



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 both authors. Author MMA conceived, designed the research and wrote the manuscript. Both authors conducted the experiments, analyzed the data, reviewed, read and approved the manuscript.

Article Information

DOI: 10.9734/AJAAR/2017/38471

Editor(s):

(1) Mustafa Turkmen, Professor, Department of Biology, Faculty of Science & Arts, Giresun University, Turkey.

Reviewers:

(1) Isela Quintero Zapata, Autonomous University of Nuevo Leon, Mexico.
(2) Zhujia Ye, Tennessee State University, USA.

Complete Peer review History: <http://prh.sdiarticle3.com/review-history/22429>

Original Research Article

Received 28th November 2017
Accepted 18th December 2017
Published 23rd December 2017

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 CO₂ 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 CO₂ 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, LT₅₀ and LT₉₀) of *S. oryzae* adults reduced significantly by the combination of tested oil and CO₂. The lethal times (LT_{90s}) were significantly the longest, when each of 25% CO₂, 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% CO₂, 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% CO₂, respectively.

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Furthermore, coriander oil was the most effective at 30°C on the lethal time of CA of 75% CO₂. It caused a reduction in LT₅₀ and LT₉₀ of CA of 75% CO₂ 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 CO₂ can be significantly increased by using it with botanical oils in insect control and this action becomes the greatest at the highest CO₂ concentration and higher temperature than lower one.

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

1. INTRODUCTION

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].

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.

These components have two groups of distinct biosynthetic origin. Terpenes and terpenoids formed the main group and the other is composed of aromatic and aliphatic components. The action has been linked to the existence of monoterpenes and sesquiterpenes which cause death of insects by inhibiting acetyl cholinesterase activity in the nervous system. However, these chemicals can work synergistically and improving their bioactivity [19,20,21,22,23,24,25]. On the other hand, CAs were recommended as an alternative to use of methyl bromide and phosphine for control of the stored product insects, carbon dioxide as natural components of the atmosphere is a safe chemical and has been permitted for using in many types of drinks and foods [26,27]. Controlled atmospheres of elevated CO₂ levels lead to spiracles to open resulting in insect death from water loss. Spiracles remain permanently open when CO₂ is above 10%. Toxic action is completely through the tracheae. Carbon dioxide has direct toxic action on the nervous system. In some cases, CO₂ can acidify the hemolymph causing membrane failure of some tissues [28,29,30,31,32,33]. Sub-lethal levels of CO₂ can have harmful effects on insect development, growth and reproduction for prolonged times. Controlled atmospheres of 60% CO₂ rapidly kill stored product insects [30,34]. Controlled atmospheres of reduced O₂ and elevated CO₂ can have an additive effect, depending on the concentrations used [27].

The temperature plays a great role in insect mortality through the exposure to CAs. In general, insect sensitivity to CAs is greater at higher temperatures due to enhancing the respiration demands [35,36].

In CA application, the time of exposure is a critical factor, the lethal atmosphere must be retained for a suitable length of time to obtain its

efficacy in insect control [37,38,39,40,41]. However, the effectiveness of CA can be enhanced by varying other parameters such as temperature when short exposure period is required [32,42,43].

A considerable effect on insect metabolism was observed for heat treatments of CA, low O₂ inhibits ATP synthesis, and high CO₂ disturbs use of ATP [44,45].

Heat combined with CAs of CO₂ 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 CO₂-enriched and botanical oils, this limits their use for protecting stored products, in which quick treatment is needed [48,49,50,51,15,41].

Therefore, the present study was conducted to investigate the effect of three botanical oils geranium, coriander and cumin on the lethal time of CO₂ 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 (CO₂) was provided as pure gases in pressurized steel cylinders. Each cylinder was connected to a pressure regular. The dilution method was used to achieve the required CO₂ concentration. CAs of 25, 50 and 75% CO₂ in the air were prepared (Fig. 1).

Table 1. Details of botanical oils used for the study

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%), β-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%), γ-terpinene (0.3%–11.2%), α-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%), γ-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%), α-phyllanderene (0.94%), acetoxylinalool (0.57%) and sabinene (0.37%) [55]

2.4 Determination of the Concentrations of CO₂ 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 CO₂

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±1°C and 20±1°C, 65±5% 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 treated with the tested oils separately as above mentioned in the botanical oils treatments. Controlled atmosphere of CO₂ concentrations, 25, 50 and 75% in the air were tested separately as mentioned before with each treatment of oil concentration, 1.5% at 20°C and 1.0% at 30°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 LT₅₀ 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].

Obtained data indicated that at 20°C, the lethal times, LT₅₀ and LT₉₀ of CA of 25% CO₂ separately were 2.48 and 12.09 days, respectively. These values decreased significantly to 1.31 and 6.14 days after the wheat grains were combined with geranium oil at 1.5% (v/w), respectively. Geranium oil gave separately lethal time values, 3.91 and 7.83 days for LT₅₀ and LT₉₀, respectively. In case of coriander oil separately, the lethal time values on the adults of *S. oryzae* were 3.24 and 5.82 days for LT₅₀ and LT₉₀, respectively. Lethal times of CA of 25% CO₂ reduced significantly to 1.08 and 4.15 days for LT₅₀ and LT₉₀, respectively, after the wheat grains were treated with coriander oil at 1.5% (v/w). When cumin oil at 1.5% (v/w) was applied on the wheat grains, lethal times of CA of 25% CO₂ on the adults of *S. oryzae* significantly decreased to 1.19 and 4.15 days for LT₅₀ and LT₉₀, respectively. However, LT₅₀ of cumin oil was separately the longest, 4.06 days. It followed by geranium and coriander oils that gave alone, 3.91 and 3.24 days for their LT_{50s}, respectively. Moreover, LT₉₀ of CA of 25% CO₂ was the longest, 12.09 days in alone treatment.

Regarding the treatments at 30°C, the lethal times were more affected than at 20 ±1°C, where the values, LT₅₀ and LT₉₀ of CA of 25% CO₂ alone were 2.06 and 6.91 days, respectively. These values significantly reduced to 0.83 and 2.34 days, respectively after the wheat grains were treated with geranium oil at 1% (v/w). This oil gave separately 1.91 and 6.51 days for its LT₅₀ and LT₉₀, respectively. In case of coriander oil, LT₅₀ and LT₉₀ of CA of 25% CO₂ significantly decreased to 0.74 and 2.45 days by the combination of the wheat grains with the oil at 1% (v/w), respectively. This oil gave alone 1.63 and 4.31 days for its LT₅₀ and LT₉₀, respectively. In cumin oil at 1% (v/w), LT₅₀ and LT₉₀ of CA of 25% CO₂ on the insect tested, significantly reduced to 0.88 and 2.69 days, respectively. While LT₅₀ and LT₉₀ of cumin oil

separately were 3.53 and 7.31 days, respectively (Table 2).

Concerning CAs of 50 and 75% CO₂, the same trend was found as in CA of 25% CO₂. But lethal time values of CA of 75% CO₂ were the shortest for *S. oryzae* adults when wheat grains were treated with the tested botanical oils. Data also revealed that coriander oil was the most effective at 30°C on the lethal time of CA of 75% CO₂. It reduced LT₅₀ and LT₉₀ of CA of 75% CO₂ 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 (Tables 3 and 4).

Moreover, the results in general indicated that the three tested botanical oils had insignificant effects among them on LT_{50s} or LT_{90s} of CA of CO₂ for the adults of *S. oryzae*.

Regarding, the insecticidal activity of the botanical oils in alone treatments of *S. oryzae* adults, they varied depending on the temperature used, concentration and type of oils. The activity order was coriander > geranium > cumin based on the recorded lethal time values.

The differences in the type of the most toxic oil might be due to differences in the active chemical constituents each of the oil that has different mode of action. The activity of the phytochemicals on stored grain pests relied on presence of active chemical groups which were easily received by direct contact with insect and causing rapid suffocation and contact toxicity.

The activity of many botanical oils might be due to monoterpenoids [19,20]. From the former studies on the insecticidal and repellent activity of monoterpenoids and phenolic acids, it can be stated that common chemicals found in plant oils, such as monoterpenoids, 1,8- cineole, alpha pinene, carvone, linalool, etc., and phenolic acids

were responsible for the insecticidal activity of these oils [24]. In addition, the geranium gave contact toxicity during 4 weeks in *T. castaneum*. The major compounds were citronellol and geranial (26.14% and 23.19%, respectively). Three other terpenes were secondary important; linalool, citronellol formate and menthone [25]. Also, previous studies revealed that the toxicity of botanical oils obtained from aromatic plants against insect pests related to several main components, such as 1.8-cineole, carvacrol, eugenol, limonene, α-pinene and thymol. For example, the botanical oils from seeds of *Coriandrum sativum*, and *Carum carvi* L. were investigated for their toxicity against *S. oryzae*, it was found that coriander contained linalool as the main product active against the insects.

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

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



Fig 1. Re-circulatory multi-flask apparatus

Table 2. Effect of tested botanical oils on the lethal time values of controlled atmosphere (CA) of 25% CO₂ 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 LT ₅₀ (days)			Lethal time and their 95% confidence limits LT ₉₀ (days)		
			Oil alone**	25% CO ₂ alone	Oil+25%CO ₂ mixture	Oil alone**	25% CO ₂ alone	Oil+25%CO ₂ mixture
Geranium	20	1.5	3.91 ^{Aa} (2.82-5.23)	2.48 ^a (2.12-2.86)	1.31 ^{Ab} (1.01-1.57)	7.83 ^{Ab} (6.17-11.98)	12.09 ^a (9.52-16.71)	6.14 ^{Ab} (4.89-8.61)
Coriander			3.24 ^{Aa} (2.55-3.89)	2.48 ^a (2.12-2.86)	1.08 ^{Ab} (0.83-1.29)	5.82 ^{Ac} (4.81-7.42)	12.09 ^b (9.52-16.71)	4.15 ^{Ac} (3.49-5.25)
Cumin			4.06 ^{Aa} (3.11-5.19)	2.48 ^b (2.12-2.86)	1.19 ^{Ac} (0.96-1.39)	8.09 ^{Aa} (6.52-11.47)	12.09 ^a (9.52-16.71)	4.15 ^{Ab} (3.53-5.16)
Geranium	30	1	1.91 ^{Aab} (0.87-2.28)	2.06 ^a (1.45-2.61)	0.83 ^{Ab} (0.59-1.00)	6.51 ^{Aa} (4.89-12.12)	6.91 ^a (5.51-11.20)	2.34 ^{Ab} (2.03-2.78)
Coriander			1.63 ^{Aa} (1.01-2.05)	2.06 ^a (1.45-2.61)	0.74 ^{Ab} (0.51-0.94)	4.31 ^{Aa} (3.76-6.75)	6.91 ^a (5.51-11.20)	2.45 ^{Ab} (2.09-3.07)
Cumin			3.53 ^{Ba} (2.28-4.19)	2.06 ^a (1.45-2.61)	0.88 ^{Ab} (0.66-1.06)	7.31 ^{Aa} (5.38-10.94)	6.91 ^a (5.51-11.20)	2.69 ^{Ab} (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% CO₂ 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 LT ₅₀			Lethal time (days) and their 95% confidence limits LT ₉₀		
			Oil alone**	50% CO ₂ alone	Oil+50%CO ₂ mixture	Oil alone**	50% CO ₂ alone	Oil+50%CO ₂ mixture
Geranium	20	1.5	3.91 ^{Aa} (2.82-5.23)	2.07 ^b (1.79-2.36)	1.07 ^{Ac} (0.46-1.31)	7.83 ^{Ab} (6.17-11.98)	7.91 ^a (6.62-9.97)	3.34 ^{Ab} (2.62-6.50)
Coriander			3.24 ^{Aa} (2.55-3.89)	2.07 ^b (1.79-2.36)	0.85 ^{Ac} (0.45-0.91)	5.82 ^{Aa} (4.81-7.42)	7.91 ^a (6.62-9.97)	2.83 ^{Ab} (2.41-3.46)
Cumin			4.06 ^{Aa} (3.11-5.19)	2.07 ^b (1.79-2.36)	1.16 ^{Ac} (0.46-1.34)	8.09 ^{Aa} (6.52-11.47)	7.91 ^a (6.62-9.97)	3.09 ^{Ab} (2.39-6.36)
Geranium	30	1	1.91 ^{Aa} (0.87-2.28)	1.32 ^a (0.99-1.61)	0.96 ^{Aa} (0.75-1.15)	6.51 ^{Aa} (4.89-12.12)	7.10 ^a (5.45-10.91)	2.87 ^{Ab} (2.46-3.56)
Coriander			1.63 ^{Aa} (1.01-2.05)	1.32 ^a (0.99-1.61)	0.73 ^{Ab} (0.54-0.89)	4.31 ^{Aa} (3.76-6.75)	7.10 ^a (5.45-10.91)	1.75 ^{Bb} (1.53-2.05)
Cumin			3.53 ^{Ba} (2.28-4.19)	1.32 ^b (0.99-1.61)	0.85 ^{Ab} (0.66-1.00)	7.31 ^{Aa} (5.38-10.94)	7.10 ^a (5.45-10.91)	2.06 ^{Bb} (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% CO₂ 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 LT ₅₀			Lethal time (days) and their 95% confidence limits LT ₉₀		
			Oil alone**	75% CO ₂ alone	Oil+75%CO ₂ mixture	Oil alone**	75% CO ₂ alone	Oil+75%CO ₂ mixture
Geranium	20	1.5	3.91 ^{Aa} (2.82- 5.23)	1.88 ^b (1.12-2.51)	0.85 ^{Ac} (0.59-1.08)	7.83 ^{Aa} (6.17-11.98)	6.17 ^a (4.99-12.38)	3.03 ^{ACb} (2.89-4.33)
Coriander			3.24 ^{Aa} (2.55-3.89)	1.88 ^b (1.12-2.51)	0.69 ^{Ac} (0.47-0.88)	5.82 ^{Aa} (4.81-7.42)	6.17 ^a (4.99-12.38)	2.29 ^{Bb} (1.97-2.74)
Cumin			4.06 ^{Aa} (3.11-5.19)	1.88 ^b (1.12-2.51)	0.81 ^{Ac} (0.58-1.00)	8.09 ^{Aa} (6.52-11.47)	6.17 ^a (4.99-12.38)	2.63 ^{BCb} (2.22-3.34)
Geranium	30	1	1.91 ^{Aa} (0.87- 2.28)	1.12 ^a (0.88-1.33)	0.74 ^{Aa} (0.53-0.89)	6.51 ^{Aa} (4.89-12.12)	4.10 ^a (3.47-5.14)	1.76 ^{Ab} (1.54-2.13)
Coriander			1.63 ^{Aa} (1.01-2.05)	1.12 ^a (0.88-1.33)	0.65 ^{Ab} (0.46-0.81)	4.31 ^{Aa} (3.76-6.75)	4.10 ^a (3.47-5.14)	1.66 ^{Ab} (1.45-1.91)
Cumin			3.53 ^{Ba} (2.28-4.19)	1.12 ^b (0.88-1.33)	0.72 ^{Ab} (0.52-0.88)	7.31 ^{Aa} (5.38-10.94)	4.10 ^b (3.47-5.14)	1.69 ^{Ac} (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 ($p = 0.05$)

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 CO₂ 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 CO₂ and botanical oil significantly reduced the time required to kill 50% of *S. oryzae* adults. Although, at 20°C, LT_{50s} of 25% CO₂, 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 CO₂, respectively. These times were shorter with the increase in CO₂ of CA from 25% to 50%. The shortest LT_{50s} for *S. oryzae* adults were 0.74, 0.65 and 0.72 days when 75% CO₂ was combined with each of geranium, coriander and cumin oils (each oil at 1% separately), at 30°C, respectively. At low CO₂ concentrations, mortality occurs due to spiracular opening and increased fumigant uptake. However, metabolic effects become a more important determinant of mortality at higher CO₂ 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 CO₂ or reductions in O₂ [37,38,41]. The greater efficacy at higher concentrations of CO₂ at low temperatures could be related to the higher solubility of CO₂ in tissues at low temperatures [29]. CO₂ 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 CO₂ 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 CO₂ 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% CO₂ + 1% O₂ + 84% N₂ and 12% CO₂ + 5% O₂ + 83% N₂) to the psocid, *Liposcelis bostrychophilus* Badonnel (Psocoptera: Liposcelidae). The present results are also in agreement with that of Saleem et al. [15] and Adel et al. [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 CO₂ with botanical oils clearly resulted in significant reductions of LT₅₀ and LT₉₀ 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 CO₂.

ACKNOWLEDGEMENTS

The authors thank all staff members of Plant Protection Department, Faculty of Agriculture, Benha University, <http://www.bu.edu.eg> for their helps and cooperation throughout the period of this research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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