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Studies on the effect of O_2 and CO_2 gases at different concentrations on the development of pulse beetle [*Callasobruchus analis*(Fabricius)] in pigeonpea*

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Abstract: Studies on the management of pulse bruchid, *C analis* (Fabricius) (Coleoptera: Bruchidae) under modified atmospheric condition was undertaken at the College of Agriculture, Raichur, Karnataka during 2010-11. Change in gas concentration was measured and there was gradual depletion of O_2 and increase of CO_2. This was mainly due to respiration of insects and grains during storage. There was no change in O_2 and CO_2 gas concentration where there was no O_2 hence there was no scope for insects as well as grains for respiration. The CO_2 concentrations of 15 and 20 per cent exposed for 45 days gave cent per cent mortality of insects wherein, no adults survived at that concentration. Further, there was no adult emergence thereby egg lying and mass loss (%) was also nil.

Key words: *Callasobruchus analis*, Carbon dioxide , Nitrogen, Oxygen, Pigeonpea

Introduction

Pigeonpea, *Cajanus cajan* (L.) is an important legume crop grown in Asia, Africa, Latin America and the Caribbean region. India is probably the primary center of origin of pigeonpea. Globally, pigeonpea has recorded 56 per cent increase in area since 1976. It is currently grown on an area of 4.8 m ha. Pigeonpea is subjected to damage in the field, as well as in the storage by bruchids, especially *Callasobruchus maculatus* (F.) (Dongre *et al.,* 1993). The larvae of the bruchid feed on the pulse seed contents reducing their degree of usefulness making them unfit either for planting or for human consumption (Ali *et al.,* 2004). Current approaches for controlling insects in stored grain rely largely on fumigation or synthetic insecticides. Yesteryear chemicals / insecticides used to control stored insects leave objectionable residues in treated commodity and generally are hazardous to handle and apply. Indiscriminate use of insecticides has led to the development of insecticide resistant strains of stored product insects as well as insecticide residue problems in food grains (Bhatia, 1990). More recently, the worldwide phase out and ban of the fumigant insecticide methyl bromide, an effective compound for killing post harvest insects, under the international agreement of the Montreal Protocol has motivated research into various alternatives to replace methyl bromide (Fields and White, 2002).

The U.S. Food Quality Protection Act of 1996 focused on evaluating all registered pesticides, with particular attention to worker and consumer exposures to chemical residues thus, reduction or elimination of residues in grain and foods was targeted by research for non chemical alternatives (Heaps, 2006). In addition to regulatory pressures for low risk control of stored-product insects, consumers and governments around the world set standards for organic food, which should be derived from raw products that are free of human-made chemicals, among other requirements (Anon, 2000). Thus, research on chemical-free or biologically based methods to control stored product insects are needed. Also, growing resistance to insecticides among insect population is reducing pesticide effectiveness (Subramanyam and Hagstrum, 1995). Cancellation of registration of almost all fumigants including methyl bromide and aluminium phosphide in many developed countries because of their possible carcinogenic effects on human beings has resulted in increased reliance on alternative ecofriendly pest management strategies such as modified atmosphere storage, use of botanical treatments, inert dusts and new insecticide molecules that have relatively low mammalian toxicity.

Modified atmosphere provide a way to eliminate insects from stored commodities without polluting the atmosphere and are safer than traditional fumigants. No harmful residues remain after the treatment of the commodity with N_2 or CO_2. Carbon dioxide is now used in several countries for the treatment of stored products, particularly grain in bulk, to control insect pests (Jay, 1984). The attraction of CO_2 in modified atmosphere treatment lies on availability, relative convenience and safety of application and the facts that it does not leave toxic residue has received the U.S. Food and Drug Administration approval for its use as a fumigant (Johnson, 1981).

Materials and methods

The experiments were conducted in the laboratories of the Department of Agricultural Entomology and Bio Control Laboratory, University of Agricultural Sciences, College of Agriculture, Raichur. Healthy seeds of pigeonpea were purchased in bulk from Karnataka State Seeds Corporation Limited, Raichur and 250 gram seeds weighed using electronic balance. Five pairs of bruchids were released into a polyethylene cover (700 gauge) containing 250 g of pigeonpea seeds (variety Maruti) which were placed in a muslin cloth so that the cloth avoids escape of insects while creating vacuum before filling the gas in the polyethylene covers. The seeds containing bruchids were exposed to the treatments detailed below by using modified atmosphere packing instrument. The

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treated seeds were kept in the labarotory condition for 45 days and observations were made after 45 days. The experiment was laid out in a Completely Randomised Block design with four replications. Details of treatment and gas concentrations are as mentioned below.

Change in the concentration of O_2 and CO_2 were checked by check mate gas analyser for that septum was stuck to the cover, which avoids loss of gas from polyethylene bag while taking readings of change in gas concentration.

After 45 days of storage the polyethylene bags were opened and the observations were recorded. Hundred seeds from the polyethylene cover were taken randomly and eggs were counted on each seed. The live and dead adult insect count was taken by using sieve and a basin and the seeds with insects were poured in a U shaped container so that adults cannot escape and the insect count was taken manually and the insects were removed and seeds were kept in a zip lock pack for further observations. Weight loss in pigeonpea was computed by using the following formula

Weight loss = Initial weight - Final weight

per cent Seed weight loss was computed by the following formula as suggested by Harris and Limblad (1978).

$$\text{Per cent weight loss} = \frac{\text{O.W.} - \text{F.W.}}{\text{O.W.}} \times 100$$

Where;

O.W. = Original weight on dry weight basis

F.W. = Final weight on dry weight basis

Germination test was conducted using four replicates of 100 seeds each in the paper (between papers) medium in the germination chamber. The germination chamber was maintained at 25±1°C temperature and 90±2% RH. At the end of fourteenth day, the number of normal seedlings in each replication was counted and the germination was calculated and expressed in percentage.

Dehydrogenase activity test for representative seeds (25) from each treatment were taken and preconditioned by soaking in water for overnight at room temperature. Seeds were taken at random and the embryos were excised. The embryos were steeped in 0.25 per cent solution of 2, 3, 5-triphenyl tetrazolium chloride solution and kept in dark for two hours at 40^{o} C for staining. The stained seeds were thoroughly washed with water and then soaked in 10 ml of two methoxy ethanol (methyl cellosolve) and kept overnight for extracting the red colour formazon. The intensity of red colour was measured using ELICO UV-VIS spectrophotometer (model SC-159) using blue filter (470 nm) and methyl cellulose as the blank. The OD value obtained was reported as dehydrogenase enzyme activity (Kittock and Law, 1968).

Results and discussion

The initial O_2 gas concentration in T_1 (Table 1) was nil and it remained same at 5, 10, 15, 20 and 25 days after treatment. Whereas, in T_2 the initial O_2 gas concentration was five per cent and there was a significant reduction in O_2 gas concentration at 5, 10, 15, 20 and 25 days after treatment, with 3.88, 2.88, 1.88, 1.75 and 1.63 per cent respectively. Similarly there was a significant reduction in O_2 gas concentration in T_3 where the initial O_2 gas concentration was 10 per cent and at 5, 10, 15, 20 and 25 days after treatment the O_2 gas concentration was reduced to 8.88, 7.75, 6.63, 6.13 and 5.13 per cent, respectively. Again there was a significant reduction in O_2 gas concentration in T_4 where the initial O_2 gas concentration was 15 per cent and at 5, 10, 15, 20 and 25 days after treatment the O_2 gas concentration was reduced to 12.88, 12.75, 11.88, 11.00 and 10.13 per cent, respectively. In treatment T_5 there was a similar result where the initial O_2 gas concentration was 20 per cent and there was a significant reduction in O_2 gas concentration due to consumption by insects and seeds at 5, 10, 15, 20 and 25 days after treatment, with a gas concentration of 16.88, 14.88, 14.38, 12.88 and 11.88 per cent respectively. Untreated control recorded the normal O_2 gas concentration of 21.88 per cent.

In T_1 the initial CO_2 gas concentration was 20 per cent and it remained same at various intervals after treatment. In T_2 the initial CO_2 gas concentration was 15 per cent and there was a significant increase in CO_2 gas concentration at 5, 10, 15, 20 and 25 days after treatment, with a gas concentration of 16.13, 17.13, 18.13, 18.25 and 18.38 per cent, respectively. Similarly there was a significant increase in CO_2 gas concentration in treatment T_3 where the initial CO_2 gas concentration was 10 per cent and at 5, 10, 15, 20 and 25 days after treatment the CO_2 gas concentration increased to 11.13, 12.25, 13.38, 13.88 and 14.88 respectively due to survival and development of insects as well as due to respiration of seeds in lower O_2 and higher CO_2 concentration. Again there was a significant increase in CO_2 gas concentration in T_4 where the initial CO_2 gas concentration was five per cent and at 5, 10, 15, 20 and 25 days after treatment the CO_2 gas concentration increased to 7.13, 7.25, 8.13, 9.00 and 9.88 per cent, respectively. In T_5 there was a similar result where the initial CO_2 gas concentration was zero per cent and there was a significant increase in CO_2 gas concentration at 5, 10, 15, 20 and 25 days after treatment, with a gas concentration of 3.13, 5.13, 5.63, 7.13 and 8.13 per cent, respectively due to survival and development of insects. The untreated control recorded the gas concentration of 0.04 per cent.

The present investigation revealed that the respiration by insects and grains released more of CO_2 which increased as the day advanced from 5 to 25 days and the CO_2 concentration increased due to utilization of O_2 for respiration and release of CO_2 and the results are in agreement with the reports of Elisabetta et al. (2009) where cent per cent mortality could be achieved within a week, even in quite moderate conditions of temperature (29 to 37^{0} C) with low O_2 (5 to 8 %). At lower O_2 and higher CO_2 concentrations metabolism level of insects become too low, combined with accumulation of toxic end products, which is a cause of stress for the insects eventually leading to death (Donahaye and Navarro, 2000; Ofuya and Reichmuth, 2002). There was decrement in O_2 concentration which was due to utilization of O_2 by insects and grains for respiration. The literature on this aspect is lacking to compare such studies

The egg count per 100 seeds revealed that in T_1 and T_2 there was no egg laying followed by T_3 with 41.00 eggs per 100 seeds. There was highest number of eggs noticed in untreated control

Table 1. Concentration levels of O_2 and CO_2 in polyethylene cover after exposing to the treatments in pigeonpea

Tr. No.	Gas combination	Oxygen gas concentration (%) Days after exposure					Carbon dioxide gas concentration (%) Days after exposure				
	N_2 : O_2 : CO_2	5	10	15	20	25	5	10	15	20	25
T_1	80: 00: 20	0.00	0.00	0.00	0.00	0.00	20.00	20.00	20.00	20.00	20.00
		(0.00)[a]*	(0.00)[a]*	(0.00)[a]*	(0.00)[a]*	(26.55)[a]*	(26.55)[a]*	(26.55)[a]*	(26.55)[a]*	(26.55)[a]*	
T_2	80: 05: 15	3.88	2.88	1.88	1.75	1.63	16.13	17.13	18.13	18.25	18.38
		(11.34)[b]	(9.75)[b]	(7.85)[b]	(7.58)[b]	(7.31)[b]	(23.67)[b]	(24.44)[b]	(25.19)[b]	(25.28)[b]	(25.37)[b]
T_3	80: 10: 10	8.88	7.75	6.63	6.13	5.13	11.13	12.25	13.38	13.88	14.88
		(17.32)[c]	(16.16)[c]	(14.90)[c]	(14.32)[c]	(13.08)[c]	(19.48)[c]	(20.48)[c]	(21.44)[c]	(21.86)[c]	(22.68)[c]
T_4	80: 15 : 05	12.88	12.75	11.88	11.00	10.13	7.13	7.25	8.13	9.00	9.88
		(21.02)[d]	(20.91)[d]	(20.15)[d]	(19.36)[d]	(18.55)[d]	(15.47)[d]	(15.61)[d]	(16.55)[d]	(17.45)[d]	(18.31)[d]
T_5	80: 20: 00	16.88	14.88	14.38	12.88	11.88	3.13	5.13	5.63	7.13	8.13
		(24.24)[e]	(22.68)[e]	(22.27)[e]	(21.02)[e]	(20.15)[e]	(10.17)[e]	(13.08)[e]	(13.70)[e]	(15.47)[e]	(16.55)[e]
T_6	Control	21.88	21.88	21.88	21.88	21.88	0.04	0.04	0.04	0.04	0.04
		(27.87)[e]	(27.87)[f]	(27.87)[f]	(27.87)[f]	(27.87)[f]	(1.12)[f]	(1.12)[f]	(1.12)[f]	(1.12)[f]	(1.12)[f]
S. Em±	0.115	0.132	0.187	0.153	0.150	0.117	0.113	0.161	0.083	0.092	
C.D. @ 1%	0.470	0.538	0.761	0.624	0.609	0.476	0.458	0.657	0.338	0.373	

Means followed by common letter do not differ significantly by DMRT at p=0.01.

* Figures in the parentheses are arc sine transformed values

(77.25) followed by T_5 (73.00) and were on par with each other. This was due to survival and development of insects in the low or nil CO_2 concentration compared to T_1 and T_2 where CO_2 concentration was 20 and 15 per cent, respectively and killed all the insects. There was no adult emergence noticed in T_1 and T_2 due to death of all the released insects which was followed by T_3 with 210.50 adults per 250 g of seeds. There was highest number of adult emergence noticed in untreated control with 259.75 adults followed by T_5 (255.00) which were on par with each other. The dead insects in T_3, T_4, T_5 and T_6 were 39.75, 43.50, 45.00 and 49.50 adults per 250 g respectively. There was no loss of weight noticed in T_1 and T_2 treatments followed by T_3 with 22.75 g of weight loss per 250 g of seeds. There was highest loss of weight in untreated control with 43.25 g followed by T_5 which was at par with untreated control with a weight loss of 41.75 g.

There was no weight loss in T_1 and T_2 due to the death of all of the insects affected by 20 and 15 per cent CO_2 concentration followed by T_3 with 9.90 per cent weight loss. There was highest weight loss in untreated control with 17.30 per cent followed by T_5 which was at par with untreated control with a weight loss of 16.80 per cent. There was no statistical significant difference noticed in respect of germination in pigeonpea which ranged from 84.75 per cent in T_4 to 86.75 per cent in T_1, indicating no effect of CO_2 even at 20 per cent.

There was no statistical significant difference noticed in dehydrogenase enzyme activity of pigeonpea which ranged

Table 2. Concentrations of O_2 and CO_2 gas on the development of pulse beetle C. analis in pigeonpea after 45 days of exposure

Tr. No.	Gas combination N_2 : O_2 : CO_2	No. of adults released	Egg count (no / 100 seeds)	Live adults (no / 250 g seeds)	Dead adults (no /250 g seeds)	Initial weight (g)	Final weight (g)	Weight loss (g)	Per cent weight loss	Germination per cent (%)	Dehydrogenase enzyme activity (OD value)
T_1	80: 00: 20	10.00	0.00	0.00	10.00	250.00	250.00	0.00	0.00	86.75	0.58
		(1.00)[a]*	(1.00)[a]*	(3.32)[a]*			(1.00)[a]*	(0.00)[a]**	(68.65)**		
T_2	80: 05: 15	10.00	0.00	0.00	10.00	250.00	250.00	0.00	0.00	85.75	0.58
		(1.00)[ab]	(1.00)[ab]	(3.32)[ab]			(1.00)[ab]	(0.00)[ab]	(67.82)		
T_3	80: 10: 10	10.00	41.00	210.50	39.75	250.00	227.25	22.75	9.90	85.50	0.59
		(6.48)[c]	(14.54)[c]	(6.38)[c]			(4.86)[c]	(18.27)[c]	(67.62)		
T_4	80: 15 : 05	10.00	50.75	251.75	43.50	250.00	218.75	31.25	12.50	85.25	0.58
		(7.91)[d]	(15.90)[d]	(6.67) [d]			(5.68)[d]	(20.69)[d]	(67.47)		
T_5	80: 20: 00	10.00	73.00	255.00	45.00	250.00	208.25	41.75	16.80	85.00	0.57
		(8.60)[e]	(16.00)[de]	(6.78)[de]			(6.54)[e]	(24.19)[e]	(67.22)		
T_6	Control	10.00	77.25	259.75	49.50	250.00	206.75	43.25	17.30	84.75	0.59
		(8.85)[ef]	(16.15)[def]	(7.11)[f]			(6.65)[f]	(24.56)[ef]	(67.01)		
S.Em±	0.066	0.118	0.040	—	—	0.096	0.429	0.824	0.013		
C.D. @ 1%	0.267	0.480	0.161	—	—	0.390	1.747	NS	NS		

NS=non-significant

* Figures in parentheses are $\sqrt{x+1}$ transformed values

** Figures in parentheses are arcsine transformed values

Means followed by common letter do not differ significantly by DMRT at p=0.01

from 0.57 in T_5 to 0.59 in untreated control. Overall results revealed that T_1 and T_2 with 20 and 15 per cent CO_2 concentration provided better control over other treatments with cent per cent mortality of insects as well as egg free seeds without loss of weight besides normal germination and dehydrogenase enzyme activity of seeds.

The results on the egg count in the present investigation revealed that due to mortality of all released adults there were no eggs in T_1 (0 % O_2 and 20 % CO_2) and T_2 (5 % O_2 and 15 % CO_2) where the CO_2 concentration was 20 and 15 per cent respectively. Whereas, in T_3, T_4 and T_5 the CO_2 concentration decreased and proportionately increased the egg laying. It was clear and evident that due to low O_2 and high CO_2 there were no adults in T_1 (0 % O_2 and 20 % CO_2) and T_2 (5 % O_2 and 15 % CO_2), where all released adults were dead when exposed to 20 and 15 per cent CO_2 for 45 days when the O_2 concentration was low (0 and 5 %) the insects were not able to respire normally. It is inferred from the results that there was no weight loss (%) in treatment T_1 (0 % O_2 and 20 % CO_2) and T_2 (5 % O_2 and 15 % CO_2) compared to 17.30 per cent weight loss in untreated control, because of the complete mortality of released insects.

The present investigations revealed that there was cent per cent mortality with zero egg laying and no weight loss (%)which is in corroboration with the results of Banks and Annis (1990) where eggs were significantly affected by 20 per cent CO_2 and at more than 20 per cent, adult insects are the most susceptible stage. For effective control, the O_2 level should be less than three per cent and preferably less than one per cent if a rapid kill is required (Navarro, 1978; Banks and Annis, 1990; Fleurat Lessard, 1990). Elisabetta *et al.* (2009) reported that cent per cent mortality could be achieved within a week, even in quite moderate conditions of temperature (29 to 37^0 C) with low O_2 (5 to 8 %). At lower O_2 and higher CO_2 concentrations metabolism level of insects became too low, combined with accumulation of toxic end products, is a cause of stress for the insects eventually leading to death (Donahaye and Navarro, 2000; Ofuya and Reichmuth, 2002).

The results revealed that there was no effect of CO_2 on germination and dehydrogenase enzyme activity. The present findings are in accordance with the results of Khan *et al.* (2002) where they reported that the controlled atmosphere did not have any consistent effect in maintaining *Citrus* seed viability. Similarly, Bera *et al.* (2004)reported that, where the storage of wheat seeds in CO_2 rich atmosphere irrespective of concentrations and periods, showed no adverse effect on germinability, vigour and no change in dehydrogenase enzyme activity as well as molondialdehyde contents. Further, Bera *et al.* (2008) also reported that, paddy seeds can be stored safely at least up to 12 months without reduction in seed viability under modified atmospheric storage up to 80 per cent CO_2.

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