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Synergistic Effect of Phosphine and Carbon Dioxide on the Mortality of *Tribolium castaneum* (Coleoptera: Tenebrionidae) in Paddy

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Abstract

Phosphine (PH_3) is widely used as a fumigant for the control of stored product pests. Indiscriminate use of PH_3 leads to the development of resistant strains. Thus, an experiment was conducted in a laboratory scale fumigation chamber to determine the synergistic effect of carbon dioxide (CO_2) and PH_3 on the mortality of *Tribolium castaneum* during fumigation of paddy. PH_3 gas generation varies depending upon grain moisture content and dosage of PH_3 used for fumigation treatment. Thus, the maximum PH_3 concentration with respect to saturation time was achieved when paddy were treated with 98% CO_2 + 3 ppm PH_3 at 17% moisture content, leading to a quick mortality of different life stages of *T. castaneum* with minimum LT_{50} and LT_{99} value. Among the different stages of *T. castaneum*, pupal stage was more resistant to all the treatments compared to larva and adult stages. PH_3 residues in all the treatments were below the recommended level and it can be considered as safe for consumption.

Keywords: paddy, moisture, phosphine, generation rate, mortality, *T. castaneum*

1. Introduction

India handles more than 200 million tons (MT) of food grains annually. These grains need to be preserved using the available fumigants. Paddy and wheat are commonly stored in warehouses in large quantities and are often infested with *T. castaneum* due to inappropriate storage and handling. To prevent infestation grains are generally fumigated with aluminum phosphide tablets for more than two decades. However, due to unscrupulous and continuous usage of aluminum phosphide resulted in high level pest resistance to phosphine in stored products (Mau et al., 2012). Mixture of CO_2 and PH_3 is considered as a potential fumigant for the management of stored product pests (Leelaja et al., 2007; Valmas & Ebert, 2006). Carbon dioxide (CO_2) a potential gaseous fumigant toxic to insects at high concentration and requires large exposure time for achieving mortality of all the stage3s of insects (Hasan et al., 2016). Many studies show that the addition of Co2 to PH3 enhances the toxicity of PH3 and reduces the dose required to kill insects (Ren et al., 1994). The combination of CO_2 with PH_3 increases the penetration rate of the fumigant, acts as the propellent for delivering PH_3, enhances the respiration rate of insects and is more effective than existing solid formulations available in the market (Leesch, 1992). A combination of CO_2 and PH_3 is available in the trade name of ECO_2FUME® and are used in countries such as Australia, New Zealand, North America and Europe (Tumambing et al., 2012). It consists of cylinderized compressed gas formulation containing a mixture of 2% PH_3 by weight (2.6% by volume) in CO_2 (98% by weight). Another recently introduced fumigant for the management of stored product pest is $VAPORPH_3OS$ (99.3 % pure PH_3 mixed with air). These formulations are considered to be safe, effective and are user friendly for fumigating food and non-food commodities (Meenatchi & Alagusundaram, 2014). But due to stringent rules in importing and the non-availability of any other chemicals for fumigation other than PH_3, none of the fumigants were registered in India. At this juncture, a study was undertaken by using PH_3 and CO_2 combinations to evaluate the synergistic effect of PH_3 with respect to saturation time, PH_3 generation in paddy and mortality of *T. castaneum*.

2. Materials and Methods

2.1 Conditioning Paddy

The paddy was procured with the initial moisture content of 12.5% wet basis (w.b.) and conditioned to the required moisture content of 11, 14 and 17%. To obtain lower moisture content, samples were spread in thin

layer under shade for natural air drying for about 8 h. To increase the moisture content, samples were conditioned by adding required amount of distilled water and mixed well by passing through a screw conveyor. The amount of water to be added to increase grain moisture content was calculated by using the following Equation (1). The conditioned grain samples were stored in sealed polythene bags in a deep freezer at -5±2 ℃.

Amount of water added,

$$Kg = W_s X \frac{M_f - M_i}{100 - M_f} \quad (1)$$

Where, W_s: Sample weight (Kg); M_f: Final moisture content (% w.b.); M_i: Initial moisture content (% w.b.)

2.2 Culturing of Test Insects

T. castaneum was cultured and maintained at the Storage Engineering Laboratory at IIFPT, Thanjavur. Insects were cultured at 30 °C and 70% RH. Different stages of insects such as pupa, larva and adults were maintained separately to carry out mortality studies.

2.3 Lab Model Fumigation Set up for Toxicity Studies

Phosphine fumigation lab model set up was designed and fabricated at Indian Institute of Food Processing Technoloy (IIFPT). It consists of a circular outer acrylic cylindrical tube with the dimension of 37 × 27 cm (ht × Ø) which is pasted in a flat acrylic at the bottom (Figure1). Inside the fumigation chamber, four compartments were made by partitioning it with wire mesh and were fitted in a stand. Fumigation cups of 4 × 6 cm (ht × Ø) were made by cutting small acrylic tubes and were pasted with fine wire mesh at the bottom. The top of the fumigation setup was covered with PVC end cap to make it air tight. Rubber septum was placed at the top cover for injecting PH_3. Provisions were made for injecting and releasing CO_2 from the cylinder (Meenatchi et al., 2015). Required volume of PH_3 gas was calculated and injected into the fumigation chamber using Hamilton syringe through the rubber septum located at the top of the fumigation chamber. The 98% CO_2 in air was continuously flowed through the chamber.

Figure 1. Acrylic fumigation chamber

2.4 Measurement of PH_3 Concentration

Phosphine concentration was measured using 250 PM+ portable phosphine monitor (Uniphos Envirotronic Pvt Ltd, India) at every 4 h interval for a period of 120 h.

2.5 Bioassay Studies

Fifteen grams of the conditioned paddy (11, 14 and 17% moisture content) were taken in fumigation cups and 10 insects of various life stages were released. The fumigation cup was covered with muslin cloth. After treatment, the insects were transferred into paddy and then kept in environmental chamber maintained at 25 °C and 60% RH. The number of different life stages of dead *T. castaneum* was recorded after fumigation at different time intervals and the mortality percentage was calculated.

In addition to PH_3 generation and saturation time, a similar set of experiments were carried out to know the effect of various combinations of fumigant on insect mortality at different time intervals. In this study, 98% CO_2 with 3, 2, and 1 ppm PH_3 and 3 ppm PH_3 alone was used as a control. From this data LT_{50} and LT_{99} value of various life stages of *T. castaneum* were calculated. All experiments were repeated thrice.

2.6 Determination of PH_3 Residues in the Fumigated Paddy

The fumigated paddy was placed in a tray for ventilation. After 5 days, 15 g of treated paddy was taken in a 500 ml conical flask filled with 150 ml distilled water. Then, the flask was sealed using a stopper with silicone septum, 5 ml of HCL was injected through the syringe in the sampling adopter. The flask was kept in an ultrasonic wave-cleaner and shaken for 5 min and subsequently allowed to stand for 30 min. The headspace analysis was done using Gas Chromatography (GC-14B, Shimadzu Technologies, Columbia, USA).

2.7 Data Analysis

The experimental data were fitted to a linear equation, $Y = aX + b$, where Y is the PH_3 concentration and X is the fumigation time. The results were statistically analysed using Factorial Completely Randomized Design (FCRD) with replication at $p < 0.01$ and $p < 0.05$ level of significance through AGRES software (7.01). Fisher's least significant difference (LSD) test was followed to determine highly significant difference between the treatments and paddy moisture content. Also, probit analysis was carried out for statistically analyzing the mortality of various life stages of *T. castaneum* (LT_{50} and LT_{99}) using polo plus software (V. 2.0).

3. Results and Discussion

3.1 Phosphine Concentration and Saturation Time

Saturation time is the extent to which maximum PH_3 concentration generated at a particular point of time. PH_3 concentration and saturation time with respect to different treatments and moisture content were depicted in Table 1. It has been observed that at 11% moisture content, maximum PH_3 concentration of 322 ppm was recorded with the corresponding saturation time of 73 h in the treatment T_1 followed by T_2 and T_4 with the PH_3 concentration of 284 ppm and 270 ppm at the saturation time of 61 h respectively. Treatment T_3 (98% CO_2 + 1 ppm PH_3) recorded lowest PH_3 concentration of 218 ppm with a saturation time of 55 h. At 14% moisture content, maximum PH_3 concentration of 340 ppm with the saturation time of 67 h was observed in T_1 followed by T_2, T_4 and T_3 (Table 1). Similarly, at 17% moisture content maximum PH_3 concentration of 351 ppm with the saturation time of 45 h was recorded. It could be explained by understanding that at higher grain moisture content elevated PH_3 concentration released in shorter period. From our studies, it can be concluded that paddy treated with 98% CO_2 + 3 ppm PH_3 was considered to be the effective treatment which enhanced PH_3 concentration due to the addition of CO_2. Comparing all the treatments, least PH_3 concentration value of 204 ppm was recorded at 11% moisture content in the treatment T_3. Several studies in the past confirmed that PH_3 concentration remains higher with the addition of CO_2 (Daolin et al., 2004). Thus, the increase in PH_3 concentration and higher grain moisture content influences the concentration at the time of fumigation. F values of PH_3 peak concentration are highly significant ($P \leq 0.01$) with respect to moisture contents, and treatments as given in the Table 2.

Table 1. Effect of different treatments (*i.e.* T_1-(98% CO_2+ 3 ppm PH_3); T_2-(98% CO_2+ 2 ppm PH_3); T3-(98% CO_2 + 1 ppm PH_3); T4-(3 ppm PH_3)) and grain moisture content on PH_3 generation with respect to saturation time. Values are expressed as Mean±standard deviations

Treatments	Moisture content (11% wet basis)		Moisture content (14% wet basis)		Moisture content (17% wet basis)	
	Saturation time (h)	[PH_3] (ppm)	Saturation time (h)	[PH_3] (ppm)	Saturation time (h)	[PH_3] (ppm)
T_1	73±2.31	322±0.57	67±2.31	340±1.00	45±2.31	351±1.00
T_2	61±2.31	284±1.00	59±2.31	287±1.00	49±2.31	290±1.10
T_3	57±2.31	204±0.74	57±2.31	235±1.00	55±2.31	218±1.53
T_4	61±2.31	270±1.00	64±4.62	295±1.00	53±1.15	296±1.60

Table 2. Analysis of variance for PH_3 generation rate and concentration with respect to saturation time ($p < 0.01$)

Variables	df	Sum of Square	Mean Square	F
PH_3				
Treatment (T)*	3	52050.97	1024.11	635.65
Moisture (M)*	2	2048.22	17350.32	10769.16
TM* (Treatment × Moisture)	6	2079.77	346.62	215.14
Error	24	38.66	1.61	1.0
Total	35	56217.63	56217.63	996.96
PH_3 generation rate				
Treatment (T)*	3	17.78	5.92	8470.41
Moisture (M)*	2	9.22	4.61	6586.77
TM* (Treatment × Moisture)	6	5.74	0.95	1368.12
Error	24	0.01	0.0007	1.00
Total	35	32.77	0.93	1337.64

3.2 Phosphine Generation Rate

The residual concentration of the PH_3 as a function of time was fitted in linear regression to determine the PH_3 generation rate. From Figure 2, it can be concluded that at 17% moisture content, T_1 recorded highest PH_3 generation rate of 8.32 ppm/h as compared to other treatments. Similarly, at 14 and 11 % moisture content maximum PH_3 generation rate of 5.60 and 4.89 ppm/h observed in T_2 and T_1 respectively. PH_3 generation rate was lower in paddy treated with 98% CO_2 + 1 ppm PH_3. Among the three moisture levels tested, T_1 at 17% moisture content recorded highest PH_3 generation rate. This could be explained by understanding that PH_3 generates faster at higher moisture levels and the presence CO_2 enhances PH_3 toxicity due to synergistic effect. The other factors influences PH_3 gas generation includes temperature, moisture content and brand of fumigant used (Banks, 1991). Comparing to low moisture grain, high moisture grain stimulates higher PH_3 gas generation (Reed & Pan, 2000). Thus, the difference in moisture levels and treatments given to the paddy significantly ($p < 0.01$) affected the PH_3 generation rate as given in the Table 2.

3.3 Phosphine Concentration With Respect to Fumigation Time

Aluminum phosphide reacts with the moisture present in the atmosphere and produces PH_3 gas. This reaction starts slowly, then gradually increases and later declines when the aluminum phosphide is spent. Rate of PH_3 decomposition during fumigation also varies depending upon the moisture and atmospheric conditions (Figure 3). From figure, it has been observed that there was a gradual decrease in PH_3 concentration after attaining saturation point in all the treatments of paddy. Adequate moisture is required for the release of the definite toxicant or PH_3 in aluminum phosphide. It is clearly shown that the grain moisture content has direct impact on PH_3 generation. PH_3 release is enhanced at higher moisture content.

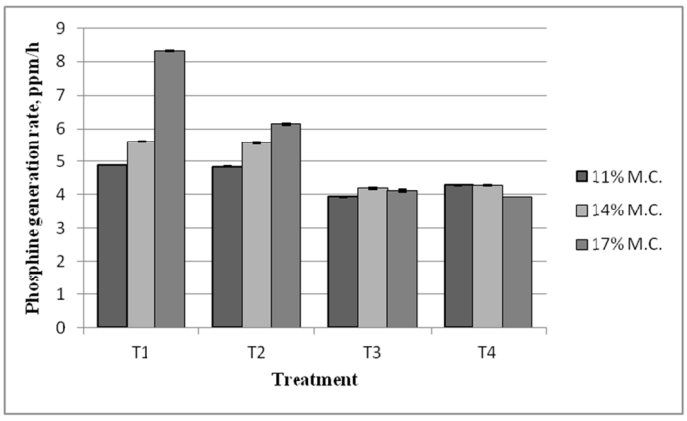

Figure 2. PH_3 generation rate in paddy fumigated with CO_2 + PH_3 at (a)11%, (b) 14% and (c) 17% moisture content (wet basis) during treatments

Note. T_1-(98% CO_2 + 3 ppm PH_3); T_2-(98% CO_2 + 2 ppm PH_3); T_3-(98% CO_2 + 1 ppm PH_3); T_4-(3 ppm PH_3).

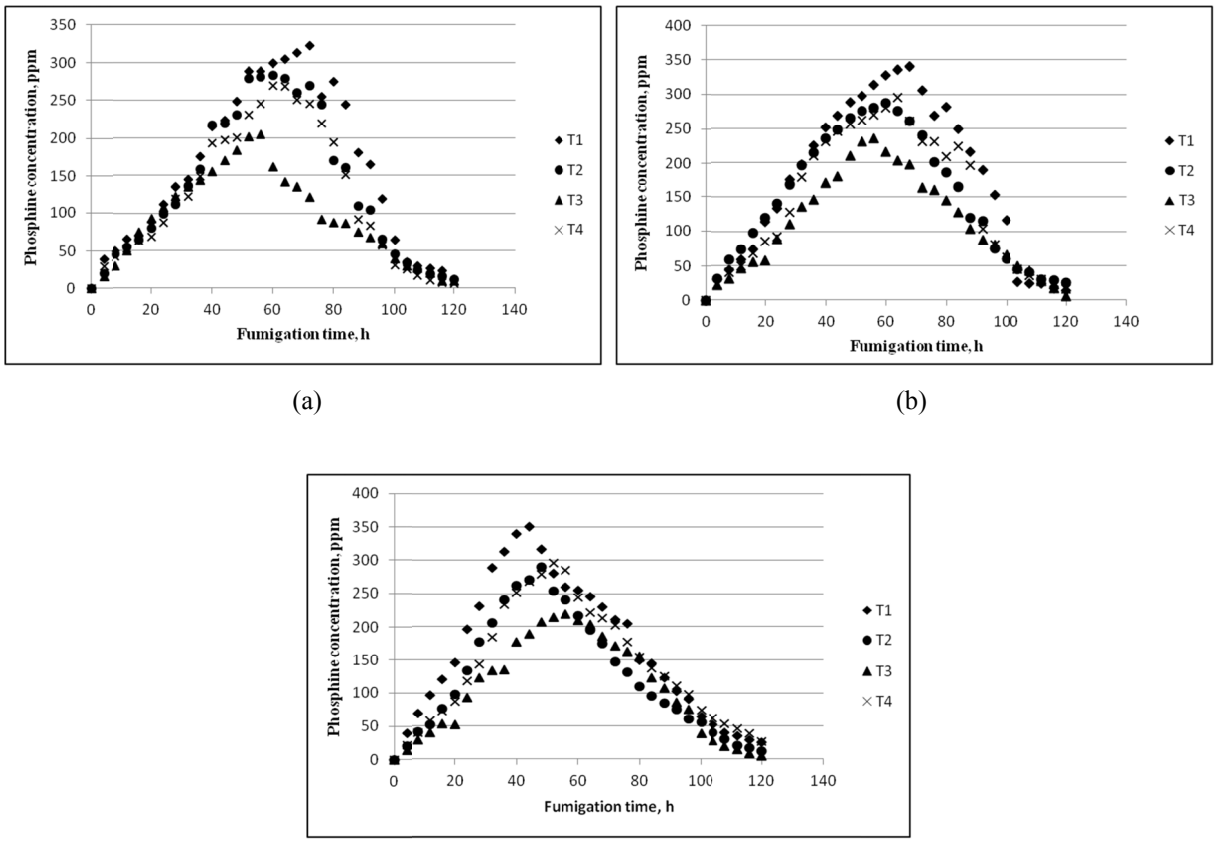

Figure 3 PH_3 concentrations in paddy fumigated with CO_2 and PH_3 at (a) 11 % (b) 14% and (c) 17 % moisture content (wet basis) during treatments

Note. T_1-(98% CO_2 + 3 ppm PH_3); T_2-(98% CO_2 + 2 ppm PH_3); T_3-(98% CO_2 + 1 ppm PH_3); T_4-(3 ppm PH_3).

3.4 Insect Mortality

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, acidification at the cellular level, and creating a lack of triglycerides for energy metabolism (Busvine, 1971; Adler, 1994; Donahaye, 1991). The LT_{99} value of *T. castaneum* in paddy at 11% moisture content was given in the Table 3. The minimum lethal time for larva was observed as 27.0 h in the treatment T_1, followed by T_2, T_4 and T_3 with the lethal time of 29.5 h, 33.7 h and 41.9 h respectively. The LT_{99} value for pupa and adult was noted as 35.5 h and 35.6 h in the treatment T_1 and T_2 respectively. LT_{50} for larva, pupa and adult were recorded as 2.9 h, 4.8 h and 1.8 h respectively. At 14% moisture content, the minimum LT_{99} value for larva was 17.1 h for the treatment T_1, followed by adult 21.7 h and pupa 36.4 h (Table 4). Similar trend was observed in paddy treated with CO_2 and PH_3 at 17% moisture content (Table 5). Among the different treatments, T_1 at 17% moisture content proved as the best treatment recorded with maximum PH_3 concentration and minimum lethal time. As the concentration of PH_3 increases time taken to achieve LT_{50} and LT_{99} decreases. Addition of CO_2 to PH_3 reduces the time and dose required for fumigation that results in quick insect mortality. Some studies have proven that the addition of CO_2 could increase the PH_3 concentration and resulted in increased toxicity levels (Donahaye & Navarro, 1989). Desmarchelier (1984) found that the mixture of phosphine and carbondioxide has synergistic effect on insect mortality as compared to either toxicant alone. The present study also confirmed that addition of CO_2 to PH_3 enhanced the insect mortality with minimum lethal time. The treatment T_1 and T_2 were significantly superior to T_3 and T_4 respectively.

Table 3. Mortality of *T castaneum* larva, pupa and adult treated with CO_2 + PH_3 (*i.e.* T_1-(98% CO_2 + 3 ppm PH_3); T_2-(98% CO_2 + 2 ppm PH_3); T_3-(98% CO_2 + 1 ppm PH_3); T_4-(3 ppm PH_3)) at 11 % moisture content at 95% of confidential limit

Stage		Slope±SE	LT_{50} (h)	LT_{95} (h)	LT_{99} (h)	X^2 (df)
Larva	T_1	2.4±0.4	2.9(2.0-3.7)	14.1(10.3-25.3)	27.0(17.1-65.9)	4.7(16)
	T_2	2.4±0.4	3.3(2.4-4.1)	15.6(11.3-28.1)	29.5(18.6-71.7)	8.6(16)
	T_3	2.3±0.4	4.2(3.2-5.2)	21.4(14.7-44.0)	41.9(24.6-118.8)	10.4(16)
	T_4	2.2±0.4	3.2(2.5-4.0)	16.9(11.9-33.4)	33.7(20.1-94.5)	8.5(16)
Pupa	T_1	2.8±0.3	5.2(4.1-6.3)	20.3(15.3-32.2)	35.5(24.1-69.2)	35.6(25)
	T_2	2.3±0.3	5.7(4.5-6.9)	28.9(20.6-51.3)	56.4(35.19-129.9)	28.8(25)
	T_3	2.4±0.3	7.3(6.1-8.5)	33.7(24.7-55.2)	63.5(41.5-127.2)	24.5(25)
	T_4	2.3±0.3	4.8(3.8-5.7)	24.3(18.3-38.3)	47.5(31.6-92.8)	13.3(25)
Adult	T_1	1.8±0.4	2.0(0.9-2.9)	15.8(10.4-40.6)	36.6(19.3-174.5)	6.7(16)
	T_2	2.1±0.4	2.8(1.7-3.6)	16.9(11.6-36.6)	35.6(20.3-118.5)	6.3(16)
	T_3	2.0±0.3	3.3(2.2-4.3)	21.8(14.2-53.5)	47.2(25.2-183.1)	8.6(16)
	T_4	1.6±0.4	1.8(0.6-2.7)	17.5(10.9-59.1)	44.7(21.2-347.9)	8.5(16)

Table 4. Mortality of *T castaneum* larva, pupa and adult treated with CO_2 + PH_3 (*i.e.* T_1-(98% CO_2 + 3 ppm PH_3); T_2-(98% CO_2 + 2 ppm PH_3); T_3-(98% CO_2 + 1 ppm PH_3); T_4-(3 ppm PH_3)) at 14 % moisture content at 95% of confidential limit

Stage		Slope±SE	LT_{50} (h)	LT_{95} (h)	LT_{99} (h)	X^2 (df)
Larva	T_1	2.6±0.4	2.3(1.5-2.9)	9.5(7.3-15.2)	17.1(11.6-36.0)	5.2(16)
	T_2	2.2±0.4	2.5(1.5-3.3)	13.4(9.6-25.2)	26.7(16.4-73.3)	6.4 (16)
	T_3	2.2±0.4	3.3(2.3-4.1)	17.8(12.4-36.3)	35.8(21.1-104.5)	10.6(16)
	T_4	2.0±0.4	2.4(1.3-3.2)	15.1(10.4-32.8)	32.3(18.4-111.9)	5.7(16)
Pupa	T_1	2.6±0.3	4.7(3.8-5.4)	20.0(15.3-30.4)	36.4(25.0-67.0)	21.7(22)
	T_2	2.2±0.3	5.1(4.1-6.2)	27.4(19.5-48.5)	54.7(33.9-125.4)	20.0(22)
	T_3	2.2±0.3	6.5(5.2-8.0)	35.1(23.2-76.0)	70.3(39.7-210.0)	25.1(22)
	T_4	2.2±0.3	4.4(3.3-5.4)	24.0(17.3-41.4)	48.3(30.3-108.7)	8.2(22)
Adult	T_1	2.0±0.4	1.5(0.6-2.3)	10.0(7.1-20.8)	21.7(12.7-80.6)	7.2(16)
	T_2	2.2±0.4	2.0(1.1-2.8)	11.3(8.2-21.3)	22.8(14.0-65.6)	8.6(16)
	T_3	2.0±0.4	2.6(1.5-3.4)	16.7(11.3-37.8)	36.1(20.1-130.5)	7.6(16)
	T_4	1.7±0.4	1.4(0.3-2.3)	12.1(7.9-41.0)	29.4(14.6-261.8)	7.9(16)

Table 5. Mortality of *T castaneum* larva, pupa and adult treated with CO_2 + PH_3 (*i.e.* T_1-(98% CO_2 + 3 ppm PH_3); T_2-(98% CO_2 + 2 ppm PH_3); T_3-(98% CO_2 + 1 ppm PH_3); T_4-(3 ppm PH_3)) at 17 % moisture content at 95% of confidential limit

Stage		Slope±SE	LT_{50} (h)	LT_{95} (h)	LT_{99} (h)	X^2 (df)
Larva	**T_1**	2.2±0.5	1.3(0.3-2.0)	6.9(2.0-5.0)	13.8(8.3-58.1)	6.4(13)
	T_2	2.0±0.5	1.3(0.4-2.1)	8.2(5.8-19.3)	17.5(10.1-84.9)	3.3(13)
	T_3	1.9±0.5	1.6(0.6-2.5)	11.3(7.6-31.8)	25.0(13.3-147.4)	6.0(13)
	T_4	1.5±0.4	1.1(0.09-2.1)	14.3(8.2-131.3)	40.3(16.2-2368.4)	8.0(13)
Pupa	**T_1**	2.4±0.3	3.8(2.7-4.8)	18.2(13.1-33.0)	34.6(21.7-84.7)	29.4(22)
	T_2	2.0±0.3	3.7(2.4-4.8)	24.6(16.4-54.3)	53.6(29.8-178.6)	25.5(22)
	T_3	2.3±0.3	5.7(4.7-6.8)	29.3(20.9-51.8)	57.4(35.8-129.7)	20.4(22)
	T_4	2.1±0.3	4.0(2.9-4.9)	23.3(16.7-41.2)	48.2(29.8-113.3)	10.5(22)
Adult	**T_1**	2.2±0.5	1.6(0.7-2.3)	8.9(6.4-19.0)	18.0(10.7-67.0)	2.7(13)
	T_2	2.2±0.5	2.0(1.0-2.7)	10.4(7.4-22.2)	20.7(12.3-71.9)	3.6(13)
	T_3	1.9±0.4	2.4(1.2-3.3)	16.4(10.4-50.8)	36.2(18.3-213.5)	7.6(13)
	T_4	2.1±0.4	1.9(0.9-2.7)	11.7(7.9-29.2)	24.6(13.6-112.3)	6.2(13)

4. Conclusion

From the above work it can be concluded that the use of combination of PH_3 and CO_2 is more effective than PH_3 fumigation alone. The mixture of CO_2 and PH_3 increased the PH_3 concentration and generation rate during fumigation. It has been observed that an application of 98% CO_2 with 3 ppm PH_3 and 2 ppm significantly ($p < 0.01$) improve the fumigation process by increasing PH_3 concentration and generation rate. Fumigating paddy with 98% CO_2 + 1 ppm PH_3 showed reduced effectiveness as compared to control treatment. 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 (Alice et al., 2015). Addition of CO_2 to PH_3 enhanced the toxicity of PH_3 and significantly reduces the LT_{50} and LT_{99} values. The average PH_3 concentration required for the mortality of *Tribolium castaneum* (adult, pupa and larva) at different grain paddy moisture content was determined. However, egg mortality has to be studied for the above mentioned treatments to prevent reinfestation of insects during storage. PH_3 residues in all the treatments including control were recorded below the recommended level (0.1 ppm). Hence it is considered as safe for consumption in all the treatments including control.

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