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ORIGINAL ARTICLE

DNA insecticides: The effect of concentration on non-target plant organisms such as wheat (*Triticum aestivum* L.)

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Abstract

The excessive use of pesticides is a problem in most parts of the world today because of their broad and unspecific target range that is considerably harmful. The accumulation of several chemical insecticide residues based on chlorpyrifos-methyl, organochlorine, different isomers of HCH, DDT etc., in *Triticum aestivum* L. plants can be dangerous. Hence, there is an urgent need to develop potential and safer alternative measures. Wheat (*Triticum aestivum* L.) is a major cereal crop grown and used for food, animal feed, beverages and furniture accessories in most parts of the world. It also serves as a host to various insect pests. Our previous studies showed the insecticidal potency and specificity of short ssDNA oligonucleotides from the inhibitor of apoptosis (IAP-2 and IAP-3) genes of *Lymantria dispar* multicapsid nuclear polyhedrosis virus (LdMNPV) against gypsy moth (*L. dispar*) larvae, a possible insect pest of non-host plants like wheat. Consequently, the present study analyzes the effects of ssDNA oligonucleotides used as DNA insecticides on wheat (*T. aestivum)* plant biomass, plant organs and some biochemical parameters as a marker of the safety margin on non-target organisms. The results obtained on plant biomass showed that groups treated with ssDNA oligonucleotides at concentrations of 0.01 pmol · μl^{-1}, 0.1 pmol · μl^{-1} and 1 pmol · μl^{-1} varied in comparison with the control group, but remained harmless to plant growth and development, while the treatment concentration of 0.001 pmol · μl^{-1} did not affect the plant biomass. The glucose, protein and phosphorous biochemical parameters, analyzed after 21 days, showed that the ssDNA oligonucleotides used were equally safe. The data obtained for the plant organs (leaves and root lengths) indicate that the phenomenon of DNA insecticides can be further studied and developed for plant protection while improving the growth of plant organs even for a non-target organism such as wheat *T. aestivum* plants.

Keywords: antisense oligonucleotides, bioprotection, DNA insecticides, IAP genes, plant protection

Introduction

Over the past years, several published reports on risks associated with pesticides based on toxicity and exposure have provided knowledgeable information for agricultural workers, farmers and a large part of the consumer population (Ecobichon 2001; Wilson and Tisdell 2001; Burger *et al.* 2008). For most pesticides, non-target groups including humans are exposed in three ways: oral, dermal and inhalation contacts (ODI contacts). Other non-target groups could be exposed to pesticides through specialized routes. Pesticide

exposure to non-target organisms frequently occurs through vital channels (Gray *et al.* 2013; Gill and Raine 2014; Lekei *et al.* 2014).

In addition, studies required for markers of pesticide toxicity and regulations for human health by the EPA (United States Environmental Protection Agency) included tests on the risks to neurons (neurotoxicity) and vulnerability interval, carcinogenicity, metabolic patterns and effects on reproduction (EPA 2009). Of note, the risks associated with the use of non-specific modern pesticides (chemical insecticides) have increasingly up-scaled health problems, from long-term neurological disorders to reproductive disorders (Grewal *et al.* 2017), and even death from suicidal ingestion because of their availability (Langley and Mort 2012) as well as agricultural losses. In developing nations, there has been increased dependence on chemical pesticides, resulting in other serious health complications and high contamination (Soares and Porto 2009; Nicolopoulou-Stamati *et al.* 2016).

For agricultural systems, pesticides have long been used to manage pests, plant pathogens and diseases. Although there is a recorded increase in plant production, the use of pesticides comes with adverse effects (Farcas *et al.* 2013; Riah *et al.* 2014). These effects have been linked to reduced populations of beneficial insect species, soil and water contamination, air pollution, and injury to non-target plants and crops. Furthermore, factors which stimulate these negative effects include pesticides' modes of action, concentrations, application methods and duration of use (Eleftherohorinos 2008).

With time, various forms of less or non-harmful pesticides, for example, DNA insecticides which are 18–20 nucleotides long, single-stranded DNA oligonucleotides, appear to reduce the high negative impacts of most existing chemical-based insecticides (Oberemok *et al.* 2013; Nyadar *et al.* 2016; Nyadar and Adeyemi 2018; Nyadar *et al.* 2018). While the mode of action has been reported (Oberemok and Nyadar 2015; Oberemok *et al.* 2017), the application of DNA insecticides is still being studied. Some published data show their potency against targeted ferocious plant-eating insects like gypsy moth *Lymantria dispar* larvae, with an emphasis on high specificity and efficiency, as potentially effective biological pesticides. The information in this manuscript does not provide a direct link between gypsy moth (*L. dispar*) and wheat (*Triticum aestivum*) plants. However, in the absence of preferred plant hosts, wheat plants may be a susceptible alternative for gypsy moth caterpillars.

In this study, we compared and analyzed the effects of three different single-stranded DNA oligonucleotides used as DNA insecticides to ascertain their safety on non-target plant organisms such as "*Triticum aestivum* L." based on biomass, length of plant organs (leaves and roots) and the effect on some biochemical parameters necessary for plant growth and development. The aim of this study was to show the harmless nature of DNA insecticides as prospective and efficient additions to the family of biological pesticides for use in plant protection.

Materials and Methods

Seed collection and treatment

Viable seeds of common wheat (*T. aestivum* L.) plants were identified and collected from the Department of Plant Physiology and Biotechnology, Taurida Academy, Crimean Federal University, Simferopol, Crimea. They were divided into four groups with three replicates each (20 seeds/replicate). The biomass was measured to determine the average before use in the experiment. Each group was labelled oligoRING, oligoAn, and oligoCpG consisting of 10 ml of 0.001 pmol · μl^{-1}, 0.01 pmol · μl^{-1}, 0.1 pmol · μl^{-1} and 1.0 pmol · μl^{-1} concentration of the target single-stranded DNA (ssDNA) oligonucleotides used for treatment, while distilled H_2O served as the control group. The seeds were immersed in the respective treatment solutions for 24 h followed by a careful rinse with distilled H_2O and a dry session before use. The weight before and after the soak period was noted, and the treated seeds were placed in a sterile Petri dish to germinate for 3 days.

Laboratory cultivation of seedlings

After 3 days, the germinated seeds were transferred into vessels containing Hoagland-Arnon substrate and hydrocultured with a 12-hour photoperiod, at a constant air temperature of 20–23°C for 3 weeks with 55–60% humidity.

ssDNA oligonucleotides design

The ssDNA oligonucleotides were designed from two inhibitors of apoptosis (IAP-2 and IAP-3) genes of LdMNPV, and the last was composed of 50% C : G ratio from CpG oligodeoxynucleotide according to information found in ICTVdb (www.ictvonline.org) by Kuzio *et al.* (1999), and BLAST (www.ncbi.nlm.nih.gov), respectively. The IAPs and oligoCpG oligonucleotides were synthesized by Evrogen (Russia). The synthesized oligonucleotides were: (1) IAP-2: 5'-TGAACTC GACGCTCTTGTCC-3' (74460-74441, antisense "oligoAn"); (2) IAP-3: 5'-CGACGTGGTGGCACG GCG-3' (135159-135142, antisense "oligoRING") and (3) oligoCpG: 5'-CGCGCGCGCGCGCGCGCG-3' (102175-102192, sense/antisense for *T. aestivum* glia-

din-B genes: random fragment). The synthesized oligonucleotides were diluted to desired concentrations with distilled water.

Biomass analysis

Biomass analysis was carried out before and after seed treatment, then sequentially after a 7 day period. The seeds and seedlings were weighed in milligrams (mg) with BTU210 (Poland) laboratory scales.

Biochemical analysis

Biochemical parameters such as glucose, protein and phosphorus were determined from the lysate of one treated seedling from each replicate of each group with biochemical reagents from PZ Cormay (Poland). Data were generated with semiautomatic biochemistry analyzer SINNOWA BS-3000m (China).

Plant organ length analysis

The length of leaves and roots of the treated plants after 21 days was analyzed with an available standard bioinformatics tool ImageJ (https://imagej.nih.gov).

Data analysis

Obtained data were analyzed with Student's t-test for group comparisons, and represented as mean ± standard error, using Microsoft Excel 2007 and STATISTICA 7.0.

Results and Discussion

Effects of ssDNA oligonucleotides on wheat (*T. aestivum*) biomass

Biomass before and after 24 hours of treatment

General observation of seed imbibition shows their viability (Hershey 1998) as indicated by hydration and expansion noted before the experiment. The average biomass of seeds before treatment was approximately 49 mg for each group and concentration. After 24 hours of treatment, their biomass approximated to 72.5 ± 3.5 (means ± SE) (Table 1).

Biomass of leaves and roots after 7 days (GS – growth stage 7)

The results show variation in the biomass of treated plants in all groups and concentrations. Notably, the decrease in the biomass of leaves for GS7 treated with oligoCpG oligonucleotides (0.1 pmol · μl^{-1}) was significant ($p < 0.05$) when compared with the water-treated control group (Fig. 1). However, there was no decrease in the biomass of roots in all groups of the experiment compared with the water-treated control group (Fig. 2).

Biomass of leaves and roots after 21 days (GS21 – growth stage 21)

Sequential analyses carried out for GS21 showed significant decrease ($p < 0.05$) in the biomass of the plant leaves for both oligoRING and oligoCpG groups

Table 1. Data analysis showing the percentage change of seed biomass before and after 24 hours of seed imbibition with treatment solution (*N* = number of seeds for each replicate/Petri-dish)

Groups	Concentration [pmol · μl^{-1}]	Mean biomass [mg] *N* = 20		Biomass increase [%]
		before treatment	after treatment	
Control	0.001	49	72	46.9
oligoRING		49	74	51.0
oligoAn		49	72	46.9
oligoCpG		49	72	46.9
Control	0.01	49	72	46.9
oligoRING		49	72	46.9
oligoAn		49	73	48.9
oligoCpG		49	76	55.1
Control	0.1	49	71	44.9
oligoRING		49	71	44.9
oligoAn		49	69	40.8
oligoCpG		49	70	42.9
Control	1	49	71	44.9
oligoRING		49	71	44.9
oligoAn		49	69	40.8
oligoCpG		49	73	48.9

(1 pmol · µl^{-1}, Fig. 3). Furthermore, the biomass of roots treated with oligoAn oligonucleotides was significantly decreased ($p < 0.05$) compared with the control group (Fig. 4).

The results indicated that exposure to ssDNA oligonucleotides affected plant (leaves and roots) biomass according to treatment concentrations. There was no significant difference observed for the control and oligo-treated groups at 0.001 pmol · µl^{-1} throughout the study on biomass. This indicated that though the ssDNA oligonucleotides interfered with the plant biomass accumulation, the effects observed at 0.01, 0.1 and 1 pmol · µl^{-1} varied and were temporary. There was no damage or stunted growth on plants throughout the study, which also validated the safety of DNA insecticides (Oberemok *et al.* 2013; Nyadar *et al.* 2016). Unlike most insecticides that are non-specific, toxic and created to kill by contact (Pimentel 2005; Aktar *et al.* 2009; Sarwar *et al.* 2015), the DNA insecticides made of unmodified antisense oligonucleotides act against target organisms (Nyadar *et al.* 2018). Based on biomass analysis, the treatment concentrations of 0.001, 0.01 and 0.1 pmol · µl^{-1} indicate a safety margin for a non-target organism such as wheat *T. aestivum*.

Effects of ssDNA oligonucleotides on the length of wheat (*T. aestivum*) plant organs

The length of leaves and roots of the treated plants were examined to evaluate the effects of the applied ssDNA oligonucleotides as a marker for good growth and overall plant health. The data generated, and analyzed results showed that the leaf lengths (average of three sprouts per group; 16 groups in total) of treated plants at GS21 of the study varied in the range of 27.9–28.9 cm for 0.001 pmol · µl^{-1}, 26.8–30.4 cm for 0.01 pmol · µl^{-1}, 25.2–32.6 cm for 0.1 pmol · µl^{-1} and 22.4–25.8 cm for 1 pmol · µl^{-1} (Fig. 5). Interestingly, the leaves treated with oligoCpG ssDNA oligonucleotides were significantly longer (by 22%, $p < 0.05$ at 0.1 pmol · µl^{-1}) than the control treated group (Fig. 5).

The data generated for (average of three sprouts per group) root lengths after 21 days, showed a variation from 14.1 to 15 cm for 0.001 pmol · µl^{-1}, 9.3–15.6 cm for 0.01 pmol · µl^{-1}, 11.9–17.7 cm for 0.1 pmol · µl^{-1} and 7.8–16.9 cm for 1 pmol · µl^{-1}. The root lengths for oligoCpG treated plants were significantly longer (by 33%, $p < 0.05$ at 0.1 pmol · µl^{-1}) than the control group (Fig. 6). Notably, the root lengths for the oligoAn treated group at 0.01 pmol · µl^{-1} concentration were short and a further comparison with the control group showed a significant difference ($p < 0.05$) of 68% (Fig. 6).

Although, there was an observable difference between the roots of the control and oligoCpG treated groups at 1 pmol · µl^{-1} concentration, it was statistically not significant compared with the control (Fig. 6). Observations of ssDNA oligoCpG oligonucleotides indicated that some ssDNA oligonucleotides may be designed to induce cell expansion, typically related to the activities of some non-enzymatic proteins found in plants and their cell walls (Cosgrove 2000; Bashline *et al.* 2014), which could regulate elongation in roots and shoots, cell division, flowering and general plant development. While there were significant changes in lengths of leaves and roots, the elongation observed in the roots of the plants treated with ssDNA oligoCpG fragment at 0.1 pmol · µl^{-1} concentrations is indicative that DNA insecticides can be studied for plant protection while improving the growth of plant organs.

Effects of ssDNA oligonucleotides on some biochemical parameters

The results obtained on the analyses of wheat *T. aestivum* seeds after 1 day (GS1) imbibition period signified a significant change in glucose concentration. The recorded data showed that groups treated with oligoRING and oligoCpG ssDNA oligonucleotides, respectively, had decreased glucose concentrations when compared to the control group. There was reduction ($p < 0.05$) of glucose concentration in the oligoRING treated group in comparison to the control group, and a glucose reduction ($p < 0.05$) in the oligoCpG group when compared to the control group at 0.001 pmol · µl^{-1} treatment concentration (Table 2). The seeds treated with ssDNA oligonucleotides at concentrations of 0.01, 0.1 and 1 pmol · µl^{-1} did not affect the glucose concentrations in any of the experimental groups. There was no change in protein concentration in the treated seeds of any of the groups and their respective treatment concentrations. However, there was a significant ($p < 0.05$) decrease in phosphorous concentration in the oligoCpG treated group at 1 pmol · µl^{-1} when compared with the control group, GS1 (Table 2).

Interestingly, no significant changes were observed in the biochemical parameters throughout GS7 (Table 2). However, a significant ($p < 0.05$) increase in phosphorus concentration was only seen at GS21 in the group treated with oligoCpG at 0.1 pmol · µl^{-1} and at 1 pmol · µl^{-1} concentrations, respectively, when compared with the control treated group (GS21, Table 2). There were no significant changes in glucose and protein concentrations throughout the experiment for GS21 (Table 2).

The immediate effects of the applied ssDNA oligonucleotides on plant safety and health were determined through biomass loss or accumulation, leaf and root lengths, and biochemical activities over the study period. The data analyzed from the increase or decrease of

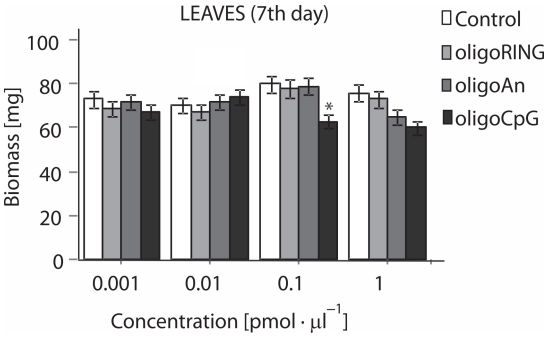

Fig. 1. The biomass of *Triticum aestivum* leaves grown at GS7. Mean ± SE are represented for 3 replicates and an asterisk (*) is marked when $p < 0.05$

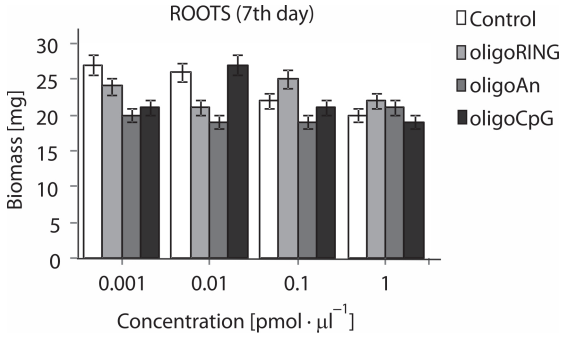

Fig. 2. The biomass of *Triticum aestivum* roots grown at GS7. Mean ± SE are represented for 3 replicates

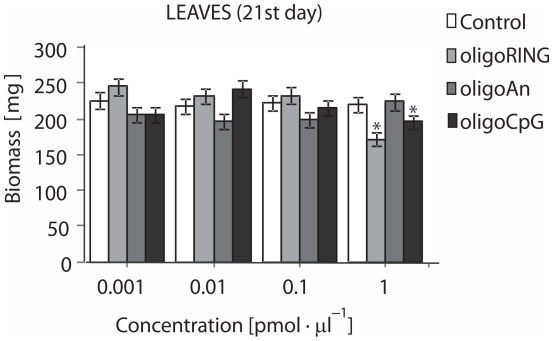

Fig. 3. The biomass of *Triticum aestivum* leaves grown at GS21. Mean ± SE are represented for 3 replicates and an asterisk (*) is marked when $p < 0.05$

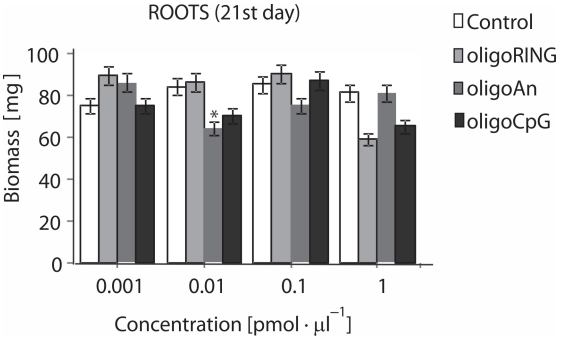

Fig. 4. The biomass of *Triticum aestivum* roots grown at GS21. Mean ± SE are represented for 3 replicates and an asterisk (*) is marked when $p < 0.05$

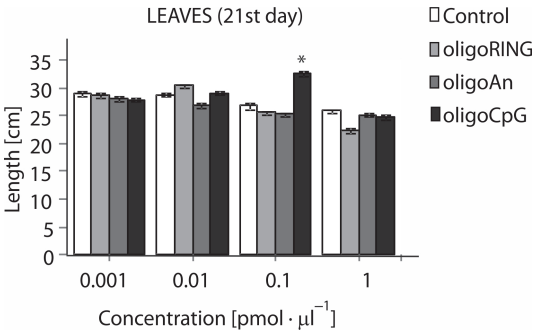

Fig. 5. The length of *Triticum aestivum* leaves grown at GS21. Mean ± SE are represented for 3 replicates and an asterisk (*) is marked when $p < 0.05$

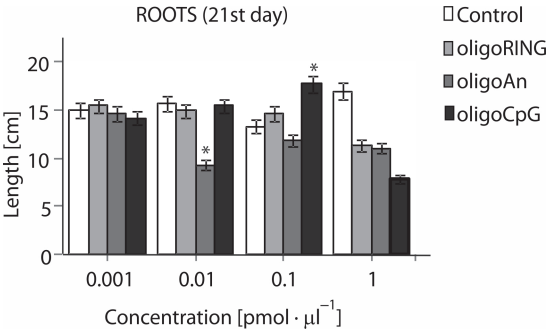

Fig. 6. The root lengths of *Triticum aestivum* grown at GS21. Mean ± SE are represented for 3 replicates and an asterisk (*) is marked when $p < 0.05$

wheat *T. aestivum* plant leaf and root biomass showed a trend of non-specific and non-harmful effects. There was an increase in biomass from the 7th day (GS7) to the 21st day (GS21) in all groups and concentrations of leaves and roots. Generally, the biomass of leaves from treated seeds with concentrations of 0.001, 0.01, 0.1 and 1 pmol · µl^{-1} of oligoRING ssDNA oligonucleotides increased by 255, 246, 199 and 136%, respectively, from the 7th day (GS7) to the 21st day (GS21). In the oligoAn ssDNA oligonucleotides group, there was an increase in the biomass of leaves from treated seed concentrations of 0.001, 0.01, 0.1 and 1 pmol · µl^{-1}, by 188, 174, 152 and 245%, respectively, from the 7th day (GS7) to the 21st day (GS21). In addition, in the oligoCpG ssDNA oligonucleotides group, the biomass of leaves from the treated seeds with concentrations of 0.001, 0.01, 0.1 and 1 pmol · µl^{-1} increased by 209, 227, 343 and 227%, respectively, from the

Table 2. Post-treatment analysis of three important biochemical parameters (concentration of glucose, protein and phosphorus) for development and survival in wheat *Triticum aestivum* plants. Mean ± SE are represented for three replicates and an asterisk (*) is marked when $p < 0.05$

S/N	Groups	Parameters [unit]	Treatment concentration [pmol · µl^{-1}]			
			0.001	0.01	0.1	1
			1 day (GS1) post-treatment			
1	Control	glucose [mmol · l^{-1}]	1.45 ± 0.14	1.09 ± 0.17	1.52 ± 0.25	1.55 ± 0.19
2	oligoRING		1.20 ± 0.18*	1.40 ± 0.31	1.90 ± 0.16	1.76 ± 0.20
3	oligoAn		1.44 ± 0.11	1.37 ± 0.19	1.60 ± 0.44	1.99 ± 0.25
4	oligoCpG		0.97 ± 0.02*	1.35 ± 0.01	1.88 ± 0.15	1.13 ± 0.22
5	Control	protein [g · l^{-1}]	0.92 ± 0.31	1.01 ± 0.27	1.41 ± 0.21	1.39 ± 0.05
6	oligoRING		1.63 ± 0.07	2.06 ± 0.33	1.53 ± 0.16	1.52 ± 0.16
7	oligoAn		2.06 ± 0.55	1.78 ± 0.26	2.02 ± 0.35	1.87 ± 0.39
8	oligoCpG		1.59 ± 0.07	1.80 ± 0.40	1.72 ± 0.05	1.56 ± 0.08
9	Control	phosphorus [mmol · l^{-1})	0.86 ± 0.23	0.74 ± 0.06	1.09 ± 0.34	1.09 ± 0.11
10	oligoRING		0.82 ± 0.11	1.11 ± 0.45	1.39 ± 0.08	1.00 ± 0.03
11	oligoAn		1.22 ± 0.10	0.80 ± 0.09	1.08 ± 0.11	1.20 ± 0.22
12	oligoCpG		0.76 ± 0.08	0.78 ± 0.09	1.15 ± 0.18	0.67 ± 0.11*
			7 days (GS7) post-treatment			
13	Control	glucose [mmol · l^{-1}]	9.86 ± 0.96	9.03 ± 0.82	10.41 ± 1.28	10.72 ± 0.26
14	oligoRING		10.55 ± 1.35	12.21 ± 1.53	9.38 ± 1.20	12.27 ± 1.11
15	oligoIAP2a		9.98 ± 0.92	12.19 ± 0.56	13.04 ± 0.13	11.68 ± 1.79
16	oligoCpG		11.66 ± 0.68	12.51 ± 0.42	11.49 ± 1.21	11.64 ± 1.24
17	Control	protein [g · l^{-1}]	2.58 ± 0.19	2.72 ± 0.22	2.90 ± 0.26	2.46 ± 0.20
18	oligoRING		3.14 ± 0.07	3.77 ± 0.12	3.44 ± 0.78	4.31 ± 0.58
19	oligoAn		4.61 ± 0.81	3.43 ± 0.13	4.35 ± 0.23	3.55 ± 0.67
20	oligoCpG		3.44 ± 0.09	4.44 ± 0.35	4.12 ± 0.33	3.64 ± 0.18
21	Control	phosphorus [mmol · l^{-1})	6.02 ± 1.34	6.32 ± 0.26	6.14 ± 0.72	5.75 ± 0.48
22	oligoRING		7.33 ± 0.37	6.48 ± 0.74	6.39 ± 0.46	6.09 ± 0.77
23	oligoIAP2a		5.48 ± 0.10	7.62 ± 0.22	6.91 ± 0.57	6.67 ± 1.28
24	oligoCpG		5.56 ± 0.58	7.00 ± 0.54	6.81 ± 0.22	5.77 ± 0.49
			21 days (GS21) post-treatment			
25	Control	glucose [mmol · l^{-1}]	0.64 ± 0.07	0.59 ± 0.08	0.68 ± 0.16	0.70 ± 0.04
26	oligoRING		0.61 ± 0.02	0.48 ± 0.04	0.68 ± 0.01	0.84 ± 0.09
27	oligoAn		0.41 ± 0.14	0.85 ± 0.19	0.48 ± 0.07	0.49 ± 0.15
28	oligoCpG		0.34 ± 0.09	0.48 ± 0.25	0.67 ± 0.07	0.47 ± 0.16
29	Control	protein [g · l^{-1}]	4.33 ± 0.56	3.51 ± 0.12	3.89 ± 0.18	4.09 ± 0.39
30	oligoRING		3.88 ± 0.21	3.66 ± 0.09	3.33 ± 0.22	4.02 ± 0.13
31	oligoAn		4.26 ± 0.66	4.36 ± 0.48	3.37 ± 0.13	3.49 ± 0.24
31	oligoCpG		4.05 ± 0.01	3.95 ± 0.13	3.60 ± 0.23	3.46 ± 0.18
33	Control	phosphorus [mmol · l^{-1}]	5.17 ± 0.30	5.69 ± 0.10	5.57 ± 0.11	5.47 ± 0.28
34	oligoRING		5.63 ± 0.07	5.76 ± 0.10	5.34 ± 0.07	5.53 ± 0.15
35	oligoAn		5.84 ± 0.29	5.66 ± 0.07	5.75 ± 0.09	5.76 ± 0.16
36	oligoCpG		5.95 ± 0.14	6.25 ± 0.17	6.61 ± 0.25*	6.56 ± 0.28*

7th day (GS7) to the 21st day (GS21) (Figs 1 and 3). The percentage change in root biomass from treated seeds with concentrations of 0.001, 0.01, 0.1 and 1 pmol · μl^{-1} from the 7th day (GS7) to the 21st (GS21) day showed increases by 270, 310, 260 and 168% in the oligoRING group, respectively. The oligoAn group increased by 330, 237, 295 and 286%, while the oligoCpG group increased by 257, 159, 314 and 242%, respectively (Figs 2 and 4). These observations indicated that the applied ssDNA oligonucleotides did not disrupt the plant mechanisms for biomass accumulation, which is an important parameter in determining plant health. DNA insecticides are specific to their target organisms, and safe for non-target organisms. However, the significant difference in the biomass of leaves and roots seen between the control and experimental groups of treated seeds on the 7th and 21st days of the study provides a basis for an extended period of investigation to ascertain the pattern and limit of biomass change.

The observed changes in lengths, and specific increase of leaf lengths by 22% (oligoCpG vs. Control) (Fig. 5), and root lengths by 33% (oligoCpG vs. Control) (Fig. 6), at 0.1 pmol · μl^{-1}, on the 21st day (GS21) suggest that DNA insecticides can be developed to enhance host plant growth and engineered to kill insect pests. Unlike most chemical insecticides that cause harm to non-target organisms and the environment, DNA insecticides are safe to non-target organisms and effective against target insects (Nyadar *et al.* 2016).

Based on these results, all concentrations were safe, and not harmful to the functions of the investigated biochemical parameters important for cell signaling, catalytic activities and other biological roles in wheat plants. The results of this analysis suggest that both oligoRING and oligoCpG ssDNA oligonucleotides induce temporary effects on glucose and phosphorus concentrations for 1 day only (GS1), and the significant increase in phosphorus concentration on the 21st day (GS21) shows that the treated plants can utilize oligoCpG ssDNA oligonucleotides at 0.1 pmol · μl^{-1} and 1 pmol · μl^{-1} concentration for plant development.

The results of the investigated biochemical parameters indicated a safe margin from the applied ssDNA oligonucleotides relative to previous studies (Oberemok *et al.* 2013). Important biomolecules, e.g. glucose, take part in supplying cell energy, development, proliferation and metabolism (Yuan *et al.* 2013; Sheen 2014). Proteins contribute to plant growth and development (Roberts *et al.* 2011; Kozuka *et al.* 2001), and phosphorus, an important macronutrient essential for some processes in plant growth, respiration, energy storage and transfer (Schatchman *et al.* 1998), was temporarily interfered with. Several studies have shown that pesticides interfere with important plant biochemical parameters. According to Chauhan *et al.* (2013) readily available pesticides, like imidacloprid (a broad spectrum insecticide), lowered ascorbic acid content, increased total protein and some enzymatic activities in potato (*Solanum tuberosum*) plants as a result of abiotic stress. This indicates that most pesticide-treated plants inadequately take up micronutrients and as a result are deficient, leading to a misbalance of important biochemical parameters. A possible interpretation of the brief interference of DNA insecticides on the treated plant biomolecules may be associated with Toll like receptors activated through CpG-rich islands (CGIs) in oligonucleotides, known for regulatory effects in both plants and animals (Imler and Zheng 2004; da Silva *et al.* 2014). Consequently, the ssDNA oligonucleotides might have affected a signal to stimulate the plant defense mechanism leading to a temporary change of investigated parameters.

Of note, this study underscores the safe nature of DNA insecticides on wheat *T. aestivum* as a model of non-target organisms, which is relative to the concentration used, indicating that low concentrations are safer for non-target plant organisms.

Conclusions

The current study found that ssDNA oligonucleotides used as DNA insecticides were generally safe for wheat *T. aestivum* seeds and seedlings. The action of the DNA insecticides based on the concentration caused temporary changes in biomass accumulation, lengths and biochemical parameters. However, there was no damage to the plant structure or its functions. Pesticide persistence is a factor to consider, because plant collection or uptake of pesticides from water, air or soil is a source of pesticide residue and phytotoxicity. Foliar application of some insecticides has been documented to adversely affect photosynthetic processes, by blocking the microscopic pores in leaves, thereby interrupting CO_2 and water vapor gas exchanges. This interruption leads to impaired photosynthetic reactions delaying plant growth, development and productivity. Some pesticides may affect several other important processes in non-target plants while not being absolutely effective against the target organism. The effect of imidacloprid was evaluated on *L. esculentum* seed germination, seedling health, strength and photosynthetic pigments by Shakir *et al.* (2016). Their results indicated reduced seed germination at the early stages of exposure, reduced plant growth, some non-specific stimulatory effects and various effects on plant pigmentation relative to the applied concentration of the pesticide. Another recent study by Bragança *et al.*

(2018) evaluated the phytotoxic impact of pyrethroid pesticides in *Cucumis sativus*. Their results showed that some pyrethroid pesticides have a negative impact on seed germination, leaf lengths and root elongation based on the applied concentrations. These observations present the sensitivity of some non-target plants of agricultural importance and contribute to expanding the understanding and development of phytotoxicity evaluation of pesticides. Our study on developing new pesticides like DNA insecticides combines most features of safe insecticidal technology. In comparison to neonicotinoids, pyrethroids, imidacloprid and other chemical insecticides, DNA insecticides are designed from insect virus inhibitors of apoptotic genes, hence their specificity to target (host-insect) organisms. Our results from laboratory studies show that DNA insecticides are safe for non-target plant organisms like *T. aestivum*. However, field trials are being considered for further assessment. The idea of DNA insecticide is tangible, as a potential resource to manage insect pest populations without harm to non-target organisms.

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