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Impact of Certain Safe Treatments on Growth, Productivity and Protection against some Insect Pests of Cowpea Grown under Thermal Stress Condition

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ABSTRACT

Climate change threatens the world food security, especially in developing countries such as Egypt because of the impact of global warming on plant diversity and productivity, even plant that is considered warm vegetation e.g., cowpea. Field and storage experiments were conducted at El-Baramoun farm and Mansoura Horticulture Research Station, Dakahlia governorate, Egypt to study the agronomical performance response of cowpea cv. Kafr El -Sheikh-1 grown under high temperature and long photoperiod conditions of late summer seasons of 2017 and 2018 to some natural and safe treatments i.e., neem oil (2.5ml/L), chitosan (CS) at 200ppm, chitosan nanoparticles (CSNPs) at 100 ppm and potassium silicate (K-silicate) at 300ppm and their interactions compared with the recommended synthetic insecticide (lannate) at 75g/100L and unprotected control (tap water). The response of infestation of the pod borer (*Etiella zinckenlla)*, as one of the determinants for yield and quality of green pods in the field and dry seeds in the store, to the assigned individual treatments and a treatment of magnetized sea water + iron (Fe) salts were also concluded. In this work, the subsequent storability and insect damage of dry seeds at the ambient temperature for 5 months were studied in terms of either the effect of prior treatments during the field experiment or the effect of post-harvest treatments i.e., natural essential oils of neem, camphor and thyme at 2.5ml/kg seed in comparison with unprotected control and synthetic insecticide (Celphos 57%) at 50mg/kg seeds.The most important results could be summarized as follows:The physiochemical characterization of chitosan nanoparticles cleared that the nanoparticles have, smooth surface, spherical shape and size about 32 nm.All protected treatments considerably differed in improving the agronomical performance over unprotected control at the two seasons. Both mixed treatments were more effective in this regard, since the treatments of neem oil+ K-silicate combined with either CSNPs or CS were superior in increasing vegetative traits, relative water and total chlorophyll contents, green pod yield (47.5% and 46.6%) and dry seed yield (82.8% and 73%), respectively over unprotected control. The magnetized sea water + Fe salts recorded the highest reduction in pod borer infestation (82.16%), followed by CSNPs (81.16%) compared with the insecticide (76.14%).Concerning the seed storability as affected by prior treatments during field experiment, mixed treatments were more superior in protecting stored seed, especially neem oil+ K-silicate+ CSNPs treatment which reduced seed damage% and infested seed% from 100% (control) to 7.15 and 6.32 % and to 12.11 and 11.14% in 1^{st} and 2^{nd} seasons, respectively. As for the effect of post-harvest treatments, Celphos 57% was the most effective treatment, since damage% and infested seeds % reduced to 0%, followed by neem and thyme oils (less than 10%).Eventually, foliar spray of neem oil+ K-silicate combined with either CSNPs or CS five times during the growing seasons may introduce integrated solutions for biotic and abiotic stresses during field and storage of cowpea. Also for the best stored seeds at the ambient temperature for 5 months, neem and thyme oils (2.5ml/kg seed) are the most recommended natural and safe applications.

Keywords: *Vigna unguiculata* L. Walp, climate change, infestation, chitosan, silicon, essential oils.

INTRODUCTION

By the end of 21^{st} century, temperature will increase between 3^{o}C – 4^{o} C in Africa roughly 1.5 times the global mean response. This will result in significant yield losses (Kiprotich *et al.*, 2015) unless key investments are made to improve agricultural productivity under climate risk. Egypt is exposed in the late summer season to high air temperature that hinders the cultivation of most vegetable crops, which will constrain agricultural production. Increasing the cultivated area of cowpea, as tropical crop, is expected in Egypt as a result of increasing the area of agricultural land left after the harvesting of winter crops and the policy of reducing the area of cultivated rice as well as being a tolerant plant to many adverse conditions and as a cheap source of protein in poor communities. Meanwhile, cowpea is exposed to biotic and abiotic stresses which are dominant during long period extended from May to October in the field and from November to April during storage. Despite the resistance of cowpea in general to many adverse environmental conditions, but some observations indicated that recent increases in air temperature have already affected production and quality of some cultivars of cowpea grown in the Delta in late summer season. Temperatures varying from 21 to 33 °C are the best for cowpea vegetative growth (Ajetomobi and Abiodun, 2010). However, for high yields with good quality seed, the dry weather and temperature (day/night) of 27.6/18.2^{o}C are preferred. When minimum air temperatures exceed 20°C, damage to reproductive processes of cowpea occurs. The extent of damage is strongly affected by photoperiod. In addition, heat stress has greater effects on both flower production and pod set under long days compared with short days, which means that heat stress may be more damaging to cowpea in sub-tropical than tropical zones (Ehlers and Hall, 1998). An increase temperature by one unit would result in a 0.027 unit decrease in cowpeas production per hectare (Kiprotich *et al.,* 2015). The results indicated negative and significant relationship between cowpea yield and temperature i.e., weak growth, poor fruit setting, abscission of the reproductive sinks and low productivity (Ahmed *et al.,* 1993; Fathy *et al.,* 2008; Ajetomobi and Abiodun, 2010 and Kiprotich *et al.,* 2015). However, increasing temperature in temperate climate may become more susceptible to afflatoxins in the relevant regions (Ajetomobi and Abiodun, 2010 and Kiprotich *et. al.,* 2015).

Pod borer (*Etiella zinckenella*), one of the most common and destructive insect, usually attacks the late cowpea plantation and causing serious crop damage, since the larvae feed on seeds only and destroy whole pods resulting in considerable losses of yield (Gehan and Abdalla, 2006). In addition, cowpea weevil (*Callosobruchus maculatus*), a bruchid that is a cosmopolitan field-to-store pest, ranked as the principal post-harvest pest of cowpea in the tropics. Seeds of unprotected cowpea had lost more than 80% after 6 months due to the damage by *C.maculatus* (Singh *et al.,* 1990). It causes substantial quantitative and qualitative losses manifested by seed perforation and reductions in weight, market value and germination ability (Tiroesele *et al.,* 2015). Fathy *et al.,* (2008) found that most pronounced damages were occurred during cowpea seed storage (90-100% perforated seeds and 60-80% weight losses) due to insect infestation.

Biochemically, heat (more than 33/20^{o}C in the field and 15-20^{o}C in the store) and insect infestation induce serious oxidative stress and ROS accumulation in high destructive and toxic levels leading to protein and cell membrane lipid oxidation, enzyme inhibition and DNA and RNA damage (Richter and Schweizer, 1997 and Dat *et al.,* 2000 and Mittler, 2002).

Some precautions must be adopted to cope with the adverse effect of climate change and some serious pests, taking into consideration the safety and economic aspects. Excessive use of chemicals and synthetic insecticides is not only expensive but also results in series problems like the development of insect resistance to insecticides, harm to other natural enemies of insects and toxic effects on plants, soil and human being (Lokanadhan *et al.,* 2012). Hence, the transition from use of synthetic products to natural ones is evident in agricultural industry. Neem oil is a natural product extracted from the seeds of neem tree (*Azadirachta indica* A. Juss) which is used mainly as a pesticide and insecticide. Neem-based products are safe and can be used in combination with other chemical and pesticide for more effectiveness as well as pests do not develop a resistance to it. Azadirachtin , one of the most active triterpenoids found in neem oil, has been reported to alter insect behavior with its repellent and anti-feedant activities to pod borer in the field and bruchid in the storage.

Chitosan (CS) is a natural polysaccharide. It is not only pollution safe, but also nourishes the plant and less costly. Chitosan $(C_6H_{11}NO_4)_n$, the N-deacetylated derivative of chitin, is preferred due to its, antioxidant, biodegradability, biocompatibility, antimicrobial and non-toxic properties (Dash *et.al.,* 2011) as well as being a kind of the waste of fish and crustaceans, which are abundant in Egypt from two seas, the Nile River, many lakes and the biggest fish farm in the Middle East i.e., Ghalioun (Abeer and Farroh, 2018). Physiologically, CS application has many vital roles in mitigating biotic and abiotic stresses, improving the plant growth and post-harvest quality of fruits and vegetables. Chitosan has been found to show strong insecticidal activity in some plant pests i.e., herbivorous insect pests, while is less harmful to carnivorous insects, reared for use in the biological control, since it has been used successfully as an ingredient in the artificial diet fed to them (Tan *et al.,* 2010). In high temperature injury, CS act as antitranspirant by promoting ABA and jasmonic acid activity and increase antioxidant contents 3.5 fold (Jail, *et al.,* 2014). CS can be more effective in temperate regions where occasional or episodic stress events occur (Iriti *et. al.,* 2009).

Nanoparticles can be generated naturally and by human activities. It started since ancient times i.e., with the beginning of glass-making in Egypt and Mesopotamia back in the thirteenth and fourteenth centuries BCE. Recently, there has been growing interest in using nanotechnology to solve agricultural problems that traditional methods have not been able to solve in order to feed more than 7 billion people on the Earth. Nano materials (less than 100 nm in size) behave differently than the bulk one because of higher surface area to volume, which explains their easy entry into the plant, since stomata size varies with end-to-end lengths from 10 to 80 µm and width from a few to 50 µm. Furthermore, Agnihotri *et al.,* (2004) stated that they are environmentally friendly and bioactive. Sahab *et al.,* (2015) found that the effect of nano-chitosan on the mean number of eggs/female and growth percentage of *C. chinensis* insect pest in soybean were significantly decreased to 21.1±6.9 and 73.0%, respectively compared with the control under storage condition.

Moreover, silicon (Si) is non-essential element but it was the second most abundant mineral in earth's crust in terms of quantity (28.20%). It significantly increased plant growth under normal and stressed (biotic and abiotic) conditions (Ma, 2004). It enhances P, Ca and K uptake, increases chlorophyll content in leaves and alleviates heavy metal accumulation in the plant. In addition, Si mechanically strengthens the cell walls of the plants and enhances resistance to both pests and diseases (Yavas and Ünay, 2017). Under stress condition, Si compounds physiologically reduce oxidation of lipid constituents thus protect cell membranes and prevent cell loss of water. They also activate antioxidant enzymes, such as catalase and superoxide dismutase and stimulate proline formation, the amino acid that has high ability to resist high temperature injury.

There's no doubt in presence a whole natural magnetic field for the earth which influences everywhere. The different negative side effects of the recent technologies represent the main challenges to plants, animals and human being. Li *et al.*, (2005) studied the potential of electromagnetism technology as a physical control method against some pests e.g., ants and cockroaches. They reported that this method is better because it is economic, safe and does not cause pollution and residues.

As for seed storage, the natural and botanical pesticides are safe and potent alternative to synthetic insecticides. They result from secondary metabolism in the plants. The toxicity of a large number of essential oils and their constituents has been evaluated against a number of bruchid pests (Chinwada and Giga, 1993; Keita, *et al.,* 2000; Mahfuz and Khalequzzaman, 2007 and Fathy *et. al.,* 2008).

The aim of this study is to show how to mitigate the adverse effects of the global warming on the production of cowpea (Kafr El-Sheikh-1 cv.) by enhancing pod and seed yields with high quality and to estimate the efficiency of some natural materials on the pod borer, *Etiella zinckenlla* in the field and on *Callosobruchus maculatus* in the store compared with synthetic insecticide.

MATERIALS AND METHODS

Two field and storage experiments were conducted at El-Baramoun farm (+ 7m altitude, 30° 11′ N latitude and 28° 26′ E longitude)and Mansoura Horticulture Research Station, Dakahlia governorate, Egypt during the two successive seasons of 2017/2018 and 2018/2019 to study the effect of some safe field treatments (neem oil, CS, CSNPs, K-silicate and their interactions) and some natural essential oils as storage treatments (neem, camphor and thyme oils) compared with the recommended pesticides on cowpea (*Vigna unguiculata* L.), Kafr El-Sheikh-1 cv. grown under biotic and abiotic stress conditions.

Meteorological data:

Plants were grown under high temperature conditions, since the air temperature rises to more than 40°C for several successive days. Figure (a) shows the mean maximum and minimum records during the late summer seasons of 2017 and 2018 at Mansoura weather station according to the Central Laboratory for Agricultural Climate (CLAC).

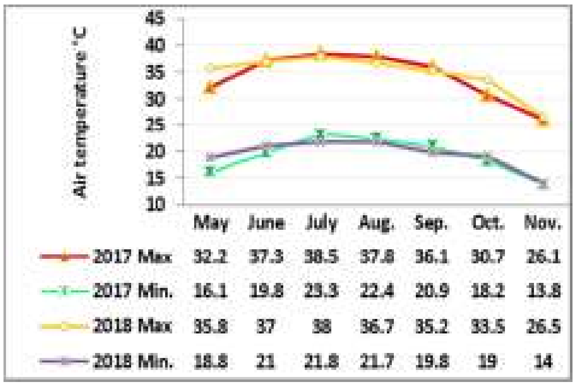

Fig. a. Monthly means of air temperature in El-Mansoura metrological station during 2017 and 2018.

The first experiment:

The field experiments:

Field experiments were laid out in an area of about 1/3 feddan. Cowpea seeds were sown on 28th May in hills at 20 cm apart on both sides of ridge 3m long and 60 cm wide and about 1m planted area was left around each plot to avoid any treatment drifts. Each treatment had 6 ridges; half of them were for the yield of green pods, whereas the remaining half was used for dry seeds yield.

Plants were foliar sprayed 5 times to the drip point, with different assigned treatments, the first one was at 20 days after sowing and repeated every 15 days. All cultural practices for cowpea were followed according to the instruction laid down by Egyptian Ministry of Agriculture.

Neem oil (70% azadirachtine) was purchased from Sigma-Aldrich (Bornem, Belgium) and K-silicate (K_2SiO_3: 11% K_2O and 22%SiO_2) from Technogreen Co., Egypt. It is very essential to add the emulsifier to neem oil and stirred well before adding water. In addition, chitosan was dissolved in 0.5% (v/v) acetic acid. Also, it is preferable to dissolve K- silicate in warm water first before preparing the assigned concentration.

Chitosan materials:

Chitosan (CS): (molecular weight 50,000-190,000 Da, degree of deacetylation, 75-85% and viscosity: (20-300 cP), glacial acetic acid and sodium tripolyphosphate (TPP). All the chemicals used in this study were purchased from Sigma-Aldrich, USA chemical company and used without further purification.

Preparation of chitosan nanoparticles

Chitosan nanoparticles (CSNPs) were prepared by ionic gelation method (Calvo *et al.,* (1997) with some modifications. Full details of CSNPs preparation is given by Abeer and Farroh (2018).

A complete randomized block design with three replicates was adopted in this experiment and the treatments are as follows :

1- Control treatment (tap water)
2- Insecticide (Lannate, 75g/100 L)
3- Neem oil (2.5 ml/L)
4- Chitosan (200ppm)
5- Chitosan nanoparticles (100ppm)
6- K- silicate (300ppm)
7- Neem oil (2.5 ml/l) + Chitosan (200ppm)+ K-silicate (300ppm)
8- Neem oil (2.5 ml/l) + Chitosan nanoparticles (100ppm) + K-silicate (300ppm)

Field experiment data:

1- Plant growth, relative water content and total chlorophyll content:

- Three plants from each treatment were randomly taken at 55 days after sowing and some vegetative traits were recorded: plant height (cm), number of branches, leaf area (cm2) and leaf specific weight (LSW) (mg/ cm2) according to Ferre and Forshey (1988) as follows:

$$\mathbf{LSW} = \frac{\mathbf{Leaf\ dry\ weight(mg)}}{\mathbf{Leaf\ area\ (cm2)}}$$

- For water relationship, fresh weight of six leaf slides sample from each replicate was recorded then was soaked with distilled water into Petri dish for 24 hr. and turgid weight was recorded then it was dried for 48 hr. and the dry weight was measured. Relative water content (RWC) was measured according to Taiz and Zeiger (1998) using the following formula:

$$\mathbf{RWC\%} = \frac{\mathbf{Fresh\ weight - Dry\ weight}}{\mathbf{Turgid\ weight - Dry\ weight}} \times \mathbf{100}$$

- Total chlorophyll was determined as described by Makeen *et al.,* (2007).

2-Yield and its components:

- **Green pod yield:**

Number of pods/plant was recorded. Samples of healthy twenty pods of each plot were used for recording average pod weight (g), pod length (cm). Marketable characteristics for pod were defined as good shape and good health state. Total green pod weight of half area was used to calculate green pod yield as ton/fed and marketable yield percentage as the following formula:

$$\mathbf{Marketable\ green\ pod\ \%} = \frac{\mathbf{Marketable\ green\ pod\ yield\ (ton/fed)}}{\mathbf{Total\ green\ pod\ yield\ (ton/fed)}} \times \mathbf{100}$$

Relative response percentages for green pod yield based on the average of the two seasons are calculated as following:

$$\boldsymbol{Relative\ green\ pod\ yield\ \%} = \frac{\boldsymbol{Total\ green\ pod\ yield\ (ton/fed) - control\ pod\ yield\ (ton/fed)}}{\boldsymbol{contorl\ yield\ (ton/fed)}} \times \mathbf{100}$$

- **Dry seed yield:**

Number of seeds/pod and weight of 100 seeds (g) were recorded. The pods of the remaining area were harvested when fully ripe and allowed to dry after harvest then total seed yield (kg/fed.) was calculated.

Relative response percentage for seed yield based on the average of the two seasons is calculated as following:

$$\textit{Relative dry seed yield}\% = \frac{\textit{Total seed yield (ton/fed)} - \textit{control seedyield (ton/fed)}}{\textit{contorl yield (ton/fed)}} \times 100$$

3- Data of *Etiella zinckenlla* infestation:

Regular samples of larvae were collected and examined to record the percent of reduction in larval infestation after 7, 10 and 14 days from foliar spraying of the following treatments:

- Control treatment (tap water)
- Insecticide (Lannate, 75g/100 L)
- Neem oil (2.5 ml/L)
- Chitosan (200ppm)
- Chitosan nanoparticles (100ppm)
- K-silicate (300ppm)
- Magnetic water+ Fe salts which is prepared as follows:

Ferric salts (20gm ferric sulphate and 20gm ferrus chloride) were added to sea water with proportion 1:1 before magnetization to increase the efficiency of sea water, the magnetization was carried out at Plant Protection Research Institute. The magnetic flux was measured with Magnetizing Battery apparatus which was 180 ml tesla. Infestation reduction percentage was calculated according to Hendrson and Tilton formula, (1955) as follows:

$$\textit{Reduction (mortality)}\,\% = \left[\left(\frac{Cb}{Ca} \times \frac{Ta}{Tb}\right).^{-1}\right] \times 100$$

Where:

C_b= number of alive pest individuals in control before treatment
C_a= number of alive pest individuals in control after treatment.
T_a= number of alive pest individuals after treatment.
T_b= number of alive pest individuals before treatment.

The second experiment:

The storage experiment:

Two storage experiments were, adopted to completely randomized design (CRD) with three replications, as follows:

a. Effect of the prior treatment during field experiment:

As for the seed storage affected by the prior field treatments, 200g of clean, healthy and unbroken seeds from each plot were stored in clean and dry plastic jar ($500cm^3$) with air permeable lid for 5 months under laboratory condition. The treatments were labeled as in the field treatment.

b. Post- harvest storage experiment:

Seeds of control plant (untreated area) were used for this storage experiment. Essential oils were applied at rate of 2.5ml/kg seed. Camphor and thyme were purchased as pure oil from El- Gommhoria Co., Egypt. Oil was dissolved in 200 ml acetone then 40ml of the resulting emulsion was mixed well with 200g seeds and left till acetone evaporating. Seeds were shaken to ensure uniform coverage and stored in plastic jars described previously for the same period (El-Gamal, 2017).

The storage treatments were:

1. Control (without any treatments)
2. Insecticide (Celphos 57% , Al- phosphide, at 50mg/kg seed)
3. Neem oil (2.5ml/ L)
4. Camphor oil (2.5ml/ L)
5. Thyme oil (2.5ml/ L)

Storage parameters:

Weight loss%, damage seed%, perforated infested seeds (due to penetration of cowpea weevil) and pest tolerance% were calculated at the end of storage period (150 days) according to the following formulas:

$$\textbf{Weight loss}\,\% = \frac{\textbf{initial seed weight} - \textbf{final seed weight}}{\textbf{initial seed weight}} \times 100$$

$$\textbf{Damage seeds}\,\% = \frac{\textbf{No. of brocken and rotten seeds}}{\textbf{Total No. of seeds}} \times 100$$

$$\textbf{Infested seed}\,\% = \frac{\textbf{No. of infested seeds}}{\textbf{Total No. of seeds}} \times 100$$

$$\textbf{Pest tolerance}\,\% = \frac{\textbf{No. of total seeds} - \textbf{No. of infested seeds}}{\textbf{Total No. of seeds}} \times 100$$

Statistical analysis:

Data were statistically subjected to analysis of variance (ANOVA) by CoStat statistical analysis system (Version 6.303, CoHort, USA, 1998-2004). Mean comparisons were performed using Duncan's Multiple Range Tests at 5% (Waller and Duncan, 1969).

RESULTS AND DISCUSSION

1- Physiochemical characterization of Nanoparticles:

Physical and chemical properties of chitosan nanoparticles could be shown through:

a- X-Ray diffraction (XRD) pattern of chitosan nanoparticles:

Figure 1 shows X-Ray powder diffraction patterns of CSNPs are shown in. No peak is found in the diffractograms. CSNPs are comprised of a dense network structure of interpenetrating polymer chains cross-linked to each other by TPP counter ions (Tang *et al.,* 2003). The XRD implicated greater disarray in chain alignment in the nanoparticles after crosslinks.

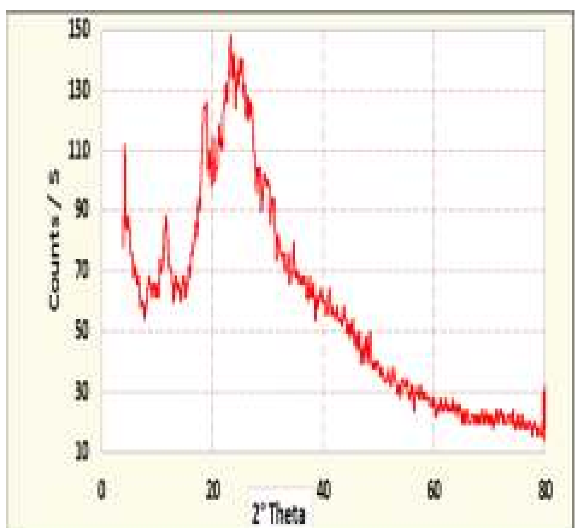

Fig. 1. X-ray powder diffraction patterns of CSNPs

b- Dynamic Light Scattering (DLS) Analysis:

DLS was used for measuring hydrodynamic diameter in the nanometer range. The size of CSNPs was 32 nm and zeta potential 42 mV (Fig. 2)

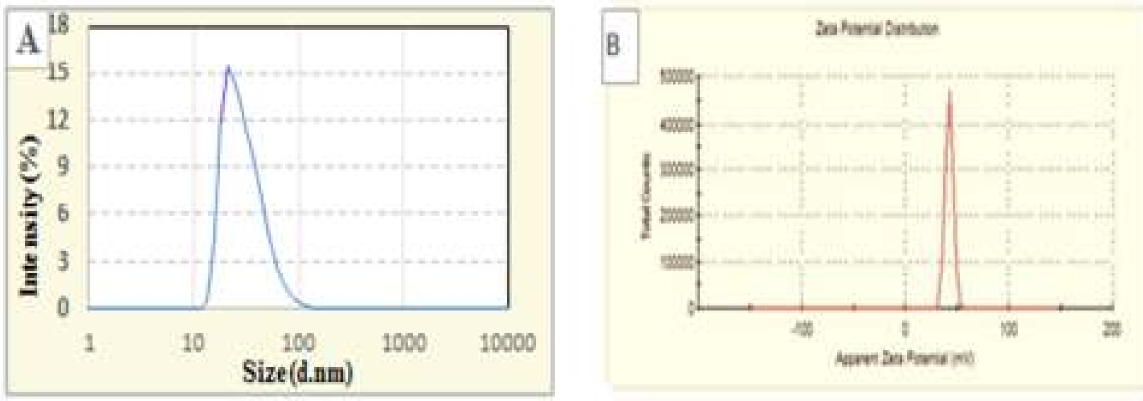

Fig. 2. DLS analysis of CSNPs. Particle size (A), and Zeta potential (B)

c- TEM analysis result:

Information on the particle shape and the determination of particle size were given by transmission electron microscope (TEM). Figure 3 shows typical TEM micrograph of the chitosan nanoparticles. The nanoparticles have smooth surface, nearly spherical shape and size range about 32 nm .

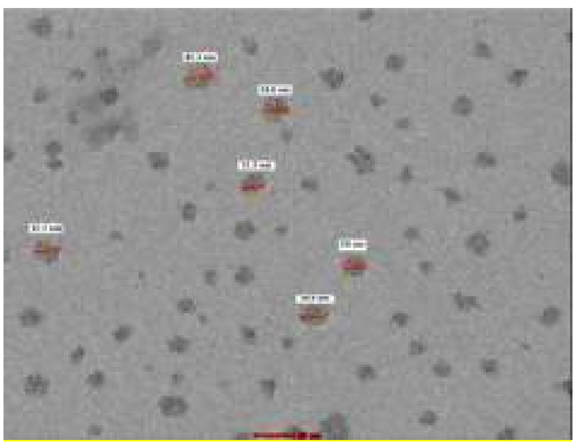

Fig. 3. TEM image of chitosan nanoparticles.

2- The effect of field treatments on growth and yields of green pod and dry seed:

a- Vegetative growth traits, relative water content and total chlorophyll content:

Although cowpea is a warm crop, it is adversely affected by photothermal condition of late summer season in Egypt i.e., high temperature (above 40^{o}C for several successive days), high relative humidity (more than 80%) and long photo period (from 12.5 to 14h.). Accordingly, Table 1 clears that plant height, number of branches/plant, Leaf specific weight (LSW), relative water content (RWC) and total chlorophyll content were decreased in control plants, whereas they were significantly enhanced in all protected treatments. Generally, the combined treatments were more pronounced, especially the treatment of neem oil+ CSNPs+ K-silicate, since the highest values were recorded compared with the control and other treatments.

The sensitivity of photosynthesis and photosystem II (PSII) may be resulted from the detrimental effects of high temperature injury on chloroplast membranes (Bernacchi *et al.*, 2001), in addition to increase plant respiration rates above the rate of photosynthesis (Hatfield and Prueger, 2015) which consequently cause the reductions in biomass accumulation and crop yield. Furthermore, the result of SLW was in agreement with Niinimets (1995) who stated that the increase in SLW due to protected treatments can be attributed to an increase in soluble carbohydrates. Foliar spray with neem oil (5% and 15%) increased root and shoot length and weight of inoculated shisham seedling. This natural oil enhances plant growth due to its antioxidantal ameliorative and insecticidal protective effects against leaf and pod borers (Rajput *et al.,* 2011).

Moreover, CS increased both leaf area and chlorophyll content which reflected on increasing significant amounts of dry weight. (Sheikha and Malki, 2011). Mona (2015) proved that CS stimulates bean plant growth by enhancing cell division similar to gibberellins. Dhoke *et al.,* (2013) found that the bioabsorption, selective uptake and transport of nanoparticles by plants have been high reactive due to more specific surface area, more density of reactive areas or increased reactivity of these areas on the particles surface. CSNPs can be easily absorbed through the leaf stomata then the phloem sieve tubes and transferred with the sugar flow to the shoots and roots due to pressure differentials between source (leaves) and sink (e.g., growing shoot apex). Hasaneen *et al.,* (2016) also mentioned that via plasmodesmata, radial transport from cell to cell occurs. Under stress condition, foliar spray with CSNPs improved vegetative growth variables and RWC% (Zayed *et al.*, 2017 and Abeer and Farroh, 2018) and activated the plant defense response by higher activities of peroxidase, superoxide dismutase, polyphenol oxidase and phenylalanine ammonialyase (Pal and Saharan, 2018). Water relation trait, RWC, reflects plant health in a certain environment. Si application normalized it to some extent under stress and normal conditions (Abdul Sattar *et al.,* 2016) and increased leaf area and chlorophyll in cowpea leaves (Mary and Aery, 2009). Silicon application enhances plant growth under stress condition due to improving antioxidant activities such as SOD (superoxide dismutase), APX (ascorbate peroxidase), DHAR dehydroascorbate), GPX (guaiacol peroxidas) and CAT (catalase) (Yavas and Unay, 2017).

Table 1. Effect of different individual or mixed compounds of applied treatments on vegetative growth traits of cowpea during the late summer seasons of 2017 and 2018

Variables / Treatments	Plant height (cm)		NO. of branches/plant		LSW (mg/ cm^2)		RWC (%)		Total chlorophyll (mg/g f.w.)	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
Control (tap water)	50.4 e	51.0f	3 c	3.6bc	10.02 e	10.08d	75.64 d	79.64e	0.93c	0.74d
Lannate (75g/100l)	49.9 e	50.2f	3 c	3.33bc	10.06 e	10.10d	76.13 d	75.13f	0.92c	0.77d
Neem (2.5ml/l)	52.9 d	53.6 de	3 c	3.67bc	10.14 e	10.20d	82.74 c	84.11d	0.93c	0.78d
CS (200ppm)	54.7c	55.2 cd	3.7 bc	3.67bc	10.51 d	10.65c	84.36bc	85.85cd	0.93c	0.82d
CSNPs (100ppm)	55.5 c	56.3c	4 ab	3.67bc	10.74 c	10.89c	85.99 ab	86.66bc	0.94c	0.95cd
K-silicate (300ppm)	52.9 d	53.2 e	3.7 bc	3c	10.79 c	10.79c	83.35cd	85.68cd	9.28c	1.16c
Neem+ CS+ K-silicate	62.3 b	61.2b	4.3 ab	4b	11.07 b	11.41b	86.29ab	88.62ab	1.10b	1.80b
Neem+ CSNPs+K-silicate	66.5 a	64.5a	4.7 a	5a	12.69 a	12.52a	88.02a	90.35a	1.64a	2.31a

Neem: neem oil; CS: bulk chitosan; CSNPs: chitosan nanoparticles; LSW: Leaf specific weight.
Values having an alphabetical letter in common within column do not statistical differ.

b- Green pod yield:

Data in Table 2 and Fig.4 reveal that all assigned treatments increased the green pod yield and its component compared with unprotected control. The mixed treatment of neem oil+ CSNPs+ K-silicate gave the highest records followed by Neem+ CS+ K-silicate, however, no significant differences in number of green pods/plant and total green pod yield/fed were observed between both treatments. Moreover, the highest records of the relative response of green pod yield was obtained with the mixed treatment of neem oil+ K-silicate+ CSNPs (47.5%), followed by the treatment of neem oil+ K-silicate+ CS (46.6%) (Fig.5).

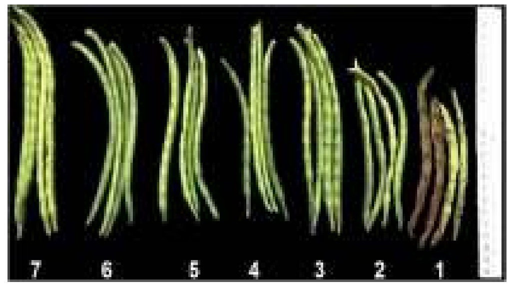

Fig. 4. A photograph of cowpea pods, Kafr El-Sheikh-1 cv., shows the effect of foliar spraying of 1: control (tap water); 2: insecticide (lannate); 3: nanochitosan; 4: chitosan; 5: K-silicate; 6: neem oil+ chitosan + K-silicate; 7: neem oil+ nanochitosan + K-silicate under thermal stress condition of 2017 and 2018.

Table 2. Effect of different individual or mixed compounds of applied treatments on green pod yield of cowpea during the late summer seasons of 2017 and 2018

Parameters / Treatments	No. of pods/plant		Average pods weight (g)		Pod length (cm)		Marketable yield % of green pod		Total green pod yield(ton/fed)	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
Control	14.3h	15.3g	26.66f	28.10f	15.3e	16.3d	33.4oh	32.03g	2.15f	2.15e
Lannate)	25.7e	28.0f	36.40c	36.60c	15.6de	17.0c	74.90b	74.13c	2.94b	2.97b
Neem	26.7d	29.0e	29.10e	33.60e	15.8cd	16.0d	45.22g	50.47f	2.77c	2.77c
CS	30.0c	33.0b	35.00d	35.20d	16.0c	16.7c	60.28f	60.71e	2.70d	2.72c
CSNPs	31.3b	32.0c	35.50d	35.90cd	16.1c	16.2d	63.05d	62.52d	2.79c	3.02b
K-silicate	31.0b	30.7d	35.40d	35.70d	16.0c	16.0d	61.79e	62.00d	2.53e	2.72e
Neem+ CS+ K-silicate	35.0a	35.7a	45.01b	45.60b	20.0b	21.0 b	73.90c	77.26b	3.140a	3.17a
Neem+CSNPs+K-silicate	35.3a	36.0a	46.67a	47.00a	21.1a	22.6 a	76.86a	80.11a	3.15a	3.19a

Neem: 2.5ml/L neem oil; CS: 200ppm bulk chitosan; CSNPs: 100ppm chitosan nanoparticles; K-silicate: 300ppm; fed: 4200m^2.
Values having an alphabetical letter in common within column do not statistical differ.

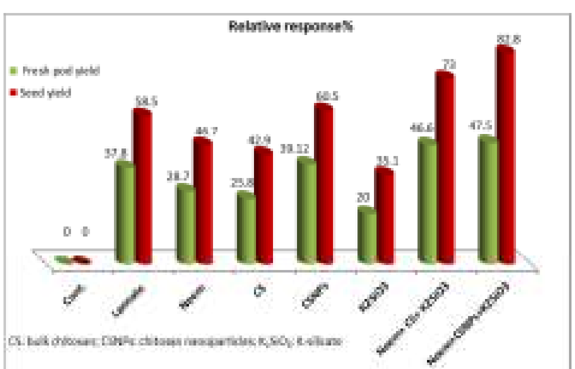

Fig. 5. Relative response percentage of green pod and dry seed yields of cowpea plant grown under biotic and abiotic stresses (average of the two seasons)

Ahmed *et al*., (1993) interpreted reproductive failure of cowpea under heat stress (33/30^{o}C) by limitations in carbohydrate supplies, especially peduncle sugars and decreases in photosynthetic rates. They also explained that pollen sterility in cowpea is caused by early degeneration of the tapetum tissue (a specialized layer of nutritive cells located between the sporangenous tissue and the anther wall). Ajetomobi and Abiodun (2010) found that high night temperatures (above 17 °C) can cause poor pod set due to earlier flowering and flower abscission in some cowpea cultivars. The decrease in yield of unprotected control was largely occurred due to the high air temperature, especially during flowering and fruit set (Fig. a).While, the promotive effects on green pod yield may be attributed to the effect of applied treatments on vegetative traits, water status and chlorophyll content (Table 1) and on pod borer infestation as shown later in Tables 4 and 5. CS oligosaccharides increased flower numbers in passion fruit (Utsunomiya and Kinai, 1994). In addition, Mondal *et al.,* (2012) attributed the yield increase in sprayed okra with 100 and 125ppm CS to production of higher number of fruits/plant and increased fruit size. Improvement in fruit weight and overall yield of tomato subjected to CS treatment may be due to high production of phenolic compound and phytoalexins (Jail *et al.*, 2014). Abeer and Farroh (2018) found that CSNPs gave the highest yield of tomatoes fruit grown under high temperature injury.

c- Dry seed yield:

Table 3 shows that seed yield of cowpea exposed to biotic and abiotic stress conditions have been severely affected while those protected by some natural substances or chemical pesticide gave higher records, except for 100seed weight in the 2nd season where lannate treatment did not reach to the significance at 5% level and no significant increases were observed in this variable among the remaining treatments. In addition, the highest seed yield/ fed was significantly obtained by the neem+ K-silicate+ CSNPs in both seasons (716.10 and 829.44, respectively). Fig. 5 also shows that the dry seed yield was increased from 35.1 to 82.8% over control due to protected treatments.

Biotic and abiotic stresses accelerate plant life cycle which reducing their chances of obtaining high yields and good qualities. Generally, grain yield was positively correlated with number of pods per peduncle and per plant. Most lines of cowpea exposed to heat stress induced reductions in number of seeds per pod in hot long days, even though there were no strong indications of carbohydrate source limitations (Ehlers and Hall, 1998). However, the increase in seed yield due to stimulants and anti-stress treatments is expected, since these treatments improved vegetative traits, water status and chlorophyll content (Table 1) and yield of pods as number and weight (Table 2) and reduced the infestation of pod borers (later in Tables 4 and 5).

Table 3. Effect of different individual or mixed compounds of applied treatments on dry seed yield of cowpea during the late summer seasons of 2017 and 2018

Variables / Treatments	No. of seeds/pod		100 seeds weight (g)		Total seed yield (kg/fed.)	
	S1	S2	S1	S2	S1	S2
Control (tap water)	9.0 d	9.3f	15.07 d	15.23 b	409.53 h	435.40g
Lannate (75g/100l)	11.1b	11.3d	14.81 d	15.42 b	636.72 d	702.30c
Neem (2.5ml/l)	10 .0c	11.0e	15.00 d	17.85 a	586.18 e	645.70d
CS (200ppm)	11.2b	12.0c	15.00 d	18.15 a	573.33 f	633.94e
CSNPs (100ppm)	12.0a	12.3b	15.50 c	18.41 a	657.58 c	697.95c
K-silicate (300ppm)	12.0a	11.0f	15.85 b	18.10 a	546.28 g	595.19f
Neem+CS+K-silicate	12.0a	12.5a	18.10 a	18.30 a	688.15 b	771.25b
Neem+CSNPs+K-silicate	12.3a	12.0c	18.03 a	18.08 a	716.10 a	829.44a

Neem: neem oil; CS: bulk chitosan; CSNPs: chitosan nanoparticles; fed: 4200m^2.

Values having an alphabetical letter in common within column do not statistical differ.

3- Effect of applied treatments on *Etiella zinckenlla*:

The results in Table 4 indicates that the total reduction caused by the insecticide (lannate) was relatively high against *Etiella zinckenlla* after 7 days from treatment, followed by the magnetic sea water + Fe salts followed by CSNPs which recorded reduction proportion nearest to the reduction caused by the insecticide. Data in the same table also show the reduction proportion after 10 days from treatment. The reduction caused by CS treatment was relatively high as the treatment of the insecticide, while CSNPs and magnetic sea water + Fe salts caused reduction proportion more than the insecticide.

Table 4. Effect of assigned treatments on infestation reduction percentage of *Etiella zinckenlla* after 7, 10 and 14 days from treatment:

Treatment	1st replicate			2nd replicate			3rd replicate			4th replicate			Treat. efficiency		
	Pest No.		Red. %	Pest No.		Red. %	Pest No.		Red. %	Pest No.		Red. %	Pest No.		Total
	Before	After		Before	After		Before	After		Before	After		Before	After	Red. %
After 7 days															
Lannate	7	4	71.4	4	3	91.67	4	3	62.5	7	4	93.65	5.5	3.5	79.82
Neem	1	2	0	1	7	22.22	1	2	0	1	8	11.11	1	4.8	8.33
K-silicate	5	8	20	2	8	55.56	5	5	50	2	9	50	3.5	7.5	43.89
CS	4	8	0	9	9	88.89	8	2	87.5	5	7	84.44	6.5	6.5	65.21
CSNPs	2	3	25	2	1	94.44	2	1	75	2	1	94.44	2	1.5	72.22
MSW+Fe	8	6	62.5	2	5	72.22	7	3	78.57	3	4	85.19	10	4.5	74.62
Control	4	8		1	9		4	8		1	9		2.5	8.5	
After 10 days															
Lannate	7	4	79.2	4	4	90	4	4	60	7	4	93.5	5.5	4	80.68
Neem	1	2	27.27	1	4	60	1	2	20	1	5	44.44	1	3.3	37.93
K-silicate	5	5	63.64	2	5	75	5	5	60	2	5	72.22	3.5	5	67.72
CS	4	5	54.55	9	5	94.44	8	4	80	5	4	91.11	6.5	4.5	80.03
CSNPs	2	2	63.64	2	1	95	2	1	80	2	0	100	2	1	84.66
MSW+Fe	8	6	72.73	2	4	80	7	3	82.86	3	2	92.59	10	3.8	82.05
Control	4	11		1	10		4	10		1	9		2.5	10	
After 14 days															
Lannate	7	7	50	4	1	96.43	4	5	37.5	7	6	87.76	5.5	4.75	67.92
Neem	1	0	100	1	1	85.71	1	0	100	1	1	85.71	1	0.5	92.86
K-silicate	5	2	80	2	2	85.71	5	0	100	2	1	92.86	3.5	1.25	89.64
CS	4	4	50	9	3	95.24	8	2	87.5	5	4	88.57	6.5	3.25	80.33
CSNPs	2	0	100	2	2	85.71	2	1	75	2	2	85.71	2	1.25	86.61
MSW+Fe	8	3	81.25	2	1	92.86	7	1	92.86	3	1	95.24	10	1.5	89.80
Control	4	8		1	7		4	8		1	7		2.5	7.5	

Lannate: 75g/ 100L; Neem: 2.5ml/L neem oil; CS: 200ppm bulk chitosan; CSNPs: 100ppm chitosan nanoparticles; K-silicate: 300ppm; CS: MSW+ Fe: magnetic sea water+ Fe salts.

These results were in agreement with Sabbour (2016). However, after 14 days, data indicated that the reduction caused due to the treatment of neem oil was very high followed by magnetic sea water + Fe salts, K-silicate, CSNPs and CS, respectively.

Data in table 5 show the mean of total reduction after the three scan which indicates that, the magnetic sea water + Fe salts recorded the highest reduction (82.16%), followed by CSNPs (81.16%) and they were the most effective than the insecticide which recorded 76.14%.

Table 5. Mean of total reduction percentage of *Etiella zinckenlla* infestation as affected by assigned treatments:

Treatments	Concentration	Mean reduction of 1st scan	Mean reduction of 2nd scan	Mean reduction of 3rd scan	Mean of total reduction
Insecticide	75g/ 100L	79.82	80.68	67.92	76.14
Neem oil	2.5 ml/L	8.33	37.93	92.86	46.37
K-silicate	300ppm	43.89	67.72	89.64	68.08
Chitosan	200ppm	65.21	80.03	80.33	75.19
Nano Chitosan	100ppm	72.22	84.66	86.61	81.16
Magnetic sea water+ Fe salts		74.62	82.05	89.80	82.16

Practically, it should be noted that the treatment of magnetic sea water + Fe salts is still under study where it needs some modifications because of the harmful effects of high salinity of sea water on the plants. The results were in agreement with Hussein et al., (2017) which recorded that magnetic sea water was more effective against *Tetranychus urticae*. Also, Sabbour (2016) indicated that nano chitosan was more effective in controlling *Schistocerca gregaria*.

4- Storage behavior:

a- Effect of the prior field treatments on dry seed storability:

Concerning the effect of the prior field treatments on storability and cowpea weevil infestation after 150 days, table 6 demonstrates that all individual and mixed treatments gave a considerable protection i.e., significant decreases in weight loss, damage and infested seed percentages compared with unprotected control. However, the synthetic pesticide used in the field gave the highest protection during the storage followed by both mixed treatments, alternatively.

Table 6. Effect of the prior field treatments on seed storability during 2017/2018 and 2018/2019

Treatments	Weight loss%		Damage seed %		Infested seed %	
	S1	S2	S1	S2	S1	S2
Control (tap water)	66.0a	63.19a	100a	100a	100a	100a
Lannate (75g/100l)	9.73h	8.13h	5.1h	3.7h	12.79h	12.18h
Neem oil (2.5ml/l)	26.11c	24.50c	10.27e	10.60e	22.11e	23.2d
CS (200ppm)	27.23b	25.00b	14.11c	13.08c	36.66b	32.12b
CSNPs (100ppm)	22.81e	24.06d	15.9b	13.22b	25.28d	26.17c
K-silicate (300ppm)	23.77d	22.17e	11.02d	10.80d	36.25c	22.13e
Neem+ CS+ K-silicate	9.73g	10.22g	6.37g	5.83g	17.90f	17.05f
Neem+CSNPs+Ksilicate	10.66f	10.50f	7.15f	6.32f	15.11g	15.14g

CS: bulk chitosan; CSNPs: chitosan nanoparticles.
Values having an alphabetical letter in common within column do not statistical differ.

Fig. 6 shows that the pesticide gave the highest pest tolerance % followed by the mixed and individual treatments, respectively.

Logically, protecting the pods against insect infestation in the field reflects on the reduction of seed infestation by weevil in the store. In general, resistance against insect infestation may be resulted from physical supports such as hard or lignified seed coat or cell wall and/or biochemical attributes. Chinwada and Giga (1993) reported that neem oil was very effective against pulse beetles till sixteen weeks due to oviposition reduction. Mahfuz and Khalequzzaman (2007) found that neem oil reduced *Callosobruchus maculatus* oviposition, allowed no adult emergence and prevented insect development during cowpea storage. When neem oil applied at the rate of 2.5 ml/kg, eggs hatching%, progeny emergence and seed damage were reduced. Also they reported that mortality of these pulse beetles was more than 99%. In addition, CS can be used in integrated pest management (IPM), since it is less harmful to non-target insects than conventional insecticides. It caused 100% mortality of larvae, whereas it was used in artificial diet fed to carnivorous insects (Sahab *et al.*, 2015). Insecticidal activity of CSNPs showed highest effect against *Callosobruchus maculatus* as the means number of eggs deposited/female were decreased to 20% (Sahab *et al.,* 2015). Silicon can be integrated with pest managed practices. It plays an important role as mechanical barrier against insect pest (in monocots) and as a physiological resistant, i.e., production of tannic and phenolic compounds (Laing *et al.,* 2006).

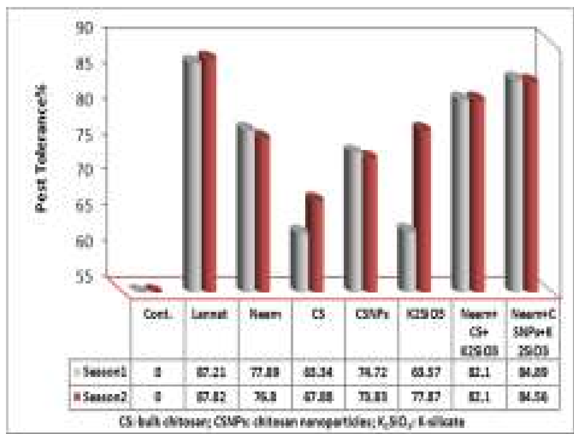

Fig. 6. Pest tolerance% of stored cowpea seeds as affected by field treatments

b - Effect of post- harvest treatments on dry seed storability:

Concerning the post-harvest treatments, it is worth mentioning that there has not been any weevil infestation till the middle of storage period (75 days) in all treatments, while it reached about 40% in uncontrolled seeds (Fig.7).

Table 7 and Fig.8 clearly show the response of storability and infestation of cowpea seed to some essential oils. Data clear that the lowest percentages of weight loss, damage, infested seed and the highest pest tolerance% were obtained by insecticide followed by neem oil and thyme oil, alternatively after 150 days of storing.

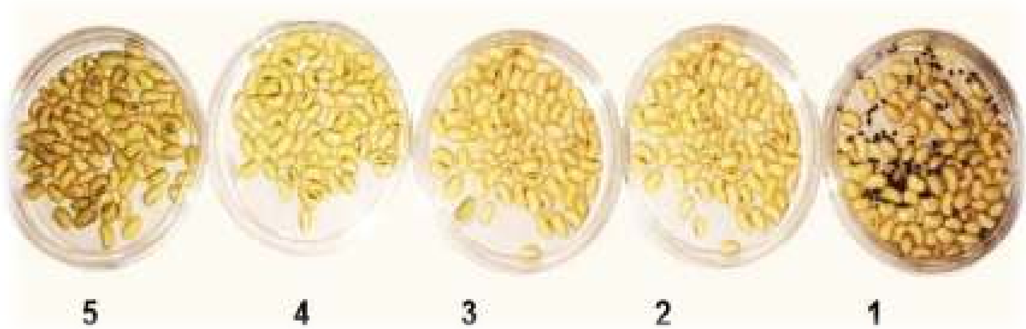

Fig. 7. A photograph of cowpea, Kafr El-Sheikh-1 cv., shows the effect of some essential oils of 1: control (without protection); 2: insecticide (Celphos 57%); 3: neem oil; 4: Camphor oil; 5: thyme oil on seed infestation by *Callosobruchus maculatus* after 75 days from storing.

Table 7. Effect of some essential oils treatment on *Callosobruchus maculatus*after after 150 days from cowpea seed storage during 2017/2018 and 2018/2019

Treatments	Weight loss %		Damage seed %		Infested seed %	
	S1	S2	S1	S2	S1	S2
Control (without protection)	66.00a	63.19a	100a	100a	100a	100a
Insecticide (Celphos 57%)	0.4d	0.2e	0e	0e	0.51e	0.33e
Neem oil (2.5ml/kg)	3.16c	2.54d	5.04d	5.87c	9.33d	9.40c
Camphor oil (2.5ml/kg)	3.77b	3.60b	8.32b	6.50b	11.30b	10.80b
Thyme oil (2.5ml/kg)	3.00c	2.79c	6.19c	5.00d	9.73c	9.22d

Values having an alphabetical letter in common within column do not statistical differ.

These botanical oils evolved their insecticidal effects via production of toxic monoterpenoids which act as insect antifeedants, repellents and oviposition. Chinwada and Giga, (1993), Keita, *et al.,* (2000), Mahfuz and Khalequzzaman, (2007) and Fathy *et. al.,* (2008) cited the insecticidal effects of numerous essential oils and botanicals in safe controlling of many pests.

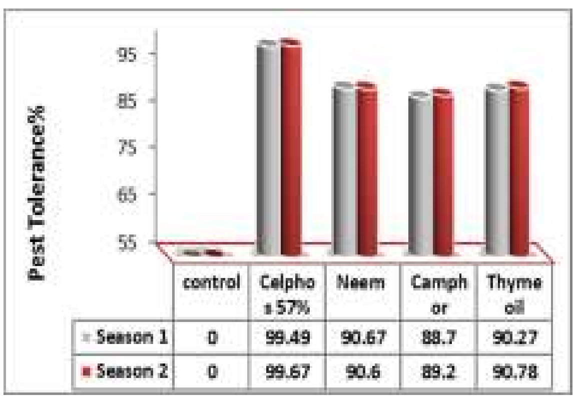

Fig. 8. Pest tolerance% of stored cowpea seeds as affected by some essential oils

CONCLUSION

Safe and natural substances hold promise in enhancing productivity and control of cowpea as compared with chemicals and insecticides which are costly and hazardous. The results of the present studies would suggest that under biotic stress i.e., insect infestation and abiotic stresses i.e., high temperature and long photoperiod, plant growth, pod and seed yields and seed protection were significantly increased by field applications of neem oil, chitosan, chitosan nanoparticles and K-silicate, especially when mixed . In addition, post-harvest applications of neem, camphor and thyme oils protected seed yield in the store.

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تأثير بعض المعاملات الآمنة على الإنتاجية و الوقاية من بعض الآفات الحشريه فى اللوبيا الناميه تحت ظروف اللإجهاد الحرارى

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يهدد التغير المناخى الأمن الغذائى العالمى خاصة الدول النامية مثل مصر نتيجة تأثير إرتفاع درجة حرارة الأرض على التنوع و الإنتاج النباتى حتى تلك النباتات التى تعتبر نباتات مناطق دافئه مثل اللوبيا. يؤدى إرتفاع درجة الحرارة عن الحد الحرج كما هو الحال فى العروة الصيفية المتأخرة بمصر الى ضعف النمو و انخفاض محصول وجودة القرون بالاضافه الى الإصابة بدودة قرون اللوبيا. أجريت تجربتان حقليتان و تخزينيتان بمزرعة البرامون ومحطة بحوث البساتين بالمنصورة لدراسة إستجابة نبات اللوبيا صنف كفر الشيخ- 1 المنزرع فى الموسم الصيفى المتأخر للعامين 2017 و 2018 بإستخدام كل من زيت النيم (2.5 مل/لتر), الكيتوزان بتركيز 200ppm, النانو كيتوزان بتركيز 100 ppm, سليكات البوتاسيوم بتركيز300ppm بالإضافة الى التفاعل بينها مقارنة بكل من الكنترول (ماء الصنبور) و معاملة المبيد الكيماوى الموصى به (Lannate) بتركيز 75جم/100 لتر, بالإضافه الى دراسة الإستجابة للإصابه بدودة قرون اللوبيا (*Etiella zinckenlla*) بإعتبارها أحد العوامل المؤثرة على محصول و جودة القرون الخضراء والبذور الجافة وذلك بإستخدام المعاملات الفرديه السابقة والمعامله بماء البحر الممغنط والمضاف إليه أملاح الحديد. كما إمتدت الدراسة لتشمل القابلية للتخزين ونسبة تلف البذور المخزنة فى درجة حرارة المخزن لمدة 5 أشهر و ذلك من حيث تأثير المعاملات السابقة أثناء الحقل أو تأثير معاملات ما بعد الحصاد بالزيوت الطبيعية (النيم والكافور والزعتر) كل بتركيز 2.5 مل / كجم بذور مقارنة بكل من معاملة الكنترول(بدون معاملة) ومعاملة المبيد الكيماوى (Celphos 57 ٪) بتركيز 50ملجم/ كجم بذور.كانت أهم النتائج كالآتى :-تباينت نتائج جميع المعاملات فيما بينها فى تحسين الآداء المحصولى سواء النمو الخضرى أو محصول القرون الخضراء و البذرة الجافة و معدل إصابة القرون، كما أنها ظلت الأعلى مقارنة بالكنترول فى الموسمين. تفوقت المعاملتان الثلاثيتان (معاملة زيت النيم + سليكات بوتاسيوم بالاضافة الى نانو كيتوزان أو الكيتوزان) حيث إرتفعت قيم الصفات الخضرية والمحتوى النسبى للماء والكلوروفيل الكلى بالنبات كما زاد محصول القرون الخضراء (47.5% و46.6 %) والبذور الجافه بنسبة (82.8% و 73%) على التوالى.أعطت معاملة الماء الممغنط + أملاح الحديد أعلى نسبة لإنخفاض الإصابة بدودة قرون اللوبيا (82.16%), تلتها معاملة النانوكيتوزان (81.16) حيث كانتا أكثر فعالية من مبيد اللانيت (76.14).فيما يتعلق بالقابلية للتخزين تأثرا بمعاملات الحقل ، تفوقت المعاملتان الثلاثيتان خاصة معاملة زيت النيم + نانو كيتوزان+ سليكات البوتاسيوم حيث إنخفضت النسبة البذور التالفة من 100% فى معاملة المقارنه (ماء الصنبور) الى 7.15 و 6.32% ونسبة الإصابة الى 12.11 و 11.14 % للموسمين على التوالى. أما بالنسبه لمعاملات ما بعد الحصاد, كانت المعاملة بالمبيد (Celphos 57٪) الأكثر فعالية ، حيث إنخفضت نسبة التلف والإصابة فى البذور من 100% الى 0% ، تليها معاملة زيت الزعترو زيت النييم (من 100٪ إلى أقل من 10 ٪).بصفة عامه يمكن التوصية برش اللوبيا المنزرعة خلال الموسم الصيفى المتأخر بزيت النيم (2.5مل/لتر) + سليكات بوتاسيوم (300ppm) بالاضافة الى النانوكيتوزان (100ppm) أو الكيتوزان (200ppm) خمس مرات خلال موسم النمو للحصول على أفضل إنتاجية للقرون الخضراء والبذور الجافة وكذلك أفضل قابلية لتخزين بذور، كما يعتبر زيت النييم وزيت الزعتر (2.5 مل / كجم بذور) أكثر التطبيقات الطبيعية والآمنة لتخزين البذور لمدة 5 أشهر على درجة حرارة المخزن.