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The Effectivity Of Silver Nanoparticles Prepared By Jujube *Ziziphus* sp. Extract Against Whitefly *Bemisia tabaci* Nymphs.

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ABSTRACT

The activity and efficacy of silver nanoparticles prepared by jujube leaf aqueous extract to control whitefly in protected culture. Results indicated aqueousr extract of jujube leaves contains natural reduction coating and stabilizer compounds involved in synthesis of silver nanoparticles. Nanoparticles tests were applied to whitefly infested Al-Mustakbal eggplant hybrid grown in a greenhouse at plant protection department/ college of Agricultural Engineering Sciences/ university of Baghdad during Spring growing season in 2017.Seasonal abundance data of white fly nymphs indicated that the insects were shown during the early stages of crop growing when reached an average of 2.3 nymph per 1 cm^2 of leaf. Insect prevalence continued at high densities to the end of growth season. Three concentrations (1000, 2000 and 3000) ppm were applied to tests of silver nanoparticles prepared by jujube relative activity. Results showed silver nanoparticles could decrease population density of whitefly nymphs based on concentration used. It reached 100,100, 100, 90 and 80% highest activity percentages after 1, 3, 7, 12 and 21 days after treatment at 3000 ppm concentration. Whereas the lowest activity percent reached 73.1% 21 days after treatment at 1000 ppm concentration.
Keywords: Nanotechnology, greenhouse, plant extracts, *Ziziphus* sp., whitefly.

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INTRODUCTION

Solanaceous crops are grown worldwide due to their economic important. They and have a high nutrition value. The family Solanaceae includes 2300 species most of them are economic crops including eggplant *Solanium melongena* L. It has been thought that eggplant was originated in South East Asia. In Iraq, eggplant is extensively grown in both protected and open field culture in most provinces. It is attacked by several pests causing economic losses including whitefly, aphids, leaf miners, cotton cutworms and two spot mites (Husni et al., 1976, Doganlar et al., 2002, Kathiar et al., 2018). White fly is one of the most harmful pest and has a wide host range of plants as it attacks about 500 different plant species including vegetables, fruits and ornaments. Its prevalence through the year in both protected and open fields. Whitefly has been reported a s a key pest both in tropical and sub-tropical areas including Asia, Africa, the Mediterranean Basin, Northern and Southern, France, Italy and Turkey. It has been thought that it was originated in India or Pakistan; however it was described for the first time in Greece on tobacco plant (Sengonca, 1975, Gennadius, 1889). Damages caused by whitefly could be through direct feeding on plant sap. Indirect damage occurs through producing honeydew which results in leaf stickiness and fungal growth. Thus, that can affect photosynthesis. Damage may occur through whitefly ability to transmit 128 virus species resulting leaf yellowing and dropping, weaken plants and affecting both production quality and quantity (Greathead, 1986, Brown America, 1994, Jones, 2003, Hogenhout et al., 2008).

Many methods were used to control this insect including chemical pesticide, eco-friendly bio-pesticides (e.g. spinosad), plant extract (e.g.azadirachtin) , microbial pathogens like *Beauveria bassiana* and *Metarhizium Banisopliae*, predators such as *Serangium parcesetosum*, fertilizers and organic nutrints (potassium fertilizer) and other controlling methods (Aboud et al., 2006 Abdel-Razek et al., 2017, AL-Khazraji et al., 2018).

Nano technology is a new revolutionary technology applied to different aspects like analytical chemistry, engineering, biotechnology and agriculture. One of the possible reasons to apply this technique in plant protection is to deliver active ingredient of pesticides to targeted area. Other reasons are coating of pesticide molecules, controlling active ingredient release and improving bio pesticide, properties (Ghormade et al., 2011). Increase the solubility of active ingredients (especially those with low dissolved ability) and the protection of active ingredient against degradation due to temperature, rain and other factors are one of the aims to use Nano-pesticides formulations (Reeves, 2014).

Most country started to follow green agriculture rather than chemical based agriculture. Many published works have referred to the successful use of nanoparticle materials and ecofriendly bio-pesticides against pests such as the use of *Zanthoxylum rhoifolium* leaf oil prepared with nanoparticles and neem oil nano-formulations(Mattosoet et al., 2005, Barik et al., 2008, Carvalho et al., 2012,Arumugam et al.,2015, Christofoli et al., 2015).

Due to the economic losses caused by whitefly and rapid pesticide resistance ability, it is vital to find new methods to control whitefly and decrease its damage. This study was aimed to test the affectivity and efficacy of silver nanoparticles prepared by jujube *Ziziphus* sp. extract to control whitefly in greenhouses.

MATERIALS AND METHODS

Silver nanoparticles preparation

Silver nanoparticles were prepared according to Babu et al. (2014) with slight modifications as follows:

Preparation of jujube *Ziziphus* sp. leaf aqueous extract

Fresh insect and disease free jujube leaves were selected and rinsed with tap water to remove dirt. Further cleaning was performed using deionized water for 30 minutes, then wet leaves were air dried for 2 h. Leaves were chopped into small pieces using sterilized scissors. About 100 mg of leaves was weighted in 1000ml flask then volume was adjusted to 1000 ml with deionized water. The mixture was heated for 10 min at 60 ° C using magnetic hot plate stirrer. Mixture was cooled and filtered using filterer (watmann no 1) and Büchner funnel. The filtrate was collected in proofed flask and incubated in dark condition at 5 °C±1 till to use.

Silver nanoparticles preparation using jujube *Ziziphus* sp. leaf water extract

Water solution of silver nitrate was prepared at 50 mM concentration. To prepare silver nanoparticles, 100 ml of water extracted jujube leaves was added gradually to 900 ml of silver nitrate solution. While adding, the mixture was heated at 65°C and stirred using magnetic hot plate stirrer. Color change of the solution, indicating biological reduction and silver nanoparticle formation, was observed. The mixture was cooled, transferred to a sealed and proofed glass flask and incubated in dark at 5 °C±2 till use. Optical properties of silver nanoparticles, jujube leaf extract and silver nitrate were measured using model Shimadzu UV-VIS spectrophotometer. Crystallite size of silver nanoparticles was determined using X-ray diffraction. Fourier transform infrared spectrometer (FTIR) analysis was carried out to determine the nature of the capping agents in each of these leaf extracts and dried Ag NPs.

Field experiment was applied to Al-Mustakbal eggplant hybrid grown in a 5 mx 36m greenhouse located at plant protection department/ college of Agricultural Engineering Sciences -university of Baghdad-Al-Jadryaa during spring season 2017. Eggplant was cultivated in 10 furrows at 5 terracesandthe spacing between two plants was 50 cm. Agriculture practices were followed according to Al-Nuaimi et al., (1999) to serve the plants. To study seasonal abundance of whitefly nymphs, 10 plant leaves with 3 replicates were collected randomly each week on regular basis. Sampling started since infection seen up to the end of plant growing season. Leaf samples were collected in labeled plastic bags and transferred to the laboratory. Eggplant leaves were examined using light microscope to count nymph number per $1cm^2$ of total leaf area. Randomized Complete Block design (RCBD) was applied to determine the activity percentage of silver nanoparticle to control whitefly *Bemisia tabaci* Greenhouse land was divided into 3 blocks. Each block was divided into 4 experimental unites contained 7 plants each. Three concentrations (1000, 2000 1nd 3000 ppm) of silver nanoparticle prepared by jujube leaf extract were applied using 2 L hand spryer. Three replicates were used for each treatment and water treatment control was used for comparison. Five leaves with 3 replicates were collected from each treatment one day before treatment and 1, 3, 7, 12 and 21 days after treatment. The number of whitefly nymphs per 1 cm^2 of total leaf area was calculated. The relative activity percentage of silver particle was calculated based on Henderson and Tilton (1955) equation as follows:

$$\text{Corrected \%} = \left(1 - \frac{\text{n in Co before treatment} * \text{n in T after treatment}}{\text{n in Co after treatment} * \text{n in T before treatment}}\right) \times 100$$

RESULTS AND DISCUSSION

Silver nanoparticles preparation using jujube *Ziziphus* sp. leaf water extract

When adding water extracted jujube leaves at 65 °C color changing to dark brown were observed. This color indicated biological reduction process occurred and silver nanoparticles were formed from silver ions 15 min after heating at 65 °C. UV spectral analysis of silver nanoparticle scored the highest absorbance value at 413.91 nm wavelength compared to water extracted jujube leaves and silver nitrate that scored the values at 254.40 and 221 nm wavelengths, respectively (figs 1-3). Color change due to biological reduction process occurred because of the presence biochemical molecules in jujube leaves water solution such as alcoholic, phenolic polysaccharides and protein compounds (Lu et al., 2014). Jujube leaves water solution contains natural reduction substances act as biological reductive agents, coat and stabilizer silver nanoparticles, which protect nanoparticles against aggregation and accumulation and keep nanoparticle properties (Lu et al., 2014, Ibrahim, 2015).

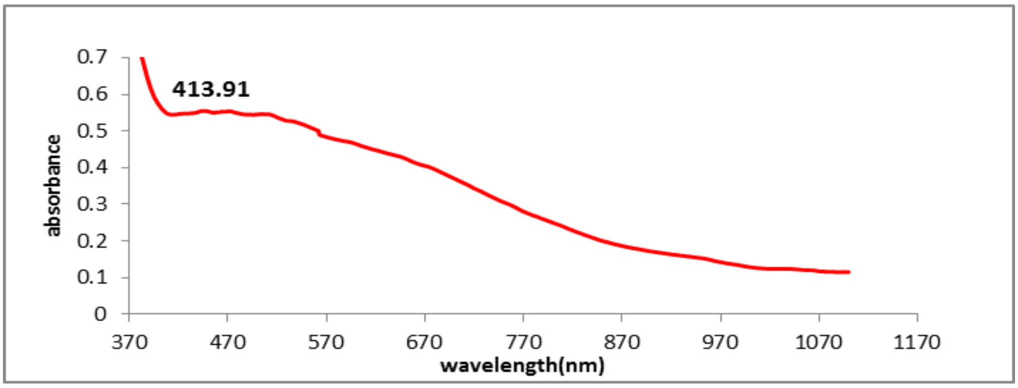

Fig 1: ultraviolet absorbance values of silver nanoparticles produced by jujube *Ziziphus* sp. leaf water solution

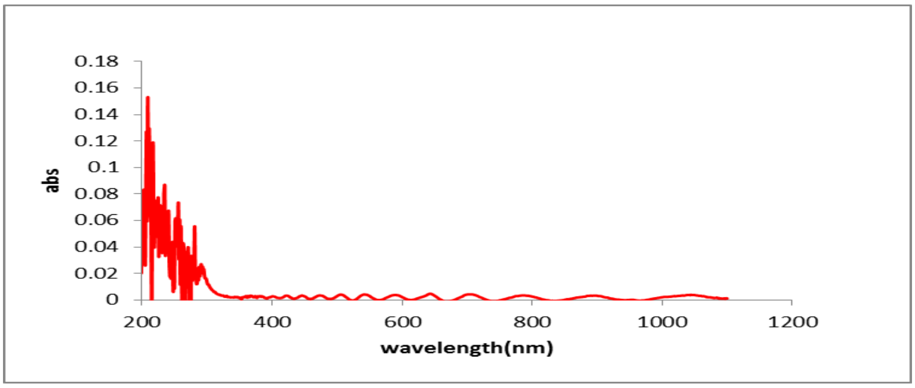

Fig 2: Ultraviolet absorbance values of jujube *Ziziphus* sp. leaf water solution

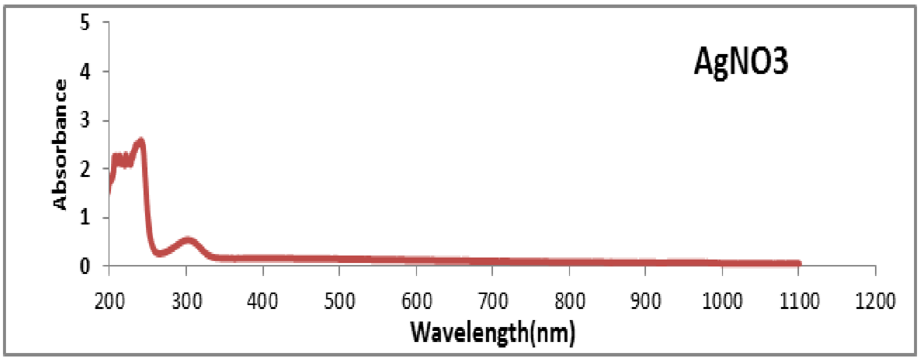

Fig 3: Ultraviolet absorbance values of silver nitrate

Infra-Red (IR) spectroscopy of jujube *Ziziphus sp* leafextract and silver nanoparticles

Infra-Red (FTIR) spectroscopy of jujube *Ziziphus* sp. leaf water extract (Fig 4) showed high content of active groups based on absorbency value of silver nanoparticles at different wavelengths. Absorbency at (3728.40-3888.49) indicates presence of alcoholic, phenolic and carbohydrate groups. Wavelength at3327.21 indicates presence of amino group, whereas it indicates presence of alkane C-H group. Further O=O=C groups are present at 2258.64 – 2360.87nm wavelength. In addition, anhydrate groups were recorded at 1772.58 nm wavelength. High active group content may have the major role of silver nanoparticles coating, fixation and reduction. Infrared spectroscopy of silver nanoparticle powder prepared by jujube leaf water extract solution (Fig 5) shows carboxyl, anhydrite, aldehyde, phenolic, amide and alcohol groups were the most active groups that involved in biological reduction, coating and fixation of silver nanoparticle prepared. This was clearly shown in absorbency values at (2389.80-2310.), (1801.51-1755.22), (2829.57) and (3280.92-3894.28), respectively. It refers to the importance these groups to reduce silver ions to form silver nanoparticles in addition to coating and fixation.

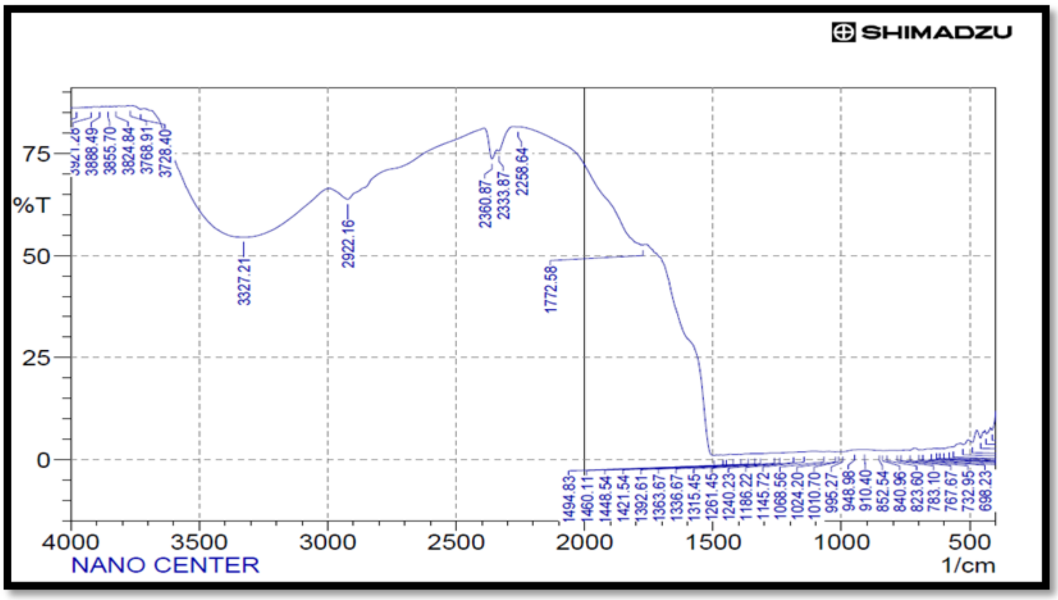

Fig 4: Infrared spectroscopy of jujube *Ziziphus sp* leaf water solution

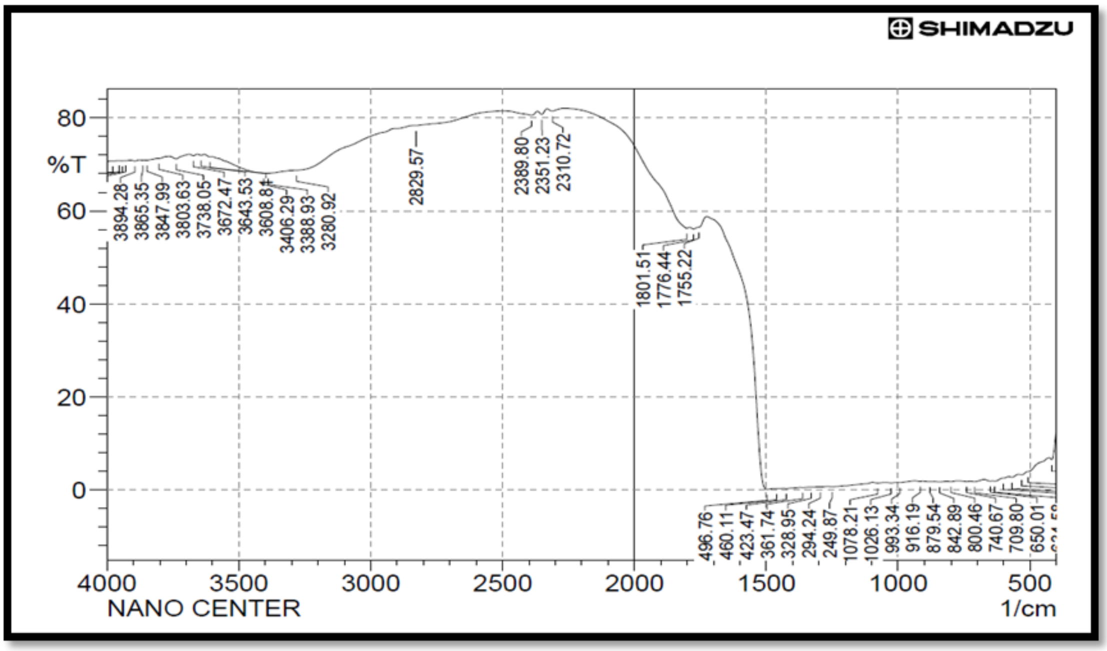

Fig 5: Infrared spectroscopy of silver nanoparticle prepared

Determination of crystallite size of silver nanoparticles using X-ray diffraction (XRD)

XRD examination (fig 6) showed high peak at 111 surface and 32º angle. In addition, further peaks were shown at 200, 220 and 311 and angles 38º, 65º and 77º, respectively. Crystallite size of silver nanoparticles scored an average of 44.67 nm indicating the nature of silver nanoparticle prepared by jujube leaf water solution.

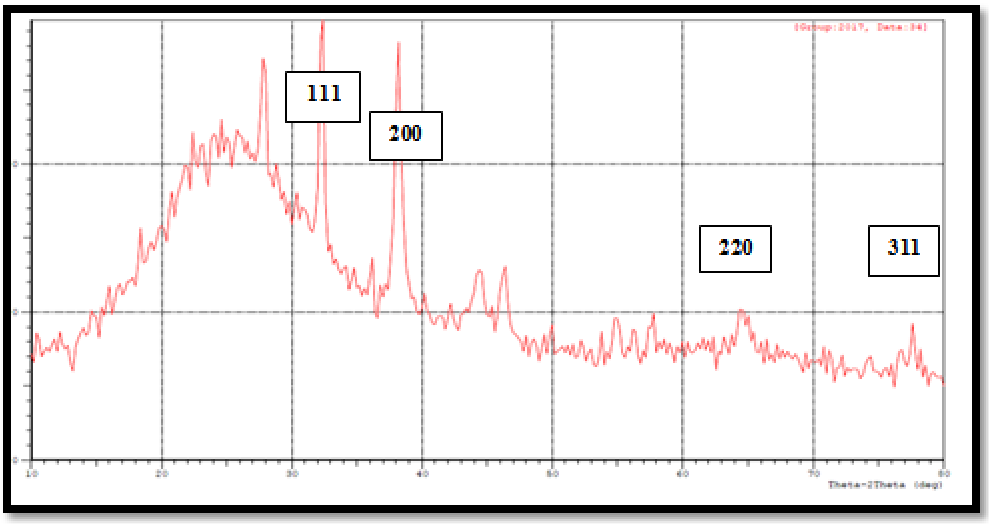

Fig 6: X-ray diffraction of silver nanoparticles prepared by jujube leaf water solution

Seasonal abundance of whitefly nymphs on eggplant crop

Population density of whitefly nymphs on eggplant was low in the beginning of plant growth stage (Fig 7). It reached 2.3 nymph/ 1 cm^2 plant leaf, gradually increased then decreased to 1.6 nymph/ 1 cm2 plant leaf in the 7th of May 2017. This could be resulted due to the activity of natural enemies, temperature and relative humidity effects in the growing area during that period. Then, nymph numbers increased to reach the highest population density in the 11th of June, 2017 which was 10.3nymph/ 1 cm^2 eggplant leaf. It started decreasing at the latest plant growth stages, especially at leaf yellowing and drying stage to score the lowest nymph population density in the 30th of July, 2017 with 0.4 nymph/ 1 cm^2 plant leaf. Similar results were obtained by MohdRasdi et al., (2009) who indicated that whitefly infestation on eggplant grown during January to May, 2003 started at the beginning of crop planting with low population densities. The highest population density was 50 nymphs per 6 leaves of a single plant during the 7th week after planting when fruits stated forming. Then it started decreasing gradually during the last growth stage of the crop. It was concluded the decreasing of population density of the insect is attributed to the decreasing of nutrition contents in plant leaves and they become not suitable for laying eggs. Dahatonde et al., (2014) revealed whitefly population density abounded during the 3rd week of eggplant cultivation which scored 7.27 whitefly/ 3 leaves for a single plant. Whereas, the lowest population density was 1.73 whitefly/ 3 leaves at the end of plant growing season which was in the 19th week of planting.

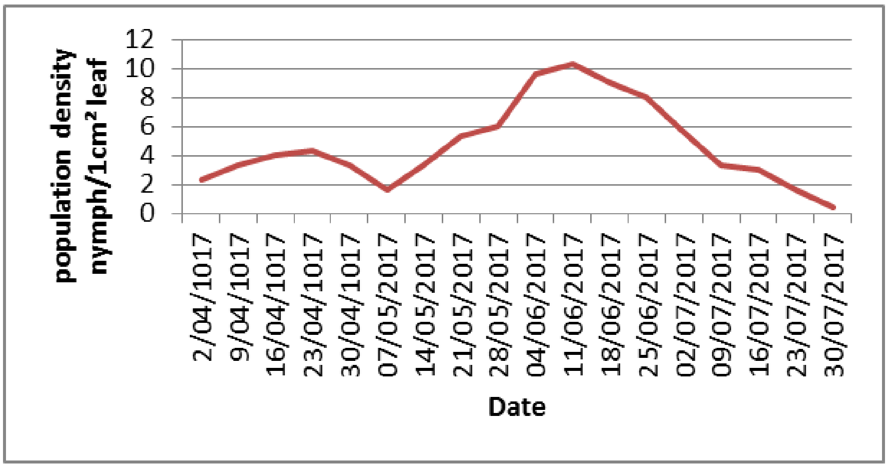

Fig 7: population density of the whitefly nymphs on eggplant

The relative activity of silver nanoparticles prepared by jujube *Ziziphus sp* leaf extract against whitefly *Bemisiatabaci*nymphs

Results presented in table (1) show silver nanoparticles prepared had a highly effective activity at all concentrations when decreased population density of whitefly nymphs throughout different period times. The 2000 and 3000 ppm concentrations scored relative activity up to 100%, whilst the relative activity of the 1000 ppm concentration was 94.4% three days after treatment. The lowest relative activity was 21 days after treatment which reached 73.1, 78.7 and 80% at concentration 1000, 2000 and 3000 ppm, respectively. It is concluded that nanotechnology is highly effective to control insect and thus, can be included with integrated pest management. Similarly, Christofoli et al., (2015) referred to the affectivity of *Zanthoxylum rhoifolium* leaf oil extraction and oil extraction prepared with nanoparticles on whitefly biology when results showed decreasing in eggs and nymphs ratio up to 95%. Results presented by Khooshe-Bast et al., (2016) confirmed that *Trialeurodes vaporariorum* adults treated with oxide nanoparticles (ZnO NPs) caused 73.3 and 81.6% mortality at 15 and 20 mg/L^{-1}concentrations, 24 h after treatment under greenhouse conditions. According to Rouhani et al. (2012) the reason of effect that nanoparticles have the ability to interact with insect bio activities as they can penetrate plasma membrane and break many of biomolecules such as enzymes. In addition they cause Coagulation of proteins and plasma membrane to lose its function then cell death. Naresh et al. (2012) indicated the effect of silver Nano-particles is due to particles ability to penetrate the cuticle and cells then interact with physiological processes including molting.

Table 1: Relative activity of silver nanoparticles prepared by jujube leaf extract against whitefly nymphs

concentration	Relative activity%					Average
	After 1 days	After 3 days	After 7 days	After 12 days	After 21 days	
1000ppm	100.0	94.4	84.2	80.0	73.1	86.4
2000ppm	100.0	100.0	88.9	86.5	78.7	90.8
3000ppm	100.0	100.0	100.0	90.0	80.0	94.0
average	100.0	98.1	91.0	85.5	77.3	90.4

L.S.D for concentrations: 9.32
L.S.D for treatment time: 10.27
L.S.D for concentrations X treatment time: 17.56

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