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Study on modified atmosphere with effect of elevated levels of CO_2 concentrations and exposure periods on adult mortality of cigarette beetle (*Lasioderma serricorne* Fabricius) in turmeric (*Curcuma longa* Linnaeus)

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Abstract
An experiment was conducted to find out the effect of CO_2 concentrations on *Lasioderma serricorne*, by transferring twenty freshly released adults to airtight plastic containers of 250 grams capacity separately and directly exposed to eight different concentrations of CO_2 *viz.*, 10, 20, 30, 40, 50, 60, 70 and 80 per cent with different exposure periods of 1, 5, 10, 15, 20, 25, 30, 35, 40 and 45 minutes. After exposure to various CO_2 concentrations, the adults were placed in plastic jars with disinfested healthy cured turmeric rhizomes. The mortality was observed daily and per cent adult mortality was calculated. The results revealed that both concentrations as well as exposure periods had significant effect on the adult mortality. The findings confirmed that exposure of *L. serricorne* adults to 70 and 80 per cent CO_2 for 45 min were considered as the best treatments for control of adult cigarette beetles which resulted in adult mortality at one and four days after treatment, respectively and these treatments can be recommended for effective management of the cigarette beetle.

Keywords: Turmeric, cigarette beetle, CO_2 and modified atmosphere

1. Introduction

Turmeric is a rhizomatous herbaceous perennial plant belonging to the ginger family (Zingiberaceaae), botanically known as *Curcuma longa* Linnaeus, originated from Tropical south Asia (India). Various insects have been recorded on dry turmeric, which belong to the order coleoptera, include cigarette beetle (*Lasioderma serricorne* Fab.), drugstore beetle (*Stegobium paniceum* L.), red flour beetle (*Tribolium castaneum* Herbst), lesser grain borer (*Rhyzopertha dominica* Fab.), saw toothed grain beetle (*Oryzaephilus surinamensis* L.) and coffee bean weevil (*Araecerus fasciculatus* DeG.). Among all these insects, the cigarette beetle is serious. The damage loss by cigarette beetle in turmeric in terms of quantitative weight loss at three and six months after storage was recorded as 7.15 and 22.75% (Vidya and Awaknavar, 2004) [23]. Very little work has been done on the study of modified atmosphere with effect of elevated levels of CO2 concentrations and exposure periods on adult mortality of cigarette beetle (Lasioderma serricorne Fabricius) in turmeric (Curcuma longa Linnaeus). In view of serious losses in storing the turmeric from the infestation a search for the management practice is required. Use of modified atmosphere through the introduction of carbon dioxide (CO_2) has been considered as one of the safest methods to control storage pests. Hence, the present investigation were being conducted to know the use of modified atmosphere with elevated levels of CO_2 against managing the *L. serricorne*

2. Materials and Methods

2.1 Effect of elevated levels of CO_2 on adult mortality of *L. Serricorne*

Modified atmosphere studies using elevated levels of CO_2 was carried out against *L. serricorne* at Agriculture Research Station, Anantapur, Andhra Pradesh in specially designed airtight containers and the required concentration of CO_2 was released into the airtight containers with the help of carbon dioxide cylinder (Model no. BRG 0/1) fitted with an outlet tube containing a nozzle and a needle.

The required concentration of CO_2 to be released into the container was regulated with the help of a pressure nob fitted at the top of the CO_2 cylinder. The required concentration of CO_2 was released into the container with a pressure of 2 kg cm^{-2} from CO_2 cylinder.

2.2 Design of airtight container

Adult mortality studies were done in airtight plastic containers of 250 g capacity consisting of two perforations of 3 mm diameter which serve as an inlet and outlet holes and they were fitted with nylon tubes of 3 mm diameter. Rubber corks of 2.95 mm diameter which exactly seal the inlet and outlet tubes were used to plug them after filling the containers with desired concentrations of CO_2 and thus the entire system was made airtight after releasing the CO_2 into the containers.

2.3 Injection of CO_2 into airtight containers

Before introducing the CO_2 into the airtight container, the air present in the airtight container was flushed out by opening the outlet present at the top of the container and then it was closed with rubber cork and then the desired concentration of CO_2 was introduced into the airtight container through the inlet located at bottom of the container by injecting the needle of CO_2 cylinder. After injecting the gas, the inlet and outlet tubes were closed at one stroke using rubber corks to prevent escape of CO_2 from the container.

2.4 Determination of CO_2 concentration in containers after injection

To ensure whether the desired concentration of CO_2 released in the plastic containers was maintained or not was checked by using CO_2/O_2 analyzer (PBI Dansensor, PBI 200616, Denmark) which measures the CO_2 concentration in the containers. For determination of CO_2, the analyzer was calibrated with atmospheric air (20.9 per cent O_2 and 0.03 per cent CO_2) and later the needle of the analyzer was introduced into the top outlet tube of the air tight container and the measuring button of the CO_2/ O_2 analyzer was pressed. The concentrations of CO_2 and O_2 present in the air tight container was displayed on screen within 10 seconds which helps in determining the concentration of CO_2 present in the containers.

2.5 Data Collection

To find out the effect of CO_2 concentrations on *L. serricorne*, twenty freshly released adults were transferred to airtight plastic containers of 250 grams capacity separately and directly exposed to eight different concentrations of CO_2 *viz.*, 10, 20, 30, 40, 50, 60, 70 and 80 per cent with different exposure periods of 1, 5, 10, 15, 20, 25, 30, 35, 40 and 45 minutes. After exposure to various CO_2 concentrations, the adults were placed in plastic jars with disinfested healthy cured turmeric rhizomes. The mortality was observed daily and per cent adult mortality was calculated by using the following formula. The data were subjected to square root and angular transformation values wherever necessary and analyzed by adopting completely randomized design (CRD).

$$\text{Per cent adult mortality} = \frac{\text{Number of adults dead}}{\text{Total number of adults released}} \times 100$$

3. Results and Discussion

3.1 Effect of elevated levels of CO_2 on adult mortality

The results obtained from the studies on adult mortality of *L. serricorne* exposed to different concentrations of CO_2 and different exposure periods are presented in Table 1. The adult mortality of *L. serricorne* exposed to different concentrations of CO_2 after 1 minute of exposure period indicated that the mean adult mortality of the cigarette beetle observed in different concentrations of CO_2 varied from 2.14 to 41.42 per cent with 10 per cent CO_2 concentration recorded as least effective and 80 per cent CO_2 concentration recorded as highly effective in increasing the adult mortality of the test insect which was significantly different from each other treatments. The results pertaining to adult mortality of *L. serricorne* exposed to elevated levels of CO_2 after 5 minutes of exposure period indicated that the mean per cent adult mortality was significantly high in 80 per cent CO_2 (47.38) followed by 70 per cent (43.80) and 60 per cent (40.23).

The results pertaining to adult mortality of *L. serricorne* exposed to elevated levels of CO_2 after 5 minutes of exposure period indicated that the mean per cent adult mortality was significantly high in 80 per cent CO_2 (47.38) followed by 70 per cent (43.80) and 60 per cent (40.23).

Table 1: Effect of elevated levels of CO_2 concentrations and exposure periods on mean adult mortality of *L. serricorne*

CO_2 Concentration (%)	Per cent adult mortality										
	Days after treatment (DAT)										
	1 min	5 min	10 min	15 min	20 min	25 min	30 min	35 min	40 min	45 min	Mean
10	2.14 (8.41)	3.80 (11.24)	5.95 (14.11)	8.09 (16.52)	12.14 (20.38)	16.19 (23.71)	20.95 (27.23)	25.47 (30.30)	32.14 (34.52)	42.38 (40.60)	16.92 (22.71)
20	6.42 (14.68)	8.57 (17.00)	10.95 (19.31)	14.76 (22.56)	19.52 (26.21)	24.52 (29.66)	31.90 (34.37)	41.19 (39.90)	46.19 (42.79)	56.19 (48.53)	26.02 (29.51)
30	13.33 (21.40)	19.52 (26.21)	26.90 (31.23)	33.80 (35.53)	38.57 (38.37)	43.09 (41.01)	46.67 (43.07)	51.42 (45.80)	55.71 (48.26)	66.42 (54.57)	39.54 (38.56)
40	16.67 (24.07)	24.76 (29.83)	30.71 (33.64)	41.19 (39.91)	46.19 (42.79)	52.85 (46.61)	57.85 (49.50)	61.90 (51.86)	65.71 (54.13)	71.42 (57.67)	46.92 (43.02)
50	23.09 (28.71)	35.23 (36.39)	40.47 (39.49)	46.42 (42.93)	52.14 (46.21)	65.71 (54.14)	72.38 (58.27)	78.80 (62.56)	82.61 (65.33)	89.76 (71.31)	58.66 (50.55)
60	28.80 (32.44)	40.23 (39.35)	46.47 (43.07)	55.00 (47.85)	63.33 (52.71)	71.42 (57.66)	77.61 (61.74)	84.76 (66.99)	90.71 (72.25)	93.57 (75.29)	65.21 (54.96)
70	34.04 (35.68)	43.80 (41.42)	51.19 (45.66)	58.57 (49.91)	70.47 (57.06)	80.47 (63.75)	85.23 (67.38)	89.04 (70.65)	93.33 (75.01)	96.90 (79.93)	70.31 (58.61)
80	41.42 (40.04)	47.38 (43.48)	57.85 (49.50)	66.90 (54.86)	76.90 (61.25)	86.90 (68.76)	91.19 (72.71)	95.47 (77.69)	98.09 (82.30)	99.52 (86.74)	76.16 (63.62)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)

Mean	18.43 (23.28)	24.81 (27..61)	30.07 (31.13)	36.08 (34.92)	42.14 (38.80)	49.02 (43.28)	53.75 (46.50)	58.76 (50.00)	62.72 (53.20)	68.46 (57.50)	
	CD (P=0.05)					SE(m)					
Concentration (F_1)	0.54					0.18					
Exposure period (F_2)	0.46					0.15					
Interaction (F_1 X F_2)	1.58					0.52					
CV (%)	2.76										

Figures in parentheses are angular transformed values

Exposure of *L. serricorne* adults to different concentrations of CO_2 up to 10 minutes of exposure period indicated that the highest CO_2 concentration of 80 per cent showed 57.85 per cent of mean adult mortality and found significantly superior to other concentrations of CO_2.

The *L. serricorne* adults subjected to exposure period of 15 minutes to different concentrations of CO_2 followed the same trend similar to that of 10 minutes exposure period with the mean adult mortality at higher concentrations of CO_2 *viz.*, 70 and 80 per cent showed 58.57 and 66.90 per cent and differed significantly from each other.

The results on adult mortality of *L. serricorne* exposed to elevated levels of CO_2 after 20 minutes of exposure period revealed that the mean per cent adult mortality was highest (76.90) in 80 per cent CO_2 treatment which was significantly superior to all other concentrations of CO_2.

The *L. serricorne* adults when exposed for 25 minutes to different concentrations of CO_2 showed that the mean per cent of adult mortality was highest (86.90) in 80 per cent CO_2 treatment which was significantly superior to all other concentrations of CO_2.

The adult mortality of *L. serricorne* exposed to different concentrations of CO_2 after 30 min of exposure period indicated that among all the concentrations, 80 per cent concentration was proved to be significantly superior over other treatments with the mean per cent adult mortality was highest (91.19) in 80 per cent CO_2 concentration and differed significantly from other treatments.

The results on adult mortality of *L. serricorne* exposed to elevated levels of CO_2 after 35 minutes of exposure period showed that among all the concentrations the highest concentration of 80 per cent CO_2 recorded more mean mortalityfollowed by the other higher concentration of CO_2 *i.e*., 70 per cent with the mean per cent of adult mortality was found to be significantly the highest at 80 per cent CO_2 concentration (95.47) followed by 70 per cent (89.04) and these two treatments were significantly different from each other.

The *L. serricorne* adults subjected to longer exposure periods of 40 minutes showed that the mean per cent mortality of adults obtained with 80 per cent CO_2 concentration (98.09) was significantly superior over other treatments.

The adult cigarette beetles exposed to longer exposure periods of 45 minutes significantly caused adult mortality at different concentrations of CO_2 showed that the mean per cent adult mortality observed at 10 (42.38), 20 (56.19), 30 (66.42) and 40 (71.42) per cent CO_2 concentrations differed significantly from each other. The next higher concentrations of 50 and 60 per cent CO_2 recorded 73.33 and 83.33 per cent of adult mortality at one day after treatment and cent per cent mortality after seven and six days of treatment, respectively. At 70 per cent CO_2 concentration, 88.33 per cent adult mortality was observed at one day after treatment and it increased to 100 per cent of adult mortality by fourth day. The higher concentration of 80 per cent CO_2 was significantly superior over all the other treatments by recording cent per cent adult mortality at two days after treatment. The mean per cent adult mortality (99.52) was highest in 80 per cent CO_2 concentration and was significantly superior over other treatments.

The overall mean adult mortality studies obtained with different concentrations and different exposure periods of CO_2 stated that among the different concentrations, significantly the lowest mean adult mortality was obtained with 10, 20, 30 and 40 per cent CO_2 concentrations with 16.92, 26.02, 39.54 and 46.92 per cent of adult mortality, respectively which were significantly different from each other. Exposure of adults to the higher concentrations *viz.*, 50, 60 and 70 per cent showed significant variation by recording the adult mortality ranging from 58.66 to 70.31 per cent. Among all the treatments, the highest mean per cent adult mortality of 76.16 was obtained with 80 per cent concentration and significantly superior over all other treatments.

The effect of various exposure periods also showed significant variation in adult mortality. Exposure of adults to lowest period of 1, 5, 10 and 15 minutes recorded the lowest per cent adult mortality ranging from 18.43 to 36.08. Increase in exposure periods resulted in increased mortality rates. The mean mortality of high exposure periods of 20, 25 and 30 minutes ranged from 42.14 to 53.75 per cent. Significantly the highest adult mortality of 68.46 per cent was recorded with longest exposure periods of 45 min followed by 62.72 and 58.76 per cent obtained with 40 min and 35 min exposure periods, respectively.

The interaction effect of concentrations and exposure periods also showed significant variations in adult mortality. Among all the interactions, exposure of adult insects to highest 80 per cent concentration for 45 minutes resulted in 99.52 per cent of adult mortality and exposure to next higher concentrations of 50, 60 and 70 per cent at high exposure period of 45 minutes resulted in significant mortality ranging from 89.76 to 96.90 per cent, while exposure to low concentrations (10, 20, 30 and 40 per cent) and low exposure period (1minute) resulted in significantly the lowest adult mortality (2.14, 6.42, 13.33 and 16.67 per cent, respectively).

The overall findings obtained from adult mortality studies of *L. serricorne* when exposed to various concentrations and exposure periods of CO_2 (Table 1) indicated that the concentrations of CO_2 as well as exposure periods had significant influence on adult mortality and increasing the exposure period from 1 minute to 45 minutes drastically reduced the time required to cause the mortality of adults. Hence it was inferred from the data that exposure of *L. serricorne* adults to 80 and 70 per cent CO_2 concentrations for 45 minutes exposure period which resulted in almost complete mortality of adults at one and four days after treatment, respectively. The results were in agreement with Toshihiro Imai (2014) [20] and Toshihiro Imai and Naoto (2012) [21] who reported that controlled atmosphere with reduced oxygen levels and higher CO_2 concentration was effective measure for controlling the cigarette beetle, *Lasioderma serricorne*. Adler *et al*. (2000) [1] reported that the different developmental stages of *L. serricorne* when exposed for high CO_2 concentration at higher exposure resulted in 100 per cent mortality of all insects. Ulrichs *et al*. (1997) [22] reported that high carbon dioxide concentration at 38.5 minutes of exposure period resulted in 95 per cent mortality of *L. serricorne*. Studies revealed that a reduction in O_2 content to 3 per cent or lower, or an increase in CO_2 content to 60 per cent or above were effective for the control of most of the storage pests (Navarro, 2006) [18]. Similar findings reported by Jyothsna (2014) [13], Krishnaveni (2012) [15] where exposure of stored pests like *Carrydon serratus* and *Callosobruchus chinensis* to 80 per cent CO_2 concentration at high exposure period resulted in complete mortality of the insects. Yadav and Mahla (2002) [24] reported that increase in the CO_2 concentrations with increase in the exposure periods increased mortality of *T. granarium* and achieved hundred per cent mortality of this pest at 70 per cent and above concentration of CO_2 at higher exposure periods. Caril *et al*. (2010) [6] reported that *S. zeamais* adults when exposed to different CO_2 concentrations of 20, 40, 60 and 80 per cent CO_2, the mortality increased with increase in concentration and exposure time and high mortality observed in case of 80 per cent CO_2 concentration.

Bera *et al*. (2004) [4] stated that modified atmosphere system involving CO_2 concentration ranging from 20 to 80 per cent in paddy effectively controlled rice weevil and lesser grain borer. Krishnamurthy *et al*. (1993) [14] used 80 per cent of CO_2 concentration to get 100 per cent mortality of *T. castaneum* and *S. oryzae* adults while Ofuya and Reichmuth (1993) [19] concluded that the mortality of *C. maculates* was significantly influenced by CO_2 concentration and exposure period. Zhou *et al.* (2000) [25] found that elevated levels of CO_2 reduced the O_2 consumption of *Platynota staltana*. They found that O_2 consumption rate was decreased by 62 per cent at 20 per cent CO_2 and by 73 per cent at 79 per cent CO_2. Empirical mortality data showed that levels of CO_2 toxicity to insects were generally above 20 per cent (Banks and Annis, 1990, [2] Carpenter and Potter, 1994, Mitcham *et al*., 1997 [17], Zhou *et al.*, 2001). Carbon dioxide can initially have a narcotic effect leading to knock down (Edwards and Batten, 1973) [8]. Most insects were more easily killed with higher CO_2 concentrations (Jay, 1984) [12].

The high mortality of *L. serricrone* adults obtained with high CO_2 concentrations and prolonged exposure periods could be attributed to the following effects. Mechanism of carbon dioxide toxicity to arthropods can be better understood if we were familiar with the terms such as Hypoxia, Anoxia and Hypercarbia. Mitcham *et al*. (2006) [17] in their review article stated that the term hypoxia loosely applies to an environment comprising < 21% oxygen. Anoxic atmospheres represent entire absence of O_2, whereas hypercarbic atmosphere comprises very high carbon dioxide concentration. Controlled atmospheres usually involve increasing the concentration of carbon dioxide to obtain hypoxic or hypercarbic atmosphere within a storage structure.

Although insects being a tiny creature requires small amount of oxygen and are capable to tolerate hypoxia yet reduced oxygen concentration has been found fatal (Hoback and Stanley, 2001) [10]. Because growth and development of insects gradually slows down with reduction in O_2 level and ultimately ceases under anoxia. The elevated CO_2 affects the respiration of insects by reducing the oxidative phosphorylation and inhibits the respiratory enzymes such as succinate dehydrogenase (Edwards, 1968) [7] and malic enzyme (Fleurat-Lessard, 1990) [9]. Reduced oxidative phosphorylation leads to reduced ATP generation. Carbon dioxide poisoning inhibits O_2 utilization by specific enzymes, such as succinic dehydrogenase, or causes a weak oxidative metabolism resulting in accumulation of toxic products such as lactate, pyruvate, and succinic acid (Bell, 1984) [3]. Zhou *et al*. (2001) (26) suggested that elevated CO_2 could increase the permeability of membranes. Therefore, the failure of membrane function under hypercarbia could result from both energy insufficiency and increased membrane permeability. It is more likely that the decreased energy supply under metabolic arrest cannot meet the need of maintaining a more permeable membrane due to elevated CO_2. Carbon dioxide has also been shown to increase intercellular Ca^{+2} ion concentration by decreasing the pH (Lea and Ashley, 1978) [16]. According to Hochachka (1986) [11], a high concentration of Ca^{+2} in the cytosol can cause the cell and mitochondrial membranes to become more permeable leading to cell damage or death. Very important effect of raised concentrations of CO_2 is prolonged opening of the spiracles, which leads to desiccation and mortality (Bursell, 1974) [5].

4. Conclusion

The present findings confirmed that exposure of *L. serricorne* adults to 70 and 80 per cent CO_2 for 45 min were considered as the best treatments for control of adult cigarette beetles and these treatments can be recommended for effective management of the cigarette beetle and this treatment was approved by US Food and Drug administration as a fumigant for safe application in food grains against stored grain pests

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