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YIELD, QUALITY AND LEAVES ANATOMY STRUCTURE OF SPRING ONION SPRAYED BY NANOCOMPOSITE TO CONTROL *THRIPS TABACI*

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Abstract

Onion (*Allium cepa* L.) is considered as one of the important crops for exportation and local market in Egypt. Onion thrips, *Thrips tabaci* (L.), is an important insect pest onion that induces damages on onion by feeding on leaves and decrease its quality and ability for exportation. The present study was performed in an open field at the Experimental Station of Faculty of Agriculture, Cairo University during two winter seasons of 2015/2016 and 2016/2017 to examine the effect of copper chlorophylline and its nano compsites as alternative sources of pesticides on reduction in population of *T.tabaci*, plant growth, chlorophyll content ,total soluble solids, marketable yield and leaves anatomy structure of spring onion. Copper chlorophyllin (Cu-chl) and its two nanocompsites (silver and graphin) with two concentrations (10^{-3} and 10^{-4}ml/L) were used against *T.tabaci* in the open onion field. A recommended rate of malathion (1ml/L) was used as insecticidal reference. The control plnts was sprayed with water. The results showed that the highest concentration of all treatments induced the highest reduction % relative to control. The foliar spray with nano silver at 10^{-3} concentration had the highest value of chlorophyll content, marketable yield, plaside, calcium and magnisum content. Total soluble solids were increased with the highest concentration in all treatments. The spony cell became larger and succulent in malathione. It could be concluded that the highest concentration (10^{-3} ml/L) of the copper chlorophyllin and two its nanocompsites (Silver and Graphen) can be recommended in controlling the onion thrips, Thrips tabaci.

Key words : Growth, marketable yield, copper cholrophylline, nanocompsites, Thrips reduction and minerals content.

Introduction

Onion (*Allium cepa* L.) is considered a well-known traditional medicinal plant that has been used for its putative nutritional and health benefits for centuries. It is highly consumable world wide vegetable. Potentially, it is an essential chemo-preventive food item that enhance dietary health and decrease cancer risks. Onions have potential against inflammatory, cholesterol, cancer and antioxidant properties because it contains phenolics and flavonoids (Ravi, 2016). Onion thrips, *Thrips tabaci* Lindeman (1889) (Thysanoptera: Thripidae) is an important insect pest onion worldwide (Diaz-Montano *et al*., 2011 and Gill *et al*., 2015). *Thrips tabaci* induces damages on onion by feeding on leaves which lead to small and less-valuable bulbs (Fournier *et al*., 1995). Thrips also transfers economically virus plant disease to onion that causes Iris yellow spot (Gent *et al*., 2006) and bacterial center rot (Dutta *et al*., 2014), while its feeding injury can increase expansion and severity of a fungal pathogen that causes purple blotch (McKenzie *et al*., 1993). In addition, new information has been created to enhance *T. tabaci* control in onion through applying insecticides depend on action thresholds for maximum efficacy and efficiency (Nault and Shelton, 2010), following specific sequences of insecticide products used during the season (Byrne and Szendrei, 2013; Nault *et al*., 2014 and Reitz, 2014) and using surfactants coapplied with insecticides (Nault *et al*., 2013). Furthermore, *T.*

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tabaci is a well known for developing resistance to insecticides and intensive insecticide use will likely result in resistance to one or various modes of action that would lead to decrease control (Shelton *et al*., 2003 and 2006).

Photosensitizing is a tool has ability to manage several various of insects by activate sunlight or artificial light (Ben Amor and Jori, 2000 and Moreno *et al*., 2001). This concept is based on photodynamic reactions. These are oxygen-dependent photosensitized reactions in which a substrate (here, a biomolecule) is destroyed only in the presence of a chromophoric molecule called a photosensitizer that absorbs light (Kohen *et al*., 1995). Photo insecticides have been successfully used in agriculture to control insect pests, such as *Liriomyza bryoniae* (Luksiene *et al*., 2007), flesh fly (Abdelsalam *et al*., 2014) and *Ceratitis capitates* (Berni *et al*., 2009).

Nanocomposites are inducing concern from the scientific community due to their outstanding activity in relation to bulk materials (Naddeo *et al*., 2015). This dignigied activity can be attributed to the extra ratio surface-volume area and unique physico-chemical, mechanical and electronic properties of nanoparticles (Somasundaran *et al*., 2010). Nanotechnology is a promising field of interdisciplinary research. It opens up a expanded array of opportunities in various fields like insecticides, pharmaceuticals, electronic and agriculture. The management of insect pests through the formulations of nanomaterials-based insecticides is included. Traditional strategies like integrated pest management used in agriculture are insufficient and application of chemical pesticides have adverse effects on animals and human beings apart from the decrease in soil fertility. Therefore, nanotechnology would provide green and efficient alternatives for the control of insect pests in agriculture without harming the nature (Dimetr and Hussein, 2016). The improvement in nanotechnology have permit its application to agriculture and the food industry and particularly in agriculture are becoming popular (Ruttkay Nedecky *et al*., 2017). On the other hand, its utility is still bounded because of shortage of information about the toxicity and environmental fate of nanomaterials (Narayanan *et al*., 2012 and RuttkayNedecky *et al*., 2017), also the little interest of uses of nanotechnology in plant sciences compared to nanomedicine and nano pharmacology (Wang *et al*., 2016).

The aim of the current study was to evaluate the efficiency of copper chlorophylline and nano copper (silver and graphiene) as alternative for controlling *T. tabaci*, as well as their effects on plant growth, yield, and quality of spring onion.

Materials and Methods

Study site

This study was conducted at the Experimental Station of the Faculty of Agricultural, Cairo University, Giza, Egypt (30°01'32.5"N & 31°11'33.0"E) during two successive winter seasons of 2015/2016 and 2016/2017.

Preparation of the nanocomposite photosensitizer

Copper chlorophyllin (Cu-chl) and two nanocompsites were used. Both silver and graphen nanoparticles were selected to be used in the formation of the natural extract porphyrin-based photosensitizer nanocomposite. Electrostatic deposition method was used for grafting the copper photosensitizers over the two nanoparticles (silver and graphine) to form the required nanocompsites:

Copper chlorophylline /Silver (Cu-Chl/ Ag) nanocomposite and Copper chlorophylline /grapheme (Cu-Chl/ GO) nanocomposite.

Two concentrations of each nanopesticide (10^{-3} and 10^{-4} ml/L) were prepared using water for dilution from stock solutions. The concentrations of stock solutions were determined using the inductively coupled plasma mass spectrometry.

Characterization of the nanocompsites

The size, morphology and composition of the two tested nanocomposites were determined by high resolution transmission electron micrograph (JEOL 20100) (HR-TEM) and x-ray diffraction (XRD).

Experiment layout

Onion cv. Giza 6 purchased from Agricultural Research Center, Giza, Egyptwas chosen for this study. Onion seeds were sown in nursery on the 1st October 2015 in first season and 7th October 2016 in the second season. The mixture of peatmoss: vermiculite (1:1v) were used for seedling production. The seedlings were transplanted to the open field on 20 and 27 November in the first and second season, respectively. Drip irrigation was used, the recommened N, P and K (75 Kg N, 66 Kg P_2O_5 and 50 Kg K) were added according to the recommendation of Egyptiane Ministry of Agriculture, and the other agricultural practices were done similarly as practiced except insecticide application. The experimental plot area was 9.6m^2 (8m length and 1.20 m width, each plot contains two rows. Seedlings were planted at each sides of drip irrigation line. The number of treatments were 8 with three replicates (24 plots). The experimental design were arranged in randomized complete block design with three replicates.

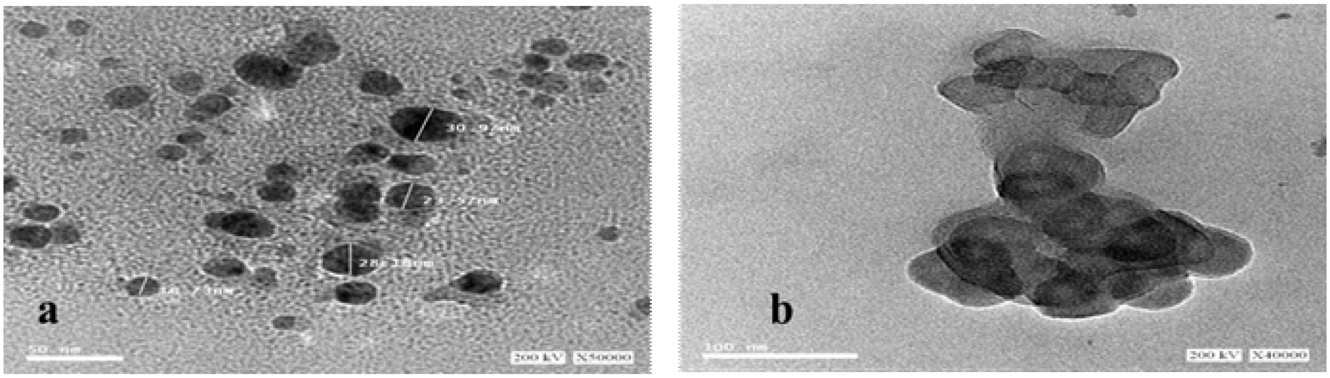

Fig. 1 : TEM image of a) Cu-chl/Ag b) Cu-chl/Go.

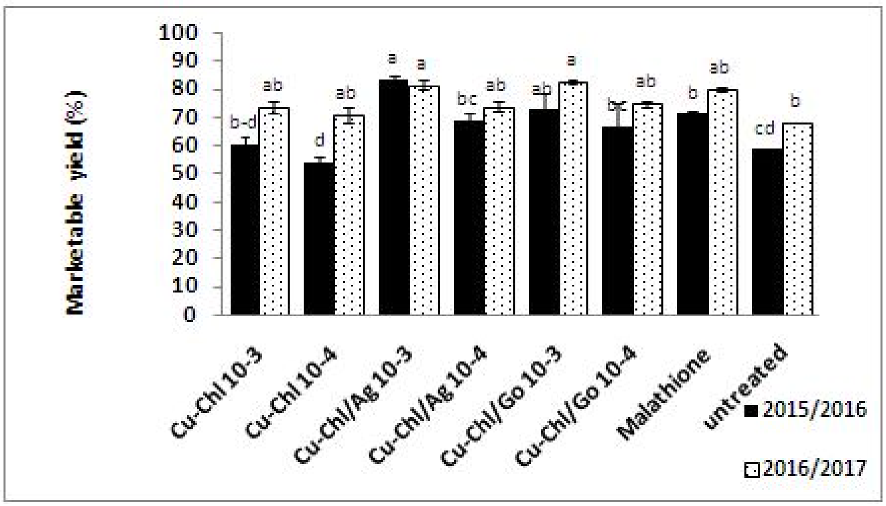

Fig. 2 : Marketable yield of onion sprayed with photosynthetizer and nano copper compounds in 2015/2016 and 2016/2017.

Treatments

Copper chlorophyllin (Cu-chl) and two nanocompsites (Ag and Go) with two concentrations (10^{-3} and 10^{-4} ml/L) were used against *T. tabaci* in the open field. A recommended rate of malathion (1ml/L) was used as insecticidal reference. The control treatment was sprayed with water. The onion plants in the open field were sprayed two times for each season. The first spraying was on the 15th day post transplanting. The second spraying was on the 2nd week post the 1st spraying.

Data recorded

Reduction percentage in population of *T. tabaci*

The numphs and adults of thrips were counted together before spraying and 1st, 3rd and 5th days post application in the first season, and after the 1st and 3rd day after spraying in the second season and calculate the reduction percentage by using Henderson and Tilton equation formula (Henderson and Tillton, 1955). The samples included ten plants which were randomly selected from each plot.

Vegetative growth, chlorophyll content and total soluble solids

During the harvest stage (50 days after sowing date), 15 randomly plants were taken from each plot to measure plant length and number of leaves per plant. Leaf cholorophyll content were determined according to Nornai (1982). In brifly disks (2.5 cm^2 area) were taken and ground with 10 cm^3 of N, N – dimethyl formamide and stored for 48 h at 5ºC under dark condition. The absorbance of the supernatant was measured at 470, 647 and 663 µm by using sperctophotometer (Model-Beckman Du 7400) and chlorophyll was calculated as mg/100g FW. Total soluble solid (T.S.S) was determined by using Atago, hand refractometer N1 (Japan).

Marketable yield %

All the plants from each plot were harvested at harvest time to calculate the marketable yield % for each treatment. The plants were sorted into healthy and infected. The healthy plants are not shown to have any silver plotches. The marketable yield was calculated by dividing the number of healthy plants/ total plants number in each treatment.

Leaves anatomy structure

Antomy structure was determined at Research Laboratories Center at Faculty of Agriculture Cairo University. Onion leaves specimens were fixed in F.A.A. (10 ml formalin, 5 ml glacial acetic acid, 35 ml distilled water and 50 ml ethyl alcholol 95%) for at least 48 h. Then selected materials were washed in 50% ethyl alcohol dehydrated in anormal butyl alcohol series, embedded in paraffin wax of melting point 56°C, sectioned to a thickness of 20 microns, double stained with safranin and fast green, cleared in xylem and mounted in Canada balsam (Nassar and Sahhar, 1998) slides were analysed microscopically and photomicrograhed (LEICA ICC50 HD).

Mineral content

Mineral content of Zinc (Zn), Magnesium (Mg), Cupper (Cu) and Calcium (Ca) were measured in plant leaves in the lab of National Research Centre in Dokki, Giza, Egypt. The wet digestion of 0.2 g plant material with sulphuric and percholoric acids was carried out by adding concentrated sulphuric acid (5 ml) to the samples and the mixture was heated for 10 min then 0.5 ml percholoric acid was added and heating continued till a clear solution was obtained. The digested solution was quantitatively transferred to a 100 ml volumetric flask using deionized water as reported by Piper (1950). Concentration of Ca, Mg, Zn and Ca in plant samples were determined using atomic absorption spectrophotometer with air-acetylene and fuel (pye Unicam, model SP-1900, US).

Statistical analysis

Data were statistically analysed using analysis of varience (ANOVA), with the means separated using Duncan Multiple Range crierion ($P \leq 0.05$) between all treatment by using Mstat-c program (2.1 version). Reduction percentage in thrips infestation corresponding to each treatment were calculated using means of alive insects for each treatment comparing with those alive in untreated control group by equation of Henderson and Tillton (1955).

Results and Discussion

Data in fig. 1 showed the formation of a chlorophyllin coating over the silver nanoparticles and graph in sheet forming the composite Cu-Chl/ Ag and Cu-Chl/ Go nanocomposite.

Reduction in population of *Thrips tabaci*

Table 1 shows the average reduction percentages in the population of onion thrips *Thrips tabaci* that sprayed two times with copper photosynthetizers (Cu-chl) and nanocomposites (Cu-chl/Ag and Cu-chl/Go) in open field during the two successive seasons 2015/2016 and 2016/ 2017. A significant decline in the population of thrips was observed at 1, 3 and 5 days post spraying with 10^{-3} and 10^{-4} of Cu-chl, Cu-chl /Go and Cu-chl/Ag. All treatments significantly reduced the population of thrips compared to the control during all investigated days. The reduction percentages were gradually increased with the increasing of concentration in all compounds. In the first season, after the first day of spray, the high concentration (10^{-3}) of Cu-chl/Ag was the most effective achieving 78.8% reduction in the population of thrips than the high concentration of Cu-chl alone (70.6%) or Cu-chl/Go (71.55%). On the third day post spray malathione gave an extending effect on the reduction population compared with the high concentration of Cu-chl/Ag. The calculated reduction percentage revealed that malathione exhibited reduction 91.41% higher than Cu-chl/Ag10^{-3} (83.94%). Five day post spray, the high concentration of copper chlorophylline recorded the highest reduction population (85.56%).

In the second season: on the first day post spray, high concentration of Cu-chl, Cu-chl/Ag, Cu-chl/Go and malathion exhibited approximately the same level of reduction (65.11% ,66.82, 66.23 and 66.70) respectively. On the third day post spray the high concentration (10^{-3}) of Cu-chl/Ag achieved the highest reduction (85.20%) than all other treatments.

Table 2 shows average reduction percentages in the population for each spray of onion thrips *T. tabaci* that sprayed with copper photosynthetizers (Cu-chl) and nanocomposites (Cu-chl/Ag and Cu-chl/Go) in open field during the two successive seasons of 2015/2016 and 2016/2017. In the first season in the first spray the average reduction percentage revealed that the high concentration (10^{-3}) of Cu-chl/Ag, Cu-chl, resulted 84.81% and 70.9% reduction in population as well as both concentrations of Cu-chl/Go and malathione had the same level of reduction in the insect population (76.11%, 69.87%, 83.72%, respectively). In the second spray, the foliar spray with high concentration of Cu-chl, Cu-chl/Ag, Cu-chl/Go and malathione achieved high reduction population percentages (78.66, 71.10, 69.30 and 82.66), respectively. While, in the first spray of the second season, all tested concentrations of nano copper induced significant increase in reduction population compared to both concentrations of copper chlorophylline. The spray with Cu-chl/Ag, Cu-chl/Go with their two concentrations and malathione gave the highest reduction in insect population percentage (83.8%, 61.9%, 77.58%, 59.3%, and 73.47%). While two concentrations of the copper

Table 1 : The average reduction percentages of onion thrips Thrips tabaci sprayed two times with photosynthetizers and nano copper compounds in open field during the two successive seasons 2015/2016 and 2016/2017.

Days post spray						
2015/2016		2016/2017				Treatment
3rd	1st	5th	3rd	1st	Rate	
66.52±0.77 b	65.11±2.09 a	85.56±2.75 a	64.58±2.17 d	70.65±0.45 b	10^{-3}	Cu-Chl
62.00±2.33 bc	39.50±3.75 c	65.65±0.27 c	59.01±0.844e	55.15±2.26 d	10^{-4}	Cu-Chl
85.20±0.14a	66.83±1.21a	70.42±0.61b	83.94±2.54b	78.89±1.11a	10^{-3}	Cu-Chl/Ag
61.23±1.22bc	48.30±1.69b	47.21±0.20d	30.95±2.77g	64.48±2.64c	10^{-4}	Cu-Chl/Ag
54.30±2.97c	66.23±3.62a	71.89±3.44b	74.65±0.82c	71.55±1.86 b	10^{-3}	Cu-Chl/Go
59.95±5.16bc	50.57±1.05b	61.05±0.51c	50.33±0.85f	41.48±0.55e	10^{-4}	Cu-Chl/Go
66.03±2.53b	66.70±2.44a	74.75±1.28b	91.41±0.51a	82.91±1.02a	1cm/L	Malathione
7.92	**7.22**	**4.73**	**5.28**	**5.16**		**L.S.D(0.05)**

Table 2 : The average reduction percentages of onion thrips *Thrips tabaci* for each spraying with photosynthetizers and nano copper compounds in open field during the two successive seasons 2015/2016 and 2016/2017.

2016/2017		2015/2016		Rate	Treatment
2nd spray	1st spray	2nd spray	1st spray		
76.05±6.88 a	55.98±7.92 bc	78.66±3.14 a	70.99±8.44 ab	10^{-3}	Cu-Chl
52.88±7.17 bc	49.12±8.06 c	60.39±5.22 ab	58.72±11.02 b	10^{-4}	Cu-Chl
67.78±1.53 ab	83.8±9.3 a	71.10±7.15 a	84.81±4.60 a	10^{-3}	Cu-Chl/Ag
47.598.21 bc	61.9±0.74 abc	38.85±11.40 bc	55.31±8.48 b	10^{-4}	Cu-Chl/Ag
43.03±5.95 c	77.58±12.88 ab	69.30±4.27 a	76.11±3.92 ab	10^{-3}	Cu-Chl/Go
51.21±7.32 bc	59.3±1.90 abc	32.04±7.51 c	69.87±3.84 ab	10^{-4}	Cu-Chl/Go
60.08±3.66 abc	73.47±2.03 abc	82.66±10.34 a	83.72±9.90 a	1cm/L	Malathione
18.64	**24.19**	**21.83**	**20.17**		**L.S.D(0.05)**

Table 3 : Plant length (cm) and number of leaves of sprayed onion with photosynthetizers and nano copper compounds in 2015/2016 and 2016/2017.

Treatment	Rate	Plant length (cm)		Number of Leaves	
		2015/2016	2016/2017	2015/2016	2016/2017
Cu-Chl	10^{-3}	48.87±0.59 a	45.53±0.39 ab	4.80±0.11 ab	4.20±0 ab
Cu-Chl	10^{-4}	42.40±1.19 b	44.40±1.47 ab	4.66±0.06 ab	4.23±0.0 ab
Cu-Chl/Ag	10^{-3}	47.63±0.23a	46.43±1.10 a	4.80±0.11ab	4.40±0.15 a
Cu-Chl/Ag	10^{-4}	45.23±0.49ab	43.10±0.58 b	4.63±0.08ab	4.00±0bc
Cu-Chl/Go	10^{-3}	45.03±0.03ab	44.53±0.29ab	4.86±0.13 a	4.460.06± a
Cu-Chl/Go	10^{-4}	44.17±1.73ab	42.30±0.57b	4.73±0.13ab	4.13±0.13abc
Malathione	1cm/L	46.97±2.75ab	45.27±0.50ab	4.60±0.11ab	4.40±0.2 a
Untreated		46.50±0.97ab	44.10±0.78ab	4.46±0.06 b	3.83±0.16b
L.S.D(0.05)		**4.20**	**2.97**	**0.32**	**0.34**

chlorophylline had the lowest reduction in insect population. In the second spray of the second season the high concentration of Cu-chl, Cu-chl/Ag and Malathione exhibited the same level of reduction in insect.

These results were in agreement with Rouhani *et al*. (2012), who found various toxicity mechanism of Ag on *Callosobruchus maculate*, such mechanism of reactive oxygen types, oxidative stress, membrane disruption, protein unfolding and or inflammation. Furthermore the Oocytes of *C. parvum*, which treated with Ag and Cu nanoparticles became inactive and this materials changed its wall viability (Saad *et al*., 2015). Exposure to high lethal concentration (20 mg L^{-1}) of silver nanoparticles induced heat shock and also generation of free oxygen radical in the larva of *Drosphila melongosta*; this oxidative stress subsequently result in DNA damage and ultimately apoptosis which lead to

Table 4 : Chlorophyll content (mg/100g f.w) and Soluble Solids content (prix) of onion sprayed with photosynthetizers and nano copper compounds in 2015/2016 and 2016/2017.

Treatment	Rate	Chlorophyll (mg/100g f.w)		Soluble solids content (prix)	
		2015/2016	2016/2017	2015/2016	2016/2017
Cu-Chl	**10^{-3}**	12.20±1.02 c	11.83±0.6 c	8.16±0.20 ab	8.30±0.20 a
Cu-Chl	**10^{-4}**	12.11±1.47 c	11.59±1.31 c	7.84±0.18 bcd	7.50 ±0.05 bc
Cu-Chl/Ag	**10^{-3}**	30.74±1.11 a	31.87±1.25 a	8.83±0.26 a	7.86±0.36ab
Cu-Chl/Ag	**10^{-4}**	18.52±1.40 b	20.08±0.199 b	7.48±0.17bc	7.53±0.13bc
Cu-Chl/Go	**10^{-3}**	32.42±0.87 a	30.43±0.41 a	8.06±0.43bc	7.86±0.43ab
Cu-Chl/Go	**10^{-4}**	18.59±1.79 b	20.06±1.33 b	7.15±0.15 de	7.53±0.15bc
Malathione	**1cm/L**	8.89±0.02cd	11.47±0.49 c	7.38±0.10cde	7.36±0.130.12bc
Untreated		7.68±0.75 d	11.20±0.67 c	6.72±0.04e	7.23 ±0.12c
L.S.D (0.05)		**3.78**	**2.74**	**0.70**	**0.55**

Table 5 : Anatomical structure of leaves onion sprayed with photosynthetizers and nano copper compounds.

Spongyìm	Pallisade (μm)	Epidermis (μm)	Rate	Treatment
115.0± b	92.22±9.72 c	30.21± bc	**10^{-3}**	**Cu-Chl**
122.1± b	133.6±13.5abc	25.11± bc	**10^{-4}**	**Cu-Chl**
124.0±13.9 b	177.1 ±24.83a	34.85±1.49ab	**10^{-3}**	**Cu-Chl/Ag**
102.7±13.69 b	122.8±19.98bc	35.40±1.53ab	**10^{-4}**	**Cu-Chl/Ag**
110.9±7.93 b	120.4±22.78bc	23.71±23.7 c	**10^{-3}**	**Cu-Chl/Go**
136.7±20.15 b	98.91±6bc	26.80 ±2.03bc	**10^{-4}**	**Cu-Chl/Go**
303.5±117.1 a	151.2±11.14ab	40.53±6.74 a	**1cm/L**	**Malathione**
157.5±26.48 b	112.0±11.65bc	42.92±1.90 a		**untreated**
135.2	**49.56**	**9.40**		**L.S.D at (0.05)**

Table 6 : Anatomical structure of leaves onion sprayed with photosynthetizers and nano copper compounds.

Vascular width (μm)	Vascular lengh (μm)	Xylem (μm)	Rate	Treatment
86.96 ±4.75ab	140.2±33.66	30.98±2.65 ab	**10^{-3}**	**Cu-Chl**
72.51±6.52 ab	161.6± 45.67	29.98± 9.24ab	**10^{-4}**	**Cu-Chl**
72.13 ±22.53ab	159.9±89.87	18.48 ±5.53ab	**10^{-3}**	**Cu-Chl/Ag**
55.87±10.03 b	106.8±13.95	13.34±2.74 b	**10^{-4}**	**Cu-Chl/Ag**
73.99±13.21 ab	133.5± 42.44	25.58 ±10.75ab	**10^{-3}**	**Cu-Chl/Go**
95.25 ±16.35ab	228.4±93.37	33.87 ±13.24ab	**10^{-4}**	**Cu-Chl/Go**
74.48±17.84 ab	164.8±77.55	38.46±7.28 a	**1cm/L**	**Malathione**
108.5±12.38 a	184.1± 38.50	31.10 ±5.34ab		**untreated**
43.42	**N.S**	**21.43**		**L.S.D (0.05)**

death (Panacek *et al*., 2011) and reduce protein synthesis and gonadotrophin release (Benelli, 2018). Moreover, Berni *et al*. (2009) found that phloxine B (photoinsecticides) had a toxicity on larva of *Ceratitis capitata* when the insects were exposed to light. The mode of action of photodynamic type insectidial agent could be due to 1): Inhabitation of feeding by damaging of memberans of midgut wall (Fondren *et al*., 1978), 2): Changes in membrane permeability (Weaver *et al*., 1976), 3): Occurrence of lethal energy stress in instead by change levels of water and protein mass (Broome *et al*., 1976) and 4): Induction of physiological and morphological abnormalities to larval, pupal and adult stage (Pimprikar *et al*., 1979 and Fairbrother *et al*., 1981).

Plant growth

Table 3 shows that the highest plant length was recorded from plants treated with foliar spray of Cu-chl/Ag 10^{-3} in both seasons (47.63 and 46.43 cm). Morever, treatment of Cu-chl 10^{-3} showed the highest value of plant length (48.87cm) compared to all other treatments in 2015/2016 season. The highest number of leave was obtained from onion plants treated with Cu-chl/Go 10^{-3}

(4.86 cm) in 2015/2016 compared to the control plants. Cu-chl/Go 10^{-3} and malathion treatments recorded the highest values of number of leaves in 2016/2017 season (4.46 and 4.40), respectively. In general, the highest concentration (10^{-3}) of phototsynthetizer and nanocomposite recorded the highest values of plant growth (plant length and number of leave) compared to the lowest concentration (10^{-4}) in both seasons. This increment in plant growth may be due to the enhancement of photosynthesis process, which lead to high chlorophyll content by Cu-chl, Cu-chl/Ag and Cu-chl/Go treatments (table 4). These results were in agreement with Morteza *et al.* (2013), who reported that copper nanoparticles enhanced seed germination and seedling growth. Copper plays an important role in many metabolic processes such as photosynsthesis, plant growth, respiration and development (Fernandes *et al*., 1991). It also, required as a structural and catalytic component of many enzymes and proteins. It has been known that the Cu ions in small concentrations are able to stimulate the plant growth and play the role as microelement (Karlsson *et al*., 2009 and Wierzbicka and Obidzinska, 1998).

Total cholorophyll content and soluble solid content (SSC)

The highest concentration (10^{-3}) of Cu-chl/Ag and Cu-chl /Go achieved the highest total cholorophyll content in both seasons respectively (30.74, 32.42) in 2015 /2016 and (31.87, 30.4) in 2016/2017 season (table 4). Soluble solid content was significantly affected by the treatments (Table 4). Foliar spray with Cu-chl/Ag 10^{-3} recorded the highest value of soluble solid content (8.83 %) in 2015/ 2016 which were insignificant with Cu-chl 10^{-3} (8.16). In 2016/2017 the highest values of soluble solid content (8.30 and 7.86%) were recorded by Cu-chl 10^{-3} and Cu-chl/ Ag 10^{-3}, respectively. Copper nanoparticles are important for plants because it leads to increasing of photosynthetic activity by modulating fluorescence emission, enhanching the electron transport chain in the dark phase and participating in the metabolism of carbon and nitrogen (Pradhan *et al*., 2015). Recent research reported that the application of Cu NPs at low concentration (0.05 to 1.0 mg L^{-1}) in the soil or by seed imbibition increase the seedling growth, chlorophyll and carotenoid content (Shah and Belozerova, 2009 and Pradhan *et al*., 2015) and enhancing photosynthesis, electron transport, photo reduction activity of PSII and oxygen evolution (Lei *et al*., 2007 and Giraldo *et al*., 2014).

Marketable yield

Fig. 2 indicates a significant increment in marketable yield as a result of treatment by copper chlorophyllin and nanocomposite. The highest concentration of cu-ch/ Ag (10^{-3}) achived the highest marketable yield (83.33 and 81.7%) in 2015/2016 and 2016/2017, respectively. In addition, the foliar spray with cu-chl /Go 10^{-3} and malathion had the highest values of martketable yield in 2016/2017. These results might be due to that the high concentration (10^{-3}) of Cu-chl/Ag and cu-chl /Go had the highest reduction population of thrips in the both seasons. Morevere, the silver patches in these treatment were the lowest composed to all other treatments (Tables 1 and 2).

Anatomy structure

Data in table 5 indicated that anatomical structure of onion leave was affected by foliar spray with copper chlorophyllin, nanocopper composite and malathion compared to untreated. The both concentrations of Cu-chl and Cu-chl/ Go reduced the epidermis cell (30.21,25.11, 23.71 and 26.8 μm) compared to untreated plants (42.92 μm). The foliar spray with cu-ch/Ag 10-3 and malathion (Fig. 3b and d) increased the elongation of Pallisade cell (177.1 and 151.2 μm) respectively compared to control (Fig. 3e). Copper chlorophyllin and nanocomposite induced malformation of Pallisade cell compared to untreated plants. The Pallisade cell had a close and regular arrangement in untreated plants but the foliar spray with copper chlorophyllin and nanocomposite increased the intercellular space between Pallisade cell compared to untreated plants (fig. 3). Foliar spray with malathion induced cell enlargement and succulent of spongy cell compared to all other treatments (table 5 and fig. 3d) and it reached to 303.5 μm relative to 157.2 μm in untreated plants. Malathion exhibited an exciting and promising increase for spongy tissue which compensate the decrease for Pallisade which more affected by the spraying of malathion for controlling the thrips. There is no significant differences between all treatment on xylem and vascular length compared to untreated plants (table 6). It is shown a slightly decrease in a vascular width in all treatment compared to untreated but with no significant differences except for Cu-chl/ Ag10^{-4}, which recorded the lowest value of vascular width (table 6). These results were in agreement with Khodakovskaya *et al*. (2012), who found that the nanoparticles when interact with plants induce many morphological and physiological changes based on their properties and chemical composition, size and surface covering. Size of Cu-chl/Ag is the smallest size (35 nm) compared to all other composite which, lead it to enter the cell and induced changes. In the leaves, nanoparticle can pass through the stoma, entering the vascular system of the leaves, and then transferred to other parts of

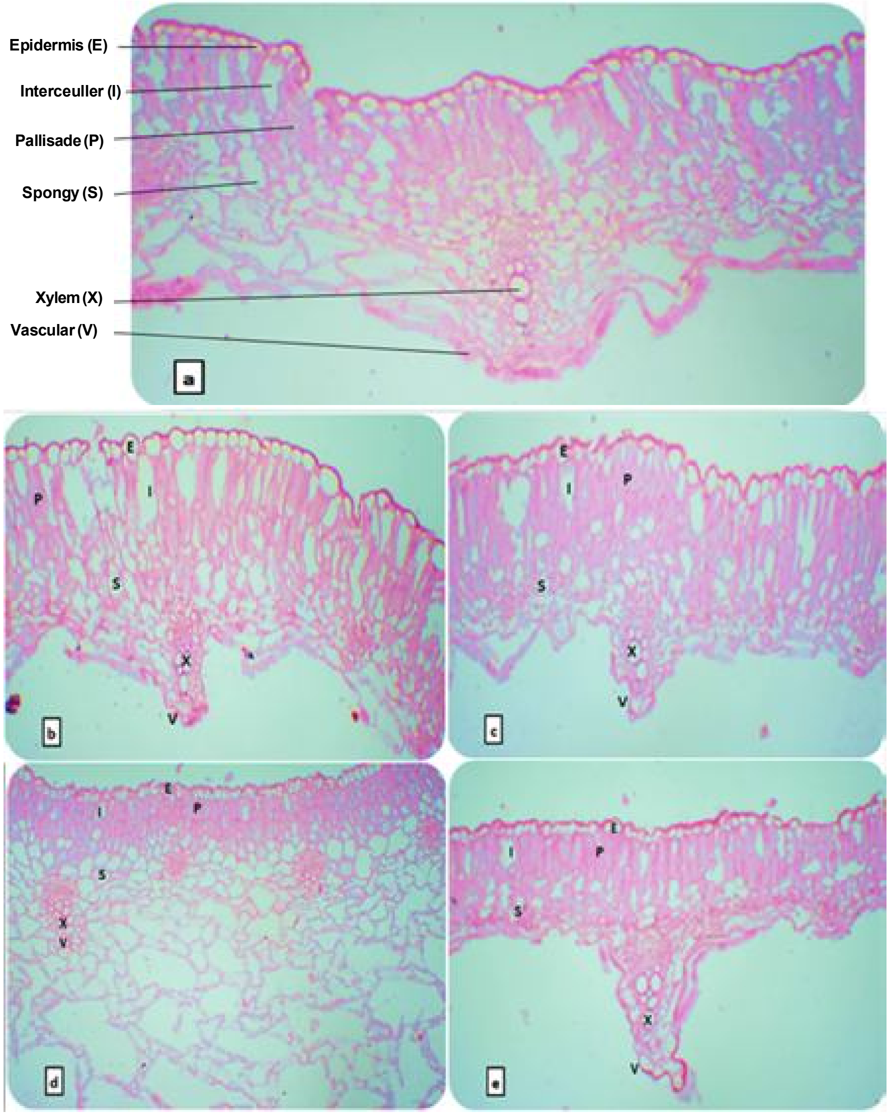

Fig. 3 : Transverse section through median poration of onion leave sprayed by photosynthetizers and nano copper compounds a): Cu-chl 10^{-3}, b): Cu-chl /Ag 10^{-3}, c): Cu-chl /Go 10^{-3}, d): Malathion and e): untrated.

through the phloem (Zhang *et al*., 2015; Servin *et al*., 2015; Shi *et al*., 2014 and Le *et al*., 2014). It is obvious from data mentioned before that treatment with nanocopper for high concentration exhibited a highest malformation for both Pallisade tissue and this may be related to high concentrate which consider a toxic heavy metals. These results agree with Asli and Neumann (2009), who reported that the toxicity of nanoparticls on plants does not necessarily require penetration into the plant, such as aggregation on the root surface, for example, alone leads to toxic effects by causing physical damage. Moreover, Ag nanoparticls at concentrations up to 1000 µg/mL induced cell wall damage and vacuoles in rice and consequently changed cell structure (Mazumdar and Ahmed, 2011).

Chemical content

Data in tables 7 and 8 shows that the foliar spray with copper photositizer and copper nano particles had significant effect on onion nutrient content in both seasons. The treatments with Cu-chl /Go 10^{-3} and Cu-chl 10^{-3} significantly increased leaves zn content (18.80 and 18.50 ppm) in 2015/2016 and (19.93, 19.63ppm) in 2016/2017 compared to control treatment. Data indicate that the lowest concentration of all treatments had the lowest values of Cu content and these values are equal to control treatment in 2015/2016, while in 2016/2017 season all the treatment recorded increase in leaves Cu content except malathion treatment. Cu-chl/Ag 10^{-3} foliar application significantly increase the Cu content (12.13 ppm) in 2015/2016, while Cu-chl/GO 10^{-3} and Cu-chl 10^{-3} had the highest Cu content (12.53 and 14.40 ppm,

Table 7 : Effect of photosynthetizer and nano copper compounds on onion mineral contents during 2015/2016 season.

Mineral contents (ppm) 2015-2016				Rate	Treatment
Ca	Mg	Cu	Zn		
0.98±0.06a	0.32±0.01abc	9.43±0.20 bc	18.5 ±0.05ab	**10^{-3}**	**Cu-Chl**
0.67± 0.06	0.24±0.008bc	8.43 ±0.49c	16.20±0.63 d	**10^{-4}**	**Cu-Chl**
1.25±0.08	0.33 ±0.56abc	12.13±0.43 a	17.23±0.74cd	**10^{-3}**	**Cu-Chl/Ag**
1.04 ±0.09	0.21± 0.02bc	8.55±0.37 c	14.83 ±0.08e	**10^{-4}**	**Cu-Chl/Ag**
1.18 ±0.02	0.30 ±0.05abc	10.03±0.26 b	18.80±0.17 a	**10^{-3}**	**Cu-Chl/Go**
0.88±0.00	0.19±0.02 c	8.93± 0.14c	18.15± 0.10abc	**10^{-4}**	**Cu-Chl/Go**
0.91±0.18	0.30 ±0.03ab	10.20 ±0.34b	17.30 ±0bcd	**1cm/L**	**Malathione**
1.20 ±0.42	0.39±0.07 ab	8.90±0.17 c	16.50 ±0.41d		**Untreared**
N.S	**0.14**	**0.92**	**1.20**		**L.S.D (0.05)**

Table 8 : Effect of photosynthetizer and nano copper compounds on onion mineral content during 2016-2017.

Mineral contents (ppm) 2016-2017				Rate	Treatment
Ca	Mg	Cu	Zn		
1.56 ±0.17bcd	0.9 ±0.09b	11.23±0.14 bc	19.63 ±0.77a	**10^{-3}**	**Cu-Chl**
1.300 ± 0cd	0.86 ±0.04	7.300±0.34 e	13.73± 1.05b	**10^{-4}**	**Cu-Chl**
2.65±0.13 a	1.18 ±0.10a	14.40±0.77a	15.63± 0.54b	**10^{-3}**	**Cu-Chl/Ag**
1.93±0.43a-d	0.87± 0.005b	11.1±0.63 bc	14.77±0.39 b	**10^{-4}**	**Cu-Chl/Ag**
1.93± 0.44a-d	0.8±0.04 bc	12.53± 0.08ab	19.93±0.43a	**10^{-3}**	**Cu-Chl/Go**
2.300±0.2ab	0.66± 0.003c	10.27±1.18 c	15.17±0.69 b	**10^{-4}**	**Cu-Chl/Go**
1.21±0.06 d	0.88±0.20 b	9.55±0.72 cd	15.33±0.20b	**1cm/L**	**Malathione**
2.10± 0.08abc	0.92 ±0.10b	7.93±0.37 de	15.0±0.46 b		**Untreated**
0.75	**0.18**	**2.0**	**1.79**		**L.S.D (0.05)**

respectively) in 2016/2017. There were insignificant differences among all treatments with high concentration and malathione or control in the first season on leaves Mg content. On the other hand, Cu-chl/GO 10^{-4} had the lowest value of leaves Mg content in the both seasons. Cu-chl/Ag 10^{-3} had the highest value of Mg content (1.18 ppm) in the second season compared to all other treatments. No significant differences were observed for Ca content relative to control treatment in the both seasons except the application of malathione had the lowest values of Ca content in 2016/2017 only. Uptake of essential elements increased in plants treated by nanoparticles, which increased photosynthetic performance (Wang *et al*., 2013).

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