

DEPARTMENT OF CROP SCIENCE
FACULTY OF AGRICULTURE
UNIVERSITY OF SCIENCE AND TECHNOLOGY
KUMASI - GHANA.

QUALITY OF OCIMUM GRATISSIMUM TREATED SOYABEAN
SEEDS UNDER DIFFERENT STORAGE CONDITIONS.

A THESIS SUBMITTED TO THE BOARD OF POSTGRADUATE STUDIES
OF THE UNIVERSITY OF SCIENCE AND TECHNOLOGY,
KUMASI, IN PARTIAL FULFILMENT OF THE
MASTER OF PHILOSOPHY (PLANT PATHOLOGY) DEGREE.

LIBRARY
UNIVERSITY OF SCIENCE AND TECHNOLOGY

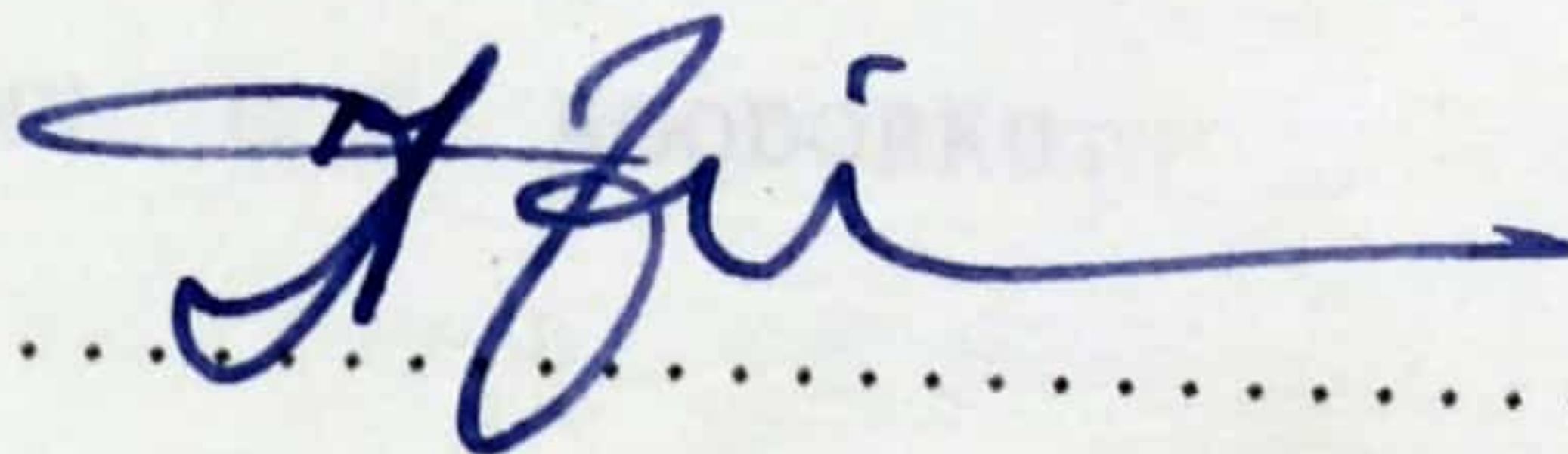
BY

CHARLES KODIA KWOSEH

MAY, 1994.

C E R T I F I C A T I O N

This is to certify that this research was carried out by Charles K. Kwoseh of the Department of Crop Science, University of Science and Technology, Kumasi, Ghana.

.....


Supervisor

Dr. F.A. Ofori

Plant Pathologist, U.S.T.

Kumasi, Ghana.

LIBRARY
UNIVERSITY OF SCIENCE AND TECHNOLOGY

THIS THESIS IS DEDICATED TO

MR. C.K. AGODORKU.

LIBRARY

UNIVERSITY OF SCIENCE AND TECHNOLOGY

ACKNOWLEDGEMENT

I wish to express my profound gratitude to Dr. F.A. Ofori, Senior Lecturer of the Department of Crop Science and my supervisor for his constant interest, valuable suggestions, constructive criticisms and support during the planning and execution of this research.

I am indeed very grateful to Dr. R.T. Awuah of the Department of Crop Science, University of Science and Technology, Kumasi for his guidance and assistance during the course of this work.

My special thanks also go to Mr. Ralph Ahiabu of Ghana Atomic Energy Commission, Accra for his advise and inspiration. I am also grateful to Miss Naomi Danso, Mr. Abasey, Mr. Joe Lartey, Messrs. J.K. Oklu and Solomon Kpabi as well as the entire workers of the Plant Pathology Laboratory of the Department of Crop Science for their immense assistance.

My sincere gratitude to Mrs. Agnes Ankomah of Crops Research Institute, Kumasi for her help in the analysis of my data.

Many thanks to Mr. Emmanuel Osei-Mensah of the Department of Agricultural Economics and Farm Management who typed this thesis.

Finally, my appreciation goes to the Almighty God for his continued divine protection.

LIBRARY
UNIVERSITY OF SCIENCE AND TECHNOLOGY

ABSTRACT

Soyabean seeds are often associated with problems of germination and plant establishment as a result of rapid loss of viability caused by mycoflora during storage. In this study, attempt was made to isolate and identify the mycoflora associated with stored Anidaso and Bengbie seeds and to ascertain a possible storage method aimed at arresting rapid deterioration and subsequent loss of seed viability.

Ocimum gratissimum leaf and seed powders were both prepared by milling 200g each of sun-dried leaves and seeds. The leaves and seeds were sun-dried for 2 weeks before milling. The powders were used immediately after preparation. Freshly harvested hand-threshed soyabean seeds were surface disinfected with 2.5% Sodium hypochlorite and 40% ethanol and dried in forced-air oven. Anidaso and Bengbie seeds treated with both powders and Dithane M-45 were stored in heat-sealed polythene bags and paper bags under ambient conditions for 9 months. Nine (9) genera of fungi were isolated and their percentage occurrence recorded. The main genera of fungi isolated were *Aspergillus*, *Penicillium*, *Fusarium* and *Mucor*. The location of seed-borne mycoflora affected seed viability especially in seeds which had their embryo infected with fungi. Increase in seed moisture as well as storage period directly affected the rate of fungal occurrence as well as decline in seed germination.

The *O. gratissimum* leaf powder, when used to treat seeds which were stored in polythene bags, suppressed the rapid deterioration of the seeds and maintained viability. This compared favourably with Dithane M-45 treated seeds stored in

sealed polythene bag.

TABLE OF CONTENTS

	PAGE
Title.....	i
Certification of supervisor.....	ii
Dedication.....	iii
Acknowledgement.....	iv
Abstract.....	v
Table of Contents.....	vii
List of Tables.....	ix
List of Figures.....	x
CHAPTER 1 : INTRODUCTION.....	1
CHAPTER 2 : LITERATURE REVIEW.....	5
2.1 Effect of seed maturity on its quality and viability.....	5
2.2 Effect of environmental and physiological factors on seed quality and viability.....	6
2.3 Effect of mechanical injury on seed quality and viability.....	12
2.4 Effect of genetic make-up on quality and viability of seed.....	14
2.5 Effect of seed moisture content and temperature on quality and viability.....	15
2.6 Effect of seed health status on quality and viability.....	18
2.6.1 Location of seed mycoflora.....	21
2.6.2 Common fungal metabolites.....	22
2.7 Effect of chemical seed treatment on quality and viability of seeds.....	24

2.7.1	Effect of hot-water treatment on seed quality and viability.....	25
2.7.2	Treatment of seed with microwaves for control of seed-borne pathogen.....	26
2.7.3	Effect of biologically treated seeds on quality and viability.....	27
2.7.4	Effect of plant parts on seed mycoflora....	28
2.8	Effect of storage containers on seed quality and viability.....	30
CHAPTER 3 : MATERIALS AND METHODS.....		35
3.1	Preparation of leaf and seed powders of <i>Ocimum gratissimum</i>	35
3.2	Source of soyabean seeds.....	35
3.3	Disinfection of seeds.....	35
3.4	Determination of seed moisture.....	35
3.5	Germination test of seeds.....	36
3.6	Isolation of mycoflora from soyabean seeds.....	37
CHAPTER 4 : EXPERIMENTAL.....		38
4.1	Effect of seed treatment on seed germination....	39
4.2	Effect of seed treatment on seed mycoflora infection.....	40
CHAPTER 5 : RESULTS AND OBSERVATIONS.....		41
CHAPTER 6 : DISCUSSION.....		69
CHAPTER 7 : CONCLUSION.....		76
	Recommendations.....	77
	REFERENCES.....	78
	APPENDICES.....	90

LIST OF TABLES

TABLE		PAGE
1	Per cent germination of Anidaso and Bengbie before storage in vitro.....	41
2	Effect of seed treatments and containers on seed germination in Anidaso and Bengbie in vitro under ambient conditions.....	43
3	Effect of seed treatments and containers on seed germination in Anidaso and Bengbie in vivo under ambient conditions.....	48
4	Per cent moisture content of Anidaso under different storage conditions.....	53
5	Per cent moisture content of Bengbie under different storage conditions.....	55
6	Seed health testing on Anidaso and Bengbie seeds during the storage period.....	58
7	Per cent of total fungal occurrence on Anidaso seeds under different storage conditions.....	62
8	Per cent of total fungal occurrence on Bengbie seeds under different storage conditions.....	62
9	Location of seedborne mycoflora in Anidaso seeds after nine months storage under different storage conditions.....	67
10	Location of seedborne mycoflora in Bengbie seeds after nine months storage under different storage conditions.....	68

LIST OF FIGURES

FIGURE		PAGE
1	Effect of storage containers on germination of Anidaso and Bengbie in vitro (control).....	44
2	Effect of <i>Ocimum</i> leaf powder treated seeds and storage containers on germination of Anidaso and Bengbie in vitro.....	45
3	Effect of <i>Ocimum</i> seed powder treated seeds and storage containers on germination of Anidaso and Bengbie in vitro.....	46
4	Effect of Dithane M-45 treated seeds and storage containers on germination of Anidaso and Bengbie in vitro.....	47
5	Effect of storage containers on germination of Anidaso and Bengbie in vivo (control).....	49
6	Effect of <i>Ocimum</i> leaf powder treated seeds and storage containers on germination of Anidaso and Bengbie in vivo.....	50
7	Effect of <i>Ocimum</i> seed powder treated seeds and storage containers on germination of Anidaso and Bengbie in vivo.....	51
8	Effect of Dithane M-45 treated seeds and storage containers on germination of Anidaso and Bengbie in vivo.....	52
9	Graph showing moisture content of Anidaso under different storage conditions.....	54
10	Graph showing moisture content of Bengbie under different storage conditions.....	57

11	Percentage fungal occurrence in Anidaso under different storage conditions.....	63
12	Percentage fungal occurrence in Anidaso under different storage conditions.....	64
13	Percentage fungal occurrence in Bengbie under different storage conditions.....	65
14	Percentage fungal occurrence in Bengbie under different storage conditions.....	66

CHAPTER ONE

I N T R O D U C T I O N

Soyabean (*Glycine max* (L.) Merril), a member of the family Leguminosae, sub-family papilionadae, is the world's leading grain legume crop. It is a native of eastern Asia. World production is approximately seventy-five million tonnes per annum, of which 60% is produced in the United States of America (Allen, 1983).

Soyabean has a great potential for easing the problem of protein malnutrition in the developing countries. In many tropical developing countries, legumes supply most of the plant proteins and are not only the main, but also the cheapest source of dietary proteins in areas where meat is scarce and too expensive for a large proportion of the population. The composition of dry soya seed is as follows: water 5.0 - 9.4%, protein 29.6 - 50.3%, fat 13.5 - 24.5%, carbohydrate 14.0 - 23.9%, fibre 2.8 - 6.3% and ash 3.3 - 6.4% (Purseglove, 1966).

The amount of soy protein is approximately twice that of cowpea or lima beans and four times that of cereals (Awuku et al., 1991). The protein quality is rich in lysine, which is deficient in cereals but relatively low in methione and theonine (Luckman, 1971 and ICRISAT report, 1989).

Soyabean is also rich in unsaturated oil and contains about seven per cent of Omega - 3 (alpha-linolenic) fatty acid. Recent research indicates that the poly-unsaturated fatty acids in human diet may be beneficial for cardiovascular health (Kinsella, 1988).

The importance of soyabean lies in its food value. It is a major source of protein of high biological value, energy, minerals, vitamins and roughage. It may also improve soil fertility when used as green manure and cover crop. Soyabean forms a component part in the manufacture of paints, cosmetics and plywood. About 40% of the world's supply of edible vegetable oil comes from soyabean, 80% of which is used in the manufacture of margarine, salad oil, cooking oils and shortening. It may be used in traditional dishes such as tuubani, koose, tuo zaafi, apapransa, kenkey, palmnut and plain soup, banku, jollof, gravy and fufu. In northern Ghana it is particularly popular for making dawadawa, a flavouring for soups and stews. It can also be used as an ingredient of weanimix or nutrimix. Soyabean residues after oil extraction are excellent protein concentrates for poultry and livestock feed (Awuku *et al.*, 1991).

About 90% of all food crops grown on earth are propagated by seed. Soyabean is one of the major important crops produced in the world which is attacked by a wide range of diseases most of them seedborne.

In Ghana, there has been an urgent need to find sources of protein other than animal protein which our resource-poor rural communities can afford. Cultivation of the crop however, has not found favour with the farming communities.

The lack of enthusiasm on the part of farmers to cultivate the crop is mainly due to problems associated with germination of seeds and plant establishment as a result of rapid loss of viability

caused by mycoflora during storage. A number of factors, such as seed quality, seed size, the stage of maturity of the crop at harvest, threshing method and storage conditions, determine the germinability of the seed (Singh, 1976; FAO report, 1982 and George, 1985). Germinability is also influenced by the genetic make-up of the crop since there is variation in viability of different crops and even among varieties of a particular crop (IITA report, 1976).

Storage condition seems to be the major factor affecting the viability of the soyabean seed in Ghana. The most common storage practice adopted by the majority of farmers is the ambient storage in open or sealed containers without any chemical treatment. Information on storage of pulse seeds to preserve their viability and vigour from harvest to next planting season and for carryover purposes is of prime importance in any seed production programme. Also, knowledge of the primary site or location of infection of the pathogen and the production of toxic metabolites in the seed is vital in evolving suitable control measures (Gopinath and Shetty, 1985).

Modernization of agriculture has led to greater dependence on various agro-chemicals, some of which are hazardous pesticides. According to a recent estimate by the World Health Organization (WHO), approximately 750,000 people are taken ill and up to 14,000 die every year as a result of pesticide poisoning (Chengappa, 1989). As well as causing environmental pollution, chemicals are becoming increasingly expensive. Realising therefore, that there

is a strong need to find an alternative to pesticides, some recent studies have shown the efficacy of essential oils, extracts and powders of some local wild plants in controlling fungal deterioration of some crops in storage. They are non-toxic and readily available (Purseglove, 1985 and Gbile, 1986).

Objective of this Thesis

Considering the hazards associated with the use of chemicals and the recent upsurge in the cost of chemicals it is imperative to look into other possible means of treating seeds before storage to avoid rapid deterioration.

The objective of this Thesis, therefore, is to investigate into the effects of *Ocimum gratissimum* powders prepared from both leaves and seeds on the maintenance of the quality of soyabean seeds during storage since this would yield useful information for soyabean breeders, agronomists and farmers.

CHAPTER TWO

LITERATURE REVIEW

The importance of the period and conditions of storage on the viability and quality of soyabean [*Glycine max* (L.)] seeds is very crucial. A major constraint to soyabean production in the tropics is seed deterioration and loss of viability during storage. Haferkamp et. al (1958) observed that factors which influence longevity of seeds included the species or kinds of seeds, the degree of maturity, the moisture content at harvest, the method of harvesting and processing of the seeds as well as the storage conditions. Roberts (1972) considered, temperature, moisture content and oxygen pressure as major factors which influenced longevity of seeds during storage. Other factors included seed health status, seed treatment with chemicals and storage containers.

2.1 Effect of seed maturity on its quality and viability

George (1985) observed that, the stage of maturity at harvest affected the quality and viability of seeds. Seeds from immature pods were severed from their source of sustenance leading to death of such seeds. Over-mature pods on the other hand were liable to pests and disease attack in the field as well as loss of seeds through dehiscence of dried pod if left to overstay on the plants. According to Austin (1963), the state of maturity of seed crops at harvest can partly account for the variation in viability.

Peterson (1942) and Schwabe (1963) observed that full germination capacity and viability were not attained prior to the attainment of full maturity where there was no further increase in dry weight. Walker (1933) in Manitoba, United States of America harvested a sweet corn variety, Burbank Golden Bantan, at various ages from 13 to 55 days after silking in an attempt to determine whether full germination and viability were attained before dry seed maturity. Results of the experiment showed that maximum germination capacity and field emergence were attained when seed weight had reached 70% of its maximum, after which there was no significant increase or decrease in per cent seed germination or viability.

Chauchan and Bhandari (1971) studied pod development in Okro (*Abelmoschus esculentus* L.) Moench and suggested that for seed production, pods should be harvested 30 days after anthesis, when pods were fully matured and dry. Per cent germination from such pods was over 80%.

2.2 Effect of environmental and physiological factors on seed quality and viability

According to IITA report (1974) and Singh (1975), environmental factors such as soil temperature, carbon dioxide and oxygen concentration are known to influence germination, growth and yield of crop. In addition to environmental factors, hereditary factors and their interaction with the environment affect the performance of crop plant.

FAO report (1982) on plant production and protection pointed out that seed quality was maximum at physiological maturity and the time between physiological maturity and harvest represented a period of seed storage in the field. During this period, quality is lost largely as a result of seed infection by fungi. According to the report, delayed harvesting resulted in an increase infection of seed by field fungi in Temperate, Subtropical and Tropical zones. The report also pointed out that repeated drying and wetting of matured seeds prior to harvesting had an adverse effect on seed quality. Under extremely high moisture conditions, seeds may germinate within pods before harvest. The report also stated that the manner in which seed were harvested, processed and stored might affect seed quality. Cool, dry condition during harvest period was also observed to favour good seed quality and warm, wet weather with drought tension caused small green seeds. The report also made mention of the length of the rainy season on the quality of the soyabean in Nigeria. Good quality seeds were produced during the shorter rainy seasons. In practice, many farmers in Nigeria plant soyabeans late in the major rainy season so as to avoid excess moisture at maturity. According to Osafo (1977), even though yield of soyabean was lower in minor season, better seed quality was produced than in the major rainy season. He recommended that for good quality seed production soyabean should be cultivated in the minor season possibly under irrigation.

Chen (1978) considered physiological deterioration of seeds during storage as one of the main factors that prevented normal germination and vigorous growth of seed plants. He reported that during storage of seed under ambient conditions, enzymatic reactions result in biochemical changes after a considerable period. Fungi may be a consequence not the principal cause of seed deterioration during storage. According to Allen (1983), seedborne fungi play a subsidiary role to physiological factors which is enhanced by high temperature and moisture content in soyabean seed deterioration during storage.

High temperatures are known to have effect on germination and seedling emergence of soyabean. Dadson (1974) reported that the major problem of poor field emergence of soyabean was high soil temperature. Mercer-Quarshie (1975), in a study at Kwadaso, Ghana, observed that during March and April, the usual planting time in the major season, soil temperatures in the afternoon are quite high and could be an important factor in poor seedling emergence. According to FAO report (1982), optimum soil temperature for germination of soyabean was between 25 and 37°C and a constant temperature of 40°C appeared to be near maximum for germination. The report also pointed out that daily exposures of soyabean to temperatures in the range of 38 to 44°C for four to eight hours markedly reduced seedling elongation. According to the report, soil temperatures at planting time in most tropical and subtropical areas usually exceed 20°C and under these conditions, emergence could occur within three to five days.

Quasem and Christensen (1958) reported that seed deterioration measured as invasion of seed by storage fungi, decrease in germination percentage and increase of discoloured germs was proportional to moisture content, temperature, time and the degree of invasion by storage fungi. They observed that low temperature was effective as low moisture content of seed in preventing damage by the fungi. According to Christensen and Kaufmann (1965), most fungi are sensitive to high temperature and are killed when exposed to such conditions. However, some fungi like *Aspergillus flavus* Link and *A. candidus* Link can withstand temperatures as high as 45°C. An IITA report (1982) considered high temperature and humid conditions of the tropics conducive to the growth, reproduction and dissemination of most seed-borne phytopathogens. Under these conditions soyabean seed does not do well, and even good seed will start to deteriorate after six months. IITA report (1977) on seed viability studies observed that deterioration in seed viability during storage was a problem in lowland humid tropics where ambient temperature and humidity are high. These hastened the decline in seed viability.

Laude (1962) observed that heat stress experienced by the mother plants at seedling stage can affect the dormancy of the seeds harvested from the mature plants.

FAO report (1982) have shown that when soil moisture, soil temperature and planting depth were optimum, soyabean seedlings emerged four to five days after sowing. However, excessive soil moisture hindered germination, probably because of restricted

oxygen movement to the seed. Low soil moisture also retarded germination unless the seed could achieve a moisture content of about 50%. According to Anon (1959), germination tests revealed only the percentage of the seeds that are viable under near-ideal conditions and there was usually a considerable discrepancy between results of germination tests in the laboratory and emergence of seeds in the field. Austin (1963) observed that variation in germination and viability in the field was the direct or indirect result of variation in the weather before and at harvest and that hot, dry periods generally gave good seeds.

Grabe and Metzger (1970) reported that varieties that failed to emerge in compacted soils had brittle hypocotyl which broke at the stress point between emerging hypocotyl and the buried hypocotyl. Dadson (1974) observed that soil temperature had little influence on hypocotyl elongation. He observed that at 40 and 42°C, seeds under laboratory condition could still emerge hence temperature alone may not be the only limiting factor. Soil crusting was also an important factor since there was a high seedling emergence under mulching. Boakye-Boateng and Hume (1975) observed that soyabean emergence in the humid tropics was poor due to poor seed germination. The large cotyledons had difficulty pushing through the crusted soil surface. In their minor season trials, three soyabean varieties were mulched and percentage emergence of seedling in all the varieties were high. Verma and Kohnke (1951) reported that mulching the soil surface with plant material can lower soil temperature, decrease crusting, conserve soil moisture

and reduce soil erosion.

According to Kaul and Bekhon (1977), shading has been found to increase rate of emergence of 19 soyabean lines and also slightly increased emergence.

IITA report (1974) pointed out that optimum planting depth of soyabean was between two and a-half to five centimetres and the most appropriate depth depended on soil type and soil moisture. In the report, germination and emergence studies of soyabeans and Lima beans showed that they were highly sensitive to planting depth. Soyabean seedling emergence also depended on variety but decreased with increased planting depth.

According to Fause and Christensen (1966), high relative humidity increased seed moisture by absorbing moisture from the environment which favoured the growth of storage moulds and reduced seed viability. Neergaard (1977) reported that at relative humidity ranging from 65% to 90%, there was equilibrium between seed moisture content which favoured the growth of most storage fungi. Christensen and Kaufmann (1965) observed that by this equilibrium there was movement of water vapour between the seed and its surrounding atmosphere. A moist seed thus gave up moisture to the atmosphere while dry seed or food absorbed water from the atmosphere. They reported that in stored grains or seeds which was not artificially aerated, there was slow movement of air, set up by differences in temperature between different portions of the bulk resulting in moisture transfer. According to Allen (1983), relative humidity of the air in the store was also important since

seed at a low moisture content picked up moisture from the air with high relative humidity. Burlison *et al.* (1940) observed that soyabeans near the surface in bin decreased appreciably in germination within one year from harvest. Within two years, germinability of beans in the bin four feet below the surface also decreased. Relatively rapid loss of viability at the surface was attributed to greater absorption of moisture.

Milner (1954) observed that the relative humidity of the inter-seed atmosphere rather than the actual moisture content of the seeds determined the susceptibility of soyabeans to moulding. He believed that the critical moisture content with respect to storage for different seed species are those which are in hygroscopic equilibrium with a relative humidity of about 75% and which over a moderate time interval were the minimal humidities required for growth of xerophytic mould contaminating the seed.

2.3 Effect of mechanical injury on seed quality and viability

Singh (1976) observed that seed quality in terms of seed weathering, embryo damage during threshing and infection were among important causes of poor germination. According to Reddy and Nusrath (1986), intact, mature and sound grains were generally not suitable for fungal invasion, while immature, shrivelled, discoloured, broken and other physically damaged seeds were highly susceptible to fungal attack. They observed that seeds usually got damaged in the fields and storage by several means such as heavy rains, floods, winds, birds, improper way of harvesting, threshing,

drying and handling. Insects may cause damage to seeds both in the fields and also during storage.

According to Allen (1983), mechanical damage may be caused by careless handling of seed during or after harvest and that some seeds could be damaged by minor hair cracks in the seed coat, such seeds are difficult to remove from the seedlot and are likely not to germinate satisfactorily. He observed that seeds which were mechanically damaged were invaded by pathogens which destroyed the seed during storage. Also, seedlot which contained a high proportion of rubbish such as broken pieces of straw or leaf as well as cracked or broken seed will not store well.

According to FAO report (1982), manual threshing resulted in less damage to soyabeans and a higher germination percentage than mechanical threshing and large seeds were readily damaged by mechanical handling. The report pointed out that low moisture seed should be handled carefully to prevent mechanical damage. It also indicated that per cent germination of soyabean seed at 12% moisture or less could be reduced as much as 10% by dropping them one and a half metres on a metal surface. The report also pointed out that pod sucking insects, such as the southern green stink bug, *Nezara viridula* (L.) could affect soyabeans directly by reducing yield and seed quality. Damage to the seeds resulted in lower germination percentage, decreased vigour, smaller seed size, reduced oil content and reduced storability.

2.4 Effect of genetic make-up on quality and viability of seed

According to IITA report (1976), although smaller-seeded lines showed better ability to withstand prolonged storage than large-seeded lines, germinability in seeds was controlled by the genetic constitution of the seed. The 1976 report of the institute pointed out that the storability of seed in a genetically segregating population seems to be governed to a degree by the genotype of the mother plant from which the seed was derived.

FAO report (1982) and IITA report (1980) indicated that there was substantial variation among tropically adapted genotypes for soyabean seed quality characteristics. According to FAO report of 1982, genotypes of soyabean with the best field emergence were small in size with the lowest incidence of seedborne fungi. In IITA report (1980) small-seeded genotypes and those with hard seed tend to have the greatest resistance to weathering. It also reported that hardseededness is a potentially valuable trait for reducing the effect of field weathering as well as seed moisture fluctuation which could occur with alternate wetting and drying in the field. The report indicated that seeds of hardseeded genotype maintained 80% viability for up to nine weeks following maturity, whereas an adapted variety grown under identical conditions maintained 80% viability for only three to four weeks. The report also pointed out that hard seeds absorbed less moisture from light rains and heavy dews, thus, mature unharvested seeds underwent less swelling and shrinking known to reduce quality and viability by causing internal mechanical damage and increased respiration and

pathogen activity. Further, stored seeds that had hard seed coats responded less to fluctuations in atmospheric humidity.

Singh (1976) and IITA report (1980) pointed out that as in the case of resistance to field weathering, genetic variations exist for storability in ambient tropical and subtropical environment. The tendency for improved storability was pointed out by FAO report of 1982 to be associated with high initial germination, small seed size and high percentage of hardseed. Singh (1976) observed that small seeded soyabeans maintained viability in storage better than large-seeded ones.

According to FAO report of 1982 on Production and Protection, the small seed required less moisture for germination and soyabean varieties which were small seeded tend to emerge better under conditions of soil stress.

Allen (1983) observed that many seed samples which had poor appearance grew satisfactorily when sown but larger, plumper, blight-looking seeds were preferred. He indicated that uniformly sized seed-lot gave a more uniform crop than ungraded seeds. According to IITA report (1976), numerous breeding lines with good storability have been recovered at IITA, Nigeria from crosses of high-yielding types with parents having good storability.

2.5 Effect of seed moisture content and temperature on quality and viability

The occurrence of fungi in or on the seeds or grains depends on their ability to survive and proliferate under the extreme dry conditions of seed storage. Seed moisture has been considered to

be the prime factor in colonization of seed by fungi. According to Roberts (1972), the lower the temperature and moisture content of the seed, the longer the period of viability.

Kelly (1988) devised two simple rules for stored seeds. These state that for each one per cent reduction in moisture content, the storage life of the seed is doubled and for every five degrees celsius lowering of storage temperature, the storage life of the seed is doubled. These rules operated with seed moisture content between five and 14% and with temperatures from zero to 50°C and were independent of each other.

According to Toole and Toole (1946), soyabeans with 13.5% moisture content maintained at 30°C failed to germinate after five months, whereas full germinability was maintained after ten years of storage at minus ten degrees celsius. Soyabeans maintained at 20°C with 18% moisture content lost all viability in five to nine months, whereas with eight to nine per cent moisture content, 90% germination was obtained after five years of storage.

Tervet (1945) showed that high moisture content and high storage temperatures favoured incidence of fungal attack particularly *Aspergillus* species. Christensen and Kaufmann (1969) observed that soyabean seeds died rapidly if stored with a moisture content just below that which permitted storage fungi to grow and at 25°C. James et al. (1967) observed that soyabeans with 11.4% moisture lost viability when stored for 34 weeks at 32.2°C and those at 21.1°C showed signs of incipient deterioration. Following three months storage under room temperature (21.1°C and 30% RH) the

two lots at 7.92% and 11.4% moisture dropped in viability to 42 and 55%, respectively.

From the age index formula (Huckil, 1963) based on storage temperature and seed moisture, soyabean seeds will lose their ability to sustain commercial production unless the seeds were stored below 12% moisture content in moisture-proof containers or kept under refrigeration. This observation is in agreement with Dorworth and Christensen (1968) that high seed moisture content and high temperature during storage decreased germinability.

According to Kelly (1940), once fungi have invaded grain, they will continue to grow in it at a lower moisture content than they otherwise would. As a result, the grain absorbs moisture much more readily and loses it slowly than sound grains. According to FAO report (1982), high moisture in stored seeds increased respiration and reduced viability. It was observed that when moisture content was above 14%, moulds grew on soyabean seed, and when it was above 18% heating occurred. Kelly (1988) reported that, in general, seed which had between 10 and 14% moisture content maintained viability until the next sowing season while seeds with less than ten per cent moisture content kept for longer periods. Christensen and Kaufmann (1965), studying the fungus invasion and deterioration of wheat stored at low temperatures and moisture contents of 15 and 18%, observed that wheat with a moisture content up to 16% could be stored safely for as long as 19 months at temperature of minus five degrees celsius.

Dorworth and Christensen (1968) stored soyabean with initial moisture contents of 12.1%, 14.7%, 16.5% and 18.3% at temperatures of 15, 25 and 30°C. Germination percentage of the beans stored at 15°C remained above 95% for 24 weeks at all moisture contents except those with 18.3% moisture content. At 20°C, germination generally remained above 90% for those at the lower moisture contents, but decreased sharply in those stored at 16.5% and 18.3% moisture content. At 20°C, germination generally remained above 90% for those at the lower moisture contents, but decreased sharply in those stored at 16.5% and 18.3% for 20 weeks. In those at 25 and 30°C, germination percentage decreased with increasing moisture content and time.

2.6 Effect of seed health status on quality and viability

According to Allen (1983), deterioration of seed proceeds inexorably at a rate dependent on temperature and water content, starting as the seed matures and continuing through harvest and storage to sowing and germination. In soyabean, the process of deterioration precedes death by a longer period than in many crops.

Dwivedi and Shukla (1990) reported that variation in number and kind of fungal species associated with different seed samples might be largely attributed to difference in agro-climatic conditions favourable or unfavourable to the development, germination, multiplication, perpetuation and dispersal of respective fungal spores. Allen (1983) observed that species of fungi which may play a role in seed decay prior to harvest include

Colletotrichum truncatum Schw., *Cercospora kikuchii* (Matsumoto and Tomoyasu), *Macrophomina phaseolina* (Tassi) Goid and *Fusarium* species. He also reported that *C. kikuchii* may be antagonistic to *Phomopsis* in both pods and seed and suggested that might explain why purple-stained seed caused by *C. kikuchii* gave higher germination than unstained seed, when the latter had a higher incidence of *Phomopsis*.

According to Dwivedi and Shukla (1990), some fungal flora associated with gram seeds were pathogenic, causing decrease in plant stand. They observed that these fungi gained entry into dry seeds through mechanical injuries and were also transmitted as superficial contaminants. According to Bedi and Jhooty (1986), *Aspergillus flavus* Link ex. Fries previously considered as storage mould infected kernels of maize before storage.

Reddy and Nusrath (1985) observed that field and storage fungi on sorghum (*Sorghum bicolor* (L.) Moench) have been worked out in India and other countries in relation to different parameters such as climatic conditions, time of fungal invasion, stage of grain maturation, pest infestation and type of storage. *Aspergillus* represented the maximum number of species in all types of earheads of sorghum and also contributed to a high percentage of total fungal flora. *Curvularia*, *Fusarium* and *Penicillium* species were frequent in the compact earheads as compared with those of normal and open type earheads. Bilgrami et al. (1976) reported that fungal invasion caused deterioration in the quality of pulses during storage. According to Dwivedi and Shukla (1990), a total of

20 species of fungi belonging to *Aspergillus*, *Fusarium* and *Penicillium* were found associated with gram seeds.

Asthana et al. (1989) observed that during storage, fruits and seeds become heavily infested with fungi which lead to their spoilage. Christensen and Kaufmann (1965) contended that storage fungi caused a decrease in maize seed germination by damaging seed embryo or inducing biochemical changes in them. Gopinath and Shetty (1985) showed that *Fusarium moniliforme* Sheldon was associated with seed rot, germination inhibition and seedling rot in sorghum seed. Similar observations were made by Tripathi (1974). According to Singh (1990), apart from saprophytes and storage fungi, many important pathogens have been recorded in seeds viz: *Botryodiplodia theobromae* Pat., *Drechslera maydis* (Nisik) Subram. and Jain, *F. moniliforme*, *Nigrospora oryzae* Sub. and Jain and *Trichoconiella padwickii* (Ganguly) Jain in rice, *Alternaria alternata* (Fries) Keissler and *Ascochyta rabei* (Pass.) Labrousse in chickpea. He pointed out that most of these fungi were seed transmitted, causing heavy losses and adverse effect on seed germination. According to FAO report (1982), increased seed infection by fungi was always associated with rapid deterioration. Tuite (1959) reported of a low occurrence of storage fungi in fresh seeds and grains and observed that storage fungi were able to utilize metabolic water and that too much water was detrimental to their growth. Mulivex and Tuite (1970) observed that some fungi such as *Penicillium* occur both in the field and in storage. Chenule and Vir (1979) observed that *Fusarium*, *Curvularia*,

Alternaria, *Penicillium* and *Aspergillus* species were associated with post-harvest deterioration of grains. Singh and Chohan (1987), working on stored cowpeas, detected *A. niger* Van Tieghem, *A. terreus* Thom, *Curvularia verruculosa* Tandon and Bilgrami ex. M.B. Ellis, *F. moniliforme*, *Penicillium crustosum* Thom, *Rhizoctonia bataticola* Butl. and *Rhizopus arrhizus* Fischer. Del Prado and Christensen (1952) reported that under normal storage condition of feed and food *Fusarium*, *Helminthosporium* and *Alternaria* species may die within few months.

2.6.1 Location of seed mycoflora

According to Gopinath and Shetty (1985), knowledge of the primary site of infection of the pathogen and the location of the production of toxic metabolites is vital in evolving suitable control measures. They also observed that 50% of the *Fusarium* infection recorded in their study on the location of *Fusarium* species on the seeds of sorghum cultivars was found to be internally seedborne. They reported that in sorghum seeds, *F. oxysporum* Schlecht. (endahl) infection may be seedborne or restricted to the outer layers of the pericarp. They also recorded *F. solani* (Mart.) Appel and Wr. in the pericarp and endosperm only and not in the embryo. Basu-Chandhury and Pal (1981) also observed a high incidence of infection by various *Fusarium* species in sunnhemp (*Crotalaria juncea* L.) and the three seed components, pericarp, endosperm and embryo, showed heavy *Fusarium* infection.

In a study on the location of *Macrophomina phaseolina* in sunflower seeds, Sadashivalah et al. (1986) reported of a high infection in the pericarp compared to the seed coat. However, the endosperm and the embryo were free from infection. They also reported the occurrence of the pathogen on the inner surface of both pericarp and seed coat. According to Dhyani et al. (1990), the infection of *Phoma destructiva* Plowr. in red pepper and bell pepper was 16 and 20% in seedcoat and ten and 13% in endosperm respectively.

Neergaard (1977) observed that in loose smut of barley caused by *Ustilago nuda* (Jens.) Rostr., blossom infection led to the establishment of dormant mycelium in the embryo and in covered smut caused by *U. hordei* (Pers.) Langerh. The fungi occurred only as contaminant on the surface of the seed. He also discovered that *Drechslera graminea* (Rabenh. ex. Schlecht) Shoemaker, the cause of barley leaf stripe, occurred as dormant mycelium within the pericarp.

According to Christensen (1951), fungi known to cause deterioration of moist stored wheat were found as dormant spores on the surface of the seed and as dormant mycelium within the pericarp.

2.6.2 Common fungal metabolites

Seed with high moisture content are metabolically active. Dwivedi (1990) reported of higher phenol levels in deteriorated seeds as a result of fungal invasion than in healthy ones.

According to Prasad (1987), besides cytological and biochemical changes in the seed, seed-borne fungi could also suppress germination. Crisan (1973) demonstrated that aflatoxin B₁ was the most toxic among all mycotoxins and restricted the growth of plants by inhibiting seed germination, seedling growth and other physiological processes of the plant. Aflatoxin also inhibited chlorophyll synthesis in maize seedlings which resulted in virescence or albinism in seedlings.

Singh and Prasad (1983) observed that a large number of fungi caused several biochemical changes in sesamum seed. According to Gopinath and Shetty (1985), various *Fusarium* species produced toxins which were phytotoxic and potential health hazards. Tripathi (1974), Basu-Chaudhary and Pal (1981) attributed reduced seed germination in sorghum to mycotoxins production by pathogens present in the embryo. Gopinath and Shetty (1985) pointed out that although, *Fusarium* was considered to be a soil-borne pathogen, its seed-borne nature has been established. They also observed wilting and inhibition of germination of coriander (*Coriandrum sativum* L.) seedlings and seeds due to toxic metabolite produced by *F. oxysporum* f. sp. *coriandrii* Schlecht. *F. moniliforme* also produced toxic metabolite with pectic and cellulolytic properties which played a very important role in stalk rot symptoms of cereal plants. In addition, it reduced germination percentage by killing the seed embryo. Dwivedi (1990) also reported that in infected purple grains of rice, the phenolic content was high and concluded that higher levels of phenolic compounds soon after infection might

be responsible for seed deterioration.

2.7 Effect of chemical seed treatment on quality and viability of seeds

Advancement in agrotechnical measures embraces the intelligent use of chemical treatment of a crop from the seed stage so as to protect the seeds from various micro-organisms which cause diseases resulting in low germination with subsequent poor establishment in the field. Asthana *et al.* (1989) reported that most fungicides have adverse effects on seed germination and plant health. They also reported of similar effects on human health. However, Vadivelu *et al.* (1985) reported of the usefulness of fungicidal treatment in minimising loss in viability and vigour in stored seeds of various crops.

Bansal and Sobti (1990) used Thiram to check the incidence of *A. flavus* and *A. niger* in groundnut. Sinha *et al.* (1984) in a study on the effect of fungicidal seed treatment on germination of triticale seeds observed that fungicides improved germination probably due to inactivation of fungi associated with seeds. In their study, seeds treated with Thiram, Duter, Benlate, Bavistin, Dithane M-45, Baytan and Dithane Z-78 gave significantly higher emergence than the untreated seeds.

According to Dhyani *et al.* (1990), Thiram was effective in controlling *Phoma destructiva* in chilli in both petri plate and pot experiments.

According to Singh and Monga (1985), several seed dressing chemicals, dilute nitric acid and hot water treatment have eradicated seedborne inoculum of rice seed. However, some of these chemicals in organic solvents have also been used successfully. Vaidya and Vir (1989) pointed out that *A. niger* and *A. flavus* spoiled stored groundnut and reduced its quality and quantity of oil during pathogenesis. However, post-harvest treatment of groundnut with propionic acid and sodium metabisulphite as spray and dip were effective against these fungi.

FAO report (1982) indicated that in soyabeans, most internally seed-borne fungi were restricted primarily to the seed coat, with relatively little penetration into the cotyledons, thus fungicides which were able to penetrate the seed coat would effectively control most seedborne fungi of the crop. It also reported increased germination percentage and field emergence in poor quality seed treated with fungicides capable of penetrating the seed coat.

2.7.1 Effect of hot-water treatment on seed quality and viability

Heating of seeds in order to control seed-borne pathogens has been known for decades. According to Gokte and Mathur (1992), hot-water treated paddy seeds improved germination compared with control samples. This was attributed to seed-borne flora and fauna which were eliminated from the seed due to the treatment. However, after 12 months of storage, very little reduction in viability was observed.

However, hot-water treatment has draw backs namely,

- i) the heat travels slowly, takes more time in reaching the centre of the seed, and
- ii) drying of the treated seed to the required moisture level.

According to Hansen (1990), heating seed by warm, dry air in an oven is also difficult because of the slow penetration of the heat into the centre of the bulk of seed.

2.7.2 Treatment of seed with microwaves for control of seed-borne pathogen

According to Hansen (1990), microwaves are easily absorbed by biological materials resulting in heating. They penetrate into the centre of the seed and consequently, all parts are heated simultaneously. As a result of these advantages, attempts have been made to control seed-borne pathogens by the use of microwaves. So far, dry seeds of maize, rice and cowpea with infections of *F. moniliforme*, *Pyricularia oryzae* Cav. and other fungi, have been treated at temperatures up to 75°C for about 40 min. With such microwave treatment, it has been possible to reduce the incidence of viable infection in seed up to 96% and germination retained at a fairly high levels. The success of the use of microwaves has been limited because microwaves are equally absorbed by the pathogens and the seed. Uncontrolled heating may result in loss of germination of seed.

2.7.3 Effect of biologically treated seeds on quality and viability of seeds

Anon (1987) viewed biological control of plant pathogens as disease control by the use of natural or modified organisms, genes or gene products.

According to Gabriel and Cook (1990), *Pseudomonas fluorescence* (Flügge) Migula, applied as a seed treatment, was able to control *Gaeumannomyces graminis* var. *tritici* Walker of wheat and was comparable to chemical control. They also pointed out the use of *Bacillus cereus* Frankland and Frankland to control *Phytophthora megasperma* Drechsler in soyabeans and alfalfa. In their field tests, soyabean seeds coated with the protecting isolate of *B. cereus* were protected against *P. megasperma*.

Gabriel and Cook (1990), also observed that species of *Trichoderma* and *Gliocladium* were very effective in reducing soil-borne pathogen. The high degree of effectiveness was attributed to the ability of the two organisms to suppress a large number of plant pathogens by employing all three arsenal of antagonism, namely competition, antibiosis and parasitism.

Harman et al. (1980) reported that biologically treated seeds have to tide over for a few months from the processing to utilization of the seed for sowing. When *Trichoderma*-coated radish seeds were stored at room temperature (20 - 25°C) the *Trichoderma* remained viable for two to 14 months, depending on the species and isolates.

Pandey (1982) reported that several workers have studied the effects of plant parts especially leaves on seed mycoflora. Sobti and Bansal (1990) also indicated the antimicrobial property in some plant extracts and looking at the cost factor of fungicides and their toxic hazards, some non-toxic plant extracts which are easily available to farmers are being exploited as alternative. According to Fawcett and Spencer (1970) and Beye (1978), some products of plant origin have shown potentials as non-phytotoxic and easily biodegradable fungal toxicants.

Pandey (1982) indicated that leaf extracts of *Datura alba* L. and *Canabis sativa* L. efficiently reduced seed mycoflora population in *Eleusine coracana* (L.) Gaertn. seeds stored for one year. The fungi found associated with *E. coracana* were *A. niger*, *A. flavus*, *Drechslera rostrata* Drechslera, *Penicillium chrysogenum* Thom, *Curvularia lunata* (Walker) Boedijn and *Monilia* species. Apart from the extracts controlling the seed mycoflora, a higher seed germination was recorded after such treatment.

According to Vir and Sharma (1986), extracts from *Azadirachta indica* L. exhibited antimicrobial activity against *Fusarium moniliforme*, *Aspergillus niger* and *Macrophomina phaseolina*. Bansal and Sobti (1990) also pointed out that neem leaf extract was at par with Thiram and Dithane M-45 in reducing the incidence of *A. flavus* and *A. niger* and also increased germination of groundnut seed in vitro. They attributed this to the presence of certain antifungal substances in the extract. Odelola and Okorosobo (1988) observed

the presence of saponin and tannin in the extracts of two Nigerian *Diospyros* species. Kishore *et al.* (1989) observed that Chenopodium oil was 1.5 times more effective than Benlate, twice as effective than Dithane Z-78, three times better than Thiovit, and four times more effective than Captan. Jain and Jain (1972) also observed during antifungal studies of some volatile oils and their combinations that the inhibitory effects of the oils were comparable with those of the selected standard substances (including Benzoic acid 1%, Hamycin 0.5%, Cetrимide 1%, Salicylic acid). Clocimum oil was found by Tripathi *et al.* (1986) to be more effective fungicide than Dithane M-45, Blitox -50 and Bavistin.

Recent studies by Asthana *et al.* (1989) showed the efficacy of the essential oils of *Ocimum adscendens* Willd in controlling fungal deterioration during storage of chilli seeds and this was attributed to the fungitoxic properties of the oils. According to them, seeds of chilli (*Capsicum annum* L.) treated with the essential oil of *O. adscendens* as well as synthetic fungicides Bavistin, Blitox -50 and Dithane M-45, were stored in jute bags and tin containers for 12 months. The oil protected the seed completely from fungal development and was more efficacious than the synthetic fungicides. The oil did not show any adverse effect on seed germination and seedling growth of *C. annum*. They also reported that, the oils of Cedrus, Citrus and *O. canum* could not check the appearance of *Penicillium* and *Rhizopus* species but *O. adscendens* eliminated these fungi at very low concentrations.

Dikshit et al. (1983) dressed seeds of *Corriandrum sativum* (L.) and *Foeniculum vulgare* Mill. with essential oil of cedrus at 0.1, 0.3 and 0.5 per cent concentration (v/w) and stored the samples for 12 months. They observed that the 0.1 per cent concentration was able to protect the seeds of both spices up to 75% and 60% respectively. Dubey et al. (1983) recorded 80% seed protection from these two spices with the oils of *Citrus medica* (L.) and *O. canum* at the same concentration.

According to Chandra et al. (1982), the essential oil of *Ageratum conyzoides* L. could protect chilli seeds from fungi deterioration up to six months at 0.1 per cent concentration. Asthana et al. (1989) observed that *Aspergillus awamori* Nak., *A. nidulans* Winter, *A. ochraceus* Wilhelm, *A. ruber* (Spieckmann and Bremer) Thom and Church, *Cladosporium* and *Fusarium* species were susceptible to ocimum oil as their appearance was completely checked even at low concentrations of the oil. However, the oil did not affect seed germination, seedling growth and general health of the plant.

Vadivelu et al. (1985) reported that seed protectants of plant origin with Thiram and moisture-vapour proof containers used for carrying over the seeds of Bengal gram considerably augmented the shelf life of the seed.

2.8 Effect of storage containers on seed quality and viability

According to Allen (1983), the production of good quality soyabean seed and maintenance of its viability during storage have

proved difficult in the humid tropics. Seed deterioration is often the major factor limiting the spread of soyabean production in tropical regions. However, various pathogens have also been implicated in seed deterioration prior to harvest and during storage.

Vadivelu et al. (1985) reported that viability of seeds in storage was influenced by moisture content as well as storage containers. They also reported that rapid deterioration of Bengal gram seeds of the cultivar CO.1 in moisture-vapour pervious containers might be due to the effect of temperature and relative humidity of the storage atmosphere of the seeds kept in open storage conditions. This was also evident from the studies of Roos and Monalo (1971) with bean seeds.

According to Singh (1990), in India, maize, rice, wheat, chickpea and millet were grown and stored in different kinds of traditional containers made of mud, dung, bamboo and rice straw. He observed that high humidity and poor storage practices provided congenial environment for microbial attack and the dominant fungi found in the produce were species of *Aspergillus*, *Fusarium* and *Penicillium*.

Vadivelu et al. (1985) reported of the advantage of sealed containers for seed storage. They also reported that seeds of *Phaseoli vulgaris* L. stored in plastic bags gave very high germination even after two years. Boakye-Boateng and Hume (1975) studied the effect of storing soyabean seeds in cloth or polythene bags on germination at ambient temperature and relative humidity of

76%, in an air-conditioned room (22°C, 59% RH) and in a cold room (2°C, 89% RH). Seeds having a moisture content of 11.2%, 14.5% and 17.8% were stored at 22°C and two degrees celsius. Seeds in polythene bags at 22°C or two degrees celsius and 11.2% moisture each did not lose viability during 50 weeks of storage. According to Asthana et al. (1990), dried chilli fruits and seeds are commonly stored for seven to 12 months for retail sale as a spice or as seed stock for sowing in India. Chillies are still stored in gunny (jute) bags, without any treatment with fungicides. They observed that during storage, the fruits and seeds became heavily infested with fungi resulting in spoilage. According to Evans (1957), seed stored in sealed containers maintained viability longer than seeds stored in open containers. In his study, soyabean seeds of six varieties were stored at ambient temperature (25 - 28°C) in open or sealed polythene bags. After open storage for five months, five of the six varieties had less than ten per cent germination, but one variety TGM - 395 retained 51% germination.

FAO report (1982) pointed out that there was no moisture exchange when seeds with seven per cent moisture content were stored. The viability of the seeds stood at 80% for nine months under ambient temperatures under tropical conditions. However, seeds with a moisture content of ten per cent in sealed cans began losing viability in three months and did not emerge after six months.

According to Allen (1983), small quantities of dried seed could be stored in moisture-proof containers such as sealed heavy duty polythene bags, or in sealed cans. When high density polythene bags are considered for sealed storage, they ought to be thick enough to effectively prevent moisture exchange. Care must be taken to heat-seal the bags and to prevent punctures.

FAO report (1982) indicated storability of seed was largely determined by its inheritance, pre-storage history and the conditions and length of storage. The report pointed out the effect of length of storage and storage temperature on soyabean emergence at International Institute of Tropical Agriculture, Ibadan, Nigeria, for good quality seeds of 69 lines harvested in December, 1975, and stored in paper bags under ambient temperature of 20°C and 75% relative humidity or in a cold room at 19°C and 70% relative humidity. With increasing length of storage time, seedling emergence declined more rapidly for seeds stored under ambient conditions than at 19°C. After 219 days of storage under ambient conditions only four lines gave more than 50% emergence.

According to the report, where atmospheric humidity is constantly or intermittently high seed should be stored in rooms where the humidity is low. However, if such storage areas are not available, seed with less than ten per cent moisture should be stored in polythene-lined bags and moisture-impervious containers. According to the report, appropriate containers include steel bins, tin cans, glass jars and bags made of materials such as aluminized polyester or high density polythene.

The report also suggested that, for long-term economics and effectiveness, air-conditioned storage is preferred to sealed bags for soyabean storage in tropical and sub-tropical areas. Adequately air-conditioned storage should maintain a temperature of 22°C or less for a storage period of eight to nine months.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Preparation of leaf and seed powder of *Ocimum gratissimum*

Powders of both leaf and seeds of *O. gratissimum* were prepared by milling 200g each of sun-dried seeds and leaves separately in a milling machine. The leaves and seeds were dried for two weeks before milling. The milled materials were not passed through any mesh. The powders were used immediately after preparation.

3.2 Source of soyabean seeds

Two varieties of soyabeans - Anidaso and Bengbie (Wonderful beans) obtained from Crops Research Institute, Fumesua - Kumasi, were multiplied during the 1992 major season. The pods were considered to be matured when the leaves turned yellow and began to drop and the pods were dry. The matured pods were harvested manually and were hand threshed.

3.3 Disinfection of seeds

Surface disinfection of seeds was done by flooding the seeds with 2.5% Sodium hypochlorite followed by 40% ethanol. Disinfected seeds were dried in forced-air oven.

3.4 Determination of seed moisture

A modified Zeleny's technique (1961) was used in determining the moisture content of seeds. Twenty grams of seeds were dried in

a forced-air oven at 100°C for 1 hour. The seeds were ground and further dried at 70°C for 24 hours to remove excess moisture. The difference in weight was the estimated moisture content of the seed. The per cent moisture content was calculated as follows:

$$\text{Per cent moisture content} = \frac{\text{Initial moisture} - \text{Final moisture}}{\text{Initial moisture}} \times 100$$

Moisture content of seeds were taken prior to storage and at three months intervals.

3.5 Germination test of seeds

Germination tests were carried out before storage and at three months intervals during storage for the nine months storage period. Both in vitro and in vivo tests were conducted. In the in vitro studies, the blotter technique was used where seeds were plated in sterilized 9-cm petri dishes lined with three layers of moist filter papers method recommended by International Seed Testing Association (I.S.T.A.). In the in vivo studies, seeds were sown in 30cm clay pots filled with sterilized sandy soil. The soil was sterilized with 5% formalin. The pots were not pasteurized. The seeds were sown to a depth of 2cm and the pots copiously watered. Unpasteurized soil was not used for comparison. Scoring for germinated seeds started from 5 days after sowing till the tenth day. Ten seeds were plated in each petri dish and twenty were sown in each clay pot. In the in vitro tests, a seed was considered germinated when it produced a radicle within ten days and in the in

vivo studies, a seed was considered germinated when its cotyledons emerged within ten days.

Percentage germination was calculated as follows:

$$\text{Per cent germination} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds expected to germinate}} \times 100$$

3.6 Isolation of mycoflora from soyabean seeds

Fungi were isolated from soyabean seeds by either plating whole or seed components on Tomato Juice Agar. The component plating method (Neergaard, 1977) was used to determine the location of the mycoflora in infected seeds. The seeds were incubated for two weeks. In the component plating, seeds of the two varieties of soyabean were soaked separately in sterilized distilled water contained in McCartney bottles for 16 hours after which the various parts of the seeds - pericarp (testa), endosperm (cotyledon) and the embryo portions were dissected aseptically and plated.

CHAPTER FOUR

EXPERIMENTAL

Chemical seed treatment is one of the methods used to free some vegetable seeds of pathogenic organisms. This treatment can therefore lead to low incidence of diseases, increase in emergence and germination of low quality seeds (FAO report, 1982). However, synthetic chemicals could be phytotoxic and injurious to embryo of some seeds or grains (Gupta and Cholan, 1969). Seed treatment with products of biological origin may provide some answer to the phytotoxic effects of synthetic chemicals.

Experiments described below were done to determine the effects of *O. gratissimum* seed and leaf powders and Dithane M-45 on:

- a) Seed germination of two varieties of soyabean after certain periods of storage in two different containers.
- b) Mycoflora infections of the two varieties of soyabean during storage.

One margarine tin each of the two varieties of soyabean Anidaso and Bengbie were treated with 25g each of *O. gratissimum* leaf and seed powders and Dithane M-45. The powders and the chemical remained on the soyabean seeds for the nine months storage period. The seeds were stored separately in sealed paper bags and high density polythene bags. Untreated seeds in the two different containers served as controls. There were eight treatments for each of the two varieties of soyabeans.

- i) Untreated seeds in sealed polythene bag
- ii) Untreated seeds in sealed paper bag
- iii) *O. gratissimum* leaf powder treated seeds in sealed polythene bag
- iv) *O. gratissimum* leaf powder treated seeds in sealed paper bag
- v) *O. gratissimum* seed powder treated seeds in sealed polythene bag
- vi) *O. gratissimum* seed powder treated seeds in sealed paper bag
- vii) Dithane M-45 treated seeds in sealed polythene bag
- viii) Dithane M-45 treated seeds in sealed paper bag.

The sealed bags were left on laboratory shelves under room condition (23 - 27.5°C).

4.1 Effect of seed treatment on seed germination

The effect of treated seeds and control in the two containers on germination in vitro was determined by plating 400 seeds in 4 replicates of 100 seeds each on moist filter paper. Ten seeds were plated in each petri-dish and incubated at room temperature (23 - 27.5°C). The number of germinated seeds was recorded from three days after plating to the tenth day.

In the in vivo studies, 400 seeds each in 4 replicates of treated seeds and control were sown in clay pots filled with moist sandy soil. Twenty seeds were sown in each pot and incubated under ambient conditions in the plant house. The seeds were sown to a

depth of 2cm. Counting of germinated seeds started from five days after sowing to the tenth day. Percentage germination was then calculated as described in the materials and methods.

4.2 Effect of seed treatment on seed mycoflora infection

Treated and untreated seeds were randomly selected for the isolation of seed-borne mycoflora. Whole seeds from treated seeds and control were plated separately on Tomato juice agar. Five seeds were plated in each petri dish. It was incubated at room temperature for two weeks. The mycoflora associated with the seeds were isolated and identified with the aid of a light compound microscope and percentage fungal occurrence recorded.

In the component plating method, the testa, endosperm and embryo were plated on Tomato juice agar to determine the location of mycoflora. Five seed components were plated separately and incubated for two weeks. The mycoflora associated with each seed component were identified and recorded. The presence of fungi was designated + and absence as -.

CHAPTER FIVE

RESULTS AND OBSERVATIONS

Per cent germination of Anidaso and Bengbie used for the gemination tests in vitro before storage was 89% and 86% respectively (Table 1).

Table 1 : Per cent germination of Anidaso and Bengbie before storage in vitro.

	Cultivars	
	Anidaso	Bengbie
No. of seeds	400	400
No. germinated	356	344
Mean (%) germination	89	86

The viability of seeds treated with 25g each of *O. gratissimum* seed and leaves powders as well as untreated seeds and stored in either polythene bags or paper bags declined slightly after three months of storage irrespective of seed moisture levels. The rate of decline varied among treatments. The polythene bag was found to be better for storing soyabean seeds with and without seed treatment for the nine months period. The viability of seeds stored in polythene bag without treatment varied from 68% to 79% and 64% to 72% in vitro for Anidaso and Bengbie respectively; and 67% to 76% for Anidaso and 63 to 70% for Bengbie in vivo.

The seed protectants of plant origin as well as Dithane M-45 and polythene bag used for carrying over the seeds augmented the shelf life of the seeds. Generally, seeds treated with *Ocimum gratissimum* leaf powder and stored in polythene bag recorded high seed viability. Viability of seeds treated with *O. gratissimum* leaf powder and stored in polythene bag varied from 84% to 87% in vitro and 82% to 85% in vivo for Anidaso and that of Bengbie varied from 78% to 83% in vitro and 66% to 74% in vivo (Tables 2 and 3, also see Figs. 1 - 8). There was significant difference ($P < 0.05$) among treatments both in vitro and in vivo. Results of analysis of variance testing difference in per cent germination are provided in Appendix 1A, 1B, 2A and 2B.

Table 2 : Effect of seed treatments and containers on seed germination in Anidaso and Bengbie in vitro under ambient conditions.

Treatments	Containers	Per cent germination (%) after certain storage periods (months)					
		3		6		9	
		Anidaso	Bengbie	Anidaso	Bengbie	Anidaso	Bengbie
Control	Polythene bag Paper bag	79 72	72 65	72 63	69 59	68 57	64 54
<i>Ocimum</i> leaf powder	Polythene bag Paper bag	87 79	83 76	87 75	80 71	84 71	78 68
<i>Ocimum</i> seed powder	Polythene bag Paper bag	81 72	79 69	79 69	74 63	75 62	69 59
Dithane M-45	Polythene bag Paper bag	85 77	81 79	83 74	79 72	80 73	75 70

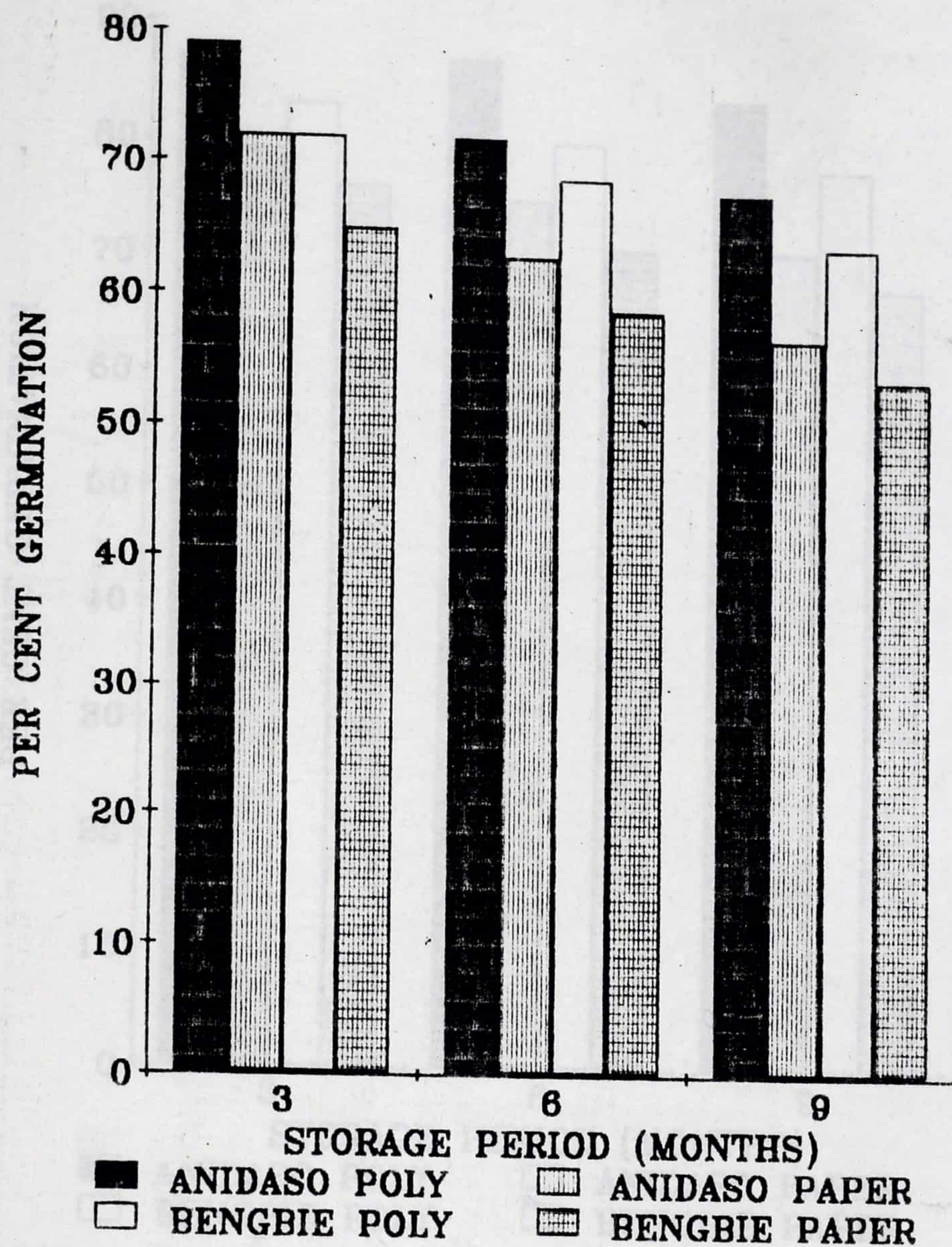


FIG. 1. EFFECT OF STORAGE CONTAINERS ON GERMINATION OF ANIDASO AND BENGBIE IN VITRO (CONTROL).

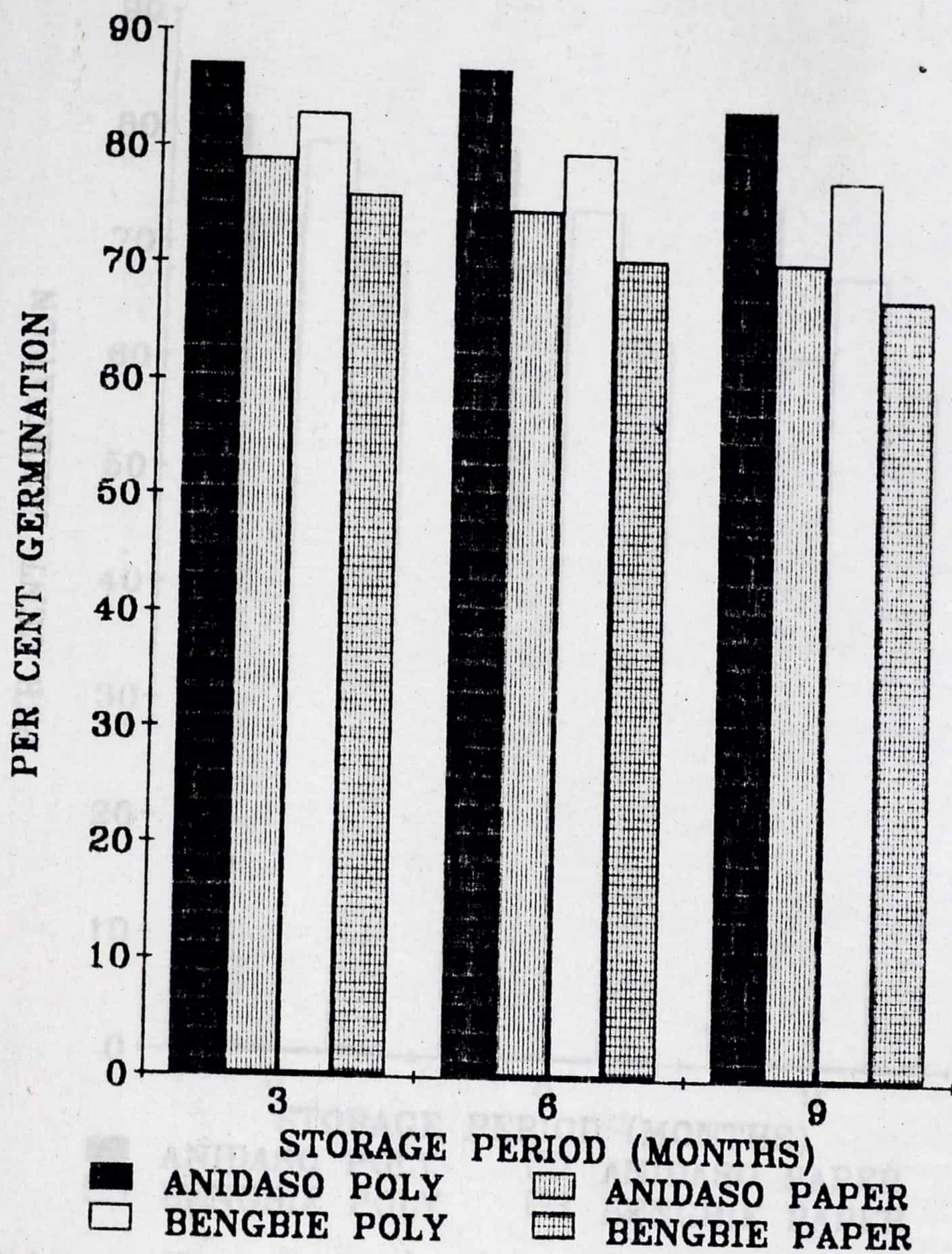


FIG. 2. EFFECT OF OCTMUM LEAF POWDER TREATED SEEDS AND STORAGE CONTAINERS ON GERMINATION OF ANIDASO AND BENGBIE IN VITRO.

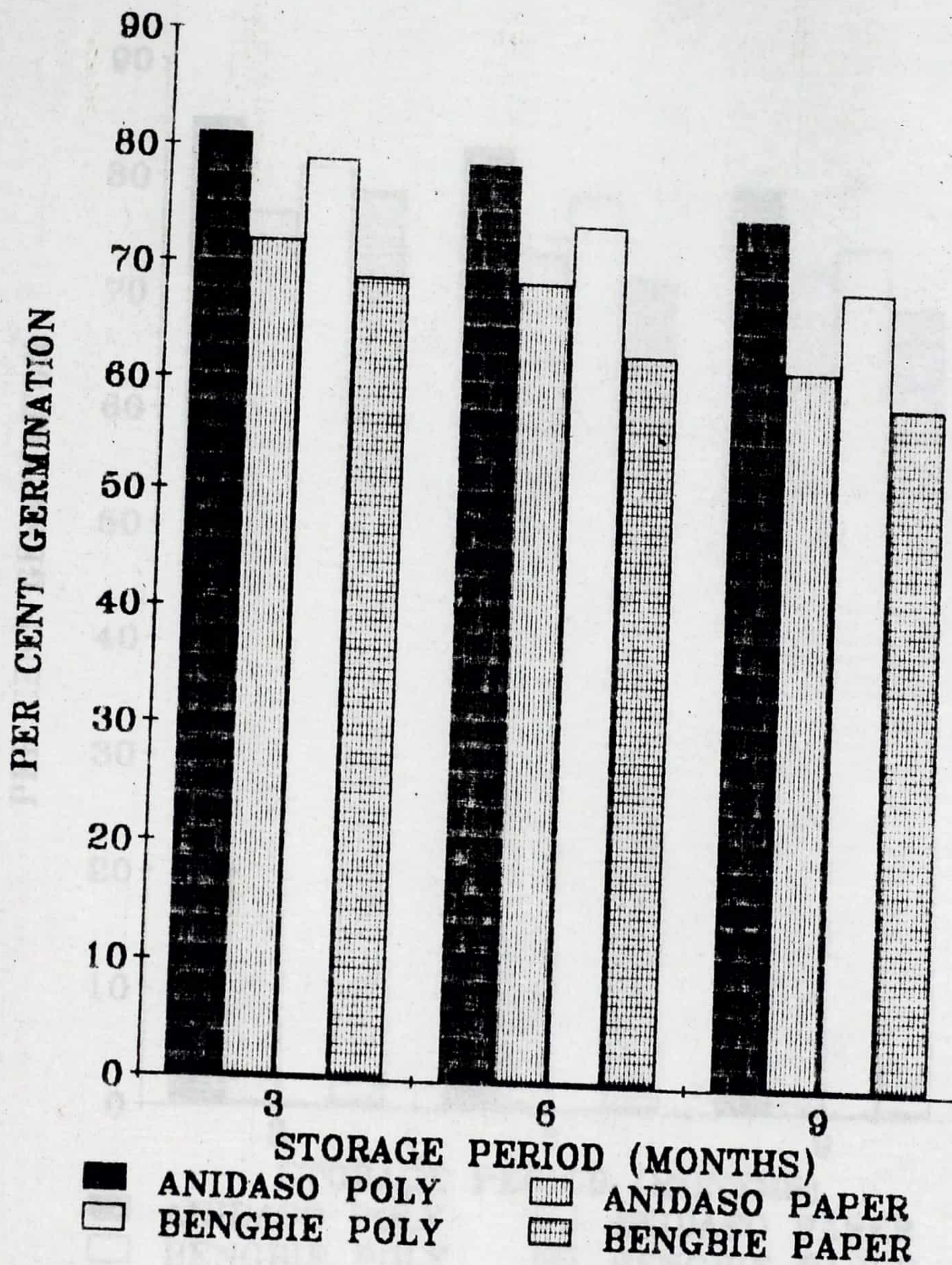


FIG. 3. EFFECT OF OCIMUM SEED POWDER TREATED SEEDS AND STORAGE CONTAINERS ON GERMINATION OF ANIDASO AND BENGBIE IN VITRO.

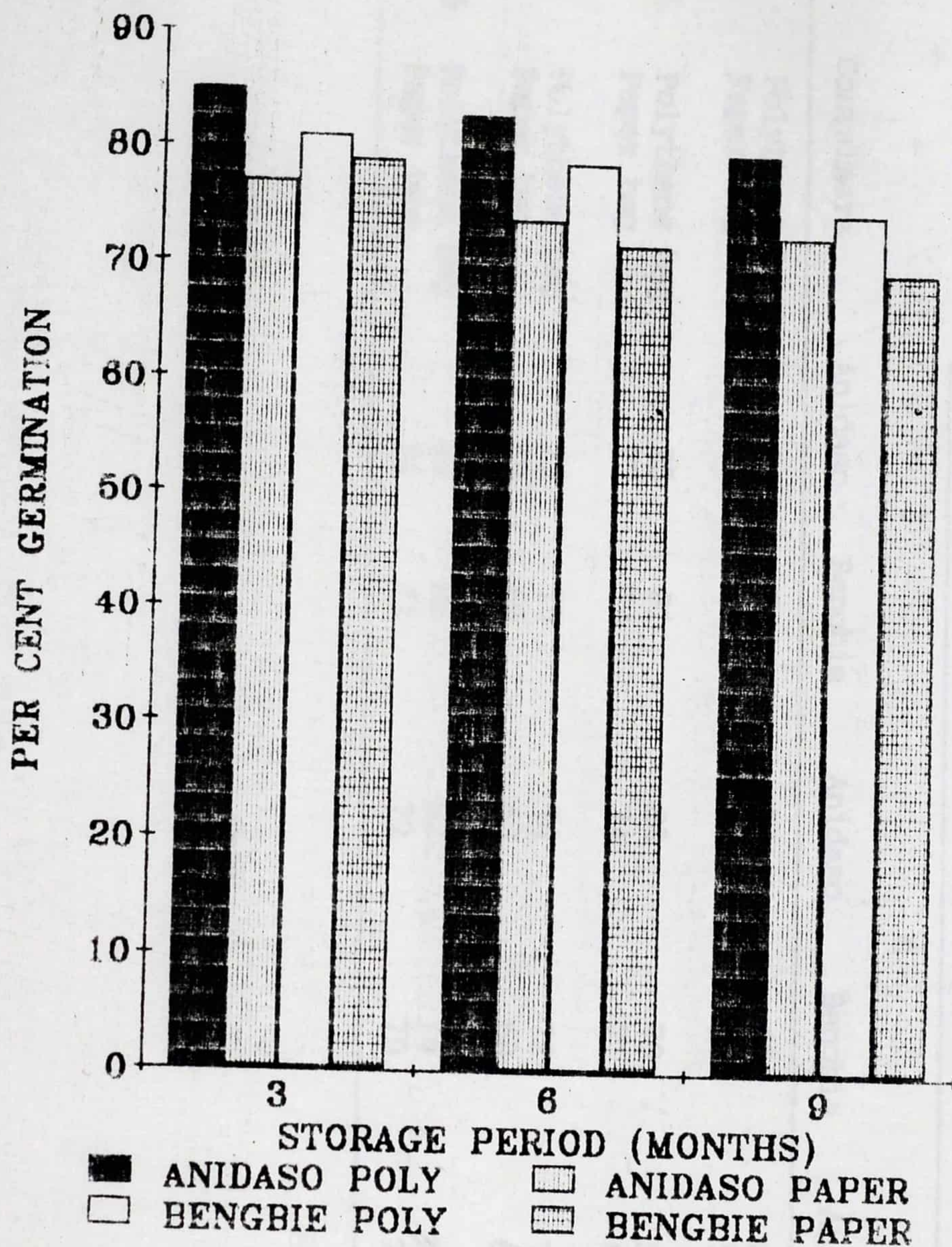


FIG. 4. EFFECT OF DITHANE M-45 TREATED SEEDS AND STORAGE CONTAINERS ON GERMINATION OF ANIDASO AND BENGBIE IN VITRO.

Table 3 : Effect of seed treatments and containers on seed germination in Anidaso and Bengbie in vivo under ambient conditions.

Treatments	Containers	Per cent germination (%) after certain storage periods (months)					
		3		6		9	
		Anidaso	Bengbie	Anidaso	Bengbie	Anidaso	Bengbie
Control	Polythene bag Paper bag	76 70	70 61	70 62	67 56	67 56	63 52
<i>Ocimum</i> leaf powder	Polythene bag Paper bag	85 77	81 74	83 74	79 71	82 69	76 66
<i>Ocimum</i> seed powder	Polythene bag Paper bag	79 69	75 66	74 63	72 60	72 60	68 57
Dithane M-45	Polythene bag Paper bag	83 74	80 71	82 72	76 70	80 71	74 67

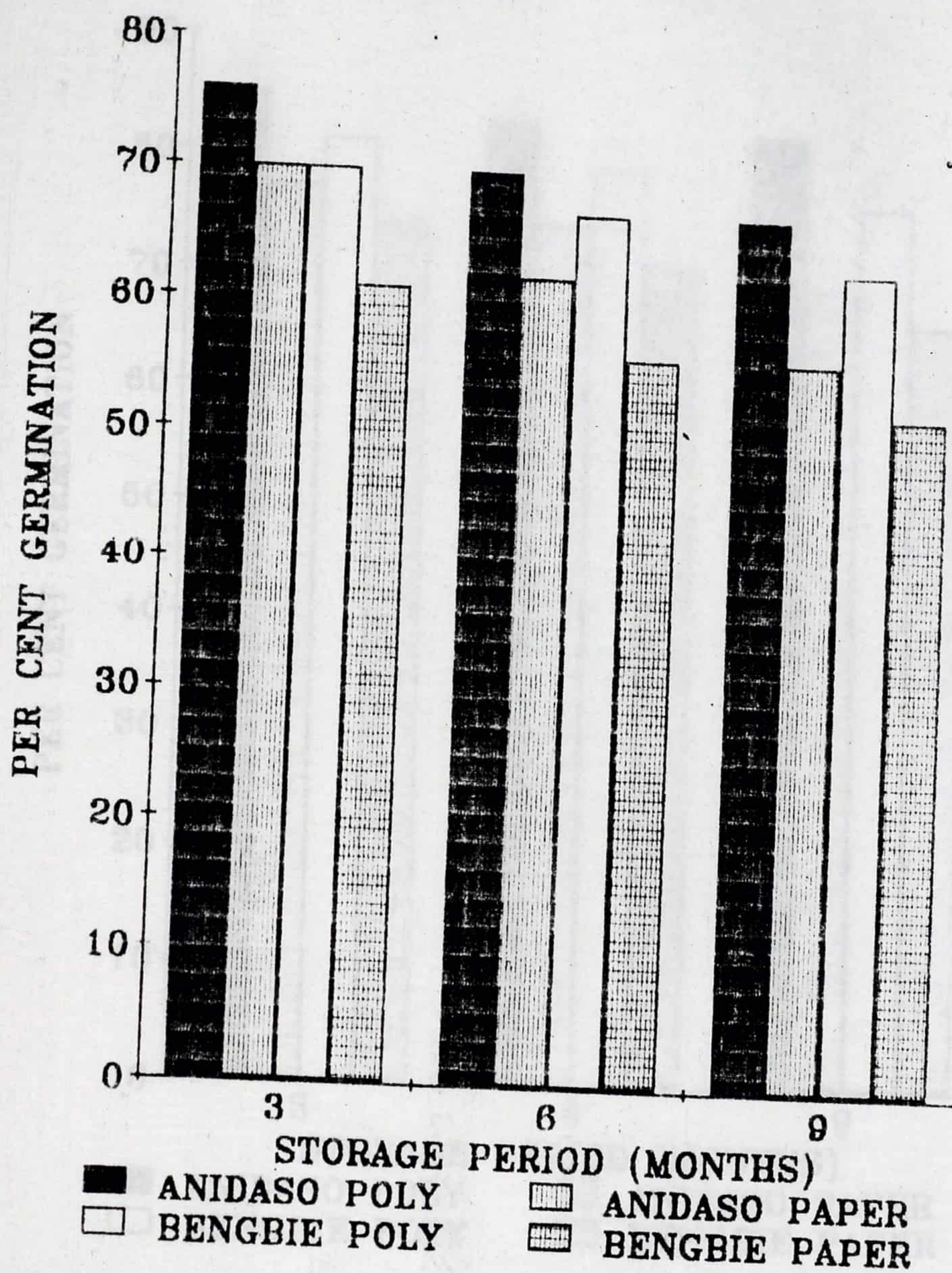


FIG. 5. EFFECT OF STORAGE CONTAINERS ON GERMINATION OF ANIDASO AND BENGBIE IN VIVO (CONTROL).

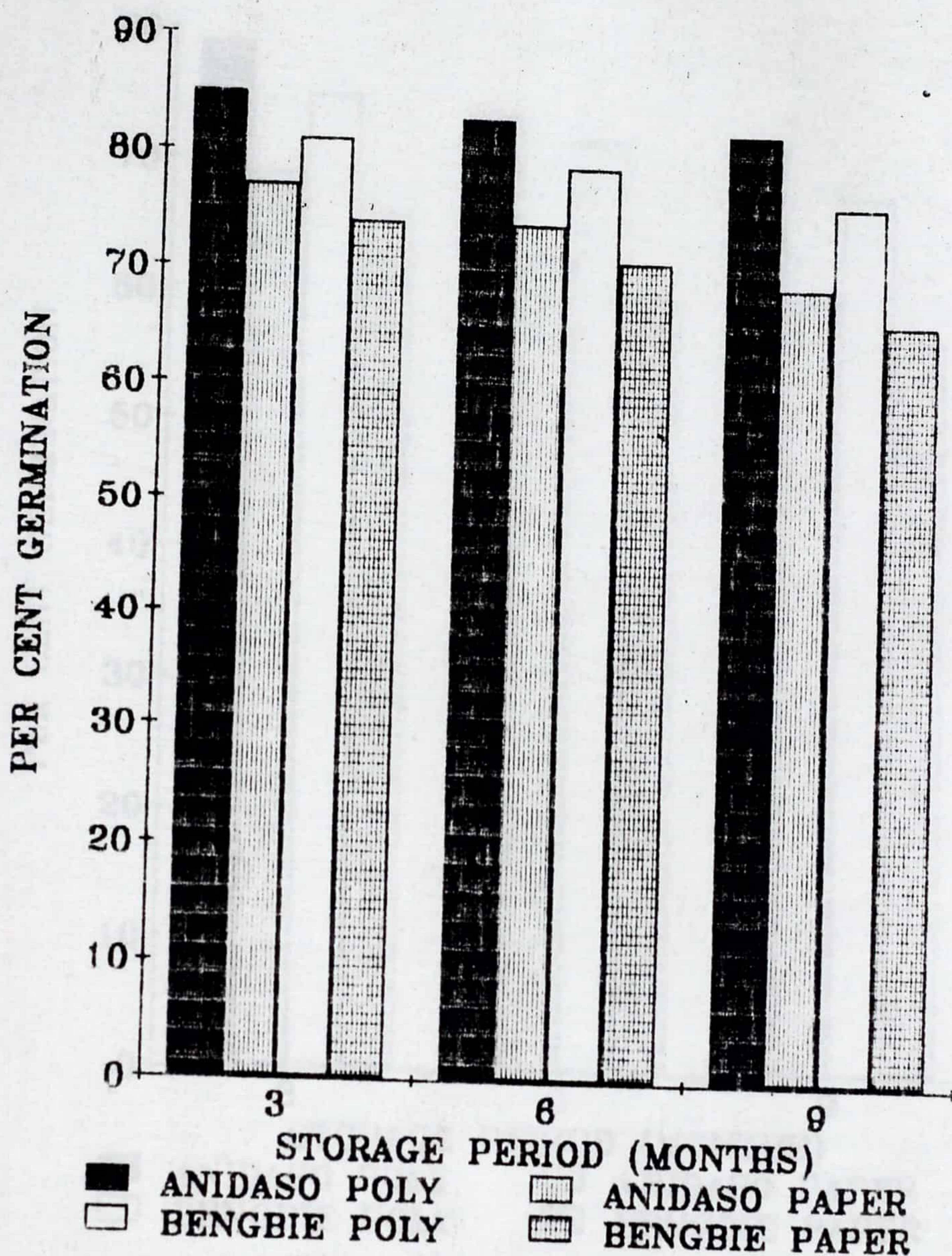


FIG. 6. EFFECT OF OCIMUM LEAF POWDER TREATED SEEDS AND STORAGE CONTAINERS ON GERMINATION OF ANIDASO AND BENGBIE IN VIVO.

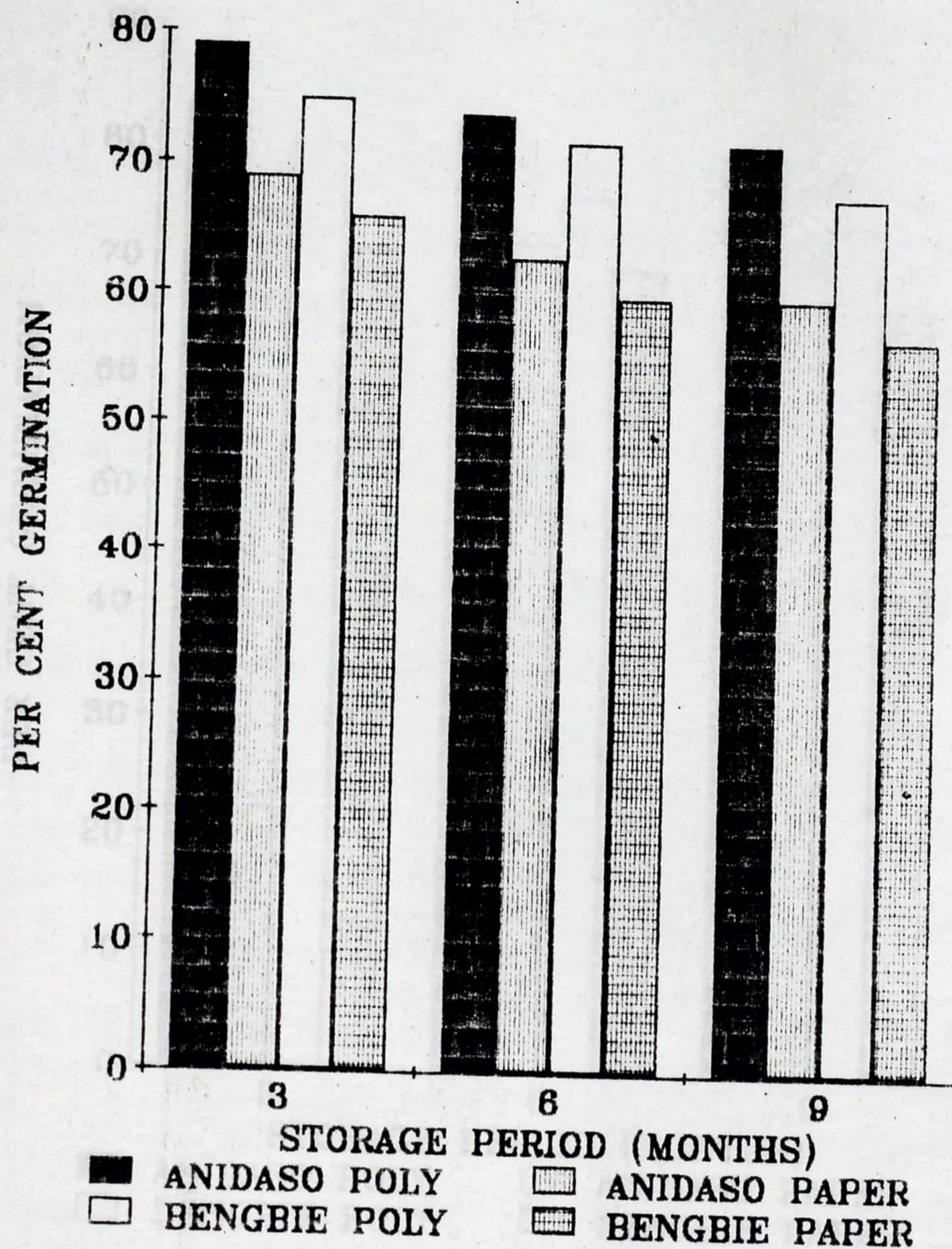


FIG. 7. EFFECT OF OCIMUM SEED POWDER TREATED SEEDS AND STORAGE CONTAINERS ON GERMINATION OF ANIDASO AND BENGIE IN VIVO.

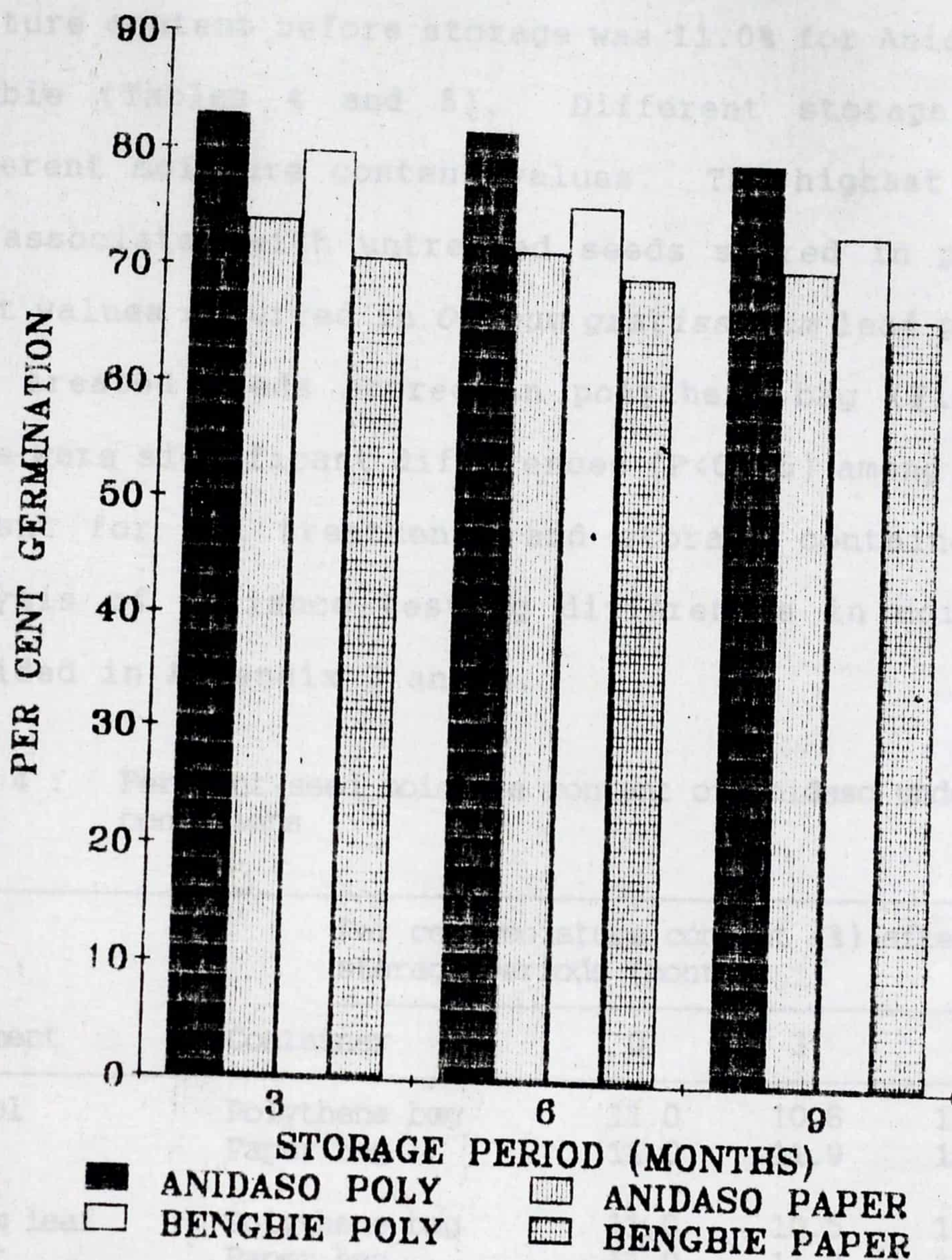
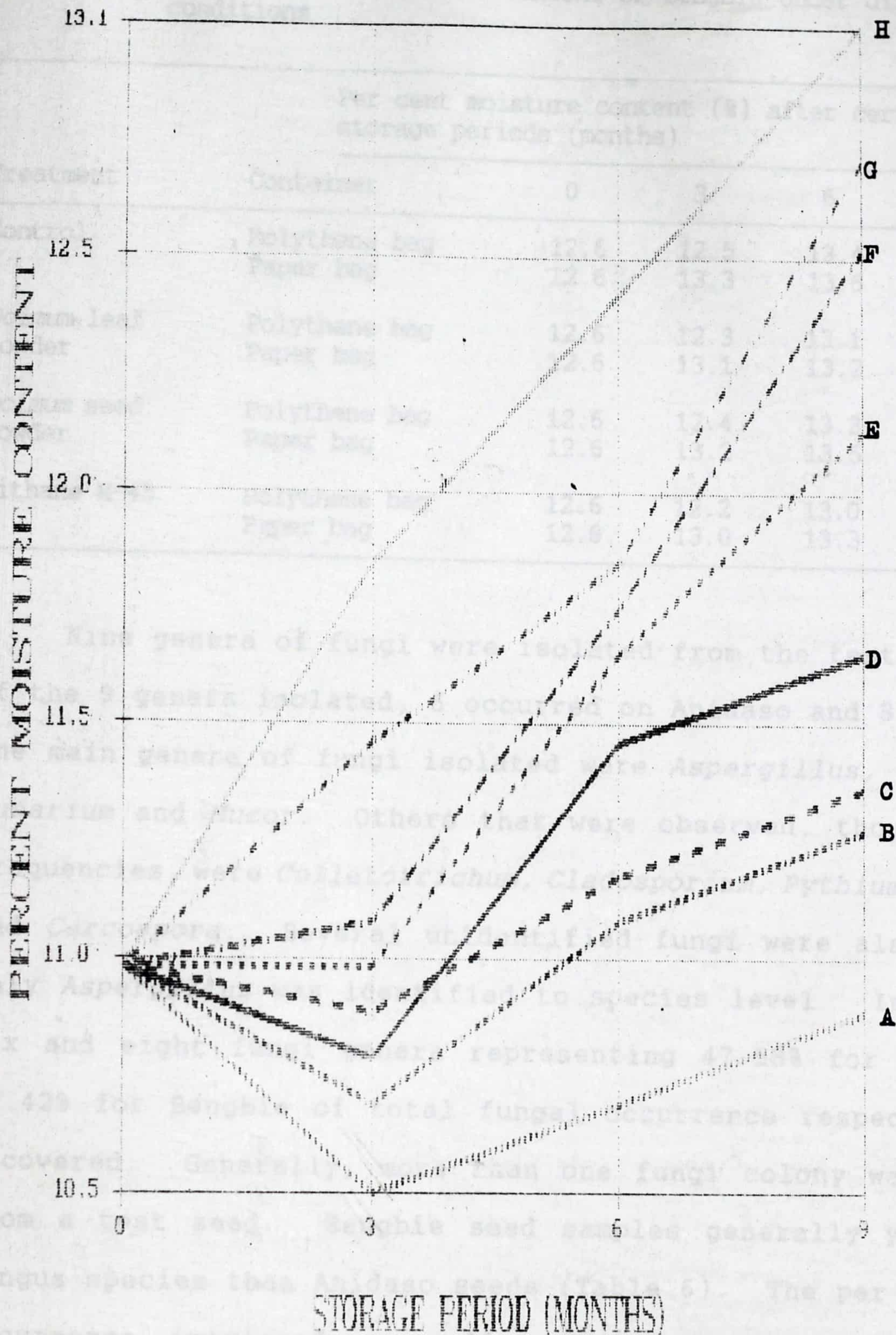


FIG. 8. EFFECT OF DITHANE M-45 TREATED SEEDS AND STORAGE CONTAINERS ON GERMINATION OF ANIDASO AND BENGIE IN VIVO.

The levels of moisture content of treated and untreated soyabean seeds ranged from 10.5% to 13.1% for Anidaso and 12.2% to 13.9% for Bengbie during the nine month storage period. The seed moisture content before storage was 11.0% for Anidaso and 12.6% for Bengbie (Tables 4 and 5). Different storage conditions gave different moisture content values. The highest moisture content was associated with untreated seeds stored in paper bag and the least values occurred in *Ocimum gratissimum* leaf powder and Dithane M-45 treated seeds stored in polythene bag (Figures 9 and 10). There were significant differences ($P < 0.05$) among the seed moisture content for all treatments and storage containers. Results of analysis of variance testing differences in moisture levels are provided in Appendix 3 and 4.

Table 4 : Per cent seed moisture content of Anidaso under different storage conditions

Treatment	Container	Per cent moisture content (%) after certain storage periods (months)				LSD
		0	3	6	9	
Control	Polythene bag	11.0	10.8	11.5	11.7	0.17
	Paper bag	11.0	11.9	12.5	13.1	0.02
<i>Ocimum</i> leaf powder	Polythene bag	11.0	10.5	10.7	10.9	0.20
	Paper bag	11.0	11.0	11.7	12.2	0.01
<i>Ocimum</i> seed powder	Polythene bag	11.0	10.9	11.2	11.4	0.09
	Paper bag	11.0	11.5	11.9	12.8	0.35
Dithane M-45	Polythene bag	11.0	10.7	11.1	11.3	0.19
	Paper bag	11.0	11.1	11.8	12.6	0.07



- | | |
|-----------------------------|------------------------------|
| A = <u>Ocimum</u> leaf poly | E = <u>Ocimum</u> leaf paper |
| B = Dithane M-45 Poly | F = Dithane M-45 paper |
| C = <u>Ocimum</u> seed poly | G = <u>Ocimum</u> seed paper |
| D = Control Poly | H = Control paper |

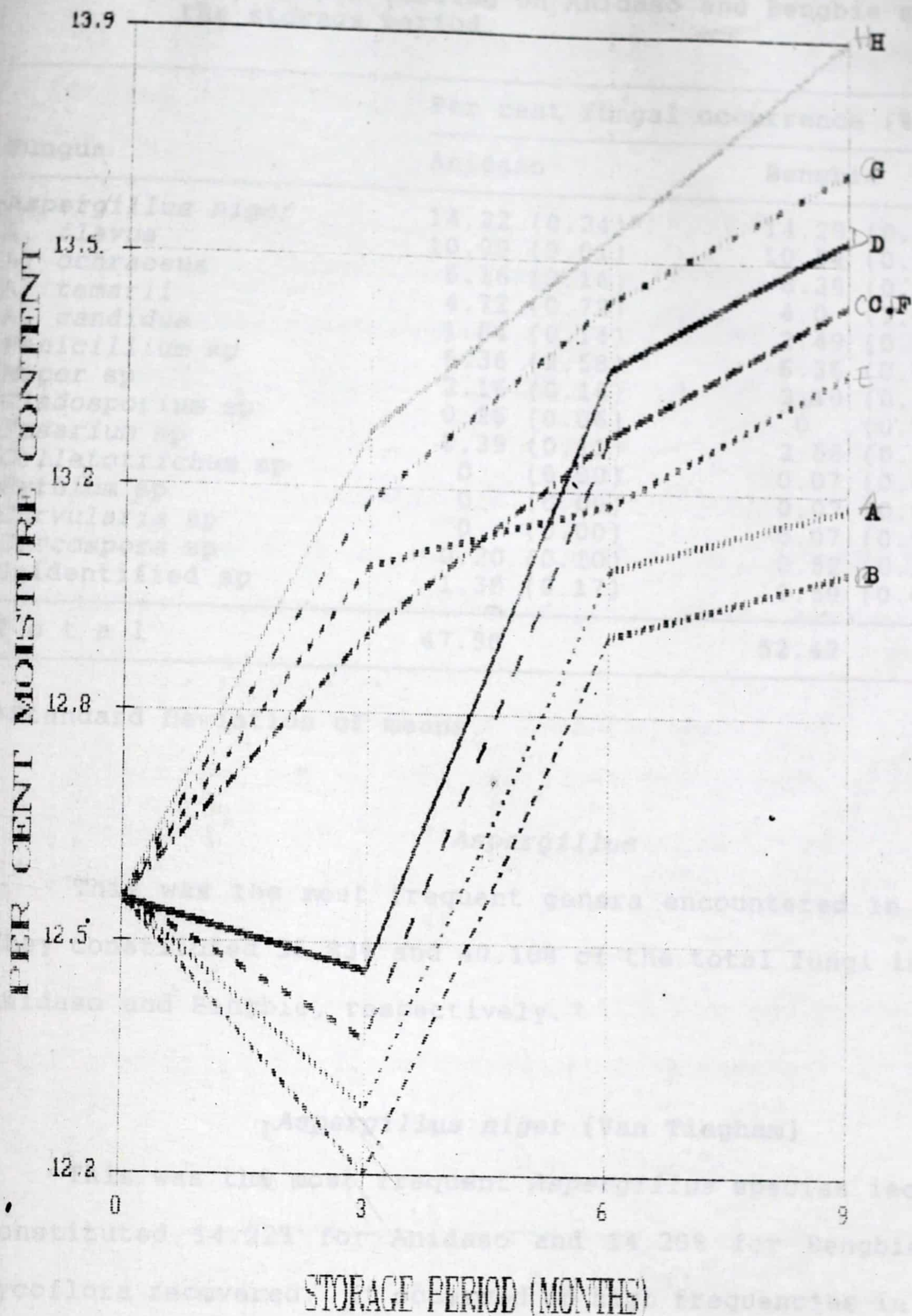
FIG. 9. Graph showing Moisture Content of Anidaso under different storage conditions.

Table 5 : Per cent seed moisture content of Bengbie under different storage conditions

Treatment	Container	Per cent moisture content (%) after certain storage periods (months)				LSD
		0	3	6	9	
Control	Polythene bag	12.6	12.5	13.4	13.6	0.01
	Paper bag	12.6	13.3	13.6	13.9	0.27
<i>Ocimum</i> leaf powder	Polythene bag	12.6	12.3	13.1	13.2	0.10
	Paper bag	12.6	13.1	13.2	13.4	0.08
<i>Ocimum</i> seed powder	Polythene bag	12.6	12.4	13.3	13.5	0.19
	Paper bag	12.6	13.2	13.5	13.7	0.20
Dithane M-45	Polythene bag	12.6	12.2	13.0	13.1	0.06
	Paper bag	12.6	13.0	13.3	13.5	0.11

Nine genera of fungi were isolated from the test seeds. Out of the 9 genera isolated, 6 occurred on Anidaso and 8 on Bengbie. The main genera of fungi isolated were *Aspergillus*, *Penicillium*, *Fusarium* and *Mucor*. Others that were observed, though in lower frequencies, were *Colletotrichum*, *Cladosporium*, *Pythium*, *Curvularia* and *Cercospora*. Several unidentified fungi were also observed. Only *Aspergillus* was identified to species level. In the assay, six and eight fungi genera representing 47.58% for Anidaso and 52.42% for Bengbie of total fungal occurrence respectively were recovered. Generally, more than one fungi colony was recovered from a test seed. Bengbie seed samples generally yielded more fungus species than Anidaso seeds (Table 6). The per cent fungal occurrence increased generally with increasing seed moisture content. There was significant difference ($P < 0.01$) among the frequencies of fungal occurrence during the storage period (See

Appendix 5 and 6).



A = Ocimum leaf poly
 B = Dithane M-45 poly
 C = Ocimum seed poly
 D = Control poly

E = Ocimum leaf paper
 F = Dithane M-45 paper
 G = Ocimum seed paper
 H = Control paper

FIG. 10 : Graph showing Moisture Content of Bengbie under different storage conditions.

Table 6 : Seed health testing on Anidaso and Bengbie seeds during the storage period.

Fungus	Per cent fungal occurrence (%)	
	Anidaso	Bengbie
<i>Aspergillus niger</i>	14.22 (0.24)*	14.28 (0.28)
<i>A. flavus</i>	10.09 (0.01)	10.94 (0.06)
<i>A. ochraceus</i>	6.16 (0.16)	8.39 (0.89)
<i>A. tamarii</i>	4.72 (0.72)	4.0 (1.00)
<i>A. candidus</i>	1.64 (0.14)	2.49 (0.51)
<i>Penicillium</i> sp	6.36 (2.58)	6.36 (0.00)
<i>Mucor</i> sp	2.16 (0.16)	2.10 (0.40)
<i>Cladosporium</i> sp	0.26 (0.06)	0 (0.00)
<i>Fusarium</i> sp	0.39 (0.11)	2.56 (0.44)
<i>Colletotrichum</i> sp	0 (0.00)	0.07 (0.01)
<i>Pythium</i> sp	0 (0.00)	0.07 (0.01)
<i>Curvularia</i> sp	0 (0.00)	0.07 (0.01)
<i>Cercospora</i> sp	0.20 (0.10)	0.52 (0.21)
Unidentified sp	1.38 (0.17)	0.59 (0.01)
T o t a l	47.58	52.42

*Standard Deviation of means.

Aspergillus

This was the most frequent genera encountered in the study. They constituted 36.83% and 40.10% of the total fungi isolated for Anidaso and Bengbie, respectively.

Aspergillus niger (Van Tiegham)

This was the most frequent *Aspergillus* species isolated. It constituted 14.22% for Anidaso and 14.28% for Bengbie of total mycoflora recovered. It occurred in high frequencies in both seeds (Table 6).

Aspergillus Flavus (Link)

This was the second most abundant species and made up of 10.09% and 10.94% of total mycoflora isolates for Anidaso and Bengbie, respectively.

Aspergillus ochraceus (Wilhelm)

Anidaso and Bengbie seeds recorded 6.16% and 8.39% of total fungal occurrence respectively of this species.

Aspergillus tamarii (Kita)

It made up 4.72 and 4.0% of total fungi isolated for Anidaso and Bengbie, respectively though its occurrence was less frequent.

Aspergillus candidus (Link)

Only 1.64% for Anidaso and 2.49% for Bengbie of total fungi were isolated.

Penicillium species

It accounted for 6.36% each for Anidaso and Bengbie of total mycoflora isolated. It occurred mostly on *Aspergillus*-free seeds.

Fusarium species

It represented a small percentage of 0.39% for Anidaso and 2.56% for Bengbie of total mycoflora isolates. *Fusarium* species appeared only on seeds free of *Aspergillus* species.

Mucor species

It constituted 2.16% for Anidaso and 2.10% for Bengbie of total mycoflora isolated. It occurred in low frequencies in all seed samples.

Cladosporium species

It constituted 0.26% of overall mycoflora population. Occurrence was only found in Anidaso and at very low frequencies.

Colletotrichum, *Pythium* and *Curvularia* species

These species were encountered in isolated cases and constituted 0.07% each of entire mycoflora recovered. They were associated with only Bengbie.

Unidentified fungi

They were suspected to be of the order Mucorales. They represented 1.38% for Anidaso and 0.57 for Bengbie of overall mycoflora isolated in the study.

Mycoflora were recovered from all treated and untreated seeds stored in polythene and paper bags in the study. There was significant difference ($P < 0.05$) among treatments and storage containers as well as length or time of storage (see appendix 5 and 6). Fungal occurrence on test samples varied from 0.95% to 11.02% for Anidaso during the storage period. The lowest fungal occurrence of 0.95% was recorded in the third month on *Ocimum* leaf

powder treated seeds stored in polythene bags. This increased to 1.93% in the sixth month and to 2.34% by the ninth month. Dithane M-45 treated seeds stored in polythene bag recorded the lowest fungal occurrence throughout the storage period except in the first three months. The highest fungal occurrence was recorded on untreated seed stored in paper bag (Table 7, Figures 11 and 12).

The fungi recovered from Bengbie seeds varied from 1.38% to 9.38%. The lowest fungal occurrence after the nine month storage period were 2.0% and 2.13% for *Ocimum* leaf powder and Dithane M-45 treated seeds stored in polythene bag respectively. Untreated Bengbie seeds stored in paper bag recorded the highest fungal occurrence of 9.38% (Table 8, Figures 13 and 14).

Table 7 : Per cent total fungal occurrence on Anidaso seeds under different storage conditions.

Treatment	Container	Per cent fungal occurrence (%) after certain storage periods (months)			
		3	6	9	LSD
Control	Polythene bag	2.20	2.76	3.31	0.47
	Paper bag	6.89	9.64	11.02	1.25
<i>Ocimum</i> leaf powder	Polythene bag	0.95	1.93	2.34	0.71
	Paper bag	3.03	4.13	4.41	0.22
<i>Ocimum</i> seed powder	Polythene bag	3.17	4.13	5.37	0.91
	Paper bag	4.27	6.06	6.75	0.56
Dithane M-45	Polythene bag	1.23	1.52	2.07	0.26
	Paper bag	3.44	4.13	5.23	0.68

Table 8 : Per cent total fungal occurrence on Bengbie seeds under different storage conditions.

Treatment	Container	Per cent fungal occurrence (%) after certain storage periods (months)			
		3	6	9	LSD
Control	Polythene bag	2.88	3.13	3.25	0.11
	Paper bag	6.50	8.75	9.38	0.60
<i>Ocimum</i> leaf powder	Polythene bag	1.63	1.63	2.00	0.00
	Paper bag	2.75	3.63	4.38	0.85
<i>Ocimum</i> seed powder	Polythene bag	3.13	4.00	5.50	0.87
	Paper bag	5.00	5.75	7.13	0.72
Dithane M-45	Polythene bag	1.38	1.50	2.13	0.10
	Paper bag	4.63	4.88	5.13	0.25

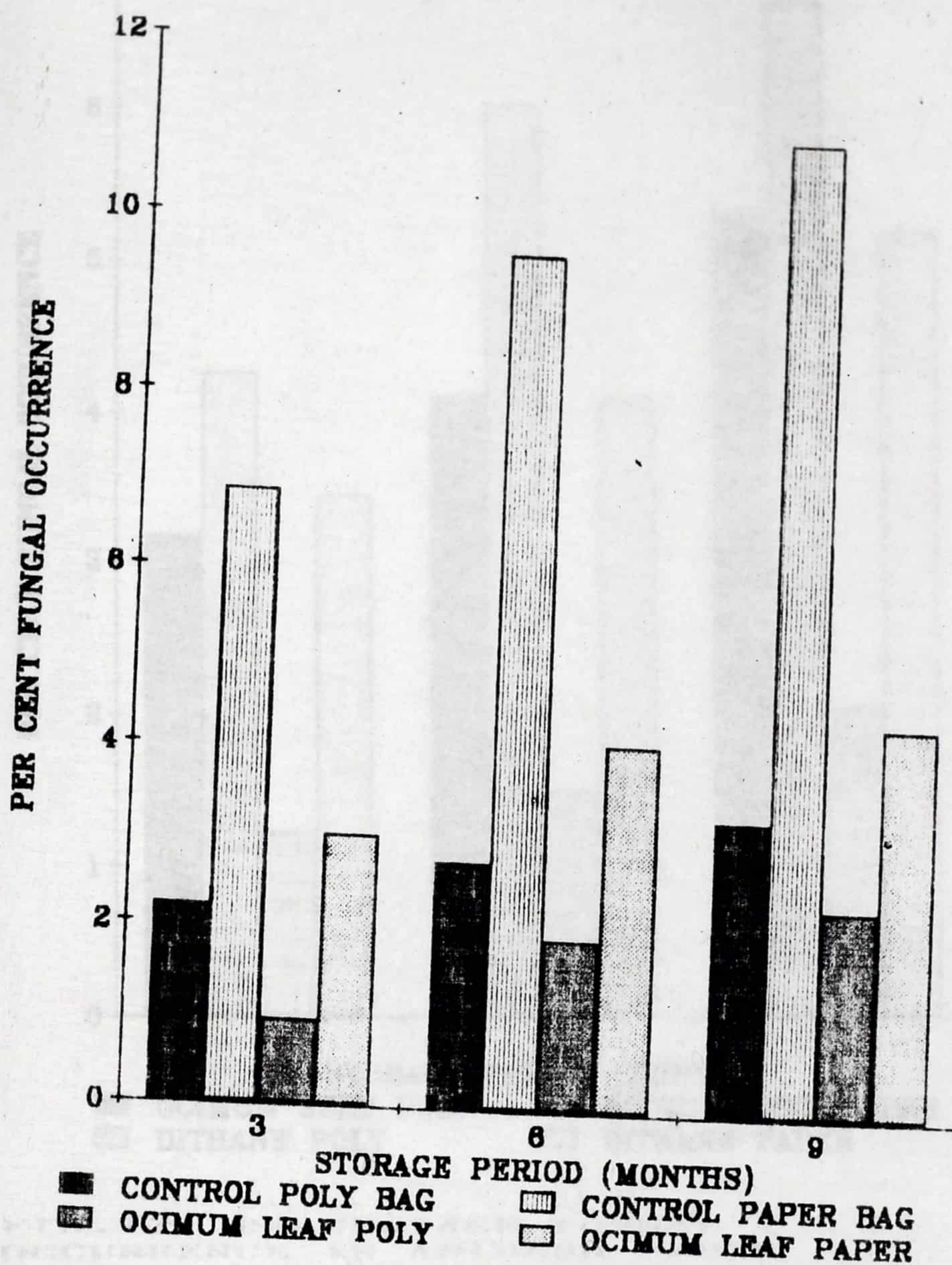


FIG. 11. PERCENTAGE FUNGAL OCCURRENCE IN ANIDASO UNDER DIFFERENT STORAGE CONDITIONS.

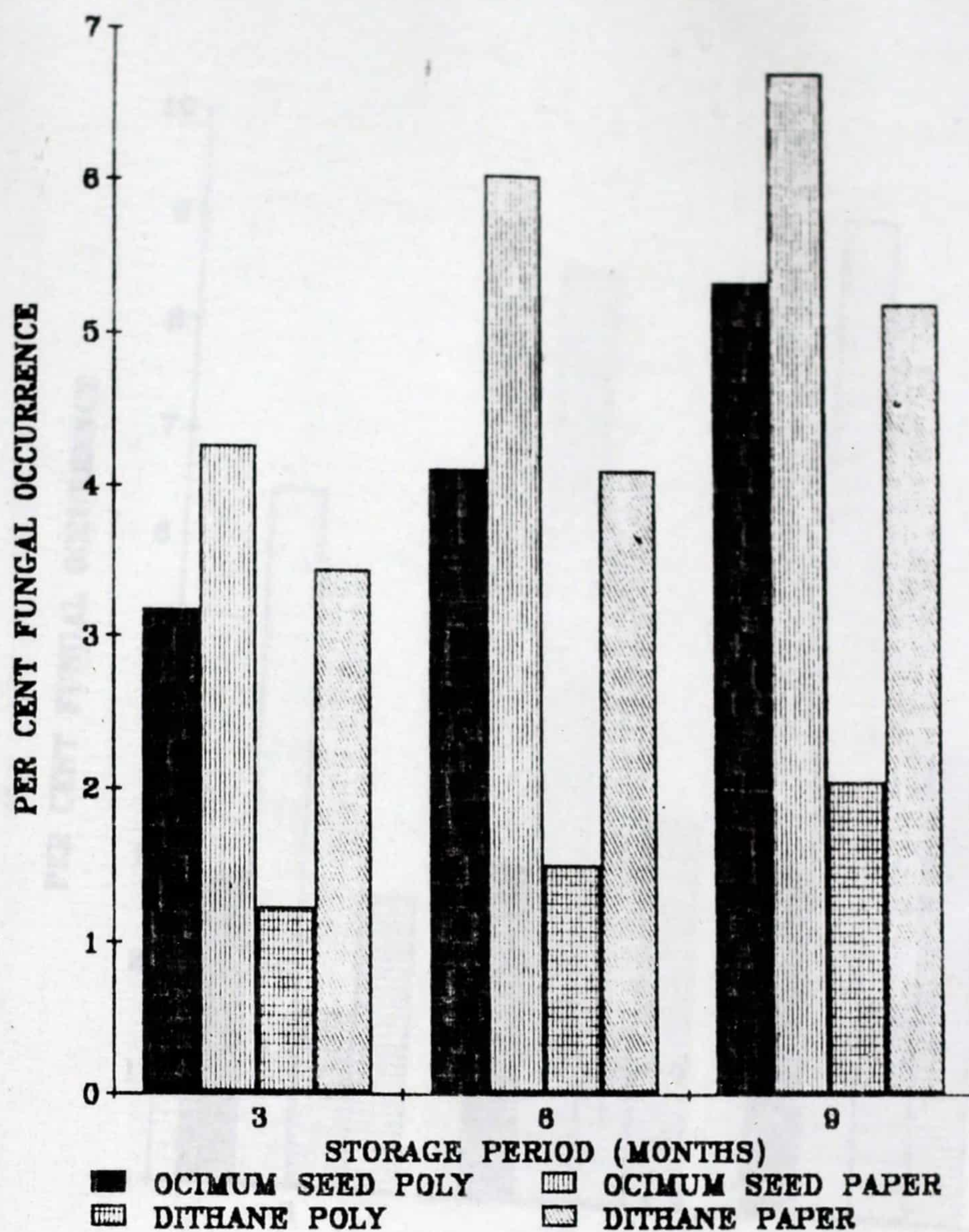


FIG. 12. PERCENTAGE FUNGAL OCCURRENCE IN ANIDASO UNDER DIFFERENT STORAGE CONDITIONS.

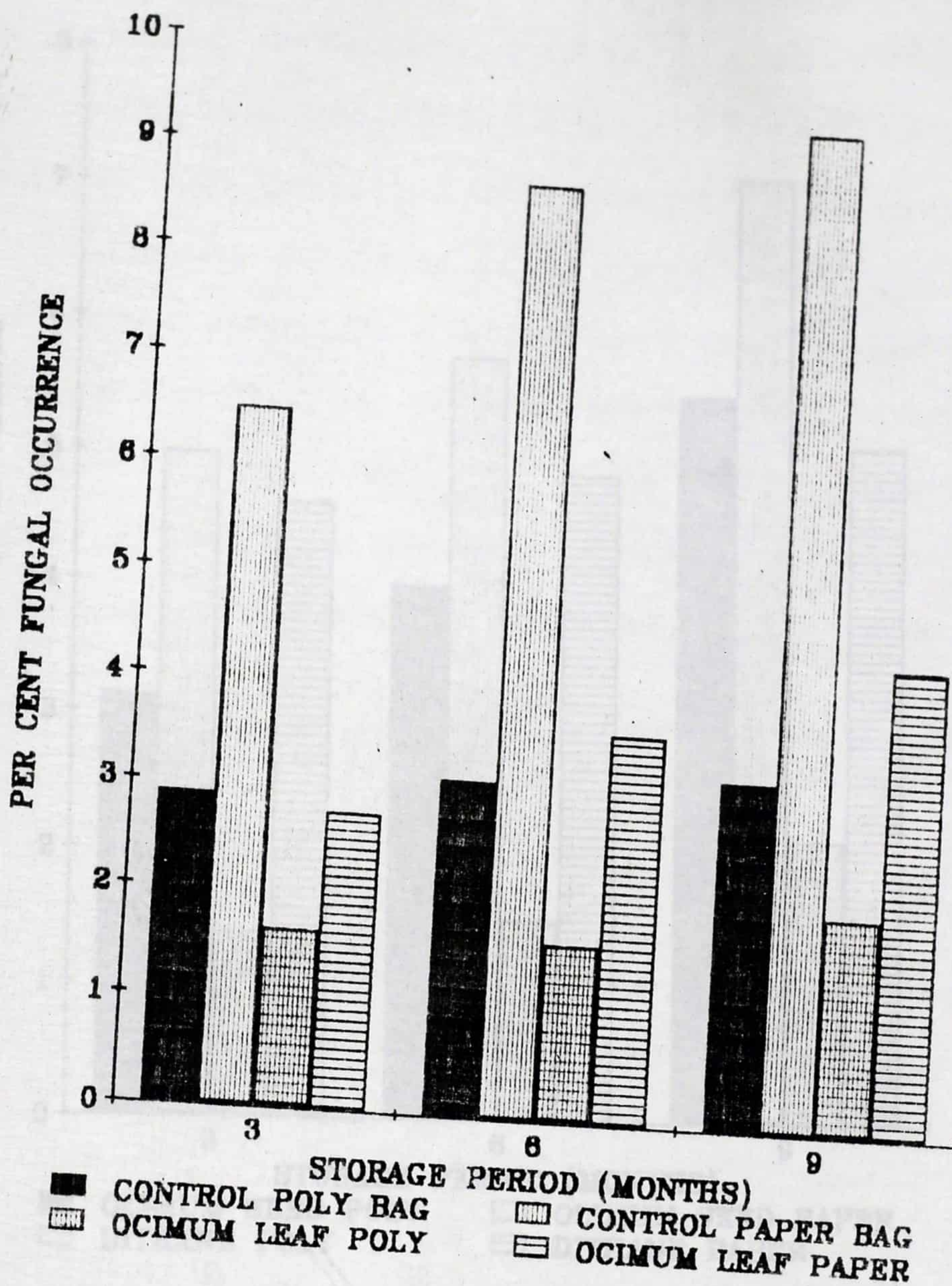


FIG. 13. PERCENTAGE FUNGAL OCCURRENCE IN BENG BIE UNDER DIFFERENT STORAGE CONDITIONS.

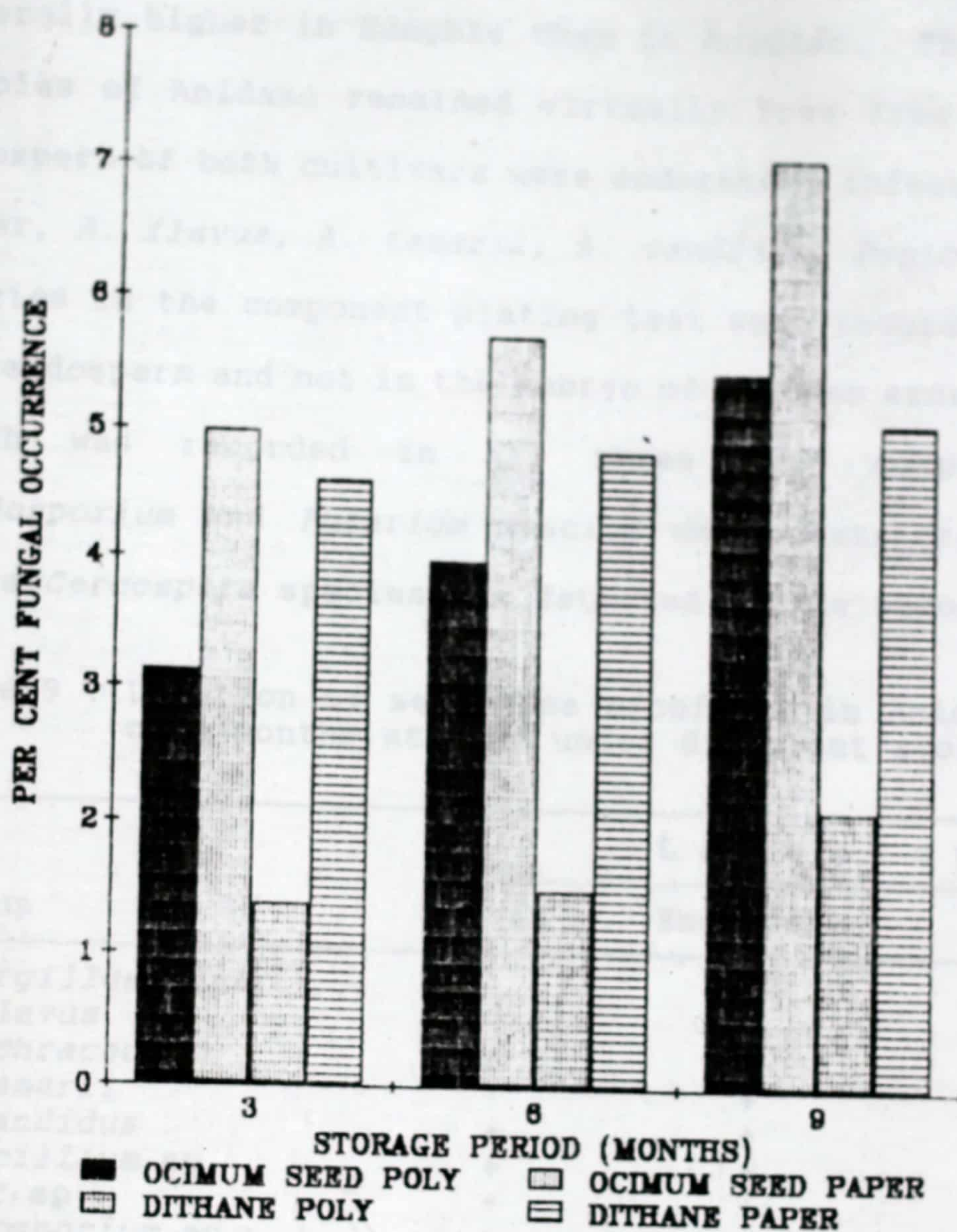


FIG. 14. PERCENTAGE FUNGAL OCCURRENCE IN BENGIBIE UNDER DIFFERENT STORAGE CONDITIONS.

The highest mycoflora infection in seed component parts of both Anidaso and Bengbie were located in the testa as compared to the embryo (Tables 9 and 10). The fungi species on testa were generally higher in Bengbie than in Anidaso. The embryo of seed samples of Anidaso remained virtually free from infection. The endosperm of both cultivars were moderately infested. *Aspergillus niger*, *A. flavus*, *A. tamaraii*, *A. candidus*, *Penicillium* and *Mucor* species in the component plating test were recorded in both testa and endosperm and not in the embryo of Anidaso except *Mucor* species which was recorded in all three seed components studied. *Cladosporium* and *Fusarium* species were restricted to the testa while *Cercospora* species was detected in the endosperm (Table 9).

Table 9 : Location of seedborne mycoflora in Anidaso seeds after nine months storage under different storage conditions

Fungus	L o c a t i o n		
	Testa	Endosperm	Embryo
<i>Aspergillus niger</i>	+	+	-
<i>A. flavus</i>	+	+	-
<i>A. ochraceus</i>	+	-	-
<i>A. tamaraii</i>	+	+	-
<i>A. candidus</i>	+	+	-
<i>Penicillium</i> sp	+	+	-
<i>Mucor</i> sp	+	+	+
<i>Cladosporium</i> sp	+	-	-
<i>Fusarium</i> sp	+	-	-
<i>Colletotrichum</i> sp	-	-	-
<i>Pythium</i> sp	-	-	-
<i>Curvularia</i> sp	-	-	-
<i>Cercospora</i> sp	-	+	-
Unidentified sp	+	-	-

+ Present
- Absent

In the component plating test of Bengbie, *A. niger*, *A. ochraceus*, *Penicillium*, *Mucor* and *Fusarium* species were recorded in all three components; testa, endosperm and embryo. *A. tamaraii* was recorded in the testa and endosperm only while *A. candidus*, *Pythium* and *Cercospora* species were restricted to the testa and *Curvularia* species to the embryo (Table 10).

Table 10: Location of seedborne mycoflora in Bengbie seeds after nine months storage under different storage conditions

Fungus	L o c a t i o n		
	Testa	Endosperm	Embryo
<i>Aspergillus niger</i>	+	+	+
<i>A. flavus</i>	+	-	+
<i>A. ochraceus</i>	+	+	+
<i>A. tamaraii</i>	+	+	-
<i>A. candidus</i>	+	-	-
<i>Penicillium</i> sp	+	+	+
<i>Mucor</i> sp	+	+	+
<i>Cladosporium</i> sp	-	-	-
<i>Fusarium</i> sp	+	+	+
<i>Colletotrichum</i> sp	+	-	-
<i>Pythium</i> sp	+	-	-
<i>Curvularia</i> sp	+	-	+
<i>Cercospora</i> sp	+	-	-
Unidentified sp	+	+	-

+ Present

- Absent

CHAPTER SIX

DISCUSSION

Seed-borne fungi may occur as saprophytes or pathogens on seeds or they may be distributed as inoculum within the seed tissue. They may also be found on the surface of the seed and as contaminant in the form of infected plant debris mixed with seed (Neergaard, 1977).

The mycoflora isolated from Anidaso and Bengbie seeds in the experiment included species of *Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium*, *Curvularia*, *Cercospora*, *Mucor*, *Colletotrichum*, *Pythium* and other unidentified species. The occurrence of these fungi might be due to the high humidity and storage conditions which provided a congenial environment for microbial attack as was also observed by Singh (1990). However, Bengbie seeds recorded higher fungal infection than Anidaso seed partly because seed moisture content of Bengbie was higher. This observation agrees with a report by Christensen and Kaufmann (1969) that a difference of only 0.2% seed moisture content makes a difference in the rate of growth of storage fungi and in the damage they cause in a given length of time.

In the experiment, it was observed that *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus ochraceus* and *Penicillium* species were probably the four main fungi responsible for the deterioration of Anidaso and Bengbie seeds in storage. Generally, these fungi occurred in higher frequencies in Bengbie seeds than in Anidaso

seeds (see table 6). These organisms are storage fungi and require certain moisture level to grow as was reported in many seeds and grains by Christensen and Kaufmann (1969). Their growth was therefore favoured more in Bengbie seed than in Anidaso seed most probably due to the initial seed moisture content of 12.6% prior to storage which might have favoured their growth condition. According to Neergaard (1977), *Aspergillus* and *Penicillium* species are dominant species of storage fungi. He also observed that the fungi occurred in large amounts in seeds because they are able to withstand dehydration. The high incidence of *Aspergillus niger* on the seeds was in agreement with the study by Fields and King (1962) who found it as an important fungus of groundnuts.

Fusarium and *Cladosporium* species were isolated in low frequencies (Table 6). These fungi were reported by Christensen and Kaufmann (1965) as typical field fungi which require high moisture levels for growth, a condition not satisfied by the seeds used for the study. *Fusarium* species were most often isolated from seeds free from *Aspergillus* species which was in conformity to observations by Joffe (1973); that *Fusarium* was suppressed whenever *Aspergillus niger* occurred due to its antagonistic effect. *Penicillium* species also occurred in *Aspergillus*-free seeds because whenever *Penicillium* species made their appearance, *Aspergillus* species was suppressed which is in agreement with findings of Bhadraia and Ramarao (1987) who observed an antagonistic association between *Penicillium* and *Aspergillus* species. These organisms, in addition to *Mucor*, *Colletotrichum*, *Pythium*,

Curvularia and *Cercospora* species, might have been present in the seed initially but might have died or was suppressed due to storage condition and suppressive competition from other well adapted storage fungi. The suppression of these fungi may also be attributed to the seed treatment by the *Ocimum* leaf and seed powders as well as Dithane M-45 which concurs with the findings of Asthana et al. (1989). Del Prado and Christensen (1952) observed that *Fusarium* may die within a few months under storage conditions through autolysis.

According to Christensen and Kaufmann (1969), field fungi may discolour seeds, cause death of the ovules, shrivelling of the seeds or kernels, weakening or death of the embryo. There was a decrease in germination of Anidaso and Bengbie seeds both in vitro and in vivo with increased storage period and this may be attributed to the invasion of the seeds by storage fungi which might have caused depletion of seed nutrient, inflicting starvation of the seeds, concurring with the observation made by Sempio (1959). Christensen and Kaufmann (1965) also reported a decrease in seed quality and heating accompanied by reduction in seed germination percentage or complete seed spoilage caused by storage fungi.

There was a difference between results of seed germination in vitro and in vivo (see table 2 and 3) of Anidaso and Bengbie and the discrepancy might be due to soil and plant house conditions as observed by Anon (1959). Seed viability reduced after three months storage, partly because increasing length of storage might have

resulted in a decline in seed germination under ambient conditions due to aging as observed in IITA report of 1982. Sao et al. (1990) also observed that oxygen uptake increased with prolonged storage and increased relative humidity; resulting in loss of dry weight and seed germination in radish. The difference in storability and germination may also be associated with genetic make up of the seeds (FAO report, 1982).

Fusarium moniliforme was associated with seed rot, germination inhibition and seedling rot as observed by Shetty and Gopinath (1985). Similar observations were made by Tripathi (1974) and Anahosur and Hedge (1980). Embryo infection by *Fusarium* species in Bengbie seeds might have accounted for the lower percentage seed germination. In addition, *Fusarium* can also reduce per cent seed germination by killing the seed embryo as observed by Shetty and Gopinath (1985). Vidyasekaran et al. (1970) also reported reduction in germination of rice seeds due to *Fusarium* species. A high incidence of embryo infection by various *Fusarium* species in Bengbie seeds was also observed in Sunnhemp seeds by Basu-Chaudhary and Pal (1981). All the three seed components of Bengbie variety showed heavy *Fusarium* infection and this suggested the infective ability of the pathogen. However, other seed-borne pathogens were also noticed (Table 10).

Aspergillus species were also recorded in all three components of seed and this probably might have contributed to decline in seed germination because they are mainly responsible for germ damage in seeds or grains as reported by Christensen and Kaufmann (1969).

There was a significant increase in seed moisture content; probably due to slow movement of air, set up by difference in temperature between different portions of the seedlot resulting in moisture transfer as observed by Christensen and Kaufmann (1969). Also, like all living things, the storage fungi break down much of their food into carbon dioxide and water, and so they are likely to increase the moisture content of the seeds they infect.

The paper bag containers registered high seed moisture content as well as high fungi occurrence. This was probably because, the paper bags are not water and vapour proof hence, there might have been exchange of moisture from the environment to the seeds through the container. However, sealed polythene bag containers recorded higher percentage seed germination as well as lower total fungi incidence because it might have effectively prevented moisture exchange as reported by Allen (1983).

Results suggested that in general, seeds stored at low moisture content were able to store better. The results also showed that viability of seeds in storage was influenced by the moisture content and storage container as also reported by Kelly (1988).

The seed protectants of plant origin as well as moisture and vapour proof containers used for carrying over the seed considerably augmented the shelf life of the seeds. Significant differences were recorded among treatments of different storage period and types of containers.

The deterioration in the viability of the seeds in moisture and vapour pervious containers might be due to the effect of temperature and relative humidity of storage atmosphere on the seeds as was evident from the studies of Vadivelu et al. (1985) with soyabean and Roos and Monalo (1971) with bean seeds. This also occurs with the observation made by Vadivelu et al. (1985) that rapid deterioration of gram seeds in moisture pervious containers was due to the effect of temperature and relative humidity of the storage atmosphere. The fact that treated seeds recorded higher seed germination in the study, bears testimony to the deleterious effect of fungi on viability of seeds in storage. The seeds treated with *Ocimum gratissimum* leaf powder recorded generally higher seed viability values because the leaves are believed to contain the most fungitoxic component, eugenol (81.24%). According to Tripathi et al. (1986), eugenol is a broad spectrum fungitoxic compound. Other components include phenol cymene (5.2%), geraniol (4.04%) and linanyl acetate (2.20%). Santra (1973) also reported that *O. gratissimum* was rich in Thymol, a phenolic terpene responsible for the strong antifungal activity. Pandey (1982) also observed the effectiveness of leaf extracts of medicinal plants and their usefulness to control seed mycoflora and increased seed germination. However, Tervet (1945), Maude (1966) and Singh and Maurya (1972) reported on usefulness of fungicidal treatment in minimising the loss in viability and vigour in stored seeds of various crops.

The fact that the treated and untreated seeds stored in moisture and vapour proof containers (sealed polythene bags) registered comparatively higher values for viability indicated that the seed moisture content was controlled in sealed polythene storage bags. Vadivelu et al. (1985) reported on the advantage of sealed container for seed storage. They also observed that seeds of *Phaseolus vulgaris* stored in plastic bags gave very high germination even after two years. The results of the study agreed with findings of Evans (1957) that soyabeans stored in sealed polythene bags recorded or retained high percentage seed germination. This also concurs with the study by Asthana et al. (1989) in which the oil of *Ocimum adscendens* protected the seeds of *Capsicum annuum* for up to 12 months.

CHAPTER SEVEN

CONCLUSION

Xerophytic fungi *Aspergillus* species and *Penicillium* species were the principal storage fungi and responsible for seed deterioration in Anidaso and Bengbie.

An increase in seed moisture as well as storage time directly affected the rate of fungal invasion as well as a decline in the rate of seed germination.

The location of mycoflora was found to affect seed viability especially if it was deeply seated in the seed. It was also observed that, the activity of storage fungi as well as moisture-pervious paper bag container escalated moisture exchange of the seeds, with the storage environment through the container resulting in a decline in seed viability. On the other hand, seeds treated with *Ocimum gratissimum* leaf powder and stored in moisture-impervious sealed polythene bags were protected hence, the rapid deterioration of the seed was suppressed and seed viability was maintained. This compared favourably with Dithane M-45 treated seeds stored in sealed polythene bags. Biocontrol requires minimum input, provides a long-term and sustainable check on rapid build up of pest population, environmentally safe and cost effective on long term basis as well as an effective control measure comparable to chemical treatment (Gabriel and Cook, 1990).

Various members of the *Ocimum* genus especially *O. gratissimum* grow very well in the Southern part of Ghana. *O. gratissimum* has been employed in folkloric medicine for diarrhoea and flavourants for food (Gbile, 1986). The extracts have no mammalian toxicity since they are utilized in local medical preparations for oral consumption as well as in foods (Purseglove, 1985 and Gbile, 1986). The method of obtaining the powders is relatively simple and is suited to the economy of a developing country such as Ghana, therefore it should be used as a preservative or seed protectant for soyabean storage in sealed polythene bags.

6.1 Recommendations

Soyabean seeds and other grains should be treated with *O. gratissimum* leaf powder and stored in sealed polythene bag.

The *O. gratissimum* leaves should be ground to be very fine, sieved and an appropriate adjuvant like oil should be added.

The *O. gratissimum* treated seeds should be stored in heavy-density polythene bag and heat sealed. Care must also be taken to prevent punctures on the polythene bags.

REFERENCES

- Allen, D.J. 1983. Disease resistance in crop improvement. The pathology of tropical food legume. John Wiley and Sons Ltd. New York. 413pp.
- Anahosur, K.H., and Hedge, R.K. 1980. Head moulds of sorghum, their effect on seed germination and in vitro evaluation of fungicides. Mysore Journal of Agricultural Science. 14: 60-63.
- Anon, 1959. International Rules for Seed Testing. Proceedings of International Seed Testing Association. 24: 475-584.
- Anon, 1987. Biological control in managed ecosystems 1987 research briefing. National Academy of Sciences. Washington, D.C., U.S.A. National Academy Press. Pages 1-12.
- Asthana, A., Dixit, K., Tripathi, N.N., and Dixit, S.N. 1989. Efficacy of *Ocimum* oil against fungi attacking chilli seeds during storage. Tropical Science. 29: 15-20.
- Austin, R.B. 1963. Effects of environment before harvesting on viability; In: Viability of seeds (1 ed) Chapman and Hall, London. Pages 114-149.
- Awuku, K.A., Bresse, G.K., Ofosu, G.K., and Baiden, S.O. 1991. Soya bean. Agriculture and environmental studies for senior secondary school. Pages 112-115.

- Bansal, R.K., and Sobti, A.K. 1990. An economic remedy for the control of two species of *Aspergillus* on groundnut. *Indian Phytopath.* 43: 451-452.
- Basu-Chaudhary, K.C., and Pal, A.K. 1981. Infection of sunnhemp seed with *Fusarium* spp. *Seed Science and Technology.* 9: 729-732.
- Bedi, J.S., and Jhooty, J.S. 1986. Incidence of seedborne *Aspergillus flavus* and resistance to its invasion in maize cultivars. *Indian Phytopath.* 39: 473-474.
- Beye, F. 1978. Insecticides from the vegetable kingdom. *Plant Research Dev't.* 7: 13-31.
- Bhadraia, B., and Ramarao, P. 1987. Fungi from stored sorghum seed. *Indian phytopath.* 40: 401-404.
- Boakye-Boateng, K.B., and Hume, D.H. 1975. Effects of storage conditions on germination of soyabean. *Ghana Journal Agric. Sc.* 8(2): 109-114.
- Bilgrami, K.S., Prasad, T., Jamaludidin and Roy, R.K. 1976. Deterioration in the quality of pulses during storage. *Indian Phytopath.* 29: 374-377.
- Burlison, W.L., Van Doreu, C.A., and Hackleman, J.C. 1940. Eleven years of soybean investigation. *Illinois Agric. Expt. Sta. Bull.* 462: 123-167.
- Chandra, H., Mall, H.V., and Tripathi, N.N. 1982. Protectant activity of *Ageratum* oil against fungal deterioration of chilli seeds during storage. *J. Indian Bot. Soc.* 61(suppl): 19.

- Chauchan, K.S., and Bhandari, Y.M. 1971. Pod development and germination in okro. *Indian Journal of Agric. Sc.* 41(10): 852-856.
- Chen, S.S.C. 1978. Application of volatile radioactive tracer to detect metabolic activities in dry wild oat seeds; *In: Dry Biological System* (1 ed.). Academic Press, New York. Pages 175-185.
- Chenule, V.V., and Vir, D. 1979. Seed mycoflora, its role in grain spoilage and production of mycotoxins. *Bull. Grain Technol.* 17: 148-157.
- Chengappa, R. 1989. Poison in your food. *India Today*. 15 June, 1989.
- Christensen, C.M. 1951. Fungi on and in wheat seed. *Cereal Chemistry*. 28: 408-415.
- Christensen, C.M., and Kaufmann, H.H. 1965. Deterioration of stored grains by fungi; *In: Ann. Rev. Phytopath.* 3: 69-84.
- Christensen, C.M., and Kaufmann, H.H. 1969. The role of fungi in quality loss. *Grain storage*. Univ. of Minnesota, Minneapolis. 153 pp.
- Crisan, E.V. 1973. Effect of aflatoxin on germination and growth of lettuce. *Appl. Microbiol.* 25: 342-347.
- Dadson, R.B. 1974. Report on soyabean trials at Legon - 1974.
- Del Prado, F.A., and Christensen, C.M. 1952. Grain storage studies 12. *Cereal Chem.* 29: 456-462.

- Dhyani, A.P., Sati, M.C., and Khulbe, R.D. 1990. Seedborne fungi of chilli with special reference to pathogenicity and control of *Phoma destructiva*. Indian Phytopath. 43(1): 110-113.
- Dikshit, A., Dubey, N.K., and Dixit, S.N. 1983. Cedrus oil - a promising storage fungitoxicant. J. Stored Prod. Res. 19: 152-162.
- Dorworth, C.E., and Christensen, C.M. 1968. Influence of moisture content, temperature and storage time upon changes in fungus flora, germinability and fatty acid values of soybeans. Phytopathology. 58: 1457-1459.
- Dubey, N.K., Bhargava, K.S., and Dixit, S.N. 1983. Protection of stored food commodities from fungi by essential oils of *Ocimum canum* and *Citrus medica*. Int. J. Tropical Plant Diseases. 1: 177-179.
- Dwivedi, S.N. 1990. Changes in the concentration of total phenolic compounds in grain seeds as influenced by fungal invasion during storage. Indian Phytopathology. 43: 96-98.
- Dwivedi, S.N., and Shukla, T.N. 1990. Mycoflora of gram seed in different agro-climatic regions and their pathology. Indian Phytopath. 43(1): 98-99.
- Evans, G. 1957. The viability over a period of fifteen years of severely dried rye grass seed. J. Brit. Grassland Soc. 22: 286-289.
- F.A.O. Report, 1982. Soyabean protection in the tropics. F.A.O. Plant production and protection paper 4. Pages 1-222.

- Fause, H.A., and Christensen, C.M. 1966. Invasion by fungi of rice stored at moisture contents of 14.5 and 15.5. *Phytopath.* 56: 1162-1164.
- Fawcett, C.H., and Spencer, D.M. 1970. Plant chemotherapy with natural products. *Ann. Rev. Phytopath.* 8: 409-418.
- Fields, R.W., and King, T.H. 1962. Influence of storage and deterioration of stored pea seeds. *Phytopathology.* 52: 336-339.
- Gabriel, C.J., and Cook, R.J. 1990. Biological control of plant pathogens. *Plant Protection Bulletin.* 38(2): 95-99.
- Gbile, O.Z. 1986. Ethnobotany, taxonomy and conservation of medicinal plants. The state of Medicinal plants research in Nigeria. Pages 13-19.
- George, R.A.T. 1985. Stage of Harvesting. Vegetable Seed Production. Longman Group Ltd., London. Pages 64-65.
- Gokte, N., and Mathur, V.K. 1992. Viability of hot-water treated paddy seeds under different storage conditions. *Plant Protection bulletin.* 40(1-2): 36-37.
- Gopinath, A., and Shetty, H.S. 1985. Occurrence and Location of *Fusarium* species in Indian sorghum seed. *Seed Science and Technol.* 13: 521-528.
- Grabe, D.F., and Metzger, R.B. 1970. Soyabean hypocotyl elongation and seedling emergence. *Crop Sc.* 9(3): 331-333.
- Gupta, V.K., and Cholan, T.S. 1969. Post harvest treatment of groundnut kernels with fungicides and their viability. *Journal Research.* 8: 331-334.

- Haferkamp, M.E., Smith, L., and Nolan, R.A. 1958. Studies on aged seeds. I. Relation of seed age to germination and longevity. Agron. Journal. 45: 434-437.
- Hansen, H.J. 1990. Treatment of seed with micro waves for control of seed-borne Pathogens. Seed Pathology News No: 20. D.G.I.S.P., Denmark. Page 11.
- Harman, G.E., Chet, I., and Baker, R. 1980. *Trichoderma hamatum* effects on seed and seedling disease induced in radish and Pea by *Pythium* spp and *Rhizoctonia solani*. Phytopath. 70: 1167-1172.
- Huckil, W.V. 1963. Storage of seeds. Proc. Int. Seed Test. Assoc. 28: 871-873.
- ICRISAT Report. 1989. Uses of Tropical Grain Legumes. Proceedings of a consultants meeting. ICRISAT center, India. Page 12.
- IITA Report (1974, 1976, 1977, 1980 and 1982). Grain Legume Improvement Programmes.
- Jain, S.R., and Jain, M.R. 1972. Antifungal studies on some indigenous volatile oils and their combinations. Planta Medica. 22: 136-139.
- James, E.L., Bass, N., and Clark, D.C. 1967. Varietal differences in longevity of vegetable seeds and their response to various storage conditions. Amerc. Soc. Hort. Sci. 91: 521-528.
- Joffe, A.Z. 1973. *Fusarium* species on groundnut kernels and in groundnut soil. Plant and Soil. 38: 439-446.

- Kaul, J.N., and Bekhon, H.S. 1977. Effect of shading on emergence of soyabean. World Crops and Livestock Nov/Dec.
- Kelly, A.F. 1940. Methods of ventilating wheat in farm storage. USDA Circular 5441.
- Kelly, A.F. 1988. Seed. Seed Production of Agricultural Crops. Longman Scientific and Technical, Longman Group U.K. Ltd., England. Pages 1-227.
- Kinsella, J.E. 1988. Food lipids and fatty acids: importance in food quality, nutrition and health. Food Technology 42(10): 124-145.
- Kishore, N., Dixit, S.N., and Dubey, N.K. 1989. Fungitoxic studies with, *Chenopodium ambrosioides* for control of damping off in *Phaseolus aureus* (Moong) caused by *Rhizoctonia solani*. Tropical Science. 28: 171-176.
- Laude, H. 1962. Fresh seed dormancy in annual grasses. Calif. Agric. 16(4): 3.
- Luckman, W.H. 1971. Insect pests of soyabeans. World Farming. Vol. 13, 5 June 1971.
- Mercer-Quashie, H. 1975. Soyabean in Ghana. INTSOY Series. International Agric. Pub.
- Milner, M. 1954. Biological process in stored soyabeans In: Soyabean and soyabean products (1 ed). Interscience. Pages 483-501.
- Mulivex, P.B., and Tuite, J.F. 1970. Temperature and relative humidity requirements of species *Penicillium* isolate from yellow dent corn. Mycologia. 62: 75-89.

- Neergaard, P. 1977. Seed Pathology Vol. 1. MacMillan Press Ltd., London. 839 pp.
- Odelola, H.A., and Okorosobo, V.I. 1988. Preliminary investigation of *in vitro* antimicrobial activity of two Nigerian *Diospyros* spp. (Ebenaceae). African Journal of Medicine and Medical Sciences. 17: 167-170.
- Osafo, D.M. 1977. Effect of population density on yields of two soyabean varieties in Ghana forest zone. Expl. Agric. Vol. 13: 235-240.
- Pandey, K.N. 1982. Antifungal activity of some medicinal plants on stored seeds of *Eleusine coracana*. Ind. Phytopath. 35: 499-501.
- Peterson, D.F. 1942. Duration of receptiveness of corn silks. J. Amer. Society Agron. 34: 369-371.
- Prasad, B.K. 1987. Effect of seed extract of Coriander inoculated with *Aspergillus* on the hydrolytic enzymes of food reserve. Indian Phytopath. 40(1): 105-106.
- Purseglove, J.W. 1966. *Glycine max* (L.) Merr. Tropical Crops Dicotyledons, Vol. 1. Pages 265-272.
- Purseglove, J.W. 1985. Lemon and citronella grass. Tropical Crops Monocotyledons, volumes 1 and 2 combined. ELBS edition, London. Pages 137-141.
- Quasem, S.A., and Christensen, C.M. 1958. Influence of moisture content, temperature and time on deterioration of stored corn by fungi. Phytopath. 48: 544-549.

- Reddy, B.N., and Nusrath, M. 1985. Mycoflora in relation to earhead and grain in sorghum. *Indian Phytopath.* 38(4): 751-753.
- Reddy, B.B., and Nusrath, M. 1986. Relationship between physical damage of the grain and the occurrence of mycotoxins in sorghum. *Indian Phytopath.* 39: 471-473.
- Roberts, E.H. 1972. Storage environment and the control of viability. *Viability of seeds.* Chapman and Hall. Pages 14-52.
- Roos, E.E., and Monalo, J.R. 1971. Testing vigour of beans following unfavourable conditions. *Hort. Sci.* 6: 347-348.
- Sadashivalah, A.S., Ranganathaiah, K.G., and Nanje, G.D. 1986. Seed health testing of *Helianthus annuus* with special reference to *Macrophomina phaseolina*. *Indian Phytopath.* 39: 445-446.
- Santra, D.K. 1973. Role of natural products - potential of medicinal plants in Ghana. *The Ghana Pharmaceutical Journal.* 1(3): 145.
- Sao, R.N., Singh, R.N., Narayan, N., Singh, S.P., Kumar, S., and Prasad, B.K. 1990. Respiration and viability of stored radish seeds due to seedborne *Aspergillus flavus*. *Indian Phytopath.* 43(2): 197-200.
- Schwabe, W.W. 1963. Studies in vernalisation of cereals. *Ann. Bot.* 27: 671-683.

- Sempio, C. 1959. The host has survived; In: Plant Pathology vol. 1 (1ed.): 277-312. Academic Press. New York and London.
- Singh, B.B. 1976. Breeding soyabean varieties for the tropics; In: Expanding the use of soyabeans. INTSOY series No. 10. Univ. of Illinois, Urbana-Champaign, U.S.A.
- Singh, B.K., and Prasad, T. 1983. Effect of seed-borne fungi on the physico-chemical properties of sesamum seed oil. J. Indian Bot. Soc. 62: 48-53.
- Singh, I., and Chohan, J.S. 1987. Seedborne fungi on cowpea. Indian Phytopath. 27:239.
- Singh, J.N., and Maurya, M.L. 1972. Effect of storage conditions on germination of soyabean seeds. Bull. Grain Tech. 10: 158-167.
- Singh, K. 1990. Health of seeds and grains produced in tribals of Rajasthan in India. Seed Pathology News. No. 20. DGISP. Denmark. Page 3.
- Singh, R.A., and Monga, D. 1985. New methods of seed treatment for eliminating *Xanthomonas campestris* pv. *oryzae* from infected rice seed. Indian Phytopathology. 38(4): 629-631.
- Singh, T.P. 1975. Grain Legume improvement programme. IITA report. Pages 1-10.
- Sinha, A.P., Thapliyal, P.N., and Agarwal, V.K. 1984. Black point of triticales : occurrence and effect of fungicidal seed treatment on germination. Ind. Phytopath. 37(2): 249-250.

- Tervet, I.W. 1945. The influence of fungi on stored seed viability and seedling emergence of soyabeans. *Phytopath.* 35: 3-15.
- Toole, E.H., and Toole, V.K. 1946. Relation of temperature and seed moisture to viability of stored soybean seeds. *USDA Cir.* 753.
- Tripathi, R.K. 1974. Head fungi of sorghum, Phytotoxins and their effects on seed germination. *Indian Phytopath.* 27:499-501.
- Tripathi, R.D., Banerji, R., Sharma, M.L., Balasubrahmanyam, V.R., and Nigam, S.K. 1986. Toxicity of essential oil from a new strain of *Ocimum gratissimum* against Pathogenic fungi. *Hort. Abstract.* 56: 288.
- Tuite, J.F. 1959. Low incidence of storage moulds in freshly harvested seeds of soft red winter wheat. *Plant Disease Repr.* 43: 470.
- Vadivelu, K.K., Karivaratharaju, T.V., and Ramakrishnan, V. 1985. Influence of seed treatments, seed moisture content and containers on the viability of CO.1 Bengal Gram (*Cicer arietinum*) seeds. *Seed Research Vol.* 13(4): 38-44.
- Vaidya, A., and Vir, D. 1989. Changes in the oil in stored groundnut due to *Aspergillus niger* and *A. flavus*. *Indian Phytopath.* 42(4): 525-529.
- Verma, A.B.S., and Kohnke, H. 1951. Effect of organic mulches on soil condition and soybean yield. *Soil Sci. vol.* 72: 149-156.

Vidyasekaran, P., Subramanian, C.L., and Govindaswamy, C.V. 1970.

Production of toxins by seedborne fungi and its role in paddy seed spoilage. Indian Phytopath. 23: 518-528.

Vir, D., and Sharma, R.K. 1986. Efficacy of fungicide evaluation of neem oil for control of plant pathogens.

Horticultural Abstract. 56(9): 775.

Walker, J. 1933. The suitability of immature sweet corn for seed. Sci. Agric. 13:642-645.

Zeleny, L. 1961. Ways to test seeds for moisture content.

U.S.D.A. Year book. Pages 443-447.

APPENDICES

APPENDIX 1A : Analysis of variance of percentage germination of Anidaso after nine months storage in vitro.

Source	Degree of freedom	Sum of squares	Mean square	F-value	Prob.
Replication	2	520.29	260.145	17.530*	.047
A	7	2151.33	307.333	20.710*	.000
B	1	192.00	192.000	12.938*	.000
AB	7	20.00	2.857	0.193NS	
Error	6	89.04	14.840		
Total	23				

*Significant at 5%
N.S. = Not significant

APPENDIX 1B : Analysis of variance of percentage germination of Bengbie after nine months storage in vitro.

Source	Degree of freedom	Sum of squares	Mean square	F-value	Prob.
Replication	2	497.86	248.93	16.21*	.041
A	7	2048.51	292.644	19.06*	.023
B	1	176.33	176.330	11.49*	.049
AB	7	18.00	2.571	0.17NS	
Error	6	92.12	15.353		
Total	23				

*Significant at 5%
N.S. = Not significant

APPENDIX 2A : Analysis of variance of percentage germination of Anidaso after nine months storage in vivo.

Source	Degree of freedom	Sum of squares	Mean square	F-value	Prob.
Replication	2	323.65	161.825	5.30*	.034
A	7	2867.81	409.687	13.43*	.024
B	1	205.33	205.330	6.73*	.043
AB	7	161.19	23.027	0.76NS	
Error	6	183.06	30.510		
Total	23				

*Significant at 5%
N.S. = Not significant

APPENDIX 2B : Analysis of variance of percentage germination of Bengbie after nine months storage in vivo.

Source	Degree of freedom	Sum of squares	Mean square	F-value	Prob.
Replication	2	209.63	104.815	4.18NS	.307
A	7	2545.33	363.619	14.51*	.000
B	1	196.33	196.33	7.83*	.029
AB	7	139.33	19.904	0.79NS	
Error	6	150.38	25.063		
Total	23				

*Significant at 5%
N.S. = Not significant

APPENDIX 3 : Analysis of variance of percentage moisture content of Anidaso.

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-Value	Prob.
Replication	3	31.38	10.458	1.00NS	
Treatment	1	153.13	153.125	14.64*	.031
Error	3	31.38	10.458		
Total	7	215.88			

*Significant at 5%

N.S. = Not significant.

APPENDIX 4 : Analysis of variance of percentage moisture content of Bengbie.

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-Value	Prob.
Replication	3	1.38	1.458	1.00NS	
Treatment	1	392.00	392.000	235.20*	.000
Error	3	5.00	1.667		
Total	7	402.00			

*Significant at 1%

N.S. = Not significant.

APPENDIX 5 : Analysis of variance of percentage fungal occurrence on Anidaso

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-Value	Prob.
Replication	2	14.85	7.423	23.62*	.000
Treatment	7	126.11	18.016	57.33*	.000
Error	14	4.40	0.314		
Total	23	145.36			

*Significant at 1%

APPENDIX 6 : Analysis of variance of percentage fungal occurrence on Bengbie

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-Value	Prob.
Replication	2	7.56	3.782	12.69*	.000
Treatment	7	100.91	14.415	48.36*	.000
Error	14	4.17	0.298		
Total	23	112.64			

*Significant at 1%