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Effect of Controlled Atmosphere Storage on Adults and Immature Stages of *Rhizopertha dominica* (Fab.)

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ABSTRACT

Effect of controlled atmosphere parameters like carbon dioxide, oxygen concentration and period of storage were studied with 40, 50 and 60% concentrations of carbon dioxide; 16, 12, 8, and 4% oxygen, and the remaining as nitrogen. A setup was developed for production of gas mixtures for controlled atmosphere storage studies. The gases were metered separately, and mixed together in a gas mixing chamber generating the desired gas composition. The containers were purged with gas mixture after introducing counted number of adults or immature stages *Rhizopertha dominica* (Fab.), and were kept at 30^{o}C in a temperature controlled chamber. Complete mortality of adults of was obtained in 5 days with 40% carbon dioxide at 16% oxygen level. With the reduced oxygen level of 8%, cent percent mortality of adults was achieved in 3 days. Complete mortality of eggs and early instar larvae were obtained with 40% carbon dioxide concentration in 9 days. Late instar larvae and pupae required 50% carbon dioxide concentration with 9 and 12 day treatment, respectively, for complete control. The control of all the life stages of *R.dominica* required a treatment with 50% carbon dioxide concentration for 12 days.

Keywords: *Controlled atmosphere storage, insect control, grain storage, insect mortality*

India has one of the biggest central procurement, warehousing and food grain distribution networks in the world. The quality and quantity of food grains is deteriorated during storage due to insect, pests, birds and rodents, as also by fungi. The post harvest losses in cereals comprising of on-farm losses, transportation and storage losses were estimated to be 3.9 to 6% (Anon, 2011).

In India, fumigation is mainly done using phosphine, which replaced liquid fumigants such as ethylene dichloride-carbon tetrachloride mixture and ethylene dibromide (Rajendran and Narasimhan, 1994). However, fumigation process using chemicals is restricted due to contamination of environment, health hazards to public and work force. The cancellation of registration of fumigants due to their carcinogenic effect, toxic effect, environmental hazards and difficulty in maintaining very low residue levels after treatments led to more studies and increased the usage of environment friendly non-chemical methods of preservation of grains like Controlled Atmosphere Storage (CAS).Among the stored products, insect pests that attack paddy, the lesser grain borer, *Rhyzopertha dominica* (Fabricius), the primary borer pose a major threat to farmers.

MATERIALS AND METHODS

Storage Container

A gas tight container with an inlet, outlet and a gas sampling port was fabricated (Fig. 1). It consisted of a round-necked glass jar with 300 ml capacity resting on a 2 mm thick mild steel plate, on which a neoprene rubber cushion was fixed. The container was covered with another mild steel plate with neoprene rubber gasket affixed to it for air tightness. Both the plates had small holes at corners, through which 150 mm long bolts were inserted and tightened to hold the glass jar between the cover plates. Three holes were made at centre of the top cover plate. Brass nipples with rubber gaskets were connected in two holes and tightened with nuts. These were used as inlet and outlet for the purged gases. Two extra soft rubber tubes were connected to these nipples. The container was closed after purging of gases by using pinch clips. To the third hole, silicon septum was fitted using brass fittings. Gas samples could be drawn through this silicon septum using a syringe for analysis. A mica sheet partition was provided in the glass storage container with 10 mm gap at the bottom. This divided the container into two sections with inlet on one side and the outlet and the sampling port on the other side, making the purging more effective with complete flushing of air from

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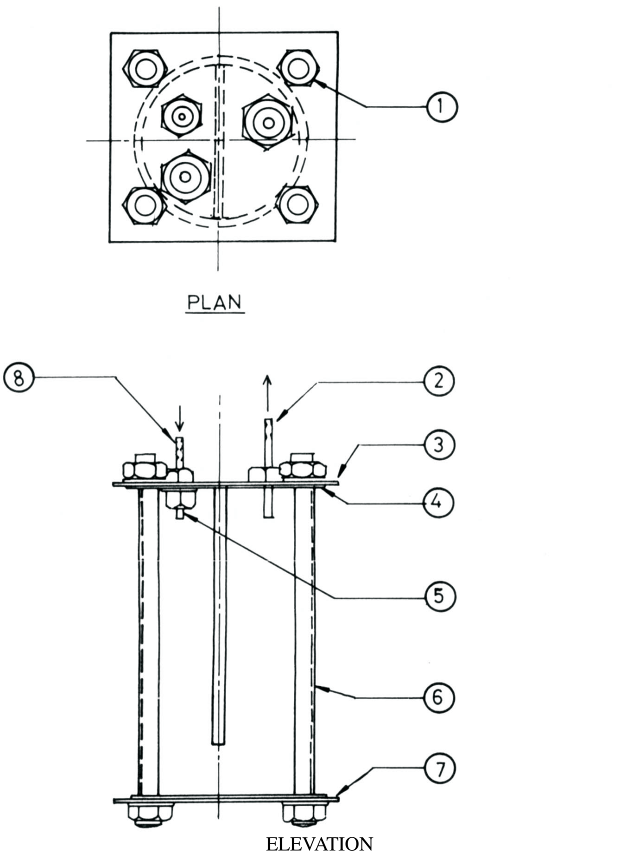

1. HALF THREADED, 6 mm ϕ BOLT AND NUG
2. HOSE NIPPLE, 6 mm ϕ (GAS INLET)
3. M.S. COVER PLATE, (88 × 88 × 1.45 mm)
4. NEOPRENE RUBBER WASHER, 7.2 mm ϕ, 1.5 mm thickness
5. SILICON RUBBER SEPTUM PORT
6. GLASS CONTAINER, 62 mm ϕ, 118 mm height
7. BASE PLATE (88 × 88 × 1.45 mm)
8. HOSE NIPPLE, 6 mm ϕ (GAS OUTLET)

Fig. 1: Schematic diagram of sample container

the container with gas flowing from the top of the inlet side to the bottom and through the gap at the bottom to the outlet on the other side. It also facilitated complete purging of intergranular space between the grains in the container. Hundred numbers of such containers were fabricated and used in the study.

Gas Mixture

Carbon dioxide, nitrogen and oxygen gases were precisely metered from their cylinders through the respective flow meters, and mixed thoroughly by using a gas-blending device. The gas coming out of the blender was used for purging the storage containers. The setup was developed for producing the desired gas ratios for controlled atmosphere storage study (Fig.2). The containers were closed after purging using pinch clips on the inlet and outlet tubes. For the experiment, different compositions of gas mixtures with carbon dioxide at 40, 50 and 60% and oxygen at 16, 12, 8 and 4% concentrations were used, the rest being nitrogen. Thoroughly mixed gas coming out of the gas blender was passed through the inlet tube of the container till the flow of gas was felt through the outlet.

Raw Material

Good quality paddy (cv. CO-47) was obtained immediately after harvest from the Central farm of Tamil Nadu Agricultural University, Coimbatore. It was cleaned thoroughly, and placed in a deep freezer maintained at -18°C in batches of 10 kg sealed in polyethylene bags for 10 days to destroy any prior infestation. The moisture content of paddy used for the study was 10% (dry basis).

A container was filled with 0.1 kg of paddy, and counted numbers of *R.dominica* adults or immature stages were introduced. To keep the gas concentration constant

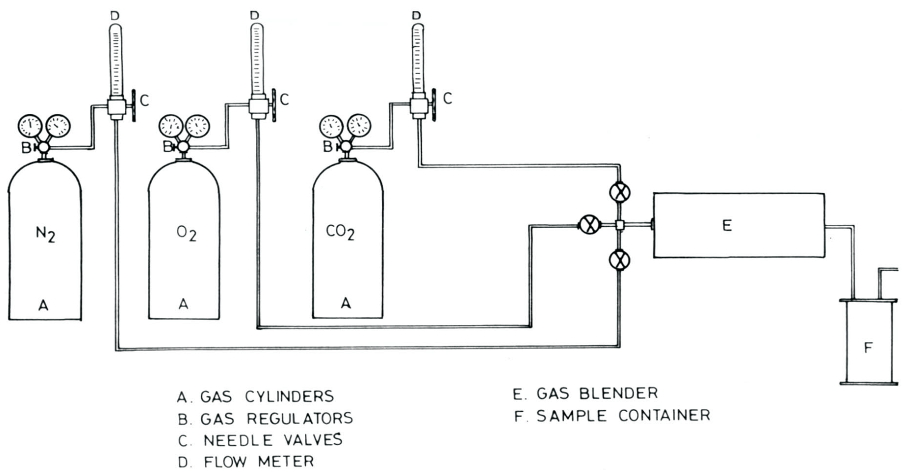

Fig. 2: Gas mixing and purging system for controlled atmosphere storage of paddy

throughout the study, the containers were purged every alternate day. The closed containers after purging were kept at constant temperature of (30±1°C) in a BOD incubator fitted with an electronic controlled thermostat for accurate temperature control. The purged samples were observed for 1, 2, 3, 5, 7, 9, 12, 15 and 18 days depending on the life stages of insects introduced in the sample.

Collection and Multiplication of *R.dominica*

Adults of *R.dominica* were collected from infected stored grains, and were multiplied in paddy grains in laboratory. Immature stages of *R. dominica* were obtained by collecting adult insects of *R.dominica,* and allowed to oviposition for egg laying in uniformly spread paddy in trays for two days. Paddy that contained insect eggs was then mixed thoroughly and divided into four equal portions for eggs, early instar larvae, late instar larvae and pupae obtained by keeping for respective development periods. The eggs were allowed to develop into various life stages by keeping for the required period, and then the respective life stages were used for the study. For *R. dominica*, the developmental periods for various life stages are five to six, seventeen and seven to eight days for egg, larva and pupa respectively (Hill, 1990).

Assessment of Mortality

Mortality of insect pest was calculated by taking the ratio of live insects after treatment to the total number of insects introduced. Live insects were counted and removed after opening the lid. Observation was continued for three more days to ensure mortality as the insects might be at a stage of anoxia produced by high carbon dioxide concentration. In case of life stages of insects, number of adults emerged was recorded after allowing time for adult emergence. The percentage reduction in adult emergence was calculated as follows.

$$R = [1- (E_t / E_c)] *100 \quad \dots (1)$$

Where,

R = Reduction in adult insect pest emergence, percent,

E_t = Average number of adults emerged in treatment, and

E_c = Average number of adults emerged in control.

Percentage mortalities obtained were analysed using factorial Completely Randomized Block Design (CRD) and the means were compared by Duncans Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Mortality of Adults of *Rhizopertha dominica*

Among the carbon dioxide levels tested, 60% carbon dioxide level gave a mean adult mortality of 96.1%, and was on par with 50% carbon dioxide level with 95.6% mean adult mortality for different periods of storage tested. Use of 40% carbon dioxide level gave a significantly lesser mean adult mortality of 78.4 per cent. Among the oxygen levels tested, 4 and 8% levels were found to be at par with 93.8 and 91.7% mean adult mortalities with different carbon dioxide concentrations. The oxygen level of 12% gave a mean adult mortality of 90.2%, which was significantly lesser than 4% oxygen level. Use of 16% oxygen level gave the least mean adult mortality of 84.6 per cent. Among the periods of storage tested, five-day treatment gave a maximum mean adult mortality of 100% for the carbon dioxide and oxygen levels tested. The three-, two- and one-day treatments gave significantly lower mean adult mortalities of 96.9, 88.1 and 75.2%, respectively.

As seen from Table 1, the interaction between the carbon dioxide and the period of storage was found to be significant at 1% level besides storage days, level of carbon dioxide and oxygen. Maximum mean adult mortality of 100% was obtained for all the three carbon dioxide concentrations

Table 1. Intreactions among variables on mortality of adults of *Rhizopertha dominica*

	Treatment						
Parameter	**D (Storage days)**	**C (CO_2 level)**	**O (O_2 level)**	**D x C**	**C x O**	**D x O**	**D x C x O**
SED	1.774	1.536	1.774	3.072	3.072	3.548	6.145
	(1.663)	(1.440)	(1.662)	(2.880)	(2.880)	(3.325)	(5.760)
	3.567	3.089	3.567	6.178	6.178	7.134	12.356
CD	(3.343)**	(2.895)**	(3.343)**	(5.791)**	(5.791) NS	(6.687) NS	(11.582) NS

Note: Values in parenthesis are arc sine transformed values
C1 = 40% CO_2, C2 = 50% CO_2, C3 = 60^ CO_2;
O1 = 16% O_2, O2 = 12% O_2, O3 = 8%, O4 = 4% O_2
*** Significant at 1 % level, * Significant at 5% level, NS- Not significant*

tested for five-day treatment. With three-day treatment, mean adult mortalities was 93.8, 98.1 and 98.8% for 40, 50 and 60% carbon dioxide levels, respectively, which were at par. The lowest mean adult mortality of 49.4% was obtained for one-day treatment with 40% carbon dioxide. The mean adult mortality was 49.4% for one-day treatment with 40% carbon dioxide, and mortality increased to 87.5% when carbon dioxide level was raised from 40% to 50% level. Similarly, for two-day treatment the mean adult mortality was 70.6%, which increased to 96.9% as carbon dioxide level increased from 40 to 50% for one-day treatment. The reason for the spurt in mortality with 10% increase in carbon dioxide might be due to relative tolerance of the adults of *R.dominica* to concentrations up to 40% carbon dioxide. The tolerance might have been greatly reduced as carbon dioxide concentration further increased resulting in strong interaction between the parameters. The interaction between carbon dioxide and oxygen, oxygen and period of storage, as also between period of storage, oxygen and carbon dioxide were found to be non-significant.

Complete (100%) mortality of adults of *R.dominica* occurred with 5-day treatment and all three carbon dioxide concentrations at all levels of oxygen under test (Fig.3). With three day exposure, 12% oxygen and 50 and 60% carbon dioxide levels as well as 8 and 4% oxygen at the three carbon dioxide levels resulted in 100% mortality of

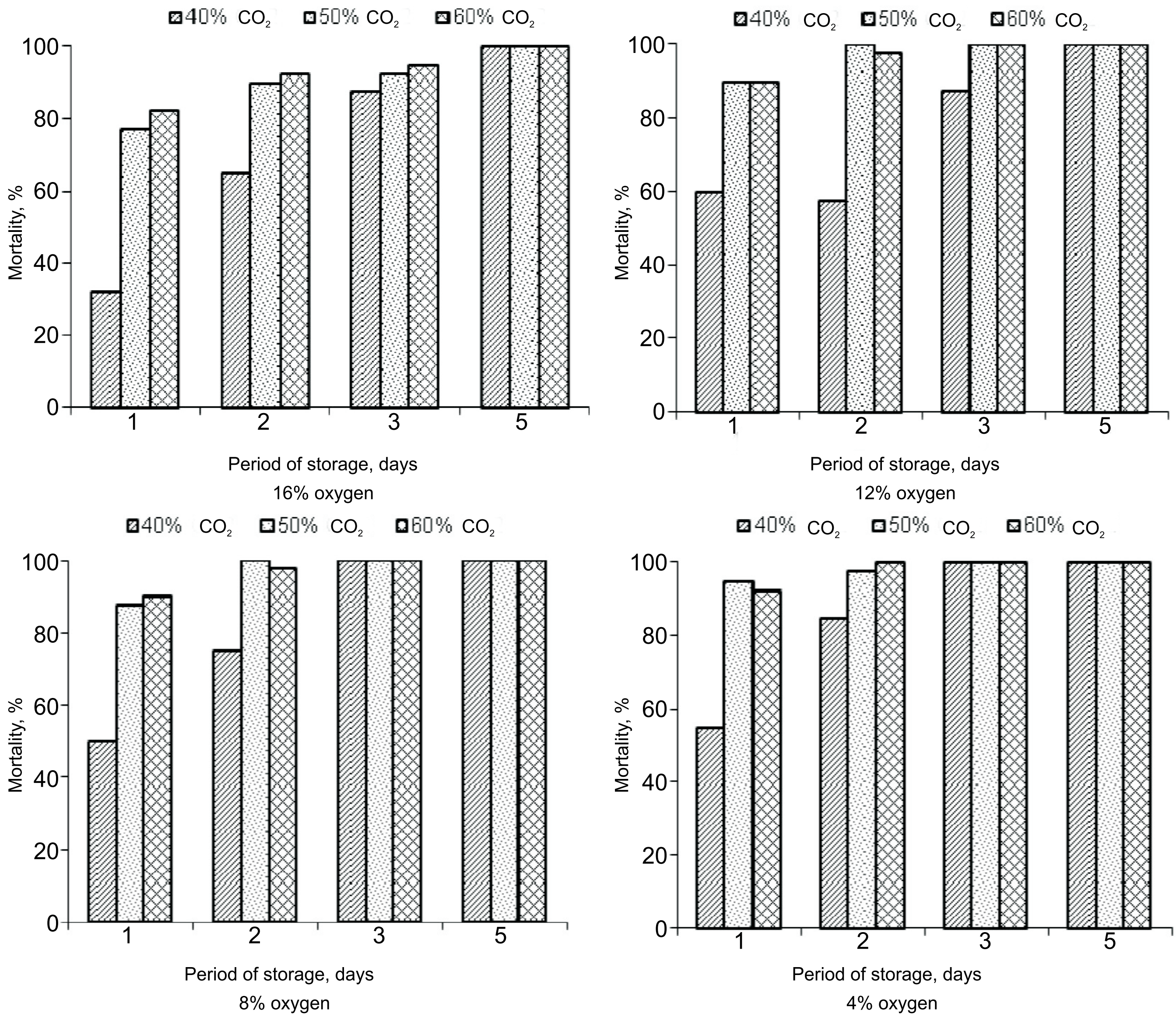

Fig.3: Effect of controlled atmosphere storage on mortality of adults of *Rhizopertha dominica* at indicated levels of oxygen

adults. In order to obtain full control of adults of *R.dominica*, five-day treatment with 40% carbon dioxide and above with oxygen levels up to 16% could be used. A three-day exposure to 40% carbon dioxide and above with 8% oxygen or less also resulted in 100% mortality of adults of *R. dominica*.

of carbon dioxide might have produced the synergistic effect. The trends observed by them with 6 and 8% oxygen levels were same as in the present study. Oxygen might be above the critical value to impart insect mortality by itself and under these conditions, the percentage of carbon dioxide might be the only factor influencing mortality to a greater extent.

Table 2. Mean comparison of treatments on *Rhizopertha dominica* in controlled atmosphere storage of paddy

Stage of insect	Treatment			
	T1	T2	T3	Mean
Emergence from eggs	71.6 (61.2)	78.7 (69.2)	83.1 (72.0)	77.8 (67.5)
Emergence from early instar larvae	81.8 (68.7)	84.9 (72.5)	89.8 (78.6)	85.5 (73.3)
Emergence from late instar larvae	59.1 (50.9)	70.7 (60.9)	75.6 (65.8)	68.4 (59.2)
Emergence from pupae	58.5 (51.0)	71.5 (62.1)	73.3 (63.8)	67.8 (59.0)

Note : Values in parenthesis are arc sine transformed values

T1 : 40% CO_2, 12% O_2, 48% N_2; T2 : 50% CO_2,10% O_2, 40% N_2; T3 : 60% CO_2, 8% O_2, 32% N_2

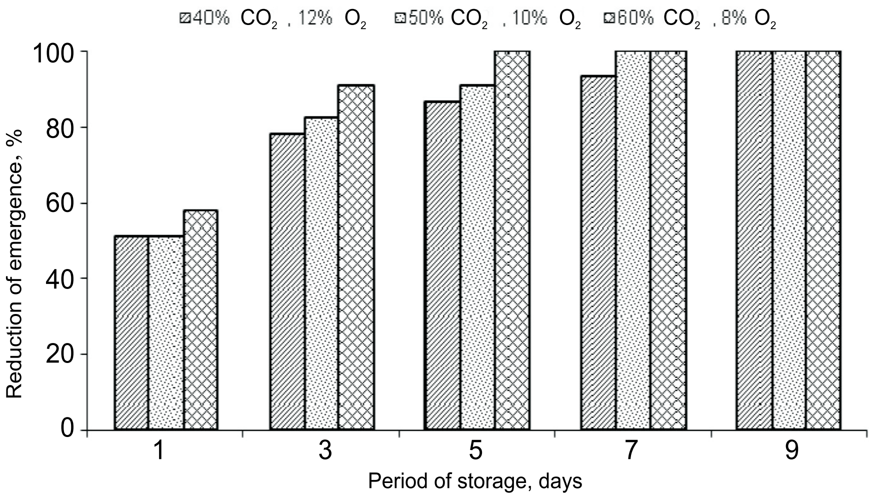

Fig. 4: Effect of controlled atmosphere storage on percentage reduction in adult emergence from eggs of *Rhizopertha dominica*

The results of the present study were in agreement with that of Jay (1971), who reported 95% control of most stored product grain pests after four-day exposure to carbon dioxide concentration of 60 per cent. The results were also in agreement with that of Calderon and Navarro (1980) who reported that 30% carbon dioxide concentration produced 100% mortality of *R. dominica* adults in 96 h. The synergistic effect between carbon dioxide and oxygen as reported by the above authors was not observed in the present study. This might be due to relatively higher levels of oxygen and carbon dioxide used in the present study compared to the lower levels of oxygen (2-8%) and carbon dioxide (0-30%) used by others. The studies referred to above mainly observed the synergistic effect for 2% level of oxygen, which might be lethal to adults, and an addition

Immature Stages of *Rhizopertha dominica*

Effect on eggs

The effect of controlled atmosphere on eggs of *R. dominica* was studied using three concentrations of carbon dioxide, namely 40, 50 and 60% and the rest of gas as in natural air. The treatments were carried out for one, three, five, seven and nine days. Carbon dioxide concentrations of 60% and 50% were found to be at par, and 40% carbon dioxide was significantly lower in effectiveness (Table 2). Among the periods of storage tested, nine-day treatment was most effective with 100% egg mortality at all carbon dioxide concentrations tested (Fig.4), followed by significantly lower effective treatment of seven-day that produced a mean reduction in adult emergence of 96.3% for various carbon dioxide concentrations . One-day treatment was the least effective, which produced a mean reduction in adult emergence of 41.5 per cent. In order to achieve cent percent mortality of eggs, seven-day treatment with 50% carbon dioxide or nine-day treatment with 40% carbon dioxide could be used.

The study on eggs of *R.dominica* was in agreement with the results of Goncalves *et al*. (2000), who reported that carbon dioxide concentrations of 30% and above killed all eggs in ten-days of exposure. Another study by Calderon and Navarro (1980) revealed that only 96% egg mortality could be achieved in 96 h exposure to 30% carbon dioxide concentration. It was also reported that even at oxygen concentrations below 8%, egg mortality did not increase by the addition of 20% carbon dioxide in four days of exposure.

Early instar larvae of *Rhizopertha dominica*

Carbon dioxide concentration of 60% was found to give maximum reduction of 89.8% in adult emergence. Significantly, lower mortalities of 84.9 and 81.8% were obtained at 50 and 40% carbon dioxide levels, respectively (Table 2). Among the periods of storage tested, nine-day treatment was best with cent percent reduction in adult emergence for all three carbon dioxide levels. The seven-day treatment was found to be at par with nine-day treatment. On the other hand, five,- three- and one-day treatments produced significantly lower reduction in adult emergence. A five-day treatment with 60% carbon dioxide also produced cent percent reduction in adult emergence. With 50 and 40% carbon dioxide concentrations, longer periods of storage of seven and nine days, respectively, was required for 100% reduction in adult emergence (Fig.5). In order to control early instar larvae of *R. dominica*, carbon dioxide concentration of 60, 50 or 40% could be used with five-, seven- and nine- day treatments, respectively.

The results were similar to the findings of Goncalves *et al.* (2000), who observed that adequate control of all larval stages required carbon dioxide concentration of 50% and above. The findings of Annis and Morton (1997) and Adler (1994) that early instar larvae were very much susceptible to carbon dioxide when the concentration increased from 40% to 65% also held good with the present study.

Late instar larvae of *Rhizopertha dominic*

Carbon dioxide concentration of 60% was found to produce the maximum mean reduction (75.6%) in adult emergence, followed by significantly lower reduction (70.7%) in emergence at 50% concentration. With carbon dioxide concentration of 40%, least mean reduction in adult emergence of 59.1% occurred for different period of storage (Table 2). A nine-day treatment was best with mean reduction in adult emergence of 95.6% for carbon dioxide concentrations tested, followed by significantly lower mean reduction in emergence of 89.6, 79.3, 49.6 and 28.1% with seven, five, three and one days of storage, respectively. Complete (100%) reduction in adult emergence occurred with 50 and 60% carbon dioxide concentrations for nine day storage period, while with 40% carbon dioxide concentration only 86.7% reduction in emergence took place (Fig.6). With 40% carbon dioxide treatment, one-day storage was least effective with only 20% reduction in adult emergence. No interaction effects were seen between the carbon dioxide concentrations tested and the period of storage. In order to control late instar larvae of *R. dominica*, 50 or 60% carbon dioxide could be used for storage period of nine days.

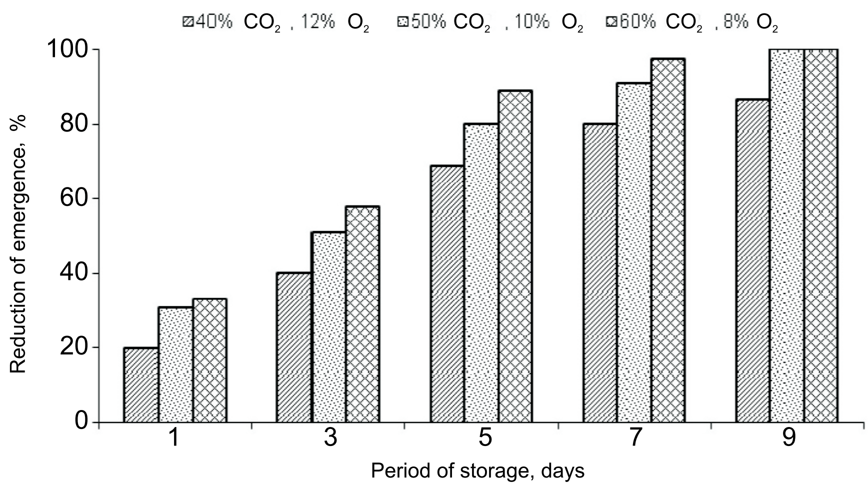

Fig. 5: Effect of controlled atmosphere storage on percentage reduction in adult emergence from early instar larvae of *Rhizopertha dominica*

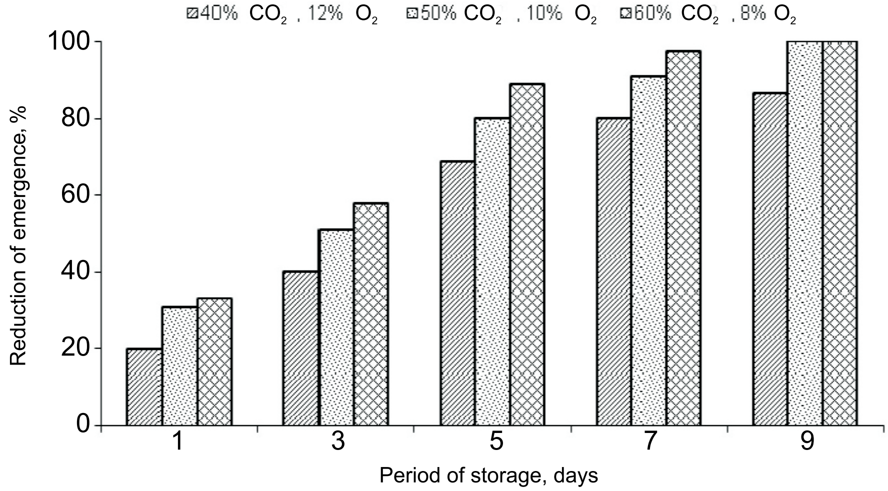

Fig. 6: Effect of controlled atmosphere storage on percentage reduction in adult emergence from late instar larvae of *Rhizopertha dominica*

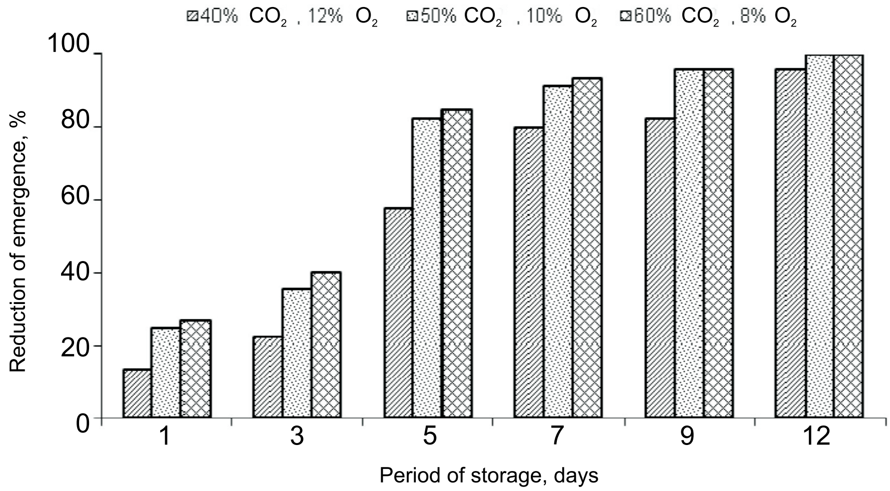

Fig. 7: Effect of controlled atmosphere storage on percentage reduction in adult emergence from pupae instar larvae *Rhizopertha dominica*

The results of the present study were in conformity with the results of Goncalves *et al*. (2000) that for adequate control of all larval stages of *R. Dominica* it required 50% carbon dioxide, or above. The finding was also similar to the report by Storey (1980) that 72-192 h were required for complete mortality of larvae of *R. dominica*.

Pupae of *Rhizopertha dominica*

The effects of three (40, 50 and 60%) carbon dioxide concentrations with rest of the gas as in air were used for the control of pupae of *R. dominica*. The periods of storage tested were one, three, five, seven, nine and twelve days. Carbon dioxide concentration of 50 and 60% was found to be at par with mean reduction in adult emergence of 73.3 and 71.5%, respectively. Carbon dioxide concentration of 40% resulted in significantly lower mean reduction in adult emergence of 58.5 per cent. Among the periods of storage, 12-day treatment gave a mean reduction in adult emergence of 98.5%, followed by a lower mean reduction in adult emergence of 91.1% for nine-day treatment. The mean percentage reductions in adult emergence for seven, five, three and one day treatments were 88.1, 74.8, 32.6 and 21.5%, respectively (Table 2). There was no interaction effect observed between the period of storage and carbon dioxide concentrations. With 50 and 60% carbon dioxide concentrations during 12-day storage period, 100% reduction in emergence of pupae of *R. dominica* was achieved (Fig.7). Over 95% reduction in adult emergence was observed with 40% carbon dioxide treatment with an exposure period of 12-days as well as 50 and 60% carbon dioxide concentration with an exposure period of nine days. In order to control pupae of *R. dominica*, 12-day treatment with 50% carbon dioxide was required.

The findings of the present study on pupae was in good agreement with the results of Goncalves *et al.* (2000) that pupae is the most tolerant stage of *R. Dominica,* and 100% mortality of pupae stage was obtained when 60% carbon dioxide was used for 15 days. The results were similar to the result of Storey (1980), who indicated requirement of 144 to 216 h for complete (100%) mortality of pupae of *R. dominica* exposed to less than one per cent oxygen and 9 to 9.5% carbon dioxide atmosphere.

Optimum Gas Concentration

The adult and different immature stages varied in susceptibility to controlled atmosphere storage, and the adults were found to be most susceptible, followed by early larvae and eggs. The late larvae were found to be more resistant than eggs, and pupae were found to be the most tolerant among all life stages. Hence, treatment with 50% carbon dioxide concentration for 12 days of storage should be used for controlling all stages of *R. Dominica*, which was effective to control pupae.

CONCLUSIONS

1. Five-day treatment with carbon dioxide concentration of 40% and above with oxygen levels up to 16% could be used for full control of adults of *R.dominica*. A three-day exposure with carbon dioxide concentration of 40% and above with 8% oxygen or less was sufficient for 100% mortality of adults.
2. For 100% mortality of eggs of *R. dominica*, seven-day treatment with 50% carbon dioxide or nine-day treatment with 40% carbon dioxide (with balance as in air) could be used.
3. To control early instar larvae of *R. dominica*, carbon dioxide concentration of 60, 50 or 40% (with balance as in air) could be used with five-, seven- and nine-day treatments, respectively.
4. For late instar larvae of *R. dominica*, 50% carbon dioxide (with balance as in air) could be used with nine-day treatment.
5. To control pupae of *R. dominica*, 12-day treatment with 50% carbon dioxide (with balance as in air) was required. This treatment could control all stages of *R. Dominica.*

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