

**Influence of some botanical oils on effectiveness of controlled atmosphere of elevated  
carbon dioxide on adults of *Sitophilus oryzae***

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***Authors' contributions***

*This work was carried out in collaboration between all authors. MMA conceived, designed  
the research and wrote the manuscript. All authors conducted the experiments, analyzed  
data, reviewed, read and approved the manuscript.*

## ABSTRACT

Botanical oils and controlled atmospheres (CAs) are natural, safe and eco-friendly alternatives of chemical fumigants for management of insect pests infesting stored grain. Oil effects of geranium, coriander and cumin were investigated on the bioactivity of CAs of three elevated CO<sub>2</sub> concentrations (25, 50 and 75% in the air) on *S. oryzae* adults at 20 and 30°C. Bioassay tests were conducted for botanical oil and CO<sub>2</sub> each alone and as a binary combination. Experiments of CAs were performed using a re-circulatory multi-flask apparatus. Results indicated that the required times to kill 50 or 90% (lethal times, LT<sub>50</sub> and LT<sub>90</sub>) of *S. oryzae* adults reduced significantly by the combination of tested oil and CO<sub>2</sub>. The lethal times (LT<sub>90s</sub>) were significantly the longest, when each of 25% CO<sub>2</sub>, geranium, coriander and cumin (each oil was at 1.5% v/w on wheat grains) were tested separately, at 20°C. These times were 12.09, 7.83, 5.82 and 8.09 days in treatments of 25% CO<sub>2</sub>, geranium, coriander and cumin (each of oil was at 1.5% separately on wheat grains) at 20°C, respectively. These times significantly reduced to 6.14, 4.15 and 4.15 days, when each of geranium, coriander and cumin oils were separately combined with 25% CO<sub>2</sub>, respectively. Furthermore, coriander oil was the most effective at 30°C on the lethal time of CA of 75% CO<sub>2</sub>. It caused a reduction in LT<sub>50</sub> and LT<sub>90</sub> of CA of 75% CO<sub>2</sub> from 1.12 and 4.1 days to the shortest lethal times 0.65 and 1.66 days for the adults of *S. oryzae*, respectively. It was concluded that efficacy of CO<sub>2</sub> can be significantly increased by using it with botanical oils in insect control and this action becomes the greatest at the highest CO<sub>2</sub> concentration and higher temperature than lower one.

**Keywords:** *Coriander; cumin; geranium; lethal time; re-circulatory multi-flask apparatus.*

## 1. INTRODUCTION

2. The main problem in storage products all over the world are the insect pests which cause a loss may amount to 30% in quantity and quality of the stored products [1,2,3,4]. The rice weevil, *Sitophilus oryzae* is a serious and severe insect pest of stored cereals and their products, resulting in considerable economic damage to the stored wheat grain, rice and maize [5,6]. Management of insect pests of stored-products depends heavily on the use of synthetic pesticides, such as pyrethroids, carbamates, organophosphates, methyl bromide and phosphine. However, the injudicious use of these toxicants led to various problems including toxic residues, the environmental contamination and the insect resistance [7]. Methyl bromide is completely phased out as ozone depleting chemical [8,9]. Phosphine has many troubles are closely connected to its use, such as negative effects on the human health and most stored product insects have a resistance development against phosphine although the control of insect pests of stored products still mainly relies on its application [10,11,12]. Alteration of traditional synthetic pesticides by bio-rational ones is commonly accepted and practical technique worldwide [13].

3. Botanical oils may offer a safe source of compounds to manage the insect pests, being relatively eco-friendly, and an excellent alternative to persistent synthetic insecticides, harmless to beneficial organisms and the development of insect resistance will be more slowly [14,15,16,17]. The mode of action of botanical oils was partially attributed to interference in normal respiration leading to insect suffocation. Another hypothesis was that the oil infiltration under the operculum may prevent respiration or disrupt the water balance [18]. The oils are very complex of natural molecule mixtures, and are hydrocarbons with a diversity of functional groups, about 20 to 60 compounds at different concentrations, characterized by two or three major components at relatively high concentrations (20 to 70%) compared to others components that existing in trace amounts. In general, the major components define the biological properties of the botanical oils.

- 81 4. These components have two groups of distinct biosynthetic origin. Terpenes and  
82 terpenoids formed the main group and the other is composed of aromatic and aliphatic  
83 components. The action has been linked to the existence of monoterpenes and  
84 sesquiterpenes which cause death of insects by inhibiting acetyl cholinesterase activity  
85 in the nervous system. However, these chemicals can work synergistically and  
86 improving their bioactivity [19,20,21,22,23,24,25]. On the other hand, CAs were  
87 recommended as an alternative to use of methyl bromide and phosphine for control of  
88 the stored product insects, carbon dioxide as natural components of the atmosphere is  
89 a safe chemical and has been permitted for using in many types of drinks and foods  
90 [26,27]. Controlled atmospheres of elevated CO<sub>2</sub> levels lead to spiracles to open  
91 resulting in insect death from water loss. Spiracles remain permanently open when  
92 CO<sub>2</sub> is above 10%. Toxic action is completely through the tracheae. Carbon dioxide  
93 has direct toxic action on the nervous system. In some cases, CO<sub>2</sub> can acidify the  
94 hemolymph causing membrane failure of some tissues [28,29,30,31,32,33]. Sub-lethal  
95 levels of CO<sub>2</sub> can have harmful effects on insect development, growth and  
96 reproduction for prolonged times. Controlled atmospheres of 60% CO<sub>2</sub> rapidly kill  
97 stored product insects [30,34]. Controlled atmospheres of reduced O<sub>2</sub> and elevated  
98 CO<sub>2</sub> can have an additive effect, depending on the concentrations used [27].
- 99 5. The temperature plays a great role in insect mortality through the exposure to CAs. In  
100 general, insect sensitivity to CAs is greater at higher temperatures due to enhancing  
101 the respiration demands [35,36].
- 102 6. In CA application, the time of exposure is a critical factor, the lethal atmosphere must  
103 be retained for a suitable length of time to obtain its efficacy in insect control  
104 [37,38,39,40,41]. However, the effectiveness of CA can be enhanced by varying other  
105 parameters such as temperature when short exposure period is required [32,42,43].

7. A considerable effect on insect metabolism was observed for heat treatments of CA, low O<sub>2</sub> inhibits ATP synthesis, and high CO<sub>2</sub> disturbs use of ATP [44,45].
8. Heat combined with CAs of CO<sub>2</sub> can significantly reduce treatment time for insect control [46,47]. But long exposure time is required to control the insects with the natural components of CAs of CO<sub>2</sub>-enriched and botanical oils, this limits their use for protecting stored products, in which quick treatment is needed [48,49,50,51,15,41].
9. Therefore, the present study was conducted to investigate the effect of three botanical oils geranium, coriander and cumin on the lethal time of CO<sub>2</sub> for *S. oryzae* adults at two different temperature degrees.

## 2. MATERIALS AND METHODS

### 2.1 Insects

Laboratory strain of the rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) was used as an adult stage in these experiments. This insect was reared in glass jars (approx. 250 ml), each jar contained (about 200 g) wheat kernels (variety Shandaweel1) and covered with muslin cloth and fixed with a rubber band. Insect cultures were kept under controlled conditions of 28±1°C and 65±5% RH in the rearing room of the laboratory. Wheat grains were treated by freezing at -18°C for 2 weeks before application to eliminate any possible infestation by any insect species [52]. The moisture content of the grains was around 14%. Three hundred of *S. oryzae* adults (1-2 weeks old) were introduced into the jars for laying eggs then kept at 28±1°C and 65±5% RH. Three days later, all insects were separated from the food, and the jars were kept again at the controlled conditions in the rearing room. This procedure was repeated several times in order to obtain a large number of the adults needed to carry out the experiments during this study.

### 2.2 Botanical oils

Botanical oils used for the present study were bought from Guvadant Swaziland Company (Table 1).

### 2.3 Gas used

Carbon dioxide (CO<sub>2</sub>) was 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<sub>2</sub> concentration. CAs of 25, 50 and 75% CO<sub>2</sub> in the air were prepared (Fig 1).

### 2.4 Determination of the concentrations of CO<sub>2</sub> gas

Carbon dioxide concentration was monitored using gas Analyzer model 200-600 (GOW-MAC Instrument Co., USA).

### 2.5 Bioassay tests

#### 2.5.1 Botanical oils

Two concentrations of each tested oil, 15 and 10% (v/v) were prepared in acetone. One milliliter from each concentration was taken and added to 10g wheat grains to obtain 1.5 and 1 % (v/w) for using in the experiments at 20±1 and 30±1°C, respectively. Thirty insects were added to each treatment and incubated at two aforementioned temperatures. Three replicates were used for each treatment. Only acetone was added for control treatment. Insect mortality was recorded after 1, 2, 3, 5, 7 and 14 days of the initial treatment.

#### 2.5.2 Controlled atmospheres of CO<sub>2</sub>

Batches of 30 *S. oryzae* adults were placed in wire gauze cages (14 mm diam. and 45 mm long), filled with about 10 g wheat grains and the cages were closed with rubber stoppers. The cages were then introduced into the 0.55 L gastight Dreshel exposure flasks. Insects in the flasks were treated for different exposure periods (1, 2, 3 and 4 days) at  $30\pm1^{\circ}\text{C}$  and  $20\pm1^{\circ}\text{C}$ ,  $65\pm5\%$  RH. After the desired exposure periods, the flasks were aerated and transferred into Petri dishes and kept at the above mentioned conditions prior to mortality assessment.

### 2.5.3 Controlled atmospheres and botanical oils combination

Batches of 30 *S. oryzae* adults were placed in wire gauze cages, filled with 10 g wheat grains which were treated with the tested oils separately as above mentioned in the botanical oils treatments. Controlled atmosphere of  $\text{CO}_2$  concentrations, 25, 50 and 75% in the air were tested separately as mentioned before with each treatment of oil concentration, 1.5 % at  $20^{\circ}\text{C}$  and 1.0 % at  $30^{\circ}\text{C}$ .

### 2.6 Statistical analysis

Mortality percentages were corrected by Abbott's formula [56]. A probit computer program of Noack and Reichmuth and Finney was used to determine the lethal times for the insecticides [57,58]. A significant difference between  $\text{LT}_{50}$  values was based on overlap of 95% confidence intervals [59].

## 3. RESULTS AND DISCUSSION

Generally, many investigators confirmed the fact, that the limiting factor of developing an effective insecticidal CA or botanical oil applications is the long exposure time required to achieve the control of stored product insects [48,49,51,15,41].

178 Obtained data indicated that at 20°C, the lethal times, LT<sub>50</sub> and LT<sub>90</sub> of CA of 25% CO<sub>2</sub>  
179 separately were 2.48 and 12.09 days, respectively. These values decreased significantly to  
180 1.31 and 6.14 days after the wheat grains were combined with geranium oil at 1.5% (v/w),  
181 respectively. Geranium oil gave separately lethal time values, 3.91 and 7.83 days for LT<sub>50</sub> and  
182 LT<sub>90</sub>, respectively. In case of coriander oil separately, the lethal time values on the adults of *S.*  
183 *oryzae* were 3.24 and 5.82 days for LT<sub>50</sub> and LT<sub>90</sub>, respectively. Lethal times of CA of 25%  
184 CO<sub>2</sub> reduced significantly to 1.08 and 4.15 days for LT<sub>50</sub> and LT<sub>90</sub>, respectively, after the  
185 wheat grains were treated with coriander oil at 1.5% (v/w). When cumin oil at 1.5% (v/w) was  
186 applied on the wheat grains, lethal times of CA of 25% CO<sub>2</sub> on the adults of *S. oryzae*  
187 significantly decreased to 1.19 and 4.15 days for LT<sub>50</sub> and LT<sub>90</sub>, respectively. However, LT<sub>50</sub>  
188 of cumin oil was separately the longest, 4.06 days. It followed by geranium and coriander oils  
189 that gave alone, 3.91 and 3.24 days for their LT<sub>50</sub>s, respectively. Moreover, LT<sub>90</sub> of CA of  
190 25% CO<sub>2</sub> was the longest, 12.09 days in alone treatment.

191 Regarding the treatments at 30°C, the lethal times were more affected than at 20 ± 1°C, where  
192 the values, LT<sub>50</sub> and LT<sub>90</sub> of CA of 25% CO<sub>2</sub> alone were 2.06 and 6.91 days, respectively.  
193 These values significantly reduced to 0.83 and 2.34 days, respectively after the wheat grains  
194 were treated with geranium oil at 1% (v/w). This oil gave separately 1.91 and 6.51 days for its  
195 LT<sub>50</sub> and LT<sub>90</sub>, respectively. In case of coriander oil, LT<sub>50</sub> and LT<sub>90</sub> of CA of 25% CO<sub>2</sub>  
196 significantly decreased to 0.74 and 2.45 days by the combination of the wheat grains with the  
197 oil at 1% (v/w), respectively. This oil gave alone 1.63 and 4.31 days for its LT<sub>50</sub> and LT<sub>90</sub>,  
198 respectively. In cumin oil at 1% (v/w), LT<sub>50</sub> and LT<sub>90</sub> of CA of 25% CO<sub>2</sub> on the insect tested,  
199 significantly reduced to 0.88 and 2.69 days, respectively. While LT<sub>50</sub> and LT<sub>90</sub> of cumin oil  
200 separately were 3.53 and 7.31 days, respectively (Table 2).

201 Concerning CAs of 50 and 75% CO<sub>2</sub>, the same trend was found as in CA of 25% CO<sub>2</sub>. But  
202 lethal time values of CA of 75% CO<sub>2</sub> were the shortest for *S. oryzae* adults when wheat grains  
203 were treated with the tested botanical oils. Data also revealed that coriander oil was the most  
204 effective at 30°C on the lethal time of CA of 75% CO<sub>2</sub>. It reduced LT<sub>50</sub> and LT<sub>90</sub> of CA of  
205 75% CO<sub>2</sub> from 1.12 and 4.1 days to the shortest lethal times 0.65 and 1.66 days for the adults  
206 of *S. oryzae*, respectively (Tables 3 and 4).  
207 Moreover, the results in general indicated that the three tested botanical oils had insignificant  
208 effects among them on LT<sub>50s</sub> or LT<sub>90s</sub> of CA of CO<sub>2</sub> for the adults of *S. oryzae*.  
209 Regarding, the insecticidal activity of the botanical oils in alone treatments of *S. oryzae*  
210 adults, they varied depending on the temperature used, concentration and type of oils. The  
211 activity order was coriander > geranium > cumin based on the recorded lethal time values.  
212 The differences in the type of the most toxic oil might be due to differences in the active  
213 chemical constituents each of the oil that has different mode of action. The activity of the  
214 phytochemicals on stored grain pests relied on presence of active chemical groups which were  
215 easily received by direct contact with insect and causing rapid suffocation and contact  
216 toxicity.

217 The activity of many botanical oils might be due to monoterpenoids [19,20]. From the former  
218 studies on the insecticidal and repellent activity of monoterpenoids and phenolic acids, it can  
219 be stated that common chemicals found in plant oils, such as monoterpenoids, 1,8- cineole,  
220 alpha pinene, carvone, linalool, etc., and phenolic acids were responsible for the insecticidal  
221 activity of these oils [24]. In addition, the germanium gave contact toxicity during 4 weeks in  
222 *T. castaneum*. The major compounds were citronellol and geranial (26.14% and 23.19%,  
223 respectively). Three other terpenes were secondary important; linalool, citronellol formate  
224 and menthone [25]. Also, previous studies revealed that the toxicity of botanical oils obtained  
225 from aromatic plants against insect pests related to several main components, such as 1.8-  
226 cineole, carvacrol, eugenol, limonene,  $\alpha$ -pinene and thymol. For example, the botanical oils  
227 from seeds of *Coriandrum sativum*, and *Carum carvi* L. were investigated for their toxicity  
228 against *S. oryzae*, it was found that coriander contained linalool as the main product active  
229 against the insects.

230 On the other hand, the lethal times of the tested oils for *S. oryzae* adults significantly varied  
231 with the temperature. It was observed that at the same concentration of the botanical oil, its  
232 lethal time was shorter at higher temperature compared to the lower for the tested insect. The  
233 results of this study agree with previous studies which showed that at 20°C the mortality of *S.*  
234 *oryzae* adults was low even at low spinosad doses, while at 30°C, spinosad was extremely  
235 effective on the treated wheat [60].

236

237 The increase in temperature from 20 to 30°C remarkably increased the insect mortality, even  
238 at the lowest dose rate used. In contrast with pyrethroids which are generally less effective at  
239 high temperatures while the reverse is true for OPs [61], when the results of the present study  
240 are interpolated with those from the previous studies using carbon dioxide, the widely  
241 reported findings that the insect control requires longer exposures to carbon dioxide especially  
242 at lower temperatures [40,41,51].

Our results indicated that the lethal exposure period for the tested insect at 30°C was significantly shorter. When the lethal time data for *S. oryzae* adults of CA of CO<sub>2</sub> in alone treatments and as a binary mixture with the botanical oils in this study were examined, it gives the clear indication that the combined action of CA of CO<sub>2</sub> and botanical oil significantly reduced the time required to kill 50% of *S. oryzae* adults. Although, at 20°C, LT<sub>50s</sub> of 25% CO<sub>2</sub>, geranium, coriander and cumin (each oil at 1.5% separately) were 2.48, 3.91, 3.24 and 4.06 days in alone treatments, respectively. These became 1.31, 1.08 and 1.19 days when each of geranium, coriander and cumin oils were combined with 25% of CO<sub>2</sub>, respectively. These times were shorter with the increase in CO<sub>2</sub> of CA from 25% to 50%. The shortest LT<sub>50s</sub> for *S. oryzae* adults were 0.74, 0.65 and 0.72 days when 75% CO<sub>2</sub> was combined with each of geranium, coriander and cumin oils (each oil at 1% separately), at 30°C, respectively. At low CO<sub>2</sub> concentrations, mortality occurs due to spiracular opening and increased fumigant uptake. However, metabolic effects become a more important determinant of mortality at higher CO<sub>2</sub> concentrations [31]. Furthermore, it was observed that the increases in time and temperature during CA exposure had a greater effect on mortality of the insects than increases in CO<sub>2</sub> or reductions in O<sub>2</sub> [37,38,41]. The greater efficacy at higher concentrations of CO<sub>2</sub> at low temperatures could be related to the higher solubility of CO<sub>2</sub> in tissues at low temperatures [29]. CO<sub>2</sub> is lethal by causing rapid water loss through opened spiracles [28,32]. Triacylglycerols are an important source of energy and for water regulation as well. Depletion of triacylglycerol levels could influence water regulation and lead to desiccation [33].

In general the results of the combined action of CO<sub>2</sub> and botanical oils are in accordance with the results of El-lakwah et al. [62,63] and Mohamed [41], who proved an additive and potentiation effects of plant extracts or oils under CA of CO<sub>2</sub> treatments against adults of *S. oryzae*. Also, Wang et al. [50] found the peel oils of *Citrus spp.* and *Eucalyptus citriodora* Hook at 10 and 20 µl/l doses were more toxic in presence of two different CAs (15% CO<sub>2</sub> + 1% O<sub>2</sub> + 84% N<sub>2</sub> and 12% CO<sub>2</sub> + 5% O<sub>2</sub> + 83% N<sub>2</sub>) to the psocid, *Liposcelis bostrychophilus* Badonnel (Psocoptera: Liposcelidae). The present results are also in agreement with that of Saleem et al. and Adel et al. [15,16] who reported that the botanical oils alone had a potential to control the stored grain insects at higher concentrations of oils and for longer exposure time. However, the results obtained from our studies show that the use of CO<sub>2</sub> with botanical oils clearly resulted in significant reductions of LT<sub>50</sub> and LT<sub>90</sub> values for adults of *S. oryzae*. This combination appears to have a synergistic effect on the insect tested.

#### 4. CONCLUSION

The combination of carbon dioxide with botanical oil has a safe, environment friendly and effective potential to control the stored grain insects for short times especially with the increase in temperature and concentration of CO<sub>2</sub>.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Sinha R N, Watters F L. Insect pests of flour mills, grain elevators, and feed mills and their control. Agriculture Canada Publication 1776E, Canadian Government Publication Centre, Ottawa, Canada; (1985).
2. Madrid F L, White N D G, Loschiavo S R. Insects in stored cereals and their association with farming practices in southern Manitoba. *Can Entomol.* (1990);122:515–523.
3. Talukder F A. Plant products as potential stored product insect management agents—a mini review *Emir J Food Agric.* (2006);18:17–32.
4. Rajendran S, Sriranjini V. Plant products as fumigants for stored-product insect control. *J Stored Prod Res.* (2008);44:126–135.
5. Arabi F, Moharramipour S, Sefidkon F. Chemical composition and insecticidal activity of essential oil from *Perovskia abrotanoides* (Lamiaceae) against *Sitophilus oryzae* (Coleoptera: Curculionidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Int. J Trop Insect Sci.* (2008);28:144–150.
6. Chu S S, Liu S L, Jiang G H, Liu Z L. Composition and toxicity of essential oil of *Illicium simonsii* Maxim (Illiciaceae) fruit against the maize weevils. *Rec Nat Prod.* (2010);4:205–210.
7. Isman M B. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu Rev Entomol.* (2006);51:45–66.
8. Shaaya E, Kostjukovski M, Eilberg J, Sukprakarn C. Plant oils as fumigants and contact insecticides for the control of stored-product insects. *J Stored Prod Res.* (1997);33:7-15.
9. Tayoub G, Alnaser A A, Ghanem I. Toxicity of two essential oils from *Eucalyptus globulus* Labail and *Origanum syriacum* L. on Larvae of Khapra beetle. *Int J Med Arom Pl.* (2012);2:240–245.

- 314 10. Ogendo J, Kostyukovsky M, Ravid U, Matasyoh J, Deng A, Omolo E, Kariuki S, Shaaya  
315 E. Bioactivities of *Ocimum gratissimum* L. oil and two of its constituents against five  
316 insect pests attacking stored food products. J Stored Prod Res. (2008);44:328–334.
- 317 11. Lorini I, Collins P J, Daglish G J, Nayak M K, Pavic H. Detection and characterization of  
318 strong resistance to phosphine in Brazilian *Rhyzopertha dominica* (F.) (Coleoptera:  
319 Bostrichidae). Pest Manag Sci. (2007);63:358–364.
- 320 12. Nayak N K, Holloway J C, Emery R N, Pavic H, Bartlet J, Collins P J. Strong resistance  
321 to phosphine in the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera:  
322 Laemophloeidae): its characterisation, a rapid assay for diagnosis and its distribution in  
323 Australia. Pest Manage Sci. (2012);69:48–53.
- 324 13. Logan J W M, Cowie R H, Wood T G. Termite (Isoptera) control in agriculture and  
325 forestry by non-chemical methods: a review Bull Entomol Res. (1990);80:309–330.
- 326 14. Rajashekar Y, Bakthavatsalam N, Shivanandappa T. Botanicals as Grain Protectants.  
327 Psyche. (2012);1–13.
- 328 15. Saleem S, Hasan M, Sagheer M, Sahi S T. Insecticidal activity of essential oils of four  
329 medicinal plants against different stored grain insect pests. Pakistan J Zool.  
330 (2014);46:1407–1414.
- 331 16. Adel I, Seada M A, Abo Arab R, Seif A I. Efficacy of three local Egyptian essential oils  
332 against the rice weevil, *Sitophilus oryzae* (coleoptera: curculionidae) and the cowpea  
333 weevil, *callosobruchus maculatus* (coleoptera: bruchidae). Egypt J Exp Biol (Zool.).  
334 (2015);11:95–105.
- 335 17. Jayakumar M, Arivoli S, Raveen R, Tennyson S. Repellent activity and fumigant toxicity  
336 of a few plant oils against the adult rice weevil *Sitophilus oryzae* Linnaeus 1763  
337 (Coleoptera: Curculionidae). J Entomol Zool Stud. (2017);5:324–335.
- 338 18. Messina F J, Renwick J A A. Effectiveness of oils in protecting stored cowpea from the  
339 cowpea weevil (Coleoptera: Bruchidae). J Econ Entomol. (1983);76:634–636.

- 340 19. Waliwitiya R, Isman M B, Vernon R S, Riseman A. Insecticidal activity of selected  
341 monoterpenoids and rosemary oil to *Agriotes obscurus*. (Coleoptera: Elateridae). J Econ  
342 Entomol. (2005);98:1560–1565.
- 343 20. Tong F, Coats J R. Effects of monoterpenoid insecticides on [3H]-TBOB binding in house  
344 fly GABA receptor and 36 Cl<sup>-</sup> uptake in American cockroach ventral nerve cord. Pestic  
345 Biochem Physiol. (2010);98:317–324.
- 346 21. Houghton P J, Ren Y, Howes M J. Acetylcholinesterase inhibitors from plants and fungi.  
347 Nat Prod Rep. (2006);23:181–199.
- 348 22. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils-a  
349 review Food Chem Toxicol. (2008);46:446–475.
- 350 23. Nerio L S, Olivero-Verbel J, Stashenko E. Repellent activity of essential oils: a review  
351 Bioresour Technol. (2010);101:372–378.
- 352 24. Rani P U. Fumigant and contact toxic potential of essential oils from plant extracts against  
353 stored product pests. J Biopest. (2012);5:120–128.
- 354 25. Gonzalez J O W, Gutierrz M M, Ferrero A A, Banol B F. Essential oils nanoformulations  
355 for stored product pest control-characterization and biological properties. Chemosphere.  
356 (2014);100:130–138.
- 357 26. Nakakita H, Kawashima K. A new method to control stored-product insects using carbon  
358 dioxide With high pressure followed by sudden pressure loss In: Highley E, Wright EI,  
359 Banks HJ, Champ BR, ed., Proceedings of the 6<sup>th</sup> International Working Conference on  
360 Stored-product Protection, Canberra, Australia; (1994).
- 361 27. Mitcham E, Martin T, Zhou S. The mode of action of insecticidal controlled atmospheres.  
362 Bull Entomol Res. (2006);96:213–222.
- 363 28. Jay E G, Cuff W. Weight loss and mortality of three life stages of *Tribolium castaneum*  
364 (Herbst) when exposed to four modified atmospheres. J Stored Prod Res. (1981);17:117–  
365 124.

- 366 29. Yacoe M E. Effects of temperature, pH, and CO<sub>2</sub> tension on the metabolism of isolated  
367 hepatic mitochondria of the desert iguana, *Dipsosaurus dorsalis*. *Physiol Zool*.  
368 (1986);59:263–272.
- 369 30. Nicolas G, Sillans D. Immediate and latent effects of carbon dioxide on insects. *Annu Rev*  
370 *Entomol.* (1989);34:97–116.
- 371 31. Janmaat A F, de Kogel W J, Woltering E J. Enhanced fumigant toxicity of p-cymene  
372 against *Frankliniella occidentalis* by simultaneous application of elevated levels of carbon  
373 dioxide. *Pest Manag Sci.* (2001);58:167–173.
- 374 32. Ofuya T I, Reichmuth C. Effect of relative humidity on susceptibility of *Callosobruchus*  
375 *maculatus* (Fabricius) (Coleoptera: Bruchidae) to two modified atmospheres. *J Stored Prod*  
376 *Res.* (2002);38:139–146.
- 377 33. Wang J J, Zhao Z M. Accumulation and utilization of triacylglycerol and polysaccharides  
378 in *Liposcelis bostrychophila* (Psocoptera: Liposcelidae) selected for resistance to carbon  
379 dioxide. *J Appl Entomol.* (2003);127:107–111.
- 380 34. White N DG, Jayas D S, Muir W E. Toxicity of carbon dioxide at biologically producible  
381 levels to stored product beetles. *Environ Entomol.* (1995);24:640–647.
- 382 35. Banks J, Fields P. Physical methods for insect control in stored-grain ecosystems. In Jayas  
383 DS, White NDG, Muir WE (eds.), *Stored Grain Ecosystems*. Marcel Dekker, New York;  
384 (1995).
- 385 36. Mbata G N, Phillips T W. Effects of temperature and exposure time on mortality of  
386 stored-product insects exposed to low pressure. *J Econ Entomol.* (2001);94:1302–1307.
- 387 37. Carpenter A. A comparison of the responses of aphids and thrips to controlled  
388 atmospheres. Vol. 1: *CA Technology and Disinfestation Studies*. Postharvest Horticulture  
389 Series, No 15, University of California, Davis; (1997).

- 390 38. Nilson T L, Sinclair B J, Roberts S P. The effects of carbon dioxide anesthesia and anoxia  
391 on rapid coldhardening and chill coma recovery in *Drosophila melanogaster*. J Insect  
392 Physiol. (2006);52:1027–1033.
- 393 39. Leong E C W, HO J H. Effects of carbon dioxide on the mortality of *Liposcelis*  
394 *bostrychophila* Bad. and *Liposcelis entomophila* (End.) (Psocoptera: Liposcelididae). J  
395 Stored Prod Res. (1995);31:185–190.
- 396 40. Banks H J, Annis P C, Rigby G R. Controlled atmosphere storage of grain: the known and  
397 the future Proc. 5th Int. Working Conf. on Stored-Product Protection, Bordeaux, France,  
398 September 1990 (ed. Fleurat-Lessard, F. and P. Ducom); (1991).
- 399 41. Mohamed H H. Studies on the efficacy of some natural materials against certain stored  
400 product insects [master's thesis]. Benha University, Egypt; (2016).
- 401 42. Mbata G N, Reichmuth C. The comparative effectiveness of different modified  
402 atmospheres for the disinfestations of bambarra groundnuts, *Vigna subterranean* (L)  
403 Verde, infested by *Callosobruchus subinnotatus* (Pic) (Coleoptera: Bruchidae). J Stored  
404 Prod Res. (1996);32(1):45–51.
- 405 43. Navarro S. The use of modified and controlled atmospheres for the disinfestation of stored  
406 products. J Pest Sci. (2012); doi:10.1007/s10340-012-0424-3.
- 407 44. Donahaye E J, Navarro S. Comparisons of energy reserves among strains of *Tribolium*  
408 *castaneum* selected for resistance to hypoxia and hypercarbia, and the unselected strain. J  
409 Stored Prod Res. (2000);36:223–234.
- 410 45. Neven G L, Hansen D L. Effects of temperature and controlled atmospheres on codling  
411 moth metabolism. Ann Entomol Soc Am. (2010);103:418–423.
- 412 46. Locatelli D P, Daotio E. Effectiveness of carbon dioxide under reduced pressure against  
413 some insects infesting packaged rice. J Stored Prod Res. (1993);29:81–87.

- 414 47. Adler C. Influence of temperature on the efficacy of nitrogen or carbon dioxide  
415 fumigations against insect pests in stored grain. Mitt Dtsch Ges Allg Angew Entomol.  
416 (1995);1:269–272.
- 417 48. Annis P C. Towards rational controlled atmosphere dosage schedules: a review of current  
418 knowledge Proc. 4th Int. Working Conf. on Stored-Product Protection, Tel Aviv, Israel,  
419 September 1986 (ed. Donahaye E and Navarro S); (1987).
- 420 49. Newton J. Carbon dioxide as a fumigant to replace methyl bromide in the control of  
421 insects and mites damaging stored products and artefacts. Proceedings of the First  
422 International Conference on Urban Pests. Wildey K B, Robinson W H.; (1993).
- 423
- 424 50. Wang J J, Tsai J H, Ding W, Zhao Z M, Li L S. Toxic effects of six plant oils alone and in  
425 combination with controlled atmosphere on *Liposcelis bostrychophilus* (Psocoptera:  
426 Liposcelididae). J Econ Entomol. (2001);94:1296–1301.
- 427 51. Azab M, Darwish A, Mohamed R, Sanad M. Comparative efficacy of controlled  
428 atmospheres against two stored product insects. J Crop Prot. (2013); 2:343–353.
- 429 52. El-lakwah F A, Azab M M, Ebrahim W M K. Influence of temperature on the  
430 effectiveness of controlled atmospheres of various carbon dioxide concentrations or high  
431 nitrogen content on two stored products insects. Annals Agric Sci Moshtohor.  
432 (2004);42:839 850.
- 433 53. Sharopov S F, Zhang H, Setzer N W. Composition of geranium (*Pelargonium graveolens*)  
434 essential oil from Tajikistan. Am J Essent Oil Nat Prod. (2014);2:13–16.
- 435 54. Raal A, Arak E, Orav A. Chemical composition of coriander seed essential oil and their  
436 conformity with EP standards. J Agric Sci. (2004);15:234–239.
- 437 55. Moghadam L R A Essential oil of the seeds of *Cuminum cyminum* L. (Apiaceae). Bull  
438 Env Pharmacol Life Sci. (2015);3:161–163.
- 439 56. Abbott W S. A method of computing the effectiveness, of an insecticide. J Econ Entomol.  
440 (1925);18:265–267.

57. Noack S, Reichmuth C H. Einrechnerisches Verfahren zur Bestimmung von beliebigen Dosiswerten eines Wirkstoffes aus empirischen Dosis-Wirkungs-Daten. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Fortswirtschaft, Berlin-Dahlem, Heft; (1978).
58. Finney D J. Probit analysis. 3rd. Eddn, Cambridge University press: Cambridge, UK; (1971).
59. Aydin H, Gürkan M O. The efficacy of spinosad on different strains of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). Turk J Biol. (2006);30:5-9.
60. Athanassiou C G, Kavallieratos N G, Yiatilis A E, Vayias B J, Mavrotas C S, Tomanovic Z. Influence of temperature and humidity on the efficacy of spinosad against four stored grain beetle species. J Insect Sci. (2008);8:60–69.
61. Johnson D L. Influence of temperature on toxicity of two pyrethroids to grasshoppers (Orthoptera: Acrididae). J Econ Entomol. (1990);83:366–373.
62. El-lakwah F A, Azab M M, Abdel-Latif A M. Efficacy of lupine seed extract with and without controlled atmospheres against some stored product insects. Annals Agric Sci Moshtohor. (2001);40:1283–1294.
63. El-lakwah F A, Abd-El-Aziz A E, Azab M M. Effectiveness of petroleum ether extracts of dill and cumin seeds alone and under modified atmospheres against Khapra beetle larvae – *Trogoderma granarium* Everts. 2<sup>nd</sup> International Conference, Plant Protection Research Institute, Cairo, Egypt; (2002).

| Common Name | Scientific Name               | Family                 | Components                                                                                                                                                                                                                                                                                                              |
|-------------|-------------------------------|------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Geranium    | <i>Pelargonium graveolens</i> | Geraniaceae            | Citronellol (37.5%), geraniol (6.0%), caryophyllene oxide (3.7%), menthone (3.1%), linalool (3.0%), $\beta$ -bourbonene (2.7%), iso-menthone (2.1%), geranyl formate (2.0%), cis-rose oxide (1.9%), geranyl tiglate (1.8%), and 2-phenylethyl tiglate (1.5%) [53]                                                       |
| Coriander   | <i>Coriandrum sativum</i>     | Apiaceae\ Umbelliferae | Linalool (58.0–80.3%), $\gamma$ -terpinene (0.3%–11.2%), $\alpha$ -pinene (0.2%–10.9%), p-cymene (0.1%–8.1%), camphor (3.0%–5.1%) and geranyl acetate (0.2%–5.4%) [54]                                                                                                                                                  |
| Cumin       | <i>Cuminum cyminum</i>        | Apiaceae               | Thymol (40.65%), $\gamma$ -terpinene (24.51%), b-pinene (5.38%), a-pinene (3.47%), camphene (2.31%), terpinene- 4 – ol (2.00%), cuminaldehyde (1.79%), a-thujene (1.45%), a-terpinolene (1.17%), myrcene (1.07%), limonene (1.04%), $\alpha$ -phyllanderene (0.94%), acetoxyllinalool (0.57%) and sabinene (0.37%) [55] |

**Table 2.** Effect of tested botanical oils on the lethal time values of controlled atmosphere (CA) of 25% CO<sub>2</sub> for the adults of *S. oryzae* at 20±1 and 30±1 °C and 65±5% RH.

| Botanical oil | Temperature (°C) | Oil concentration % (v/w) | Lethal time and their 95% Confidence Limits<br>LT <sub>50</sub> (days) |                                  |                                   | Lethal time and their 95% Confidence Limits<br>LT <sub>90</sub> (days) |                                    |                                   |
|---------------|------------------|---------------------------|------------------------------------------------------------------------|----------------------------------|-----------------------------------|------------------------------------------------------------------------|------------------------------------|-----------------------------------|
|               |                  |                           | Oil alone**                                                            | 25% CO <sub>2</sub> alone        | Oil+25% CO <sub>2</sub> mixture** | Oil alone**                                                            | 25% CO <sub>2</sub> alone          | Oil+25% CO <sub>2</sub> mixture** |
| Geranium*     | 20               | 1.5                       | 3.91 <sup>Aa</sup><br>(2.82-5.23)                                      | 2.48 <sup>a</sup><br>(2.12-2.86) | 1.31 <sup>Ab</sup><br>(1.01-1.57) | 7.83 <sup>Aab</sup><br>(6.17-11.98)                                    | 12.09 <sup>a</sup><br>(9.52-16.71) | 6.14 <sup>Ab</sup><br>(4.89-8.61) |
| Coriander*    |                  |                           | 3.24 <sup>Aa</sup><br>(2.55-3.89)                                      | 2.48 <sup>a</sup><br>(2.12-2.86) | 1.08 <sup>Ab</sup><br>(0.83-1.29) | 5.82 <sup>Aac</sup><br>(4.81-7.42)                                     | 12.09 <sup>b</sup><br>(9.52-16.71) | 4.15 <sup>Ac</sup><br>(3.49-5.25) |
| Cumin*        |                  |                           | 4.06 <sup>Aa</sup><br>(3.11-5.19)                                      | 2.48 <sup>b</sup><br>(2.12-2.86) | 1.19 <sup>Ac</sup><br>(0.96-1.39) | 8.09 <sup>Aa</sup><br>(6.52-11.47)                                     | 12.09 <sup>a</sup><br>(9.52-16.71) | 4.15 <sup>Ab</sup><br>(3.53-5.16) |
| Geranium*     | 30               | 1                         | 1.91 <sup>Aab</sup><br>(0.87-2.28)                                     | 2.06 <sup>a</sup><br>(1.45-2.61) | 0.83 <sup>Ab</sup><br>(0.59-1.00) | 6.51 <sup>Aa</sup><br>(4.89-12.12)                                     | 6.91 <sup>a</sup><br>(5.51-11.20)  | 2.34 <sup>Ab</sup><br>(2.03-2.78) |
| Coriander*    |                  |                           | 1.63 <sup>Aa</sup><br>(1.01-2.05)                                      | 2.06 <sup>a</sup><br>(1.45-2.61) | 0.74 <sup>Ab</sup><br>(0.51-0.94) | 4.31 <sup>Aa</sup><br>(3.76-6.75)                                      | 6.91 <sup>a</sup><br>(5.51-11.20)  | 2.45 <sup>Ab</sup><br>(2.09-3.07) |
| Cumin*        |                  |                           | 3.53 <sup>Ba</sup><br>(2.28-4.19)                                      | 2.06 <sup>a</sup><br>(1.45-2.61) | 0.88 <sup>Ab</sup><br>(0.66-1.06) | 7.31 <sup>Aa</sup><br>(5.38-10.94)                                     | 6.91 <sup>a</sup><br>(5.51-11.20)  | 2.69 <sup>Ab</sup><br>(2.31-3.34) |

\* Different lowercase letters within each row of each lethal time value indicate significant differences ( $p = 0.05$ )

\*\* Different uppercase letters within each column of each tested temperature indicate significant differences ( $p = 0.05$ )

**Table 3.** Effect of tested botanical oils on the lethal time values of controlled atmosphere (CA) of 50% CO<sub>2</sub> for the adults of *S. oryzae* at 20±1 and 30±1 °C and 65±5 % RH.

| Botanical oil | Temperature (°C) | Oil concentration % (v/w) | Lethal time (days) and their 95 % Confidence Limits |                                  |                                     | Lethal time (days) and their 95 % Confidence Limits |                                   |                                     |
|---------------|------------------|---------------------------|-----------------------------------------------------|----------------------------------|-------------------------------------|-----------------------------------------------------|-----------------------------------|-------------------------------------|
|               |                  |                           | LT <sub>50</sub>                                    |                                  |                                     | LT <sub>90</sub>                                    |                                   |                                     |
|               |                  |                           | Oil alone **                                        | 50 % CO <sub>2</sub> alone       | Oil+50 % CO <sub>2</sub> ** mixture | Oil alone **                                        | 50 % CO <sub>2</sub> alone        | Oil+50 % CO <sub>2</sub> ** mixture |
| Geranium *    | 20               | 1.5                       | 3.91 <sup>Aa</sup><br>(2.82-5.23)                   | 2.07 <sup>b</sup><br>(1.79-2.36) | 1.07 <sup>Ac</sup><br>(0.46-1.31)   | 7.83 <sup>Aab</sup><br>(6.17-11.98)                 | 7.91 <sup>a</sup><br>(6.62-9.97)  | 3.34 <sup>Ab</sup><br>(2.62-6.50)   |
| Coriander *   |                  |                           | 3.24 <sup>Aa</sup><br>(2.55-3.89)                   | 2.07 <sup>b</sup><br>(1.79-2.36) | 0.85 <sup>Ac</sup><br>(0.45-0.91)   | 5.82 <sup>Aa</sup><br>(4.81-7.42)                   | 7.91 <sup>a</sup><br>(6.62-9.97)  | 2.83 <sup>Ab</sup><br>(2.41-3.46)   |
| Cumin *       |                  |                           | 4.06 <sup>Aa</sup><br>(3.11-5.19)                   | 2.07 <sup>b</sup><br>(1.79-2.36) | 1.16 <sup>Ac</sup><br>(0.46-1.34)   | 8.09 <sup>Aa</sup><br>(6.52-11.47)                  | 7.91 <sup>a</sup><br>(6.62-9.97)  | 3.09 <sup>Ab</sup><br>(2.39-6.36)   |
| Geranium *    | 30               | 1                         | 1.91 <sup>Aa</sup><br>(0.87-2.28)                   | 1.32 <sup>a</sup><br>(0.99-1.61) | 0.96 <sup>Aa</sup><br>(0.75-1.15)   | 6.51 <sup>Aa</sup><br>(4.89-12.12)                  | 7.10 <sup>a</sup><br>(5.45-10.91) | 2.87 <sup>Ab</sup><br>(2.46-3.56)   |
| Coriander *   |                  |                           | 1.63 <sup>Aa</sup><br>(1.01-2.05)                   | 1.32 <sup>a</sup><br>(0.99-1.61) | 0.73 <sup>Ab</sup><br>(0.54-0.89)   | 4.31 <sup>Aa</sup><br>(3.76-6.75)                   | 7.10 <sup>a</sup><br>(5.45-10.91) | 1.75 <sup>Bb</sup><br>(1.53-2.05)   |
| Cumin *       |                  |                           | 3.53 <sup>Ba</sup><br>(2.28-4.19)                   | 1.32 <sup>b</sup><br>(0.99-1.61) | 0.85 <sup>Ab</sup><br>(0.66-1.00)   | 7.31 <sup>Aa</sup><br>(5.38-10.94)                  | 7.10 <sup>a</sup><br>(5.45-10.91) | 2.06 <sup>Bb</sup><br>(1.81-2.45)   |

\* Different lowercase letters within each row of each lethal time value indicate significant differences ( $p = 0.05$ )

\*\* Different uppercase letters within each column of each tested temperature indicate significant differences ( $p = 0.05$ )

**Table 4.** Effect of tested botanical oils on the lethal time values of controlled atmosphere (CA) of 75% CO<sub>2</sub> for the adults of *S. oryzae* at 20±1 and 30±1°C and 65±5% RH.

| Botanical oil | Temperature (°C) | Oil concentration % (v/w) | Lethal time (days) and their 95 % Confidence Limits<br>LT <sub>50</sub> |                                  |                                   | Lethal time (days) and their 95 % Confidence Limits<br>LT <sub>90</sub> |                                   |                                    |
|---------------|------------------|---------------------------|-------------------------------------------------------------------------|----------------------------------|-----------------------------------|-------------------------------------------------------------------------|-----------------------------------|------------------------------------|
|               |                  |                           | Oil alone**                                                             | 75% CO <sub>2</sub> alone        | Oil+75% CO <sub>2</sub> mixture** | Oil alone**                                                             | 75% CO <sub>2</sub> alone         | Oil+75% CO <sub>2</sub> mixture**  |
|               |                  |                           |                                                                         |                                  |                                   |                                                                         |                                   |                                    |
| Geranium*     | 20               | 1.5                       | 3.91 <sup>Aa</sup><br>(2.82- 5.23)                                      | 1.88 <sup>b</sup><br>(1.12-2.51) | 0.85 <sup>Ac</sup><br>(0.59-1.08) | 7.83 <sup>Aa</sup><br>(6.17-11.98)                                      | 6.17 <sup>a</sup><br>(4.99-12.38) | 3.03 <sup>ACb</sup><br>(2.89-4.33) |
| Coriander*    |                  |                           | 3.24 <sup>Aa</sup><br>(2.55-3.89)                                       | 1.88 <sup>b</sup><br>(1.12-2.51) | 0.69 <sup>Ac</sup><br>(0.47-0.88) | 5.82 <sup>Aa</sup><br>(4.81-7.42)                                       | 6.17 <sup>a</sup><br>(4.99-12.38) | 2.29 <sup>Bb</sup><br>(1.97-2.74)  |
| Cumin*        |                  |                           | 4.06 <sup>Aa</sup><br>(3.11-5.19)                                       | 1.88 <sup>b</sup><br>(1.12-2.51) | 0.81 <sup>Ac</sup><br>(0.58-1.00) | 8.09 <sup>Aa</sup><br>(6.52-11.47)                                      | 6.17 <sup>a</sup><br>(4.99-12.38) | 2.63 <sup>BCb</sup><br>(2.22-3.34) |
| Geranium*     | 30               | 1                         | 1.91 <sup>Aa</sup><br>(0.87-2.28)                                       | 1.12 <sup>a</sup><br>(0.88-1.33) | 0.74 <sup>Aa</sup><br>(0.53-0.89) | 6.51 <sup>Aa</sup><br>(4.89-12.12)                                      | 4.10 <sup>a</sup><br>(3.47-5.14)  | 1.76 <sup>Ab</sup><br>(1.54-2.13)  |
| Coriander*    |                  |                           | 1.63 <sup>Aa</sup><br>(1.01-2.05)                                       | 1.12 <sup>a</sup><br>(0.88-1.33) | 0.65 <sup>Ab</sup><br>(0.46-0.81) | 4.31 <sup>Aa</sup><br>(3.76-6.75)                                       | 4.10 <sup>a</sup><br>(3.47-5.14)  | 1.66 <sup>Ab</sup><br>(1.45-1.91)  |
| Cumin*        |                  |                           | 3.53 <sup>Ba</sup><br>(2.28-4.19)                                       | 1.12 <sup>b</sup><br>(0.88-1.33) | 0.72 <sup>Ab</sup><br>(0.52-0.88) | 7.31 <sup>Aa</sup><br>(5.38-10.94)                                      | 4.10 <sup>b</sup><br>(3.47-5.14)  | 1.69 <sup>Ac</sup><br>(0.48-2.02)  |

\* Different lowercase letters within each row of each lethal time value indicate significant differences ( $p = 0.05$ )

\*\* Different uppercase letters within each column of each tested temperature indicate significant differences ( $p = 0.05$ )



**Fig 1. Re-circulatory multi-flask apparatus**