Susceptibility of different life stages of *Callosobruchus maculatus* and *Callosobruchus chinensis* to ECO_2FUME gas and its impact on cowpea seeds quality

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Abstract

ECO_2FUME gas is an alternative to toxic phosphine fumigant and as a quarantine treatment for the control of a particularly recalcitrant pest, Callosobruchus maculatus and Callosobruchus chinensis. This gas was used to fumigate stored cowpea piles under gas-proof sheets to assess its performance against different developmental stages of *Callosobruchus maculatus* and *Callosobruchus chinensis*. The mortality was determined on four developmental stages of *C. maculatus* and *C. chinensis*, employing ECO_2FUME at different concentrations 25, 30, 40, and 50 g/m^3 for 3-days. All stages of both insect species in packed cowpea stacks were completely controlled at 3-days when applied with an ECO_2FUME application rate of 50 g/m^3. Cases of pupae of *C. maculatus* and *C. chinensis* exhibit the highest resistance than other stages, with 78.2 and 73.93% mortality respectively, at 40 g/m^3 after 3-days post-exposure to ECO_2FUME. Suppression of F_1 generation was obtained after fumigation with the same concentration (50 g/m^3). Quality (in terms of cowpea germination) and all chemical constituents of cowpea seeds were non significantly ($P \leq 0.05$) affected by the fumigation concentration of 50 g/m^3.

Introduction

The cowpea, Vigna unguiculata (L.), is a high-nutritive legume that is widely cultivated for human and animal consumption. Cowpeas have a high nutritional value due to their high protein, carbohydrate, fat, sodium, potassium, and iron content in dry seeds (**Hall 2004)**. The most important and common pests of stored cowpea seeds in many parts of the world, as well as in Egypt, are *Callosobruchus maculatus* (F.) and *Callosobruchus chinensis* L. (Chrysomelidae: Bruchinae). Through their postharvest feeding and reproductive activity, these insects target stored cowpea seeds and other legumes, contaminate afflicted seeds, and cause physical damage and quality loss **(Ali et al. 2005; Musa and Adeboye 2017)**. They are responsible for considerable economic loss and consequent weight and germination reduction in stored cowpea **(Vales et al. 2014)**. Fumigants are the most common tools for the management of these insects **(Akinkurolere et al. 2006)**. Many fumigators today rely on pesticide sprays or tablets, such as magnesium phosphide and aluminum phosphide. Regardless of the fact that stored goods insects are becoming increasingly resistant to phosphine (Mau et al. 2012; Nayak et al. 2013, Corrêa et al. 2014, Manivannan 2015; Nguyen et al. 2016; Jagadeesan and Nayak 2017;

Konemann et al. 2017) that has far-reaching consequences in terms of grain biosecurity and global trade (Norwood 2017). Although effective, these products can pose safety, environmental and performance challenges, resulting in higher treatment costs and posing regulatory hurdles.

Carbon dioxide (CO_2) is a gaseous fumigant that can be toxic to insects at high concentrations and takes a long time to kill all stages of insects **(Hasan et al. 2016)**. CO_2 has features that make it an ideal candidate for co-fumigation with PH_3. It facilitates the equivalent distribution of PH_3 throughout the grain mass **(Constantin et al. 2020)**, ensuring that insects are exposed to both gases simultaneously. In addition, simultaneous exposure to the two gases can cause increased toxicity, minimize the survival of

insects, thereby decreasing tolerance and resistance levels to PH_3 that vary substantially among insect species their different life stages **(Jagadeesan and Nayak 2017; Venkidusamy et al. 2018; Cato et al. 2019)**. CO_2 as well prevent the flammability of PH_3, which is important to an occupational safety **(Constantin et al. 2020)**.

With the advent of ECO2FUME cylinderized gas fumigants, a gas formulation having a mixture of 2% PH3 by weight (2.6 percent by volume) in CO2 (98 percent by weight) offers an alternative treatment option that addresses limitations posed by other offerings on the market. ECO2FUME has little amount of phosphine and become a non-flammable and ready-to-use gas mixture (Tumambing et al. 2012). For fumigating food and non-food commodities, this formulation is safe, effective, and easy to apply **(Meenatchi and Alagusundaram 2014)**.

In this study, we aim to find alternative fumigants that offer treatment for the control of a particular pest, *Callosobruchus maculatus*, and *Callosobruchus chinensis*. Moreover, to establish the response of different developmental stages of *C. maculatus* and *C. chinensis* to different concentrations of ECO2FUME gas at 30 °C. Furthermore, the most effective ECO2FUME concentration in storage for determining the quality of cowpea seeds by germination, hardness, cooking time, and chemical composition was assessed.

Materials And Methods

The field application of ECO_2FUME gas was conducted in El-Baharia Oasis Shona, Giza Governorate, Egypt.

2.1. Materials:

ECO_2FUME gas cylinders (Fumigant gas produced by CYTEC, Canada), piles of 240 Jute bags each of 100 kg cowpea seeds, protective clothes, silo check (PH_3 detector), sealing materials, weight digital scale and plastic sheets (14x20m).

2.3. Insect cultures:

Cultures of the cowpea beetle; *Callosobruchus maculatus* (F.) and pulse beetle *Callosobruchus chinensis* (L) were reared on cowpea seeds in the Stored Grains pest Research Department, Plant Protection Research Institute, Agriculture Research Center (ARC), Giza, Egypt. Both insects were reared on cowpea seeds in glass jars. Adult male and female insects were placed in each jar to lay eggs and covered with muslin by a rubber band to prevent insect escape. The jars containing insects were incubated at 28±2 °C and 75±5% RH for 1 week. Then the parent adults were sieved out and discarded. Different stages of insects such as eggs, larvae, pupae and adults were maintained separately to carry out mortality studies. To collect newly deposited eggs of *C. maculatus* and *C. chinensis*, adults were maintained on cowpea in ventilated plastic cages. At different periods of time, eggs of known age (after 1-5 days), larvae

(after 7-18 days), pupae (after 21-23 days), or adults (3-days after emergence) were obtained for treatments **(Wong-Corral et al. 2013)**.

2.4. Fumigation procedures:

Three piles of 240 Jute bags each of 100 kg cowpea seeds. From the stock cultures maintained in the rearing room, cloth bags (10x16 cm) each contained 10 g cowpea seeds infested with one of the different stages of *C. maculatus* and *C. chinensis* eggs, larvae, pupae and adults (30 individuals for each bag in case of adult) were prepared and introduced into the pile and distributed in the four directions (North, South, Middle and West).The total numbers of bags for each concentration of ECO_2FUME gas were 48 bags; 12 bags for each direction (North, South, Middle and West). The pile was covered exactly and tightly with a plastic sheet 14x20m. After sealing the place of fumigation where of the gas cylinder was introduced inside the pile and the gas cylinder was put on platform balance to calculate the required dose. Four doses of ECO_2FUME (25, 30, 40 and 50 g/m^3) were used. After 3-days of exposure to ECO_2FUME gas, the piles were aerated, and the cloth bags contain adults were inspected directly for adult mortality and corrected according to Abbott's formula **(Abbott 1925)**. Bags of the other insect stages were incubated at 28±2 °C and 75±5% RH until beetles emerge (F_1 progeny). The percentage of insects' reduction was estimated. Similar numbers of cloth bags of insect stages were distributed in another cowpea seeds pile using the same procedures without ECO_2FUME gas to be used as a control.

3. Effect of ECO_2FUME gas on quality and chemical constituents of cowpea seeds:

The effect of ECO_2FUME gas at 50 g/m^3 on quality (germination, Hardness, relative humidity and cooking time) and chemical constituents (protein, lipids, carbohydrate, moisture and ash) contents of fumigated and nonfumigated cowpea seeds were studied at both zero time and after 3-months of storage.

Twenty-five cowpea seeds (fumigated and nonfumigated) were put into each dish on top of the moist paper. The three dishes were placed under the lights to allow the seeds' germination. After 7-days, the numbers of germinated seeds were counted and expressed as percent germination.

Hardness and relative humidity testing were carried out by the Penetrometer system (Digital Force Gauge Model FGN-20G, Nidec-Shimpo Corporation Jap.) and grain moisture meter (DRAMINSKI SA Owocowa 17, 10-860 Olsztyn-Poland) respectively. Two hundred of (fumigated and nonfumigated) cowpea seeds were soaked for 1 h in tap water. Afterward, they were placed in an aluminum pot with 2000 ml of water. The average cooking time (min) for three replicates was recorded.

Protein, lipids, carbohydrate, moisture and ash contents of fumigated and nonfumigated cowpea seeds were determined according to the method of **AOAC (1990)**.

4. Statistical analysis:

Percentages of adult mortality were calculated using the initial number of individuals placed in each cage. In the case of eggs, larvae, or pupae, the mean number of emerged adults in the control treatments was utilized as the initial number of individuals when calculating the mortality rate. For statistical analysis, the average percent mortalities of the tested insects were calculated and corrected using Abbott's formula (**Abbott 1925)**. Toxicity values (LC_{50} and LC_{99}) were calculated by probit analysis **(Finney 1971)** using Ldp-line software to obtain the toxicity regression lines. Obtained results were analyzed using one-way analysis of variance (ANOVA) in **SAS (Anonymous 2003)**. All percentages were Arcsine transformed before analysis. To elucidate the general differences between the two pests, stages and different ECO_2FUME gas concentrations factorial analysis was conducted using Proc ANOVA in SAS. Means were compared by Tukey's HSD (*P*=0.05 level) in the same program.

Results

Different concentrations of ECO_2FUME gas were evaluated against the different stages of *C. maculatus* and *C. chinensis* in cowpea piles under gas-proof cover at 30°C, stored at El-Baharia Oasis Shona, Giza Governorate, Egypt. The effects of various concentrations of ECO_2FUME gas on the mortality of the different developmental stages of *C. maculatus* and *C. chinensis* are presented in Table 1. The results revealed that the mortality rate of different developmental stages is directly proportional to the concentrations of the ECO_2FUME gas; hence mortality rates for all different developmental stages of *C. maculatus* and *C. chinensis* exposed to different concentrations of ECO_2FUME gas increased with increasing the gas concentrations. We observed that all different developmental stages of *C. maculatus* and *C. chinensis* in the vials, treated with 50 g/m^3 for 3-days were dead after fumigation reaching 100% mortality, indicating that this concentration is effective in controlling all life stages of *C. maculatus* and *C. chinensis*. No significant differences were observed among the mortality of different developmental stages of *C. maculatus* treated with 25 and 30 g/m^3 ECO_2FUME gas for 3-days compared to the vials treated with 40 and 50 g/m^3 ECO_2FUME gas for 3-days ($P < 0.05$). However, it was observed a significant difference among the mortality of different developmental stages of *C. chinensis* post-exposure to 20, 30, 40 and 50 g/m^3 ECO_2FUME gas for 3-days ($P > 0.05$). These findings were supported by other studies on the insecticidal activity of ECO_2FUME gas against other stored product insects.

Table 2 shows the results of the factorial analysis for the overall trend between the two pests, stages, and exposure concentrations. The exposure concentrations had a significant effect on *C. maculatus and C. chinensis* mortality ($F=201.87$ and $p=0.0001$). The exposure concentrations were the most influential component, with a substantial influence. Neither pests nor stages had a significant effect.

Lethal concentration values and parameters of mortality regression line *C. maculatus* and *C. chinensis* at different developmental stages 3-day post-exposure to different concentrations of ECO_2FUME gas are presented in Table 3. The efficacy of ECO_2FUME varies depending on the life stage of insects within their life cycle. The results showed that the ECO_2FUME concentration required to obtain 50% mortality of *C.*

maculatus adult, larvae, pupae and egg were 20.38, 29.23, 31.71 and 31.76 g/m^3 respectively. While it was 21.25, 27.95, 31.54 and 28.44 g/m^3 for the adult, larvae, pupae and egg of *C. chinensis* respectively. The obtained correlation coefficient values of regression lines of the two tested insects indicated a high significant correlation between the ECO_2FUME gas concentrations and mortality percentages (table 3).

Adult survivorship from two-days old eggs of *C. maculatus* and *C. chinensis* at different concentrations of ECO_2FUME gas in cowpea seeds are depicted in Table 4. Treatment of two-days old eggs of *C. maculatus* and *C. chinensis* with different concentrations of ECO_2FUME caused a significant reduction in the progeny of both insect species after 3-days of exposure compared with progeny production in the control treatment ($P < 0.05$). The number of *C. maculatus* progeny was 58.0, 48.0, 69.0 and 103.0 in control while the numbers of progeny were 45.0, 32.0, 10.0 and 00.0 at ECO_2FUME concentrations of 25, 30, 40 and 50 g/m^3, respectively. Similarly, the treatment with ECO_2FUME at concentrations of 25, 30, 40 and 50 g/m^3 reduced the progeny numbers of *C. chinensis* to be 60.0, 70.0, 15.0 and 00.0 compared with 91.0, 171.0, 81.0 and 44.0 in control. It was also clear that the treatment with ECO_2FUME induced a higher reduction in the progeny of *C. chinensis* than *C. maculatus*. The concentration level of 40 g/m^3 caused the highest reduction in the progeny production of *C. maculatus* and *C. chinensis* from treated two-days old eggs was 85.51 and 81.5% respectively. While 50 g/m^3 was able to prevent adult emergence completely in both insects. It is obvious that the rate of failure to get adult emergence increased with increasing ECO_2FUME gas concentrations in all stages that have been treated.

Adult survivorship from treated larvae of *C. maculatus* and *C. chinensis* at different concentrations of ECO_2FUME gas in cowpea seeds is presented in Table 5. All ECO_2FUME gas concentrations were effectively caused a significant reduction in emerging adults from treated larvae of *C. maculatus* and *C. chinensis* ($P < 0.05$). When the larvae of *C. maculatus* and *C. chinensis* were treated at 25, 30 and 40 g/m^3 ECO_2FUME gas caused a reduction of 36.6, 46.3, 83.1% and 39.8, 63.1, 78.6% respectively, when compared to the control treatment. *C. maculatus* and *C. chinensis* larvae treated at 50 g/m^3 of ECO_2FUME gas showed 100% mortality based on the adult emergence, indicating that the concentration 50 g/m^3 resulted in non-completion of the development of immature stages of *C. maculatus* and *C. chinensis*.

Adult survivorship from treated pupae of *C. maculatus* and *C. chinensis* with different concentrations of ECO_2FUME gas in cowpea seeds is depicted in Table 6. It was observed a significant difference among the number of adults who emerged from treated cowpea seeds with 25, 30 and 40 g/m^3 ECO_2FUME gas for 3-days compared to the untreated seeds ($P < 0.05$). Whereas the concentration of 50 g/m^3 of ECO_2FUME gas success to achieve complete protection by preventing adults emerging from treated pupae 3-days post-exposure. It was confirmed that 50 g/m^3 of ECO_2FUME gas was effective in controlling all life stages of *C. maculatus* and *C. chinensis* 3-days post-exposure at 30°C.

The effect of ECO_2FUME gas at 50 g/m^3 on some properties of cowpea seeds (germination%, relative humidity, hardness and cooking time) of treated and non-treated cowpea seeds at initial application and after 3 months of storage are presented in Tables 7. All ECO_2FUME treatments induced a non-significant effect on germination%, relative humidity, hardness and cooking time of treated cowpea seeds at initial application and after 3-months of storage compared with control treatment ($P < 0.05$). The average germination percentage in both fumigated and nonfumigated cowpea seeds at zero time was 100.0%. This indicates that the ECO_2FUME gas at 50 g/m^3 had no effect on germination percentage at zero time. But after 3-months of storage, the average germination percent of cowpea seeds, whether fumigated or nonfumigated decreased but the decrease in nonfumigated samples was higher. This indicates that the ECO_2FUME gas at 50 g/m^3 had improved cowpea seeds germination after storage.

The average hardness of the nonfumigated samples was 54.38 N, and that for fumigated samples was 54.16 N. Neither fumigated at 50 g/m^3 nor storage for 3-months significantly changes the hardness of cowpea seeds (table 7). Applying ECO_2FUME gas at 50 g/m^3 caused a non-significant effect on relative humidity, hardness and cooking time of treated cowpea seeds at initial application and after 3 months of storage compared with control treatment ($P < 0.05$) (Table 7).

The effect of ECO_2FUME gas at 50 g/m^3 on the major chemical constituents of cowpea seeds of fumigated and nonfumigated cowpea seeds at zero time and after 3-months of storage are presented in Table 8. In general, the results showed that protein and carbohydrates contents were slightly increased, and the lipid, moisture and ash contents were slightly decreased in fumigated cowpea seeds with ECO_2FUME gas at 50 g/m^3 at zero time and after 3-months of storage. Maximum increase for protein (0.047 and 0.1%) and carbohydrates (0.15 and 0.07%) was detected in treated seeds with ECO_2FUME gas at 50 g/m^3 at zero time and after 3 months of storage, respectively, compared with nonfumigated cowpea seeds. Also, the maximum decrease of lipid (0.12 and 0.13%), moisture (0.19 and 0.00%) and ash (0.23 and 0.26%), respectively, was observed at 50 g/m^3 at zero time and after 3 months of storage compared with nonfumigated cowpea seeds. The results indicate that there was no significant effect of fumigation at this concentration level either at zero time or after 3 months of storage at ambient temperature and humidity ($P < 0.05$). From our results, fumigation using ECO_2FUME gas at 50 g/m^3 did not significantly affect the major chemical constituents or properties of cowpea ($P < 0.05$).

Discussion

Different concentrations of ECO_2FUME gas were evaluated against the different stages of *C. maculatus* and *C. chinensis* in cowpea piles under gas-proof cover at 30 °C. The results revealed that the mortality rate of different developmental stages is directly proportional to the concentrations of the ECO_2FUME gas; hence mortality rates for all different developmental stages of *C. maculatus* and *C. chinensis* exposed to different concentrations of ECO_2FUME gas increased with increasing the gas concentrations. For instance, **Amin et al. (2020)** reported that the efficacy of ECO_2FUME gas was increased as the

concentration increased furthermore, a dose of 50 g/m^3 induced 100% mortality of all insect stages after 3-days of treatment. Insects are stressed by the increased levels of CO_2, which allows lower levels of phosphine to be more effective in achieving 100% mortality of all life stages including the egg stage in shorter periods of time. Increased carbon dioxide accelerates the penetration rate of the fumigant and enhances the respiration rate of insects thereby making them more susceptible to phosphine **((Leesch 1992; Chadda et al. 2004)**. Complete mortalities were achieved for the adults and immature stages of *Ephestia cautella*, *Ephestia calidella* and *Oryzaephilus surinamensis* after fumigation with ECO_2FUME gas 3-days post-exposure **(Mohamed and Sayed 2017)**. The results of the fumigation trials of mixed-age cultures of *Sitophilus zeamais, Tribolium castaneum* and *O. surinamensis* in packed rice stacks were completely controlled for all stages at 2 and 3-days when applied with an ECO_2FUME application rate of 50 g/m^3 **(Kengkanpanich et al. 2018)**. For the management of stored commodities pests, a mixture of CO_2 and PH_3 is being evaluated as a viable fumigant **(Leelaja et al. 2007; Valmas and Ebert 2006)**. Many studies show that the addition of Co_2 to PH_3 enhances the toxicity of PH_3 and reduces the dose required to kill insects **(Constantin et al. 2020)**. A recent study against mixed-age populations of PH_3-resistant *Rhyzopertha dominica* indicated that the toxicity of PH_3 was enhanced up to 28-fold when it was combined with 30% CO_2 **(Manivannan et al. 2016)**. The exposure period required for killing all the immature stages of *O. surinamensis*, *Lasioderma serricorne*, *Plodia interpunctella* can be reduced to 1-day from 5-days when PH_3 is used in combination with 24% of carbon dioxide **(Hartsell et al. 2005)**, and these findings are also in agreement with that of **Constantin et al. (2020)** reported that an observed enhancement in toxicity toward the rusty grain beetle, *Cryptolestes ferrugineus* with the PH_3+CO_2 mixture was consistent. The most likely explanation for this enhanced toxicity of phosphine comes from two physiological responses to CO_2 exposure: one of them, low concentrations of CO_2 possibly increase aerobic energy metabolism through higher oxygen uptake **(Kashi and Bond 1975)** which was well known to enhance phosphine toxicity **(Bond et al. 1967; Kashi 1981 a, b)** and another explanation is at concentrations above 15%, CO_2 stimulates the opening of spiracles **(Matthews and White 2011).** facilitating more gaseous exchange **(Mitcham et al. 2006)** that could favor increased phosphine uptake in tissues. The presence of CO_2 is also essential during fumigation which causes suffocation to insects and results in quick mortality of insects in modified atmospheric storage **(Sujeetha et al. 2015)**.

By changing a few factors like concentration, can change the insecticidal effect of ECO_2FUME. Our results showed that the concentration level had the premier impact on the mortality for the two pests at various developmental stages 3-days post-exposure to ECO_2FUME gas with Neither pests nor stages had a significant effect (**Amin et al. 2020)**.

According to lethal concentration values and parameters of the mortality regression line, *C. maculatus* and *C. chinensis* adults were more ECO_2FUME sensitive than the other stages, which required treatment of 45.31 and 41.10 g/m^3, respectively to reach 99% mortality after 3-day post-exposure to ECO_2FUME gas followed by eggs which required treatment of 53.51 and 57.12 g/m^3, respectively to reach 99% mortality after 3-day post-exposure to ECO_2FUME gas. The adults of *C. maculatus* are the most susceptible with

regard to the developmental states during which they are exposed, and these adults demonstrate high activity and sensitivity to hypoxia. Similarly, the corium is soft in young eggs, which can leak water and oxygen during exposure to a controlled atmosphere, with higher mortality and susceptibility in mature eggs. This is due to the high respiratory activity in the formation of larvae **(Iturralde et al. 2016).**

The most ECO_2FUME-tolerant stages of *C. maculatus* and *C. chinensis* were pupae and larvae, which required treatment of 62.24, 59.40 g/m^3 and 73.30, 65.17 g/m^3, respectively to reach 99% mortality after 3-day post-exposure to ECO_2FUME gas. Admixtures of phosphine with CO_2 resulted in reducing the lethal concentrations to achieve increasing mortality of *R. dominica* **(Manivannan et al. 2016)**. The obtained results are in harmony with the findings of other investigators on the efficacy of combinations of phosphine plus carbon dioxide against some stored product insects. Adults of *C. maculatus*, *R. dominica* and *Sitophilus oryzae* proved to be the most susceptible stage post-exposure to mixtures of phosphine and carbon dioxide at 30ºC **(El-Lakwah et al. 1992a; 1992b and 1992c)**, respectively. The diverse responses of different life stages of *C. maculatus* could be due to the variation in their respiration rates, differences in body size of life stages and the sex of adults **(Iturralde et al. 2016)**. A considerable relationship exists between the respiratory rate and the body mass of insects. Pupal states have a low oxygen demand, the former being most tolerant to hypoxia due to their metabolic rate, which is slow compared with other stages, as noted in a study on *Callosobruchus subinnotatus* **(Mbata et al. 2000).** The increased tolerance of larvae and pupae to ECO_2FUME gas could be due to the lower respiration rates in these life stages **(Hoback and Stanley 2001)**. Thus, a high mortality rate of adults was observed compared to the other stages of *C. maculatus* and *C. chinensis* even at the same concentration and exposure time. Moreover, larvae and pupae of *C. maculatus* and *C. chinensis* are surrounded by the seed material shielded from the external atmosphere providing an additional layer of obstruction to the ECO_2FUME gas. The integrity of the outer layer and metabolic status of the insect's stage are some of the defining factors that make some individuals more susceptible to ECO_2FUME than others **(McDonough et al. 2011)**. Phosphine and CO_2 formulation can be an effective fumigant when applied even though different levels of sensitivity occur as a function of insect species and life stage **(Hartsell et al. 2005)**.

The treated cowpea seeds, having eggs, larvae and pupae showed 100% mortality 3-days post-exposure with 50 g/m^3 of ECO_2FUME gas indicating that the treatment schedule was effective in eliminating all life stages of the *C. maculatus* and *C. chinensis*. Similar results were obtained by **Perera et al. (2018)** reported that dose/ time regimes of ECO_2FUME can be recommended for the fumigation of rice for the control of *S. oryzae*, at 700 ppm (50 g ECO_2FUME /m^3)/ 36 h. **Meenatchi et al. (2018)** reported that the mixture of PH_3 and CO_2 significantly affects the mortality of various life stages of *T. castaneum* the synergistic effect of CO_2 on phosphine toxicity is further supported by the fact that, CO_2 exerts lethal effects on insects causing their death by dehydration at the cellular level and creating a lack of triglycerides for energy metabolism. Complete mortality of all stages of *Ephestia Cautella, Ephestia Calidella* and *O. surinamensissin* after the application of 50 g/m^3 of ECO_2FUME **(Mohamed and Sayed 2017)**.

ECO_2FUME gas at 50 g/m^3 had no effect on some properties of cowpea seeds (germination%, relative humidity, hardness and cooking time). ECO_2FUME gas at 50 g/m^3 had no effect on germination percentage at zero time, however, had improved cowpea seeds germination after the storage period (3 months). **Mekali et al. (2013)** indicated no loss in germination on employing CO_2 of <20%. The increase in the concentration of CO_2 in CA treatments and exposure time benefits the vigor of chickpea germination **(Iturralde et al. 2016)**. **Saha et al. (2015)** obtained similar values to those of the control as in this study using 89.5% ambient CO_2.

Overall, the results showed that protein and carbohydrates contents were slightly increased, and the lipid, moisture and ash contents were slightly decreased in fumigated cowpea seeds with ECO_2FUME gas at 50 g/m^3 at zero time and after 3 months of storage. In consistence with our results, no negative effects were identified to fruit quality (physical, chemical and sensory analysis) after the treatment, storage and shelf life in green pepper fruit treated with phosphine (ECO_2FUME) for 24 h at 500, 1000 and 2000 ppm **(Ertürk et al. 2018)**. The quality of grains is not affected by treatment with a CO_2-rich atmosphere and the application meets the requirements of organic markets **(Annis and Graver 1991)**.

Conclusions

Our study provides information about the insecticidal efficacy of ECO_2FUME gas for the management of *C. maculatus and C. chinensis* in infested cowpea seeds. As indicated by the results of this study, exposure with 50 g/m^3 of ECO_2FUME gas indicated that the treatment was effective in eliminating all life stages of the two insects, prevented progeny production and improved germination of the cowpea seeds and increased the major chemical constituents of cowpea seeds (protein and carbohydrates) after 3-months of 50 g/m^3 of ECO_2FUME application.

Declarations

Author Contribution:

Manar Yousef Amin, Abeer Omar Abotaleb and Refaat Abdelshafı Mohamed conceived, designed the experiments and contributed material. Manar Yousef Amin and Refaat Abdelshafi Mohamed conducted experiments. Abeer Omar Abotaleb analyzed data, conducted the statistical analysis and wrote the manuscript. All authors revising the manuscript.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Statements and Declarations

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Ethics declarations

No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with an unregulated invertebrate species.

Conflict of interest

The authors declare that there is no conflict of interest regarding this study.

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Tables

Tables 1-8 are in the supplementary files section.

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