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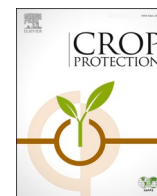


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Combinations of insecticides with carbon dioxide for the management of *Sitophilus oryzae* (Linnaeus 1763) on stored wheat: An approach to reduce the application rates of insecticides

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ABSTRACT

Efficacy of insecticides chlorpyrifos-methyl (CH), deltamethrin (DE) and spinosad (SP) was evaluated alone and in binary combinations with carbon dioxide (CA) against *Sitophilus oryzae* (L.). Mortality of *S. oryzae* adults was recorded after 3, 7 and 14 days, and progeny production and wheat weight loss were assessed after 90 days for all treatments. In individual treatments, the mortality of *S. oryzae* adults was 69.5, 79.8, 50.7 and 61.4% after 14 days of exposure to the highest concentration of CA (30%), CH (0.5 mg/kg), DE (1.0 mg/kg) and SP (0.5 mg/kg), respectively. Mortality of *S. oryzae* adults was higher in combined treatments at all tested rates than individual treatments. Mortality of adults of 100% was obtained in combined treatments of CH (0.5 mg/kg) + CA (30%) after 3 days and DE (1.0 mg/kg) + CA (30%) after 14 days of exposure. Combinations of SP + CA were more effective than CH + CA and DE + CA as 100.0% mortality was achieved in combined treatments of SP at 0.25 mg/kg with CA at 20 and 30%, and SP at 0.5 mg/kg with CA at 20 and 30% after 3 days of exposure. Progeny production of *S. oryzae* was decreased significantly in all treatments compared with untreated wheat grains after 90 days of exposure. High rates induced progeny reduction of 100% and complete protection of wheat grains against damage caused by *S. oryzae* after 90 days. Moreover, germination percentages of wheat seeds treated with CH at 0.5 mg/kg, DE at 1.0 mg/kg, CA at 30% and CH at 0.5 mg/kg + CA at 30% were 90.6, 86.7, 86.7 and 90.0, respectively. Our results indicate that the combination of insecticides with CA appears promising as an approach for the control of *S. oryzae*.

1. Introduction

Wheat is one of the most important grain crops in Egypt and worldwide (Shewry, 2007). The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), is a primary stored product insect for stored cereals, such as wheat, barley, corn and rice (Padin et al., 2002; Follett et al., 2013; Mehta et al., 2021). This weevil infests stored grains and causes excessive loss in grain weight and reduces quality and germination of grains (Gomes et al., 1983; Mebarkia et al., 2010; Saad et al., 2018).

Management of stored product insects by chemical insecticides and fumigants is a common tactic worldwide. These chemicals are applied on stored commodities as grain protectants and can protect grains for several months (Daglish et al., 1996; Golić et al., 2018; Rumbos et al.,

2018; Tsaganou et al., 2021). However, the frequent use of high application rates of these substances can be associated with an increase in resistance of insects and detrimental effects on the environment, animals and humans (Nayak et al., 2005; Daglish, 2008). Therefore, alternative management tools, such as modified atmosphere, inert dusts, and bio-pesticides, should be considered. Additionally, good stewardship of crop protectants is recommended; for example, the rotation of crop protectants with different modes of action and specific combinations with alternative management tools should be considered to reduce usage and extend the life span of insecticides.

Modified atmosphere with carbon dioxide (CA) is an environmentally friendly, economical and safe technique for stored product insect management and useful alternative to chemical fumigants used in several developed countries (Navarro, 2012; Iturralde-García et al.,

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2020). The efficacy of CA has been reported against *S. oryzae* (Annis and Morton, 1997), *Sitophilus zeamais* Motchulsky and *S. oryzae* (Carvalho et al., 2012), *S. zeamais* and *Tribolium castaneum* (Herbst) (Noomhorm et al., 2013), *Trogoderma granarium* Everts and *S. zeamais* (Vassilakos et al., 2019) and *Oryzaephilus surinamensis* (L.) (Hashem et al., 2021).

The combination of insecticides with other insect control management methods can help to reduce application rates and to increase the efficacy of insecticides at low rates against stored product insects. Insecticides such as spinosad combined with inert dusts, entomopathogenic fungi and monoterpenes (Chintzoglou et al., 2008; Vayias et al., 2009; Machekano et al., 2017; Gad et al., 2020, 2021; Abdelgaleil et al., 2021) and deltamethrin with inert dusts (Ceruti and Lazzari, 2005; Karimzadeh et al., 2021) provide higher efficacy against stored product insects than application of the insecticide alone. Recently, Gad et al. (2022a) found that treatment of cowpea seeds with two inert dusts combined with carbon dioxide (CA) was more effective against *Callosobruchus maculatus* and *C. chinensis* than individual treatments. The present study continues this approach by evaluating the efficacy of chlorpyrifos-methyl (CH) (organophosphate), deltamethrin (DE) (pyrethroid), spinosad (SP) (spinosyn, microbial-derived insecticide) alone and in binary combinations with carbon dioxide (CA) against *S. oryzae* on wheat grains. The effects of insecticides and CA on adult mortality, progeny production and damage caused by *S. oryzae* on wheat grains were studied.

2. Materials and methods

2.1. Insects

A stock culture of *S. oryzae* adults was obtained from Plant Protection Research Institute, Giza, Egypt. The insect colony has been maintained in our laboratory for over 14 years without exposure to insecticides in glass jars (13 cm diameter × 17 cm height) covered by fine mesh cloth to allow proper ventilation. The rice weevil was reared on whole wheat grains (var. Giza 168) at 28 ± 2 °C, $65 \pm 5\%$ r.h. and continuous darkness. Uninfested wheat grains (var. Giza 168) free of pesticides were obtained from Faculty of Agriculture farm, Alexandria, Egypt. For bioassays, unsexed adults (two weeks old) were used. The sieve (#12 mesh) was used to separate the weevils from wheat grains. All experiments

were carried out under the same insect rearing conditions.

2.2. Tested insecticides and carbon dioxide

Chlorpyrifos-methyl (97%) and spinosad (98%) were purchased from Dow AgroSciences LLC (Indianapolis, USA) and deltamethrin (97%) was obtained from Alexandria for Chemicals Company (Alexandria, Egypt). Modified atmosphere with carbon dioxide (CA) was provided as pure gas in pressurized steel cylinders (Alexandria Industrial Gases, Mirghem, Alexandria, Egypt) (Fig. 1) and concentrations of CA were monitored using a carbon dioxide gas analyzer model 200–600 (Gow-Mac-Instrument Company, USA), according the method described by Gad et al. (2022a).

2.3. Bioassays

The insecticides and carbon dioxide were first tested as individual treatments against *S. oryzae* adults on wheat grains. Three insecticides were tested at different low-application rates; chlorpyrifos-methyl (recommended rate = 6 mg/kg, Arthur, 1992) at 0.1, 0.25 and 0.5 mg/kg, deltamethrin (recommended rate = 1–2 mg/kg, Kavallieratos et al., 2015) at 0.2, 0.5, and 1.0 mg/kg and spinosad (recommended rate = 1.0 mg/kg, Hertlein et al., 2011) at 0.1, 0.25 and 0.5 mg/kg, while CA was tested at concentrations of 10, 20 and 30%. Acetone was used to prepare stock solutions of insecticides. The combinations of each insecticide and CA were also assessed. For each treatment, samples (50 g) of wheat grains (var. Giza 168) with moisture content of 10.9% were treated with the above insecticides. Each sample was placed in a glass jar (180 ml) and treated individually with 1 ml of stock solution of insecticide to attain final application rates as mentioned above. All jars were then manually shaken for 2 min to attain equal distribution of the insecticides on grain mass. The samples were left for 30 min to ensure complete evaporation of the solvent. An additional series of samples were treated with 1 ml acetone alone and served as control. Thirty adults of *S. oryzae* were then released in each jar either in the individual treatments or in the combined treatments with CA (Gad et al., 2022b). The jars prepared for combined treatments of insecticides with CA and CA alone were transferred to Dreshel flasks (glass bottle of 3 L capacity) and treated with concentrations of CO₂ gas (10, 20 and 30%). After

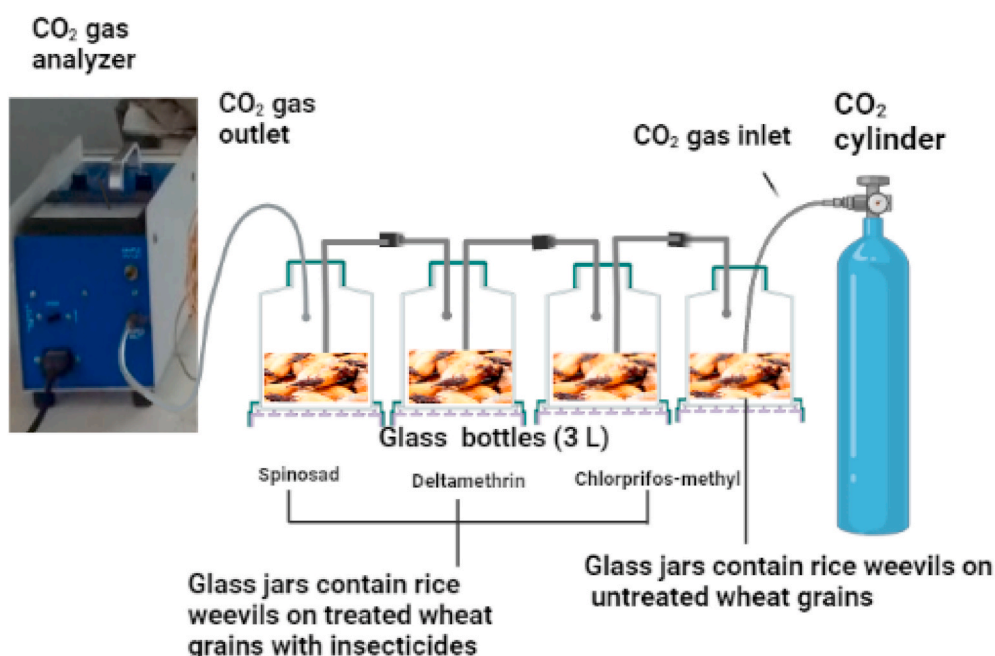


Fig. 1. Schematic diagram of CO₂ treatment setup.

achieving these CO₂ concentrations at gas outlet, the flasks were kept tightly sealed for 48 h, and the flasks were then opened and aerated. The flasks were covered with fine mesh cloth to prevent insect escape and to maintain appropriate ventilation until the end of experiments. All jars were then placed in incubators at 28±1 °C and 65 ± 5% r.h. Twelve individual and 27 combined treatments are described in Table 1. Three replicates were used for each treatment, with one flask of 30 adults for each replicate. The experiment was repeated independently twice. The mortality of *S. oryzae* adults was recorded after 3, 7 and 14 days of exposure. Adults (dead and alive) were then removed from wheat grains after 14 days. Jars were left at the same conditions for an additional period of 90 days. Progeny numbers were then counted and the following formula was used to determine the reduction (%) in the number of progeny after 90 days:

$$\% = (1 - x/y) \times 100$$

where x = the number of adults emerging in the treatment; y = the number of adults emerging in the control.

The treated and untreated wheat grains were sieved and the powder was discarded. The weight of remaining wheat grains in treatments and control was recorded to obtain the weight loss percentages after 90 days. The weight loss percentage was calculated from the following formula:

$$\% \text{ weight loss} = ((W_u - W_i) / W_u) \times 100$$

where W_u = weight of uninfested wheat seeds; W_i = weight of infested wheat seeds of control and treatment.

The germination of wheat grains treated with highest rates of each insecticide, CA and their combinations was tested after 90 days of treatment. One hundred seeds with four replicates from each treatment were placed in glass Petri dishes lined with moistened filter papers and kept for 8 days. The numbers of germinated seeds were recorded and germination percentages (GP) were calculated from this equation:

$$GP = g/t \times 100$$

where g = number of germinated wheat seeds; t = total number of wheat seeds.

2.4. Data analysis

Statistical analysis was conducted by using the software SPSS 21.0 (SPSS, Chicago, IL, USA). The percentages of adult mortality and wheat weight loss and seed germination were arcsine transformed prior to analysis. All data were submitted to one-way analysis of variance (ANOVA). Mean separations were performed by Tukey's HSD test at a significance level < 0.05.

3. Results

3.1. Effect of treatments on mortality of *S. oryzae* adults

The effects of individual and combined treatments of insecticides and

CA on the mortality of *S. oryzae* adults are shown in Tables 2–4. The mortality of *S. oryzae* adults was 56.8 and 69.5% at 3 and 14 days after exposure to the highest concentration of CA (30%), respectively. For individual treatments with insecticides at the highest application rates of CH (0.5 mg/kg), DE (1.0 mg/kg) and SP (0.5 mg/kg), the mortalities of adults were 34.2, 22.2 and 12.9% at 3 days after exposure and increased to 79.8, 50.7 and 61.4% at 14 days after exposure, respectively. In general, all combinations of DE with CA increased adult mortality significantly than DE alone 14 days after treatment. Similarly, combinations of SP with CA at 20 and 30% were significantly more toxic than SP alone to *S. oryzae* adults 14 days after treatment. Complete mortality of *S. oryzae* adults (100.0%) was achieved with CH at 0.5 mg/kg and DE at 1.0 mg/kg with 30% of CA at 3 and 14 days after exposure, respectively (Tables 2 and 3). Complete mortality was also achieved in the combinations of SP at 0.25 mg/kg with 20 or 30% of CA at 3 days after exposure (Table 4).

3.2. Effect of treatments on progeny production of *S. oryzae*

All individual and combined treatments of the three insecticides (CH,

Table 2

Mean mortality (%±SE) of *Sitophilus oryzae* adults after exposure for 3, 7 and 14 days to wheat grains treated with chlorpyrifos-methyl (CH), carbon dioxide (CA) and their combinations at different application rates.

Treatment CH (mg/kg), CA (%)	Adult mortality (% ± SE)		
	3 days	7 days	14 days
Control (0.0)	0.0 ± 0.0e	1.1 ± 0.8g	3.3 ± 0.1f
CH (0.1)	27.9 ± 0.2d	55.3 ± 1.9def	64.8 ± 4.8bcde
CH (0.25)	31.8 ± 5.2d	62.3 ± 6.4bcdef	69.1 ± 4.1bcde
CH (0.5)	34.2 ± 0.6d	70.2 ± 0.1bcdef	79.8 ± 0.6bcde
CA (10)	25.6 ± 0.8d	33.3 ± 1.4f	44.4 ± 2.0e
CA (20)	42.2 ± 1.6cd	46.7 ± 2.7ef	54.4 ± 2.1de
CA (30)	56.8 ± 1.0bcd	60.3 ± 2.6cdef	69.5 ± 2.5bcde
CH (0.1)+ CA (10)	30.8 ± 0.8d	56.3 ± 2.0def	61.5 ± 1.8cde
CH (0.25)+ CA (10)	39.6 ± 1.0cd	77.0 ± 0.5bcde	84.4 ± 0.2abcd
CH (0.5)+ CA (10)	33.6 ± 0.2d	77.6 ± 3.9bcde	84.7 ± 0.5abcd
CH (0.1)+ CA (20)	57.7 ± 1.5bcd	81.1 ± 3.7bcde	85.6 ± 4.2abcd
CH (0.25)+ CA (20)	81.6 ± 8.5 ab	86.4 ± 6.9abcd	90.3 ± 5.0abc
CH (0.5)+ CA (20)	85.2 ± 5.3 ab	90.3 ± 4.1abc	91.8 ± 4.3 ab
CH (0.1)+ CA (30)	79.9 ± 6.0bc	84.6 ± 6.8abcd	85.6 ± 6.2abcd
CH (0.25)+ CA (30)	84.8 ± 5.5 ab	91.8 ± 3.7 ab	92.5 ± 3.8 ab
CH (0.5)+ CA (30)	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
F	20.4	17.9	17.4
P	<0.01	<0.01	<0.01

Mean values in each column with different letter(s) are significantly different ($P < 0.05$, $df = 15, 32$).

Table 1

Concentrations of chlorpyrifos-methyl (CH), deltamethrin (DE), spinosad (SP) and CO₂ (CA) used in this study.

Chlorpyrifos-methyl (CH) (mg/kg)	Deltamethrin (DE) (mg/kg)	Spinosad (SP) (mg/kg)	CO ₂ (CA) (%)	Chlorpyrifos-methyl + CO ₂ (CH+CA)	Deltamethrin + CO ₂ (DE+CA)	Spinosad + CO ₂ (SP+CA)
0.1	0.2	0.1	10	0.1 + 10	0.2 + 10	0.1 + 10
0.25	0.5	0.25	20	0.25 + 10	0.5 + 10	0.25 + 10
0.5	1.0	0.5	30	0.5 + 10	1.0 + 10	0.5 + 10
				0.1 + 20	0.2 + 20	0.1 + 20
				0.25 + 20	0.5 + 20	0.25 + 20
				0.5 + 20	1.0 + 20	0.5 + 20
				0.1 + 30	0.2 + 30	0.1 + 30
				0.25 + 30	0.5 + 30	0.25 + 30
				0.5 + 30	1.0 + 30	0.5 + 30

Table 3

Mean mortality (%±SE) of *Sitophilus oryzae* adults after exposure for 3, 7 and 14 days to wheat grains treated with deltamethrin (DE), carbon dioxide (CA) and their combination at different application rates.

Treatment DE (mg/kg), CA (%)	Adult mortality (% ± SE)		
	3 days	7 days	14 days
Control (0.0)	0.0 ± 0.0f	1.1 ± 0.8h	3.3 ± 0.1f
DE (0.2)	7.9 ± 0.6e	8.0 ± 0.5gh	44.9 ± 2.4e
DE (0.5)	13.0 ± 0.4e	22.5 ± 1.2 fg	45.6 ± 1.4e
DE (1.0)	22.2 ± 1.0de	40.0 ± 0.2ef	50.7 ± 0.5e
CA (10)	25.6 ± 0.8de	33.3 ± 1.4ef	44.4 ± 2.0e
CA (20)	42.2 ± 1.6cd	46.7 ± 2.7ef	54.4 ± 2.1e
CA (30)	56.8 ± 1.0c	60.3 ± 2.6de	69.5 ± 2.5de
DE (0.2)+ CA (10)	39.8 ± 5.3cd	75.6 ± 5.9cd	83.5 ± 3.9cd
DE (0.5)+ CA (10)	59.1 ± 1.5c	77.3 ± 0.2bcd	84.2 ± 3.6cd
DE (1.0)+ CA (10)	63.7 ± 2.6bc	78.8 ± 3.1bcd	89.2 ± 2.9bcd
DE (0.2)+ CA (20)	84.3 ± 0.2 ab	90.9 ± 0.8abc	95.8 ± 0.4abc
DE (0.5)+ CA (20)	85.8 ± 2.1 ab	92.8 ± 0.6abc	95.9 ± 0.6abc
DE (1.0)+ CA (20)	93.9 ± 2.9a	96.5 ± 1.8a	97.2 ± 1.9 ab
DE (0.2)+ CA (30)	88.2 ± 4.6a	93.5 ± 2.6 ab	96.9 ± 2.2 ab
DE (0.5)+ CA (30)	89.8 ± 0.7a	94.9 ± 0.7 ab	97.0 ± 0.1 ab
DE (1.0)+ CA (30)	94.3 ± 1.8a	98.4 ± 1.2a	100.0 ± 0.0a
F	59.2	61.9	54.0
P	<0.01	<0.01	<0.01

Mean values in each column with different letter(s) are significantly different (P < 0.05, df = 15, 32).

Table 4

Mean mortality (%±SE) of *Sitophilus oryzae* adults after exposure for 3, 7 and 14 days to wheat grains treated with spinosad (SP), carbon dioxide (CA) and their combinations at different application rates.

Treatment SP (mg/kg), CA (%)	Adult mortality (% ± SE)		
	3 days	7 days	14 days
Control (0.0)	0.0 ± 0.0g	1.1 ± 0.8g	3.3 ± 0.1g
SP (0.1)	4.5 ± 0.2f	9.0 ± 0.4f	20.9 ± 0.6f
SP (0.25)	10.6 ± 0.4f	14.3 ± 0.4f	38.9 ± 4.0ef
SP (0.5)	12.9 ± 1.4f	30.9 ± 2.1e	61.4 ± 4.9cde
CA (10)	25.6 ± 0.8e	33.3 ± 1.4e	44.4 ± 2.0e
CA (20)	42.2 ± 1.6cd	46.7 ± 2.7de	54.4 ± 2.1de
CA (30)	56.8 ± 1.0c	60.3 ± 2.6cd	69.5 ± 2.5cd
SP (0.1)+ CA (10)	35.8 ± 2.2de	63.7 ± 1.3c	72.2 ± 1.1cd
SP (0.25)+ CA (10)	42.1 ± 6.7cd	72.5 ± 5.2c	79.2 ± 2.8c
SP (0.5)+ CA (10)	42.7 ± 2.6cd	74.3 ± 2.1c	79.5 ± 2.6bc
SP (0.1)+ CA (20)	82.5 ± 0.2b	89.1 ± 0.2b	95.1 ± 0.5 ab
SP (0.25)+ CA (20)	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
SP (0.5)+ CA (20)	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
SP (0.1)+ CA (30)	85.5 ± 0.8b	90.6 ± 0.2b	96.2 ± 1.6 ab
SP (0.25)+ CA (30)	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
SP (0.5)+ CA (30)	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
F	308.2	223.5	84.9
P	<0.01	<0.01	<0.01

Mean values in each column with different letter(s) are significantly different (P < 0.05, df = 15, 32).

DE and SP) and CA significantly decreased progeny of *S. oryzae* compared with untreated wheat grains (393.3 ± 6.2) 90 days after exposure (Table 5). Complete progeny inhibition of *S. oryzae* was recorded in combinations of 0.5 mg/kg of CH + 30% of CA (0.0 ± 0.0), 1.0 mg/kg of DE + 30% of CA (0.6 ± 0.2) and 0.5 mg/kg of SP + 30% of CA (0.0 ± 0.0).

3.3. Effect of treatments on wheat grain loss caused by *S. oryzae*

All individual and combined treatments of three insecticides and CA significantly reduced weight loss and damage of treated wheat grains after 90 days compared with untreated grains (28.1%) except treatments with SP at 0.1 and 0.25 mg/kg (Table 6). The combination of insecticides and CA at the highest application rates were more efficient in protection of treated wheat grains than each insecticide alone or CA

alone. The full protection of wheat grains with 0.0% weight loss was obtained in the mixtures of CH (0.5 mg/kg) + CA (30%), DE (1.0 mg/kg) + CA (30%), SP (0.25 mg/kg) + CA (20%), SP (0.5 mg/kg) + CA (20%), SP (0.25 mg/kg) + CA (30%) and SP (0.5 mg/kg) + CA (30%).

3.4. Effect of treatments on wheat seed germination

Percentages of wheat grain germination treated with the individual and binary mixtures of insecticides (CH, DE and SP) and CA at highest rates are shown in Fig. 2. Treatments with CH (0.5 mg/kg), DE (1.0 mg/kg), CA (30%) and CH (0.5 mg/kg) + CA (30%) caused significant reduction in germination of wheat grains, while other treatments had no or slight effect on germination.

4. Discussion

The insecticidal efficacy of insecticides (chlorpyrifos-methyl, deltamethrin and spinosad), and carbon dioxide alone against stored product insects has been reported by several researchers (Carvalho et al., 2012; Noomhorm et al., 2013; Athanassiou and Kavallieratos, 2014; Golić et al., 2018; Vassilakos et al., 2019; Karimzadeh et al., 2021). However, this is the first study on the effectiveness of combinations between these insecticides and CA for the control of *S. oryzae* on stored wheat. Our results demonstrated that treatment of wheat grains with three insecticides (CH, DE and SP) and CA alone induced moderate adult mortality of *S. oryzae*, but the combinations of insecticides and CA at the highest tested doses caused strong adult mortality. The full adult mortality was achieved in the highest combinations of CH or SP with CA. Although there are no reported studies on the efficacy of the combinations of tested insecticides with CA against stored product insects, the combination of the insecticides with other control methods, such as inert dusts and natural products increased the potential of insecticides when applied at low rates for the control several stored product insects (Chintzoglou et al., 2008; Vayias et al., 2009; Gad et al., 2020; Karimzadeh et al., 2021). Other studies have reported on the efficacy of combinations of natural products or natural methods, such as inert dusts and CA, against stored grain insects. For instance, El-Lakwah et al. (2001) demonstrated that the combination of diatomaceous earth (2000 mg/kg) + CA (30%) induced full mortality of *Rhyzopertha dominica* (F.) on wheat grains while single treatment with diatomaceous earth and CA caused 76.0 and 78.0% mortality, respectively, after 5 days of exposure. El-Lakwah and Gharib (2005) showed that the binary combinations of diatomaceous earth (0.3 and 0.6% w/w) and CA (30, 60 and 80%), increased mortalities of *S. oryzae*, *R. dominica*, *Tribolium castaneum* (Herbst) adults and *T. granarium* larvae compared to separate treatments. Likewise, Gad et al. (2022a) reported that the combined treatment of zeolite (1000 mg/kg) and CA (20%) caused complete mortality of *Callosobruchus maculatus* (F.) and *C. chinensis* L. adults after 3 days of exposure. The strong insecticidal activity of the combined insecticides with CA treatments observed here may be due to the joint toxic action between insecticides and CA. The insecticides cause their toxic effect by interaction with nervous system of insects, mainly on nerve pulse transmission (Tan et al., 2005; Bajracharya, 2013; Mangas et al., 2017). Moreover, CA causes an anesthetic effect directly on the nervous system and pure CA has an inhibitory effect on the bioelectrical responses of the nervous system and induces depolarization of the neurons (Clark and Eaton, 1983; Nicolas and Sillans, 1989). It has also been suggested that exposure to high carbon dioxide atmospheres causes insect death as the result of desiccation and exhaustion of triglyceride energy reserves rather than by the prolonged narcotic effect of anaesthesia, or the accumulation of toxic end products (Ofuya and Reichmuth, 2002). However, Janmaat et al. (2001) argued that heavy narcotic and metabolic effects occurring at high CO₂ concentrations are more important determinants of insect mortality.

The current study also revealed that the progeny production of *S. oryzae* decreased significantly in all individual and binary treatments

Table 5

Mean progeny production (No. of adults \pm SE) and reduction (%) of *Sitophilus oryzae* after 90 days of exposure to wheat grains treated with chlorpyrifos-methyl (CH), deltamethrin (DE), spinosad (SP), carbon dioxide (CA) and their combinations at different application rates.

Treatment CH (mg/kg), CA (%)	Progeny production (\pm SE)		Treatment DE (mg/kg), CA (%)	Progeny production (\pm SE)		Treatment SP (mg/kg), CA (%)	Progeny production (\pm SE)	
	No. progeny / 50g	PR (%)		No. progeny / 50g	PR (%)		No. progeny / 50g	PR (%)
Control (0.0)	393.3 \pm 6.2a	0.0	Control (0.0)	393.3 \pm 6.2a	0.0	Control (0.0)	393.3 \pm 6.2a	0.0
CH (0.1)	96.4 \pm 2.4b	75.4	DE (0.2)	126.7 \pm 1.2b	72.2	SP (0.1)	176.7 \pm 6.2b	61.2
CH (0.25)	84.3 \pm 7.5bc	78.5	DE (0.5)	121.6 \pm 4.7b	73.3	SP (0.25)	166.6 \pm 6.3bc	63.4
CH (0.5)	24.7 \pm 1.4ghi	93.	DE (1.0)	100.0 \pm 8.2bc	78.0	SP (0.5)	133.3 \pm 2.4cd	70.7
CA (10)	104.3 \pm 3.1b	73.5	CA (10)	104.3 \pm 3.1bc	73.5	CA (10)	104.3 \pm 3.1de	73.5
CA (20)	77.3 \pm 2.8bcde	80.3	CA (20)	77.3 \pm 2.8cd	80.3	CA (20)	77.3 \pm 2.8ef	80.3
CA (30)	60.0 \pm 7.1cdef	84.7	CA (30)	60.0 \pm 7.1de	84.7	CA (30)	60.0 \pm 7.1fg	84.7
CH (0.1)+ CA (10)	78.7 \pm 5.5bcd	79.9	DE (0.2)+ CA (10)	38.7 \pm 1.4ef	90.2	SP (0.1)+ CA (10)	96.7 \pm 6.7def	75.4
CH (0.25)+ CA (10)	41.7 \pm 9.6fgh	89.4	DE (0.5)+ CA (10)	25.0 \pm 2.0fg	93.6	SP (0.25)+ CA (10)	83.7 \pm 5.7ef	78.7
CH (0.5)+ CA (10)	39.0 \pm 0.4fgh	90.1	DE (1.0)+ CA (10)	16.0 \pm 0.7fg	95.9	SP (0.5)+ CA (10)	75.3 \pm 7.0ef	80.8
CH (0.1)+ CA (20)	50.0 \pm 2.0defg	87.3	DE (0.2)+ CA (20)	38.0 \pm 0.8ef	90.3	SP (0.1)+ CA (20)	29.0 \pm 7.2gh	92.6
CH (0.25)+ CA (20)	46.0 \pm 2.1efg	92.4	DE (0.5)+ CA (20)	16.7 \pm 0.6fg	98.6	SP (0.25)+ CA (20)	2.0 \pm 0.8h	99.5
CH (0.5)+ CA (20)	10.7 \pm 0.9hi	97.3	DE (1.0)+ CA (20)	15.0 \pm 0.4fg	96.2	SP (0.5)+ CA (20)	0.7 \pm 0.2h	99.8
CH (0.1)+ CA (30)	52.3 \pm 1.7cdefg	86.7	DE (0.2)+ CA (30)	13.3 \pm 0.6fg	96.6	SP (0.1)+ CA (30)	9.6 \pm 3.1h	97.5
CH (0.25)+ CA (30)	23.0 \pm 1.6ghi	94.2	DE (0.5)+ CA (30)	5.0 \pm 3.5g	98.7	SP (0.25)+ CA (30)	0.0 \pm 0.0h	100.0
CH (0.5)+ CA (30)	0.0 \pm 0.0i	100.0	DE (1.0)+ CA (30)	0.6 \pm 0.2g	99.8	SP (0.5)+ CA (30)	0.0 \pm 0.0h	100.0
F	212.5			337.5			161.1	
P	<0.01			<0.01			<0.01	

Mean values in each column with different letter(s) are significantly different ($P < 0.05$, $df = 15, 32$), PR = Progeny reduction.

Table 6

Mean weight loss (% \pm SE) of wheat grains after 90 days of treatment with chlorpyrifos-methyl (CH), deltamethrin (DE), spinosad (SP), carbon dioxide (CA) and their combinations at different application rates.

Treatment CH (mg/kg), CA (%)	Weight loss (%) \pm SE)	Treatment DE (mg/kg), CA (%)	Weight loss (%) \pm SE)	Treatment SP (mg/kg), CA (%)	Weight loss (%) \pm SE)
Control (0.0)	28.1 \pm 0.7a	Control (0.0)	28.1 \pm 0.7a	Control (0.0)	28.1 \pm 0.7a
CH (0.1)	12.3 \pm 0.9b	DE (0.2)	12.7 \pm 0.5b	SP (0.1)	20.7 \pm 1.1 ab
CH (0.25)	5.2 \pm 0.9de	DE (0.5)	12.3 \pm 1.0b	SP (0.25)	19.7 \pm 0.4abc
CH (0.5)	2.3 \pm 0.1def	DE (1.0)	9.7 \pm 0.7bc	SP (0.5)	14.6 \pm 0.6bcd
CA (10)	11.1 \pm 1.1bc	CA (10)	11.1 \pm 1.1b	CA (10)	11.1 \pm 1.1bcde
CA (20)	6.1 \pm 0.4cd	CA (20)	6.1 \pm 0.4cd	CA (20)	6.1 \pm 0.4def
CA (30)	5.1 \pm 0.9de	CA (30)	5.1 \pm 0.9de	CA (30)	5.1 \pm 0.9ef
CH (0.1)+ CA (10)	5.6 \pm 1.2de	DE (0.2)+ CA (10)	4.8 \pm 0.1def	SP (0.1)+ CA (10)	9.7 \pm 0.7cde
CH (0.25)+ CA (10)	2.9 \pm 0.6def	DE (0.5)+ CA (10)	1.9 \pm 0.1efg	SP (0.25)+ CA (10)	7.7 \pm 1.7def
CH (0.5)+ CA (10)	2.7 \pm 0.1def	DE (1.0)+ CA (10)	1.4 \pm 0.3gh	SP (0.5)+ CA (10)	8.2 \pm 1.7def
CH (0.1)+ CA (20)	3.3 \pm 0.1def	DE (0.2)+ CA (20)	2.6 \pm 0.6defg	SP (0.1)+ CA (20)	2.1 \pm 0.5 fg
CH (0.25)+ CA (20)	2.9 \pm 0.1def	DE (0.5)+ CA (20)	1.8 \pm 0.1efg	SP (0.25)+ CA (20)	0.0 \pm 0.0g
CH (0.5)+ CA (20)	1.3 \pm 0.2f	DE (1.0)+ CA (20)	1.7 \pm 0.1 fg	SP (0.5)+ CA (20)	0.0 \pm 0.0g
CH (0.1)+ CA (30)	2.8 \pm 0.7def	DE (0.2)+ CA (30)	0.8 \pm 0.1ghi	SP (0.1)+ CA (30)	1.5 \pm 1.0g
CH (0.25)+ CA (30)	1.8 \pm 0.1ef	DE (0.5)+ CA (30)	0.3 \pm 0.2hi	SP (0.25)+ CA (30)	0.0 \pm 0.0g
CH (0.5)+ CA (30)	0.0 \pm 0.0g	DE (1.0)+ CA (30)	0.0 \pm 0.0i	SP (0.5)+ CA (30)	0.0 \pm 0.0g
F	40.3		74.8		42.0
P	<0.01		<0.01		<0.01

Mean values in each column with different letter(s) are significantly different ($P < 0.05$, $df = 15, 32$).

of insecticides and CA compared with untreated wheat grains after 90 days of exposure. Moreover, progeny of *S. oryzae* was strongly suppressed at the higher rates of combined treatments of insecticides and

CA. Several studies reported the effect of individual treatments of insecticides on the progeny production of stored product insects (Daglish et al., 1996; Huang and Subramanyam 2007; Kavallieratos et al., 2015; Dissanayaka et al., 2020). Likewise, the effect of CA on progeny production of stored product insects has previously been studied. For instance, Carli et al. (2010) examined the effect of CA on the progeny production of *Sitophilus weevils* and noted that treatment with CA at 80% induced complete inhibition of progeny after 30 days of exposure. Vassilakos et al. (2019) showed that exposure of *S. zeamais* adults to CA at a concentration of 70% for 4 days caused complete inhibition of progeny. Also, Ingabire et al. (2021) found that an increase in concentration of CA to (40%) decreased the progeny of *C. maculatus* to 0.2 beetles per treatment compared to 17.5 beetles in control after 30 days of treatments. Gad et al. (2022a) found that the binary combinations of 1000 mg/kg of zeolite or kaolin and CA (20%) caused complete progeny inhibition of *C. chinensis* after 60 days. The current study also indicated that full progeny inhibition of *S. oryzae* was obtained in the treatments with the highest application rates of binary combinations of tested insecticides and CA after 90 days. This complete inhibition of progeny production of *S. oryzae* is due to the high rate of adult mortality achieved by these combined treatments leading to no offspring production (Arthur, 1996; Athanassiou et al., 2005).

The binary mixtures of insecticides and CA at the rates of 0.5 mg/kg for CH and SP or 1.0 mg/kg for DE with 30% for CA were more efficacious in protection of treated wheat grains from loss and damage caused by *S. oryzae* for 90 days than the single treatments with insecticides or CA. Also, these treatments had little impact on seed germination. These results were supported by several reports indicated that combinations of insecticides and CA have potential to protect stored cereals and legumes against damage caused by stored product insects (Chintzoglou et al., 2008; Vayias et al., 2010; Shivaraja et al., 2013; Dissanayaka et al., 2020; Gad et al., 2022a).

The use of conventional insecticides for the management of stored product insects has advantages, including quick toxic action against target insects and high reduction of progeny and consequently satisfactory protection of treated commodities. However, drawbacks of conventional insecticides include the development of resistance, toxic residues in treated commodities and environmental pollution. On the other hand, modified atmosphere with carbon dioxide is non-toxic to non-target organisms, leaves no toxic residues in treated commodities and has less harmful impact in the environment. However, the effects of CA are slower than those of conventional insecticides and are effective

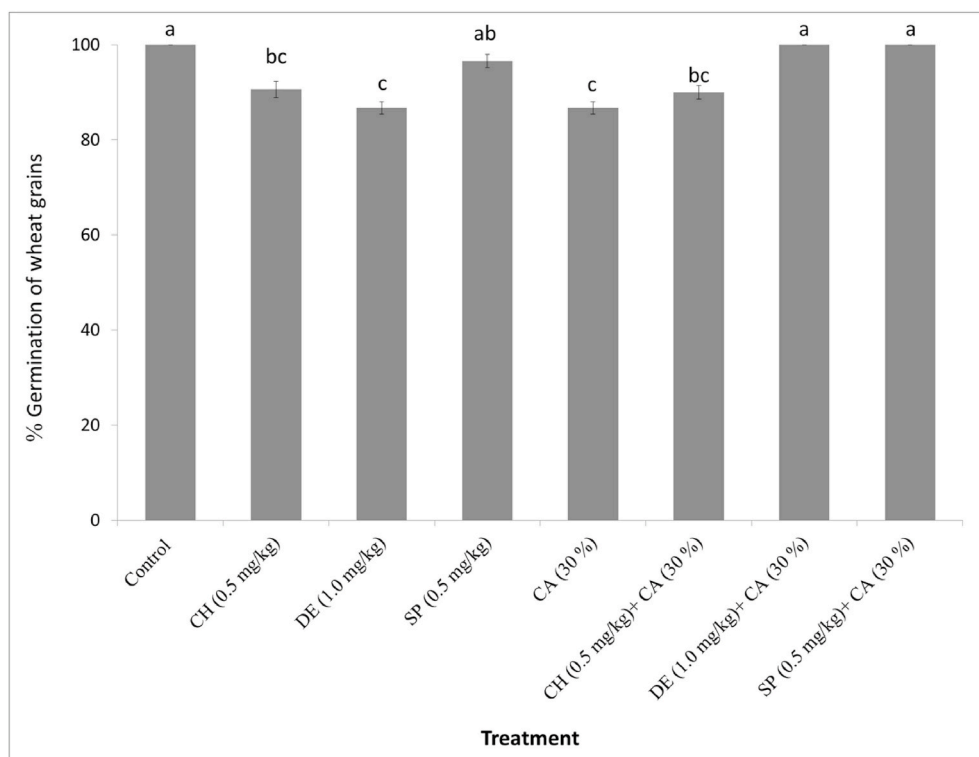


Fig. 2. Effect of individual and binary treatments with chlorpyrifos-methyl (CH), deltamethrin (DE), spinosad (SP) and carbon dioxide (CA) on germination of wheat grains after 90 days ($F = 22.3$; $P < 0.01$; $df = 7, 16$).

only at high concentrations. Therefore, the combination of insecticides with CA as shown in this study increases the insecticidal activity and gives satisfactory control of *S. oryzae* in stored wheat at lower application rates of insecticides. These findings indicate that the use of insecticide/CA combinations has potential to decrease application rates, commodity contaminations and pollution.

In conclusion, the effectiveness of insecticides (chlorpyrifos-methyl, deltamethrin and spinosad) at low application rates was significantly increased when combined with carbon dioxide against *S. oryzae*. Therefore, treatments with binary mixtures of insecticides and CA could be successfully used for the protection of stored wheat against *S. oryzae* for 90 days. However, more work is needed at a larger scale to validate these results and for the approach to be considered as an alternative tool within IPM strategies for the control of this insect pest.

Author contributions

Mohamed S. Al-Anany; Methodology, Investigation, Data curation; Formal analysis. **Hassan A. Gad**; Conceptualization; Data curation; Methodology; Investigation, Writing - original draft. **Sara E. El-Deeb**; Methodology, Investigation, Data curation; Formal analysis. **Samir A. M. Abdelgaleil**; Conceptualization; Validation; Visualization; Writing - original draft; Writing - review and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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