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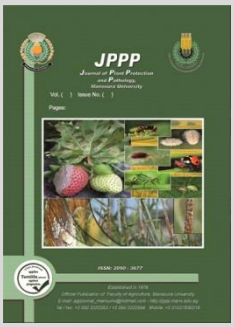
Effectiveness of Silver Nanoparticles Botanically and Chemically Synthesized as well as Chitosan against Root-Knot Nematode *Meloidogyne javanica* in Vitro

Hassan, H. M.*; A. S. M. H. El Roby and Wafaa S. A. Mohamed

Plant Protection Department, Faculty of Agriculture, Minia University, Minia, Egypt.



ABSTRACT

The bioassay of some Silver nanoparticles AgNP synthesized botanically against *Meloidogyne javanica* J2. was conducted. All materials were prepared and evaluated at the concentrations of 100, 50, 25 and 12.5µl/50 ml of nematode suspension. Mortalities% of *M. javanica* J2 were recorded after 96 hrs. The obtained data explained the increasing of *Meloidogyne javanica* J2 with the increasing of the material concentrations. AgNP prepared by oleander extract gave mortality of *M. javanica* J2 ranged between 37.1and 93.25% with the low and high concentrations. The LC_{50} was 15.31µl/50 ml. Nanoparticles of silver by chamomile cause 34.30 and 91.33% mortality of 2nd nematode juvenile with the low and high concentrations. The LC50 was 18.72µl/50 ml. The Ag NP synthesized by thyme cause 14.3 and 91.0% mortality of *M. javanica* J2 with the low and high concentrations, respectively. LC_{50} was 37.71µl/50 ml. The formulation of AgNP by ginger gave mortality of 52.0 and 92.0%. LC_{50} was 11.11. Silver nanoparticles formulated by jojoba surpassed other botanical formulation causing 59.55 and 95.84% mortality with the low and high concentrations, respectively and the LC_{50} was 10.24µl/50ml. Chemical preparation of AgNP cause mortality of 40.8 and 82.0% at low and high concentrations. The LC_{50} was 18.88. The effectiveness of chitosan at the low molecular weight at the concentrations of 100, 50, 25 and 12.5µl/50ml was tested revealing that at low and high concentrations the mortality ranged between 72.34 and 96.8 %, respectively. The estimated LC_{50} was 5.16µl/50 ml. These results explain the superiority of chitosan over other materials.

***Keywords*:** AgNP, Root-knot nematodes, *Meloidogyne jsvanica*, Chitosan.

INTRODUCTION

Phyto-nematodes caused significant and remarkable damage to most agricultural crops reducing the yield and quality, causing loss valued at over $75 billion per annum in tropical and sub-tropics (Luc *et al* 2005). The worldwide crop loses resultant from nematode infection could be estimated by 20.6 % (Sasser & Freckman, 1987). Nematodes account for an estimated 14% of all worldwide plant losses, which translates into almost 100 billion dollars annually. By far, root-knot nematodes are the most common and destructive nematode pathogens. They produce some of the most dramatic symptoms and can considerably reduce crop yields. Root-knot nematodes are found in all agricultural regions worldwide. They can survive in temperate climates and can destruct crops grown in the tropics. Most root-knot nematodes also have extremely wide host ranges. Although it is difficult to make sure the number of hosts for any one root-knot nematode species, it is likely that some root-knot nematodes can survive on hundreds of different plant species. This can make the control a root-knot nematode problem challengingly, particularly if the nematode can survive on weeds. In addition, root-knot nematodes have repeatedly been shown to prepare their host plants to infection by other crop pathogens, increasing the potential for crop loss (Mitkowski & Abawi , 2003)

The new recent advances are to use new approaches for overcome the problem of the pesticides pollution such as the use of green chemistry including plant extracts as one of chemical nematicides alternative. Also nanotechnology is considered the best measure for reducing the hazards of chemical nematicides. (Hassan, 1999; Hassan, 2004;Khan *et al*.,2008 Shawky *et al*.,2010;Nour El-Deen & Darwish, 2011; Singh, 2011; Mervat *et al*. 2012; Ardakani, 2013;Cromwell *et al*., 2014; Nour El Deen *et al*, 2014; El-Sayed and Mahdy,2015; Surega 2015 ; Nassar, 2016;Maggie, *et al*. 2016;Mahmoud *et al*., 2016; Abbassy *et al*,. 2017; Soliman *et al*., 2017; Nour El-Deen & El-Deeb, 2018;Nazir *et al*.,2019; Richa *et al*., 2020 and Danish *et al*. 2021). The present study aims to test in vitro the effect of silver nanoparticles prepared botanically by some plant extracts as well as AgNp prepared chemically and Chitosan

MATERIALS AND METHODS

AgNP mediated by some plant extracts:

Plant leaves or roots of oleander, chamomile, thyme, ginger and jojoba were washed with distilled water to remove debris and soils. Dried in a vacuum oven for 3 h.. A portion of 250g was crushed in electric blender with adding 200 ml of distilled water during crushing. The extract was soaked for a half hour in water bath at 70°C. This extract was centrifuged at 1000 rpm for ten minute. The supernatant was taken then30 ml of the supernatant of the extract was added to 100 ml of 3 mM AgNO3. The solution was mixed well and kept in a shaker incubator for overnight at 37 °C. Once dark brown color was formed that indicating the formation of silver nanoparticle.

* **Corresponding author.**
E-mail address: dr_hassan_m_hassan2000@yahoo.com
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Visual observation and UV–visible spectroscopy

Noble metals are known to show unique optical properties due to the property of surface plasmon resonance (SPR) (Bindhu & Umadevi, 2013). The formation of silver nanoparticles was monitored with color change and UV–Vis spectroscopy. The color of the reaction mixture started changing to yellowish brown within 10 min and to reddish brown after 1 h, indicating the generation of silver nanoparticles, due to the reduction of silver metal ions Ag^+ into silver nanoparticles Ag° via the active molecules present in the plant extracts (Ahmad *et al*., 2003). This color is attributed to the excitation of SPR. Silver nanoparticles was obtained at around λ 433 nm (Mulvaney, 1996).

Synthesis of AgNP chemically:

AgNP were prepared by chemical route using a citrate reduction method (Mulfinger *et al*., 2007). Briefly, 50 ml aqueous solution of 1 mM $AgNO_3$ was heated to boiling temperature. After that, 5 ml of 10 mM trisodium citrate aqueous solution was added dropwis under vigorous stirring until the color of the solution changed to pale yellow. The stirring was continued for another 15 min, and the sample was allowed to cool to room temperature.

Chitosan tested in this experiment:

Chitosan clear viscous at the low molecular weight 161.61 Kd. (Kd. = 1000g/ mol) and degree of deacetylation 92% Dd., was obtained from Naqaa for Nanotechnology co.

Method of application:

Concentrations of 100, 50, 25 and 12.5 µl per 50 ml of nematode suspension were bioassayed in Petry dishes contained 500 of root knot nematode J2.These concentrations interprets to 2000,1000, 500 and 250 ppm . Mortality was observed under the stereo microscope, considering dead those that don't reacted when touched with a probe. Mortality percentages were corrected according to Abbot' formula (1925). LC25, LC 50 and LC90 were calculated with Finney 1971. Toxicity index of the tested materials was estimated according to the following formula,

Toxicity index = LC50 of the most effective compound/LC50 of the tested compound × 100 (Sun 1950).

RESULTS AND DISCUSSION

Bioassay study:

All treatments showed that the increasing of nanoparticles concentration increased the mortality of 2nd J of *M. javanica* nematode this result is in agreement with the finding of Khalil *et al*. (2018) with M. incognita nematode.

Bioassay of the efficiency of AgNo3 nano particles with oleander plant extract against Root-knot nematodes was illustrated in Table (1) whereas four concentrations were used at 100, 50, 25 and 12.5 microliter / 50 ml of nematode suspension. Corrected mortality of the 2nd juveniles of *M. javanica* caused by using the low and the high concentration ranged between 37.0 to 93.25%, respectively. The slope of the LCP line of AgNo3 nano-particles with oleander extract was positive with the value of 2.06, this value is high and that mean the rate of change of *y* (mortality) is changing more twice as fast as concentration. The LC_{50} was 15.31 µl /50 ml. of nematode suspension (Fig. 1).

The corrected mortality of *M. javanica* exposed to AgNo3 nano-particles synthesized by chamomile was slightly less than oleander recording 91.33 and 34.30% with the high and the low concentrations, 100 and 12.5 microliter, respectively. Slope of the LCP line by using Silver nanoparticles synthesized by chamomile was positive with slope value of 1.92 this value is high and that mean the rate of change of *y* (mortality) is changing nearly twice as fast as concentration. The LC_{50} was 18.72 µl /50 ml. of nematode suspension (Table 2 & Fig. 2).The Silver nanoparticles synthesized by thyme gave mortality ranged between 14.3 to 91.0 % with the concentrations of 12.5 and 100 microliter/ 50 ml nematode suspension, respectively. The estimated slope of this material was 2.69. This means that the rate of change of *y* (mortality) is changing nearly twice and half as fast as concentration. The LC_{50} was 37.71µl /50 ml. of nematode suspension as shown in Table (3) and Fig. (3).

Data in Table (4) and Fig. (4) explained that the Silver nanoparticles synthesized by ginger gave mortality ranged between 52.0 to 92.0 % with the concentrations of 12.5 and 100 microliter/ 50 ml nematode suspension, respectively. The estimated slope of this preparation of ginger was 1.45. This means that the rate of change of *y* (mortality) is changing nearly one and half as fast as concentration. The LC_{50} was 11.11 µl /50 ml. of nematode suspension. The last preparation of AgNo3 nano particles with extract of Jojoba plant against Root-knot nematodes, *M. javanica* caused mortality ranged between 59.55 and 95.84 with the concentration of 12.5 and 100 microliter/ 50 ml of nematode suspension. The estimated slope of this preparation was 1.74. This means that the rate of change of *y* (mortality) is changing more than one and half as fast as concentration. The LC_{50} was 10.24 µl /50 ml. of nematode suspension as shown in (Table, 5 & Fig. 5). The superiority of jojoba for controlling root-knot nematode in this research is supported by the results reported by Mervat *et al*. (2012).

We can concluded that the closeness of the ginger and jojoba preparations slope values as well as the slopes of LCP lines of oleander and chamomile means that the two preparations behaved similarity and these two preparations have same mode of action as shown in Table (5).

Testing of AgNo3 nano particles prepared chemically against Root-knot nematodes, *M javanica* caused mortality ranged between 40.80 and 82.00 with the concentration of 12.5 and 100 microliter/ 50 ml of nematode suspension. The estimated slope of this preparation was 1.29. This means that the rate of change of *y* (mortality) is changing more than one as fast as concentration. The LC50 was 18.88 µl /50 ml. of nematode suspension (Table, 6 & Fig. 6).

Data in Table (7) and Fig. (7) show the effect of Chitosan prepared in nano phase on Root-Knot nematodes. This preparation caused mortality ranged between 72.34 to 96.8 % at the low and high concentrations 12.5 and 100 µl / 50 ml of nematode suspension and the LCP line slope was1.40 that mean the rate of change of y (mortality) is changing nearest one and half as fast as concentration. The LC_{50} was 5.16 µl /50 ml. of nematode suspension. The estimated toxicity indices of these nano-technique preparations were 33.7, 27.6, 13.7, 46.4, 50.4, 27.3 and 100 % with AgNo3 prepared with oleander, chamomile, thyme, ginger, jojoba, AgNo3 prepared chemically and Chitosan, respectively. This result shows the superiority of Chitosan (Ti 100%) than AgNP prepared by jojoba, ginger and oleander whereas the estimated toxicity indices by these extracts were 50.4, 46.4 and 33.7 %, respectively while the AgNo3 prepared chemically or by chamomile gave moderate toxicity

index (27.3 & 27.6 %). The silver nanoparticle composed by thyme retreated to the last toxicity index value recording 18.6 % (Table 8 & Fig. 8). El-Sayed and Mahdy (2015) Tested chitosan against *M. javanica* in vitro and reported that both low and high molecular weight chitosan significantly affected the larvae mortality of *M. javanica* at all evaluated dilutions concentrations compared to control with superiority of low molecular weights of Chitosan.

The advantage of using AgNPs that for their wide spectrum effective against other pathogens such bacteria and viruses (Lara *et al*.,). They reported that Ag+ ions and Ag-based compounds are toxic to microorganisms, possessing strong biocidal effects on at least 12 species of bacteria. Also they mentioned that nanoparticles bind with a viral envelope glycoprotein and inhibit the virus by binding to the disulfide bond regions of the CD4 binding domain within the HIV-1 viral envelope glycoprotein gp120,

Table 1. Bioassay of the efficiency of AgNo3 nano particles with oleander plant extract against Root-knot nematodes

AgNo3 nano particle with Oleander extract	Concentration (X)	Log (X)	Aveg. corrected Mortality	Probit
	100.0	2.000	93.25	6.51
	50.0	1.698	89.62	6.25
	25.0	1.397	71.67	5.56
	12.5	1.096	37.00	4.67
control		0.0	0.0	0.0

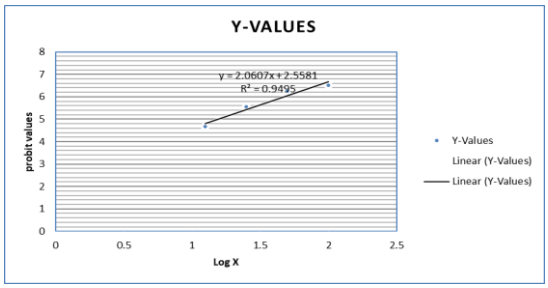

Fig. 1. LCP line of the effect of of AgNo3 nanoparticle with AgNo3 nanoparticles with extract of Oleander plant against Root-knot nematode(Slope=2.06)

Table 2. Bioassay of the efficiency of AgNo3 nano particles with the extract of Chamomile plant against Root-knot nematodes

AgNo3 nano particle with Chamomile extract	Concentration (X)	Log (X)	Aveg. corrected Mortality	Probit
	100.00	2.000	91.33	6.36
	50.0	1.698	79.25	5.82
	25.0	1.397	63.00	5.33
	12.5	1.096	34.30	4.60
control		0.0	0.0	0.0

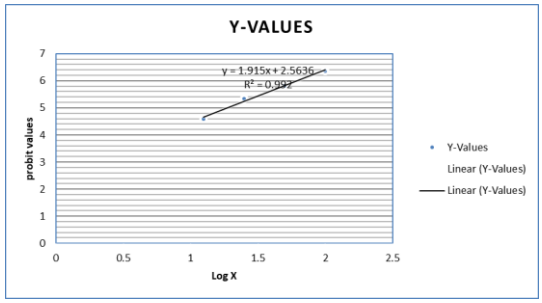

Fig. 2. LCP line of the effect of AgNo3 nanoparticles with extract of Chamomile plant against Root-knot nematode (Slope =1.92)

Table 3. Results of Bioassay of the efficiency of AgNo3 nanoparticle with AgNo3 nanoparticles with extract of Thyme plant against Root-knot nematodes

AgNo3 nanoparticle with Thyme extract	Concentration (X)	Log (X)	Aveg. corrected Mortality	Probit
	100.00	2.000	91.00	6.34
	50.0	1.698	56.50	5.16
	25.0	1.397	23.00	4.26
	12.5	1.096	14.30	3.93
control		0.0	0.0	0.0

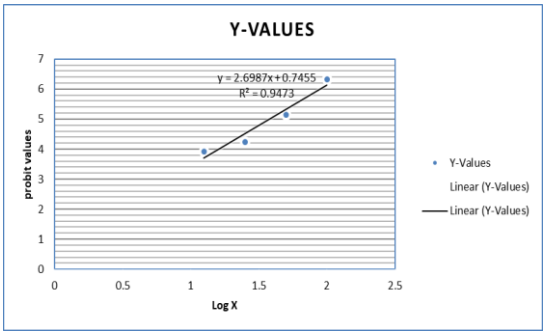

Fig. 3. LCP line of the effect of AgNo3 nanoparticles with extract of Thyme plant against Root-knot nematode (Slope =2.69)

Table 4. Results of Bioassay of the efficiency of AgNo3 nanoparticle with AgNo3 nano particles with extract of Ginger plant against Root-knot nematodes

AgNo3 nano particle with Ginger extract	Concentration (X)	Log (X)	Aveg. corrected Mortality	Probit
	100.00	2.000	92	6.41
	50.0	1.698	81	5.88
	25.0	1.397	72	5.58
	12.5	1.096	52	5.05
control		0.0	0.0	0.0

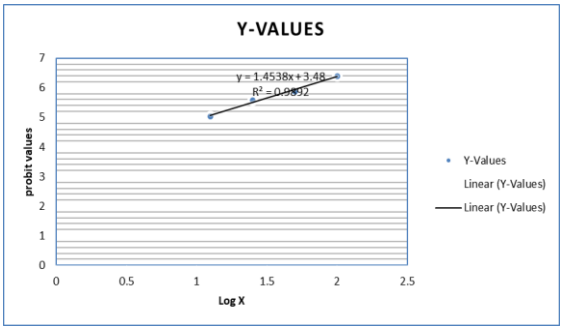

Fig. 4. LCP line of the effect of AgNo3 nanoparticles with extract of Ginger plant against Root-knot nematode (Slope= 1.45)

Table 5. Results of Bioassay of the efficiency of AgNo3 nanoparticle with AgNo3 nano particles with extract of Jojoba plant against Root-knot nematodes

AgNo3 nanoparticle with Jojoba extract	Concentration (X)	Log (X)	Aveg. corrected Mortality	Probit
	100.00	2.000	95.84	6.73
	50.0	1.698	89.41	6.26
	25.0	1.397	69.00	5.50
	12.5	1.096	59.55	5.24
control		0.0	0.0	0.0

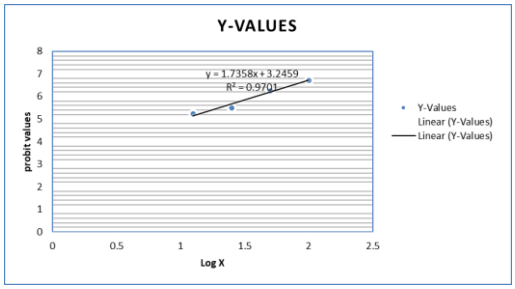

Fig. 5. LCP line of the effect of AgNo3 nanoparticles with extract of Jojoba plant against Root-knot nematode (Slope=1.74)

Table 6. Results of Bioassay of the efficiency of AgNo3 nanoparticle prepared chemically against Root-knot nematodes

	Concentration (X) UL (3mlmol)/ 5 ml	Log (X)	Aveg.corrected Mortality	Probit
AgNo3 nano particle with Chemical prpared	100.0	2.000	82.0	5.92
	50.0	1.698	72.0	5.58
	25.0	1.397	56.18	5.15
	12.5	1.096	40.80	4.76
	control			

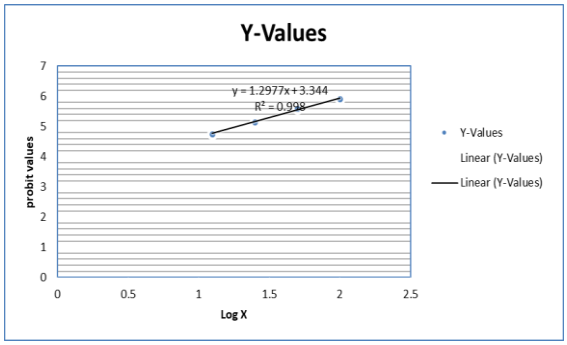

Fig. 6. LCP line of the effect of of AgNo3 nanoparticle prepared chemically against Root-knot nematodes Slope=1.28

Table 7. Results of Bioassay of the efficiency of chitosan nanoparticle prepared chemically against Root-knot nematodes

	Concentration (X) UL(1ppm) mlmol)/5 ml	Log (X)	Aveg. corrected Mortality	Probit
Chitosan	100.0	2.17	96.8	6.88
	50.0	2.000	90.42	6.29
	25.0	1.698	82.57	5.93
	12.5	1.397	72.34	5.59
	control			

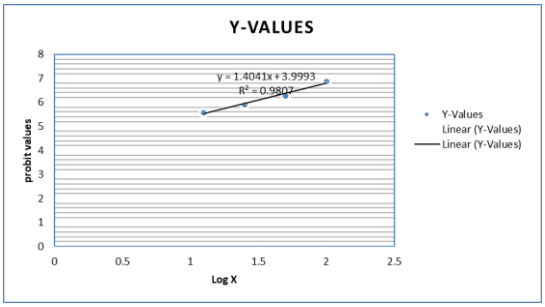

Fig. 7. LCP line of the effect of Chitosan against Root-knot nematode Slope 1.40

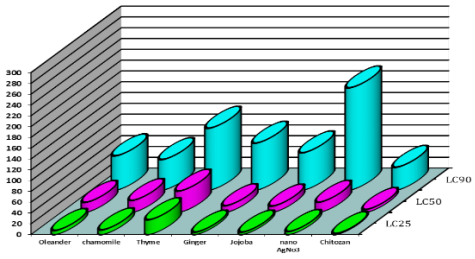

Fig. 8. LC25, LC50 and LC90 of AgNo3 Nano particles with extract of different plants when bio- assayed against Root-knot nematode *M. javanica* calculated with Finney 1971

Table 8. LC25, LC50±se and its fiducial limits and slope values and LC90 of AgNo3 Nano particles with extract of different plants when bio assayed against Root-knot nematode *M. javanica* calculated with Finney 1971

Treatments	LC25	LC50±se	fiducial limits of LC50		LC90	Slope	X^2	Toxicity index%
			Upper	lower				
Oleander	7.24	15.31±0.4	21.49	11.5	63.99	2.06	0.29	33.7
Chamomile	8.36	18.72 ±0.56	33.49	16.18	87.23	1.92	0.99	27.6
Thyme	21.29	37.71 ±0.71	54.76	22.6	112.41	2.69	2.6	13.7
Ginger	3.84	11.11 ±35	16.5	8.2	84.33	1.45	0.22	46.4
Jojoba	4.21	10.24 ±33	15.3	7.6	55.97	1.74	1.46	50.4
AgNo3 nano particle	5.75	18.88 ±0.20	46.36	4.83	183.00	1.29	2.12	27.3
Chitosan	1.72	5.16±0.28	19.79	6.35	42.10	1.40	0.51	100

Characterization of Silver Nanoparticles (Visual observations)

UV-Vis spectra analysis was indicated in Figure (9) showing that the curve of the absorbance of the nanoparticles synthesized by oleander behaved in the same trend with ginger whereas the 1st peak was 1.863 (a.u.) and realized by wave length of 450 nm with oleander and as for ginger preparation the 1st peak was 1.902 with the same wave length. The 2nd peak with oleander and ginger recorded 1.93 and 1.889 (a.u.) at the wave length 600.

Chamomile and jojoba behaved similarity in uv-vis spectra analysis whereas the 1st peaks of these AgNP preparations absorbance were at 430 nm., recording 1.596 and 1.087 and the 2nd peak was with wavelength 650 nm., recording 0.914 and 0.676 (a. u.), respectively. AgNP with thyme gave one peak (1.904) of absorption with the wave length 450nm. Agno3 NP prepared chemically recorded one peak of absorption (0.600) at the wave length 200 nm. On the other hand the peak of absorption (0.600) with chitosan also was at wave length 200 nm . Aziz *et al*. (2017) reported that the UV-Vis spectrum shows distinguishable surface plasmonic resonance (SPR) absorption peaks at about 430 nm for the samples containing different amount of silver salt. The SPR peaks indicate the existence of silver nanoparticles. Plasmonic nanoparticles are particles whose electron density can couple with electromagnetic radiation of wavelengths

that are far larger than the particle due to the nature of the dielectric-metal interface between the medium and the particles: unlike in a pure metal where there is a maximum limit on what size wavelength can be effectively coupled based on the material size.

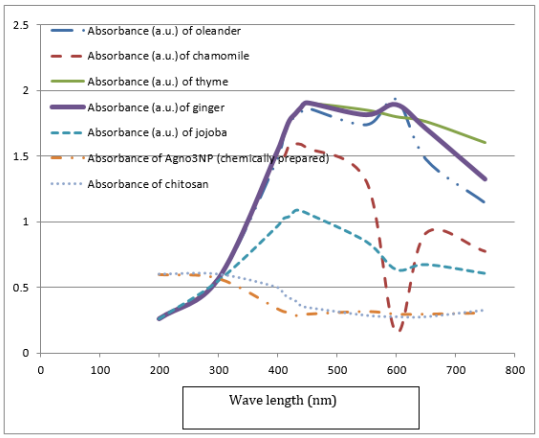

Fig. 9. Chamomile and jojoba uv-vis spectra analysis.

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تأثير الفضة النانوميترية المحضرة نباتياً وكيميائياً بالإضافة إلى الكيتوزان ضد نيماتودا تعقد الجذور *Meloidogyne javanica* فى المختبر

حسن محمد حسن ، أحمد صلاح محمد حسين الروبى ، وفاء صابر عبد الوهاب محمد

قسم وقاية النبات –كلية الزراعة- جامعة المنيا

أوضحت نتائج الإختبار الحيوى فى المختبر أن زيادة تركيز المواد النانوميترية اعطى زيادة فى نسب موت العمر اليرقى الثانى لنيماتودا تعقد الجذور وأن التركيزات المختبرة مع كل المواد هى ١٠٠،٥٠،٢٥،١٢,٠٥ ميكروليتر / ٥٠ مل معلق نيماتودى واعطى التحضير النانوميترى للفضة بواسطة الدفلة معدل تراوح بين ٣٧,٠ ، ٩٣,٢٥ عند تركيزات ١٢,٥ ، ١٠٠ ميكروليتر / ٥٠ مل وكانت قيمة التركيز القاتل ل ٥٠ % هو ١٥,٣١ ميكروليتر/ ٥٠ مل. كذلك فإن تحضير الفضة النانوميترية بواسطة شيح البابونج أعطى أعلى موت ٩١,٣٣ وأقل موت ٣٤,٣٠ عند تركيزى ١٠٠ و١٢,٥ ميكروليتر / ٥٠ مل وأن التركيز القاتل ل ٥٠ % هو ١٨,٧٢ ميكروليتر/ ٥٠ مل. وأعطى مستحضر الفضة النانوميترى بواسطة الزعتر نسب موت تراوحت بين ١٤,٣ و ٩١,٠ % عند أقل وأعلى تركيز مختبر والتركيز القاتل ل ٥٠ % كان ٣٧,٧١ ميكروليتر/٥٠ مل . تراوحت نسب الموت عند أقل وأعلى تركيز لمستحضر الفضة النانوميترى بواسطة الزنجبيل بين ٥٢,٠ ، ٩٢,٠ % وأن التركيز القاتل ل ٥٠% هو ١١,١١ ميكروليتر/ مل. أظهرت نتائج استخدام الجوجوبا لتحضير الفضة النانوميترية تفوقاً ملحوظاً بين المستخلصات النباتية حيث تراوحت نسب الموت عند أقل تركيز وأعلى تركيز بين ٥٩,٥٥ و ٩٥,٨٤ % وكان التركيز القاتل ل٥٠ % هو ١٠,٢٤ ميكروليتر / ٥٠ مل . بإختبار الفضة النانوميترية المحضرة كيميائاً نجد أن نسب الموت ليرقات نيماتودا تعقد الجذور عند أقل تركيز وأعلى تركيز كانتا ٤٠,٨ و ٨٢,٠% وأن قيمة التركيز القاتل ل ٥٠% من كان١٨,٨٨ ميكروليتر / ٥٠ مل.كذلك فقد تم إختبار تأثير الكيتوزان منخفض الوزن الجوزيئى بنفس التركيزات السابقة ١٠٠، ٥٠، ٢٥، ١٢,٥ ميكروليتر/ ٥٠ مل وأوضحت النتائج تفوق هذه المادة تفوقاً ملحوظاً على باقى المواد المختبرة حيث كان نسب الموت المسجلة عند أعلى تركيز وأقل تركيز هى ٩٦,٨ و ٧٢,٣٤ % على التوالى وكان التركيز القاتل ل ٥٠% من النيماتودا ٥,١٦ ميكروليتر / ٥٠ مل وبالتالى فقد تم حساب درجات السمية لمستحضرات الفضة النانوميترية بواسطة الدفلة، شيح البابونج، الزعتر، الزنجبيل والجوجوبا وأيضا الفضة النانوميترية المحضرة كيميائياً بالإضافة إلى الكيتوزان لتكون نسب السمية ٣٣,٧ ، ٢٧,٦ ، ١٣,٧ ، ٤٦,٤ ، ٥٠,٤ ، ٢٧,٣ و ١٠٠ % على التوالى