report of Sugar Crops Council, December 2018). The industrial demand for sugarbeet is still increasing, which provides a higher price, thus incentivizing many farmers to plant more beets (reached 255,000 ha) GAIN (Global Agricultural Information Network Report, 2020). As sugarbeet production growing, challenges augmenting, among these challenges in newly reclaimed soils the root knot nematodes are included within the genus *Meloidogyne* Goldi, important polyphagous assemblage of extremely adapted obligate plant pathogens (Abad *et al.*, 2003).

**Corresponding Author:** Gohar I.M.A., Sugar Crops Research Institute, Department of Sugar crops disease and pests Research, Agricultural research center, 12619, Giza, Egypt.  
E-mail: ibrahimgohar121@gmail.com

Root knot nematodes interrupt the physiology of the plant and able to cause economic importance great losses in production and quality of sugarbeet crop (Gohar and Maareg, 2005). Chemical nematicides are usually preferred for their effective control; though, their excessive and continues use caused direct toxicity to predators, pollinators, fish and man, had adverse effects on soil health and environment and cause poor soil fertility, productivity and pesticides residues in products. The problems associated with nematicides application turned the workers vision to focus new alternative agents for nematode management programs. Emerging fields such as nanotechnology and biotechnology demonstrate extensive promising pathways for managing PPNs through minimized production inputs and maximized crop yield. In addition, both fields have changed the entire scenario of the agricultural sector with a high potential to conceive products under a healthy and friendly environment Ansari *et al.* (2020).

The field of nanotechnology has grown up extremely from its beginning to influence all kinds of organic and inorganic materials to the extreme Nano scales, typically less than 100 nm (Abraham *et al.* 2008). The most outstanding feature of nanoparticles or nanomaterial is a large surface-to-volume ratio, which provides a crossing layer between the materials themselves and their close environment. In addition, the extraordinary surface-to-volume ratio increases the rate of chemical and biochemical activities (Dubchak *et al.* 2010). In the twenty-first century, the increasing progression of nanotechnology in agriculture has gained a great consideration worldwide since it can be applied to any system of agriculture involved in crop farming through a potential and well-ordered release and targeted source of agrochemicals toward plant parasitic nematodes PPNs. Nano-biotechnology plays a major role in the production of efficient and eco-friendly NPs using “natural bioresources” such as microorganisms and plant natural extracts (Khan *et al.* 2009). Although several methods are used to produce silver nanoparticles (Ag-NPs), the inclusion of toxic chemical substances cannot be avoided in their common method of synthesis, the chemical approach (Hardman 2006). Therefore, a considerable number of research is being carried out to synthesize them using plant extracts as the biological base to maintain “clean,” “nontoxic,” “harmless,” and “eco-friendly green chemistry.” There are quite a lot of journal papers that claim to reveal that plant extracts have nematicidal and nematostatic properties (Nour El-Deen *et al.* 2014; Khan *et al.* 2017; Singh *et al.* 2017). Silver NP (Ag-NP) is one of the most utilized nanomaterials that have emerged as a superior product to control Phytonematodes. Silver NPs possess sufficient conductivity, have a good catalytic attribute with pronounced antimicrobial activity, and are chemically stable (Nour El-Deen and Bahig Ahmed El-Deeb 2018; Roh *et al.* 2009; Chen *et al.* 2007; Setua *et al.* 2007). Lim *et al.* (2012) have demonstrated that Ag-NPs cause oxidative stress in the cells of nematodes. However, the plant based Ag-NPs has revealed a very significant control of *M. incognita* by reducing number of galls and egg mass and resulted in improved growth and fresh weight of tomato (Nour El-Deen and Bahig Ahmed El-Deeb 2018; Fouda *et al.* 2020 ). Similar research was conducted by Abbasy *et al.* (2017) to evaluate the nematicidal activities of leaf extracts of *Conyza dioscoridis*, *Melia azedarach*, and *Moringa oleifera* against eggs and second-stage juveniles (J2s) of *Meloidogyne incognita* using crude extracts in different solvents and their Ag nanoformulations. The phytochemical based synthesis of AgNPs showed enriched nematicidal activity affecting J2 and eggs up to the levels of 5 and 2 times respectively while “rugby” was the reference nematicide and that was most toxic against *M. incognita*.

The objective of this study was to evaluate quantitative and qualitative effects of different concentrations of high throughput microcrystalline cellulose decorated silver nanoparticles (AgNPs) as an eco-nematicide on the pathogenicity and reproductive of root-knot nematode, *M. incognita* infecting susceptible sugarbeet variety through outdoors pots experiment.

## Materials and Methods

### 1. Planting the tested seeds

Planting of tested seeds of sugarbeet *Beta vulgaris*, subsp. *vulgaris* was Polat monogerm commercial variety obtained from the certified group of sugarbeet varieties own by Alexandria Sugar Company. Its origin Germany characterized as diploid × diploid (CECD 2019). Sugarbeet, Polat categorized as susceptible to root-knot nematodes based on Canto-Saenz host suitability designations modified for sugarbeet by Gohar *et al.* (2013).

## 2. Preparing of pots for planting:

The soil was obtained from fields of Sabahia Agricultural Research Station, Alexandria, Egypt. It was clay soil with heavy texture and mixed with pure sandy soil in ratio of 2:1, then sterilized chemically by Formaldehyde (Formalin) 1 gallon of commercial formaldehyde (about 37% strength) mixed with 30 gallons water. Drench soil at 10 liters/sq. meter. Water heavily afterwards and covered by plastic sheet. Removed plastic after 48 hours to get rid of formalin residue and work up the soil as soon as it is dry and left 10-14 days before sowing (make a simple test germination first). Pots (35 cm diameter) were filled with 5 kg soil mixed soil for each season, pots were used for planting sugarbeet seeds and filled with soil (5 kg) to relating plant growth or/and yield components per plant in the judgment of sugarbeet tested variety for response to experienced factors (Ag-NPs concentrations and times of application), after 180 days termination). Sowing was done in outdoors of Sabahia Agricultural Research Station Alexandria, Egypt. Two weeks old well established and healthy seedlings of sugarbeet variety were thinned to two plants per pot before inoculation.

## 3. The nematode inoculation

Roots of Okra (*Abelmoschus esculentus* L., Moench 'Lady's finger') infested with root-knot nematodes were collected from West Nubaria district. The root-knot nematodes species were identified as *Meloidogyne incognita* (Treub) Chitwood, with the help of perennial pattern as described by Taylor and Netscher (1974). The okra plants were up-rooted and the egg masses were picked as described by (Hartman and Sasser 1985). One hour before inoculation, Nematode inoculum of 4000 *M. incognita* eggs per pot according to (Gohar and Maareg 2009) - approximately 400 eggs 250 cm<sup>3</sup> soils. Inoculum was dispersed into three punctures (approximately 2.5 cm deep) and covered with soil. Pots were watered immediately following inoculation. The plants were then watered regularly along with proper nutrition.

## 4. Quantitative and qualitative Effects of different Nano silver concentrations

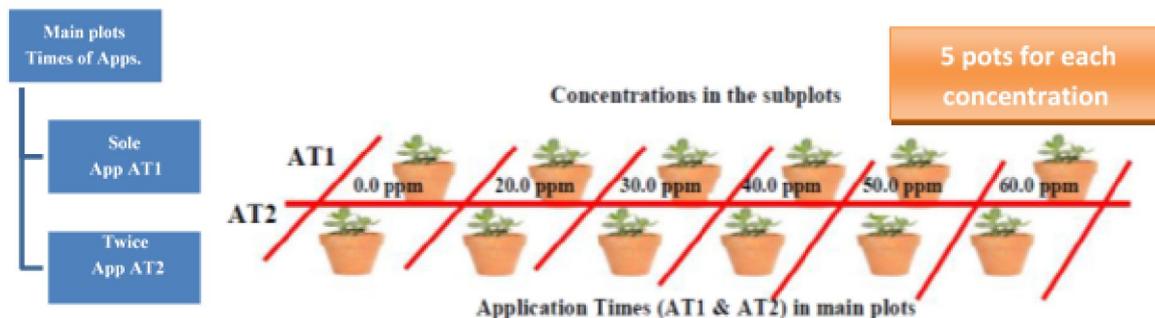
The experiment was conducted in 35 cm. pots under outdoor screen-house conditions. All treatments of all experiments were replicated four times. Sugarbeet seeds were sown in pots containing sterilized sandy loam soil.

## 5. Preparation of silver:

Nanoparticles (Ag-NPs) and their concentration was done as described by Fouada *et al.* (2020), Ag-NPs was prepared in very high concentration. Ag-NPs prepared using such technique has many advantages such as: absence of organic or solvents, scaling up thru using high concentration of silver precursor and utilization of environmentally benign polymer; Microcrystalline Cellulose (MCC). At the beginning, the bulk Ag-NPs colloidal solution is diluted to 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 80 and 100 ppm. Based on laboratory test results the chosen tested concentrations were 20, 30, 40, 50, and 60 ppm of Ag-NPs with four replicates/ each concentration.

## 6. Experiment set up:

Experiment design was split plot design with four replications. Each replicate had 12 pots/ concentration drenched for each of the previous Ag-NPs concentrations (0.0 – 60 ppm/ml) with total of 60 pots replicate<sup>-1</sup>. The 60 pots were divided into groups each represented the main plot to allocate application time, divided to subplots to allocate concentrations of Ag-NPs. Each pot received 4000 J2s. Check treatments were represented by untreated Ag-NP-pots supplied with nematodes only. Pots that received 4000 *M. incognita* j2s after full emergence of sugarbeet seedlings (two weeks) and received 100 ml of the respective concentration. Times of adding concentration were; synchronizing with inoculation by j2s one time only (AT1) to the first main plot and the second main plot received AgNPs concentrations as two times, one applied synchronizing with inoculation by j2s and the second time with the same amount of each concentration later after four weeks (AT2). The experiment was terminated after 180 days. Also, Ethoprop granules 10% (Mocap<sup>®</sup> 10% G) [0-ethyl S,S-dipropyl phosphorodithioate] is a non-systemic, non-fumigant nematicide and soil insecticide which has been registered since 1967 for use on tobacco in the United States. The rate of application was 1.2 kg a.i. feddan<sup>-1</sup> used as a relatively comparable tool. Every pot received 0.04 g a.i.



Layout of one replicate (R)

### 7. Evaluate the efficacy % of experimented concentrations of Ag-NP against root-knot nematode:

The numbers of nematodes in the pot's soil and disease severity % were determined. The nematode numbers in the soil were detected by Baermann funnel apparatus Barker (1985). A hold tightly rubber tube was placed beneath the funnel, and a piece of window screen (or similar material) was placed in the opening of the funnel; the funnel was placed into a holder; a tissue-paper wrapped soil sample was placed onto the screen material then add water to the funnel setup until the screen and soil sample were immersed; incubate overnight (or longer if desired). Finally, the first couple of drops of water from the bottom of the tube were gathered by slowly releasing the clamp on the tubing, and then the density of larvae was examined under the microscope. For the disease severity % detection, the gall indices were recorded at the termination of experiments on the scale rating used was as follows: 0 = no galls; 1 = 1 to 2 galls; 2 = 3 to 10 galls; 3 = 11 to 30 galls; 4 = 31 to 100 galls; and 5 = >100 galls per root system (Maareg *et al.*, 2005). The six-control strategies efficacy was calculated as described by (Xue *et al.* 2009).

Disease severity, drop rate and control strategies efficacy were calculated as following:

$$\text{Disease severity} = \left[ \frac{\sum \text{Class frequency} \times \text{Score of rating class}}{\text{total number of plants investigated} \times \text{Maximal disease index}} \times 100\% \right]$$

Drop rate =

$$\frac{\text{the number of J2 in control treatment} - \text{the number of J2 in strategy management treatment}}{\text{the number of J2 in control treatment}} \times 100\%$$

Ag-NP concentrations efficacy =

$$\frac{\text{disease severity in control treatment} - \text{disease severity in strategy treatment}}{\text{disease severity in control treatment}} \times 100\%$$

**Relative efficacy %** for a given AgNP concentration defined as relating the specific efficacy% for that concentration to efficacy% of a standard nematicide.

$$\text{Relative efficacy\%} = \frac{\text{Efficacy of AgNP concentration\%}}{\text{Efficacy of a standard nematicide\%}} \times 100\% \text{ Talavera-Rubia et al. (2020).}$$

### 8. The impact of Ag-NP concentrations on the yield of sugarbeet as roots and sugar g/plant:

The technological characters on the basis of total soluble solids (T.S.S) percentage was measured in the fresh roots by hand refractometer, sucrose percentage was determined according to Le Docte as described by Mc Ginnis (1982) and purity percentage was calculated as a ratio between sucrose% and T.S.S%. Sugar yield plant-1 was calculated by multiplied sucrose% X root weight.

### 9. Data Recorded:

1) Biological yield plant<sup>-1</sup>(g)

- 2) Root yield plant<sup>-1</sup>(g).
- 3) Top yield plant<sup>-1</sup>(g).
- 4) T.S.S. %
- 5) Sucrose %
- 6) Sugar yield plant<sup>-1</sup>(g) = [Root yield plant<sup>-1</sup>(g) × Sucrose %]
- 7) Purity % =  $\frac{\text{Sucrose \%}}{\text{T.S.S.\%}} \times 100\%$  ; According to the equation given by Süheri, (2007).
- 8) Avoidable loss % for root and yield plant<sup>-1</sup>(g):

The avoidable loss has been calculated by adopting the formula adopted by Jagdishwar Reddy (2001) as given on below.

Avoidable loss (%) =  $\frac{Y-Y1}{y1} \times 100\%$  the formula adopted by Jagdishwar Reddy (2001). Where,

Y = Mean yield in sprayed pots.

Y1 = Mean yield in unsprayed pots

### Statistical analysis:

Data of the experienced growing season subjected to analysis of variance (ANOVA) according to Steel and Torrie (1981) using MSTAT version 4 (1987), followed by testing significant differences among the means of different treatments were separated by Duncan's Multiple Range Test at 0.05 probability according to Duncan (1955).

## Results and Discussion

### 1- Impact of Ag-NPs concentrations on root-knot nematode; *Meloidogyne incognita* number in soil, reproductive factor and knot disease severity% of roots sugarbeet in pots experiment:

The effects of certain concentrations of high throughput microcrystalline cellulose rooted on silver nanoparticles (Ag-NPs) were studied on root-knot nematode; *Meloidogyne incognita* number in soil, reproductive factor and knot disease severity% for sugarbeet roots in pots experiment (outdoors). Sugarbeet variety, Polat was used in this study whereas categorized as susceptible variety to *M. incognita* according to two susceptibility tests i.e. Adapted Quantitative scheme for assignment of Canto - Saenz's host suitability – resistance Designations (AQSCS) for sugarbeet/RKN Gohar *et al.* (2013) after Sasser *et al* (1984) and the other method used as a verification was the modified host parasite index (MHPI) also according to Maareg *et al.*, (2009). The trials were done as pots experiments at outdoors as described in materials and methods chapter with multiple concentrations of Ag-NPs by two ways of applications along with synthetic organo-phosphorus Ethoprop granules 10% (Mocap® 10% G) as a standard comparable nematicide.

### 2. Juveniles' number in soil:

Results in Table (1) verified effects of various concentrations of Ag-NPs on root- knot nematode *M. incognita* J<sub>2</sub> number/g soil, knot disease severity%, J<sub>2</sub> drop rate% and disease severity drop rate% (efficacy%) in sugarbeet plants. Numbers concerning the nematode (J<sub>2</sub>) number/ g soil under efficacy of different Ag-NPs concentrations revealed that number of J<sub>2</sub>/ g soil was significantly at P ≤ 0.05 varied by concentration. Increasing Ag-NPs concentration from 0 to 60 ppm/ml diminished J<sub>2</sub> number from 6.5 down to 1.7 J<sub>2</sub>/ g soil. The Ag-NPs concentration of 60 ppm/ml produced the significant lowest J<sub>2</sub> number (1.7 J<sub>2</sub>/ g soil) than the five other Ag-NPs concentrations, followed by 50 ppm/ml (2.1) and 40 ppm/ml (2.4 J<sub>2</sub>/ g soil) (Table, 1). Also, from Table (1), data revealed that application times of Ag-NPs concentrations, if it was one time (AT1) or two times (AT2) as designated in materials and methods had significant impact at P ≤ 0.05 on J<sub>2</sub> number / g soil. The AT2 maintained lower number (2.7 J<sub>2</sub>/ g soil) than AT1 (3.2 J<sub>2</sub>/ g soil). Regarding interaction between Ag-NPs concentrations and application times (AT), records in the same Table (6) showed that the best obtained result was at 60 ppm/ml with AT2 (1.4 J<sub>2</sub>/ g soil) followed by 50 ppm/ml with AT2 (1.8 J<sub>2</sub>/ g soil) followed with 40 ppm/ml with AT2 (1.8 J<sub>2</sub>/ g soil) without significance among them. All abovementioned records whether for Ag-NPs concentrations, application times and interaction between them were with clear significance at P ≤ 0.05 from control treatment (0.0 ppm/ml – 6.5 J<sub>2</sub>/ g soil).

### 3. Reproductive Factor (RF) $P_f/P_i$ :

Also, findings in Table (1) demonstrate the effect of different concentrations of Ag-NPs on reproductive factor (RF) of root-knot, nematode *M. incognita*. As RF expresses the multiplication rate of a nematode Sasser *et al* (1984), and governed by the degree of host suitability or numerous biotic and/or abiotic factors or by control measures according to the degree of their efficiency. The most significantly effective Ag-NPs concentrations at  $P \leq 0.05$  on RF were orderly 60 ppm/ml (1.1) followed by 50 ppm/ml (1.3) then 40 ppm/ml concentration (1.5). On the other hand, application times (AT) of Ag-NPs concentrations had significant effect on RF, whereas AT2 achieved 1.9 and AT1 recorded RF value of 2.2. Concerning the impact of interaction between Ag-NPs concentrations and application times (AT) on RF, records revealed that the best values for RF found on concentration 60 ppm/ml of Ag-NPs with AT2 (0.9) followed by 50 ppm/ml with AT2 (1.1) followed by 40 ppm/ml with also AT2 (1.3 RF). All records showed significance among them at  $P \leq 0.05$  (Table, 1).

**Table 1:** Impact of Ag-NPs concentrations on root-knot nematode; *Meloidogyne incognita* number in soil, reproductive factor and knot disease severity% for sugarbeet roots in pots experiment.

Concentrations of Ag-NPs	Root-knot nematode ( $J_2$ ) number/ g soil			Reproductive Factor (RF) $P_f/P_i^X$			Root-knot disease Severity%		
	Time of AgNP application (AT)								
	AT1 <sup>Y</sup>	AT2 <sup>Z</sup>	Mean	AT1	AT2	Mean	AT1	AT2	Mean
20 ppm/ml	4.2	3.6	3.9	2.6	2.3	2.4	33.6	28.8	31.2
30 ppm/ml	3.1	2.8	3.0	1.9	1.8	1.8	24.8	22.4	23.6
40 ppm/ml	2.8	2.0	2.4	1.8	1.3	1.5	22.4	16.0	19.2
50 ppm/ml	2.3	1.8	2.1	1.4	1.1	1.3	18.4	14.4	16.4
60 ppm/ml	2.0	1.4	1.7	1.3	0.9	1.1	16.0	11.2	13.6
0 ppm/ml *	6.6	6.3	6.5	4.1	3.9	4.0	52.8	50.4	51.6
Ethoprop G 10%**	1.1	1.1	1.1	0.7	0.7	0.7	8.8	8.8	8.8
Mean	3.2	2.7	2.9	2.2	1.9	2.0	25.3	21.7	23.5
<b>L.S.D.<sub>0.05</sub></b>									
Time of Ag-NP application (AT)	0.17			0.11			1.4		
Concentrations of Ag-NPs (Conc.)	0.30			0.12			1.8		
(AT) × (Conc.)	0.54			0.13			1.5		

\*Control "1" = without Ag-NPs; \*\* Chemical nematicides as Control "2"; Y = One application time; z = Two application time  $P_i = 1.6 (J_2) / g \text{ soil}$

### 4. Root-knot disease severity%

Presented results in Table (1) indicated that Ag-NPs concentrations had significant ( $P \leq 0.05$ ) effect on disease severity%. All Ag-NPs concentrations significantly reduced the disease severity% as compared with control treatment (51.6%); Ag-NPs concentration of 60 ppm/ml had the lowest disease severity% (13.6%), followed by 50 ppm/ml (16.4%) then 40 cm Ag-NPs concentration (19.2%), with significant differences among them. As well as the two application times (AT) showed distinguished effect on disease severity% compared with control treatment plus there was significance between them at  $P \leq 0.05$ . AT2 application time soared AT1 by disease severity 3.6% lower. The interaction between Ag-NPs concentrations and application times (AT) - (Ag-NPs concentrations × AT) was significant records for disease severity%. The highest reduction (11.2%) was noted at 60 ppm/ml concentration with AT2 followed by 50 ppm/ml with AT2 (14.4%), followed by AT2 (16.0%) which equaled the value of interaction effect obtained by concentration of 60 ppm/ml with AT1 treatment (16.0%). In short, the effects of certain concentrations of high throughput microcrystalline cellulose rooted on silver nanoparticles (Ag-NPs) as a factor and application time as another factor with regard to their interaction, produced significant effect on root-knot nematode; *Meloidogyne incognita* number in soil, reproductive factor and knot disease severity% for sugarbeet roots in pots experiment at  $P \leq 0.05$ . Data in Table (1) demonstrate that as concentrations raising from 0.0 to 60.0 ppm/ml the potential nematicidal activity of Silver Nanoparticles (Ag-NPs) against the Root-Knot Nematode, *M. incognita* increases. As well as

two times of application made additive impact on the abovementioned nematode parameters the one-time application of Ag-NPs concentrations. Concerning the comparison between nematicidal activity of Ag-NPs concentrations and control treatment (0.0 ppm/ml Ag-NPs), along with synthetic organo-phosphorus Ethoprop granules 10% (Mocap® 10% G) as a standard comparable nematicide, the findings in Table (6) showing promising gains. The studied Ag-NPs concentrations above 40 ppm/ml achieved records approached of that achieved by Ethoprop for most investigated nematode parameters. These findings are with agree with those obtained by Maggie *et al.* (2016) indicated that Ag-NPs either alone or combined with nematicides had positive effect on managing *M. incognita* as decreased all nematode parameters. Nassar (2016) showed that the phytochemical based synthesis of Ag-NPs enriched nematicidal activity affecting J2 and eggs up to the levels of 5 and 2 times respectively while “rugby” was the reference nematicide and that was most toxic against *M. incognita*. The study revealed that the toxicity of all extracts either inhibited nematode activity or caused death, depending on the concentration. Also, Bernard *et al.* (2019) study potential nematicidal activity of silver nanoparticles against the root-knot nematode, *M. incognita* and concluded that there was dramatic difference in nematode numbers between treated control groups.

### 5. Damage index (gall index GI):

Results in Table (2) denoted the significance effect of Ag-NPs concentrations and application times (AT) on damage of sugarbeet roots induced by root-knot nematode, *M. incognita* as gall index (GI) at  $P \leq 0.05$ . The best Ag-NPs concentration was 60 ppm/ml where it reduced significantly GI to 1.6 clearly than control treatment (0 ppm/ml) which was 4.1. Also application time AT2 recorded 2.8 GI lesser than AT1 (3.0 - GI). Regarding interaction between Ag-NPs concentration and ATs, the best values were noticed at 60 ppm/ml concentration with AT2 (1.4) followed by 60 ppm/ml with AT1 (1.8) then 50 ppm/ml concentration with AT2 (2.0). Notably there is relation between concentration levels and degree of GI as concentration cumulative from 0.0 to 0.60 ppm/ml GI dropping sharply, also AT2 had a potential effect on GI obvious in all levels of concentration. Results of Ag-NPs are positive and promising as it achieves values close to those recorded by Ethoprop in reducing GI. Baronia *et al.* (2020) revealed that in glasshouse assays in soilless system of rice cultivation, 1 µg/ml concentration of Ag-NP applied directly to the trays achieved significant suppression of root gall formation. The effective dosage to kill nematodes in field soil assays was determined to be 3 µg/ml, which is lower than the value of 150 µg/ml reported in the literature. The results indicate that Ag-NP has effective nematicidal activity against *M. graminicola* in rice. Entsar (2016) investigated nematicidal effects of silver nanoparticles on root-knot nematodes, *M. incognita* in laboratory and greenhouse and found all the concentrations of Ag-NP inhibited the nematode growth (gall and egg formation and final population) and eggs hatchability.

**Table 2:** Impact of Ag-NPs concentrations on damage index (gall index) for sugarbeet roots infested by root-knot nematode; *Meloidogyne incognita* in pots experiment.

Time of AgNP application (AT)	Damage index gall index (GI)**							Ethoprop G 10%**	Mean
	Concentrations of Ag-NPs								
	20 ppm/ml	30 ppm/ml	40 ppm/ml	50 ppm/ml	60 ppm/ml	0 ppm/ml *			
AT1 <sup>y</sup>	3.8	3.2	2.8	2.4	1.8	4.2	1.0	3.0	
AT2 <sup>z</sup>	3.6	3.0	2.6	2.0	1.4	4.0		2.8	
Mean	3.7	3.1	2.7	2.2	1.6	4.1		3.0	

**L.S.D<sub>0.05</sub>**  
 Time of Ag-NP application (AT) 0.16  
 Concentrations of Ag-NPs (Conc.) 0.14  
 (AT) × (Conc.) 0.17

\*Control “1” = without Ag-NPs \*\* Chemical nematicides as Control “2” \*\*\*gall index (GI); 1 = 1 – 2 galls; 2 = 3 – 10 galls; 3 = 11 – 30 galls; 4 = 31 100 galls; 5 = 101 galls and above according to (Taylor and Sasser 1978). Y = One application time; z = Two application time  
 Drop Rate % and efficacy %:

Drop rate % is parameter measures the ratio of population reduction that takes place after a treatment it can be deduced as mentioned in the material and methods, it is considered as a tool of

evaluation for pests or diseases control. Data in Table (3) illustrates impact of Ag-NP concentrations and times of application on root-knot nematode, *Meloidogyne incognita* as drop rate% and efficacy% in sugarbeet pots experiment. The most promising drop rate % for second juvenile larvae ( $J_2$ s) in soil of pots recorded at 60 ppm/ml Ag-NP concentration (73.7%) followed by 50 ppm/ml concentration (68.3%) followed by 40 ppm/ml concentration (62.9%). Also application times revealed significant difference between them at  $P \leq 0.05$  where, AT2 achieved 65.5 drop rate% soaring AT1 (60.9%). Concerning interaction between Ag-NP concentrations and times of application (ATs), the best positive results were obtained at 60 ppm/ml concentration with AT2 (77.8) followed by 60 ppm/ml at AT1 (69.7%) followed by 50 ppm/ml at AT2 (71.4%). These findings are in agreement with those obtained by Entsar (2016) who stated that Numbers of *M. incognita*  $J_2$  extracted from soil samples were reduced when the soil was treated with Ag-NP, and there was an correlation between Ag-NP concentration and this reduction. Lower concentrations (20, and 40 ppm/ml/ml) of Ag-NP reduced the number of  $J_2$  extracted from the soil, galls, and eggs formation but not significantly while the rate of reproduction reduced. Control efficacy % of root-knot nematodes, *M. incognita* by applying AgNP concentrations and times of application AgNP concentrations and number of application times is demonstrated in the same Table (3). The highest efficacy % was recorded by 60 ppm/ml (65.1%) and for application time was noticed at AT2 (58.3%). The most effective interaction on efficacy % was found 60 ppm/ml concentration with AT2 (77.1%) followed by the same concentration with AT1 (53.1%). All obtained values of efficacy % were with significance among them at  $P \leq 0.05$  except for values obtained by 50 ppm/ml and 60 ppm/ml at AT1 (53.1 and 50.0%, respectively). Efficacy % increases by increasing Ag-NP concentrations and this concedes with those results obtained by Baronia *et al.* (2020) who stated that 0.1  $\mu\text{g/ml}$  of Ag-NP proved ineffective even up to 72 hr. but juvenile mortality started at 0.2  $\mu\text{g/ml}$ , and it increased with increasing concentration of Ag-NP and time, but 100% mortality was achieved at concentrations of 2  $\mu\text{g/ml}$  and above after 24 hr. The interaction of time and concentration of Ag-NP was significant ( $p \leq 0.05$ ). Also, Entsar (2016) confirmed that all the concentrations of Ag-NP inhibited the nematode growth (gall and egg formation and final population) and eggs hatchability. However, the high concentrations of 200 ppm/ml, 500 ppm/ml, and 1500 ppm/ml were more significant in their effect on *M. incognita*.

**Table 3:** Impact of Ag-NP concentrations on root-knot nematode, *Meloidogyne incognita* as drop rate% and efficacy% in sugarbeet pots experiment.

Concentrations of Ag-NPs	Drop rate %			Efficacy %		
	Time of Ag-NP application (AT)					
	AT1 <sup>Y</sup>	AT2 <sup>Z</sup>	Mean	AT1	AT2	Mean
20 ppm/ml	36.4	42.9	39.6	27.7	36.6	32.1
30 ppm/ml	53.0	55.6	54.3	44.9	35.7	40.3
40 ppm/ml	57.6	68.3	62.9	38.3	59.2	48.8
50 ppm/ml	65.2	71.4	68.3	51.0	48.4	49.7
60 ppm/ml	69.7	77.8	73.7	53.1	77.1	65.1
0 ppm/ml *	0.0	0.0	0.0	0.0	0.0	0.0
Ethoprop G 10%**	83.3	83.3	83.3	93.0	93.0	93.0
Mean	60.9	66.5	63.7	51.3	58.3	54.8
L.S.D <sub>0.05</sub>						
(AT)		3.7		3.2		
Ag-NPs (Conc.)		6.2		5.7		
(AT) × (Conc.)		11.2		9.6		

\*Control "1" = without Ag-NPs; \*\* Chemical nematicides as Control "2" Y = One application time; z = Two application time .

## 6. Evaluating relative efficacy%:

Relative efficacy % for a given Ag-NP concentration defined as relating the specific efficacy% for that concentration to efficacy% of a standard nematicide (Talavera-Rubia *et al.*, 2020). Data in Table (4) revealed that 60 ppm/ml Ag-NP concentration recorded the highest relative efficacy (70%) and was 55.3% for application time (AT2). Also, interaction between Ag-NP concentration and application times (AT) had potential effect on relative efficacy%. Interaction of 60 ppm/ml concentration with AT2

achieved 82.9% followed by interaction between the 50 ppm/ml concentration with AT2 (63.6%) then 57.1% by 60 ppm/ml at AT1. As Ag-NP concentration increases the efficacy of Ag-NPs comes close to that of standard nematicides, this is consistent with those obtained by Maggie *et al.* (2016) concluded that the juvenile's mortality was increased with the increase of concentration. The highest concentration 90% of Ag-NPs showed increasing in mortality with exposure time. This observation of Ag-NPs was like the behavior of both nematicides fenamiphos and Oxamyl on mortality for highest concentration 90% and prolonged exposure time.

**Table 4:** Relative efficacy of Ag-NP concentrations on controlling root-knot nematode, *Meloidogyne incognita* in sugarbeet pots experiment.

Concentrations of Ag-NPs	Relative Efficacy %		
	Time of Ag-NP application (AT)		
	AT1 <sup>y</sup>	AT2 <sup>z</sup>	Mean
20 ppm/ml	29.8	39.3	34.6
30 ppm/ml	48.2	38.4	43.3
40 ppm/ml	41.2	52.1	52.4
50 ppm/ml	54.8	63.6	53.4
60 ppm/ml	57.1	82.9	70.0
0 ppm/ml *	0.0	0.0	0.0
Ethoprop G 10%**	100.0	100.0	100.0
Mean	46.2	55.3	50.7
<b>L.S.D<sub>0.05</sub></b>			
Time of Ag-NP application (AT)	3.2		
Concentrations of Ag-NPs (Conc.)	6.2		
(AT) × (Conc.)	4.1		

\*Control "1" = without Ag-NPs; \*\* Chemical nematicides as Control "2"; Y = One application time; z = Two application time

## 7. Impact of Ag-NPs concentrations on Root yield plant<sup>-1</sup>(g), Top yield plant<sup>-1</sup>(g) and sugar yield plant<sup>-1</sup>(g) in sugarbeet infested with root-knot nematode, *Meloidogyne incognita* in pots experiment:

### 7.1. Root yield plant<sup>-1</sup>(g)

Data illustrated in Table (5) revealed the 60 and 50 ppm/ml Ag-NPs concentration achieved the highest root yield plant<sup>-1</sup>(g), (880.7 and 848.7 g plant<sup>-1</sup>, respectively) without significance at  $p \leq 0.05$ , followed by 40 ppm/ml (798.1 g plant<sup>-1</sup>) with significant difference with control treatment (0.0 ppm/ml). also application time (AT2) was better than (AT1) whereas they were 690.3 and 686.3 g plant<sup>-1</sup>, respectively without significance between them. The distinguish interaction effect of Ag-NPs concentrations and ATs on root yield plant<sup>-1</sup>g was at 60 ppm/ml with AT2 (892.2 g) followed by 869.3 g at 60 ppm/ml with AT1 followed by 855.6 g plant<sup>-1</sup> at 50 ppm/ml with AT2 without significance among them at  $p \leq 0.05$ .

### 7.2. Top yield plant<sup>-1</sup>(g):

From the same Table (5) it can be noticed that best effect of Ag-NPs concentration on top yield plant<sup>-1</sup> (g) was 60, 50 and 40 ppm/ml orderly where they were 388.0, 381.0 and 371.0 g plant<sup>-1</sup>, respectively without significance among them at  $p \leq 0.05$ . There was no significance effect for ATs on top yield plant<sup>-1</sup> (g). Also, there were no significant differences for the interaction effect of Ag-NPs concentrations with AT1 and/or AT2 after 40 ppm/ml Ag-NPs.

### 7.3. Sugar yield plant<sup>-1</sup>(g):

Findings in Table (5) showed the finest effect of Ag-NPs concentration on sugar yield plant<sup>-1</sup> (g) was obtained by 60 ppm/ml (168.7 g plant<sup>-1</sup>) followed by 50 ppm/ml (176.0 g plant<sup>-1</sup>) then at 40 ppm/ml (160.1 g plant<sup>-1</sup>) with notably significance differences among the at  $p \leq 0.05$  and higher than that recorded at control treatment (101.6 g plant<sup>-1</sup>). There was significant difference between ATs for sugar yield plant<sup>-1</sup> (g). There are discriminate effect for interaction between Ag-NPs concentrations and application times (ATs) in comparison with control treatment, in the lead, values show that 60 ppm/ml with AT2 and AT1 achieved 189.3 and 184.0 g plant<sup>-1</sup>, respectively without significance difference

between them at  $p \leq 0.05$  followed by 50 ppm/ml with the same manner at AT2 and AT1. Briefly, all sugarbeet yield components i.e. Root yield plant<sup>-1</sup>(g), Top yield plant<sup>-1</sup>(g) and sugar yield plant<sup>-1</sup>(g) of infested sugarbeet plant with root-knot nematode, *M. incognita*, affected positively as Ag-NPs concentrations get greater especially greater than 40 ppm/ml, also two times of application (AT2) achieved valued numbers of yield components than that achieved by on time application (AT1) as shown in Table (5).

**Table 5:** Impact of Ag-NP concentrations on Root yield plant<sup>-1</sup>(g), Top yield plant<sup>-1</sup>(g) and sugar yield plant<sup>-1</sup>(g) in sugarbeet

Concentrations of Ag-NPs	Root yield plant <sup>-1</sup> (g)			Top yield plant <sup>-1</sup> (g)			sugar yield plant <sup>-1</sup> (g)		
	Time of Ag-NP application (AT)								
	AT1 <sup>y</sup>	AT2 <sup>z</sup>	Mean	AT1	AT2	Mean	AT1	AT2	Mean
20 ppm/ml	704.6	710.5	707.6	356.0	358.0	357.0	111.0	118.8	114.9
30 ppm/ml	716.4	738.0	727.2	360.0	364.0	362.0	126.6	141.2	133.9
40 ppm/ml	759.5	800.7	798.1	368.0	374.0	371.0	155.8	164.5	160.1
50 ppm/ml	841.8	855.6	848.7	380.0	382.0	381.0	173.3	178.6	176.0
60 ppm/ml	869.3	892.2	880.7	384.0	392.0	388.0	184.0	189.3	186.7
0 ppm/ml *	686.3	690.3	688.3	348.0	349.1	348.6	101.2	102.0	101.6
Ethoprop **	915.0			400.0			194.7		
Mean	762.4	781.2	775.1	366.0	369.9	367.9	142.0	149.1	145.5
<b>L.S.D.<sub>0.05</sub></b>									
(AT)		45.0		21.3			8.2		
(Conc.)		45.1		21.5			8.7		
(AT) × (Conc.)		45.4		21.3			8.5		

\*Control "1" = without Ag-NPs; \*\* Chemical nematicides as Control "2"; Y = One application time; z = Two application time

### 8. Impact of Ag-NP concentrations on Sucrose, total soluble solids (T.S.S) and purity percentages of sugarbeet infested with root-knot nematode, *Meloidogyne incognita* in pots experiment:

Results in Table (6) elucidate the effects of different Ag-NPs concentrations and times of application on main technological characters i.e. Sucrose, total soluble solids (T.S.S) and purity percentages of sugarbeet infested with root-knot nematode, *Meloidogyne incognita* in pots experiment.

#### 8.1. Sucrose %:

The most influencing Ag-NP concentration on sucrose % was 60 ppm/ml as achieved 23.1% followed by 50 ppm/ml (20.7%) then 40 ppm/ml concentration (18.3%) with significance among them at  $p \leq 0.05$ . application times (AT1 & AT2) didn't make any significant difference between them at  $p \leq 0.05$ . regard to effect of interaction between Ag-NP concentrations and application times (ATs) on sucrose%, AT2 and AT1 at 60 ppm/ml recorded the highest values (23.2 and 23.0 %, respectively) without significance between them at  $p \leq 0.05$ , followed by AT2 and AT1 at 50 ppm/ml as achieved 21.2 and 20.2%, respectively without significance between them. There are significance differences for the effect on sucrose % between interactions took place at 60 and 50 ppm/ml (Table, 6).

#### 8.2. T.S.S. %:

Data of total soluble solids percentage (T.S.S. %) in the same Table (6) made known that there are significance differences at  $p \leq 0.05$  among effects of different Ag-NPs concentrations and application times (ATs) on T.S.S. %. The uppermost values are detected at 60 and 50 ppm/ml (24.7 and 23.3%, respectively), without significance between them at  $p \leq 0.05$  but with significance with other tested Ag-NPs (0.0, 20.0, 30.0 and 40 ppm/ml). For the effect of application times (ATs) on T.S.S. % findings showed that AT2 and AT1 recorded the highest values (21.6 and 20.8%, respectively) without significance at  $p \leq 0.05$ . Interaction effect of different Ag-NPs concentrations and application times (ATs) on T.S.S. % shown powerfully in 60 ppm/ml Ag-NPs concentration at both application times, AT2 and AT1 (24.8 and 24.6, respectively) without significance at  $p \leq 0.05$ . In this concern of interaction, 50 ppm/ml ranked second with two insignificant value between them at AT2 and AT1 (23.7 and 22.8%, respectively), but by significance at  $p \leq 0.05$  with those took place at 60 ppm/ml.

### 8.3. Purity %:

Findings in Table (6) demonstrate effects of different Ag-NPs concentrations and times of application on juice purity % sugarbeet pulps. Concentration of 60 and 50 ppm/ml achieved the highest values of purity % (93.5 and 89.0%, respectively) without difference between the at  $p \leq 0.05$ , but with significance with lower Ag-NPs concentrations. There were no significance differences at  $p \leq 0.05$  among subordinate concentrations (20, 30 and 40 ppm/ml) for purity %. Times of application (ATs) didn't make any significance differences at  $p \leq 0.05$ . Considering effect of interaction for Ag-NPs concentrations and times of application on juice purity %, the higher values took place from concentration 40 to 60 ppm/ml almost equally at AT2 or AT1, the same trend observed at the lower Ag-NPs concentrations (0, 20 and 30 ppm/ml) and times of application.

Table (6) illustrates the avoidable loss percentage in roots and sugar yields plant<sup>-1</sup>(g) over control. Also, it was calculated by obtaining yield from Ag-NP concentrations treatments and untreated pots. The avoidable loss has been calculated by adopting the formula adopted by Jagdishwar Reddy (2001). It is evident from the data presented in Table (6) that the percentage of avoidable loss in roots and sugar yields plant<sup>-1</sup>(g) was greater at the higher Ag-NP concentrations i.e. goes greater as concentration goes higher. There are significance differences at  $p \leq 0.05$  among tested Ag-NP concentrations the superior values for avoidable loss in roots and sugar yields plant<sup>-1</sup>(g) found at 60 ppm/ml (21.8 and 45.6%, respectively). Also, effect of application times (TAs) showed the AT2 influenced avoidable loss in roots and sugar yields plant<sup>-1</sup>(g) with significant difference greater than AT1 as shown in Table (7) as they are 13.0 % for roots yields plant<sup>-1</sup>(g) and 32.7% for sugar yields plant<sup>-1</sup>(g). Effect of Ag-NP concentrations  $\times$  application times (ATs) on avoidable loss % of roots and sugar yields plant<sup>-1</sup>(g) which recorded 22.6 % at 60 ppm/ml for roots yields plant<sup>-1</sup>(g) with AT2 and differed significantly ( $p \leq 0.05$ ) at AT1 (21.1%). Whereas, 46.1% avoidable loss for sugar yields plant<sup>-1</sup>(g) at 60 ppm/ml with AT2 and 45.0%, with AT1 without significance between them at  $p \leq 0.05$  (Table, 7).

**Table 6:** Impact of Ag-NP concentrations on Sucrose, total soluble solids (T.S.S) and purity percentages of sugarbeet infested with root-knot nematode, *Meloidogyne incognita* in pots experiment.

Concentrations of Ag-NPs	Sucrose %			T.S.S. %			Purity%		
	Time of Ag-NP application (AT)						AT1	AT2	Mean
	AT1 <sup>y</sup>	AT2 <sup>z</sup>	Mean	AT1	AT2	Mean			
20 ppm/ml	14.7	15.1	14.9	17.8	18.2	18.0	82.6	83.0	82.8
30 ppm/ml	15.4	16.0	15.7	18.2	19.4	18.8	84.6	82.5	83.5
40 ppm/ml	18.2	18.4	18.3	20.6	21.7	21.2	88.3	84.8	86.6
50 ppm/ml	20.2	21.2	20.7	22.8	23.7	23.3	88.6	89.5	89.0
60 ppm/ml	23.0	23.2	23.1	24.6	24.8	24.7	93.5	93.5	93.5
0 ppm/ml *	14.7			20.0			73.5		
Ethoprop G 10%**	23.2			25.0			92.8		
Mean	18.3	18.8	18.5	20.8	21.6	21.2	87.5	86.6	87.1
<b>L.S.D.<sub>0.05</sub></b>									
Time of Ag-NP application (AT)		1.33		1.43			5.03		
Concentrations of Ag-NPs (Conc.)		1.34		1.61			5.00		
(AT) $\times$ (Conc.)		1.35		1.44			5.08		

\*Control "1" = without Ag-NPs; \*\* Chemical nematicides as Control "2"; Y = one application time; z = two application time.

Findings illustrated in Tables (1 & 2) concerning nematode parameters i.e. root-knot nematode; *Meloidogyne incognita* numbers in soil, reproductive factor (RF), knot disease severity% and damage index (gall index GI), evidently showed that as Ag-NP concentrations get higher the effects get powerful, so the case of application times, two times of Ag-NPs concentrations (AT2) gave better results than one time (AT1). The potential of Ag-NPs concentrations and application times (ATs) represented as respectable reduction in all abovementioned nematode parameters. Obviously, this proves the

nematicidal effects on root-knot nematode; *Meloidogyne incognita*, mainly juvenile larvae physiologically and biologically, hence reduction takes place in numbers invading roots, numbers in soil, gall numbers (GI) and reproduction of nematodes (RF). These observations are in consistency with suggestions about these effects which may be due Ag-NPs mode of action is not specific but associated with multiple cellular mechanisms including ATP synthesis, membrane permeability, and response to oxidative stress in both of eukaryotic cells (Roh *et al.*, 2009; Ahamed *et al.*, 2010; Lim *et al.*, 2012). The initial studies on the toxicity of Ag-NP involved free-living species *Caenorhabditis elegans* (Roh *et al.*, 2009; Meyer *et al.*, 2010) and *Panagrellus redivivus* (Mahmoud *et al.*, 2016). Oxidative stress-related PMK- 1 P38 MAPK activation was reported as a mechanism for toxicity of Ag-NP to *Caenorhabditis elegans* (Lim *et al.*, 2012). Hassan *et al.* (2016) observed degradation. Fouda *et al.* (2020) concluded that Ag-NPs could be successfully used as eco-nematicide for Root-knot nematodes; *Meloidogyne incognita* with a recommended dose of 20–40 ppm that is acquired higher M% and caused many aberrations during the different growth stages of *M. incognita*.

Also results in the same mentioned Tables (1) are in constancy with those obtained by Maggie *et al.* (2016) indicated that highest concentration 90% achieved highest percentage of juveniles mortality which were 95, 87, 98, and 100% of Ag-NPs, polyvinylpyrrolidone (PVP), fenamiphos and oxamyl, respectively. While mortality in control treatment was 1.5%. The study involved that Ag-NPs showed degradation in cell wall under laboratory conditions. Assessment included root of tomato seedling and showed optimistic effect for Ag-NPs. The pathogen signs such as galls, egg-masses, developmental stages, rate of buildup and nematodes in 250 g soil had defined. Correspondingly, Baronia *et al.* (2020) found in glasshouse assays in soilless system of rice cultivation that 1 µg/ml concentration of Ag-NP applied directly to the trays achieved significant suppression of root gall formation. The effective dosage to kill nematodes in field soil assays was determined to be 3 µg/ml, which is lower than the value of 150 µg/ml reported in the literature.

**Table 7:** Impact of Ag-NP concentrations on Percentage of avoidable loss in Root yield plant<sup>-1</sup>(g) and sugar yield plant<sup>-1</sup>(g) on sugarbeet infested with root-knot nematode, *Meloidogyne incognita* in pots experiment.

Concentrations of Ag-NPs	Percentage of avoidable loss					
	Root yield plant <sup>-1</sup> (g)			Sugar yield plant <sup>-1</sup> (g)		
	AT1 <sup>y</sup>	AT2 <sup>z</sup>	Mean	AT1	AT2	Mean
20 ppm/ml	2.6	2.8	2.7	2.4	8.8	5.5
30 ppm/ml	4.2	6.5	5.3	20.0	27.7	24.1
40 ppm/ml	9.6	13.8	11.7	35.0	38.0	36.5
50 ppm/ml	18.5	19.3	18.9	41.6	42.9	42.2
60 ppm/ml	21.1	22.6	21.8	45.0	46.1	45.6
0 ppm/ml *	0.0	0.0	0.0	0.0	0.0	0.0
Ethoprop G 10%**	25.0			48.0		
<b>Mean</b>	11.2	13.0	12.1	28.8	32.7	30.8
<b>L.S.D<sub>0.05</sub></b>						
<b>Time of AgNP application (AT)</b>		0.65		1.67		
<b>Concentrations of Ag-NPs (Conc.)</b>		0.70		1.79		
<b>(AT) × (Conc.)</b>		0.76		1.90		

\*Control “1” = without Ag-NPs; \*\* Chemical nematicides as Control “2”; Y = One application time; z = Two application time

Results in Tables (3) demonstrated efficacy evaluation of Ag-NPs concentrations to suppress root-knot nematode, *M. incognita* by several means as validation tool, i.e. Drop rate% as an indicator for reduction assessment for *M. incognita* in the soil (capability of a treatment to reduce nematode numbers), efficacy% as an evaluation of concentration treatments proportioned to control treatment and relative efficacy % to assess effectiveness of concentration treatments proportioned to standard nematicide treatment. The shown data confirmed that all mentioned efficacy assessment procedures increase in their efficiency as Ag-NPs concentration raise up to the studied level in this study (60

ppm/ml), also application of Ag-NPs concentration two times (AT2) more efficient for suppressing *M. incognita* more than one time application (AT1). Bernard *et al.* (2019) stated that after Ag-NPs treatment, dead nematodes exhibited the typical “banana shape” curvature predominately displayed in treated groups. At the 1-min exposure interval, a significant difference ( $P < 0.01$ ) in living and dead nematodes between treated and control groups were observed. The 24 and 48 hr Ag-NPs exposure groups showed 100% mortality in comparison to the controls. Exposure intervals in Bernard’s study represent the effect of times application (ATs) in this study. Findings of efficacy in the same Tables (8 and 9) with agreement with those obtained by Entsar (2016) who stated that numbers of *M. incognita* J2 extracted from soil samples were reduced when the soil was treated with Ag-NP, and there was correlation between Ag-NP concentration and these reduction. Lower concentrations (20, and 40 ppm/ml) of Ag-NP reduced the number of J2 extracted from the soil, galls and eggs formation but not significantly while the rate of reproduction reduced significantly compared to the non-treated plots. The concentrations 200.500 ppm were more effective in all nematode parameters especially gall formation and the final population. While the most effective concentration which effected significantly on all the nematode parameters was 1500 ppm of Ag-NP.

From Tables (3 and 4) which concerning efficacy showing that as efficacy%, drop rate% and relative efficacy% of a given Ag-NPs concentration as approaches in its number with that achieved by synthetic standard nematicide (Ethoprop 10%G) 65% or above, it proves a satisfied efficacy as Ag-NPs. And this may be linked with what approved by Maggie *et al.* (2016) who indicated that silver nanoparticles either alone combined with nematicides fenamiphos or oxamyl had positive effect on controlling *M. incognita*. All treatments decreased the nematode parameters. The most effective treatment was Ag-nanoparticles which showed the same results of control. While, the combined treatment fenamiphos: Ag-nanoparticles (1:1) recorded the lowest number of galls, egg-masses, developmental stages, rate of buildup and number of nematodes in 250 soil g compared with control infested with nematode treatment. While the rest of treatments were varied in their effects and this reflected on the rate of buildup. The treatment with fenamiphos recorded 3.59 buildup rate followed by Oxamyl. PVP and Oxamyl: Ag-nanoparticles (1:1) which recorded 2.21, 0.4 buildup rate, respectively.

Toxicity of sub lethal doses of AgNP to nematodes could result in reproduction inhibition with 20; 40 ppm/ml of AgNP, (Meyer *et al.*, 2010) obtained the same data up to 50 ppm. This suggests the AgNP effect may be subtle and chronic at low concentrations applied in the field and this is agreed with the present data. AgNP has a nematicidal activity which may provide an alternative to high-risk chemical nematicides. Findings in Tables (5 and 6) illustrated clearly the impacts of AgNPs application on yield and yield quality. Data revealed enhancement took place in yield components and its quality as AgNPs concentrations increase up to 60 ppm/ml and application twice (AT2), that is because of suppression of nematodes which due to their endoparasitic mode of living and feeding, root knot nematodes interrupt the physiology of the plant and able to cause great losses in production and quality of sugarbeet crop (Gohar and Maareg, 2005), thus as AgNPs concentration succeed to hinder plant parasitic nematodes from invading roots, plants maintain good health expressed as high yielding and high quality. This is in agreement with finding obtained by Entsar (2016) who concluded that adding the silver nanoparticles increased the plant growth (even when adding nematode infection with the AgNPs) or at least was equal to which obtained in the control. Findings in Table (7) gave an illustration about avoidable loss % in roots and sugar yields as an outcome of applying Ag-NPs to control *M. incognita* infested sugarbeet plant. Avoidable loss % increase positively as Ag-NPs concentration increases. Avoidable loss % was greater in sugar yield plant<sup>-1</sup>(g) than in root yield plant<sup>-1</sup>(g), that’s mainly since the loss in sugar yield is greater than it in root yield (Gohar and Maareg, 2005; Gohar *et al.* 2013). Interaction effect between Ag-NPs concentrations and times of applications (AT1 and AT2) showed that there was no significance at  $p \leq 0.05$  for the high concentrations (50 and 60 ppm/ml).

The entire studied elements i.e. Ag-NPs concentrations and times of applications (AT1 and AT2) that were employed in this investigation proved enhancements as illustrated by obtained results in root-knot nematode parameters (Tables: 1-4) or/and in sugarbeet yield and quality parameters (Tables 5-7), along with confirmations by other authors (Gohar *et al.* 2009; Agami *et al.* 2010; Gohar *et al.*, 2012; Gohar *et al.* 2014; Maareg *et al.* 2018 and Michalska-Klimczak *et al.* 2018). Percentage of efficacy of nematode control by AgNPs in sugarbeet pots experiment is an outcome of the efficiency and success of each of the elements used in the study and their impact on root-knot nematode or/and sugarbeet yield parameters.

## Conclusions

Efficacy percentage of root-knot nematodes control by means of Ag-NPs concentrations in sugarbeet pots experiment considered the most authentic mean to draw and recommend successful tested control strategy using Ag-NPs. Applying Ag-NPs directly to infested sugarbeet pots achieved significant suppression at  $p \leq 0.05$  of root-knot nematode, *M. incognita* in terms of reducing numbers in soil, reproductive factor and knot disease severity%; efficacy % that proportionated to untreated pots and relative efficacy% that proportionated to pots treated with Ethoprop 10% G get higher potential as of Ag-NPs of concentrations get higher; times of application [one (AT1) and twice (AT2)] contributed in making significant differences. AT2 enhanced the efficacy of Ag-NPs at low concentration (under 50 ppm/ml) and occasionally above 50 ppm/ml; effects of different Ag-NPs concentrations and times of application on yield components i.e. Root yield plant<sup>-1</sup>(g), Top yield plant<sup>-1</sup>(g) and sugar yield plant<sup>-1</sup>(g) of infested sugarbeet plant with root-knot nematode, *M. incognita*, were related to degree of Ag-NPs concentration to suppress nematodes activity; different concentrations of Ag-NPs increased yield components i.e. Root yield plant<sup>-1</sup>(g), Top yield plant<sup>-1</sup>(g) and sugar yield plant<sup>-1</sup>(g) even at low concentration (20 ppm/ml) in comparison with control treatment (0.0 ppm/ml). The same trend for quality as Sucrose, total soluble solids (T.S.S) and purity percentages of sugarbeet infested with root-knot nematode, *Meloidogyne incognita* in pots experiment and avoidable loss percentage in roots and sugar yields plant<sup>-1</sup>(g) as an economic expression responded positively to different levels of Ag-NPs concentrations and to time of application AT2 in low concentration < 50 ppm/ml. This study has demonstrated a potential environmentally friendly alternative for the management of the root-knot nematodes. Based on the obtained results, the study recommends using Ag-NPs concentrations to manage root-knot nematode, *M. incognita* in low concentration  $\leq 40$  ppm/ml by two applications with 30 days interval or high concentration  $\geq 50$  ppm sole application. Applying Ag-NPs as a nematicide has become a popular area of research because of the lowered risks and hazards associated with the handling, compatibility with IPM procedures and implementation associated with its use and its economic feasibility. Use of Ag-NPs for control of plant-parasitic nematodes is a novel idea; however, as seen with many studies in different cropping systems there is a lot of variability and in consistencies associated with their use. Future work should focus on the soil environment and how it affects the efficacy of these Ag-NPs treatments. Possibilities are that these Ag-NPs treatments may be beneficial for certain regions and soil types or under controlled conditions such as irrigated fields. Our current research suggests that host status is the most influential effect on the RKN and that the Ag-NPs treatments are either short lived in the soil or are not moving with the root system. Sugarbeet Ag-NPs treatments provided excellent early season protection against RKN.

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