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Effectiveness of two inert dusts in conjunction with carbon dioxide for the control of *Callosobruchus maculatus* and *C. chinensis* in stored cowpea seeds

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ABSTRACT

The insecticidal potential of two inert dusts, zeolite (ZE) and kaolin (KA), and carbon dioxide (CA) and their binary combinations was evaluated against *Callosobruchus maculatus* (F.) and *C. chinensis* L. (Coleoptera: Chrysomelidae: Bruchinae). The inert dusts were applied on cowpea seeds at 100, 500 and 1000 mg/kg, and CA was tested at 10 and 20% for 12 h. The adult mortality was counted after 1, 3 and 7 days of treatment, and progeny production and weight loss of cowpea seeds were examined after 60 days. The adult mortality of both insect species exposed to cowpeas treated with individual and binary treatments increased with the increase of concentration and exposure time. In general, the adult mortality was higher in binary treatments than individual treatments. The full adult mortality (100.0%) was observed in combined treatment (ZE (1000 mg/kg) + CA (20%)) after 1 and 3 days in the case of *C. chinensis* and after 3 days in the case of *C. maculatus*. Similarly, the combined treatments of CA (20%) with three rates of ZE (100, 500 and 1000 mg/kg), and CA (20%) with three rates of KA (100, 500 and 1000 mg/kg) induced 100% mortality of both insect species after 7 days except for CA (20%) + KA (100 mg/kg) against *C. chinensis*. Progeny of *C. maculatus* and *C. chinensis* was significantly reduced in individual and binary treatments compared with untreated cowpea seeds. The highest application rates of the binary mixtures of inert dusts with CA induced strong progeny reduction and complete protection of cowpea seeds against damage caused by both insect species for 60 days. Our results indicated that the binary treatments of inert dusts (ZE and KA), with CA seems to be a promising alternative to synthetic insecticides for the control of bruchid insects on stored legumes.

1. Introduction

Cowpea is a major source of food in Africa and different regions around the world. It is very important legume for human and animal, because it is a cheap source of proteins (Langyintuo et al., 2003; Diouf, 2011; Murdock et al., 2012). *Callosobruchus maculatus* (F.) and *C. chinensis* L. (Coleoptera: Chrysomelidae: Bruchinae) are key insect pests of stored cowpea and other grain legumes. These insects attack the stored legumes and cause damage and loss in quality and quantity of seeds as well as reduce seed germination, particularly at heavy infestation (Sharma, 1984; Edde and Amatobi, 2003; Lopes et al., 2018). The control of *C. maculatus* and *C. chinensis* and other stored product insects is regularly based on the application of synthetic insecticides. However, increasing public awareness about the risks of these chemicals on human health and environment as well as the outbreak of insect resistance encouraged the search for new management tools (Daglish, 2008). Thus, alternative strategies have been examined and used for insect management in stored grains, such as modified atmospheres, ozone fumigation, inert dusts and natural products (Divya et al., 2016; Gad et al., 2021a,b).

Modified atmosphere with carbon dioxide gas treatment is a safe and environmentally friendly method of insect control in a large number of raw and manufactured products. In several developed countries, carbon dioxide has been adopted as feasible alternative to methyl bromide (Fleurat-Lessard, 1990). The insecticidal efficacy of carbon dioxide (CA) against bruchid insects has been documented in several studies (Ofuya and Reichmuth, 1994, 2002; Cheng et al., 2013; Pascua et al., 2021).

Inert dusts are commonly used as grain protectants against stored product insects. These inert materials, such as diatomaceous earths (DE),

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natural zeolite (ZE) and kaolin (KA) are nontoxic and safe for humans and animals (Arnaud et al., 2005; Losic and Korunic, 2018; Ziaee et al., 2019). Several studies indicated that the inert dusts were very effective against stored product insects when applied at high rates (>1000 mg/kg) (Golob, 1997; Korunic, 1998). But application of the inert dusts at higher rates negatively affected on bulk density and the physical properties of grains (Korunic et al., 1996). To overcome these problems, it has been suggested that the use of inert dusts in combination with other insect management tools or products. This approach decreases application rates and increases the efficacy of inert dusts against stored product insects. The use of inert dusts combined with other methods, such as bioinsecticides (Athanassiou et al., 2016; Abdelgaleil et al., 2021; Ali et al., 2021; Wakil et al., 2021), low and high temperature (Rojht et al., 2010; Karimzadeh et al., 2020) and modified atmosphere (El-Lakwah et al., 2001; El-Lakwah and Gharib, 2005) has been reported. These combinations have higher effectiveness against stored product insects than the use of individual methods. However, no information is available on the effectiveness of combinations between inert dusts and carbon dioxide (CA) against *C. maculatus* and *C. chinensis*. Therefore, the aim of this study was to investigate the efficacy of two inert dusts, (ZE and KA), carbon dioxide (CA) and their binary mixtures against *C. maculatus* and *C. chinensis* in stored cowpea seeds under laboratory condition. The effect of inert dusts and CA and their combinations on adult mortality, progeny production and damage in cowpea seeds caused by both insect species were assessed.

2. Materials and methods

2.1. Insects and culture maintaining

Adults of *Callosobruchus maculatus* and *C. chinensis* were obtained from cultures reared for several generations on cowpea seeds (*Vigna unguiculata* var. Dokki 331) with 9.7% moisture content under laboratory conditions at 28 ± 1 °C with 65 ± 5% RH and a photoperiod of 12:12 h light-dark in 1-L wide-mouth glass jars. The cowpea seeds were frozen at −20 °C for 7 days to avoid possible previous infestations. All experiments were conducted using sexed 1–2 days old adults.

2.2. Tested inert dusts (ZE and KA)

The two inert dusts, natural zeolite (ZE) and kaolin (KA), used in this study were purchased from Al-Ahram Mining Co. (Giza, Egypt). The ZE and KA were characterized at the Central Laboratory (XRF LAB), Egyptian Mineral Resources Authority (EMRA), Ministry of Petroleum, Egypt. The chemical composition of ZE is SiO_2 (68.15%), Al_2O_3 (12.30%), CaO (3.95%), K_2O (2.80%), Fe_2O_3 (1.30%), MgO (0.90%), Na_2O (0.75%), and TiO_2 (0.20%). Kaolin is consisted of SiO_2 (47.60%), Al_2O_3 (36.85%), TiO_2 (1.56%), Fe_2O_3 (0.40%), CaO (0.11%), MgO (0.01%), Na_2O (0.01%) and K_2O (0.01%) (Abdelgaleil et al., 2021). The inert dusts were sieved through a 325 mesh sieve before use and the particle size average was <45 μm.

2.3. Carbon dioxide (CO_2) gas treatment equipment

CO_2 (CA) experiments were conducted in a modified atmospheres unit at the Department of Plant Protection, Faculty of Agriculture, Benha University Qalyubia, Egypt. CA was provided as pure gas in pressurized steel cylinders (Fig. 1). The insects were exposed to two CA concentrations (10 and 20%) for 12 h. The treatments were carried out in Dreshel flasks (glass bottles of 3 L capacity) according the method described by Hashem et al. (2012). The Dreshel flasks were sealed after reaching to the required concentration. CA concentration was monitored using a carbon dioxide gas analyzer model 200–600 (Gow-Mac-Instrument Company, USA).

2.4. Bioassays

The insecticidal activity of the two inert dusts and carbon dioxide were tested individually against *C. maculatus* and *C. chinensis* on cowpea seeds. The inert dusts (ZE and KA) were tested at 100, 500 and 1000 mg/kg (Abdelgaleil et al., 2021) and carbon dioxide (CA) was evaluated at two concentrations 10 and 20% (Ingabire et al., 2021). The binary combinations of the inert dusts and CA were also evaluated. Lots (50 g) of clean and infestation-free cowpea seeds, variety Dokki 331, with 9.7% moisture content, were weighed and placed into 180 ml-glass jars. Then, cowpea seeds in each jar were treated with ZE or KA at application rates of 100, 500 and 1000 mg/kg. The jars were shaken manually for 2 min to ensure uniform coverage of seeds with inert dusts before introducing adults of *C. maculatus* or *C. chinensis* in the individual treatments or mixing with CA in the case of binary mixtures. After cowpea seed treatment with inert dusts, *C. maculatus* or *C. chinensis* sexed adults (10 ♀ + 10 ♂) were separately introduced into each jar. The jars include cowpea seeds treated with inert dusts in case binary mixtures and untreated seeds in case CA alone were transferred to the Dreshel flasks then

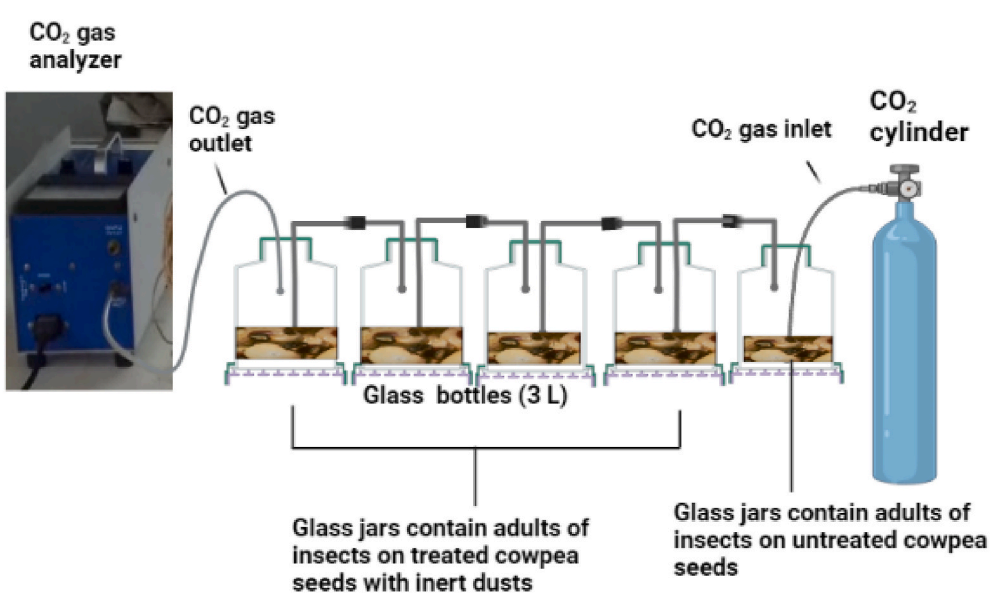

Fig. 1. Schematic diagram of CO_2 treatment setup.

exposed to different concentrations (10 and 20%) of CO_2 gas. After 12 h of exposure to CA, the flasks were opened and aerated. Then, all jars were transferred to incubators (28 ± 1 °C and 65 ± 5% RH). Eight individual treatments and twelve binary mixtures were carried out as described in Table 1. Each treatment was replicated three times. The numbers of dead adults were counted after 1, 3 and 7 days of treatment. Then, all the dead and alive adults were removed from cowpea seeds and all jars were returned to incubator at the same conditions to assess the progeny of both insect species after 60 days. Progeny numbers were counted and the following formula was used to determine the reduction percentage in the number of progeny after 60 days of treatment:

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

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

The treated and untreated cowpea seeds were sieved and weighed to obtain the weight loss percentages after 60 days. The weight loss percentage was calculated from the following formula:

$$\% \text{ weight loss} = ((Wu - Wi)/Wu) \times 100$$

where Wu = weight of uninfested cowpea seeds; Wi = weight of infested cowpea seeds of control and treatment.

2.5. Germination test of cowpea seeds

One hundred seeds with three replicates from cowpea seeds treated with the two inert dusts, CA and their combinations at highest application rates after 60 days 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 cowpea seeds; t = total number of weight of cowpea seeds.

2.6. Data analysis

The data of adult mortality, weight loss and germination of cowpea seeds were transformed using arcsine before analysis. All data were subjected to One-way analysis of variance using SPSS 21.0 software (SPSS, Chicago, IL, USA). Duncan's Multiple Range Test (DMRT) was used to detect differences between mean values at 0.05 significance level.

Table 1
Concentrations of inert dusts, kaolin (KA) and zeolite (ZE), and CO_2% (CA) used in this study.

Zeolite (mg/kg)	Kaolin (mg/kg)	CO_2 (% for 12 h)	Zeolite (mg/kg) + CA (% for 12 h)	Kaolin (mg/kg) + CA (% for 12 h)
ZE1 (100)	KA1 (100)	CA1 (10)	ZE1 (100) + CA1 (10)	KA1 (100) + CA1 (10)
ZE2 (500)	KA2 (500)	CA2 (20)	ZE2 (500) + CA1 (10)	KA2 (500) + CA1 (10)
ZE3 (1000)	KA3 (1000)		ZE3 (1000) + CA1 (10)	KA3 (1000) + CA1 (10)
			ZE1 (100) + CA2 (20)	KA1 (100) + CA2 (20)
			ZE2 (500) + CA2 (20)	KA2 (500) + CA2 (20)
			ZE3 (1000) + CA2 (20)	KA3 (1000) + CA2 (20)

3. Results

3.1. Effect of inert dusts and CO_2 on adult mortality of *C. maculatus* and *C. chinensis*

Effect of individuals and binary combinations of inert dusts (ZE and KA) and CA on the adult mortality of *C. maculatus* and *C. chinensis* is presented in Tables 2 and 3. The adult mortality of both insect species was increased in all treatments with the increase both the application rate and exposure time. In the individual treatments, ZE and KA at 1000 mg/kg caused the highest insecticidal activity with adult mortality of 82.2 and 80.9% for *C. maculatus*, and 86.4 and 72.4% for *C. chinensis* after 7 days, respectively. The individual treatment with CA at 20% for 12 h caused 75.9 and 84.6% mortality after 7 days for *C. maculatus* and *C. chinensis*, respectively. In general, the binary treatments induced higher toxicity to the adults of both insect species than individual treatments. The full mortality (100.0%) of *C. maculatus* adults was achieved in cowpea seeds treated with ZE (1000 mg/kg) + CA (20%) after 3 days. While, treatments with ZE (1000 mg/kg) + CA (10%), ZE (100 mg/kg) + CA (20%), ZE (500 mg/kg) + CA (20%), KA (100 mg/kg) + CA (20%), KA (500 mg/kg) + CA (20%) and KA (1000 mg/kg) + CA (20%) caused 100% mortality of this insect after 7 days of exposure (Table 2). Moreover, the full adult mortality of *C. chinensis* was obtained

Table 2
Mean mortality (% ± SE) of *Callosobruchus maculatus* adults after 1, 3 and 7 days of exposure to treated cowpea seeds with different application rates of zeolite (ZE), kaolin (KA), CO_2 (CA) and their combinations.

Treatment (Concentration, mg/kg or %)	Mean adult mortality (%) ± SE		
	1 day	3 days	7 days
Control (0.0)	0.0 ± 0.0j	0.0 ± 0.0k	0.0 ± 0.0h
ZE1 (100)	2.1 ± 1.5j	23.1 ± 0.7j	62.7 ± 2.4 fg
ZE2 (500)	10.1 ± 0.4ghi	35.2 ± 1.5hi	83.9 ± 2.2bcd
ZE3 (1000)	12.2 ± 1.2fgh	55.3 ± 0.4g	82.2 ± 4.8bcd
KA1 (100)	6.7 ± 2.4i	41.1 ± 2.1hi	51.1 ± 4.4g
KA2 (500)	8.3 ± 0.6hi	69.4 ± 1.1def	72.8 ± 1.4defg
KA3 (1000)	18.9 ± 0.8ef	73.7 ± 3.9cde	80.9 ± 7.7cdef
CA1 (10%)	9.3 ± 2.6hi	44.4 ± 2.3h	66.1 ± 2.8efg
CA2 (20%)	17.3 ± 0.4efg	41.5 ± 1.1hi	75.9 ± 8.7cdef
ZE1 + CA1	22.1 ± 3.4de	54.6 ± 5.1g	93.4 ± 0.4abc
ZE2 + CA1	19.6 ± 0.7ef	80.4 ± 0.7c	95.6 ± 1.6 ab
ZE3 + CA1	35.6 ± 1.7c	92.1 ± 1.4b	100.0 ± 0.0a
ZE1 + CA2	23.5 ± 0.5de	76.7 ± 1.5cd	100.0 ± 0.0a
ZE2 + CA2	31.5 ± 1.1cd	81.0 ± 2.8c	100.0 ± 0.0a
ZE3 + CA2	48.1 ± 1.3b	100.0 ± 0.0a	100.0 ± 0.0a
KA1 + CA1	11.1 ± 0.8fgh	31.1 ± 0.8ij	86.4 ± 1.0bcd
KA2 + CA1	15.2 ± 2.1efgh	63.6 ± 4.9 fg	92.6 ± 2.6abc
KA3 + CA1	17.2 ± 0.7efg	64.7 ± 2.5efg	93.2 ± 0.9abc
KA1 + CA2	22.3 ± 0.5de	61.2 ± 1.4 fg	100.0 ± 0.0a
KA2 + CA2	53.1 ± 1.3 ab	62.7 ± 0.7 fg	100.0 ± 0.0a
KA3 + CA2	63.1 ± 1.4a	92.3 ± 1.7b	100.0 ± 0.0a
F	33.3	87.2	24.6
P	<0.01	<0.01	<0.01

Within each column, mean values followed by the same letter are not significantly different, $P < 0.05$, df = 20, 42.

Table 3
Mean mortality (% ± SE) of *Callosobruchus chinensis* adults after 1, 3 and 7 days of exposure to treated cowpea seeds with different application rates of zeolite (ZE), kaolin (KA), CO_2 (CA) and their combinations.

Treatment (Concentration, mg/kg or %)	Mean adult mortality (%) ± SE		
	1 day	3 days	7 days
Control (0.0)	0.0 ± 0.0h	0.0 ± 0.0g	0.0 ± 0.0i
ZE1 (100)	5.2 ± 0.1g	25.9 ± 0.3f	53.7 ± 0.9h
ZE2 (500)	5.9 ± 0.1 fg	39.2 ± 1.4ef	85.6 ± 0.4cdef
ZE3 (1000)	11.1 ± 0.5efg	70.4 ± 5.7cd	86.4 ± 2.8bcdef
KA1 (100)	4.9 ± 0.1g	34.4 ± 0.4ef	59.8 ± 3.1gh
KA2 (500)	6.8 ± 1.0 fg	47.6 ± 4.4def	67.3 ± 7.1fgh
KA3 (1000)	9.7 ± 0.2efg	59.9 ± 2.1cde	72.4 ± 1.3fgh
CA1 (10%)	12.8 ± 2.3efg	46.6 ± 5.9def	72.2 ± 5.6fgh
CA2 (20%)	37.2 ± 6.7c	60.9 ± 7.6cde	84.6 ± 0.8def
ZE1 + CA1	15.9 ± 3.0defg	54.4 ± 7.8cdef	77.4 ± 8.7efg
ZE2 + CA1	30.2 ± 3.6cd	60.2 ± 8.8cde	86.4 ± 9.6abcde
ZE3 + CA1	29.2 ± 1.5cd	62.5 ± 7.7cde	94.9 ± 1.8abcd
ZE1 + CA2	77.8 ± 9.8b	89.6 ± 7.4 ab	100.0 ± 0.0a
ZE2 + CA2	68.5 ± 4.4b	93.3 ± 4.7a	100.0 ± 0.0a
ZE3 + CA2	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a
KA1 + CA1	17.2 ± 2.8def	60.0 ± 8.2cde	74.9 ± 6.2fgh
KA2 + CA1	22.2 ± 1.6cde	73.8 ± 8.5cd	96.3 ± 2.6 ab
KA3 + CA1	27.0 ± 2.1cd	81.3 ± 1.3bc	100.0 ± 0.0a
KA1 + CA2	38.7 ± 6.9c	75.3 ± 3.6cd	95.5 ± 3.2abc
KA2 + CA2	30.2 ± 1.1cd	75.2 ± 1.8cd	100.0 ± 0.0a
KA3 + CA2	77.7 ± 0.5b	98.1 ± 1.3a	100.0 ± 0.0a
F	41.7	14.1	19.9
P	<0.01	<0.01	<0.01

Within each column, mean values followed by the same letter are not significantly different, $P < 0.05$, df = 20, 42.

at concentration of 20% of CA with ZE (1000 mg/kg), after 1 day of exposure, CA (20%) with two rates of ZE (100 and 500 mg/kg), KA (1000 mg/kg) + CA (10%), KA (500 mg/kg) + CA (20%) and KA (1000 mg/kg) + CA (20%), after 7 days of treatment (Table 3).

3.2. Effect of inert dusts and CO_2 on progeny production of *C. maculatus* and *C. chinensis*

Treatment of cowpea seeds with ZE, KA, CA and their binary mixtures resulted significant reduction in progeny production of both insect species after 60 days of treatment at all tested rates compared with mean progeny production in the control of *C. maculatus* (515.0 ± 18.7) and *C. chinensis* (360.6 ± 7.5), respectively (Table 4). The lowest progeny of *C. maculatus* was found in treatments with mixtures 1000 mg/kg of ZE+ 20% of CA (76.7 ± 1.2) and 1000 mg/kg of KA+ 20% of CA (34.0 ± 1.5). Furthermore, strong and complete suppression of *C. chinensis* progeny was observed at binary combinations 1000 mg/kg of ZE with 20% of CA (2.3 ± 0.9) and 1000 mg/kg of KA with 20% of CA (0.0 ± 0.0) after 60 days of treatment.

3.3. Effect of inert dusts and CO_2 on damage and weight loss in cowpea seeds caused by *C. maculatus* and *C. chinensis*

All individual and combined treatments of inert dusts (ZE and KA) and CA reduced damage and weight loss of treated cowpea seeds after 60 days in comparison with untreated seeds (Table 5). Also, the combinations of inert dusts and CA were more effective in protection of seeds than individual treatments. Percent of weight loss of untreated seeds was 35.2 and 28.3% for *C. maculatus* and *C. chinensis*, respectively, after 60 days. The complete cowpea seed protection with weight loss of 4.6 and 1.0% in the case *C. maculatus* and weight loss of 0.0 and 0.0% in the case of *C. chinensis* was observed in seeds treated with ZE or KA (1000 mg/kg) in combination with 20% of CA. Cowpea seeds treated with these binary mixtures were intact and free from damage caused by both insects for 60 days.

Table 4
Progeny production (mean number of adults/jar±SE) and reduction (%) of *Callosobruchus maculatus* and *Callosobruchus chinensis* after 60 days of exposure to treated cowpea seeds with different application rates of zeolite (ZE), kaolin (KA), CO_2 (CA) and their combinations.

Treatment (Concentration, mg/kg or %)	Progeny production of *C. maculatus* (±SE) after 60 days		Progeny production of *C. chinensis* (±SE) after 60 days	
	No. progeny/50g	Progeny reduction (%)	No. progeny/50g	Progeny reduction (%)
Control (0.0)	515.0 ± 18.7a	0.0	360.6 ± 7.5a	0.0
ZE1 (100)	370.0 ± 12.3bcd	28.2	240.0 ± 4.3b	33.4
ZE2 (500)	351.7 ± 9.2cde	31.7	182.0 ± 4.9cde	49.5
ZE3 (1000)	206.7 ± 4.7g	59.9	95.3 ± 1.9ghi	73.6
KA1 (100)	376.4 ± 9.5bc	26.9	248.0 ± 8.2b	31.2
KA2 (500)	309.8 ± 17.7ef	39.8	218.7 ± 1.0bc	39.3
KA3 (1000)	156.0 ± 5.4hi	69.7	132.0 ± 6.4efg	63.4
CA1 (10%)	275.0 ± 8.2f	46.6	200.0 ± 4.1bcd	44.5
CA2 (20%)	220.0 ± 8.1g	57.3	166.0 ± 2.5def	53.9
ZE1 + CA1	317.3 ± 7.2e	38.4	150.3 ± 3.9def	58.3
ZE2 + CA1	195.0 ± 10.2gh	62.1	95.0 ± 3.5ghi	73.6
ZE3 + CA1	115.0 ± 6.1ij	77.7	42.7 ± 1.0ijk	88.2
ZE1 + CA2	180.0 ± 2.0gh	65.1	115.0 ± 2.0fgh	68.1
ZE2 + CA2	100.7 ± 2.1j	80.5	56.3 ± 0.9ij	84.4
ZE3 + CA2	76.7 ± 1.2j	85.1	2.3 ± 0.9k	99.4
KA1 + CA1	410.0 ± 7.1b	20.4	127.0 ± 10.8 fg	64.8
KA2 + CA1	330.0 ± 10.8de	35.9	72.0 ± 1.2hi	80.0
KA3 + CA1	120.0 ± 7.0ij	76.7	57.0 ± 2.9ij	84.2
KA1 + CA2	210.0 ± 4.1g	59.2	130.3 ± 12.5efg	63.8
KA2 + CA2	82.3 ± 1.0j	84.0	17.7 ± 1.0jk	95.1
KA3 + CA2	34.0 ± 1.5k	93.4	0.0 ± 0.0k	100
F	84.9		29.5	
P	<0.01		<0.01	

Within each column, mean values followed by the same letter are not significantly different, $P < 0.05$, df = 20, 42.

3.4. Effect of treatments on germination of cowpea seeds

Germination percentages of cowpea seeds treated with ZE, KA, CA

Table 5
Mean weight loss (% ± SE) of cowpea seeds after 60 days of treatment with different application rates of zeolite (ZE), kaolin (KA), CO_2 (CA) and their combinations.

Treatment (Concentration, mg/kg or %)	Weight loss (% ± SE) after 60 days	
	Callosobruchus maculatus	Callosobruchus chinensis
Control (0.0)	35.2 ± 1.5a	28.3 ± 3.4a
ZE1 (100)	21.8 ± 0.4bc	20.3 ± 0.2b
ZE2 (500)	18.6 ± 0.8cde	19.7 ± 0.1b
ZE3 (1000)	14.4 ± 1.7de	4.7 ± 0.5def
KA1 (100)	19.9 ± 0.2bcd	13.8 ± 0.5c
KA2 (500)	16.4 ± 1.9cde	13.2 ± 2.4c
KA3 (1000)	7.3 ± 0.7gh	12.7 ± 0.5c
CA1 (10%)	17.1 ± 1.8cde	13.6 ± 0.3c
CA2 (20%)	13.9 ± 0.8de	7.0 ± 0.4d
ZE1 + CA1	16.0 ± 1.7cde	6.5 ± 0.8de
ZE2 + CA1	13.8 ± 2.4ef	4.7 ± 0.2def
ZE3 + CA1	6.7 ± 0.6gh	1.8 ± 0.1g
ZE1 + CA2	9.1 ± 0.3 fg	4.5 ± 0.1def
ZE2 + CA2	5.6 ± 0.4gh	1.7 ± 0.1g
ZE3 + CA2	4.6 ± 0.1hi	0.0 ± 0.0h
KA1 + CA1	25.7 ± 1.2b	3.7 ± 0.5defg
KA2 + CA1	19.5 ± 2.2cde	3.6 ± 0.9efg
KA3 + CA1	6.7 ± 0.7gh	2.5 ± 0.1 fg
KA1 + CA2	14.4 ± 1.7de	4.7 ± 0.6def
KA2 + CA2	2.4 ± 0.2ij	0.7 ± 0.5h
KA3 + CA2	1.0 ± 0.1j	0.0 ± 0.0h
F	27.5	42.2
P	<0.01	<0.01

Within each column, mean values followed by the same letter are not significantly different, $P < 0.05$, df = 20, 42.

and their mixtures at the highest application rates are presented in Fig. 2. The results showed no adverse effects of treatments on the cowpea seed germination compared with untreated seeds.

4. Discussion

The insecticidal efficacy of individual treatments with inert dusts and carbon dioxide (CA) on bruchid insects has been described by several researchers (Ofuya and Reichmuth, 2002; Mahdi and Khalequzzaman, 2012; Lü et al., 2017; Ingabire et al., 2021; Pascua et al., 2021). However, this is the first report on the effectiveness of binary treatments of inert dusts and CA for the control of *C. maculatus* and *C. chinensis* in stored cowpea seeds. Our results indicated that treatment of cowpea seeds with two inert dusts (ZE and KA) and carbon dioxide (CA) induced pronounced mortality of *C. maculatus* and *C. chinensis* adults. The results also showed that ZE was more toxic than KA against both insect species. The higher toxicity of ZE may be due to the high SiO2 content (68.15%) in this inert dust compared with KA (SiO2 content = 47.60%). In agreement with this finding, several researchers stated that the insecticidal efficacy of inert dusts depends on SiO2 content (Korunic, 1998; Ziaee and Ganji, 2016; Liška et al., 2017). In addition, the combined treatments of two inert dusts with CA were more toxic than individual treatments against both insect species. These findings were matched with previous studies demonstrated that the inert dusts had the ability to enhance the efficacy of CA against stored product insects, such as El-Lakwah et al. (2001) who tested DE (2000 mg/kg), CA (30%) and their combination against *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) in wheat grains and found that the binary treatment (2000 mg/kg of DE + 30% of CA) caused complete mortality (100.0%) of adults compared with DE alone (76.0%) and CA alone (78.0%) after 5 days of exposure. Furthermore, El-Lakwah and Gharib (2005) studied the toxicity of DE with two application rates (0.3 and 0.6% w/w) alone and under 30, 60 and 80% of CA against adults of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *R. dominica*, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and larvae of *Trogoderma granarium* (Everts) (Coleoptera: Dermestidae) and noted that the combined treatments of DE and CA generally enhanced mortalities of all tested insects compared with individual treatments. This enhancement of insecticidal efficacy of inert dusts was also observed with other insect management products. For instance, Khoobdel et al. (2019) stated that the binary mixtures of DE and entomopathogenic *Beauveria bassiana* Balsamo (Vuillemin) (Hypocreales: Cordycipitaceae) increased mortality of *C. maculatus* adults more than DE or fungus alone. Karimzadeh et al. (2020) demonstrated that combinations of ash or kaolin with high temperatures caused higher toxicity than the individual treatments to *C. maculatus*. Gad et al. (2021b) showed that treatment of cowpea seeds with mixture DE (500 mg/kg) + spinosad (0.5 mg/kg) enhanced adult

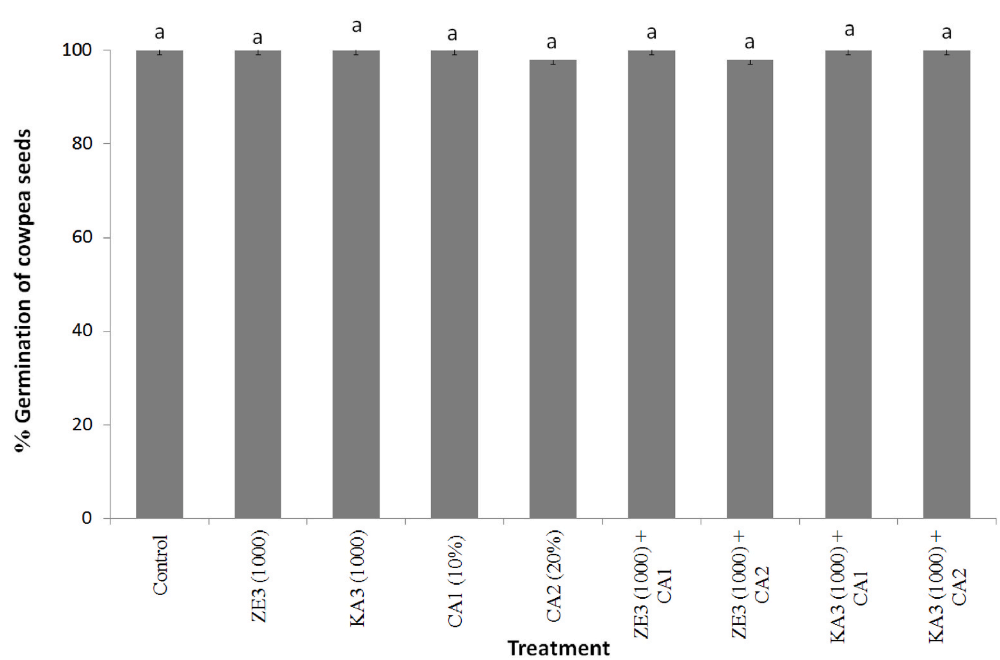

Fig. 2. Effect of different treatments of zeolite (ZE), kaolin (KA), CO_2 (CA) and their combinations on germination of cowpea seeds after 60 days of exposure ($P < 0.05$, F = 0.875, df = 8, 18).

mortality of *C. maculatus* and *C. chinensis* than individual treatments. The highest mortality of *C. maculatus* and *C. chinensis* adults observed in combined treatments in this study is due to the synergistic effect of inert dusts and CA on water loss from insect body or dehydration. The inert dusts cause their effect through abrasion of the insect cuticle and absorption of the epicuticular lipids leading to insect death by desiccation (Ebeling, 1971). High concentration of CA causes longer opening of the spiracles of insects allowing rapid loss of water and desiccation (Jay et al., 1971).

Our results demonstrated that the progeny production of *C. maculatus* and *C. chinensis* was significantly reduced in all individual and combined treatments of inert dusts and CA after 60 days. All treatments decreased progeny production of *C. chinensis* than *C. maculatus*. Progeny production was completely suppressed at the highest application rates, particularly in case of the mixtures of 20% of CA with 1000 mg/kg of ZE or KA on *C. chinensis* (Table 4). In accordance with these findings, several reports indicated that the inert dusts or CA had the ability to reduce progeny of stored product insects. Vassilakos et al. (2019) showed that treatment of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) with CA (70%) for 4 days caused complete suppression of progeny. Ingabire et al. (2021) observed that concentration of CA (40%) reduced progeny of *C. maculatus* (0.2 beetles) compared with control (19.0 beetles), after 30 days of exposure and concluded that the increase of CA concentration was the key factor behind reduction of *C. maculatus* progeny. Also, Abdelgaleil et al. (2021) found that application of DE at 1000 mg/kg reduced the progeny of *C. chinensis* (82.3%) on cowpea seeds after 45 days of treatment. The inhibition of progeny of *C. chinensis* and *C. maculatus* exposed to cowpea treated by individual and combined treatments of inert dusts and CA may be due to the quick death of adults after treatments or to effect of these substances on eggs and newly hatched neonate larvae (Athanassiou et al., 2005).

In conclusion, our study indicated the possibility to achieve complete mortality of *C. maculatus* and *C. chinensis* adults in cowpea seeds by the combinations of inert dusts (ZE and KA) and CA within 7 days of treatment. Furthermore, the binary mixtures of ZE or KA with CA were highly effective seed protectants as they strongly inhibited insect progeny and protected cowpea seeds from damage caused by bruchid insects for 60 days. Based on these findings, mixtures of ZE or KA with CA could be used as an effective method for the control of *C. maculatus* and *C. chinensis* on cowpea seeds without harmful effects resulting from treatment with chemical insecticides. This strategy would help to protect legumes during storage and offer safe food free from insecticide residues.

CRediT author contribution statement

Hassan A. Gad: Conceptualization; Data curation; Methodology; Investigation, Writing – original draft. Sara E. El-Deeb: Methodology, Investigation, Data curation, Formal analysis. Fathia S. Al-Anany: Methodology, Investigation, Data curation; Formal analysis. Samir A. M. Abdelgaleil: Conceptualization; Validation; Visualization; Writing – original draft; Writing – review & 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.

The authors H. A. Gad, S. E. El-Deeb, F. S. Al-Anany and S. A. M. Abdelgaleil report no conflicts of interest to be declared.

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