EVALUATION OF IMPROVED GRAIN STORAGE PRACTICES FOR THE MANAGEMENT OF GROUNDNUT BRUCHID *Caryedon serratus* OLIVIER (COLEOPTERA: BRUCHIDAE)

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B. Sc. (Ag.)

MASTER OF SCIENCE IN AGRICULTURE

(ENTOMOLOGY)

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BY

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THESIS SUBMITTED TO THE
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2016

DECLARATION

I**, Ms. Y. SWATHI,** hereby declare that the thesis entitled **"EVALUATION OF IMPROVED GRAIN STORAGE PRACTICES FOR THE MANAGEMENT OF GROUNDNUT BRUCHID *Caryedon serratus* OLIVIER. (COLEOPTERA: BRUCHIDAE)"** submitted to the PROFESSOR JAYASHANKAR TELANGANA STATE AGRICULTURAL UNIVERSITY for the degree of **Master of Science in Agriculture** is the result of original research work done by me. I also declare that any material contained in the thesis has not been published earlier in any manner.

Place: Hyderabad **(Y. SWATHI)**

Date: 18-7-16 **I. D. No. RAM/14-32**

CERTIFICATE

Ms. Y. SWATHI has satisfactorily prosecuted the course of research and that the thesis entitled **"EVALUATION OF IMPROVED GRAIN STORAGE PRACTICES FOR THE MANAGEMENT OF GROUNDNUT BRUCHID *Caryedon serratus* OLIVIER. (COLEOPTERA: BRUCHIDAE)"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has been previously submitted by him for a degree of any University.

Place: Hyderabad **(P.RAJANIKANTH)**

Date: 18-7-16 Chairperson

CERTIFICATE

This is to certify that the thesis entitled **"EVALUATION OF IMPROVED GRAIN STORAGE PRACTICES FOR THE MANAGEMENT OF GROUNDNUT BRUCHID *Caryedon serratus* OLIVIER. (COLEOPTERA: BRUCHIDAE)"** submitted in partial fulfilment of the requirements for the degree of **Master of Science** in Agriculture of the Professor Jayashankar Telangana State Agricultural University, Hyderabad is a record of the bonafide original research work carried out by **Ms. Y. SWATHI** under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all assistance received during the course of the investigations have been duly acknowledged by the author of the thesis.

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LIST OF ABBREVIATIONS

AF	:	*Aspergillus flavus*
CFU	:	Colony forming units
Cm	:	Centimeter
CRD	:	Completely Randomized Design
ELISA	:	Enzyme-linked immunosorbent assay
et al.	:	and other
etc.	:	and so on
Fig.	:	Figure
g	:	gram
i.e.	:	that is
Kg	:	Kilogram
$KMNO_4$	:	Potassium permanganate
L	:	litre
m	:	Metre
M	:	Molar
M.ha	:	Million hectares
mg	:	milligram
ml	:	millilitre
mm	:	millimetre
Mt	:	Million tonnes
N	:	Normal
NA	:	Nutrient Agar
ng	:	nano gram
nm	:	nano metre
No.	:	Number
$^{\circ}C$	:	Degree Centigrade
PDA	:	Potato Dextrose Agar
Pg	:	Picogram
pH	:	Hydrogen ion concentration
Spp.	:	Species
SC	:	Soluble concentrate
viz.,	:	namely
µg/Kg	:	Micrograms per kilogram

%	:	per cent
@	:	At the rate of
±	:	plus or minus
µg	:	Microgram (s)
µL	:	Micro litre
&	:	And
Ppm	:	Parts pert million
Fig	:	Figure
CO_2	:	Carbon dioxide
O_2	:	Oxygen
G	:	Gram
SEm	:	Standard error of mean

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ABSTRACT

Groundnut (*Arachis hypogaea* Linn.) is an important food legume and an oilseed crop in India and is cultivated in an area of 5.53 million ha with a production of 9.67 million tons and productivity of 1750 kgha^{-1} (FAO, 2013-14). Groundnut production is usually hampered by several biotic and abiotic constraints during pre-harvest which to a greater extent are tackled by integrated management approaches while in the post-harvest, storage is a big challenge especially for farmers as groundnut bruchid, *Caryedon serratus* (Olivier) causes severe damage to groundnut pods when stored in improper storage conditions.

In view of the importance of groundnut bruchid during storage, laboratory studies on **"Evaluation of improved grain storage practices for the management of Groundnut Bruchid, *Caryedon serratus* Olivier. (Coleoptera: Bruchidae)"** were conducted at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad. Studies were contemplated on estimation of oxygen requirement to bruchid, performance of triple layer bag as a tool of hermetic storage technology to manage bruchid, *Aspergillus flavus* growth and aflatoxin contamination in groundnut pods stored at 10 and 14 per cent moisture regimes and effect of *C. serratus* and *A. flavus* on changes in important biochemical constituents *viz.,* oil, protein and fatty acid composition of groundnut kernels under different storage conditions.

The mode of action of hermetic storage on groundnut bruchid was investigated to determine the actual requirement of oxygen by the bruchid and to understand the dynamics of change in oxygen and carbon dioxide concentrations and its effects on insects. The results revealed that a single bruchid from egg to pupal stage used approximately 39.97 ml of oxygen and simultaneously produced 26.21 ml of carbon dioxide. It is observed that any reduction in level of oxygen availability (hypoxia) than the requirement and increase in level of carbon dioxide concentration (hypercarbia)

than optimum level, in storage conditions caused cessation of feeding and eventually death of insects.

The performance of triple layer plastic bag as source of hermetic storage technology was evaluated for managing groundnut bruchid, *C. serratus* and storage fungi, *A. flavus* by storing groundnut pods with 10 and 14 per cent moisture contents and observing the development of bruchid and fungus at 2, 4 and 6 months of storage. The results revealed that groundnut pod with varying moisture contents stored in triple layer bag for a period of six months recorded 100 per cent mortality of bruchids and retained seed integrity significantly better than the pods stored in traditional bags such as jute, polythene and jute bag treated with insecticide. The percentage of damaged pods and test weight in triple layer plastic bag were unchanged at 10% (76.9g) and 14% (77.93g). The impermeability of triple layer plastic bag impeding diffusion of gases or exchange of gases with outer environment caused decrease in oxygen and increase in carbon dioxide concentrations and resulted in total cessation of egg development, larval feeding and ultimately caused adult mortality.

In contrast, the traditional bags recorded 94 per cent of pod damage, 22 per cent weight loss of pods and ultimately recorded reduction in test weight of pods to be 56.67 grams. This loss to stored pods is attributed to the fact that availability of congenial conditions in traditional storage bags which promoted insect growth leading to increase in number of eggs up to 312, emergence holes up to 73.63, number of pupae up to 29.43 and a massive increase in live insects up to 97 for every 100 pods.

The germination percentage after two and four months of storage was found to be reduced to 75.00- 58.33 per cent and 57.66 - 37.00 per cent respectively in traditional bags. Significantly low reduction in germination per cent to 85 and 77 was observed in triple layer plastic bags after two and four months of storage periods, respectively. No germination of seed was recorded after six months of storage in all types of bags. The loss in germination percentage was due to influence of abiotic factors like high relative humidity and high moisture contents and biotic factors like high insect activity and high rate of storage fungal growth in traditional bags compared to triple layer plastic bags.

The triple layer plastic bags recorded minimum aflatoxin accumulation compared to traditional bags. It is quantified that only 11.99μgkg^{-1} and 14.01μgkg^{-1} of aflatoxin was accumulated in the pods containing 10 and 14 per cent moisture, respectively when stored for two months. Similarly 456.0μgkg^{-1} and 700.23μgkg^{-1} of aflatoxins were observed after four months and 2444.46μgkg^{-1} & 2701.93μgkg^{-1} was recorded after six months of storage from pods containing 10 and 14 per cent moisture contents, respectively. The maximum aflatoxin content of 5093.53μgkg^{-1} was recorded in traditional jute bag at 14 per cent moisture. The low production of aflatoxins in triple layer plastic bags is due to low oxygen availability and reduced insect activity. It is observed that apart from oxygen availability and insect activity the presence of moisture within the pods also governed production of aflatoxin, thus pods stored at 10 per cent moisture recorded less aflatoxin compared to pods stored at 14 per cent moisture however a gradual increase in aflatoxin content was observed with increase in storage period in all the bags.

The impact of insect activity and aflatoxin accumulation in storage and their effect on important biochemical constituents of stored groundnut pods was investigated. The results revealed that among fatty acids significant minimum decrease in linoleic acid and oleic acid and increase in palmitic and stearic acid was recorded in pods stored in triple layer plastic bags compared to traditional bags. Similarly a significant reduction in total oil and protein contents was recorded in pods stored in traditional bags

compared to triple layer plastic bags. It is observed that the undesirable changes in biochemical constituents were more in traditional bags compared to triple layer plastic bags and also in the produce stored at higher moisture content (14%) compared to produce sufficiently dried (10%) and stored. It was also found that the undesirable changes increased with increase in duration of storage period.

It is concluded from the present investigation that the triple layer plastic bags using hermetic technology efficiently managed insect pests and mycotoxin producing storage fungi compared to traditional storage bags. The study also revealed that triple layer plastic bags protected the biochemical constituents and germination of the stored seed and could be best alternative for traditional storage bags for short and medium term storage, provided the produce is sufficiently dried (<10%) before storage.

Chapter I

INTRODUCTION

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INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important food legume and an oil seed crop belongs to the family Leguminosae (Beghnin and Sewadah, 2003). It is also known as peanut, earthnut, goober, pindar, manila nut etc. It is cultivated in many of the tropical, subtropical and temperate countries of the world (Halima 2000). Groundnut is ranked as 13th most important food crop and 4th most important oilseed crop covering an area of 25.4 M.ha, globally with production of 45.2 Mt and productivity of 1.77 tonnes ha^{-1}(FAOSTAT, 2013).In India it is cultivated in an area of 5.53 M.ha with annual production of 9.67 MT and productivity of 1750 kg ha^{-1}which makes India second largest producer after China (FAO, 2013-14). In Telangana state, groundnut is grown in an area of 0.21 M.ha with annual production of 0.355 MT and productivity of 1320 kg ha^{-1} (Statistical year book of Telangana 2015). Groundnut is semi perishable and has 44-56% oil and 22-30% protein on a dry seed basis and is a rich source of minerals (P, Ca, Mg, K) and vitamins (E, K, B group) (Savage and Keenan, 1994).

Groundnut is usually stored as pods (unshelled form) and in kernels (shelled form)for different uses. Generally the harvested produce is stored by farmers, processors, seed agencies and other oil extraction units for about 6-9 months before final use (Azeemoddin 1993). In India, storage losses of groundnut range between 10% and 15% (Ranga Rao *et al*., 2010). Its quality and quantity is reduced during storage and post-harvest due to several insect pests such as groundnut bruchid, *Caryedon serratus* (Olivier); pod sucking bug, *Elasmolomus sordidus* (F.) and red flour beetle, *Tribolium castaneum* (Herbst) etc. Apart from insect pests different mycoflora belonging to storage fungi *viz., Aspergillus flavus* and *Aspergillus parasiticus* also reduces the quality by producing secondary metabolites known as aflatoxins. These aflatoxins can even pose serious health hazards in humans and animals upon consuming the contaminated food and feed.

Among the insect pests, groundnut bruchid *C. serratus* (Olivier) is an economically important storage pest and cause severe damage to groundnut pods when stored in improper storage conditions. Though most of the storage insect pests attack kernels, *C. serratus* is the only major pest of groundnut that infests unshelled pods as well as kernels. In India, *C. serratus* was first reported to be infesting groundnut round the year in Andhra Pradesh and Tamilnadu in 1914 (Fletcher, 1914).

It is estimated that post-harvest losses in groundnut range between 10 to 25% of the total production, of which 83% of damage was by bruchids alone, when stored for a period of 8-13 months under unprotected conditions (Dick 1987). Insect infestation not only causes direct loss to the produce, but also creates entry points to the fungal colonization especially storage fungi belonging to *Aspergillus* group.

It is because of these post-harvest losses farmers sell their produce immediately after harvest and fetch marginal profits in spite of scope for achieving higher market price for the produce if stored for a little longer time. However, the storage of the produce has to be done following safe post-harvest management practices including use of proper storage structures, maintaining moisture content of 8-12%, temperature of 25-30^{o}C and relative humidity of 65% (Pattee and Young, 1982) which play a major role in storing any produce for longer duration without any damage. Above all the safe post-harvest management practice at farmer level involves use of no or low chemical insecticides on the stored produce.

The traditional storage structures available to farmers are made of mud *i.e., kanaja, sanduka and gummi*, which require frequent plastering with mud and dung and highly prone to attack of non-insect pests like rodents. The other traditional structures like underground pits effectively control the insect pests as they create airtight environment but the produce is liable to theft by thieves and also need to have proper drainage facilities during rainy season. An improved traditional storage tool currently being used by farmers on a large scale includes jute bags as they are easy to handle but they are highly porous in nature and absorbs moisture and allows free exchange of gases from atmosphere leading to attack of pests. Further, plastic polythene bags do not absorb the moisture but they are sensitive to sunlight and deteriorate the produce. Metallic bins, as they are more resistant to insect attack by creating closed environment but require more space to storage.

Considering the limitations associated with traditional and improved storage structures a more recent technique was developed known as controlled atmosphere storage technique which works on the principle of hermetic storage technology targeting the respiratory biology of a living organism.

Every living organism requires oxygen to survive by inhalation of oxygen with exhalation of carbon dioxide to continue its metabolic activities. In pest management perspective, the amount of oxygen required to complete the life cycle of an insect is essential to estimate what percent depletion of oxygen prompts the insect to die due to hypoxia (reduced levels of oxygen) and hypercarbia (increased levels of carbon

dioxide). In this way it is useful to devise management practices that avoid the usage of insecticides as they leave hazardous residues on stored products.

The controlled atmosphere storage technology was found to give good control of storage insect pests without usage of chemical insecticides but, the very draw back about the technology was creation of a modified environment by changing the gas compositions artificially using vacuum cylinders in the storage structures and thus making the technology non-practical at farmer's level.

An improvement of the above technology following the hermetic storage principles is the use of triple layer plastic bags developed recently by Purdue University, USA under the Bean/Cowpea Collaborative Research Support Program (CRSP). These triple layer plastic bags provided an improved alternative for insecticide-free, long-term storage of common beans with minimal grain damage (Murdock *et al*., 2003).

The safe storage of a particular produce for considerable period depend up on the fact the probable damage the produce is prone due to biotic or abiotic factors during storage. Fundamentally it is to be taken into consideration that for any grain ecosystem, the most important abiotic conditions influencing biotic activity *viz.,* insect attack, mould growth and mycotoxin production are water activity, temperature and gas composition (Magan *et al.*, 2004).

Hence, a comprehensive study was planned to evaluate the triple layer bag against the groundnut bruchid *C. serratus* with the following objectives to understand the respiratory biology of *C. serratus* which forms a basis to know the dynamics of hermetic management associated with the low cost triple layer plastic bags made up of high density polyethylene. The study was also aimed to determine the improved storage technology following hermetic storage principles on insect colony development, mould formation and aflatoxin build up which often influence the nutritional composition of the groundnut kernels.

Objectives of investigation:

1. To determine the Oxygen requirement of *Caryedon serratus* at different stages of its life cycle.
2. To study the performance of triple layer bags as a source of hermetic storage technology for management of *Caryedon serratus* (Olivier).

3. To study the aflatoxins build up in different storage bags at 10% and 14% moisture regimes.
4. To study the effect of *Caryedon serratus* and *Aspergillus flavus* infestation on total oil and fatty acid composition of groundnut kernels under different storage conditions.

Chapter II

REVIEW OF LITERATURE

Chapter II

REVIEW OF LITERATURE

Groundnuts stored after harvest are prone to severe infestation by different insect pests. It is documented that more than 100 insect species infests groundnuts in storage (Ranga Rao *et al.,* 2010) of which the groundnut bruchid, *Caryedon serratus* (Olivier), is considered the major pest of unshelled groundnut pods. It penetrates intact pods, infests the kernels and provides entry for microorganisms leading to development of aflatoxins. Several researches opined that the degree of pest infestation depends on some of the key factors which include post-harvest management practices, types of storage conditions and have proposed various biorational methods of pest management. Among various biorational approaches designed for storage pest management, the latest approach is adoption of hermetic technology of pest management by use of triple layer plastic bags. The available literature pertaining to the required moisture content of groundnut pods for safe storage, the biological oxygen requirement of *C. serratus*, use of triple layer plastic bags following hermetic storage technology for management of *C. serratus* and its impact on biochemical constituents is here with reviewed in this chapter under the following headings.

2.1 Nature and extent of damage by *C. serratus*

Okeke (1986) reported that groundnut bruchid damaged pods up to 83 per cent under ambient conditions when groundnut stored unprotected for about 8-13 months.

Dick (1987) recorded 20 per cent damage of unshelled groundnuts by *C. serratus* when stored for a period of five months in an oil mill warehouse in Andhra Pradesh.

Singal and Toky (1990) revealed that *C. serratus* infestation on groundnut pods caused loss in weight, increased heat and moisture development within heaps or stacks due to insect activity and thereby promoted mould growth which adversely affected the germination potential of seed and lowered the quality of oil by increased levels of free fatty acids.

Sontakke *et al*. (1992) reported the infestation of groundnut pods for the first time in godowns of Western Orissa by the bruchid *C. serratus* during 1989 and revealed

that the larvae attacked the kernels by penetrating the shells and fed on the cotyledons by making a large excavation.

Kapadia (1994) recorded 45 per cent damage of groundnut seeds equivalent to 65.00 per cent loss in weight due to groundnut seed beetle *C. serratus*.

Kumari *et al*. (2002) reported 77.10 per cent damage to groundnut pods, 67.80 per cent damage to kernels and per cent weight loss reported by them was 55.10 and 52.30 per cent in kernels and pods respectively. It was also recorded a 3.50% reduction in oil content and 3.10% increase in free fatty acid content in the variety TMV-2 due to infestation by *C. serratus.*

Radadia (2003) revealed that minimum infestation of 1.33% was recorded in the month of August and maximum during April (43.71%) on the basis of larval holes examined on groundnut pods of variety GG-2.

Shukla and Rathore (2007) reported that *C. serratus* cause 17-47% of the pod damage in godowns of Rajasthan.

Nesci *et al*. (2011) reported that the pods and kernels damaged by bruchids were further prone to aflatoxin contamination making it unfit for human consumption.

Harish *et al*. (2012) reported that eight pairs of adult bruchids per 100 g pods can cause 70-80 % damage in stored groundnuts.

Oaya *et al*. (2012) reported that *C. serratus* caused 90% pod damage and 60% weight loss of stored groundnut pods in a span of six months.

2.2 Oxygen requirement for development of storage insects

Krishnamurthy *et al.* (1986) observed complete mortality of insects within seven days due to toxicity of CO_2 when adults of *Sitophilus granarius, Tribolium castaneum, Oryzaephilus surinamensis, Cryptolestes ferrugineus* and *Rhyzopertha dominica* were exposed to simulated atmospheres containing low oxygen (0.5-2.6%) and increased carbon dioxide (10-30%) with a balance of nitrogen at 20°C and 70% relative humidity.

Ramesh Babu *et al*. (1991) found that maximum mortality of adults (99%) and eggs (85%) of *C. ferrugineus* was obtained at high CO_2 (88-91.70%) and low O_2 (0-0.50%), when exposed for a period of 96 hours.

White and Jayas (1993) exposed adults of *T. castaneum* and *C. ferrugineus* to 34 per cent CO_2 and 15 per cent O_2 at a temperature decreasing from 18 to 10^0 C and 29 per cent CO_2 and 3 per cent O_2 at decreasing temperatures of 25 to 20^0 C and observed 100 per cent mortality of target pests at 34 per cent CO_2 in two weeks.

El- Lakwah *et al*. (1994) used 23, 46 and 68 per cent CO_2 against *S. granarius* and *Callosobruchus chinensis* at 26 ± 1^oC and 60 ± 5% RH and observed *C. chinensis* to be more susceptible to changes in carbon dioxide concentrations compared to *S. granarius.*

White *et al*. (1995) reported that exposure of insects to low levels of CO_2 (7.5-19.2%) for prolonged periods sharply increase immature and adult mortality.

Mbata *et al*. (1996) observed 100 per cent mortality of eggs and adults of both *C. chinensis* and *C. subinnotatus* when exposed to an inert atmosphere of 100 per cent carbon dioxide at a temperature of 32^o C and a humidity of 70 per cent.

Mannad *et al*. (1999) demonstrated that CO_2 generated from dry ice and circulated with a vacuum pump at a concentration of 51 per cent at 20°C caused 100 per cent mortality of *C. ferrugenius* after 10 days.

White and Jayas (2003) reported that living organisms in storage (insects, fungi, and grain) consumed oxygen during respiration, reducing it from near 21 per cent in air to 1 to 2 per cent and released carbon dioxide raising it from an ambient 0.035 per cent to near 20 per cent.

Emekci *et al*. (2004) reported that low levels of oxygen (5%) caused metabolic stress in insects due to increased respiration which resulted in death of insects.

Conyers and Bell (2007) worked out minimum oxygen requirement of five coleopteran storage insects *C. ferrugineus, O. surinamensis, S. granarius, S. oryzae and T. castaneum* and revealed that the modified atmosphere containing oxygen less than 5% and carbon dioxide more than 10-20% at a temperature of 20 - 25^oC and relative humidity of 75 - 85 per cent caused poor emergence of adults and sometimes led to adult mortality.

Chiappini *et al*. (2009) achieved 100 per cent mortality of *T. confusum* within a week when the insect was subjected to controlled atmosphere containing low oxygen content of 5-8 per cent at moderate conditions of temperature ranging 29-37^0C.

Chenga *et al.* (2012) observed impact of hypoxia and hypercarbia conditions on cowpea bruchids, when exposed to two different combinations of O_2/CO_2 concentrations *viz.*, 10% O_2 + 10% CO_2, and 2% O_2 + 18% CO_2 and recorded egg to adult mortality at 2% O_2+ 18% CO_2 concentration.

Murdock *et al*. (2012) estimated that 8.9 ml of oxygen required for completion of life cycle by an individual cowpea bruchid, *C. maculatus* and further demonstrated cessation of feeding activity by *C. maculatus* due to drop in oxygen concentration by about 2-3 per cent.

Bell *et al.* (2014) reported that death of insect species and their immature stages occurred at temperature above 25^0 C either with less oxygen (1 per cent) or with increased carbon dioxide (60-80 per cent) concentration.

2.3 Use of triple layer plastic bags following hermetic storage technology for the management of storage pests

Triple layer bags were developed by Professor Larry Murdock in 1987 at Purdue University, in association with Cowpea CRSP (Cowpea Collaborative Research Support Program) and USAID team of researchers to combat bruchid infestation on cowpea in Cameroon.

De Lima (1990) reported that hermetic storage technology act as non-chemical means of grain protection against insect pest and storage fungi.

Weyel and Wegener (1996) revealed that triple layer plastic bags caused mortality of insects by creating hypoxia and hypercarbia conditions in the storage bags, thus leading to accumulation of metabolic toxins which are harmful to the insects. Similar findings was also put forth by Mbata and Reichmuth, (1996) who observed death of egg, larvae and pupae of storage insects in triple layer plastic bags due to reduction in level of oxygen and production of high levels of carbon dioxide.

Adler *et al*. (2000) suggested triple layer plastic bags following hermetic storage technology as an important tool in integrated management of storage pests since it uses no chemicals and does not leave pesticide residues.

Bulaong and Dharmaputra (2002) revealed that the triple layer bags effectively checked the production of mycotoxin in the stored produce by preventing the mould

growth in the bags when compared to traditional storage structures like gunny bags, metal drums etc.

White and Jayas (2003) termed the use of triple layer bags for storage pest management following hermetic principle as a "green", chemical-free technology and suggested it as effective method of managing storage insects and fungi.

Quezada *et al*. (2006) reported that hermetic technology works by creating an airtight seal in which oxygen levels dramatically decrease within days through insect, fungal or seed respiration.

Moussa *et al.* (2009) reported that triple layer bag plastic bags as an economically, simple and effective technology for reducing the storage losses.

Baribusta *et al.* (2010) suggested use of triple layer plastic bags for long term storage of maize grains for the control of *Prostephanus truncates* due to their simplicity, durability, low cost with proper thickness and its manufacture using high density polythene consisting three layers of which inner two layers acting as oxygen barriers and outer layer is a normal polypropylene woven sack providing strength to the unit.

Jones *et al.* (2011) proposed that the triple layer bag can be reused for the purpose of storage.

Anankware *et al.* (2012) proposed that the triple layer plastic bag technology works on principle of creation of modified atmospheres that hinder survival of life forms either by vacuum hermetic fumigation, gas hermetic fumigation or bio-generated modified atmosphere.

Njoroge *et al.* (2014) explained that impermeability to gases by triple layer bags caused drastic fall down of oxygen content and increase of carbon dioxide due to respiration by grains, insect and fungus thus resulted in inactiveness, cessation of feeding and finally death of insects by asphyxiation.

2.3.1 Effect of triple layer bags on storage pests and impact on seed germination

Yakubu *et al*. (2010) recorded higher mortality of maize weevil, *S. zeamais*, infesting stored maize in triple layer bags at low temperature and low seed moisture level as it created hypoxia (depleted levels of oxygen) conditions sooner than the grains stored at high temperatures and moistures.

Hell *et al*. (2010) observed 100 per cent mortality of maize storage pests *P. truncates S. zeamais, Cathartus quadricollis* and *Tribolium* sp when slightly infested maize seed from field was stored in triple layered bags for a period of 3-6 months.

Omondi *et al.* (2011) studied the impact of triple layer bag on seed germination and revealed that the seeds stored in triple layer plastic bags maintained the germination percentage of 85 per cent up to 9 months when compared to traditional storage gunny bag where the germination reduced to 14-76 per cent within 3 months.

Sanon *et al*. (2011) revealed that cow pea stored in triple layer plastic bags consisting of two inner layers made of high density polythene with a thickness of 100 µm effectively managed *C. maculatus* for about seven months.

Baoua *et al*. (2012) studied the performance of once used triple layer bag to test its reusability with that of a freshly woven bag and observed that once used triple layer plastic bag recorded 99 per cent mortality of adults and total death of larvae when compared to freshly woven bags.

Anankware *et al.* (2013) compared the effectiveness of triple layer bags with that of jute and polythene bags against maize weevil, *S.zeamais* and observed increased level of seed damage, weight loss in jute bags followed by polythene bags and highest percent mortality of *S. zeamais*, seed germination was recorded in triple layer bags.

Anankware and Bonu-Ire (2013) revealed that the effectiveness of triple layer bags in controlling major storage pests (*S. zeamais* and *P. truncatus)* infesting maize in storage is due to the reduction of oxygen levels from 21 to 5 per cent within 22 days that hinders insects respiratory metabolism.

Sudini *et al*. (2015) reported that bruchid damage pod was less in triple layer bags (21.3%) compared to cloth bags (92.7%) under artificial inoculation with groundnut bruchids and *A. flavus*.

Affognon *et al*. (2014) recorded no significant change in weight of common beans (*Phaseolus vulgaris* L.) stored for a period of six months in Purdue Improved Crop Storage (PICS) bags artificially infested with common bean bruchid, *Acanthoscelides obtectus.* However, they recorded a reduction in weight of untreated beans stored in normal bags up to 33.6 per cent and beans treated with grain protectant, Actellic Super® up to 19.10 per cent.

Baoua *et al.* (2014) reported higher weight loss poor mortality of storage insects *P. truncatus, S. zeamais* and reduction in germination percentage of maize stored for a period of 6.5 months in woven polypropylene bags but recorded 95-100 per cent mortality of storages pests with no significant loss weight and a germination percentage of 90.5 when stored in triple layer plastic bags.

Baoua *et al*. (2014) compared the mean insect population, per cent germination after storing Bambara groundnut (*Vigna subterranean*) slightly infested with *C. maculatus* and *C. subinotatus* for a period of seven months in triple layer bags and woven polypropylene bags and observed mean population of bruchids to be 309 and 251 per 500g of pods in polypropylene bags and triple layer bags respectively while the per cent germination was found to be 34.8 per cent in polypropylene bag and 89.3 per cent in triple layer bags.

Cugala *et al*. (2014) evaluated the performance of triple layer bag and polypropylene bag in managing *P. truncates*, a serious storage pest of maize, for a period of six months by storing the untreated and Actellic treated (grain protectant) maize seed and found triple layer plastic bags alone and in combination with grain protectant recorded no insect development and no loss in weight. It was also observed that the maize seed treated with grain protectant and stored in polypropylene bags recorded 107.3 per cent increase in insect number and 4.8 per cent loss in weight while untreated seed stored in polypropylene bags recorded 701.8 per cent increase in insect population and 46.3 per cent weight loss.

Murdock *et al*. (2014) evaluated the performance of triple layer plastic bags against *C. maculatus* infesting mung bean and pigeonpea and observed no significant weight loss of produce compared to seed stored in woven polypropylene bags which recorded 26.2 per cent loss in weight and seed treated with Actellic (grain protectant) and stored in woven polypropylene bags where the 13 per cent loss of weight was observed.

Sarr *et al*. (2014) compared the effectiveness of different storage structures *viz.,* triple layer plastic bags, metal drums and seed treated with insecticide in managing groundnut bruchid, *C. serratus* and *T. castaneum* in storage and found higher infestations in metal drums (71 insects/100grains) and insecticide treated seeds (36 insects per 100grains) when compared to triple layer plastic bags (0 insect per

100grains). The per cent seed germination was recorded similar in metal drums (74.7%) and triple layer plastic bags (75.7%).

Martin *et al*. (2015) observed 50 per cent damage of wheat grain due to *S. oryzae* infestation when stored in woven polypropylene bags for a period of six months and no damage or weight loss was observed when stored in triple layer plastic bags.

2.4 Impact of biotic and abiotic factors on fungal development in stored produce

Dickens and Pattee (1966) detected highest level of aflatoxin at 85 per cent RH and 32°C in groundnut samples and revealed that moisture, relative humidity and temperature play an important role in build up of *Aspergillus* in stored products.

Sanders *et al*. (1968) reported slow multiplication of fungi and thereby reduced level of aflatoxin production in peanuts inoculated with *A. flavus* and maintained in an atmosphere consisting of 60 per cent CO_2, 20 per cent O_2, and 20 per cent N_2, when compared with aflatoxin of peanuts stored in normal air at 25^{o} C and 90% relative humidity.

Wallace (1973) proposed that moisture play an important role in build up of moulds in stored produce and hence the cereals are to be dried to less than 13.5 per cent moisture level and oil seeds to be dried to less than 7.8 per cent moisture level to protect from mould growth.

Christensen *et al*. (1977) reported that moisture level higher than 7 per cent in the presence of temperatures ranging from 21^{o} C - 37^{o} C and relative humidity ranging from 83-85 per cent facilitated fungal growth in storage.

Proper drying of grains after harvest to less than or equal to 7 per cent moisture levels is ideal to prevent growth of fungi, including aflatoxigenic strains was suggested by Heathcote and Hibbert, (1978).

Prasad *et al.* (1998) reported that incidence of fungal infection and severity of damage by fungi in storage depend on storage temperature, seed moisture content, relative humidity and type of fungal species or isolate.

Navarro *et al.* (1989) suggested 7.5 per cent of kernel moisture content, 10°C of temperature and 65 per cent of relative humidity to be optimal for bulk storage for

groundnut and further revealed that increase in temperature from 10°C to 20°C and relative humidity from 65 per cent to 85 per cent resulted in loss of germination to 20 per cent in a span of 80 days.

Lynch and Wilson (1991) studied the interaction between storage insects and fungi and proposed that insects transmitted *A. flavus* in storage which led to multiplication of fungi in storage.

Kennedy and Devereau (1994) observed increased build up of *A. flavus* in jute bags and suggested high porous nature and ability to absorb moisture from environment by jute bags to be the key factors for increased fungal build up.

McDonald (2004) described seed deterioration as an undesirable and detrimental attribute of stored products and proposed that losses in seed quality occur during field weathering, harvesting and storage.

Chapin *et al.* (2004) opined that pod damage due to insect or by any other means leads to invasion of saprophytic fungi and result in aflatoxin production.

Aliyu and Kutama (2007) revealed that inappropriate processing and storage conditions lead to attack of several fungi (*Rhizopus* sp, *Penicillium* sp, *A. niger* and *A. flavus*) and insects (*C. macualtus, C. serratus, Tribolium* sp etc.) on groundnut because of its complex food matrix with rich amount of fat, protein and fibre contents.

Magan and Aldred (2007) demonstrated that the growth of *A. flavus* is comparatively low at less than 14% moisture levels.

Oh *et al*. (2008) revealed that temperature ranging from 22.6° C to 27.0° C and relative humidity ranging from 23.3-44.2 per cent resulted in increased growth of *A. candidus, A. flavus, A. fumigatus* and *Penicillium* sp in storage.

Nesci *et al*. (2011) revealed that storage insects act as vectors for entry of fungi, *A. flavus* in peanuts thus result in development of aflatoxins and recorded highest levels of aflatoxins (68.86 and 69.12 μg/kg) in groundnut pods stored for a period of six months in ware houses.

Bhushan *et al*. (2013) observed rapid development of *A. flavus* from the freshly harvested sorghum grains containing 14, 16 and 18 per cent moisture content stored in polythene bags when compared to seed containing 10 and 12 per cent of moisture.

Rani *et al.* (2013) studied the optimum seed moisture content and temperature for safe storage of pinto beans and reported that pinto beans can be safely stored at 12 and 14 per cent moisture content, at a temperature of 10 and 20^0C without effecting appreciable seed germination, seed coat colour, and microbial infection for about 16 weeks.

Mutegi *et al.* (2013) observed 7.3 per cent and 13.4 per cent higher levels of aflatoxin contamination in peanut stored for a period of six months in polypropylene and polyethylene bags respectively when compared to jute bags.

Williams *et al*. (2014) observed no accumulation of aflatoxins in maize seed containing 12 and 15 per cent moisture stored in triple layer plastic bags but found little accumulation after one to two months only in seed containing 18 and 21 per cent of moisture. In contrast the accumulation was found in all the moisture regimes when stored in poly propylene bags.

Waliyar *et al.* (2015) suggested different factors *viz.,* harvesting, drying, and storage methods as well as final moisture content before storing the products, insect damage, and physical damage cause development of aflatoxin content in groundnut kernels.

2.5 Impact of storage conditions on development of mycotoxins in stored produce

Saur *et al*. (1984) reported that pre-harvest insect infection led to increased production of aflatoxin in maize during storage due to metabolic heat produced by insect pests which was favourable for multiplication of fungi.

Ellis *et al*. (1994) studied the combined effect of water activity, storage temperature and gaseous composition in a storage container on *A. flavus* and aflatoxin production and reported maximum growth of *A. flavus* and aflatoxin production in groundnut occurred when water activity (a_w), storage temperature and headspace oxygen was 0.97, 25°C and 10% respectively. They further revealed that the aflatoxin build up occurred in storage with increased levels of carbon dioxide but in presence of oxygen only.

Bankole *et al*. (1996) reported that 196 isolates of *A. flavus* infested pigeon pea when stored in jute sacks and iron bins for a period of five months and observed 48 per

cent of the isolates produced mycotoxins of which the more number of isolates recorded from jute sacks produced mycotoxins compared to the isolates recorded from iron bins.

Hell *et al*. (2000) described the importance of moisture on aflatoxin production in storage and revealed increased moisture content in storage environment will result in increased levels of aflatoxin production.

Proctor *et al*. (2004) reported that produce containing higher quantities of nitrogen and simple sugars produced more aflatoxins due to fungal infection in storage at optimal temperature of 28^{o} C and pH of 4.5.

Rahmianna and Yusnawan (2007) reported that increased storage period resulted in increased level of mycotoxins in stored produce due to more physical damage by insects.

Nakai *et al*. (2008) revealed that temperature of 32-33^{o}C, water activity of 0.83 to 0.97 and moisture content greater than 14 per cent resulted in production of aflatoxin by *A. flavus* in storage.

Jubeen *et al*. (2012) observed higher production of aflatoxin (158.67 µg kg^{-1}) in peanut samples maintained at 16±3% moisture level compared to the samples maintained at 10±3% moisture (46.77 µg kg^{-1}).

3.0 Influence of biotic and abiotic factors on biochemical changes in stored produce

Ramamurthy and Karivaratharaju (1989) noticed decrease in germination percentage, oil and protein content and an increase in free fatty acid content in stored groundnut kernels.

Pomeranz (1992) reported reduction in the fat quality of peanut infested by *A. flavus* in storage and proposed that *A. flavus* released hydrolytic enzymes converting fats into free fatty acids and glycerol.

Shin *et al.* (1997) observed decrease in polyunsaturated fatty acids and increase in saturated fatty acids content in nuts stored at high moisture level.

Bulaong and Dharmaputra (2002) recorded significant increase in free fatty acid content of groundnut stored for a period of six months in jute bags and polythene bag but observed significant low free fatty acid content in groundnut stored in jute bag

doubled with thin polyethylene and jute bag doubled with thick polythene and opined that increase in *A. flavus* build up as the probable reason for increased free fatty acid content.

Bhattacharya and Raha (2002) observed decrease in carbohydrates, protein and oil content of maize, groundnut and soybean from 74.7-57%, 17-12.7% and 21-16.8%; 13-11.2%, 26-21.8% and 40-38.4%; 46-43% and 18- 17.1% respectively and increase in free fatty acid content from 1.2-2.8 per cent in groundnut and 0.90-1.92 per cent in soybean when stored for 12 months.

Chang *et al*. (2004) reported rapid deterioration of soybean occurred by auto oxidation of lipids due to storage of seed with high moisture content in presence of oxygen.

Embaby *et al.* (2006) observed reduction in carbohydrate, reducing sugar and crude fat content in legume seeds during storage due to *Fusarium oxysporum* infection.

Jain (2008) reported a rapid increase in concentration of free fatty acids in damaged groundnut kernels during storage due to fungal invasion.

Gopinath *et al.* (2011) observed biochemical changes in different varieties of red gram and green gram stored for six months and revealed that lipolytic activity of grains caused decrease in total lipids and triglycerides and increase in phospholipids, free fatty acids and peroxides. He further recorded reduction in palmitic, stearic, linoleic, linolenic acids of the total fatty acid composition but increase in oleic acid due to bio-deterioration of lipids.

Pawar (2012) suggested that the storage fungi, *A. flavus* is responsible for various biochemical changes *i.e.,* increase in amino acid content, decrease in total reducing and non-reducing sugars, vitamins and ash content of fruits.

Witulska *et al.* (2012) studied the effect of temperature and moisture content on biochemical changes of rapeseed during storage and recorded reduction of stigmasterol and brassicasterol by 17 and 28 per cent respectively when stored at 12.5 per cent moisture content and 30^{o} C temperature but higher reduction up to 73 and 63 per cent respectively was observed when stored at 15.5 per cent moisture content.

Chapter III

MATERIAL AND METHODS

Chapter III

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3.1 Location of work

The present investigation of "Evaluation of improved grain storage practices for the management of groundnut bruchid, *Caryedon serratus* Olivier. (Coleoptera: Bruchidae)" was carried out at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad and India.

3.2 Groundnut Pods

Approximately 800 Kg of freshly harvested groundnut pods with initial pod moisture in the range of 6-8 % of ICGV 02266 were obtained from Groundnut Breeding Unit of ICRISAT, Patancheru, and Hyderabad.

It is a Spanish bunch variety with maturity duration of 115-120 days. The groundnut pods were tampered with sprinkle water and kept for 24-48 hours to get desire levels of 10 % and 14 % moisture (Plate 1). The pods after achieving desired moisture contents were used for the experimental purpose by storing them in different types of storage bags.

3.2.1 Storage Bags

Four different types of storage bags *viz.*, (i) Jute bags (ii) Polythene bags (iii) Triple layer plastic bags and (iv) Jute bags treated with Spinosad were used for evaluating their efficacy in managing groundnut bruchid. Brand new jute bags and polythene bags were purchased from local market. The untreated jute bags and polythene bags were used as such for storing the groundnut pods while the jute bags treated with chemical were turned inside out before spraying with insecticide (Spinosad, Tracer 45% SC) @ 1 ppm on the inner side. Later the jute bags were shade dried and used for the experiment.

The triple layer plastic bags were manufactured locally by order at Sri Mahalakshmi Woven Sacks Pvt. Ltd., Hyderabad as per the technical specifications of the Purdue Improved Crop Storage (PICS) bags developed by Purdue University, USA. Triple layer plastic bags consists of three layers; inner and middle layers were made up of 80 micron thickness high density polyethylene (HDPE) material and do not allow

diffusion of gases (Oxygen and Carbon dioxide) while the outermost layer is a normal woven sac made up of polypropylene and provides strength for handling.

Plate 1. Groundnut pods with moisture contents of 10% and14% used for conducting experiment

3.2.2 Groundnut Bruchids (*Caryedon serratus* Olivier.)

Initial culture of groundnut bruchid, *C. serratus* (Olivier), was collected from naturally infested pods stored in the godowns of groundnut breeding unit at ICRISAT, Patancheru. The bruchid population was then multiplied under laboratory conditions at a temperature of 25±2°C and 70 % relative humidity using the groundnut pods of variety ICGV02266. The bruchids population was maintained in the plastic jars (15 cm X 10cm diameter) fitted with fine mesh lids to provide good ventilation and aeration (Plate 2). Freshly emerged day old males and females were separated by sexing and were used for conducting experiment.

Plate 2. Mass culturing of *Caryedon serratus* Olivier on Groundnut pods under laboratory conditions

3.2.2.1 Sexing of Test Insect

The females are bigger in size than male insect. The sexing of the adult beetles was done by observing the last visible segments of the abdomen. In male pygidium or sixth visible tergite projects downwards and was hidden by the elytra. The fifth visible sternite was deeply incurved and seventh tergite projects between the fifth sternite and the pygidium. In female the pygidium can be seen in dorsal view projecting beyond the elytra. The fifth sternite was fully extended and the ventral surface was more or less flat. The seventh tergite was not seen in the female (Davey, 1958) (Plate 3).

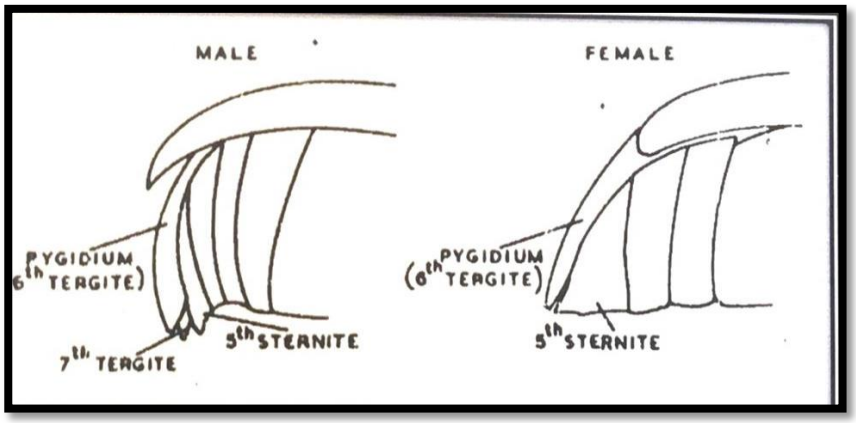


Plate 3. Sexing of male and female of *C. serratus* adult

3.2.3 Association of *Caryedon serratus* and *Aspergillus flavus* in storage

As is known that there exists an association between groundnut bruchid infestation and *A. flavus* infection in groundnut kernels and the physical damage caused by the insect paves way for fungal infection, a potentially high aflatoxin producing *A flavus* strain (*Af* 11-4) culture was obtained from Groundnut Pathology laboratory at ICRISAT and was inoculated in the form of spore suspension in the storage bags along with bruchids.

3.2.3.1 Preparation of *Aspergillus flavus* spore suspension

Spore suspension of *A. flavus* was prepared by sub culturing of initial inoculum from Petri plates having abundant growth of *A. flavus* cultured on PDA media under laboratory conditions at ambient temperatures of 28±2^{o}C. After a week of inoculation on fresh Petri plates containing PDA media, the plates with full grown *A. flavus* were selected and spore suspension was prepared by using sterile distilled water. The required spore concentration of 5 x10^{5} CFU/ml was obtained by following serial dilution technique using Haemocytometer. This preparation was done under laboratory conditions using laminar air flow chamber.

3.3 Experimental Setup

Ten kilograms of groundnut pods with moisture per cent of 10 and 14 were weighed separately and placed in each of four bags *viz*.,1) Jute bags (2) Polythene bags (3) Triple layer plastic bags and (4) Jute bags treated with Spinosad. Each of these bags was infested with 30 pairs of adult bruchids and spore suspension of *A. flavus* toxigenic strain (AF 11-4) @ 15 ml/bag. The bags were then moved gently upside and down for uniform mixing of *A. flavus* spore suspension and adult bruchids before closing the bags. The storage bags (one layer at a time starting with the inner most in the case of triple layer bags) were then tied manually by twisting the loose end of the bag around and folding it over then tying it tightly at the base of the twist and around the folded loop using a strong thread. Each of the four bags used for the experiment were replicated thrice for given moisture percentage. Hence, a total of 24 such storage bags were formed as a batch. Three such batches were formed which were tested for bruchid development and fungal build up after an interval of 2, 4 and 6 months of storage.

Complete Randomized Design (CRD) was followed for setting up the experiment where in four different bag types and two different seed moisture regimes

(10 % and 14 %) were considered as two factors influencing insect growth and multiplication and fungal growth and aflatoxin build-up (Fig 3.1).

3. 3.1 Methodology and equipments

Initial data on test weight, per cent germination and composition of biochemical constituents *viz.*, oil and free fatty acids was recorded for the pods with 10 % and 14 % moisture content just a day before the setting up of the experiment. After the experiment was set up, all the bags were closed and the data on various parameters pertaining to insect damage, fungal build up, seed characteristics and changes in biochemical composition of pods stored in different bags at different moisture regimes was recorded at every two months interval, starting with first batch consisting of 24 bags after two months, second batch bags after four months and final batch after six months of storage duration.

Table 3.1. Initial observations on biochemical constituents, per cent germination, test weight of ICGV 02266 and pod moisture before experimentation

Particulars	Value
A. Oil and Fatty acids (%)	
Oil	53.73
Protein	29
Linoleic acid	37.28
Oleic acid	57
Palmitic acid	8.2
Stearic acid	2.83
B. Moisture content (%)	
M1	10
M2	14
C. Germination percentage	
Initial Value	92
D. Test weight (g)	
10%	76.9
14%	77.93

	10% moisture			14% moisture		
2 months						
	R1	**R2**	**R3**	**R1**	**R2**	**R3**
T1						
T2						
T3						
T4						
4 months						
	R1	**R2**	**R3**	**R1**	**R2**	**R3**
T1						
T2						
T3						
T4						
6 months						
	R1	**R2**	**R3**	**R1**	**R2**	**R3**
T1						
T2						
T3						
T4						

However, CO_2 and O_2, concentrations were recorded using a Mocon PAC Check® 183 Model 325 head space analyzer (Mocon, Minneapolis, MN, USA) in all the bags at weekly intervals from the day of setting up of the experiment till the set of bags pertaining to a batch were opened as per their storage duration. Observation on O_2 and CO_2 concentrations were measured at around 10:00 a.m. on selected days by inserting needle probe of head space analyser into a triple layer bag for which a minute circular window was cut on the surface of the outer woven sac of the bag. The needle probe is then pushed into and the middle and inner layers of the Triple layer bags. The hole created on the outer layer of the bag was sealed immediately using plastic adhesive tape; no sealing was necessary in the case of the gunny bags and polythene bags (Plate 4).

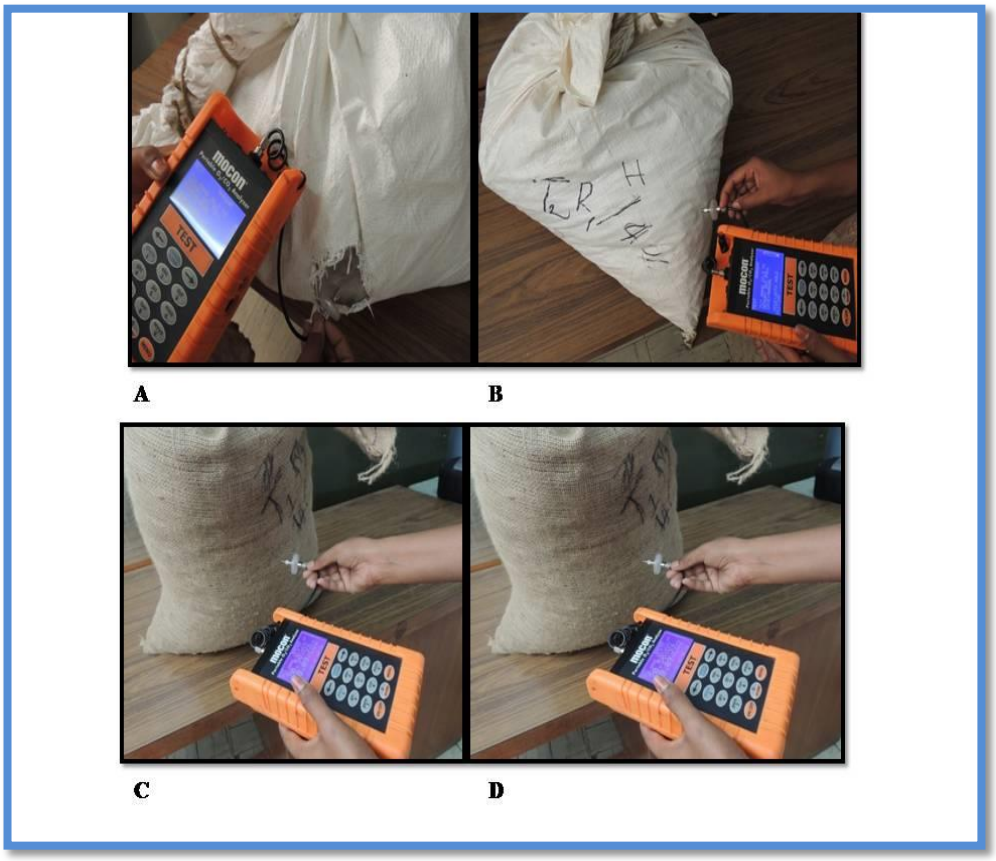


Plate 4. Estimation of gas composition in different storage bags by using hand held Mocon head space analyzer

3.3.2 Temperature, Relative Humidity

Temperature, humidity and dew points within the sealed bags were automatically recorded every hour from the beginning of the experiment till the end of the experiment, over a period of six months by placing the programmed data loggers (Lascar model EL-USB-2, Whiteparish, Wiltshire, Great Britain) in the bags (Plate 5).

One data logger was kept in the storage room to record ambient conditions. After completion of 2 months of storage duration the data loggers were removed after opening the set of bags belonging to first batch and the data on temperature and relative humidity data were downloaded using the software provided by the manufacturers. Similarly data on temperature, relative humidity and dew point were obtained from data loggers after take down of second batch of storage bags at four months duration and third batch after six months duration.

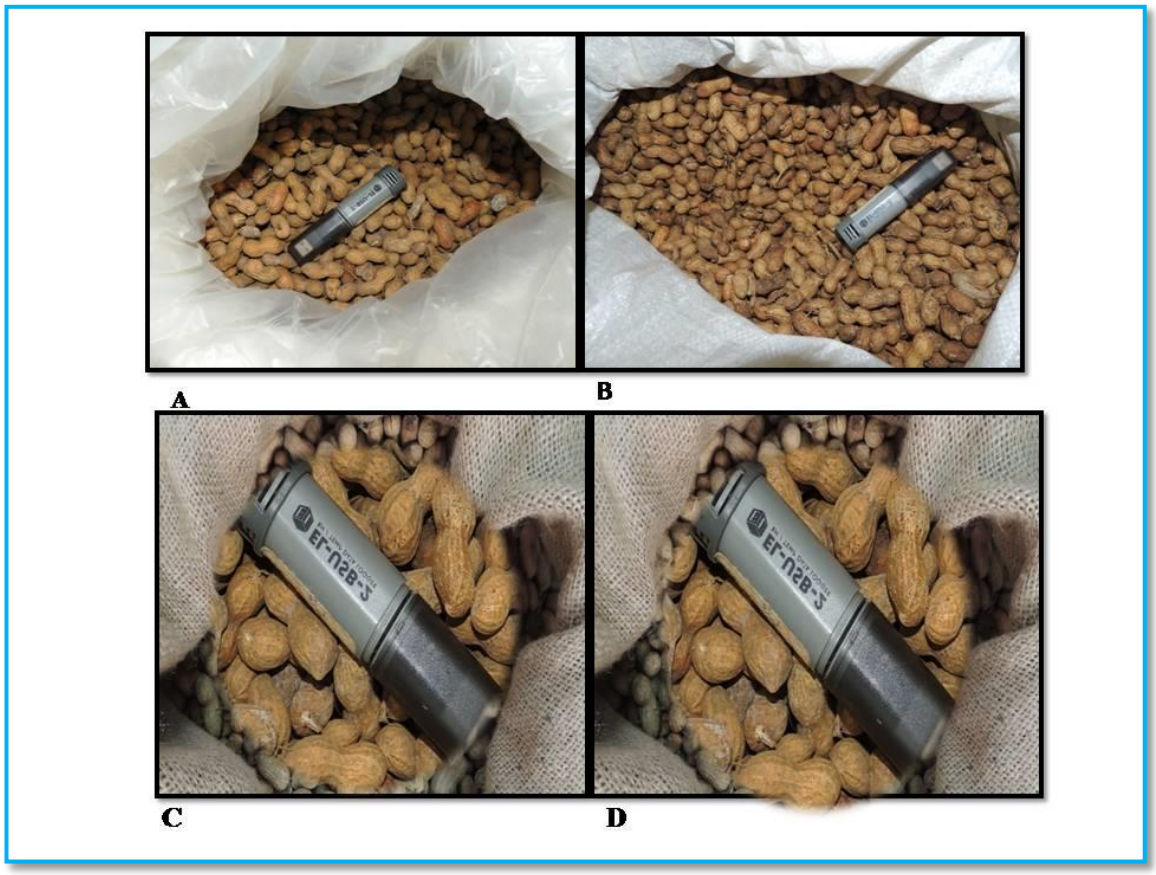


Plate 5. Recording of data on Relative humidity and Temperature in different storage bags by using Lascar model EL-USB-2, Data loggers

3.3.3 Data on seed characteristics

The data on seed characteristics *viz.,* test weight, moisture content and per cent germination was estimated initially before setting up the experiment and after every 2 months of setting up of the experiment up to a period of 6 months to determine the changes in seed characteristics due to insect and fungal attack.

The pods with initial moisture content of 10 % and 14 % stored in different storage bags were estimated for change in moisture content soon after the bags were opened after 2, 4 and 6 months of duration.

Moisture estimation was done by randomly selecting few groundnut pods and weighing them to know the initial weight. The selected pods were then placed in an aluminium dish of known weight. The pods were then dried in an oven at 105^{o}C for 17 hours, and the sample was later cooled and weighed. The moisture content was then calculated following the formula:

Per cent Moisture content $=W_1-W/ W_1-W_2$ X100

Where, W = Weight of blank aluminium dish with lid

W_1 = Weight of seed plus aluminium dish with lid before drying

W_2 = Weight of seed plus aluminium dish with lid after drying

The per cent seed germination of pods stored in different bags at different moisture regimes was calculated after 2, 4 and 6 months of storage period by randomly selecting 60-70 pods from each of the treatment bag. The selected pods were then shelled to obtain 100 seeds which were used in germination test following the procedure.

The 100 seeds so obtained were sterilized with 0.1% Mercuric chloride ($HgCl_2$) and rinsed with distilled water, placed evenly on germination paper, the paper was then rolled around the seeds, wetted with distilled water and placed in an incubator (Percival Scientific, Iowa, USA) at 25°C for 10 days. Moisture was maintained by misting with distilled water daily. After ten days the germination paper was opened to observe sprouting and development of epicotyl and hypocotyl from each seed. The seeds with good epicotyl and hypocotyl length were recorded as germinated while the rest were treated as ungerminated. Based on the observations recorded per cent germination is calculated by the formula

$$\text{Per cent seed germination} = \frac{\text{No. of seeds germinated} \times 100}{\text{Total No. of seeds}}$$

A separate set of 100 seeds were obtained from 60-70 pods selected from each of the treatment bag and was weighed to obtain 100-seed weight (test weight), which was recorded to compare the change in test weight of seeds in storage.

3.3.4 Data on insect damage

The storage bags after inculcation with known number of bruchid population and known quantity of *A. flavus* spore suspension were opened batch wise after 2, 4 and 6 months of storage and data on different parameters pertaining to insect damage and fungal build up was recorded. Three samples each of 1 Kg were drawn randomly from

upper, middle and bottom portions of each bag and counted for adult bruchid population and is finally represented as the average number of adult bruchids per Kg of the sample for respective bag. The data on number of eggs was recorded by counting the round white to pale yellow coloured eggs adhered on 100 randomly selected pods obtained from the pooled sample of three Kg drawn @ of 1 Kg from three different portions of a bag. Similarly the number of damaged pods (a pod was considered 'damaged' if one or more holes were observed) was also recorded by selecting 100 pods randomly from the pooled 3 Kg sample drawn from a three different portions of a bag.

The per cent damage, per cent mortality and per cent loss were calculated by using formulae which was given by Lale and Igwebuike (2002):

$$\text{Percentage (\%) loss} = \frac{a-b}{b} \times 100$$

a= initial weight of stored produce before starting experiment

b=Final weight of stored produce after terminating experiment

$$\text{Per cent pod damage} = \frac{\text{Number of bored pods}}{\text{Initial number of pods}} \times 100$$

3.4 Estimation of Aflatoxin build up

Aflatoxin levels were measured with four treatments which are replicated thrice before starting the storage experiment using recently harvested groundnut kernels, and at the end of each two months of storage up to six months with total of 24 samples in each time. Three replications per treatment combination (1) Jute bags as control (2) Polythene bags (3) Triple layer plastic bags (4) Jute bags treated with Spinosad. All bags have initial infestation with *A. flavus* spore suspension of 15ml/bag, 30 pairs of adult bruchids. A representative sample of approximately 100 g of groundnut kernels was collected from each treatment following an indirect competitive ELISA to quantify the aflatoxin levels (Reddy *et. al.*, 2001).

Procedure:

Coating

ELISA plates were coated with 150 µl of AFB_1 – BSA conjugate (1 µl of AFB_1 – BSA in 10 ml of 0.2 % carbonate buffer)

⇩

Incubated for overnight in refrigerator or incubator at 37^0 C for one hour

⇩

Washed the plate thrice with PBS – T20 (phosphate buffer saline – tween for 3 min)

Blocking

160 µl of 0.2 % BSA (Bovine serum albumin) added and incubated at 37^0 C one hour.

⇩

Washed the plate thrice with PBS – T 20

⇩

Dilution of antiserum in a ratio of 1:2000 in a test tube and incubated at 37^0 C.

Competition

AFB_1 standards ranging from 0.1 to 50 µg/ml were prepared in groundnut extracts (diluted to 10 %) not containing any aflatoxin. 20 g of healthy groundnut kernels free of aflatoxin were powdered and extracted in 100 ml of 70 % methanol containing 0.5 % KCl. The extract was filtered and diluted to 1:10 in PBST – BSA. This was used as a diluent for aflatoxin standards.

Simultaneously prepared pure toxin (AFB_1) by diluting with above prepared healthy groundnut (HGN) extract in a test tube

⇩

100 µl of AFB_1 (50 ng/ml) was added to first two columns of first two rows

⇩

100 µl of diluted HGN extract was added to remaining wells of first two rows

⇩

The remaining wells were loaded with 90 µl of BSA + 10 µl of sample extract to be analyzed

⇩

50 µl of incubated antiserum was loaded to each well of ELISA plate and kept in shaker for 10 min

⇩

The plate was then incubated for one hour at 37^0 C to facilitate reaction between toxin and antibody and later the plate was washed thrice in PBS – T 20

Conjugation

150 µl of substrate buffer {PNPP (P – Nitro Phenyl Phosphate) in 10 % diethylene amine} was added to each well

⇩

Simultaneously substrate was added to top left corner well as blank

⇩

Incubation was done at normal temperature in dark for colour development at 15 minutes interval

⇩

Absorbance was measured at 405 nm in ELISA reader

3.4.1 Required materials

ELISA Plate Reader (Bio-Rad)

Micropipettes: 1-40 µl, 40-200 µl and 200-1000 µl single channel pipettes, 40-200 µl multichannel pipettes (Finn pipette) were used.

ELISA plates: For high binding 'NUNC – Maxisorp^{TM}surface' plates were used (Plate 6A).

Others

AFB1 specific polyclonal antibodies

Mortar and pestle, muslin cloth, pH meter, incubator, refrigerator

Aflatoxin B1standard (Sigma A6636)

Aflatoxin B_1-BSA conjugates (Sigma A6655)

Bovine Serum Albumin (Sigma A6793)

200mg in 100 ml of PBS-Tween (0.2%)

3.4.2 Solutions of Carbonate buffer or coating buffer (pH 9.6)

Na_2CO_3	-- 1.59 g
$NaHCO_3$	--2.93 g
Distilled water	-- 1000 ml

Phosphate buffer saline (PBS), (pH 7.4)

Na_2HPO_4	-- 02.38 g
KH_2PO_4	-- 00.40 g
KCl	-- 00.40 g
NaCl	-- 16.00 g
Distilled water	-- 2000 ml

Phosphate buffered saline Tween (PBS-T)

PBS	--1000 ml
Tween-20	-- 0.5 ml

PBST-BSA

PBS-T	--100 ml
Bovine serum albumin	-- 0.2 g

3.4.3 Substrate buffer for alkaline phosphatase system

p-nitro phenyl phosphate (pNPP) chemical in tablet form (5,15 or 20 mg tablets are available) was stored at –20º C. Ten percent diethanolamine (v/v) was prepared in distilled water and stored, its pH was adjusted to 9.8 adding concentrated HCl prior to use. Para nitro phenyl phosphate (PNPP) (0.5 mg ml^{-1}) was prepared in 10% diethanolamine, pH 9.8 (for each 15 mg tablet 30 ml solution was required). Precaution was taken not to turn pNPP solution to yellow colour as that may sometimes happen due to alkaline phosphatase (ALP) contamination from skin.

3.4.4 Preparation of groundnut seed extracts

Approximately 100 g of groundnut kernels was ground to powder using a blender. The powder was titrated in 70% methanol (v/v,70 ml of absolute methanol in 30 ml distilled water) containing 0.5% KCl (proportion used in 100 ml for 20 g seed) in a blender, until the mixture was thoroughly homogenized. The extract was then transferred to a conical flask and shaken for 30 min at 300 rpm. The extract was filtered through Whatman No. 4filter paper and diluted at 1:10 in PBS-Tween (1 ml extract and 9 ml of buffer). A simple liquid-liquid cleanup and concentration (5:1) procedure was adopted prior to ELISA, for estimation of lower levels of AFB_1 (<10 μg Kg^{-1}). Twenty ml of methanol extract 10 ml of distilled water and 20 ml of chloroform were mixed in a separating funnel and used for cleanup. After vigorous shaking for a minute, the lower chloroform layer was collected and evaporated to near desiccation in water bath at 60° C. Four ml of PBS-Tween containing 7% methanol was added to the residue obtained after desiccation and was used for analysis by ELISA.

AFB_1-BSA conjugate was prepared in carbonate coating buffer at 100 ng ml^{-1} concentrations, and 150 μl of the diluted AFB_1-BSA was dispensed to each well of ELISA plate. The plate was incubated in a refrigerator overnight or at 37°C for one hour.

The plates were washed thrice using PBS-Tween, with a gap of 3 minutes between each wash (To inhibit non-specific binding of antibodies and thereby giving false positive reaction). BSA (0.2%) was prepared in PBS-Tween was added at 170 µl per each well of ELISA plate and incubated at 37°C for 1h.The plates were washed in three changes of PBS-Tween, allowing 3 min between each wash.

3.4.5 Preparation of Aflatoxin B_1 standards

Healthy groundnut seed extract was prepared as mentioned previously. Aflatoxin B_1 standards (using 1:10 healthy groundnut seed extract) were diluted at concentrations ranging from 100ng to 10 picogram in 100µl volume.

3.4.6 Procedure of ELISA

Fifty µl of antiserum was added to each dilution of aflatoxin standards (100µl) and groundnut seed extract (100µl) intended for analysis. To facilitate reaction between the toxins present in the sample with antibody, the plate containing the mixture of aflatoxin samples (100 µl) and antiserum (50 µl) was incubated for 1 h at 37°C. Entire process was carried out in 96-well micro plate and there was no need to pre-incubate the toxin and antibody mixture in separate tubes.

The plate was washed in three changes of PBS-tween allowing for 3min for each wash. Goat anti-rabbit IgG (1:4000 dilution) was prepared and labelled with alkaline phosphatase, in PBS-Tween containing 0.2% BSA. To each well, 150µl was added and incubated for 1h at 37°C. The plate was washed in three changes of PBS-Tween allowing for 3 min for each wash. Substrate solution (p-nitro phenyl phosphate prepared in 10% diethanollamina buffer, pH 9.8) (150µl) was added and incubated for 1h at room temperature. Absorbance (optical density) was measured at 405 nm in a micro plate reader (iMark Micro plate Reader, BIO-RAD) (Plate 6B).

Using the values obtained for aflatoxin B_1 standards a curve was drawn with the help of computer software, taking aflatoxin concentrations on the X-axis and optical density values on the Y-axis. Amount of aflatoxin present in the sample was calculated using the formula mentioned below.

$$\text{AFB1 } (\mu g/kg) = \frac{A \times D \times E}{G} \text{ Or } \frac{A \times E}{C \times G}$$

A = AFB_1 concentration in diluted or concentrated sample extract ($ngml^{-1}$)

D = Time dilution with buffer

C = Times concentration after clean up

E = Extraction solvent volume used (ml)

G = Sample weight (g)

3.5 Estimation of oil and fatty acid content

The effect of bruchid infestation and fungal build up on oil and fatty acid composition of groundnut pods with different moisture per cent stored in different bags was estimated by using Near Infrared Reflectance Spectroscopy (NIRS; model XDSRCA, FOSS Analytical AB, Sweden, Denmark) a non-destructive method of estimating biochemical constituents (Plate 7).

The initial oil and fatty acid composition of groundnut seeds at two different moisture regimes was estimated a day before setting up of the experiment by drawing a representative sample of pods which were shelled to obtain approximately 70-100 grams of kernels. The kernels so obtained were placed in a small rectangular cup of the NIRS equipment and allowed to scan. The scanned sample was then analysed by the equipment and data for total oil content and compositions of different fatty acids *viz.*, Palmitic acid, Stearic acid, Oleic acid, Linoleic and Protein content etc. was displayed by the equipment on its monitor which was recorded.

Similarly the data on oil content and fatty acid composition of pods stored in four different types of bags at two different moisture regimes was recorded after opening the first, second and third batch of bags at two, four and six months of storage periods respectively.A pooled sample of 3 Kg was drawn from each of the bag with one Kg each from top, middle and bottom portions of the bag. Approximately 70-100 g of shelled kernels were obtained from the pooled sample which was used for analysing in NIRS. The initial data before setting up of the experiment was compared with data obtained at two, four and six months of storage period in different types of bags and at different moisture levels was analysed following suitable statistical method.

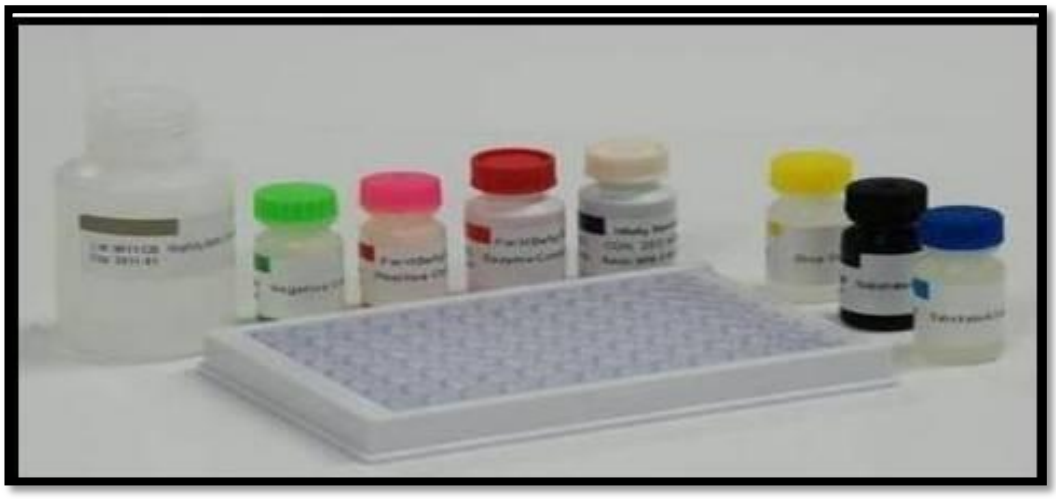


Plate 6A. Elisa kit used for estimation of aflatoxin content by Indirect Competitive Elisa method

Plate 6B. BIO-RAD (i Mark Micro plate Reader) used for the estimation of absorbance of toxin accumulation in ELISA plate Indirect Competitive Elisa method

Plate 7. Near Infrared Reflectance Spectroscopy (NIRS) used for determination of oil and fatty acids in groundnut kernels

3.6 Estimation of Oxygen requirement of *Caryedon serratus*

The total oxygen requirement for completion of entire life cycle i.e., from the day an egg is laid till its emergence as adult from pupa was estimated by using air tight septum bottles (Sigma Aldrich) (Plate 8A). Ten pods each containing single zero day old egg was placed one each in air tight septum bottles of 240 ml volume and ten similar bottles with a normal pod without egg was placed. The septum bottles were kept in dark place at temperature of 25±2°C and 70 % relative humidity. The initial oxygen and carbon dioxide concentrations in the septum bottles were recorded and there after the changes in O_2 and CO_2 concentrations were measured at every 12 hours interval using a Mocon PAC Check® Model 325 head space analyser (Mocon, Minneapolis, MN, USA) (Plate 8B). The mean data obtained from the ten check septum bottles was subtracted from the mean data obtained from the ten septum bottles containing eggs so as to avoid the respiration performed if any by the pod. The septum bottles were regularly observed to determine the transformation of egg to larva, larva to pupa and pupa to adult so as to quantify the amount of oxygen consumed by the insect for transforming from one particular stage to the next. The quantification was done by subtracting the initial oxygen concentration from the concentration of oxygen measured just after the transformation of an insect stage. Similarly the CO_2 concentration generated was quantified by subtracting the concentration of the CO_2 measured just after the transformation of the insect from the initial CO_2 concentration. The data obtained from all the ten septum bottles was recorded and mean value generated from it was used for calculation. Similar pattern was followed to determine the quantity of oxygen consumed and Carbon dioxide released by the insect while transforming from one stage to the other. The entire data was summed up to determine the total quantity of oxygen used by the insect for completion of life cycle and was represented in the form of volume. The O_2 and CO_2 concentrations were converted in to volume by using the following formula.

Respiratory quotient value is calculated by using formula (Jakobsen and Thorbek, 1993)

$$RQ = CO_2 \text{ produced (ml)} / O_2 \text{ consumed (ml)}$$

Plate 8A. Septum bottles used for estimation of oxygen requirement of *C.* serratus

Plate 8B. Estimation of gas composition in septum bottles by using hand held Mocon head space analyser

3.7 Statistical analysis

The data on the observations made were analyzed statistically by applying the technique of analysis of variance for factorial completely randomized design and significance was tested by F-test (Snedecor and Cochran, 1967). Critical difference for examining treatment means for their significance was calculated at 1 per cent level of probability.

Chapter IV

RESULTS AND DISCUSSION

Chapter -IV

RESULTS AND DISCUSSION

A comprehensive study was carried out to evaluate different storage bags for their ability to manage groundnut bruchid, *Caryedon serratus* and storage fungi without effecting the seed germination and bio chemical compositions. The results obtained from the experiment entitled "**Evaluation of improved grain storage practices for the management of Groundnut Bruchid *Caryedon serratus* Olivier."** is presented in this chapter under different headings.

The results obtained during the study on different aspects such as total oxygen requirement to complete groundnut bruchid life cycle, performance of triple layer bag to manage groundnut bruchid in comparison to other storage bags, aflatoxin accumulation during storage, effect of *C. serratus* and *A. flavus* on oil content and fatty acid changes in stored kernels of groundnuts at different moisture levels (10% and 14%) with bi-monthly observations are presented in this chapter. The results are summarized in tables and illustrated through figures wherever appropriate and essential. The results obtained are discussed with possible reasons and correlated with similar findings.

4.1 Oxygen requirement for completion of life cycle of groundnut bruchid (*C. serratus*)

The oxygen requirement by groundnut bruchid for completion of each stage of its life cycle was studied. The results showed that a single bruchid consumed about 5.44 ml of oxygen for completion of egg to first instar stage, releasing 2.9 ml of carbon dioxide while it consumed a highest quantity of 32.97 ml of oxygen for its development from first instar to final instar releasing 22.68 ml of carbon dioxide. Relatively low quantity of oxygen (1.56 ml) was used by bruchid for its development from final instar to pupal formation with release of 0.63 ml of carbon dioxide (Table 4.1 and Fig.4.1). A total of 39.97 ml of oxygen was consumed by the bruchid for its development from egg to pupa and simultaneously released 26.21 ml of carbon dioxide during the process.

The respiratory quotient (RQ) calculated from the data obtained on the quantity of oxygen consumed and carbon dioxide released at each stage showed the highest RQ value of 0.68 for the development stage starting from first instar to final instar while

low RQ value was recorded for formation of pupa from final instar larva. An RQ value of 0.53 was recorded for the development of bruchid from its eggs to first instar.

Table 4.1 Oxygen requirement to groundnut bruchid, *C. serratus* to completion of different stages life cycle

Particulars	Duration	O2 Consumed (ml)	CO2 Produced(ml)	Respiration Quotient
Egg period	5-7	5.44	2.90	0.53
Larval period	15-18	32.97	22.68	0.68
Pupal period	12-14	1.56	1.00	0.63

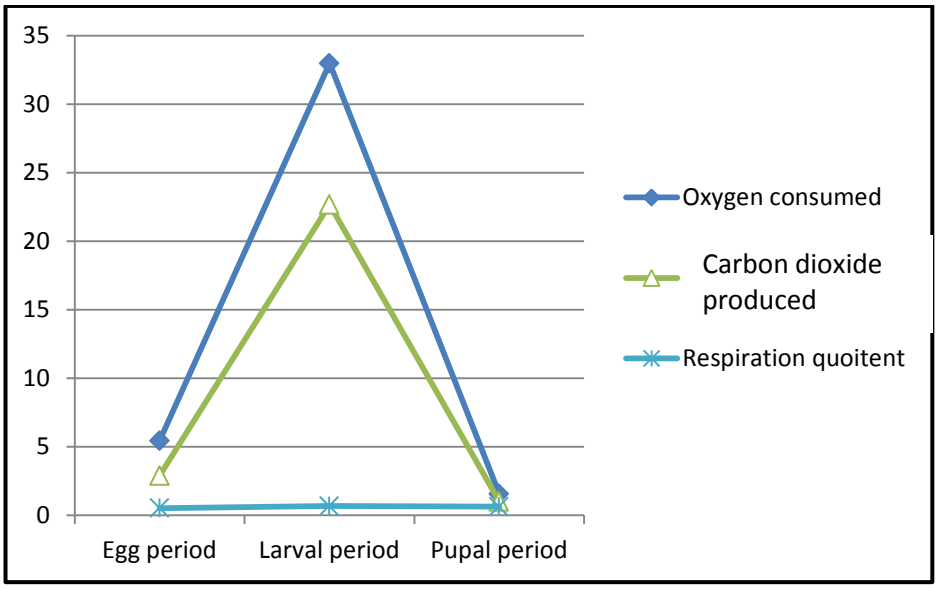


Fig 4.1. The quantity of oxygen consumed and correspondence released at different developmental stages of groundnut bruchid, *Caryedon serratus*

The record of highest RQ value during larval development compared to egg and pupal development was due to production of lipids from carbohydrates was proposed by Nielsen *et al*. (1993). Bhattacharya *et al.* (2003) opined that the insects obtained the energy by burning the carbohydrates and transformed it into lipids during active feeding larval stages where in they consumed high oxygen resulting in higher RQ values.

The results obtained in the study helped to understand the mode of action of hermetic storage and its effect on groundnut bruchid survival. The dynamic changes in the O_2 and CO_2 levels that occur within a sealed storage container cause conditions of hypoxia (reduction in availability of oxygen) and hypercarbia (increase in carbon dioxide concentrations) which result in cessation of feeding there by growth and development, ultimately leading to death of insect. The level at which the conditions of

hypoxia or hypercarbia occur can be determined with the knowledge of actual oxygen requirement by the insect and its availability in the container.

However, few studies have revealed that many of the storage insects continue feeding normally at extremely high levels of carbon dioxide, the levels which are far above those that would kill a mammal within seconds. Thus, it is to be understood clearly, that feeding activity falls in response to the drop in O_2 and not to the rise in CO_2 concentrations. These findings support use of triple layer bags as source of hermetic technology rather than a modified atmosphere storage technology where in high concentrations of carbon dioxide is pumped in to achieve insect mortality.

The findings which propose hypoxia conditions leads to insect mortality gains support from the fact that oxygen insufficiency causes two major problems in insects. First, the insect finds itself unable to utilize oxidative metabolism to form the ATP essential for normal body functions of maintenance, growth, development and movement, etc. Second, the low levels of O_2 propel development of reactive oxygen species (ROS) such as superoxide anion and hydrogen peroxide, within insect body that can damage membranes and interfere with proteins and enzymes of various metabolic activities. The only way to circumvent this happening by the insect is to shut down all major metabolic activity, thereby reducing the levels of the ROS species (Margam, 2009).

Thus it is suggested from the above facts that conditions leading to hypoxia result in much faster insect mortality than hypercarbia. The triple layer plastic bag which creates hypoxia and hypercarbia simultaneously will be a better option for adopting it in storage pest management following hermetic technology.

4.2 Performance of triple layer plastic bags in the management of Groundnut bruchid (*C. serratus* Olivier).

Infestation of groundnut pods/kernels with insect pest and inappropriate moisture levels during storage result in pod/kernel damage. The insect infestation further leads to build-up of storage fungi ultimately cause losses in quantity and quality such as loss of seed weight, germination and alterations in biochemical compositions of stored product. The performance of triple layer plastic bag in comparison with traditional bags was studied to determine the effectiveness of triple layer plastic bag in managing *C. serratus*. Data was collected on the insect parameters which include number of eggs laid, number of emergence holes, number of pupae, and number of live adults and also the insect activity causing percent damage and percent weight loss of stored product.

4.2.1(a) Effect of storage bags and pod moisture contents on production of eggs by bruchid (No. of bruchid eggs/100 pods)

The pod samples drawn from the triple layer plastic bags at the end of 2, 4 and 6 months storage showed presence of no eggs at all and these bags were significantly different ($P<0.01$) from other bags under study (Fig 4.2). Highest number of eggs 167.4, 255.5 and 283.08 were recorded on pods stored in jute bag after 2, 4 and 6 months of storage period respectively while the jute bags treated with spinosad recorded 146.21, 220.30 & 233.30 eggs respectively after 2, 4 and 6 months of storage period. Among the traditional bags the polythene bags recorded an egg count of 131.98, 201.11 & 216.58 after 2, 4 and 6 months of storage respectively (Table 4.2).

The results also indicated significant effect ($P< 0.01$) of pod moisture content during storage on number of eggs laid by bruchids. The maximum number of eggs 138.65, 201.79 & 286.11 were recorded after 2, 4 and 6 months of storage respectively on pods containing 14 per cent moisture compared to the pods stored at 10 per cent moisture which recorded an egg count of 84.15, 136.59 & 202.53 at 2,4 and 6 months of storage respectively.

The data on cumulative effect of moisture and bag type on egg production by bruchids showed absence of eggs on both 10 per cent and 14 per cent groundnut pods stored in triple layer plastic bags after 2, 4 and 6 months of storage. The triple layer plastic bags were thus found to be significantly better in preventing insect activity (egg production) compared to traditional bags where in the maximum number of eggs 131and 203.8 after 2months, 226.86 and 283.63 after 4months and 254.16 and 312 after 6months of storage at 10 and 14 per cent moisture levels were observed in traditional jute bag.

The jute bag treated with spinosad recorded relatively less number of eggs compared to jute bags at 10 and 14 per cent moisture levels of pods with an egg count of 108.8 and 183.63, 166.56 and 274.23 and 180.76 and 285.83 respectively after 2, 4 and 6 months of storage period. The lowest number of eggs at 10 and 14 per cent moisture levels were recorded in polythene bags compared to other traditional bags with an egg count of 96.80 and 167.16, 152.93 and 249.30 and 172.60 and 260.50 respectively 2, 4 and 6 months of storage.

Observations on different storage periods revealed that maximum numbers of eggs were recorded on the pods after 6 months of storage followed by 4 and 2 months storage.

Table 4.2 Effect of storage bags and pod moisture levels of groundnut on egg laying by *C. serratus* at different storage periods

Average number of eggs laid by *C. serratus* on groundnut pods (100) in different storage bags at 10 and 14 per cent moisture levels				
		2 Months	4 Months	6 Months
Per cent moisture	10	84.15a	136.58 a	202.53 a
	14	138.65b	201.79 b	286.11 b
S.E(m)		0.28	0.07	0.48
C.D(P=0.01)		1.17	0.3	2.09
Bag type	Triple layer bag	0 a	0a	0a
	Polythene bag	131.98 b	201.11 b	216.58 b
	Jute bag treated with Spinosad	146.21c	220.39c	233.30c
	Jute bag	167.40d	255.24d	283.08d
S.E(m)		0.4	0.1	0.60
C.D(P=0.01)		1.65	0.43	2.56
Interaction (Moisture x Bag type)				
10%	Triple layer bag	0a	0a	0a
	Polythene bag	96.80 b	152.93 b	172.60 b
	Jute bag treated with Spinosad	108.80c	166.56c	180.76c
	Jute bag	131.00d	226.86d	254.16d
14%	Triple layer bag	0a	0a	0a
	Polythene bag	167.16e	249.30e	260.50e
	Jute bag treated with Spinosad	183.63f	274.23f	285.83f
	Jute bag	203.80g	283.63g	312.00g
S.E(m)		0.56	0.14	0.84
C.D(P=0.01)		2.34	0.61	3.63

*Values followed by the same letter are not significantly different

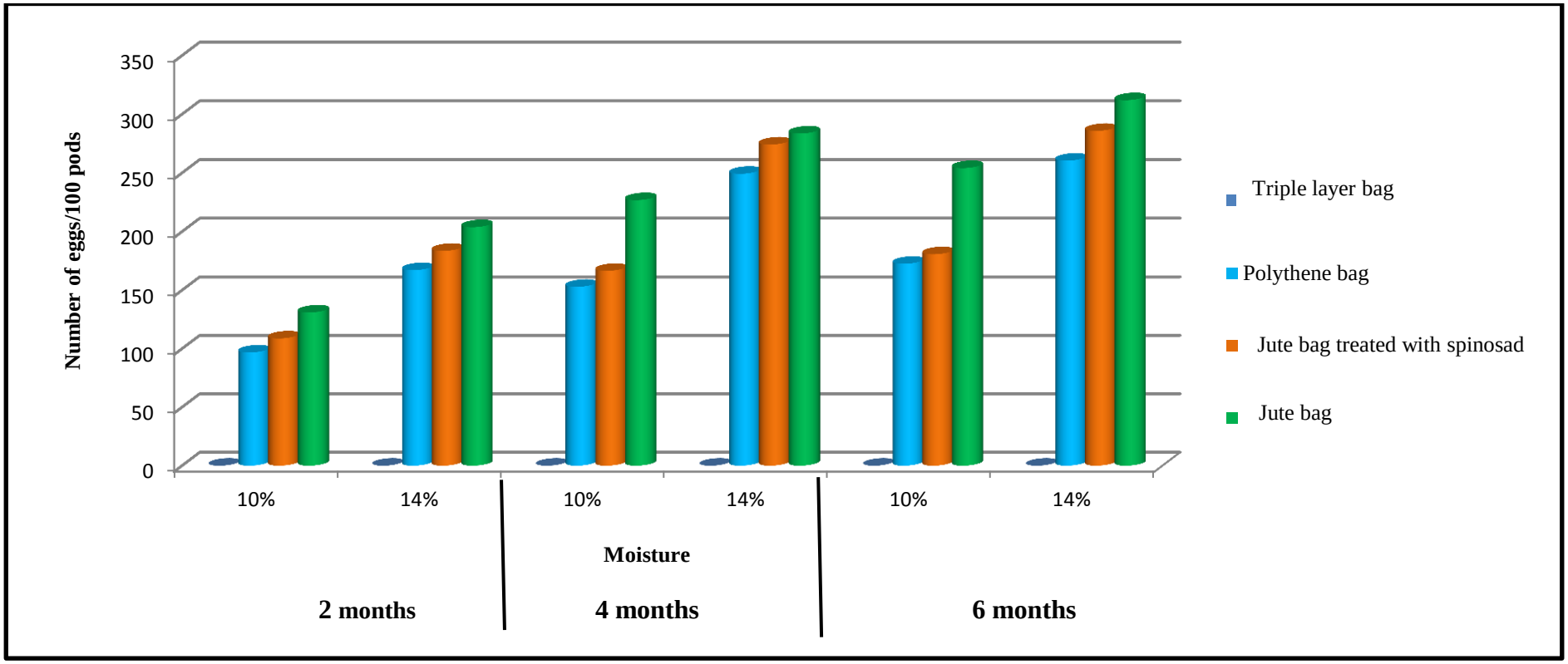


Fig 4.2. Effect of bag type and pod moisture content on egg laying by *C. serratus* on groundnut pods after 2, 4 and 6 months of storage

4.2.1(b) Effect of storage bags and pod moisture contents on adult emergence of bruchids (No. of emergence holes/100 pods)

Data pertaining to the number of emergence holes on groundnut pods after damage by bruchids in storage at different set of experiments of 2, 4 and 6 months storage was presented in Table 4.3 and illustrated in Fig. 4.3.

Results clearly indicated significant effect ($P< 0.01$) of pod moisture content during storage on the number of insect emergence holes as the highest numbers were recorded in pods, stored at 14 per cent moisture (13.63, 49.52 & 51.63) compared to pods stored at 10 per cent moisture (8.86, 28.65 & 32.50)after 2, 4 and 6 months of storage.

The results on effect of bag type on insect emergence showed, triple layer plastic bag to be significantly ($P<0.01$) effective in managing insect activity with recorded of nil emergence holes compared to other treatment bags. The maximum number of emergence holes among traditional bags were recorded in jute bag stored groundnut pods (16.98 after 2 months, 61.70 after 4 months & 66.04 after 6 months) followed by jute bag treated with spinosad (15.65,50.16& 54.1) and polythene bag (12.35,44.49& 48.13) at different sets of 2, 4 and 6 months in storage.

The results on interaction of moisture and bag type on bruchid emergence hole was found significant ($P<0.01$) at different sets of storage periods.

The results showed triple layer plastic bags recorded no emergence holes at 10 and 14 per cent moisture levels at 2, 4 and 6 months of storage and were found to be significantly different from traditional bags.

The data on number of emergence holes in traditional bags after 2 months storage indicated that the jute bag (20.44) and jute bag treated with spinosad (19.31) were recorded on par with each other at 14% moisture, while polythene bag (14.77) at 14 per cent moisture and jute bag (13.52) at 10% moisture were found to be on par with each other. Polythene bag (9.92) and jute bag treated with spinosad (11.99) were significantly differ ($P< 0.01$) with each other at 10% moisture.

Data pertaining to number of emergence holes after 4 months storage showed the polythene bag (61.55), jute bag treated with spinosad (65.99) were recorded on par with each other at 14% moisture, jute bag treated with spinosad intern on par with jute

bag (70.55) and remaining treatment bags at 10% moisture were significantly different ($P< 0.01$) with each other.

Data on number of emergence holes after 6 months storage results the polythene bag (65.73), jute treated spinosad (67.16) were recorded on par with each other at 14% moisture and remaining treatments were significantly differ ($P< 0.01$) with each other.

Table 4.3 Effect of storage bags and pod moisture levels of groundnut on development of *C. serratus* at different storage periods

Average number of emergence holes on groundnut pods (100) in different storage bags at 10 and 14 per cent moisture levels				
		2 Months	4 Months	6 Months
Per cent moisture	10	8.86a	28.65a	32.50a
	14	13.63b	49.52b	51.63b
S.E(m)		0.17	0.65	0.3
C.D(P=0.01)		0.72	2.72	1.24
Bag type	Triple layer bag	0a	0a	0a
	Polythene bag	12.35b	44.49b	48.13b
	Jute bag treated with Spinosad	15.65c	50.16c	54.10c
	Jute bag	16.98d	61.70d	66.04d
S.E(m)		0.24	0.93	0.42
C.D(P=0.01)		1.02	3.85	1.75
Interaction (Moisture x Bag type)				
10%	Triple layer bag	0a	0a	0a
	Polythene bag	9.92b	27.44b	30.53b
	Jute bag treated with Spinosad	11.99c	34.33c	41.03c
	Jute bag	13.52d	52.86d	58.46d
14%	Triple layer bag	0a	0a	0a
	Polythene bag	14.77d	61.55e	65.73e
	Jute bag treated with Spinosad	19.31e	65.99ef	67.16e
	Jute bag	20.44e	70.55f	73.63f
S.E(m)		0.35	1.31	0.6
C.D(P=0.01)		1.44	5.44	2.48

*Values followed by the same letter are not significantly different

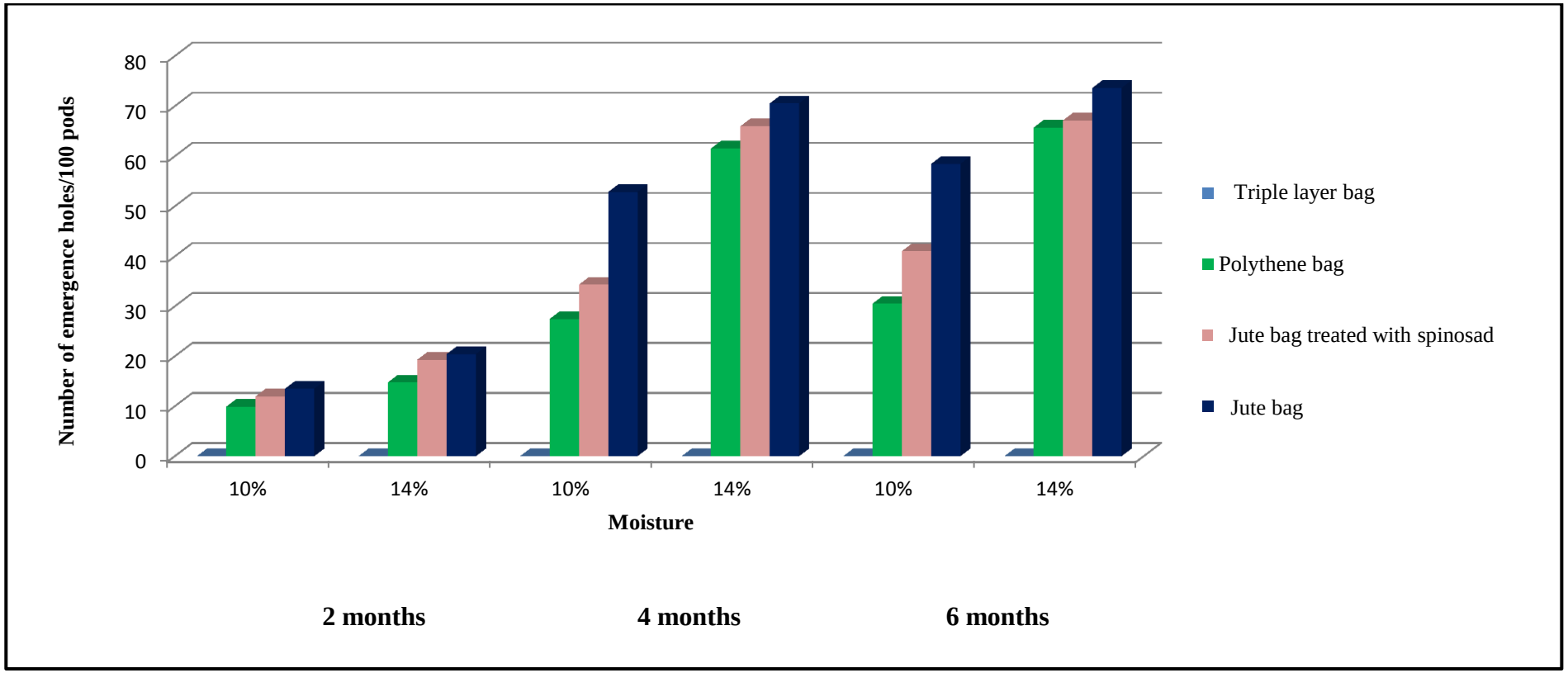


Fig 4.3. Effect of bag type and pod moisture content on emergence of *C. serratus* groundnut pods stored for 2, 4 and 6 months period

4.1.1(c) Effect of storage bags and pod moisture contents on pupal development of bruchids (No. of pupae/100 pods)

The effect of storage bags, pod moisture contents alone and their combined effect on development of bruchid pupa in storage at 2, 4 and 6 months of storage is presented in Table 4.4

The results clearly indicated the significant effect (P<0.01) of pod moisture content during storage on pupal development of bruchids. Significant higher number of pupae were formed in bags containing pods stored at 14 per cent moisture (5.85, 11.21 & 19.09) while less number of pupal formation was noticed in pods stored at 10 per cent moisture contents (4.53, 8.67 & 15.30) at 2, 4 and 6 months of storage.

The results also established significant effect (P<0.01) of bag types on pupal development with triple layer plastic bag showing no development of pupae after 2, 4 and 6 months of storage. Significant increase in pupal formation in traditional storage bags with maximum number of pupae in jute bag (9.25 after 2 months, 17.66 after 4 months & 25.60 after 6 months) followed by jute bag treated with spinosad (6.58, 12.18 & 21.91) and polythene bag (4.94, 9.94 & 21.30) were observed after 2,4 and 6 months storage.

The combined effect of moisture and bag type on pupal formation showed no pupal formation in triple layer bags at 10 and 14 per cent moisture contents at 2, 4 and 6 months of storage. However, significant (P<0.01) number of pupae were observed in traditional bags at different sets of storage periods.

Jute bags recorded 9.06 and 9.44 pupae per 100 pods at 10 and 14 per cent moisture contents which were found to be on par with each other after 2 months of storage period. The polythene bag and jute bag treated with spinosad containing pods at 14 per cent moisture recorded 6.92 and 7.06 number of pupae respectively after 2 months of storage period and were found to be on par with each other. The polythene bag with pods stored at 10 per cent moisture content recorded significantly less number of pupae (2.96 per 100 pods) compared to other traditional bags after 2 months of storage period.

Table 4.4 Effect of storage bags and pod moisture levels of groundnut on formation of pupae by *C. serratus* at different storage periods

Average number of pupae on groundnut pods (100) in different storage bags at 10 and 14 per cent moisture levels				
		2 Months	4 Months	6 Months
Per cent moisture	10	4.53a	8.67a	15.30a
	14	5.85b	11.21b	19.09b
S.E(m)		0.07	0.32	0.17
C.D(P=0.01)		0.30	1.35	0.73
Bag type	Triple layer bag	0a	0a	0a
	Polythene bag	4.94b	9.94b	21.30b
	Jute bag treated with Spinosad	6.58c	12.18c	21.91b
	Jute bag	9.25d	17.66d	25.60c
S.E(m)		0.1	0.46	0.25
C.D(P=0.01)		0.42	1.91	1.04
Interaction (Moisture x Bag type)				
10%	Triple layer bag	0a	0a	0a
	Polythene bag	2.96b	7.23b	19.70b
	Jute bag treated with Spinosad	6.10c	10.56c	19.75b
	Jute bag	9.06e	16.89e	21.77c
14%	Triple layer bag	0a	0a	0a
	Polythene bag	6.92d	12.66cd	22.88cd
	Jute bag treated with Spinosad	7.06d	13.81d	24.08d
	Jute bag	9.44e	18.42e	29.43e
S.E(m)		0.14	0.65	0.35
C.D(P=0.01)		0.6	2.7	1.47

*Values followed by the same letter are not significantly different

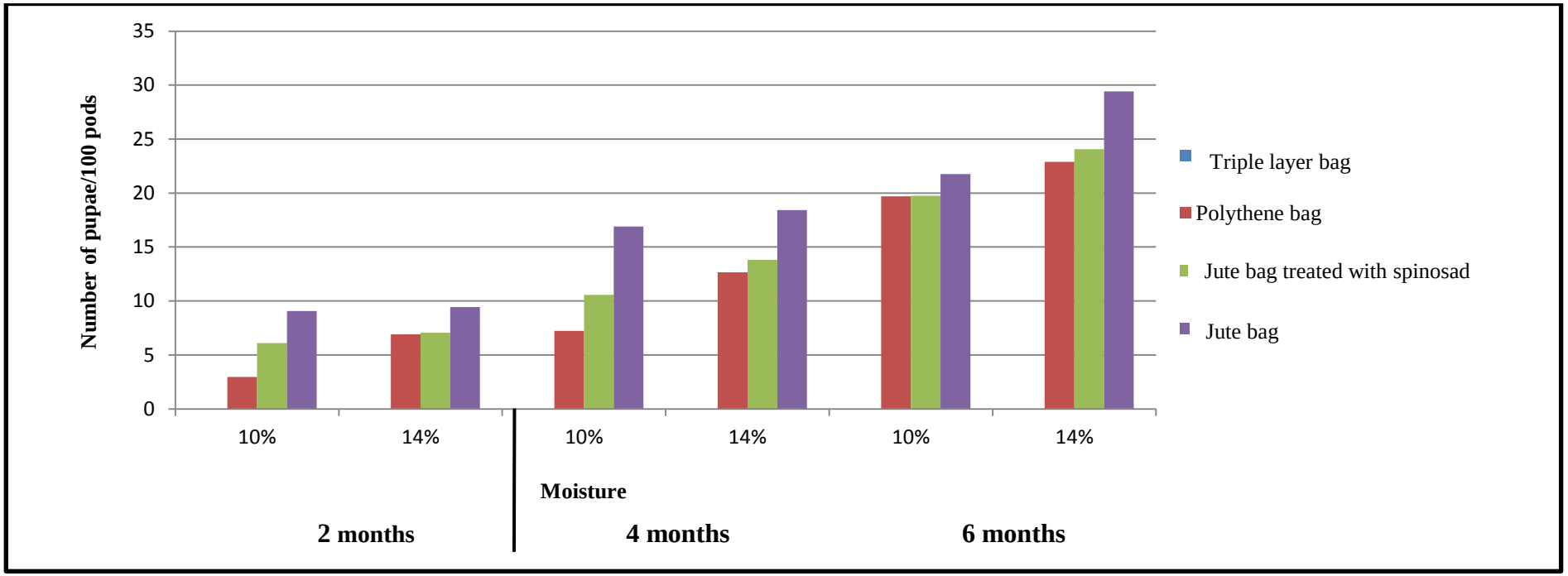


Fig 4.4. Effect of bag type and pod moisture content on development of *C. serratus* pupae groundnut pods stored for 2, 4 and 6 months period

The jute bag treated with spinosad with pods at 10 per cent moisture and polythene bag with pods at 14 per cent moisture recorded 10.56 and 12.66 pupae per 100 pods and found to be on par with each other after four months of storage. The jute bag with pods at 10 (16.89) and 14 per cent moisture contents on the other hand recorded significantly on par number of pupae 16.89 and 18.42 respectively. The polythene bag with pods at 14 per cent moisture recorded significantly on par number of pupae (12.66) with the pods stored in jute bag treated with spinosad (13.81) at the same level of moisture., while the polythene bag with pods at 10 per cent moisture recorded significantly less number of pupae (7.23) among the traditional storage bags after four months of storage.

The results on effect of bag type and moisture contents of the pods on pupal formation after six months of storage revealed that the polythene bag and jute bag treated with spinosad containing pods at 10 per cent moisture level recorded 19.7 and 19.75 pupae per 100 pods and were found to be on par with each other. The polythene bag and jute bag treated with spinosad recorded significant highest and on par number of pupae of 22.88 and 24.08 on pods containing 14 per cent moisture while jute bags with pods of 10 per cent moisture resulted in development of 21.77 number of pupae which was found to be on par with jute bag treated with spinosad (24.08) at 14% moisture. The jute bag with pods at 14 per cent moisture recorded significantly highest number of pupae (29.43 per 100 pods) among all the traditional bags (Fig. 4.4)

4.1.1(d) Effect of storage bags and pod moisture contents on presence of live adult bruchids (No. of adult bruchids/100 pods)

The effect of pod moisture content on development of live bruchids showed significant increase in number of live adults at 14 per cent moisture content compared to the pods stored at 10 per cent moisture after 2, 4 and 6 months of storage. An average number of 13.25, 59.83 and 70.25 live adult bruchids were recorded after 2,4 and 6 months of storage respectively in pods stored at 14 per cent moisture, while a reduced number of adults 12.33, 47.16 and 59.66 were observed in pods stored at 10 per cent moisture content(Table 4.5)

Table 4.5 Effect of storage bags and pod moisture levels on survival of *C. serratus* adults on groundnut pods at different storage periods

Average number of live adults in different storage bags at 10 and 14 per cent moisture levels				
		2 Months	4 Months	6 Months
Per cent moisture	10	12.33a	47.16a	59.66a
	14	13.25b	59.83b	70.25b
S.E(m)		0.15	0.54	0.33
C.D(P=0.01)		0.65	2.25	1.37
Bag type	Triple layer bag	0a	0a	0a
	Polythene bag	15.30b	63.00b	80.50b
	Jute bag treated with Spinosad	17.28c	72.49c	87.66c
	Jute bag	18.60d	78.50d	91.66d
S.E(m)		0.22	0.77	0.47
C.D(P=0.01)		0.92	3.19	1.94
Interaction (Moisture x Bag type)				
10%	Triple layer bag	0a	0a	0a
	Polythene bag	15.00b	52.00b	71.00b
	Jute bag treated with Spinosad	16.00b	63.66c	81.33c
	Jute bag	18.33c	73.00d	86.33d
14%	Triple layer bag	0a	0a	0a
	Polythene bag	15.60b	74.00d	90.00e
	Jute bag treated with Spinosad	18.56c	81.33e	94.00f
	Jute bag	18.86c	84.00e	97.00g
S.E(m)		0.31	1.09	0.66
C.D(P=0.01)		1.3	4.51	2.75

*Values followed by the same letter are not significantly different

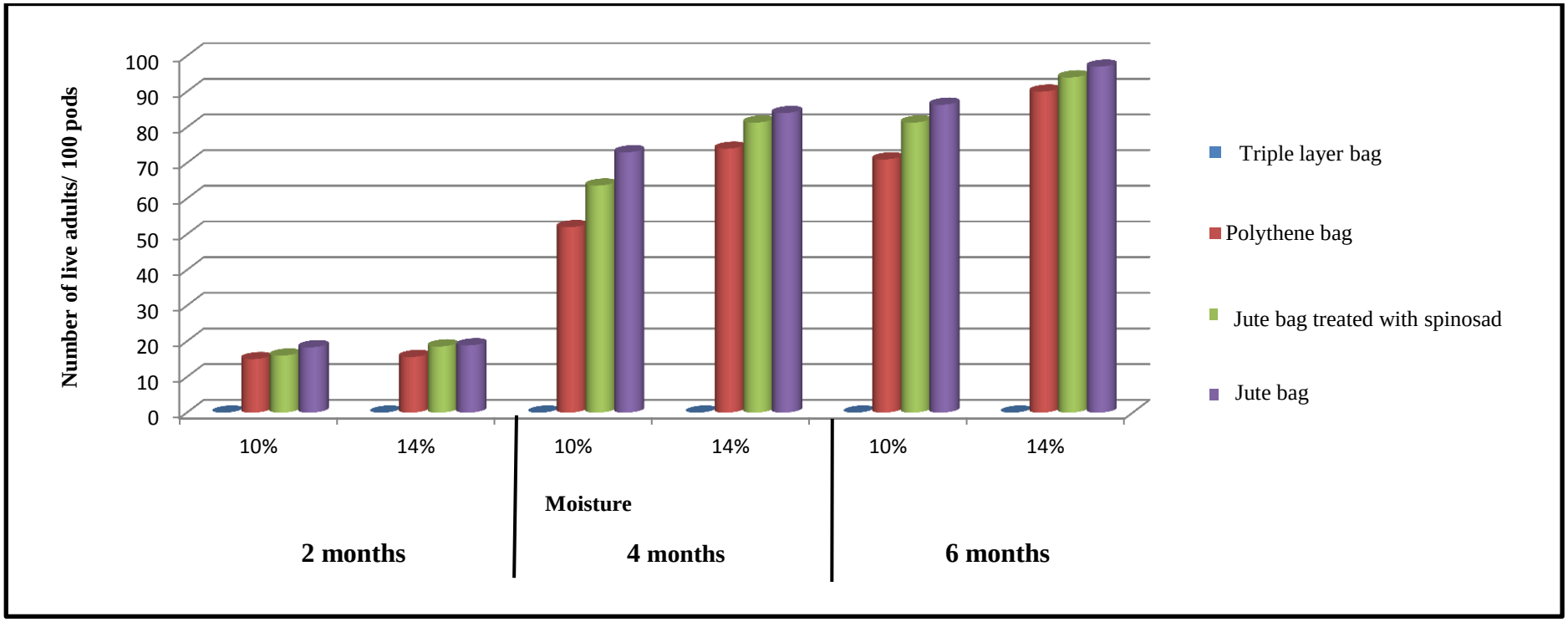


Fig 4.5. Effect of bag type and pod moisture content on survival of *C. serratus* on groundnut pods stored for 2, 4 and 6 months period

The storage bag type recorded significant effect on presence of live bruchid adults ($P< 0.01$) with the results clearly indicating complete absence of live adults in triple layer plastic bags at 2, 4 and 6 months of storage period. The polythene bags recorded significant lowest number of live adults 15.30 per 100 pods followed by 17.28 in spinosad treated jute bags and highest number 18.60 in jute bags after 2 months of storage period. Similarly the polythene bags recorded significant lowest number of live adults 63.00 followed by 72.49 in spinosad treated jute bags and highest number of 78.50 adults in jute bags after 4 months of storage. The presence of average number of live adults after six months of storage revealed significant highest number of live adults in jute bags (91.66) followed by spinosad treated jute bags (87.66) and least were observed in polythene bags (80.50).

The interaction of moisture content and bag type on the presence of live adult bruchidsat2, 4 and 6 months storage periods revealed that the triple layer plastic bags consisting pods of either 10 or14 per cent moisture recorded no live adult bruchids (Illustration in Fig. 4.5)

The polythene bag after 2 months of storage recorded15.0 and 15.6 number of live adults per 100 pods at 10 and 14 per cent moisture contents which were found to be on par with pods with 10 per cent moisture content and stored in jute bags treated spinosad (16.0). Jute bag (18.33) with pods at 10 and 14 per cent moisture and jute treated with spinosad at 14 per cent moisture recorded 18.33, 18.56 and 18.86 number of live adults after 2 months of storage and were found to be on par with each other.

The cumulative effect of bag type and moisture content on number of live bruchid adults after 4 months of storage showed presence of 73.0 live adults in jute bag with 10 per cent moisture pods which was on par with polythene bag that has 74.00 live adults at 14 per cent moisture content. The jute and jute bag treated with spinosad contained significantly highest number of live adults of 84.00 and 81.33 which were on par with each other. The polythene bag and jute bag treated with spinosad with pods at 10 per cent moisture recorded 52.0 and 63.66 number of live adults which were found be significantly low among the traditional bags with either 10 or 14 per cent of moisture contents.

The bag type and moisture content showed significant effect on live bruchid population with significant highest number of live insects 97.00, 94.00 and 90.00 respectively in jute bag, spinosad treated jute bag and polythene bag respectively on

pods with 14 per cent moisture after six months of storage. Similar trend in bag type with significant less number of live adults in jute bags (86.33) followed by jute bags treated with spinosad (81.33) and polythene bag (71.00) were observed in pods with 10 per cent moisture after six months of storage.

It is observed from the tables 4.2, 4.3, 4.4 and 4.5 that the triple layer plastic bag managed bruchid effectively with almost nil number of eggs, emergence holes, pupae and live adults compared to other traditional bags. The traditional jute bag with pods at 14 moisture recorded maximum number of eggs, emergence holes, pupae and live adults. Among the traditional bags the polythene bags with pods at 10 per cent moisture recorded minimum number of eggs, emergence holes, pupae and live adults.

The triple layer plastic bag recorded absence of live adults due to decreased levels of oxygen (hypoxia) and increased concentrations of carbon dioxide (hypercarbia) which might have shown synergistic effect on insect mortality (Banks and Annis, 1990). The triple layer plastic bag because of their impermeable nature to diffusion of gasses might have created competition for oxygen among bruchids, which lead to lesser metabolic activity and finally death of beetles and its immature stages due to asphyxiation.

The bruchid multiplication was more in jute bag due to its permeable nature for exchange of gases and diffusion of oxygen into jute bags and carbon dioxide out of bag and also moisture present in the air through the perforations in the bag. The same jute bag treated with insecticide spinosad recorded relatively less number of insect infestation compared to untreated jute bag, this could be due to effect of insecticide which might have managed the insect infestation that occurred during initial periods. However, the presence of insect infestation thereafter could be due to loss in insecticidal effectiveness after a short period as it was applied only once at the time of filling the produce and also due to the inherent property of jute bags to absorb moisture and free exchange of gasses with outer environment which might have promoted insect activity after longer periods of storage. Polythene bag due to its semi-permeable nature when exposed to environment was different from jute bags. This semi-permeable nature must have allowed little development of insect population, hampering rest of population due to insufficient oxygen. The results are in accordance with the findings of Mutungi *et al*. (2014) who recorded similar weight loss 14.5% and seed damage 71.8%in polypropylene bags working with storage of Mung bean and Pigeonpea by *Callosobruchus maculatus*.

Apart from the nature of bags the moisture content within the pod also govern the microclimate surrounding the produce and effects insect activity. The produce with high moisture content generates heat and makes environment favourable for insect and even fungal development (Harish *et al*., 2014; Baoua *et al*., 2012). This could be the probable reason for relative increase in number of eggs, pupa and live adults in pods containing high moisture content when stored in traditional bags.

4.1.2 Effect of storage bags and moisture content of pods on per cent weight loss of stored groundnut pods due to bruchid infestation

The data pertaining to effect of storage bags and per cent moisture content of groundnut pods on bruchid infestation leading to per cent weight loss of stored groundnut pods after 2,4 and 6 months of storage is presented in Table 4.6.

The effect of moisture content of the pods on per cent weight loss showed significant ($P< 0.01$) increase in per cent weight loss of produce due to increased moisture content of the produce during storage. The pods stored at 14 per cent moisture content recorded 5.16, 11.83 and 15.62 per cent weight loss when stored for 2, 4 and 6 months while significant less weight loss of produce 3.79, 9.16 and 13.08 respectively after 2,4 and 6 months of storage was recorded when stored at 10 per cent moisture content.

Results on effect of bag type on per cent weight loss due to insect infestation clearly indicated significant ($P< 0.01$) increase in per cent weight loss of pods stored in traditional bags compared to triple layer plastic bags which recorded no loss in weight due to pod damage at 2, 4 and 6 months of storage. The jute bags and spinosad treated jute bags highest recorded highest weight loss of 6.16 and 7.25 per cent and were on par with each other while the polythene bag recorded significant low per cent weight loss of 4.5 after 2 months of storage period.

The type of storage bags and pod moisture contents showed significant impact on loss in per cent weight of stored groundnut pods after 2, 4 and 6 months of storage periods. The per cent weight loss was significantly high in polythene bags(5.66) jute bags (7.66) and jute bags treated with spinosad (7.33) in pods stored at 14 per cent moisture and these bags were found on par with jute bags (6.83) consisting of pods with 10 per cent moisture. The jute bags (6.83) and jute bags treated with spinosad (5.00) consisting of pods with 10 per cent moisture were found on par with polythene bags (5.66) consisting of pods at 14 per cent moisture while the polythene bags with pods at

10 per cent moisture content recorded significant least per cent weight loss of 3.33after 2 months of storage.

Table 4.6 Effect of storage bags and pod moisture content on percent weight loss due to infestation of *C. serratus* on ground nut pods with two different moisture levels stored in four different types of storage bags

Per cent weight loss of groundnut pods in four different bags at 10 and 14 per cent moisture levels				
		2 Months	4 Months	6 Months
Per cent moisture	10	3.79 (10.60)a	9.16 (16.28)a	13.08 (19.52)a
	14	5.16 (12.40)b	11.83 (18.55)b	15.62 (21.37)b
S.E(m)		0.31	0.26	0.18
C.D (P=0.01)		1.28	1.08	0.75
Bag type	Triple layer bag	0 (4.05)a	0 (4.05)a	0 (4.05)a
	Polythene bag	4.50 (12.05) b	12.16 (20.34) b	17.91 (25.02) b
	Jute bag treated with spinosad	6.16 (14.30)c	13.50 (21.44)	19.33 (26.06) c
	Jute bag	7.25 (15.60) c	16.33 (23.82) c	20.16 (26.65) c
S.E(m)		0.44	0.37	0.25
C.D(P=0.01)		1.81	1.53	1.07
Interaction ((Moisture x Bag type))				
10%	Triple layer bag	0 (4.05)a	0 (4.05)a	0 (4.05)a
	Polythene bag	3.33 (10.34)b	10.0 (18.43)b	16.33 (23.83)b
	Jute bag treated with spinosad	5.00 (12.88)bc	11.00 (19.33)b	17.66 (24.85)b
	Jute bag	6.83 (15.15)cd	15.66 (23.30)c	18.33 (25.34)bc
14%	Triple layer bag	0 (4.05)a	0 (4.05)a	0 (4.05)a
	Polythene bag	5.66 (13.76)cd	14.33 (22.24)c	19.5 (26.20)cd
	Jute bag treated with spinosad	7.33 (15.70)d	16.00 (23.55)c	21.00 (27.27)de
	Jute bag	7.66 (16.06)d	17.00 (24.34)c	22.00 (27.96)e
S.E(m)		0.62	0.52	0.36
C.D(P=0.01)		2.57	2.16	1.51

*Values followed by the same letter are not significantly different

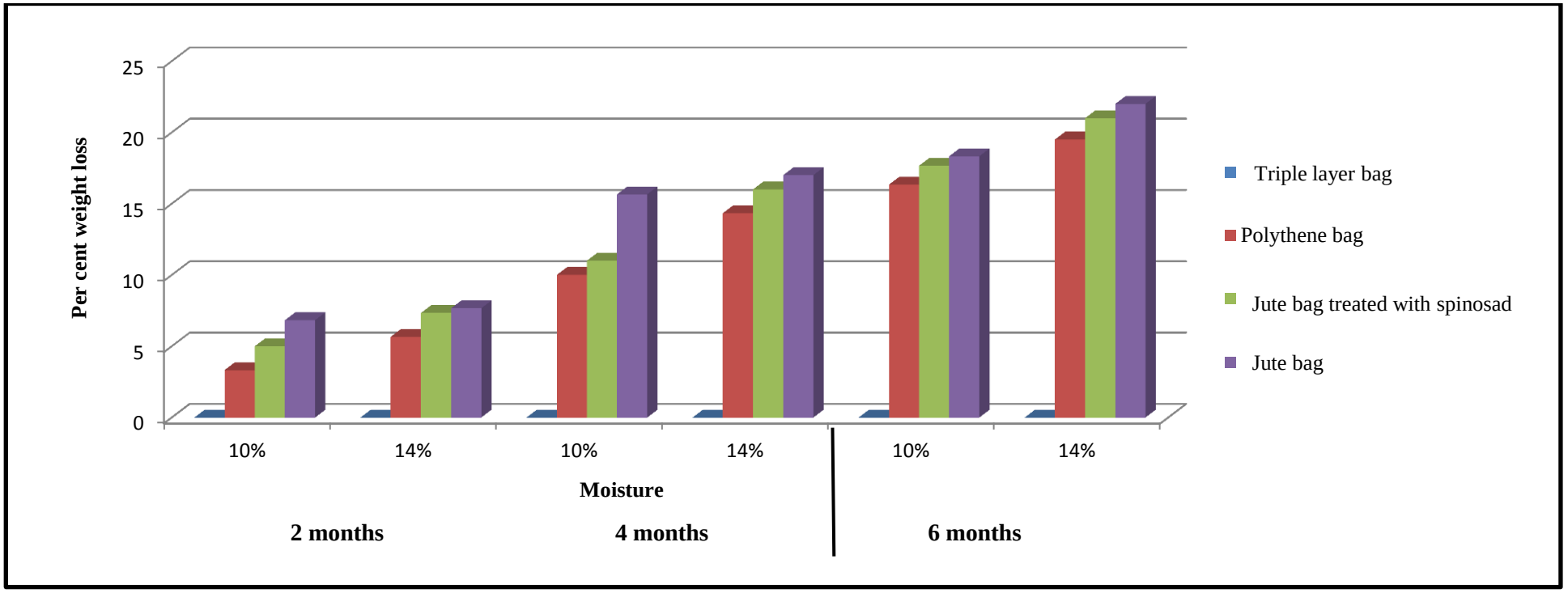


Fig 4.6. Effect of bag type and pod moisture content on per cent weight loss of groundnut pods due to infestation of *C. serratus* after 2, 4 and 6 months of storage

After four months of storage the jute bags with pods of 10 moisture content recorded significant highest per cent weight loss of 15.66 and was found to be on par with polythene bags (14.33), jute bag (17.00) and jute bag treated with spinosad (16.00) containing pods with 1 per cent moisture. The pods with 10 per cent moisture stored in polythene bags and spinosad treated jute bags recorded significant low per cent weight loss of 10.00 and 11.00 respectively.

The jute bag and spinosad treated jute bag recorded significant highest per cent weight loss of 21.00 and 22.00 in pods stored at 14 per cent moisture content, while the polythene bag and jute bag treated with spinosad with per cent weight loss of 19.5 and 21.00 in pods stored at 14 per cent moisture were found to be on par with each other. The jute bag with pods at 10 per cent moisture recorded per cent weight loss of 18.33 which was on par with per cent weight loss of pods with 14 per cent moisture content stored in polythene bag. The pods with 10 per cent moisture content stored in polythene bag, jute bag treated with spinosad and untreated jute bag recorded 16.33, 17.66 and 18.33 per cent weight loss which were found to be on par with each other and recorded significantly low per cent weight loss among traditional bags after 6 months of storage.

Among four different types of storage bags the jute bag at higher moisture levels of 14 per cent recorded highest percent weight loss of groundnuts at 2, 4 and 6 months of storage period (Fig. 4.6).

4.1.3 Effect of storage bags and moisture content of pods on per cent damage of stored groundnut pods due to bruchid infestation

Results on the effect of moisture content of stored produce and bag type clearly indicated significant effect ($P< 0.01$) of pod moisture content on the damage of groundnut pods inflicted by bruchids during storage. Significantly high percent damage was observed at 14 per cent pod moisture with 33.91% after 2 months, 61.66% after 4 months &70.08% after 6 months of storage. The pods stored with10 per cent moisture content recorded relatively less pod damage of 28.41% after 2 months, 58.75% after 4 months & 62.83% after 6 months of storage (Table 4.7 and Fig 4.7).

Results also indicate the significant effect ($P< 0.01$) of bag type used for storage on per cent damage inflicted by bruchids. No per cent damage due to bruchid infestation was recorded in triple layer plastic bag while significant high per cent damage of 46.33 was recorded in jute bags. The spinosad treated jute bag recorded 43.33 per cent pod damage comparatively little lower than jute bag and lowest per cent damage of 35.00

was recorded in polythene bags after 2 months of storage period. Similar trend with increased level of per cent pod damage was found in storage bags after 4and 6months of storage. The polythene bag, jute bag treated with spinosad and untreated jute bag recorded 71.00, 84.16 and 85.66 per cent pod damage respectively after 4months of storage while at 6months the same bags recorded 85.16, 88.83 and 91.83 per cent pod damage.

The interaction of pod moisture content and bag type was found to effect significant percent damage of stored groundnut pods at different storage periods.

After 2 months of storage, the untreated jute bag recorded significant high per cent damage of pods stored at 14 per cent moisture. The treated jute bag at 10 and 14 per cent pod moisture, untreated jute bag with 10 per cent pod moisture and polythene bag with 14 per cent pod moisture recorded 42.00, 44.66, 43.66 and 44.66 per cent pod damage and were found to be on par with each other. The spinosad treated jute bag with 10 per cent pod moisture and polythene bag with 14 per cent pod moisture recoded a pod damage of 42.00 and were on par with each other. The polythene bag consisting of pods with10 per cent moisture content recorded significantly less pod damage of 28.00 percent which was the lowest among the traditional bags used for storage.

The untreated jute bag and spinosad treated jute bag recorded significantly high per cent pod damage of 86.33 and 88.66 at 14 per cent pod moisture and were found to be on par with each other. Similarly the untreated and treated jute bags with pod moisture content of 10 per cent recorded pod damage of 82.66 and 82.00 per cent respectively and were on par with each other. The polythene bag at 10 and 14 per cent pod moisture recorded 70.33 and 71.66 per cent pod damage which were on par with each other after four months of storage.

The polythene bag, jute bag and jute bag treated with spinosad at 14 per cent pod moisture recorded significant high per cent pod damage of 93.00, 93.33 and 94.00 and were on par with jute bag with 10 per cent pod moisture (84.33%). The spinosad treated and untreated jute bags with 10 per cent moisture pods were found to be on par with each other with a per cent pod damage of 84.33 and 89.66. The polythene bag recorded significant low per cent pod damage of 77.33 with 10 per cent pod moisture among traditional bags after six months of storage.

Table 4.7 Effect of storage bags and pod moisture content on per cent damage of groundnut pods due to infestation of *C. serratus* with two different moisture levels stored in four different types of storage bags

Per cent damage in groundnut pods in different storage bags at 10 and 14 per cent moisture levels				
		2 Months	4 Months	6 Months
Per cent moisture	10	28.41 (29.43)a	58.75 (47.83) a	62.83 (51.00) a
	14	33.91 (32.70)b	61.66 (50.16) b	70.08 (57.45) b
S.E(m)		0.2	0.32	0.67
C.D(P=0.01)		0.86	1.34	2.76
Bag type	Triple layer bag	0 (4.05)a	0 (4.05)a	0 (4.05)a
	Polythene bag	35.00 (36.16)b	71.00 (57.42)b	85.16 (68.12)b
	Jute bag treated with spinosad	43.33 (41.16)c	84.16 (66.60)c	88.83 (70.96)bc
	Jute bag	46.33 (42.90)d	85.66 (67.92)c	91.83 (73.76)c
S.E(m)		0.30	0.45	0.94
C.D(P=0.01)		1.22	1.90	3.91
Interaction (Moisture x Bag type)				
10%	Triple layer bag	0.00 (4.05)a	0.00 (4.05)a	0.00 (4.05)a
	Polythene bag	28.00 (31.94)b	70.33 (57.00)b	77.33 (61.57)b
	Jute bag treated with spinosad	42.00 (40.40)cd	82.00 (65.00)c	84.33 (66.71)bc
	Jute bag	43.66 (41.36)d	82.66 (65.40)c	89.66 (71.68)cd
14%	Triple layer bag	0.00 (4.05)a	0.00 (4.05)a	0.00 (4.05)a
	Polythene bag	42.00 (40.40)cd	71.66 (57.84)b	93.00 (74.68)d
	Jute bag treated with spinosad	44.66 (41.93)d	86.33 (68.30)d	93.33 (75.22)d
	Jute bag	49.00 (44.42)e	88.66 (70.44)d	94.00 (75.85)d
S.E(m)		0.41	0.65	1.34
C.D(P=0.01)		1.72	2.68	5.53

*Values in the parenthesis are angular transformed values

*Values followed by the same letter are not significantly different

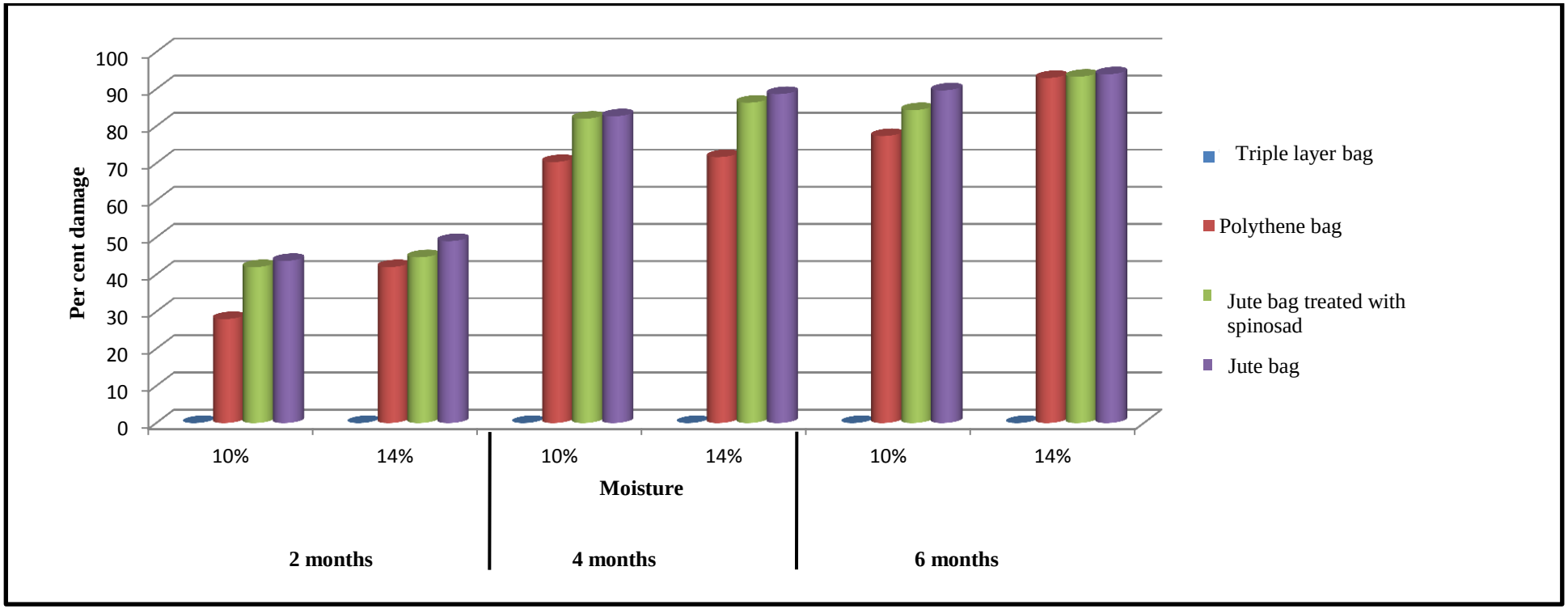


Fig 4.7. Effect of bag type and pod moisture content on per cent damage of groundnut pods due to infestation of *C. serratus* after 2, 4 and 6 months of storage

The highest damage and loss was observed in jute bag stored groundnuts at 14 per cent moisture followed jute bag treated with spinosad and polythene bag. Similar trend was also observed in pods with 10per cent moisture, but the per cent weight loss and per cent damage was relatively less compared to pods stored with 14 per cent moisture. The loss and damage were more in jute bag, jute bag treated with spinosad and polythene bag due to inherent nature of bag which promoted congenial conditions for insect growth compared to triple layer plastic bag as discussed earlier. The microclimate in the bag surrounding the pods and insects, initial and final moisture contents, high temperatures and low relative humidity and duration of storage periods are the possible factors influencing the status of stored produce. The results are in accordance with investigations on maize storage pest by Martinez *et al*. (2000).

The jute bag treated with spinosad did not stop the multiplication of *C. serratus* in groundnut. The reason could be single application of the insecticide is inappropriate where the storage re-infestation and cross infestation are occur. These results are similar with earlier investigations on *Callosobruchus maculatus* resistance towards Pirimiphos-methyl insecticide (Dasback *et al*., 2009).

The triple layer plastic bag prevented the survival of *C. serratus* and halted the pod damage and percent weight loss. A number of mechanisms were responsible for reduction of insect survival under hermetic storage. The air tight conditions primarily create the hypoxia (reduced levels of oxygen) and hypercarbia (increased levels of carbon dioxide) inside the bag. The lethal action of CO_2 increases the solubility of body fluids, which subsequently lowers the pH. A drop in pH creates lesions in the cell membrane of larvae and adult insects, which cause cellular integrity (Nielsen, 2001). These results were similar with Mutungi *et al*. (2014).

4.1.4 Test weight of stored groundnut kernels collected from different storage bags consisting different moisture levels of pods kept for different storage periods

Data pertaining to test weight of stored groundnut pods collected from different storage bags with bruchids infestation kept for 2, 4 and 6 months storage was presented in Table 4.8 and Fig. 4.8.

Results clearly indicate the significant effect ($P< 0.01$) of storage bag on per cent damage of groundnut pods. Test weight of stored groundnut kernels did not change

significantly in triple layer plastic bags (77.41 g) even after different sets of storage periods.

Table 4.8 Effect of storage bags and moisture levels on test weight of groundnut pods at different storage of periods

Test weight of groundnut pods in different storage bags at 10 and 14 per cent moisture levels				
		2 Months	4 Months	6 Months
Per cent moisture	10	74.01a	69.89a	64.76a
	14	70.35b	69.27b	63.05b
S.E(m)		0.25	0.09	0.13
C.D(P=0.01)		1.05	0.4	0.56
Bag type	Triple layer bag	77.41a	77.41a	77.41a
	Polythene bag	72.38b	69.16b	60.86b
	Jute bag treated with Spinosad	71.15b	66.23c	59.16c
	Jute bag	67.78d	65.51	58.20d
S.E(m)		0.36	0.13	0.19
C.D(P=0.01)		1.50	0.55	0.80
Interaction (Moisture x Bag type)				
10%	Triple layer bag	76.90ab	76.90b	76.90a
	Polythene bag	74.96bc	70.70c	61.93b
	Jute bag treated with Spinosad	73.16c	66.40e	60.50c
	Jute bag	71.03d	65.56f	59.73c
14%	Triple layer bag	77.93a	77.93a	77.93a
	Polythene bag	69.80d	67.63d	59.80c
	Jute bag treated with Spinosad	69.13d	66.06ef	57.83d
	Jute bag	64.53e	65.46f	56.66e
S.E(m)		0.51	0.19	0.27
C.D(P=0.01)		2.10	0.78	1.13

*Values followed by the same letter are not significantly different

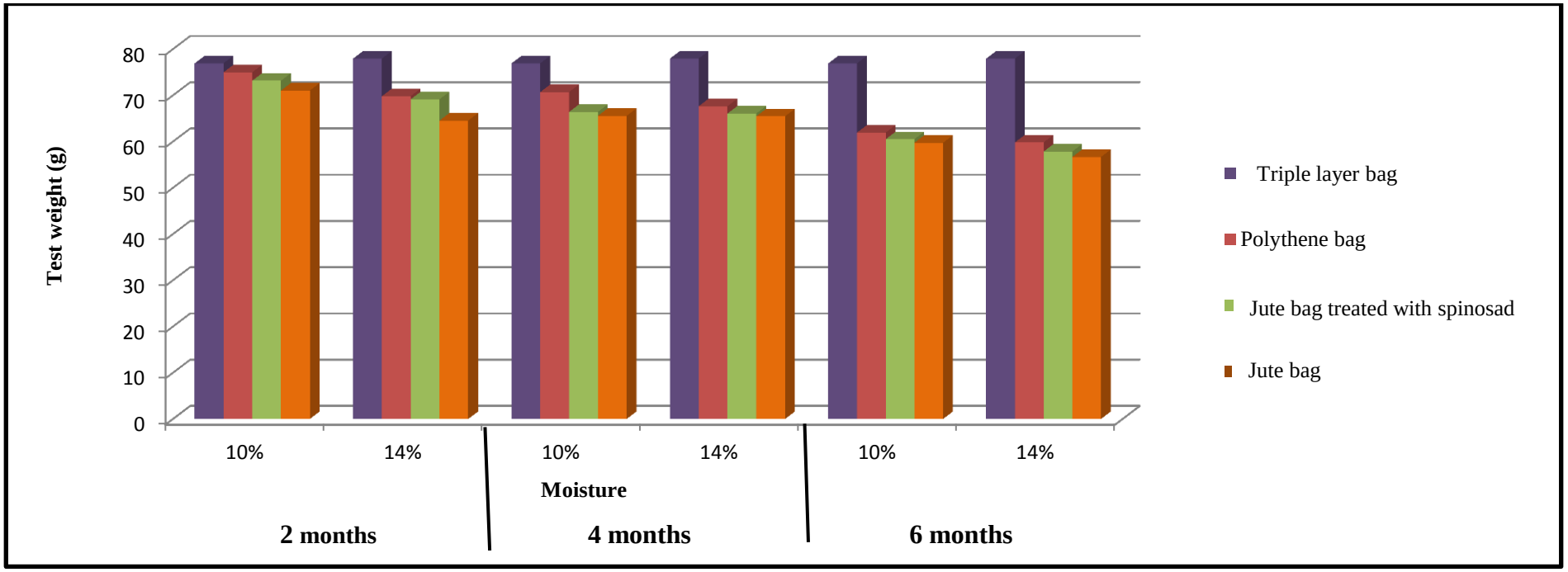


Fig 4.8. Effect of bag type and pod moisture content on test weight of groundnut pods due to *C. serratus* after 2, 4 and 6 months of storage

Maximum loss in 100 seed weight was recorded in jute bag (67.78g, 65.51g & 58.20g) followed by jute bag treated with spinosad (71.15g, 66.23g &59.16g) and polythene bag (72.38g, 69.16g & 60.86g) which were significantly differ ($P< 0.01$) with each other at different sets of storage periods such as 2, 4 and 6 months.

Results also indicate the significant effect ($P< 0.01$) of pod moisture content during storage. Minimum changes in test weight was recorded at 10% moisture (74.01g, 69.89g & 64.76g) in comparison to 14%moisture (70.35g, 69.27g & 63.05g) for 2, 4 and 6 months of storage.

Interaction of moisture content and bag type reveals that no change in the 100 seed weight was observed in triple layer bag at 10% (76.9g) and14% (77.93g) moisture even after 2, 4 and 6 months storage.

After 2 months storage, triple layer plastic bag (76.90g & 77.93g) at 10% and 14% were recorded on par with each other. No significant differences were observed between triple layer plastic bag (76.90g) and polythene bag (74.96g) at 10% moisture; polythene bag (74.96g) and jute bag treated with spinosad (73.16g) at 10% moisture. Jute bag (71.03g) at 10% moisture and polythene bag (69.80g), jute treated with spinosad (69.13g) at 14% moisture were recorded on par with each other. Maximum loss in test weight was recorded in jute bag (64.53g) consisting 14% moisture pods which was significantly differ ($P< 0.01$) from all other treatments.

After 4 months storage the triple layer plastic bag (76.90g, 77.93g), polythene bag (70.70g, 67.63g) polythene bag (70.70g)were recorded significantly differ ($P<0.01$) with other treatments at 10 and 14% moisture. Jute treated spinosad and jute bag (66.40g, 66.06g & 65.56g, 65.46g at 10 and 14% moisture were recorded on par with each other. Jute treated with spinosad (66.06g) was intern on par with the jute bag (65.46g) at 14% moisture.

After 6 months storage the triple layer plastic bag at 10% moisture (76.90g) and 14 (77.93g) were recorded on par with each other. Jute treated spinosad (60.50g), jute bag (59.73g) at 10% and polythene bag (59.80g) were recorded on par with each other at 10 and 14% moisture. Polythene bag (61.93g) at 14% moisture was recorded on par with each other. Jute treated spinosad (57.83g) and jute bag (56.66g) were significantly differ with each other.

Overall bag type, moisture content and their interaction reveal that the triple layer plastic bag with artificial inoculation of *A.flavus* and *C. serratus* was recorded no change in test weight at different moisture levels at different sets of storage periods.

Groundnut pods stored in polythene bag followed by jute bag treated with spinosad and jute bag alone, with artificial infestations of *A.flavus* and *C. serratus*, were recorded maximum weight loss at 14% moisture with comparison of 10%. Insect multiplication was more in jute bag; jute bag treated with spinosad and polythene bag due to high moisture content, free flow of gases from outside environment and increased temperatures which are favourable conditions for insects' multiplication. In comparison of different set of storage periods maximum test weight loss were recorded after 6 months storage followed by 4 months and 2 months storage. In triple layer bags test weight did not change significantly compared to the initial weight due to the death of insects by hypercarbia (increased levels of CO_2) and hypoxia (reduced levels of O_2). These results are almost similar with the earlier studies conducted on the efficacy of triple layer bags (Vales *et al*., 2014 and Sudini *et al.,* 2015).

4.2.6 Changes in gas composition (O_2 and CO_2) in storage bags at different moisture levels and at different sets of storage periods

Gas composition changes in different storage bags at different moisture levels and at different sets of storage periods presented in Table 4.9 and illustrated in Fig. 4.9.

Results clearly indicate the effect of bag type on changes in gas composition in stored groundnut pods. Triple layer plastic bag significantly differing ($P<0.01$) from other bags with maximum drop in oxygen levels to the extent of 1.66%, 1.47% & 1.36% and maximum increase in carbon dioxide levels to the extent of 10.66 %, 11.07% & 12.38% after 2, 4 & 6 months of storage respectively. Oxygen changes observed in polythene bag after 2 (20.62%), 4 (20.34%) and 6 (20.28%) months of storage was significantly ($P<0.01$) differ from jute bag treated with spinosad and jute bag. On the other hand, jute bag treated with spinosad and jute bag were recorded on par with each other after 2 (20.28%, 20.27%), 4 (19.99, 19.93%) and 6 (19.72%, 19.68%) months of storage respectively. Carbon dioxide changes in polythene bag, jute bag treated with spinosad and jute bag were recorded on par after 2 (0.04, 0.06 & 0.06%), 4 (0.06, 0.1 & 0.14%) and 6 (0.09, 0.12 & 0.18%) months of storage.

Table 4.9 Changes in gas composition (O_2 and CO_2) in storage bags at different moisture levels at different sets of storage periods

Oxygen and carbon dioxide changes (%)							
Storage period		2 Months		4Months		6 Months	
Moisture (Mean)		O_2	CO_2	O_2	CO_2	O_2	CO_2
Per cent moisture	10	15.75a	2.66 a	15.52 a	2.77 a	15.32 a	3.11 a
	14	15.66b	2.75 b	15.35 b	2.91 b	15.20 b	3.27 b
C.D		0.08	0.02	0.05	0.13	0.09	0.16
S.E (m)		0.02	0.006	0.01	0.03	0.02	0.04
Treatments (Mean)							
Bag type	Triple layer bag	1.66 a	10.66 a	1.47 a	11.07 a	1.36 a	12.38 a
	Polythene bag	20.62 b	0.04 b	20.34 b	0.06 b	20.28 b	0.09 b
	Jute + Spinosad	20.28c	0.06 b	20.00 c	0.10 b	19.72 c	0.12 b
	Jute bag	20.27c	0.06b	19.93c	0.14b	19.68c	0.18b
C.D		0.12	0.03	0.08	0.18	0.13	0.23
S.E(m)		0.03	0.009	0.02	0.04	0.03	0.05
Interaction							
10%	Triple layer bag	1.81 a	10.51 a	1.68 a	10.86 a	1.51 a	12.09 a
	Polythene bag	20.63 c	0.04 c	20.36 c	0.05 c	20.31 c	0.08 c
	Jute + Spinosad	20.29d	0.05c	20.09d	0.08c	19.74d	0.12c
	Jute bag	20.28d	0.06c	19.96e	0.12c	19.72d	0.17c
14%	Triple layer bag	1.51 b	10.81 b	1.26b	11.28 b	1.21 b	12.68 b
	Polythene bag	20.62c	0.05c	20.33c	0.08c	20.26c	0.10c
	Jute + Spinosad	20.27d	0.07c	19.92e	0.13 c	19.71d	0.13c
	Jute bag	20.26d	0.07c	19.90e	0.16c	19.65d	0.19c
S.E(m)		0.04	0.01	0.03	0.06	0.04	0.08
C.D (P=0.01)		0.17	0.05	0.11	0.26	0.2	0.33

*Values followed by the same letter are not significantly different

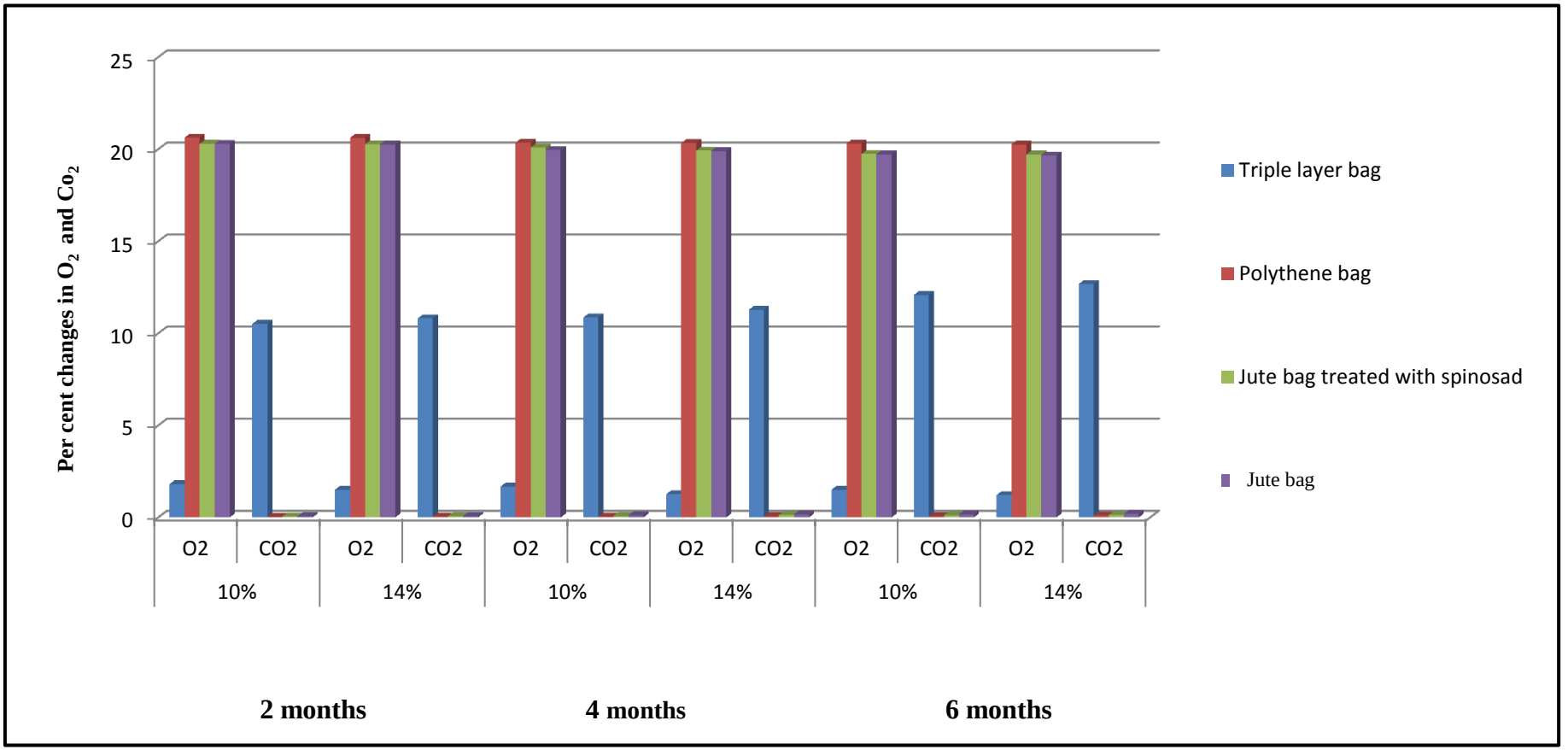


Fig 4.9. Effect of bag type and pod moisture content on gas composition changes in different storage bags after2,4 and 6 months

Results also indicate the significant effect ($P< 0.01$) of pod moisture content during storage on gas composition changes. The maximum decrease in oxygen and maximum increase in carbon dioxide content was recorded at 14% moisture varied from 15.66%, 15.35%, & 15.2% and 2.75%, 2.91% & 3.28% in comparison to 10% moisture 15.75%, 15.51% & 15.32% and 2.66%, 2.77% & 3.11%after 2, 4 and 6 months storage respectively.

Interaction effect of moisture content and bag type on gas composition changes in storage bags showed maximum decrease in O_2 and increase in CO_2 content at 14%moisture varied with different storage periods i.e., 1.51%, 1.26% &1.21% and 10.81%, 11.28% & 12.68% when it was compared with 10% moisture the readings were 1.81%, 1.68% & 1.51% and 10.51%, 10.86% & 12.09% after 2, 4 and 6 months respectively.

Polythene bags were on par with each other at 10% and 14% after 2 (20.63%, 20.62%), 4 (20.36%, 20.33%) and 6 (20.31%, 20.26%) months storage respectively. And also jute bag treated with spinosad and jute bag were on par at 10% and 14% moisture after 2 (20.29, 20.28 and 20.27, 20.26%), 4 (20.07, 19.96 and 19.92, 19.90) and 6 (19.74, 19.72 and 19.71, 19.65%) months storage respectively. However, triple layer plastic bags were significantly differ ($P<0.01$) from other treatments.

After 2, 4 and 6 months of storage, three bag types such as jute, jute with spinosad treatment and polythene bags were not significantly differ with each other in changes in carbon dioxide levels both at 10% and 14% moisture contents. However, the triple layer plastic bag was significantly differing ($P<0.01$) from other treatments in changes in carbon dioxide levels at 10%as well as14% moisture for different storage periods.

Triple layer plastic bag recorded maximum reduction in oxygen levels and maximum increase in carbon dioxide levels due to its impermeable nature that did not allow the exchange of gases from outside environment. This creates hypercarbia (increased levels of CO_2) and hypoxia (decreased levels of O_2) conditions inside the bag. Also groundnut pods inside the bag utilize the oxygen for respiration. Oxygen content did not change significantly in jute bag, jute bag treated with spinosad and polythene bag. Carbon dioxide content also did not change significantly in all these bags as CO_2 is released into external environment due to their porous nature of

packaging materials. These results are similar with earlier investigation conducted on pigeonpea storage by Vales *et al*. (2014).

Triple layer plastic bag recorded maximum reduction of oxygen and increase of carbon dioxide due to aerobic metabolism by insects inside the bags. Respiration by pods also leads to drop in O_2 and rise in CO_2. Utilization of O_2 by insects and grains for respiration leads to reduction of O_2 and increase of CO_2. These results are similar with earlier investigations conducted on cowpea storage by Murdock *et al*. (2003) and on groundnut storage by Sudini *et al*. (2015).

O_2 and CO_2 concentrations were remain more or less same of the concentrations of the environment in conventional storage bags used in the study such as jute bag, jute bag treated with spinosad and polythene bag. This is mainly due to the porous nature of the bags which makes them permeable to gases from outside environment. But in the case of triple layer bags, which are impermeable, respiration by insects leads to increase in CO_2 and decrease in O_2 concentrations. These results are similar with earlier investigations by Martin *et al*. (2015) on wheat storage.

CO_2 and O_2 concentrations were varied according to the moisture content as at high moisture (14%) content CO_2 release and O_2 utilization were more in comparison to at 10% moisture due anaerobic respiration by pods and insects under sealed conditions as internal pressure was increased which leads to maximum utilization of O_2 at high moisture content. These results are similar with earlier investigations on maize hermetic storage by Weinberg *et al*. (2008).

Reduction of oxygen and increase of carbon dioxide concentrations in triple layer bags could be due to the temperatures inside these bags. Temperatures were markedly cooler compared to the infested woven bags. This difference is probably related to the reduced level of oxygen available in the triple layer bag. These results are similar with investigation conducted on grain storage by Denmead and Bailey (1966).

In another study it was found that reduced O_2 levels could be due to a locally concentrated insect population eventually leads to falling O_2 levels in other parts of the bag. These results were similar with the earlier investigations carried out on mode of action of triple layered PICS bags by Murdock *et al*. (2014).

4.2.7 Changes in germination percentage of stored groundnut pods at different moisture levels at different sets of storage periods

Percent germination changes in different storage bags at different moisture levels and at different sets of storage periods presented in Table 4.10 and illustrated in Fig 4.10.

Table 4.10 Effect of storage bags and pod moisture levels on per cent germination of groundnut kernels at different storage periods

Per cent changes in germination in different storage bags at 10 and 14 per cent moisture levels				
		2 Months	4Months	6 Months
Per cent moisture	10	73.00 (59.08)a	48.66 (37.44)a	0 (4.05)a
	14	54.16 (47.59)b	30.25 (22.71)b	0 (4.05) a
S.E (m)		0.78	0.47	0
C.D(P=0.01)		3.20	1.95	0
Treatments (Mean)				
Bag type	Triple layer bag	81.66 (64.81)a	63.833 (53.23)a	0 (4.05) a
	Polythene bag	65 (53.95)b	37.833 (37.77)b	0 (4.05) a
	Jute bag treated with Spinosad	60.9 (51.57)b	30.167 (33.21)c	0 (4.05) a
	Jute bag	46.6 (43.00)c	26.00 (30.07)d	0 (4.05) a
S.E (m)		1.10	0.67	0
C.D(P=0.01)		4.55	2.77	0
Interaction (Moisture x Bag type)				
10%	Triple layer bag	85.00 (67.37)a	77.00 (61.34)a	0 (4.05) a
	Polythene bag	75.00 (60.05)b	57.66 (49.39)c	0 (4.05) a
	Jute bag treated with Spinosad	73.66 (59.12)b	43.66 (41.33)d	0 (4.05) a
	Jute bag	58.33 (49.78)c	37.00 (37.44)d	0 (4.05) a
14%	Triple layer bag	78.33 (62.26)ab	68.00 (55.53)b	0 (4.05) a
	Polythene bag	55 (47.86)c	27.66 (31.70)e	0 (4.05) a
	Jute bag treated with Spinosad	48.34 (44.02)c	25.00 (29.98)e	0 (4.05) a
	Jute bag	35 (36.22)d	15.00 (22.71)f	0 (4.05) a
S.E (m)		1.56	0.95	0
C.D(P=0.01)		6.45	3.91	0

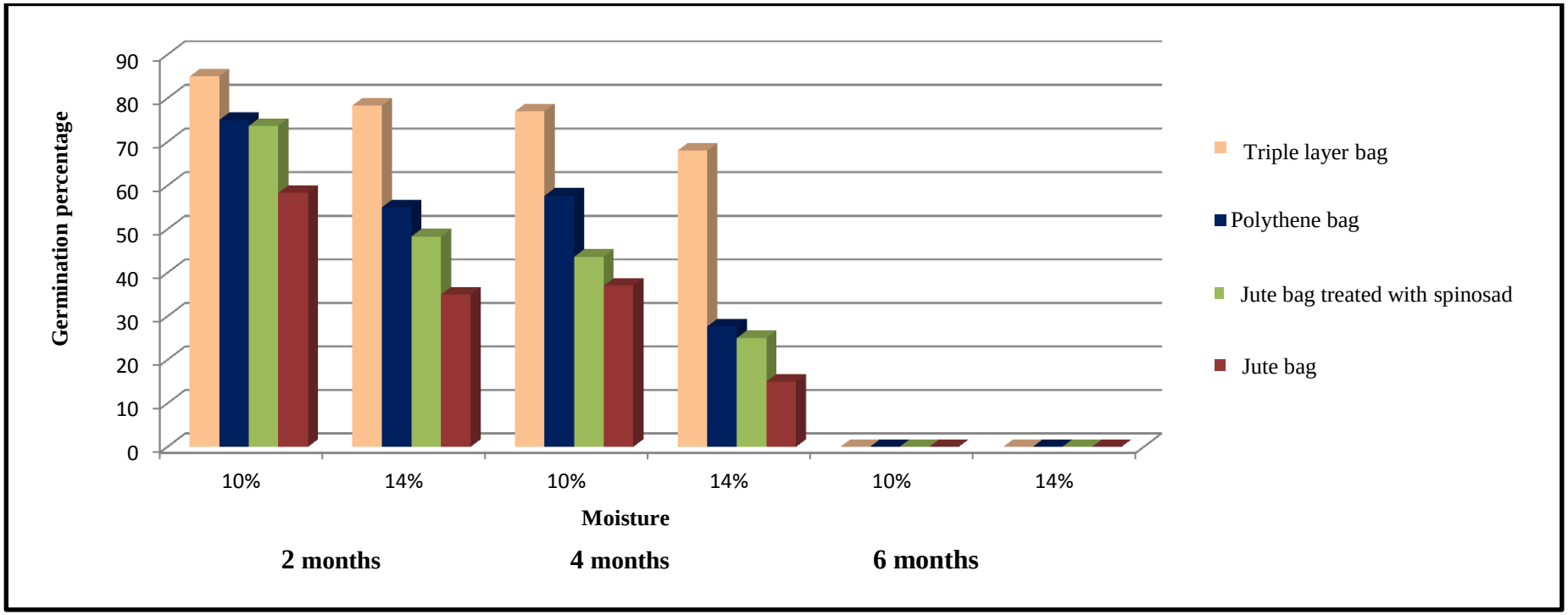


Fig 4.10. Effect of bag type and pod moisture content on per cent germination of groundnut kernels stored for 2, 4 and 6 months per

Results clearly indicate the effect of bag type on changes in germination percentages in stored groundnut pods. Maximum and minimum changes in germination percentage were recorded in jute bag (46.66, 26%) and triple layer plastic bag (81.66, 75%) after 2 and 4 months storage respectively and were significantly differ ($P<0.01$) from all other treated bags. After 2 months storage polythene bag (65%) and jute bag treated with spinosad (61.0%) were recorded on par with each other. After 4 months storage all treated bags were significantly differ ($P<0.01$) with each other. Results also indicate the significant effect ($P< 0.01$) of pod moisture content during storage on changes in percent germination. Maximum reduction changes were recorded at 14% moisture levels of 54.16 and 33.91% in comparison to 10% moisture levels of 73.0, 53.83 after 2 and 4 months storage respectively.

Interaction effect of moisture content and bag type on changes in germination percentage in storage bags results in maximum reduction in germination percentage were recorded at 14% moisture in comparison to 10% moisture.

After 2 months storage triple layer plastic bag (85% and 78.33%) at 10 and 14% moisture were recorded on par with each other. Triple layer plastic bag (78.33%) at 14% moisture was intern on par with polythene bag (75.0%) at 10% moisture. Polythene bag (75.0%) and jute bag treated with spinosad (73.66%) were recorded on par with each other at 10% moisture. Jute bag (58.33%) at 10% moisture and jute treated spinosad (48.33%), jute bag (35%) at 14% moisture were recorded on par with each other.

After 4 months storage the triple layer plastic bag (77 and 68%) consisting 10% and 14% moisture content pods recorded 77% and 68% germination respectively and were significantly differ with each other and also with other treated bags. Jute bag treated with spinosad (43.66%) and jute bag (37.0%) were recorded on par with each other consisting 10% moisture pods. Polythene bag (27.66%) and jute bag treated with spinosad (25.0%) were recorded on par with each other consisting 14% moisture pods. Jute bag consisting 14% moisture pods recorded lowest germination (25%) and significantly differs from all other treatments.

After 6 months storage complete loss in germination percentage was observed in all treatment bags at moisture levels of 10% and 14%.

Maximum reduction in percent germination was observed in the pods stored in jute bag, jute bag treated with spinosad and polythene bag with 14% and 10% moisture content pods. This might be due to high moisture content hastening the growth and

multiplication of fungi and insects. After 6 months of storage the pods were with completely damaged kernels and were recorded nil germination percentage in comparison to 2 and 4 months storage. These results are similar with earlier investigation done on maize storage by Njoroge *et al*. (2014).

After 2 and 4 months storage the triple layer bag recorded reduced levels of percent germination and complete loss in germination percentage after six months storage might be due to inherent loss of embryo vigour, high relative humidity and moisture content. These results are similar with earlier investigations done on cereal seed storage by Guberac *et al*. (2003).

4.2.8 Temperature and Relative humidity

The data pertaining to changes in temperatures and relative humidity in storage bags was given in Table 4.11and illustrated in Fig. 4.11.

The data loggers measured temperature and relative humidity inside each treatment combinations at different moisture levels of 10 and 14% every 24 hours. However, we presented the data and plotted based on bimonthly intervals of averages at 10% and 14% moisture. The comparison does not have statistical power and should only be considered as trends.

The data loggers recorded higher temperatures and low relative humidity in jute bag after 2 months (32.25°C, 46.8% & 32.18°C, 49%), 4months (33°C, 44.41% & 32.2°C, 47.41%) and 6 months (33.18°C, 43.41%& 32.56°C, 46.66%) of storage at 14% moisture in comparison to 10% moisture. Minimum temperature and maximum relative humidity recorded in triple layer plastic bag at 10% (29°C, 81.25%) moisture in comparison to 14% (30.18°C, 80.75%) which was not changed much from 2, 4 and 6 months storage.

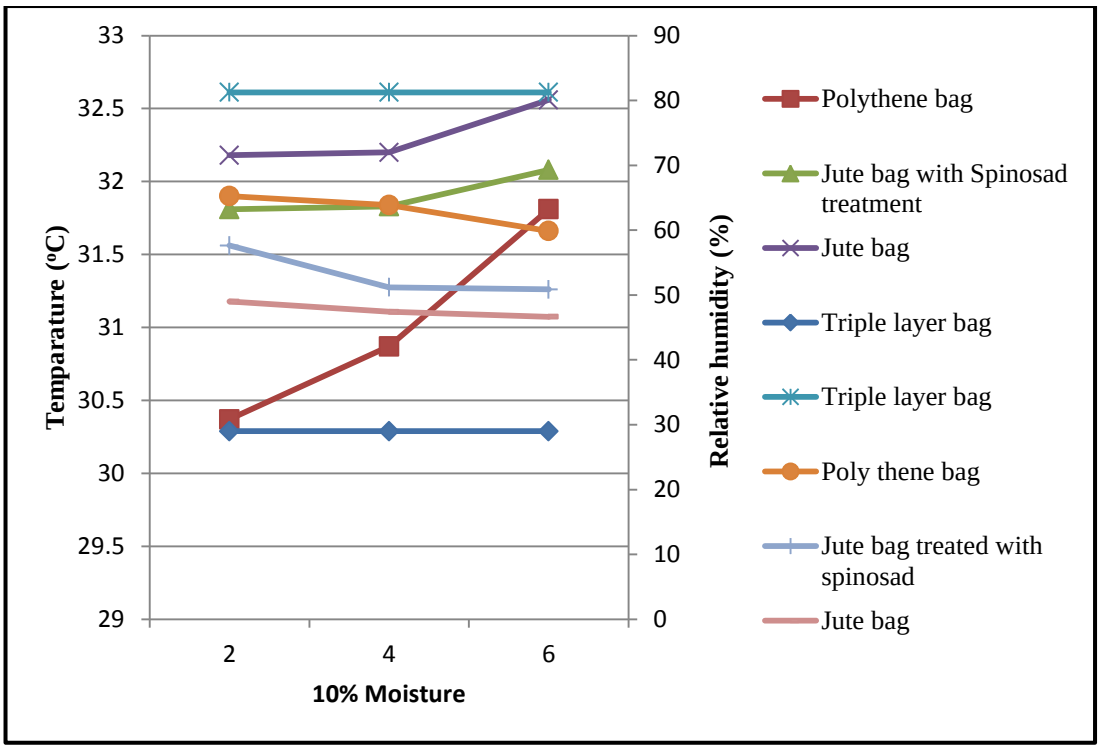
Maximum temperature and minimum relative humidity was recorded in jute bag followed by jute bag treated with spinosad and polythene bag at 14% moisture in comparison to 10% moisture after 6 months storage followed by 4 and 2 months storage.

The traditional jute bag, jute bag treated with spinosad and polythene bag were recorded maximum changes in temperature and relative humidity due to their porosity of bag structure; provide only slight restriction to O_2 movement across the bag walls. Access to oxygen in these bags allowed the insects to respire freely and leads to

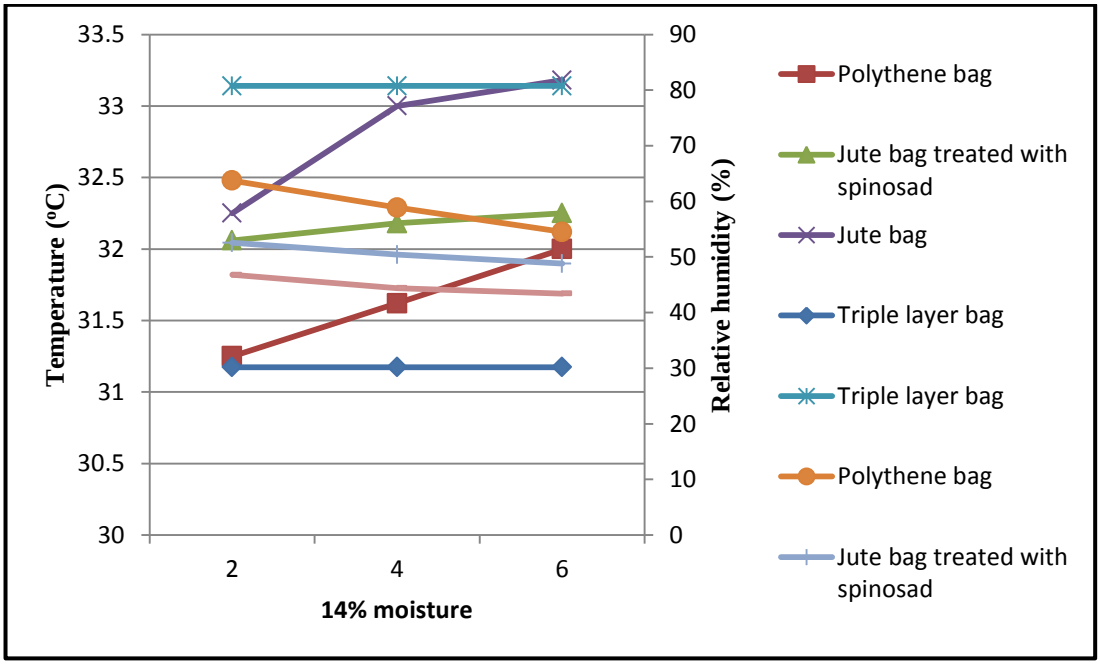
multiplication of insects and mold fungi and also bag material allowing moisture to be lost to the outside during drier winter months thus produce significantly more heat and reduced levels of relative humidity respectively. These results are similar to the investigations conducted on rice storage (Martin *et al*., 2015) and pigeonpea storage (Vales *et al.*, 2014).

Table 4.11 Mean temperature and relative humidity in different storage bags with pods of 10 and14 per cent moisture at 2, 4 and 6 months of storage

	2 Months			
	10%		14%	
	Temp (^{o}C)	R.H (%)	Temp (^{o}C)	R.H (%)
Triple layer bag	29	81.25	30.18	80.75
Polythene bag	30.37	65.25	31.25	63.75
Jute bag treated with spinosad	31.81	57.62	32.06	52.56
Jute bag	32.18	49	32.25	46.8
	4 Months			
	10%		14%	
Triple layer bag	29	81.25	30.18	80.75
Polythene bag	30.87	63.87	31.62	58.87
Jute bag treated with spinosad	31.83	51.18	32.18	50.43
Jute bag	32.2	47.41	33	44.41
	6 Months			
	10%		14%	
Triple layer bag	29	81.25	30.18	80.75
Polythene bag	31.81	59.87	32	54.5
Jute bag treated with spinosad	32.08	50.87	32.25	48.81
Jute bag	32.56	46.66	33.18	43.41



(A)



(B)

Fig 4.11.Mean Temperature and Relative humidity in different storage bags with pods of 10 and 14 per cent moisture at 2, 4 and 6 months of storage

4.2.9 Moisture content

The data pertaining to moisture content of the pods in different storage bags was given in Table 4.12 and illustrated in Fig. 4.12.

Table 4.12 Changes in moisture content of pods during storage in different types of bags

	Moisture (%)					
Bag type	2 months		4 months		6 months	
Triple layer bag	10	14	10	14	10	14
Polythene bag	9.25	6.02	5.33	5.33	5.33	5.33
Jute bag treated with spinosad	9.25	6.02	5.33	5.33	5.33	5.33
Jute bag	9.25	6.02	5.33	5.33	5.33	5.33

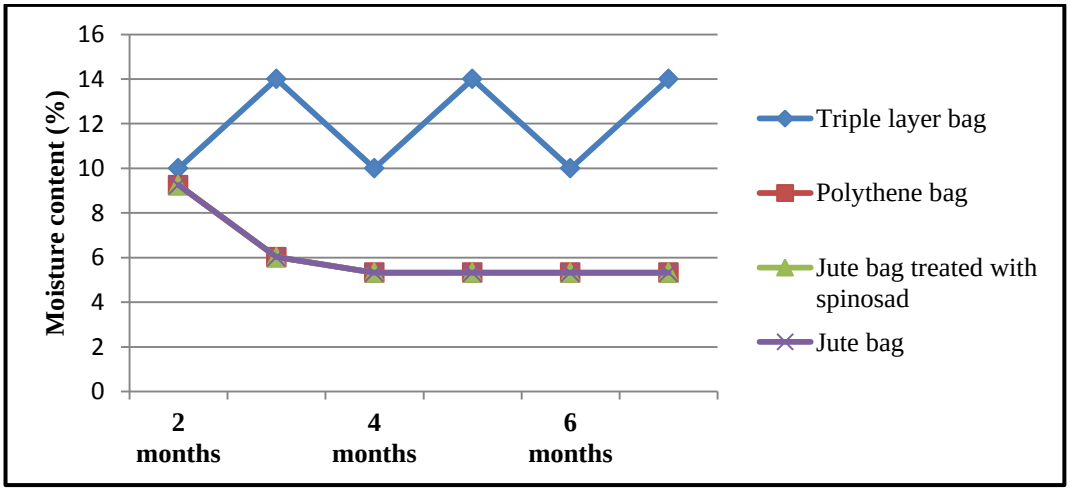


Fig 4.12.Changes in moisture content of pods during storage in different types of bags

Maximum decrease in moisture content was observed in jute bag with initial14% moisture pods in comparison to 10% moisture. However, the moisture content was more or less constant in triple layer bags after 2, 4 and 6 months of storage.

After 2 months, jute bag, polythene bag and jute bag treated with spinosad recorded minimum changes in moisture content 10% (9.25%) in comparison to 14% (6.02%).

After 4 and 6 months, jute bag, jute bag treated with spinosad and polythene bag did not differ significantly but differed at 10 and 14% moisture levels. As at 10%

moisture levels the moisture reduction was 5.33% and at 14% it was 4% due to higher rate of growth and multiplication of insects and fungi.

The decreased moisture content in treatment bags other than triple layer plastic bag could be due to decreased ambient relative humidity levels of the air during the drier winter months. These investigations are similar with earlier investigations on rice storage by Martin *et al* (2015).

The triple layer plastic bag appeared to retain the initial pod moisture of 10% and 14% and preserved it against changes related to seasonal variations outside environment due to its two impermeable HDPE liners inside the bag. They are essentially water tight, retaining existing water in the bag when it is tied shut. By contrast, the jute bag and other bag types are essentially open to the ambient air and in time slowly equilibrate with the outside environment. These results are similar with earlier investigations on maize storage by Edoh Ognakossan *et al* (2013).

4.3 Aflatoxins accumulation in groundnut pods stored in different storage bags with10% and 14% moisture contents

Aflatoxins accumulation in groundnut pods stored in different storage bags with 10% and 14% moisture levels for different storage periods presented in Table 4.13 and illustrated in Fig 4.13.

Results indicate the effect of bag type on changes in aflatoxin content in stored groundnut pods. The highest accumulation of toxins observed in pods stored in jute bag (4676.01, 1913.78 & 135.2 μgkg^{-1}) followed by jute bag treated with spinosad (4094.35, 1600.65 & 94.17 μgkg^{-1}) and polythene bag (3810.23, 1400.35 & 69.75 μgkg^{-1}) after 6, 4 and 2 months storage respectively. On the other hand minimal accumulation of toxins observed in triple layer plastic bag (2573.2, 578.06 & 13 μgkg^{-1}) after 6 months followed by 4 and 2 months respectively. All treated bags were significantly differing with each other after 2, 4 and 6 months of storage.

Results also indicate the significant effect ($P< 0.01$) of pod moisture content during storage on changes in aflatoxin content in stored groundnut pods. Maximum accumulation of toxin was observed at 14% moisture (128.2, 1687.15 & 4291.7 μgkg^{-1}) after 2 months of storage followed by 4 and 6 months in comparison to 10% moisture content pods (27.7, 1059.01 & 3285.19 μgkg^{-1}) respectively.

Interaction of moisture content and bag type was found significant with reference to aflatoxin content changes at different moisture levels at different storage periods.

Table 4.13 Effect of *C. serratus* and *A. flavus* on aflatoxin builds up in groundnut kernels stored in different bags at different moisture levels with different storage periods

Aflatoxin content (µgkg^{-1}) in different storage bags at 10 and 14 per cent moisture levels				
		2 Months	4Months	6 Months
Per cent moisture	10	27.70 (5.12)a	1059.04 (31.77)a	3285.22 (57.03)a
	14	128.20 (10.56)b	1687.40 (40.24)b	4291.7 (65.06)b
S.E(m)		0.02	0.48	0.01
C.D (P=0..01)		0.1	1.98	0.05
Bag type	Triple layer bag	13.00 (3.67)a	578.11 (23.85)a	2573.26 (50.71)a
	Polythene bag	69.45 (7.65)b	1400.35 (36.86)b	3810.23 (61.40)b
	Jute bag treated with Spinosad	94.16 (9.03)c	1600.64 (39.71)c	4094.35 (63.75)c
	Jute bag	135.19 (11.01)d	1913.78 (43.60)d	4676.01 (68.31)d
S.E(m)		0.03	0.68	0.02
C.D(P=0..01)		0.14	2.8	0.08
Interaction (Moisture x Bag type)				
10%	Triple layer bag	11.99 (3.53)a	456.00 (21.33)a	2444.46 (49.44)a
	Polythene bag	17.90 (4.29)c	938.60 (30.60)c	3036.10 (55.10)c
	Jute bag treated with Spinosad	29.17 (5.44)d	1230.56 (35.03)d	3401.71 (58.32)d
	Jute bag	51.76 (7.23)e	1611.00 (40.14)e	4258.50 (65.26)e
14%	Triple layer bag	14.01 (3.81)b	700.23 (26.38)b	2701.93 (51.98)b
	Polythene bag	121.00 (11.02)f	1862.10 (43.13)ef	4584.36 (67.71)f
	Jute bag treated with Spinosad	159.16 (12.63)g	1970.73 (44.40)f	4787.00 (69.19)g
	Jute bag	218.63 (14.80)h	2216.56 (47.06)f	5093.53 (71.37)h
S.E(m)		0.04	0.96	0.02
C.D(P=0..01)		0.2	3.96	0.11

*Values in the parenthesis are square root transformed values

*Values followed by the same letter are not significantly different

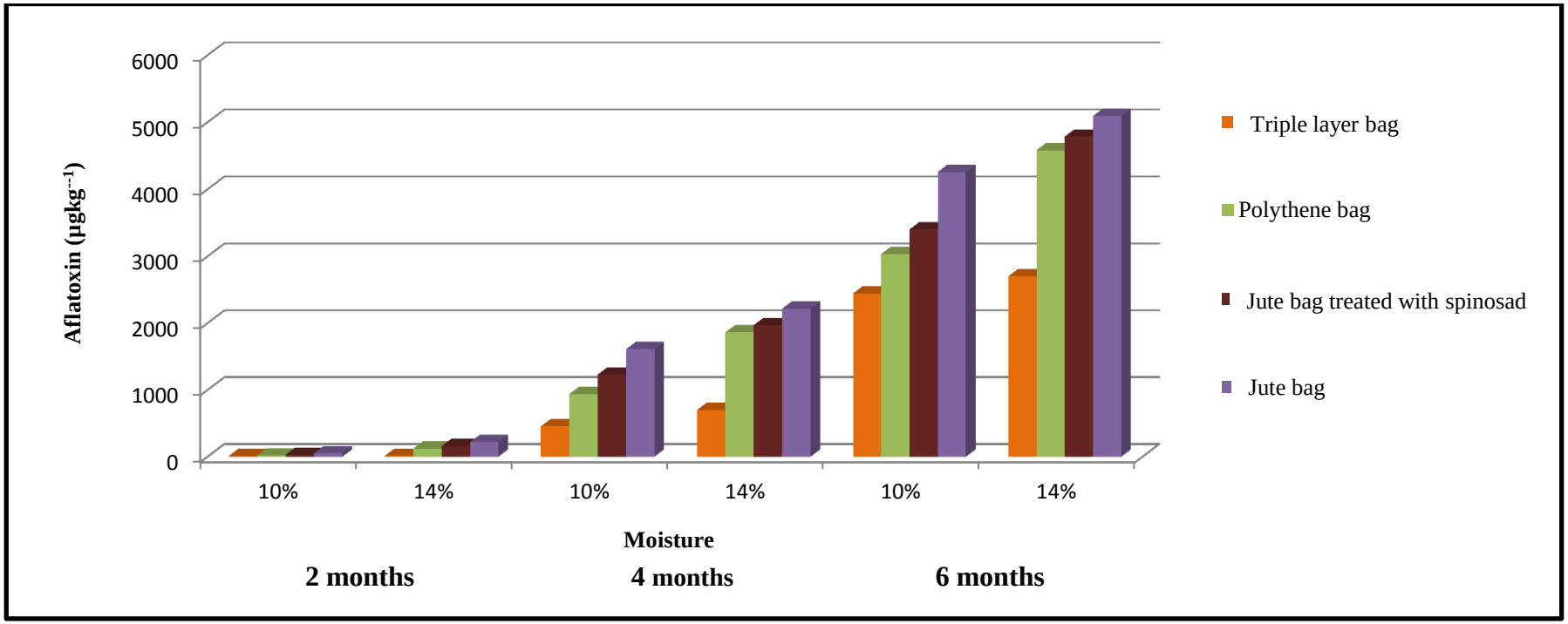


Fig. 4.13. Effect of bag type and pod moisture content on aflatoxin content of groundnut kernels after 2, 4 and 6 months of storage

After 2 and 6 months storage, all treated bags were significantly differing with each other at 10% and 14% moisture. After 4 months storage, jute bag (1611.01µgkg^{-1}) at 10% and polythene bag (1862.10µgkg^{-1}) at 14% moisture were recorded on par with each other. Polythene bag (1862.10µgkg^{-1}) was intern on par with jute bag treated with spinosad (1970.73µgkg^{-1}) and jute bag (2216.56µgkg^{-1}) which was also on par with each other.

Less aflatoxin accumulation was observed in triple layer plastic bag after 2, 4 and 6 months storage compared to other bag treatments. However, the aflatoxin build up was higher at 14% moisture (14.01, 700.23 & 2701.93 µgkg^{-1}) compared to 10% (11.99, 456.0 & 2444.46 µgkg^{-1}).

The maximum content of aflatoxin were recorded in jute bag at 14% moisture *i.e.*, 218.63, 2216.56 & 5093.53 µgkg^{-1}at 2, 4 and 6 months of storage, respectively followed by jute bag treated with spinosad and polythene bag. Then, the readings were also recorded at 10% moisture showed that maximum aflatoxin were recorded in jute bag *i.e.*, 121, 1862.10 & 4584.36 µgkg^{-1}at 2, 4 and 6 months of storage respectively followed by jute bag treated with spinosad and polythene bag.

Aflatoxin accumulation observed in triple layer plastic bag was low compared to other bags in the study. This could be due to increased levels of CO_2, reduced levels of $O_2$and less insect activity inside the bags which are detrimental to the fungal growth and aflatoxin production. But considerable amounts of aflatoxin content were observed in pods consisting 14% moisture compared to 10% moisture. This clearly indicates that the moisture content along with relative humidity and temperature plays an important role in molds fungi growth and aflatoxin production in groundnut storage. However, in an earlier study conducted on maize storage for 1 and 2 months using Purdue Improved Crop Storage (PICS) bags, there was no aflatoxin content observed in the moisture contents of 12-21% (Williams *et al*., 2014).

The highest amount of aflatoxin content was recorded in jute bag, jute treated spinosad and polythene bag due to absorption of moisture and temperature from external environment where ambient humidity was much higher in external environment which were favourable conditions to fungal multiplication in stored groundnut. These results in this direction are in agreement with the observations made by Njoroge *et al*. (2014).

The lowest amount of aflatoxin was recorded at 10 and 14% moisture at 2 months of storage due to high CO_2 and high and low moisture contents are the factor that limits the growth of fungi for short duration periods. These results are similar with investigation conducted on peanut storage before drying by Mosley *et al*. (1971).

The highest amount of aflatoxin was detected in artificial inoculated jute bag followed by jute bag treated with spinosad and polythene bag consisting 14% moisture and 10% moisture groundnuts. This could be due to high *A. flavus* growth and more insect multiplication at high moisture content than at low moisture content. The damaged pods are more prone to build up of aflatoxin than undamaged pods; bruchids play a role in enhancing the infection by aflatoxigenic molds. These results are similar with investigations conducted on groundnut storage by Sudini *et al.* (2015).

The high levels of aflatoxin content was recorded at 6 months after storage than 2 and 4 months storage as longer storage period enhances the build up of aflatoxins under conducive environmental conditions. These results are similar with investigations conducted on maize by Udoh *et al.* (2000).

4.4 Effect of *C. serratus* and *A.flavus* infestation on total oil content and fatty acid composition of groundnut kernels with different moisture levels in different storage bags for different sets of storage periods

The insects act as vectors in spreading the diseases or spores from one part to other parts. Insect contaminated with *A.flavus* in stored groundnuts was recorded maximum content of aflatoxin and ultimately leads to changes in oil and fatty acid composition of groundnut kernels which were unfit for human consumption.

The fungal population was increased during storage periods at high moisture levels in comparison to that of low moisture levels. The growth of *Aspergillus* is more in peanut samples due to better adaptation of these fungi to this substrate throughout storage, but *Aspergillus* growth was low at 10%moisture in comparison to 14-20% (Ghosh *et al*., 1996).

4.4. (a) Changes in linoleic acid content in different storage bags at different moisture levels at different sets of storage periods

The results on linoleic acid changes at different sets of storage periods are presented in Table 4.14 and illustrated in Fig.4.14.

Results indicate the effect of bag type on changes in linoleic acid content in stored groundnut kernels After two, four and six months storage the triple layer plastic bag was recorded less linoleic acid change (34.92%, 33.66% and 31.54%) and was significantly differ ($P<0.01$) from other conventional treated bags. Polythene bag (30.7%, 30.02%) and jute bag treated with spinosad (30.03%, 29.49%) were recorded on par with each other and also jute bag (28.0%, 27.17%) were recorded significant differ with each other after 2 and 4 months storage respectively. After six months storage jute bag treated with spinosad (27.43%) and jute bag (26.87%) were recorded on par with each other. Maximum reduction of linoleic acid content was recorded in jute bag 29.95%, 27.17% and 26.87% after 2, 4 and 6 months storage respectively.

Results also indicate the significant effect ($P<0.01$) of pod moisture on linoleic acid content were observed during storage. The less reduction in linoleic acid content were observed at 10% moisture (31.86, 31.29 &28.88%) in comparison to 14% moisture (31.12, 28.88 & 27.27%) after 2, 4 and 6 months storage respectively

Among all the treatments at different sets of 2, 4 and 6 months storage periods the triple layer bag gave fewer changes in linoleic acid content. Maximum changes observed in jute bag followed by jute bag treated with spinosad and polythene bag. Maximum changes occur at six months storage than 2 and 4 months storage.

After 2, 4 and 6 months storage the interaction between moisture content and type of bag reveal that the minimum changes in linoleic acid content recorded in triple layer bag at 10% (36.01, 34.93 & 32.05) and 14% (33.83, 32.4 & 31.03) moisture were recorded significantly differ with all other treatments.

After 2 months storage jute bag (25.53%) at 14% moisture was significant differ with maximum reduction of linoleic acid content in comparison of all other treated bags. Polythene bag (31.03 %) jute bag treated with spinosad (30.65%) and jute bag were recorded on par with each other at 10% moisture. Jute bag treated with spinosad (30.65%) intern on par with jute bag (30.48%) at 10% moisture and polythene bag (30.38%) at 14% moisture. Jute bag (30.48%) at10% moisture and polythene bag

(30.38%) was recorded intern on par with jute bag treated with spinosad (29.95%) at 14% moisture.

Table 4.14 Effect of storage bags and pod moisture content on per cent change in linoleic acid content at different storage periods

Per cent change in linoleic acid in different storage bags				
		2 Months	4Months	6 Months
Per cent moisture	10	32.04a	31.29a	30.13a
	14	29.92b	28.88b	27.26b
S.E (m)		0.06	0.11	0.11
C.D (P= 0.01)		0.28	0.48	0.47
Bag type	Triple layer bag	34.92a	33.66a	31.54a
	Polythene bag	30.70b	30.02b	28.96b
	Jute bag treated with Spinosad	30.30b	29.49b	27.43c
	Jute bag	28.00c	27.17c	26.86c
S.E (m)		0.09	0.16	0.16
C.D(P= 0.01)		0.4	0.7	0.67
Interaction (Moisture x Bag type)				
10%	Triple layer bag	36.01a	34.93a	32.05a
	Polythene bag	31.03c	30.96c	29.92c
	Jute bag treated with Spinosad	30.65cd	29.99cd	29.74cd
	Jute bag	30.48cde	29.30d	28.83de
14%	Triple layer bag	33.83b	32.40b	31.03b
	Polythene bag	30.38d	29.09de	28.00e
	Jute bag treated with Spinosad	29.95e	29.00e	25.13f
	Jute bag	25.53f	25.05f	24.89f
S.E (m)		0.13	0.23	0.23
C.D(P= 0.01)		0.56	0.97	0.95

*Values followed by the same letter are not significantly different

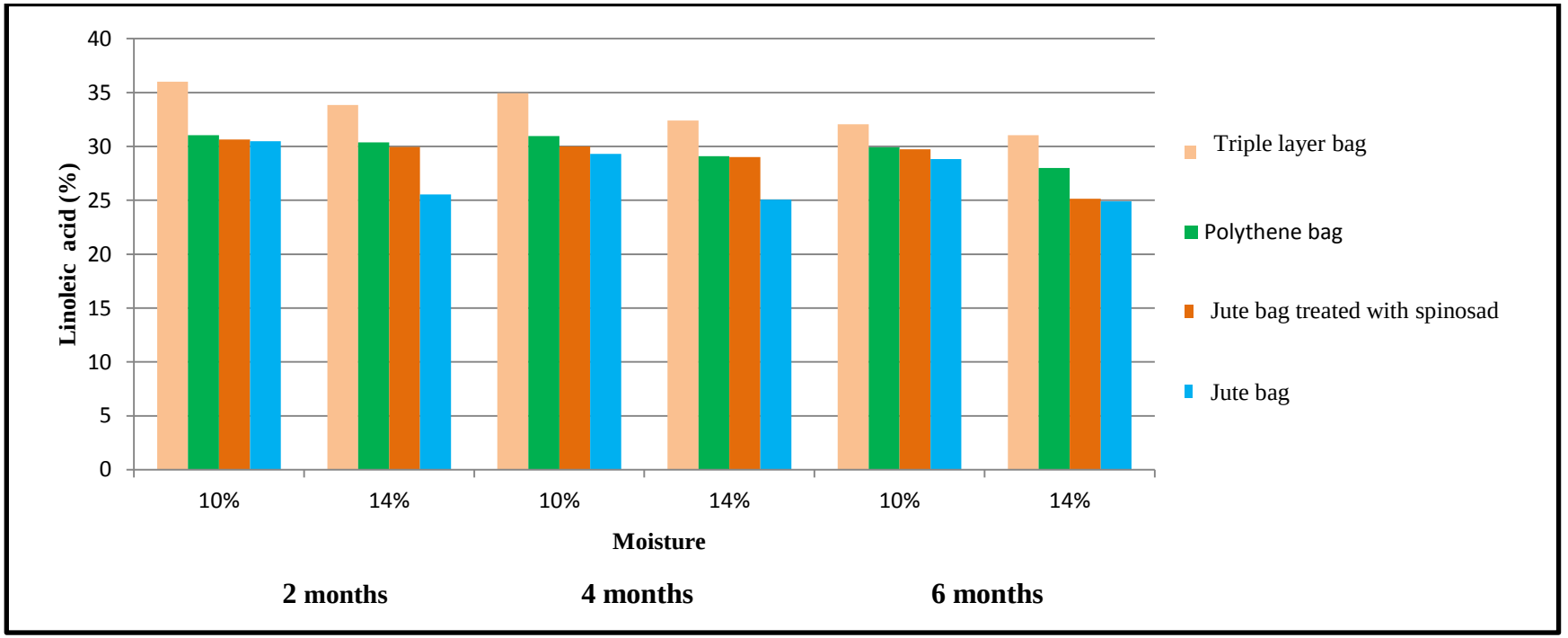


Fig 4.14. Effect of bag type and pod moisture content on linoleic acid content of groundnut kernels stored for 2, 4 and 6 months period

After 4 months storage the jute bag (25.05%) was significantly differ in maximum changes in linoleic acid content at 14% moisture. Polythene bag (30.96%), jute bag treated with spinosad (29.99%) was recorded on par with each other and jute bag treated with spinosad was intern on par with jute bag (29.3%) at 10% moisture. Jute bag at percent moisture of 10 and polythene bag (29.09%) at 14% moisture were recorded on par with each other which was intern on par with jute bag treated with spinosad (29.0%) at 14% moisture.

After six months storage the polythene bag (29.92%), jute bag treated with spinosad (29.74%) were recorded on par with each other while jute bag treated with spinosad was intern on par with the jute bag (28.83%) at 10% moisture. Jute bag at 10% moisture was intern on par with the polythene bag (28.0%) at 14% moisture. Jute bag treated with spinosad (25.13%) and jute bag (24.90%) were on par with each other at 14% moisture.

4.4. (b) Changes in oleic acid content in different storage bags at different moisture levels at different sets of storage periods

The results on oleic acid changes at different sets of storage periods are presented in Table 4.15 and illustrated in Fig. 4.15.

Results indicate the effect of bag type on changes in oleic acid content in stored groundnut kernels. The triple layer plastic bag (55.73, 55 and 54 %) was recorded minimum changes in oleic acid content. Maximum reduction was observed in jute bag (50.96, 50.12 and 48.44%) stored kernels in comparison of other treatments after 2, 4 and 6 months storage respectively. After 2 and 4 months storage polythene bag (52.71, 52%) and jute bag treated with spinosad (52.02, 51.22%) were recorded on par with each other. After 6months storage all treated bags were significantly differ ($P<0.01$) with each other.

Results also indicate the significant effect ($P<0.01$) of pod moisture content during storage on oleic acid content changes. The maximum changes in oleic acid content were found at 14% moisture (51.47, 50.38 & 49.79%) in comparison of 10 (54.22, 53.80 &52.07%) after 2, 4 and 6 months storage respectively.

Interaction effect of moisture and bag type on oleic acid changes in stored kernels reveal that maximum changes were observed in jute bag followed by jute bag treated with spinosad and polythene bag at 14% moisture in comparison to 10%

moisture. Triple layer bag recorded little changes in oleic acid content at different sets of storage periods.

Table 4.15 Effect of storage bags and pod moisture content on per cent change in oleic acid content at different storage periods

Per cent changes in oleic acid content in different storage bags with different moisture levels				
		2 Months	4 Months	6 Months
Per cent moisture	10	54.22a	53.80a	52.07a
	14	51.47b	50.38b	49.79b
S.E(m)		0.08	0.24	0.14
C.D(P= 0.01)		0.34	1.02	0.61
Bag type	Triple layer bag	55.73a	55.00a	54.00a
	Polythene bag	52.71b	52.02b	51.01b
	Jute bag treated with Spinosad	52.00b	51.22bc	50.27b
	Jute bag	50.96d	50.12c	48.44c
S.E(m)		0.11	0.35	0.2
C.D(P= 0.01)		0.48	1.45	0.86
Interaction (Moisture x Bag type)				
10%	Triple layer bag	55.90a	55.50a	54.20a
	Polythene bag	54.66b	53.63ab	52.00b
	Jute bag treated with Spinosad	53.34c	53.14b	51.86b
	Jute bag	53.00c	52.93b	50.23c
14%	Triple layer bag	55.56a	54.50ab	53.80a
	Polythene bag	50.76d	50.42c	50.02c
	Jute bag treated with Spinosad	50.66d	49.30cd	48.68d
	Jute bag	48.92e	47.31d	46.66e
S.E(m)		0.16	0.5	0.3
C.D(P= 0.01)		0.7	2.05	1.22

*Values followed by the same letter are not significantly different

*Values followed by the same letter are not significantly different

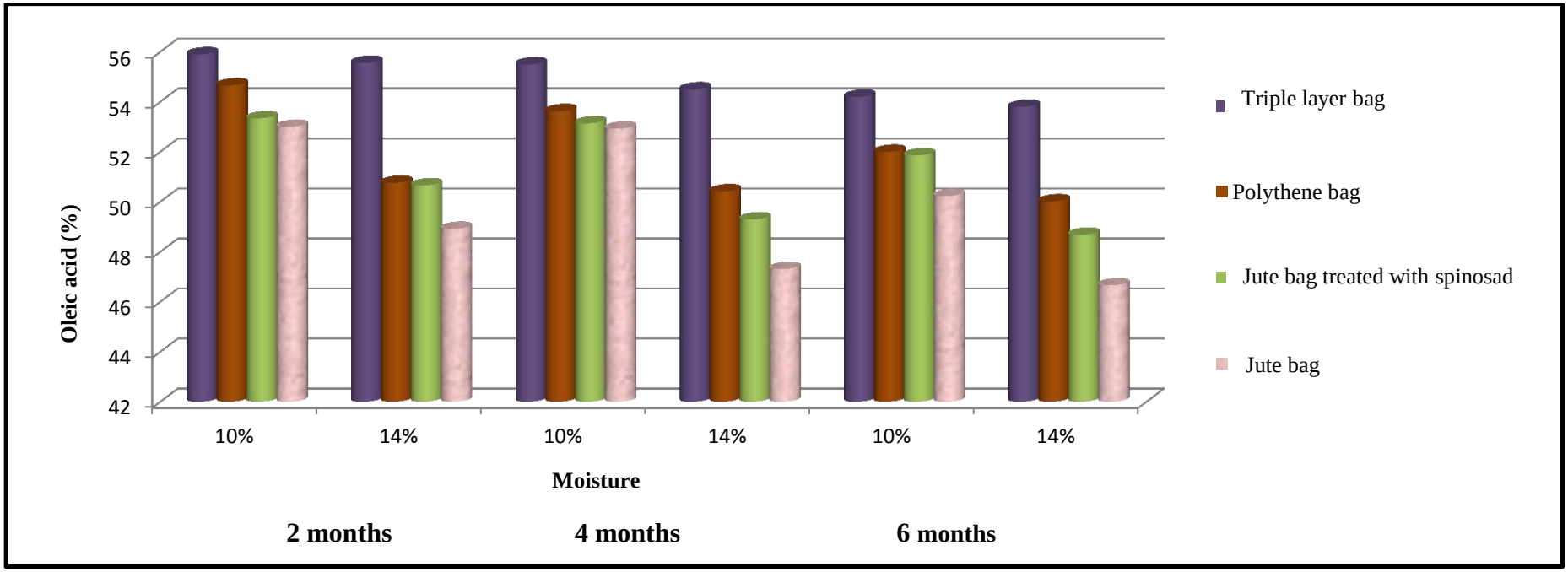


Fig 4.15. Effect of bag type and pod moisture content on oleic acid content of groundnut kernels stored for 2, 4 and 6 months of period

The triple layer bag at 10 and 14% moisture were recorded minimum changes in oleic acid content after 2 (55.9 & 55.56%), 4 (55.5 & 54.5) and 6 (54.2 & 53.8%) months storage which were recorded on par with each other respectively and significant different from other treatments.

After 2 months of storage, jute bag treated with spinosad (53.34%) and jute bag (53%) at10% moisture. Polythene bag (50.76%) and jute treated spinosad (50.66%) at 14% moisture were recorded on par with each other.

After 4 months of storage the polythene bag (53.63%) was recorded intern on oar with jute bag treated with spinosad (53.14%) and jute bag (52.93%) at 10% moisture. Polythene bag (50.42%) and jute bag treated with spinosad (49.3%) were recorded on par with each other at 14% moisture where as jute bag treated with spinosad was recorded intern on par with jute bag (47.31%) at 14% moisture.

After 6 months storage the polythene bag (52%) and jute bag treated with spinosad (51.86%) were recorded on par with each other at 10% moisture. Jute bag (50.23%) at 10% moisture and polythene bag (50.02%) at 14% moisture were recorded on par with each other. Jute bag treated with spinosad (48.68%) and jute bag (46.66%) were recorded significantly differ with each other at 14% moisture.

From Tables 4.14 and 4.15 reveal that linoleic and oleic acid content was decreased at different sets of two, four and six months storage. Maximum decrease was observed at six months storage in comparison to two and four month's storage. Maximum changes occur at 14% moisture in jute bag followed by jute bag treated with spinosad and polythene bag in comparison to 10% moisture, where as minimum changes observed in triple bag stored kernels due bay type and moisture effect. As triple layer plastic bag protect the storable product from external environment against oxygen, relative humidity and temperature as its bag structure with three layers would not allow the external oxygen, relative humidity and temperature and a proportional decrease in oxygen and increase in carbon dioxide with no changes in moisture under sealed conditions were major factors in changes in acid content (Weinberg *et al*., 2008).

In jute bag, jute bag treated with spinosad and polythene bags were gain the external oxygen moisture, relative humidity and temperature. Major changes in linoleic and oleic acid changes occur these bags due to insect and fungal multiplication by utilizing the oxygen which was available and also increased pod moisture, relative

humidity and temperature dictate microorganisms and insect degrade the storage groundnut pods (Magan *et al*., 2003).

Linoleic and oleic acid content decreased due to huge multiplication of fungi due to insect damage to the pods while insect damaged emergence pods allowed more atmospheric oxygen into the pods. Kernel lipases and fungal build up increase the acidity of kernels. Lipid deterioration occurs due to increased peroxide value by direct autocatalytic attack of atmospheric oxygen. At high moisture levels during storage, seed lipoxygenase activity increases the lipid deterioration. Due to lipolytic activity of pods and fungi results in lipid deterioration which further leads to decrease in linoleic and oleic acid content in stored kernels. The present experimental results are in par with the earlier investigations and changes in acid content due to lipid deterioration during storage by activity of insect and fungus in presence of moisture, relative humidity and temperature (Gopinath *et al.,* 2011).

4.4. (c) Changes in palmitic acid content in different storage bags at different moisture levels at different sets of storage periods

The results on palmitic acid changes at different sets of storage periods are presented in Table 4.16 and illustrated in Fig 4.16.

Results clearly indicate the significant effect ($P<0.01$) of pod moisture content during storage on palmitic acid content changes. Maximum changes observed at 14% moisture (11.99, 14.67 & 15.21%) in comparison to 10% moisture (9.65, 13.57 & 14.32%) after 2, 4 and 6 months storage respectively.

Results also indicate the effect of bag type on changes in palmitic acid content in stored groundnut kernels. Maximum increase in palmitic acid content was recorded in jute bag (15.75, 14.88 &12.52%) followed by jute bag treated with spinosad (14.96, 14.62 & 11.42%) and polythene bag (14.61, 14.38 & 10.09%) and minimum changes observed in triple layer plastic bag (13.75, 12.61 &9.26%) after 6 months storage followed by 4 months and 2 months storage respectively. All treated bags were significantly differing ($P<0.01$) with each other after 2, 4 and 6 months storage period.

Interaction of moisture and bag type was found significant ($P<0.01$) regarding to palmitic acid changes at different moisture levels at different storage periods.

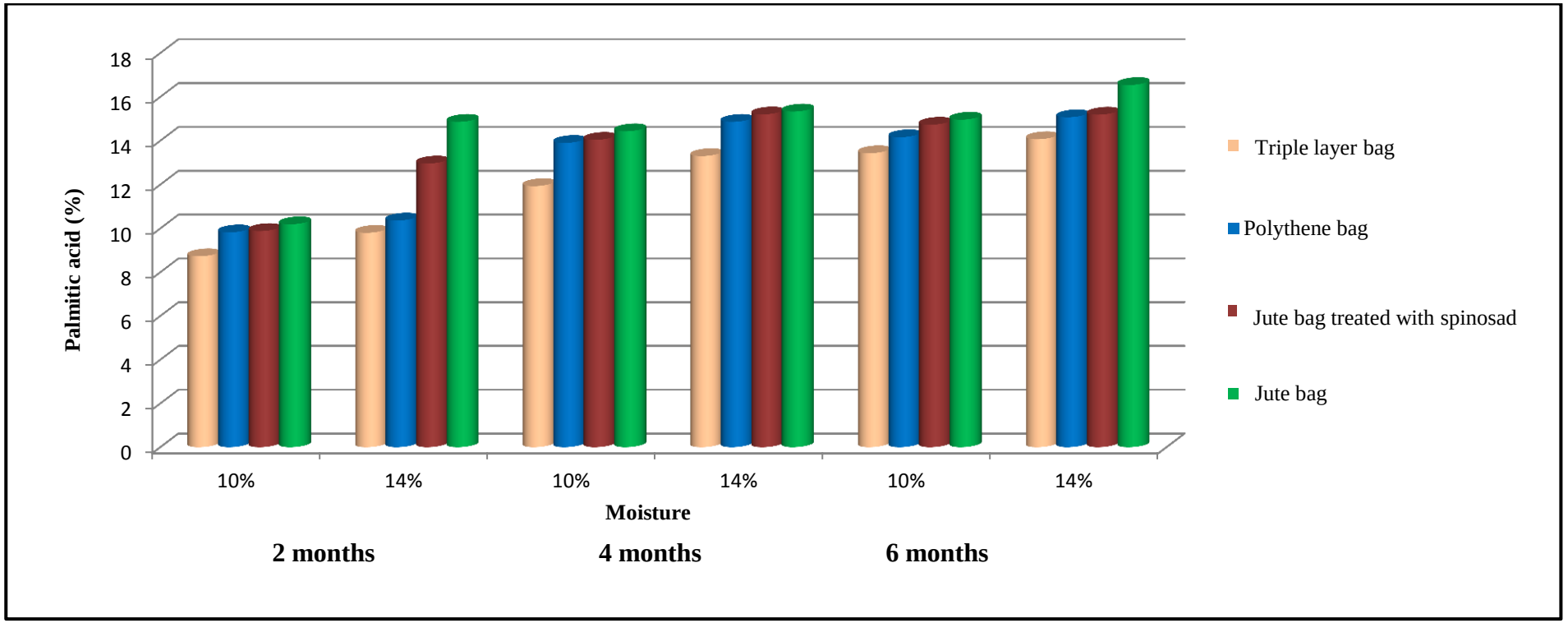
After 2, 4 and 6 months storage periods the triple layer plastic bag was recorded significantly differ from all other treatments with little change in palmitic acid content

but more changes recorded after 6 months storage (13.43, 16.54%) followed by 4 months (11.93, 13.3%) and 2 months storage (8.73, 9.8%) at 10 % moisture and at 14% moisture respectively.

Table 4.16 Effect of storage bags and pod moisture content on per cent change in palmitic acid content at different storage periods

Per cent changes in palmitic acid content in different storage bags at different moisture levels				
		2 Months	4Months	6 Months
Per cent moisture	10	9.65a	13.57a	14.32a
	14	11.99b	14.67b	15.21b
S.E(m)		0.03	0.03	0.01
C.D(P= 0.01)		0.12	0.13	0.06
Bag type	Triple layer bag	9.26 a	12.61a	13.75a
	Polythene bag	10.09 b	14.38b	14.61b
	Jute bag treated with Spinosad	11.42c	14.62c	14.96c
	Jute bag	12.52d	14.88d	15.75d
S.E(m)		0.04	0.04	0.02
C.D(P= 0.01)		0.18	0.2	0.08
Interaction (Moisture x Bag type)				
10%	Triple layer bag	8.73a	11.93a	13.43a
	Polythene bag	9.82b	13.90c	14.16b
	Jute bag treated with Spinosad	9.88b	14.04c	14.73c
	Jute bag	10.19c	14.43d	14.96d
14%	Triple layer bag	9.8b	13.30b	14.07b
	Polythene bag	10.36c	14.86e	15.07d
	Jute bag treated with Spinosad	12.96d	15.20f	15.19e
	Jute bag	14.86e	15.33f	16.54f
S.E(m)		0.06	0.06	0.028
C.D(P= 0.01)		0.25	0.27	0.11

*Values followed by the same letter are not significantly different



4.16. Effect of bag type and pod moisture content on palmitic acid content of groundnut kernels stored for 2, 4 and 6 months of period

After 2 months storage, the polythene bag (9.82%), jute bag treated with spinosad (9.88%) at 10% moisture and triple layer plastic bag (9.8%) at 14% moisture were recorded on par with each other. Jute bag (10.19%) at 10% moisture and polythene bag (10.36%) at 14% moisture were recorded on par with each other. Jute bag treated with spinosad (12.96%) and jute bag (14.86%) at 14% moisture were recorded significantly differ ($P<0.01$) with each other.

After 4 month storage, the jute bag (14.43%) at 10% moisture and triple layer bag (13.30%) and polythene bag (14.86%) at 14% moisture were significantly ($P<0.01$) differ from all other treatments. Polythene bag (13.90%) and jute bag treated with spinosad (14.04%) at 10% moisture were recorded on par with each other. Jute bag treated with spinosad (15.20%) and jute bag (15.33%) at14% moisture were recorded on par with each other.

After 6 months storage the jute bag treated with spinosad (14.73%, 15.19%) at 10% and 14% moisture and jute bag (16.54%) at 14% moisture were significantly ($P<0.01$) differ from other treatments. Jute bag (14.96%) at 10% moisture and polythene bag (15.07%) at 14% moisture were on par with each other.

4.4. (d) Changes in stearic acid content in different storage bags at different moisture levels for different storage periods

The results on stearic acid changes at different sets of storage periods are presented in table 4.17 and illustrated in Fig. 4.17.

Results indicate the effect of bag type on changes in stearic acid content in stored groundnut kernels. Maximum increase observed in jute bag (3.73, 3.67 & 3.57%) followed by jute bag treated with spinosad (3.67, 3.60 & 3.41%) and polythene bag (3.54, 3.52 & 3.32%) after 6, 4 and 2 months storage respectively. On the other hand, minimum changes were observed in triple layer plastic bag (3.06, 3.29 & 3.32%) after 6 months of storage followed by 4 and 2 months. All treated bags were significantly ($P<0.01$) differing with each other after 2, 4 and 6months.

Results also indicate the significant effect ($P<0.01$) of pod moisture content during storage on stearic acid content changes. Maximum changes observed at 14% moisture (3.46, 3.6 & 3.66%) in comparison to 10% moisture (3.21, 3.43 &3.47%) after 2 months followed by 4 months and 6 months storage respectively.

Interaction of moisture and bag type was found significant (P<0.01) regarding to stearic acid changes at different moisture levels at different storage periods.

Table 4.17 Effect of storage bags and pod moisture content on per cent change in stearic acid content at different storage periods

Per cent changes in stearic acid content in different storage bags at different moisture levels				
		2 Months	4Months	6 Months
Per cent moisture	10	3.21a	3.43a	3.47a
	14	3.46b	3.60b	3.66b
S.E(m)		0.02	0.01	0.005
C.D(P= 0.01)		0.08	0.04	0.02
Bag type	Triple layer bag	3.06a	3.29a	3.32a
	Polythene bag	3.32b	3.52b	3.54b
	Jute bag treated with Spinosad	3.41b	3.59c	3.67c
	Jute bag	3.57c	3.67d	3.73d
S.E(m)		0.03	0.016	0.007
C.D(P= 0.01)		0.11	0.05	0.026
Interaction (Moisture x Bag type)				
10%	Triple layer bag	3.05a	3.27a	3.30a
	Polythene bag	3.12ab	3.41b	3.44b
	Jute bag treated with Spinosad	3.28bc	3.52c	3.56c
	Jute bag	3.40cd	3.54c	3.58c
14%	Triple layer bag	3.06a	3.31a	3.34a
	Polythene bag	3.52d	3.63d	3.64d
	Jute bag treated with Spinosad	3.54d	3.67d	3.79e
	Jute bag	3.74e	3.81e	3.88f
S.E(m)		0.04	0.02	0.009
C.D(P= 0.01)		0.17	0.08	0.04

*Values followed by the same letter are not significantly different

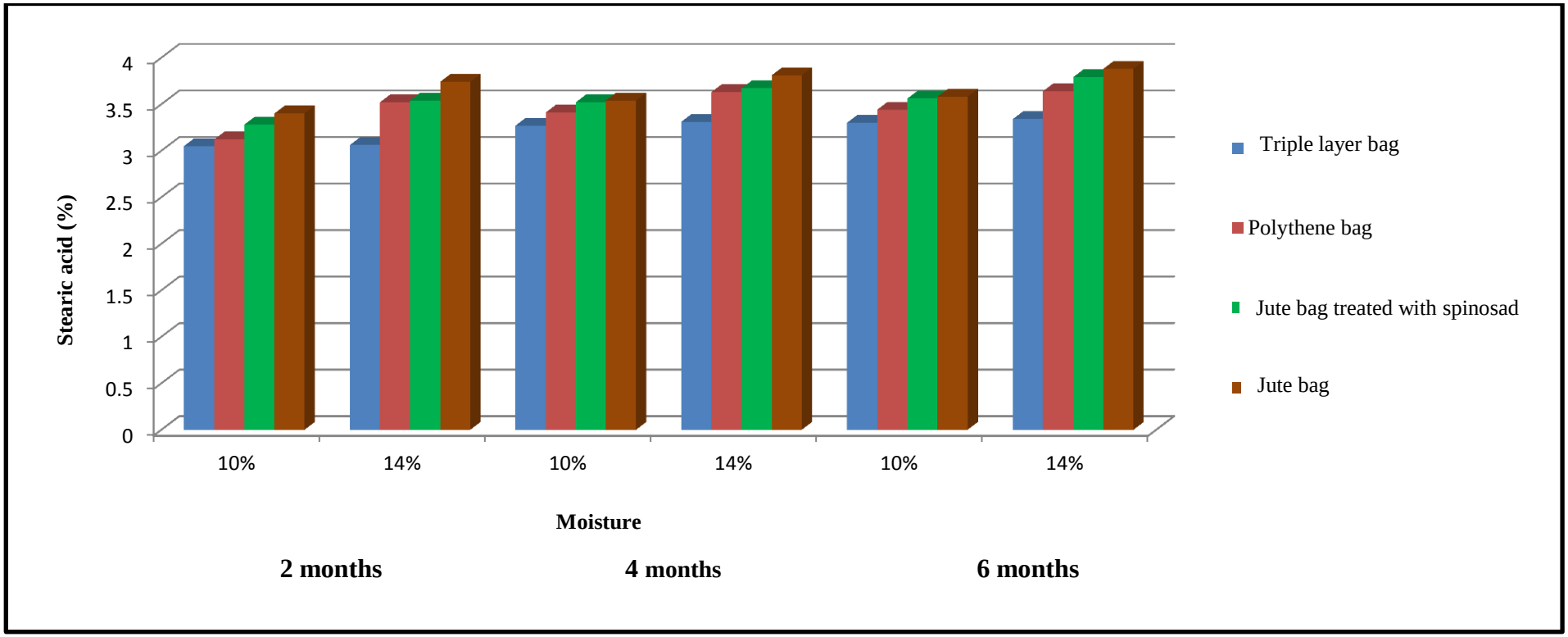


Fig. 4.17. Effect of bag type and pod moisture content on stearic acid content of groundnut kernels stored for 2, 4 and 6 months of period

After 2, 4 and 6 months storage, pods stored in the triple layer plastic bags (3.05, 3.27 & 3.30%) recorded minimum changes in stearic acid at 10% in comparison to 14% moisture (3.06, 3.31 & 3.34) were recorded on par with each other and also significantly differ (P<0.01) from other treatments. Jute bags (3.74, 3.81 &3.88%) at 14% moisture was recorded maximum increase in stearic acid content after 2, 4 and 6 months storage respectively and also significantly (P<0.01) differ from other treatments.

After 2 months, triple layer plastic bags (3.05%) consisting 10 and 14% moisture content pods were recorded on par with polythene bag (3.12%) at 10% moisture. Polythene bag (3.12%) intern on par with the jute bag treated with spinosad (3.28%) at 10% moisture, jute bag treated with spinosad (3.28%) was intern on par with jute bag (3.40%) at 10% moisture. Jute bag (3.40%) at 10% moisture was intern on par with polythene bag (3.52%) and jute treated spinosad (3.54) at 14% moisture.

After 4 months, polythene bag (3.41%) at 10% moisture and jute bag (3.81%) at 14% moisture were significantly (P<0.01) differ from other treatments. Jute bag treated with spinosad (3.52%) and jute bag (3.54%) at 10% moisture was recorded on par with each other. Polythene bag (3.63%) and jute bag treated with spinosad (3.67%) at 14% moisture were recorded on par with each other.

After 6 months, jute treated spinosad (3.56%) and jute bag (3.58%) at 10% moisture were recorded on par with each other. Polythene bag (3.44%) at 10% moisture and polythene bag (3.64%), jute treated spinosad (3.79%) and jute bag (3.88%) at 14% moisture were significantly (P<0.01) differ with each other.

From table 4.16 and 4.17 as previously mentioned that the triple layer plastic bag reveals little changes in stearic and palmitic acid content due to the effect of moisture and increased carbon dioxide contents.

Jute bag, jute treated spinosad and polythene bag were able to absorb the moisture content, relative humidity and temperature from external environment. These facilities are favourable to multiply the fungi. The increase in free fatty acids (palmitic and stearic acid) due to lipolytic activity of fungus as lipids are triglycerides and their hydrolysis leads to formation of free fatty acids and glycerol which cause the deterioration of kernels (Roberts *et al.,* 1987). The maximum increase in saturated free fatty acids (stearic and palmitic) observed in the pods stored at higher moisture (14%) levels in comparison to the pods with low moisture (10%). This could be majorly due to

decrease in oxidation index (∑ UFA/∑ SFA) in storage periods at different moisture levels. Decrease in polyunsaturated fatty acids and increase in saturated fatty acids was more pronounced for nuts adjusted at high moisture levels. These results were in agreement with Bhatti *et al*. (2012).

Increase in the content of free fatty acids from lipids occurs by the action of lipase and phospholipase enzymes present in the soybeans or produced by the associated microflora, which contribute to the breaking of ester linkages of triglycerides. These results are similar with earlier investigations conducted on benagalgram by Modgil & Metha. (1996).

4.4. (e) Changes in oil content in different storage bags at different moisture levels at different sets of storage periods

The results on changes in oil content at different sets of storage periods are presented in table 4.18 and illustrated in Fig. 4.18.

Results indicate the effect of bag type on changes in total oil content in stored groundnut kernels. Maximum decrease observed in the pods stored in jute bag (50.12, 50.73 & 51.06%) followed by jute bag treated with spinosad (50.75, 51.23 & 51.68%) and polythene bag (50.82, 51.61 & 51.92%) after 2, 4 and 6 months storage respectively. On the other hand, minimal variations observed in the pods stored in triple layer plastic bag (52.2, 53.0 & 53.12%) after 6 months of storage followed by 4 and 2 months of storage. All treated bags were significantly differing ($P<0.01$) with each other after 2, 4, and 6 months.

Results clearly indicate the significant effect ($P<0.01$) of pod moisture content during storage on changes in oil content. Maximum changes observed at 14% moisture (51.37, 50.86 & 50.35%) after 2 months of storage followed by 4 and 6 months in comparison to 10% (52.52, 52.41 & 51.59%).

After 2, 4, and 6 months, triple layer bags consisting pods with 10% (53.5, 53.4 & 52.6%) and 14% (52.75, 52.6 & 51.8%) moisture were recorded significantly from other treatments.

After 2 months, the total oil content in the 14% moisture content pods collected from polythene bag (51.14%) and jute bag treated with spinosad (51.0%) was similar without any significant change. Remaining all treatments was significantly ($P<0.01$) differing with each other. However, maximum reduction of oil content was observed in

the pods from jute bag (50.6%) at 14% followed by jute treated spinosad (51.0%) and polythene bag (51.14%) and followed by jute bag (51.53%), jute bag treated with spinosad (52.36%) and polythene bag (52.7%) at 10% moisture.

Table 4.18 Effect of storage bags and pod moisture content on per cent change in oil content at different storage periods.

Per cent changes in oil content in different storage bags at different moisture levels				
		2 Months	4Months	6 Months
Per cent moisture	10	52.52a	52.40a	51.59a
	14	51.37b	50.85b	50.35b
S.E(m)		0.02	0.02	0.02
C.D(P= 0.01)		0.08	0.08	0.08
Bag type	Triple layer bag	53.12a	53.00a	52.20a
	Polythene bag	51.92b	51.60 b	50.82b
	Jute bag treated with Spinosad	51.68c	51.20c	50.75b
	Jute bag	51.06d	50.70 d	50.12c
S.E(m)		0.03	0.03	0.03
C.D(P= 0.01)		0.11	0.11	0.11
Interaction (Moisture x Bag type)				
10%	Triple layer bag	53.50a	53.40a	52.60a
	Polythene bag	52.70b	52.60b	51.60c
	Jute bag treated with Spinosad	52.36c	52.20c	51.50c
	Jute bag	51.53d	51.40d	50.68d
14%	Triple layer bag	52.75b	52.60b	51.80b
	Polythene bag	51.14e	50.60e	50.05e
	Jute bag treated with Spinosad	51.00e	50.20f	50.01e
	Jute bag	50.60f	50.00g	49.57f
S.E(m)		0.04	0.04	0.04
C.D(P= 0.01)		0.14	0.17	0.14

*Values followed by the same letter are not significantly different

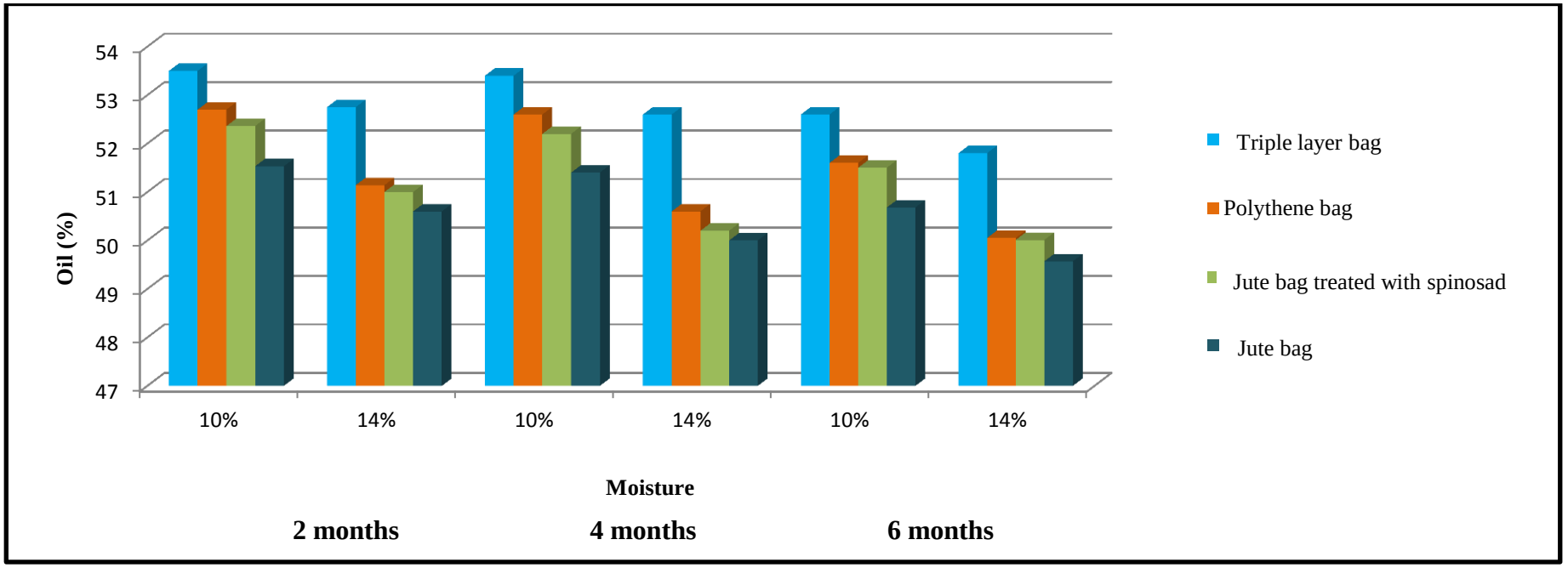


Fig 4.18. Effect of bag type and pod moisture content on oil content of groundnut kernels stored for 2, 4 and 6 months of period

After 4 months, polythene bag (52.62%) with 10% moisture pods and triple layer plastic bag (52.6%) with 14% moisture pods were recorded on par with each other. Jute bag treated with spinosad (50.2%) and jute bag (50.06%) at 14% moisture was recorded on par with each other. Jute bag treated with spinosad (52.26%) and jute bag (51.4%) at 10% moisture and polythene bag (50.6%) at 14% moisture were significantly ($P<0.01$) differing from other treatments. Maximum reduction of oil content was observed on jute bag (50.06%) at 14%moisture followed by jute bag treated with spinosad (50.2%) and polythene bag (50.6%) and followed by jute bag (51.4%), jute bag treated with spinosad (52.26%) and polythene bag (52.62%) at 10% moisture.

After 6 months, polythene bag and jute treated spinosad were recorded on par with each other at 10% moisture as well as at 14% moisture. Other treatments were significantly ($P<0.01$) differing with each other. Maximum reduction of oil content was observed in the pods stored in jute bag (49.57%) at 14% moisture followed by jute bag treated with spinosad (50.01%), polythene bag (50.05%). In the case of pods stored at 10% moisture, jute bag (50.68%), jute bag treated with spinosad (51.50%) and polythene bag (51.60%).

Reduction in oil content, in jute bag, jute treated spinosad and polythene bags, was majorly due to heavy insect infestation, insect feeding and *A. flavus* fungal infestation. This is mainly because of favourable conditions inside these three types of bags such as free flow of oxygen from outside environment, relative humidity, temperatures and food source. Further such conditions bring changes in free fatty acid content as saturated fatty acids were increased and unsaturated fatty acids were decreased during storage by hydrolysis of lipids by invading fungi in stored kernels.

Oil content reduction intern interrelated with saturated and unsaturated fatty acid content. Oil content was reduced more at 14% moisture in comparison to 10% due to high populations of insects and fungal growth. These results are supported by pervious investigations of Kashinath Bhattacharya and Subrata Raha (2002).

4.4. (f) Changes in protein content in different storage bags at different moisture levels at different sets of storage periods

The results on changes in protein content at different sets of storage periods are presented in Table 4.19 and illustrated in Fig 4.19.

Results clearly indicate the effect of bag type on changes in protein content in stored groundnut kernels. Maximum decrease in protein content observed in the pods stored in jute bag (19.7, 20.09 & 24.68%) followed by jute bag treated with spinosad (20.32, 22.04 & 25.52%) and polythene bag (21.69, 22.31 & 28.15%). Whereas minimal changes observed in protein content of pods stored in triple layer plastic bags (24.18, 24.4 & 29.74%) after 6 months of storage followed by 4 and 2 months respectively. All treated bags were significantly differing ($P<0.01$) with each other at 2, 4 and 6months.

Results also indicate the significant effect ($P<0.01$) of pod moisture content during storage on changes in protein content. Maximum changes observed at 14% moisture (24.99, 21.06 & 20.73%) in comparison of 10% moisture content (29.05, 23.35 & 22.21%) after 2 months storage followed by 4 and 6 months storage respectively.

After 2, 4 and 6 months storage, triple layer plastic bags at 10% moisture (30.07, 24.63 & 24.46%) and 14 (29.41, 24.17 & 23.9%) were recorded significantly differ ($P<0.01$) from other treatments.

After 2 months storage, the jute treated spinosad (28.62%) and jute bag (28.52%) at 10%moisture were recorded on par with each other. Remaining all treatments was significantly differing ($P<0.01$) with each other. However maximum decrease in protein content was recorded on jute bag (20.84%) at 14% moisture followed by jute bag treated with spinosad (22.43%) and polythene bag (27.3%) and followed by jute bag (28.52%), jute bag treated with spinosad (28.62%) and polythene bag (29.0%) at 10% moisture.

After 4 months storage, the polythene bag (23.92%) and jute bag treated with spinosad (23.63%) were on par with each other at 10% moisture. Triple layer plastic bag (24.17%) at 14% moisture was intern on par with polythene bag (23.92%) and jute bag treated with spinosad (23.63%). Jute bag (21.23%) at 10% moisture and polythene bag (20.7%) at 14% moisture were on par with each other. Polythene bag (20.7%) was intern on par with jute bag treated with spinosad (20.45%) at 14% moisture.

Maximum decrease in protein content was recorded in jute bag (18.95%) at 14% moisture followed by jute treated spinosad (20.45%) and polythene bag (20.7%) in comparison to jute bag (21.23%), jute bag treated with spinosad (23.63%) and polythene bag (23.92%) at 10% moisture.

Table 4.19 Effect of storage bags and pod moisture content on per cent change in protein content at different storage periods

Per cent changes in protein content in different storage bags at different moisture levels				
		2 Months	4Months	6 Months
Per cent moisture	10	29.05a	23.35 a	22.21 a
	14	24.99b	21.06 b	20.73 b
S.E(m)		0.02	0.08	0.04
C.D(P= 0.01)		0.09	0.34	0.17
Bag type	Triple layer bag	29.74a	24.40a	24.18a
	Polythene bag	28.15b	22.31b	21.69b
	Jute bag treated with Spinosad	25.52c	22.04b	20.32c
	Jute bag	24.68d	20.09c	19.70d
S.E(m)		0.03	0.11	0.05
C.D(P= 0.01)		0.13	0.48	0.24
Interaction (Moisture x Bag type)				
10%	Triple layer bag	30.07a	24.64a	24.46a
	Polythene bag	29.00c	23.92b	22.94c
	Jute bag treated with Spinosad	28.62d	23.63b	20.76d
	Jute bag	28.52d	21.23c	20.70d
14%	Triple layer bag	29.41b	24.18ab	23.90b
	Polythene bag	27.30e	20.70cd	20.45d
	Jute bag treated with Spinosad	22.43f	20.45d	19.88e
	Jute bag	20.84g	18.95e	18.70f
S.E(m)		0.04	0.16	0.08
C.D(P= 0.01)		0.2	0.68	0.34

*Values followed by the same letter are not significantly different

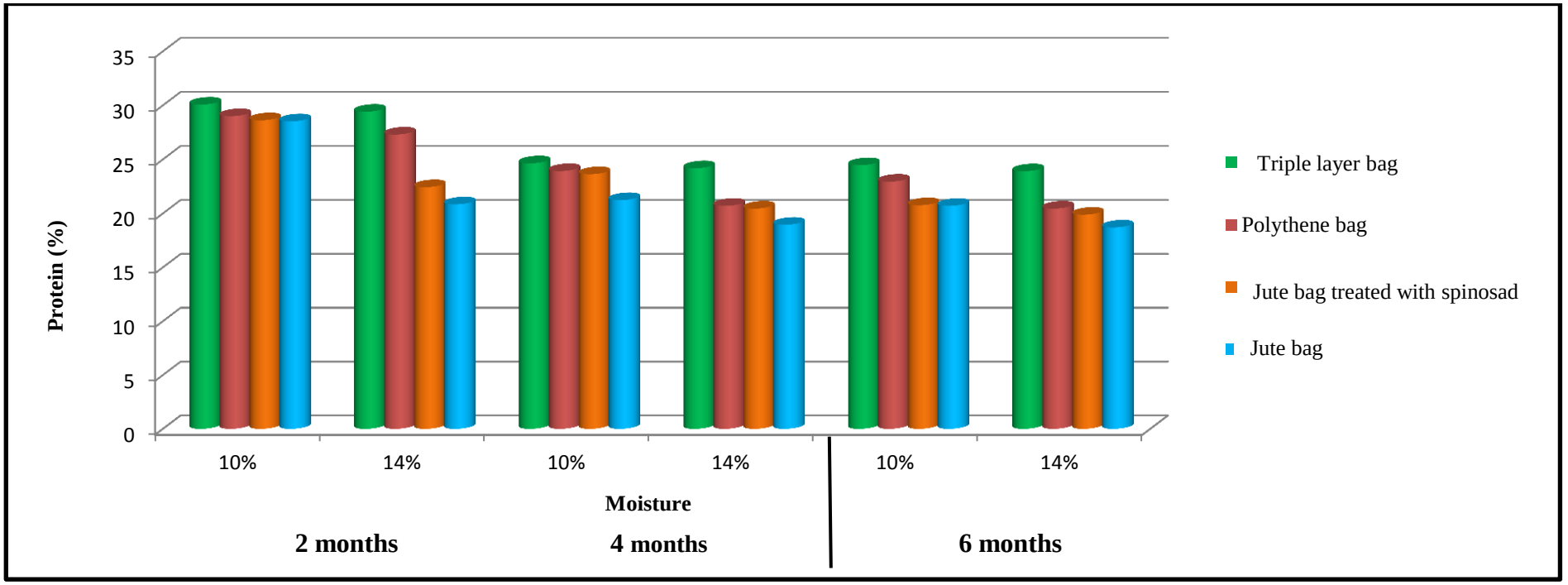


Fig. 4.19. Effect of bag type and pod moisture content on protein content of groundnut kernels stored for 2, 4 and 6 months of period

After 6 months jute bag treated with spinosad (20.76%), jute bag (20.7%) at 10% moisture and polythene bag (20.45%) at 14% moisture were on par with each other. Remaining all treatments was significantly differing with each other. Maximum decrease in protein content was recorded on jute bag (18.70%) at 14% moisture followed by jute bag treated with spinosad (19.88%) and polythene bag (20.45%) in comparison to jute bag (20.7%), jute bag treated with spinosad (20.76%) and polythene bag (22.93%) at 10% moisture.

In triple layer plastic bag little changes occur in protein reduction at different moisture levels at different storage periods due to increased carbon dioxide which was toxic to fungal development and insect multiplication but due to moisture some content fungal mycoflora was observed in triple layer plastic bag due to this little changes observed in reduction of protein content in triple layer bag stored kernels.

Protein content was decreased after 2, 4 and 6 months of storage at different moisture levels as highest reduction was observed at higher moisture levels due to increased conditions of moistures in jute, jute bag treated with spinosad and polythene bag.

Among the treatments the decrease in protein content was more in treatments having higher moisture because higher moisture content in stored pods favoured proteolytic activity of invading fungi in comparison of low moisture treatments. Due to proteolysis and formation of simpler compounds such as amino acids, this could be utilized by invading fungi. Similar results are obtained in previous investigations conducted by Butt *et al* (2004).

Changes in protein content was in triple layer plastic bag in comparison to all other treated conventional bags due to its structure ability reduce the fungal and insects development. These results are in close agreement with the results obtained by Upadhyay *et al*. (1994).

Protein content was decreased in stored kernels due to fungus development via insect multiplication as fungus lipolytic activity by lipase enzyme leads to utilization of protein and sugar as substrate for their growth. These results are similar to the investigations conducted on nutritional changes in oilseeds in storage by Chavan (2011).

Reduction in protein content and increase of free fatty acids was observed in groundnut pods stored with higher moisture content which further creates high temperatures. These conditions usually create favourable environment for insect multiplication and mold fungi growth in storage materials. These results are in agreement with earlier investigation on pinto beans by Rani *et al*. (2013)

ChapterV

SUMMARY AND CONCLUSIONS

Chapter V

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Groundnut in India is usually stored in the form of unshelled pods as well as kernels. Both these forms are vulnerable to attack by several insect pests. The most commonly reported and economically significant storage pest of groundnut is groundnut bruchid, Caryedon serratus. Bruchid infestation in storage ultimately leads to both quantitative (weight loss) and qualitative (mould fungi growth) losses. The recommended pest management practices include specific chemicals as fumigants that are not in vogue due to serious health hazards, residue effects and due to development of insecticide resistance. These bottle necks in the use of chemical pesticides necessitate for alternative to chemical control methods for protection of stored grains and their products. Creation of a modified environmental condition by changing the gas composition inside the storage structure was proved as a non-chemical alternative in managing the storage pests. The very drawback about technology was creation of modified environment by changing gas composition artificially using vacuum cylinders in storage structures and thus making the technology non- practical at farmer's levels. An improved technology in this direction that works on the principle of hermetic storage technology is the use of triple layer plastic bag an alternative for insecticide free, long-term storage of produce after harvest.

The effectiveness of these triple layer plastic bags in managing storage insects and fungi without effecting the biochemical constituents and seed germination of the stored produce was studied in the present investigation "Evaluation of improved grain storage practices for the management of Groundnut Bruchid Caryedon serratus Olivier. (Coleoptera: Bruchidae)" under laboratory conditions. Apart from evaluating the triple layer plastic bags the fundamental studies on respiratory biology of C. Serratus were also carried out to determine the actual requirement of oxygen by a bruchid to complete its life cycle and quantity of carbon dioxide released by the bruchid during the process of respiration.

The studies were carried out using the facilities at ICRISAT, Patancheru. The study on respiratory biology of C. serratus was done by placing a pod containing single freshly laid egg in septum bottle and determining the changes in oxygen and carbon dioxide using a hand held Mocon head space analyzer.

The effectiveness of triple layer plastic bag was evaluated along with three traditional storage bags viz., polythene bag, jute bag and jute bag treated with 2 ppm of insecticide spinosad. All these bags contained 10 kg of groundnut pods at 10 and 14 per cent moisture levels which were artificially infested with C. serratus and high aflatoxin producing strain of Aspergillus flavus culture solution. Each of the bag type was considered as a treatment and was replicated thrice for the two different moisture contents. A total of 24 such bags formed as a set and three such sets were formed which were further evaluated for insect damage, fungal growth, seed germination and biochemical constituents by opening each set at 2, 4 and 6 months of storage.

The results obtained from the above studies are summarized here under.

The study on respiratory biology of C. Serratus revealed that a bruchid required about 39.97 ml of oxygen for its development from egg to pupal stage and simultaneously released 26.21 ml of carbon dioxide. The respiratory quotient (RQ) calculated from the data obtained on quantity of oxygen utilized and carbon dioxide produced at different life stages of bruchid showed highest RQ value of 0.68 for the development of bruchid from first instar to final instar.

The study on management of groundnut bruchid on pods with 10 and 14 per cent of moisture using different storage bags viz., triple layer plastic bag, polythene bag, jute bag and jute bag treated with spinosad revealed supremacy of triple layer bags in restricting the per cent damage, weight loss of stored produce, test weight and germination percentage compared to traditional bags (Polythene bag, jute bag treated with spinosad and jute bag). The traditional jute bags recorded highest pod damage up to 94 per cent, maximum loss in weight up to 22 per cent and lowest test weight of 56.67 g.

The triple layer plastic bags recorded 100 per cent mortality of insects but the traditional bags recorded highest number of eggs up to 312, emergence holes up to 73.63, pupae up to 29.43 and a massive increase in live insects up to 97 for every 100 pods.

The data on oxygen and carbon dioxide concentrations inside triple layer plastic bag revealed effectiveness of triple layer plastic bag in managing bruchid and restricting the multiplication of fungi due to creation of hypercarbia conditions i.e., increased levels of CO2 concentrations ranging from 10.51 to 12.68 per cent in a storage period of

2-6 months. Similarly, development of hypoxia conditions such as availability of reduced level of oxygen concentrations ranging from 1.21 to 1.81 per cent in a storage period of 2-6 months.

The triple layer plastic bags recorded significant low reduction in germination percentage after four months of storage that ranged between 77-85 per cent and higher reduction in germination per cent was found in traditional bags that ranged between 37.00- 57.66 per cent after four months of storage. Absolute loss of seed germination was observed in all types after six months of storage.

The results on aflatoxin accumulation in 10 per cent and 14 per cent moisture pods stored in triple layer plastic bags revealed acceptable levels of aflatoxin production of 11.99 µg kg -1 &14.01 µg kg-1 respectively after 2 months of storage suggesting their usage for short term storage of pods even with high moisture contents. However, higher levels of aflatoxin accumulation, of 456.0 µg kg-1 & 700.23 µg kg-1after 4 months and 2444.46 µg kg -1& 2701.93 µg kg-1 after 6 months, with 10% and 14% moisture pods suggest proper drying (up to 8%) is essential for longer term storage. On the other hand, jute bags and polythene bags were found unsuitable to store groundnut pods even for shorter period (2 months) as they recorded much higher levels of aflatoxin contamination even at low moisture content of 10 per cent.

The results on impact of insect infestation and fungal activity on changes in biochemical constituents of stored produce revealed that among fatty acids significant minimum decrease in linoleic acid and oleic acid and increase in palmitic and stearic acid was recorded in pods stored in triple layer plastic bags compared to traditional bags. Similarly a significant reduction in total oil and protein contents was recorded in pods stored in all the three types of traditional bags compared to triple layer plastic bags.

CONCLUSIONS

Based on the results obtained from the studies on the use of different storage bags in managing groundnut bruchid C. serratus, the following conclusions were drawn

- The groundnut bruchid C. serratus requires certain quantity of oxygen for growth, development and completion of its life cycle. Creation of either hypoxia or hypercarbia will lead to death of insects.

- The triple layer plastic bag working on principle of hermetic storage created hypoxia and hypercarbia conditions within a short period of time thus effectively checked insect multiplication reducing pod damage and weight loss of produce.

- The minimum aflatoxin content was recorded in triple layer plastic bag due to creation of hypoxia and hypercarbia conditions. The moisture content of the produce play a key role apart from oxygen availability and insect activity in production of aflatoxin, thus pods stored at 10 per cent moisture recorded less aflatoxin compared to pods stored at 14 per cent moisture.

- Germination percentage was completely reduced after six months of storage in all the bags. The loss of germination in triple layer plastic bag too due to high relative humidity and moisture content likely affected embryo vigour and in the case of traditional bags, it was majorly due to biotic factors such as insect activity and fungi infestation.

- The minimal changes occurred in total oil and fatty acid composition of groundnut pods stored in triple layer bags compared to other bags suggesting the quality did not deteriorate in a storage period of six months.

- It is concluded from the present investigation that the triple layer plastic bags using hermetic technology efficiently managed insect pests and mycotoxin producing storage fungi compared to traditional storage bags. The study also revealed that triple layer plastic bags protected the biochemical constituents and germination of the stored seed and could be best alternative for traditional storage bags for short and medium term storage, provided the produce is sufficiently dried (<10%) before storage.

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LITERATURE CITED

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