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TOXICITY AND JOINT ACTION OF CUMIN SEEDS EXTRACT WITH CERTAIN CONTROLLED ATMOSPHERES AGAINST STORED-PRODUCT INSECTS

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ABSTRACT

A petroleum ether extract of cumin seeds (*Cuminum cyminum* L.) was investigated in the laboratory, alone and under controlled atmospheres (CA) of 25% $C0_2$, 50% CO_2, and 99.8% N_2 for its toxic effect on the rice weevil, *Sitophilus oryzae* (L.), lesser grain borer, *Rhyzopertha dominica* (F.) and red flour beetle, *Tribolium castaneum* (Herbst). Results indicated relatively high adult mortality levels for S. *oryzae and R. dominica* at the highest concentration (10%) of cumin seed extract 2 weeks after treatment. In contrast, very low mortality levels were attained with T *castaneum*. Inhibition of F_1 progeny was much higher than mortality especially at higher concentrations. Cumin seeds extract under CA was more effective than the extract alone. The combined action of 2.5% and 5% concentrations + CO_2 showed an additive or synergistic effect on the three insect species at various exposure periods. Co-toxicity resulting from CA of 99.8% N_2 + 2.5% or 5% extract concentrations produced synergistic or additive effects on *S. oryzae* and T *castaneum* for all exposure periods. However, in the case of *R. dominica,* an antagonistic effect was observed for all exposure periods with 99.8% N_2 + 2.5% extract. With 99.8% N_2 + 5% extract, the antagonistic effect for a short exposure period of 2 days changed to an additive effect at longer periods (3 and 5 days).

INTRODUCTION

For their effectiveness in controlling pests, controlled (CA) or modified atmosphere treatments (MA) depend on removal of life-supporting oxygen (O_2) or the addition of toxic levels of carbon dioxide (CO_2), or a combination of the two. Such atmospheres have the advantage of leaving no chemical residues in the commodity (Mitsuda *et al.,* 1973). The time required to kill pests with CAs depends mainly upon the species concerned, its life stage, temperature, relative humidity (r.h.) and the composition of atmosphere. Oxygen concentrations around 1% or less are frequently

recommended for insect control, but some species can be controlled using O_2 concentrations as high as 4% (Reichmuth, 1986).

Natural plant products are presently in the focus of research efforts because of their mammalian safety and efficacy (Schmutterer, 1989). Darwish, (1997) investigated the effectiveness of the extracts of neem fruits and datura leaves under CAs against some stored-products insects. El-Lakwah *et al.,* (1998) revealed that when adults of the lesser grain borer *Rhyzopertha dominica*, (F.) and active fourth instar larvae and diapausing larvae of the Khapra beetle *Trogoderma granarium* (Everts) were exposed in the laboratory to wheat grains treated with Neemazal-T at 0.125, 0.25, 0.5 and 1% concentrations for 1, 2, 3, 5, 10 and 14 days, results showed low mortality values even after 14-days of exposure. At the highest concentration, the recorded mortalities were 22.9%, 20% and 13% for active, diapausing larvae of *T. granarium* and *R. dominica* adults, respectively. When both insect species were exposed to CAs of different CO_2 concentrations and CAs with nearly pure nitrogen at 25ºC and 60% r.h., *T. granarium* active larvae were more sensitive than the diapausing larvae. A decrease in CO_2 concentration and an increase in O_2 concentration of the CA decreased mortality of *T. granarium* larvae and *R. dominica* adults.

The toxic effects of extracts of datura fruits, dill seeds and morgan leaves in petroleum ether alone and mixed with CAs of 12.5% CO_2 in air or 99% N_2 were investigated by Mohamed (1999) against the cowpea beetle, *Callosobruchus maculatus* (F.). Findings on co-toxicity values, revealed pronounced synergistic effects for the plant extracts with the CO_2 enriched CA: at 10 and 5% for the datura extract; at 5, 2.5 and 1.25% for the dill extract, and at 1.25% concentration for the morgan extract. Also, additive effects were observed with mixtures of 99.9% N_2 and plant extracts at all the tested concentrations. Thus it is clear that the toxicity of the various plant extracts was increased when the insects were treated under CAs of enhanced CO_2 or high N_2 concentrations. Several investigators have studied the insecticidal effects of different plant extracts against stored-product pests (Darwish, 1992 and 1997; El-Lakwah *et al*., 1993; Mohamed, 1997 and 1999; Ismail, 1997; Ismail and Habiba, 1997). Also, it is well known that CO_2 possesses fairly high toxicity to insect pests especially those that infest stored grains (Tunç *et al*., 1982; El-Lakwah *et al.,* 1992 and 1994; Hashem and Reichmuth, 1993; Hashem *et al.,* 1993; Ofuya and Reichmuth, 1993).

This paper reports on a laboratory study of the toxic effect of a petroleum-ether extract of cumin seeds alone and under CAs against some stored-product insects.

MATERIALS AND METHODS

Experimental insects

The rice weevil, *Sitophilus oryzae* (L.), and the lesser grain borer, *R. dominica* were reared on wheat kernels, while the red flour beetle, *Tribolium castaneum* (Herbst) was reared on wheat flour under laboratory conditions (25±1ºC and 55±5 % r.h.); all

were from stock cultures held for several years in the Stored Product Laboratory of the Department of Plant Protection, Faculty of Agriculture, Moshtohor, Zagazig University, Egypt. Wheat grains and wheat flour were subjected to freezing at -18ºC for 2 weeks before being used in order to eliminate any possible infestation by other insect species. Grain m.c. was ~ 14%.

Cumin seed extract

Cumin (*Cuminum cyminum* L.) seeds contain 2.5-4% volatile oil. This contains 25-35% of aldehydes- cuminic aldehyde pinene and □-terpinol (Evans, 1994). The cumin product was ground into a fine powder, then extracted with petroleum ether at 50ºC under reduced pressure as described by Su (1985).

Bioassay tests

Effect of cumin extract on tested insects: Concentrations of 10, 5, 2.5 and 1.25 (w/v) were prepared from the stock solution. Ten grams of wheat grains or crushed wheat were treated with cumin extract in petri dishes by adding 1 mL of each concentration to the media to give final concentrations of 1.0, 0.5, 0.25 and 0.125 % (w/w). Batches each of 30 adult insects (1-2 weeks old) of *S. oryzae, R. dominica* and *T. castaneum* were introduced to the treated petri dishes. Three replicates for each treatment at different exposure intervals were used. Treated insects were kept at 25±1ºC and 55±5% r.h. Insect mortalities were recorded after 2, 3, 5, 7 and 14 days of exposure.

Reduction in F_1-progeny was calculated 60 days after treatment according to the following equation:

$$\% \text{ Reduction} = \frac{\text{No. adults emerged (control) - No. adults emerged (treatment)}}{\text{No. adults emerged (control)}} \times 100$$

Effect of modified atmospheres: CO_2 and N_2 were provided as pure gases in pressurized steel cylinders. Each cylinder was connected to a pressure regulator. The dilution method was used to achieve the required CO_2 concentration. For the atmosphere of nearly pure N_2, the valve of the N_2 cylinder was opened for two minutes in order to flush the Dressel exposure flask with the gas. CAs of 25% CO_2, 50% CO_2 in air and 99.8% N_2 were prepared and concentrations were measured. CO_2 was monitored using Gas Analyzer model 200-600 (Gow-Mac-Instrument Co., USA). N_2 concentration was determined by measuring the O_2 content with an Oxygen Analyzer 572, Servomex, England.

Batches of 30 adult insects were placed in wire gauze cages (14 mm diam. and 45 mm long), filled with about 10 g wheat grains or crushed wheat, and the cages were closed with rubber stoppers. The cages were then introduced into the 0.55-L gas-tight Dressel exposure flasks. Insects in these flasks were treated for different exposure periods at 25±1ºC and 55±5% r.h. After the desired exposure periods, the

flasks were aerated and the insects were transferred into petri dishes and kept at 25±1ºC and 55±5% r.h. prior to mortality assessment.

Effect of cumin extract under modified atmospheres: Wheat grains or crushed wheat (10 g) were treated with cumin extract at concentrations of 0.25 and 0.5% as described above. Thirty insects were introduced into each cage (together with the wheat). Insects inside the cages were transferred into Dressel-flasks, and exposed to the above-mentioned CAs. Tests were conducted at 25±1ºC and 55±5% r.h. for different exposure periods, and insect mortalities were assessed as described above.

Calculation of joint action

For the evaluation of the joint action of cumin extract with the CAs, the following equation was adopted as reported by Mansour *et al*. (1966):

$$\text{Co-toxicity factor} = \frac{\text{Observed mortality \% - Expected mortality \%}}{\text{Expected mortality \%}} \times 100$$

This factor was used to classify the results into three categories. A positive factor of 20 or more meant potentiation (synergistic effect), a negative factor of -20 or less meant antagonism, and any intermediate value, i.e. between +20 and -20 was considered as an additive effect.

RESULTS AND DISCUSSION

Toxicity of the plant extract

The effects of cumin seeds extract on mortality and reduction in F_1-progeny of the tested insects are summarized in Table 1. These data clearly reveal that cumin extract was more toxic to adults of *S. oryzae* and *R. dominica* than *T. castaneum*. The toxic effects of cumin extract on the first two species was concentration- and time-dependent over the test period of two weeks. The highest recorded mortalities were found to be 97.8 and 100% after two weeks exposure period at the highest tested concentration (1%) of the extract for *S. oryzae* and *R. dominica,* respectively. However, the same extract showed only slight mortality (11.3%) to *T. castaneu*m at the same exposure period and the highest concentration.

Also, results in Table 1 showed a pronounced adverse effect of cumin extract on F_1-progeny. Inhibition of F_1-progeny was much higher than mortality values at the two higher cumin extract concentrations. Reduction in F_1 progeny ranged from 42.1 to 100%; 0.0 to 100% and 16.5 to 72.2% at different concentrations of cumin extract for *S. oryzae*, *R. dominica* and *T. castaneum*, respectively.

TABLE 1
Effects of petroleum ether extract of cumin seeds on mortalities and reduction in F_1-progeny of three stored-product insect species

Concentration % (w/w)	Adult mortalities after indicated days					F_1 progeny after 60 days	Reduction in F_1-progeny %
	2	3	5	7	14		
Sitophilus oryzae							
1.0	32.2	42	67.6	82.2	97.8	0.0	100
0.5	11	17.6	49.7	63	76.7	1.7	87.2
0.25	3.3	7.8	26.5	32.1	68.9	6.3	52.6
0.125	3.3	3.3	18.9	30	46.7	7.7	42.1
Control	0.0	2.2	3.3	4.4	4.4	13.3	-
Rhyzopertha dominica							
1.0	77.5	83.3	87.8	97.8	100	0.0	100
0.5	21.1	31.1	56.7	71.1	90.4	2	91.0
0.25	6.7	11.1	20	43.3	87.8	14.7	32.3
0.125	3.3	3.3	6.7	28.9	71.1	21.7	0.0
Control	0.0	2.2	7.8	7.8	10	21.7	-
Tribolium castaneum							
1.0	0.0	0.0	0.0	3.3	11.3	29.7	72.2
0.5	0.0	0.0	0.0	3.3	10.2	66.7	37.7
0.25	0.0	0.0	0.0	1.1	6.7	77	28.0
0.125	0.0	0.0	0.0	1.1	10	89.3	16.5
Control	0.0	0.0	0.0	0.0	0.0	107	-

Toxicity of different modified atmospheres

Effects of CAs of 25% CO_2, 50% CO_2 and 99.8% N_2 on adult mortalities of *S. oryzae*, *R. dominica* and *T. castaneum*, are presented in Table 2. From the Table it is clear that mortality levels were concentration dependent since the 50% CO_2 CA resulted in mortality levels of 100, 76.4 and 84.4% after 2-day exposure of the three insect species. Mortalities of all three species reached 100% after one week exposure to the two CO_2 enriched CAs. The Table also shows that the CA of 99.8% N_2 caused relatively high mortalities of the tested insects; mortalities after 2 days were 36.7, 95.8 and 70% in the same order as above for the three insect species. Mortality reached 100% after 5 days exposure for *S. oryzae* and *R. dominica*, but only after 14 days for *T. castaneum*. CAs containing different levels of CO_2 resulted in decreasing numbers of emerging adults and reduction in F_1-progeny (Table 3). Such effects were concentration and time dependent. The reductions of F_1-progeny were 43.5 to 100%, 67.3 to 100% and 68.9 to 100% as a result of using 25% CO_2 over two weeks for *S. oryzae*, *R. dominica* and *T. castaneum*, respectively. The reductions in F_1-progeny using 50% CO_2 ranged from 86.9 to 100%, 75 to 100% and 72.4 to 100% respectively.

TABLE 2
Effects of different controlled atmospheres on mortalities of three stored-product species

CAs (CO_2 : N_2 : O_2 in % v/v)	Adult mortalities after indicated days				
	2	3	5	7	14
Sitophilus oryzae					
25 : 60 : 15	33.8	73	96.7	100	100
50 : 40 : 10	100	100	100	100	100
0 : 99.8 : 0.2	36.7	53	100	100	100
Control	0.0	0.0	3.3	6.7	6.7
Rhyzopertha dominica					
25 : 60 : 15	71.3	96.7	100	100	100
50 : 40 : 10	76.4	100	100	100	100
0 : 99.8 : 0.2	95.8	97.6	100	100	100
Control	3.3	3.3	3.3	6.7	8.3
Tribolium castaneum					
25 : 60 : 15	72.6	100	100	100	100
50 : 40 : 10	84.4	100	100	100	100
0 : 99.8 : 0.2	70	79	83.1	91.2	100
Control	0.0	0.0	0.0	6.7	6.7

Toxic effect of cumin extract under modified atmospheres

The effectiveness of cumin extract under CAs with enriched CO_2 against *S. oryzae*, *R. dominica* and *T. castaneum* is shown in Table 4. The data clearly indicates that the test insects were more sensitive to cumin extract when used under CAs in comparison with exposure to the extract alone. The lower (0.25%) level of cumin extract under 25% CO_2 resulted in mortalities of 57.3, 100 and 86.7% after 2 days exposure for *S. oryzae*, *R. dominica* and *T. castaneum*, respectively. Also, complete mortalities of all three species was found after 2-days exposure to 0.5% cumin extract under 50% CO_2.

The effect of cumin extract under CO_2 enriched CAs on the number of emerging adults and reduction in F_1- progeny is shown in Table 5. The cumin extract under 25% CO_2 resulted in a drastic decrease in the number of emerging adults and this effect was time-dependent. Its effect on reduction in F_1-progeny reached 100% after 2 weeks of exposure, and emergence of adults was completely stopped. Also, a total reduction in F_1-progeny of all three species was found as a result of using the 0.5% cumin extract and 50% CO_2 for 2 days exposure.

Table 6 shows the effect of cumin extract under 99.8% N_2 on mortalities of *S. oryzae*, *R. dominica* and *T. castaneum* adults. *S. oryzae* was the most sensitive followed by *T. castaneum* and *R. dominica* when exposed to the 0.25% cumin extract under 99.8 %N_2. The observed mortalities were 84.0, 66.7 and 31.1% respectively for these species, after 2 days of exposure. This effect was time-dependent reaching 100% mortality after 5, 7 and 14 days for *S. oryzae*, *T. castaneum* and *R. dominica*, respectively.

TABLE 3
Effects of controlled atmospheres on number of emerged adults and reduction in F_1-progeny

CAs (CO_2 : N_2 : O_2 in % v/v)	Exposure period (days)	No. of emerged adults	% Reduction in F_1 progeny
	Sitophilus oryzae		
	2	13.0	43.5
	3	7.0	65.6
25 : 60 : 15	5	3.0	86.9
	7	1.0	95.7
	14	0.0	100
	2	3.0	86.9
	3	0.0	100
50 : 40 : 10	5	0.0	100
	7	0.0	100
	14	0.0	100
Control		23	-
	2	10.2	45.5
	3	8.3	55.6
0 : 99.8 : 0.2	5	0.0	100
	7	0.0	100
	14	0.0	100
Control		18.7	-
	Rhyzopertha dominica		
	2	4.7	67.3
	3	2.2	84.4
25 : 60 : 15	5	0.0	100
	7	0.0	100
	14	0.0	100
	2	3.0	75.0
	3	1.7	88.3
50 : 40 : 10	5	0.3	97.9
	7	0.0	100
	14	0.0	100
Control		14.3	-
	2	1.7	97.7
	3	0.0	100
0 : 99.8 : 0.2	5	0.0	100
	7	0.0	100
	14	0.0	100
Control		74	-
	Tribolium castaneum		
	2	6.0	68.9
	3	2.0	89.6
25 : 60 : 15	5	0.3	98.4
	7	0.0	100
	14	0.0	100
	2	5.3	72.4
	3	1.0	94.8
50 : 40 : 10	5	0.0	100
	7	0.0	100
	14	0.0	100
Control		19.3	-
	2	0.0	100
	3	0.0	100
0 : 99.8 : 0.2	5	0.0	100
	7	0.0	100
	14	0.0	100
Control		46	-

TABLE 4
Effect of petroleum ether extract of cumin seeds under controlled atmospheres (CAs) on mortalities of three stored-product insect species

CAs (CO_2 : N_2 : O_2 in % v/v) + cumin extract	Adult mortalities after indicated days				
	2	3	5	7	14
Sitophilus oryzae					
25 : 60 : 15 + 0.25 % extract	57.3	84.3	100	100	100
50 : 40 : 10 + 0.5 % extract	100	100	100	100	100
Control	3.3	4.5	6.7	6.7	6.7
Rhyzopertha dominica					
25 : 60 : 15 + 0.25 % extract	100	100	100	100	100
50 : 40 : 10 + 0.5 % extract	100	100	100	100	100
Control	3.3	3.3	3.3	3.3	3.3
Tribolium castaneum					
25 : 60 : 15 + 0.25 % extract	86.7	100	100	100	100
50 : 40 : 10 + 0.5 % extract	100	100	100	100	100
Control	0.0	6.7	6.7	6.7	6.7

The effect of cumin extract on adult mortality was concentration-dependent for *S. oryzae* and *R. dominica* but a slight change in mortality of *T. castaneum* was revealed at different exposure intervals to 0.5% cumin extract under 99.8% N_2, the observed mortalities being 100, 72.2 and 69.2% for *S. oryzae*, *R. dominica* and *T. castaneum*, respectively after 2 day-exposure. The results in Table 7 showed the adverse effect of 0.25% cumin extract under 99.8% N_2 on the number of emerged adults since no emergence was observed after 2, 5 and 7 days for *S. oryzae*, *T. castaneum* and *R. dominica*, respectively. This effect was even more evident at the 0.5% extract level. Another striking observation is the fact that these treatments resulted in high levels of reduction in F_1- progeny especially when 0.5% extract was used. These reductions ranged from 90.1 to 100% and 99.3 to 100% for *R. dominica* and *T. castaneum*, respectively over the first 3 and 5 days of exposure to the 0.5% extract under 99.8% N_2. However, complete reduction in F_1-progeny was also observed for *S. oryzae* exposed to both levels of cumin extract.

The joint toxic actions of cumin extract with CAs of 25% and 50% CO_2 and 99.8% N_2 are shown in Tables 8 to 11. Results in Table 8 revealed that the 0.25% extract under 25% CO_2 produced pronounced additive effects for all three species at all exposure periods except 2 days which produced synergistic effects for *S. oryzae* and *R. dominica* only.

Meanwhile, the values of the co-toxicity factor resulting from 50% CO_2 and 0.5% extract revealed additive effects for all insect species (Table 9). The co-toxicity values resulting from the 99.8% N_2 and 0.25% cumin extract, produced an antagonistic effect with *R. dominica* at various exposure periods. However, an additive effect was observed with *T. castaneum* at different exposure periods. In the case of *S. oryzae*, a synergistic effect was found after 2 and 3 days. This effect turned into an additive effect at the 5-day exposure period (Table 10). The joint action of

99.8% N_2 with 0.5% cumin extract is shown in Table 11. This treatment resulted in a pronounced synergistic effect for *S. oryzae* after 2 and 3 days exposure, but showed an additive effect after 5 days exposure. However, the same treatment did show an additive effect for *T. castaneum* and *R. dominica* at the different exposure intervals except after 2 days for *R. dominica* when an antagonistic effect was noticed.

TABLE 5
Effect of cumin extract under certain CAs of CO_2 on number of emerged adults and reduction in F_1-progeny of three stored-product insect species

CAs (CO_2 : N_2 : O_2 in % v/v) + extract (% w/w)	Exposure periods (days)	No. of emerged adults	% Reduction in F_1-progeny
	Sitophilus oryzae		
	2	8.0	65.2
	3	4.0	82.6
25 : 60 : 15 + 0.25 % extract	5	2.0	91.3
	7	0.0	100
	14	0.0	100
	2	0.0	100
	3	0.0	100
50 : 40 : 10 + 0.5 % extract	5	0.0	100
	7	0.0	100
	14	0.0	100
Control		23	-
	Rhyzopertha dominica		
	2	0.0	100
	3	0.0	100
25 : 60 : 15 + 0.25 % extract	5	0.0	100
	7	0.0	100
	14	0.0	100
	2	0.0	100
	3	0.0	100
50 : 40 : 10 + 0.5 % extract	5	0.0	100
	7	0.0	100
	14	0.0	100
Control		14.3	-
	Tribolium castaneum		
	2	3.0	84.5
	3	2.0	89.6
25 : 60 : 15 + 0.25 % extract	5	0.0	100
	7	0.0	100
	14	0.0	100
	2	0.0	100
	3	0.0	100
50 : 40 : 10 + 0.5 % extract	5	0.0	100
	7	0.0	100
	14	0.0	100
Control		19.3	-

TABLE 6
Effect of cumin extract under CA of 99.8 % N_2 on mortalities of three stored-product insect species

Treatments	Adult mortalities after indicated days				
	2	3	5	7	14
	Sitophilus oryzae				
99.8 N_2 + 0.25 % extract	84	95.5	100	100	100
99.8 N_2 + 0.5 % extract	100	100	100	100	100
Control	0.0	0.0	3.3	3.3	6.7
	Rhyzopertha dominica				
99.8 N_2 + 0.25 % extract	31.1	46	54.3	83.1	100
99.8 N_2 + 0.5 % extract	72.2	90.3	100	100	100
Control	3.3	3.3	3.3	6.7	6.7
	Tribolium castaneum				
99.8 N_2 + 0.25 % extract	66.7	75.2	80.0	100	100
99.8 N_2 + 0.5 % extract	69.2	78.9	83.2	100	100
Control	0.0	0.0	0.0	3.3	6.7

The results obtained are in agreement with the findings of other investigators (Su, 1989; El-Lakwah *et al.,* 1992, 1994, 1995 and 1996; Abdel-Latif, 1999; Mohamed, 1997 and 1999). The exposure of certain stored-product insects to CAs and plant extracts of neem and datura were investigated by Darwish (1997). Mortalities of adults or larvae were increased when tested insects were treated with the two extracts in the presence of CAs at various exposure periods. The co-toxicity factor increased gradually to change from the antagonistic action at lower exposure periods and extract concentrations, into potentiating or synergistic action at the longest exposure periods and the highest extract concentration. There are numerous studies with regard to joint action of two compounds, a major concern being whether they interfere with each other's activity or induce a detoxication reaction, or both (Hewlett, 1960; Dubois, 1961; Mansour *et al.,* 1966). If both reactions are encountered the potentiation or additive effect could depend on the degree of interference between the different reactions. More studies are needed to clarify the principal mechanism responsible for the potentiating activity resulting from the joint use of CAs and botanical insecticides.

In conclusion, the efficacy of either the CO_2 enriched or N_2 CAs alone or in combination with cumin extract against *S. oryzae*, *R. dominica* and *T. castaneum* adults was concentration- and time-dependent. Also, treatment of the insects with the cumin extract under the tested CAs clearly produced higher mortalities than that of each CA alone and proved to be effective against the insect species under study. Thus, this method could be considered as a potential alternative to methyl bromide for stored-product pest control in the framework of IPM programs.

TABLE 7
Effect of cumin extract under CA of 99.8 N_2 on number of emerged adults and reduction in F_1-progeny of three stored-product insect species

Treatments	Exposure periods (days)	No. of emerged adults	% Reduction in F_1-progeny
	Sitophilus oryzae		
	2	0.0	100
	3	0.0	100
99.8 N_2 + 0.25 % extract	5	0.0	100
	7	0.0	100
	14	0.0	100
	2	0.0	100
	3	0.0	100
99.8 N_2 + 0.5 % extract	5	0.0	100
	7	0.0	100
	14	0.0	100
Control		18.7	-
	Rhyzopertha dominica		
	2	23.0	68.9
	3	4.0	94.6
99.8 N_2 + 0.25 % extract	5	0.7	99.0
	7	0.0	100
	14	0.0	100
	2	7.3	90.1
	3	0.0	100
99.8 N_2 + 0.5 % extract	5	0.0	100
	7	0.0	100
	14	0.0	100
Control		74	-
	Tribolium castaneum		
	2	4.7	89.8
	3	1.0	97.8
99.8 N_2 + 0.25 % extract	5	0.0	100
	7	0.0	100
	14	0.0	100
	2	0.3	99.3
	3	0.3	99.3
99.8 N_2 + 0.5 % extract	5	0.0	100
	7	0.0	100
	14	0.0	100
Control		46	-

TABLE 8
Joint action of CA of 25 % CO_2 and 0.25 % cumin extract against three stored-product insect species

Exposure period (days)	Adult mortalities			Co-toxicity factor	Type of Joint action
	25 % CO_2 alone	0.25 % cumin seed extract alone	25 % CO_2 + 0.25 % cumin seed extract		
		Sitophilus oryzae			
2	33.8	3.3	57.3	54.5	s
3	73	7.8	84.3	4.3	d
5	96.7	26.5	100	0.0	d
		Rhyzopertha dominica			
2	71.3	6.7	100	28.2	s
3	100	11.1	100	0.0	d
5	100	20	100	0.0	d
		Tribolium castaneum			
2	72.6	0.0	86.7	19.4	d
3	100	0.0	100	0.0	d
5	100	0.0	100	0.0	d

s = synergistic effect; d = additive effect

TABLE 9
Joint action of CA of 50 % C O_2 and 0.5 % cumin extract against three stored-product insect species

Exposure period (days)	Adult mortalities			Co-toxicity factor	Type of Joint action
	50 % CO_2 alone	0.5 % cumin seed extract alone	50 % C O_2 + 0.5 % cumin seed extract		
		Sitophilus oryzae			
2	100	11.0	100	0.0	d
3	100	17.6	100	0.0	d
5	100	49.7	100	0.0	d
		Rhyzopertha dominica			
2	76.4	21.1	100	2.6	d
3	96.7	31.1	100	0.0	d
5	100	56.7	100	0.0	d
		Tribolium castaneum			
2	84.4	0.0	100	18.5	d
3	100	0.0	100	0.0	d
5	100	0.0	100	0.0	d

d = additive effect

TABLE 10
Joint action of CA of 99.8 % N_2 and 0.25 % cumin extract against three stored-product insect species

Exposure period (days)	Adult mortalities			Co-toxicity factor	Type of Joint action
	99.8 % N_2 alone	0.25 % cumin seed extract alone	99.8 % N_2 + 0.25 % cumin seed extract		
		Sitophilus oryzae			
2	36.7	3.3	84.0	110	s
3	53.0	7.8	95.5	57.1	s
5	100	26.5	100	0.0	d
		Rhyzopertha dominica			
2	95.8	6.7	31.1	-68.9	a
3	97.6	11.1	46.0	-54.0	a
5	100	20.0	54.3	-45.7	a
		Tribolium castaneum			
2	70.0	0.0	66.7	-4.7	d
3	79.0	0.0	75.2	-4.8	d
5	83.1	0.0	80.0	-3.7	d

s = synergistic effect; a = antagonistic effect; d = additive effect

TABLE 11
Joint action of CA of 99.8 % N_2 and 0.5 % cumin extract against three stored-product insect species

Exposure period (days)	Adult mortalities			Co-toxicity factor	Type of Joint action
	99.8 % N_2 alone	0.5 % cumin seed extract alone	99.8 % N_2 + 0.5 % cumin seed extract		
		Sitophilus oryzae			
2	36.7	11.0	100	109.6	s
3	53.0	17.8	100	41.6	s
5	100	49.7	100	0.0	d
		Rhyzopertha dominica			
2	97.8	21.1	72.2	-27.8	a
3	95.6	31.1	90.3	-9.7	d
5	100	56.7	100	0.0	d
		Tribolium castaneum			
2	70.0	0.0	69.2	-1.1	d
3	79.0	0.0	78.9	-0.13	d
5	83.1	0.0	83.2	0.12	d

s = synergistic effect; a = antagonistic effect; d = additive effect

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