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Toxicity of N-alkyl Derivatives of Chitosan Obtained from Adult of *Chrotogonus trachypterus* (Orthoptera, Acrididae) against the Wheat, Cabbage and Oleander Aphid (Hemiptera: Aphididae) Species

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Abstract

Chitosan and its derivatives have received attention as alternatives to pesticides in agriculture. Insects are a good source for chitosan isolation. In the present study, chitosan was obtained from *Chrotogonus trachypterus* (Orthoptera, Acrididae), and its N-alkyl derivatives were synthesized. Experiments were conducted to assay their aphicidal activity against three aphid species. All derivatives had a higher aphicidal action (> 98%) than pure chitosan (> 15.2%) against aphid species. N-(3-phenyl butyl) chitosan and N-(ethyl butyl) chitosan were the most and least active derivatives. Results confirmed that the chemical modification of chitosan increased the aphicidal activity. A comparison of the aphicidal activities confirmed that N-alkyl derivatives of commercial chitosan had more toxic effects on aphid species than derivatives of grasshopper chitosan. No significant differences were observed between the two groups of commercial and *C. trachypterus* chitosan derivatives. This encourages us to introduce N-alkyl derivatives of grasshopper chitosan as a promising alternative source of aphicides in future.

Keywords: Chitosan, N-alkyl derivatives, Insecticide, *Aphis nerii, Schizaphis graminum, Brevicoryne brassicae, Chrotogonus trachypterus*.

1. Introduction

Chitin is a polymeric component present in the skeletal structure of arthropods, algae, crustaceans and fungi (Podile and Neeraja, 2011). Chitin and cellulose are two linear biopolymers; in their chemical structures, monomeric units of *N*-acetyl-2-amino-2-deoxy-d-glucose are connected by β-(1-4)-glycosidic bonds. Chitin has been extracted and characterized from a limited number of insects such as Lepidoptera (Zhang *et al*., 2000; Paulino *et al*., 2006), Hymenoptera (Nemtsev *et al*., 2004; Majtan *et al*., 2007; Marei *et al*., 2015; Kaya *et al*., 2016), Diptera (Ai *et al*., 2008), Homoptera (Sajomsang and Gonil, 2010), Coloeptera (Marei *et al*., 2015; Liu *et al*., 2012), and Orthoptera species (Marei *et al*., 2015; Kaya *et al*., 2014b; Kaya *et al*., 2015c).

Due to the insolubility of chitin in most solvents, modifications of chitin's structure are conducted to obtain its derivatives, such as chitosan, which, in ambient conditions, is more soluble in water and dilute aqueous organic acids, like acetic acid and formic acid (Toffey *et al*., 1996). Chitosan is formed by partial deacetylation of chitin, and preparation of its derivatives results in improved solubility in general solvents (Liu *et al*., 2012). Currently, commercial chitosan is primarily obtained from crustaceans, such as crab, and shrimp. However, such sources are unavailable in arid and semi-arid areas. Thus, finding new sources of chitosan is important, and pest insects may be a promising source to this end.

Previous researches suggested that reductive alkylation of chitosan with aldehydes or ketones could result in the interesting biological activities against some insect pests (Rabea *et al*., 2003; Rabea *et al*., 2006). In the present study, we obtained natural chitosan by deacetylation of chitin that was extracted from the grasshopper *Chrotogonus trachypterus* (Orthoptera, Acrididae). This grasshopper is found abundantly in the Sistan region (Zabol, Iran) and causes economic losses of seedlings of barley, wheat and vegetables. The present study aims to

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synthesize N-alkyl derivatives of chitosan of both commercial (low molecular mass) and *C. trachypterus* sources and to examine their insecticidal activities on aphids including *Aphis nerii* Boyer de Fonscolombe, *Schizaphis graminum* Rondani, and *Brevicoryne brassicae* Linnaeus.

2. Materials And Methods

2.1. Chemicals

Low molecular weight (3.60×10^5 g/mol) chitosan and all chemicals were purchased from Sigma Aldrich (Spain) and used without further purifications.

2.2. Isolation of Chitin from Adult of C. trachypterus

Adults of *C. trachypterus* were captured in April-July 2015 in wheat fields of Sistan region (Sistan va Baluchestan, Zabol, Iran). The adults of grasshoppers were starved for 48 hours to eliminate their gut contents and were then killed by freezing at -20 °C. The killed specimens were washed with distilled water and dried at room temperature. The samples were air-dried at 50°C for two days. Then, the air-dried specimens were pulverized using a mortar and stored at 4°C. In the step of demineralization, 5 grams of the powdered grasshoppers were treated with 1 M HCL (250 mL, 60 min, 75°C). The demineralized samples were washed and filtered several times to reach neutrality. The next step was deproteinization, in which the samples were treated with 1 M NaOH (250 mL, 24 h, 80°C), followed by filtering and washing with distilled water to obtain a neutral pH. The samples were decolorized by treating the precipitate with 1% potassium permanganate (100 mL, 2 h). The obtained light chitin was rinsed with distilled water several times to reach neutrality and was dried in an oven (24 h, 50°C) (Kaya *et al*., 2014a).

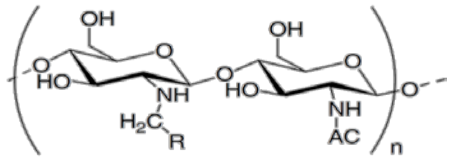
2.3. Chitosan Preparation

The purified chitin was refluxed in 50% NaOH (100°C, 5 h). The samples were rinsed many times until neutralization. The chitosan samples were dried at 50°C (24 h). To purify, the chitosan structures were dissolved in 2% acetic acid and re-precipitated in 20% NaOH. The chitosan samples were washed to obtain a neutral pH. The described potentiometric titration by Abdou *et al.* (2008) and Marei *et al*. (2015) was used to measure the degree of deacetylation (DD) of *C. trachypterus* chitosan (Ct.c). Furthermore, the molar mass of Ct.c was determined using a solvent system (0.2 M of NaCl, 0.1 M of acetic acid), as described by Erdogan and Kaya (2016). The molar mass of Ct.c was determined with data on the intrinsic viscosity and using the Mark-Houwink equation (Wang *et al*., 1991).

2.4. Synthesis of N-alkyl Chitosan Derivatives

The N-(alkyl) derivatives of both commercial and *C. trachypterus* chitosans (Cc and Ct.c, respectively) were synthesized using the method described by Kim *et al.* (1997). Eighteen nmol of chitosan (3 grams calculated as glucose amine unit) was dissolved in 300 mL 1% (v/v) glacial acetic acid. One equivalent of aromatic aldehydes (2-ethyl butyraldehyde, n-tridecanal, phenyl acetaldehyde, diphenyl acetaldehyde, 3-phenyl butyraldehyde) was separately added to the chitosan solution while stirring for 60 minutes at room temperature. Next, 1 M aqueous NaOH was added drop-wise to adjust the solution's pH to 4.5. 10% (w/v) NaBH4 (1.5 equivalents to the aldehyde) was added to this solution and stirred for 90 minutes at room temperature. To precipitate N-(alkyl) derivatives, the pH of the solution was adjusted to 10. The precipitate was neutralized by rinsing it with distilled water many times. Finally, the precipitates of N-(alkyl) derivatives were soxhlet-extracted with 1:1 (v/v) ethanol/diethyl ether for 48 hours, and the residues were oven-dried overnight at 60°C. Table (1) shows a series of *N*-Alkyl Chitosan (NAC) derivatives with their chemical structure.

Table 1. Chemical structure of NAC derivatives



Compound abbreviation	R	Compound name
NAC-1	$CH_3CH(C_2H_5)CH_2$	N-(ethyl butyl) chitosan
NAC-2	$CH_3(CH_2)_{11}$	N-tridecanyl chitosan
NAC-3	$(C_6H_5)CH_2$	N-(2-phenyl ethyl) chitosan
NAC-4	$(C_6H_5)_2CH$	N-(2, 2-diphenyl ethyl) chitosan
NAC-5	$CH_3CH(C_6H_5)CH_2$	N-(3-phenyl butyl) chitosan

2.5. Aphid Sampling and Rearing

To initiate the aphid cultures, the aphids (*A. nerii*, *S. graminum*, and *B. brassicae*) were originally collected from randomly selected fields in the suburbs of Zabol, Iran during spring 2015. The aphid colonies were reared in a $20\times15\times10$ cm^3 container under constant temperature in greenhouse conditions (26±2°C, 65±5% RH, 16:8 L:D). Aphids were reared for 2-3 generations (Wille and Hartman, 2008) in the laboratory before the insecticidal tests were carried out.

2.6. Bioassay Tests

Leaf-dip and plant systemic methods developed by Badawy and El-Aswad (2012) were used to assay the insecticidal activities of chitosan derivatives. N-alkyl chitosan derivatives of Cc and Ct.c were dissolved in 0.5% (w/v) aqueous acetic acid (50 mL). Then, a series of concentrations (200, 400, 600, 800, and 1000 mg/L) were prepared by dilution of the stock solutions.

In the leaf-dip method, fresh leaves of host plants of each aphid species were dipped in the chitosan derivatives for 30 seconds. The treated leaves were air-dried at room temperature (30-60 minutes) and then placed petri dishes (9 cm diameter) on filter papers (Whatman no. 1). A fine brush was used for transferring 25 wingless adult aphids from a stock culture to each petri dish. In the control, the leaves were treated with distilled water. The treatments were kept at 26±2°C, 65±5% RH and 12:12 L: D. Aphid mortality was recorded at 24 and 48 hours post treatment. Aphids, which were unable to move after the treatments, were scored as dead (Badawy and El-Aswad, 2012).

To study the insecticidal effects of chitosan derivatives on *A. nerii*, in the plant systematic method, branches of oleander plant were put in conical flasks containing the experimental concentrations (200, 400, 600, 800, and 1000 mg/L) of the derivatives. In addition, to assay the insecticidal activity of chitosan derivatives on *S. graminum*, and *B. brassicae*, the different concentrations of chitosan derivatives were added to hydroponically grown wheat (Moon *et al*., 1995). Thirty newly matured females of aphid species were transferred from the stock culture on the upper side of the leaves of the plant hosts (Badawy and El-Aswad, 2012). Distilled water was used in the control treatment. The treatments were kept under the conditions described above. All experimental bioassays were repeated for three replications.

To compare the efficiency of Cc and Ct.c, all experiments were first conducted with a series of Ct.c concentrations. Afterward, the maximum and minimum percentages of aphid mortalities with concentrations of Ct.c were obtained. The same concentrations of NAC derivatives were synthesized and tested on the aphids.

2.7. Statistical Analysis

Under a complete randomized design, data were compared by one-way analysis of variance (ANOVA) using SPSS (Statistical Package for Social Sciences, USA) software (version 21.0). Differences between treatment means were established using Student- Newman-Keuls (SNK) test (Snedecor and Cochran, 1989). Differences at $p \leq 0.05$ were considered to be statistically significant.

3. Results

3.1. Chitin and Chitosan Characterization

An amount of 9.1% Chitin was isolated from adult of *C. trachypterus*. Degree of deacetylation (DD) of chitosan from *C. trachypterus* was found to be 97%, which is considerably pure for chitosan. In addition, chitosan samples obtained from adult *C. trachypterus* had a molar mass of 8.1 kDa.

3.2. Insecticidal Activities of N-alkyl Chitosan Derivatives

Table (2) shows the insecticidal activity of N-alkyl derivatives of *C. trachypterus* chitosan against the tested aphid species using the leaf dip and systemic methods. Control CA (chitosan and acetic acid) showed 2.3-10.2% and 6.4-15.2% mortality in leaf dip and systemic bioassays, respectively. Mortalities of 0.0-4.1% were observed when aphides were treated with CW (chitosan and distilled water). Likewise, chitosan derivatives that had their NH2 group substituted with an alkyl group exhibited a range of aphicidal activity between 10.3-98.9% against the treated aphid species (Table 2). Our results showed that *N*-(3-phenyl butyl) chitosan (NAC-5) was the most active derivative in the leaf dip (67.1%, and 89.2% after 24 and 48 h of treatment, respectively) method, as well as in the systemic method (98.9%) against *B. brassicae* at 1000 mg/L. In addition, *N*- tridecanyl chitosan (NAC-2) showed a mean mortality of 47.5%, 68.3%, and 88.87% against all treated aphid species in the leaf dip (24 and 48 h post treatment) and systemic bioassay methods, respectively. *N*-(2, 2- diphenyl ethyl) chitosan (NAC-4) and *N*-(2-phenyl ethyl) chitosan (NAC-3) showed moderate aphicidal activity with a range 34-67% in both bioassay methods. While *N*-(ethyl butyl) chitosan (NAC-1) was found to be the least efficient for killing the aphids in the leaf dip method (26.5-47.5% 24 and 48 h post treatment, respectively), its systematic effect on aphids interestingly showed mortalities higher than 69% at 1000 mg/L.

Table 2. Aphicidal activity of N-alkyl derivatives of chitosan obtained from adults of *Chrotogonus trachypterus* against aphid species

Treatment	Concentration mg/L	Leaf dip method 24 h			Leaf dip method 48 h			Systemic effect method 48 h		
		Mortality % ± SE			Mortality % ± SE			Mortality % ± SE		
		Aphid 1	*Aphid 2*	*Aphid 3*	*Aphid 1*	*Aphid 2*	*Aphid 3*	*Aphid 1*	*Aphid 2*	*Aphid 3*
CW	0	0.0±0.0 l	0.0±0.0 l	0.4±0.0 m	1.00±0.4 m	2.5±0.01 n	1.8±0.4 l	2.9±1.21 i	4.1±0.5 j	3.0±0.9 i
CA	0	2.3±1.2 k	5.6±0.0 k	4.5±1.1 kl	3.7±0.3 l	10.2±2.5 m	9.8±0.1 jk	6.4±2.2 h	14.4±1.8 i	15.2±0.7 h
NAC-1	200	10.3±3.2 ij	12.7±2.2 ij	8.1±0.5 k	18.6±1.8 jk	20.3±0.8 jk	13.0±2.0 ij	51.0±0.0 e	57.4±1.1 ef	55.1±0.1 e
	400	10.6±5.0 ij	19.4±4.1 hi	10.5±0.0 jk	21.1±0.0 jk	26.4±1.4 j	15.1±0.3 ij	50.2±1.3 e	61.2±0.0de	56.3±1.4 e
	600	15.1±2.4 hi	21.0±1.8 hi	12.9±1.4 jk	33.2±1.5 ij	41.1±0.0 fg	18.0±0.5 ij	54.0±1.0 e	62.3±2.4de	57.5±0.5 e
	800	24.0±1.9efg	26.2±1.9gh	20.1±0.0 hi	38.0±2.1ghi	49.6±2.4def	24.3±1.1 i	60.3±0.0 d	68.0±0.0 c	63.7±0.0cd
	1000	25.5±2.8efg	30.2±3.0 f	24.0±0.6gh	46.0±1.0 fg	56.8±1.9 d	39.9±0.9 fg	72.9±2.1bc	75.9±0.1 c	69.0±0.3bc
NAC -2	200	19.3±1.2 gh	21.4±0.9 hi	17.0±1.7 ij	31.4±0.0 ij	49.0±0.2def	32.2±2.1gh	60.0±1.2 d	70.9±1.1 c	51.7±0.0 f
	400	23.0±0.8efg	31.9±2.0 f	25.4±0.0gh	40.9±2.1gh	52.7±0.0 de	39.7±0.5 fg	73.9±1.5bc	74.0±1.5 c	59.0±0.6de
	600	30.5±0.0 e	39.0±2.7de	39.2±2.2cd	50.0±1.5 ef	61.7±1.1 c	43.7±0.0 f	75.0±0.0bc	76.5±2.0 c	63.1±0.5cd
	800	33.7±0.1 de	46.0±1.4cd	49.0±3.0bc	55.8±1.3 de	66.1±2.3 c	50.0±1.4de	84.7±0.1 b	83.5±0.0 b	75.0±1.3bc
	1000	40.9±3.5 c	50.8±2.1bc	50.8±0.4 b	63.7±0.0 cd	72.3±0.0 b	68.9±0.1bc	85.6±0.0 b	94.0±2.3 a	87.0±0.1ab
NAC-3	200	12.6±1.2 hi	13.0±1.1 ij	15.0±0.9 jk	29.9±0.0 ij	32.6±1.5 hi	30.8±0.9gh	45.9±1.7 ef	50.0±0.5 fg	50.1±1.1 f
	400	18.3±2.6 gh	22.5±0.3 hi	20.9±2.1 hi	37.5±1.4 hij	39.5±1.5 h	35.5±1.2 fg	50.8±2.1 e	52.5±1.7 fg	54.6±0.7 e
	600	26.2±2.1efg	25.1±0.1gh	21.8±3.0 hi	41.1±0.5 gh	44.0±2.1 ef	36.0±1.0 fg	51.0±0.0 e	59.4±2.0 ef	62.8±0.3cd
	800	31.7±1.4 de	29.6±0.0 fg	32.1±0.0 ef	50.3±0.5 ef	63.0±0.0 cd	43.1±0.5 f	52.8±0.5 e	64.4±0.5de	70.9±0.1bc
	1000	31.9±0.9 de	32.4±0.1 f	38.4±1.9cd	59.4±2.2 d	69.6±0.9 c	51.3±0.0de	61.9±3.0 d	67.5±0.5 d	74.2±0.0bc
NAC-4	200	13.2±0.1 hi	15.1±0.5hij	15.5±2.0 jk	30.6±2.1 ij	42.8±0.7 fg	34.4±0.0 fg	35.9±1.7fg	49.0±0.5gh	44.1±1.1 g
	400	15.0±2.0 hi	18.0±1.4 hi	17.1±1.1 ij	39.5±0.3 gh	44.0±1.4 ef	40.1±0.0 fg	40.8±2.1 f	52.5±1.7 fg	50.6±0.7 f
	600	15.6±3.1 hi	28.5±0.0gh	25.5±1.9gh	42.8±0.0 gh	52.1±2.1 de	50.5±0.5de	41.0±0.0 f	59.4±2.0 ef	54.8±0.3 e
	800	29.1±0.9 e	35.6±0.5 ef	30.4±0.5 ef	52.1±1.4 ef	67.0±0.0 c	55.2±1.7 d	42.8±0.5 ef	64.4±0.5 d	60.9±0.1cd
	1000	37.5±4.1 d	40.5±0.1de	41.7±0.0cd	60.0±0.9 d	72.2±3.8 b	62.5±1.2 c	54.9±3.0 e	67.5±0.5 d	64.2±0.0cd
NAC-5	200	54.9±4.0 b	41.5±3.0de	34.1±1.0de	67.9±1.9 bc	62.7±2.4 cd	55.4±1.2 d	71.0±2.2bc	65.2±0.3 d	61.3±0.2cd
	400	57.8±3.0 b	57.6±1.1 b	48.2±0.8bc	70.1±0.0 b	72.5±0.0 b	60.0±0.6 c	79.0±0.0 b	74.8±0.5 c	70.1±0.6bc
	600	62.0±2.5 a	59.0±0.0 a	55.8±0.0 b	81.0±2.3 a	77.9±1.5 b	62.2±0.5 c	85.1±1.4 b	84.1±1.0 b	79.4±0.0 b
	800	65.3±1.4 a	60.2±2.1 a	59.5±1.4 a	87.2±1.3 a	80.0±0.0 a	72.5±0.0 b	90.3±0.1 a	85.6±1.1 b	80.3±1.1 b
	1000	67.1±3.3 a	62.0±2.3 a	60.7±0.2 a	89.2±0.0 a	81.3±0.2 a	85.7±1.0 a	98.9±0.9 a	92.1±0.9 a	90.0±1.7 a
df		26.49	26.49	26.49	26.49	26.49	26.49	26.49	26.49	26.49
p-value		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Aphid ***1***: *Brevicoryne brassicae*; *Aphid* ***2***: *Schizaphis graminum*; *Aphid* ***3***: *Aphis nerii*; CW: chitosan and distilled water; **CA:** chitosan and acetic acid; **NAC-1:** *N*-(ethyl butyl) chitosan; **NAC -2:** *N*-tridecanyl chitosan**; NAC -3:** *N*-(2-phenyl ethyl) chitosan**; NAC -4:** *N*-(2,2-diphenyl ethyl) chitosan **; NAC -5:** *N*-(3-phenyl butyl) chitosan; Data are expressed as mean percentages ±SE of three replicates; Values followed by the same letter within a column are not significantly different ($P \leq 0.05$) according to Student-Newman-Keuls (SNK) test; df – degree of freedom.

4. Discussion

4.1. Chitin Content (%) of C. trachypterus

Our results showed that the dry weight of chitin isolated from *C. trachypterus* was 9.1%. In previous studies, chitin content isolated from different insect species varied between 6% and 36%; the maximum chitin yield was obtained from cicada sloughs (Sajomsang and Gonil, 2010). In addition, the dry weights of chitin isolated from *Apis melifera* (Hym. Apidae), *Calosoma rugosa* (Col., Carabidae), and *Holotrichia parallela* (Col., Scarabaeidae) were 2.5%, 5.0%, and 15%, respectively (Marei *et al*., 2015; Liu *et al*., 2012). Kaya *et al*. (2014b) reported that the yield of chitin from seven grasshopper species varied between 5.3% and 8.9%. The desert locust (*Schistocerca gregaria* F., Acrididae) and the Mexican katydid (*Pterophylla beltrani* B. & B., Tettigoniidae) had 12.2% and 11.8% chitin, respectively (Marei *et al*., 2015; Torres-Castillo *et al*., 2015). Kaya *et al*. (2015a) compared chitin structures derived from three *Vespa* species (Hym., Vespidae) and found that the chitin contents of *V. crabro* L., *V. orientalis* L. and *V. germanica* F. were 8.3%, 6.4%, and 11.9%, respectively.

The dry weight of chitin isolated from female and male of grasshopper species, such as *Celes variabilis*, *Decticus verrucivorus*, *Melanogryllus desertus*, and *Paracyptera labiata*, was found to be 4.71–11.84% (Kaya *et al*., 2015c). In addition, it was confirmed that the yield of chitin varied between insect developmental stages and sexes. In the grasshopper *Dociostaurus maroccanus* (Acrididae), the chitin contents of the adults and nymphs were reported to be 14% and 12%, respectively (Erdogan and Kaya, 2016). The adult Colorado potato beetles (*Leptinotarsa decemlineata*, Chrysomelidae) and its larvae reported to yield 20% and 7% chitin, respectively (Kaya *et al*., 2014a). In another study, Kaya *et al*. (2015b) studied the physicochemical properties of isolated chitin from the body of a butterfly species (*Argynnis pandora*, Nymphalidae). The results confirmed that the chitin isolated from the wings was much higher than other body parts, except the wings (22% and 8%, respectively). They hypothesized that the surface morphology of chitin is highly related to the body part of insect.

4.2. Chitosan Characterization

Pure chitosan samples (DD= 97%) were obtained from *C. trachypterus*. This reveals that chitin was deacetylated to chitosan. The different DD influences biological, physicochemical and mechanical properties of chitosan. The DD value of chitosan isolated from the nymph of *D. maroccanus* was as 64% and 22%, respectively (Erdogan and Kaya, 2016).

Chitosan samples of *C. trachypterus* had molar mass of 8.1 kDa. Depending on the initial source of chitosan (crab, fungi, insect, shrimp, etc.) and the preparation method, the molar mass (or molecular weight, MW) of chitosan can show a decrease or increase in line with the significant increase or decrease in the degree of deacetylation (reviewed in Yuan *et al*., 2011). Artemia, crab, and shrimp produced chitosan samples with molar mass of 450-570, 483-526, and 2.20 kDa, respectively (Erdogan and Kaya, 2016; Tajik *et al*., 2008; Yen *et al*., 2009; Kucukgulmez *et al*., 2011). In other studies, the molar mass of obtained chitosan from adults of insect pests including *D. maroccanus*, *A. mellifera*, and *L. decemlineata* were found to be 7.2, 200-250, and 2.722 kDa, respectively (Nemtsev *et al*., 2004; Kaya *et al*., 2014a; Erdogan and Kaya, 2016). Chitosan samples with low molecular mass were commonly used in agriculture (gene transferring, plant protection), medicine (biomedical engineering, drug and vaccine delivery), and food production (seed-coating technology) (Erdogan and Kaya, 2016; Yen *et al*., 2009). Thus, it can be suggested that *C. trachypterus* chitosan, like other low molar mass samples, could be used effectively in these areas.

4.3. Insecticidal Activities of N-alkyl Chitosan Derivatives

The present study indicate that chitosan, without any substitution, was the least effective among the tested compounds. When chitosan derivatives were assayed, the aphicidal activity increased significantly compared to the control treatments (CW and CA). Chitosan (CA and CW) showed a low insecticidal activity against aphid species, but its chemical modification led to an increase in activity, especially for *N*-(3-phenylbutyl) chitosan (NAC-5) and *N*-tridecanylchitosan (NAC-2). In line with our findings, Rabea *et al*. (2006) demonstrated that *N*-(3-phenylbutyl) chitosan and *N*-tridecanyl chitosan were the most active chitosan derivatives when added to the artificial diet of *Spodoptera littoralis* Boisd (Lep., Noctuidae) larvae. It is suggested that chitosan and its derivatives probably block air from the insect cuticle by forming a layer on that surface, or inducing chitinases' activity in the insect body, thereby causing insecticidal activity.

Despite our findings of low the efficiency of chitosan (CW and CA) against aphid species, Zhang *et al*. (2003) reported that chitosan was an active insecticide against *Plutella xylostella* (Lep., Plutellidae) and homopterous insects with mortalities higher than 70%. Their study demonstrated that the insecticidal activity of chitosan to *P. xylostella* was higher than that of *S. exigua* at 3g/L concentration of chitosan (72% and 40%, respectively). In addition, the mortality of aphid species (*Rhopalosiphum padi* L., *Metopolophium dirhodum* Walker, and *Aphis gossypii* Glover) was 60-80%. Interestingly, in that study, the aphicidal activity was found to be higher than 90% against *Hyalopterus pruni* (Goffroy) on flowers, while *Sitobion avenae* (Fabricius) and *Myzus persicae* (Sulzer) showed a lower susceptibility to the aphicidal activity of chitosan (Yen *et al*., 2009). Similar to their findings, our results showed variable efficiency of NACs against different species of aphids. NAC-5 was insignificantly very potent in killing the treated aphid species (*A. nerii*, *S. graminum*, *B. brassicae*) in the present study. Other NAC derivatives showed a slightly higher effect against *A. nerii*, and *S. graminum* in comparison with *B. brassicae*. The reason that the aphicidal activities of these NACs on *B. brassicae* were lower than those of the same NACs did against *A. nerii*, *S. graminum* is not clear, but it may be because of the powdery cover on the external structure of its body, which might decrease the efficiency of NACs.

In the present study, aphicidal activity was significantly increased in the leaf dip bioassay at 48 h post treatment in comparison to 24 h after treatment. It was of great interest that higher than 88% mortality against aphids was obtained in systemic bioassays with NAC derivatives (NAC-5, NAC-2). The finding of both studies confirmed that in the leaf dip method, aphids feeding on treated leaves for 24 and 48 h were significantly affected; this suggests that oral uptake is essential for aphid control. The aphicidal activity by systemic bioassay confirmed that chitosan derivatives are primarily translocated in the plant phloem, which passively transports mainly water in an acropetal, i.e., upward movement. After the chitosan molecule moved into the plant, the aphids died and the treatments protected the plant (Erdogan and Kaya, 2016).

Rabea *et al*. (2014) showed that chitosan derivatives including N-(4-propyl benzyl) chitosan, *N*-(3,4-methylenedioxy benzyl) chitosan and *N*-(2-chloro, 6-flouro benzyl) chitosan possessed the toxic action of the males and females of *Ceratitis capitata* (Wiedemann, Diptera: Tephritidae) after 24 and 48 h of feeding under laboratory conditions. Time-lapse data of chitosan or its derivatives showed a fair amount of increase in insecticidal activity.

Derivatives of chitosan, including *N*-benzyl, *N*-butyl, *N*-dodecyl, and *N*-octyl chitosan, were evaluated for their activity against *S. littoralis*. Among them, both derivatives of *N*-benzyl chitosan, including *N*-(*p*-isopropyl benzyl) chitosan and *N*-(*o*-nitro benzyl) chitosan, caused significant mortalities of 46%. The most active compound, *N*-(2-chloro-6-fluorobenzyl) chitosan showed 100% mortality again this pest (Rabea *et al*., 2003).

The minimum and maximum percentages of aphid mortalities when treated with N-alkyl derivative of commercial chitosan (Sigma) demonstrated that due to the initial sources of chitosan, NAC derivatives of Cc (including NAC-1′, NAC-2′, NAC-3′, NAC-4′, and NAC-5′) had more insecticidal effects on aphid species when compared to N-alkyl derivatives of *C. trachypterus* chitosan. Although NAC derivatives of Cc caused a slightly higher percentage of aphid mortalities, the data were statistically insignificant. To the best of our knowledge, there are no studies examining the insecticidal activity of derivatives of chitosan obtained from insect sources. However, if NAC derivatives are to be encouraged and incorporated into pest control, especially for sucking insects, it is important to understand the acute effects that such derivatives may have on the behavior and physiology of insects under greenhouse or field conditions.

5. Conclusion

In the present study, chitin and chitosan were derived for the first time from the adults of *C. trachypterus*. The dry weight of the chitin structure of *C. trachypterus* was in the same range as the isolated chitin from other grasshopper species. Because of the large number of individuals in the invasive population, this grasshopper species could be used as a good source for chitosan preparation. Degree of deacetylation of *C. trachypterus* was found to be 97%, which was higher than chitosans isolated from other initial sources such as fungi and crustaceans. Our findings suggested that the efficiency of NAC derivatives seems promising because of their more specific mode of action towards aphids, especially by the systemic method of bioassay. It can be suggested that their mechanisms of NAC derivatives on specific pest species require additional study in the future.

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