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EFFICIENCY OF KATEL-SOUS AND BLACK PEPPER SEEDS DUSTS UNDER MODIFIED ATMOSPHERES AGAINST SOME STORED PRODUCT INSECTS

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ABSTRACT

These experiments were conducted inside gastight steel bins, each of about 0.5 m^3 volume and filled with approximately 450 kg wheat grains. The bins were situated on the ground of the agricultural experimental farm of the Faculty of Agric., Moshtohor, Tukh, Qalyubia. Grain temperature was 26±2 ºC. Modified atmospheres (MAs) tested were 99% N_2 and 30±5% CO_2. Tested dusts used were katel-sous dust and powdered black pepper seeds. Tested insects were the adults of *Sitophilus oryzae* L., *Rhizopertha dominica* F., *Tribolium castaneum* Herbst., and active and diapausing larvae of *Trogoderma granarium* Everts. Obtained results indicated that katel-sous was more active than black pepper to all tested insects. Diapausing larvae of *T. granarium* were the least sensitive species to all the test dusts and MAs, followed by *T. castaneum* adults. The sensitivity of the tested insects to dusts or MAs varied according to insect species. The efficacy of the tested MA of CO_2 against the tested insects was greater than the MA of N_2. Percentage mortality of all tested insects was dependent on concentration and exposure period. The results also showed that the efficacy and combined action of tested dusts under MA of N_2 or CO_2 were pronounced additive, potentiation and antagonistic effects according to dusts concentration, exposure period and insect species.

INTRODUCTION

Post-harvest losses are often more significant than crop losses which occur in the field. In Africa as much as 20-50% of the grain can be lost in maize and pulses because of insects' infestations (FAO, 1985). In addition to the poisonous gases that are used for stored-products' protection, the normal gases of the atmosphere can be altered to achieve control. The use and manipulation of natural components of the atmosphere, e.g. oxygen, nitrogen and carbon dioxide, to preserve food is referred to as modified atmospheres. Modified atmospheres techniques are widely used in the storage of perishable commodities such as grain, vegetables, cut flowers, etc. to control some insects in these products (Navarro and jay, 1987; Ofuya and Reichmuth, 1993; Hashem and Reichmuth, 1994; El-Lakwah *et al*., 1997; Mohamed, 1999 and El-Lakwah *et al*., 2004). The most extensive use of modified atmospheres for insect control is on grain and similar commodities. The atmospheres are modified by adding various levels of carbon dioxide and high levels of nitrogen. Frequent application of insecticides by methods that did not completely kill an insect infestation developed an insect population that became increasingly resistant to several insecticides and many effective insecticides have been banned for health and environmental reasons and only a few new insecticides have been developed to replace them. For these reasons there is a renewed interest in physical control methods even though most of these methods may be more expensive and not as effective in

eliminating or preventing an infestation as are chemical treatments. Non-silica dusts, such as rock phosphate, have been used in Egypt (Fam *et al.* 1974) and lime (calcium oxide) provides some control (Golob and Webley 1980). The main advantage of using inert dusts is their safety to humans and animals. Inert dusts in stored grains can provide continuous protection from insect infestations and do not affect the baking quality of wheat. Inert dusts kill insects by causing moisture to move out of the insect's body. The dust either scratches through the insect's waxy integument that normally prevents excessive loss of moisture to the dry grain and intergranular air. There is a common tradition among farmers at the subsistence level to use natural plant products such as black pepper dusts instead and these alternatives appear to have increased their importance.

The aim of the present investigation is to evaluate the effect of a modified atmosphere (CO2 or N2) associated with two tested materials (katel-sous dust and black pepper dust) against some stored product insects.

MATERIALS AND METHODS

Tested insects:

Laboratory strains of the rice weevil (*Sitophilus oryzae* L.), the lesser grain borer (*Rhizopertha dominica* F.) and the red flour beetle (*Tribolium castaneum* Herbst.) were used as adlut stage in these experiments, in addition to active and diapausing larvae of Khapra beetle (*Trogoderma granarium* Everts).

Insects rearing:

Tested insect species were reared in glass jars of approx. 250 ml volume, each jar contained about 200 g. of wheat kernels for *S*. *oryzae*, *R*. *dominica* and *T*. *granarium* or wheat flour in case of *T*. *castaneum* jars were covered with muslin cloth fixed with rubber band. Insect cultures were kept under controlled conditions of 26±2 °C and 55±5% RH in the rearing room of the laboratory. Active and diapausing larvae of 0.8±0.2 mg/l and 2.7±0.2 mg/l, respectively were used for the bioassay tests. The used active larvae were those of 3rd and 4th instars. Diapausing larvae were collected from a roll paper which had been placed on the top of the culture media. Diapausing larvae are known to be more tolerant to fumigants, inert gases and insecticides than active larvae taken from culture media (Bell *et al.*, 1984). Wheat grains were well treated by freezing at –18 ˚C for two weeks before application to eliminate any possible infestation by any other species. The moisture content of the grains was about 14%.

Gases used:

-Carbon dioxide was provided as a pure gas in a pressure cylinder and monitored using a CO_2 Gas-Analyzer model 200-600 (Gow-Mac Instrument Co., USA).

- Nitrogen was provided as a pure gas of more than 99% gas in a pressure cylinder and monitored using an Oxygen Analyzer 572, Servomex, England.

Tested materials:

-Katel-sous dust: Grain protectant contains 84% calcium triphosphate plus 16% sulpher, produced by Kafr El-Zayat Company for

Pesticides, Egypt, mixed with the grains at its recommended rate (1%) and half of it (0.5 w/w).

-Black pepper seeds(*Piper nigrum* L.): Obtained from herb shop, ground well in an electric mill, sieved using a 300 mesh sieve and kept in a clean glass jar. The pulverized seed powder was tested at 1 and 2 % w/w concentrations.

Bioassay tests:

The appropriate amount of the above mentioned dusts to give the required concentrations was mixed thoroughly with wheat grains or wheat flour. Batches of 30 adult insects (1-2 week-old) or 30 active and diapausing larvae of *T. granarium* were confined in the cloth bags (10x16 cm) each containing 50 g. treated or untreated wheat kernels for *S*. *oryzae*, *R*. *dominica* and *T*. *granarium* or wheat flour in case of *T*. *castaneum*. Each bag was closed well by a rubber band and placed into the steel bins (each of 0.5 m^3 volume). All tests were triplicated.

Purging of the gases inside the bin:

After introducing the insect samples inside the bin, and to obtain 30% CO_2 concentration, the cylinder of CO_2 was connected with the upper valve of the bin through a polyethylene tube. The valve of the cylinder was opened for one and half minute, while the bottom valve of the bin was opened and closed after one minute from the initial purging of gas.

For achieving modified atmosphere of high nitrogen content (99% N_2), the upper valve of the bin was opened for 5 minutes after the connection of N_2-cylinder with bin, while the bottom bin valve was opened for two minutes, after which it was closed tightly.

Fumigation procedure:

Insect samples were fumigated with the different treatments for different exposure periods (2, 3, 5, 7 and 14 days) inside gastight steel bins during summer time at temperature ca. 26±2ºC and 55±5% RH.

Post-fumigation procedure:

Aeration was carried out after exposure periods, the mortality of the insect adults was determined after 48 hrs.

Calculation of Joint Action:

For the evaluation of the joint action of katel-sous dust and powdered black pepper seeds under modified atmospheres, the following equation was adopted as reported by Mansour *et al.* (1966):

$$\text{Co-toxicity Factor} = \frac{(\%\text{Observed Mortality} - \%\text{Expected Mortality})}{\%\text{Expected Mortality}} \times 100$$

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

RESULTS AND DISCUSSION

Mortality percentages after treatments indicated that the efficacy of tested dusts and CO2 or N2 when each of them was used separately or in mixtures (the dusts under MA of CO2 or N2) was increased with extending the time of exposure with all the insect species.

A- Efficacy and Combined Action of Tested Dusts Under MA of 30±5% CO_2 to Various Insect Species at Grain Temperature of 26± 2 ºC.

The results in table (1) present the efficacy and combined action of black pepper dust under MA of 30±5% CO_2 to the adults of *S*. *oryzae*, *R*. *dominica* and *T*. *castaneum* as well as active and diapausing larvae of *T. granarium* at grain temperature of 26± 2 ºC and 55± 5% RH. Mortality percentages resulting from mixtures of black pepper dust and CO_2 were higher than those obtained from each treatment alone with the adults of *S*. *oryzae*, *R*. *dominica* and *T*. *castaneum* while mortality percentages resulting from the mentioned mixtures for the active and diapausing larvae of *T. granarium* were lower than mortalities resulted from CO_2 alone. Black pepper dust at 1% and 7days-exposure gave 65, 43 and 5% mortality with the adults of *S*. *oryzae*, *R*. *dominica* and *T*. *castaneum*, respectively. While, those were 28 and 38 as well as 6 and 8% after 14 days post-treatment at the low and high black pepper dust concentration for the active and diapausing larvae of *T. granarium*, respectively. However, mortalities resulted from CO_2 alone were 30, 6 and 3% at 2 days-exposure and these values increased to 97, 99 and 78% after 7 days-exposure for the adults of *S*. *oryzae*, *R*. *dominica* and *T*. *castaneum*, respectively. Meanwhile, these values were 25 and 16% at 5 days-exposure and increased to 77 and 66% after 14 days-exposure for the active and diapausing larvae of *T. granarium*, respectively. Treatment of black pepper dust at 2% under MA of 30±5% CO_2 resulted in complete kill after one week exposure time for *S*. *oryzae*, *R*. *dominica* and *T*. *castaneum* adults, while this combined treatment resulted in 72 and 30% mortality after two weeks exposure period with the active and diapausing larvae of *T. granarium*, respectively. Co-toxicity values resulting from the mixtures of black pepper dust at 1 and 2% plus 30±5% CO_2 indicated potentiation or additive effect at short exposure period while a negative effect was observed at longer period with *S*. *oryzae* adults. While in case of *R*. *dominica*, potentiation effect was observed at short exposure, but this effect turned to antagonistic effect at longer period. In case of *T*. *castaneum*, data indicated potentiation effect after 2 days post-treatment while after one week exposure time, this effect changed to be additive. Meanwhile, the active and diapausing larvae of *T. granarium,* using black pepper dust at 1 and 2% under MA of 30±5% CO_2 resulted in antagonistic effect at the various tested exposure periods of 5 – 14 days.

Table (2) presents the efficacy and combined action of katel-sous dust at 0.5 and 1% concentration under MA of 30±5% CO_2 to the tested insects at grain temperature of 26± 2 ºC and 55± 5% RH. The obtained mortality percentages showed that 1% katel-sous under MA of CO_2 caused 100% mortality of *S*. *oryzae* and *R*. *dominica* adults after two days exposure,

while this combined treatment resulted in 70% mortality after one week exposure period with *T. castaneum* adult. Treatment of the active larvae of *T. granarium* with katel-sous dust at the lower concentration under MA of CO_2 gave a higher mortality values (26-68%) than those of the higher concentration (20-30%). Co-toxicity values resulting from the mixtures of katel-sous dust at 0.5 and 1% plus 30±5% CO_2 indicated potentiation or additive effect with *S. oryzae* adults. While in case of *R. dominica*, potentiation or antagonistic effect was observed. Co-toxicity values of the mixtures showed a potentiation effect with the *T. castaneum* adults, but this effect turned to additive effect after one week exposure period. In case of active and diapausing larvae of *T. granarium* co-toxicity values resulting from addition of CO_2 at 30±5% concentration to katel-sous dust at its two application rates induced antagonistic effect with all various periods of exposure.

Table (1): Efficacy and Combined Action of Black Pepper seeds Bp) Dust under MA of 30±5% CO_2 to Various Insect Species at Grain Temperature of 26± 2 ºC and 55± 5% RH.

Exposure Period (days)	% Mortality at Various Treatments					Co-toxicity Factor		Type of Joint Action	
	Bp% (W/W)		MA of 30± 5% CO_2	Mixtures of Bp+CO_2					
	1 (a)	2 (b)		(a)	(b)	(a)	(b)	(a)	(b)
S. oryzae-adults									
2	0	1	30	97	98	223	216	**s**	**s**
3	1	7	90	98	99	8	2	**d**	**d**
5	44	52	94	100	100	-28	-32	**a**	**a**
7	65	75	97	100	100	-38	-42	**a**	**a**
R. dominica-adults									
2	10	11	6	93	96	481	465	**s**	**s**
3	21	22	50	96	98	35	36	**s**	**s**
5	32	41	98	100	100	-23	-28	**a**	**a**
7	43	58	99	100	100	-30	-36	**a**	**a**
T. castaneum-adults									
2	0	0	3	21	41	425	925	**s**	**s**
3	1	1	17	32	55	78	206	**d**	**s**
5	2	3	34	43	70	19	89	**d**	**s**
7	5	7	78	91	100	10	17	**d**	**d**
T. granarium active larvae									
5	7	22	25	18	26	-44	-45	**a**	**a**
7	11	24	31	32	42	-24	-24	**a**	**a**
10	21	31	54	47	62	-37	-27	**a**	**a**
14	28	38	77	62	72	-41	-37	**a**	**a**
T. granarium diapause larvae									
5	1	2	16	4	8	-77	-56	**a**	**a**
7	1	3	26	15	21	-44	-28	**a**	**a**
10	5	5	46	16	28	-69	-45	**a**	**a**
14	6	8	66	18	30	-75	-60	**a**	**a**

a = antagonistic effect / d = additive effect / s = potentiation effect

Table (2): Efficacy and Combined Action of Katel-Sous (KS) Dust under MA of 30±5% CO_2 to Various Insect Species at Grain Temperature of 26± 2 ºC and 55± 5% RH.

Exposure Period (days)	% Mortality at Various Treatments					Co-toxicity Factor		Type of Joint Action	
	KS% (W/W)		MA of 30± 5% CO_2	Mixtures of Ks+CO_2					
	0.5 (a)	1 (b)		(a)	(b)	(a)	(b)	(a)	(b)
S. oryzae-adults									
2	1	2	30	97	100	213	213	**s**	**s**
3	2	4	90	98	100	7	6	**d**	**d**
5	17	24	94	100	100	-10	-12	**d**	**d**
7	33	45	97	100	100	-23	-30	**a**	**a**
R. dominica-adults									
2	61	71	6	95	100	42	30	**s**	**s**
3	83	90	50	97	100	-27	-29	**a**	**a**
5	93	98	98	100	100	-48	-49	**a**	**a**
7	95	99	99	100	100	-49	-50	**a**	**a**
T. castaneum-adults									
2	0	1	3	10	25	233	800	**s**	**s**
3	1	1	17	53	41	194	278	**s**	**s**
5	8	18	34	96	55	129	92	**s**	**s**
7	15	33	78	100	70	8	-10	**d**	**d**
T. granarium active larvae									
5	18	22	25	26	20	-40	-47	**a**	**a**
7	32	41	31	28	27	-56	-43	**a**	**a**
10	37	43	54	48	28	-55	-51	**a**	**a**
14	46	64	77	68	30	-55	-54	**a**	**a**
T. granarium diapause larvae									
5	6	21	16	15	52	-32	-46	**a**	**a**
7	15	32	26	20	84	-51	-54	**a**	**a**
10	23	42	46	22	96	-68	-68	**a**	**a**
14	28	53	66	24	98	-75	-75	**a**	**a**

a = antagonistic effect / d = additive effect / s = potentiation effect

B- Efficacy and Combined Action of Tested Dusts Under MA of 99% N_2 to Various Insect Species at Grain Temperature of 26± 2 ºC.

Data of the efficacy and combined action of black pepper dust at 1 and 2% under MA of 99% N_2 to the adults of *S. oryzae*, *R. dominica* and *T. castaneum* as well as active and diapausing larvae of *T. granarium* at grain temperature of 26± 2 ºC and 55± 5% RH. are given in table (3). The results shows that, using MA of N2 alone or combined with the black pepper dust followed a time-dependent phenomenon. Generally, the results revealed that the combined treatment of black pepper dust under MA of 99% N_2 enhanced the resulted mortalities of all the tested insects at all exposure periods except with *T. granarium* active larvae after two weeks exposure period. Co-toxicity values resulting from the mixtures indicated mostly potentiation effects or sometimes additive actions with all the insects at various periods of exposure.

Table (3): Efficacy and Combined Action of Black Pepper (Bp) Seeds Dust under MA of 99% N_2 to Various Insect Species at Grain Temperature of 26± 2 ºC and 55± 5% RH.

Exposure Period (days)	% Mortality at Various Treatments					Co-toxicity Factor		Type of Joint Action	
	Bp% (W/W)		MA of 99% N_2	Mixtures of Bp+N_2					
	1 (a)	2 (b)		(a)	(b)	(a)	(b)	(a)	(b)
S. oryzae-adults									
2	0	1	25	96	98	284	277	**s**	**s**
3	1	7	58	98	100	66	2	**s**	**s**
5	44	52	94	100	100	-28	-32	**a**	**a**
7	65	75	100	100	100	0	0	**d**	**d**
R. dominica-adults									
2	10	11	8	38	48	111	153	**s**	**s**
3	21	22	11	91	93	184	182	**s**	**s**
5	32	41	15	93	94	98	68	**s**	**s**
7	43	58	48	96	100	6	-6	**d**	**d**
T. castaneum-adults									
2	0	0	2	28	40	1300	1900	**s**	**s**
3	1	1	3	36	46	800	1050	**s**	**s**
5	2	3	6	75	81	838	800	**s**	**s**
7	5	7	24	85	88	193	193	**s**	**s**
T. granarium active larvae									
5	7	22	3	43	50	330	100	**s**	**s**
7	11	24	8	48	51	153	59	**s**	**s**
10	21	31	45	58	65	-12	-15	**d**	**d**
14	28	38	94	81	83	-34	-37	**a**	**a**
T. granarium diapause larvae									
5	1	2	5	38	46	533	557	**s**	**s**
7	1	3	6	45	46	543	411	**s**	**s**
10	5	5	15	51	60	155	200	**s**	**s**
14	6	8	66	65	73	-10	-1	**d**	**d**

a = antagonistic effect / d = additive effect / s = potentiation effect

Table (4) shows the results of efficacy and combined action of katel-sous dust at 0.5 and 1% under MA of 99% N_2 to the tested insects at grain temperature of 26± 2 ºC and 55± 5% RH. Mortality percentages apparently indicated that the efficacy of katel-sous dust was concentration-dependent, treatment of the all tested insects with katel-sous dust at the two applied concentrations under the above mentioned MA of N_2 gave considerably higher mortality values than those of each component alone at various exposure periods. Co-toxicity values of the mixtures showed mostly potentiation or additive effects with the four insect species at the different periods of exposure.

Katel-sous dust at 1% under MA of 99% N_2 resulted in complete kill after two days exposure period with the adults of *S. oryzae*, while these combined treatment resulted in complete kill after 5 and 14 days exposure period with *T. castaneum* adults and active larvae of *T. granarium*, respectively. 1% katel-sous under MA of CO_2 caused 100% mortality of *R. dominica* adults after two days exposure period, while the same combined treatment caused 98% mortality of diapausing larvae of *T. granarium* after 14 days exposure period. Generally, the results revealed that katel-sous dust at 1% was more active than the black pepper dust to all tested insect species.

Table (4): Efficacy and Combind Action of Katel-Sous (KS)) Dust under MA of 99% N_2 to Various Insect Species at Grain Temperature of 26± 2 ºC and 55± 5% RH.

Exposure Period (days)	% Mortality at Various Treatments					Co-toxicity Factor		Type of Joint Action	
	KS% (W/W)		MA of 99% N_2	Mixtures of Ks+N_2					
	0.5 (a)	1 (b)		(a)	(b)	(a)	(b)	(a)	(b)
S. oryzae-adults									
2	1	2	25	98	100	277	270	**s**	**s**
3	2	4	58	100	100	67	61	**s**	**s**
5	17	24	94	100	100	-10	-15	**d**	**d**
7	33	45	100	100	100	0	0	**d**	**d**
R. dominica-adults									
2	61	71	8	60	63	-13	-9	**d**	**d**
3	83	90	11	95	98	1	-3	**d**	**d**
5	93	98	15	98	100	-9	-12	**d**	**d**
7	95	99	48	100	100	-30	-32	**a**	**a**
T. castaneum-adults									
2	0	1	2	95	97	4650	3133	**s**	**s**
3	1	1	3	97	98	2325	2350	**s**	**s**
5	8	18	6	98	100	600	317	**s**	**s**
7	15	33	24	100	100	156	75	**s**	**s**
T. granarium active larvae									
5	18	22	3	53	53	152	112	**s**	**s**
7	32	41	8	55	60	41	50	**s**	**s**
10	37	43	45	68	78	-17	-11	**d**	**d**
14	46	64	94	82	100	-41	-37	**a**	**a**
T. granarium diapause larvae									
5	6	21	5	50	51	355	96	**s**	**s**
7	15	32	6	51	56	143	47	**s**	**s**
10	23	42	15	55	71	45	25	**s**	**s**
14	28	53	66	77	85	-18	-29	**d**	**d**

a = antagonistic effect / d = additive effect / s = potentiation effect

Similar results were observed by Darwish, (1997); Mohamed, (1999) and El-Lakwah *et al*. (1998, 2000a and 2000b) in their experiments on the combined action of plant extracts in presence of various atmospheres of either CO_2 or N_2. Also El-Lakwah *et al*. (2002) reported that the combined action of Dill and Cumin seed extracts under modified atmospheres of various carbon dioxide concentrations or modified atmospheres of very high nitrogen content resulted in either synergistic or additive effects with active and diapausing larvae of *T*. *granarium* at different periods of exposure.

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فاعلية مسحوق قاتل سوس ومسحوق بذور الفلفل الأسود تحت الأجواء المعدلة ضد بعض حشرات الحبوب المخزونة

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اجريت هذه الدراسة بغرض تقييم فعالية تأثير نوعين من المساحيق وهي مسحوق قاتل سوس ومسحوق بذور الفلفل الأسود علي الحشرات الكاملة لكل من سوسة الأرز, ثاقبة الحبوب الصغرى وخنفساء الدقيق الصدئية, وكذلك اليرقات النشطة والساكنة لخنفساء الصعيد وذلك تحت الأجواء المعدلة من غاز ثاني أكسيد الكربون ٣٠±٥% وغاز النيتروجين ٩٩% عند درجة حرارة الحبوب ٢٦±٢م°.

أجريت التجارب داخل صوامع محكمة الغلق حجم كلا منها ١/٢م٣ وكانت هذه الصوامع ممتلئة بحوالي ٤٥٠ كيلو جرام من حبوب القمح وتم وضع الصوامع داخل مزرعة كلية الزراعة بمشتهر.

و قد أوضحت النتائج أن مسحوق قاتل سوس كان أعلي تأثيرا من مسحوق الفلفل الأسود علي جميع الحشرات تحت الدراسة، كذلك تباينت حساسية الحشرات المختبرة للمسحوقين وكذلك للأجواء المعدلة علي حسب نوع الحشرة.

كما أوضحت النتائج أن اليرقات الساكنة لخنفساء الصعيد كانت الأقل حساسية لتأثير كلا المسحوقين وكذلك الأجواء المعدلة سواء من ثاني أكسيد الكربون أو النيتروجين عند استخدام كلا منهما منفردا ثم تلاها في الأقل حساسية الحشرات الكاملة لخنفساء الدقيق الصدئية.

وقد أشارت النتائج كذلك إلي أن فعالية غاز ثاني أكسيد الكربون كانت أعلي من فعالية غاز النيتروجين عند استخدام كلا منهما منفردا، كذلك أظهرت النتائج أن نسبة الموت للحشرات تحت الدراسة توقفت علي تركيز المسحوق المستخدم, كما زاد معدل الموت بزيادة فترة التعريض.

كذلك أوضحت النتائج أن التأثير المشترك للمساحيق المختبرة تحت الأجواء المعدلة من كل من غازي ثاني لأكسيد الكربون والنيتروجين إما تأثير تقوية أو إضافة أو تضاد، وكان هذا التأثير متوقفا علي نوع الحشرة، تركيز المسحوق وكذلك فترة التعريض.