

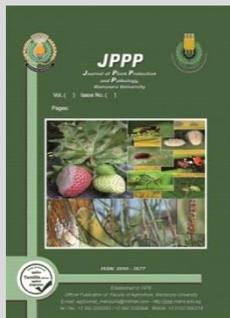
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A Novel Approach of Chitosan and Its Derivatives Bioactivity Against The Pinworm *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae)

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ABSTRACT

The present study was carried out to evaluate the relative insecticidal and biological activity of chitosan and its derivatives against the 2nd larval instar of *T. absoluta* compared to the unprecedented insecticide chloroantrilprole (Coragen) using standardized method of bioassay under laboratory conditions. The larvacidal activity for the different treatments against 2nd instar larvae of *T. absoluta* were predestined. Also, effect of the tested agents on growth inhibition, antifeedant and life-cycle of the tested insect were determined. The results revealed that chitosan nanoparticles (NPs) was the most effective treatment against 2nd larval instar and eggs deposited per gravid female of *T. absoluta*. while, chitosan hydroxyapatite nanoparticles (NPs) was the highest effective treatment on growth inhibition and antifeedant. All tested agents disrupted life cycle of the tested insect under laboratory conditions. Therefore, this study suggests the possible using of chitosan and its derivatives as safe alternatives to conventional insecticide and compatible with integrated pest management practices.

Keywords: Chitosan; Nanoparticles; *Tuta absoluta*; Biology

INTRODUCTION

The tomato crop occupies a very important place in world agriculture; it is grown all over the world even cold areas (FAOstat, 2015). The tomato culture is procumbent to discrete restriction such herbivores pests which mark in the output alteration of the total productivity (Van Eck *et al.* 2006). Among the harmful insects *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) which one of the most important pests of tomato worldwide (Desneux *et al.*, 2011; Campos *et al.*, 2014 and 2017; Biondi *et al.*, 2018). South America considered as the origin of *Tuta absoluta* Meyrick and source of the most subversive pests which squander tomato crop (Biondi *et al.*, 2018). Due to it have a accelerate outbreaks rate which often times surpass the economic tolerable threshold, its larvae can feed on all parts of the plant such as leaves, stems, flowers and fruits give rise to tomato yield loss (Moreira, 2005 ; Niedmann and Meza-Basso, 2006).

Currently, chemical control is the common trend for *T. absoluta* control. Furthermore, it has harmful effects on environment and natural enemies beside water and soil contamination (Qessaoui *et al.*, 2019; Mohanny *et al.*, 2020). The endophytic behavior of the larva (being found in the mesophyll of leaves) makes it hard for management of *T. absoluta* (Retta and Berhe, 2015). Also, the hyper extensive, repeated and indiscriminate application enhanced resistance for many groups of recommended insecticides, (Siqueira *et al.*, 2000; Roditakis *et al.*, 2015; Silva *et al.*, 2016).

Chitosan is a natural polysaccharide prepared by the N-deacetylation of chitin (Sabbour, 2019); it has significant biological and chemical properties such as biodegradability minimizing the noxious effects of

pesticides, biocompatibility where shield the toxic pesticide impact reducing the phytotoxicity and bioactivity help in leaching pesticides residues (Goodwin *et al.*, 2007; Sharma *et al.*, 2019) the small size, in the nanometer regime, enhances the penetration on the plant cell wall and cuticle, which in turn increasing the agrochemical uptake (Zhang, 2018).

Nanoparticles materials synthesis is terribly researched because of its wide several potential applications (Harris *et al.*, 2001). It considered a common technique advances as an alternative to chemical conventional insecticides (Martinelli *et al.*, 2014; Abdellatif *et al.*, 2016; Maluin and Mohd, 2020). Therefore, beneficial pesticides in the control of plant diseases have an economical returning (Jo *et al.*, 2009). Abundant studies on the potential insecticidal activity of chitosan and its derivatives in crop protection have been investigated (Oerke and Dehne, 2004 ;Kaur *et al.*, 2012; El-Mohamady *et al.*, 2014; Maluin *et al.*, 2019).

The objective of this study are: 1. investigate the insecticidal and biological activity of chitosan and its derivatives against the tested pinworm. 2. The effects of tested agents on growth inhibition, antifeedant effect through seven days and

MATERIALS AND METHODS

Tested insect

The tomato pinworm were reared on tomato leaves under laboratory conditions 22±2C° and RH 60-70% according to Galdino *et al.* (2011). Bioassays were performed with the second instar larvae of *T. absoluta* and they were obtained from a laboratory where they were

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raised on tomato leaves, at the Pesticides Department, Damietta University, Egypt.

Tested compounds

Chitosan bulk (Cs. Bulk) with purity of 99.99%, degree of deacetylation (DDA 90 %), Chitosan phosphite (Cs. Phi), with purity of 99.99%, Chitosan piperonil butoxide (Cs. PBO) which the synergist loaded with chitosan by 1:1, Chitosan nanoparticles (CS. NPs) with purity of 99.99%, size of 100 nm, degree of deacetylation (DDA 89 %) and molecular weight (2.27×10⁵) g/mol, Chitosan hroxyapatite nanoparticles (Cs. Ha-NPs), with empirical formula [Cs. Ca₅(PO₄)₃(OH)], purity of 99.99% and size of 80 nm. All previous agents were obtained from Egypt Nanotech Company limited, Cairo, Egypt. Chlorantraniliprole with trade name of Coragen 24% SC, it was supplied by BAYER Company.

Bioassay

To evaluate the larvicidal activity of the tested materials on the 2nd larval instar of *T. absoluta*, ten homogenous larvae were put in 9 cm diameter petri dish for each replicate according to the thin dry film technique described by Grafton-Cardwell and Hoy (1986). Four replicates were made for each treatment. Acetone was used as a solvent, while check treatment was executed using acetone only. The petri dishes were stored in a cabinet at 25±4°C, 60±4% RH, 16 hour photoperiod. After 24 h. the dead larvae were counted. The larvae were considered dead when no movement was observed after touch with a fine brush. The obtained data were statistically analyzed according to Finney (1971). Incorporated diet method where used to evaluate growth inhibition and antifeedant effect of the tested agents against the tested insect. Untreated diet was provided to control. All treatments treated diet by 4g (a.i) / kg diet, divided and placed in petri dishes. After seven days of continuous feeding, larval growth inhibition was assayed relative to the control based on larval weight gain through 7 days of feeding. The growth inhibition was calculated from the following equation:

$$\text{Growth inhibition \%} = \frac{CL - T L}{CL}$$

Where: CL: weight of larvae earned in the control
 TL: weight of larvae earned in the treatment

The percentage of feeding inhibition was determined after 7 days by the formula of Abivardi and Benz (1984)

$$\text{Antifeedant (\%)} = \frac{c-T}{c} \times 100$$

Where: C: weight of diet consumed in untreated control
 T: weight of diet consumed in treatment

Biology test

All the tested treatments at different concentration were prepared at their recommended doses and evaluated

Table 1. The larvicidal activity of the tested treatments against 2nd instar larvae of *T. absoluta*

Treatment	LC ₅₀ (ppm)	Confidence limit		Potency levels	Slope value	Toxicity index
		Lower	Upper			
Cs. Bulk	54.87	27.01	98.28	1.89	0.34	36.30
Cs. Phi	52.78	34.91	69.41	1.96	0.89	37.74
Cs. NPs	19.92	10.02	28.48	5.20	0.80	100.00
Cs. Ha-NPs	103.74	47.72	154.28	1.00	0.79	19.20
Cs. PBO	67.32	37.79	105.39	1.54	0.49	29.59
ChN	29.89	15.03	42.72	3.47	0.82	66.64

Abbreviation: Chitosan bulk (Cs. Bulk); Chitosan Phosphite (Cs.Phi); Chitosan nanoparticles (Cs.NPs); Chitosan hydroxyapatite nanoparticles (Cs..Ha-NPs); Chitosan piperonil butoxide (Cs.PBO); Chlorantraniliprole (ChN).

by adopting leaf residue method under laboratory conditions.

To assay the residual effect of each tested agent at LC₂₅ level on adult of *T. absoluta*, the technique advised by (Halder *et al.*, 2007 and 2011) was used. Ten adult females of *T. absoluta* with known age were placed on each tomato leaflets disc of size 3 cm tomato leaflets was wrapped with cotton moistened with distilled water. The treated leaf discs were transferred singly to each petri dish (9 cm diameter × 1.8 cm depth). After dipping in LC₂₅ concentration of each tested treatments for 20s and then air-dried, According to numbers of eggs deposited per gravid female through 5 days were assessed individually on different discs later. The number of hatched eggs was also counted 5 days after egg deposition. Malformed larvae's were accounted after complete egg hatching malformed pupae's, emerged adults and malformed adults were accounted by observation through the insect development. Assessment was conducted at 25+2°C with 16 hours photoperiod. Each treatment was replicated four times.

Statistical analysis

Abbott's formula (1925) was used to correct % mortality

$$\text{Mortality (\%)} = \frac{\text{Mortality \% of treatment} - \text{mortality \% of control}}{100 - \text{Mortality \% of control}} \times 100$$

The toxicity lines were statistically analyzed according to Litchfield and Wilcoxon (1949).

Statistical analysis of all data was carried out according to ANOVA and Duncan's multiple range test (Duncan 1955).

RESULTS AND DISCUSSION

The larvicidal activity of the tested treatments against 2nd larval instar of *T. absoluta*

The larvicidal activity of chitosan bulk, chitosan phosphite, chitosan nanoparticles and chitosan hydroxyapatite nanoparticles, chitosan loaded with piperonil butoxide compared to chlorantraniliprole against *T. absoluta* is shown in Table (1). Chitosan nanoparticles was the most active treatment after 24h with value of 19.92 ppm followed by Ch N, Cs. Phi, Cs. Bulk and Cs. PBO with values of 29.89, 52.78, 54.87 and 67.32ppm, respectively. While, chitosan hydroxyapatite nanoparticles was the least treatment with value of 103.74 ppm. Figure (1) indicated to Chitosan nanoparticles was the most toxicity compound against 2nd larval instar of *T. absoluta*. While, chitosan hydroxyapatite nanoparticles. The obtained results in (Table 1) indicated that the bioactivity of chitosan and its derivatives against *T. absoluta* gave a satisfied results compared to the chemical insecticide.

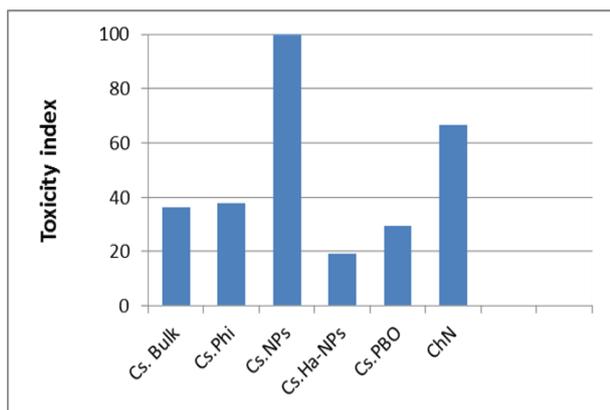


Fig. 1. Toxicity index of the tested treatments on the 2nd instar larvae of *Tuta absoluta* relative to the most effective treatment is Cs. NPs (NPs).

The obtained results was in agreement with Sabbour and Solieman (2016), who found that chitosan nanoparticles was more effective than chitosan bulk for different instars; newly hatched larvae, 1st larval instars, 2nd larval instars, 3rd larval instars, adult males and adult females of *T. absoluta* respectively. Also, Sahab *et al.* (2015) proved that nano-chitosan poly-acrylic acid (CS-g-PAA) showed highest effect against the three insect of soybean. At the same trend, Badawy and El-Aswad, (2012) investigated the promising insecticidal activity of chitosan nanoparticles at different concentrations against cotton leafworm *Spodoptera littoralis* and oleander aphid *Aphis neri*. The insecticidal activity of chitosan diethyl phosphate has been demonstrated against *Myzus persicae* due to high systemic and hydrolysis of chitosan diethyl phosphate (Cabrera *et al.*, 2002). This supposing support our finding because of chitosan phosphite is more systemic and hydrolysis than chitosan diethyl phosphate. Furthermore, it's considered to be super technology preferred than conventional insecticides in the test insect control which feed on the inner parts of the foliage of plants, their small size, short life cycle, and high fecundity often permit dramatic rises in population sizes. Our finding meet with Barrera-Necha *et al.* (2018) whose confirmed that chitosan bulk, chitosan nanoparticles and chitosan loaded botanical extracts against *Alternaria spp* and *Colletotrichum gloeosporioides* had a potent pesticidal activity. Also, Zhang *et al.* (2003) submitted evidence for

the bioactivity of chitosan against *Plutella xylostella* and *Helicoverpa armigera* respectively. Sahab *et al.* (2015) found that the nano chitosan have an insecticidal effect against *Aphis gossypii* under laboratory and field conditions. Sabbour and Solieman (2014) reported that nano-bio pesticides application increase the productivity of the olive fruits under field conditions. Also, Sabbour and Solieman (2016) control *Tuta absoluta* by nano-chitosan and results showed a reduction in the infestation numbers. Sabbour, (2016) found a loss of the insect population numbers when used the nano chitosan against *schistocerca gergaria* under laboratory and field conditions. Our obtained results supposed that chitosan nanoparticles was the most effective against the tested insect. It's may be due to the higher charge (more stability) and smaller size (easier cell penetration) of chitosan nanoparticles compared to the tested agents. This in agreement with Kheiri *et al.* (2017) whose demonstrated chitosan nanoparticles at low molecules was more effective than high molecules. The nanoformulations enhanced the solubility and stability with a slow and stable release of the insecticides (Feng and Peng, 2012). The obtained results implied that the tested materials had a promising effect on the pinworm pest relative to control.

Biochemical trial of the tested treatments against 2nd larval instar of *T. absoluta*

The incorporation of chitosan treatments into the diet reduced the mean weight gain of *T. absoluta* larvae compared to the control as shown in Table (2). For growth inhibition assessment at 7 days. Cs. Ha-NPs was the most effective in larval weight reduction (84.06 % growth inhibition) compared to all treatments. Cs. NPs, Ch N, Cs. Phi and Cs. PBO have a mild growth inhibition at seven days with values of 59.18, 58.68, 49.42 and 45.85 respectively. While, Cs. Bulk was the lowest treatment with value of 34.10.

For antifeedant percentage, Table (2) and Fig (2) refer to Cs. Ha-NPs was the most effective treatment with value of 87.64 followed by Ch N, Cs. NPs, Cs. PBO and Cs. Phi with values of 71.78, 64.09, 56.81 and 52.73 respectively. While, Cs. Bulk was the least treatment with value of 36.12.

Table 2. Growth inhibition and Antifeedant activity (%) of the tested treatments against 2nd instar larvae of *T. absoluta*

Treatment	Growth inhibition (%) through 7 days of feeding							Antifeedant (%) After 7 days
	1	2	3	4	5	6	7	
Cs. Bulk	10.43	12.19	14.77	21.41	25.65	25.65	34.10	36.12
Cs. Phi	12.58	18.34	22.52	25.41	31.36	36.30	45.85	52.73
Cs. NPs	25.80	28.06	32.94	38.98	45.08	48.87	59.18	64.09
Cs. Ha-NPs	19.78	25.82	33.51	40.10	60.38	71.97	84.06	87.64
Cs. PBO	15.43	18.67	23.06	26.41	33.55	40.14	49.42	56.81
Ch N	17.58	23.13	27.47	31.31	36.81	53.29	58.68	71.78

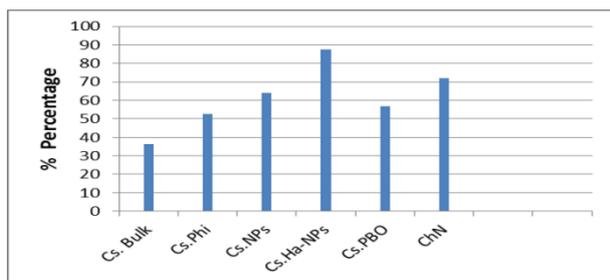


Fig. 2. Antifeedant percentage of the tested treatments against the 2th instar larvae of *Tuta absoluta*.

Recently, the investment of a biological activity of chitosan, chitosan polymers and chitosan derivatives to control agricultural pests as received more attention. In the current dissertation, chitosan hydroxyapatite nanoparticles (chitosan metal-complex) was the most effective treatment in growth inhibition and antifeedant against the tested insect. Chitosan has been shown to have the best chelating properties among natural polymers (Varma *et al.*, 2004). Effective groups (NH₂) are responsible for the metal-complex chelation where bonding with calcium apatite. Hydroxyapatite is the hydroxyl end member of the complex apatite group. End member in mineralogy is a mineral that is at the extreme end of a mineral series in terms of purity. Metal (apatite) was tested as a trial to found safe alternatives to conventional insecticides in controlling *T. absoluta*. This agree with Poschenrieder *et al.*(2006) who confirmed that agents including metals had a positive effect in phytosanitary treatments; they had supposed that metals induced a self-defense mechanism against pathogens, xenobiotics and herbivores. Minerals often can be described as solid solutions with varying compositions of some chemical elements, rather than as substances with an exact chemical formula. There may be two or more end members in a group or series of minerals. The combination had a wide variance and depends on the ion type and pH of the solution. Metal ion like a bridge connected one or more chains of chitosan through interacting with -OH and -NH₂ groups (Badawy and El-Aswad, 2012). The bio activity of chitosan metal complex proved against cotton leaf worm *S. littoralis* as antifeedant and growth inhibition (Badawy *et al.*, 2005; Rabea *et al.* 2005 and 2006; Badawy, 2008). After Rabea *et al.* (2006)

assayed 17 chitosan derivatives at a rate of 5 g (a.i) /kg diet against *S. littoralis*, found strongly weight loss in the test insect after 4 days of feeding. In addition, the activity of chitosan derivatives against several plant-feeding insects was tested (Zhang *et al.*,2003). The present study referred to the incorporation of chitosan compounds into the diet reduced the mean weight gain of *T. absoluta* larvae. Cs. Ha-NPs showed a high activity on larva growth where was the most active among chitosan derivatives in the inhibition of larva growth and antifeedant activity. The binding between chitosan and metal ion gave it the properties of disinfection (Wang *et al.*, 2004; Mekahlia and Bouzid, 2009; Higazy *et al.*, 2010) bindings are likely to leave some potential donor atoms free and these free donor atoms enhance biological activity (Wang *et al.*, 2005). The toxicity thresholds of different metals in the diamondback moth fed with artificial diets containing metal salts (Coleman *et al.*, 2005 and Jhee *et al.*, 2006). The study was mainly aimed at recording of different biological parameters of *T. absoluta* in its new habitat. There is a possibility of change in the growth and developmental parameters when an insect occupies a new area. The current results suggested that the tested materials got a discriminate Antifeedant against the pinworm pest relative to control.

Biology

Data in Table (3) and Fig. (3) indicated that Cs. NPs was the most effective treatment on eggs deposition of *T. absoluta* throughout five successive days with total value of 36.77 egg deposited / gravid female followed by Cs. NPs, ChN, Cs. Bulk, Cs. PBO and Cs. Ha-NPs with total values of 52.78, 65.02, 69.20, 72.95 and 77.59, respectively. Table (4) and Fig. (4) showed that the tested treatments disrupted life-cycle of the insect such as percentage of egg hatching; malformed larvae, malformed pupae, emerged adults and malformed adults significantly decreased after chitosan derivatives and chloroanatrilprole treatments (Table 4). Chloroanatrilprole and Cs. NPs were the most effective treatments on eggs hatching, malformed larvae, malformed pupae, emerged adults and malformed adults among others relative to control .

Table 3. Effect of the tested treatments on *T. absoluta* egg deposition

Treatment	No. of egg deposited / gravid female/day					Total	Mean /day
	1 st day	2 nd day	3 rd day	4 th day	5 th day		
Cs. Bulk	17.64 ⁱ ± 0.03	16.88 ^g ± 0.94	14.48 [±] 0.12	11.14 ^l ± 0.12	9.06 ^o ± 0.10	69.20 ^d	13.84 ^d
Cs. Phi	14.22 ⁱ ± 0.12	13.58 ^l ± 0.42	10.26 ^m ± 0.07	8.51 ^p ± 0.08	6.21 ^r ± 0.02	52.78 ^f	10.55 ^f
Cs. NPs	11.10 ^l ± 0.31	10.76 ^m ± 0.58	7.14 ^q ± 0.11	4.54 ^s ± 0.08	3.23 ^t ± 0.02	36.77 ^g	7.35 ^g
Cs. Ha-NPs	17.90 ^f ± 0.49	17.65 ^f ± 0.63	15.74 ^h ± 0.09	13.47 ^j ± 0.05	12.83 ^k ± 0.04	77.59 ^b	15.52 ^b
Cs. PBO	18.78 ^e ± 0.34	16.65 ^g ± 0.15	14.2 ⁱ ± 0.15	12.89 ^k ± 0.20	10.43 ^m ± 0.03	72.95 ^c	14.59 ^c
Ch N	16.60 ^g ± 0.11	15.48 ^h ± 0.95	12.82 ^k ± 0.15	11.36 ^l ± 0.11	9.15 ^o ± 0.09	65.02 ^e	13.10 ^e
Control	25.21 ^a ± 0.21	23.60 ^b ± 0.71	22.62 ^c ± 0.05	19.73 ^d ± 0.03	18.71 ^e ± 0.03	109.47 ^a	21.99 ^a

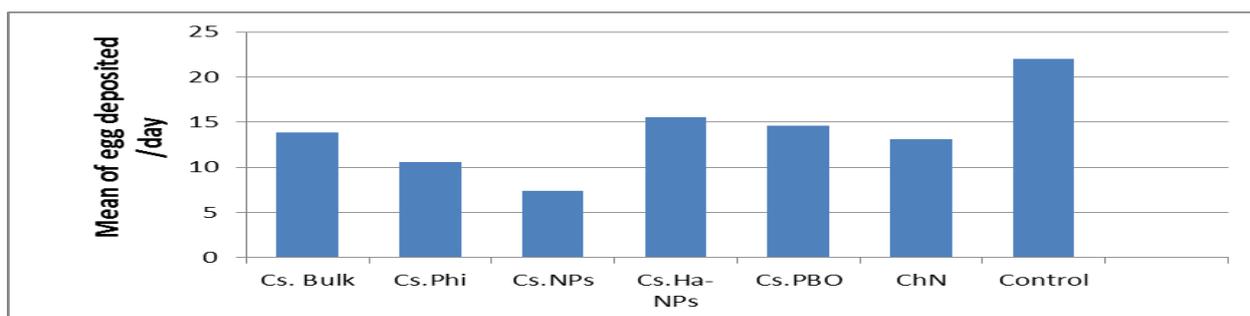


Fig. 3. Effect of the tested treatments on egg deposited of *Tuta absoluta*

Table 4. Biological effect of the tested treatments in life-cycle of *T. absoluta*

Treatment	% Egg hatching	%larvae mortality	%Malformed Larvae	%Malformed pupae	%emerged adults	% malformed adults
Cs. Bulk	22.32	78.12	83.98	80.12	9.42	87.01
Cs.Phi	11.19	86.17	88.80	82.14	6.76	77.91
Cs.NPs	5.88	95.18	99.00	100.00	0.00	0.00
Cs. Ha-NPs	9.11	92.83	90.05	91.11	4.65	88.34
Cs. PBO	18.76	84.22	88.33	93.19	4.07	89.99
ChN	8.47	96.80	97.65	100	0.00	0.00
Control	98.03	-	-	-	100	-

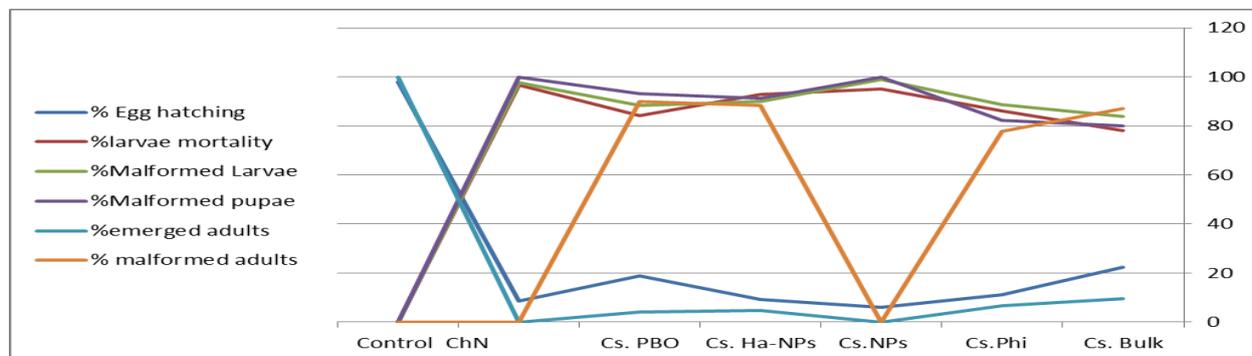


Fig. 4. Biological effect of the tested treatments in life-cycle of *T. absoluta*

The biological cycle is completed in 29-45 days depending on environmental conditions (EPPO, 2005), while 29-38 days under laboratory conditions (Halder *et al.*, 2019). Females laid eggs at oviposition period by 5-6 days (Polat *et al.*, 2016). These finding counterparts with our investigation. The adults survived for 40days at 10°C, 16 days at 19°C and 17 days at 23°C According to studies by Cuthbertson *et al.*, (2013). Gravid females laid eggs mostly singly or some time in small groups on young leaves followed by tender stems and green fruits (Duartei *et al.*, 2015). The obtained results were in accordance with Halder *et al.* (2019) who reported that egg ranges per gravid female with an average of 79.40. Pereyra and Sánchez (2006) who found that females laid an average of 132.78 eggs at 25°C and Erdogan and Babaroglu (2014) found that females laid 41.16 eggs on average. The decrease in the number of eggs is related to host aging as well as reduced temperature (Uchoa-Fernandes *et al.*, 1995; Cuthbertson *et al.*, 2013). Our finding meet with Sabour, (2018) who reported that chitosan bulk and chitosan nanoparticles significantly decreased number of *Pegomyia hyoscami* eggs deposited per gravid female per day compared to control. Sabour, (2016) recorded that the number of eggs laid / female of *Schistocerca gregaria* significantly decreased after treated with chitosan and nano-chitosan as compared to the control. Sahab *et al.* (2015) found the means number of eggs deposited /female of *Aphis gossypii* were significantly decreased to 20.9±9.1 and 28.9±9.2 eggs/female respectively as compared to 97.3±4.9 and 90.3±4.9 eggs/female in the control when treated by chitosan and its derivatives, respectively under laboratory and semi field condition. They added that the pervious effects also were observed against *Callosobruchus maculatus*. The percentage of egg hatching was decreased, larval mortality, malformed pupae and malformed adults significantly raised in case of *Pegomyia hyoscami* treated with chitosan and nano chitosan as reported by Sabour, (2018). Determination of the side effect of chitosan and chitosan – metal complex

nanoparticles on biological properties of *T. absoluta* is crucial for the correct timing of pest control applications. Controlling the spread of *T. absoluta* is essential for tomato production and determination of its biological parameters is an important step towards developing successful Integrated Pest Management strategies.

CONCLUSION

Because *T. absoluta* develop resistance, finding novel compounds to eradicate *T. absoluta* is an ongoing process. The scope of this thesis is an attempt to eliminate the use of IPM program and come over the disadvantages of conventional insecticides by evolution the insecticidal and biological activity of chitosan and its derivatives against *T. absoluta* under laboratory conditions.

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مقاربة جديدة للنشاط الحيوي للشيتوزان ومشتقاته ضد الدودة الدبوسية توتا أبلولوتا

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أجريت الدراسة الحالية لتقييم الفعالية النسبية للمبيدات الحشرية والنشاط البيولوجي للشيتوزان ومشتقاته ضد الطور اليرقي الثاني لـ توتا أبلولوتا مقارنة بالمبيد الحشري غير المسبوق كوراجين (كلوراناترابرول) باستخدام الطرق القياسية للتقييم الحيوي تحت الظروف المعملية. تم تقييم النشاط الإبادي للمعاملات المختلفة ضد الطور اليرقي الثاني من توتا أبلولوتا. كما تم تحديد تأثير العوامل المختبرة على تثبيط النمو ومضاد التغذية ودورة حياة الحشرة المختبرة. أظهرت النتائج أن جسيمات الشيتوزان النانوية (نانوبارتكل) كانت أكثر المعاملات فاعلية ضد الطور اليرقي الثاني ووضع البيض لكل أنثى حامل من توتا أبلولوتا. بينما كانت الجسيمات النانوية للشيتوزان هيدروكسي أباتيت (نانوبارتكل) هي أعلى معاملة فعالة في تثبيط النمو ومضاد التغذية. أدت جميع العوامل المختبرة إلى تعطيل دورة حياة الحشرة المختبرة في ظروف معملية. لذلك، تقترح هذه الدراسة إمكانية استخدام الشيتوزان ومشتقاته كبديل آمن للمبيدات الحشرية التقليدية ومتوافقة مع ممارسات مكافحة المتكاملة للأفات.