

**ECOFRIENDLY STRATEGIES FOR MANAGEMENT OF *Sitophilus oryzae* (L.)
(CURCULIONIDAE: COLEOPTERA) IN STORED MAIZE**

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DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

CENTRE FOR PLANT PROTECTION STUDIES

TAMIL NADU AGRICULTURAL UNIVERSITY

COIMBATORE – 641 003

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Thesis submitted in part fulfillment of the requirements for the award of the degree of
Master of Science (Agriculture) in Entomology to the Tamil Nadu Agricultural University,
Coimbatore - 641 003

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2008

CERTIFICATE

This is to certify that the thesis entitled “**Ecofriendly strategies for management of *Sitophilus oryzae* (L.) (Curculionidae: Coleoptera) in stored maize**” submitted in part fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE) IN ENTOMOLOGY** to the Tamil Nadu Agricultural University, Coimbatore, is a record of bonafide research carried out by **THIRU. N. SRITHAR**, under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

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CONTENTS

Chapter No.	Title	Page No.
1.	INTRODUCTION	1
2.	REVIEW OF LITERATURE	4
3.	MATERIALS AND METHODS	21
4.	RESULTS	42
5.	DISCUSSION	77
6.	SUMMARY	92
7.	REFERENCES	

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(N.Srithar)

ABSTRACT

Ecofriendly strategies for Management of *Sitophilus oryzae* (L.) (Curculionidae: Coleoptera) in stored maize

By

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**Degree : Master of Science (Agriculture) in
Agricultural Entomology**

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Maize is ravaged by a large number of insects in field as well as storage. Among the insect pests infesting stored maize the rice weevil, *Sitophilus oryzae* (L.) (Curculionidae: Coleoptera) is the most serious one and it attacks almost all important cereals under storage conditions with mild infestation in the field as well. It is therefore, imperative to save the stored maize from this serious pest.

Insecticides used to control stored insects leave objectionable residues in treated commodity and generally are hazardous to handle and apply. Some of the stored product insects have also developed resistance to these insecticides. This has resulted in increased reliance on alternative physical and ecofriendly control methods such as modified atmosphere storage treatments which will not contaminate the environment, but bring about remarkable control of storage pests.

Experiments were hence conducted in laboratory to find out the effective concentration of CO₂ and period of exposure to bring about control of the rice weevil without affecting the quality of maize grain and seed using air tight containers. Effect of botanicals and inert dust treatment on insect control was studied in laboratory by treating and storing with botanicals and inert dusts for various periods. Newer and safer insecticides were screened as seed and fabric treatment against storage pests and their effect on seed quality also studied. The effect of insect damage on grain and seed quality was estimated in terms of

biochemical composition and seed germination, respectively. Attempts were also made to find out effective natural enemies against this pest.

Increasing CO₂ concentration in storage atmosphere to 60 per cent resulted in 100 per cent mortality of larvae of *S. oryzae* at 4 days of exposure time on maize seeds. The LC₅₀ and LC₉₅ values for larvae were 23.3 and 47.5% CO₂ for an exposure time of 4 days. The LC₅₀ and LC₉₅ values for adult rice weevils were 15.1 and 45.0% CO₂ concentrations when exposed for 4 days. In the long term storage study no adults emerged in treated seeds up to 120 DAT at 60 and 70% CO₂ concentrations and seed germination remained unaffected.

Among the new insecticide molecules evaluated as pre-storage seed treatment on maize seeds, deltamethrin 1ppm and lufenuron 5ppm caused high mean per cent larval mortality of 86.7 and 78.9, respectively. The adult mortalities caused by deltamethrin 1ppm and thiamethoxam 2 ppm were as high as 66.7 and 63.3 per cent, respectively on 2 DAT on maize. Storage bags treated with deltamethrin 100 ppm and lufenuron 100 ppm recorded nil adult population upto 120 DAT.

The mortality of larvae of *S. oryzae* due to seed treatment with botanicals and inert dusts was above 90% in maize seeds treated with sweet flag rhizome powder 5g/kg, rice husk ash 5g/kg and diatomaceous earth (DE) 2.5g/kg of seeds. The mortality of adults of *S. oryzae* due to botanicals seed treatment was 66.7, 53.3 and 23.3 per cent in CO1 maize seeds treated rice husk ash 5 g/kg, DE 2.5g/kg and sweet flag rhizome powder 5g/kg of seeds on 4 DAT. The mean per cent seed damage was less than 1% and adult population nil in seeds treated with DE 2.5g/kg, rice husk ash 5g/kg and sweet flag rhizome powder 5g/kg on 120 DAT in the long term study.

Two parasitoids belonging to families Pteromalidae and Bethylidae, Hymenoptera were recorded from insect cultures of *S.oryzae*. Biological control potential of these parasitoids has to be worked out.

The nitrogen content increased in *S.oryzae* damaged maize grains compared to uninfested grains indicating the increase in uric acid content due to insect excreta in infested grains. There was a reduction of 7.5 and 9.8% in carbohydrate content and 4.5 and 4.4% starch content in CO1 and COH(M) 5 maize grains damaged by *S.oryzae* which clearly indicated deterioration in nutritional status of insect damaged maize grains.

ABBREVIATIONS

%	: Per cent
ai	: active ingredient
CD	: Critical Difference
cm	: centimeter
CO ₂	: carbon dioxide
col	: column
Conc	: concentration
CRD	: completely randomized design
CV	: Coefficient of Variation
D	: Dust
DAT	: days after treatment
EC	: Emulsifiable concentrate
FCRD	: factorial completely randomized design
g	: grams
G	: Granule
Hg	: Mercury
h	: Hours
ISTA	: Indian Seed Testing Association
kg	: kilogram
LC ₅₀	: Lethal concentration to kill 50 per cent of test population
LC ₉₅	: Lethal concentration to kill 95 per cent of test population
LD ₅₀	: Lethal dose to kill 50 per cent of test population
lit	: litre
ml	: milliliter
mm	: millimeter
N ₂	: Nitrogen
O ₂	: oxygen
°C	: Degrees centigrade
ppm	: parts per million
Qty	: quantity
RH	: Relative Humidity
SC	: Suspension Concentrate
SE(d)	: Standard Error
SLPM	: standard litre per minute

LIST OF TABLES

SNO	Title of table	Page No
	Review of Literature	
2.1.	Mortality of storage pests under modified atmospheric storage	
2.2.	Comparative efficacy of CO ₂ and N ₂	
2.3.	Studies on different CO ₂ sources and combinations on mortality of insects	
2.4.	Effect of CO ₂ treatment on insect species and life stages	
2.5.	Development of resistance in storage pests to insecticides	
2.6.	Efficacy of insecticides as seed / grain protectants	
2.7.	Studies on the effect of insecticidal treatment of storage bag fabric	
2.8.	Use of Plant products in stored product insect management	
	Results	
4.1	Effect of CO ₂ treatment on the mortality of larval stage of <i>S. oryzae</i> in maize	
4.2	Probit analysis of CO ₂ concentration Vs insect mortality relationship	
4.3	Effect of CO ₂ treatment on adult mortality of <i>S. oryzae</i> in stored maize	
4.4	Effect of treatment CO ₂ on adult population of <i>S. oryzae</i> in stored maize (Variety CO 1)	
4.5	Effects of CO ₂ seed treatment on per cent damage by <i>S. oryzae</i> in stored maize	
4.6	Effect on CO ₂ treatment on germination of seeds in stored maize	
4.7	Effect of seed with insecticide treatment on larval mortality of <i>S. oryzae</i> in stored maize seeds	

SNO	Title of table	Page No
4.8	Effect of seed treatment with insecticides on adult mortality of <i>S. oryzae</i> in stored maize seeds	
4.9	Effect of seed treatment with insecticides on adult population of <i>S. oryzae</i> in stored maize (Variety CO 1)	
4.10	Effect of seed treatment with insecticides on per cent damage by <i>S. oryzae</i> in stored maize (Variety CO1)	
4.11	Influence of seed treatment with insecticide on germination of seeds in stored maize	
4.12	Impact of storage bag with insecticides treatment on adult population of <i>S. oryzae</i> in stored maize seeds	
4.13	Influence of storage bag treatment with insecticides on per cent damage caused by <i>S. oryzae</i> in stored maize	
4.14	Influence of storage bag treatment with insecticides on seed germination caused by <i>S. oryzae</i> in stored maize	
4.15	Effect of seed treatment with botanicals and inert dust on the larval mortality of <i>S. oryzae</i> in maize	
4.16	Effect of treatment with botanical or inert dust on adult mortality of <i>S. oryzae</i> in maize (Variety CO 1)	
4.17	Effect of treatment with botanicals and inert dust on adult population of <i>S. oryzae</i> in stored maize (Variety CO 1)	
4.18	Effect of treatment with botanicals and inert dust on per cent damage caused by of <i>S. oryzae</i> in stored maize (Variety CO 1)	
4.19	Effect of treatment with botanicals and inert dust seed germination caused by of <i>S. oryzae</i> in stored maize (Variety CO 1)	
4.20	Comparison of multiplication of <i>S. oryzae</i> and its parasitoids	
4.21	Carbohydrate content of maize grains at different level of insect infestation	
4.22	Starch content of maize grains at different level of insect infestation	
4.23	Nitrogen content of maize grains at different level of insect infestation	

LIST OF FIGURES

SNO	Title of Figure	Page No
	Results	
4.1	Effect of CO ₂ treatment on the larval mortality of <i>S. oryzae</i>	
4.2	Effect of CO ₂ treatment on the adult mortality of <i>S. oryzae</i>	
4.3	Relationship between CO ₂ concentration and mortality of <i>S. oryzae</i> adults for different periods of exposure on maize CO1	
4.4	Relationship between CO ₂ concentration and mortality of <i>S. oryzae</i> adults for different periods of exposure on maize COH(M)5	
4.5	Relationship between CO ₂ concentration and mortality of different life stages of <i>S. oryzae</i> on maize CO1	
4.6	Relationship between CO ₂ concentration and mortality of different life stages of <i>S. oryzae</i> on maize COH(M)5	
4.7	Effect of seed treatment with insecticides on the larval mortality of <i>S. oryzae</i>	
4.8	Effect of seed treatment with insecticides on the adult mortality of <i>S. oryzae</i>	
4.9	Effect of seed treatment with botanicals and inert dust on the larval mortality of <i>S. oryzae</i>	
4.10	Effect of seed treatment with botanicals and inert dust on the adult mortality of <i>S. oryzae</i>	
	Discussion	
5.1	Effect of CO ₂ treatment on adult population of <i>S. oryzae</i> in stored maize	
5.2	Effect of CO ₂ treatment on per cent damage caused by <i>S. oryzae</i> in stored maize	
5.3	Effect of insecticide treatment on adult population of <i>S. oryzae</i> in stored maize	
5.4	Effect of insecticide treatment on per cent damage caused by <i>S. oryzae</i> in stored maize	
5.5	Effect of storage bag treatment with insecticides on adult population of <i>S. oryzae</i> in stored maize	

SNO	Title of Figure	Page No
5.6	Effect of storage bag treatment with insecticides on per cent damage caused by <i>S. oryzae</i> in stored maize	
5.7	Effect of seed treatment with botanicals and inert dust on adult population of <i>S. oryzae</i> in stored maize	
5.8	Effect of seed treatment with botanicals and inert dust on per cent damage caused by <i>S. oryzae</i> in stored maize	

LIST OF PLATES

SNO	Title of Plate	Page No
3.1	Photomicrograph of the test insects <i>S. oryzae</i>	
3.1a	Weevil reared on maize	
3.1b	Weevil reared on sorghum	
3.1c	Aedeagus of adult male weevils protruding out	
3.1d	Smooth dorsal surface of aedeagus characteristic of <i>S. oryzae</i>	
3.2a	Mass culturing of <i>S. oryzae</i> on maize grains	
3.2b	Germination test by roll towel method	
3.3	Evaluation of CO ₂ treatment against <i>S. oryzae</i> on maize grains	
3.3a	Filling CO ₂ in airtight container	
3.3b	Experimental setup – container with maize seeds filled with CO ₂	
3.4	Management methods against <i>S. oryzae</i> Experimental setup	
3.4a	Surface treatment of seeds with botanicals	
3.4b	Surface treatment of seeds with insecticides	
3.4c	Treatment of storage bags with insecticides	
4.1	Photometric of parasitoids recorded from <i>S. oryzae</i> culture	
4.1a	Belonging to family Pteromalidae	
4.1b	Belonging to family Bethylidae	

CHAPTER I

INTRODUCTION

Maize (*Zea mays*, L.) is the staple starchy diet for many groups of people and the main constituent of livestock feeds in many countries. In India, maize is emerging as the third most important crop after rice and wheat. Besides its uses as human food and animal feed, maize has its diversified uses in starch industry, corn oil production, baby corns, popcorns, etc., the potential of maize for exports has increased its demand. In 2006, maize area under cultivation in world, its production and productivity were 145 million ha, 695 million tonnes and 4.8 tonnes / ha respectively. In India these figures were 7.6 million ha, 14.7 million tonnes and 1.9 tonne / ha respectively (Source: FAOSTAT, FAO). In Tamil nadu the maize area, production and productivity figures for 2005-06 were 202 thousand ha, 294 thousand tonnes and 1.2 tonne /ha (Season and Crop Report 2005-06, Govt. of Tamil Nadu). Its area and production are increasing year after year in Tamil Nadu and the whole nation.

Insect pests have been a threat to food and seed since man started growing crops. Grains are stored for varying periods to ensure proper and balanced distribution throughout the year. Insect pests cause heavy losses of stored grain quantitatively and qualitatively throughout the world (Madrid *et al.*, 1990). The protection of stored grains from insect pests is of considerable importance since they are capable of causing severe infestation and damage in a short period (Allotey, 1991). Post harvest losses of food grains in India are estimated to be around 10 per cent of the total production, of which losses during storage alone are estimated to be 6.58 per cent (Gahukar, 1994). In India annual storage losses was estimated as 13.98 million tonnes of food grain worth Indian Rupees 6845 crores every year (Mesech, 1990). Out of this, food grain losses due to insects alone account for nearly Rupees 1275 crores.

In South India, the rice weevil *Sitophilus oryzae* (L.) is the most serious storage pest on stored maize grains and seeds. Maize weevil, *Sitophilus zeamais* (Motsch.) has also been reported as a serious pest in India and abroad. Reports in Southern India on *S. zeamais* are mainly based on the size of the insect and not on any other taxonomic character. But in our

studies, identification of the insects based on male genitalia indicated the presence of only *S. oryzae* and not *S. zeamais* infestation in stored maize. The weevil specimen were sent to Division of Entomology, IARI, New Delhi for identification who identified them to be only *S. oryzae*. The size of the *S. oryzae* adults reared on maize grains was much greater than when reared on rice or sorghum.

Current approaches for controlling insects in stored grain rely largely on fumigation or synthetic insecticides. Yesteryear chemicals / insecticides used to control stored insects leave objectionable residues in treated commodity and generally are hazardous to handle and apply. Indiscriminate use of insecticides has led to the development of insecticide resistant strains of stored product insects as well as insecticide residue problems in food grains (Bhatia, 1990). Also, growing resistance to insecticides among insect population is reducing pesticide effectiveness (Subramanyam and Hagstrum, 1995). Further, the recent cancellation of registration of almost all fumigants including methyl bromide and aluminum phosphide in many developed countries because of their possible carcinogenic effects on humans has resulted in increased reliance on alternative ecofriendly pest management strategies such as modified atmosphere storage, use of botanical treatments, inert dusts and new insecticide molecules that have relatively low mammalian toxicity.

Modified atmosphere have many advantages viz., they provide a way to eliminate insects from stored commodities without polluting the atmosphere and are safer than traditional fumigants. No harmful residues remain after the treatment of the commodity with N₂ or CO₂. Carbon dioxide (CO₂) is now used in several countries for the treatment of stored products, particularly grain in bulk, to control insect pest (Jay, 1984). Recent development in its use with the sheeted stack methods indicates its value as an alternative non toxic control method to the use of fumigants in long term storage of bagged grain. The attraction of CO₂ as a modified atmosphere (MA) treatment lies on availability, relative convenience and safety of application and the facts that it does not leave toxic residue has received the U.S. Food and Drug Administration (FDA) approval for its use as a fumigant (Johnson 1981).

Use of botanicals or plant products is a promising alternative to insecticide treatment in storage pest management (Mulungu *et al*, 2007). Evaluating the efficacy of locally available botanicals will aid in effective storage pest management.

Inert dusts act as physical toxins of stored product pests when mixed with stored grains/ seeds (Fields and Korunic, 2000). It is essential to find out the effective inert dust and its dose to manage *S. oryzae* in stored maize.

Insecticides still remain a means of quickly reducing the pest population and minimising loss to grains and seeds. It is hence essential to evaluate newer and effective insecticide molecules which exhibit low mammalian toxicity to protect seeds through pre-storage seed treatment and safer insecticides to protect grains through fabric treatments. Taking into consideration the above said facts, investigations were carried out on ecofriendly strategies for managing *S. oryzae* with the following objectives.

Objectives:

1. To study the effect of increasing CO₂ concentrations in storage atmosphere on *S. oryzae* attacking stored maize and its effect on quality of grains / seeds
2. To find the effectiveness of botanicals as pre-storage treatment of maize grains for management of *S. oryzae*
3. To manage *S. oryzae* on stored maize by fabric treatment of storage bags with new insecticide molecules
4. To protect maize seeds from *S. oryzae* through surface treatment of seeds with new insecticides.

CHAPTER - II

REVIEW OF LITERATURE

The research work done related to management of storage pests with carbon dioxide, insecticides, botanicals and inert dusts and the biochemical changes taking place in seeds due to insect damage are reviewed in light of the research work presently undertaken.

2.0. Modification of storage atmosphere:

The normal storage atmosphere (or earth's atmosphere) contains 78% nitrogen (N₂), 21% oxygen (O₂) and 0.03% carbon dioxide (CO₂). In grain storage, insects can be controlled by decreasing O₂ or increasing CO₂ or N₂ concentration in the atmosphere thereby interfering with the normal respiration of insects. This is achieved by modified atmospheric storage, controlled atmospheric storage or airtight storage.

In case of modified atmosphere, the storage atmosphere is modified by introducing CO₂ or N₂ replacing O₂. Controlled atmosphere is precisely maintaining the composition of selected gases such as CO₂, O₂ and N₂ at specified concentration under normal pressures or under partial vacuum.

Airtight or hermetic storage of grains / seeds does not involve introducing any gas but just making storage conditions airtight. This leads to decrease in available O₂ and increase in CO₂ due to respiration and metabolism of the seeds in turn leading to mortality of insects. (Banks, 1981; White *et al.*, 1993).

2.1. CO₂ treatment to manage stored pests:

In the past few years, considerable effort has been placed on the development of control measures for stored product insects that would replace or supplement conventional fumigants and insecticide. A portion of this effort has been directed toward controlling these insects by modification of storage atmospheres through introduction of carbon dioxide. Controlled atmospheric storage protects stored food products against damage without leaving toxic residues. In recent years, workers in several countries (Jay and Pearman, 1973; Banks and Annis, 1977) have demonstrated the efficacy of atmospheres containing high level of

nitrogen (N₂) or carbon dioxide (CO₂) in controlling stored product pest generally. Gas concentrations and exposure times to cause >95% mortality of storage insects was assessed by Banks and Fields (1995) and Jayas *et al.*, (1995). Spratt (1975) and Calderon and Navarro (1979) showed that combinations of reduced O₂ with increased CO₂ levels were more effective in controlling insects than a change in the concentration of only one of the gases. The literature available on the effect of different gases under controlled/ modified atmosphere on the morality of different stored product pests are given in Table 2.1.

Table 2.1. Mortality of storage pests under modified atmospheric storage

Sl. No.	Insect species	Gas, concentration and their effect	Reference
1.	<i>Sitophilus zeamais</i> (Mots.)	10% O ₂ , 10% CO ₂ and 80%N ₂ at 30° C,71% RH resulted in a delay in development of about 11 days	Spratt (1979)
2.	<i>S. zeamais</i>	All stages of insect were killed in an atmosphere containing 5% or less of O ₂	Bailey (1957)
3.	<i>Sitophilus oryzae</i> (L.)	All stages of insects were killed in an atmosphere containing 4% or less of O ₂	Bailey (1957)
4.	<i>Sitophilus</i> sp	Mortality was increased when the level of CO ₂ was increased from 40 - 75%	Navarro <i>et al.</i> (1985)
5.	<i>Sitophilus</i> sp	Steep mortality curves observed when exposed to increased carbon dioxide levels	Spratt (1979)
6.	<i>S. oryzae</i> and <i>Rhizopertha dominica</i> (Fab.)	Emergence of immature stages stopped completely by exposing them for 2 weeks to 60 per cent CO ₂ and low O ₂ (< 12%) at high relative humidities (> 50%), and at 2 and 16°C	Jay (1984)
7.	<i>Ephestia cautella</i> (Walker.)	Eggs did not hatch when adults of both species were exposed to 96% CO ₂ for 2h daily for 6 days at 27° C	Press and Flaherty (1973)

Sl. No.	Insect species	Gas, concentration and their effect	Reference
8.	<i>E. cautella</i>	Mortality was correlated with loss in weight due to desiccation and the build up of lactate in the haemolymph when exposed to high CO ₂	Navarro and Calderon (1974)
9.	<i>Cryptolestes ferrugineus</i> (Stephens)	Maximum mortality of adults (99%) and eggs (85%) were obtained at high CO ₂ (88 - 91.7%) and low O ₂ (0-0.5%), over an exposure period of 96 hours	Ramesh Babu <i>et al.</i> (1991)
10.	Stored product insects	Exposure of 4 days to 60% CO ₂ in air effective for control of most stored product insects	Jay (1971)
11.	Non diapausing larvae of storage pests	Mortality was 100% at 60% CO ₂ with 21% O ₂ after 17 days at 30°C and 72%RH	Bailey (1965)
12.	Various insect species and stages	All stages of insects were killed in an atmosphere containing 2% or less of O ₂ .	Bailey (1957)

2.2. Comparative efficacy of CO₂ and N₂

The comparative efficacy of CO₂ and N₂ against storage pests are reviewed in Table 2.2.

Table 2.2. Comparative efficacy of CO₂ and N₂

Sl.No.	Insect species	Comparison of CO ₂ and N ₂ gases	Reference
1.	<i>E. cautella</i>	The egg hatching was lower in CO ₂ than in N ₂ irrespective of exposure period showing a toxic effect of CO ₂ in the absence of oxygen.	Bell <i>et al.</i> (1980)
2.	<i>Tribolium castaneum</i> (Herbst.)	Both mortality and weight loss of all life stages were small when insects were exposed to an atmosphere of 97 per cent N ₂ , but weight loss was generally small and mortality was large for those insects exposed to 97 per cent CO ₂	Jay and Cuff (1981)
3.	<i>Oryzaephilus mercator</i> (Fauvel.) and <i>E. cautella</i>	Hundred percent CO ₂ atmospheres was significantly faster than N ₂ in controlling adults	Varma and Wahdi (1978)
4.	Major stored product insects	High CO ₂ atmospheres generally killed insects faster than high nitrogen atmospheres, and that CO ₂ concentration of 60 per cent could be allowed to fluctuate as low as 35 per cent to get good insect control	Jay (1980)
5.	Internal feeders	Sorption of CO ₂ by grain or oilseeds might make it more effective than N ₂ against species whose immature stages feed inside the kernel.	Jay (1980)
6.	Navel orangeworm	Hundred percent CO ₂ reduced the time required for 95 per cent mortality by 10 hours compared with 100 per cent nitrogen treatment	Brandle <i>et al.</i> (1983)

2.3. CO₂ sources and combinations

CO₂ has been used in combination with other chemicals. The literature available on CO₂ sources and combinations are reviewed in Table 2.3.

Table 2.3. Studies on different CO₂ sources and combinations on mortality of insects

Sl.No.	Insect species	CO ₂ sources and combinations	Reference
1.	<i>Callosobruchus chinensis</i> (L.)	Biogas (60 per cent methane and 40 per cent carbon dioxide) from cow dung could be used as fumigant	Mohan and Gopalan (1992)
2.	<i>C.chinensis</i>	Biogas is a good CO ₂ source and has a quick knock down effect and resulted in complete mortality of adults in 16 hours	Subramanya <i>et al.</i> (1994)
3.	<i>Lasioderma serricorne</i> (F.) and <i>T. castaneum</i>	In insects which were tolerant to allyl acetate, higher mortality was reported due to allyl acetate + CO ₂ combinations and that CO ₂ could be used as an adjuvant for allyl acetate	Leelaja <i>et al.</i> (2007)

2.4. Effect on insect species and life stages

It has been observed that different species and different stages respond differently to CO₂ treatment. The information available on effect of CO₂ on insect species and stages are compiled and given in Table 2.4.

Table 2.4. Effect of CO₂ treatment on insect species and life stages

Sl.No.	Insect species	Impact on life stages/ species	Reference
1.	<i>Sitophilus</i> sp.	Pupae more tolerant than other stages when tested against a range of CO ₂ concentrations (15 - 100%)	Annis and Morton, (1997)
2.	<i>E. cautella</i>	At 90% N ₂ and 10% CO ₂ , the eggs survived longer than any other stages	Storey (1975)
3.	<i>E. cautella</i>	All the eggs aged within 1 h died when exposed to CO ₂ or N ₂ for 24hrs at 25°C	Bell <i>et al.</i> (1980)
4.	<i>L. serricorne</i>	Eggs, larvae, and pupae were killed in 4 days while the adults required 8 days when exposed to 30°C	Shunmugam <i>et al.</i> (1993)
5.	Storage pests	Granary weevil > rice weevil > red flour beetle > Indian meal moth > rusty grain beetle in the order of increasing susceptibilities to controlled atmospheres	Banks and Fields (1995)

Sl.No.	Insect species	Impact on life stages/ species	Reference
6.	Most stored product insects	The adults more susceptible to controlled atmospheres than the larvae, pupae, and egg	Jayas <i>et al.</i> (1995)

2.5. Storage Relative Humidity

The relative humidity (RH) of an atmosphere was found by Jay (1971) to have an important effect in insect mortality in controlled atmospheric storage. This mortality was attributed to water loss and eventual desiccation that occurs when insects open spiracles in response to either low oxygen when exposed to high N₂ atmospheres or to high CO₂ concentration. Navarro (1978) showed that low relative humidity could enhance mortality of stored-product insects under low oxygen conditions, this interaction was not consistent among species and humidity had less of an effect at oxygen levels close to 1.0%, as used in his experiments.

2.6. Storage Temperature

The effect of modified atmospheres is dependent on the ambient temperature. Soderstrom *et al.* (1992) concluded that, heat combined with controlled atmospheres increased mortality of *T. castaneum* larvae when temperatures greater than 38°C were combined with carbon dioxide enriched or oxygen deficient atmospheres. Jay (1984) reported that the exposure time for 99 per cent mortality of *T. castaneum* was reduced from 24 hours to 6 hours when temperature increased from 32 to 38°C. No egg hatched after the shortest exposure period of 2 days at either temperature. Pupae were all killed within 6 days, which was the shortest exposure period at 30°C, but 60% survived to become fertile adult after 3 days exposure at 20°C (Spratt, 1985).

2.7. Exposure time

Modified atmospheres need longer period for insect mortality as compared with some chemical fumigants. Ramesh Babu *et al.* (1991) reported that the mortality of adults and eggs of *C. ferrugineus* almost linearly increased with exposure time in high CO₂ (>68%) and low

O₂ (<9.2%) atmospheres. Girish (1978) found that 40-64 h exposures were required at 35°C for kill of *T. granarium* Everts larvae using 1.1% O₂ and 0-15% CO₂ in N₂.

2.8. Other factors that affect effectiveness of modified atmosphere storage

The effectiveness of modified atmosphere in controlling insects is dependent on various abiotic (gas composition, gas pressure, relative humidity, temperature and length of exposure) and biotic (insect species, life stage and size and distribution of infestation) factors (Jayas *et al.*, 1991). The moisture content, gas concentration gradients within a grain bulk, efficiency of the gas generating unit, the problems related to the sorption of CO₂ on the grain and leakage rate of storage structure are other aspects that must be taken into account for modified atmosphere storage (Jay, 1986).

Different insect species require different atmospheres for their control. Even within the insect species, different strains or populations require different atmospheres for their control depending on their physiology, environmental conditions, temperature and relative humidity (Ramesh Babu *et al.*, 1989). Hence, it is not possible to generalize the results obtained from controlled atmosphere studies of one species to another. Bailey and Banks (1980) summarized the order of susceptibility of some of the main grain insects to various controlled atmospheres.

2.9. Effect of modified atmospheric storage on mycoflora and aflatoxin in stored products

The use of controlled atmospheres (CA) for insect control in the bulk storage of grain can have the added benefit of controlling fungal growth and mycotoxin production. Atmospheres high in CO₂ are more effective against fungi than those which exclude O₂ by replacement with N₂. Although most fungi require some O₂ for growth, many spoilage species are efficient scavengers and are capable of near normal growth in O₂ concentration <1%. Atmospheres with about 20% CO₂ generally inhibit mould growth, but >80% CO₂ may be required to prevent fungal deterioration of commodities with high moisture contents. Some *Fusarium*, *Aspergillus*, and *Mucor* spp. are particularly tolerant of high levels of CO₂.

Mycotoxin production is more sensitive than fungal growth to low O₂ and high CO₂; 20-60% CO₂ prevented or reduced production by some *Fusarium*, *Aspergillus* and *Penicillium* spp. Reduction of O₂ content is less effective in preventing mycotoxin formation (Hocking, 1988).

After 90 d storage in a modified atmosphere (MA) (CO₂:O₂:N₂ in the ratio 20:20:60, and 90% RH), only a few groundnut seeds showed visible moulding compared with an untreated control (seeds stored in ambient air) in which seeds were completely colonized after 30 d. No visible moulding occurred when groundnuts were stored in MA with additional treatment with 0.2% w/w propionic acid (PA). All seeds were colonized after 60 d when treated with PA only. The percentage of infected seeds increased during storage in all treatments except MA + PA when it decreased. Incidence of *Aspergillus flavus* decreased in all treatments. *A. niger* decreased in all treatments except MA. In contrast, *Penicillium* spp. increased in all treatments except MA (Patkar et al., 1991).

2.10. Studies on the effect of insecticidal seed treatment on *Sitophilus* sp.

Insecticidal treatment of seeds and grains is an important method of management of storage insects in different parts of the world. Due to prolonged use of certain insecticides in storage, some of the insect pests have been reported to have developed resistance to these insecticides. A review of the insecticide resistance developed in storage pests is provided in Table 2.5.

Table 2.5. Development of resistance in storage pests to insecticides

Sl. No.	Insect species	Insecticide	Result	Reference
1.	<i>S. oryzae</i> , <i>R. dominica</i> , <i>T. castaneum</i> , and <i>C. ferrugineus</i> , and psocids	Aluminium Phosphide	Resistance was reported in the major pest species	Daglish and Bengston, (1998)
2.	Many stored-product pests, including <i>S. oryzae</i>	Phosphine	Insects have developed resistance due to wide application	Georgiou (1991)
3.	<i>R. dominica</i> and some key stored-wheat insects	Chlorpyrifos-methyl	Resistance reported to diminish effectiveness	Arthur (1992)
4.	Stored grain insects	Organophosphorus insecticides	Resistance was detected in Brazilian populations	Champ and Dyte (1978) and Pacheco <i>et al.</i> (1990)

In India treatment of grains meant for consumption is not permitted. However, those meant for seed purpose are being treated prior to storage or sowing. Information available on management of storage insects using surface treatment of seeds / grains with insecticides are reviewed in Table 2.6.

Table 2.6. Efficacy of insecticides as seed / grain protectants

Sl. No.	Insect species and host	Insecticide	Result	Reference
1.	<i>S. zeamais</i>	Deltamethrin and Pirimiphos-methyl	These are good alternatives to Malathion to which storage pests have developed resistance worldwide	Santos (1988); Braga <i>et al.</i> (1991)
2.	<i>S. zeamais</i>	Fenoxycarb	Causes reduction of oviposition, affects hidden stages and has adult toxicity	Letellier <i>et al.</i> (1995)
3.	<i>S. zeamais</i>	Deltamethrin	Very effective and recommended for use in West Africa	Krall (1987)
4.	<i>S. zeamais</i> on shelled maize	Permethrin 3.3 and Pirimiphos methyl 17.7ppm	Successfully controls all storage pests and is recommended in East Africa	Golob (1988)
5.	<i>Sitophilus sp</i> on wheat	Pirimiphos methyl	Vegetable oils combined with Pirimiphos methyl at half dose was as effective as Pirimiphos methyl alone	Tembo and Murfitt,(1995)
6.	<i>Sitophilus sp</i>	Juvenile Hormone Analogues (JHAs)	The insects are considerably less sensitive to JHAs	Edwards and Short (1984)
7.	<i>R dominica</i> , <i>S oryzae</i> , <i>T castaneum</i> , <i>C ferrugineus</i> and <i>Plodia interpunctella</i> (Hubner) on wheat	Pirimiphos-methyl at 4–8 mg/kg	Provided excellent control	Fangneng Huang and Subramanyam (2005)

Sl. No.	Insect species and host	Insecticide	Result	Reference
8.	<i>Tribolium</i> and <i>Sitophilus</i> species	Organo phosphorus insecticides	More effective than Pyrethroids such as Permethrin.	Davis (1981)
9.	<i>Callosbruchus maculatus</i> (F.)	Pirimiphos methyl at 5ppm	Controlled for 24 weeks.	Taylor and Evans (1980)
10.	<i>C. chinensis</i>	DeltaDust (0.05% a.i. Deltamethrin)	One hundred per cent mortality of cowpea weevil resulted within 24 hours	Yadav (1997)
11.	Storage pests on cowpea seeds	Malathion 5D and 10D each 10g/kg	Significant increase in germination, root and shoot length, dry weight of seedling vigour index over untreated seeds	Ravindranath, <i>et al</i> , (1990)
12.	<i>R. dominica</i> in Wheat	Deltamethrin 2.5 WP 40mg/kg of seeds (1ppm)	Seed treatment gave complete protection for 6 months of storage without hampering viability	Patil, <i>et al</i> . (2004)
13.	<i>P. interpunctella</i> and <i>R. dominica</i>	Pirimiphos methyl	Progeny not produced, negligible kernel damage (1%) in wheat	Fangneng Huang and Subramanyam (2005)
14.	Stored product insects	Pirimiphos methyl	A viable grain protectant at rates of 4–8 mg/kg	Fangneng Huang and Subramanyam (2005)
15.	Storage pests on cereals	Deltamethrin, Pirimiphos methyl, Fenitrothion and Etrimfos	Gave good control when grain treated and stored for 16 and 24 weeks	Nicola and Evans (1985)

Sl. No.	Insect species and host	Insecticide	Result	Reference
16.	Five major stored product insects on stored wheat	Pirimiphos-methyl at 4–8 mg/kg	A potential grain protectant	Fangneng Huang and Subramanyam (2005)
17.	Many storage pests	Spinosad	Persist on grain and effective (>98% mortality of adults) for 6-12 months without loss of activity at 1 mg/kg on wheat and corn	Subramanyam <i>et al.</i> (2004)

2.11. Studies on the effect of insecticidal fabric treatment

In addition to treatment of grains/ seeds with insecticides, work has been conducted on treatment of storage bags with insecticides. Table 2.7 provides a review of these works.

Table 2.7. Studies on the effect of insecticidal treatment of storage bag fabric

SNo	Insects	Insecticides	Effectiveness	Reference
1.	<i>S. oryzae</i> and <i>R.dominica</i> and larvae of <i>Trogoderma granarium</i> (Everts)	Pirimiphos methyl treated gunny bags	Protected the cereal seeds for few months against adults and larvae	Sukhdev and Chahal (1975)
2.	<i>S. oryzae</i> , <i>R. Dominica</i> and <i>Corcyra cephalonica</i> (Stainton)	Jute fabric treatment with Deltamethrin of wheat storage bags	Gave excellent protection against the major insect pests and seed germination remained high 97 per cent	Yadav (1997)
3.	<i>S. oryzae</i> , <i>R. dominica</i> , <i>T. castaneum</i>	Jute fabric treatments with Deltamethrin at 20 mg / m ²	Protected seeds of wheat and maize for up to 6-9 months, sorghum and chickpea for up to 3 months	Yadav and Singh (1994)

SNo	Insects	Insecticides	Effectiveness	Reference
4.	Stored product insects	Jute bag dipped in 1% Malathion, BHC and DDT	Gave 80% protection from insects upto 3 months of storage	Joshi and Kaul (1966)
5.	Wheat in <i>R. dominica</i>	Malathion, Pirimiphos methyl treated bags	Insect damage was 75.55 and 10.50% respectively compared to untreated control where damage was 88.32% after 15 months storage	Bareth and Guptha (1989)
6.	Major storage pest	Deltamethrin jute bag treatment plus seed treatment	The germination of seed was not affected upto 15 months of storage and insect infestation less than 0.5% as per minimum seed certification standard	Bareth and Guptha (1989); Sinha, (1993)

2.12. Plant products in stored product insect control

Research on the evaluation of local plants for plant protection is very necessary to help farmers use locally available, environmentally friendly products to limit post-harvest losses of their produce.

Pereira and Wohlgemuth (1982) reported that in a storage ecosystem, uses of plant products were found to be more advantageous over synthetic insecticides. Biorational pesticides like botanicals were identified as an ideal component of integrated pest management (Hodges, 1984). Regnault Roger and Hamraoui (1993) inferred that botanical insecticides were found to be highly useful to farmers for the better post-harvest storage of the harvested food commodities. Prakash and Rao (1997) reported that higher plants were found to be a rich source of novel insecticides. The research works carried out on the use of botanicals for storage pest management are reviewed in Table 2.8.

Table 2.8. Use of Plant products in stored product insect management

SNo	Insects	Botanicals	Effectiveness	Reference
1.	<i>S. zeamais</i>	<i>Lantana camara</i> (L) <i>Chromolaena odorata</i> (L) and. <i>Elaeis guineensis</i> (Jacq)	The mortality rate increased with the concentration of these essential oils	Bouda, <i>et al.</i> (2000)
2.	<i>S. zeamais</i>	Powder of <i>L. camera</i> and <i>T. vogelii</i> (Hook F) 5.0% (w/w)	Effective in reducing the damage	Ogendo <i>et al.</i> (2004)
3.	<i>S. oryzae</i>	Turmeric powder at four per cent concentration	After a storage period of three months, mortality of adults were 80 per cent in treated rice	Harish Chander <i>et al.</i> (1991)
4.	<i>Sitophilus</i> sp	<i>Acorus gramineus</i> (Sol.) rhizome powder	Recorded 57% mortality in laboratory condition	Chan Park <i>et al.</i> (2003)
5.	<i>T. castaneum</i> and <i>S. oryzae</i>	Acetone extracts of <i>Murraya koenigii</i> (L.) and turmeric, <i>Curcuma longa</i> (L.) rhizomes at one per cent level as bag treatments curry leaf,	Good control of was achieved. Both extracts exhibited good repellency even after three months	Ahuja <i>et al.</i> (2000)
6.	Storage pests of wheat and rice	turmeric (<i>C. longa</i>) powder	Common practice in the household in South-Asian countries	Chatterjee (1980)
7.	<i>Sitotroga cerealella</i> (Olivier) <i>R. dominica</i> and <i>S. oryzae</i>	<i>Aegle marmelos</i> (L) leaves powder two per cent (w/w) admixed with rice	Reduced the level of damage in the store during six months storage period	Ambika devi and Mohandas (1982)
8.	Cowpea <i>C. chinensis</i>	<i>Psidium guineense</i> (Swartz.)	Powder had the highest percentage mortality as well as reduced the number of adult emergence than any of the plant materials	Adedire and Ajayi (1996)

SNo	Insects	Botanicals	Effectiveness	Reference
9.	<i>C. chinensis</i> on khesari (<i>Lathyrus sativus</i> L.)	Mixing dry neem leaf powder at the rate of 2 milligram per 100 gram seed	The seeds could be effectively protected from infestation	Singh (2003)
10	<i>T. castaneum</i> .	<i>Aphanamixis polystachya</i> (Wall.)	That seed extracts highly toxic to the adults	Talukder and Howse (1993)
11	<i>T. castaneum</i> on wheat kernels	Eight per cent powder of fruits of China berry, <i>Melia azadiracta</i> (L.)	Complete mortality was achieved	Mohamed <i>et al.</i> (1994)
12	<i>T. castaneum</i>	Azadirachtin incorporated in wheat flour at one ppm and above	Reduced growth and survival of adults	Mukherjee and Ramachandran (1989)
13	<i>T. granarium</i> larvae	<i>Citrus limon</i> leaf powder two per cent (w/w) admixed with wheat	Reduced the percentage of damage	Jood <i>et al.</i> (1993)
14	<i>Zabrotes subfasciatus</i> (Boheman)	Powdered leaves of <i>Ocimum canum</i> (Sims). which contain linalool at 1 per cent	Suppressed oviposition and killed all the adult beetles within 48h	Weaver <i>et al.</i> (1994)
15	<i>Prostephanus truncatus</i> (Horn)	<i>Acorus calamus</i> (L) oil 0.01 per cent (w/w)	Significantly reduced the amount of damage	Schmidt and Streloke (1994)
16	<i>R. dominica</i>	Ethanol extract of neem seed kernel	Inhibited F1 progeny	Rahim (1998)
17	<i>S. cerealella</i> and <i>R. dominica</i>	Extract of <i>Azadirachta indica</i> (L) one per cent admixed with rice	Significantly reduced the per cent of damage upto six months	Ambika devi and Mohandas (1982)
18	<i>Acanthoscelides obtectus</i> (Say) and <i>Zabrotes subfasciatus</i> (Boheman)	Dried, milled leaves of <i>Tetradenia riparia</i> (Hochst.) two per cent (w/w) admixed with beans	Significantly decreased the oviposition after an exposure period of six days	Dunkel <i>et al.</i> (1991)

SNo	Insects	Botanicals	Effectiveness	Reference
19	<i>Z. subfasciatus</i>	<i>T. riparia</i> dried milled leaves ten per cent (w/w) admixed with pinto beans	Reduced the oviposition by 40 per cent and reduced the egg hatch by 50 per cent	Weaver <i>et al.</i> (1992)
20	Major stored insects	<i>Piper guineense</i> (Schumach. & Thonn.), <i>Capsicum frutescens</i> (L.), <i>Nicotiana tabacum</i> (L), and <i>Xylopia aetiopica</i> (Duanl.)	Caused the high percentage mortality of 79, 75, 71 and 70% mortality respectively	Asawalam and Adesiyan (2001)

2.13. Effect of inert dusts for management of *S. oryzae*

The efficacy of Diatomaceous earth (DE) to control stored product pests were found to depend on different factors such as insect species, commodity, grain moisture, and temperature (Fields and Korunic, 2000). DE kills insects by adhering to and abrading the insect cuticle, thereby adsorbing lipids in the epicuticle and causing death due to water loss and desiccation (Subramanyam and Roesli, 2000). Korunic and Fields, (1995) observed that only 24h were required to achieve 100% mortality of *C. ferrugineus* with 300 ppm of DE, while applying the same dosage under the same conditions to *T. castaneum* required 21 days for 100% mortality. Diatomaceous earth and artificial silica dusts have been reported to be effective alternatives to chemical pesticides for control of insects pests in stored products. E.g. when applied to grain or to the walls of storage facilities (McLaughlin, 1994).

2.14. Biochemical losses in stored gains due to insect damage

In wheat grain the total sugars, reducing sugars, uric acid, and the major nutrients level change at increased storage periods. In stored ground nut, protein content is reported to increase with insect infestation which was due to addition of frass and faeces produced by insects including certain nitrogenous compound like quinine and thiamine (Ananth *et al.* 1985; Uma Reddy, 1981). Hira *et al.* (1988) reported an increase in protein content of wheat grain during insect infestation.

Many investigators have reported a decrease in free amino acids and free amino nitrogen during storage of rice and jowar (Pushpamma and Uma Reddy 1979). There was a gradual decrease in limiting amino acids in stored paddy and jowar, the decrease being greater in lysine content than in other amino acids, even when stored under controlled conditions (Uma Reddy, 1981).

Increased concentration of uric acid, non protein nitrogen compound has been reported by Darshan *et al.* (1992). Lypolitic activities of moulds in turn increase fatty acid and fat acidity and change in non reducing sugars (Pushpamma and Uma Reddy, 1979).

According to Darshan *et al.* (1992), the carbohydrate concentration decreases at increased storage periods. Carbohydrates of bajra and groundnuts increased on storage for six months, the mean values being 24.2 and 26.1 per cent, respectively (Sathyavathi Devi, 1978).

2.15. Parasitoids attacking stored product beetle pests

Many Hymenopteran parasitoids belonging to Pteromalidae; Chalcidoidea namely *Anisopteromalus calandrae* (Howard), *Lariophagus distinguendus* Forster, *Pteromalus spp*, *Theocolax elegans* (Westwood) and *Dinarmus spp.* were reported to attack Coleopteran pests of stored products. They are reported as late larval and pupal parasitoids. These paraitoids drill into the seed and sting the immature host and oviposit. The immature stage of parasitoid develops outside the host (Brower, 1991; Peck, 1963). In India, the parasitoid *A.calandrae* was recorded on *C.chinensis* infesting chickpea grains in the mid hill zone of Himachal Pradesh (Nirmala Devi and Devi, 1996). *Dinarmus colemani* which is minute, metallic coloured and hind tibiae with a single apical spur has been reported parasitize *Sitophilus sp* throught India. (Narshimam *et al.* 1997).

Holepyris sylvanidis (Brethes) (Bethylidae) is reported as a cosmopolitan ectoparasitoids of *O. surinamensis*, *S. oryzae*, *T. castaneum*, *T. confusum* and *C. ferrugineus*. Stejskal *et al* (2002) suggested that the bio-control of Coleopterans can be accomplished by an augmentation of one or two species of polyphagous parasitoids (*e.g.L. distinguendus*, *A. calandrae* or *H. sylvanidis*). *H. sylvanidis* finds a place in the list of natural enemies catalogued at PDBC Bangalore (Narshimam *et al.* 1997).

CHAPTER III

MATERIALS AND METHODS

The experimental procedures adopted for studies on use of CO₂, insecticides, botanicals and inert dusts for the management of rice weevil, *S. oryzae* in maize storage are elaborated in this chapter.

Location of experiment

Department of Agricultural Entomology and

Seed Entomology Lab, Seed Centre, TNAU, Coimbatore.

Crop and variety used for the study

Freshly harvested maize seeds with high initial germination percentage and low moisture were obtained the Department of Millets and Central farm, TNAU Coimbatore and ARS, Bhavanisagar. Seeds of the following crop/ variety were used in the storage experiments.

Crop	Maize	Germination %	Moisture %
Variety	CO 1	95	9.5
Hybrid	COH(M) 5	94	9.8

3.1. Establishment of identity of test insect, *S.oryzae*

Adult beetles from maize and sorghum grain samples were collected from different sources namely, Department of Millets, TNAU, Coimbatore, and maize grain wholesale market, Coimbatore. The size of the insects collected on maize were larger (length 3.76 ± 0.20 mm and width 1.10 ± 0.09 mm) compared to those collected on sorghum which were smaller (length 3.02 ± 0.17 mm and width 0.92 ± 0.07 mm) (Plate 3.1a, b) when measured using an image analyzing system (Model: WinDIAS). These insects were dissected for aedeagus character of male genitalia. The aedeagus of *S.oryzae* is described to have smooth and convex dorsal surface while that of *S. zeamais* has a central ridge between two longitudinal depressions on its dorsal surface (Rees, 1996). In our insect culture, all the insects possessed smooth and convex dorsal surface of aedeagus (Plate 3.1c,d) which is characteristic of

S.oryzae. The insects were also sent to Dr. V.V. Ramamurthy, Principal Scientist, Insect taxonomy section of the Division of Entomology, IARI, New Delhi for identification. All the insects were identified to be *S.oryzae* based genitalic structures, female abdominal sternites and rostrum. Hence the test insects are described only as *S. oryzae* in this thesis.

3.1.1. Mass culturing of *Sitophilus oryzae*:

Mass culturing of *S. oryzae* was done at the Seed Entomology Laboratory of Seed Centre, TNAU, Coimbatore. The *Sitophilus oryzae* adults were collected from stored maize grains from sources as mentioned in 3.1 and utilized for mass culturing work.

Fresh maize seeds were disinfested by keeping at - 20°C for 48h in deep freezer in moisture proof container to kill all existing insects. About 200 g of the disinfested maize seeds were placed in 600 ml plastic containers, into which approximately 50 pairs of freshly emerged adults were introduced. The containers were covered with muslin cloth and placed in dark to facilitate maximum oviposition maintained at a room temperature of $30 \pm 5^{\circ}\text{C}$ and $70 \pm 5\%$ RH throughout the period of study. After about 25 - 30 days, the adults that started emerging from the culture were utilized for the maintenance of sub cultures (Plate 3.2 a). Subculturing of this weevil was done at 20 days intervals so that a continuous supply of insects for experiments was ensured. For getting uniform aged adults, 100 g of fresh and disinfested seeds were exposed to 20 pairs of insects and allowed for 20 days for egg laying. All the adult insects were removed after 20 days and uniform aged adults were harvested 25 days later. Various sub cultures were maintained for conducting experiments on larva and adult mortality studies.

3.2. Studies on the effect of CO₂ treatment on *Sitophilus oryzae*

3.2.1. Materials used

- Carbon dioxide cylinder with initial pressure - 150 kg/cm²
- Pressure gauge - Two gauges, one for measuring cylinder pressure and another for indicating the outlet valve pressure.

- Flow meter - Flow rate of CO₂ indicated in terms of Standard Liters Per Minute (SLPM) when outlet pressure is 1kg/cm².
- Oxygen analyzer - Nucon 101 model (capable of measuring 0-25% O₂).
- Carbon dioxide cylinder, flow meter, pressure gauge and oxygen analyzer were supplied by Nucon Engineer, New Delhi- 20.
- Airtight plastic container (1000 ml capacity)
- Nylon tubes, Rubber cork, Teflon tape and superglue
- Sieves (3 mm)

3.2.2. Design of airtight container

In airtight plastic containers of 1.05 lit volume, two perforations of 3 mm diameter were made for inlet and outlet and nylon tubes of 3 mm diameter were inserted. Rubber corks of 3 mm diameter which exactly seal the inlet and outlet tubes airtight were used to plug them after filling CO₂. The entire system was made airtight with Teflon tape and superglue (Anabond[®]).

3.2.3. Injection of CO₂ into airtight container

Carbon-dioxide from cylinder was introduced into the airtight container through the 3 mm inlet hole at the bottom of the container, keeping the outlet at the top of the container open to flush out air from the container Plate 3.3 a). The quantity of CO₂ and the time required to inject the required quantity of CO₂ were calculated using the formula given in 3.2.4. After injecting the gas, the inlet and outlet were closed at one stroke using rubber corks to prevent escape of CO₂ from the container. The concentration of CO₂ in the container was determined and verified using methods described in 3.2.5.

3.2.4. Estimation of CO₂ to be injected

The volume as well as concentration of CO₂ to be injected into the plastic containers was arrived at based on White *et al.* (1993) with modifications to suit the experiment. .

i. The Volume of CO₂ to be added in ml (V_c) is calculated as follows

$$V_c = \left[\frac{D_c}{100 - D_c} \right] \times V_a$$

where

V_c = Volume of CO₂ to be added in ml

D_c = Desired concentration of CO₂ (%)

V_a = Vol. of free space in container above the seeds including intergranular space (ml)

ii. The time required in seconds (T_c) to achieve desired CO₂ concentration at 1 SLPM is calculated as follows

$$T_c = \frac{V_c}{1000} \times 60$$

Where

T_c = Time required in seconds to open CO₂ cylinder at a flow rate of 1 SLPM to achieve desired CO₂ concentration

V_c = Volume of CO₂ to be added in ml

The volume of CO₂ to be injected/ released (V_c) and the time of injection required (T_c) to achieve desired CO₂ concentration calculated by the above formulae are shown in Table 3.1.

Table 3.1. Estimation of volume and time of injection of CO₂

D_c	V_c	T_c
Desired Concentration of CO ₂ (%)	Vol. of CO ₂ to be added (ml) *	Time (sec) of Injection of CO ₂ at 1SLPM
0	0	0
10	108	7
20	244	15
30	418	25
40	650	39

50	975	59
60	1463	88
70	2275	137

- Vc calculated taking Va= 0.975 lit (Vol of free space in the container above 100 g seeds)

3.2.5 Determination of CO₂ concentration in containers after injection

The concentration of CO₂ in the containers was determined using two different methods as described below.

i. Determination of CO₂ concentration using O₂ analyzer

Oxygen analyzer (Model: Nucon 101) was used to measure the O₂ concentration in the containers. The atmospheric O₂ concentration of 21 per cent gets decreased inside the container proportionally when CO₂ concentration is increased. Thus, the O₂ concentration was measured and a factor was worked to determine the CO₂ concentration as follows.

$$C = \left(1 - \frac{y}{k}\right) \times 100$$

where

C = CO₂ concentration

y = O₂ concentration

k = constant (O₂ concentration in atmosphere *i.e.* 21%)

Table 3.2 shows the CO₂ concentration determined in containers using this method initially, 2 and 4 days after injection.

Table 3.2. Determination of CO₂ concentration in container using O₂ analyzer

S.No	Injected CO ₂ %	O ₂ concentration measured (%)			CO ₂ concentration worked out (%)		
		Initial 0 days	2 days	4 days	Initial 0 days	2 days	4 days
1.	0	0	0	0	0	0	0
2.	10	18.9	19	19.1	10.0	9.5	9.0
3	20	16.7	16.9	17.2	20.5	19.5	18.1
4	30	14.6	14.8	15.1	30.5	29.5	28.1
5	40	12.6	12.8	13.1	40.0	39.0	37.6
6	50	10.4	10.8	11.1	50.5	48.6	47.1
7	60	8.5	8.8	9.1	59.5	58.1	56.7
8	70	6.3	6.6	7.1	70.0	68.6	66.2

ii. Gravimetric method

Gravimetric method of determining CO₂ concentration was used for the first time in this laboratory. This method was used to cross verify the CO₂ concentrations determined by previous method. Moreover since oxygen analyzer took more time to stabilize and show correct reading, this method was preferred.

Under atmospheric pressure, CO₂ is heavier than air. The mass of CO₂ is 1.87 g / lit while that of air is only 1.2 g / lit. Considering this mass difference, the concentration of CO₂ was estimated based on the increase in weight of the container after injection of CO₂.

The following formula was used to determine CO₂ concentration based on the increase in weight of the container

$$C = \frac{d}{0.67Va} \times 100$$

where

C= Concentration of CO₂ in % Vol

Va = Volume of free space available above seeds in the container in L

d = Increase in mass after injecting CO₂ in grams

0.67 is a constant and denotes the mass difference in grams between 1 lit CO₂ and air under atmospheric pressure (1.87 – 1.20). Table 3.3 shows CO₂ concentration determined in the container by gravimetric method initially, 2 and 4 days after injecting CO₂.

Table 3.3. Determination of CO₂ concentration in container by gravimetric method

S.No	Injected CO ₂ %	Difference in mass after injecting CO ₂ (g)			CO ₂ concentration determined (%)		
		Initial	2 days	4 days	Initial	2 days	4 days
1.	0	0.000	0.000	0.000	0.0	0.0	0.0
2.	10	0.068	0.065	0.061	9.9	9.5	8.9
3	20	0.137	0.134	0.130	19.9	19.5	18.9
4	30	0.200	0.197	0.194	29.1	28.7	28.2
5	40	0.275	0.270	0.265	40.0	39.3	38.6
6	50	0.352	0.340	0.329	51.2	49.5	47.9
7	60	0.408	0.398	0.390	59.4	57.94	56.8
8	70	0.482	0.470	0.455	70.1	68.4	66.2

3.2.6. Effect of CO₂ fumigation on the mortality of *S. oryzae*

Design: FCRD

Total number of replications: 3

Number of factors: 3

Factor 1 – CO₂ Treatment

T₁ – 10% CO₂

T₂ – 20% CO₂

T₃ – 30% CO₂

T₄ – 40% CO₂

T₅ – 50% CO₂

T₆ – 60% CO₂

T₇ – 70% CO₂

T₈ – 0% CO₂ (control)

Factor 2 - Exposure period:

P₁ - 2 days

P₂ - 4 days

Factor 3 - varieties

V₁ - CO1 variety

V₂ - COH(M) 5 Hybrid

3.2.7. Methodology

Seeds were kept at -20°C for 48h in deep freezer in moisture proof container to kill all existing insects. The seeds thus disinfested were used to study the effect of CO₂ on the mortality of different stages of *S. oryzae*.

3.2.8. Larval stage

One hundred grams of disinfested seeds was taken in zip lock polythene bags (19x13 cm, 200 gauge) and 20 uniform aged adults were allowed for twenty days for egg laying. All the adults were removed after 20 days, and transferred to airtight containers and exposed to CO₂ fumigation. Two sets of entire treatments and replications were used for 2 and 4 days of

exposure. At the end of 2 or 4 days of exposure period, the treated seeds transferred to insect proof polythene bags and stored until observations on adult emergence were recorded.

Observations were made on the adult emergence, thirty days after egg laying. The per cent larval mortality was calculated following Howe (1971).

Per cent larval mortality = 100 - Percent larval survival

$$\text{Per cent larval survival} = \frac{\text{No. of adults emerged in treatment}}{\text{Total no of adults emerged in control}} \times 100$$

3.2.9. Adult stage

One hundred grams of disinfested seeds were taken in airtight plastic containers and twenty uniform aged adults were released into each and exposed to various CO₂ concentrations for two and four day's period. The per cent adult mortality was calculated by using the following formula.

$$\text{Per cent adult mortality} = \frac{\text{No. of adults dead}}{\text{No. of adults released}} \times 100$$

3.2.10. Effect of CO₂ treatment on storability of seeds

The treatment details adopted was same as 3.2.6 except for exposure period where 4 days alone was adopted. Freshly harvested and untreated seeds of maize (100 g) were taken in airtight plastic containers and exposed to different concentrations of CO₂ for 4 days. Seeds were taken out after 4 days exposure period and placed in zip lock polythene bags (19x13 cm) to find the effect of CO₂ on the multiplication and damage caused by field carry over *S. oryzae* in storage. The following observations were made at monthly intervals for four months;

- i. Adult populations in 100g seeds
- ii. Number of bored seeds in 100 seeds
- iii. Germination of seed (Roll towel method).

Number of live and dead adults was counted. The dead insects were removed while live insects were returned to the sample. The number of live insects returned to the sample was deducted during next observation.

Per cent damage was calculated by taking a random sample of 100 seeds and counting the number of seeds with bore holes caused by *S. oryzae* adult emergence. Seed germination was tested by using roll towel method (Plate 3.2b).

3.3.0. Studies on the effect of pre-storage seed treatment with botanicals and inert dusts on *S. oryzae*

3.3.1. Effect on mortality of *S. oryzae*

Botanicals found safer to humans and environment were selected for pre storage seed treatment. Botanicals namely *Acorus calamus*(L.) rhizome powder (Sweet flag), *Curcuma longa*(L.) (Turmeric powder), *Azadirachta indica*(L.) leaf powder (neem) and *Vitex negundo*(L.) leaf powder (nochi) and inert dusts namely Diatomaceous earth (DE), rice husk ash were included for treatment of seeds. Malathion was used as a standard check.

Total number of treatments: 8

Total number of replications: 3

Design: FCRD

Treatment details.

Treatments		Conc. g/ kg of seeds
T ₁	Sweet flag rhizome powder (<i>Acorus calamus</i> (L.)	5
T ₂	Turmeric powder (<i>Curcuma longa</i> (L.)	5
T ₃	Neem leaf powder (<i>Azadirachta indica</i> (L.)	5
T ₄	Nochi leaf powder (<i>Vitex negundo</i> (L.)	5

T ₅	Diatomaceous earth	2.5
T ₆	Rice husk ash	5
T ₇	Malathion	500 (ppm)
T ₈	Untreated (control)	

The experimental setup is shown in Plate 3.4a.

3.3.3. Larval stage

One hundred grams of disinfested seeds were taken in zip lock polythene bags (19x13 cm) and 20 uniform aged adults were released for egg laying. Adults were removed after 20 days of egg laying. Then seeds were treated with botanicals and inert dusts at recommended quantity. The treated seeds were kept in polythene bags. Daily observation on F1 progeny, adult emergence was recorded until fresh adult emergence stopped. The per cent larval mortality was calculated (Howe, 1971) based on the number of adults emerged as described in 3.2.8.

3.3.4. Adult stage

One hundred grams of disinfested seeds were treated with different botanicals mixed and transferred to zip lock polythene bags and twenty uniform aged adults were released. The per cent adult mortality was recorded after 4 and 8 days. The per cent mortality was calculated as described in 3.2.9.

3.4.0. Studies on the effect of pre-storage insecticidal seed treatment on *S. oryzae*

3.4.1. Effect on mortality of *S. oryzae*

Newer insecticides that are reported to be relatively safer and having low mammalian toxicity were selected for surface treatment of seeds. Deltamethrin and malathion recommended in many states of India for managing storage pests were included in addition to four new compounds namely spinosad, lufenuron, thiamethoxam, and emamectin benzoate. Pirimiphos methyl which is widely recommended for storage pest management in many parts of the world was also included

Total number of treatments: 8

Total number of replications: 3

Design: FCRD

Treatment details

Treatments		Conc. of a.i. ppm	Qty of formulation/ mg or µl/ kg seed
T ₁	Spinosad (Tracer) 45 SC	2	4.4
T ₂	Lufenuron (Cigna) 5 EC	5	100
T ₃	Thiamethoxam (Cruiser) 70WS	2	2.9
T ₄	Pirimiphos methyl (Actellic) 50 EC	10	20
T ₅	Emamectin benzoate (Proclaim) 5G	2	40
T ₆	Deltamethrin (Decis) 2.8 EC	1	35.7
T ₇	Malathion (Killers) 50EC	500	1000
T ₈	Control		

The experimental setup is shown in Plate 3.4b.

3.4.2. Larval stage

One hundred grams of disinfested seeds were taken in zip lock polythene bags (19x13 cm) and 20 uniform aged adults were released for egg laying. Adults were removed after 20 days of egg laying. Twenty days later the seeds were treated with insecticides at recommended dose by mixing in 5ml water/ kg seeds. The insecticide treated seeds containing larval stage were shade dried and kept in polythene bags. Observations were made on the adult emergence, thirty days after egg laying. The per cent larval mortality was calculated (Howe, 1971) based on the number of adults emerged as described in 3.2.8.

3.4.3. Adult stage

One hundred grams of disinfested seeds were treated with different insecticidal solutions shade dried and transferred to zip lock polythene bags and twenty uniform aged adults were released. The per cent adult mortality was recorded after 2 and 4 days. The per cent mortality was calculated as described in 3.2.9.

3.4.4. Effect of pre-storage insecticidal seed treatment on storability of seeds

One hundred grams of freshly harvested maize seeds were treated with insecticidal solutions, shade dried, kept in insect proof polythene bags for 4 months and the observations as mentioned in 3.2.10 were made at monthly intervals.

3.5.0. Studies on the effect of insecticidal fabric treatment on *S. oryzae*

3.5.1. Effect on mortality of *S. oryzae*

Design: FCRD

Factor 1- Insecticidal treatments (seven insecticides and a control)

Total number of replications: 3

Treatment details

Treatments		Conc. of a.i. ppm	Conc., of formulation (%)
T ₁	Spinosad (Tracer) 45 SC	100	0.02
T ₂	Lufenuron (Cigna) 5 EC	100	0.20
T ₃	Thiamethoxam (Cruiser) 70WS	100	0.02
T ₄	Pirimiphos methyl (Actellic) 50 EC	200	0.04
T ₅	Emamectin benzoate (Proclaim) 5G	100	0.20
T ₆	Deltamethrin (Decis) 2.8 EC	100	0.36
T ₇	Malathion (Killers) 50EC	5000	1.0

The experimental setup is shown in Plate 3.4c.

Insecticide solutions of required concentrations were prepared by serial dilution method. The cloth bags were dipped in the insecticide solution so as to drench them completely. The cloth bags were then shade dried, filled with grain and stored at ambient conditions.

3.5.3. Larval stage

One hundred grams of disinfested seeds were taken in zip lock polythene bags (19x13 cm) and 20 uniform aged adults were released for egg laying. Adults were removed after 20 days of egg laying. Cloth bags were treated with various insecticidal solutions by dip method and seeds containing eggs were placed in these bags. Larval mortality caused by insecticidal fabric treatment were worked by Howe, 1971 by counting the adults that emerge.

3.5.4. Effect of fabric treatment with insecticides on storability of seeds

One hundred grams of freshly harvested maize seeds were taken in a cloth bag which was treated with various insecticidal solutions by dip method as mentioned 3.5.1 and kept in storage room. Observation as described under 3.2.10 were recorded at monthly interval

3.6.0. Per cent seed germination and moisture estimation

Seed germination was assessed by following Roll towel method and moisture content was estimated by oven dry method as recommended by ISTA (1999).

3.7.0. Record and identification of parasitoids

One hundred grams of freshly harvested and dried seeds were placed in 600 ml plastic containers covered with muslin cloth and placed in dark to facilitate multiplication of carried over rice weevil population and its natural enemies. Five such containers were maintained.

The following observations were recorded at monthly intervals,

- i. Population of *S. oryzae*

ii. Population of parasitoids

The natural enemies were identified based on description available in literature.

3.8.0. Biochemical estimation of stored maize

3.8.1. Seed sample preparation

Seeds of three levels of insects infestation namely no infestation, medium infestation and high infestation (0, 26 and 43% damaged grains in case of CO1 and 0, 27, 45% damaged grains in case of COH(M) 5 maize) from maize grain treated with 70, 20 and 0% CO₂ treatments 90 days after treatment were taken for biochemical analysis.

Seed sample were dried to uniform moisture level by drying them in oven at 70°C for 1h in a hot air oven. The seeds were ground using a high speed blender and the finely powdered sample was used for biochemical estimation.

3.8.2. Determination of total carbohydrate by anthrone methods

Principle

Carbohydrates are first hydrolyzed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green coloured product with an absorption maximum at 630nm.

Procedure:

One hundred mg of the finely powdered seed sample was weighed into a boiling tube and was hydrolyzed by keeping in a boiling water bath for three hours with 5 ml of 2.5N HCl and cooled to room temperature. Later it was neutralized with solid sodium carbonate until the effervescence ceased. The volume was made up to 100ml and centrifuged. From the supernatant, 0.5 and 1ml of aliquots were collected for analysis.

Standard glucose stock solution was prepared by dissolving 100mg glucose in 100ml water. Then working standard was prepared by diluting 10ml of stock to 100ml with water. From the working standard, 0.0, 0.2, 0.4, 0.6, 0.8, 1.0 ml were taken in separate test tubes.

Volume of all test tubes including the sample tubes was made up to one ml by adding distilled water.

Anthrone reagent was prepared by dissolving 200mg of anthrone in 100 ml of ice cold H_2SO_4 . Then 4 ml of anthrone reagent was added to each of the tubes including standards, samples and blank. They were heated for 8 minutes in a boiling water bath. Later they were cooled rapidly and the OD value at 630 nm was recorded in a UV - V7S – spectrophotometer 117 (systronics). A standard graph was drawn by plotting concentration of the standard in mg of glucose / ml on X axis versus absorbance on Y axis. From the graph the amount of carbohydrate present in the sample was calculated as follows.

Calculation

$$\begin{aligned} &\text{Amount of carbohydrate present in 100mg of the sample} \\ &\qquad \text{mg of glucose per ml sample} \\ &= \frac{\text{Volume of the test sample in ml}}{\text{Volume of the test sample in ml}} \times 100 \end{aligned}$$

3.8.3.Estimation of starch by anthrone method

Principle

The sample is treated with 80% alcohol to remove sugars and then starch is extracted with perchloric acid. In hot acidic medium, starch is hydrolyzed to glucose and dehydrated to hydroxymethyl furfural. This compound forms a green coloured product with anthrone, which is measured at 630 nm.

Procedure

One hundred mg of the sample was homogenized in 80% hot ethanol to remove sugars. It was centrifuged and the sediment was retained. Later the sediment was washed repeatedly with 80% hot ethanol till the washing did not give colour with anthrone reagent (Anthrone reagent was prepared by dissolving 200mg anthrone in 100ml of ice cold 95% sulphuric acid). The sediment was dried well over a water bath. To the sediment 5.0ml of

water and 6.5 ml of 52% perchloric acid was added and extracted at 0°C for 20 min. It was centrifuged and the supernatant was taken. The extraction was repeated one more time using fresh perchloric acid and centrifuged. The supernatant were pooled together and made up to 100ml. From this 0.1ml were pipetted out and the volume made up to 1ml with water. Glucose standard having serially increased glucose concentration were prepared as describe in 3.8.2. Four ml of anthrone reagent was added to each tube and heated for eight min. in a boiling water bath. After cooling rapidly, the OD value was recorded in a spectrophotometer at 630 nm.

Calculation

Amount of starch present in 100mg of the sample

$$= \frac{\text{mg of glucose per ml sample}}{\text{Volume of the test sample in ml}} \times 100$$

3.8.4. Estimation of total nitrogen (by Micro Kjeldhal method)

Principle

A known weight of the powdered sample is treated with diacid mixture as to oxidize the organic matter and bring the mineral elements in the solution.

Procedure

One g of sample was taken in a 100 ml conical flask. About 10 – 15 ml of diacid mixture (sulphuric acid and perchloric acid in the ratio of 5:2) was added and the mouth of the flask was covered with a funnel. The contents of the flask were digested over a sand bath till a clear solution is obtained. The contents of the flask were transferred to a 250 ml volumetric flask using minimum quantity of water and the volume was made to 250 ml. Ten ml of diacid extract was pipetted out into a distillation flask. Twenty five ml of 2% boric acid was pipetted out into an ice tumbler / breaker and two drops of double indicator (bromocresol green methyl red) was added and kept at the delivery end of the distillation setup. Ten ml of

40% NaOH was added to the distillation flask and the distilled contents were collected in 2% boric acid. The completion of distillation was tested with moistened red litmus paper. Absence of blue colour indicates that all ammonia has been distilled. The delivery tube was washed with distilled water and the washings were collected in ice tumbler. The contents were titrated with 0.1N sulphuric acid and the end point was change of blue colour to green.

$$A \times 0.00028 \times 100 \times 100$$

$$\text{Amount of nitrogen in 1.0 g of sample} = \frac{\text{—————}}{10 \times 10}$$

A= volume of H₂SO₄ consumed in ml

1ml of 0.1N H₂SO₄ = 0.00028 g of Nitrogen

3.9.0 Statistical analyses

The data obtained from different experiments were analyzed for Analysis of variance as described by Panse and Sukhatme (1985). Per cent values were subjected to Arcsine transformation and insect counts were transformed to square root transformation before doing analysis. The critical differences (CD) were calculated at 5 per cent probability level.

The dose mortality relationships in case of CO₂ and time mortality treatments were subjected to probit analysis as per Finney (1971), and calculated using a excel based probit analysis software (Srinivasan, 2004).

CHAPTER IV

RESULTS

The results of the experiments conducted on the management of *S. oryzae* infesting stored maize with CO₂, insecticide, botanical and inert dust treatments are presented in this chapter.

4.1. Effects of CO₂ on management of *S. oryzae*

4.1.1. Larval mortality

It was found that CO₂ at 60 and 70% concentrations recorded 100 per cent mortality of larvae of *S. oryzae* at 4 days of exposure time when tested with both CO1 and COH(M)5 maize seeds. At 50% CO₂ concentration, the mean mortality was 97.6 and there was not much variation among the variety and hybrid. Untreated control did not record any mortality. There was a corresponding increase in mortality with increase in CO₂ concentration (Table 4.1, Fig 4.1).

The LC₅₀ and LC₉₅ values for larvae were 23.3 and 47.5% CO₂ when tested on variety CO1 for an exposure time of 4 days. On COH(M)5 hybrid the LC₅₀ and LC₉₅ values were 21.4 and 46.1% CO₂ respectively (Table 4.2). The comparisons of periods of exposure on the relationship between CO₂ concentration and mortality of *S.oryzae* adults on CO1 and COH(M)5 maize seeds respectively are shown in Fig 4.3 and 4.4.

4.1.2. Adult mortality

At 40% concentration of CO₂ the per cent adult mortality was 83.3 and 96.7 for 2 and 4 days exposure, respectively with CO1 maize variety. These values were 86.7 and 96.7 with respect to COH(M)5 hybrid. There was a gradual increase in mortality from 10 to 40% CO₂ concentrations. There was not much variation in adult mortality between 2 and 4 days exposure period. The adult mortality reached 100% at 50% CO₂ concentration when exposed for 2 or 4 days (Table 4.3).

The LC₅₀ and LC₉₅ values for adult rice weevils were 15.1 and 45.0% CO₂ when tested on variety CO1 for an exposure period of 4 days. For an exposure period of 2 days, the

LC₅₀ and LC₉₅ values were 19.6 and 83.1% CO₂. On COH(M)5 hybrid the LC₅₀ and LC₉₅ values were 13.9 and 41.6% CO₂ respectively when exposed for 4 days (Table 4.2). For an exposure period of 2 days, the LC₅₀ and LC₉₅ values were 18.3 and 67.7% CO₂ respectively. The comparisons of relationship between CO₂ concentration and mortality of larval and adult stages of *S. oryzae* on CO1 and COH(M)5 maize seeds respectively are shown in Fig 4.5 and 4.6.

4.2. Effect of CO₂ on storability of seeds

4.2.1. Adult emergence

In the long term storage study that was conducted to find out the storability of CO₂ treated seeds the adult population was recorded at monthly intervals. At 60 and 70% CO₂ concentrations there was no adult emergence recorded up to 120 DAT. At 50% concentration, the adult populations were 0.0, 4.0, 8.7 and 12.3 on 30, 60, 90 and 120 DAT respectively. On 120 DAT the adult populations were 84.3, 82.0, 55.0, 32.0, 27.0 and 12.3 in untreated control, 10, 20, 30, 40 and 50% CO₂ concentrations respectively (Table 4.4).

4.2.2. Per cent damage to seeds

The per cent damage caused by *S. oryzae* was 56.7 % in untreated control on 120 DAT while it was nil damage in case of 60 and 70% CO₂ concentration (treated for 4 days and stored air tight for rest of the period). Seeds exposed to CO₂ concentrations namely 10, 20, 30, 40 and 50% showed 37.3, 30.3, 26.3, 17.3 and 8.0 per cent damage respectively on 120 DAT. Similar trend was noticed on other days of observation namely 30, 60 and 90 DAT (Table 4.5).

4.2.3. Germination test

The initial germination of maize seeds was 95 per cent. The germination was not affected by the CO₂ treatment over a period of four months storage period after treatment. However the germination of seeds was reduced due to insect infestation in lower CO₂ concentrations. The mean per cent germination of seeds on 120 DAT was 83.3, 90.0 and 91.7

in 50, 60 and 70% CO₂ concentration while untreated recorded 38.3 per cent seed germination (Table 4.6).

4.3. Studies on the management of *S. oryzae* through pre-storage seed treatment with insecticides

4.3.1. Effect on mortality of different stages of *S. oryzae*

4.3.1.1. Larval mortality

The mortality of larvae of *S. oryzae* due to insecticidal seed treatment, calculated based on adult emergence was 100 per cent for Malathion 500 ppm in both CO1 and COH(M) 5. This was followed by deltamethrin 1ppm and lufenuron 5ppm which showed 87.7 and 79.9 per cent mortality, respectively in the variety CO1. The larval mortality caused by these insecticides was 85.7 and 77.8 per cent respectively in the hybrid COH(M)5 (Table 4.7; Fig 4.7). Thiamthoxam 2 ppm caused only 20.0 and 20.5 per cent larval mortality when tested on CO1 and COH(M) 5 maize, respectively. In untreated control nil mortality was recorded.

4.3.1.2. Adult mortality

The mortality of adults of *S. oryzae* due to insecticidal seed treatment, calculated based on mortality of released adults at 2 DAT was 100 per cent for Malathion 500 ppm followed by deltamethrin 1ppm, thiamethoxam 2 ppm which showed 66.7 and 63.3 per cent mortality respectively (Table 4.8; Fig 4.8). Lufenuron 5 ppm caused only 26.7 per cent mortality. Untreated control recorded nil mortality. The mortality of adults on 4 DAT was 100 per cent in all the treatments except lufenuron 5ppm which recorded 63.7 per cent mortality.

4.3.2. Effect of insecticides on storability of seeds

4.3.2.1. Population of adults

Thirty days after treatment, malathion 500 ppm and deltamethrin 1 ppm recorded the lowest adult population of 8.3 and 9.3, respectively and proved best among all the chemicals

which were followed by thiamethoxam 2 ppm and emamectin benzoate 2 ppm which registered 10.3 adults each (Table 4.9). However among the insecticidal treatments, the maximum adult population (17.3) was recorded in pirimiphos methyl 10 ppm treated seeds. Untreated control registered a maximum adult population of 23.0.

Sixty days after treatment, the minimum adult populations of 0.7 and 3.3 were recorded in malathion 500 ppm and deltamethrin 2 ppm, respectively. The adult populations considerably decreased on 60 DAT in most of the insecticides treatments except in pirimiphos methyl 10 ppm and spinosad 2 ppm treated seeds where they increased to 18.0 and 16.3 respectively. Untreated control registered an adult population of 45.0.

On 120 DAT, nil or minimum adult population was observed in seeds treated with lufenuron 5 ppm, malathion 500 ppm and deltamethrin 1 ppm. Emamectin benzoate 2 ppm and thiamethoxam 2 ppm registered 2.7 and 3.3 adults respectively. In the seeds treated with spinosad 2ppm and pirimiphos methyl 10 ppm, 16.7 and 19.0 adults were found while untreated control 83.0 adults were recorded (Table 4.9).

4.3.2.2. Per cent damage

On 120 DAT, seeds treated with malathion 500 ppm and deltamethrin 2 ppm recorded minimum per cent damage of 0.3 and 1.3 respectively while lufenuron 5 ppm, thiamethoxan 2 ppm and emamectin benzoate 2 ppm registered 1.3, 2.0 and 2.3 per cent damage respectively. Spinosad 2 ppm and pirimiphos methyl 10 ppm recorded high damage level of 13.3 and 16.0 per cent while untreated control recorded 56.7 per cent (Table 4.10).

4.3.2.3. Seed germination:

Seed germination recorded 120 DAT was highest in malation 500 ppm and deltamethrin 1 ppm treated seeds (90.0 and 85.0) followed by lufenuron 5 ppm and emamectin benzoate 2 ppm which where all on par. Germination was low in spinosad (70.0 per cent) and thiamethoxan 2 ppm (70.0) while untreated control recorded only 52.9 per cent on 120 DAT (Table 4.11).

4.4. Studies on the management of *S. oryzae* through fabric treatment of storage bags

4.4.1. Adult population

Storage bags treated with deltamethrin 100 ppm and lufenuron 100 EC 5 ppm recorded nil adult population on 120 DAT in maize CO1 seeds. Emamectin benzoate 100 ppm and thiamethoxam 100 ppm bag treatment recorded slightly higher populations of 0.7 and 3.0 adults. Pirimiphos methyl and spinosad recorded significantly high adult population of 25.7 and 24.3. Untreated control recorded 83.3 adults (Table 4.12)

4.4.2. Per cent damage

Per cent seed damage caused by *S. oryzae* on 120 DAT was 2.3 for Malathion followed by deltamethrin which recorded 2.7 per cent. Emamectin benzoate 2 ppm and lufenuron 5 ppm registered 3.7 and 5.0 per cent damage. Spinosad, pirimiphos methyl and thiamethoxam treated seeds recorded very high seed damage and untreated control registered 55.7 per cent damage (Table 4.13).

4.4.3. Seed germination

Per cent seed germination was maximum in case of malathion 50 EC (91.7). This was followed by deltamethrin and lufenuron which recorded at 88.3 and 78.3 respectively. Spinosad, pirimiphos methyl treatments and untreated control recorded low seed germination possibly due to high damage to seeds by *S. oryzae* (Table 4.14).

4.5. Studies on management of *S. oryzae* through pre-storage seed treatment with botanicals and inert dusts

4.5.1. Effect on mortality of different stages of *S. oryzae*

4.5.1.1. Larval mortality

The mortality of larvae of *S. oryzae* due to seed treatment with botanicals and inert dusts, calculated based on adult emergence was maximum in CO1 maize seeds treated with sweet flag rhizome powder 5g/kg of seeds, rice husk ash 5g/kg of seeds and diatomaceous earth 2.5g/kg of seeds which registered 96.8, 95.2, and 93.7 per cent mortalities respectively (Table 4.15; Fig 4.9). Dried nochi leaf powder 5g/kg, neem leaf powder 5g/kg seed and

turmeric rhizome powder 5g /kg seed recorded only 22.2, 26.9 and 30.1 per cent larval mortalities respectively. The standard insecticidal check, malathion 500 ppm recorded 100 per cent mortality. On COH(M) 5 maize, similar trend was noticed and there was no significant difference between the variety and the hybrid with respect to larval mortality caused by botanicals.

4.5.1.2. Adult mortality

The mortality of adults of *S. oryzae* due to botanicals seed treatment, calculated based on mortality of released adults was 66.7 per cent for rice husk ash at 5 g/kg of seed followed by diatomaceous earth 2.5g/kg of seeds and sweet flag rhizome powder 5g/kg of seeds which registered 53.3 and 23.3 per cent mortality respectively at 4 DAT. Turmeric powder, neem leaf powder and nochi leaf powder registered low larval mortalities of 13.3, 3.3 and 3.3 per cent.

On 8 DAT, seeds treated with rice husk ash 5g/kg of seeds and diatomaceous earth 2.5g/kg of seeds registered 76.7 and 63.3 per cent adult mortality respectively which were on par. Sweet flag rhizome powder 5g/kg of seeds and turmeric powder 5 g/kg seeds registered 33.3 and 30.0 per cent and were on par with each other. Nil mortality was recorded in untreated control (Table 4.16; Fig 4.10).

4.5.2. Effect of seed treatment with botanicals on storability of seeds

4.5.2.1. Adult population

Thirty days after treatment, the adult population in seeds treated with sweet flag rhizome powder 5g/kg, diatomaceous earth 2.5g/kg and rice husk ash 5g/kg of seeds was 2.3, 2.3 and 2.7, respectively and proved best among all the botanicals/ inert dusts (Table 4.17). However maximum adult population of 16.7 was recorded in Nochi leaf powder 5g/kg of seeds.

The adult population gradually decreased with time in treated seeds and on 120 DAT, the adult population was on par and minimum in seeds treated with diatomaceous earth

2.5g/kg, rice husk ash 5g/kg and sweet flag rhizome powder 5g/kg of seeds which registered 0.0, 0.0, 0.3 adults respectively and proved best among all the botanicals/ inert dusts.

4.5.2.2. Per cent damage

The mean per cent damage to seeds was minimum in seeds treated with diatomaceous earth 2.5g/kg, rice husk ash 5g/kg and sweet flag rhizome powder 5g/kg which recorded 0.4, 0.5 and 1.0 per cent damage, respectively. Nochi leaf powder, turmeric powder and neem leaf powder all at 5g/kg registered high per cent seed damages of 24.2, 17.2 and 13.7 per cent respectively while untreated control recorded 38.4 per cent damage (Table 4.18).

4.5.2.3. Seed germination:

Germination was highest in rice husk ash, diatomaceous earth and sweet flag rhizome powder treated seed (86.7, 85.0 and 85.0 per cent) was recorded. Germination was low in nochi leaf powder (46.7 per cent) and turmeric rhizome powder (45.0) while untreated control recorded only 43.3 per cent on 120 DAT (Table 4.19).

4.6. Record of parasitoids attacking *C.chinensis*

Two parasitoids attacking *S. oryzae* were recorded from the insect culture. One of the parasitoid was identified to be belonging to the family Pteromalidae, super-family Chalcidoidea under order Hymenoptera (Plate 4.1a). The adult female parasitoid measured approximately 2.5 mm in length while the male was slightly smaller.

The other parasitoid was identified as belonging to the family Bethyridae, super-family Chrysidoidea order Hymenoptera (Plate 4.1b).

Freshly harvested seeds were stored in laboratory and observed for multiplication of parasitoids and the host insect. It was found that on 60 and 120 days of storage the population of the Pteromalid parasitoid adults was 5.4 and 9.2, respectively and that of Bethyrid parasitoid adult was 4.6 and 5.8 while that of *S. oryzae* adult was 45.2 and 83.2, respectively (Table 4.20). At any given point of time, the population of these two species was not sufficient enough to bring about a significant reduction in the host population.

4.7. Biochemical losses during storage

4.7.1. Carbohydrate content of grains

The per cent carbohydrate content in CO 1 maize grains analyzed by anthrone method showed that there was a reduction in carbohydrate content in the infested grains compared uninfested grains. Uninfested grains contained 65.6 per cent carbohydrate while grains with 43% damage due to insects had 60.7 per cent carbohydrate which corresponded to 7.5 per cent reduction in carbohydrate content. There was 5.9 per cent reduction in carbohydrate in grains showing 26% insect damage due to grains (Table 4.21).

In COHM5 maize grains there was a similar reduction in carbohydrate content in the infested grains compared uninfested grains. Uninfested grains contained 65.3 per cent carbohydrate while grains with 45% damage due to insects had 58.9 per cent carbohydrate which corresponded to 9.8 per cent reduction in carbohydrate content. There was 2.1 per cent reduction in carbohydrate in grains showing 27% insect damage due to grains. There was a significant negative correlation between insect damage and carbohydrate content of seeds (Table 4.21).

4.7.2. Starch content

In CO1 maize, there was reduction in starch content as the *S. oryzae* infestation increased. Uninfested grains contained 41.9 per cent starch while grains with 43% damage due to insects had 40 per cent starch which corresponded to 4.5 per cent reduction in starch content. Grains having 26% damage showed 1.9 per cent reduction in starch content compared to uninfested grains (Table 4.21).

In COH(M) 5 maize, uninfested grains contained 41.2 per cent starch while grains with 45% damage due to insects had 39.4 per cent starch which corresponded to 4.4 per cent reduction in starch content. There was 3.4 per cent reduction in starch content in grains with 27% insect damage due to insects (Table 4.22).

4.7.3. Nitrogen content

As the grains were infested with *S oryzae* there was an increase in the nitrogen content of the grains. CO1 maize with 43% insect damage had 2.3% more nitrogen content than grains showing no damage. COH(M) 5 maize grains with 45 and 27% grain damage showed 40.4 and 19.1% increase in nitrogen content, respectively than uninfested grains (Table 4.23).

Table 4.2. Probit analysis of CO₂ concentration Vs insect mortality relationship

Host seed and Stage of insect	Duration of exposure	LC ₅₀ CO ₂ %	95% Fiducial limits		LC ₉₅ CO ₂ %	95% Fiducial limits		χ^2 (p=0.05)	Equation (Y=bx+a)
			LL	UL		LL	UL		
Maize Variety CO 1									
Larva	4 days	22.3	20.9	23.9	47.5	42.5	53.1	5.22	y = 5.13x– 1.83
Adult	2 days	19.6	17.4	22.1	83.1	60.0	115.0	2.36	y = 2.66x + 1.56
	4 days	15.1	13.5	16.9	45.0	37.2	54.5	5.30	y = 3.75x + 0.59
Maize F1 Hybrid COH(M)5									
Larva	4 days	21.4	20.0	23.0	46.1	41.3	51.4	1.58	y = 4.95x – 1.55
Adult	2 days	18.3	16.4	20.5	67.7	51.9	88.2	1.28	y = 2.95x +1.28
	4 days	13.9	12.4	15.6	41.6	34.5	50.1	2.32	y = 3.67x + 0.80

Table 4.1. Effect of CO₂ treatment on the mortality of larval stage of *S. oryzae* in maize

Treatment		Concentration of CO ₂ in storage atmosphere	Per cent larval mortality after exposure of Maize varieties to CO ₂ for 4 days *		
			Variety CO 1	F1 Hybrid COH(M) 5	Mean
T ₁	CO ₂	10%	6.4 (14.5)	6.4 (14.4)	6.4 (14.4)
T ₂	CO ₂	20%	37.0 (37.4)	41.3 (40.0)	39.1 (38.7)
T ₃	CO ₂	30%	69.2 (56.3)	74.5 (59.7)	71.9 (58.0)
T ₄	CO ₂	40%	91.8 (73.6)	93.6 (75.6)	92.7 (74.6)
T ₅	CO ₂	50%	98.3 (85.7)	96.8 (81.6)	97.6 (83.6)
T ₆	CO ₂	60%	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
T ₇	CO ₂	70%	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
T ₈	Control		0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Mean			62.8 (55.9)	64.1 (56.4)	
			Treatments	variety	Interaction
CD (0.05)			3.9	1.9	5.6
SE(d)			1.96	0.98	2.77
CV%			5.70%		

* Larval mortality worked out based on reduction in adult emergence in treatment compared to untreated control

Figures in parenthesis are arcsine ($\sin^{-1} \sqrt{p}$) transformed value

Table 4.3. Effect of CO₂ treatment on adult mortality of *S. oryzae* in stored maize

Treatment			Concentration of CO2 in storage atmosphere			Per cent mortality of adults on different maize varieties and after different periods of exposure to CO ₂					
						Maize Variety CO1		Maize Hybrid COH(M) 5		Mean	
						2 days	4 days	2 days	4 days		
T ₁	CO ₂	10%	23.3 (28.8)	30.0 (33.2)	23.3 (28.8)	33.3 (35.2)	27.5 (31.5)				
T ₂	CO ₂	20%	50.0 (45.0)	60.0 (50.9)	53.3 (46.9)	66.7 (54.8)	57.5 (49.4)				
T ₃	CO ₂	30%	63.3 (52.8)	83.3 (66.1)	70.0 (57.0)	86.7 68.9)	75.8 (61.2)				
T ₄	CO ₂	40%	83.3 (66.1)	96.7 (83.9)	86.7 (68.9)	96.7 (83.9)	90.8 (75.7)				
T ₅	CO ₂	50%	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)				
T ₆	CO ₂	60%	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)				
T ₇	CO ₂	70%	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)				
T ₈	Control		0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)				
Mean			65.0 (57.8)	71.3 (63.0)	66.7 (58.9)	72.9 (64.1)					
			Treatments	Periods	Variety	VxD	DxT	VxT	VxDxT		
CD (0.05)			3.1	1.6	1.6	2.2	4.4	4.4	6.2		
SE(d)			1.56	0.78	0.78	1.10	2.20	2.20	3.12		
CV%			6.26%								

Figures in parenthesis are arcsine ($\sin^{-1} \sqrt{p}$) transformed value.

Table 4.4. Effect of treatment CO₂ on adult population of *S. oryzae* in stored maize
(Variety CO 1)

Treatment	Concentration of CO ₂ in storage atmosphere	Adults population at different periods after treatment
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			30DAT	60DAT	90DAT	120DAT	Mean
T ₁	CO ₂	10%	20.0 (4.5)	39.0 (6.3)	59.3 (7.7)	82.0 (9.1)	50.1 (6.9)
T ₂	CO ₂	20%	12.7 (3.6)	30.3 (5.5)	48.0 (6.9)	55.0 (7.4)	36.5 (5.9)
T ₃	CO ₂	30%	7.0 (2.7)	19.7 (4.5)	32.0 (4.4)	32.0 (5.6)	22.7 (4.3)
T ₄	CO ₂	40%	3.3 (1.9)	9.3 (3.1)	25.7 (5.1)	27.0 (5.2)	16.3 (3.9)
T ₅	CO ₂	50%	0.0 (0.7)	4.0 (2.1)	8.7 (3.0)	12.3 (3.6)	6.2 (2.4)
T ₆	CO ₂	60%	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)	0.0 ((0.7)	0.0 (0.7)
T ₇	CO ₂	70%	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)	0.0 (0.7)
T ₈	Control		21.0 (4.6)	40.3 (6.4)	51.7 (7.0)	84.3 (9.2)	51.7 (7.0)
Mean			8.0 (2.4)	17.8 (3.7)	29.3 (4.6)	36.6 (5.2)	

	Treatments	Periods	Interaction
CD (0.05)	0.4	0.3	0.8
SE(d)	0.20	0.14	0.40
CV%	12.53%		

Figures in parenthesis are square root ($\sqrt{x+0.5}$) transformed value.

Table 4.5. Effects of CO₂ seed treatment on per cent damage by *S. oryzae* in stored maize (Variety CO 1)

Treatment			Per cent damage at different period after treatment				
			30 DAT	60 DAT	90 DAT	120 DAT	Mean
T ₁	CO ₂	10%	17.3 (24.6)	24.0 (29.3)	32.7 (34.8)	37.3 (37.7)	27.8 (31.6)
T ₂	CO ₂	20%	13.3 (21.4)	19.3 (26.1)	25.7 (30.4)	30.3 (33.4)	22.2 (27.8)
T ₃	CO ₂	30%	11.7 (19.9)	14.0 (21.9)	18.0 (25.1)	26.3 (30.9)	17.5 (24.5)
T ₄	CO ₂	40%	10.0 (18.4)	11.7 (19.9)	14.3 (22.2)	17.3 (24.6)	13.3 (21.3)
T ₅	CO ₂	50%	3.7 (11.0)	4.3 (12.0)	4.7 (12.4)	8.0 (16.4)	5.2 (12.9)
T ₆	CO ₂	60%	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
T ₇	CO ₂	70%	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
T ₈	Control		21.3 (27.5)	33.3 (35.2)	42.7 (40.8)	56.7 (48.8)	38.5 (38.1)
Mean			9.7 (15.4)	13.3 (18.1)	17.2 (20.7)	22.0 (23.9)	
			Treatments		Periods		Interaction
CD (0.05)			0.4		0.3		0.9
SE(d)			0.22		0.16		0.44
CV%			2.72%				

Figures in parenthesis are arcsine ($\sin^{-1} \sqrt{p}$) transformed value.

Table 4.6. Effect on CO₂ treatment on germination of seeds in stored maize (Variety CO 1)

Treatment			Per cent seed germination at different period after treatment				
			30 DAT	60 DAT	90 DAT	120 DAT	Mean
T ₁	CO ₂	10%	71.7 (57.8)	68.3 (55.8)	61.7 (51.7)	51.7 (46.0)	63.3 (52.8)
T ₂	CO ₂	20%	76.7 (61.1)	71.7 (57.8)	66.7 (54.7)	61.7 (51.7)	69.2 (56.4)
T ₃	CO ₂	30%	80.0 (63.4)	73.3 (58.9)	71.7 (57.8)	68.3 (55.8)	73.3 (59.0)
T ₄	CO ₂	40%	85.0 (67.2)	80.0 (63.4)	76.7 (61.1)	75.0 (60.0)	79.2 (62.9)
T ₅	CO ₂	50%	91.7 (73.2)	91.7 (73.2)	88.3 (70.0)	83.3 (65.9)	88.8 (70.6)
T ₆	CO ₂	60%	95.0 (77.1)	91.7 (73.2)	91.7 (73.2)	90.0 (71.6)	92.1 (73.8)
T ₇	CO ₂	70%	95.0 (77.1)	93.3 (75.0)	93.3 (75.0)	91.7 (73.2)	93.3 (75.1)
T ₈	Control		65.0 (53.7)	61.7 (51.7)	56.7 (48.8)	38.3 (38.3)	55.4 (48.1)
Mean			82.5 (66.3)	79.0 (63.6)	75.8 (61.6)	70.0 (57.8)	
Treatments			Periods		Interaction		
CD (0.05)			2.0		4.0		
SE(d)			1.00		2.00		
CV%			3.95%				

Figures in parenthesis are arcsine ($\sin^{-1} \sqrt{p}$) transformed value.

Table 4.7. Effect of seed with insecticide treatment on larval mortality of *S. oryzae* in stored maize seeds

Treatment		Conc ppm a.i.	Per cent mortality of larval on different maize variety / hybrid*		
			Variety CO1	Variety COH(M) 5	Mean
T ₁	Spinosad 45 SC	2	55.3 (48.1)	55.5 (48.2)	55.4 (48.1)
T ₂	Lufenoron 5 EC	5	79.9 (63.4)	77.8 (61.9)	78.9 (62.7)
T ₃	Thiamethoxam 70 WS	2	20.0 (26.5)	20.5 (26.8)	20.2 (26.7)
T ₄	Primiphos methyl 50 EC	10	56.9 (49.0)	57.0 (49.0)	57.0 (49.0)
T ₅	E. benzoate 5 SG	2	58.4 (49.9)	60.3 (50.9)	59.3 (50.4)
T ₆	Deltamethrin 2.8 EC	1	87.7 (69.5)	85.7 (67.8)	86.7 (68.7)
T ₇	Malathion 50 EC	500	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
T ₈	Control		0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Mean			57.3 (49.6)	57.1 (49.3)	
Treatments		Variety		Interaction	
CD (0.05)		2.5		3.5	
SE(d)		1.22		1.72	
CV%		4.01%			

* Larval mortality worked out based reduction in adult emergence in treatment compared to untreated control

Figures in parenthesis are arcsine ($\sin^{-1} \sqrt{p}$) transformed value

Table 4.8. Effect of seed treatment with insecticides on adult mortality of *S. oryzae* in stored maize seeds

		Per cent mortality of adults on different maize variety / hybrid						
		Conc ppm a.i.	Variety CO1		Variety COH(M) 5		Mean	
Treatment			2 days	4 days	2 days	4 days		
T ₁	Spinosad 45 SC	2	60.0 (50.8)	100.0 (90.0)	56.7 (48.8)	100.0 (90.0)	79.2 (69.9)	
T ₂	Lufenoron 5 EC	5	26.7 (31.0)	63.7(53.5)	23.3 (28.8)	53.3 (46.9)	40.0 (38.9)	
T ₃	Thiamethoxam 70 WS	2	63.3(52.8)	100.0 (90.0)	60.0 (50.9)	100.0 (90.0)	80.8 (70.9)	
T ₄	Primiphos methyl 50 EC	10	36.7 (37.2)	100.0 (90.0)	33.3 (35.2)	100.0 (90.0)	67.5 (63.1)	
T ₅	E. benzoate 5 SG	2	56.7 (48.8)	100.0 (90.0)	53.3 (46.9)	100.0 (90.0)	77.5 (68.9)	
T ₆	Deltamethrin 2.8 EC	1	66.7 (54.8)	100.0 (90.0)	63.3 (52.8)	100.0 (90.0)	82.5 (71.9)	
T ₇	Malathion 50 EC	500	86.7 (68.9)	100.0 (90.0)	86.7 (68.9)	100.0 (90.0)	93.3(79.4)	
T ₈	Control		0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	
Mean			49.6 (43.0)	82.1 (73.6)	47.1 (41.5)	81.7 (73.4)		
Treatment		Variety	Period	VxD	DxT	VxT	VxDxT	
CD (0.05)		2.2	1.1	1.1	1.5	1.5	3.1	4.3
SE(d)		1.08	0.54	0.54	0.76	3.05	1.53	2.2
CV%		4.57%						

Figures in parenthesis are arcsine ($\sin^{-1} \sqrt{p}$) transformed value

Table 4.9. Effect of seed treatment with insecticides on adult population of *S. oryzae* in stored maize (Variety CO 1)

Treatment		Conc. ppm a.i.	Population of adults at different periods after treatment with insecticides				
			30 DAT	60 DAT	90 DAT	120 DAT	Mean
T ₁	Spinosad 45 SC	2	12.3 (3.6)	16.3 (4.1)	21.0 (4.6)	16.7 (4.1)	16.6 (4.1)
T ₂	Lufenoron 5 EC	5	15.7 (4.0)	7.7 (2.9)	0.3 (0.9)	0.0 (0.7)	5.9 (2.1)
T ₃	Thiamethoxam 70 WS	2	10.3 (3.3)	7.0 (2.7)	4.0 (2.1)	3.3 (1.9)	6.2 (2.5)
T ₄	Primiphos methyl 50 EC	10	17.3 (4.2)	18.0 (4.3)	22.7 (4.8)	19.0 (4.4)	19.2 (4.4)
T ₅	E. benzoate 5 SG	2	10.3 (3.3)	5.3 (2.4)	3.6 (2.0)	2.7 (1.8)	5.5 (2.4)
T ₆	Deltamethrin 2.8 EC	1	9.3 (3.1)	3.3 (1.9)	1.0 (1.2)	0.3 (0.9)	3.5 (1.8)
T ₇	Malathion 50 EC	500	8.3 (2.9)	0.7 (1.0)	0.3 (0.9)	0.3 (0.9)	2.4 (1.4)
T ₈	Control		23.0 (4.8)	45.0 (6.7)	64.0 (8.0)	83.0 (9.1)	53.7 (7.2)
Mean			13.3 (3.7)	12.9 (3.3)	14.6 (3.1)	15.7 (2.9)	
			Treatments		Periods		Interaction
CD (0.05)			0.1		0.1		0.3
SE(d)			0.07		0.07		0.14
CV%			5.19%				

Figures in parenthesis are square root ($\sqrt{x+0.5}$) transformed value.

Table 4.10. Effect of seed treatment with insecticides on per cent damage by *S. oryzae* in stored maize (Variety CO 1)

Treatment		Conc, ppm a.i.	Per cent damage				
			30DAT	60DAT	90DAT	120DAT	Mean
T ₁	Spinosad 45 SC	2	4.7 (12.5)	13.7 (21.7)	11.3 (19.7)	13.3 (21.4)	10.7 (18.8)
T ₂	Lufenoron 5 EC	5	5.7 (13.7)	5.0 (12.9)	5.2 (13.2)	5.4 (13.4)	5.3 (13.3)
T ₃	Thiamethoxam 70 WS	2	2.3 (8.7)	2.7 (9.3)	2.3 (8.7)	2.0 (7.9)	2.3 (8.7)
T ₄	Primiphos methyl 50 EC	10	8.3 (16.8)	13.0 (21.1)	13.0 (21.1)	16.0 (23.6)	12.6 (20.6)
T ₅	E. benzoate 5 SG	2	2.7 (9.3)	3.0 (9.9)	3.3 (10.5)	2.3 (8.7)	2.8 (9.6)
T ₆	Deltamethrin 2.8 EC	1	1.7 (7.3)	1.0 (4.6)	1.3 (6.5)	1.3 (6.5)	1.3 (6.2)
T ₇	Malathion 50 EC	500	0.7 (3.8)	0.7 (3.8)	1.3 (6.5)	0.3 (1.9)	0.7 (4.03)
T ₈	Control		21.0 (27.3)	34.3 (35.9)	42.7 (40.8)	56.7 (48.8)	38.7 (38.2)
Mean			5.9 (12.4)	9.2 (14.9)	10.01 (18.45)	12.2 (20.4)	
			Treatments		Periods		Interaction
CD (0.05)			1.31		0.92		2.61
SE(d)			0.65		0.46		1.31
CV%			11.20%				

Figures in parenthesis are arcsine ($\sin^{-1} \sqrt{p}$) transformed value

Table 4.11. influence of seed treatment with insecticide on germination of seeds in stored maize

Treatment		Conc., ppm a.i.	Per cent seed germination on exposure to different period				
			30 DAT	60 DAT	90 DAT	120 DAT	Mean
T ₁	Spinosad 45 SC	2	76.7 (61.1)	73.3 (58.9)	76.7 (61.1)	70.0 (56.8)	74.2 (59.5)
T ₂	Lufenoron 5 EC	5	86.7 (68.7)	81.7 (64.7)	81.7 (64.7)	80.0 (63.4)	82.5 (65.4)
T ₃	Thiamethoxam 70 WS	2	86.7 (68.7)	85.0 (67.2)	78.3 (62.3)	70.0 (56.8)	80.0 (63.8)
T ₄	Primiphos methyl 50 EC	10	83.3 (66.0)	80.0 (63.4)	78.3 (62.3)	71.7 (57.9)	78.3 (62.4)
T ₅	E. benzoate 5 SG	2	90.0 (72.0)	83.3 (66.0)	78.3 (62.3)	73.3 (58.9)	81.3 (64.8)
T ₆	Deltamethrin 2.8 EC	1	93.3 (75.2)	88.3 (70.1)	85.0 (67.4)	85.0 (67.4)	87.9 (70.0)
T ₇	Malathion 50 EC	500	95.0 (79.5)	93.3 (75.2)	90.0 (72.0)	90.0 (72.0)	92.1 (74.7)
T ₈	Control		61.7 (51.8)	55.0 (47.9)	53.3 (46.9)	41.7 (40.2)	52.9 (46.7)
Mean			84.2 (67.9)	80.0 (64.2)	77.7 (62.4)	72.7 (59.2)	
			Treatments	Periods		Interaction	
CD (0.05)			2.6	1.8		5.2	
SE(d)			1.29	0.91		2.59	
CV%			4.87%				

Figures in parenthesis are arcsine ($\sin^{-1} \sqrt{p}$) transformed value

Table 4.12. Impact of storage bag with insecticides treatment on adult population of *S. oryzae* in stored maize seeds

Treatment		Conc ppm a.i.	Population of adults after different periods of treatment with insecticidal bag				
			30 DAT	60 DAT	90 DAT	120 DAT	Mean
T ₁	Spinosad 45 SC	100	12.7 (3.6)	20.3 (4.6)	26.0 (5.1)	24.3 (5.0)	20.8 (4.6)
T ₂	Lufenoron 5 EC	100	15.7 (4.0)	6.3 (2.6)	0.3 (0.9)	0.0 (0.7)	5.6 (2.1)
T ₃	Thiamethoxam 70 WS	100	9.7 (3.2)	7.0 (2.7)	3.0 (1.9)	3.0 (1.9)	5.7 (2.4)
T ₄	Primiphos methyl 50 EC	200	12.7 (3.6)	19.0 (4.4)	23.3 (4.9)	25.7 (5.1)	20.2 (4.5)
T ₅	E. benzoate 5 SG	100	7.7 (2.9)	7.0 (2.7)	2.3 (1.7)	0.7 (1.1)	4.4 (2.1)
T ₆	Deltamethrin 2.8 EC	100	0.3 (0.9)	0.3 (0.9)	0.3 (0.9)	0.0 (0.7)	0.3 (0.8)
T ₇	Malathion 50 EC	1000	13.7 (3.8)	4.7 (2.3)	0.0 (0.7)	0.0 (0.7)	4.6 (1.9)
T ₈	Control		21.7 (4.7)	40.0 (6.4)	61.0 (7.8)	83.3 (9.2)	51.5 (7.0)
Mean			11.8 (3.3)	13.1 (3.3)	14.5 (3.0)	17.1(3.0)	
Treatments		Periods		Interaction			
CD (0.05)		0.1		0.1		0.3	
SE(d)		0.06		0.04		0.13	
CV%		5.24%					

Figures in parenthesis are square root ($\sqrt{x+0.5}$) transformed value.

Table 4.13. Influence of storage bag treatment with insecticides on per cent damage caused by *S. oryzae* in stored maize

Treatment		Conc. ppm a.i.	Per cent damage				Mean
			30 DAT	60 DAT	90 DAT	120 DAT	
T ₁	Spinosad 45 SC	100	10.7 (19.1)	15.7 (23.3)	20.3 (26.80)	24.7 (29.8)	2.2 (8.0)
T ₂	Lufenoron 5 EC	100	2.0 (7.9)	2.3 (8.7)	2.7 (9.36)	2.7 (9.36)	2.4 (9.2)
T ₃	Thiamethoxam 70 WS	100	13.7 (21.7)	17.7 (24.8)	18.7 (25.60)	18.7 (25.6)	17.2 (24.4)
T ₄	Primiphos methyl 50 EC	200	12.3 (20.6)	8.3 (16.8)	6.7 (14.95)	5.0 (12.9)	8.1 (16.3)
T ₅	E. benzoate 5 SG	100	2.3 (8.6)	3.0 (9.9)	3.7 (11.02)	3.7 (11.0)	3.2 (10.1)
T ₆	Deltamethrin 2.8 EC	100	1.0 (4.6)	2.3 (8.7)	2.7 (9.36)	2.7 (9.4)	2.2 (8.0)
T ₇	Malathion 50 EC	1000	0.7 (3.8)	0.7 (3.8)	0.7 (3.83)	0.7 (3.8)	0.7 (3.8)
T ₈	Control		21.3 (27.5)	31.3 (34.0)	42.0 (40.40)	55.7 (48.2)	37.6 (37.5)
Mean			8.0 (14.2)	10.2 (16.3)	12.1 (17.6)	14.2 (18.7)	
			Treatments		Periods		Interaction
CD (0.05)			1.4		0.9		2.7
SE(d)			0.68		0.48		1.36

CV%

10.30%

Figures in parenthesis are arcsine ($\sin^{-1} \sqrt{p}$) transformed value

Table 4.14. Influence of storage bag treatment with insecticides on seed germination caused by *S. oryzae* in stored maize

Treatment		Conc. ppm a.i.	Per cent seed germination at different period after treatment				
			30 DAT	60 DAT	90 DAT	120 DAT	Mean
T ₁	Spinosad 45 SC	100	68.3 (55.8)	63.3 (52.7)	51.7 (46.0)	50.0 (45.0)	58.3 (49.9)
T ₂	Lufenoron 5 EC	100	78.3 (62.3)	75.0 (60.1)	78.3 (62.3)	78.3 (62.3)	77.5 (61.7)
T ₃	Thiamethoxam 70 WS	100	70.0 (56.8)	61.7 (51.8)	61.7 (51.8)	60.0 (50.8)	63.3 (52.8)
T ₄	Primiphos methyl 50 EC	200	81.7 (64.7)	81.7 (64.7)	55.0 (47.9)	53.3 (46.9)	67.9 (56.0)
T ₅	E. benzoate 5 SG	100	86.7 (68.9)	71.7 (57.9)	73.3 (58.9)	71.7 (57.9)	75.8 (60.9)
T ₆	Deltamethrin 2.8 EC	100	95.0 (79.5)	86.7 (68.7)	88.3 (70.1)	88.3 (70.1)	89.6 (72.1)
T ₇	Malathion 50 EC	1000	98.3 (85.7)	91.7 (73.4)	91.7 (73.4)	91.7 (73.4)	93.3 (76.5)
T ₈	Control		60.0 (50.8)	56.7 (48.8)	51.7 (46.0)	43.3 (41.2)	52.9 (46.7)
Mean			79.8 (65.6)	73.5 (59.8)	69.0 (57.0)	67.1 (55.9)	
Treatments			Periods		Interaction		
CD (0.05)			2.6		5.2		
SE(d)			1.29		2.58		
CV%			5.16%				

Figures in parenthesis are arcsine ($\sin^{-1} \sqrt{p}$) transformed value

Table 4.15. Effect of seed treatment with botanicals and inert dust on the larval mortality of *S. oryzae* in maize

			Per cent larval mortality		
Treatment		Conc. g/kg seeds	Variety CO 1	F1 Hybrid COH(M) 5	Mean
T ₁	Sweet flag rhizome powder	5	96.8 (84.0)	96.7 (81.5)	96.8 (82.7)
T ₂	Turmeric powder	5	30.1 (33.3)	28.9 (32.5)	29.5 (32.9)
T ₃	Neem leaf powder	5	26.9 (31.2)	29.0 (32.6)	27.9 (31.9)
T ₄	Nochi leaf powder	5	22.2 (28.1)	25.7 (30.4)	23.9 (29.2)
T ₅	Diatomaceous Earth	2.5	93.7 (75.6)	96.8 (81.6)	95.3 (78.6)
T ₆	Rice husk ash	5	95.2 (77.4)	96.8 (81.6)	96.0 (79.5)
T ₇	Malathion	500ppm	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
T ₈	Control		0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Mean		58.1 (52.4)	59.3 (53.8)	
			Treatments	variety	Interaction
CD (0.05)			5.1	2.6	7.2
SE(d)			2.52	1.26	3.56
CV%			8.39%		

Figures in parenthesis are arcsine ($\sin^{-1} \sqrt{p}$) transformed value

Table 4.16. Effect of treatment with botanical or inert dust on adult mortality of *S. oryzae* in maize (Variety CO 1)

Treatment			Per cent mortality of adults after different periods of treatment with botanicals and inert dusts		
			Exposure period		
			4 day	8 day	Mean
T ₁	Vasambu rhizome powder	5	23.3 (28.8)	33.3 (35.2)	28.3 (32.0)
T ₂	Turmeric powder	5	13.3 (21.1)	30.0 (33.0)	21.7 (27.1)
T ₃	Neem leaf powder	5	3.3 (6.1)	13.3 (21.1)	8.3 (13.6)
T ₄	Nochi leaf powder	5	3.3 (6.1)	6.7 (12.3)	5.0 (9.2)
T ₅	Diatomaceous Earth	2.5	53.3 (46.9)	63.3 (52.8)	58.3 (49.8)
T ₆	Rice husk ash	5	66.7 (54.8)	76.7 (61.2)	71.7 (58.0)
T ₇	Malathion	500 ppm	96.7 (83.9)	96.7 (83.9)	96.7 (83.9)
T ₈	Control		0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Mean			32.5 (31.00)	40.0 (37.4)	
			Treatments	Periods	Interaction
CD (0.05)			7.9	3.9	11.2
SE(d)			3.89	1.95	5.50
CV%			19.79%		

Figures in parenthesis are arcsine ($\sin^{-1} \sqrt{p}$) transformed value

Table 4.17. Effect of treatment with botanicals and inert dust on adult population of *S. oryzae* in stored maize (Variety CO 1)

Treatment		Conc. g/kg seeds	Population of adults at different periods after treatment with botanicals and inert dust				
			30 DAT	60 DAT	90 DAT	120 DAT	Mean
T ₁	Sweet flag rhizome powder	5	2.3 (1.7)	1.0 (1.2)	0.7 (0.7)	0.3 (0.3)	1.1 (0.9)
T ₂	Turmeric powder	5	15.7 (4.0)	32.3 (5.7)	32.7 (32.7)	44.0 (44.0)	31.2 (21.6)
T ₃	Neem leaf powder	5	12.7 (3.6)	28.3 (5.4)	30.3 (30.3)	32.0 (32.0)	25.8 (17.8)
T ₄	Nochi leaf powder	5	16.7 (4.4)	34.7 (5.9)	39.3 (39.3)	47.0 (47.0)	34.4 (24.1)
T ₅	Diatomaceous Earth	2.5	2.3 (1.7)	2.0 (1.6)	0.0 (0.00)	0.0 (0.0)	1.1 (0.8)
T ₆	Rice husk ash	5	2.7 (1.8)	1.7 (1.5)	0.3 (0.3)	0.0 (0.0)	1.2 (0.9)
T ₇	Malathion	500ppm	0.0 (0.7)	0.0 (0.7)	0.0 (0.0)	0.0 (0.0)	0.0 (0.3)
T ₈	Control		24.3 (4.9)	40.0 (6.4)	64.0 (64.0)	84.0 (84.0)	53.1 (39.8)
Mean			9.6 (2.8)	17.5 (3.5)	20.9 (20.9)	25.9 (25.9)	
Treatments			Periods		Interaction		
CD (0.05)			0.1		0.1		0.2
SE(d)			0.06		0.04		0.12
CV%			4.35%				

Figures in parenthesis are square root ($\sqrt{x+0.5}$) transformed value.

Table 4.18. Effect of treatment with botanicals and inert dust on per cent damage caused by of *S. oryzae* in stored maize (Variety CO 1)

Treatment		Conc. g/kg seeds	Per cent damage				
			30DAT	60DAT	90DAT	120DAT	Mean
T ₁	Sweet flag rhizome powder	5	2.7 (9.4)	0.7 (3.8)	0.3 (1.9)	0.3 (1.9)	1.0 (4.25)
T ₂	Turmeric powder	5	12.3 (20.6)	15.7 (23.3)	17.7 (24.8)	23.0 (28.6)	17.2 (24.3)
T ₃	Neem leaf powder	5	8.7 (17.1)	12.7 (20.8)	14.7 (22.5)	19.0 (25.8)	13.7 (21.6)
T ₄	Nochi leaf powder	5	15.3 (23.0)	22.3 (28.2)	26.0 (30.6)	33.0 (35.1)	24.2 (29.2)
T ₅	Diatomaceous Earth	2.5	0.7 (3.8)	0.7 (3.8)	0.3 (1.9)	0.0 (0.0)	0.4 (2.4)
T ₆	Rice husk ash	5	0.7 (3.8)	0.7 (3.8)	0.3 (1.9)	0.3 (1.9)	0.5 (2.9)
T ₇	Malathion	500ppm	0.7 (3.8)	0.7 (3.8)	0.0 (0.0)	0.0 (0.0)	0.3 (1.9)
T ₈	Control		20.7 (27.0)	34.3 (35.9)	42.3 (40.6)	56.3 (48.6)	38.4 (38.0)
Mean			7.7 (13.6)	10.9 (15.4)	12.7 (15.5)	16.5 (17.7)	
			Treatments		Periods		Interaction
CD (0.05)			1.7		1.2		3.4
SE(d)			0.85		0.59		1.69
CV%			13.48%				

Figures in parenthesis are arcsine ($\sin^{-1} \sqrt{p}$) transformed value

Table 4.19. Effect of treatment with botanicals and inert dust seed germination caused by of *S. oryzae* in stored maize (Variety CO 1)

	Treatment	Dose g/kg	Per cent seed germination on exposure to different period				
			30 DAT	60 DAT	90 DAT	120 DAT	Mean
T ₁	Sweet flag rhizome powder	5	88.3 (70.1)	86.7 (68.7)	86.7 (68.7)	85.0 (67.4)	86.7 (68.7)
T ₂	Turmeric powder	5	71.7 (57.9)	65.0 (53.8)	46.7 (43.1)	45.0 (42.1)	57.1 (49.2)
T ₃	Neem leaf powder	5	91.7 (73.4)	85.0 (67.4)	56.7 (48.8)	55.0 (47.9)	72.1 (59.4)
T ₄	Nochi leaf powder	5	65.0 (53.8)	55.0 (47.9)	48.3 (44.0)	46.7 (43.1)	53.8 (47.2)
T ₅	Diatomaceous earth	2.5	90.0 (72.0)	86.7 (68.7)	86.7 (68.7)	85.0 (67.2)	87.1 (69.1)
T ₆	Rice husk ash	5	93.3 (75.2)	86.7 (68.9)	86.7 (68.9)	86.7 (68.9)	88.3 (70.5)
T ₇	Malathion 50EC	500ppm	95.0 (79.5)	93.3 (75.2)	93.3 (75.2)	93.3 (75.2)	93.8 (76.3)
T ₈	Control		61.7 (51.8)	56.7 (48.8)	50.0 (45.0)	43.3 (41.2)	52.9 (46.7)
	Mean		82.1(66.7)	76.9 (62.4)	69.4 (57.8)	67.5 (56.6)	
Treatments			Periods		Interaction		
CD (0.05)			2.7		1.9		
SE(d)			1.38		0.97		
CV%			5.41%				

Figures in parenthesis are arcsine ($\sin^{-1} \sqrt{p}$) transformed value

Table 4.20. Comparison of multiplication of *S. oryzae* and its parasitoids

Storage period (days)	Population of <i>S. oryzae</i>	Population of Pteromalid	Population of Bethylid
	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
30	22.4 $\pm$ 1.7	3.8 $\pm$ 0.4	2.6 $\pm$ 0.3
60	45.2 $\pm$ 3.2	5.4 $\pm$ 0.5	4.6 $\pm$ 0.4
90	64.0 $\pm$ 4.3	7.8 $\pm$ 0.7	5.4 $\pm$ 0.5
120	83.2 $\pm$ 5.5	9.2 $\pm$ 0.4	5.8 $\pm$ 0.3

Table 4.21. Carbohydrate content of maize grains at different level of insect infestation

CO1	Level of damage	Carbohydrate % *	% reduction from uninfested
	Grains with 43% damage	60.7 $\pm$ 1.25	7.5
	Grains with 26% damage	61.7 $\pm$ 1.62	5.9
	Grains with 0% damage	65.6 $\pm$ 2.12	0
COH(M)5			
	Grains with 45% damage	58.9 $\pm$ 1.78	9.8
	Grains with 27% damage	63.9 $\pm$ 1.35	2.1
	Grains with 0% damage	65.3 $\pm$ 1.89	0.0

* Mean $\pm$ standard deviation of three readings

Table 4.22. Starch content of maize grains at different level of insect infestation

CO1	Level of damage	Starch % *	% reduction from uninfested
	Grains with 43% damage	40.0±1.24	4.5
	Grains with 26% damage	41.1±1.61	1.9
	Grains with 0% damage	41.9±0.82	0.0
COH(M)5			
	Grains with 45% damage	39.4±0.78	4.4
	Grains with 27% damage	39.8±1.12	3.4
	Grains with 0% damage	41.2±0.98	0.0

Table 4.23. Nitrogen content of maize grains at different level of insect infestation

CO1	Level of damage	Nitrogen % *	% increase over control
	Grains with 43% damage	2.21±0.12	2.3
	Grains with 26% damage	2.21±0.08	2.3
	Grains with 0% damage	2.16±0.05	0.0
COH(M)5			
	Grains with 45% damage	3.3±0.12	40.4
	Grains with 27% damage	2.8±0.16	19.1
	Grains with 0% damage	2.35±0.17	0.0

* Mean ± standard deviation of three readings

CHAPTER V

DISCUSSION

The results from the research work carried on ecofriendly strategies for the management of *S. oryzae* in stored maize are discussed in this chapter.

5.1. Effects of CO₂ on management of *S. oryzae*

Experiments conducted to find out the effects of CO₂ at various concentrations of 10 to 70 per cent under two different exposure periods namely 2 & 4 days on the mortality of *S. oryzae* life stages revealed that fumigation with CO₂ can be utilized as an effective tool in managing this pest in storage.

It was found that when maize seeds were exposed to CO₂ at 60 and 70% concentrations for a period of four days, 100 per cent mortality of larvae of *S. oryzae* was recorded. There was a corresponding increase in mortality with increase in CO₂ concentration from 10 to 50%. This finding is in agreement with Jay, (1971) who found that exposure to 60% CO₂ for 4 days was sufficient to control all stages of stored product pests.

The LC₅₀ values for *S. oryzae* larvae were 23.3 and 21.4% CO₂ when tested on maize variety CO1 and hybrid COH(M)5 respectively for an exposure time of 4 days. However Girish (1964) found that LC₅₀ of CO₂ against *T. granarium* larvae was 40 per cent. Sridhar (2007) reported that the LC₅₀ of CO₂ against *C. chinensis* to be 33.7% for an exposure period of 4 days. Hence *S. oryzae* larvae are found having low LC₅₀ values and hence are more susceptible to CO₂ than the other storage pests reported.

For an exposure period of 2 days, the LC₉₅ values of *S. oryzae* adults was 83.1% CO₂ indicating that 2 days exposure period needs very high CO₂ concentrations which are practically difficult to achieve. The per cent adult mortality reached 100% when exposed for 4 days to 50% CO₂ concentration. The LC₅₀ and LC₉₅ values for adult rice weevils were as low as 15.1 and 45.0% CO₂ when tested on variety CO1 for an exposure period of 4 days. The above findings

clearly indicate that adults are more susceptible to CO₂ than the larvae. One hundred per cent mortality of larvae of *S. oryzae* was achieved at 60% CO₂ concentrations while that of the adults were killed at 50% itself. The LC₅₀ at 4 days exposure period for adults and larvae was 15.1 and 23.3% respectively showing that adults are killed at much lower CO₂ concentration than the adults. These results confirmed the earlier findings (Jayas *et al.*, 1995) that adults were more susceptible and were killed even within 2 days of exposure at 60 per cent CO₂. Sridhar (2007) also observed that larvae of *C. chinensis* were more tolerant to CO₂ treatment than the adults.

5.1.1. Effect of CO₂ on storability of seeds

There was a gradual build up of adult population when observed at 30 days interval in seeds treated with CO₂ at 10 to 50% concentration levels. At 60 and 70% CO₂ concentrations there was no adult populations recorded up to 120 DAT (Figure 5.1 and 5.2).

CO₂ treatment does not leave any residual effect on the seeds to cause further mortality. As the CO₂ concentration is increased it lead to elimination of increasing proportions of insects and their stages in the grain. Once the treatment for a period of four days is completed the seeds are stored in insect proof bags. At lower CO₂ concentrations since all the insects are not eliminated, there is a gradual build up of population. At high CO₂ concentrations since all insects and stages are eliminated further build up is totally prevented. This situation is different from treatment of seeds with insecticides where residues of insecticides remain on seeds and mortality of further generations take place leading to gradual reduction in population during first few months of storage but insecticides do not cause total elimination of insects as done by CO₂. Owing to this reason CO₂ treatment at sufficient concentrations to eliminate all insects in stored grains / seeds is a better method to manage storage pests than insecticidal treatments, in addition to its advantage of being eco-friendly.

Krishnamurthy *et al.* (1993) found 100 per cent mortality of *T. castaneum* and *S. oryzae* adults at 60 per cent CO₂. Spratt (1985) reported that at 60 per cent CO₂ concentration all the adults of *T. granarium* were killed within 6 days. Sridhar (2007) found that exposing

pulse seeds to 70% CO₂ concentrations for four days helped to store the seeds free from pulse beetle for a period of more than 120 days. These findings add support to our results that CO₂ treatment at 60% concentration for 4 days can kill all stages of *S. oryzae* and keep the maize seeds free from insects for long periods when stored in insect proof containers.

5.2. Studies on the management of *S. oryzae* through pre-storage seed treatment with insecticides

5.2.1. Effect on mortality of *S. oryzae*

Among the new insecticide molecules evaluated as pre-storage seed treatment on CO 1 and COH(M) 5 maize seeds, deltamethrin 1 ppm and lufenuron 5 ppm caused high mean per cent larval mortality of 86.7 and 78.9, respectively while thiamethoxam 1 ppm caused low larval mortality of 20.2%. Almost all the insecticides tested except lufenuron caused 100% mortality of *S. oryzae* adults within four days of treatment. Krall (1987) reported that deltamethrin was effective and hence recommended for use in West Africa. Yadav (1997) and Patil *et al.* (2004) also found the usefulness of deltamethrin at 1 ppm for management of *C. chinensis* and *R. dominica*, respectively.

Subramanyam *et al.* (2004) reported that spinosad at 1 mg / kg caused >98% mortality of many storage pests of wheat. However, in our experiment spinosad caused 55.4% mortality of larvae and 100% mortality of adults. Our experiments also indicated that lufenuron was more effective against larval stages than the adult stages. This could be due to the reason that lufenuron is a chitin synthesis inhibitor belonging to benzoyl phenyl urea group which acts while the insects are in their early stages of growth such as the larval stage. In the full grown or adult stage the chitin synthesis inhibitor does not act and hence its efficacy is not pronounced.

Thiamethoxam 2 ppm caused low larval mortality of 20.2% but high adult mortality of 100% on 4 DAT. Since the insecticide remains on the surface of the seeds and kill all emerging

adults, this chemical can also be effectively utilized for management of *S. oryzae* like other chemicals tested.

Malathion 500 ppm which was used as a standard check, even though effective at this high concentration has to be replaced with newer molecules which act at low concentrations of 1 to 5 ppm to combat the problem of insecticide resistance and environmental pollution. Champ and Dyte (1978) and Pacheco *et al.* (1990) reported insecticide resistance in stored product insects to organophosphorus insecticides. Insecticide resistance was observed in Karnal populations of *R. dominica* against all four insecticides tested namely dichlorvos, deltamethrin, malathion and phosphine (Saxena *et al.*, 1999). Thus it is essential to find out new molecule to which insects do not exhibit resistance.

5.2.2. Effect of insecticides on storability of seeds

Observations recorded on adult population, per cent seed damage and seed germination up to 120 DAT indicated that the use of new insecticide molecules can be an important component in managing *S. oryzae* in storage. Insecticides namely deltamethrin 1 ppm, emamectin benzoate 2 ppm, thiamethoxam 2 ppm and lufenuron 5 ppm proved very effective since the adult population in the treated seeds gradually decreased with time and was nearly zero on 120 DAT (Fig 5.3). The per cent damage also did not increase in treated seeds with time showing the effectiveness of the insecticides (Fig 5.4). Santos (1988) and Braga *et al.* (1991) found those deltamethrin and pirimiphos methyl are good alternatives to malathion to which storage pests have developed resistance worldwide.

Fangneng Huang and Subramanyam (2005) found that pirimiphos methyl at 4 to 8 ppm concentration provided excellent control of stored product pest on wheat. However, in our experiment spinosad 2 ppm and pirimiphos methyl 10 ppm treated seeds showed high adult population (16.7 and 19.0 adults / 100g seeds on 120 DAT) and there was an increase in the per cent damaged seed with time indicating that these insecticide are not as effective as the other insecticides mentioned above.

The seed germination remained high in deltamethrin 1 ppm, emamectin benzoate 2 ppm and lufenuron 5 ppm treated seeds. These insecticides did not have any effect directly on the seed germination but helped in control of the insects which indirectly prevented loss of germination. Patil *et al.* (2004) recorded the effectiveness of deltamethrin 1 ppm in providing complete protection from *R. dominica* on wheat for 6 months of storage without loss of viability which is in support of our studies.

5.3. Studies on the management of *S. oryzae* through fabric treatment of storage bags

5.3.1. Effect of fabric treatment on storability of seeds

The effectiveness of treating seed storage bags with insecticides was evaluated based on per cent damage caused, adult population and seed germination. Storage bag treatment with insecticides namely deltamethrin 100 ppm, emamectin benzoate 100 ppm, thiamethoxam 100 ppm and lufenuron 100 ppm caused reduction in adult population and recorded almost nil population on 120 DAT (Fig 5.5). The per cent damage was also minimum and seed germination high in these treatments (Fig 5.6). Yadav (1997), Yadav and Singh (1994) Bareth and Guptha (1989) and Sinha (1993) used insecticides for storage bag treatment and found that deltamethrin treatment was very effective against various storage pests.

Our experiment indicated that spinosad 100 ppm and pirimiphos methyl 200 ppm bag treatment were not as effective as the other insecticides mentioned since high adult population, per cent damage and low seed germination were recorded in these two treatments. However Sukhdev and Chahal (1975) and Bareth and Guptha (1989) observed that primiphos methyl was effective against many storage pests as storage bag treatment. The reason for low level of effectiveness of primiphos methyl during our experiment could be due to the development of cross resistance to organophosphorus compounds in *S. oryzae* due to the use of high concentration of malathion.

5.4. Studies on management of *S. oryzae* through pre-storage seed treatment with botanicals and inert dusts

Botanicals and inert dusts are non toxic and considered ecofriendly. Hence four botanicals / plant products namely sweet flag rhizome powder (*A. calamus*), Turmeric powder (*C. longa*), neem leaf powder (*A. indica*), nochi leaf powder (*V. negundo*) all at 5g / kg and inert dusts diatomaceous earth 2.5g / kg and rice husk ash 5g / kg seeds were tested as surface treatment of maize grains / seeds. Among them both the inert dusts and sweet flag rhizome powder worked well and produced high larval mortality of more than 90%. The inert dusts caused more than 60% mortality of adults in the treated seeds 8 DAT. Chan Park *et al*, (2003) recorded 57% mortality of *Sitophilus sp* due to treatment with *A. gramineus* rhizome powder. Harish Chander *et al*. (1991) found that 80% of adults were killed when rice grain were treated with turmeric powder. However, in our studies, turmeric powder, neem leaf powder and nochi leaf powder produced low levels (less than 30%) of larval and adult mortalities. The experiment to find the suitability of these agents for long term storage of seeds showed that the inert dusts diatomaceous earth 2.5g / kg and rice husk ash 5g / kg seeds and sweet flag rhizome powder 5g / kg seeds registered low adult population, per cent seed damage and high seed germination (Fig 5.7 and 5.8). These agents can hence be used as ecofriendly alternative to insecticide treatment of seeds / grains. Korunic and Fields (1995) observed that only 24h were required to achieve 100% mortality of *C. ferrugineus* with 300 ppm of DE which is in agreement with our studies. McLaughlin (1994) suggested that DE can be an effective alternative to chemical pesticides for control of insect pest in stored products. Schmidt and Streloke (1994) found that *A. calamus* oil 0.01 per cent (w/w) significantly reduced the amount of damage measured based on maize dust produced by *Prostephanus truncatus* (Horn) which corroborates with our results. Thus, botanicals and inert dusts can be effectively and safely be used as seed / grain protectants during storage.

5.5. Record and Identification of parasitoids on *S. oryzae*

In the present study, two parasitoids attacking *S. oryzae* were recorded and identified to belong to the families Pteromalidae and Bethliidae, Hymenoptera based on taxonomic characters and earlier records in India and abroad. The population of these two parasitoids put together was around 20 – 25% per cent of the host population at any given time indicating the reduction of an equivalent per cent of host population. Even though preliminary observations indicated that these parasitoids do not cause sufficient reduction in host population, further studies are required to establish the biocontrol potential of these parasitoids against *S. oryzae*. Our studies reinforce the earlier findings by Stejskal *et al.* (2002) who suggested multi biocontrol agent strategy by augmentation of one or two species of polyphagous parasitoids (e.g. *Lariophagus distinguendus*, *Anisopteromalus calandrae* or *Holepyris sylvanidis*) to tackle multi species pest attack on stored products. The most frequent parasitoids attacking storage pests in Greece were *A. calandrae* followed by *H. sylvanidis* (Eliopoulos *et al.* 2002).

5.6. Biochemical changes during insect infestations

The carbohydrate content of insect damaged maize grains decreased by 7 to 10% when about 45% of the grains were damaged when compared to uninfested grain. The decrease in carbohydrate content can be attributed to increase in frass and waste materials in the infested grains due to insect attack. Darshan *et al.* (1992) reported that carbohydrate content decreased with increased storage period. However Sathyavathi Devi (1978) reported increase in carbohydrate content by 24.2 and 26.1%, respectively when bajra and ground nut stored for six months.

Starch content decreased by 4 to 5% when maize grain were infested to a level of 45%. The reduction in the starch content was comparatively lesser than the reduction in total carbohydrate content indicating that simple sugars present in the total carbohydrate decrease at a faster rate due to insect attack than starch.

The total nitrogen content increased from 2.35% in uninfested grains to 3.3% in 45% damaged grains. The increase in nitrogen content can be attributed to addition of insect's excreta which contain primarily uric acid which is a nitrogenous compound. Ananth *et al.* (1985) reported an increase in nitrogenous compounds like quinine and thiamine due to addition of frass, urine and faeces in insects infested grains which agree with our findings.

The changes in biochemical composition of untreated grains can be attributed to deterioration of maize grains due to insect damage while no biochemical changes can be attributed directly to the CO₂ treatment which only helped to protect the grains from insects.

CHAPTER VI

SUMMARY

Findings of the research carried out on the ecofriendly strategies for the management of *S. oryzae* in stored maize are summarized below

- Increasing CO₂ concentration in storage atmosphere to 60 per cent resulted in 100 per cent mortality of larvae of *S. oryzae* at 4 days of exposure time when tested in CO 1 and COH(M)5 maize seeds.
- The LC₅₀ and LC₉₅ values for larvae were 23.3 and 47.5% CO₂ when tested on variety CO1 for an exposure time of 4 days. On COH(M)5 hybrid the LC₅₀ and LC₉₅ values were 21.4 and 46.1% CO₂, respectively.
- When the adults of *S. oryzae* were exposed to 50 per cent CO₂ concentrations in storage atmospheres for 2 days, 100 per cent mortality resulted.
- The LC₅₀ and LC₉₅ values for adult rice weevils were 15.1 and 45.0% CO₂ concentrations in storage atmospheres when tested on variety CO1 for an exposure period of 4 days. On COH(M)5 hybrid the LC₅₀ and LC₉₅ values were 13.9 and 41.6% CO₂ concentrations, respectively when exposed for 4 days.
- In the long term storage study no adults emerged up to 120 DAT at 60 and 70% CO₂ concentrations. The percent damage caused by *S. oryzae* was nil in these concentrations. The mean per cent germination of seeds on 120 DAT was 90.0 and 91.7 in 60 and 70% CO₂ concentration while untreated recorded 38.3 per cent seed germination.
- Among the new insecticide molecules evaluated as pre-storage seed treatment on CO 1 and COH(M) 5 maize seeds, deltamethrin 1ppm and lufenuron 5ppm caused high mean per cent larval mortality of 86.7 and 78.9, respectively while thiamethoxam 1ppm caused low larval mortality of 20.2 %.

- The adult mortalities caused by deltamethrin 1ppm and thiamethoxam 2 ppm were as high as 66.7 and 63.3 per cent respectively on 2 DAT on CO1 maize. lufenuron 5 ppm caused a low adult mortality of 26.7% indicating that lufenuron being a chitin synthesis inhibitor works better on larval stage than adult stage. Similar results were obtained when observed on 4 DAT and when tested on COH(M) 5 maize.
- In the long term storage study, nil or minimum adult population and per cent damage were observed in maize seeds treated with newer insecticides namely lufenuron 5 ppm and deltamethrin 1 ppm and were on par with malathion 500 ppm on 120 DAT. Spinosad 2 ppm and pirimiphos methyl 10 ppm recorded high adult populations of 16.7 and 19.0 /100g seeds and damage levels of 13.3 and 16.0% respectively.
- Seed germination recorded 120 DAT was highest in deltamethrin 1 ppm treated seeds (85.0%) followed by lufenuron 5 ppm and emamectin benzoate 2 ppm which were all on par.
- Storage bags treated with deltamethrin 100 ppm and lufenuron 100 ppm recorded nil adult population and per cent seed damage and maximum seed germination, on 120 DAT in maize CO1 seeds which proved that these insecticides can be effectively used in storage bag treatments. Storage bag treatments with pirimiphos methyl 200 ppm and spinosad 100 ppm recorded significantly high adult population of 25.7 and 24.3 showing their ineffectiveness.
- The mortality of larvae of *S. oryzae* due to seed treatment with botanicals and inert dusts was maximum in CO1 maize seeds treated with sweet flag rhizome powder 5g/kg, rice husk ash 5g/kg and diatomaceous earth 2.5g/kg of seeds which registered 96.8, 95.2, and 93.7 per cent mortalities respectively.

- The mortality of adults of *S. oryzae* was 66.7, 53.3 and 23.3 per cent in CO1 maize seeds treated with rice husk ash 5 g/kg, diatomaceous earth 2.5g/kg and sweet flag rhizome powder 5g/kg of seeds on 4 DAT.
- The mean per cent seed damage was less than 1% and adult population nil in seeds treated with diatomaceous earth 2.5g/kg, rice husk ash 5g/kg and sweet flag rhizome powder 5g/kg on 120 DAT in the long term study.
- Two parasitoids belonging to families Pteromalidae and Bethyridae, Hymenoptera were recorded from insect cultures of *S.oryzae*. Biological control potential of these parasitoids has to be worked out.
- There was a reduction of 7.5 and 9.8% carbohydrate content and 4.5 and 4.4% reduction in starch content in insect damaged CO1 and COH(M) 5 maize grains, respectively which clearly indicated the loss of nutrients and deterioration of quality of insect damaged grains.
- The nitrogen content increased by 2.3 and 40.4% in insect damaged CO1 and COH(M) 5 maize grains respectively compared to uninfested grains indicating the increase in uric acid content due to insect excreta in infested grains.

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ABSTRACT
Ecofriendly strategies for Management of *Sitophilus oryzae* (L.) (Curculionidae: Coleoptera) in stored maize

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Maize is ravaged by a large number of insects in field as well as storage. Among the insect pests infesting stored maize the rice weevil, *Sitophilus oryzae* (L.) (Curculionidae: Coleoptera) is the most serious one and it attacks almost all important cereals under storage conditions with mild infestation in the field as well. It is therefore, imperative to save the stored maize from this serious pest.

Insecticides used to control stored insects leave objectionable residues in treated commodity and generally are hazardous to handle and apply. Some of the stored product insects have also developed resistance to these insecticides. This has resulted in increased reliance on alternative physical and ecofriendly control methods such as modified atmosphere storage treatments, use of botanicals, safe insecticides and biocontrol which will not contaminate the environment, but bring about remarkable control of storage pests. The following are the findings obtained from experiments conducted on ecofriendly management of *S. oryzae* in stored maize.

Increasing CO₂ concentration in storage atmosphere to 60 per cent resulted in 100 per cent mortality of larvae of *S. oryzae* at 4 days of exposure time when tested on maize seeds. The LC₅₀ and LC₉₅ values for larvae were 23.3 and 47.5% CO₂ when tested on variety CO1 for an exposure time of 4 days. The LC₅₀ and LC₉₅ values for adult rice weevils were 15.1 and 45.0% CO₂ concentrations. In the long term storage study no adults emerged in treated seeds up to 120 DAT at 60 and 70% CO₂ concentrations and seed germination remained unaffected.

Among the new insecticide molecules evaluated as pre-storage seed treatment on maize seeds, deltamethrin 1ppm and lufenuron 5ppm caused high mean per cent larval mortality of 86.7 and 78.9 respectively. The adult mortalities caused by deltamethrin 1ppm and thiamethoxam 2 ppm were as high as 66.7 and 63.3 per cent respectively on 2 DAT on maize. Storage bags treated with deltamethrin 100 ppm and lufenuron 100 ppm recorded nil adult population upto 120 DAT.

The mortality of larvae of *S. oryzae* due to seed treatment with botanicals and inert dusts was above 90% in maize seeds treated with sweet flag rhizome powder 5g/kg, rice husk ash 5g/kg and diatomaceous earth 2.5g/kg of seeds. The mortality of adults of *S. oryzae* due to botanicals seed treatment was 66.7, 53.3 and 23.3 per cent in CO1 maize seeds treated rice husk ash 5 g/kg, diatomaceous earth 2.5g/kg and sweet flag rhizome powder 5g/kg of seeds on 4 DAT.

Two parasitoids belonging to families Pteromalidae and Bethylidae, Hymenoptera were recorded from insect cultures of *S.oryzae*. Biological control potential of these parasitoids has to be worked out.

The nitrogen content increased in *S.oryzae* damaged maize grains compared to uninfested grains indicating the increase in uric acid content due to insect excreta in infested grains. There was a reduction of 7.5 and 9.8% in carbohydrate content and 4.5 and 4.4% starch content in CO1 and COH(M) 5 in *S.oryzae* damaged maize grains which clearly indicated deterioration in nutritional status of insect damaged maize grains.

Plate 4.1: Photomicrograph of parasitoids recorded from
S. oryzae culture

a. Belonging to family Pteromalidae



b. Belonging to family Bethyidae

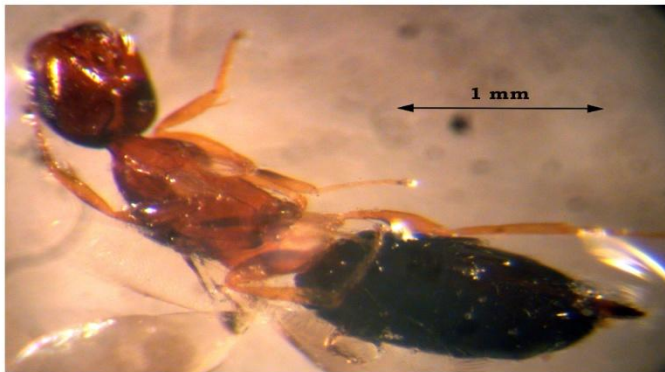


Figure 4.7: Effect of seed treatment with insecticides on the larval mortality of *S. oryzae*

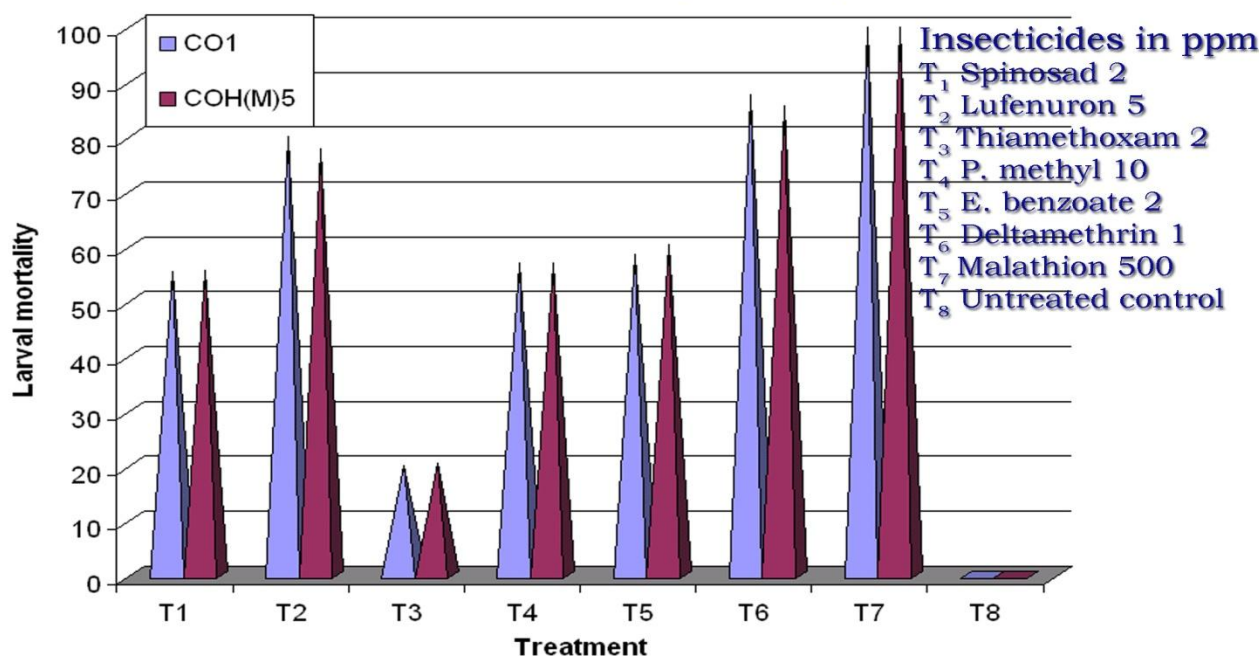
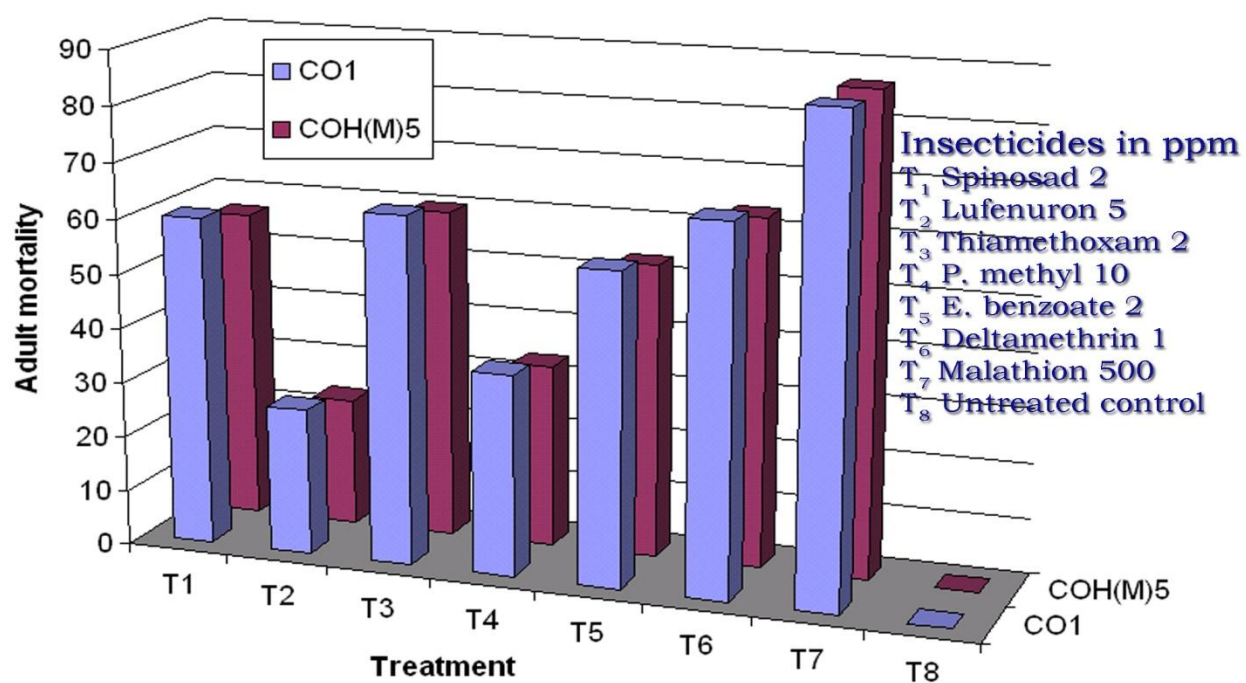


Figure 4.8: Effect of seed treatment with insecticides on the adult mortality of *S. oryzae*



**Plate 3.4 Management methods against *S. oryzae* -
Experimental set up**

a. Surface treatment of seeds with botanicals



b. Surface treatment of seeds with insecticides



c. Treatment of storage bags with insecticides



Plate 3.2 a: Mass culturing of *S. oryzae* on maize grains



Plate 3.2 b: Germination test by roll towel method



Plate 3.3: Evaluation of CO₂ treatment against *S. oryzae* on maize grains

a. Filling CO₂ in airtight container



b. Experimental set up - container with maize seeds filled with CO₂



Figure 4.1: Effect of CO₂ treatment on the larval mortality of *S. oryzae*

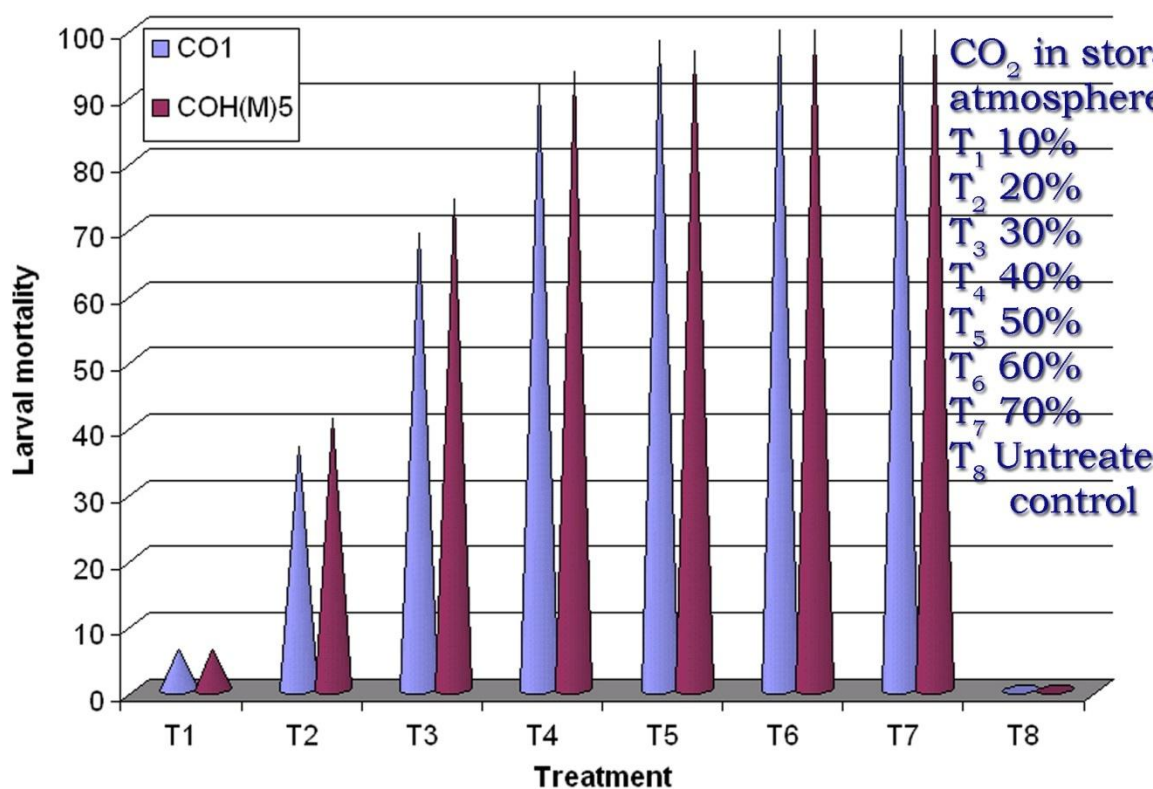


Figure 4.2: Effect of CO₂ treatment on the adult mortality of *S. oryzae*

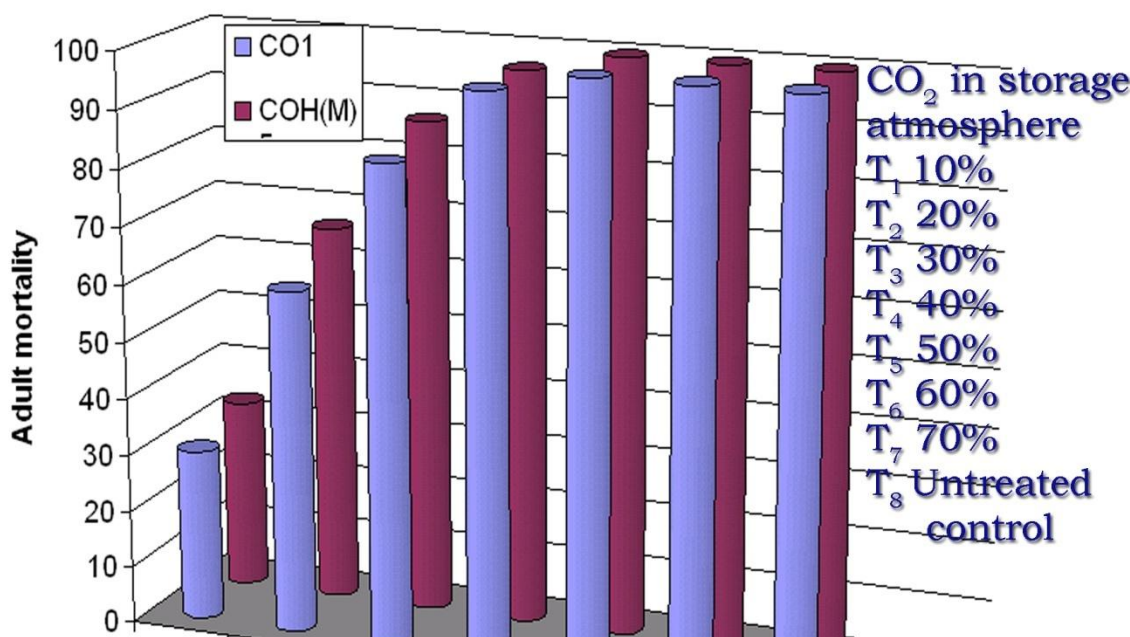


Figure 4.9: Effect of seed treatment with botanicals and inert dust on the larval mortality of *S. oryzae*

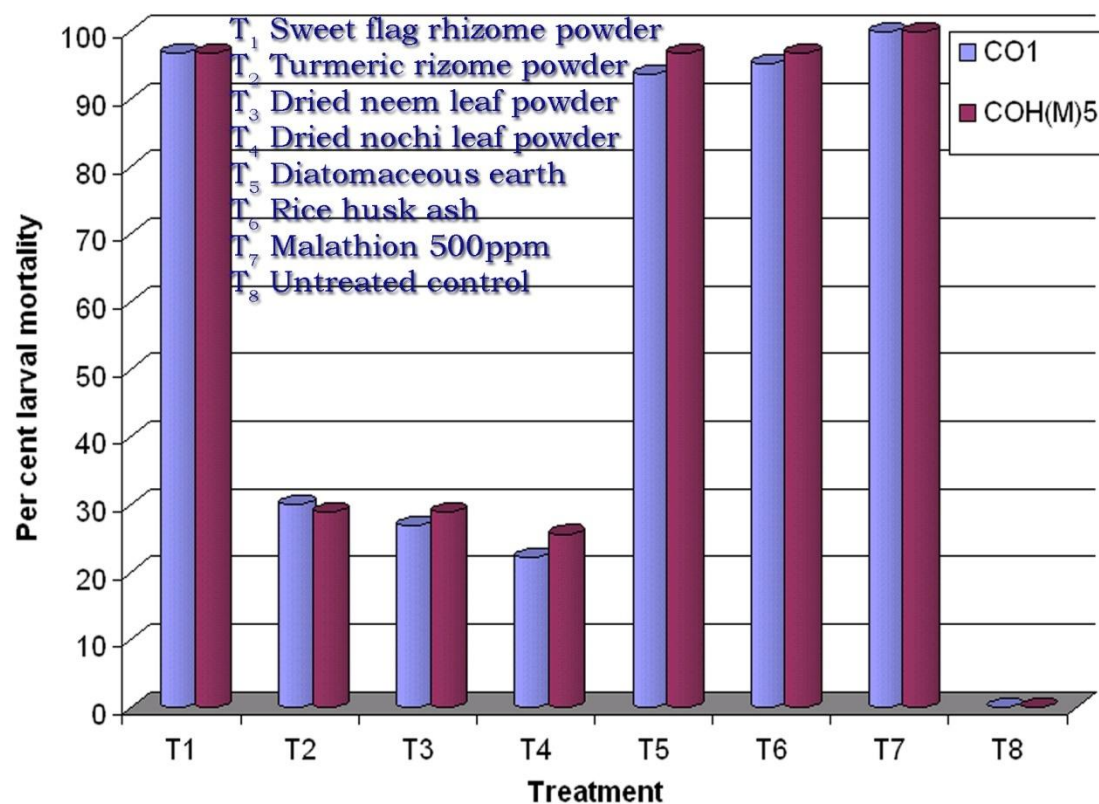


Figure 4.10: Effect of seed treatment with botanicals and inert dusts on the adult mortality of *S. oryzae*

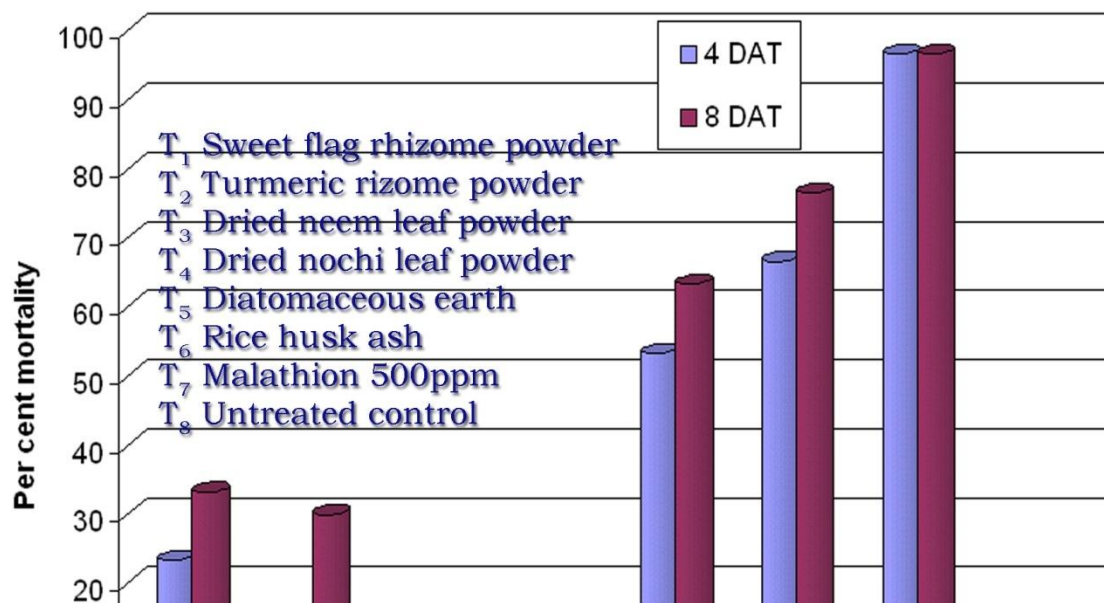


Figure 5.8: Effect of seed treatment with botanicals and per cent damage caused by *S. oryzae* in stor

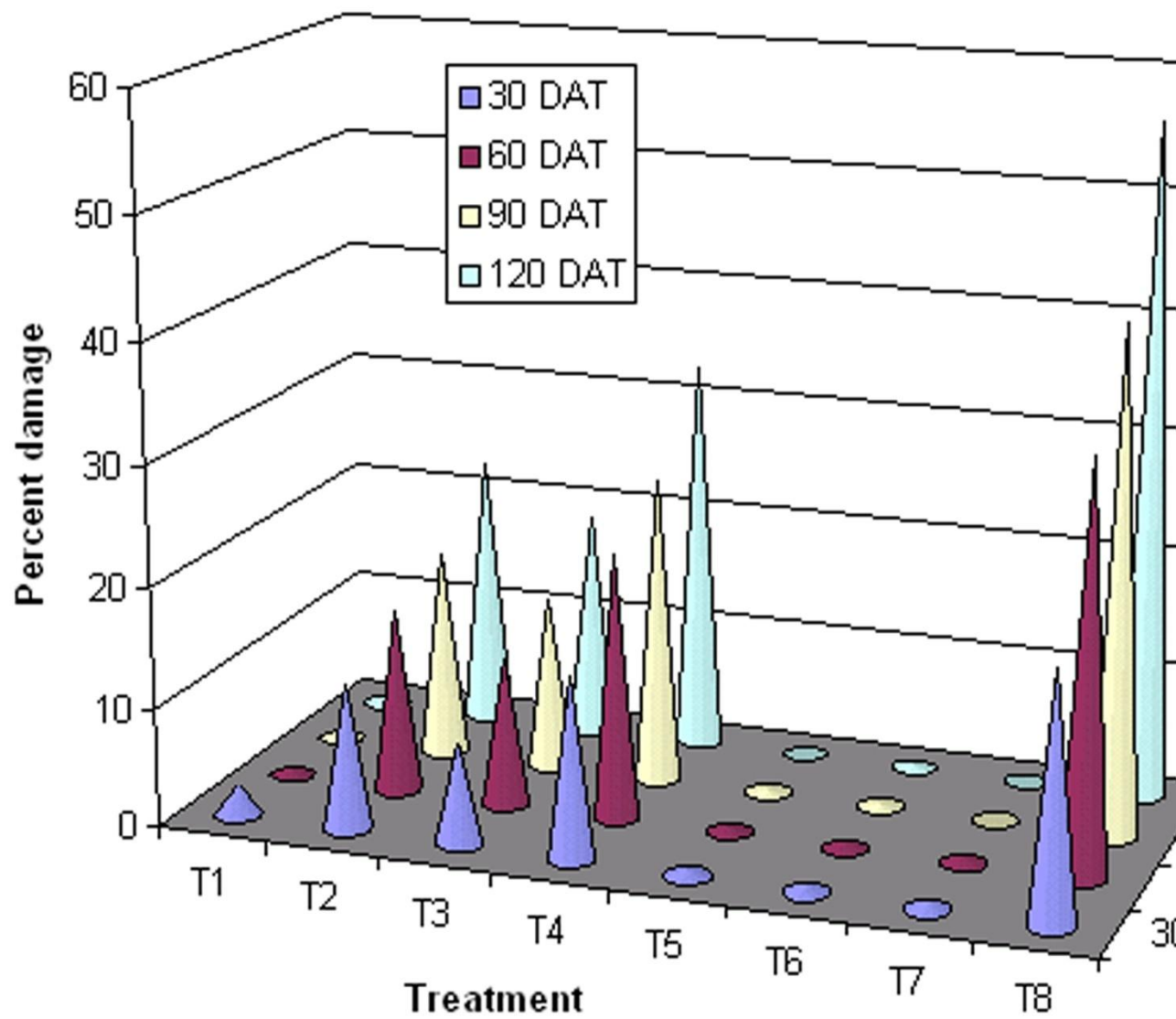


Figure 5.7: Effect of seed treatment with botanicals and inert dust on adult population of *S. oryzae* in stored maize

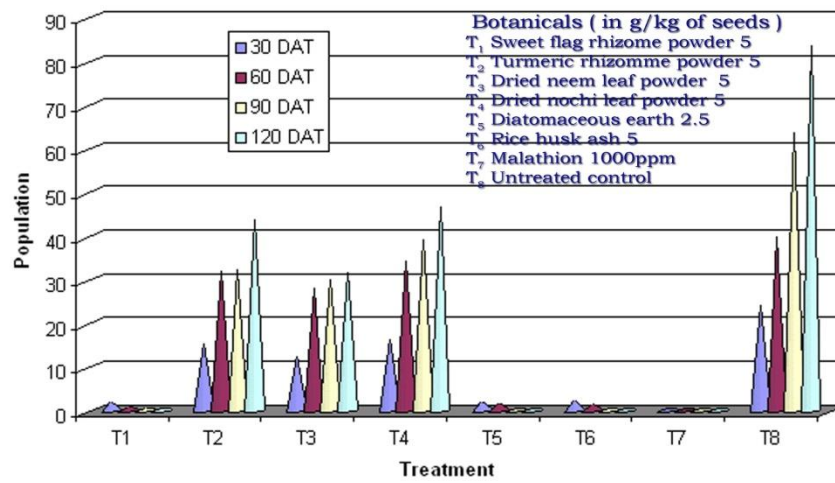


Figure 5.6: Effect of storage bag treatment with ins
damage caused by *S. oryzae* in stored m

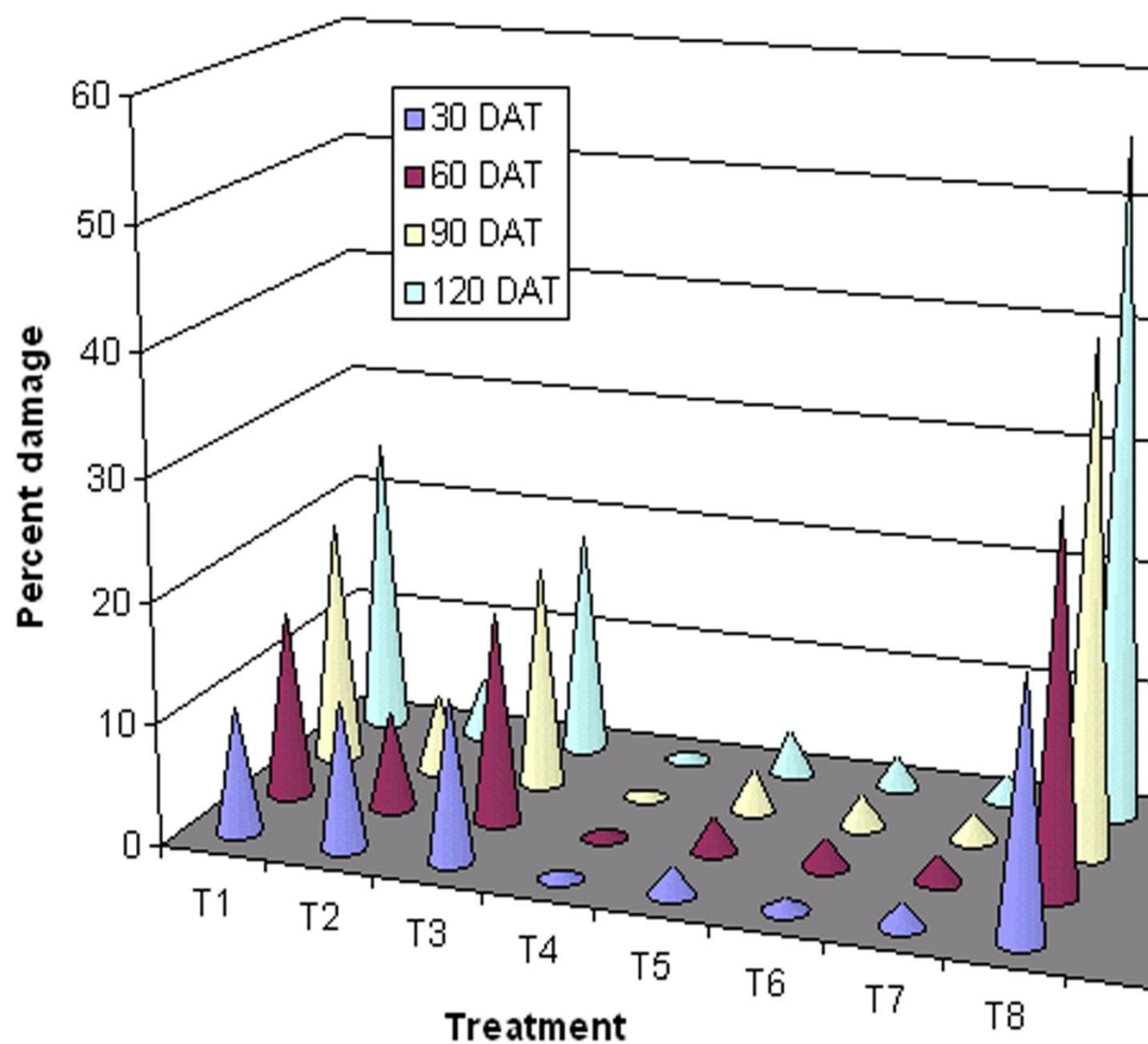


Figure 5.5: Effect of storage bag treatment with insecticides on adult population of *S. oryzae* in stored maize

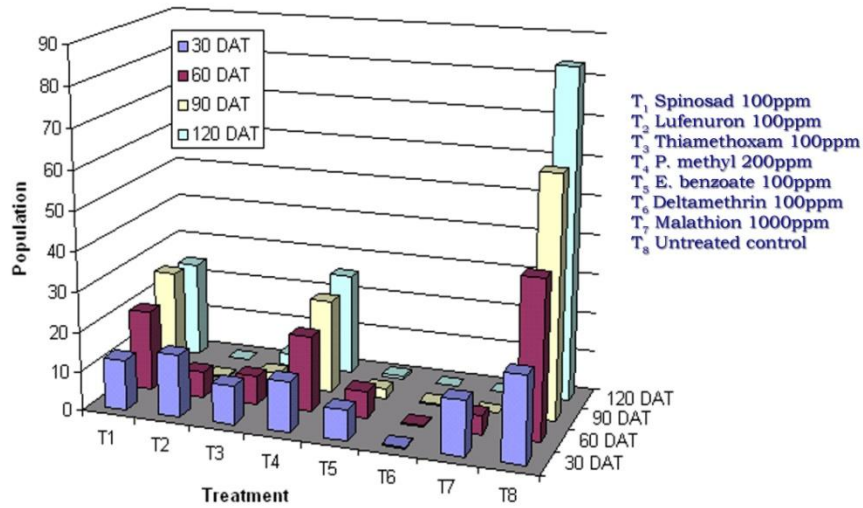


Figure 5.4: Effect of insecticide treatment on percent damage caused by *S. oryzae* in stored maize

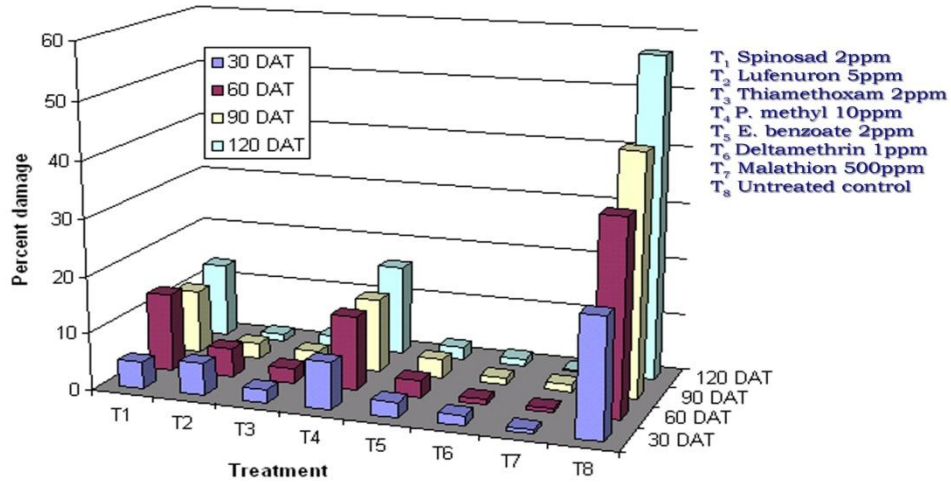


Figure 5.3: Effect of insecticide treatment on adult population of *S. oryzae* in stored maize

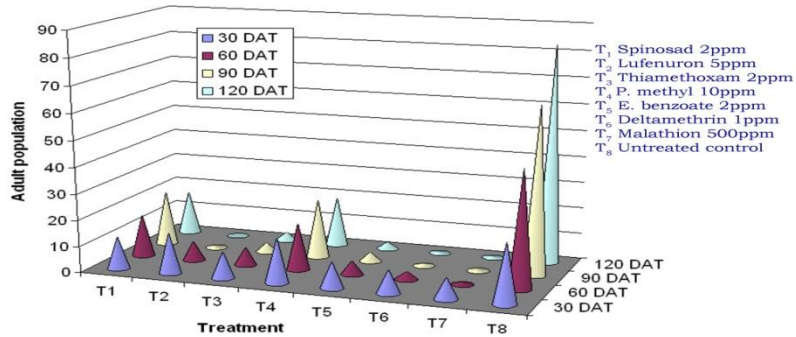


Figure 5.2: Effect of CO₂ treatment on per cent damage caused by *S. oryzae* in stored maize

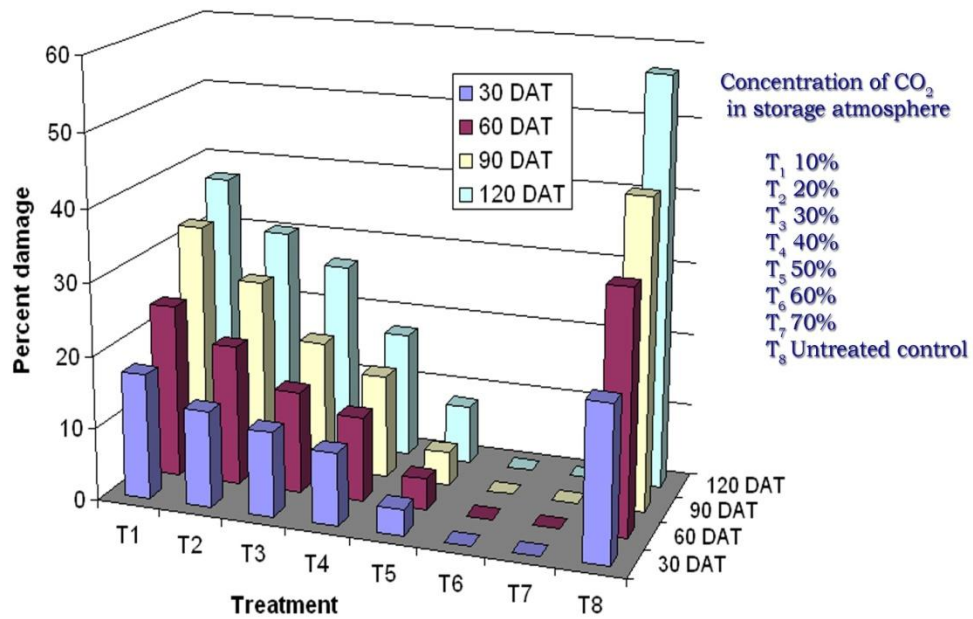


Figure 5.1: Effect of CO₂ treatment on adult population of *S. oryzae* in stored maize

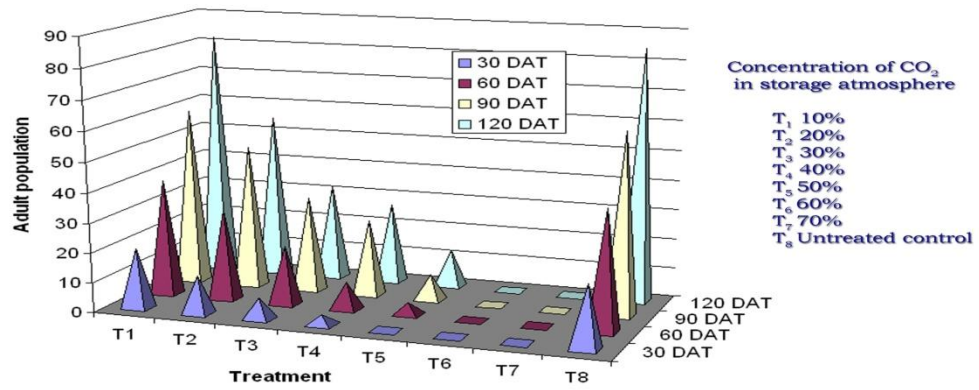


Figure 4.5 Relationship between CO₂ concentration and mortality different life stages of *S. oryzae* on maize CO1

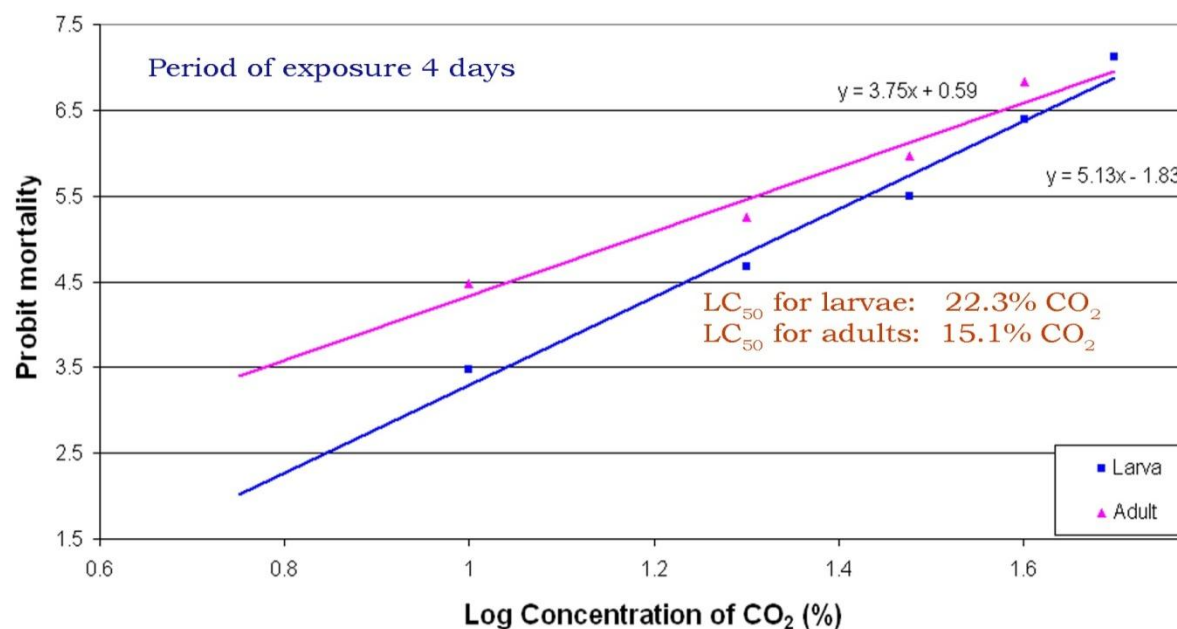


Figure 4.6 Relationship between CO₂ concentration and mortality different life stages of *S. oryzae* on maize COH(M) 5

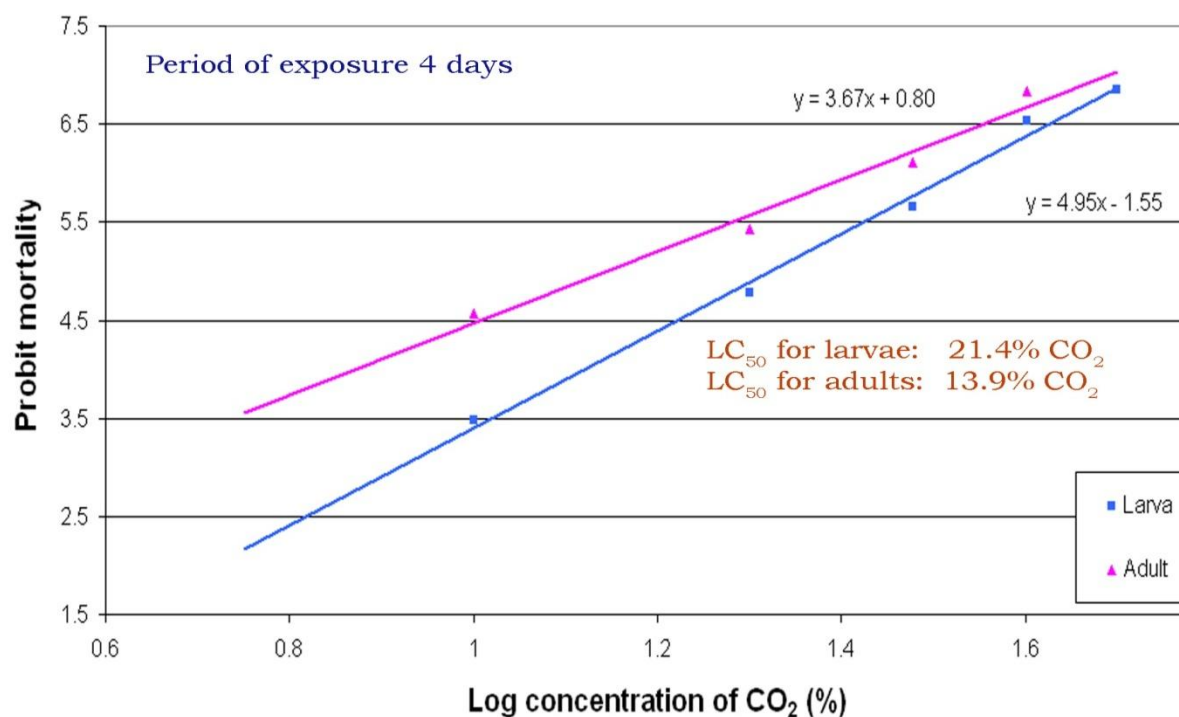


Figure 4.3. Relationship between CO₂ concentration and mortality of *S. oryzae* adults for different periods of exposure on maize CO1

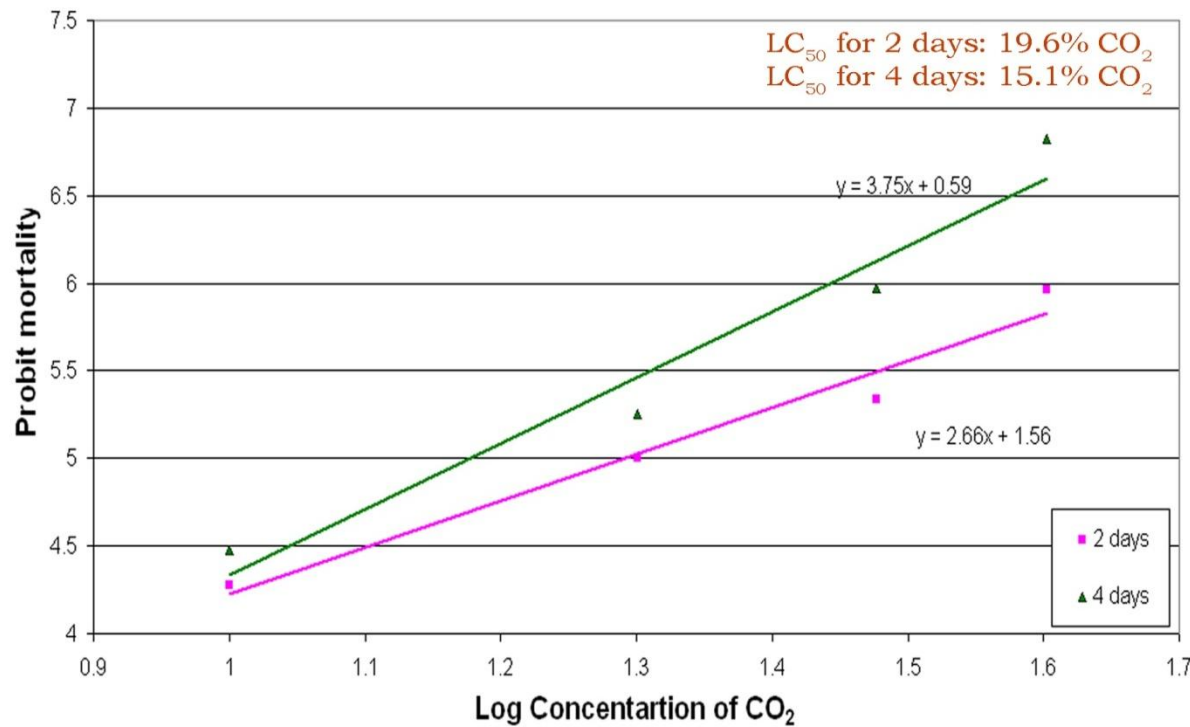


Figure 4.4. Relationship between CO₂ concentration and mortality of *S. oryzae* adult for different periods of exposure on maize COH(M)5

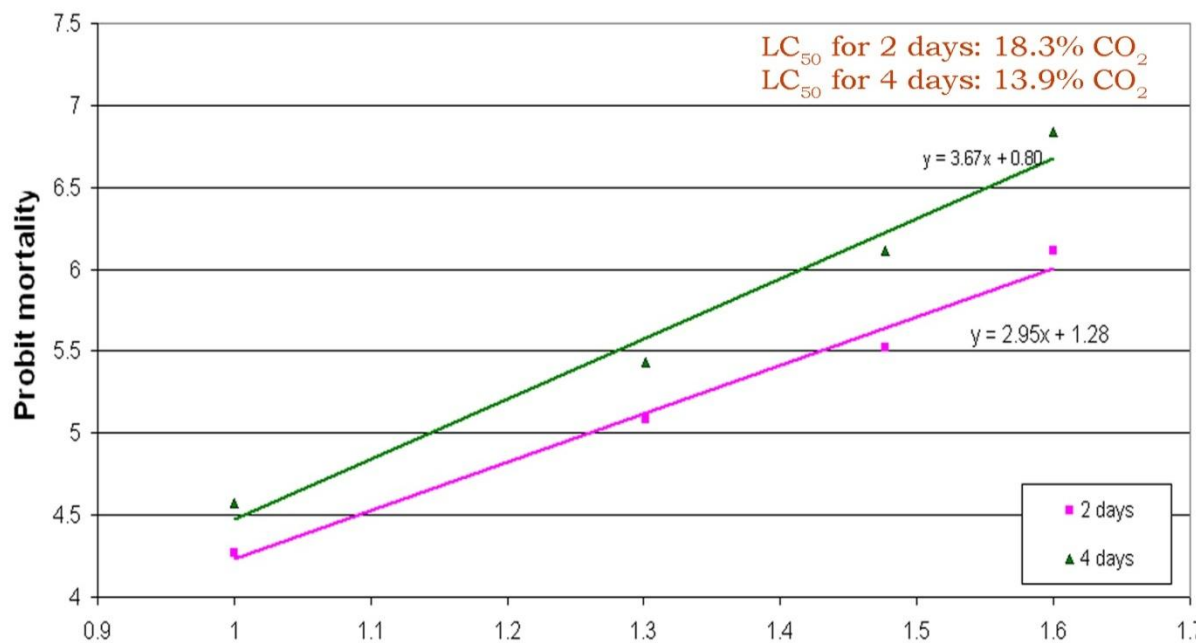
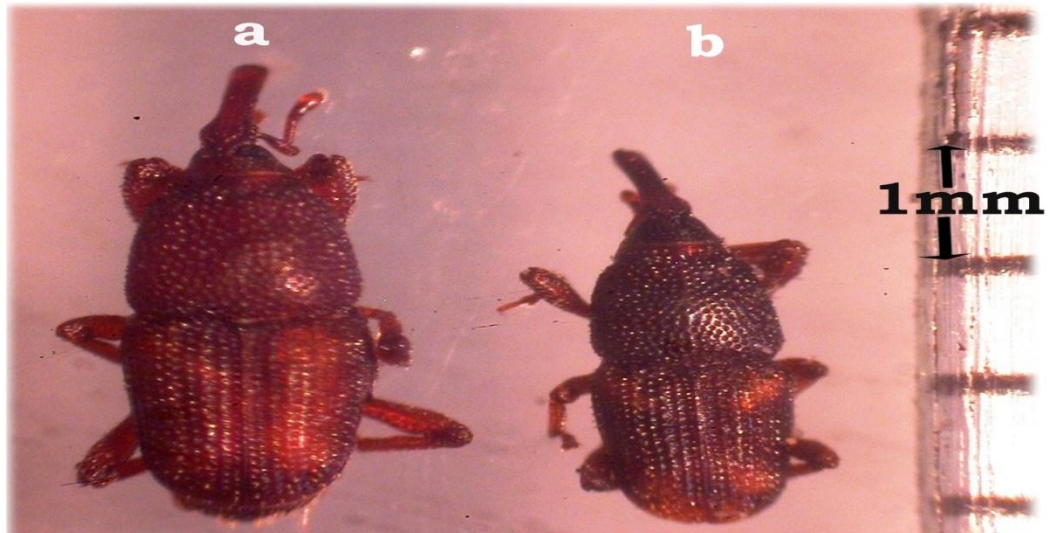


Plate 3.1: Photomicrograph of the test insects *S. oryzae*

a. Weevil reared on maize

b. Weevil reared on sorghum



c. Aedeagus of adult male weevils protruding out

d. Smooth dorsal surface of aedeagus characteristic of *S. oryzae*

