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Application of Ethyl Formate as a Postharvest Fumigant for Dry Cocoa Beans under a Semi Commercial Trial

[1]H. Asimah, [3]L. Albert and [2]A.B. Idris
[1]Malaysia Cocoa Board, CITC, Lot PT 12621, 71800, Nilai, Negeri Sembilan, Malaysia
[2]Center for Insect Systematics, Faculty of Science and Technology, National University of Malaysia, Bandar Baru Bangi, Selangor, Malaysia
[3]Malaysian Cocoa Board, Wisma SEDCO, Kota Kinabalu, Sabah, Malaysia

Corresponding Author: H. Asimah, Malaysia Cocoa Board, CITC, Lot PT 12621, 71800, Nilai, Negeri Sembilan, Malaysia

ABSTRACT

Fumigant toxicity of Ethyl Formate (EF) against four major cocoa storage pests was carried out on a semi commercial trial of two metric ton of cocoa beans under sheet without forced air circulation. The EF with concentration from 76-380 g L^{-1} were studied against pupae, larvae and adult of *Cocyra cephalonica* and *Ephestia cautella* (moth) and *Tribolium castaneum* and *Lasioderma serricorne* (beetles). These stored pests were inserted into a wire mesh tubes 6×1.5 centimeters (cm) and then arranged in a 25×2.5 cm polyethylene tubes and then inserted inside gunny sacks and exposed to the EF gas for 40 h with surrounding temperature and relative humidity (R.H.) recorded throughout the trial of 26±0.3-34±0.4°C and 65±0.4-91±0.2%, respectively. While, the temperature inside the tarpaulin was between 24±0.3-30±0.2°C and the R.H. as minimum of 42±0.4% and maximum of 63±0.3%. The result indicated that complete mortality for all stages of insects was detected at minimum concentration of EF (190 g L^{-1}). Different stages and insect species gave difference response to the toxicity level of EF. However, the LC_{99} value for larvae and pupae of all stages of four insects tested showed no significant different among species except for adults of *T. castaneum* and *L. serricorne*. For LC_{50} value for larvae of *L. serricorne*, *E. cautella* and *L. serricorne* pupae are relatively more susceptible to EF than other two species. The estimated probit regressions were well fitted to the response as the chi-square values for larvae, pupae of four pest and two adults pests tested were not significant at 5% level of confident. The residue of EF was not found in whole beans, nibs and cocoa beans. As such the EF was successfully developed as a fumigant for treatment of pests control for dry cocoa beans.

Key words: Ethyl formate, fumigant, cocoa beans, postharvest, insects

INTRODUCTION

Insect pests of stored products are responsible for considerable economic losses to stored products. Eleven insects' species were found in cocoa beans during storage in East Malaysia warehouses. Quality and weight changes of cocoa beans during storage were attributed to insects and mold infestation. Findings indicated that the weight decreased by approximately 1.2% in spite of increases in the beans moisture content. The weight losses was attributed principally to insects infestation, namely by *Tribolium castaneum* and *Ahasverus advena* and other 9 insects species collected during the investigation. Insects and mold infestation as well as free fatty acids increased in cocoa beans upon prolong storage. Findings, also indicated that the cocoa liquor and butter quality were affected by the insects and mold infestation (Asimah and Lopez, 2000). Sivapragasam

(1990) has found thirteen insect pests species in stored cocoa beans in Peninsular Malaysia. Five major species were *T. castaneum*, *Oryzaephilus* spp., *Lasioderma serricorne*, *Ephestia cautella* and *Corcyra cephalonica*. Economic losses of beans weight and quality were mainly attributed by insects' infestation.

Methyl bromide is an ozone depleter, the usage is mandated by United Nation and ceased for use in 2005 for developed countries under the terms of Montreal Protocol. For developing countries, 10 year grace period for technology transfer is provided and was restricted since 2014 (TEAP, 2000). Therefore alternative fumigants should be developed for dry cocoa beans.

The Ethyl Formate (EF) is a colourless liquid with a boiling point of 54.1°C and pleasant aromatic odour. The EF has been used as a fumigant for stored products in general since many years (Cotton and Roark, 1928; Roark and Cotton, 1929) for dried fruit (Simmons and Fisher, 1945; Vincent and Lindgren, 1972; Hilton and Banks, 1997). The EF has been reevaluated and accepted as fumigant replacing methyl bromide for stored grains (Desmarchelier *et al*., 1998). It exist naturally in a range of products, including vegetables products, animals products and also products from stored grain (Desmarchelier, 1999). Since, EF is safe and naturally exist in many products, therefore it is suitable to be developed as a fumigant for dry cocoa beans.

Fruits naturally produce volatile compounds, such as EF, which are important for aromatic and flavor characteristics (Nursten, 1970). Plant volatiles, such as EF have been shown to have insecticidal properties (Vincent and Lindgren, 1971; Aharoni *et al*., 1987; Rohitha *et al*., 1993). One of the benefit of using volatiles, such as EF as fumigant that residues left on treated commodities are only in trace amount (Muthu *et al*., 1984; Desmarchelier and Ren, 1999). The EF is also known as a solvent and is used as a flavoring agents in the food industry. It is naturally present in certains fruits, wine and honey and range of raw and processed food from 0.05-1 mg kg^{-1} (Vu and Ren, 2004). In India, laboratory tests on the effect of EF as fumigants against insects' pest of food commodities and field trials on bagged cereals, spices, pulses, dry fruits and oilcakes have been successfully done (Muthu *et al*., 1984).

This study focused on investigating the EF as an alternative fumigant for dry cocoa beans replacing methyl bromide and evaluate of the EF residue in the cocoa beans.

MATERIALS AND METHODS

Insects culture and rearing

Insects culture: Two species of moths and two species of beetles were used for fumigant testing, namely *C. cephalonica*, *E. cautella*, *T. castaneum* and *L. serricorne*. All insects were collected from infested dry cocoa beans from warehouses in Malaysia.

Artificial diet: Rice bran and ground cocoa were used as artificial media. A total of 20 g of rice bran and 20 g of ground cocoa bean was weighed and transferred into media bottle and microwaved. A pinch of yeast and 10 mL of glycerol were added. Glycerol was only added in *E. cautella* and *C. cephalonica* diet.

Rearing insects in artificial diet: Glass media bottles, glass rod and filter funnels were microwaved for about 10-15 min before used. Adults *T. castaneum* and *L. serricorne* were separated by sieving and transferred into a new media bottle containing artificial diet. Insects rearing were done at room temperature of 27±3ºC and relative humidity of 60-90%. Insects at different life stages: Pupae, larva and adults were collected for fumigant testing.

For moths rearing, adults of *C. cephalonica* and *E. cautella* were collected from the same cocoa warehouses and then reared in media bottles containing artificial diet. Moth adults were collected from the media bottles and then transferred to glass jars containing cotton wool soaked with honey for egg laying. The jars were previously heated in an oven for about 10 min at temperature of 70°C before used. Cotton soaked with honey solution (2:10 v/v) was put into the laying jar as food for insects. After 4 days, eggs were collected and sterilized by soaking with $HgCl_2$ solution (1 M) for about 10 min and then rinsed with distilled water. The dried eggs were then transferred to media bottles containing artificial diet and placed on shelf at temperature 27±3°C and relative humidity of 60-90%. Adults, pupae and larva were then collected for fumigant testing.

Fumigation trial using EF: Two metric tonnes of dry cocoa beans (62.5 kg of dry cocoa beans packed in jute bag each) were used for trial. The beans were uniform in size with bean count of 103 beans per 100 g, well fermented with fermentation index of 97.4% and moisture content of 6.5-6.9%. A total of 32 bags containing 62.5 kg dry beans each were placed under tarpaulin made of canvas as shown in Fig. 1. Polyvinylchloride PVC piping was used as the frame of the fumigation space. Tarpaulin was layered on to the piping frame and on the pallets at the bottom of the gunny sacks. These two layers of tarpaulin were sealed to avoid leaking and gases lose during fumigation.

Bioassays were conducted by placing different stages of insects (larva, pupae and adults) were placed in insects tubes (1.5×6 cm o.d.) and each tubes contains different stages of insects were placed in a 6 cm wire mesh tubes. These tubes were then placed in a 25×2.5 cm polyethylene tube and inserted into the gunny sacks at depth of 3 feets and position at bottom (0.3 m from bottom), middle (1 m from bottom) and top (0.3 m from top) of the arranged gunny sacks under the air proof tarpaulin. However for *C. cephalonica* and *E. cautella*, the adult were not tested because the life span of adult is less than a week (3-4 days) and died upon putting inside the tubes. The bioassay samples were retrieved at the end of fumigation period. The live and dead insects were counted, removed and the remaining mixed ages culture was later incubated at 25°C and 55-70% R.H. The mortality count was recorded after 40 h exposure to EF.

Liquid EF was introduced in four containers and placed at four different positions (at each edge on the floor) under the tarpaulin to produce EF vapor. To complete the trial, six different volumes

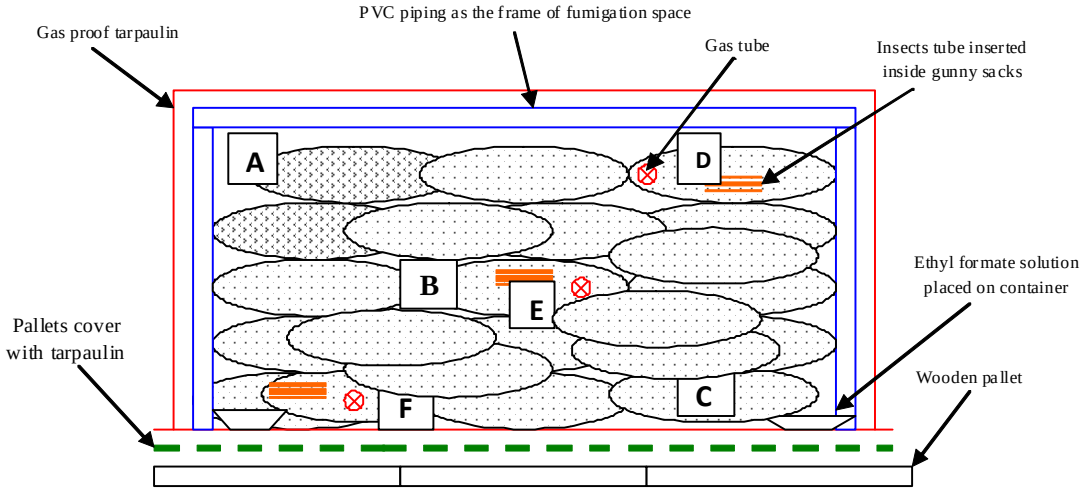

Fig. 1: Layout of cocoa bags inside canvas during fumigation. The diagram shows the position of gas tubing (inside gunny sacks, D, E and F and outside gunny sacks A, B and C) and insects' tubes

of EF solutions were applied with new test bags of cocoa beans. The volumes were applied from 660, 726, 1045, 1320, 1650 and 3300 mL to give EF concentration of 76.1, 83.71, 120.49, 152.19, 190.24 and 380.48 g L^{-1} respectively onto fumigation space of 7.74 m^3 (Fig. 1). Concentration of EF was calculated based on fumigation space, volume of EF used, purity and specific gravity of EF. Gas concentration inside gunny sacks were measured at three different locations (top, middle and bottom) and also outside gunny sacks beneath tarpaulin (top, middle and bottom) as shown in Fig. 1. The EF released in reducing vaporization condition for about 40 h. The gas concentrations were recorded using Fumiscope model version 5.0 (Moon Trading, Malaysia MTFC-S/NO:728). The fumiscope is capable of reading gases up to 749, 750 ppm. The temperature and relative humidity were recorded using a data logger Ebro EBI 20-IF (Germany) with memory/capacity of 8000 measured data. For comparison purpose, a set of control trial was conducted concurrently without using fumigant. A new stack (2 t) of cocoa bags are also used for control during each dosage trial.

Sample preparation for EF residue analysis in fumigated sample: All chemicals and standard used were of analytical grade. The purity of EF used was 97%, with a specific gravity of 920 g L^{-1}.

Extraction of EF in samples and determination of EF: Cocoa beans treated with EF were deshelled. The nib, shell and whole beans were ground separately and 15 g of each ground sample was transferred into a sealed amber bottle (50 mL) containing 25 mL of ammonium nitrate solution (1 M). This method is based on Vu and Ren (2004). The sealed amber bottles were shaked on a shaker at room temperature (25±3°C) for 24 h. After 24 h the mixture was transferred into 1.5 mL centrifuge tube and centrifuged at 5 g×14,000 rpm (Eppendorf centrifuge 5810 R). Then top layer was filtered using 0.45 μm syringe filter. A total of 10 μL aliquot of headspace over the centrifuge extraction solution was injected directly into gas chromatograph (GCMSD, Shimadzu. The GC system: The HP 6890 series, USA), the GC selective detector equipped with FID. Separation was achieved on a 0.25 μm film megabore capillary columns DB FFAP (J and W 125-3212) (30 m (length) ×0.25 mm (i.d.) in an oven temperature of 70°C for 12 min.

The level of EF was calculated on the basis of peak area. The peak areas were calibrated periodically using standard EF. Standard EF solutions were prepared from 0.001-0.5 ppm. The EF in all samples were determined using scan and SIM modes. Method used was described by Vu and Ren (2004).

Data analysis: Toxicity effect of the EF was determined by correcting the mortality data according to Abbott (1925) and then analyzed by probit analysis (Finney, 1971). Data obtained from each zero dose control and concentration-mortality responses were subjected to probit analysis by using SPSS software to determine LC_{50} (Lethal Concentration 50), LC_{99} (Lethal Concentration 99) and their respective 95% confidence intervals. Two LC_{50} values were considered significantly different if their 95% confidence limits did not overlap. All LC_{99} values for larvae and pupae were not significantly different if their 95% confidence limits overlapped except for two adults moths tested.

RESULTS

The results of probit analysis of EF concentration application on four different types of larvae species is presented in Table 1. Results show that the toxicity effect of EF was not significantly different among the larva of all insects species as the fiducial limit for LC_{50} values were all overlapped.

Table 1: Summary of probit analysis of larvae and pupae of four types of insects species against ethyl formate concentration

Insects species	n[a]	Slope±SE	LC_{50} (g L^{-1})	Fiducial limit	LC_{99} (g L^{-1})	Fiducial limit	Chi sq[c]
Larvae							
T. castaneum	45	4.905±0.876	90.73	80.53-99.52[a]	270.42	206.34-468.07[a]	3.776[ns]
L. serricorne	45	5.876±1.074	76.19	65.52-83.58[a]	189.59	154.94-286.19[a]	0.989[ns]
C. cephalonica	45	4.864±0.973	75.30	61.87-84.08[a]	226.49	175.13-400.72[a]	1.065[ns]
E. cautella	45	3.196±0.854	72.62	48.55-85.47[a]	388.21	240.05-1751.26[a]	0.612[ns]
Pupae							
T. castaneum	45	6.412±0.961	91.00	83.45-97.99[a]	209.81	174.15-292.20[a]	3.600[ns]
L. serricorne	45	4.739±1.070	62.24	47.49-75.30[b]	202.01	156.74-379.25[a]	2.375[ns]
C. cephalonica	45	5.040±1.434	75.10	58.10-83.64[ab]	217.37	155.99-680.86[a]	0.252[ns]
E. cautella	45	2.824±0.834	71.65	42.37-85.97[ab]	477.61	266.17-4119.71[a]	3.054[ns]

[a]Number treated, excluding controls, Fiducial limit give the 95% confidence range, [c]Chi-square (chi-square is significant, $p<0.05$ and ns: Not significant at $p>0.05$)

Table 2: Summary of probit analysis of adult of two types of insects species against ethyl formate concentration

Insects species	n[a]	Slope±SE	LC_{50} (g L^{-1})	Fiducial limit	LC_{99} (g L^{-1})	Fiducial limit	Chi sq[c]
T. castaneum	45	6.190±0.940	91.60	0-0[a]	217.50	0-0[a]	5.778[ns]
L. serricorne	45	4.378±0.923	74.99	59.73-84.66[b]	254.94	189.54-510.23[b]	2.182[ns]

[a]Number treated, excluding controls, Fiducial limit give the 95% confidence range, [c]Chi-square (chi-square is significant, $p<0.05$ and ns: Not significant at $p>0.05$). Adults for EC and CC were not tested toxicity against EF due to very short life cycle (3-5 days)

For the pupae, results showed that there was a significant different in LC_{50} value of EF among insects species (Table 1). The EF was more toxic to *L. serricorne* pupae with LC_{50} value was significantly lower than LC_{50} value of the other species. However the toxicity effect of EF was not differently significant among pupae of insects tested at LC_{99}.

There was a significantly different of the toxicity effect of EF on adult of *T. castaneum* and *L. serricorne* (Table 2). The LC_{50} (91.60 g L^{-1}) is needed to kill 50% of *T. castaneum* adult population compared to adult (74.99 g L^{-1}) of *L. serricorne*. For adult stage, only *T. castaneum* and *L. serricorne* were tested for toxicity against EF since *C. cephalonica* and *E. cautella* adults have very short life cycles, too delicate and died upon putting inside the wire mesh tubes.

All the estimated probit regression fitted well to the response as the chi-square values in two adults species were not significant at 5% level. The chi-square values for four larvae and pupae are also not significant at 5%. Therefore, it can be concluded that all stages of insects species used for experiments are homogenous.

In the initial phase of fumigation, there were significant differences in the level of EF vapor between top, middle and bottom position of stacks (Fig. 2a-f). At earlier stage, there was no significant difference in EF gas levels between the inside and outside the gunny sacks and also between bottom, middle and top of gunny sacks. However, after 400 min, the concentration of EF vapor remains constant between inside and outside gunny sacks. The EF vapor concentration using 660, 726, 1045 and 1320 mL were more stable and remained constant (Fig. 2a-c) compared to fumigation using 1650 and 3300 mL (Fig. 2d-f). This indicates that with higher dosage of fumigants, the gas released and distribution of EF was more saturated and perturbed. The results also indicates that the gas concentration of EF was higher inside gunny sacks compared to outside gunny sacks (Fig. 2e-f). However, the EF vapor concentration reached optimum condition and remain constant at 1600 min in each fumigations and it was remained till the end of fumigation at 40 h (Fig. 2a-f).

Table 3 indicates the Concentration×time product (Ct) calculated for position of the middle outside (MO) gunny sack under sealed tarpaulin with average constant value of EF gas concentration. The Ct product is estimated simply by multiplying the concentration by exposure time and expressed in g h L^{-1}. The cumulative Ct product then has been calculated by adding together the component Ct products obtained from the average concentration of sequential

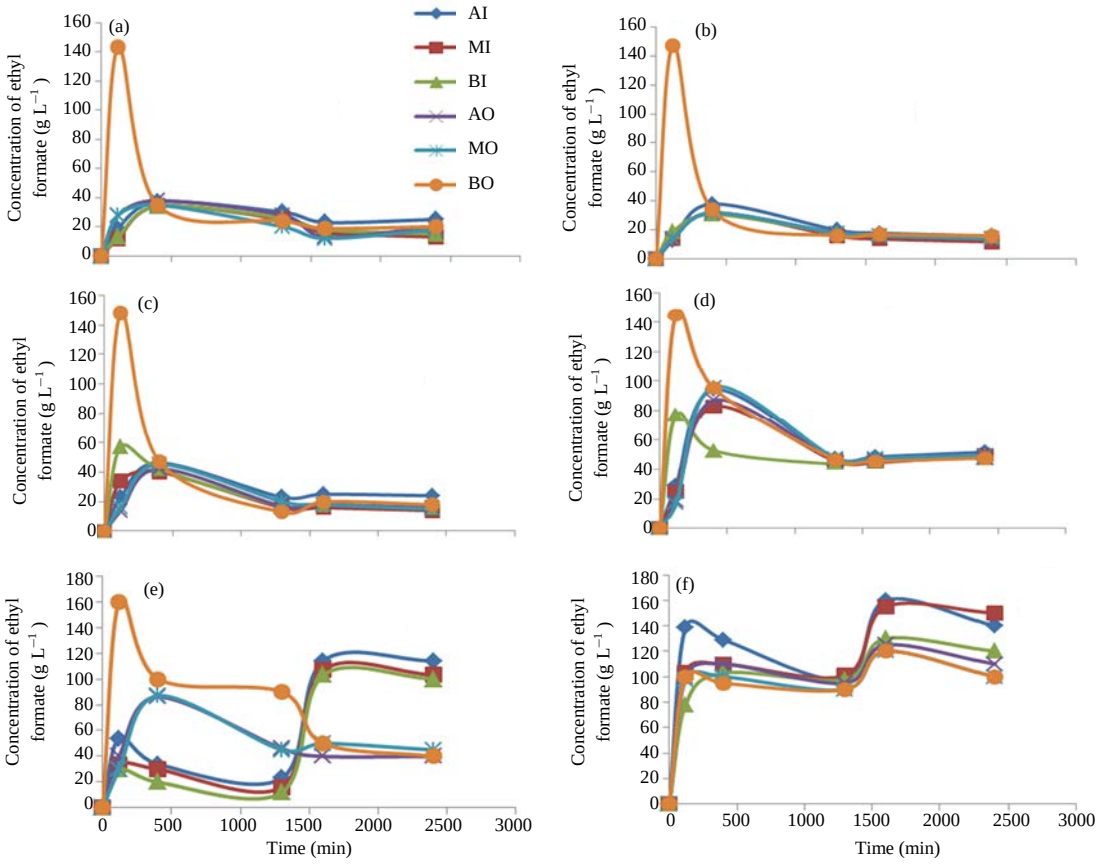

Fig. 2(a-f): Changes in the concentration of ethyl formate during fumigation under tarpaulin for different volume of ethyl formate added, (a) 660 mL, (b) 726 mL, (c) 1045 mL, (d) 1320 mL, (e) 1650 mL and (f) 3300 mL. AI: Top inside, MI: Middle inside, BI: Bottom inside, AO: Top outside, MO: Middle outside, BO: Bottom outside

observation and multiplying by the time interval between them. To produce the best approximation of true total Ct products is obtained with a large number of concentration observations in an exposure. However, in this study practical constrains limit the number of observations that can be made. Only Ct products outside middle position under sheet (tarpaulin) was calculated as stated in Table 3. For higher doses (1650 and 3300 mL) EF used for fumigation have resulted in not even distribution of EF vapour (Fig. 2e-f). The finding indicated that from time of 6-26 h, the Ct product increased and then reduced gradually and finally at 40 h its remained constant in almost all concentration applied. The Ct product reached maximum at 26 h and complete mortality was obtained for all stages of insects (Table 3).

The outside (surrounding) temperature and R.H. recorded throughout the trial were 25±0.4°C and 65-91%, respectively. However, the data logger inside the tarpaulin had recorded temperature as low as 24±0.3°C and maximum at 30±0.2°C and the R.H. as minimum of 42±0.4% and maximum of 63±0.3%, respectively (Fig. 3). This indicated that the EF vapour inside the tarpaulin caused a lower temperature and R.H. The temperature and r.h were maintained within the range in all trials (Fig. 3).

Table 3: Volume of EF used and Ct product calculated for position of the middle outside gunny sack (MO) with average value of EF gas concentration under sealed tarpaulin

Volume of EF (mL)	Time (h)	Time step (h)	Concentration of EF (g L^{-1})	Ct product (g h L^{-1})	Cumulative Ct (g h L^{-1})
660	0	-	-	-	-
	2	2	28	0	0
	6	4	35	125.22	125.22
	26	20	19	515.75	640.97
	40	14	19	266.0	906.97
726	0	-	-	-	-
	2	2	14	0	0
	6	4	34	87.27	87.27
	26	20	17	480.43	568.1
	40	14	16	230.89	798.99
1045	0	-	-	-	-
	2	2	16	0	0
	6	4	42	103.69	03.69
	26	20	20	579.66	683.35
	40	14	18	265.63	948.98
1320	0	-	-	-	-
	2	2	17	0	0
	6	4	95	160.75	160.75
	26	20	46	1322.12	1482.87
	40	14	48	657.85	2140.72
1650	0	-	-	-	-
	2	2	30	0	0
	6	4	87	204.35	204.35
	26	20	50	1319.09	1523.44
	40	14	45	664.08	2187.52
3300	0	-	-	-	-
	2	2	98	0	0
	6	4	100	395.98	395.98
	26	20	120	2190.89	2586.87
	40	14	110	1608.48	4195.35

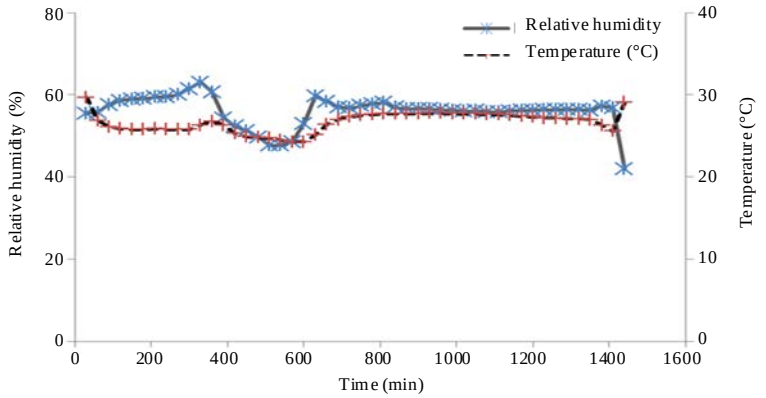

Fig. 3: Temperature and relative humidity recorded inside the tarpaulin during fumigation

The EF standard graph was established using standard EF dilutions, which was prepared using standard EF solutions from 0.01-0.5 ppm. The detection limits were from minimum 0-0.001 mg kg^{-1} up to 0.05 mg kg^{-1}. Calibration was done periodically prior to injection into GC. Table 4 shows the ethyl formate residue detected in whole beans, nibs and shell of the cocoa beans tested. The results also indicated that there was no residue found in all fumigated samples.

Figure 4-5 indicate the mortality percentage against EF concentration for all stages of insects for namely *C. cephalonica*, *E. cautella*, *T. castaneum* and *L. serricorne*. These four pests are

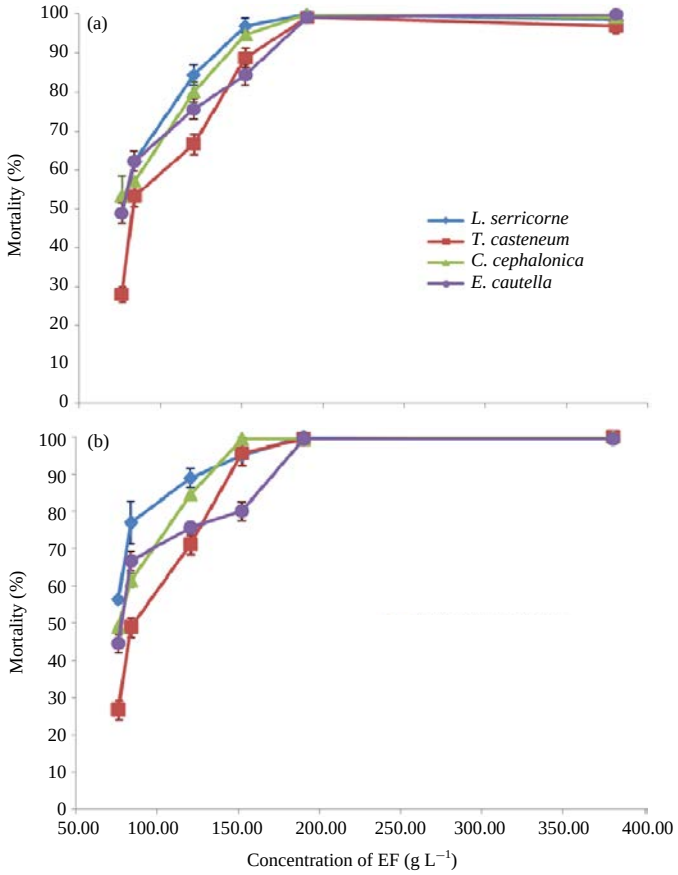

Fig. 4(a-b): Mortality of (a) Larvae and (b) Pupae of *T. castaneum*, *L. serricorne*, *C. cephalonica* and *E. cautella*

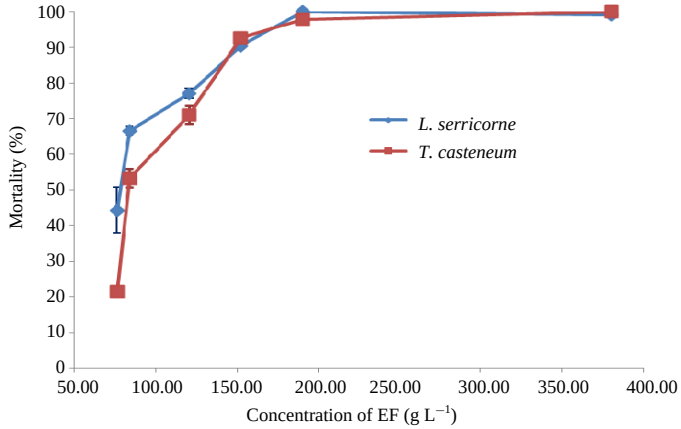

Fig. 5: Mortality of adult of *C. cephalonica*, *E. cautella*, *T. castaneum* and *L. serricorne*

very serious cocoa pest especially when the conditions are conductive for their development, such as high moisture content. However the moisture of the cocoa beans used in this trial was 6.5-6.9%. At 190 g L^{-1} of EF applied for all stages of insects were killed rapidly.

Table 4: Ethyl formate residue in whole bean, nibs and shells of fumigated cocoa beans

Dose (mL)	Concentration (g L^{-1})	Samples no.	Whole bean concentration (mg kg^{-1})	Nib concentration (mg kg^{-1})	Shell concentration (mg kg^{-1})
660	76.1	A Control	n.d	n.d	n.d
726	83.71	B Control	n.d	n.d	n.d
1045	120.49	C Control	n.d	n.d	n.d
1320	152.19	D Control	n.d	n.d	n.d
1650	190.24	E Control	n.d	n.d	n.d
3300	380.48	F Control	n.d	n.d	n.d

n.d: Not detected

DISCUSSION

The EF was toxic to larvae of all insects species studied as indicated with lower LC_{50} and LC_{99} value across species (Table 1). However, EF seems to be more toxic to *E. cautella* than the other species as indicated by relatively high LC_{99} value and its LC_{50} value very much lower than *T. castaneum*. Interestingly that *E. cautella* showed more tolerance (slope, 3.196) to EF compared to others and this is in agreement to the values of LC_{50} and LC_{99}. Therefore, the results of the study indicate that EF can effectively control larva of all insects' tested. Results also showed that there were no significant differences in LC_{50} and LC_{99} among insects species as they have indicated by the overlapping of fiducial limit. However, *T. castaneum* has relatively high LC_{50} values than the others while, *E. cautella* has much higher mortality. The slope of *L. serricorne* is steeper than other (Table 1). The chi-square is not significant at $p>0.05$ (Table 1-2), indicates that all samples are homogenous (no variable between individual insects tested).

Table 1 summaries the toxicity effect of EF against pupae of all insects species. The slope values were varied ranging from 2.824-6.412 indicating much heterogeneity in the response of pupae to EF treatment. The LC_{50} of pupae of *L. serricorne* was significantly different compared to other species, indicating *L. serricorne* pupae are more sensitive to toxicity of EF. However, the LC_{50} value for *T. castaneum* pupae was 91 g L^{-1} and less susceptible to EF. The LC_{99} of *E. cautella* gave the highest value, 477.61 g L^{-1} and but there were no significant different between them.

However, *T. castaneum* gave higher slope (6.190) compared to *L. serricorne* (4.378). Although, LC_{99} value for *L. serricorne* was significant ($p<0.01$) than *L. serricorne* and likewise for LC_{50} value, the slope was higher than of *T. castaneum* and this indicates that *T. castaneum* gave higher LC_{50} value than *L. serricorne* (Table 2).

Mean mortality percentage of all insects' stages increases as the fumigants concentrations increases (Fig. 4-5). Results also indicated that complete mortalities were obtained at minimum concentration of 190 g L^{-1} for adult, pupae and larvae of all insect species. Figure 4 and Table 1 also showed that the LC_{99} value of larvae and pupae of all insects species except *T. castaneum* increases as the concentration of fumigant increases. The adult's mortality of *T. castaneum* and *L. serricorne*, also indicated the same pattern. However LC_{99} and LC_{50} for all insects species shows no significant different among insects species irrespective of insects stages except for pupae of *L. serricorne* or between adult of *L. serricorne* and *T. castaneum*.

EF dosing can caused perturbed and not even distribution of EF vapour especially for dosing at higher concentration such as 1650 and 3300 mL (Fig. 2e-f). Table 4 shows that dosing at 1650 mL of EF, Ct product valued almost doubled 2190.89 g h L^{-1} (Table 4). Naked eye observation of all stages insects treated with EF with 3300 mL shows insects were killed and the carcass was burn. Insects death presumably occurs during treatment as a consequence of prolong and intense narcosis. These observations can be supported by the toxic action of inert gases which was first described by Ferguson and Hawkins (1949) and later by Johnson and Quastel (1953) and Carpenter (1954), they have mentioned that these toxic gases can caused narcotic effects and destruction of cell membranes.

Table 3 indicates that a Ct product valued 1322.12 g h L^{-1} (1320 mL) is sufficient to kill insect's pest but to be more efficient 1650 mL or 190 g L^{-1} is recommended for future application. However, in previous study by Muthu *et al*. (1984), adult of *T. castaneum* was found to be most susceptible but pupae was the most tolerant stage to EF at 300-400 g m^{-3}. Mean while, Desmarchelier *et al*. (1998) has reported that 90 g t^{-1} of EF on wheat in a 55 t bin can of mixed culture of adults and larvae of *T. castaneum* can have complete control and total mortality. Ren and Mahon (2006) has also reported that fumigation trial of EF with 85 g t^{-1} to wheat, split faba beans and sorghum with two dosage application in unsealed metal bins kill rapidly of all stages of *Sitophilus oryxae*, *Rhyzopertha dominica* and *T. castaneum* within two days.

The residue of EF were not found in whole beans, shell and nibs of cocoa beans treated with fumigant. Salt solutions such as ammonium nitrate was used in the extraction of fumigant residue since they are likely inhibitors of hydrolytic enzymes. According to Desmarchelier *et al*. (1998), EF in stored grains can be determined from headspace concentration over grains in organic solvent such as methanol or propanol. There is no specific detector for EF. It can be detected by a Flame Ionization Detector (FID) but the use of organic solvent can cause interference, especially, when measuring low levels of EF. Salts solutions were more commonly selected as likely inhibitors of hydrolytic enzymes. Therefore in this study ammonium nitrate was used for extraction. Vu and Ren (2004) used ammonium nitrate solution to perform extraction in Australian wheat, barley, oat and canola and natural EF was determined with a Gas Chromatograph equipped with a flame ionization detector.

The rapid breakdown of EF also ensures that there are no issues of chemical residue on treated products. There are no withholding periods after treatment (Ren and Desmarchelier, 2001; Vu and Ren, 2004).

CONCLUSION

EF was successfully tested as a fumigant for pests control for dry cocoa beans. From the probit analyses we found that *L. serricorne* is the most sensitive insect of all stages against EF, while the other species are less sensitive but still affected by EF and can be controlled. It is more economical since shorter time and less chemical required for post harvest treatment. As experience in this study and previous trials records from literatures, EF has advantages in term of workers and environment safety. It is highly recommended for replacement of methyl bromide which is ozone depleter and was phased out in 2014. End point complete mortality was reached with minimum dosage of 190 g L^{-1} with exposure of 40 h. Therefore, EF has a good potential as fumigant for cocoa beans and can be introduced for usage in the cocoa industry.

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