# INSECT CONTROL IN SELECTED SPICE PRODUCTS USING CARBON DIOXIDE

A THESIS SUBMITTED TO THE UNIVERSITY OF MYSORE
FOR THE AWARD OF THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN ZOOLOGY

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May 2001

## DECLARATION

I hereby declare that this thesis entitled " **INSECT CONTROL IN SELECTED SPICE PRODUCTS USING CARBON DIOXIDE"** submitted to the UNIVERSITY OF MYSORE for the award of the degree of DOCTOR OF PHILOSOPHY in Zoology, is the result of the research work carried out by me in the Department of Food Protectants and Infestation Control, Central Food Technological Research Institute, Mysore, under the guidance of Dr. S. Rajendran, scientist E II, during the period 1996 to 2001.

I further declare that the results of this work have not been previously submitted for any other degree or fellowship.

N. GUNASEKARAN

Date: 28.05.2001

Place: Mysore

# CERTIFICATE

I hereby certify that this thesis entitled "INSECT CONTROL IN SELECTED SPICE PRODUCTS USING CARBON DIOXIDE" submitted by Mr. N. Gunasekaran for the degree of DOCTOR OF PHILOSOPHY in ZOOLOGY, University of Mysore, is the result of the research work carried out by him in the Department of Food Protectants and Infestation Control, Central Food Technological Research Institute, Mysore, under my guidance and supervision during 1996 to 2001.

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## ACKNOWLEDGEMENT

It is my pleasure to express my gratitude to Dr. S. Rajendran, scientist E II, Food Protectants and Infestation Control Department, Central Food Technological Research Institute, Mysore, for the valuable guidance and constant help throughout the course of this investigation.

My grateful acknowledgements are to Dr.N.G.K Karanth, Head, Food Protectants and Infestation Control Department, CFTRI, Mysore. It is my privilege to express my thanks to Dr. V. Prakash, Director, CFTRI for providing all facilities.

My special thanks to Mr. Jan van S. Graver, senior scientist, Division of Entomology, Stored Grain Research Laboratory, CSIRO, Australia for providing relevant literature . My sincere thanks to Dr.V. Baskaran, scientist, Biochemistry and Nutrition department for his help in proximate analysis, Mr. N. Muralidharan, FPIC department for his help in conducting the experiments and Mrs. Sowbhagya, PPSFT department for her advice. I also thank Mrs. Indiramma, Mrs. Vijayalakshmi, Mr. Rajesh Matche and Mr. Pandian, Food Packaging Technology department for their help in studies of packaging materials. In addition the help rendered by Mr. R. Ravi,Sensory Science department for statistical analysis is greatly acknowledged.

I express my special thanks to Dr. H. M. Shivaramaiah, Mr. Harish for their help in making this thesis, and all my colleagues of FPIC department for extending help in all respects.

Finally, I wish to place on record the indebtedness to my beloved parents Mr. T. Nagamuthu and Mrs. Veghuthaniam, sisters, brother, father in-law, mother in-law, sisters in-law , wife and daughter for their affectionate support and inspiration provided to me.

**(N.GUNASEKARAN)**

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## INTRODUCTION

Spices are defined as "the flavoured or aromatic substances of vegetable origin obtained from tropical or other plants commonly used as condiments or employed for other purposes on account of their fragrances, preservative or medicinal qualities" (Anonymous 1996). They are available as seeds, fruits, bark, flowers, and as bulbs or rhizomes (Table 1.1). Spices serve as important condiments in culinary. They play a significant role in the treatment of several disorders in humans because of their therapeutic properties. Spices are consumed in small quantities in every day diet of humans. They have been used in all categories of food industry involving meat, fish, vegetable products, bakery products and convenience foods. They have exceptionally high levels of minerals and vitamins, which play an important role in human nutrition. Use of spices in every day food in reasonable amounts cannot, but help to meet cumulatively the needs of vitamins and minerals of the human body (Mahindru, 1994).

World trade of spices during 1995-96 has been estimated to be 0.55 million tonnes worth US $ 1873 millions and is projected to be 0.63 million tonnes worth US $ 2000 millions by 2001 AD. India is the largest producer, consumer and exporter of spices in the world. India's contribution to the world trade of spices is of the order of 37% by volume and 12% by value. On an average, 8 to 10% of the total production in India is exported. India exported about 0.20 million tonnes of spices during 1995-96

valued at US $ *221.14* millions, and about 0.22 million tonnes during 1997-98 valued at US $338 millions (Table 1.2). Export of spices during 1998-99 made an all time record earning foreign exchange of US $ 398.90 millions.

Considering the importance of spices/spice products in getting valuable foreign exchange, it is essential to look into the status of the pest problem and the various options for controlling the pests. Insects are the principal agents of spoilage in stored spices and their products followed by fungi and other microbes. Altogether, the pest activity not only leads to loss of dry matter, and quality, but also produces pathogenic toxins. The exact losses in stored spices, whole or powdered, due to insects and other pests in India or elsewhere have not been quantified. However, the problem has assumed significance from the point of view of quality standards in national and international markets.

Table 1.1 Different types of spices

| **Common Name** | **Scientific Name** |
|---|---|
| **Seeds**<br>Anise | *Pimpinella anisum* |
| Coriander | *Coriandrum sativum* |
| Cumin | *Cuminum cyminum* |
| Dill | *Peucedanum graveolens* |
| Fenugreek | *Trigonella foenum* |
| Nutmeg | *Myristica fragans* |
| Poppy | *Papaver somniferum* |
| **Fruits**<br>Capsicum | *Capsicum annum* |
| Cardamom | *Elletaria cardamomum* |
| Caraway | *Carum carvi* |
| Celery | *Apium graveolens* |
| Fennel | *Foeniculum vulgare* |
| Pepper | *Piper nigrum* |
| **Bulbs and Rhizomes**<br>Garlic | *Allium sativum* |
| Ginger | *Zingiber officinale* |
| Onion | *Allium cepa* |
| Turmeric | *Curcuma longa* |
| **Others**<br>Basil | *Ocimum basilicum* |
| Chicory | *Cichorium intybus* |
| Cinnamon | *Cinnamomum zeylanicum* |
| Clove | *Eugenia aromatic* |
| Mace | *Myristica fragrans* |
| Marjoram | *Origanum marjorana* |
| Mint | *Mentha viridis* |
| Rosemary | *Rosmarinus officinalis* |
| Saffron | *Crocus sativus* |
| Sage | *Salvia officinalis* |
| Thyme | *Thymus vulgaris* |

Table 1.2 Production, consumption and export trend of spices in India

| Year | Total production (million tonnes) | Total consumption (million tonnes) | Total export (million tonnes) |
|---|---|---|---|
| 1994-95 | 2.3 | 2.14 | 0.155 |
| 1995 -96 | 2.5 | 2.20 | 0.203 |
| 1996-97 | 3.0 | 2.30 | 0.225 |
| 1997-98 | 2.5 | 2.20 | 0.228 |
| 1998-99 | | 2.28 | 0.210 |

# INSECT PESTS

Different types of spices and spice products are attacked by both beetle and moth pests (Table 1.3). Among the insect pests the drugstore beetle or spice beetle, *Stegobium paniceum* Lin. and the cigarette beetle, *Lasioderma serricorne* Fab. are important (Pruthi, 1980; Butani, 1984; Rahman Rezaur et al., 1982 ; Yadav 1989; Srinath 1984).

***Lasioderma serricorne*** : *L. serricorne* is widespread throughout the tropics and subtropics (Aitken 1975 ). This beetle is well adapted to survive on a wide range of foodstuffs. The two factors that limit the distribution of the pests are low temperature and humidity. Adults of *L. serricorne* are light to dark brown in colour and of 2.0 - 3.7 mm size. Adult size and colour may vary according to the type of food and conditions of temperature and humidity encountered during development. Adult is oval in shape with head and thorax bent downward giving a humped or convex appearance. The antennae are serrate, with a saw-like appearance and are the same thickness from base to tip. These can be used as an identification feature, since they differ from the typical anobiid antennae which form a broad, three segmented club. Females are larger than males. The adults fly during late afternoon, at dusk and darkness, but sometimes in daylight on warm dull days.

Females directly oviposit on to dried material (on an average 110 eggs/female). Eggs are pearly white, with spines at the end of the egg from which the larva emerges. Eggs hatch in 6 - 8 days and eggshell is eaten by newly emerged larva. There are 4 larval instars before pupation.

**Table 1.3 Insect pests of stored spices and spice products**

| INSECT | COMMODITY |
|---|---|
| **Beetles** | |
| *Lasioderma serricorne* | Coriander and its products, Turmeric. Ginger, Chillies, Cardamom, Garlic bulb, and Black pepper |
| *Stegobium paniceum* | Turmeric, Chillies, Coriander, Ginger, Cardamom, and Black pepper |
| *Tribolium castaneum* | Chillies,Turmeric, Black pepper, Cardamom, Coriander, and Ginger. |
| *Tribolium confusum* | Chillies |
| *Trogoderma granarium* | Coriander and its products |
| *Tenebroides mauritanicus* | Chillies, Ginger, Turmeric, Cardamom |
| *Araecerus fasciculatus* | Ginger, Turmeric, Cardamom |
| *Rhyzopertha dominica* | Chillies, Turmeric, Coriander, Ginger Black pepper, and Cardamom |
| *Carpophilus spp.* | Garlic bulb, and Turmeric |
| *Cryptolestes spp.* | Turmeric, and Garlic bulb |
| *Oryzaephilus surinamensis* | Turmeric, and Black pepper |
| *Attagenus dimidiatus* | Turmeric |
| *Cryptoblabes gnidiella* | Garlic bulb |
| *Sitophuus oryzae* | Turmeric, Black pepper, Cardamom, and |
| **Moths** | Coriander Chillies |
| *Sitotroga cerealella* | |
| *Ephestia cautella* | Chillies, and Turmeric |
| *Corcyra cephalonica* | Turmeric |

The larvae are creamy or greyish white and thickly covered with fine hairs that appear as light brown on fully-grown individuals. The newly hatched larvae are extremely active but

they tend to penetrate deeply into loosely held commodities. Fully-grown larvae stop feeding and form a pupal case, which is made of food and waste material, cemented together by secretion produced by midgut. After pupal period, which varies between 4 - 1 2 days, the adult remains in the pupal case to harden and becomes sexually mature and then emerges. The adults have short life of 2 - 7 weeks depending on larval food type, temperature and humidity. The total life cycle under optimum conditions takes about 4 1 - 5 8 days ( Ashworh, 1993).

***Stegobium paniceum:*** *S. paniceum,* virtually a cosmopolitan species, infesting various types of plant and animal products including spices. They have intracellular symbiotic yeasts in their hindguts (mycetomes) which make them independent of external source of vitamin B. Adults of *S. paniceum* are uniform brown in colour, and of 2.5 mm size and cylindrical in form. The adult resembles the cigarette beetle. However, it can be distinguished by the three segmented antennal club. Adult female lays about 75 eggs on foodstuffs during its life. There are five or six larval instars; the last of which constructs a cocoon in which it pupates. In powdery foodstuffs, the larvae usually form a ball or cell which will become its cocoon. After eclosion, the adults will remain in the cocoon for variable periods before emerging. Some individuals do not construct cocoons. There is no obvious external sexual distinction in the adult excepting that females are generally heavier than males. Adults do not feed. The life cycle is about 40 days and the adult lives for 13 - 65 days depending on ambient conditions ( Lefkovitch, 1967).

It has been observed that high moisture content in the spices and spice products favours development of insect pests and mould growth. In dry spice products insect infestation leads to heating and moisture condensation which is conducive for spoilage by micro organisms (Seenappa, 1979). Pest activity results in contamination of products with insect fragments, excreta (uric acid), pathogenic microbes and mycotoxins. Consequently, the quality, shelf life and markeability of spices/spice products are affected and thus it becomes an economically important issue. Hence, spices/spice products should be protected from the attack of insect pests and other depredating agents in order to meet the quality standards and lor regulatory limits with respect to pest contaminants and pesticides. In India, as per the Agmark Grade Specifications (1994 ) under the Agricultural Produce-Grading and Marking Act (1937), all the spices and their products should be free from insect infestation, insect fragments and mould growth. The maximum allowable limit for uric acid, which is an indicator for insect infestation, is 100 mg/kg under the PFA Act (1954).

## INSECT CONTROL METHODS

### FUMIGATION

Among the insect control methods applicable to stored spices/spice products, fumigation, irradiation and use of heat treatment are important. Fumigation involves, application of chemicals in the gaseous phase that act against pest organisms. Currently, spices/spice products are disinfested with phosphine or methyl bromide fumigants (Table 1.4). Ethylene oxide is used primarily for controlling micro flora in spices.

**Table 1.4 Fumigants used for controlling insects and microbes in spices/spice Products**

| Fumigant | Availability | Residue limits (ppm) | |
|---|---|---|---|
| | | **Codex Alimentarius** | **PFA ACT** |
| PHOSPHINE ($PH_3$) | l)Solid formulations of aluminium and magnesium phosphides<br>2) Cylinder based formulation with $CO_2$ or $N_2$ | 0.10 (whole grains);<br>0.01 (milled products) as phosphine | 0.0 (whole grain)<br>0.0(milled products) |
| METHYL BROMIDE ($CH_3$ Br) | Methyl bromide with or without 2% chloropicrin in cylinders and cans | 50 (whole grains)<br>50 (milled products) ( as total bromide from all sources)<br>5 (whole grain);<br>1 (in milled products)<br>as methyl bromide | 25(whole grains)<br>25 (milled products) as totall bromide from all Sources<br>— |
| ETHYLENE OXIDE ($(CH_2)_2$ 0) | 1:9 and 9:1 with $CO_2$ in cylinders | 50 ppm as ethylene oxide;<br>100 ppm as ethylene chlorohydrin / bromohydrin | |

*PHOSPHINE*

Phosphine is a low molecular weight, low boiling point compound that diffuses rapidly and penetrate deeply into materials such as large bulks of grain or tightly packed materials. It is a colourless, inflammable gas, which in pure form is odourless. But it gives a smell, similar to garlic due to the presence of impurities when generated from the formulations. The essential properties of phosphine are listed in Table 1.5. Phosphine is liberated usually from metallic phosphides, namely aluminium or magnesium phosphide preparations when exposed to atmospheric moisture.

$$AlP + 3H_2O \rightarrow PH_{3\uparrow} + Al(OH)_3$$

$$Mg_3P_2 + 6H_2O \rightarrow 2PH_{3\uparrow} + 3Mg(OH)_2$$

The aluminium and magnesium phosphides are formulated as tablets, pellets or granules in sachets. These formulations contain additional materials such as ammonium carbonate, ammonium bicarbonate, urea and paraffin to regulate release of fumigant and suppress flammability. Phosphine as a fumigant has many desirable properties such as ease in handling, less residue problems and low cost.

Phosphine is very toxic to all forms of animal life, and ranks as one of the most toxic fumigants of stored product insects. It is slow acting against stored product insect pests. The fumigant is effective at very low concentrations if the exposure time is long enough. The toxicity of phosphine to insects declines as the temperature falls to 15°C or less, so that longer exposure periods are required to exert its effect. Phosphine is not recommended for use below 10°C.

**Table 1.5 Essential properties of phosphine**

| | |
|---|---|
| Alternate name | Hydrogen phosphide |
| Chemical formula Odour | $PH_3$<br>Odourless upto 200 ppm. Carbide or garlic-like odour due to impurities present |
| Boiling point | -87.4° C |
| Freezing point | -133.5° C |
| Molecular weight | 34.04 |
| Specific gravity ; gas (air =1) | 1.214 |
| liquid (water at 4° C = 1) | 0.746.90 |
| Latent heat of vaporization | 102.6 cal/g |
| Lowest explosion point | 1.79% by volume in air |
| Solubility in water | 26cc/100mlat17°C |
| Pertinent chemical properties | Reacts with copper and precious metals. |
| Natural vapour pressure at different temperatures | |
| 0°C | 21.6atmos |
| 20° C | 34.2 atmos |
| 30°C | 42.0 atmos |
| 40° C | 51.9 atmos |

Phosphine has an inhibitory effect on insect respiration and is unique in that it is toxic to insects only when oxygen is present. The action of phosphine is potentiated by carbon dioxide (Bond 1984).

Reports indicate that phosphine can be used for treatment of spices and spice products (pruthi, 1980; Muthu and Majumder 1974). A dosage of 2 to 4 aluminium phosphide tablets (2 to 4g phosphine) per tonne of commodity for not less than 5 days has been used against spices and spice products. At the end of fumigation the spent powder (ash) comprising mainly metal hydroxide and 3 to 5% unreacted metal phosphide are removed and disposed off after deactivation. Cylinder-based phosphine formulation, i.e. about 2 - 3% phosphine by weight in carbon dioxide and nitrogen is also available in some countries such as Australia, Germany, USA and UK. At present most of the stored product insects including *L. serricorne* have been reported to have developed different degrees of resistance to phosphine and the problem appears to be more acute in the tropical region (Subramanyan and Hagstrum 1996).

***METHYL BROMIDE***

Methyl bromide is a highly poisonous, colourless and odourless gas. It is chemically stable, non-explosive, free from fire risks. The essential properties are listed in Table 1.6. It is marketed in cylinders in different capacities in 1 and 1.5 lb cans with or without the warning agent, chloropicrin at 2 % w/w.

Methyl bromide is highly toxic to a wide range of insect pests. Unlike phosphine, this fumigant is effective against insects even at lower temperatures (10° C or less). The gas is relatively less effective than ethylene oxide against microbes (Bond 1984).

Methyl bromide is recommended for fumigating spices and spice mixes generally at 24 - 48g/$m^3$ with an exposure period of 24 - 48 hrs under normal atmospheric conditions. It is particularly suitable for vacuum fumigation of processed and packed products including spice powders (Muthu and Majumder 1974). Methyl bromide been declared as a serious ozone depletor with an Ozone Depleting Potential (ODP) 0.7 (UNEP 1995). Repeated treatments of spice products with the fumigant may ca discolouration and loss of essential oils (Pruthi, 1980). Spices fumigated under atmospheric conditions with methyl bromide at 96 g/$m^3$ for 12 hr showed inorganic bromide residues exceeding the permissible limits of 50 ppm (Reeves et al., 1985).

**Table 1.6 Essential properties of methyl bromide**

| | |
|---|---|
| Alternative name | Monobromomethane |
| Chemical formula | $CH_3Br$ |
| Odour | Nil at low concentration; strong musty or sickly sweet at high concentration |
| Boiling point | 3.6° C |
| Freezing point | -93° C |
| Molecular weight | 94.95 |
| Specific gravity; gas (air =1) | 3.27 at 0° C |
| liquid (water at 4° C = 1) | 1.732 at 0°C |
| Flammability | Non-flammable |
| Solubility in water | 1.34g/l00ml at 25°C |
| Pertinent chemical properties | Powerful solvent of organic materials. In pure form, non-corrosive to metals. Liquid reacts with aluminium |
| Method of evolution as fumIgant | From steel cylinders under natural or added pressure. Also dispensed from 1 or 1.5 lb cans. |
| Natural vapour pressure at different temperature | |
| 0°C | 690 mm Hg |
| 10° C | 1006 mm Hg |
| 20° C | 1390 mm Hg |
| 25° C | 1610 mm Hg |

*ETHYLENE OXIDE*

Ethylene oxide is an inflammable, colourless gas with irritating odour, which is detectable only at high concentrations (Table 1.7). It is supplied in cylinders at 1: 9 and 9:1 admixture with carbon dioxide gas. Ethylene oxide is used primarily for sterilisation of spices although during the treatment insects are also controlled (Bond 1984).

In rare instances, ethylene oxide fumigation affected the quality of spices. The volatile oil content was decreased after ethylene oxide treatment of certain spices. Fumigation of cloves by ethylene oxide at dosages of 550, 750, 1000 $g/m^3$ for 6 hr at 20-25° C under reduced pressure resulted in 1.7 to 2.2 % decrease in the volatile oil content (Coretti and Inal 1969). Vajdi and Periera (1973) observed a similar effect in black pepper and allspice exposed for 16 hr (dosage not clear). The treatment affected the colour of black pepper. Formation of ethylene chlorohydrin residues in ethylene oxide treated spices has been reported (Gerhardt and Ladd-Effio 1983). Kuruppu et al., (1985) in their study found that the antioxidative properties of the spices such as marjoram, nutmeg, paprika and black pepper were not affected by ethylene oxide treatment.

Ethylene oxide is a suspected carcinogen and it forms chlorohydrin or bromohydrin residues, which are also carcinogenic. Hence, the use of ethylene oxide has been discontinued in many developed nations.

coriander, cumin, turmeric and chillies treated by gamma radiation at 10 kGy and stored for 6 months proved to retain good microbiological quality (Alam et al.1992).

The effect of irradiation on the physical appearance, chemical quality and sensory characteristics has been examined by many workers (Bachman & Gieszczynska 1973; Byun et al., 1983; Lescano et al .,1991 ). In general, no adverse effect has been observed in spices irradiated at the recommended dosage of 10 kGy. Nevertheless, there are a few reports on the changes in the quality of spices following treatment (Table 1.8). The yield of volatile oil constituents in spice mixtures, white pepper, nutmeg and ginger increased after irradiation of the commodities at the dosage of 2-25 kGy (Tjaberg, et al. 1972; Bachman and Gieszczynska 1973). The γ-terpene content increased in dried thyme treated at 30 kGy (Venskutonis et al. 1996). Loss of major flavour components such as anethole, anisaldehyde and chavicol in anise, anisaldehyde in fennel and β-pinene and cineol in black pepper occurred after irradiation treatment at 10-kGy (Farag et al. 1996). In the black pepper irradiation resulted in conversion of monoterpene hydrocarbons to terpenes. Lescano et al. (1991) observed that the pH of the ground ginger, turmeric and garlic powder was affected by irradiation treatment at 10-30 kGy.

Colour and appearance play a vital role in determining the consumer acceptance of spices. Studies so far have indicated that the appearance and sensory qualities of irradiated spices/spice products remain unaffected (Narvaiz et a1 1989, Szabad and Kiss, 1979, Lescano et aI, 1991; lnal et al. 1975). Nevertheless, consumer unawareness,

lack of well-defined standards, lack of significant data on wholesomeness of irradiated foods, and cost of the process are some of the limiting factors affecting the wide use of the process (Thakur and Singh 1995).

## HEAT TREATMENT

Insect control in food commodities including spices by heat treatment has been considered since long time. However, the insulating effect of spices in bulk greatly increases the time required for heat treatment and therefore is expensive. Furthermore, the treatment time is critical as excess heat may affect the quality of spice, for example colour and pungency in capsicum (Govindarajan, 1985). Farag et al., (1996) however noticed that in spices such as black pepper, fennel, coriander, anise and turmeric heat treatment at 70° C for 15 minutes did not affect the quality. The microbial load was reduced partly by the application of heat. Sterilisation of spices with pulsed steam, in which actually the steam condenses on the spice particles, helped to eliminate microorganisms with minimum adverse effects on flavour or aroma (Leife, 1992). Subsequently, the spices need to be dried to eliminate moisture absorbed by the steam treatment. Dehne and Bogi (1993) reported that pasteurisation of spices could be done by high frequency and microwave treatments as an alternative to irradiation and fumigation. Following high frequency treatment at 30KW, 27.12 MHz for 15-30 min, loss in essential oil content was noted in ground cinnamon, crushed white and black pepper, caraway (whole), and marjoram leaves. The loss was more in ground spices than in whole spices.

Thermal regulating methods generally cause one or the other undesirable changes in the treated products. Hence heat treatment can be supplemented or combined with other control measures. Thakur and Singh (1995) suggested that irradiation could be used in combination with the other methods such as cryogenic temperature, modified

atmosphere or vacuum packing. However all these processes are labour intensive, time consuming and expensive.

## CONTROLLED ATMOSPHERES

Due to residue problems in foodstuffs, adverse effects on environment, regulatory restrictions and consumer aversion towards pesticides there is an increased emphasis on non-chemical methods of preserving food commodities (Shjejbal, 1980; Ripp, 1984; Donahaye and Navarro,1987; Highly et al., 1994). In this context controlled atmospheres wherein the normal composition of atmospheric air i.e. 21% oxygen, 0.03% CO2 and 78% nitrogen is altered appropriately, have been used for the control of insect and other pests. Accordingly an atmosphere containing more than 35% CO2 known as carbon dioxide atmosphere and the atmosphere containing less than 1% oxygen i.e. low oxygen atmosphere are lethal to insects. This modified atmosphere (MA) or controlled atmosphere (CA) storage has been shown to be promising in creating lethal conditions for insects and fungi in stored food commodities. In recent years, there has been growing interest on the use of controlled atmospheres to manage insect pests in stored grain. This, method is already used for disinfestations and storage of food grains in Australia and Indonesia (Annis, 1987; Ripp et al., 1990). However, the application of carbon dioxide-rich atmosphere for Insect control and storage of spices has not been studied.

*CARBON DIOXIDE*

Carbon dioxide has been known to be toxic to insects since long and has received considerable attention in disinfesting stored products. It is a nonflammable, colourless gas with a pungent smell at high concentrations . It is 1.5 times as heavy as air and liquifies at -56.6°C at a pressure of 5.11 atmosphere. It is supplied either as liquid maintained by its own vapour pressure (58.3 $Kg/cm^2$ at 30°C ), or as solid 'dry ice' subliming at -78.5°C (Table 1.9). The normal concentration of $CO_2$ in air is about 0.03%. The hygienic standard (i.e. the concentration to which a worker may be continually exposed without ill effects) in many countries is 0.5%. Concentrations in the range of 2 to 5% cause a noticeable increase in the rate of breathing, from 5-10% breathing becomes laborious and at 10% it can voluntarily be endured for only a few minutes. At high concentrations, $CO_2$ acts as an asphyxiant and can paralyze the respiratory centre altogether. Exposure to 12 - 15% CO2 causes unconsciousness, while 25% will lead to death in a few hours. Recovery from exposure to high concentrations of CO2 is generally complete, with no long term effects on health.

The benefits of airtight storage, in terms of preserving grain quality, have been recognized for many years. Generally, $CO_2$-rich atmospheres shown to have no detrimental effects on overall storability of grain (Banks 1981). In storage of fresh fruits and vegetables the $CO_2$-rich atmospheres have shown beneficial effects such as reduction of respiration and inhibition of ethylene production and action, retardation of colour

change and softening, maintenance of nutritional composition, reduction of physiological disorders and inhibition of decay (Ke and Kader 1992). $CO_2$-rich atmosphere is effective in limiting the growth of many moulds, yeasts and bacteria in stored products, and preserves grain quality and does not adversely affect the chemical composition of stored grain (Bell and Armitage 1992; Jayas et al. 1991). The adequately packaged baked products in $CO_2$ atmosphere seems to have reduced rate of staling (Seiler 1983; Knorr and Tomlins 1985). Physical appearance, cooking quality and palatability of cooked rice has not been affected when rice was stored under $CO_2$ for more than six months (Sukprakaran et al. 1990; Gras and Bason, 1990). Similarly, no significant change in blistering of peel and sugar formation in dates was observed even after 4.5 months of storage under enriched $CO_2$ atmosphere (Donahaye et al. 1999). Altogether, no deleterious quality effects have been noticed on barley, rice (brown and white) rhye, soy beans, and wheat stored in $CO_2$ atmosphere (Annis and Graver 1991). However, high-CO2 atmospheres shown to have very little effect on the quality of milled rice, maize and barley ( Gras and Bason 1990). Neverthless germination is likely to be affected by $CO_2$-rich atmospheres. A reduction in viability of seeds of paddy rice, maize, wheat, soybean, parsely, paprika, lettuce, kale, onion and grass seed stored under $CO_2$ atmosphere has been reported (Hamel 1990; Gras and Bason 1990).

Of late, techniques have been developed to treat stored products with $CO_2$ under high pressure (10 to 20 bar). The major advantage in application of $CO_2$ under high pressure is that the exposure period is drastically reduced to less than an hour (Reichmuth and Wohlgemuth 1994).

**Table 1.9 Essential properties of Carbon dioxide**

| | |
|---|---|
| Formula | $CO_2$ |
| Molecular weight | 44 |
| Boiling point (sublimation) | -78.5° C |
| Specific gravity of gas (air =1) | 1.51 |
| Gas density at 30° C, 1 atm ($Kg/m^3$) | 1.732 |
| Specific volume at 30° C, 1 atm ($m^3/Kg$) | 0.548 |
| Flammability limits in air | Non-flammable |
| Solubility in water v/v | 0.76% |
| Specific gravity at 30° C,60 % R.H. (liquid, Kg/L) | 0.93 |

## CONCLUSION

Stored spices and spice products are attacked by insect pests such as *Stegobium paniceum* and *Lasioderma serricorne.* In addition, these commodities are contaminated with moulds and other microbes. These pests are controlled either by fumigation or irradiation treatment. Fumigants currently used for controlling insects and micro organisms in spices have problems such as insect resistance, residues, carcinogenicity and environmental hazards. Irradiation, though accepted by regulatory authorities, is not widely used due to constraints in terms of economic costs

and consumer acceptance. Carbon dioxide treatment though adopted for storage and preservation of other agricultural commodities, the technique is yet to be applied for the protection of spices. In general, studies on pest control in stored spices and spice products are very limited when compared to other food commodities like cereals, pulses and oilseeds. Therefore, there is a need for detailed investigation on the application of $CO_2$ -rich atmospheres for disinfestation and disinfection of stored spices.

# EFFECT OF INSECT INFESTATION ON CHEMICAL COMPOSITION OF SPICES AND SPICE PRODUCTS

## INTRODUCTION

During storage spices and spice products are attacked by insect pests, which consume, contaminate and make the products favourable for growth of other spoilage microorganisms leading to quality and quantity loss. The effect of insect infestation on nutritive value or proximate composition of stored cereals and pulses have been widely studied. Changes in chemical composition and nutritional value of field bean (Venkat Rao et al. 1960a); wheat flour (Venkat Rao et al. 1960b); maize and cowpea (Rajan et al. 1975), sorghum (Pant and Susheela 1977); corn, greengram, bengalgram and redgram (Daniel et al.1977 ; Modgil and Mehta 1996); wheat, maize and sorghum, (Jood 1990) and in cassava chips (Prem Kumar et al. 1996) have been reported. In addition, the level of uric acid, was monitored to indicate the degree of infestation in bengalgram and redgram (Daniel et al. 1977; Modgil and Mehta 1996) corn, maize, blackgram, field beans, redgram, greengram, cowpea, and wheat flour (Swaminathan 1977, Modgil and Mehta 1994). However information about the changes in proximate or chemical composition and uric acid level in spices and spice products due to insect pest activity is very limited. Invariably spices and spice products are also prone to insect attack, particularly by *L. serricorne* and *S. paniceum.* Hence, the effect of insect infestation on proximate composition and uric acid level of selected spice/ spice products such as turmeric rhizomes, coriander powder and *sambar* powder (mixed spice powder) was

investigated. These commodities were chosen because they are highly susceptible to insect attack. Preliminary tests revealed that that chilli powder, garam masala powder (curry powder) and turmeric powders do not support development of either *S. paniceum* or *L. serricorne.*

# MATERIALS AND METHODS

## 1. Infestation of spice/ spice products:

Market samples of turmeric rhizomes, coriander powder, and *sambar* powder, 100 g each, were taken in 250 ml culture bottles. There were 4 replicates per product. Adults, 2-3 days old and mixed sex were used for the experiment. *L. serricorne* and *S. paniceum* 30 per replicate were released into separate set of bottles. The culture bottles were incubated at 27±2°C and 70% R. H. For each species there were two sets of experiments (1 set incubated for 3 months and another for six months) with four replicates each. For each spice product and insect species a set of controls without insects (four replicates) was maintained. Three and six month infestation periods were chosen based on time required for completion of one and two generation respectively by the test insects. At the end of infestation period, the insects were removed by sieving and the samples were analyzed for proximate composition such as moisture, total protein, total fat, total ash, total carbohydrate, energy content and uric acid content by following standard procedures. In all these determinations, four replicates for each product were maintained.

**2. Proximate composition of infested spice products:**

Moisture: Spice samples, 20 to 40 gram, were (4 replicates per product) distilled with toluene to estimate moisture content by Dean and Stark method (1920).

**Total Protein:** The total protein content was determined by the micro-Kjeldahl method (AOAC 1990). Two gram samples were digested in concentrated $H_2SO_4$ in the presence of catalyst mixture (for 8 h) until the solution became clear. The digested samples were cooled and made upto known volume (100 ml) with distilled water and aliquot (2 ml) of the solution was distilled in the micro- Kjeldahl apparatus with an excess of 40% NaOH. The ammonia liberated was trapped into 2% boric acid solution and titrated against standard HCl (N/70) using a mixed indicator and the protein content was calculated (Nx 6.25).

Total fat: The total fat content was determined using Soxhlet apparatus (AOAC 1990). The fat was extracted (10 g sample replicate ) using petroleum ether (40-60°C) and dried at 100°C to constant weight in hot air oven.

Total ash: The total ash content was estimated according to AOAC (1990). Five gram samples were taken in pre-weighed silica crucible and ignited at 450-500°C in a muffle furnace. Incineration was carried out to constant weight, and the ash was cooled and weighed.

**Total carbohydrates:** The total carbohydrate was calculated by differential method (AOAC 1990) from the moisture, total protein, fat and ash content.

Energy: The total energy contents of samples was calculated as per AOAC (1990) using protein, fat and carbohydrate content (% protein x4 + % fat x 9 + % carbohydrate x4).

**2. Determination of uric acid in insect infested spice products:**

Uric acid level in coriander powder, *sambar* powder and turmeric rhizomes infested with *L. serricorne* and *S. paniceum* for 3 and 6 months, was estimated by the method of AOAC (1995). Four gram sample of each was mixed with 5 ml glutathione solution (10 mg/ml) and left overnight. To this mixture, 25 ml of 1N NaOH (pH 9.0-9.3) was added and transferred to 100 ml glass stoppered cylinder. The solution was made upto 100 ml with sodium acetate solution (5%), then mixed, the mixing continued every 10 minutes for 1 hr. From this, 15 ml of solution was taken and centrifuged for 30 minutes at 3000 rpm. Supernatant (4 ml) of each sample was taken into 2 test tubes (named 1& 2) and 1 ml of borate buffer (0.01M, pH 9.2) was added and mixed well. To the 3$^{rd}$ test tube and test tube No.1, 5 ml of uricase solution (100 mg in 50 ml of 0.01 M sodium borate buffer) was added and mixed well. Then the content of tube No.2 and 3 were mixed and the absorbance was read immediately in spectrophotometer at 292 nm against solution in tube No. 1 as blank. Similarly, control samples of each product were also carried out as earlier and their OD was subtracted from their respective infested samples, as the

absorbance values were high in spices. From the absorbance values the uric acid content (mg/100g) was calculated using standard graph.

**Standard curve:** Uric acid standard solution (containing 100µg per ml of 5% sodium acetate) corresponding to 0-10µg uric acid per ml in final solution were taken and the procedure given above was followed. From the absorbance values standard curve was drawn by plotting absorbance versus concentration of uric acid (Figure 2.1).

**4. Statistical analysis:** All the data were subjected to analysis of variance (ANOYA) (Snedecor and Cochran, 1967) and significance if any against respective controls was accepted at 1 or 5% levels.

## RESULTS AND DISCUSSION

**Proximate composition of infested spice/spice products:**

Data on proximate composition of coriander powder, *sambar* powder and turmeric rhizomes infested with *L. serricorne* for 3 and 6 months are given in Table 2.1. The moisture content increased significantly in coriander powder (40.7 and 50.8%) and *Jambor* powder (20.6 and 25%) infested for 3 and 6 months respectively. However in turmeric rhizomes the increase was not significant ($p>0.05$) (5.4 and 6.9%). The total protein, fat, and ash content were reduced significantly ($p< 0.05$) by 9.5 and 23.6% (protein), 19 and 26.4% (fat) and 4.8 and 12.6% (ash) in coriander powder infested for 3

and 6 months respectively (Figure 2.2a) when compared with control values. Similarly, there was a significant reduction in protein (17 and 27.6%), fat (19.2 and 26.4%), and ash (27.3 and 34.8%) content in *sambar* powder (Figure 2.2b), and protein (11.8 and 27.1%), fat (8.3 and 16.6%) and ash (3.1 and 9%) content in turmeric rhizomes (Figure 2.2c) infested for 3 and 6 months respectively. There was only a slight reduction in carbohydrate and energy content in infested samples. The maximum reduction in total protein and fat occurred in coriander powder followed by *sambar* powder and turmeric rhizomes. The reduction or increase in proximate components due to *L. serricorne* was more in products infested for 6 months than in 3 months infested products.

Changes in proximate composition of the commodities was noticed following *S. paniceum* infestation for 3 and 6 months (Table 2.2). An increase in moisture content in coriander powder (41.6 and 49.2%), *sambar* powder (20.9 and 27.3%), and turmeric rhizomes (4.6 and 6.9%) was observed (Figures 2.3a, 2.3b, and 2.3c). A significant ($p<0.01$) reduction in total protein (14.3 and 30.7%), total fat (2land 32.4%), and ash (12.2 and 39%) content was noted in coriander powder (Figure 2.3a). There was a significant reduction in protein (16.3 and 31.3%), fat (19.2 and 35.8%), and ash (22.7 and 39.1%) content *of sambar* powder (Figure 2.3b); and protein (10.2 and 25.4%), fat (4.2 and 6.6%) and ash (3.0 and 7.4%) content of turmeric rhizomes (Figure 2.3c). Similar to *L. serricorne* infestation, the reduction of carbohydrates and energy contents was not significant in *S. paniceum* infested products. The data revealed that the maximum reduction in proximate composition occurred in coriander powder and *sambar* powder when compared with turmeric rhizomes. The reduction or increase in proximate

components was more pronounced in products infested for 6 months than that infested for 3 months only.

In general, the moisture content of spice/ spice products infested by *L. serricorne* or *S. paniceum* was significantly increased (upto 50%) in 6 months infested samples. Insect proliferation and resultant higher metabolic activity have contributed to the increase in moisture content. An increase in moisture content was also reported in bengalgram, fieldbean, greengram and redgram infested with pulse beetle, *Callosobrachus chinensis* (Daniel et al 1977; Shehnaz and Theophillus 1975; Modgil and Metha 1994 and 1996); sorghum infested with rust red flour beetle *Tribolium castaneum* (Pant and Susheela 1977) and blackgram infested with *Callosobruchus maculatus* F. (Gupta et al. 1981). Infestation of *L. serricorne* and *S. paniceum* reduced the level of nutrient (protein, fat, carbohydrates and ash) content of spice/ spice products (Tables 2.1 and 2.2). This observation is similar to that of Venkat Rao et al. (1960c) with sorghum grains that was infested by the rice weevil, *Sitophilus oryzae* for 6 months, with wheat infested by multiple pests (Samuels and Modgil 1999) and maize and cowpea infested with *S. oryzae* and *C. chinensis* for 5 months respectively (Rajan et al. 1975). Shehnaz and Theophilus (1975) noticed a significant loss of protein quality due to insect infestation on bengal gram and fieldbean. Observations on increased protein contents in infested pulses (Modgil and Mehta 1994 and 1996) have been reported. The increase has been due to the presence of immature stages inside the kernels/grains and the samples were analysed along with insect present. However, in the present study, insects have been removed from the samples before analysis and hence no such increase in protein

levels was noticed. Losses in vitamin content (thiamine) in pulses, maize and cowpea due to infestation have been reported by Venkat Rao et al. (1959) and Rajan et al$_e$ (1975). Jood and Kapoor (1994) observed losses in thiamine, riboflavin and niacin contents of cereals infested by the khapra beetle, *Trogoderma granarium* and the lesser grain borer, *Rhyzopertha dominica.* Further a significant decrease in essential amino acids of wheat, maize and sorghum was observed by Jood et al (1995), and Pant and Susheela (1977) due to *R. dominica, T granarium* and *T. castaneum* infestation. The nutrient losses are also evidenced in wheat (Venkat Rao et al. 1960b; Rajan et al. 1975); greengram, redgramand bengalgram (Modgil and Mehta, 1994 and 1996); bengalgram and redgram (Daniel et al. 1977) with insect infestation. In the current investigation, the decreased levels of total carbohydrates of spice products was observed following insect infestation (Table 2.2). With increase in duration of infestation, there was progressive increase in loss of not only total carbohydrates but also protein, fat and ash content A significant reduction in total carbohydrates has been noticed in cereals infested by *S. oryzae* ( Girish et at. 1975, Sharma et al 1979). Jood et al. (1993) reported that total reducing and nonreducing sugar content of cereals were affected due to infestation by *T. granarium* and *R. dominica.* The decrease in nutrient contents has been due to consumption by insects. A decrease in calorific value has also been observed, but the decrease was not significant. Similar results have been reported in greengram and redgram infested with *C. chinensis* (Modgil and Mehta 1996).

Uric acid levels in infested spice products**:**

The uric acid levels increased significantly in infested samples (Table 2.3). The uric acid level was 12 to 15% more in case of 3 months infested spices while it was 20 to 30% more in 6 months infested spice products. In general, the level of uric acid was slightly more in S. *paniceum* infested products than in *L. serricorne* infested ones. This indicates that *S. paniceum* breed fast in the tested spice/ spice products than *L. serricorne.* The uric acid level of infested samples of spice/ spice products, exceeded the maximum allowable limit fixed by Prevention of Food Adulteration (PFA) Act (1954).

Reports on uric acid level in infested spices/ spice products are very limited (Brown et al. 1982). The increased level of uric acid in 6 months infested samples over that of 3 months' batches was due to increased insect population. Similar trend was observed in cereals and legumes infested for different periods (2-6 months) (Daniel et al 1977, Swaminathan 1977), in wheat flour (Venkat Rao et at. 1960d), wheat (Samuels and Modgil l999) or in certain stored pulses (Modgil and Mehta 1994 and 1996).

**Table 2.1 Proximate composition of spice/ spice products infested with *L. serricorne* for 3 and 6 months.**

| Commodity | Moisture content (%) | Total protein (%) | Total fat (%) | Total ash (%) | Total carbobyd-rates (%) | Energy (Kcals.) |
|---|---|---|---|---|---|---|
| **3 months** | | | | | | |
| **Coriander powder** | 6.00± O.13 | 12.6±O.26 | 15.2±O.17 | 8.2±O.38 | 60.2±3.5 | 419.2 |
| Control | 8.4±O.18[8] | 11.4±O.0I | 12.2±O.05 | 7.7±O.50 | 57.9±2.5 | 396.1 |
| Infested | | | | | | |
| *Sambar* **powder** | 8.7±O.21 | 13.5±O.3 | 5.2±O.15 | 2.2±O.17 | 72.5±1:4.8 | 382.6 |
| Control | 10.5±tO.22[b] | 11.2±O.23[b] | 4.2±O.08 | 1.6±O.54[8] | 70.4±1:3.2 | 372.7 |
| Infested | | | | | | |
| **Turmeric rhizomes** | 13.0±tO.05 | 5.9±O.Ol | 2.4±O.02 | 6.6±O.00 | 72.5±1:4.3 | 333.6 |
| Control | 13.7±tO.06 | 5.2±O.00 | 2.2±O.02 | 6.4±O.00 | 72.1±1:3.2 | 330.6 |
| Infested | | | | | | |
| **6 months** | | | | | | |
| **Coriander powder** | 6.3±O.08 | 12.7±O.05 | 14.8±O.09 | 7.8±O.09 | 63.1±1:4.5 | 417.6 |
| Control | 9.5±O.05[8] | 9.7±O.1 1[8] | 10.9±O.08[8] | 6.8±O.05 | 58.4±1:3.5 | 389.3 |
| Infested | | | | | | |
| ***Santbar* powder** | 8.8±O.04 | 13.4±O.09 | 5.3±O.02 | 2.3±O.02 | 73.9±2.8 | 382.1 |
| Control | II.O±O.10[8]. | 9.7±O. 10[8] | 3.9±O.04 | 1.5±O.03 | 70.2±1:3.2 | 369.5 |
| Infested | | | | | | |
| **Turmeric rhizomes** | 13.0±O.10 | 5.9±O.10 | 2.4±O.00 | 6.7±O.10 | 73.7±3.8 | 333.2 |
| Control | 13.9±O.l0 | 4.3:tO. l0[8] | 2.0±O. l0[b] | 6.1±O.10 | 72.0±2.5 | 330.0 |
| Infested | | | | | | |

Values are mean ±SO of four analysis.
Values [8] and are significantly different from respective controls at [a]p<O.Ol [b] p<O.O5 levels.

**Table 2.2 Proximate composition of spice! spice products infested with *S. paniceum* for 3 and 6 months.**

| Commodity | Moisture content (%) | Total protein (%) | Total fat (%) | Total ash (%) | tal carbo-hydrates ( % | EnergJ (Kcals. |
|---|---|---|---|---|---|---|
| **3 months** | | | | | | |
| **Coriander powder** | 6.0±O.13 | 12.6:tO.26 | 15.2±O.20 | 8.2±O.40 | 60.2±1:3.5 | 419.2 |
| Control Infested | 8.5±O.20[8] | 10.8:tO.20 | 12.0±O. 1O[b] | 7.2±O.20 | **57.9±1:2.8** | 396.1 |
| *Sambar* **powder** | 8.6±0.21 | 13.5 ±:0.30 | 5..2±0.20 | 2.2±0.20 | 72.3±1:3.5 | 382.6 |
| Control Infested | 10.4 ±O.20[b] | 11.3±O.20 | 4.2±0.20[b] | 1.7±O.10[8] | 70.4±1:3.2 | 372.4 |
| **Turmeric rhizomes** | 13.0±1:0.10 | 5.9±O.00 | 2.4±1:0.02 | 6.6±O.00 | 72.4±1..2 | 333.6 |
| Control Infested | 13.6 ±1:0.10 | 5.3±1:0.00 | 2.3±10.00 | 6.4±0.00 | 72.1±1..1 | 331.5 |
| **6 months** | | | | | | |
| **Coriander powder** | 6.3±O.04 | 12.7±O.10 | 14.8±O.10 | 7.8±0.10 | 65.7±1:3.0 | 417.6 |
| Control Infested | 9.4±O.108 | 8.8±O.20[8]. | 10.0±0.20[8] | 6.1±O.10[8] | 58.4±1:2.0 | 388.0 |
| *Samba,* **powder** | 8.8±0.04 | 13..4±0.10 | 5.3±0.02 | 2.3±0.02 | 79.8±1:3.5 | 382.1 |
| Control. Infested | 11.2±0.10[8] | 9.2±0.10[8] | 3.4±0.10[8] | 1.4±O.01 | 70.2±1:2.0 | 366.6 |
| **Turmeric rhizomes** | 13.0±O.10 | 5.9±O.10 | 2.4±O.0 | 6.7±0.10 | 73.5±2.5 | 333.2 |
| Control Infested | 13.9±0.00 | 4.4±0.10[8] | 2.0±0.1 | 6.2±0.10 | 72.0±1.5 | 329.6 |

Values are mean 1 SD of four analysis.
Values a and **b** are significantly different from respective controls at [8] p<O.OI, [b] p<0.05 levels.

**Table 2.3 Uric acid levels in infested spice/spice products**

| Spice/ Spice products | Infestation with | Uric acid level* (mg/lOOg) following infestation for | |
|---|---|---|---|
| | | 3 months | 6 months |
| Coriander powder | (Control) | 0 | 0 |
| | *S. paniceum* | 15.0±O.26 | 30.8±O.57 |
| | *L. serricorne* | 13.9±O.15 | 27.6±O.58 |
| *bar* powder | (Control) | 0 | 0 |
| | *S. paniceum* | 13.2±O.29 | 27.6±O.11 |
| | *L. serricorne* | 12.2±O.33 | 22.7±O.24 |
| Tunneric rhizomes | (Control) | 0 | 0 |
| | *S. paniceum* | 11.9±O.39 | 21.6±O.68 |
| | *L. serricorne* | 11.6±O.19 | 2O.5±O.43 |

* Values are mean ± SD of 4 replicate analysis. The values for infested samples are significantly different from respective controls ($p<O.O5$)

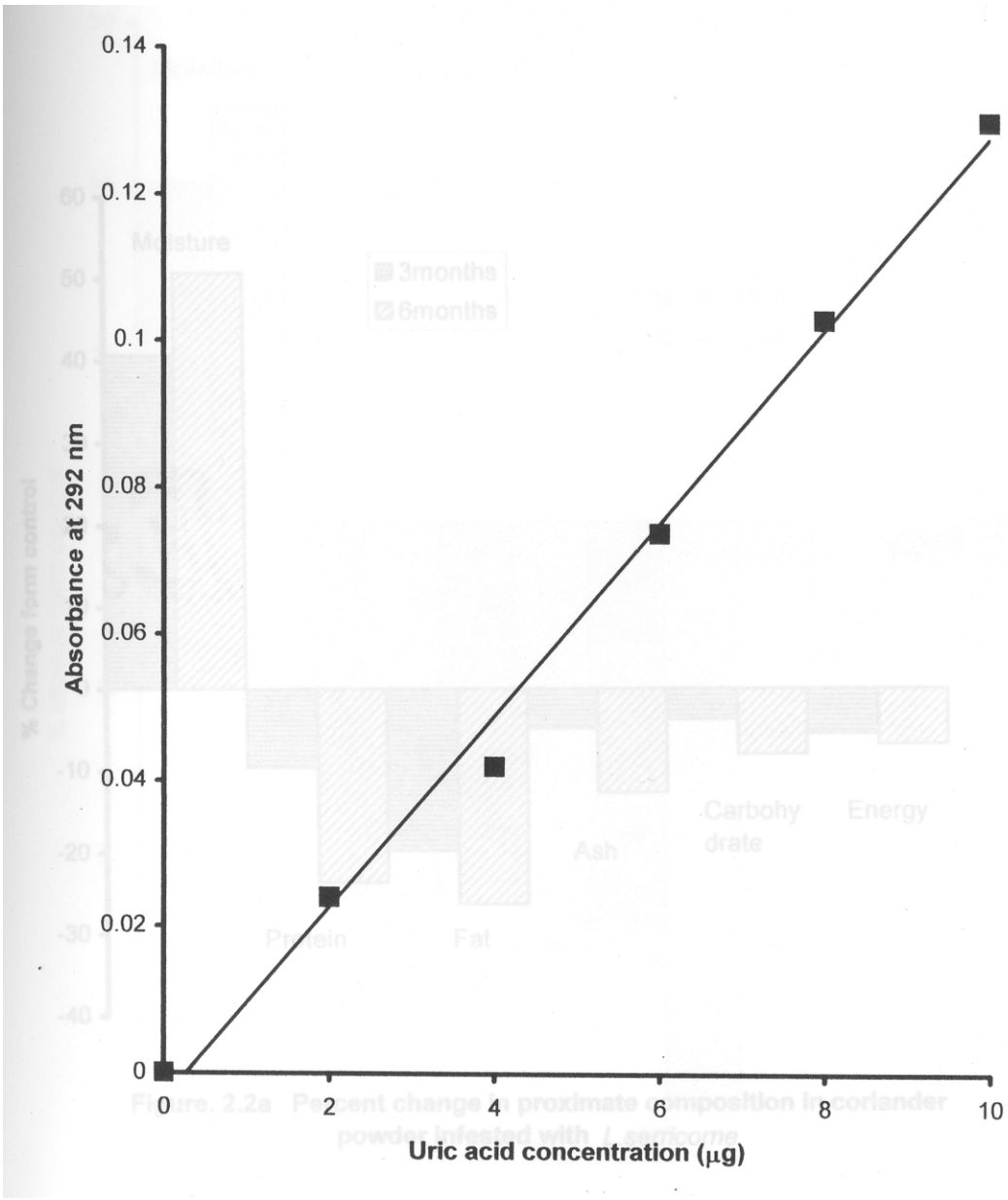



Figure 2.1 Standard Graph for uric acid

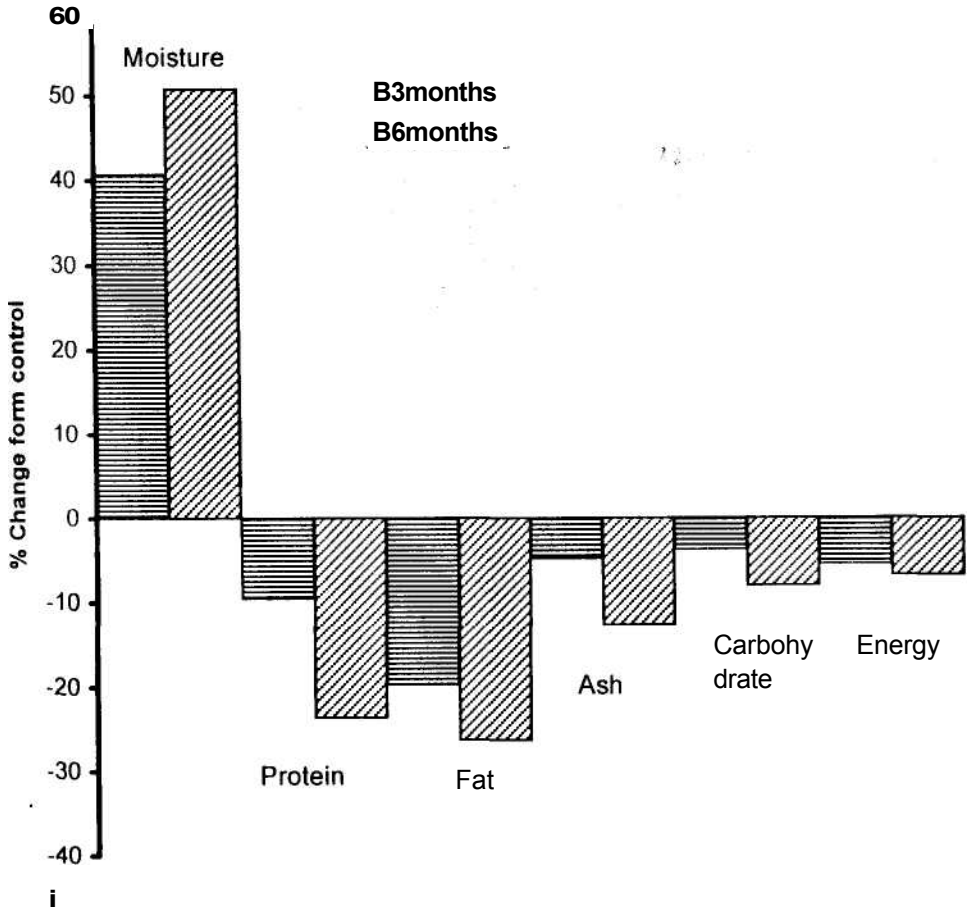



**Figure. 2.2a Percent change in proximate composition in coriander powder infested with** *L.serricorne*

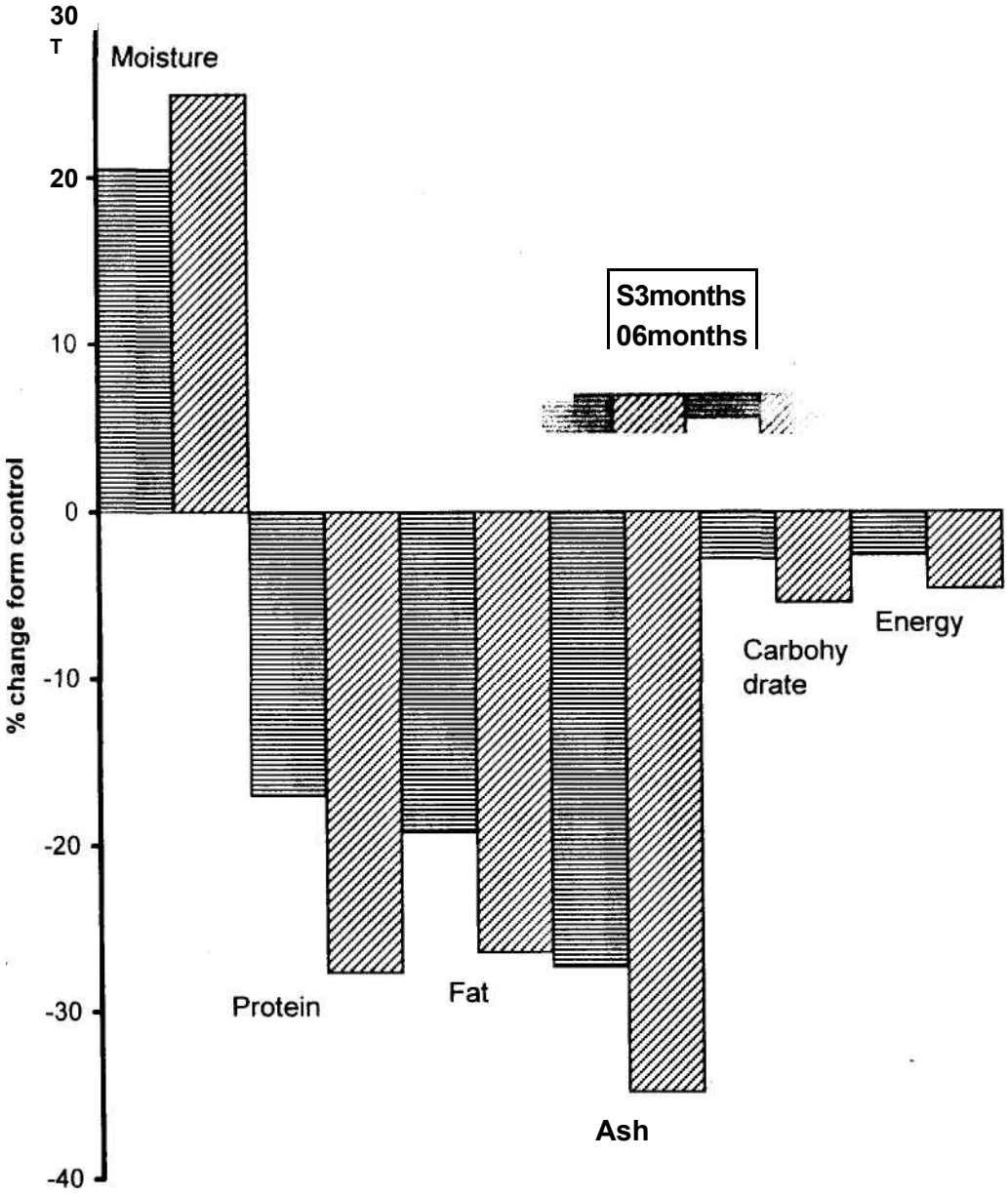



**Figure 2.2b Percent change in proximate composition of *samba,* powder infested with *L.serricorne***

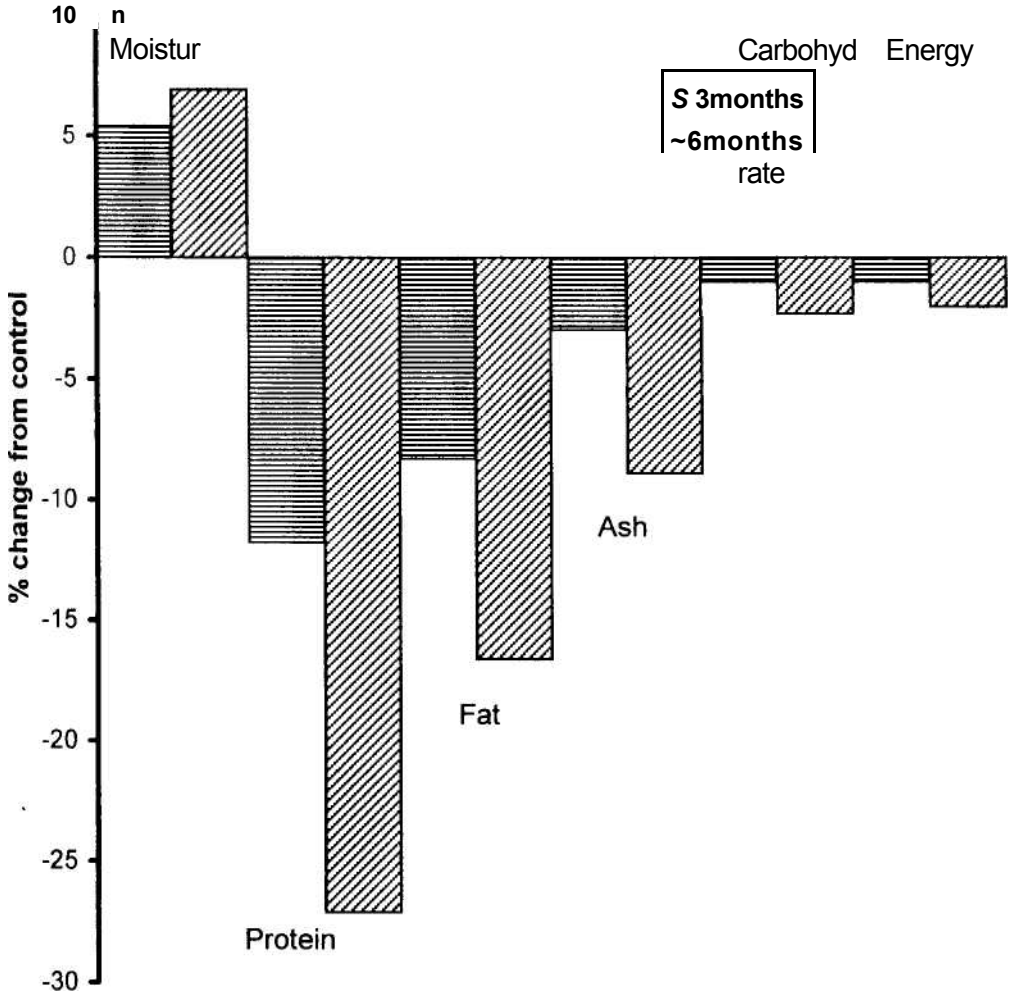



**Figure 2.2c Percent change in proximate composition in turmeric rhizomes infested with *L. serricorne***

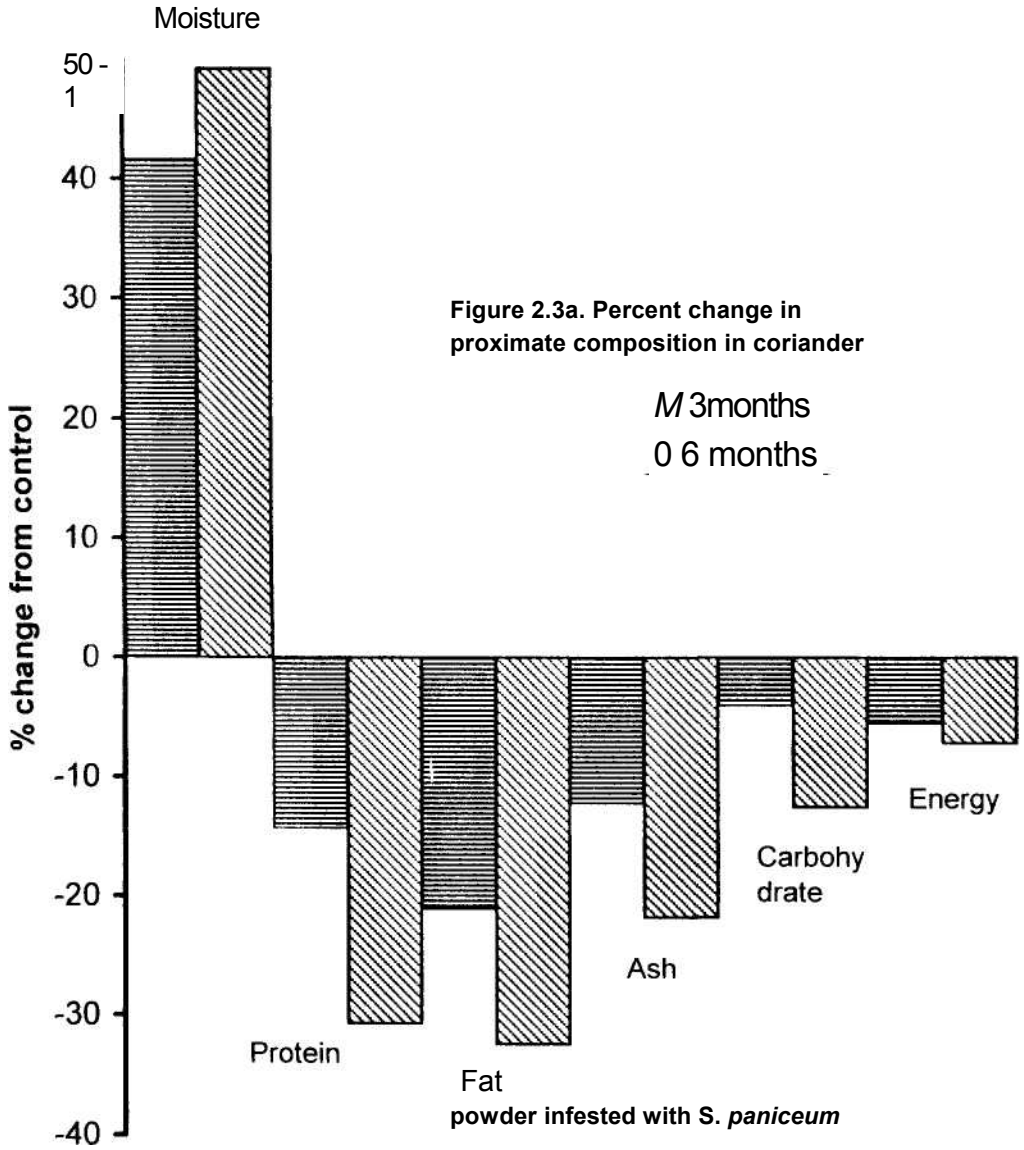



**Figure 2.3a. Percent change in proximate composition in coriander powder infested with S. *paniceum***

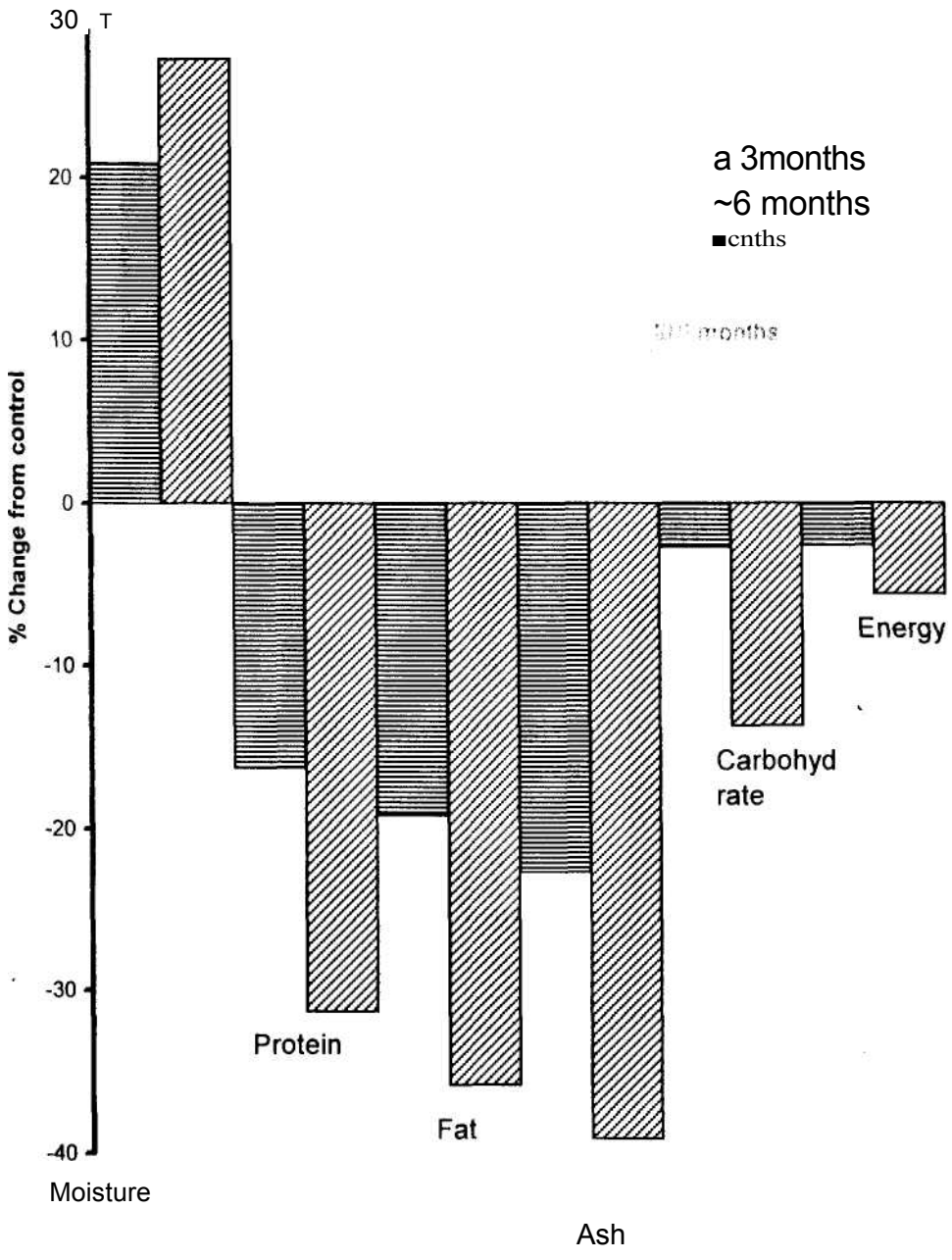



**Figure 2.3b. Percent change in proxiamte composition in *samba,* powder infested with *S.paniceum***

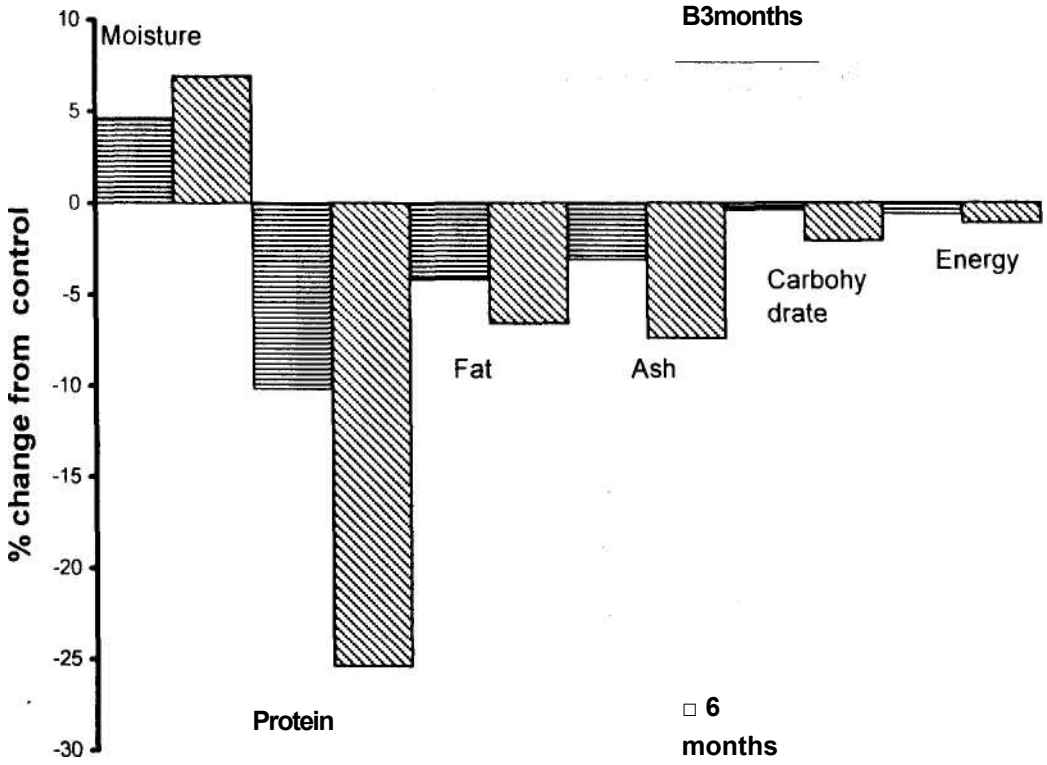



**Figure. 2.3c. Percent change in proximate composition in turmeric rhizomes infested with *S.paniceum***

# CONCLUSION

The effect of *L. serricorne* and *S. paniceum* infestation on proximate composition and uric acid level of coriander powder, *sambar* powder and turmeric rhizomes have been studied. The moisture content increased significantly after infestation in coriander powder and *sambar* powder. The total protein, fat, ash contents were reduced significantly. A slight reduction in carbohydrates and energy content in infested samples was also noticed. The maximum reduction in proximate composition occurred in coriander powder and *sambar* powder. The reduction or increase in proximate components was more pronounced in products infested for 6 months than that infested for 3 months only. The uric acid level in infested samples was noted to exceed the maximum allowable limit fixed by Prevention of Food Adulteration Act (1954).

# Chapter three

# Evaluation of toxicity of carbon dioxide against life stages of *Stegobium paniceum* and *Lasioderma serricorne*

# EVALUATION OF TOXICITY OF CARBON DIOXIDE AGAINST LIFE STAGES OF *STEGOBIUM PANICEUM* AND *LASIODERMA SERRICORNE*

## INTRODUCTION

The stored product pest management system of the future is now open to question, due to the decreasing tolerance of residues in foodstuffs and the increasing incidence of insect resistance to pesticides. Therefore, there is a pressing need to develop alternative non-chemical pest control techniques for protecting stored food commodities. In this context carbon dioxide that has long been known to be toxic to stored product insects, received considerable attention in disinfesting stored foodstuffs, particularly the durable products. The effect of controlled atmospheres on a wide range of stored product insects has been reported (Bailey and Banks 1980 Bell and Armitage 1992). Also there are several reports about the toxic effect of $CO_2$ rich atmosphere on insect pests of stored products (Annis 1987). These investigations have shown that $CO_2$ is a feasible insect control agent. Generally insects are far more tolerant of $CO_2$ exposure than mammals, and are able to survive for long periods in the presence of high concentrations of $CO_2$, particularly at lower temperatures. Bailey (1955, 1956, 1957, 1965) studied the effect of high-$CO_2$ and low-oxygen atmospheres against the beetle pests such as *Sitophilus* spp. (all stages), *Rhyzopertha dominica* (F.), *Oryzaephilus surinamensis* (L.), *Tribolium castaneum* (Herbst), *Cryptolestes ferrugineus* Everts (larvae only). It was observed that with 14 to 17 days exposures at 32° C, all the species except *Sitophilus* spp. required 60%

$CO_2$ in air for complete kill. For controlling *Sitophilus* spp. about 40% $CO_2$ proved adequate at 25-29ºC.

Further studies on *S. granarius* and *S. oryzae* (Lindgren and Vincent, 1970; Marzke et al 1970); *T. castaneum* and *R.. dominica* (Pearman and Jay,1970; Ali Niazee, 1971; Calderon and Navarro, 1979, 1980) and *O. surinamensis* and *C. ferrugineus* (Anonymous, 1985) showed that much shorter exposures could achieve the same result at these high concentration levels and at lower temperatures. However, for larvae of *Trogoderma granarium,* it was noticed that exposure period longer than 17 days was required in tests with $CO_2$ levels at or below 60% in air at < 30°C (Spratt et al 1985). The acute mortality effects of $CO_2$ on various life stages of *S.oryzae* have been studied in detail (Annis and Morton 1997). The effect of high pressure $CO_2$ (20 bar pressure) on life stages of *Plodia interpunctella, Oryzaephilus surinamensis* and *Sitophilus oryzae* have been studied by Locatelli et al., (1999). Complete mortality of larvae and pupae of *P. interpunctella* was achieved after 5 minutes while 15 minutes at 20 bar pressure was required to prevent eggs. *O. surinamensis* eggs were more tolerant that 100% kill occurred following treatment for 45 minutes while larva and pupa took 10 minutes and adults required 5 minutes for total kill.

The toxicity of $CO_2$ to insects is known to vary between species, developmental stage, and age. Immature stages are generally tolerant than adults. The toxicity is also influenced by the physical environment like temperature and humidity, and $CO_2$ concentrations. Comprehensive toxicity data for some of the stored product

insects such as the *Stegobiuum paniceum* and *Lasioderma serricome* which are the major pests of stored spices and spice products are not available. Therefore, a comprehensive study on the mortality response of life stages of *S. paniceum* and *L. serricome* was carried out at atmospheric pressure, and room temperature (27 ± 2° C). In addition, the effect of insect response to $CO_2$ treatment at a low temperature of 20°C was also investigated. Furthermore, the differences if any in the response of adults of *S. paniceum* and *L. serricorne* to constant and changing $CO_2$ concentrations was also examined.

## MATERIALS AND METHODS

Culturing of test insects:

The culture medium comprised wheat flour with 5% yeast for both *L. serricorne* and *S. paniceum* (Childs and Overby 1983). The cultures were maintained at room temperature (27 ± 2° C) and 70% r.h. Larvae (20 - 22 days old) and pupae (4 - 5 days old) were obtained from these cultures for toxicity tests. In order to collect eggs for the experiments, freshly emerged adults were released in large numbers to wheat flour that has been passed through 180 micron mesh sieve. After two days the adults were removed by sieving the flour with a sieve (pore size 600 microns). Then wheat flour was sieved through 180 micron mesh sieve so that the eggs were retained in the sieve. The collected eggs were carefully counted under a binocular microscope for the experiments.

**1. Exposure of developmental stages to $CO_2$:**

Adults of *L. serricorne* and *S. paniceum,* 2 - 3 days old were exposed to different levels of $CO_2$ separately. For each species the insects, 40 per replicate, were taken in 7x1.2 cm size glass tubes containing paper strip. In each tube a paper strip of 5 cm x 0.5 cm size was kept for the insects to hold and rest. The open ends of the tubes containing insects were covered with muslin cloth. The tubes were placed in individual test chambers (gas-wash-bottles, 500 ml capacity) for exposing the insects to different levels (%) of $CO_2$. Four replicates were maintained for each level of $CO_2$ with equal number of untreated controls. The test chambers were flushed with $CO_2$ from a cylinder ( 99% purity). Before passing into the chamber the $CO_2$ was humidified to 70% r. h. by passing through 50% v/v glycerol water solution (Figure 3.1). Immediately after flushing with $CO_2$, the $CO_2$ levels in the exposure chambers were checked with Riken Infrared Gas Analyzer (Model RI 550A, Japan Make). When the desired $CO_2$ level inside the test chambers was achieved they were sealed with rubber septa. Then, the test chambers were left undisturbed for required exposure periods of 24, 48, 72 and 96 hours at 27 ± 2° C. At the end of the exposure period, the tubes containing test insects were taken out and transferred to another set of tubes. The latter were kept at room temperature (27 ± 2° C) and at 70% r. h. Mortality was assessed after three days. The corrected mortality was calculated after taking into consideration of natural mortality in untreated controls.

Similarly, developmental stages such as eggs (30 per replicate), larvae (40 per replicate), and pupae (30 per replicate) of *S. paniceum* and *L. serricorne* were taken in

24,48, 72 and 96 hours. At the end of exposure period, the tubes containing insects were removed from the test enclosure, and kept at room temperature (27 ± 2° C) and 70% r. h. After three days, the mortality was assessed and corrected mortality (% ± SD) detennined.

3. Exposure **of adults of *S. paniceum* and *L. serricorne* to changing concentrations of $CO_2$:**

The insects were tested at a Ct product of 960% hr (40% $CO_2$ x 24 hours = 960% hr). The Ct product was achieved by three different combination of $CO_2$ concentrations and exposure periods i.e. (1) constant (2) increasing and (3) decreasing $CO_2$ concentration. Accordingly an increasing order of concentration , 30, 40, and 60% $CO_2$ for 8, 12, and 4 hours exposure respectively and a decreasing order of concentration 60, 40 and 30% $CO_2$ for 4, 12 and 8 hours exposure respectively were selected. Altogether at three concentrations the adults of *S. paniceum* and *L. serricorne* were exposed separately.

Adults 2 - 3 days old of individual species were exposed to $CO_2$ separately as described earlier. There were 40 insects per replicate and six replicates each were maintained for increasing, decreasing and constant concentrations of $CO_2$ and for controls. All the three exposures viz constant, increasing and decreasing were carried out simultaneously for each species. $CO_2$ concentrations in the test chambers were created and adults were exposed as described earlier in the case of constant exposure. At the end of first dosage/ exposure (i.e. 30% $CO_2$ for 8 hours) of increasing order, the test chambers

were again flushed with $CO_2$ and checked with Riken Infrared Gas Analyzer. At the end of second dosage exposure (i.e. 40% $CO_2$ for 12 hours), the test chambers were again flushed with $CO_2$ (i.e. 60% $CO_2$ for 4 hours) and allowed to stand for third exposure. Similarly the concentrations were maintained in decreasing order (i.e. 60, 40, and 30% $CO_2$ for 4, 12 and 8 hours respectively) by diluting $CO_2$ with air and concentrations checked again. At the end of all three types of exposures the tubes containing insects were removed and incubated at room temperature (27 ± 2° C) and at 70% r. h. After three days, mortality was assessed.

## RESULTS AND DISCUSSIONS

Data on mortality (% ± SD) of adults of *S. paniceum* and *L. serricorne* exposed to different levels of CO2 at different exposure period are given in Table 3.1 and 3.2 respectively. In general, adults of L. *serricorne* were noted to be more tolerant than *S. paniceum* adults, as the former achieved 100% mortality at 70% $CO_2$ for 48 hr exposure, while in the latter 100% mortality was recorded even at 60% $CO_2$ in 24 hr. Similarly with longer exposure period i.e. 96 hr, 100% mortality of *L. serrricorne* adults was achieved with 40% CO2, whereas in *S. paniceum* 100% mortality was achieved with 35% $CO_2$ itself. The mortality data of adults of *L. serricorne* (Table 3.2), are comparable with that of Childs and Overby (1983) who treated the insects only at selected concentrations of $CO_2$ for a fixed exposure period. In tests at high pressure CO2 (20 - 40 bar), Gerard et al (1988) achieved complete kill of stored product beetle pests including *S. paniceum* and

*L. serricorne* within 2 hr. Adults of both *S. paniceum* and *L. serricorne* were found to be more susceptible than their immature stages (Fig. 3.2 and 3.3).

Data on mortality of eggs, larvae, and pupae of *S. paniceum* and *L. serricorne* are presented in Tables 3.3 and 3.4, 3.5 and 3.6 and 3.7 and 3.8 respectively. It is evident that the pupa was the most tolerant followed by larva, egg and adult. Childs and Overby (1983) noticed in *L. serricorne* that among the selected $CO_2$ concentrations (35, 65 and 92 %), the 65% $CO_2$ was generally most lethal to all stages. It was also observed that pupae from tobacco strain were more tolerant of $CO_2$ than pupae of wheat feed strain of *L. serricorne.* When life stages of *S. oryzae* were tested against a range of $CO_2$ concentrations (15- 100%), Annis (1990) and Annis and Morton (1997) observed that pupae were more tolerant than other stages. There are several reports indicating that the older larvae and pupae of *S. granarius* and *S. oryzae* were tolerant to $CO_2$ (Lindgren and Vincent, 1970; Annis, 1987; Navarro and Jay, 1987). A similar trend was observed in tests on the life stages of *T. castaneum* and *T. confusum* at different set of temperatures (AliNiazee 1971). When all stages of *T. granarium* were exposed to 60% CO2 in air at 20 and 30°C and 60% r.h, the larva was found to be tolerant than other stages (Spratt et al, 1985). Larvae of Indian meal moth, *Plodia interpunctella* were noted to be more susceptible than *T. castaneum* adults or larvae when exposed to binary mixtures of atmospheric gases ($CO_2$, $N_2$ and $O_2$ at various temperatures (15.6, 26.7 and 37.8° C), and the mortality increased with decrease in $O_2$ concentration and increase in $CO_2$ concentration, exposure period or temperature (Harein and Press 1968). Suss et al, (1993) studied the effect of $CO_2$ on life stages of the larger grain borer, *Prostephanus*

*truncatus* at 30 ± 2° C and 60 ± 3% r.h. It was observed that larvae were more tolerant than eggs or adults. The authors were not able to conclude about the response of pupae as the results were heterogeneous.

Adults of R. *dominica, S. oryzae, O. surinamensis* and *P. interpuncte//a* were more susceptible at 20°C, than under treatment at 25±2°C. However, the eggs of *P. interpuncte//a* were more tolerant when they were exposed to $CO_2$ at low temperature (20° C) under reduced pressure (Locatelli and Daolio, 1993).

In some instances, egg was the most tolerant stage to $CO_2$. The egg stage was tolerant in the tropical warehouse moth *Ephestia caute//a* (Verma and Wadhi, 1978), the psocids *Liposce/is entomophi/us* (Leong and Ho, 1990), *Liposcelis bostrychophi/us* and *Lepinotus patruelis* (Spratt, personal communication), and in the mite *Tyrophagus putrescenitae* (Stepien, 1974). The tolerance of egg is reported to change radically during development. Press and Flaherty (1973a) observed that eggs of P. *interpunctella* upto 5 hr after oviposition were highly susceptible to $CO_2$. In *E. cautella,* when eggs aged to within 1 hr were exposed to 100% $CO_2$ or nitrogen for 24 hr, the tolerance increased sharply between 5 and 7 hr and slowly declined after 30 hr (Bell et at, 1980).

The average mortality of adults of *S. paniceum* and *L. serricorne* to a range of $CO_2$ concentration at 20°C temperature for different exposure periods have been presented in Table 3.9 and 3.10 respectively. The mortality response of *S. paniceum* and *L. serricorne* at each concentration at different exposure period has been summarized and

presented in Fig. 3.4 and 3.5 respectively. The adults of both species were noted to be more tolerant when exposed at a lower temperature of 20°C than when treated at 27 ± 2°C. Adults of *S. paniceum* showed 100% mortality at 60% $CO_2$ in 24 hr exposure at 27±2°C (Table 3.1) whereas at 20°C it required about 90% $CO_2$ and 48 hr exposure (Table3. 9) to achieve 100% mortality. Similarly, in *L. serricorne* adults 1000/0 mortality was achieved following exposure to 700/0 $CO_2$ for 48 hr (Table 3.2) at 27 ± 2° C. On the other hand at 20°C, it required treatment of insects with 90% $CO_2$ for 48 hr to achieve 100% kill (Table 3.10). The temperature dependency of $CO_2$ in its toxic action against adults and life stages of *T castaneum* and *P. interpunctella* (Harein and Press, 1968); *T granarium* (Spratt et al, 1985); *Tcastaneum* and *Tconfusum* (Aliniazee, 1971); *T castaneum, O. surinamensis, R. dominica, S. oryzae, S. zeamais, T granarium, T glabrum, T. variable* and *E. cautella* (Jay, 1984) has been reported.

The response of adults of S. *paniceum* and *L. serricorne* exposed to constant and changing concentrations of $CO_2$ is given in Table 3.11. The results indicate that the changing concentrations of $CO_2$ are more effective than constant concentration. This trend has been observed in both *S. paniceum* and *L. serricorne.* The response was more prominent in *S. paniceum* than in *L. serricorne.* This was evident when at a particular Ct product 1000/0 mortality of *S. paniceum* was achieved at increasing and decreasing $CO_2$ concentrations, but in *L. serricorne,* only 62.4 (in increasing order) and 81% (in decreasing order) kill achieved respectively (Table 3.11). Very little work has been carried out on the effect of changing concentrations of $CO_2$ against stored product insects. Dales (1990) and Dales et al, (1993) studied the effect of falling concentrations of $CO_2$

against survival and development of S. *oryzae, S. zeamais, T. castaneum and R. dominica.* They observed that complete control of pests was not achieved with changing concentrations. However the minimum effective concentration of CO2 and the degree of response of insects to $CO_2$ vary substantially between species and between developmental stages within species (Banks and Annis, 1990). In addition, the effectiveness of controlled atmospheres against insects is dependent on various abiotic factors such as gas composition, relative humidity, temperature, length of exposure and gas pressure (Jayas et al, 1991).

**Table 3.1 Susceptibility of adults of *S. paniceum* to $CO_2$ at 27±2°** C

| $CO_2$ dosage (%) | **Corrected** 1 **mortality (% ± SD*) for exposure periods of** | | | |
|---|---|---|---|---|
| | **24 hr** | **48 hr** | 72 **hr** | **96 hr** |
| 5 | 0 | 2.1±1.05 | 3.2 ±1.15 | 9.2±t1.75 |
| 10 | 0 | 3.6 ± 0.0 | **11.6 ± 1.21** | 16.7±t2.80 |
| 15 | 0 | 12.3 ± 1.95 | 19.7±t1.00 | 26.5±1.75 |
| 20 | 5.0±1.90 | 30.3 ± 1.67 | 34.6 ±1.05 | 50.3±3.96 |
| 25 | 20.0 ± 2.73 | 34.4±1.67 | 43.1±1.10 | 78.4 ±1.75 |
| 30 | 26.6 ± 2.73 | 38.5±1.67 | 60.6 ±1.21 | 90.3 ± 1.27 |
| 35 | 30.0 ± 2.73 | 49.7±1.15 | 76.0 ± 2.71 | 100 ±0.0 |
| 40 | 47.5 ±3.21 | 61.1 ±1.71 | 82.4 ± 2.05 | 100 ±0.0 |
| 45 | 82.5 ± 3.20 | 91.3 ± 1.10 | 97.8 ±1.75 | 100 ±0.0 |
| 50 | 89.2 ±1.70 | 94.8 + 1.21 | 100 ± 0.0 | 100 ±0.0 |
| 55 | 91.6 ±1.90 | 99.0 ±1.21 | 100 ±0.0 | 100 ± 0.0 |
| 60 | 100 ±0.0 | 100 ±0.0 | 100 ±0.0 | 100 ±0.0 |
| Control | (0) | (2.5 ±1.91) | (6.0 ±1.63) | (7.5 ± 3.00) |

* The data are average of 4 replicates

Table 3.3 Mortality response of eggs of *S. paniceum* exposed to $CO_2$ at 27:t2° C

| $CO_2$ dosage (%) | Corrected mortality (% ± SD*) for exposure periods of | | | |
|---|---|---|---|---|
| | 24 hr | 48 hr | 72 hr | 96 hr |
| 10 | 2.9 ± 2.82 | 6.7 ± 2.70 | 11.1 ±0.00 | 18.4 ±3.30 |
| 20 | 3.7 ±2.82 | 14.8 :t2.70 | 15.2 ±2.75 | 27.5 ± 2.05 |
| | 8.1±2.82 | 25.6 ± 2.70 | 29.1 ±2.80 | 49.2±2.80 |
| | 9.4 ±2.75 | 36.1±4.85 | 48.4 ±3.17 | 71.4 ±3.30 |
| | 12.4±2.50 | 48.6 ±3.17 | 51.4 ±2.75 | 82.6 ±3.87 |
| | 18.5±2.70 | 64.9 ±3.11 | 65.2± 2.75 | 92.8 ±2.00 |
| | 25.4±2.80 | 75.6 ±3.17 | 99.1 ±2.75 | 100 ± 0.00 |
| | 27.2 ±2.45 | 87.8 ±5.12 | 100 ± 0.00 | 100 ±0.00 |
| **90** | 53.7 ±3.34 | 99.2 ±3.11 | 100 ±0.00 | 100±0.00 |
| Control | (19.0 ±2.00) | (26.0 ±5.16) | (28.0 ±3.26) | (28.0 ± 3.26) |

The data are average of 4 replicate

Table **3.4 Mortality response of** eggs of *L semcom* exposed to CO2 at 27:t2° C

| $CO_2$ dosage (%) | **Corrected mortality** (% ± SD*) **for exposure periods** of | | | |
|---|---|---|---|---|
| | 24 hr | 48 hr | 72 hr | 96 hr |
| 10 | 0 | 0 | 11.8±2.44 | 13.2 ±3.10 |
| 20 | 3.5 ± 0.00 | 3.6 ±1.95 | 15.5 ±2.10 | 18.8 ±2.13 |
| 30 | 3.7 ±1.75 | 14.5 ±2.10 | 20.0 ± 2.98 | 42.5 ± 3.06 |
| 40 | 6.2 ± 5.45 | 35.2 ±2.07 | 34.0 ± 2.93 | 60 ± 0.00 |
| 50 | 12.1±2.07 | 47.6 ±1.85 | 50.3 ± 3.76 | 80.1 ±2.00 |
| 60 | 17.5 ±2.07 | 62.2 ±2.13 | 65.5 ±3.49 | 91.8 ± 2.00 |
| 70 | 25.5 ± 1.80 | 70.0 ± 0.00 | 74.0 ± 0.00 | 98.1±2.19 |
| 80 | 28.6 ±1.85 | 85.5 ± 1.80 | 95.5 ± 3.49 | 100 ±0.00 |
| 90 | 53.7± 2.07 | 95.0 ± 0.00 | 100 ±0.00 | 100±0.00 |
| Control | (6.6 ± 2.69) | (8.3 ± 1.91) | (8.25 ± 1.90) | (11.6 ±4.29) |

* The data are average of 4 replicates

**Table 3.5 Susceptibility of larvae of *S. paniceum* to $CO_2$ at 27±2°C**

| 1 $C0_2$ dosage (%) | **Corrected mortality (% ± SD*) for exposure periods of** | | | |
|---|---|---|---|---|
| | **24 hr** | 48 hr | 72 hr | 96 hr |
| 10 | 2.7 ± 0.0 | 3.7±2.19 | 11.8 ±2.19 | 15.7 ±1.95 |
| 20 | 3.6 ±1.80 | 4.7 ±1.90 | 21.6 ±3.63 | 44.7 ±1.90 |
| 30 | 9.9 ± 0.0 | 10.4 ±1.90 | 50.0 ±3.58 | 53.4 ± 1.90 |
| 40 | 16.2 ±1.80 | 16.9 ±3.06 | 62.3 ±3.10 | 68.5 ±1.90 |
| 50 | 17.1 ±2.94 | 23.5 ±1.90 | 75.5 ±4.90 | 82.8 ±2.19 |
| 60 | 19.8±3.44 | 29.3 ± 1.90 | 83.1 ±2.13 | 92.4 ±3.10 |
| 70 | 26.1 ±2.10 | 49.6 ±3.10 | 92.5 ±1.90 | 100±0.0 |
| 80 | 34.3 ±1.85 | 69.1 ±2.13 | 100 ±0.00 | 100±0.00 |
| 90 | 52.2 ± 3.45 | 88.8 ±1.85 | 100 ±0.00 | 100±0.00 |
| Control | (7.5 ±1.70) | (11.6 ±1.91) | (11.6±1.91) | (13.3 ±0.00) |

* The data are average of 4 replicates

**Table 3.6 Susceptibility of larvae of *L. serricorne* to $CO_2$ at 27±2°C**

| $CO_2$ dosage (%) | **Corrected mortality (% ± SD * ) for exposure periods of** | | | |
|---|---|---|---|---|
| | **24 hr** | **48 hr** | 72 hr | 96 hr |
| 10 | 0 | 0 | 3.7± 1.80 | 12.6±1.80 |
| 20 | 0 | 4.9±1.91 | 21.8 ±2.08 | 39.6±1.80 |
| 30 | 4.1 ± 1.65 | 10.8 ± 1.65 | 50. 0 ± 2.94 | 51.4 ±2.13 |
| 40 | 4.9 ±1.91 | 33.3 ±2.69 | 56.3 ±3.41 | 63.0 ±1.75 |
| 50 | 10.8 ±l.65 | 44.9 ±l.91 | 73.2 ±2.07 | 77.5 ± 1.85 |
| 60 | 16.6 ± 0.00 | 51.6 ±1.91 | 84.6 ±1.85 | 89.2 ± 0.00 |
| 70 | 18.3 ±1.93 | 58.3 ± 1.96 | 90.9 ± 2.07 | 95.5 ± 1.80 |
| 80 | 19.2 ±1.76 | 76.6 ± 2.74 | 94.5 ± 2.08 | 100 ±0.00 |
| 90 | 48.3 ± 1.96 | 81.6 ±1.91 | 100±0.00 | 100 ±0.00 |
| Control | (0) | (0) | (8.3 ± 1.91) | (8.3 ±1.91) |

* The data are average of 4 replicates

**Table 3.7 Mortality of pupae of *S. paniceum* exposed to $CO_2$ at 27±2°C**

| $C0_2$ dosage (%) | **Average mortality (% ± SD *) for exposure periods of** | | | |
|---|---|---|---|---|
| | **24 hr** | 48 hr | 72 hr | 96 hr |
| 10 | 3.4 ±1.72 | 8.8 ± 0.34 | 12.2 ± 3.56 | 26.6 ± 3.00 |
| 20 | 6.0 ±1.96 | 21.8 ±2.07 | 23.5 ± 2.25 | 27.5 ±3.10 |
| 30 | 11.9 ±1.70 | 30.0 ± 3.44 | 35.8 ±3.06 | 48.1± 3.74 |
| 40 | 16.2 ±1.96 | 40.9 ±1.80 | 50.0 ± 3.53 | 65.5 ±2.25 |
| 50 | 26.5 ± 1.96 | 50.0 ±1.80 | 62.3 ±3.10 | 81.4 ±1.95 |
| 60 | 32.5± 1.75 | 61.8 ±2.07 | 76.5 ± 1.90 | 93.2 ±1.95 |
| 70 | 38.5 ±2.73 | 74.3 ± 3.35 | 91.5 ±3.06 | 99.1 ± 1.95 |
| 80 | 49.6 ± 3.30 | 80.0 ±1.80 | 100 ±0.00 | 100 ±0.00 |
| 90 | 51.0 ±1.90 | 87.2 ± 2.07 | 100 ±0.00 | 100 ±0.00 |
| Control | (2.5 ± 1.65) | (8.3 ±1.96) | (11.6 ±1.90) | (15.0 ±1.90) |

* The data are average of 4 replicates

Table **3. 8 Mortality of pupae of *L serricome* exposed to $CO_2$ at 27±2°C**

| $CO_2$ dosage (%) | **Average mortality ( % ± SD * ) for exposure periods of** | | | |
|---|---|---|---|---|
| | **24 hr** | **48 hr** | **72 hr** | **96 hr** |
| 10 | 0 | 9.5 ±2.36 | 15.5±2.42 | 19.7 ±2.48 |
| 20 | 3.0 ±2.00 | 24.7 ± 2.05 | 55.3 ± 2.42 | 55.9 ±4.10 |
| 30 | 5.2± 2.00 | 34.1±0.00 | 64.9 ± 2.48 | 70.5 ±2.10 |
| 40 | 18.0±2.31 | 46.4 ± 5.79 | 80.6 ± 2.42 | 82.6 ±2.10 |
| 50 | 22.0 ±2.31 | 55.6 ± 2.05 | 82.9 ±2.15 | 90.7 ±2.17 |
| 60 | 30.0 ±**2.31** | 55.6 ± 2.48 | 85.1 ±2.54 | 93.6 ±2.15 |
| 70 | 37.0 ± 2.00 | 59.8 ± 2.05 | 91.6 ±3.51 | 94.7 ±2.15 |
| 80 | 41.0 ±2.00 | 64.9 ± 2.05 | 94.5 ±2.19 | 100±0.00 |
| 90 | 45.6 ±**2.31** | 70.1±3.47 | 96.7 ± 2.43 | 100±0.00 |
| Control | (0) | (3.0 ± 2.00) | (6.0 ±2.31) | (6.0 ±2.31) |

* The data are average of 4 replicates.

**Table 3. 9 Response of adults of S. *paniceum* exposed to different concentrations of $CO_2$ at** 20° C

| $CO_2$ dosage (%) | Average mortality (% ± SD[k]) for exposure periods (h) | | | |
|---|---|---|---|---|
| | **24** | **48** | **72** | **96** |
| 5 | 0 | **0** | 2.8 ±1.67 | 2.9 ±1.44 |
| 10 | 0 | 3.2 ±2.85 | 6.5 ±1.45 | 8.7 ±1.45 |
| 15 | - | - | 10.1 ±0.00 | 13.8 ±1.67 |
| 20 | 0 | 9.8 ± 2.28 | 20.9 ±1.45 | 22.6 ±1.67 |
| 25 | - | - | 23.2 ± 1.67 | 28.5 ± 1.67 |
| 30 | 0 | 16.9 ±1.61 | - | - |
| 35 | - | - | 29.7 ±1.45 | 63.5 ± 1.73 |
| 40 | 27.0 + 0.00 | 32.4 + 2.32 | - | - |
| 45 | - | - | 67.4 ±1.45 | 78.8 ±1.50 |
| 50 | 39.8 ±1.50 | 60.6 ±2.32 | - | - |
| 55 | - | - | 81.2 ±1.67 | 93.5 ± 1.50 |
| 60 | 48.7 ± 2.08 | 67.6 ±1.67 | - | - |
| 65 | - | - | 90.6 ±1.45 | 99.3 ± 1.50 |
| 70 | 49.4±3.19 | 71.8 + 2.24 | - | - |
| 75 | - | - | 94.9 ±1.45 | 100 ±0.00 |
| 80 | 55.7 ±4.36 | 88.7 ±2.28 | - | - |
| 85 | - | - | 100 ±0.00 | 100 ±0.00 |
| 90 | 77.6 ±1.35 | 100 ±0.00 | - | - |
| Control | (2.5 ± 1.80) | (11.3 ±**2.50)** | (13.7 ±**1.44)** | (14.4±1.25) |

* The data are average of 4 replicates

**Table 3.10 Response of adults of *L. serr;corne* exposed to different concentrations of $CO_2$ at 20°C**

| $CO_2$ dosage (%) | Average mortali | mortality (% ± SD *) for exposure periods (h) | | |
|---|---|---|---|---|
| | **24** | **48** | **72** | **96** |
| 5 | 0 | **0** | 2.1 ±**1.65** | 2.8 ± 0.00 |
| 10 | 0 | **0** | 5.7 ±**1.45** | 7.2 ± 1.67 |
| 15 | - | **-** | **7.8** ± 1.62 | 10.0 ±1.62 |
| 20 | 0 | **0** | 13.6 ±**1.50** | 14.2 ±1.40 |
| 25 | - | **-** | **22.8** ±**1.61** | 24.5 ±1.24 |
| 30 | 0 | 11.2 ±**1.35** | **-** | - |
| 35 | - | **-** | 28.4 ±**1.45** | 30.7 ±1.40 |
| 40 | 27.5 ± 3.21 | 33.2 ±1**.30** | **-** | - |
| 45 | - | **-** | 44.7 ±**1.61** | 49.3 ± 1.40 |
| 50 | 28.3 ±4.31 | 46.3 ±**3.52** | **-** | - |
| 55 | - | **-** | 65.9 ± 3.27 | 67.8 ±1.50 |
| 60 | 29.2 ± 5.00 | **68.3** ± 1.60 | **-** | - |
| 65 | - | **-** | 84.4 ±**1.55** | 93.6 ±1.50 |
| 70 | 42.5 ±3.15 | **84.8** ± 1.55 | **-** | - |
| 75 | - | **-** | 93.6 ±**1.40** | 100 ±0.00 |
| 80 | 62.5 ± 5.71 | **91.0** ±**1.40** | **-** | - |
| 85 | - | **-** | 100 ±0.00 | 100 ±0.00 |
| 90 | 73.3 ± 5.00 | 100 ±0.00 | **-** | - |
| Control | (0) | **(9.4** ±**7.18)** | **(11.9**±**1.25)** | (12.5 ± 0.00) |

* The data are average of 4 replicates

Table 3.**11** **Response of adults of *S. paniceum* and *L serricorne* exposed to constant and changing $CO_2$ concentrations at** 27±2°C

| Exposure | **Concentration (%)** | **Exposure period (hr)** | **Corrected mortality** (%±SD) |
|---|---|---|---|
| | | ***S. paniceum*** | |
| Constant | **40** | **24** | 48.3±l.63 |
| Increasing | **30** | **8** | 100±O.00 |
| | **40** | **12** | |
| | **60** | **4** | |
| Decreasing | **60** | **4** | 100±O.00 |
| | **40** | **12** | |
| | **30** | **8** | |
| ontrol | **-** | **-** | (4.1±l.34) |
| | | ***L. semcorne*** | |
| onstant | **40** | **24** | 40.5±O.14 |
| Increasing | **30** | **8** | 62.4±2.12 |
| | **40** | **12** | |
| | **60** | **4** | |
| Decreasing | **60** | **4** | 81.0±l.29 |
| | **40** | **12** | |
| | **30** | **8** | |
| Control | **-** | **-** | (2.8±l.42) |

* Average of 6 replicates

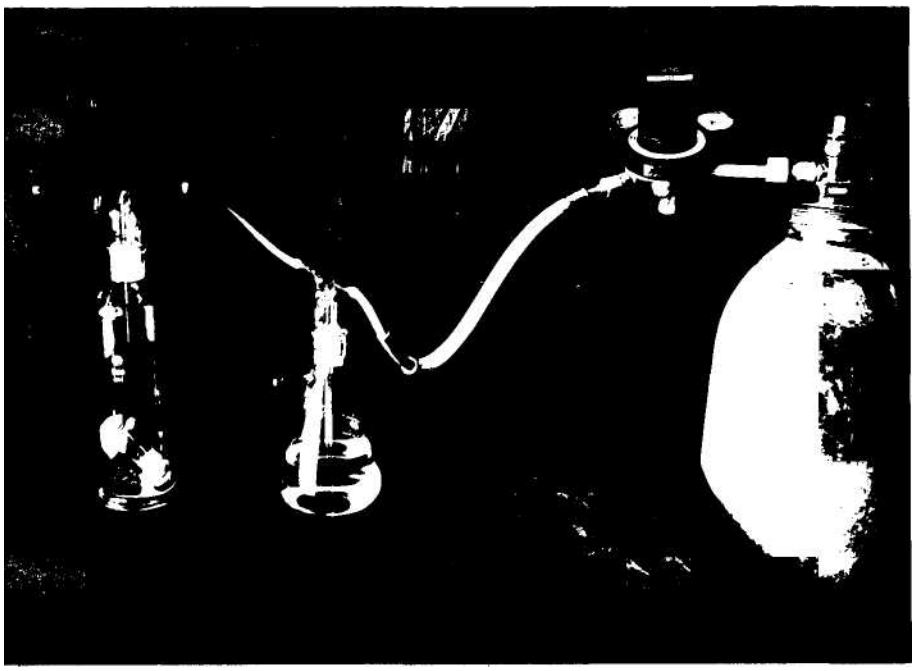
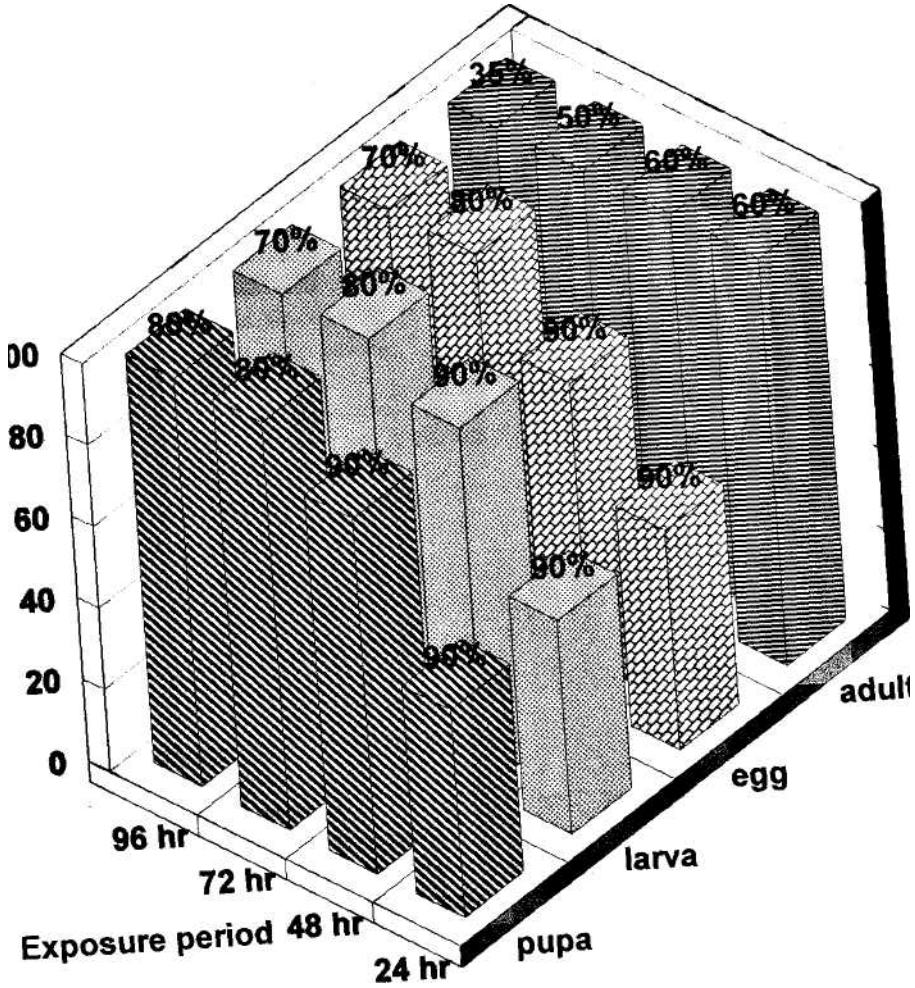


**Fig. 3.1 Dosing of test chambers with $CO_2$ discharged from a cylinder**


00
80
60
40
20
0
96 hr
72 hr
48 hr
24 hr
Exposure period
pupa
larva
egg
adul
35%
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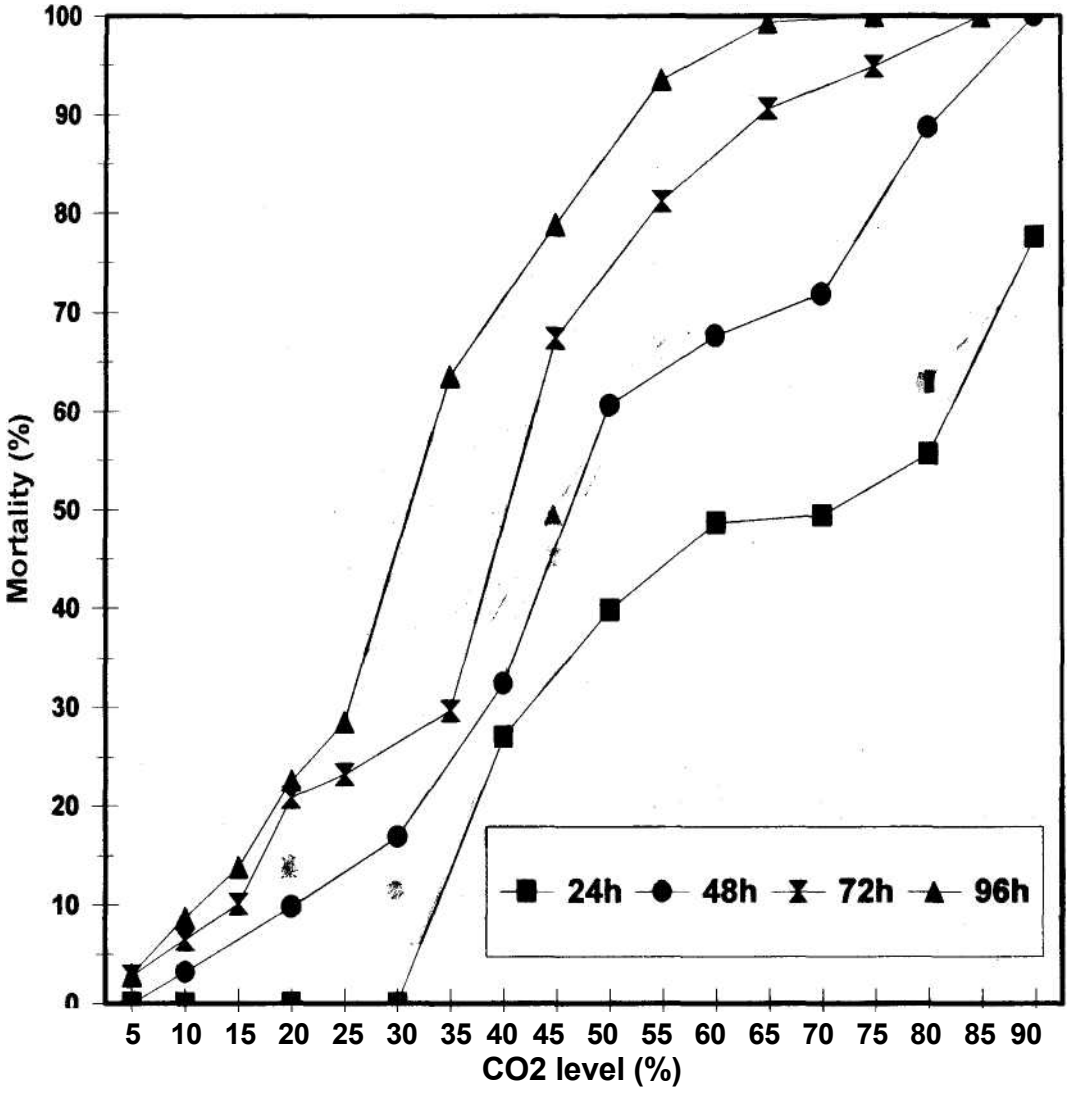



**Fig. 3.4 Morality trend in *S.paniceum* adults at 20°C and different exposure periods**

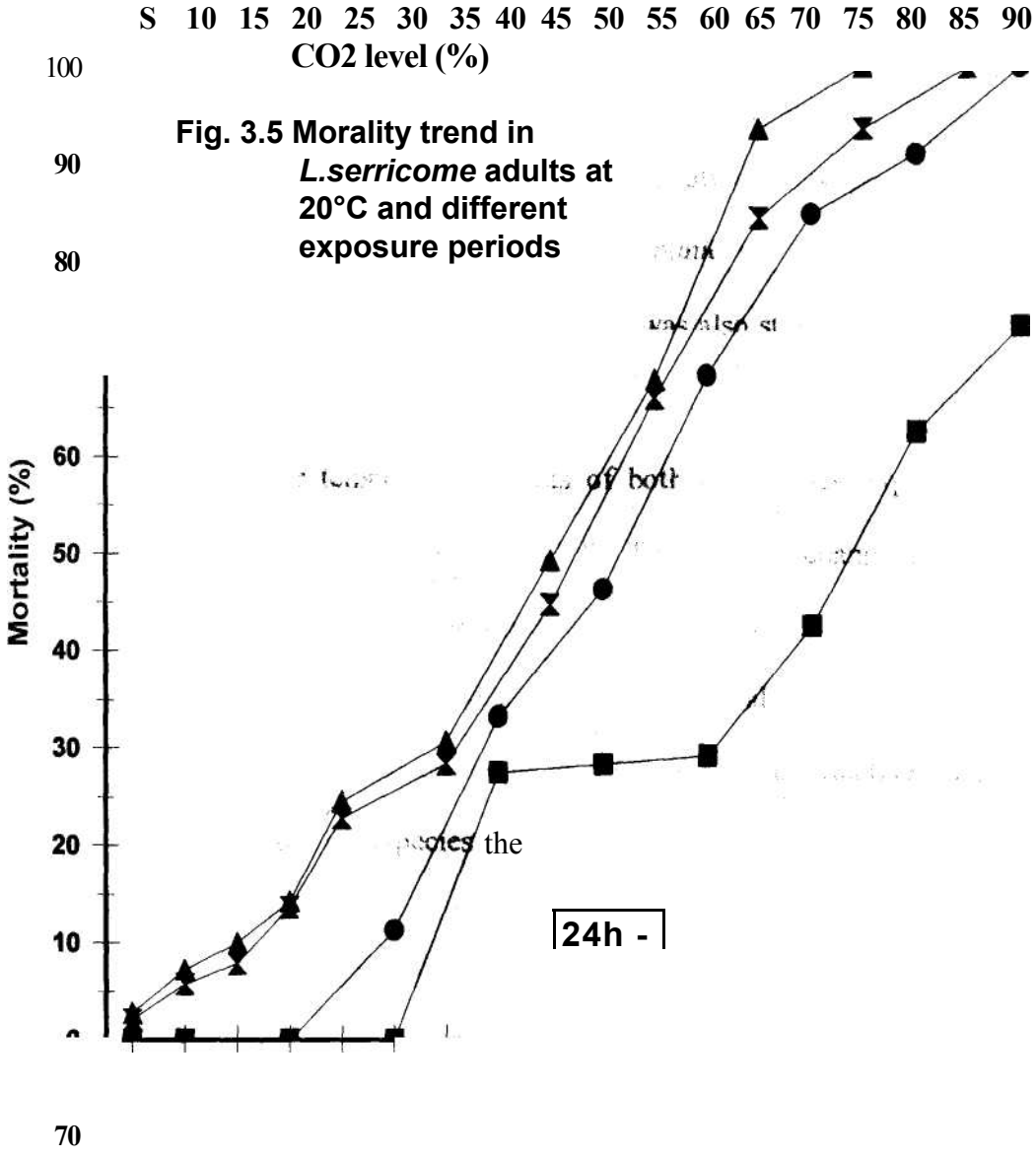



**Fig. 3.5 Morality trend in *L.serricome* adults at 20°C and different exposure periods**

# CONCLUSION

The mortality response of developmental stages of *S. paniceum* and *L. serricorne* exposed to a range of $CO_2$ concentrations for different exposure periods was investigated at 27±2°C. In both species, the pupal stage was the most tolerant followed by larva, egg and adult stages. All life stages of *S. paniceum* were relatively susceptible than that of *L. serricorne.* The mortality response of adults of *S. paniceum* and *L. serricome* against different levels of $CO_2$ at low temperature of 20°C was also studied. The adults of both species were noted to be more tolerant when exposed at 20°C than when treated at 27±2°C. Thus, at lower temperature, adults of both the species required more $CO_2$ concentration and exposure period for 100% kill. The effect of changing concentrations of $CO_2$ (stepwise increase and stepwise decrease) with a constant Ct product on mortality response of adults of *S. paniceum* and *L. serricorne* was also examined. The results indicate that the changing concentrations of $CO_2$ are more effective than constant concentration. Among the two species the mortality response was more prominent in *S. paniceum* than in *L. serricorne.*

Chapter four

# Assessment of quality parameters of spice powders treated with different fumigants

**ASSESSMENT OF QUALITY PARAMETERS OF SPICE POWDERS TREATED WITH DIFFERENT FUMIGANTS**

# INTRODUCTION

Spices and spice products are susceptible to insect pest attack and microbial contamination like any other stored products. It is essential to disinfest and disinfect these commodities in order to meet the requirements of national and international standards in terms of quality. Currently, phosphine and methyl bromide are used for disinfestation of spices and spice products and ethylene oxide is used for disinfection or microbial control of spices. In the recent years carbon dioxide has been used for insect control in stored grains and the treatment is considered as non-chemical.

Phosphine ($PH_3$), under certain conditions, may affect the quality parameters of certain commodities (Rajendran and Gunasekaran, 1995) other than spices. Repeated treatment of spice products with methyl bromide (MB) may cause discolouration and loss of essential oils (Pruthi 1980). Ethylene oxide (ETO) treatment is known to affect the volatile oil content of spices in some instances (Coretti and Inal 1969; Vajdi and Periera 1973). On the other hand, carbon dioxide ($CO_2$) -rich atmospheres has been known to preserve grain quality apart from insect control and does not affect the chemical composition of stored grain (Bell and Armitage 1992; Jayas et al. 1991). However, information on the quality parameters of spice/spice products exposed to conventional fumigants or $CO_2$ atmosphere are scanty. In the present investigation changes if any in the prime spice principles such as capsaicin content and colour value in chilli powder;

curcumin content in turmeric powder, volatile oil content in coriander powder and curry powder were examined following treatment with methyl bromide, ethylene oxide, phosphine and carbon dioxide. In addition, linalool, a major component of volatile oils of coriander powder and curry powder was also analyzed.

## MATERIALS AND METHODS

**Spice powders:**

Chilli powder, coriander powder, turmeric powder and curry powder (garam masala -powder of mixed spices) in unit packs were obtained from local market. The unit packs of each were cut open, pooled and mixed thoroughly for homogeneity before fumigation. The moisture content of the spice powders before and after treatment was determined as per Dean and Stark (1920) method. Spice powders 40 gram samples (4 replicates) for each treatment was distilled with toluene and moisture content was estimated from volume of water collected in distillation. The moisture content of the commodities before treatment were as follows: Chilli powder 6.0%, coriander powder 6.0%, turmeric powder 10% and curry powder 7.5 % (values are mean of 4 replicate analysis). There was no change in moisture content of spice powders after exposure to fumigants and $CO_2$ atmosphere.

**1. Treatment of spice powders:**

From each spice powder, 250 gram (4 replicates per treatments) lots were taken in open polyethylene bags and placed individually in 2.8L desiccators and the desiccators were closed using lids. Phosphine gas was liberated from aluminium phosphide tablet preparation in 5% sulfuric acid solution and collected in a gas burette according to FAO method (1975). From the gas burette the required quantity of phosphine gas was drawn in gas-tight syringe and injected into desiccators through the rubber septum fitted with desiccators containing spice powders. Phosphine fumigation was carried out at two dosages (commercial and exaggerated) i.e. 2 and 6 mg/L for 7 days under normal atmospheric pressure. Phosphine concentrations were checked in the desiccators immediately after dosing with a Bedfont phosphine monitor (Model EC 80). At the end of 7 days the desiccators were opened and samples after 5 hr aeration were preserved in polyethylene bags and stored at 4°C in a refrigerator until quality analysis.

For methyl bromide fumigation the required quantity of methyl bromide gas was drawn from a 0.5 Kg lecture bottle containing methyl bromide using gas-tight syringes and injected into desiccators containing spice powders. Two dosages (insecticidal and exaggerated) i. e. 24 and 80 mg/L for 24 hours at normal atmospheric pressure were used. The methyl bromide concentrations were checked immediately after dosing with a Bedfont methyl bromide monitor (Model TM 3). At the end of 24 hr the desiccators were opened and the samples after aeration were preserved in polyethylene bags and stored at 4°C in a refrigerator till quality analysis was carried out.

Ethylene oxide treatment was carried out under reduced pressure. The desiccators containing spice powders were evacuated upto 100 mm Hg negative pressure. The required quantity of gas was drawn in 500 and 1000 ml gas-tight syringes from a cylinder containing 1: 9 ethylene oxide and carbon dioxide mixture and dosed into the desiccators containing spice powders. The dosages were 100 and 640 mg/L with an exposure period of 6 hr. The treated samples were stored as mentioned above.

For carbon dioxide treatment of different spice powders, 150 gram lots from each product (6 replicates per treatment) were taken in 500 m1 gas-wash-bottles fitted with dip tube. Two dosages i. e. 60 and 90% $CO_2$ and 15 days exposures were used. From a cylinder containing (99% purity) $CO_2$, the gas was allowed to flow through gas-wash bottles at a controlled rate till the desired levels of 60 and 90%$CO_2$ were reached. $CO_2$ concentrations in the gas-wash-bottles were checked with a Riken Infrared Gas Analyser (Model RI 550 A, Japan Make). The open ends of tubes of gas- wash-bottles were sealed with rubber septa, and left undisturbed for the required exposure period (15 days). The spice powders were removed from test chambers, and stored as above till quality tests were completed.

**2. Determination of colour value and capsaicin content in chilli powder:**

For estimation of colour value in chilli powder AST A (1985), method was followed. Chilli powder, 15 gram (4 replicates per treatment) sample was extracted in a glass column (size 22 cm x 3 cm i.d.) with AR grade acetone for 6 hrs or until the last

fraction of extraction does not contain any colour following AOAC (1995). The total volume of extract collected was measured. Known volume of acetone extract was diluted suitably in acetone and the absorbance was measured in a spectrophotometer (Model: UV-160A-Shimadzu, Japan Make) at 458 nm against acetone as blank. The absorbance at 458 nm was multiplied by a factor of 61,000 to get colour values.

The capsaicin content (capsaicin and dihydrocapsaicin -components responsible for pungency) was analyzed following method of Attuquayefio and Buckle (1987) in High Performance Liquid Chromatograph (HPLC -Model LC 6A, Shimadzu, Japan Make) equipped with a dual pump, rheodyne injector, and system controller (SCL 6A) module fitted with reverse phase ODS stainless steel (CI8) column (25 X 4.6 mm i.d.) with 5 microns pore size. Ultraviolet-visible detector (6A) set at 280 nm and acetonitrile/double distilled water (63:37, v/v) as mobile phase at a flow rate of 1.5 ml/min. were used.

Known volume of acetone extract of chilli powder was taken in pre-weighed petri dish and the solvent (acetone) was evaporated in water bath and then dried in oven at 100°C for 3 hours or until the constant weight of oleoresin achieved at two consecutive observations with 1 hour intervals. Known weight (0.lg) of chilli oleoresin was dissolved in 2.0 ml of hexane and the capsaicinoids were transferred into 10 ml acetonitrile by solvent partitioning. The hexane fraction was removed and 1.0 ml aliquot of the acetonitrile extract was taken for cleanup.

A $C_{18}$ Sep-Pak cartridge was conditioned with about 5.0 ml of acetonitrile Hawed by 5.0 ml of double-distilled water. The extract (1.0 ml) was diluted with 9.0 ml of water and injected into the conditioned Sep- Pak cartridge. The capsaicinoids were then eluted with 4.0 ml of acetonitrile followed by 1.0 ml of acetonitrile containing 1 % , acetic acid and the elution was collected. Known volume (20 µl) of eluted sample was injected into HPLC. Capsaicin standard solution (60% purity, 1ml = 1mg) a mixture of capsaicin and dihydrocapsaicin was also injected. From the peak area of the standard and samples (Figure 4.1) the per cent capsaicin content was calculated considering the dilution factors.

**3. Determination of curcumin content in turmeric powder:**

After treating with different fumigants, turmeric powder, 5 gram each ( 4 replicates per treatment), was extracted in a column (size 22 cm X 3cm i.d.) with known volume of acetone for 8 hours. Total volume of extract collected was measured, from which known volume of extract was pipetted into a small beaker and the solvent .(acetone) was evaporated, further dissolved and diluted with 95% alcohol. The absorbance of alcohol solution of turmeric powder extract was measured in a spectrophotometer (Model: UV -160A-Shimadru) at 425 nm using 95% alcohol as blank. Similarly, the absorbance of known concentration of cur cumin standard solution (0.0275 mg per ml) was also measured. From the absorbance value of standard and the sample solutions, the percent curcumin content was calculated considering the dilution factors (ASTA 1985).

## 4. Determination of volatile oil content in coriander powder and curry powder and lioalool concentration in volatile oils:

Treated coriander powder and curry powder samples were distilled using Clevenger hydro distillation apparatus for their volatile oil content. Coriander powder, 200 gram each, (4 replicates per treatment) was taken in a 2 L round bottom flask and 1000 ml water was added and mixed well. A few drops of silicone antifoam solution was added to avoid bumping during distillation. Volatile oil trap was connected to the flask with a condenser. Distillation was carried out for 6 hours, and amount of volatile oil collected was red directly from the trap. In the same manner, volatile oil from curry powder was also distilled and collected. Volatile oil content was expressed as per cent (vol./wt.) ASTA (1985). The extracted oil was collected in small gas-tight vials and stored at 4°C in a refrigerator until GC analysis was carried out.

Linalool concentration of volatile oils of coriander powder and curry powder was analyzed using FISONS-Gas Chromatograph (Model 8000 series, Italy Make) with SE 30 chromato-pak column (length 3' and 1/8" i.d). The operating conditions of GC were as follows:

Carrier gas : $N_2$ at 30 ml/min.

Temperatures: Injector 150°C

Column 70°C

Detector 210°

Temperature programme: 70 (2)° C -200 (2)°C at 5°C/min.

The oil sample was diluted to 10 fold with acetone and 1 µl aliquots were injected into GC (Figure 4.2a, 4.2b and 4.3a, 4.3b). Similarly, pure linalool was diluted and injected into GC to note the retention time ( Figure 4.4.).

**5. Statistical analysis:**

The data on capsaicin content in chilli powder, curcumin content in turmeric powder and volatile oil content in coriander powder and curry powder were subjected to analysis of variance (ANOV A-one way analysis) as described in Snedecor and Cochran, (1967) and significance if any against respective controls was accepted at 5% levels.

# RESULTS AND DISCUSSION

## Colour value and capsaicin content of chilly powder

The colour value of chilli powder exposed to different fumigants are given in Table 4.1. There was a significant reduction in colour values of chilli powder after treatment, but the reduction was relatively less (1.1 and 1.2%) in $CO_2$ treated chilli powder over that of others. Vajdi and Periera (1973) also observed a reduction in colour values in ground paprika and black pepper after treatment with ethylene oxide.

The capsaicin content decreased significantly ($p< 0.05\%$) in chilli powder after exposure to ethylene oxide and methyl bromide (Table 4.1). The decrease in capsaicin content was up to 10.2 and 27.5% in chilli powder treated with ethylene oxide at 100 and 640 mg/L and 19.2 and 25.1 % in the commodity exposed to methyl bromide at 24 and 80 mg/L respectively. The decrease in capsaicin content was not significant ($p > 0.05$) in

phosphine and $CO_2$ treated chilli powder when compared with their respective controls. Literature search shows that no data is available on the effects of fumigants on chilli powder.

## Curcumin content

The curcumin content was reduced significantly ($p<0.05$) in all treatments (Table 4.2). The maximum reduction occurred in ethylene oxide (100 and 640 mg/L) treated turmeric powder (17.6 and 18.4%) and methyl bromide (24 and 80 mg/L) treated ; turmeric powder (14.7 and 20%) respectively. Carbon dioxide (60 and 90%) treated turmeric powder showed least reduction i.e. 5.2 and 6.6% of curcumin content, while the reduction was 8.2 and 11 % following phosphine fumigation. Ethylene oxide was known to affect taste and colour of turmeric and mustard seed (Plimrner, 1977).

## Volatile oil content

The volatile oil content was also reduced after exposure to methyl bromide, ethylene oxide, phosphine and $CO_2$ (Table 4.3). The reduction was more pronounced (10 to 50%) in coriander powder than in curry powder. The least reduction (10%) in oil content was noticed in $CO_2$ treated coriander powder, whereas the reduction was up to 50% when methyl bromide, ethylene oxide, and phosphine were used. On the other hand, the reduction in oil content of curry powder ranged from 6 to 17.6%. The reduction in oil content was not significant ($p>0.05$) in $CO_2$ treated curry powder while it was significant ($p<0.05$) in other cases. Coretti and lnal (1969) reported that ethylene oxide treatment

reduced volatile oil content in cloves. Vajdi and Periera (1973) also observed a similar trend in black pepper and allspice exposed to ethylene oxide (dosage not mentioned).

## Linalool concentration

The relative linalool concentrations (%) of volatile oils from fumigated coriander powder and curry powder are given in Table 4.4. Linalool concentration was reduced in volatile oils from both coriander and curry powders. The reduction was more in methyl bromide treated coriander powder (63.7 and 67.1 %) followed by ethylene oxide (24.8 and 44.8%), phosphine (21.7 and 32.6%) and it was least in $CO_2$ (10 and 11.5%) treated coriander powders. The relative linalool concentration of volatile oil was reduced significantly ($p<0.05$) in coriander powder as well as curry powder that were treated with methyl bromide, ethylene oxide and phosphine. The reduction was not significant in $CO_2$ treated curry powder or coriander powder. The maximum reduction in linalool concentration observed in oils of curry powder, treated with ethylene oxide (dosage 640 mg/L), methyl bromide (80 mg/L) and phosphine (6 mg/L) were 31.1, 22.7 and 20.6% respectively.

Table 4.1 Effect of fumigants on colour value and capsaicin content of chilli powder.

| Fumigant | Dosage | Exposure Period | Colour value | Capsaicin content (%±SD) |
|---|---|---|---|---|
| Control | - | - | 7911±33.3 | 0.323±0.003 |
| Ethylene oxide | 100 mg/L | 6 hrs | 7734± 20.5* | 0.290± 0.005* |
| | | | ( 2.2%) | (10.2%) |
| | 640 mg/L | 6 hrs | 7430±21.4* | 0.234±0.004* |
| | | | (6%) | (27.5%) |
| Control | - | - | 9938± 27.3 | 0.427 ±003 |
| Methyl bromide | 24 mg/L | 24 hrs | 8532± 21.2* | 0.345± 0.002* |
| | | | (14.1%) | (19.2%) |
| | 80 mg/L | 24 hrs | 7313± 26.3* | 0.320± 0.004* |
| | | | (26.4%) | (25.1%) |
| Control | - | - | 13744±21.2 | 0.635±0.009 |
| Phosphine | 2mg/L | 7 days | 12654±15.7* | 0.625±0.003 |
| | | | (7.9%) | (1.6%) |
| | 6 mg/L | 7 days | 12498±27.3* | 0.620± 0.003 |
| | | | (9.1%) | (2.4%) |
| Control | - | - | 8854± 36.2 | 0.455± 0.003 |
| Carbon dioxide | 60% | 15 days | 8765±21.4* | 0.448± 0.003 |
| | | | (1.1%) | (1.5%) |
| | 90% | 15 days | 8745±20.5* | 0.443± 0.005 |
| | | | (1.2%) | (2.6%) |

Values are mean of four replicate analyses. Figures in parentheses are the levels of reduction in comparis9n with respective controls. Colour values* are significantly reduced in all four types of treatments. Only ETO and MB treated samples are significantly ($p<0.05$) different from the respective controls in capsaicin content*.

Table 4.2. Effect of fumigants on curcumin content of turmeric powder

| Fumigant | Dosage | Exposure Period | Capsaicin content (%±SD)# |
|---|---|---|---|
| Control | - | - | 2.61±0.011 |
| Ethylene oxide | 100 mg/L | 6 hrs | 2.15± 0.025* 17.6%) |
| | 640 mg/L | 6 hrs | 2.13±0.052* (18.4%) |
| Control | - | - | 1.90±0.052 |
| Methyl bromide | 24 mg/L | 24 hrs | 1.62± 0.025* 14.7%) |
| | 80 mg/L | 24 hrs | 1.52± 0.035*(20%) |
| Control | - | - | 1.83±0.028 |
| Phosphine | 2mg/L | 7 days | 1.68±0.011* (8.2%) |
| | 6 mg/L | 7 days | 1.63± 0.052* (11%) |
| Control | - | - | 2.13± 0.025 |
| Carbon dioxide | 60% | 15 days | 2.02± 0.520* (5.2%) |
| | 90% | 15 days | 1.99± 0.052*(6.6%) |

Values are mean of four replicates and curcumin content* of treated samples are significantly ($p<0.05$) different from respective controls.

Figures in parentheses indicate % reduction in curcumin content against controls

# On moisture free basis

Table 4.3 The effect of fumigants on volatile oil content of coriander powder and curry powder

| Fumigant | Dosage | Exposure Period | Volatile oil contents# (%) of | |
|---|---|---|---|---|
| | | | Coriander Powder | Curry Powder |
| Control | - | - | 0.20 | 0.85 |
| Ethylene oxide | 100 mg/L | 6 hrs | 0.13* (35%) | 0.73* (14.1%) |
| | 640 mg/L | 6 hrs | 0.10* (50%) | 0.70* (17.6%) |
| Methyl bromide | 24 mg/L | 24 hrs | 0.15* (25%) | 0.73* (14.1 %) |
| | 80 mg/L | 24 hrs | 0.10* (50%) | 0.70* (17.6%) |
| Phosphine | 2 mg/L | 7 days | 0.10* (50%) | 0.73* (14.1 %) |
| | 6 mg/L | 7 days | 0.10* (50%) | 0.71* (16.5%) |
| Carbon dioxide | 60% | 15 days | 0.18* (10%) | 0.80 (5.9%) |
| | 90% | 15 days | 0.18* (10%) | 0.80 (5.9%) |

# Values are mean of 4 replicate analysis. Figures in parentheses indicate % reduction of volatile oil content against controls.

* Volatile oil contents significantly different from respective controls at $p<0.05$.

Table 4.4 Effect of fumigants on relative linalool concentration in volatile oils of coriander powder and curry powder

| Fumigants | Dosa ge | Exposure Period | Relative linalool concentration # (%) | |
|---|---|---|---|---|
| | | | Coriander powder | Curry powder |
| Control | - | - | 0.69 | 33.3 |
| Ethylene oxide | 100 mg/L | 6 hrs | 0.52* (24.8%) | 30.1* (9.8%) |
| | 640 mg/L | 6 hrs | 0.38* (44.8%) | 23.0* (31.1%) |
| Methyl | 24 mg/L | 24 hrs | 0.25* (63.7%) | 26.5*(20.6%) |
| bromide | 80 mg/L | 24 hrs | 0.23* (67.1 %) | 25.8*(22.7%) |
| Phosphine | 2 mg/L | 7 days | 0.54* (21.7%) | 29.4* (12.3%) |
| | 6mg/L | 7 days | 0.46* (32.6%) | 26.6*(20.6%) |
| Carbon dioxide | 60% | 15 days | 0.62 (10%) | 32.4 (3%) |
| | 90% | 15 days | 0.61 (11.5%) | 31.9 (4.5%) |

#Values are mean of 4 replicate analysis.

Figures in parentheses indicate % reduction of relative linalool concentration in treated samples of oils compared with controls.

* Linalool concentration significantly different from control at $p<0.05$.

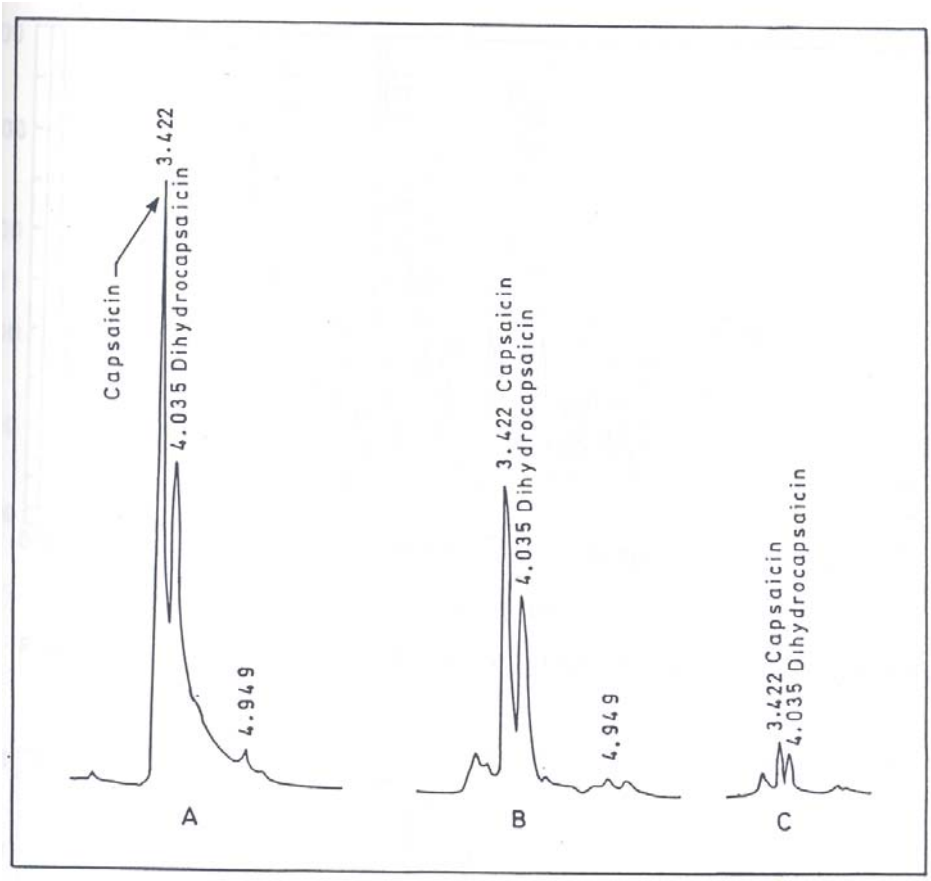



Fig. 1.1. HPLC Chromatogram of capsaicinoids

A Capsaicinoids standard

B Capsaicinoids from chilli powder (Control)

C Capsaicinoids from chilli powder (Treated)

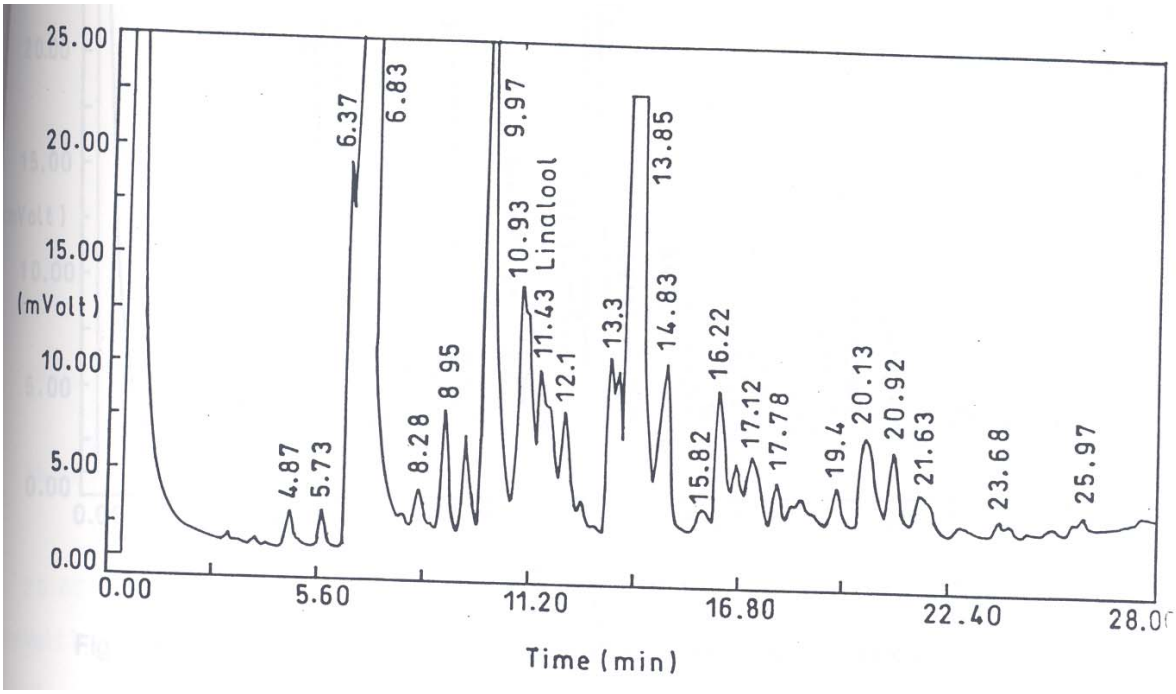



Figure 4.2a GC Chromatogram of volatile oil of coriander powder (Control)

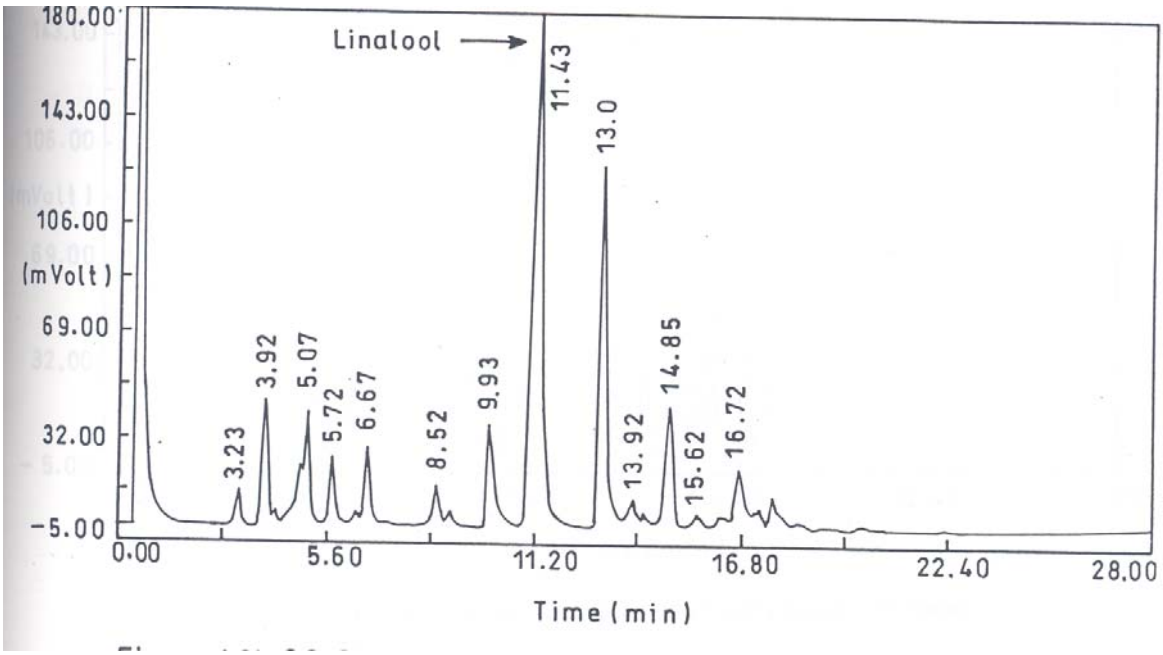



Figure 4.2b. GC Chromatogram of volatile oil of curry powder (Control)

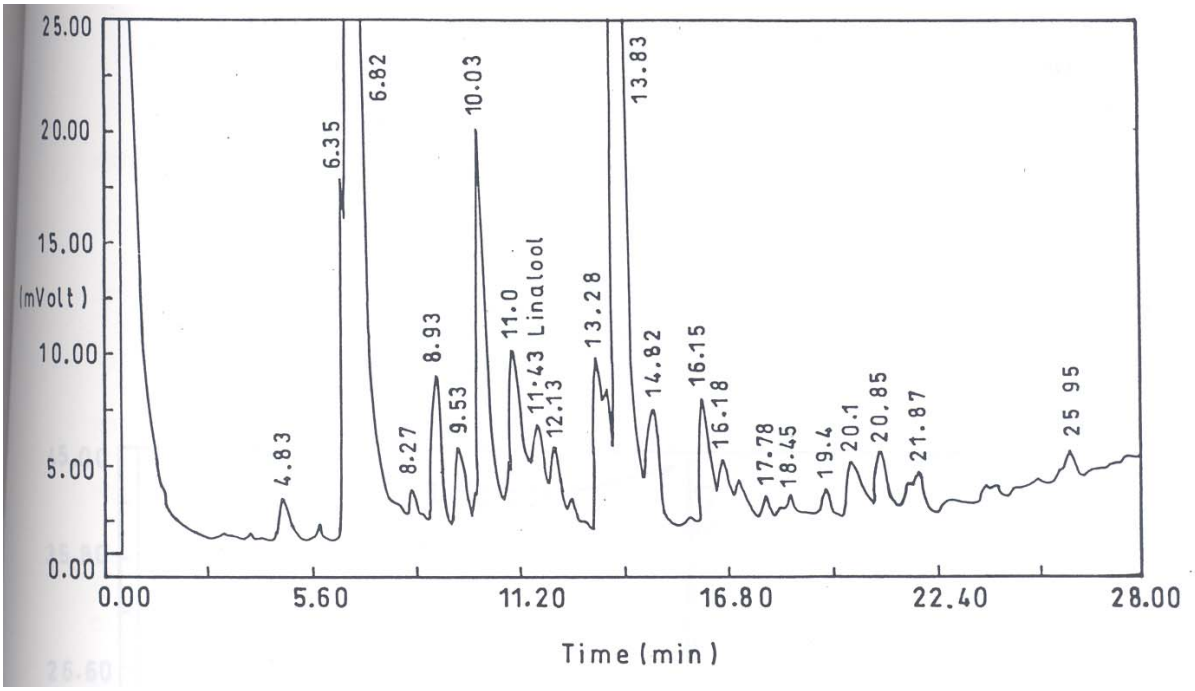



Fig 4.3a. GC Chromatogram of volatile oil of coriander powder (treated)

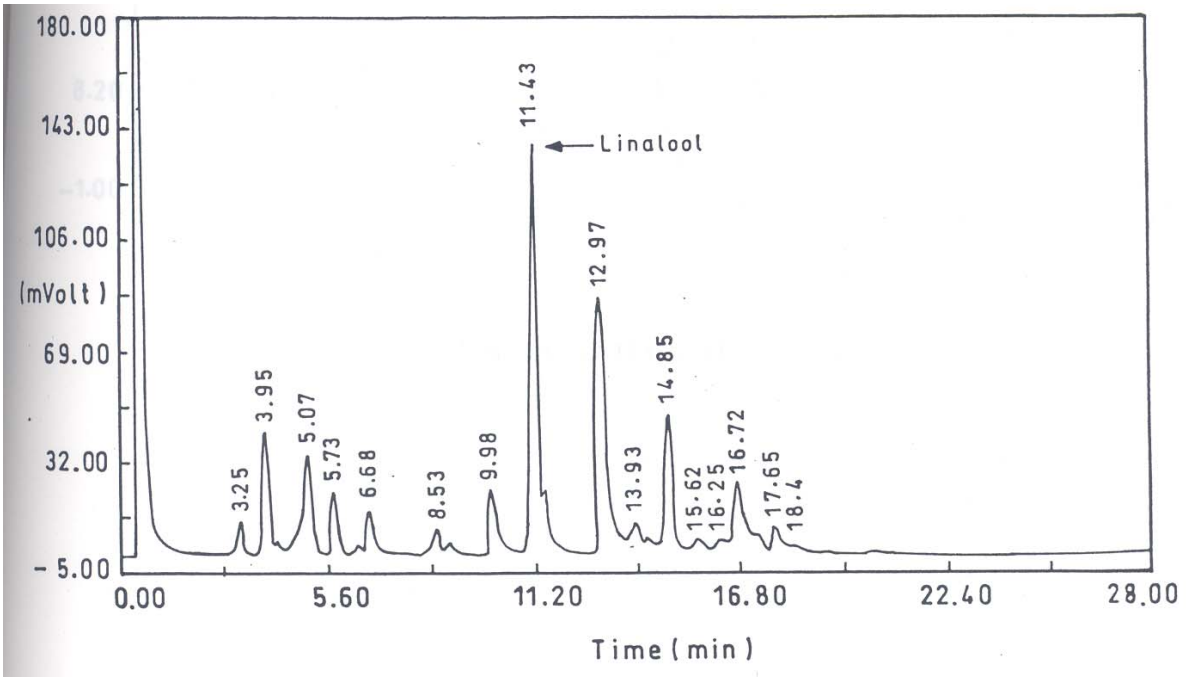



Fig 4.3b. GC Chromatogram of volatile oil of curry powder (treated)

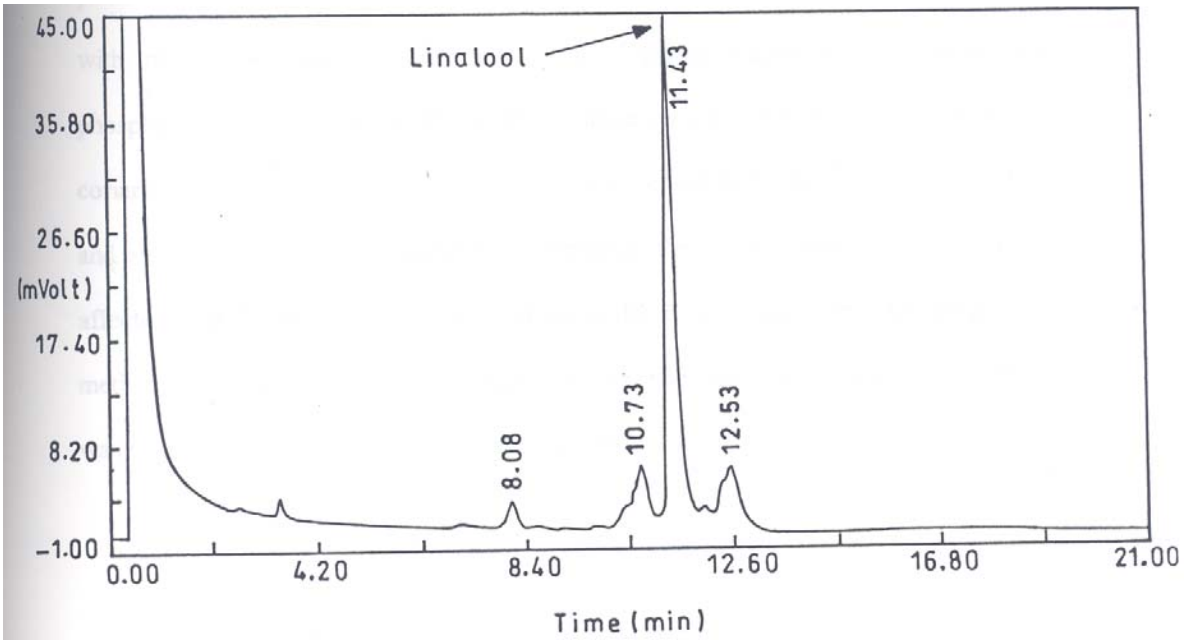



Figure 4.4. GC Chromatogram of linanol (standard)

# CONCLUSION

The effect of treatment with methyl bromide, phosphine, ethylene oxide and carbon dioxide at insecticidal and higher dosages on capsaicin content and colour value of chilli powder, curcumin content of turmeric powder, volatile oil content of coriander powder and curry powder was investigated. Linalool concentration of volatile oils of coriander and curry powders was also analysed. A significant decrease in colour value of chilli , powder after the treatment was observed. The capsaicin content in chilli powder treated with methyl bromide and ethylene oxide showed significant reduction, whereas phosphine and CO2 had least effect. The volatile oil content was significantly reduced in coriander powder and curry powder that were treated with phosphine, methyl bromide and ethylene oxide but not with $CO_2$. Similarly, the relative linalool concentration was affected significantly in volatile oils of coriander powder and curry powder exposed to methyl bromide, ethylene oxide and phosphine. The reduction of linalool concentration was more pronounced in oil from treated coriander powder than in curry powder oil.

# Chapter five

# Screening of packaging materials for carbon dioxide retention and insect resistance

# SCREENING OF PACKAGING MATERIALS FOR CARBON DIOXIDE RETENTION AND INSECT RESISTANCE

## INTRODUCTION

Carbon dioxide is a bacterial and fungal growth inhibitor (Wolfe 1980; Dixon and KeII 1989), and has insecticidal effect only when its concentration exceeds 35% (Fleurat-Lessard 1990; Adler 1994). The concentration has to be maintained for more than a week for an effective treatment of stored food commodities. The time required to kill egg and pupal stages is much longer. Processed foods packed in flexible unit packages is quite common in the markets in developing and developed countries. The packaging material used is primarily intended to serve the purpose of a barrier against moisture ingress, insect attack and to maintain the quality till it reaches the consumer. Insect infestation is not uncommon in machinery or other parts in a food processing plant. The infestation is likely to be passed on to the product during packaging. Flushing with CO2 before packing can help to control resident infestation in the product. Airtight packaging with CO2-rich modified atmosphere is becoming a current practice in the food industry for value-added dry food products such as cereal flours, rice, pasta, cereal flakes, break fast mixes, dried fruits, spices/spice products and dehydrated plants in developed countries (Fleurat-Lessard et al., 1999). The packaging film used should be i) least permeable to CO2 and ii) insect-proof. Accordingly the CO2 retention property of packaging materials such as metallised polyester/polyethylene, polyester/polyethylene, polyester aluminum foil/polyethylene, nylon based co-extruder and paper foil/polyethylene were tested.

Insect infestation is a serious problem for concern in dry packaged foods. The extent of damage of packaged food products depends on factors like initial level of insect contamination of product, the form and type of packaging materials used and the type of insect. Different insects have different penetration capacity and sensitivity to different packaging materials, with reference to prevailing environmental conditions such as temperature, and humidity (Rao et al., 1972). Insects either bore through package materials (penetrators) or enter through existing holes caused by imperfect seals or over wraps, mechanical damage, or damage resulting from previous insect infestation (invaders) (Highland, 1986). Insect-proof packaging of foodstuffs is very important for different products in the commercial channels (Newton 1988). Thus the insect control in packaged foods must include investigations of packaging materials used in modern food package and distribution systems (Mullen 1994). In this context, different packaging materials were also tested for their proofness against insect penetration. *S. paniceum* and *L. serricorne* the major insect pests attacking spices and spice products were the test organisms in these studies.

# MATERIALS AND METHODS

**1.Testing of packaging films for carbon dioxide retention**

Different types of indigenous packaging materials namely 1) metallised polyester/polyethylene, 2) polyester/polyethylene, 3) polyester aluminum foil/polyethylene, 4) nylon based co-extruder and 5) paper foil/polyethylene were

obtained from the Department of Food Packaging Technology, CFTRI. The materials were chosen based on their property to retain the flavour or aroma of spices and spice powders (Indiramrna, 1995). The thicknesses of these films as measured by a dead load micrometer in microns were: metallized polyester/polyethylene 30, polyester/polyethylene 38, polyester aluminium foil/polyethylene 60, nylon based co-extruder 100, and paper foil/polyethylene 110. From each type of film. pouches of 24 x 16 cm size were made using heat sealing machines. All the four sides of pouches were sealed except leaving a small opening at one comer to insert a 10 mm diameter rubber tube for CO2 flushing. Prior to CO2 flushing a piece of silicon rubber was stuck in each pouch at one side. This served as the spot to pierce the needle of CO2 monitor for measuring CO2 concentration in pouches without damaging the pouches. Carbon dioxide ftom a cylinder was allowed to flush (10 L/min. for 180 seconds or till desired CO2 level achieved) into the pouches through a rubber tube. Immediately after flushing with CO2 the pouches were sealed gastight. Four pouches (replicates) were maintained for each type of film. After sealing the pouches the initial CO2 concentration (%) was measured with a Systech Carbon dioxide Analyzer ( Model Portamap 2, England Make) by piercing the needle of the instrument into pouches through the silicon rubber piece already fixed. Subsequently, CO2 concentration was measured at the end of 24 hr. Then the pouches were left undisturbed at 27::t2°C for 15 days after which CO2 concentration was again measured. From the data on CO2 levels of 0 day and 1$^{st}$ day, the percent retention of $CO_2$ in 24 hrs was calculated.

**2. Testing of packaging films for insect resistance**

*S. paniceum* and *L. serricorne* cultures were maintained at 27±2°C and 70% r.h. on whole wheat flour supplemented with 5% dried yeast in the laboratory. The insect penetration test was carried out using adults, young larvae, and mature larvae separately for each species at 27:t2°C. Culture medium (wheat flour with yeast) 100 gram each was taken in bottom chamber of desiccator (0.85 L capacity). The packaging film was cut into 15cm x 15 cm size pieces. Then the film was sandwiched between the bottom chamber and lid of desiccator. Thus the film was held firmly by bottom chamber and its lid. The insects 2-3 days old adults (mixed sex) were collected from stock culture and allowed to starve for 24 hours. Then the insects, (200 in a replicate) were released on the film through a hole in the lid of desiccator (Figure 5.1). Four replicates were maintained for each type of film. In another set of experiment the adults were released along with 5 gram food ( culture medium) to know any difference in penetration between starved and fed insects. They were under regular observation for 4 weeks or until the adults were found dead. At the end of 4 weeks, the desiccators were opened, the films were removed and carefully examined for any hole made by insects, and the culture medium kept in desiccators was taken out and sieved to find out whether any adults penetrated through the film.

Similarly, in another set of experiments young larvae (7-10 days old ) and old or late larvae (20-22 days old) 100 each were released on the film separately. The larvae were provided with 5 gram food (culture medium). They were observed for 6 and 8

weeks respectively for old and young larvae. At the end, the films were examined for any hole and food kept in the bottom chamber of desiccators were checked for presence of any insects.

# RESULTS AND DISCUSSION

## 1. $CO_2$ retention of packaging materials

Among the packaging films tested, the polyester aluminium foil/polyethylene retained maximum $CO_2$ (99.2%) and polyester/polyethylene showed least retention of 95.3% $CO_2$ in 24 hr (Table 5.1). The $CO_2$ flushing and sealing of pouches was done manually in the present study. If the flushing and sealing done with standard packaging machines that work by vacuum packaging or flushing gas and sealing, the $CO_2$ retention would have been much better. According to Bell and Armitage (1992) gases permeate through sheeting materials by simple diffusion and dissolution. At present very little information is available about the $CO_2$ retention or $CO_2$ permeability of packaging materials. Fleurat-Lessard et al., (1999) believed that $CO_2$ - enriched modified atmosphere packaging can eliminate residual infestation of dry food products provided the resting time after sealing before opening would be at least 10 days with 40% of $CO_2$ concentration. These conditions were reported to be efficient to kill the most tolerant stage of Indian meal moth *Plodia interpunctella* and the confused flour beetle *Tribolium confusum* even at low temperature of 20°C. Data on $CO_2$ levels at the end of 15 days (Table 5.1) reveal that all the materials tested retained more than 40% $CO_2$ level. The

nylon based co-extruder, metallized polyester/polyethylene and polyester aluminium foil/polyethylene retained good amount of $CO_2$ concentration at >55%. Polyester/polyethylene and paper foil/polyethylene retained about 41.2 and 43.3 % $CO_2$ respectively. It is evident, therefore, that all the packaging materials studied are suitable for packaging of spice products with $CO_2$ - enriched modified atmosphere. The tested packaging materials are known to possess good barrier properties to moisture, volatile oils, flavours and prevention of pick up of foreign odour (Indiramma 1995) due to the presence of polyester or foil.

**2) Packaging materials for insect proof packaging**

All the packaging materials tested were resistant against attack by *S. paniceum* and *L. serricorne* adults as well as larvae. None of the films tested showed any hole or sign of insect bite by adults or larvae. Polyester or foil in laminate films is known to offer resistance to damage by insects (Indiramma 1995). All the packaging films tested had either polyester or foil and therefore were noted to be resistant to both *S. paniceum* and *L.serricorne.* The insect penetration test followed in this study was similar to the 'cup test' method of Gerhardt and Lindgren (1954). In their study, 14 different packaging films were tested against 11 species of stored product insects. Among the fIlms tested, S. *paniceum* penetrated cellophane film which was a single layer film and all other laminated films were resistant to *S. paniceum* adults and larvae. Aluminium foil proved to be the most resistant to insect penetration. The same trend was noticed in the present study. Wohlgemuth (1979) also observed that the polyethylene and cellophane

single layer films were susceptible for penetration by *S. paniceum* . On the other hand, *S. paniceum* larvae could penetrate a few laminated films such as traypol blue (112.5 micron), traypol white (112.5 micron), paper/polyethylene (37.5 micron) saran coated cellophane (75 micron) and saran coated cellophane/polyethylene (112.5 micron) in tests conducted at controlled temperature of 30°C and 60% r.h. (Rao et al., 1972).

Different types of laminated films were resistant to adults and larvae of both *L. serricorne* and *S. paniceum* with increase in thickness of the films. However, the penetration capacity was relatively more with *L. serricorne* than *S. paniceum* (Rao et aI., 1972). Young and matured larvae of eleven stored product insects were tested against packages made of paper, polyester, cellophane, polyethylene, polyvinyl chloride, aluminium foil and polypropylene. Larvae of the merchant grain beetle, *Oryzaephilus mercator,* the square-necked grain beetle, *Cathartus quadricollis,* and the flat grain beetle, *Cryptolestes pusillus* were unable to penetrate any of the packaging materials. The hide beetle *Dermestes maculates, Lasioderma serricorne, Ephestia cautella, Corcyra cephalonica,* and *Plodia interpunctella* larvae were able to penetrate only five of seven packaging materials tested. *L. serricorne* larvae penetrated cellophane and paper rather easily but rarely penetrated polyvinyl chloride and polyethylene films (Cline, 1978). In another instance, all stages of *L. serricorne* failed to survive in or penetrate out of vacuumized and unvacuumized polyethylene or polyester film bags (Highland, 1988). Jha and Yadav (1991) studied population build-up and insect bite of *L. serricorne* and *S. paniceum* to different packaging materials were studied. Polypropylene (25 micron) was found to be insect bite proof against both the pests, whereas *S. paniceum* did not bite low

density polyethylene of 250 micron thickness, aluminium foil laminated with LDPE (50 micron thickness) and printed polypropylene (50 micron). Apart from these, various plastic materials namely saran, polyethylene, ethylene vinyl acetate, cellophane, polyvinyl chloride, polyester, polypropylene, polyurethane, polyethylene along with paper and aluminum foil were tested against the pulse beetle *Callosobruchus maculatus,* and all the materials were found to be resistant to insect attack (Highland 1986).

**Table 5.1 Carbon dioxide retention of packaging materials**

| **Film** | | **$CO_2$ concentration** (%) **at** | | | **% $CO_2$ Retention at** | |
|---|---|---|---|---|---|---|
| **Type** | **Thickness (Micron)** | **Ohr** | 24 **hr** | 360 **hr** | **24 hr** | **360 hr** |
| Polyester/polyethylene | **38** | 66.7±1.2 | 63.5±0.4 | 41.210.6 | 95.3±1.2 | **61.811.4** |
| Paper | 110 | 66.2±1.2 | 64.1±0.5 | 43.3±0.5 | 96.8±1.1 | 65.4+1.0 |
| foil/polyethylene | | | | | | |
| Nylon based co-extruder | 100 | 67.3±0.6 | 66 2+0.4 | 56.3±0.5 | 98.4±0.8 | 83.7±1.0 |
| Metallized polyester/polyethylene | 30 | 64.2±0.7 | 63.4±0.6 | 57.5+0.4 | 98.8±0.4 | 89.5±1.2 |
| Polyester aluminum foil/polyethylene | 60 | 67.3±0.5 | 66.6±0.7 | 59.5±0.3 | 99.2±0.3 | 88.5±1.2 |

* Values are mean (±SD) of 4 replicates

**Fig. 5.1 Testing of packaging films for insect resistance**

# CONCLUSION

Carbon dioxide retention by different packaging films such as metallised polyester/polyethylene 30 micron thickness), polyester/polyethylene (38 micron), polyester aluminum foil/polyethylene (60 micron), nylon based co-extruder (100 micron) and paper foil/polyethylene (110 micron) was studied at 27±2°C. Polyester aluminum foil/polyethylene retained maximum $CO_2$ (99.2%) followed by metallised polyester/polyethylene (98.8%), and polyester/polyethylene retained least (95.3%) in 24 hr. At the end of 15 days 89.5% $CO_2$ was retained by metallised polyester/polyethylene and 88.4% by polyester aluminum foil/polyethylene. The packaging materials were also tested for resistance to insect pest attack for 4 to 8 weeks. All the packaging materials were resistant to the attack of adult and larval stages of *Stegobium paniceum* and *Lasioderma serricorne.*

# Chapter six

# Effect of carbon dioxide on development and multiplication of *Stegobium paniceum* and *Lasioderma serricorne* at sub-lethal doses

# EFFECT OF CARBON DIOXIDE ON DEVELOPMENT AND MULTIPLICATION OF *STEGOBIUM PANICEUM* AND *LASIODERMA SERRICORNE* AT SUBLETHAL DOSES

## INTRODUCTION

Apart from lethal effects on insects, CO2 - rich atmosphere may affect reproduction and developmental process at sub-lethal levels, but the extent of adverse effect, if any, depends on the dosage, the species, life stage exposed, environmental factors and the type of treatment (Bailey and Banks 1980, and Nicolas 1989). A 30 minute to 1 hour exposure to a high CO2 concentration reduced egg production and hatchability in the rust-red flour beetle *Tribolium castaneum,* confused flour beetle *T. confusum* and Indian meal moth *Plodia interpunctella* (AliNiazee and Lindgren 1970; Lum and Flaherty 1972). The duration of nymphal stage of German cockroach, *Blatella germanica* was prolonged following short exposures to CO2 (Brooks, 1957). Most of the studies were generally concerned about the sub-lethal effects of CO2 on insects that were exposed for a short period only (anaesthetic effects). However, for disinfestation and storage of food grains or other commodities with CO2-rich atmosphere, an exposure period of 10 days or more are required (Annis, 1987). Therefore it is appropriate to study the sub-lethal effects of CO2 atmosphere on stored product insects after longer exposure periods rather than at short exposures. Moreover data on sub-lethal effects of CO2 on *S. paniceum* and *L. serricorne,* the major pests on stored spices, are lacking in. In this context, the immature stages of *S. paniceum* and *L. serricorne* were exposed to CO2 at

LD50 doses to study the effect of the treatment on their development. Adults were also exposed to sub lethal doses to find out the influence of CO2 on the their multiplication.

## MATERIALS AND METHODS

1.Tests on insect development

The immature stages of *S. paniceum* and *L. serricorne* were exposed to CO2 at $LD_{50}$ doses separately. Eggs (1-2 days old), larvae (20-22 days old) and pupae (2-3 days old) were taken in 7 X 1.2 cm size glass tubes separately. There were 30 insects in a tube (replicate). In the tubes containing larvae, about 2 gram of culture medium was added to avoid starvation. The open-end of the tubes were covered with pieces of muslin cloth held by rubber rings. The tubes containing immature stages were placed individually in gas-wash-bottles (test chambers) of 500 ml capacity for CO2 treatment. LD50 doses of CO2 that were determined earlier (chapter 3) were used. Six replicates were maintained for each life stage for each exposure period with equal number of untreated controls. The test chambers containing the life stages (egg, larva or pupa kept in separate chambers) were flushed with CO2 from a cylinder and humidified to 70% r.h. by passing through 50% v/v glycerol water solution. Immediately after flushing, the CO2 levels in test chambers were checked with Riken Infrared Gas Analyzer (model: RI 550 A). When the desired CO2 level inside the test chamber was achieved, they were sealed with rubber septa using quick drying glue solution. Then the test chambers were left undisturbed at 27±2°C for required exposure period, i.e. 50, 30 and 40% CO2 with 72 hr exposure for

egg, larva and pupa of *S. paniceum* respectively. Similarly, 50, 30 and 20% CO2 with 72, 72 and 96 hr exposure for egg, larva and pupa of *L. serricome* respectively. At the end of exposure periods, the life stages were removed from the test chambers and transferred to tubes containing 5 gram of culture medium and incubated at 27±2°C and 70% r.h. They were under regular observation till adults started emerging. Adult emergence was then recorded on alternate days till no more adults emerged. The statistical analysis of the data on adult emergence in control and treated batches of respective days was carried out by Wilcoxon's two-sample rank test (Snedecor and Cochran 1967). Difference in development as evidenced by the time taken by treated insects to reach the adult stage was estimated at 5% significant level.

2. Tests for multiplication potential

Adults of *S. paniceum* and *L. serricorne,* 2-3 days old, were exposed to $CO_2$ at $LD_{50}$ doses separately. For each species 30 insects per replicate were taken in 7 x 1.2 cm size glass tubes. In each tube a paper strip of 5cm x 0.5 cm size was kept for the insects to hold and rest. The open ends of tubes were covered with muslin cloth. The tubes containing insects were placed in individual gas-wash-bottles (test chambers) of 500 ml capacity for exposing to $CO_2$. The LDso doses determined earlier (chapter 3) were used. Adults of *S.paniceum* were exposed to 40% $CO_2$ for 24 hr and *L. serricorne,* to 35% $CO_2$ for 48 hr. Six replicates were maintained for each exposure with equal number of untreated controls. The test chambers containing test insects were flushed with $CO_2$ from a cylinder as described earlier. Immediately after flushing with $CO_2$, the concentrations

in test chambers were checked. When the desired CO2 level inside the test chambers was achieved, they were sealed with rubber septa. Then, the test chambers were left undisturbed for required exposure periods at 27±2°C. At the end of exposure period the tubes containing insects were taken out and kept at 27±2°C and at 70% r.h. At the end of three days, the actual mortality was assessed and the survivors were released in 15 x 2.5 cm size tubes containing 10 gram of culture media ( wheat flour with 5% yeast). Similarly, from untreated controls equal number of survivors were released and remaining were discarded. These tubes were incubated at 27±2°C and 70% r.h. These were under regular observation till $F_1$ adults emerged. The adults emerged were recorded every alternative day till the emergence ceased. From the data on adult emergence in treated and control batches, the percent reduction in progeny production was calculated (Dunkel et al. 1990). A 't' test was carried out on data on Fi progeny to check whether there is a significant difference between control and treated batches in progeny production.

# RESULTS

**1. Influence on development**

Data on emergence following treatment of adults, eggs, larvae and pupae of *S. paniceum* with CO2 are given in Table 6.1. The relative trend in adult emergence from survivors of are presented in Figure 6.1. In general, the emergence of adults from the survivors of immature stages following CO2 treatment was delayed significantly when

compared with their respective controls. A significant decrease in the number of adults emerged on the first two days of count and a proportionate increase on the last 2 or 3 days in treated batches clearly indicate the influence of CO2 on the development of insects. This delay was evidenced by occurrence of peak emergence of adults in treatments as eggs on $5^{th}$ and $11^{th}$ day respectively in control and treated (Figure 6.1). In tests with larvae and pupae the peak emergence was observed on $5^{th}$ and $3^{rd}$ day respectively in controls while it was on $11^{th}$ and $9^{th}$ day in the treated (Figure 6.1). The total number of adults emerged from egg, larva and pupa and actual mortality occurred following treatment with their respective controls are given in Table 6.3. At $LD_{50}$ doses the actual mortality achieved was 52.1, 49.6 and 49.4% respectively in eggs, larvae and pupae.

The emergence of adults from eggs, larvae and pupae of *L. serricorne* following treatment with $LD_{50}$ doses of $CO_2$ are given in Table 6.2. The trend with reference to relative percent adult emergence at alternate days from the start of emergence are presented in Figure 6.2. In general, the emergence of adults from immature stages of L. *serricorne* were delayed significantly after the treatment. Peak emergence of adults from egg were noticed on $5^{th}$ day in controls, but in treatment the peak emergence occurred on $9^{th}$ day (Figure 6.2). Similarly, the peak emergence of adults from larva and pupa of controls were on $5^{th}$ day, while in treatment it was on $9^{th}$ day respectively (Figure 6.2). The total number of adults emerged and the actual percent mortality occurred in treated batches of egg, larva and pupa along with their respective controls are given in Table 6.4.

2. **Effect on progeny production or multiplication**

The number of progeny (FI adults) produced from treated adults of *S. paniceum* and *L.serricorne* and percent reduction in progeny are given in Table 6.5. A significant reduction in progeny of about 41.5% in *S. paniceum* and about 35.8% in *L. serricorne* was observed.

## DISCUSSION

There are several reports about the sub-lethal effects of controlled atmospheres influencing the overall fitness of the exposed population. A delay in developmental process corresponding to exposure time under high-CO2 of 46-53% was observed by Oothuizen and Schmidt (1942) in the pulse beetle, *Callosobruchus chinensis*. Intermittent exposure of *Blatella germanica,* the German cockroach to high-CO2 levels for three minutes to a week was found to slow down their development (Brooks 1957). Delay in development was also noticed in *Tribolium castaneum* exposed as pupae and larvae under pure nitrogen and CO2 (AliNiazee, 1971, 1972) and in eggs exposed to 20% CO2 (AliNiazee and Lindgren, 1970). Similar delay in development of the immature stages of granary weevil, *Sitophilus granarius* and rice weevil, *Sitophilus oryzae* under pure nitrogen and CO2 atmospheres has been observed by Lindgren and Vincent (1970). Spratt (1979a) found that the continuous exposure of the maize weevil, *S. zeamais* to 10%$0_2$, 10% $CO_2$, 80% $N_2$ at 30°C, 71% r.h. resulted in a delay in development of about

11 days. The short exposures of high CO2 concentration once a week, prolonged the duration of nymphal stage by 14-53% in *Blatella germanica* (Brooks 1957). On the other hand, the larval duration was reduced by repeated short exposures in house flies, *Musca domestica* and German cockroach *Blatella germanica* (Edwards and Battem 1973, Brooks 1957, Tanaka 1982, Tanaka 1985). In the present study, $LD_{so}$ doses of $CO_2$ prolonged the developmental period of both *S. paniceum* and *L. serricorne* when tested as eggs, larvae and pupae.

There are a few reports on the effect of $CO_2$ on reproduction in stored product insects. Press and Flaherty (1973) exposed adult moths of *Ephestia khueniella,* (Mediterranean flour moth) *E. cautella* ( Tropical warehouse moth)and *Plodia interpunctella* (Indian meal moth) to a 96% $CO_2$ atmosphere for 2 hours per day for 6 days. Significant reduction in oviposition in all three species was observed in the studies. Repeated exposures to $CO_2$ suppressed oocyte development in the ovarioles in *Tribolium castaneum* (Press et al. 1976). In *Glossina* species (tsetse fly) suppression of insemination frequency of females and reduction in insemination capability of males was observed following exposure of the insects to $CO_2$ for a short period (Moloo and Kutuza 1975). Increasing the time of exposure to 100% $CO_2$ caused progressive and significant decrease in fecundity during the first 40 hr in the pulse beetles, *Callosobruchus maculatus* (Dawson, 1995). The current study showed that progeny production was affected by $CO_2$ treatment of adults of *S. paniceum* and *L. serricorne.*

**Table 6.1 Adult emergence following treatment of eggs, larvae and pupae of *S. pan;ceum* at $LD_{so}$ doses of $CO_2$**

| Emergence days | Total number of adults emerged following treatment of | | | | | |
|---|---|---|---|---|---|---|
| | Eggs | | Larvae | | Pupae | |
| | Control | Treated | Control | Treated | Control | Treated |
| 1 | 16 | 0* | 7 | 0* | 17 | 0* |
| 3 | 32 | 0* | 31 | 0* | 58 | 0* |
| 5 | 49 | 10* | 48 | 8* | 38 | 8* |
| 7 | 28 | 9* | 34 | 12* | 23 | ′ 15* |
| 9 | 12 | 14 | 19 | 18 | 4 | 19* |
| 11 | 7 | 15* | 10 | 20* | 0 j | 16* |
| 13 | 0 | 13* | 2 | 13* | 0 | 10* |
| 15 | 0 | 7* | 0 | 12* | 0 | 3* |
| 17 | 0 | 0 | 0 | 0 | 0 | 0 |

Values are total of 6 replicates

* Significantly different from corresponding control at 5% level (Wilcoxon's two-sample rank test)

**Table 6.2 Adult emergence following treatment of eggs, larvae and pupae of *L. serricome* at $LD_{50}$ doses of $CO_2$**

| Emergence days | No. of adults emerged following treatment | | | | | |
|---|---|---|---|---|---|---|
| | Eggs | | Larvae | | Pupae | |
| | Control | Treated | Control | Treated | Control | Treated |
| 1 | 15 | 0* | 15 | 0* | 14 | 0* |
| 3 | 22 | 0* | 39 | 3* | 28 | 0* |
| 5 | 42 | 8* | 52 | 14* | 44 | 11* |
| 7 | 29 | 11* | 51 | 18* | 33 | 13* |
| 9 | 33 | 23* | 18 | 20 | 16 | 15 |
| 11 | 0 | 9* | 0 | 16* | 0 | 13* |
| 13 | 0 | 10* | 0 | 12* | 0 | 10* |
| 15 | 0 | 6* | 0 | 0 | 0 | 5* |
| 17 | 0 | 0 | 0 | 0 | 0 | 0 |

Values are total of 6 replicates

* Significantly different from respective control at 5% level (Wilcoxon's two-sample rank test)

**Table 6.3 Average of adults emerged following treatment of immature stages of S. *paniceum* at sub-lethal doses of $CO_2$.**

| Life stage treated | No. of adults emerged (x±SD) | | Actual mortality (%) |
|---|---|---|---|
| | Control | Treated | |
| Egg | **24.0±1.8** | **11.5±I.l** | 52.1 |
| Larva | **25.0±0.8** | **12.6±1.2** | 49.6 |
| Pupa | **23.1±1.0** | **11.9±1.5** | 49.4 |

LDso dose for eggs, larvae and pupae respectively were 50, 30 and 40% CO2 with 72 hr exposure period for each.

Values are mean of 6 replicates

**Table 6.4 Average of adults emerged following treatment of immature stages of *L. serricome* at sub-lethal doses of CO2.**

| Life stage treated | No. of adults emerged (x±SD) | | Actual mortality (%) |
|---|---|---|---|
| | **Control** | **Treated** | |
| Egg | 23.5±1.0 | **11.1±1.5** | 51.9 |
| Larva | 29.2±0.8 | **13.8+1.6** | 52.9 |
| Pupa | 22.5+2.1 | **11.2+1.6** | 50.4 |

$LD_{50}$ dose of egg, larva and pupa were 50, 30 and 20% $CO_2$ with 72, 72 and 96 hr exposure respectively

Values are mean of 6 replicates

Table **6.5 Progeny produced following treatment of adults of 5. *paniceum* and *L.serricorne* at $LD_{50}$ doses of CO2**

| **Insect** | **$LD_{50}$ dose (%)** | **Exposure period (hr)** | **Actual mortality** (%) | **F, progeny (Mean** ±SEM) | **% reduction in progeny production** |
|---|---|---|---|---|---|
| *S. paniceum* | **40** | **24** | 50.0±2.6 | *95.2+2.2** | 41.5 |
| Control | **-** | **-** | 0 | 162.8±1.3 | 0 |
| *L. serricorne* | **35** | **48** | 51.1±2.7 | 51.5±1.5* | 35.8 |
| Control | **-** | **-** | 0 | 80.3±3.9 | 0 |

The data are mean of 6 replicates

# % Reduction = 100 - No. of adults emerged from treatment / No. of adults emerged from control X ent

* Significantly different from respective control at 5% level ('t' test)

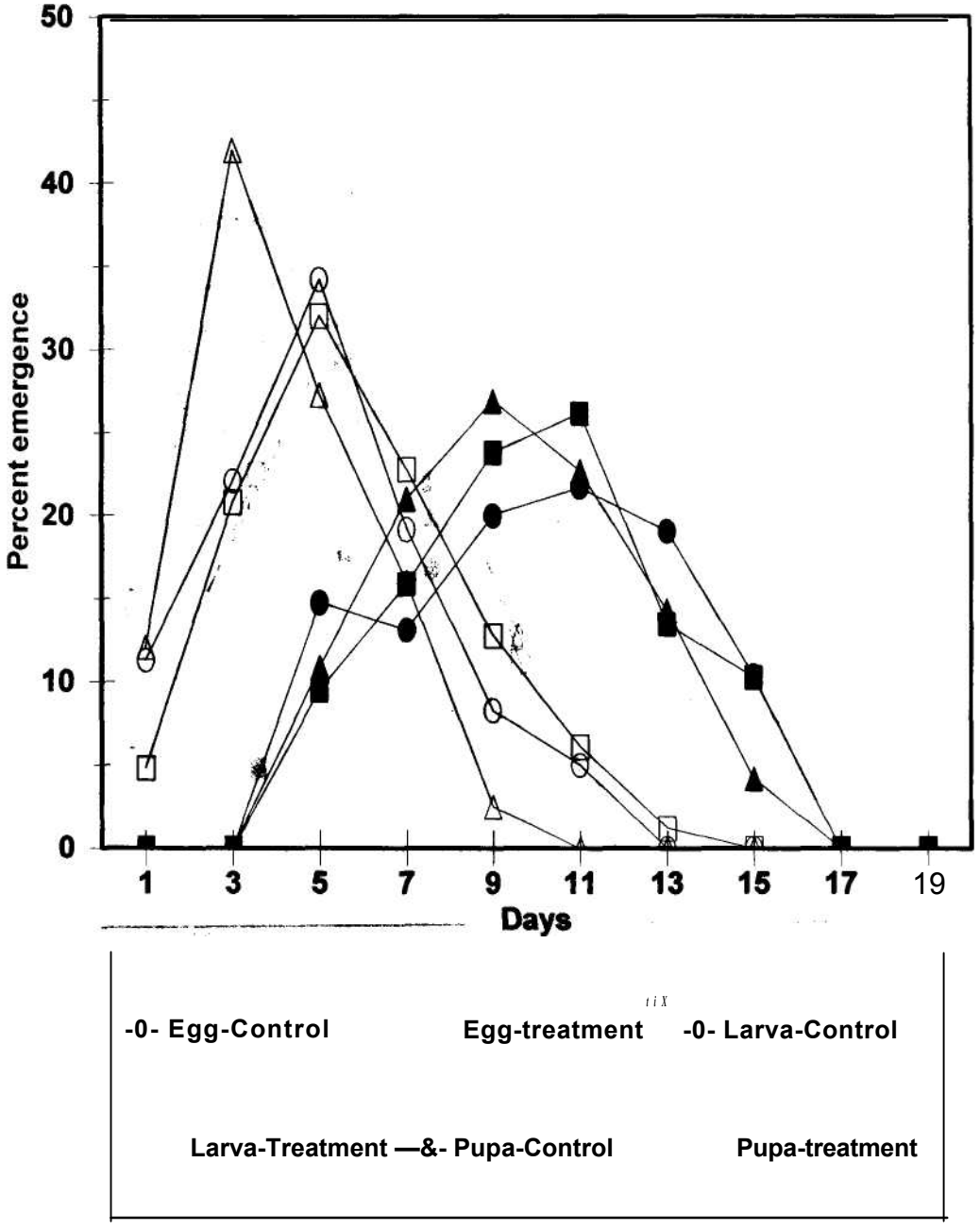



Fig. 6.1 Adult emergence trend in *S.paniceum* following exposure as immature stages at $LD_{50}$ doses of $CO_2$.

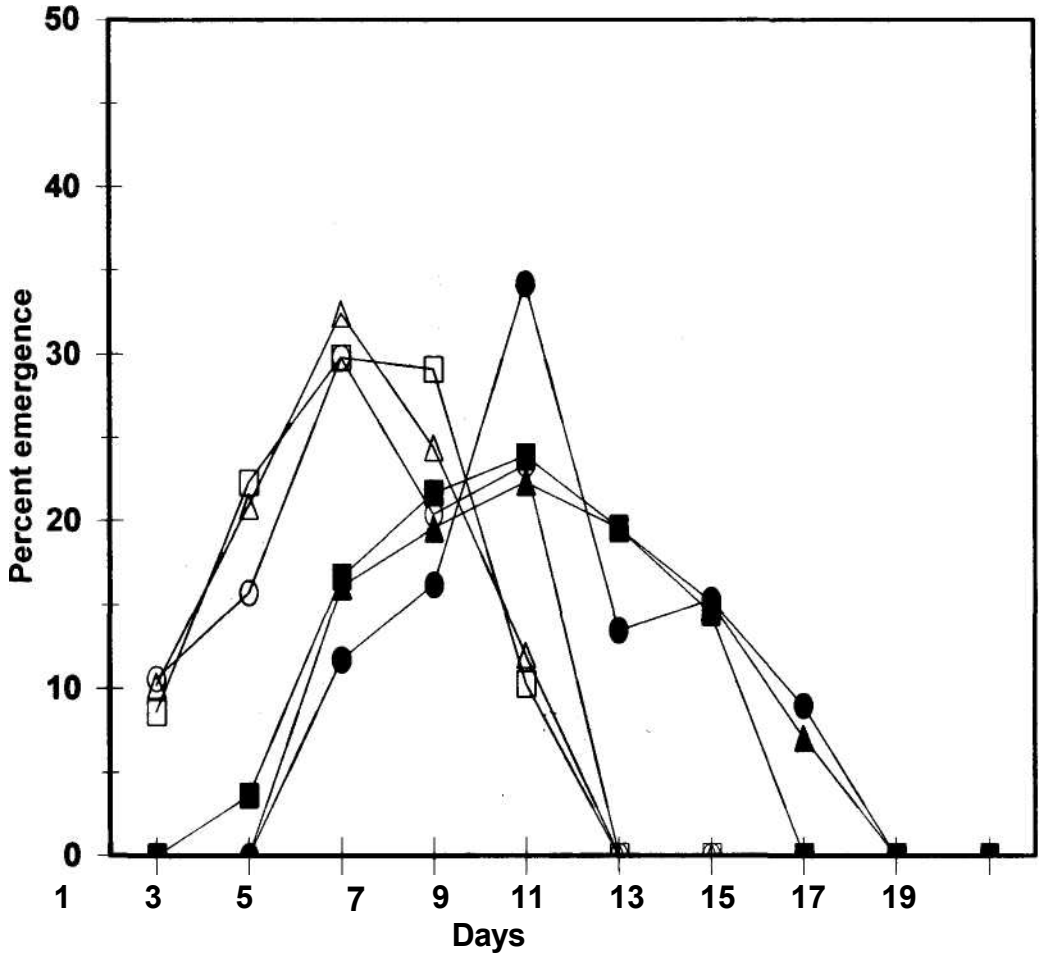



Fig. 6.2 Adult emergence trend in *Lserricorne* following exposure as immature stages at $LD_{50}$ doses of $CO_2$.

# CONCLUSION

The influence of $CO_2$ at sub-lethal doses on 1) the development of immature stages of *S. paniceum* and *L. serricorne* and 2) multiplication potential of adult stages was. studied. $CO_2$ prolonged the developmental period of the survivors as evident from the rate of adult emergence following treatment of the immature stages. Adults of *S. paniceum* and *L. serricorne* exposed to $CO_2$ produced relatively less number of progeny indicating an adverse effect of $CO_2$ on the multiplication potential of the survivors.

# Chapter seven

# Effective carbon dioxide dosage for insect control

# EFFECTIVE CARBON DIOXIDE DOSAGE FOR INSECT CONTROL

## INTRODUCTION

Data on the effect of $CO_2$ -rich atmospheres on mortality response of different life stages of several stored product insect pests are available (Jay and Cuff, 1981; Ofuya and Reichmuth, 1993; Leong and Ho, 1994, Mbata et al., 1994 and Annis and Morton, 1997). Studies on the mortality response of individual life stages of *Stegobium paniceum* and *Lasioderma serricorne* to $CO_2$ -rich atmospheres have been reported in chapter 3 of this investigation. However, there is paucity of information about the effect of $CO_2$ against the mixed-age cultures of *S. paniceum* and *L. serricorne.* Assays using mixed-age insect cultures are known to have more practical applications (Hole et al., 1976). In this context, tests were carried out against mixed-age cultures of *S.paniceum* and *L. serricorne* to arrive at effective $CO_2$ dosage.

For a successful treatment of stored food commodities using $CO_2$ - rich atmosphere, it is important that effective $CO_2$ concentration is maintained in the enclosures during the exposure periods. The maintenance of effective levels of $CO_2$ for the required exposure period depends on 1) $CO_2$ leakage rate from the treated enclosure and 2) $CO_2$ sorption by the commodities treated. Cofie-Agblor et al. (1993 and 1995) studied $CO_2$ sorption by wheat that was exposed to an initial $CO_2$ concentration of 100%. It was reported that the rate of sorption and the amount of gas sorbed were influenced by temperature and moisture content of the grain. As different commodities sorb different amounts of $CO_2$ the amount of $CO_2$ remaining in the void volume or intergranular space

after a particular dosage may vary from commodity to commodity (Mitsuda et al. 1973; Cofie-Agblor et al. 1998). Therefore, it is essential to determine sorption potential of commodities for arriving at effective dosages for insect control. There is lack of data on CO2 sorption by spice powders. Hence the sorption characteristics of different spice powders viz. chilli, coriander, turmeric and curry powders was also studied.

# MATERIALS AND METHODS

**Preparation of mixed-age cultures**

*S. paniceum* and *L. serricorne* cultures were maintained on whole wheat flour supplemented with 5% dried yeast at 27±2°C and 70% r.h. in the laboratory. Preliminary studies showed that it takes about 55 to 58 and 60 to 64 days for completion of one generation of life cycle for *S. paniceum* and *L. serricorne,* respectively. Taking into account of the time taken to complete a life cycle, about 300 freshly emerged adults (mixed sex) of *S. paniceum* were released into a bottle (No.l) containing culture medium (300 g) and incubated at the rearing conditions for 19 days. At the end of $19^{th}$ day the adults were removed through sieving. About 300 fresh adults were released into another bottle (No. 2) containing culture medium and incubated for 19 days as bottle number 1. Likewise a series culture bottles were maintained one after other for 19 days. In order to obtain a mixed-age culture, 3 bottles containing cultures in sequence i.e., bottles 1, 2 and 3 were pooled and mixed together thoroughly taking into care, not to damage the immature stages. This mixture of culture medium contained all the life stages of *S.*

*paniceum.* Mixed-age culture of *L. serricorne* was also prepared in a similar way but the bottles were set up every 21 days (instead of 19 days).

**1. Exposure of mixed-age cultures to $CO_2$**

Three different $CO_2$ concentrations of 50,75 and 90% levels each with 2, 4, 7 and 15 days exposure periods were selected for this experiment. The mixed-age insect culture i.e wheat flour containing all life stages of *S. paniceum,* 15 g each, was taken directly into individual gas-wash-bottles (test chambers). The test chambers containing mixed-age insect culture was then flushed with $CO_2$. From a $CO_2$ cylinder the gas was allowed to pass through 50% v/v glycerol water solution (before entering to the test chambers) (Figure 7.1) so that the gas is humidified to 70% r.h. Carbon dioxide concentrations, in the test chambers were checked with a Riken Infrared Gas Analyzer (Japan Make, Model RI 550 A). When the desired level of $CO_2$ was achieved inside the test chambers, the open ends of tubes of test chambers were closed using rubber septa and sealed gas tight using quick drying glue solution. Then the test chambers were left undisturbed for required exposure periods. For each $CO_2$ dosage four replicates were maintained separately for exposure periods of 2, 4, 7 and 15 days. Equal number (4) of untreated controls were maintained for each dosage/exposure period. At the end of exposure periods, the test chambers were opened. The grease around the neck was cleaned and then the mixed-age cultures were removed from test enclosure. Live adults were counted and discarded, and then the contents were transferred to test tubes (15 x 2.5 cm size). The mouth of the tubes was closed with cloth pieces held by rubber rings. The

tubes were held at 27 ± 2°C and 70% r.h. Adult emergence was recorded at weekly intervals for eight consecutive weeks since within that time the immature stages (egg, larva and pupa) reached the adult stage.

The mixed-age culture of *L. serricorne* was similarly prepared and exposed to different levels of CO2. The emergence of adults was recorded for 8 weeks as described earlier. From the data on adult emergence, the per cent emergence and per cent mortality of *S. paniceum* and *L. serricorne* were calculated.

**2. Carbon dioxide sorption by spice powder:**

Spice powders such as chilli powder, coriander powder, turmeric powder and curry powder in unit packs were obtained from local market. The unit packs of each spice powder type were cut open and pooled separately to required quantity and mixed thoroughly for homogeneity. The moisture content of the spice powders was determined before treatment according to Dean and Stark (1920) method. Representative samples 150 gram (4 replicates) per commodity were taken in 500 ml glass gas-wash-bottles (test chambers). The gas-wash-bottles containing spice powders were then flushed with CO2 (from a cylinder) through dip tube of gas-wash-bottles for 180 seconds. Preliminary studies showed that flushing the test chambers for 180 seconds was sufficient to achieve the desired CO2 levels in the test chambers. Soon after flushing, the open ends of tubes in lids of gas-wash-bottles were sealed with rubber septa using quick setting glue

solution. Similarly, a set of empty flasks (4 Numbers ) without commodity were also flushed with CO2 and sealed as described earlier to serve as controls.

The initial (0 day) $CO_2$ concentration in the test chambers containing spice powders and in control chambers was measured using Systech carbon dioxide analyzer (Model Portamap 2, England Make) by piercing the needle of instrument, through the rubber septum fitted in the tubes of test chambers (Figure 7.2). Immediately after measuring $CO_2$, the piercing point in the septum was stuck with a piece of cellophane adhesive tape to avoid any leakage of $CO_2$. $CO_2$ concentrations in all the test chambers were measured every alternative days until $16^{th}$ day. The experiments were conducted at 27±2°C. From the initial (0 day) and final ($16^{th}$ day ) $CO_2$ concentrations the per cent $CO_2$ sorption (gas loss due to sorption by products) by spice powders was calculated.

**3. Determination of effective** dosage

The experiments on determination of effective dosage were carried out at 27±2°C. Mixed-age cultures of S. *paniceum* and *L. serricorne,* 5 g each were taken separately, in glass tubes ( 7 x 1 . 2 cm size) with both ends open. The open ends of the tubes were covered with muslin cloth held by rubber rings. The tubes containing test insect cultures were placed in 500 ml gas-wash-bottles that served as test chambers. Subsequently, about 100 g of spice powders such as coriander powder, chilli powder, turmeric powder and curry powder were weighed into individual chambers. Then the test chambers were flushed with $CO_2$ discharged from a cylinder. The gas was passed through 50% v/v glycerol water solution before flushing. When the required $CO_2$ level was achieved the

open ends of tubes of test chambers were sealed with rubber septa using quick drying glue solution. The initial $CO_2$ concentration in the test chambers was measured by Systech $CO_2$ analyzer (Model Portarnap 2, England Make). For each treatment there were 4 replicates with an equal number of untreated controls. The initial $CO_2$ levels (%) were 65.1±O.3, 66.1±O.3, 66.8±O.5 and 66.5±O.4% in the test chambers containing coriander powder, chilli powder, turmeric powder and curry powder respectively. At the end of 15 days the tubes containing mixed-age cultures were removed from test chambers. Counts on live adults were recorded and adults were discarded after counting. Then the mixed-age cultures were transferred to individual tubes of 15 x 2.5 cm size, and the tubes were held at 27±2°C at 70% r.h. Number of adults emerged from the test cultures was recorded at weekly intervals for 8 consecutive weeks. From the data on adult emergence, the % kill was calculated.

## RESULTS AND DISCUSSION

### 1. Exposure of mixed-age culture to $CO_2$

Data on adult emergence from mixed-age cultures of S. *paniceum* and *L. serricorne* after treatment with $CO_2$ are given in Tables 7.1 and 7.2 respectively. Among the four exposure periods (2, 4, 7 and 15 days) with 50, 75 and 90% $CO_2$ levels tested, no emergence of adults of S. *paniceum* was observed in 7 and 15 days exposure with all the three $CO_2$ levels. At an exposure period of 2 days however, survivors were noticed in all treatments. The adult emergence was 11.7, 5.6 and 2.6% respectively in 50, 75 and 90% $CO_2$ levels in two days expos1;1re ( Figure 7.3). On the other hand, only 3.8% adult

emergence was observed in 50% $CO_2$ level with four days exposure period. There was no adult emergence at 75 and 90% $CO_2$ with an exposure period of 4 days.

With *L. serricorne,* there was 30.6, 21.3 and 13.8% adult emergence in 50, 75 and 90% $CO_2$ levels respectively at 2 days exposure. At 4 days exposure, the adult emergence was 10.4, 4.5 and 1.4% in 50, 75 and 90% $CO_2$ levels respectively (Table 7.2). There was 100% mortality in cultures exposed to 50, 75, 90% $CO_2$ levels for 7 and 15 days (Figure 7.4). The data on adult emergence reveals that *L. serricorne* is relatively tolerant than *S. paniceum* to $CO_2$ treatment.

**2. Sorption of $CO_2$ by spice powders**

Levels of $CO_2$ sorption by different spice powders over a period of 16 days are given in Table 7.3. The trend in sorption of $CO_2$ by spice powders is presented in Figure 7.5.. Coriander powder sorbed relatively more $CO_2$ (50.6%) than other spice powders that were tested. The sorption was least with curry powder (23%). According to Mitsuda et al, (1973), variation in sorption of $CO_2$ among different commodities may be due to 1) dissolution of $CO_2$ into water, 2) dissolution of $CO_2$ into fats and oils, 3) dissolution of CO2 to porous nature of commodities and 4) biological fixation of $CO_2$. A comparison of the data on lethal levels of $CO_2$ for mixed-age cultures of *S. paniceum* and *L. serricorne* ( Table 7.1 and 7.2) and the data on $CO_2$ sorption by different spice powders (Table 7.3) shows that it is possible to retain insecticidal concentration of $CO_2$ in $CO_2$ treatment of spice powders.

Wells (1954) studied $CO_2$ sorption by walnut meats and found that the amount of CO2 sorbed was more when the oil content was 68% than in fat free meats. In contrast, coriander powder with less oil content sorbed more $CO_2$ (50.6%) than curry powder which had more oil content. This may be due to porous nature of the coriander powder which is evidenced by its more voluminous nature (Mitsuda et al., 1973). Another important factor is influence of moisture content on $CO_2$ sorption. The rate of $CO_2$ sorption increased with an increase in moisture content in brown rice and wheat (Yamamoto and Mitsuda 1980; Cofie-Agblor 1993). Contrary to this, coriander powder and chilli powder with 6% moisture content sorbed more $CO_2$ than turmeric and curry powders with 10.0 and 7.5% moisture content respectively. Similar trend i.e. adsorption of CO2 decreased with increase in moisture content was noticed in paddy rice (Yamamoto and Mitsuda 1980; Diawara et al., 1987), wheat and hulless oats (Cofie-Agblor, 1998). The $CO_2$ sorption may also increase due to biological fIXation of $CO_2$ (Mitsuda et al., 1973), absorption by constituents of commodity (Wells 1954) and the reversible interaction with functional groups of protein which is assumed to contribute to retaining grain qualities (Yamamoto and Mitsuda 1980).

3. Effective $CO_2$ dosage

When mixed-age cultures were exposed to $CO_2$ in the presence of commodities, the initial $CO_2$ levels in the test chambers were about 65-66%. With this initial $CO^2$ concentration and an exposure period of 15 days, there was no adult emergence from mixed-age cultures of *S. paniceum* and *L. serricorne.* However the average number of L.

*serricome* adults emerged in untreated controls, were 44.5±1.6, 31.7±2.3, 28.4±1.5 and 26.0±1.6, when the mixed-age culture kept with coriander powder, chilli powder, turmeric powder and curry powder respectively. Similarly on an average, 46.4±2.3, 33.4±2.2, 30.2±2.0 and 28.5±1.8, *S. paniceum* adults emerged in control batches also kept with coriander powder, chilli powder, turmeric powder and curry powder respectively. Although, only 5 gram of mixed-age culture was kept along with the commodity, the adult emergence in control batches in both species greatly varied. The variations might be due to the influence of active spice principles or volatiles of these spice powders acting on life stages of these insects. The results indicate that a dosage of 65-66% CO2 with an exposure period of 15 days is adequate for controlling all the life stages of *L. serricome* and *S. paniceum* in spice powders.

Mixed-age cultures of the maize weevil *Sitophilus zeamais* in maize, *Rhyzopertha dominica* in wheat and Azuki bean weevil, *Zabrotes subfasciatus* in dried beans were exposed to $CO_2$ rich atmospheres with commodity by Santos et al., (1999). $CO_2$ concentrations of 40% with 15 days of exposure, and 50 and 60% $CO_2$ with 10 days exposure provided total kill of all stages of *S. zeamais.* A dosage of 60% $CO_2$ for 15 days and 50% for 20 days eliminated all stages of *R. dominica* and *Z. subfasciatus* respectively.

Table 7.1 Emergence of adults of *S.paniceum* in mixed-age cultures treated with $CO_2$ for different exposure periods

| $CO_2$ dosage (%) | Adults emerged at weeks | | | | | | | | | Total emerged | %Emergence | % Kill* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | | | |
| 2 days | | | | | | | | | | | | |
| Control | 35.8 | 29 | 10.3 | 9.5 | 20.5 | 10.5 | 10.8 | 26.3 | 69.5 | 222±l0.3 | | |
| 50 | 1 | 10.5 | 5.5 | 1.5 | 2.5 | 2 | 1 | 1.3 | 1.3 | 26.0±2.2 | 11.7±l.0 | 88.3±l.0 |
| 75 | 0 | 3 | 3 | 1 | 2 | 1.3 | 1 | 1 | 1 | 12.5±3.3 | 5.6±l.5 | 94.4±0.5 |
| 90 | 0 | 1.5 | 0 | 1 | 1.3 | 1.3 | 1 | 0 | 1 | 5.8±l.5 | 2.6±O.7 | 97 .4±O. 7 |
| 4 days | | | | | | | | | | | | |
| Control | 44.5 | 16 | 12.3 | 13.8 | 11.5 | 9.5 | 14.3 | 61.5 | 97.5 | 286±17.6 | | |
| 50 | 0 | 4 | 0 | 4.8 | 4.5 | 1 | 0 | 0 | 0 | 11.0±l.6 | 3.8±O.5 | 96.2±O.5 |
| 75 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100±O.0 |
| 90 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100±0.0 |
| 7 days | | | | | | | | | | | | |
| Control | 61.3 | 9 | 11.5 | 15 | 11 | 6.5 | 13.5 | 56.5 | 48.3 | 232.3±19.6 | | |
| 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100±0.0 |
| 75 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100±0.0 |
| 90 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100±0.0 |
| 15 days | | | | | | | | | | | | |
| Control | 72 | 10.8 | 19.8 | 10.5 | 11.8 | 15 | 49.3 | 53 | 43.3 | 284.8±9.2 | | |
| 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100±0.0 |
| 75 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10±0.0 |
| 90 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100±O.0 |

* Mean± SD of 4 replicates

Table 7.2. Emergence of adults of *L .serricorne* from mixed-age cultures treated with $CO_2$ at different exposure periods

| $CO_2$ dosage (%) | Adults emerged at weeks | | | | | | | | | Total emerged | %Emergence | % Kill* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | | | |
| 2 days | | | | | | | | | | | | |
| Control | 18 | 30.5 | 75 | 41.5 | 17.7 | 15 | 8.5 | 40.5 | 69 | 315±30.6 | | |
| 50 | 1 | 21.3 | 5.7 | 33.3 | 17.5 | 11.8 | 1.7 | 2 | 3 | 96.7±9.0 | 30.6±2.8 | 69.4±2.8 |
| 75 | 1 | 16.5 | 2.5 | 22.7 | 10.5 | 10 | 1.7 | 1.3 | 1.8 | 67 .3±8. 7 | 21.3±2.7 | 78.7±2.7 |
| 90 | 0 | 12 | 1.8 | 1.8 | 5.5 | 6.8 | 1.8 | 1.3 | 1.3 | 43.5±7.9 | 13.8±2.4 | 86.2±2.5 |
| 4 days | | | | | | | | | | | | |
| Control | 40.7 | 37.7 | 114.7 | 17.7 | 22.3 | 11.7 | 7 | 43 | 57.7 | 352±14.3 | | |
| 50 | 0 | 1 | 1 | 13.3 | 11.5 | 7.5 | 0 | 0 | 1.5 | 36.7±4.0 | 10.4±l.1 | 89.63±l.l |
| 75 | 0 | 1 | 1 | -2.7 | 5 | 6 | 0 | 0 | 1 | 15.7±l.5 | 4.5±0.4 | 95.5±0.4 |
| 90 | 0 | 1 | 0 | 0 | 1.7 | 2 | 0 | 0 | 1 | 5.0±l.4 | 1.4±O.4 | 98.6±0.4 |
| 7 days | | | | | | | | | | | | |
| Control | 88 | 34.7 | 123.5 | 24 | 13.7 | 9.7 | 25 | 71.7 | 83.3 | 473.7±35.1 | | |
| 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100±O.0 |
| 75 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100±0.0 |
| 90 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100±0.0 |
| 15 days | | | | | | | | | | | | |
| Control | 64.5 | 110.3 | 25.3 | 20.7 | 9.7 | 24.3 | 86.5 | 46 | 41 | 428.3±21.5 | | |
| 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100±0.0 |
| 75 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100±0.0 |
| 90 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100±0.0 |

* Mean± SD of 4 replicates

**Table 7.3 Carbon dioxide sorption by spice powders.**

| Commodity | Quantity (g) | Moisture content* (%) | CO2 concentratIon (%)* at | | % Loss of $CO_2$ due to sorptIon* |
|---|---|---|---|---|---|
| | | | 0 d a y | 1 6 t h d a y | |
| Control (empty flasks) | 150 | 6.0 | 68.0:tO.2 | 65.1:t0.1 | (4.3:t0.l) |
| Coriander powder | 150 | 6.0 | 66.1:tO.3 | 32.6:tO.8 | 50.6:tO.9 |
| Chilli powder | 150 | 10.0 | 66.5:tO.7 | 48.5:t0.5 | 27.1:tO.9 |
| Tunneric powder | 150 | 7.5 | 66.4:t0.6 | 49.2:tO.6 | 26.3:tl.2 |
| Curry powder | | | 66.9:tO.4 | 51.7:t0.2 | 23.0:tO.3 |

* Values are mean of 4 replicates

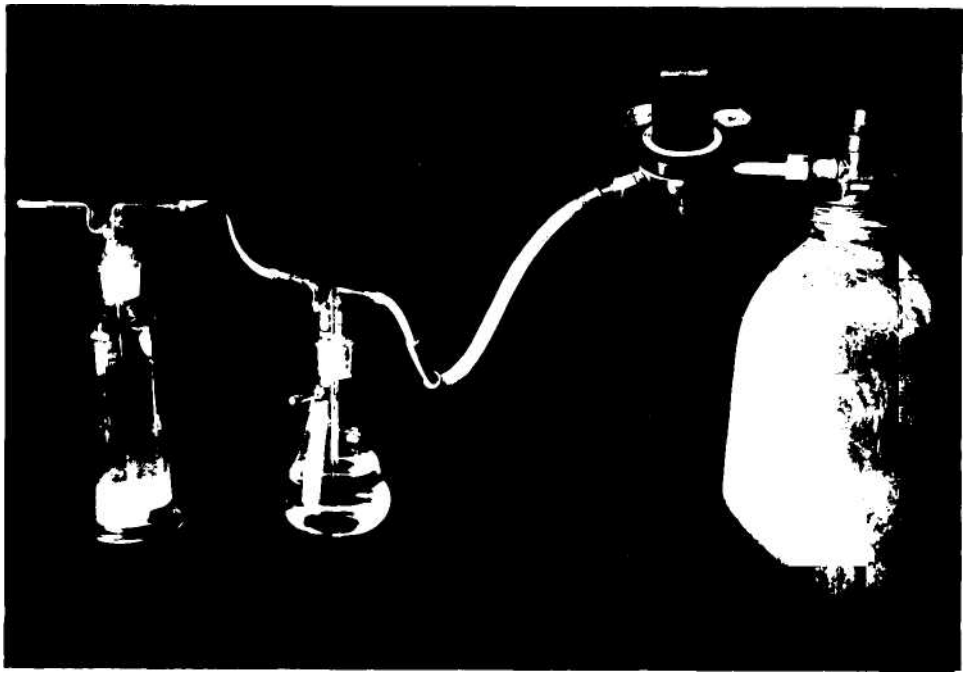


**Fig. 7.1 Exposure of mixed-age culture to carbon dioxide**

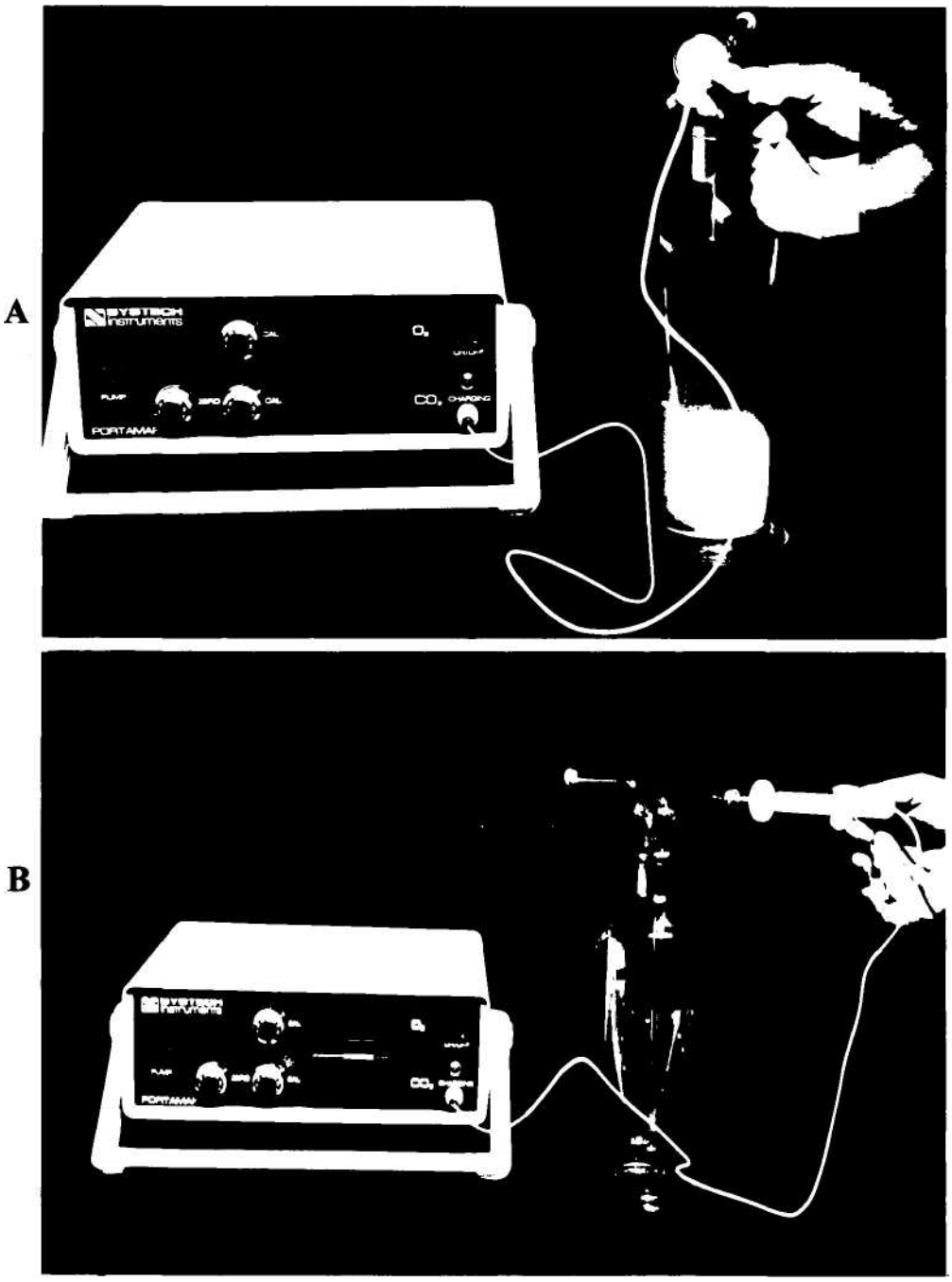



**Fig. 7.2 Carbon dioxide monitoring in test chambers (A) with and (B) without commodity (spice powder)**

**Fig. 7.3 Percent adult emergence from mixed-age culture of** *S.paniceum* treated with carbon dioxide

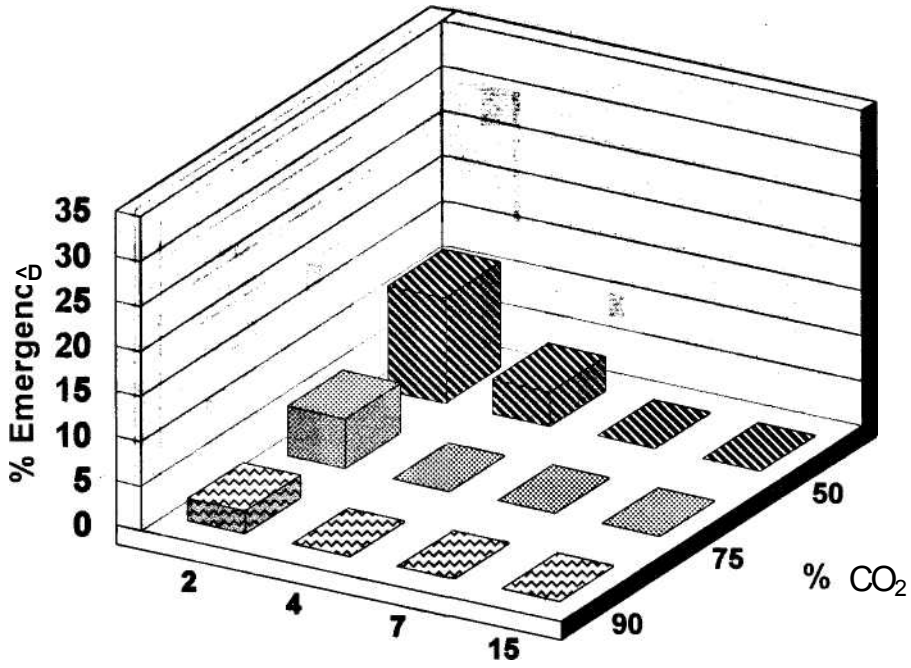
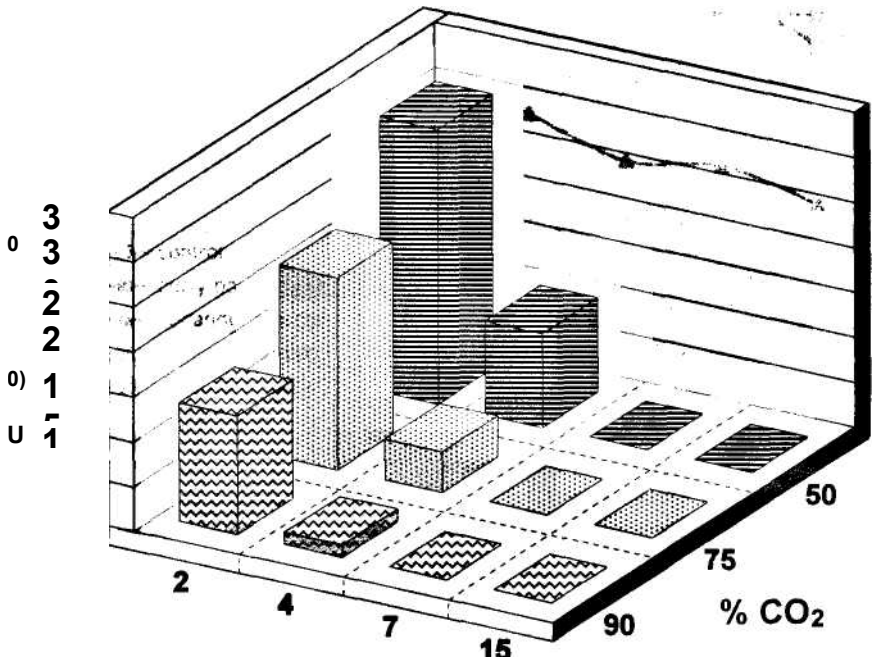


**Fig. 7.4. Percent adult** emergence from mixed-age culture of *L.serricorne* treated with carbon dioxide



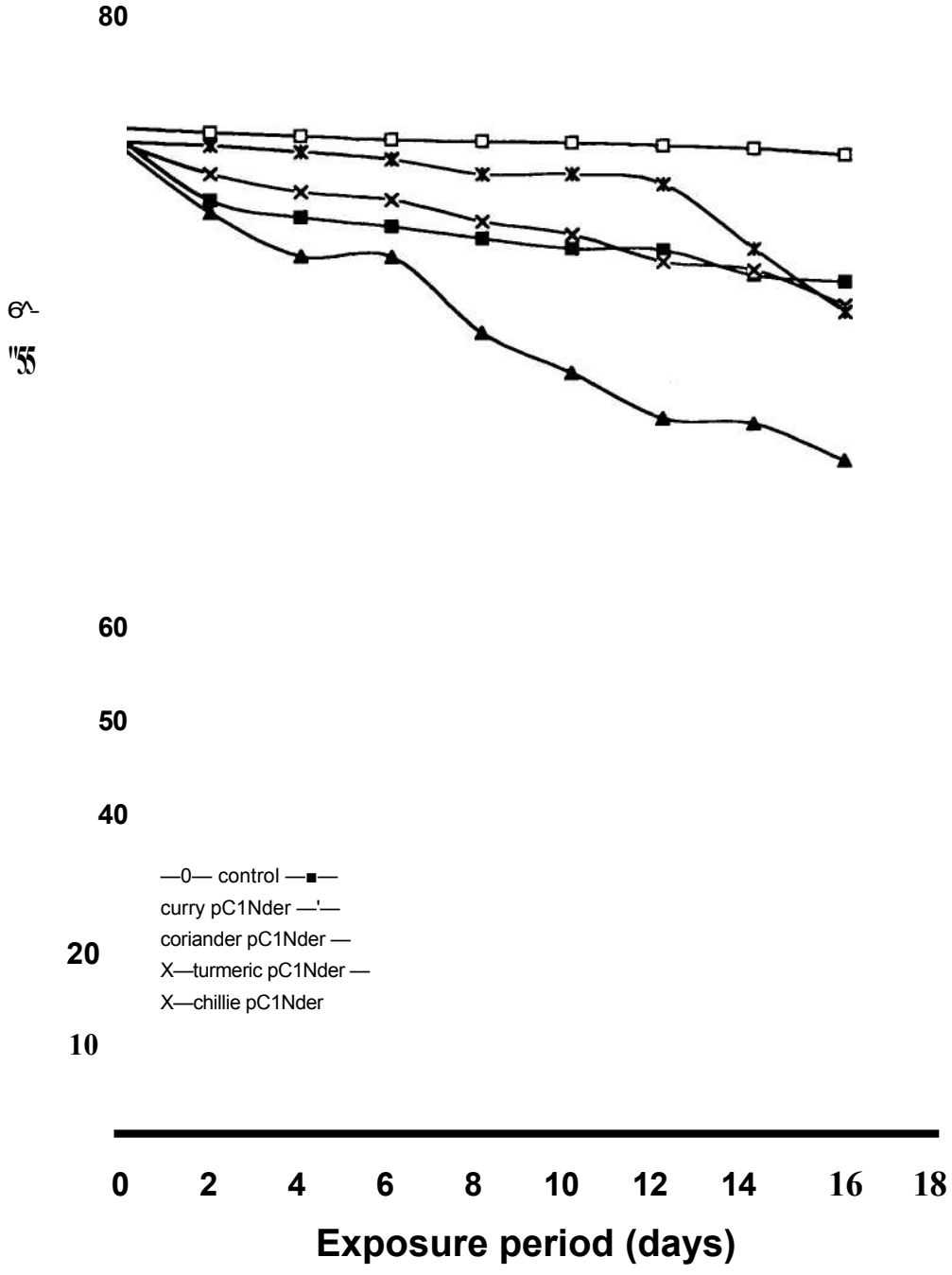



**Figure 7.5 Carbon dioxide sorption trend of different spice powders**

# CONCLUSION

Toxicity of $CO_2$ to mixed-age cultures of *S. paniceum* and *L. serricorne* and $CO_2$ sorption by different spice powders were studied. The mixed-age cultures of individual species were exposed to a range of $CO_2$ doses for different exposure periods. All life stages of *S. paniceum* were killed in 75 and 90% $CO_2$ with 4 days exposure. Similarly, the life stages of *L. serricorne* were killed at 50, 75 and 90% $CO_2$ levels with 7 days exposure. Studies on $CO_2$ sorption revealed that, coriander powder sorbed more $CO_2$ (50.6%), followed by chilli powder (27.1%), and turmeric powder (26.3%) and curry powder showed least sorption (23.0%). An effective dosage of 66% $CO_2$ with 15 days exposure for treating spice powders was determined.

# GENERAL SUMMARY

Aspects of susceptibility of the drugstore beetle, *Stegobium paniceum* and cigarette beetle, *Lasioderma serricorne* to $CO_2$-rich atmosphere, response of the pests to sublethal doses of CO2 and dosage required for controlling the insects in spice products were studied.

The effect of infestation by *S. paniceum* and *L. serricorne* on proximate composition and uric acid level of coriander powder, *sambar* powder and turmeric rhizomes was examined. The total protein, fat, ash contents were reduced significantly due to infestation. A slight reduction in carbohydrates and energy contents was also noticed. A significant increase in moisture content was observed in infested coriander and *sambar* powders. The reduction in proximate composition was prominent in coriander powder and *sambar* powder than in turmeric rhizomes. The reduction or increase in proximate components was more pronounced in products infested for six months than that infested for three months. Over a period of 6 months the uric acid level in infested samples was found to exceed the maximum allowable limit (100 mg/kg) fixed by Prevention of Food Adulteration Rules (1955).

In toxicity tests against the developmental stages of *S. paniceum* and *L. serricorne* to $CO_2$ atmospheres at 27±2°C, it was noted that pupal stage was the most tolerant. $CO_2$ at 80% produced 100% mortality of pupae of *S. paniceum* and *L. serricorne* in 72 and 96 hr exposure periods respectively. Adults when tested at 20°C were found to be more tolerant than in exposures at 27±2°C. The effect of varying or changing

concentrations of $CO_2$ (stepwise increase or decrease) on mortality response of adults showed that the changing concentrations of $CO_2$ were more effective than constant concentration. The mortality response was more prominent in *S. paniceum* than in *L., serricorne.*

Quality paran1eters of different spice powders after treatment with methyl bromide, ethylene oxide, phosphine and $CO_2$-rich atmosphere (at insecticidal and a higher dosage) were examined. A significant decrease in colour value of chilli powder and volatile oil content in coriander powder and curry powder after treatment with fumigants was observed. The capsaicin content in chilli powder treated with methyl bromide and ethylene oxide showed significant reduction. The linalool concentration and volatile oil of coriander and curry powders was also affected by all the three fumigants. The reduction of volatile oil and linalool concentration was prominent in treated coriander powder and its oil than in curry powder and its oil. $CO_2$ had the least effect on the quality parameters of the products tested.

The $CO_2$ retention property and insect-proofness of packaging materials such as metallized polyester/polyethylene (30 micron thickness), polyester/polyethylene (38 micron), polyester aluminum foil/polyethylene (60 micron), nylon based co-extruder (100 micron) and paper foil/polyethylene (110 micron) were studied at 27±2°C. Polyester aluminum foil/polyethylene retained maximum level of $CO_2$ (99.2%) in 24 hr. At the end of 15 days 89.5% $CO_2$ was retained by metallised polyester/polyethylene and 88.4% by polyester aluminum foil/polyethylene. In tests on insect-resistance or proofness, all the

packaging materials tested were noted to be resistant to the attack of adult and larval stages (starved and fed) of *S. paniceum* and *L. serricorne* in a 4 to 8 week period.

The influence of carbon dioxide on development and multiplication of *S. paniceum* and *L. serricorne* was investigated. The immature stages of *S. paniceum* and *L. serricorne* were exposed to $CO_2$ at $LD_{50}$ doses after which adult emergence was checked every week, for 8 weeks. Data on weekly emergence revealed that $CO_2$ prolonged the developmental period of the survivors. In another experiment, adults of *S. paniceum* and *L. serricorne* were exposed to $CO_2$ at $LD_{50}$ doses. The survivors produced relatively less number of progeny indicating an adverse effect of $CO_2$ on the multiplication potential of the insects.

The $CO_2$ sorption potential of spice powders viz coriander, chilli, turmeric and curry powders, and effective $CO_2$ dosage for mixed-age cultures of S. *paniceum* and *L. serricorne* were studied. Among the spice powders tested, coriander powder sorbed more $CO_2$ (50.6%) and curry powder showed least sorption (23.0%) in 16 days. The mixed-age cultures of individual species were exposed to a range of $CO_2$ concentrations at different exposure periods without commodity. All life stages of *S. paniceum* were killed in 75 and 90% $CO_2$ levels with an exposure period of 4 days and above. Similarly, the developmental stages of *L. serricorne* were killed at 50, 75 and 90% $CO_2$ levels with 7 or 15 days exposure. Further tests on mixed-age cultures in the presence of spice powders at 27±2°C revealed that a dosage of 66% $CO_2$ with 15 days exposure was necessary for controlling all stages of *S. paniceum* and *L. serricorne.*

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