Studies on the effect of O_2 and CO_2 gases at different concentrations on the development of pulse beetle *Callasobruchus analis* (Fabricius) (Coleoptera: Bruchidae) in chickpea

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ABSTRACT

Studies on the management of pulse bruchid, *Callasobruchus 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; no adults survived at that concentration. Further, there was no adult emergence thereby egg laying and mass loss (%) were also nil.

Key words: Carbon dioxide, *Callasobruchus analis*, chickpea, nitrogen, oxygen

INTRODUCTION

Chickpea [*Cicer arietinum* (Linnaeus)] is one of the important legume crops produced in Asia, Africa, Latin America and the Caribbean region. Chickpea is the first most important pulse crop and is grown under varied agro climatic conditions. Karnataka occupies an area of 0.68 m ha with a production of 0.11 m tonnes. Chickpea is subjected to damage in the field, as well as in the storage by bruchids, especially *Callasobruchus maculatus* (Fabricius) (Dongre *et al*., 1993). The pest generates exceedingly high levels of infestation even when they pass only one or two generations on the host. 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).

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 food was targeted by research for nonchemical 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 (Anonimous, 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 eco-friendly pest management strategies such as modified atmosphere storage, use of botanical treatments, inert dusts and new insecticide molecules that have relatively low mammalian toxicity.

Hermetic storage of grain was practised in ancient times in underground pits in the dry, subtropical regions of the Middle East and other dry regions of the world such as Africa and India. Underground pits for grain storage were used in Egypt during 1940s (Attia, 1948). Very old but active hermetic storages were reported to be in operation in India (Girish, 1980) and in Yemen, Somalia, Sudan, and Egypt (Kamel, 1980). Hermetic storage for generating a dynamic modified atmosphere has been demonstrated extensively in Israel and parts of Asia and Africa, and provided a means of safe storage in locations where electricity or access to gases or permanent storage structures is limited (Navarro, 2006). Toxicity responses of insects to controlled or modified atmospheres are similar to those with chemical fumigants. Modified atmosphere provides a way to eliminate insects from stored commodities without polluting the atmosphere and is 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

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products, particularly grain in bulk, to control insect pest (Jay, 1984). The attraction of CO_2 in modified atmosphere (MA) treatment lies on availability, relative convenience and safety of application and the fact that it does not leave toxic residue has received the U.S. Food and Drug Administrations (FDA) approval for its use as a fumigant (Johnson, 1981).

MATERIALS AND METHODS

The experiments were conducted in the laboratories of Department of Agricultural Entomology and Bio Control Laboratory, University of Agricultural Sciences, College of Agriculture, Raichur. Healthy seeds of chickpea were purchased in bulk from M/S KSSC Ltd., Raichur and 250 g seeds were weighed using electronic balance. Five pairs of bruchids were released into a polyethylene cover (700 gauge) containing 250 g of chickpea seeds placed in a muslin cloth so that the cloth avoided loss 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 (MAP) instrument. The treated seeds were kept under lab condition for 45 days and the following 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 concentration are as mentioned below:

T1- 80% N_2 : 00 % O_2 : 20 % CO_2; **T_2**- 80% N_2 : 05 % O_2 : 15 % CO_2; **T_3**- 80% N_2 : 10 % O_2 : 10 % CO_2; **T_4**- 80% N_2 : 15 % O_2 : 05 % CO_2; **T_5**- 80% N_2 : 20 % O_2 : 00 % CO_2; **T_6**-Untreated Control

Change in the concentration of O_2 and CO_2 were checked by Check mate Gas Analyser for the septum 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 following observations were recorded. Hundred seeds from the polyethylene cover were taken randomly and eggs were counted on each seed. After one month of storage the live and the dead adult insect count was taken by using a sieve and a basin and the seeds with insects were poured in a **U** shaped container so that adults could not 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. Seed weight loss was computed by the following formula as suggested by Harris and Limblad (1978). Germination test was conducted using four replicates of 100 seeds each in the paper (between papers) medium in the germination room. The germination room was maintained at 25 ± 1°C temperature and 90 ± 2% RH. At the end of fourteenth day of sowing, 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 was done preconditioned by soaking in water 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 the dark for two hrs at 40°C for

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

Treatments	Gas combination N_2:O_2:CO_2	Oxygen gas concentration (%)					Carbon dioxide gas concentration (%)				
		Days after exposure					Days after exposure				
		5	10	15	20	25	5	10	15	20	25
T_1	80: 00: 20	0.00 ^{a}	0.00 ^{a}	0.00^a	0.00^a	0.00^a	20.00^a*	20.00^a*	20.00^a*	20.00 ^{a}*	20.00 ^{a}*
T_2	80: 05: 15	3.63^b	2.00^b	1.38^b	0.88^b	0.63^b	16.38^b	18.00^b	18.63^b	19.13^b	19.38^b
T_3	80: 10: 10	8.50^c	6.88^c	5.88^c	5.63^c	3.88^c	11.50^c	13.13^c	14.13^c	14.38^c	16.13^c
T_4	80: 15 : 05	12.25^d	11.25^d	10.75^d	10.50^d	8.25^d	7.75^d	8.75^d	9.25^d	9.50^d	11.75^d
T_5	80: 20: 00	16.38^e	14.00^e	13.50^e	10.88^e	10.25^e	3.63^e	6.00^e	6.50^e	9.13^e	9.75^e
T_6	Control	21.88^f	21.88^f	21.88^f	21.88^f	21.88^f	0.04^f	0.04^f	0.04^f	0.04^f	0.04^f
S. Em±		0.229	0.267	1.004	0.269	0.824	0.229	0.209	0.330	0.192	0.247
CD (p=0.01)		0.933	1.089	4.085	1.093	3.354	0.932	0.850	1.344	0.783	1.004

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staining. The stained seeds were thoroughly washed with water and then soaked in 10 mL of two methoxy ethanol (methyl cello solve) and kept overnight for extracting the red color formazon. The intensity of red color 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 activity (Kittock and Law, 1968).

RESULTS AND DISCUSSION

In T_1 CO_2 and O_2 gas concentration remained the same throughout the observation whereas it varied in subsequent treatments. Oxygen concentration was decreased and carbon dioxide concentration was increased in the following treatments (Table1). In untreated control remained up to 25 days 21.8 O_2 gas concentration and 0.04 CO_2 gas concentration.

There was a decrease in O_2 concentration which was due to utilization of O_2 by insects and grains for respiration. Literature on this aspect is lacking making comparison of such studies impossible and ours seems to be first of its kind on the measurement of O_2 levels under modified atmosphere condition.

The present investigation revealed that the respiration by insects and grains releases more of CO_2 which increased as the day advanced from five 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 those 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 per centage (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

Table 2. Effect of O_2 and CO_2 gas concentrations on the development of pulse beetle *C. analis* in chickpea after 45 days of exposure

Treatments	Gas combination $N_2 : O_2 : CO_2$	Egg count (no / 100 seeds)	Live adults (no / 250 g of 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	0.00 (1.00)^{a}*	0.00 (1.00)^{a}*	10.00 (3.32)^{a}*	250.00	250.00	0.00 (1.00)^{a}*	0.00 (0.00)^{a}**	93.00 (74.70)**	0.64
T_2	80: 05: 15	0.00 (1.00)^{ab}	0.00 (1.00)^{ab}	10.00 (3.32)^{ab}	250.00	250.00	0.00 (1.00)^{ab}	0.00 (0.00)^{ab}	91.75 (73.42)	0.65
T_3	80: 10: 10	46.75 (6.91)^{c}	231.75 (15.26)^{c}	43.00 (6.63)^{c}	250.00	222.00	28.00 (5.38)^{c}	10.99 (19.25)^{c}	91.75 (73.42)	0.64
T_4	80: 15 : 05	58.75 (7.73)^{d}	268.75 (16.42)^{d}	45.00 (6.78)^{cd}	250.00	206.50	43.50 (6.67)^{d}	17.65 (24.79)^{d}	92.25 (73.93)	0.65
T_5	80: 20: 00	71.50 (8.51)^{e}	276.75 (16.67)^{e}	47.25 (6.95)^{de}	250.00	197.50	52.50 (7.31)^{e}	21.25 (27.40)^{e}	92.00 (73.66)	0.65
T_6	Control	74.50 (8.69)^{ef}	281.25 (16.80)^{ef}	52.25 (7.30)^{f}	250.00	197.50	52.50 (7.31)^{ef}	20.90 (27.19)^{ef}	91.00 (72.53)	0.65
S. Em±		0.069	0.040	0.055			0.067	0.297	1.106	0.010
CD (p=0.01)		0.280	0.162	0.222			0.274	1.208	NS	NS

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

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eventually leading to death (Donahaye and Navarro, 2000; Ofuya and Reichmuth, 2002).

The egg count per 100 seeds revealed (Table 2) that the eggs laid per 100 seeds was nil in T_1 and T_2 followed by T_3 with 46.75 eggs per 100 seeds. The highest number of eggs were noticed in untreated control (74.50 eggs/100) followed by treatment T_5 (71.50 eggs/100) where there was zero CO_2 and 20 per cent O_2, which was on par with the untreated control (74.50 eggs/100) followed by T_4 (58.75 eggs/100). 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 that killed all the insects. There was no adult emergence in T_1 and T_2 where CO_2 was 20 and 15 per cent respectively leading to death of all insects which was followed by T_3 with 231.75 adults per 250 g of seeds. The highest number of adult emergence was noticed in untreated control with 281.25 adults followed by treatment T_5 which were on par with untreated control. No loss of weight in T_1 and T_2 treatments followed by T_3 with 28.00 g of weight loss per 250 g of seeds. The highest loss of weight was observed in untreated control with 52.50 g followed by T_5 which was at par with untreated control with a weight loss of 52.50 g.

There was no statistically by significant difference in germination and dehydrogenase enzyme activity of chickpea, which ranged from 0.64 in T_1 to 0.65 in untreated control. Overall results revealed that T_1 and T_2 with 20 and 15 per cent CO_2 concentration gave 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 present investigations revealed that cent per cent mortality with zero egg laying and no weight loss (%) is in corroboration with the results of Elisabetta *et al.* (2009), where eggs were significantly affected by 20 per cent CO_2 and at more than 20 per cent, adult insects were 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. 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 percentage (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. This is a cause of stress for the insects which eventually leads to death (Donahaye and Navarro, 2000; Ofuya and Reichmuth, 2002). Larvae and adults were more susceptible while eggs and pupae were more tolerant to CO_2. A two-day exposure period was adequate to completely kill larvae and adults under all tested MAs. All eggs and pupae were killed after four days of exposure to the high CO_2 atmospheres (75% and 85%) as reported by Mohamed *et al*. (2012).

Caril *et al*. (2010) reported that no significant differences were observed in dead insects when CO_2 was used. The results of progeny indicated that from the fifth day the number of emerging insects were low at 20, 60 and 80 per cent CO_2. Complete inhibition of the insects was achieved with 30 days of exposure in CO_2 atmospheres. 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 Elisabetta *et al.* (2009) who that the controlled atmosphere did not have any consistent effect in maintaining *Citrus* seed viability. 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. Paddy seeds can be stored safely at least upto 12 months without reduction in seed viability under modified atmospheric storage upto 80 per cent CO_2 as reported by Elisabetta *et al.* (2009).

In conclusion, the five tested MAs containing 20%, 15%, 10% and 5% CO_2 varied in their lethat effects against adults of *Callasobruchus analis*. About 45 days were adequate to kill adults completely under 20% and 15% CO_2 concentration. At lower O_2 and higher CO_2 concentrations metabolism level of insects became too low, combined with accumulation of toxic end products, which causes stress for the insects eventually leading to their death.

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