Egypt. J. Plant Prot. Res. Inst. (2022), 5 (2): 149–164

Egyptian Journal of Plant Protection Research Institute

www.ejppri.eg.net

Susceptibility of different life stages of *Callosobruchus maculatus* and *Callosobruchus chinensis* (Coleoptera: Chrysomelidae :Bruchidae) to ECO_2FUME gas and its impact on cowpea seeds quality

Manar, Y. Amin; Abeer, O. Abotaleb and Refaat, A. Mohamed

Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

ARTICLE INFO

Article History

Received: 17 / 4 /2022

Accepted:23 /6 /2022

Keywords

Callosobruchus spp., ECO_2FUME gas, germination, cowpea, and seeds quality.

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* (Fabricius) and *Callosobruchus chinensis* Linnaeus (Coleoptera: Chrysomelidae:Bruchidae) . This gas was used to fumigate stored cowpea piles under gas-proof sheets to assess its performance against different developmental stages of *C. maculatus* and *C. 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 to 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* (Fabricius) and *Callosobruchus chinensis* Linnaeus (Coleoptera: Chrysomelidae :Bruchidae) . Through their postharvest feeding and reproductive activity, these insects target stores cowpea seeds and other legumes, contaminate afflicted seeds, and cause physical damage and quality loss (Ali *et al.*, 2005 and 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 and 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 and minimize the survival of insects, thereby decreasing tolerance and resistance levels to PH_3 that vary substantially among insect species and their different life stages (Jagadeesan and Nayak, 2017; Venkidusamy *et al*., 2018 and Cato *et al.*, 2019). CO_2 as well as preventing the flammability of PH_3, which is important an occupational safety (Constantin *et al*., 2020).

With the advent of ECO_2FUME cylinderized gas fumigants, a gas formulation having a mixture of 2% PH_3 by weight (2.6 percent by volume) in CO_2 (98 percent by weight) offers an alternative treatment option that addresses limitations posed by other offerings on the market. ECO_2FUME has little amount of phosphine and becomes 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).

The aim of this study was to determine the optimal dosages (Application rate) of ECO_2FUME® gas for the control of common pests of stored legumes, *C. maculatus* and *C. chinensis*. Additionally, to find out the response of different developmental stages of *C. maculatus* and *C. chinensis* to different concentrations of ECO_2FUME gas at 30°C.

Furthermore, the present study was carried out to investigate the effect of the 50 g/m^3 ECO_2FUME gas on the quality of cowpea seeds by germination, hardness, cooking time, and chemical composition.

Materials and methods

The field application of ECO_2FUME gas was conducted in El-Baharia Oasis Shona, Giza Governorate, Egypt. 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) Silo.Chek MARKII is manufactured by the CANARY COMPANY Pty Ltd, AUSTRALIA. This device detects high fumigation phosphine levels greater than 1 ppm up to 2000 ppm. The automatic sampling model (Which was used in this study) had a sample tube, which connect to the gas-sampling lines coming from the pile under fumigation and a built-in pump and battery. After connecting the sample tube with the gas-sampling lines, the key switch downturned to on. A period of up to 3 minutes was elapsed to allow the PH_3 sensor to record the final reading of PH_3 concentration. Sealing materials, weight digital scale, and plastic sheets (14x20m).

1. Insect cultures:

Two species of legume seed insects were used, *C. maculatus* and *C. chinensis* were reared on cowpea seeds in the Stored Grains Pest Research Department, Plant Protection Research Institute, Agriculture Research Center (ARC), Giza, Egypt. 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 (2 days old), larvae (After 7-8 days old), pupae (2-3 days old), or adults (3-days after emergence) were obtained for treatments (Wong-Corral *et al.*, 2013).

2. 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 the gas cylinder was introduced inside the pile and the gas cylinder was put on platform balance to calculate the required dose. Similar numbers of cloth bags of insect stages were distributed in another cowpea seeds pile using the same procedures without ECO_2 FUME gas to be used as a control.

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 containing adults were inspected directly. Bags of the other insect stages were incubated at 28±2 °C and 60±5% RH until adults emerge (F1 progeny). The percentage of insect mortality was estimated and corrected according to Abbott's formula (Abbott, 1925).

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 non-fumigated cowpea seeds were studied at both zero time and after 3-months of storage. Twenty-five cowpea seeds (Fumigated and non-fumigated) 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 non-fumigated) 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 non-fumigated 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 emerging adults in the control treatments was utilized as the initial number of individuals when calculating the mortality rate. For statistical analysis, the average percent mortality of the tested insects was calculated and corrected using Abbott's formula (Abbott, 1925). Toxicity values (LC50 and LC99) 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 and 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, 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 between the mortality of different developmental stages of *C. chinensis* post-exposure to 25, 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 of 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.

Table (1): The percentage mortality (Mean±SE) of the different developmental stages of *Callosobruchus maculatus* and *Callosobruchus chinensis* post-exposure to different concentrations of ECO$_2$FUME gas.

Conc (g/m^3)	Mortality% (Mean±SE)							
	Callosobruchus maculatus				***Callosobruchus chinensis***			
	Egg	**Larvae**	**Pupae**	**Adults**	**Egg**	**Larvae**	**Pupae**	**Adults**
0	0	0	0	0	0	0	0	0
25	19.06±0.02^{c}	36.27±0.09^{c}	27.18±0.03^{c}	72.2±0.04^{c}	34.05±0.02^{d}	39.79±0.03^{d}	22.93±0.02 ^{d}	69.99±0.02^{d}
30	33.33±0.02^{c}	46.29±0.04^{c}	33.33±0.06^{c}	87.73±0.03^{c}	59.06±0.01^{c}	58.3±0.0^{c}	49.44±0.0^{c}	91.00±0.01^{c}
40	83.06±0.01^{b}	82.48±0.01^{b}	78.2±0.0^{b}	96.6±0.0^{b}	81.49±0.02^{b}	78.57±0.01^{b}	73.93±0.02^{b}	98.87±0.1^{b}
50	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}	100±0.00^{a}

Means followed by the same letter are not significantly different at the 0.05 level using Tukey's HSD test, (P= 0.05)

Table (2): Factorial analysis of obtained data is presented in Table 1.

Factor	**Level**	**Mean**	**Factor**	**Level**	**Mean**
Pest	***Callosobruchus maculatus***	38.97±30.3^{a}	Con.	25	73.24±11.71^{a}
				30	52.81±6.02^{b}
	Callosobruchus chinensis	33.68±25.5^{b}		40	19.25±4.29^{c}
F		5.23		50	00.00±00.00^{d}
P		0.0254			
Stage	Egg	36.25±32.098^{ab}			
	Larvae	32.07±26.55^{b}	F		201.87
	Pupae	40.65±33.15^{a}	P		0.0001
F		4.58			
P		0.0138			

Means with the same letter are not significantly different.

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$_2$FUME gas are presented in Table (3). The efficacy of ECO$_2$FUME varies depending on the life stage of insects within their life cycle. The results showed that the ECO$_2$FUME 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$_2$FUME gas concentrations and mortality percentages (Table, 3).

Adult survivorship from two-days old eggs, larvae and pupae of *C. maculatus* and *C. chinensis* at different concentrations of ECO$_2$FUME 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$_2$FUME 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$_2$FUME concentrations of 25, 30, 40 and 50 g/m^3, respectively. Similarly, the treatment with ECO$_2$FUME 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 the control. It was also clear that the treatment with ECO$_2$FUME 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 85.5 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 ECO2FUME gas concentrations in all stages that have been treated.

All ECO$_2$FUME 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$_2$FUME 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$_2$FUME 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* (Table, 4). 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$_2$FUME gas for 3-days compared to the untreated seeds ($P < 0.05$). Whereas the concentration of 50 g/m^3 of ECO$_2$FUME gas success to achieve complete protection by preventing adults from emerging from treated pupae 3-days post-exposure. It was confirmed that 50 g/m^3 of ECO$_2$FUME gas was effective in controlling all life stages of *C. maculatus* and *C. chinensis* 3-days post-exposure at 30°C (Table, 4).

The effect of ECO$_2$FUME 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 Table (5). All ECO$_2$FUME 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$_2$FUME 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$_2$FUME gas at 50 g/m^3 had improved cowpea seed germination after storage.

The average hardness of the nonfumigated samples was 54.38 N, and that for fumigating 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, 5). Applying ECO$_2$FUME gas at 50 g/m^3 caused a non-significant effect on relative humidity, hardness, and the cooking time of treated cowpea seeds at initial application and after 3 months of storage compared with control treatment ($P < 0.05$) (Table, 5). The effect of ECO$_2$FUME 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 (6).

Table (3): Relative lethal concentrations (LC) of ECO_2FUME gas for both pests at different stages after 3-days post-exposure.

Tested insects	Stage	LC_{50} (g/m^3)	LC_{99} (g/m^3)	Confidence limits(g/m^3.)				Slope	r	X^2
				LC_{50}		LC_{99}				
				Lower	Upper	Lower	Upper			
Callosobruchus maculatus	Adults	20.38	45.31	16.73	22.64	39.89	57.96	6.71±1.17	0.98	0.61
	Larvae	29.23	59.40	27.76	30.57	53.34	69.36	7.55±0.74	0.98	5.98
	pupae	31.71	62.24	24.65	37.24	62.05	138.01	7.94±0.63	0.97	6.69
	Egg	31.76	53.51	30.65	32.89	49.51	59.42	10.27±0.84	0.97	5.97
Callosobruchus chinensis	Adults	21.25	41.10	18.02	23.17	36.78	51.22	8.12±1.46	0.99	0.93
	Larvae	27.95	65.17	26.13	29.50	57.97	77.12	6.33±0.62	0.93	3.35
	Pupae	31.54	73.30	29.83	33.21	63.01	92.41	6.35±0.71	0.94	1.58
	Egg	28.44	57.12	26.95	29.76	51.39	66.61	7.68±0.77	0.95	5.88

Table (4): Adult survivorship from two-day old eggs, larvae and pupae of *Callosobruchus maculatus* and *Callosobruchus chinensis* post exposure to different concentrations of ECO_2FUME gas.

Gas conc. (g/m^3)	Seeds status	No. emerging adults from treated eggs				No. emerging adults from treated larvae				No. emerging adults from treated pupae			
		C.m.	**Reduction%**	***C.c.***	**Reduction%**	***C.m.***	**Reduction%**	***C.c.***	**Reduction%**	***C.m.***	**Reduction%**	***C.c.***	**Reduction%**
25	Untreated	58^a	-	91^a	-	41^a	-	103^a	-	125^a	-	122^a	-
	Treated	45^b	22.4	60^b	34.1	26^b	36.6	62^b	39.8	101^b	19.2	103^b	15.6
30	Untreated	48^a	-	171^a	-	54^a	-	203^a	-	141^a	-	98^a	-
	Treated	32^b	33.3	70^b	59.1	29^b	46.3	75^b	63.1	94^b	33.3	48^b	51
40	Untreated	69^a	-	81^a	-	65^a	-	196^a	-	177^a	-	69^a	-
	Treated	10^b	85.5	15^b	81.5	11^b	83.1	42^b	78.6	31^b	82.5	18^b	73.9
50	Untreated	103^a	-	44^a	-	176^a	-	56^a	-	170^a	-	124^a	-
	Treated	00^b	100	00^b	100	00^b	100	00^b	100	00^b	100	00^b	100

Mean followed by the same letter are not significantly different using Tukey's HSD test, (*P*= 0.05).

C.m.* = *Callosobruchus maculatus* *C.c.* = *Callosobru-chus chinensis

Table (5): The effect of ECO_2FUME gas (Mean±SE) at 50g/m^3 on some properties of treated and untreated cowpea seeds at initial time and after 3-months of storage.

Parameters	Initial time		After storage	
	Untreated Mean±SE	Treated Mean±SE	Untreated Mean±SE	Treated Mean±SE
Germination%	100±0.0 ^{a}	100±0.0^b	89.32±0.43^a	90.68±0.5^b
Hardness	54.38±0.7^a	54.16±0.7^b	54.38±0.6^a	54.16±0.7^b
Relative humidity	6.23±0.1^a	9.03±0.1^b	6.37±0.14^a	9.23±0.15^b
Cooking time(min)	45±1.1^a	44.67±1.1^b	42.67±0.29^a	43±0.3^b

Mean followed by the same letter are not significantly different using Tukey's HSD test, (*P*= 0.05)

Table (6): The effect of ECO_2FUME gas at 50 g/m^3 on the major chemical constituents of fumigated and nonfumigated cowpea seeds at initial time and after 3-months of storage.

Constituents	Initial time		After storage	
	Untreated Mean±SE	Treated Mean±SE	Untreated Mean±SE	Treated Mean±SE
Protein	25.173±0.18^a	25.22±0.17^b	25.16±0.19^a	25.26±0.2^b
Lipid	1.67±0.13^a	1.55±0.12^b	1.64±0.04^a	1.51±0.05^b
Carbohydrates	55.97±0.3^a	56.12±0.3^b	56.3±1.22^a	56.37±0.47^b
Moisture	7.58±0.20^a	7.39±0.30^b	7.16±0.14^a	7.16±0.14^b
Ash	3.13±0.20^a	2.9±0.21^b	3.03±0.03^a	2.77±0.03^b

Mean followed by the same letter are not significantly different using Tukey's HSD test, (*P*= 0.05)

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 treating 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 ($P < 0.05$).

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 and Chadda *et al.*, 2004). Complete mortalities were achieved for the adults and immature stages of *Ephestia cautella* (Walker) , *Ephestia calidella* Guenee (Lepidoptera:Pyralidae) and *Oryzaephilus surinamensis* (Linnaeus) (Coleoptera : Silvanidae) 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 50g/m^3 (Kengkanpanich *et al*., 2018). For the management of stored commodity pests, a mixture of CO_2 and PH_3 is being evaluated as a viable fumigant (Leelaja *et al*., 2007 and 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* (F.) (Coleoptera: Bostrychidae) 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 *Oryzaephilus surinamensis* (L.) (Coleoptera : Silvanidae), *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae) and *Plodia interpunctella*

(Hübner) (Lepidoptera: Pyralidae) 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 and Kashi, 1981 a and 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) 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).

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 having 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-García *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* (L.) (Coleoptera: Curculionidae) proved to be the most susceptible stage post-exposure to mixtures of phosphine and carbon dioxide at 30ºC, respectively (El-Lakwah *et al*., 1992a , b and c). 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-García *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 more tolerant to hypoxia due to their metabolic rate, which is slow compared with other stages, as noted in a study on *C. 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 in 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 *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) 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 *E. Cautella*, *E. 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). ECO2FUME gas at 50 g/m^3 had no effect on germination percentage at zero time, however, had improved cowpea seed 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-García *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 consistent 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 *et al.*, 1991).

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. However, research efforts must be undertaken to evaluate the fumigation technology with ECO_2FUME gas to be economically feasible and compete with existing storage insect control technologies. Further studies are required in the development of commercial and continuous ECO_2FUME gas treatment and what the economic, ecological, and optimal treatment time according to the actual storage.

References

Abbott, W. S. (1925): A method of computing the effectiveness of an insecticide. J. Entomol., 18:265-267.

Akinkurolere, R.O.; Adedire, C.O. and Odeyemi, O.O. (2006): Laboratory evaluation of the toxic properties of forest anchomanes, *Anchomanes difformis* against pulse beetle *Callosobruchus maculatus* (Coleoptera: Bruchidae). Insect Sci., 13:25-29.

Ali, M.A.M.; El-Sayed, F.M.A. and El-Bishlawy, H.M.I. (2005): Damage and quantitative loss caused by *Callosobruchus maculatus* (Coleoptera: Bruchidae) to some cowpea and faba bean varieties. Egypt. J. Agric. Res., 83:563-581.

Amin, M.Y.; Aamir, M.M.I.; Mohamed, R.A. and Abd-Alla, S.M. (2020): Control of the rice weevil *Sitophilus oryzae* (Coleoptera: Curculionidae) using some insecticide alternative safe methods. Egypt. J. Plant Prot. Res. Inst., 3:535-543.

Annis, P.C.; Graver, J.V.S. and Highley, E. (1991): New operations manuals for safe and effective fumigation of grain in sealed bag-stacks. In International working Conference on Stored-Product Protection, pp. 747-755.

Anonymous (2003): SAS Statistics and graphics guide, release 9.1. SAS Institute, Cary, North Carolina 27513, USA.

AOAC (1990): Association of Official Analytical Chemistry, 15th Ed. Arlington, West Virginia, USA, Washington DC: Official methods of analysis, USA.

Bond, E. J.; Monro, H. A. U. and Buckland, C. T. (1967): The influence of oxygen on the toxicity of fumigants to *Sitophilus granarius* (L). J. Stored Prod. Res., 3:289-294.

Cato, A.; Afful, E.; Nayak, M.K. and Phillips, T.W. (2019): Evaluation of knockdown bioassay methods to assess phosphine resistance in the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Insects, 10:140.

Chadda, I.C.; Jayraj, K. and Dhuri, A.V. (2004): New method of phosphine and carbon dioxide application and its optimization. In Proceedings of the International Conference on Controlled Atmosphere and Fumigation in Stored Products. FTIC Ltd., Publishing, Israel, Gold-Coast, Australia, pp. 455-465.

Constantin, M.; Jagadeesan, R.; Chandra, K.; Ebert, P. and Nayak, M.K. (2020): Synergism between phosphine (PH_3) and carbon dioxide (CO_2): implications for managing PH_3 resistance in rusty grain beetle (Laemophloeidae: Coleoptera). J. Econ. Entomol., 113: 1999-2006.

Corrêa, A.S.; Tomé, H.V.V.; Braga, L.S.; Martins, G.F.; De Oliveira, L.O. and Guedes, R.N.C. (2014): Are mitochondrial lineages, mitochondrial lysis and respiration rate associated with phosphine susceptibility in the maize weevil *Sitophilus zeamais*?. Ann. Appl. Biol., 165:137-146.

El-Lakwah, F.A.; Saleh, M.K.; Omer, E.E. and Mohamed, R.A. (1992a): Efficiency of phosphine alone and in combination with carbon dioxide against the various stages of cowpea weevil *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Egypt. J. Appl. Sci., 7:80-98.

El-Lakwah, F.A.; Saleh, M.K.; Omer, E.E. and Mohamed, R.A. (1992b): Efficacy of phosphine, carbon dioxide and their mixtures against the various stages of lesser grain borer *Rizopertha dominica* (F.) (Coleoptera: Bostrychidae). Egypt. J. Appl. Sci., 7:99-117.

El-Lakwah, F.A.; Saleh, M.K.; Omer, E.E. and Mohamed, R.A. (1992c): Efficacy of phosphine, carbon dioxide and their mixtures against the various stages of rice weevil *Sitophilus oryzae* (L.) (Coleoptera: Curcliondae). Egypt. J. Appl. Sci., 7:203-221.

Ertürk, S.; Fatih, Ş.E.N.; Alkan, M. and Ölçülü, M. (2018): Effect of different phosphine gas concentrations against *Frankliniella occidentalis* (Pergande, 1895) (Thysanoptera: Thripidae) on tomato and green pepper fruit, and determination of fruit quality after application under low-temperature storage conditions. Turk. J. Entomol., 42:85-92.

Finney, D.F. (1971): Probit Analysis. Cambridge University Press, London. pp.256. Guedes RNC, Guedes NMP, Rosi-Denadai CA (2011) Sub-lethal effects of insecticides on stored-product insects: current knowledge and future needs. Stewart Postharvest Rev., 7:1–5.

Hall, A.E. (2004): Breeding for adaptation to drought and heat in cowpea. Eur. J .Agron. , 21:447-454.

Hartsell, P.L.; Muhareb, J.S.; Arnest, M.L.; Hurley, J.M.; McSwigan, B.J. and Deskin, R. (2005): Efficacy of a mixture of phosphine/carbon dioxide on eight species of stored product insects. Southwest Entomol., 30:47-54.

Hasan, M.M.; Aikins, M.J.; Schilling, W. and Phillips, T.W. (2016): Efficacy of controlled atmosphere treatments to manage arthropod pests of dry-cured hams. Insects, 7:44.

Hoback, W.W. and Stanley, D.W. (2001): Insects in hypoxia. J. Insect Physiol., 46:533–542.

Iturralde-García, R.D.; Borboa-Flores, J.; Cinco-Moroyoqui, F.J.; Riudavets, J.; Del Toro-Sánchez, C.L.; Rueda-Puente EO *et al.* (2016): Effect of controlled atmospheres on the insect *Callosobruchus* *maculatus* Fab. in stored chickpea. J. Stored Prod. Res., 69:78-85.

Jagadeesan, R. and Nayak, M.K. (2017): Phosphine resistance does not confer cross-resistance to sulfuryl fluoride in four major stored grain insect pests. Pest Manag. Sci., 73:1391-1401.

Kashi, K.P. (1981a) Toxicity of phosphine to five species of stored-product insects in atmospheres of air and nitrogen. Pestic. Sci., 12:116-122.

Kashi, K.P. (1981b): Response of five species of stored-product insects to phosphine in oxygen-deficient atmospheres. Pestic. Sci., 12:111-115.

Kashi, K.P. and Bond, E.J. (1975): The toxic action of phosphine: role of carbon dioxide on the toxicity of phosphine to *Sitophilus granarius* (L.) and *Tribolium confusum* DuVal. J. Stored Prod. Res., 11:9-15.

Kengkanpanich, R., Suthisut, D. and Sitthichaiyakul, S. (2018): Application of ECO_2FUMER phosphine fumigant for the complete control of major stored product insect pests in milled rice in Thailand. Julius-Kühn-Archiv., 463:618-625.

Konemann, C.E.; Hubhachen, Z.; Opit, G.P.; Gautam, S. and Bajracharya, N.S. (2017): Phosphine resistance in *Cryptolestes ferrugineus* (Coleoptera: Laemophloeidae) collected from grain storage facilities in Oklahoma, USA. J. Econ. Entomol., 110:1377-1383.

Leelaja, B.C.; Rajashekar, Y.; Reddy, P.V.; Begum, K. and Rajendran, S. (2007): Enhanced fumigant toxicity of allyl acetate to stored-product beetles in the presence of carbon dioxide. J. Stored Prod. Res., 43:45-48.https://doi.org/10.1016/j.jspr.2005.09.004

Leesch. J.G. (1992): Carbon dioxide on the penetration and distribution of phosphine through wheat. J. Econ. Entomol., 85:157-161.

Manivannan, S. (2015): Toxicity of phosphine on the developmental stages of rust-red flour beetle, *Tribolium castaneum* Herbst over a range of concentrations and exposures. J. Food Sci. Technol., 52:6810-6815.

Manivannan, S. ; Koshy, G.E. and Patil, S.A. (2016): Response of phosphine-resistant mixed-age cultures of lesser grain borer, *Rhyzopertha dominica* (F.) to different phosphine-carbon dioxide mixtures. J. Stored Prod. Res., 69:175-178.

Matthews, P.G. and White, C.R. (2011): Regulation of gas exchange and haemolymph pH in the cockroach *Nauphoeta cinerea*. J. Exp. Biol., 214:3062-3073.

Mau, Y.S.; Collins, P.J.; Daglish, G.J.; Nayak, M.K. and Ebert, P.R. (2012): The rph2 gene is responsible for high level resistance to phosphine in independent field strains of *Rhyzopertha dominica*. PLoS ONE, 7:34027.

Mbata, G.N.; Hetz, S.K.; Reichmuth, C. and Adler, C. (2000): Tolerance of pupae and pharate adults of *Callosobruchus subinnotatus* Pic (Coleoptera: Bruchidae) to modified atmospheres: a function of metabolic rate. J. Insect Physiol. 46:145-151.

McDonough, M.X.; Campabadal, C.A.; Mason, L.J.; Maier,

D.E.; Denvir, A. and Woloshuk, C. (2011): Ozone application in a modified screw conveyor to treat grain for insect pests, fungal contaminants, and mycotoxins. J. Stored Prod. Res., 47:249-254.

Meenatchi, R. and Alagusundaram, K. (2014): The current status of fumigation in India: Constrains and recent developments. Trends in Entomology, 10:97-103.

Meenatchi, R.; Alice, R. P.S.J. and Paulin, P.P. (2018): Synergistic effect of phosphine and carbon dioxide on the mortality of *Tribolium castaneum* (Coleoptera: Tenebrionidae) in Paddy. J. Agric. Sci., 10:503-510.

Mekali, J. ; Naganagoud, A.; Kapasi, M.; Sreenivas, A.G.; Nidoni, U. and Baskar, K. (2013): Management of *Rhyzopertha dominica* Fab. under modified atmospheric condition in stored sorghum. J. Entomol. Stud., 2: 34-43.

Mitcham, E.; Martin, T. and Zhou, S. (2006): The mode of action of insecticidal controlled atmospheres. Bull. Entomol. Res., 96:213-222.

Mohamed, R.A. and Sayed, A.A. (2017): Efficiency of ECO_2FUME gas against some dry and semi-dry date fruit insect pests in different stores. Ann. Agric. Sci. Moshtohor, 55:137-144.

Musa, A.K. and Adeboye, A.A. (2017): Susceptibility of some cowpea varieties to the seed beetle *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae). J. Agric. Sci. (Belgrade), 62:351-360.

Nayak, M.K.; Holloway, J.C.; Emery, R.N.; Pavic, H.; Bartlet, J. and Collins, P.J. (2013): Strong resistance to phosphine in the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae) : its characterization, a rapid assay for diagnosis and its distribution in Australia. Pest Manag. Sci., 69:48-53.

Nguyen, T.T.; Collins, P.J.; Duong, T.M.; Schlipalius, D.I. and Ebert, P.R. (2016): Genetic conservation of phosphine resistance in the rice weevil *Sitophilus oryzae* (L.). J. Hered., 107:228-237.

Norwood, C. (2017): New fumigation protocols are being developed to tackle the challenge of increasing insect resistance to current control strategies, Ground Cover. Grain Research Development Corporation, Australia.

Perera, G.T.S.; Fernando, T.N.P.; Weerasinghe, W.R.U.; Nugaliyadde, L.; Senadeera, S.P.S.K.; Wijesinghe, PRA *et al.* (2018): Fumigation standards for liquid phosphine (Eco_2 fume 2% phosphine in 98% carbon dioxide W/W) for the control of quarantine pests of rice, pineapple and bitter gourd. Trop. Agric. TurisT, 166:17-32.

Saha, S.; Chakraborty, D.; Sehgal, V.K. and Pal, M. (2015): Rising atmospheric CO_2: Potential impacts on chickpea seed quality. Agric. Ecosyst. Environ., 203:140-146.

Sujeetha, A.R.; Meenatchi, R.; Venkatesan, P. and Brimapureeswaran, R. (2015): Nitrogen based modified atmosphere for the management of Cigarette beetle, *Lasioderma sericorne* in turmeric. IJSART, 1:84-87.

Tumambing, J.; Depalo, M.; Garnier, J.P., and Mallari, R. (2012): ECO_2Fume and Vapor PH_3 OS phosphine fumigants—Global application updates. In S. Navarro, H. J. Banks, D. S. Jayas, C. H. Bell, R. T. Noyes, A. G. Ferizli, M. Emekci, A. A. Isikber, and K. Alagusundaram (Eds.), Proc. of the 9th Int. Conf. on Controlled Atmosphere and Fumigation in Stored Products, 15-19 October 2012 (pp. 363-373). Antalya, Turkey, ARBER Professional Congress Services, Turkey.

Vales, M.I.; Rao, G.R.; Sudini, H.; Patil, S.B. and Murdock, L.L. (2014): Effective and economic storage of pigeonpea seed in triple layer plastic bags. J. Stored Prod. Res., 58:29-38.

Valmas, N. and Ebert, P.R. (2006): Comparative toxicity of fumigants and a phosphine synergist using a novel containment chamber for the safe generation of concentrated phosphine gas. PLOS One 1:e130.

Venkidusamy, M.; Jagadeesan, R.; Nayak, M.K.; Subbarayalu, M.; Subramaniam, C. and Collins, P.J. (2018): Relative tolerance and expression of resistance to phosphine in life stages of the rusty grain beetle, *Cryptolestes ferrugineus*. J. Pest Sci., 91:277-286.

Wong-Corral, F.J.; Castan, E.C. and Riudaviets, J. (2013): Lethal effects of CO_2-modified atmospheres for the control of three Bruchidae species. J Stored Prod. Res., 55:62-67.