STUDIES ON THE EFFECTIVENESS OF VARIOUS INSECTICIDES AND DRYING IN CONTROLLING GRANARY WEEVIL (*Sitophilus granarius* L.) IN STORED WHEAT SEED

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ABSTRACT

This study was designed to examine the effects of various insecticidal materials and of repeated seed drying during storage on the population dynamics and survival of Granary Wevil (Sitophilus granarious L.) in wheat. The study was conducted in three parts. The first experiment examined the immediate and longer term effectiveness of the contact insecticide malathion and the fumigant phosphine along with influence of repeated drying to safe moisture contents on granary weevil infestation. Wheat seed was stored for 165 days at 25 C and 80% R.H. Treatments were examined every 30 days to study the development of Sitophilus granarius populations and associated damage to wheat seed and evaluation made of the effects of drying, malathion and phosphine on the established infestation and on seed quality. Granary weevils increased by about a factor of X 10 every 60 days and did extensive damage to chemically untreated seed. Repeated drying reduced the rate of increase but did not eliminate the insect population. Malathion dust added to infested seed severely checked insect development and when combined with drying destroyed the infestation completely. Malathion also displayed considerable residual effect and had no adverse effect on seed viability. Phosphine was found to be totally effective in eradicating an established population of granary weevils from seed without affecting seed quality.

In a second experiment malathion was sprayed onto jute squares at 2.5% and at one half and one quarter of this rate. Treated squares were stored for 90 days at 20 C, ambient RH of 70 - 90% or 30 C, ambient RH of 60 - 80% and the residual toxicity of the deposit was assayed with live insects at intervals after treatment. Malathion was also applied at 2.5% concentration to the outside of grain filled sacks which were then placed individually into large plastic bags into which adult granary weevils were introduced at 7 day intervals. After 56 days storage, counts were made of live and dead insects inside the sacks to assess protective effect of the malathion treatment. On jute squares malathion was completely effective at all concentrations and at both storage temperatures (20 C and 30 C) immediately after application and also after 7 days. Thereafter, it lost its effectiveness slowly over the next 90 days storage. In whole sack treatment malathion was found to provide only immediate protection at both temperatures and was inadequate after only a few days.

In the third experiment wheat seeds, uninfested and infested with Sitophilus granarius, were mixed with ground neem seed of each of two species of neem (Azadirachta indica and Melia azaderach) at 1 g per 20 g wheat and were stored at 25 C and 80% R.H. Seeds were examined for live and dead insects and germination assessed after 90 days storage. Little or no direct mortality of adults was recorded but there was indirect evidence of suppression of egg laying particularly with Azadirachta indica. Neem seed powder did not affect the viability of the wheat seed.

This study has clearly shown the short term residual effectiveness of malathion, the immediate eradicant action of phosphine and the poor performance of the natural insecticidal chemical in neem seed on granary weevil infestation in wheat. The results also show the maintenance of low seed moisture contents in wheat to be a practical method of reducing insect populations. The role of granary weevil in damaging seed was clearly seen by X-ray photography and by the extent of types of abnormal seedlings found in positional germination tests. In the absence of effective control Sitophilus granarius has the potential to devastate wheat seed quality in terms of both purity and germination in as little as 90 days.

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INTRODUCTION

Shortage of food is a major problem in most developing countries. Low crop yields aggravate this problem. Among the factors that increase the yield of agricultural products, the successful production and availability of high quality seed is most important. Even though many countries particularly in the humid tropics and sub-tropics, have developed the necessary technology for the production of high quality seeds they often face great difficulties in maintaining high seed quality in storage, particularly where lengthy storage periods occur between harvest and the next sowing season.

The combination of high humidity and high temperature environments are disastrous in their effects on the viability of stored seeds (Delouche et al., 1973). Some information on how high temperature and humidity affect the seed viability in storage has been provided by Islam (1984). Dehumidified cold storage is the best solution for storing most types of crop seeds. However, financial constraints in most developing countries, such as Bangladesh at present preclude the commercial use of environmentally conditioned seed stores. A common storage practice now being adopted there is to keep the moisture content of stored seed at low levels by repeated drying.

Under high humidity and temperature storage conditions insects can be one of the most important single causes of damage to seeds if infestation is not prevented or controlled (Henderson and Christensen, 1961). Hall (1970) states that at least 10% of harvested food crops are destroyed by pests in storage, and that current losses of 30% are apparently common in large areas of the world especially in some of the tropical and sub-tropical countries where the need for more food is greatest. High temperature and high relative humidity ambient conditions, are most detrimental to seed longevity in storage and at the same time provide very favourable environments for the development of insect populations. This makes safe seed storage in such countries even more difficult. The present study was undertaken to investigate some aspects of chemical control of insects in stored seed.

In Bangladesh wheat and rice seeds are generally stored on a large scale. The storage of wheat seed however, is generally considered to be more difficult. Weevils are the most common insects that infest stored seeds in Bangladesh. The insecticide malathion and

the fumigant phosphine (liberated from Phostoxin tablets) are the chemicals mainly used there to combat insect infestation of seed. However, little research has been done there on the mode of action of these chemicals or on their effects on seed quality and thus practical use depends on the recommendations of manufacturers or suppliers. This study was therefore, undertaken to investigate several aspects of the action of malathion and phosphine when used on wheat seed infested by granary weevil (Sitophilus granarius) under simulated tropical storage conditions.

The increasing cost of some insecticides and also the rapid development of resistance by insects against a particular insecticide necessitates the search for new but relatively cheap insecticides. Recently neem seed has received wide attention as a possible cheap but effective natural pesticide (Ivbijaro, 1983). Neem is readily available in most tropical and subtropical countries including Bangladesh. This study therefore, included an evaluation of the effectiveness of two botanical species of neem seed (Azadirachta indica and Melia Azedarach) as an insecticide against granary weevil.

In summary therefore, the objectives of the present study were to investigate the following aspects of chemical control of granary weevil (Sitophilus granarius L.) in stored wheat seed (Triticum aestivum).

(1) To study the development of granary weevil populations and associated damage done to wheat seed in storage.

(2) To evaluate the effect of seed drying on insect population development and on seed quality.

(3) To evaluate the effectiveness of the contact insecticide, malathion, the fumigant phosphine, and the natural pesticide neem seed, in controlling an established infestation of granary weevil in wheat seed.

(4) To study the effectiveness of malathion applied as a spray to the outside of storage sacks as a preventative measure against infestation.

(5) To investigate the effect of the above chemicals on wheat seed quality.

CHAPTER 1

LITERATURE REVIEW

INTRODUCTION

Storage of seed is an important part of seed production and marketing since deterioration of seed results from interaction between physical, chemical and biological factors. Any review of aspects of seed storage is not complete without consideration of the effects of the seed storage environment on longevity of seed. The amount of literature on the longevity of seed in storage is voluminous, although reviews of the effects of different factors on seed storage longevity have been made by Islam (1984). For this reason only a concise review on seed storage is given here with discussion being mainly confined only to the role of insects in the storage environment, and their prevention and control. Insects are considered to be the principal external agent affecting the quality and longevity of seed in storage in tropical environments.

INSECT PESTS OF STORED SEED

Seeds in store, according to Parkin (1963), are subject to attack by the same insect species as other stored food crops and products and possess as a common characteristic the ability to feed on and breed in relatively dry materials (moisture content less than about 20%). The most important insects damaging stored seeds are the immature stages of certain species of moths (Lepidoptera) and beetles (Coleoptera). Some species are primary pests in that they can attack intact seed. Others are secondary and follow on and enlarge damage caused by primary pests.

Insects damage the seed embryo (germ), endosperm or cotyledons, create contamination by their excreta and dead bodies, modify the storage environment (relative humidity and temperature) and introduce fungi. Therefore, they reduce the storability of seeds and thus their viability and vigour (Cotton, 1947; Howe, 1973; Cotton and Wilbur, 1974).

The growth, development and increase of insects depend on three factors or groups of factors; climate, food and competition with other organisms (Munro, 1966; Howe, 1972; Cotton and Wilbur, 1974; Fenemore,

1984). Fenemore (1984) states that by far the most important environmental factor is temperature because insects are so temperature dependent, though relative humidity may also be important for some insects.

The optimum temperature range for most seed infesting insects is 27 to 30 C. Temperatures above 35 C are not favourable and below 21 C development is generally retarded (Cotton, 1954; Henderson and Christensen, 1961; Howe, 1972; Parkin, 1963; Cotton and Wilbur, 1974; Christensen and Kaufman, 1974; Fenemore, 1984; Heather, 1984). Insects do not breed successfully in an environment where the relative humidity is maintained at less than 40% (i.e., for cereals less than equilibrium moisture content of 8%), or a temperature below 10 C. Most species do not tolerate prolonged temperatures above 42 C and cease feeding and become inactive between 5 C and 10 C (Henderson and Christensen, 1961; Hall, 1970). However, each insect species has a characteristic range of physical conditions for optimum development (Howe, 1965).

Under favourable conditions most insects breed very quickly, the life cycle from egg to adult being completed in a few weeks with each adult female laying a large number of eggs. Insects multiply most rapidly under warm moist condiions where a population increase factor of x 50 every 6 weeks can be predicted from the data of Birch (1953). As temperature and humidity conditions diverge from the optimum, the time taken to develop from egg to adult increases and the number of eggs laid becomes fewer thus reducing the overall rate of increase (Hall, 1970; Heather, 1984).

Granary Weevil

Granary Weevil (Sitophilus granarius L.) is a major pest of stored seeds and belongs to the order Coleoptera and family Curculionidae. The granary weevil was described and named nearly 250 years ago although it was well known long before that on account of its habit of infesting granaries (Cotton, 1941). It has been an inhabitant of houses, barns, and granaries so long that it has actually lost the use of its wings and is now strictly an indoor species. Until 1959 the generic name Calandra was used in most European literature, whereas Sitophilus was used in North America. The currently accepted generic name is now Sitophilus.

a) Description

Cotton (1941) describes granary weevil as a polished chestnut brown or blackish beetle very similar in size and appearance to the rice weevil (S. oryzae). However, the pits on the pronotum (the large plate behind the head) are oval-shaped, larger, and less compacted than those on rice and maize weevils. Granary weevils usually are larger than the other species and they do not have functional wings. The mature beetle is about 3.2 to 4.8 mm long and it has a long slender snout, or proboscis. The thorax is marked with shallow oval punctures, while the wing covers are grooved and ridged lengthwise and are uniformly brown (Herrick, 1926; Hinton and Corbet, 1963; Khan, 1948; Stephensons, 1983).

b) Life Cycle and Behaviour

Like other insects with complete metamorphosis, granary weevils have four distinct stages in their life cycle - egg, larva, pupa and adult. Hinds and Turner (1911), Winterbottom (1922), Herrick (1926), Kirkpatrick and Wilbur (1965), Khan (1948), Howe (1972), Cotton and Wilbur (1974) and Longstaff (1981) have done extensive research on ovipositional behaviour and development of Sitophilus granarius from which the following is condensed.

The adult weevil lives on average 7 - 8 months (unlike other weevils which live for only 4 to 5 months), each female laying between 300 and 400 eggs during this period (Cotton, 1947). Cotton and Wilbur (1974) describe how a female weevil chews a hole through the tough seed coat and prepares a cavity in the endosperm in which to deposit an egg. Then she inserts a long ovipositor protruding from the tip of her abdomen into the hole and deposits a small, soft, whitish egg. As the ovipositor is withdrawn, glands associated with it secrete a gelatinous material that fills the remainder of the oviposition hole not occupied by the egg. The naked eye can scarcely detect an infested kernel after the gelatinous plug has filled the cavity. The shape, hairiness, and softness of the grain as well as its position and stability, determine where eggs are laid but only one egg is usually laid in a grain so that there is no overcrowding of larvae.

Hinds and Turner (1911) state that hatching occurs on average three days from deposition of the egg at a favourable temperature. The egg hatches and the small white grub lives inside the kernel, eating out the dry inner portion.

The larva is short, fleshy, and legless. The newly hatched larva usually tunnels towards the centre of a wheat kernel until it reaches the crease then it tunnels back and forth along the crease. However, at times a newly hatched larva will feed just under the outer coat and make a pale scar that provides external evidence that a larva occupies the kernel (Winterbottom, 1922; Eastham and McCully 1943; Eastham and Segrove, 1947). If two or more eggs are deposited on the same side of the crease, the larvae will fight until one is destroyed (Sharifi and Mills (1971)). When granary weevil larvae are about half grown, they cut a passage through the crease and occupy the centre of the wheat kernel.

During its development, a weevil larva sheds its exoskeleton four times. The grub greatly increases in size after each molt, except the fourth. The width of its tunnel is indicative of the larval instar (Kirkpatrick and Wilbur, 1965). Hinds and Turner (1911), Winterbottom (1922), Cotton (1947) and Howe and Hole (1966) have shown the duration of larval stages under favourable conditions to be 1st larval stage, about 3 days; 2nd larval stage, about 4 days; 3rd larval stage, about 9 days; then a distinct prepupal stage lasting usually 1 day. The entire larval stage occupies between 16 and 17 days. The grubs eat the endosperm ravenously and convert the starch into fatty tissue, which they store throughout their bodies.

After the fourth moult, the grubs undergo a transformation to the pupa. Average duration of this stage is about six days (Winterbottom, 1922). The pupa is white with the proboscis, legs, wing pads and antennae of the future adult plainly developed. During the pupal stage, the stored fat is broken down into simpler materials which are used to build adult beetle tissues (Cotton and Wilbur, 1974).

Larval feeding and pupation are entirely inside the kernel, and the young adult, after a period of quiescence, actively bores out of the kernel (Howe, 1972). Normally, the adults remain inside their kernels a few days after emergence from the pupa before chewing an escape hole through the seed coat and emerging to the exterior. By

passing their developmental life inside a kernel, the defenseless larvae are protected from most enemies as well as from sudden changes in temperature and moisture while, at the same time, they are surrounded by an abundance of nutritious endosperm (Cotton and Wilbur, 1974). Following adult emergence there is a further maturation period (4 - 5 days at 25 C) before egg laying commences (Howe, 1972).

c) Ecology

Granary weevil is cosmopolitan in distribution although it prefers a temperate climate. Eastham and McCully (1943) and Eastham and Segrove (1947) have shown that the lower limit of temperature for multiplication of granary weevil is 15 C, while the optimal range is 26 - 30 C. Minimum R.H. is 50% and maximum rate of increase per lunar month is 15 times. Under favourable conditions the whole life cycle of granary weevil may be passed in about six weeks but the period will vary with the temperature. Compared to other insect S. granarius is quite cold hardy. The adult can live some 2.5 months at 0 C and thus is likely to overwinter in warehouses in temperate areas, whereas other weevil species rarely survive a cold winter (Howe, 1972).

The optimum constant temperature for Sitophilus species is about 30 C. At this temperature it takes about 30 days from oviposition to emergence of the adult from the cereal grain. It has been generally recognised that the development of most storage pests is slow at temperatures below 20 C, although at this temperature Sitophilus spp. have been shown to complete its life cycle in 120 days in wheat at a relative humidity of about 60%. At 15 C the developmental period of S. granarius extends to about 180 days. Sitophilus granarius is the most tolerant of Sitophilus species to low humidity though it suffers heavy mortality at 40% RH or 10.5% grain moisture content (Oxley and Howe, 1944; Hall, 1970; Howe, 1972).

The size of the Granary Weevel adult depends somewhat on the size of the grain kernel as well as on the seed species. In small grains such as millet or grain sorghum, the weevil is small, but in maize, a particularly favourable food, it attains its maximum size (Cotton and Wilbur, 1974).

Weevils rarely develop in anything except seeds. However, occasionally they develop in solid-milled cereal products such as macaroni, and they have been found breeding in tightly packed flour. Since granary weevils cannot fly, they are confined to grain stores that can be reached by walking or to which they are transported by man or machine (Cotton and Wilbur, 1974).

d) Damage to Stored Seed

Sinha, (1984) evaluated the effects of weevil infestation on the abiotic and biotic quality of stored wheat. The effect of infestations with either Sitophilus granarius or S. oryzae on the quality of wheat grain alone or with dockage was determined in laboratory studies over 20 weeks at 30 C and 70% RH. The changes in quality criteria included increases in fat acidity values, loss of seed germination, increased germ and endosperm damage, and increased fungal and bacterial infection of seed. Sharp increases in seed moisture content and dockage also occurred in infested wheat.

Feeding in the kernels results in loss in weight, loss of nutrients, conversion of the nutrients to inferior food materials, reduction of germination, reduced vigour of seeds, downgrading of market grains, and lowering of market values (Cotton and Wilbur, 1974). The weight loss in wheat seed infested with Sitophilus spp under natural temperature conditions was reported by Koura and El-Halfawy (1972) to be 32%.

Golebiwska, et al. (1977) found in their work on feeding capacity of some species of storage insects that most species, including granary weevils, fed most frequently on the germ end of the grain and less frequently on the brush end. The resultant damage had a major effect in reducing seed germination and subsequent seedling growth capacity.

CONTROL OF INSECT PESTS IN STORED SEED

Insects can be one of the most important single causes of damage to seeds after harvest if infestation is not prevented (Henderson and Christensen, 1961). Seed, a valuable product, must not be allowed to be damaged or destroyed by insects in storage. Parkin (1963) presents the view that in light of the protective measures now available such damage and loss can be due only to ignorance or negligence.

Munro (1966) classified control measures against insect infestation of stored products into five categories; (1) Hygienic measures, (2) physical and mechanical measures, (3) chemical measures, (4) biological measures and (5) legislative measures.

Knowledge of pest biology is basic to all forms of control. Biological data on population growth rates under various environmental conditions set the parameters for safe storage of seed as far as insect damage is concerned (Heather, 1984). Henderson and Christensen (1961), Parkin (1963) mention that the conditions that make for good storage of seed from the standpoint of retention of germinative capacity are equally important in helping to minimise the risk of insect damage. Thus, prevention of insect damage to seeds can best be considered from the view points of pest biology, sanitation, and control measures involving physical or chemical procedures (Heather, 1984).

Sanitation and Good Management

A principle of prevention of infestation by sanitation and good management is, as mentioned by Henderson and Christensen (1961) that clean seed is not only of better quality but also is better able to resist infestation. Seeds should therefore be clean (free from dust, broken seed, foreign seed, husks, chaff and soil) and should not be stored in untreated containers that have previously held infested materials. Preventive measures that can be taken against insect attack by sanitation or storage management are summarised by Henderson and Christensen (1961) and Heather (1984).

Physical Measures

Physical measures of control involve techniques designed to render storage conditions unsuitable for insects. Howe (1972) mentions that temperature has more influence on the rate of multiplication of pests than any other single environmental factor and, after moisture, is the key factor in seed storage. Damage by insects is therefore relatively uncommon in seeds stored dry and cool. Birch (1953) showed that for Sitophilus oryzae and Rhizopertha dominica temperatures of 15 C or below gave effective control. If there are some species which can still multiply at these temperatures

there are none which can multiply at a rate likely to cause problems. A very few species such as rusty grain beetle (Cryptolestes ferrugineus) as shown by Smith (1970) (cited by Heather, 1984) can survive sub zero temperatures if given a chance to acclimatise. For practical purposes temperatures of 12 - 15 C give adequate control of insects and temperature around 9 C will disinfest seed provided that seed moisture content is below 12% (Taylor and Elder, 1981) (cited by Heather, 1984). Extremes of temperature and seed moisture content/relative humidity will thus give total control of insects. Although these lethal conditions are frequently impractical the contributory benefits of less radical reductions should not be overlooked (Heather, 1984).

Chemical Control

Despite attention to all the factors which dispose seed in store to insect attack, there may still be a risk of infestation. Chemicals can then be used to give additional protection (Parkin, 1963).

Two main types of chemicals used in the control of insect pests of stored seeds are contact insecticides and respiratory poisons or fumigants (Cotton, 1947; Henderson and Christensen, 1961; Parkin, 1963; Hall, 1970; Howe, 1973; Heather, 1984).

A contact insecticide is a poison which is able to penetrate the insect cuticle and thereby enter the body tissue killing the contacted insects. A fumigant is a gas or vapour which is taken into the body of the insect through its respiratory system (Hall, 1970).

There are three ways in which insecticides may be used, namely as seed protectants, as residual surface treatments and as space treatments (Heather, 1984).

Fumigants may be available in solid, liquid or gaseous forms but all fumigants must be in the gaseous state before they can be effective. Due to their volatile nature, fumigants are able to penetrate a stack of bagged produce or bulk stored grain and will kill any insect infestation present (including eggs and other immature stages inside the grains). However, fumigants do not give any lasting protection against reinvasion by insects (Munro, 1966; Thompson, 1966; Lindgren and Vincent, 1966; Monro, 1969; Hall, 1970; Harein and Casas, 1974).

In the treatment of seed with chemicals to protect them from insect attack or to kill infestation which is already present, the following important factors and aspects of chemicals are generally considered.

a) Persistence

Immediately an insecticide is applied its activity starts to decline. Insecticides whose activity declines slowly are said to have long persistence or to be persistent. Those chemicals whose activity disappears quickly are of short persitence (Fenemore, 1984). Chemical breakdown of insecticide depends on the stability of the chemical itself and is influenced by moisture, light and temperature (Parkin, 1963; Hall, 1970; Harein and Casas, 1974; Fenemore, 1984).

b) Specificity

The general purpose insecticide which is effective against all pests under all conditions does not exist. All of the chemicals used in the control of pests have a certain degree of specificity (Cotton, 1947; Giles, 1965; Rowlands, 1967; Hall, 1970; Minett and Willams, 1971; Fenemore, 1984). Not only do different species of insects vary in their susceptibility, but within a species the different stages of egg, larva, pupa and adult may also vary in their reaction (Lindgren and Vincent, 1966; Howe, 1973). The effect of physical environment on the efficiency of insecticides in killing insects is also important. Thus a particular insecticide under a certain set of temperature and humidity conditons may be either more or less effective in controlling the same pest under a different set of conditions (e.g. higher temperature). This necessitates investigations to determine the most effective dose rates for different insecticides under local conditions against local strains of insect pests (Howe, 1965; Parkin, 1965; Minett et al., 1968; Tyler and Green, 1968; Hall, 1970).

c) Hazard to Humans

Toxicity to humans is the main cause of danger in using insecticides. Virtually all insecticides are to some extent toxic to higher animals, including man, as well to the pests against which they are applied, but the degree of mammalian toxicity varies greatly from

one insecticide to another (Fenemore, 1984). Toxicity is commonly expressed in terms of necessary or estimated dosage when ingested to kill 50% of a large population of the species of animal under consideration, that is, acute oral LD_{50} values (Hall, 1970; Fenemore, 1984). The main danager from fumigants is however, one of inhalation. Danger may also occur from the residues of insecticide if the treated seed is consumed by humans, animals or birds (Parkin, 1963). On the other hand, this residual effect of insecticide, that is their persistence, is of great value in protecting seed from insect attack for long periods (Munro, 1966; Parkin, 1963).

d) Danger to Seed

Toxicity (injury) by insecticide to plant life or seed is termed phytotoxicity. This aspect of deterioration of seeds by chemicals is affected by the inherent characteristics of the seed, the conditions of storage, and the properties and formulation of the insecticide used (Hall, 1970). The use of insecticides, in addition to the presence of chemical residues which may be toxic in seed, can result in odour or off flavour which detracts from seed quality (if used as food); ability of seeds to germinate may also be impaired. Any insecticidal treatment of seed must be evaluated for possible phytotoxic effects.

e) Development of Resistance in Insect Species

Parkin (1965) reported that there were already a number of proven instances of the development of resistance to contact insecticides and to a smaller extent to fumigants. Insecticide resistance is defined by Fenemore (1984) as the developed ability of an insect population to withstand an insecticide which was formerly effective. Resistance to insecticides is a very serious problem threatening the continued effective control of many important pests.

MALATHION

Chemical Properties and Formulations

Haerin and Casas (1974); The Agricultural Chemical Board (1975) and Thomson (1982) describe malathion as an organic phosphate insecticide - acaricide. Its molecular formula is $C_{10}\ H_{19}\ O_6\ PS_2$ and molecular weight 330.36. It is a clear amber liquid in physical form

(Technical ca. 95%). Its melting point is 2.8 - 3.7 C (pure); boiling point, 156 - 157 C at 1 mbar; vapour pressure, 1.6 x 10^{-4} mbar at 20 C and it is miscible with many organic solvents. Its acute oral toxicity to rats is LD_{50} 1375 mg/kg. Malathion is available in several different fomulations, such as, aerosol, emulsifiable concentrate, wettable powder, bait and non-aqueous concentrate. It is sold under several different trade names. In New Zealand it is known as Maldison.

Specificity

Miles et al. (1971) describe malathion as a contact insecticide with fairly fast action against a considerable number of species of beetles attacking cereal and other seeds if applied to give a dosage of about 10 ppm malathion and may be admixed with seed as dust or spread directly onto the seeds. It is of special value when seed damage was threatened by Oryzaephilus spp., Tribolium spp. and Sitophilus spp., but is not very effective against Lepidoptera at this rate of use. Moore and Decker (1961) however, found malathion to be effective against some Lepidoptera while McFarlane (1961) reported malathion as ineffective against Cadra cautella in Kenya.

Persistence

In some cases malathion may be preferred to some other insecticides such as lindane in so far as the potential toxic hazard to man is concerned. However, malathion has the disadvantage of a certain amount of chemical instability and thus tends to break down and become partially ineffective in certain circumstances (Parkin, 1963; Hall, 1970; Fenemore, 1984). Dust formulations may lose insecticidal activity if incorrectly formulated (Hall, 1970). The factors which increase the rate of breakdown of malathion when applied to seeds (or other stored products) are high moisture content (Watters, 1959; Strong and Sbur, 1960; Rowlands, 1967), high temperature (e.g. after artificial drying without cooling) (Gunther et al., 1958; Tyler and Green, 1968; Minett et al., 1968) and the presence of enzymes in the grain being treated (Rowlands, 1967). Malathion is also unstable when applied to alkaline surfaces such as concrete (Parkin, 1963; Hall, 1970). The rate of breakdown of

malathion is greater at increased moisture content and increased temperatures but critical moisture levels show some degree of temperature dependence and the maximum safe temperature which may determine the persistence of malathion at effective levels on grain in storage is not clearly defined. However, different investigations (e.g. Strong and Sbur, 1960; Minett et al., 1968) have observed that a moisture content of 12% is about the maximum for wheat in the temperature range of 21 C to 32 C. Information on the interaction of moisture content and storage temperature shows that dosages of malathion should be adjusted in practical storage conditions to compensate for advserse effects of seed moisture content and storage temperature. The Pesticides Board in New Zealand (formerly the Agricultural Chemicals Board) (1975) recommends repeated application of malathion for the control of insects in stored seed because of its instability.

Hazards

Malathion appears to be a relatively safe contact insecticide in relation to toxicity to humans and seeds (Parkin, 1963; Howe, 1973). A large number of insecticides have been evaluated for use in stored seed but little, if any, information is available on their effects on seed viability. Four established insecticides, DDT, pyrethroids, lindane and malathion have been widely used on seeds and if properly applied do not usually cause damage. Lochner (1962) sprayed malathion on wheat and maize at 20 p.p.m. without effect on viability but recommended a maximum moisture content of 12.5% for maize because malathion is not sufficiently effective against insects at higher moisture levels. Parkin (1963), Witt et al. (1960) stated that there was evidence indicating no harmful effect on germination of several kinds of seeds treated with high doses of malathion dust and stored for a year or more. Even if there is a risk of insecticide damage to seed, it may be less than the insect damage it prevents.

PHOSPHINE

Chemical Properties and Formulations

Phosphine (PH_3) is a fumigant commonly applied in the form of a tablet, Phostoxin, (based on aluminium phoshide) developed by Degesch of West Germany. Thomson (1981) describes the properties of Phostoxin as follows:

Phostoxin tablets contain 55% aluminium phosphide, 42% ammonium carbamate and 3% edible paraffin. Dosages recommended are 5 - 10 tablets per tonne of seeds with treatment for 3 - 7 days. Tablets decompose slowly on exposure to moisture from grain and liberate phosphine.

Phosphine is a gas, with an objectionable ammoniacal odour and boiling point, 87.4 C. It is sparingly soluble in water. It is spontaneously flammable (due to the presence of traces of other hydrides of phosphorus) in air and highly volatile. CO_2 is also released from phostoxin pellets to counteract flammability. Toxicity of phosphine is 2.8 mg/litre. LD_{50} (human) is 20 mg/kg and it is lethal to man in a few minutes.

Specificity and Residual Effect

Phosphine has been recommended to eliminate various insect pests in storage. It is effective against all insect pests in all stages of growth and also against rodents. The most important stored seed pests which are controlled by phosphine are weevils, grain beetles, grain borers, flour beetles, cadelle beetle, flour moths, and grain moths. The residue of phostoxin is non-poisonous and consists of inert aluminium hydroxide. Phosphine has no residual protective value (Monro, 1969; Reynolds et al., 1967; Lindgren et al., 1958; Thomson, 1981).

Mode of Action

Bond and Monro (1967), Bond et al. (1967), Bond et al. (1969), and Kashi and Bond (1975) did extensive work on the mode of toxic action of phosphine. Many factors in the environment of insects influence the effectiveness of fumigants. Among these factors, temperature, ambient presure, humidity, oxygen tension and the physiological condition of the insects are most important. Oxygen

appears to be essential for the absorption of phosphine by insects and under anoxic conditions phosphine is not toxic becuase it is not absorbed to any appreciable extent. On the other hand, presence of carbon dioxide potentiates the action of phosphine.

Reynolds et al. (1967) found phostoxin to achieve excellent control of heavy insect infestations in grain fumigated at high temperature. The manufacturers (Anon, 1966) recommend adjusting the length of the fumigation period according to the temperature and advocate a minimum of 3 days at 20 C or above increasing to 5 days at 15 C. Although a high seed moisture content or a high temperature during fumigation cause a reduction in viability of seed (King et al. 1960), the gas is evolved only by reaction of the tablet with the moisture in the atmosphere or seed. Parkin (1963) has stressed the fact that the rate of phosphine gerneration may be slow and finally incomplete if the moisture content of the seed is too low i.e. below about 9% for cereals.

Lindgren and Vincent (1966) investigated the toxicity of phosphine on several species of stored-product insects and their life stages under various conditions of exposure time and temperature. The order of tolerance to phosphine fumigation of the various stages of S. granarious and S. oryzae was pupa egg larva adult. From CXT (concentration x time) products they found, lower concentrations of phsphine are required to obtain an equivalent kill as the temperature increases.

Hazards

Hall (1970) states that fumigants enter the internal structures of the seed, in which some of the fumigant remains even after aeration. As a result of chemical reaction between a fumigant and seed composition, permanent residues may be formed. For example, fumigation with methyl bromide or ethylene dibromide may result in the formation of inorganic bromide residues. Monro (1969) reported that many fumigants are physiologically active compounds and may affect the seed adversely by causing:

(i) Stimulation of germination

(ii) Impairment or total loss of germination and/or

(iii) Sub-lethal injuries which cause more abnormal seedlings to be produced.

Whitney et al. (1958) and Parkin (1963) indicated that a margin of tolerance usually exists between the dosages required for insect kill and those which are liable to cause some loss of viability of retardation of seedling growth of high quality dry seeds. This margin of tolerance is dependent upon the complex interaction of several variables including the fumigant dose applied, seed moisture content, exposure period, kind of seed, fumigation temperature and the history of the seed (its age, vigour, previous fumigation etc.).

King et al. (1960) found that either a high seed moisture content or a high temperature during fumigation caused a reduction in viability. A high seed moisture content combined with a high temperature usually resulted in extensive injury.

In general, conditions which are optimum for good storage are favourable for seed fumigation, and no phytotoxicity to seed should then occur under recommended dosages (Whitney et al., 1958).

It has been reported that phosphine does not impair the germination of a wide variety of seeds, namely, wheat, sorghum, maize, cotton, grasses, ground nuts, small legumes and seeds of food legumes (Heseltine and Thompson, 1957; Lindgren et al., 1958; Strong and Lindgren, 1960; Harada, 1962; Beratlief and Alexandreson, 1964; McGregor and White, 1969; Quershi, 1965; White and Jacobson, 1972; Ahmad, 1976), but Potgieter and de Beer (1972) found that repeated fumigation with phosphine decreased field performance.

Hall (1970) points out that most fumigants, including phosphine, are highly toxic to man and should therefore, be handled only by properly trained personnel.

NEEM SEED

The potential of neem seed, a cheap but effective natural pesticide, as an alternative to the increasingly expensive and imported synthetic pesticides has recently been reported by Ivbijaro (1983). The repellency of the neem tree to locusts was first noted by Volkousky (1939) (cited by Sinha and Gulati, 1965) and was confirmed by Roonwall (1939) and Hussain et al., (1949) (cited by Sinha and Gulati, 1965).

The neem tree, Azadirachta indica A. Juss, is an evergreen tree, found in tropical and subtropical regions of Asia, Africa and Australia. The kernels contain 40 - 45% of an acrid bitter greenish-yellow to brown oil with a strongly disagreeable garlic-like odour (Jacobson, 1981; Lewis and Elvin-Lewis, 1983). In India and Bangladesh there is local use of the oil as an illuminant and in traditional medicine.

Extracts of another species of neem seed, Melia azedarach, have also been shown to have an antifeedant effect against some insects (Ruscoe, 1972).

The bitter principles of neem seed are locally known in India as nimbin, nimbinin and nimbidin. The several insect feeding deterrents isolated from neem seeds are meliantriol (Lavie et al., 1967, cited by Ruscoe, 1972); azadirachtin (Butterworth and Morgan, 1968, 1971) and salannin (Warthen, 1975). There are many reports that meliantriol from the fresh fruit of Melia azedarach and the oil of Azadirachta indica is a feeding repellant for the desert locust. However, Butterworth and Morgan (1968) report that the inhibiting substance, which they call azadirachtin is not related to meliantriol and is of notably high activity as a feeding repellant.

Specificity

Extracts from neem seeds have been found to be both a broad-spectrum repellant and an insecticide. Antifeedant activity has been noted for neem against several insect orders, including Orthoptera (Attri, 1975), Coleoptera (Saradamma et al., 1977), Lepidoptera (Warthen et al., 1978) and Diptera (Kareem et al., 1974). Additionally, insecticidal activity of neem has been noted against aphids (Goyal et al., 1971), Colorado Potato Beetle (Steets, 1976,

cited by Webb et al., 1983), Mexican Bean Beetle (Schmutterer and Rembold, 1980), Diamondback Moth (Tan and Sudderuddin, 1978), and the Gypsy Moth (Skatulla and Meisner, 1975). Neem also has shown activity against nematodes (Egunjobi and Afolami, 1976), fungi (Kher and Chaurasia, 1977), and viruss (Rai and Sethi, 1972). Neem kernel is also an ovipositional deterrent for Sopodoptera litura (F.) (Joshi and Sitaramaiah, 1979). Other biologically active compounds were reported by Kraus et al. (1980). The chemistry of extracts of neem was elucidated by Nakanishi (1975) and improved methods for its isolation and purification were developed by Uebel et al. (1979) and described by Gill and Lewis (1971) and Webb et al. (1983). Among three feeding: inhibitory triterpenods,. namely melianthriol, salannin and azadirachtin, the latter has been found to be the most effective and versatile (Ivbijaro, 1983).

Persistence

In Nigeria, maize grains mixed with dry ground seeds of neem at 0.5, 1 and 2.5 g/20 g maize were protected for 6 months in storage from damage by Sitophilus oryzae (L.) (Ivbijaro, 1983). At the highest rate neem seed prevented oviposition, at the lowest rate it markedly reduced ovipositoin, and at all the rates tested it completely halted post-embryonic development. Parent weevils placed on maize that had been treated 4 months previously with 2.5 g neem/20g maize failed to oviposit, and 76% of them died within 10 days; when adults were placed on maize treated with 1 g neem seed/20 g maize, progeny emergence was delayed for 6 months and very few new adults were produced.

Hazards

Sinha and Gulati (1965) found neem extracts not to be phytotoxic to treated crops or ornamentals. Ivbijaro (1983) reported that neem seed had no adverse effects on the viability of seed and no fungus or shrinkage was observed.

EFFECT OF STORAGE CONDITIONS ON SEED LONGEVITY

Seed is stored for a number of reasons and over various periods of time. Like any other biological material, seeds deteriorate with age. The main purpose of seed storage is to preserve or maintain the physiological quality of seed throughout the storage period by minimizing the rate of seed deterioration (Delouche et al., 1973).

The rate at which seeds lose viability during storage is dependent upon two sets of factors - those which relate to the seed itself and those relating to the conditions under which the seed is stored.

The most important consideration in seed storage is the quality of seed entering storage. High qulaity seed stores better than low quality seed. Also, longevity of seed is a chracteristic of the species or variety (Vaughan, 1969; Harrington, 1972; Delouche, 1973). The inherent longevity of seeds, the conditions to which they were exposed prior to storage, and damage sustained during pre-storage operations determine their response to a specific storage environment (Delouche, 1971; Moore, 1963). Storability of seeds is also adversely affected by mechanical injury, insect damage and plant diseases that infect seed directly or indirectly. These factors may seriously reduce seed quality as well as storability.

Assuming undamaged seeds are in storage and that they are free from undesirable admixture, it is the function of storage to provide an environment in which changes in the seeds will be held at an acceptable level. Since the seeds are living, some changes will occur under any circumstances (Hukill, 1963).

A stored seed bulk is an ecological system in which deterioration results from interaction between physical, chemical and biological factors. Some of the more important factors are temperature, moisture, oxygen level, geographical location, granary structure, physical and chemical properties of different seed types and the activities of micro-organisms, insects, mites, rodents and birds. The degree of interaction between these factors and their relative influence on the deterioration of seed in storage is highly complex (Hill, 1983).

Among environmental factors, most authors agree that the relative humidity and temperature of the storage environment are the most important, with relative humidity more important than temperature (Owen, 1956; Barton, 1961; Delouche, 1968; Christensen and Kaufman, 1969; Harrington, 1960d, 1972; Pixton, 1967; Justice and Bass, 1979).

The physiological quality of seed is affected by relative humidity in two ways (Delouche et al., 1973).

(a) Seed moisture content is a function of ambient relative humidity
(b) Infestation, growth and reproduction of both storage moulds (fungi) and insects are strongly influenced by the realtive humidity of the micro-environment in the seed mass.

Seeds are hygroscopic and absorb moisture from the atmosphere or lose moisture to it until the vapour pressure of seed moisture and atmospheric moisture reach equilibrium. The moisture content that seeds will maintain in a given atmospheric condition is known as the Equilibrium Moisture Content (EMC) or hygroscopic equilibrium.

The equilibrium moisture content varies according to the kind of seed, and to a lesser extent the prevailing temperature. The hygroscopic equilibrium of a seed at a given relative humidity decreases slowly with increasing temperature and increases slightly with increasing deterioration of seed (Delouche, 1968). EMC of seeds may not always be the same due to the hysteresis effect. When seeds lose (desorb) water and reach equilibrium at any level of RH, the EMC of seeds will be higher than if dry seeds are allowed to gain (absorb) moisture to reach equilibrium at the same RH (Harrington, 1973). Excepting all these variations, the different kinds of seed attain specific or characteristic moisture contents when exposed to different levels of atmospheric relative humidity.

The rate of degenerative or deteriorative processes in physiologically mature seed increases as seed moisture content increases. It is, therefore, necessary to store seed with a 'safe' moisture content. Since seed moisture content and ambient relative humidity are in equilibrium during the storage period, maintenance of a 'safe' moisture content requires an average level of relative

humidity in the storage environment no higher than that in equilibrium with the desired seed moisture content (James, 1967). This favourable situation can only be achieved by either storing seeds in regions where the relative humiditiy is naturally low or reducing the relative humidity to a favourable level by conditioning the storage environment. The maximum level of safe relative humidity for successful storage of seed depends, of course, on the kind of seeds the length of the storage period, and the storage temperature (Delouche, 1968; Toole, 1950; Roberts, 1974).

The growth, reproduction and activity of storage fungi and insects are also dependant on the relative humidity of the micro-environment within a seed lot. Seeds kept at a relative humidity less than 65 to 70% maintain a seed moisture equilibrium unfavourable for the growth of fungi (Christensen and Kaufman, 1969). Such conditions also eliminate the storage fungi problem regardless of other conditions of storage. Activity of some of the more serious insect pests of stored seed decreases rapidly as the relative humidity drops below 50% and reproduction stops altogether at less than 35% (Cotton, 1963). Considering the negative influence of high moisture content on the longevity of seed, Harrington (1959) rightly points out that high moisture is the greatest single cause of loss of germination in seeds. The tremendous influence of moisture content on longevity of seed is also reflected in Harrington's rule-of-thumb (Harrington, 1959): the storage life of seed being doubled for each 1% decrease in seed moisture content. This rule-of-thumb has proven to be substantially correct for many kinds of seed and for seed moisture contents in the range of 6 to 15% (Delouche et al., 1973).

Temperature is the second most important environmental factor affecting longevity of seed in storage. Within limits the storage life of seeds of vegetables, flowers, and field crops decreases as the temperature increases. The ideal temperature range for most fungal activity is 21 C to 27 C. The optimum temperature conditions for most of the major insect pests of stored seeds lies between 28 C and 35 C and the maximum above which they die or at least cannot reproduce is usually between 35 C and 45 C (F.A.O. 1961). Storage of seed at high temperatures i.e., above 45 C is obviously not possible. Below 19 - 20 C stored seed is relatively safe from

insect activities. Other conditions and factors being equal, Harrington's rule-of-thumb also states: that the longevity of seed in storage is approximately doubled for each 5.5 C reduction in storage temperature within the range of 0 to 45 C (Harrington, 1959).

Both seed moisture content/relative humidity and storage temperature reinforce and compensate each other in their effect on longevity of seed. In general, the higher the temperature the more rapid deteriortion of seed at a given moisture level. Conversely the lower the temperature the greater the tolerance to high moisture content. Seeds with moisture contents between 14 and 16% can be stored for a year or more at temperatures of 10 C or lower, while many seeds with low moisture contents of 10% or less can withstand temperatures in the range from 30 to 34 C for a similar storage period (Delouche, 1968).

In many sub-tropical and tropical countries both humidity and temperature remain high for much of the year. These high temperatures and atmospheric relative humidities are obviously the main problems for seed storage in those regions. Delouche et al. (1973) rightly states that the combination of high humidity and temperature characteristic of tropical and sub-tropical environments, is disastrous for seed storage. Most kinds of seed decrease substantially in viability and even more in vigour during only six months storage at 30 C and 75% RH. Conditioning of the storage environment to reduce the relative humidity or temperature to a favourable level is the best way to store seeds in these regions for long term storage. Otherwise, repeated drying of seeds may be carried out even for short term storage (Harrington, 1959, 1963).

CHAPTER 2

AN EVALUATION OF MALATHION DUST, FUMIGATION WITH PHOSPHINE AND SEED DRYING IN CONTROLLING AN ESTABLISHED INFESTATION OF SITOPHHILUS GRANARIUS AND THEIR EFFECT ON SEED QUALITY

INTRODUCTION

Insect pests of stored grain have certain temperature, moisture and food requirements which directly affect their abundance and hence their ability to cause significant losses in seed quality. The extent and rate of loss of such quality, particularly viability, may also be influenced by management systems designed to reduce insect activity. In particular, the drying of seed and the effects of chemical insecticides, whether these be applied as protectants or fumigants, can have an effect on seed quality and longevity.

This chapter examines the effects of Sitophilus granarius population changes, malathion and phosphine insecticides and seed drying systems, alone or in combination, on the longevity of wheat seed over a 165 day storage period at 25 C and 80% relative humidity.

MATERIALS AND METHODS

MATERIALS

Seed

Wheat seed (Triticum aestivum L.) cultivar 'Rongotea' was used in this study. 40 kg of seed was thoroughly mixed and cleaned at the Seed Technology Centre. Initial seed moisture content was 15% and the seed was therefore dried to adjust moisture content to the desired levels before use.

Insects

Granary weevils (Sitophilus granarius L.) were obtained from a culture maintained by the Department of Horticulture and Plant Health, Massey University.

Chemicals

1. Malathion (Maldison)

Malathion dust (2% concentration, marketed by the New Zealand Farmer's Fertilizer Co. Ltd, Auckland) was procured from a retail outlet and used at the manufacturer's recommended rate of 1 kg/2.5 tonne of seed.

2. Kaolin Powder (Eckaglas powder)

Non reactive Kaolin powder (manufactured by Kaolin Australia PTY Ltd) was used as an extender for the malathion. A stock of malathion dust/Kaolin mixture was made containing 24 g malathion dust and 36 g Kaolin powder.

3. Phosphine

Phostoxin tablets (manufactured by Degesch GMBH, Frankfurt AM MAIN Federal Republic of Germany) which generate phosphine when exposed to moisture were used for fumigation of seed. Again the manufacturers recommendations (10 - 20 tablets per tonne of seed or 5 - 10 tablets per m^3) were followed. Ten tablets per m^3 were used in this experiment.

Specialised Equipment

1. Vacuum Counter

A vacuum counter specially designed at the Seed Technology Centre was used to count insects (Plate 1).

2. Experimental Containers

Containers were specifically manufactured for this experiment. P.V.C. pipe of 11 cm diameter was cut into 35 cm lengths. Caps fitted with insect proof wire mesh screen were used to close both ends. These containers were designed to hold 1 kg of seed and when laid on their side to be approximately 1/2 full leaving sufficient air/seed contact for rapid moisture equilibration (Plate 2).

The containers were placed in large black plastic airtight drums of 100 l capacity. Each drum contained 12 of the smaller containers making 1 complete replicate (Plate 3).

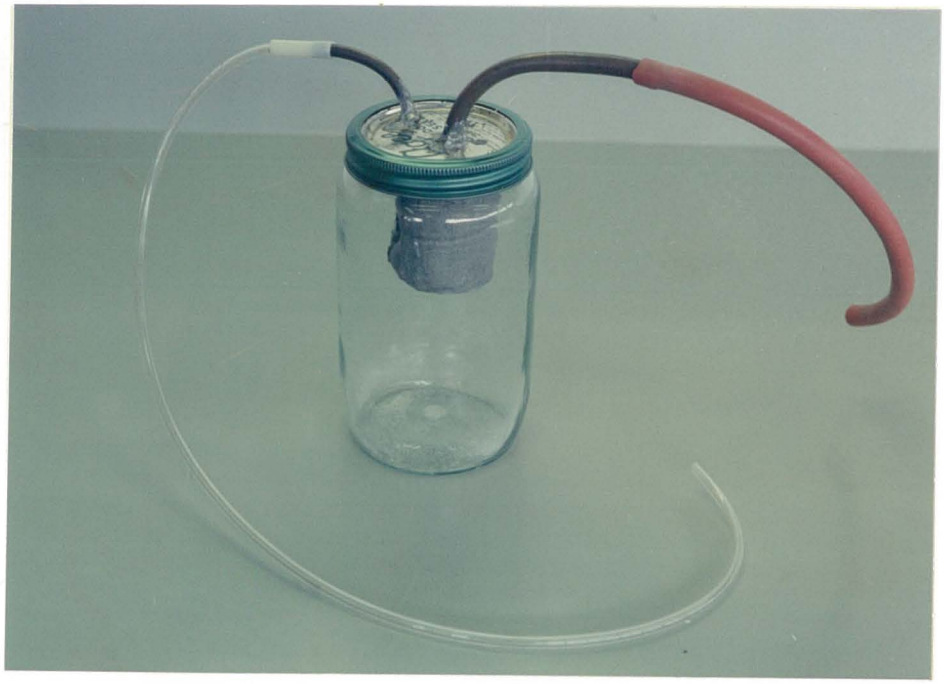


Plate 1: Vacuum insect counter

Plate 2: Containers for storing wheat seed

METHODS

Experimental Design

The experiment was a randomised complete block design with one storage environment, one species of seed, 2 insect treatments (no insect, with insect), 2 drying treatments (no drying, drying) and 3 chemical treatments (no chemical, malathion, phosphine). 3 replicates were used.

Setting Up and Seed Storage Environment

The seed bulk was divided using a Boerner Divider to produce 36 x 1 kg lots. Each lot was then placed in one of the small containers described. Each treatment receiving insects was infested with 100 adult weevils. No attempt was made to sex the weevils.

The experiment was commenced on 28.9.84 when the small containers were placed in their respective large drums. A storage environment of 25 C and 80% RH was maintained during this trial. The temperature was maintained in a thermostatically controlled room set for 25 C $\pm$ 1. Humidity control was obtained using a glycerine/water mixture of the correct proportions in each drum as employed by Hill (1965). The three drums were connected by means of plastic tubing and air was circulated between drums every 12 hours throughout the experiment in a further attempt to maintain a uniform atmosphere (Plate 4). Humidity was measured using wet and dry bulb thermometers (Plate 5) three times a week for the duration of trial.

The final samples were taken on 14.3.85 and the experiment terminated.

Chemical Treatments

Chemical treatments were carried out 30 days after the start of the experiment (this was sufficient time for insects to lay eggs inside wheat kernels and larval stages to develop but not sufficient time for the next generation of adults to emerge).

Malathion Insecticide Treatment

1 g of dilute malathion dust was added to each sample to be treated giving an effective concentration of 0.4 g malathion dust per kg seed. Containers were shaken and rotated vigorously following the addition of insecticide to ensure thorough mixing.

Plate 3: Large plastic drums used to hold one complete replicate in experiment 1.

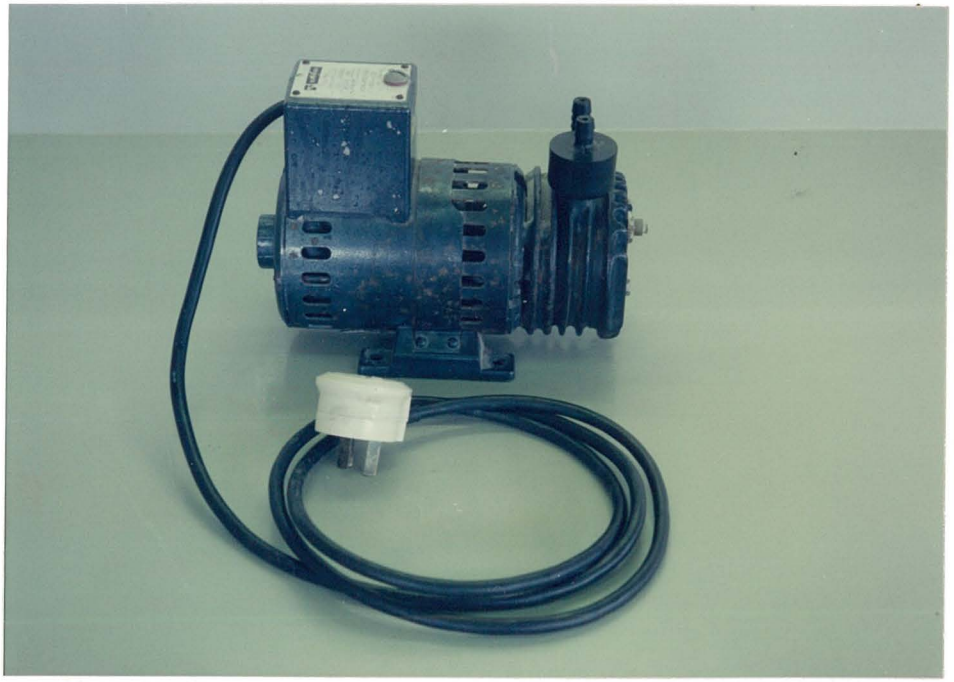


Plate 4: Pumps for cirulating air through large drums.

Fumigation Treatment

Fumigation was carried out in one big plastic drum. The volume of the drum was .135 m^3. 1 tablet of phostoxin was sufficient to fumigate the contents of the drum at the recommended rate. The 9 containers to be fumigated were first placed inside the drum and the empty space filled with barley seed. One phostoxin tablet was placed at the top of the contents of the drum which was then closed airtight. The drum was kept at 25 C for 48 hours and then aerated for 4 hours in the open air.

Drying of seed

All treatments were initially adjusted to 11% seed moisture content. Seed for drying treatments was dried on 'kiwi' mini driers whenever the samples exceeded 13% M.C. Drying was continued until seed reached 11% M.C. The regain period for seed to return to 13% MC following drying was approximately 25 days. An air temperature of 30 C was used for drying the seeds and the change in seed moisture content was checked every hour during drying by use of an Agromatic moisture meter (Plate 6) calibrated beforehand against the air oven method.

Sampling

Sampling of seed was carried out after 0, 30, 60, 90, 120 and 165 days of storage. At each sampling adult insects were counted to assess development and mortality and measurements made of seed germination and seed moisture content. Moisture of each treatment was determined every week using the Agromatic meter.

MEASUREMENTS

1. Counting of Insects

All adult insects (dead or alive) were sieved from insect infested treatments and counted immediately after chemical treatment and then at each sampling time. Counting of insects was facilitated by use of the vacuum insect counter. At the first assessment following chemical treatments all insects removed were discarded. At the following sampling times, live insects were put back into their respective containers. In later countings, when the populations of

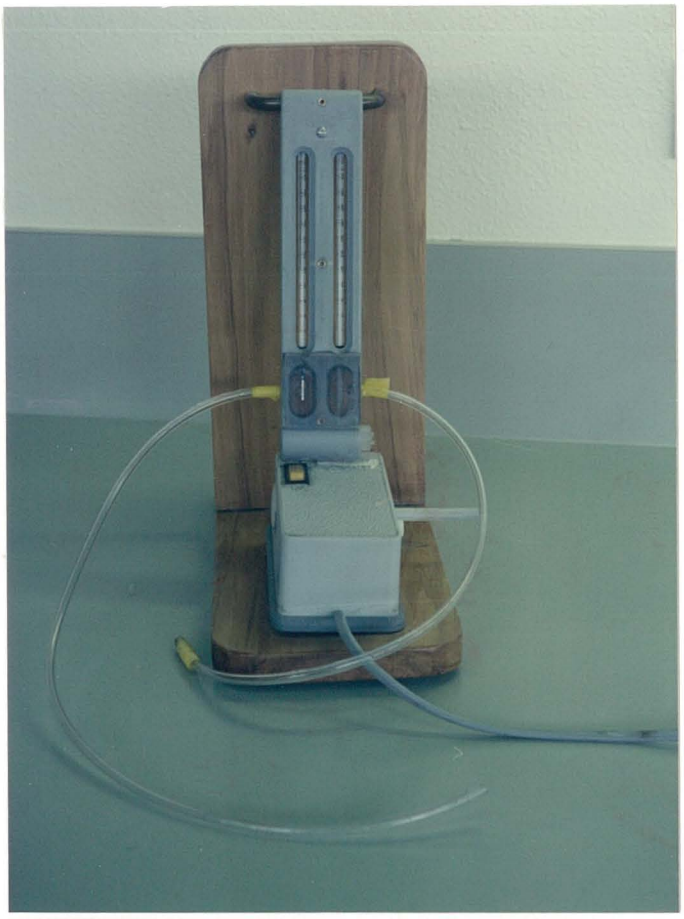


Plate 5: Aspirated wet and dry bulb thermometer.

Plate 6: Agromatic portable moisture meter.

insects increased to many thousands, the number of insects was estimated by weight. Four samples each of 100 insects were weighed. The average weight of 100 insects was found to be 0.24 g for live insects and 0.12 g for dead insects. The number of insects was then calculated using the following formula:

$$\text{No. of insects} = \frac{W}{W_1} \times 100$$

Where W = weight of 100 insects

W_1 = weight of insect population

2. Germination of Seeds

Germination tests were carried out according to standard laboratory germination methods using four replicates of 50 seeds. The rolled paper method was used at a temperature of 20 C. The first seedling count was taken on the 5th day and the final count on the 8th day.

At the first count only normal seedlings were counted and then removed. Where clearly dead seeds were identified and found to be mouldy, which could affect the germination of other seeds, they were also removed at the first count. At the end of the test, all other seeds and seedlings were examined and recorded in the appropriate category. Seedling evaluation was based on the ISTA Rules (1976). Seedlings were considered normal when they showed the capacity for continued development into normal plants as defined in the ISTA Rules. Seedlings which did not show such normal development were classified as abnormal. Abnormal seedlings included damaged seedlings, deformed seedlings, decayed seedlings, and seedlings showing any other defects as specified in the ISTA Rules (1976). The replicate results were averaged to give the mean percentage of normal and abnormal seedlings and dead seeds.

3. Moisture Content Determination

Moisture content of seed was (initially) determined by the Air Oven Method as recommended in the ISTA Rules (1976). For subsequent readings moisture content was determined by Agromatic moisture meter.

For the air oven method, seeds from each treatment were ground and duplicate samples of approximately 5 g of ground seed were placed in metal containers and kept for two hours in an air oven at 130 C.

Moisture content was calculated on a wet-weight basis where the amount of water lost in drying was divided by the initial weight of the sample using the following formula:

$$\frac{M_2 - M_3}{M_3 - M_1} \times 100$$

M_1 = weight of empty container
M_2 = weight of container plus wet seed
M_3 = weight of container plus dry seed

4. Assessment of Internal Damage to Seeds

Most damage caused by larvae and adults of S. granarius cannot be detected or evaluated by visual examination because these insects complete their life cycle inside seeds. Attempts were therefore made to assess the amount of internal damage by using X-ray photographs of seeds. X-ray photographs were taken after 60 days of storage to allow insects time to complete their life cycle inside the seeds. A Faxitron 43804N X-ray machine was used with exposure onto Polaroid film. The X-ray machine was operated using 25 KVP, and 3 Ma for 3 minutes (the time was fixed after several trials to produce the best pictures) to photograph a representative sample of 50 seeds from each treatment. Following X-ray photography, positional germination tests were conducted to compare internal seed damage, as shown by X-ray analysis, with the capacity of seeds to develop into normal seedlings.

RESULTS AND DISCUSSION

A. INITIAL QUALITY LEVEL OF WHEAT SEED

The initial quality of the wheat seed used in this study in terms of physical purity, viability and moisture content is shown in Table I.

Table 1: Initial Quality of Wheat Seed

Measurement	Value
% Purity (by weight)	99.7
% Normal germination	95.0
% Abnormal seedlings	1.0
% Dead seeds	4.0
% Moisture content (before drying)	15.0
% Moisture content (after drying)	11.0

The results clearly show the seed to be of high initial quality in terms of both purity and germination. Just as importantly, however, they also show that the seedlot contained no evidence of mechanical or insect damage and very low levels of seedling abnormality and dead seed.

B. INSECT POPULATIONS

Numbers of live and dead adult granary weevils over 165 days storage in the different treatments are given in Table 2. The patterns of development of live insect population with time in the different treatments are shown in Figure 1 (raw data in appendix 3).

1. Population Growth in Undried and Chemically Untreated Seed

In undried and chemically untreated seed (control treatment for insect development) the insect population was found to increase by about a factor of X 10 every 60 days. This result is a reflection of the statement by Howe (1972) that the growth of an insect population tends to be exponential and hence, should insects gain entry to stored seed, the build up of a population is determined by the period of storage and the suitability of the environment. The development of granary weevil is favoured by seed moisture content above 14%, (i.e., above 70% RH in storage) and an optimum temperature range of 26 - 30 C. Under favourable conditions the whole life cycle of granary weevil may be passed in about six weeks (Howe, 1972) during which time the maximum rate of increase is 15 times (Eastham and Sergove, 1947). The storage temperature in this experiment was 25 C which was just below the optimum for development of granary weevil. If, therefore, the temperature had been maintained at 26 C to 30 C instead of 25 C the increase in insect numbers may have been closer to X 15 rather than X 10.

After 165 days storage the number of live adult weevils in seed subjected to repeated drying was 22% less than in seed not dried (both without chemical treatment). This difference was highly significant. The difference between dried and undried seed started to appear after 60 days storage (Figure 1) but did not reach significance until 120 days storage (Table 2).

Table 2: Number of live and (dead) adult granary weevils in the different treatments up to 165 days of storage (mean).

Treatments		Days of storage					
		0	30	60	90	120	165
No chemical	No Drying	100.0 (0)	83.3A (17)	1066.0A (2)	1883.3Aa (17)	9313.3A (121)	11082.6Aa (748)
	Drying	100.0 (0)	85.6A (14)	861.8A (11)	1460.3A (42)	5482.0B (87)	8664.3Aa (171)
Malathion	No Drying	100.0 (0)	0.0B (100)	191.3B (745)	41.6C (512)	12.0C (26)	124.8B (119)
	Drying	100.0 (0)	0.0B (100)	106.6B (541)	13.0C (587)	4.3C (38)	0.0B (28)
Phostoxin	No Drying	100.0 (0)	0.0B (100)	0.0B (0)	0.0C (0)	0.0C (0)	0.0 (0)
	Drying	100.0 (0)	0.0B (100)	0.0B (0)	0.0C (0)	0.0C (0)	0.0B (0)
Significance		N.S	**	**	**	**	**
L.S.D.	1%		5.5	366.6	528.5	2044.4	5092.1
	5%		3.9	261.5	377.0	1458.2	3631.9

Values not followed by the same letter in vertical columns differ at P = 0.01.

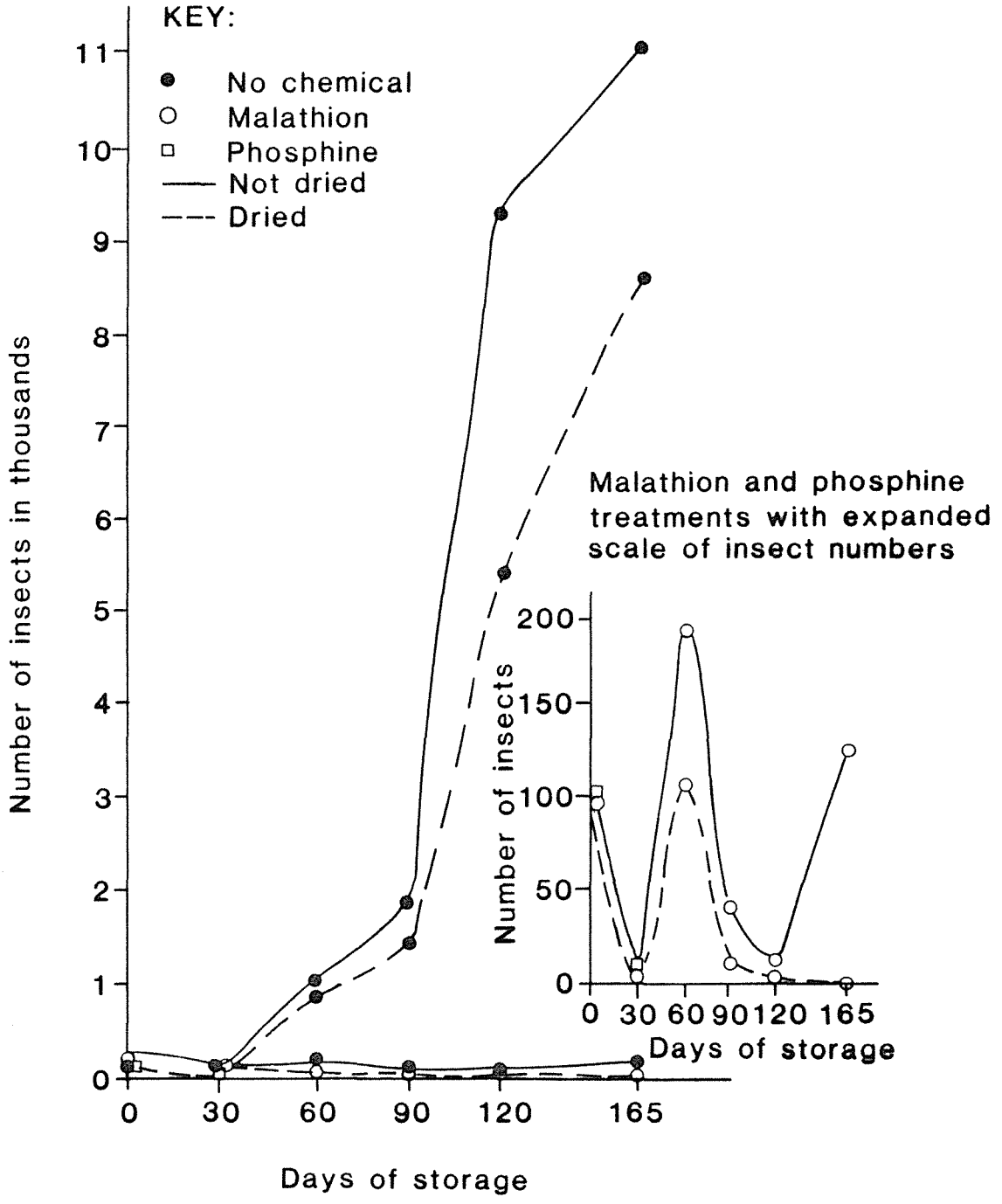
** Significant at 1% level.

Table 2a: Numbers of live and (dead) adult granary weevils in Malathion and Phosphine treatment up to 165 days storage (mean).

Treatments		Days of storage					
		0	30	60	90	120	165
Malathion	No Drying	100.0 (0)	0.0B (100)	191.3a (745)	41.6 (512)	12.0Aa (26)	124.8 (119)
	Drying	100.0 (0)	0.0 (100)	106.6ab (541)	13.0 (587)	4.3ABb (38)	0.0 (28)
Phosphine	No Drying	100.0 (0)	0.0 (100)	0.0b (0)	0.0 (0)	0.0Bc (0)	0.0 (0)
	Drying	100.0 (0)	0.0 (100)	0.0b (0)	0.0 (0)	0.0Bc (0)	0.0 (0)
Significance		NS	NS	*	NS	**	NS
L.S.D.	1%			202.3		8.4	
	5%			139.0		5.7	

Values not followed by same capital or small letter in vertical colums differ at P = 0.01 or 0.05 respectively.
** Significant at 1% level
* Significant at 5% level

FIGURE 1: INSECT POPULATION DEVELOPMENT IN DIFFERENT TREATMENTS WITH TIME



It is estimated that within the first 90 days of storage there would have been time for one full generation of granary weevil to develop but not a second. This, together with the fact that the difference in moisture content between dried and undried seed over this period was only about 3% (11% compared to 13 or 14%, Figure 2), may account for the lack of significance between the insect populations up to 90 days. In the latter part of the storage period (120 - 165 days) however, the difference in moisture content between dried and undried seed was greater (Figure 2) and furthermore, a second generation of weevils by then had time to develop. The difference between dried and undried seed then reached significance.

The result is a clear expression of the effect of drying on the development of the insect population. Development of granary weevil is favoured by high seed moisture (above 14%) within the optimum range of temperature (26 - 30 C) and any reduction of SMC from the optimum decreases the rate of development (Hall, 1970; Howe, 1972 and many others). These results confirm that repeated drying of seed, i.e. maintaining a low SMC despite high relative humidity storage conditions, can discourage but not prevent the development of insects.

2. Effect of Chemicals

a) Malathion

In malathion treated seeds, all adult granary weevils were found to be dead immediately after the insecticide was applied. This confirmed that malathion was effective in killing adults present in the seed mass at the time of application. In subsequent samplings however, some live adults and quite a large number of dead adults, were recorded (Table 2). This may be explained by the fact that immature stages of granary weevil occur inside the seed and thus are not affected by malathion on the outside of the seeds. However, when adults emerge they come in contact with the malathion and are then affected. The majority of adults from 60 days onward were therefore, dead. It also shows that malathion had considerable residual effect. However, not all adults were affected in this way and some live insects were present at every sampling from 60 days storage onwards. It seems therefore that some adults were always able to survive long enough to lay eggs inside seeds otherwise the population would

eventually have died out. In interpreting the figures in Table 2, it should be remembered that dead insects from each sampling were discarded and not replaced in the seed sample. From the fact that the population appeared to be increasing after 165 days storage it may be inferred that malathion was not then as effective as when applied. This agrees with the known property of malathion tending to breakdown chemically and thus lose its effectiveness after a period of time in certain circumstances (Parkin, 1963; Hall, 1970; Fenemore, 1984).

These findings suggest that in using malathion to 'clean up' infested seed and subsequently to keep such seed insect free, repeated application is necessary. This is in agreement with the recommendation of the Pesticides Board in New Zealand (1975).

In seed which was treated with malathion and also subjected to drying the numbers of live adult weevils were consistently less than in seed which was treated with malathion but not dried (Table 2). This interaction between malathion treatment and drying was sufficiently pronounced to result in complete elimination of the insect population after 165 days storage. This was in marked contrast to the undried malathion treatment in which the population was increasing again at this time. If repeated drying had any deleterious effect on the persistence of insecticidal action of malathion this was more than compensated by the increased mortality provided by drying.

c) Phosphine

Immediately after fumigation of seeds with phosphine all adult weevils were found to be dead. Furthermore, at the second sampling (30 days after fumigation) and at all subsequent samplings up to 165 days no adult insects were found in phosphine fumigated seed (Table 2). In comparison, in seeds not treated with any chemical (and not dried) there was a total of 1065 live adults after only 60 days storage. These adults were clearly descendents of the 100 weevils initially introduced into the seed indicating that there were about 1000 immature larvae inside the seeds at the time of chemical treatments. It may be concluded therefore, that phosphine was effective in killing not only adult weevils external to the seed but also all immature stages, including eggs, within the seed. This confirms the findings of Munro, 1966; Lindgren and Vincent, 1966;

Monro, 1969; Hall, 1970; and Harein and Casas, 1974, that fumigants such as phosphine have the ability to penetrate stacks of bagged produce or bulk grain and to kill all stages of insects present.

In practice therefore, if infested seed is fumigated and no insect is subsequently allowed to gain entry then seeds can be kept insect free.

C. SEED MOISTURE CONTENT (SMC)

Changes in moisture content of seed of treatments not subjected to drying during the course of the experiment are shown in Figure 2.

Absorption of moisture by seeds in all treatments was very slow. This was perhaps due to an effect of the containers. These were airtight (PVC pipe) and only the two ends were open but covered with closely woven wire mesh. There was also perhaps some overcrowding of the containers in the large airtight drums which might also have restricted air movement or circulation.

The SMC of the control treatment (not infested, not treated with any chemical and not dried) reached 15.5% only after 84 days storage and no further increase was observed until the end of the storage period. During this 90 - 165 day period seed moisture content was in equilibrium with the relative humidity of the storage atmosphere (80%).

Up to 56 days storage, the moisture content of seeds infested with granary weevils (but not treated with any chemical and not repeatedly dried) was similar to that of the control treatment (14.8% vs 14.4%). After that a steep increase in moisture content was observed to 18.5% after 120 days. This increase in SMC can be attributed to respiration of the insects as supported by numerous investigators (e.g. Cotton and Wilbur, 1974).

The lowest rate of increase in SMC was observed in seeds treated with malathion (both insect free and insect infested treatments). SMC increased to 15.5% in 105 days and 112 days respectively compared to 84 days in the untreated control. This slow absorption of moisture perhaps may be explained by the fact that seeds were surrounded by a mixture of malathion dust and kaolin powder thus hindering moisture absorption. From 105 days insect infested and malathion treated seeds maintained 15.5% SMC, that is, similar to that of control treatment.

FIGURE 2: CHANGES IN SEED MOISTURE CONTENT IN UNDRIED TREATMENTS

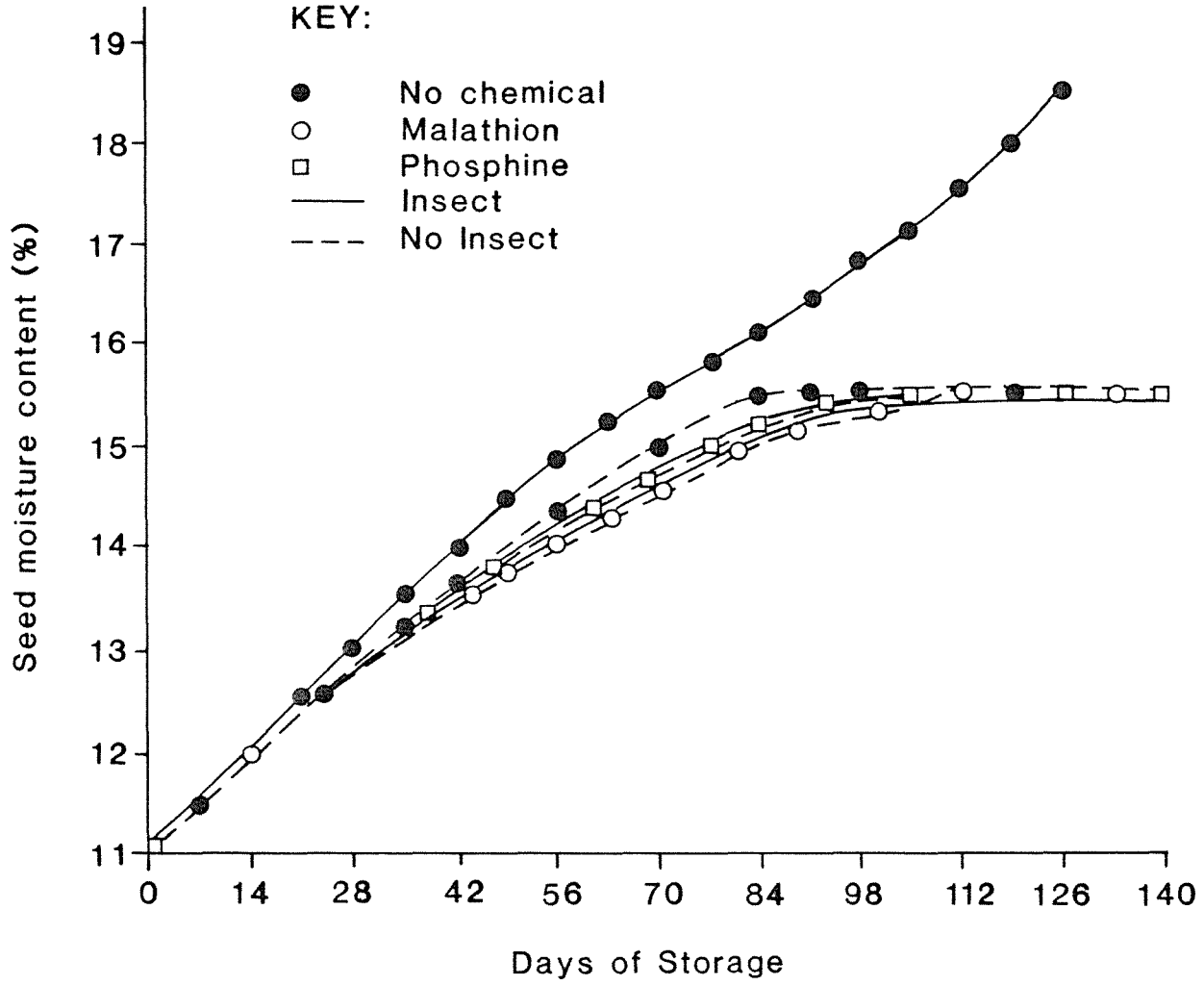
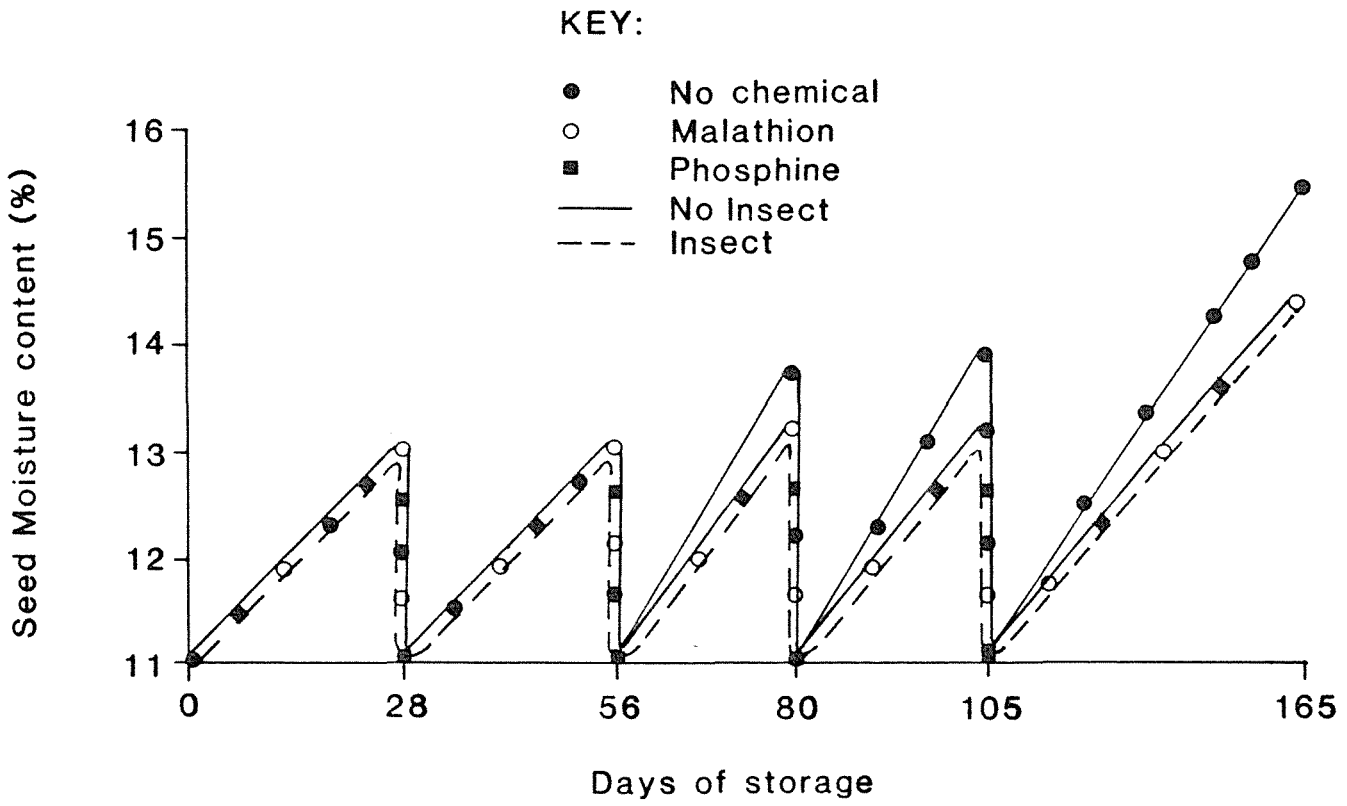


FIGURE 3: CHANGES IN SEED MOISTURE CONTENT IN DRIED TREATMENTS



This was probably because there were not enough insects to increase SMC by respiration compared with the situation in infested but chemically untreated seeds.

The changes in moisture content of seeds fumigated with phosphine (both insect infested and insect free) were closely similar to that of the control treatment. There were no insects in this infested treatment after fumigation and prior to that insects caused little damage to seeds during one month. Therefore, both insect free and insect infested seeds fumigated with phosphine behaved similarly to the control treatment.

Changes in seed moisture content of seed subjected to regular drying are shown in Figure 3.

In this case, changes in seed moisture content in all treatments were similar except for that of insect infested seeds which had not been treated with any chemical. The changes in SMC in all treatments were virtually identical up to 56 days. However, the SMC of the insect infested treatment was higher than other treatments after 77 days and 105 days (13.8% and 14% respectively compared to 13% in all other treatments). Similarly at the end of the storage period the SMC in this treatment had risen to 15.5% compared to 14.5% for other treatments. This higher SMC of insect infested and not chemically treated seed was presumably due to the respiration of insects as previously discussed.

D. NORMAL GERMINATION

The percentage of normal germination of seed from all treatments at different storage intervals is presented in Table 3 and graphically in Figure 4 (raw data in Appendix 4).

The germination percentage of the control treatment (no insect, no chemical, no drying) declined only slowly during the first 30 days of storage. Subsequently, however, germination levels fell significantly after 120 days storage. This rate of decline then accelerated with germination levels being reduced drastically to only 17% after 165 days storage.

These results agree with the statement by Delouche et al. (1973) that the combined effect of high humidity and high temperature is disastrous in its effect on the viability of stored seeds and by Toole

(1950) that most crop seeds lose viability rapidly at relative humidities approaching 80% and temperatures of 25 C to 30 C. The adverse storage temperature (25 C) and relative humidity (80%) used in the present study were obviously important in hastening the deterioration of seeds. However, because of the slow rate of absorption of moisture by seed under the conditions used in this experiment, they tended to remain at a relatively safe seed moisture content (11 - 14%) during at least the first 60 days of storage. Subsequent increase in the seed moisture content to an unsafe equilibirum moisture content then accelerated the rate of seed deterioration.

1. Effect of Drying

The mean germination percentage of wheat seed stored without insects or chemical treatment but which was subjected to repeated drying to retain a safe (11%) seed moisture content during the entire storage period was generally higher than that of seed which was not dried, allowing seed moisture content to increase to 15.5%. Although differences were small during the first 120 days of storage a significant reduction in seed germination capacity occurred from 120 to 165 days of storage.

The results show that drying had a beneficial effect on the retention of seed viability in storage. This effect was most pronounced as the length of the storage period increased. The moisture content in the 'dried' treatment was maintained at 11 to 13% during the entire storage period, while the SMC of seed which was not dried eventually increased to 15.5% from 90 days until the end of the storage period. This comparatively small difference in the maximum level of seed moisture content between dried and undried treatments during the latter part of the storage period in particular was still sufficient to result in major differences in seed longevity. The results clearly show that under high relative humidity conditions seed can be stored for a comparatively longer period if it is repeatedly dried (Harrington, 1959, 1972).

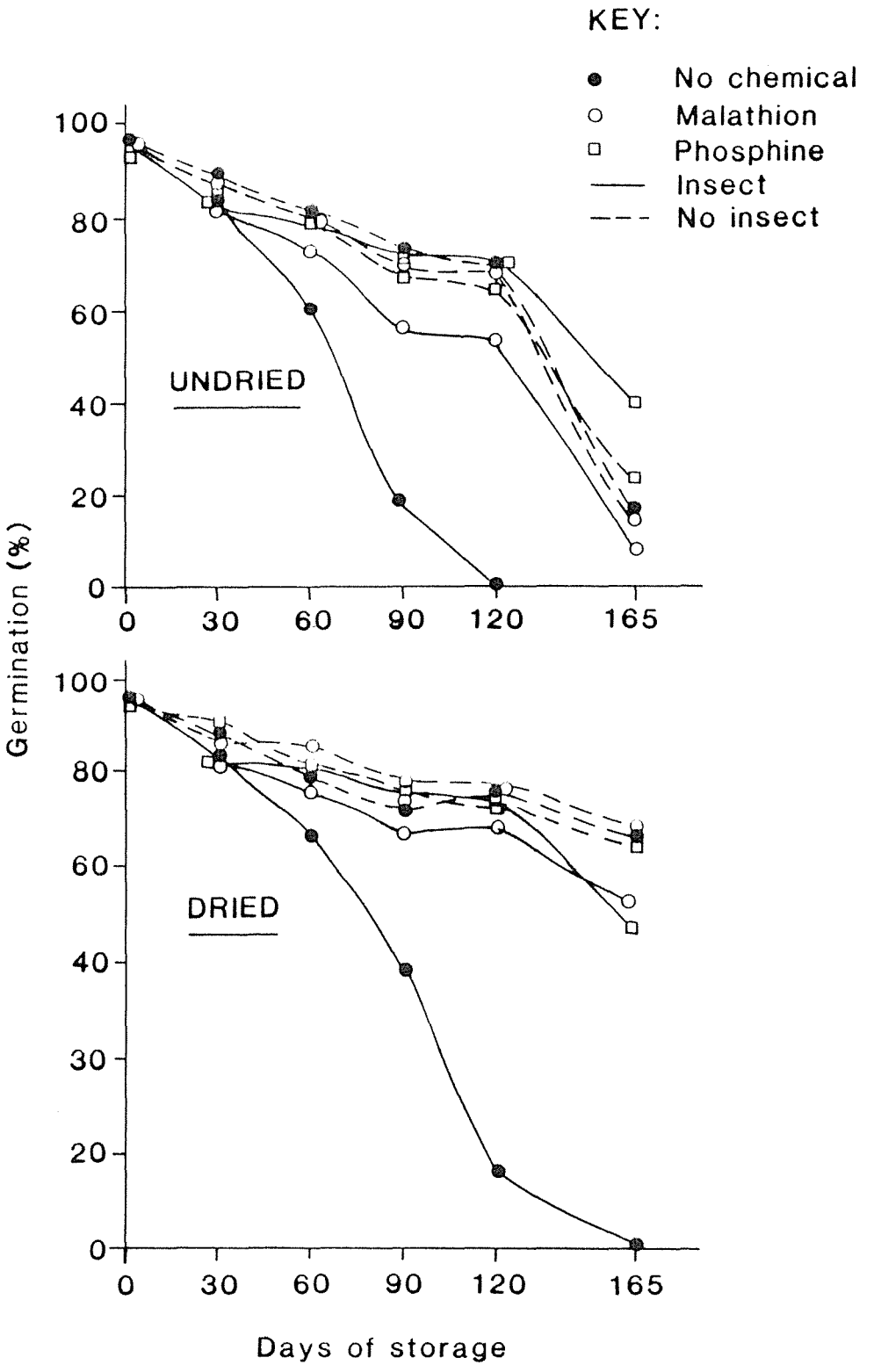
Table 3: Percentage of Normal Germination of seeds from different treatments up to 165 days of storage (mean).

Treatments			Days of storage					
			0	30	60	90	120	165
NO INSECT	No chemical	Undried	95.0	89.8	82.0AB	74.0A	70.8A	17.0BC
		Dried	95.0	88.3	80.0AB	72.7A	74.0A	67.0A
	Malathion	Undried	95.0	89.3	80.7AB	70.3AB	70.8A	16.7A
		Dried	95.0	88.3	85.0A	76.3AB	75.0A	67.3A
	Phosphine	Undried	95.0	87.8	80.8AB	68.0AB	65.0AB	23.0BC
		Dried	95.0	90.0	82.3AB	73.0A	72.8A	66.7A
INSECT	No Chemical	Undried	95.0	84.3	61.8C	19.0D	0.0D	0.0C
		Dried	95.0	83.8	69.0BC	38.0C	15.8C	0.0C
	Malathion	Undried	95.0	84.8	74.8B	56.7B	55.0B	9.0C
		Dried	95.0	83.0	76.0AB	67.3AB	68.0A	52.3A
	Phospine	Undried	95.0	83.0	78.3AB	73.0A	71.8A	40.0AB
		Dried	95.0	82.8	81.3AB	76.0A	74.8A	46.7AB
Significance				NS	**	**	**	**
L.S.D		1%			9.9	14.5	10.7	28.1
		5%			7.3	10.7	7.9	20.8

Values not followed by the same letter in vertical columns differ at P = 0.01.

** Significant at 1% level

FIGURE 4: CHANGES IN GERMINATION PERCENTAGE OF DIFFERENT TREATMENTS DURING STORAGE



2. Effect of Chemicals

The normal germination percentages of seeds from treatments free of insects but treated with either malathion or phosphine were similar to that of the control treatment during the entire storage period. This similarity occurred irrespective of whether seed had been dried or not dried during storage.

These results show that neither malathion nor phosphine had any adverse effect on seed germinability at the applied dosages and confirms similar findings by Parkin (1963) and Hall (1970).

3. Effect of Insects

Seed infested with insects but not treated chemically or subjected to drying maintained a similar germination percentage to the control treatment up to 30 days storage. However, the presence of insects subsequently resulted in a more rapid and pronounced drop in seed germination capacity from 60 days storage onwards. Seeds in this treatment lost any planting value within 90 days of storage (germination dropped to 19%) and all seeds were dead after 120 days storage. Total loss of viability at this time compared with 74% and 70% germination in seed from the control treatment.

These results reflect the fact that insects can be one of the most serious single causes of damage to seeds after harvest if infestation is not prevented (Henderson and Christensen, 1961). Upto 30 days storage there were insufficient numbers of insects (only 100 adult granary weevils) present to cause obvious damage to seeds and perhaps for that reason viability of seed of this treatment remained similar to that of control treatment. Subsequently, however, the insect population increased approximately 10-fold each month - an effect similar to that described previously by Eastham and Segrove (1947). Damage to the seed became obvious due to destruction of the germ and endosperm through ravenous feeding by immature larvae. This effect, has been previously described in detail by Cotton and Wilbur (1974) and Howe (1972). The physical damage to seeds caused by such characteristics as discolouration, mustiness, powdering and stickiness associated with insect activity is shown in Plate 10 compared with undamaged seeds from the control treatment (Plate 7). Plates 8 and 9 show the physical appearance of seeds infested with insects but which

had been treated with either malathion or phosphine. It is obvious that the physical appearance of fumigated seed is similar to that of seed from control treatment and damage to seeds treated with malathion is far less than that of insect infested and chemically untreated seeds.

4. Combined Effect of Insects and Chemicals

a) Malathion

While the germination of seeds containing insects but not treated with insecticide declined drastically and reached zero within 120 days of storage, the rate of decline in insect infested seedlots which had been dusted with malathion was significantly slower. After 120 days storage 55% of malathion treated seeds were viable compared with 70% in the control treatment. Those seeds which contained insects but which had not been dusted with malathion were all dead after 120 days storage. Even at the end of the storage period (165 days) some seeds (9%) which had been treated with malathion were still viable compared with a germination value of 17% in uninfested seed.

The results show that if insect infested seed is treated with malathion the insecticide helps to maintain viability for a comparatively longer period.

b) Phosphine

Wheat seed which had been infested with insects but which was subsequently fumigated with phosphine showed a better storage performance than malathion treated seed. This fumigation treatment was totally effective in controlling insects and allowed seed to retain germination levels similar to the control treatment for at least a 120 day storage period. The germination percentage of seed from the treatment containing insects but which had been fumigated with phosphine and not dried was significantly higher than the control treatment (40% vs 17%) after 165 days storage.

These findings show that if insect infested seeds are fumigated with phosphine, before significant injury has occurred they become totally insect free and then behave like seeds that have never been infested.

Plate 7: Physical appearance of uninfested untreated wheat seed after 120 days of storage.

Plate 8: Physical appearance of insect infested and malathion treated wheat seed after 120 days of storage.

Plate 9: Physical appearance of insect infested and phosphine fumigated seed after 120 days of storage.

Plate 10: Physical appearance of insect infested and untreated seeds after 120 days of storage.

The improved longevity of fumigated seeds compared with malathion treated seeds may be explained by the fact that phosphine kills not only adults mixed with seeds but also the immature stages of granary weevil present inside the seeds - an effect previously described by Hall (1970), Monro (1969) and Lindgren and Vincent (1966). The treatment of seed with phosphine had an abrupt and permanent effect in curtailing insect activity. Consequently, no further damage to the seed occurred after fumigation as a result of either adult insect feeding or larval development. Conversely malathion, because of its seed surface activity, did not kill the immature stages of granary weevil inside the seed. As a result, larvae continued to develop and were able to emerge as adults from seed throughout the storage period. Some of these adults were then presumably able to oviposit prior to their death following contact with malathion. This accounts for the continued damage to malathion treated seeds observed during the experiment.

One interesting aspect of these results was the significantly higher germination percentage of fumigated seeds in non-drying treatments than in the control treatment by the end of the storage period. A possible reason for this effect may be that phosphine has some fungicidal value which could destroy fungi present in seed mass. Therefore, while fungi (particularly Aspergillus and Penicillium spp.) might have caused some deterioration of seeds in the control treatment, fumigated seeds, on the other hand, were perhaps less liable to such deterioration and performed better in terms of seed storage longevity. Again in dried treatments, the germination percentage of phosphine treated seeds was quite different to that of the control treatment although slightly more rapid loss of viability occurred in lots containing insects despite phosphine treatment.

5. Combined Effects of Insects, Chemical Treatments and Drying

The germination percentage of seeds from treatments containing insects but which had been treated with either malathion or phosphine were similar, irrespective of whether seeds had been repeatedly dried or not throughout the first 120 days of the storage period. However, after 165 days storage the germination of malathion treated seeds from the dried treatments was significantly higher than from the undried

and therefore, higher seed moisture treatment. In seed lots fumigated with phosphine there was no insect activity in either the dried or undried treatments and therefore, the germination percentage of both dried and non dried but fumigated seeds were similar throughout the storage period. However, in the absence of insects, seed germination percentage of malathion treated seeds which were subjected to drying during storage was significantly higher than in undried lots after 165 days storage.

E. ABNORMAL SEEDLINGS

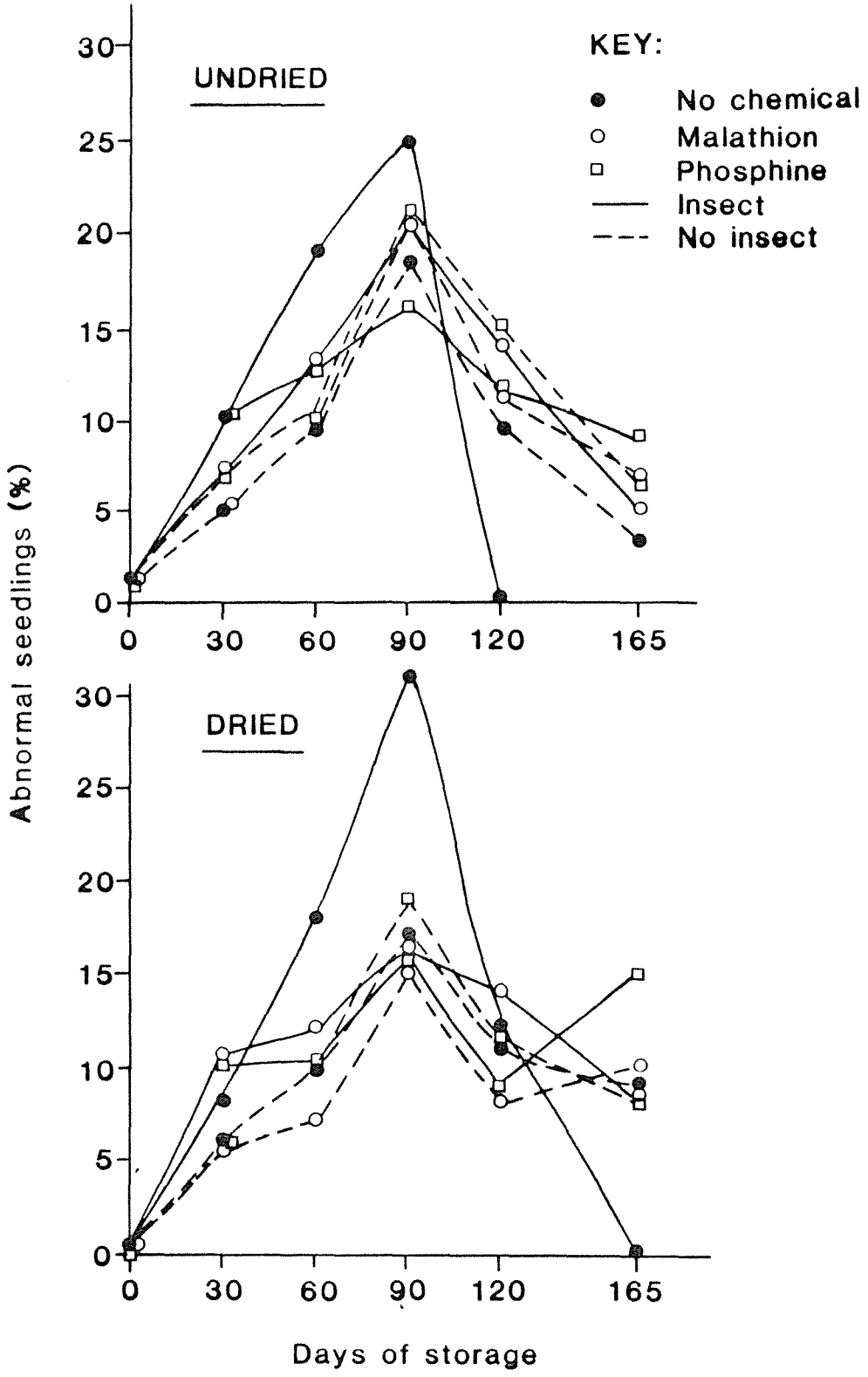
The abnormal seedlings produced from different seed treatments at different storage intervals are shown in Figure 5. (raw data in appendix 1 and 5).

It can be seen from the Figure that the occurrence of abnormal seedlings increased with the progress of storage up to 90 days before decreasing as a direct result of increasing numbers of dead seeds.

The storage temperature (25 C) and relative humidity (80%) used in the present study were sufficiently adverse to hasten deterioration in stored seed as also shown by Delouche et al. (1973), Harrington (1972) and Toole (1950). Thus, as the storage period continued, storage potential of seeds in all treatments fell as a consequence of seed deterioration. This was reflected in the pattern of change of abnormal seedlings produced, since loss of storage potential is one of the specific consequences of seed deterioration along with a decrease in germination rate, and an increase in the incidence of seedling abnormalities (Delouche and Baskin, 1973). Up to 90 days storage, seed deterioration rate increased in all treatments.

It is also evident from the Figure that the highest numbers of abnormal seedlings were produced (up to 90 days) in the treatment containing insects but which had not been treated with chemical. The reason is obvious as adult granary weevils and their immature larval stages caused major damage to seeds affecting both the endosperm and the embryo. Such activity resulted in more seedling abnormality in infested treatments. A subsequent sharp decline in abnormal seedlings with increased storage period reflected the rapid increase in dead seed numbers.

FIGURE 5: CHANGES IN ABNORMAL SEEDLING PERCENTAGE IN DIFFERENT TREATMENTS DURING STORAGE



The changes in numbers of abnormal seedling numbers produced in treatments without insects but dusted with malathion or fumigated with phosphine were similar to those of the uninfested and chemically untreated control treatments throughout the storage period. This clearly shows that neither malathion nor phosphine had any adverse effect on the level of seedling abnormalities at the dose rate applied. A similar finding has been recorded by Parkin (1963) and Hall (1970).

Towards the end of the storage period the percentage of abnormal seedlings was higher in all dried seed treatments than in respective undried treatments. This was presumably a reflection of the good effect of drying on seed storability. While in undried treatments seeds were progressively dying as a reaction to the unfavourably high humidity and high temperature conditions, in dried treatments seeds were still able to survive but continued to produce abnormal seedlings.

F. TYPES OF ABNORMAL SEEDLINGS

The main types of abnormal seedlings detected in germination tests are presented in Plates 11 and 12 and the percentages of each category of abnormals produced from individual treatments at different intervals of storage are shown in Appendix 2.

Three main types of seedling abnormalities i.e. damaged seedlings, deformed or unbalanced seedlings and decayed seedlings were found to occur apparently randomly in all seed treatments after 30 days of storage but numbers of abnormals were higher in all insect infested treatments. From 60 days onwards, abnormal seedling types v g (completely decayed seedlings) and vi a (seedlings showing short and weak growth) were produced in larger numbers than other types of abnormalities. In treatments infested with insects but which had not been treated with any chemicals and in insect infested lots which had been dusted with malathion, seedlings showing abnormalities in the coleoptile region (categories IIIa, IIIc, IIId and IIIf) were observed to occur in higher numbers than in other treatments. The categories of abnormal seedlings detected in insect infested treatments fumigated with phosphine were found to be similar to those in insect-free treatments.

The results show that up to 30 days storage seeds from all treatments retained similar levels of viability but a decline in seedling vigour had already started in all insect infested treatments as shown by increased levels of abnormal seedlings. This supports previous work which has shown that reduction in the vigour of a seed lot can occur before there is a significant decline in germination (Roberts, 1974). However, although insects might have caused some decline in seed vigour after only 30 days storage, such deterioration was not sufficiently severe to be reflected in loss of germination.

The occurrence of vi a and v g categories of abnormals in high numbers in all the treatments after 60 days storage indicated that seed in all treatments was already ageing (ISTA, 1976).

The occurrence of higher numbers of seedlings showing abnormality in the coleoptile region in insect infested seeds was presumably an indication that the larvae of granary weevil preferentially damage not only the endosperm but also the plumule of the embryonic axis. The results also indicate that neither malathion nor phosphine have any special effect on seed quality through the production of specific categories of seedling abnormalities.

G. ASSESSMENT OF INTERNAL INSECT DAMAGE TO SEED

The use of X-ray radiography to assess internal seed structures, the presence of insects in seed and the extent of structural damage caused by insect larvae has obviously advantages in the evaluation of insect population and seed quality. In the present study attempts were made to develop X-ray radiography methods which could be used to examine these factors. Photographs of wheat seed samples taken after 60 days storage clearly show the presence of weevil larvae within numbers of individual seeds. Such seeds with a larva are marked in plates 13 to 20. Even though these larvae are well formed there is little or no external evidence of seed injury at this stage. Insect infested seed damage usually becomes obvious only after emergence of the adult (Plate 10). The X-ray photographs further show that almost invariably only one larva occurred per seed. This agrees with similar findings by Sharifi and Mills (1971).

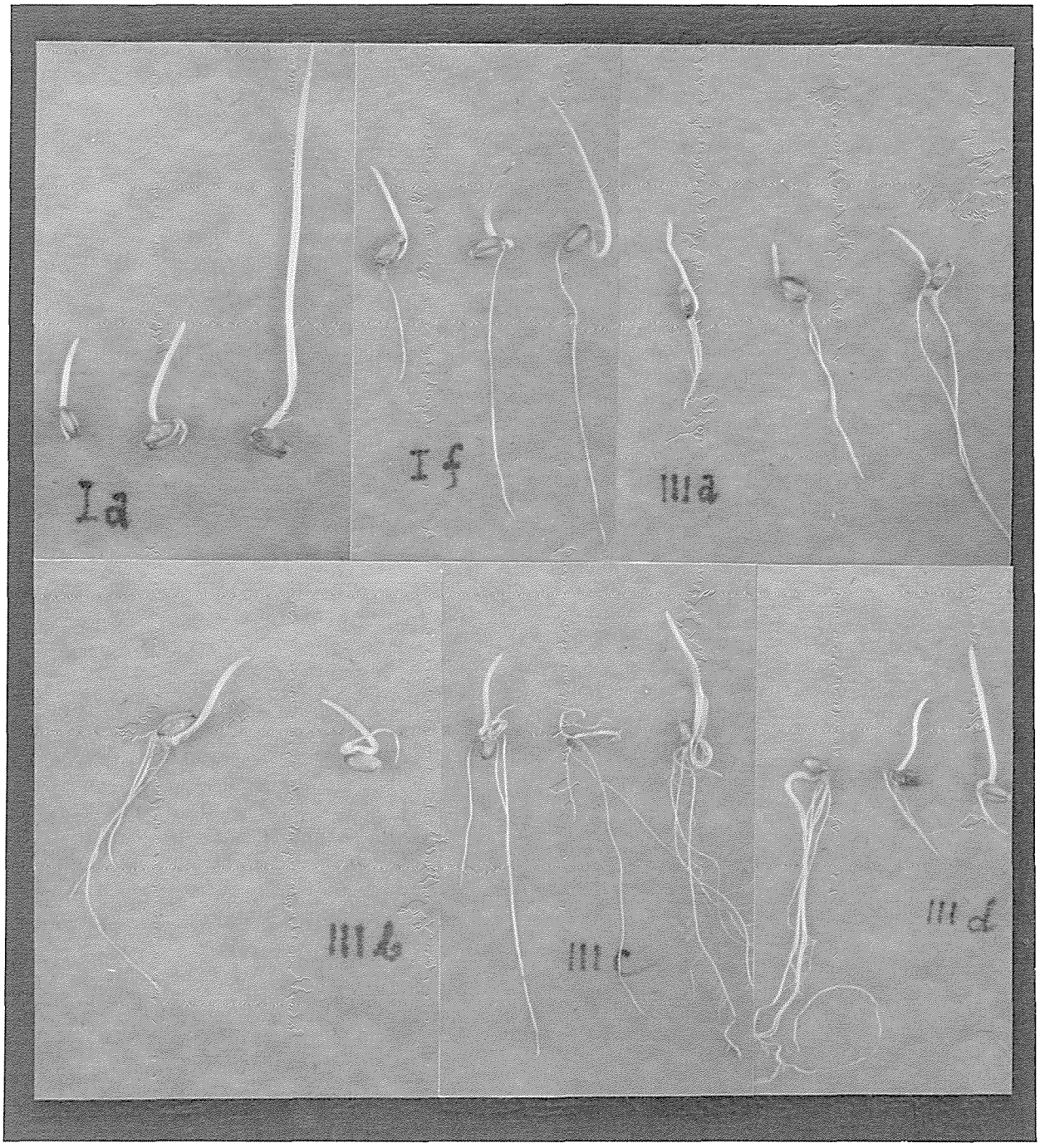


Plate 11: Abnormal types: Ia - No roots; If - Not more than one seminal root; III a - No primary leaves; III b - Short primary leaves extending less than half length of coleoptile; III c - Primary leaves shattered or split and/or split or abnormal coleoptile and III d - Coleoptile and primary leaves spindly, or pale, or watery.

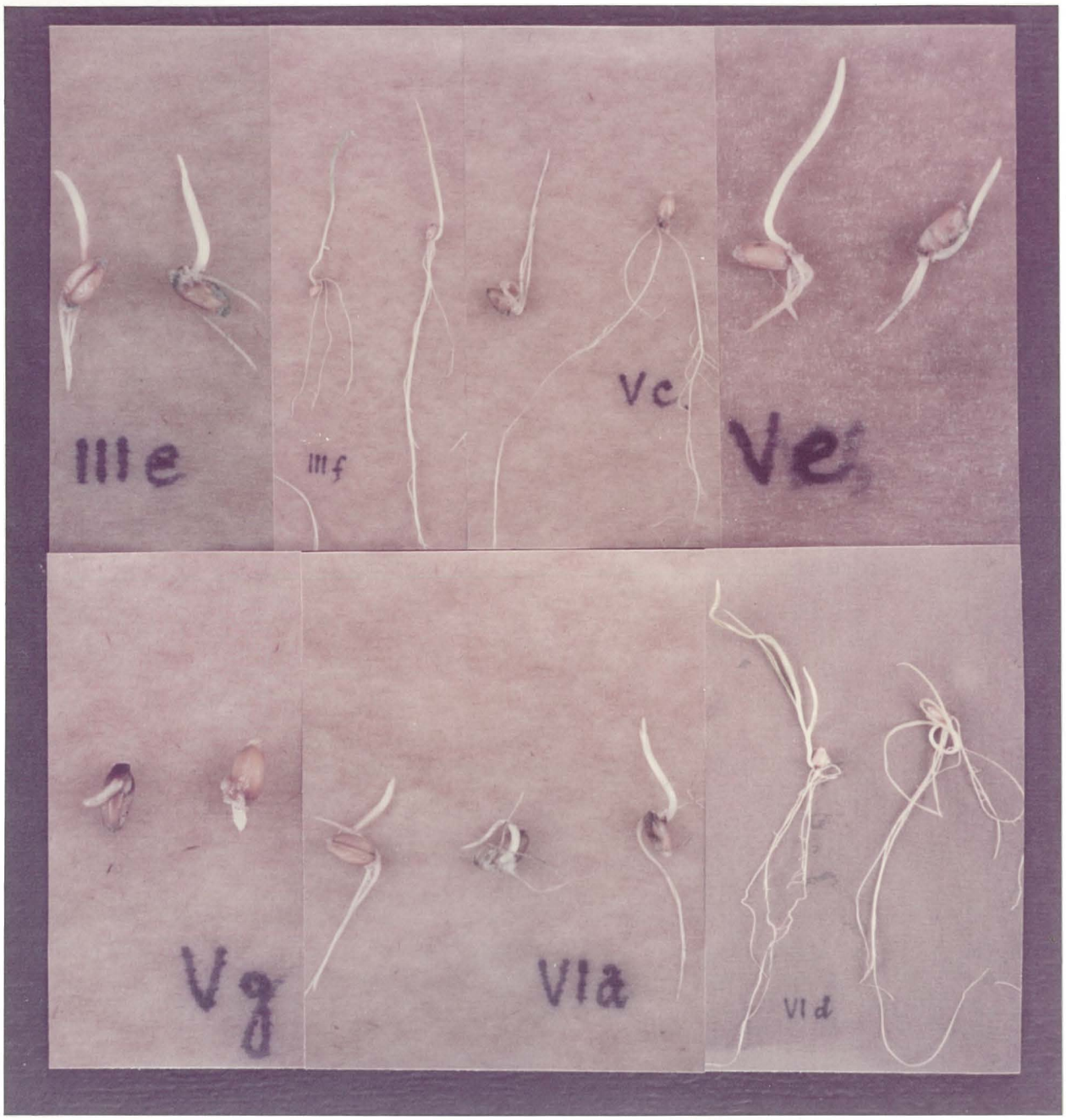


Plate 12: Abnormal types: III e - Coleoptile and primary leaves short and thick, usually with short or stunted seminal roots; III f - Coleoptile disproportionately short or missing; Vc - Decayed epicotyl or stem; Ve - Decayed seminal roots; Vg - Completely decayed seedling; VIa - Seedling short and weak, or spindly, or with unbalanced development of the main structures.

Identification key for positional germination results:

 Normal

 Abnormal

 Dead

 Seed contains larva

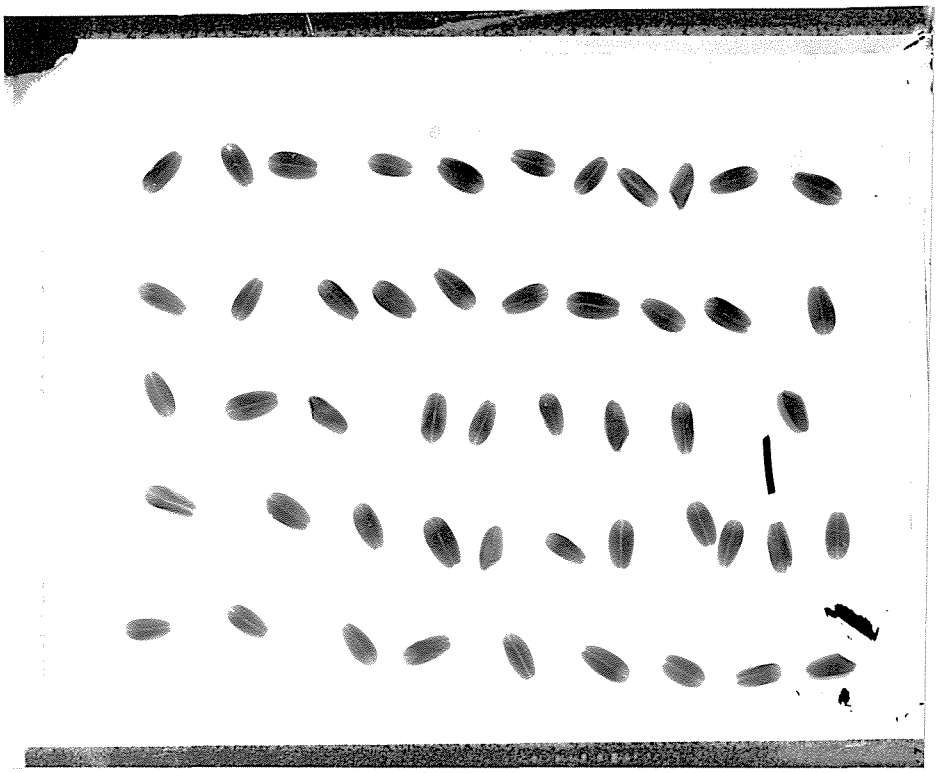


Plate 13: X-ray photographs of seeds not infested with insect, chemically untreated and not dried (control treatment) after 60 days of storage.

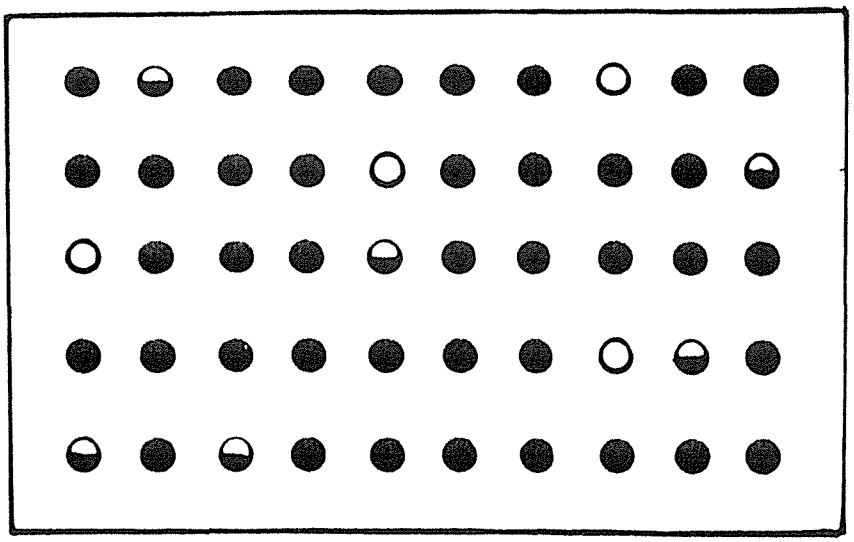


Figure 6: Positional germination results of seeds tested in same sequence as shown in Plate 13.

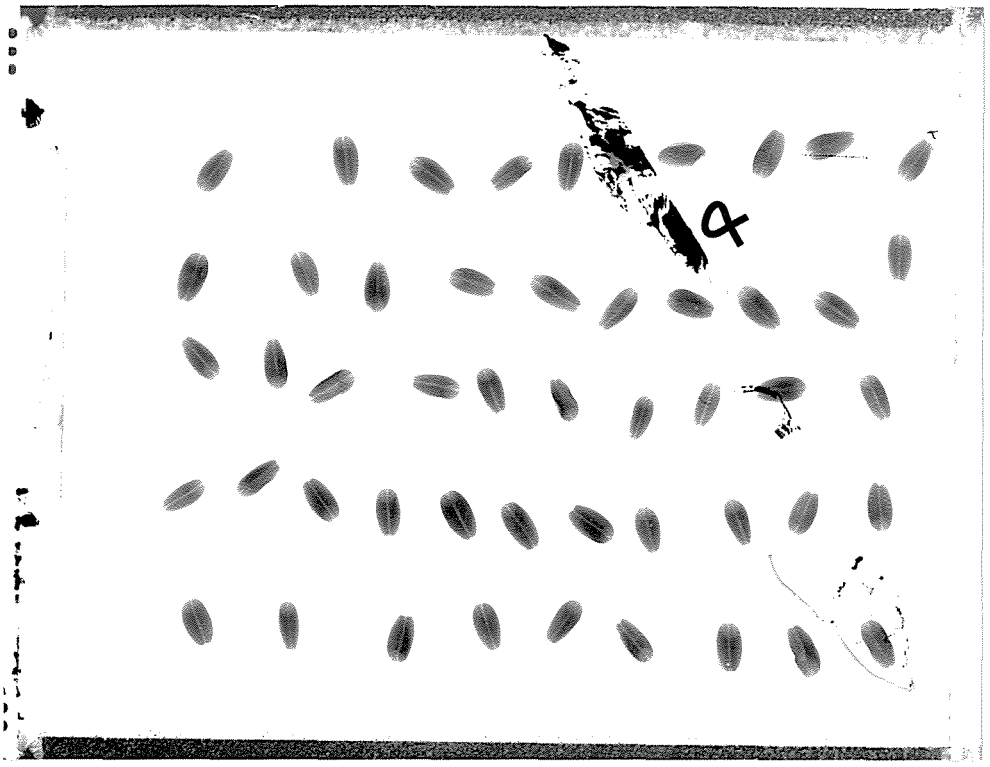


Plate 14: X-ray photographs of seeds of control treatment with repeated drying after 60 days of storage.

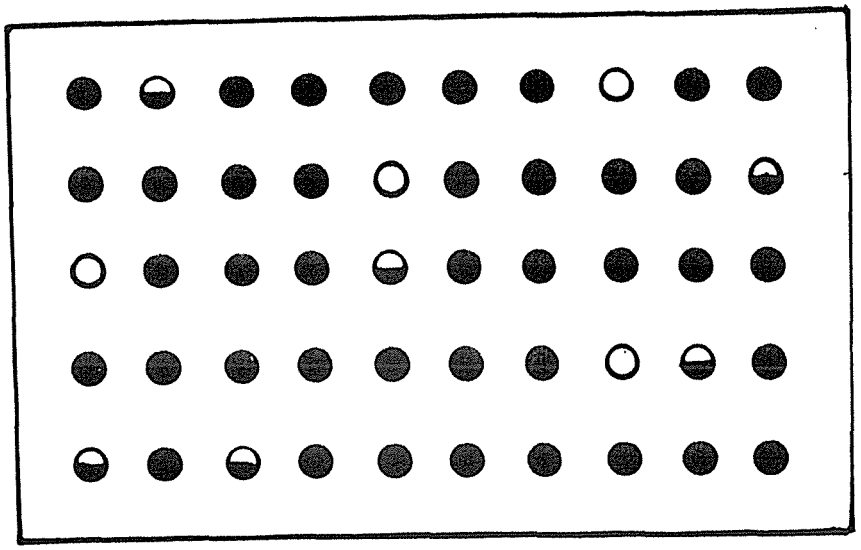


Figure 7: Positional germination results of seeds tested in the same sequence as shown in Plate 14.

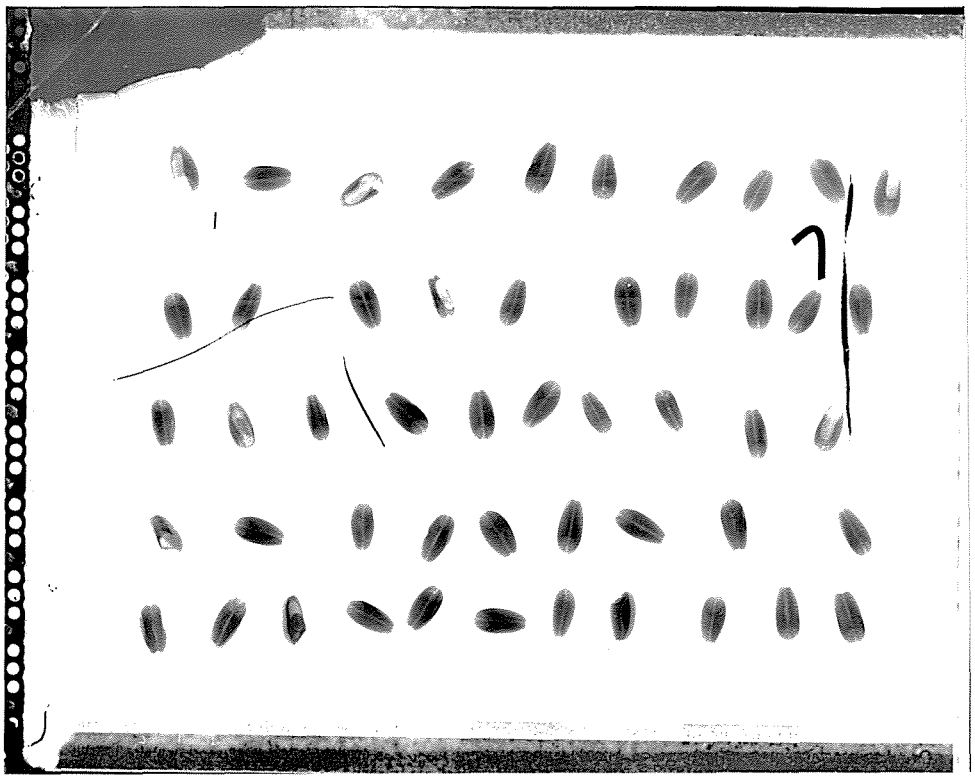


Plate 15: X-ray photographs of seeds, insect infested but not chemically treated and not dried after 60 days of storage.

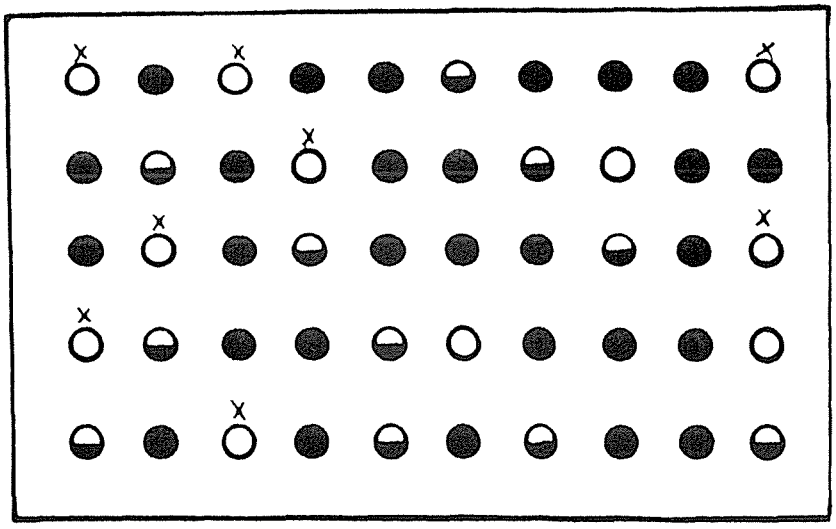


Figure 8: Positional germination results of seeds tested in the same sequence as shown in Plate 15.

Plate 16: X-ray photographs of seeds insect infested, chemically untreated and dried after 60 days of storage.

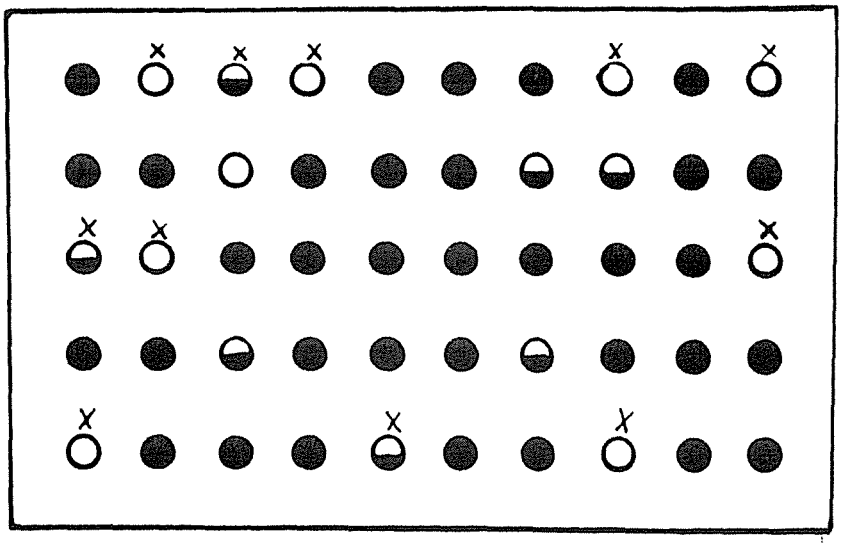


Figure 9: Positional germination results of seeds tested in the same sequence as shown in Plate 16.

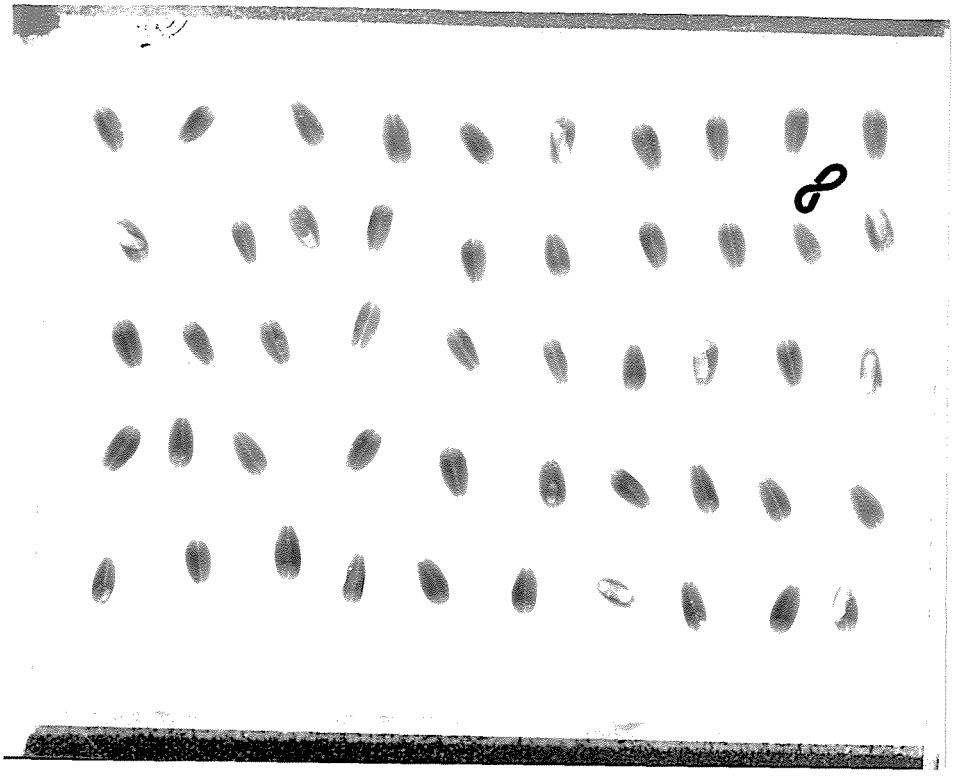


Plate 17: X-ray photographs of seeds, insect infested but treated with malathion and not dried after 60 days of storage.

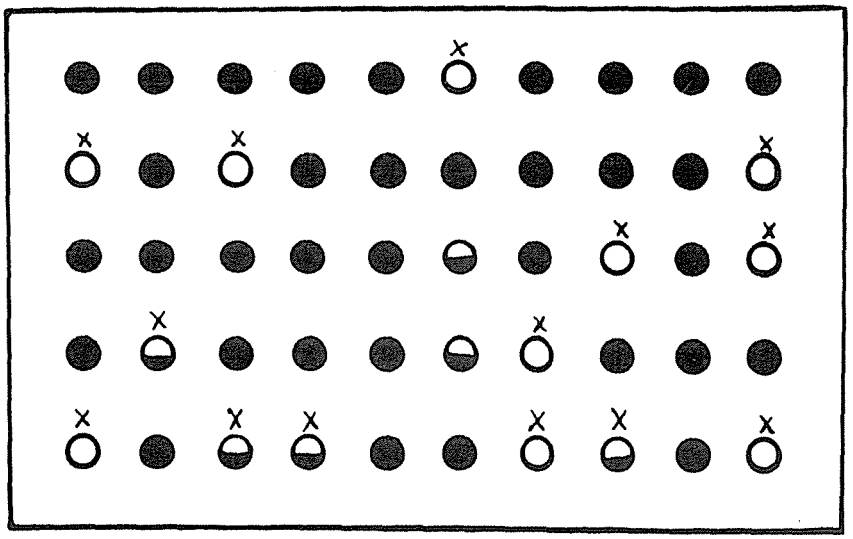


Figure 10: Positional germination results of seeds tested in the same sequence as shown in Plate 17.

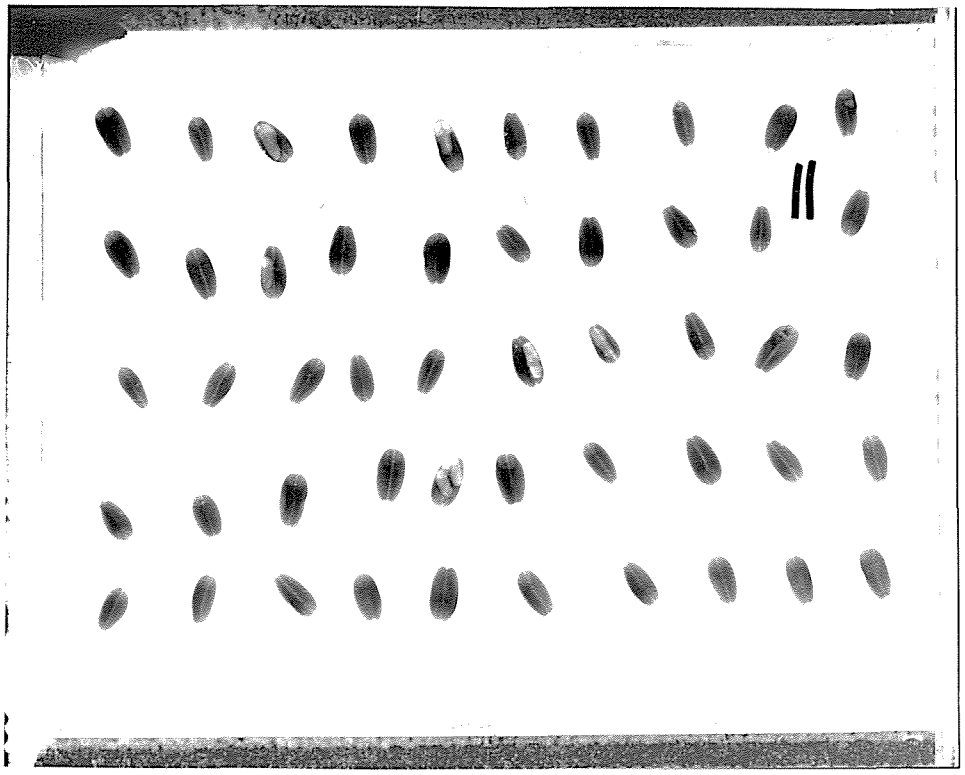


Plate 18: X-ray photographs of seeds, insect infested but treated with malathion and dried after 60 days of storage.

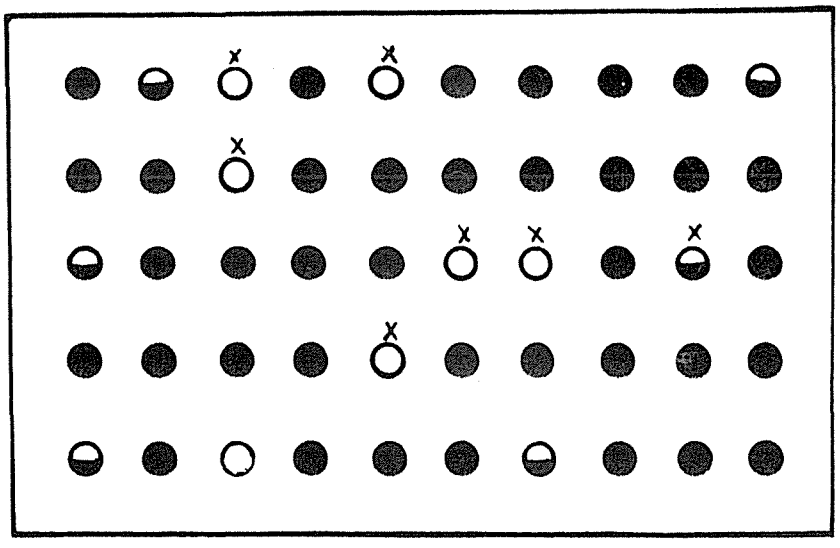


Figure 11: Positional germination results of seeds tested in the same sequence as shown in Plate 18.

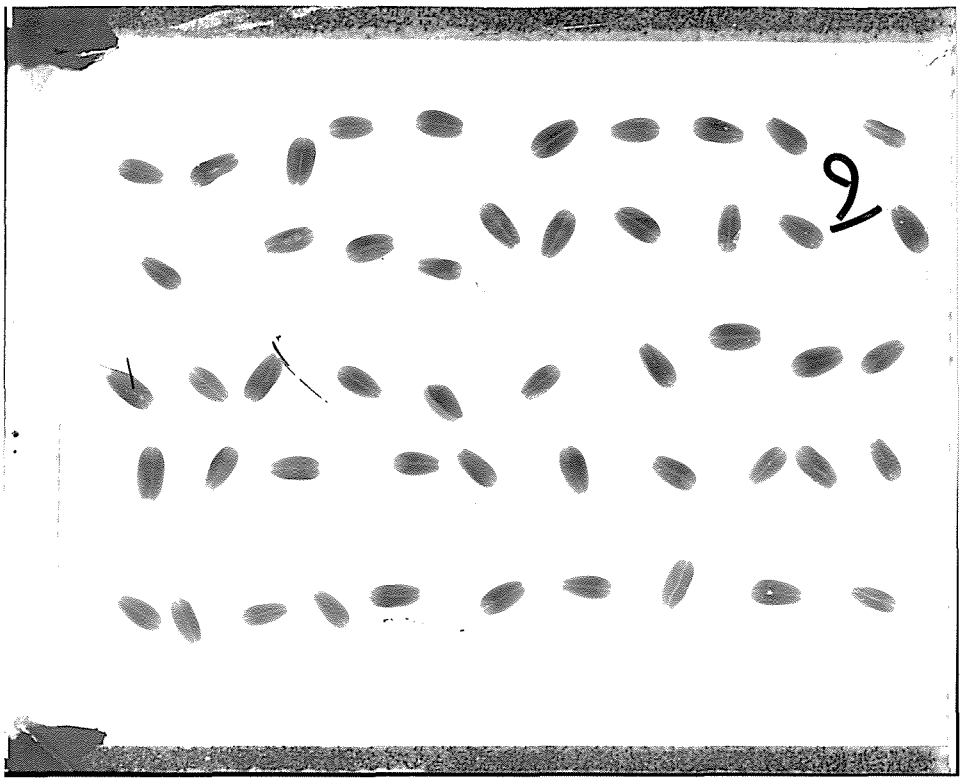


Plate 19: X-ray photographs of seeds, insect infested, fumigated with phosphine and not dried after 60 days of storage.

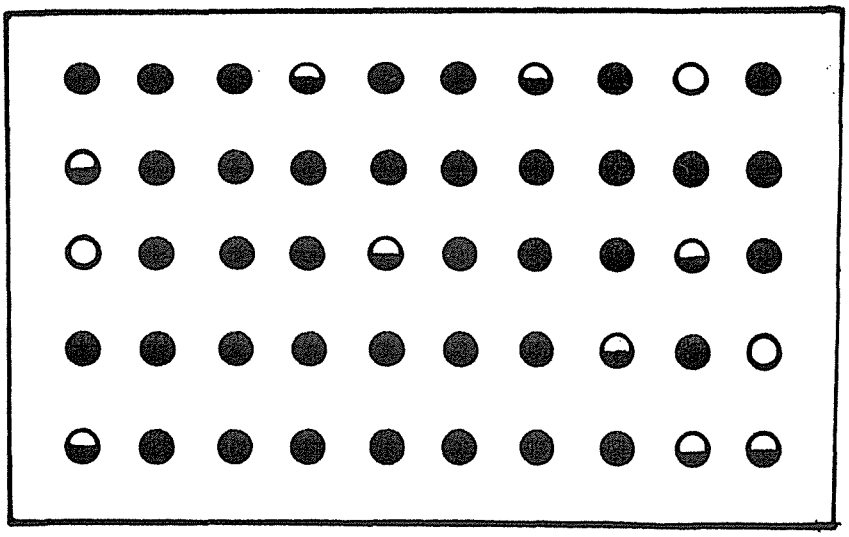


Figure 12: Positional germination results of seeds tested in the same sequence as shown in Plate 19.

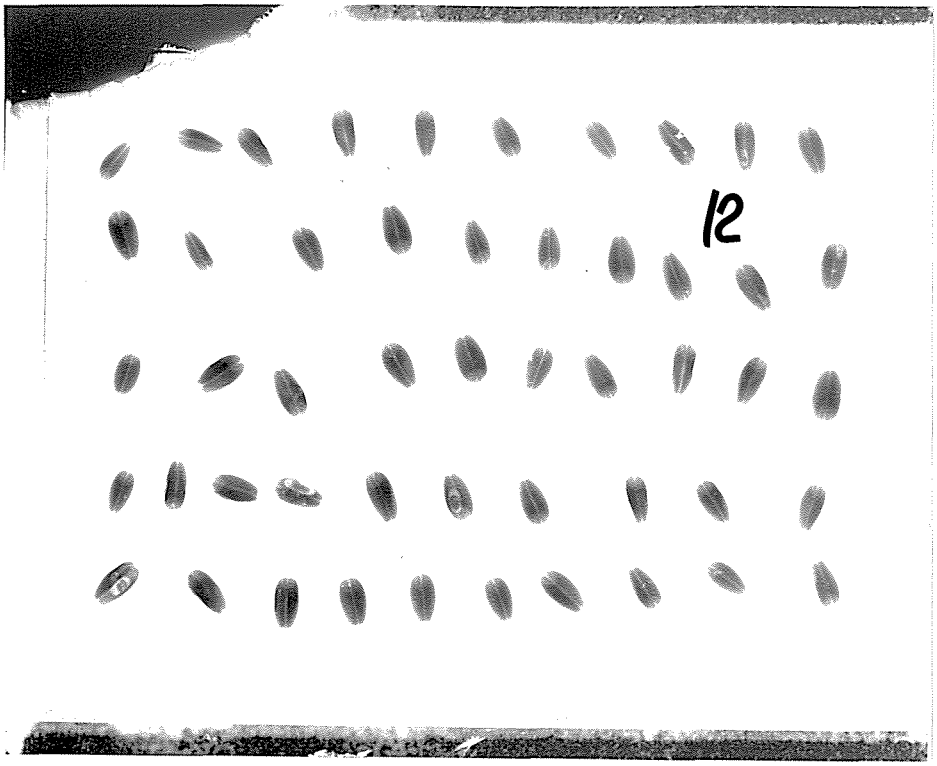


Plate 20: X-ray photographs of seeds, insect infested, fumigated with phosphine and dried after 60 days of storage.

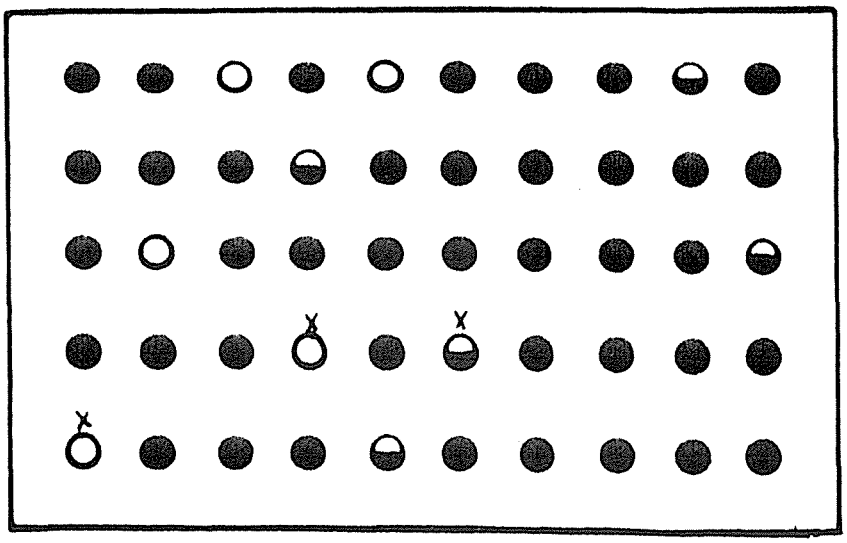


Figure 13: Positional germination results of seeds tested in the same sequence as shown in Plate 20.

Presence of a larva inside a seed is itself evidence of internal injury to the seed. However, in some seeds white areas around the larvae is visible in photographs. These are obviously empty space in the seed indicating tissue eaten by the larvae. The presence of larvae or injury in seed were found to occur predominantly in the region of the seed embryo.

It is evident from the photographs that as expected there was no internal injury to seeds in insect-free treatments. In all other cases involving the presence of insects, and even when seed was subsequently treated with insecticide or fumigant, presence of larvae was detected following X-ray radiography. Seeds with larvae or insect injuries obviously occurred more in treatments containing insects which had not been treated with any chemical. It can also be seen that the larvae in infested treatments are larger and that the damaged area in seed is more extensive than in other treatments. There were also considerable numbers of seeds containing larvae in the treatment containing insects but subsequently dusted with malathion, the number of larvae, and their size and the extent of internal damage being similar to those in infested but not chemically treated seed lots. In seed containing insects which had been subsequently fumigated with phosphine variable results were obtained. No seeds containing larvae or showing insect damage occurred in undried seed lots. However, larvae were visible in three seeds in the dried and phosphine treatment. The size of these larvae was much smaller than larvae in other treatments.

The use of X-ray radiography has clearly shown that insects cause extensive internal damage to seeds although their presence may not be visible externally to the naked eye. If infested seeds are treated with a contact insecticide such as malathion the extent of damage is reduced since the insecticide kills adult insects in the seed mass but those larvae inside the seeds are unaffected and continue to develop until they emerge as adults. This explains why the size of larvae in malathion treated seed was similar to those in untreated seeds. The photographs further reveal that when infested seeds are fumigated with phosphine further damage to seed ceased completely. Insects might already have laid some eggs and the eggs may have developed to the larval stage before fumigation but when fumigation was carried out

immature stages including eggs and larvae inside the seed along with adults in the seed masss were killed. There is therefore, no sign of visible insect damage following X-ray radiography in insect infested seeds fumigated with phosphine. The small size larvae detected by X-ray photographs in three seeds from fumigated and dried treatment suggests that they were not fully developed and presumed to be dead.

Results of X-ray photographs become more valuable in conjuction with positional germination tests. The figures beneath each plate show the results of such tests with seeds being placed on the germination substrata in the same sequence as they had been X-ray photographed.

It is evident from these results that most internally damaged seeds containing larvae were either dead or produced abnormal seedlings. However, in insect-free treatments, some abnormal seedlings and dead seeds were also recorded. These seedling abnormalities and dead seeds presumably occurred as a result of natural deterioration processes in seeds caused by the adverse storage conditions (25 C, 80% RH) used in this experiment. Such seedling abnormalities and dead seeds also occurred in insect infested treatments in the seeds exhibiting no internal injury and the absence of larvae following X-ray radiography. It is further evident from the categorisation of abnormal seedling types (Table 4) that seedling abnormalities occurred more extensively as a result of coleoptile damage in insect damaged seeds than in uninfested seeds.

In X-ray photographs the presence of larvae or insect injury was more concentrated in the embryo region suggesting that the softer embryo tissue was more preferred by both larvae and adults. The finding that the plumule of the embryonic axis was more readily affected and resulted in the production of larger numbers of abnormal coleoptile damage support this finding and agrees with similar conclusions by Golebiwska et al. (1977).

Table 4: Categories* of abnormal seedlings of wheat seed detected in positional germination test after X-ray photography.

Treatments			Numbers of abnormal types out of 50 seed										Total
			Root		Coleoptile						Decay		Oth.	
			Ia	If	IIIa	IIIb	IIIc	IIId	IIIe	IIIf	Vc	Vg	VIa	
No Insect	No Chemical	Undried	1				1					1	3	6
		dried		1				1					2	4
Insect	No Chemical	Undried		1	2	1	2			2		1	1	10
		dried		1	2	1	2					1		7
	Malathion	Undried	1		1		1		1		1	1		6
		dried		1	1		2					1	1	6
	Phosphine	Undried		1	1	1	2		1		1	1	1	9
		dried	1	1				1					2	5

* Categories of abnormal seedlings are shown in Plate 11 and 12.

CHAPTER 3

EFFECTIVENESS OF MALATHION APPLIED AS SPRAY TO THE OUTSIDE OF STORAGE SACKS AS A PREVENTATIVE MEASURE AGAINST INFESTATION

INTRODUCTION

Once a widely used insecticide, malathion use is now decreasing due perhaps to its rather short persistence and to the development of resistance in some insect species. However, in many developing countries, such as Bangladesh, malathion is still widely used. In the preceding Chapter it was shown that malathion could provide effective control of an established insect population in a seed mass particularly when combined with regular drying. The present experiment aimed to determine whether malathion could be used to protect stored seed from insect invasion by applying it to the outside of storage sacks. A further aim was to assess how long such malathion treatment remained effective and thus to determine the time necessary for retreatment.

MATERIALS AND METHODS

MATERIALS

Seed

Seed of wheat (Triticum aestivum L.) cultivar 'Rongotea' of 1984 harvest (as used in preceding study) stored at 5 C was used in the present study. The moisture content of seed when used was 15%.

Insects

Granary weevils (Sitophilus granarius L.) as previously, were derived from a culture maintained by the Department of Horticulture and Plant Health, Massey University.

Insecticide - Malathion

Maldison 50, as marketed by the New Zealand Farmers' Fertilizer Co. Ltd, Auckland was procured from a retail trade outlet. Active ingredient was 500 g/litre (50%) malathion and formulation an emulsifiable concentrate. The recommended dose for residual treatment of malathion in seed stores is 2.5%, i.e., 50 ml Malathion 50/litre. In the present study, treatment was made of whole sacks at 2.5% and in the case of jute squares at 2.5% and at half and quarter of this concentration.

Experimental Containers and Equipment

Jute sacks of 1 kg capacity, jute squares of the same material as the sacks cut to approximately 30 x 30 cm, large polythene bags, rubber bands and vacuum counter as used in preceding study. A pneumatic hand sprayer was used to apply the insecticide to the sacks and jute squares.

METHODS

Experimental Design

The experiment was designed in two parts:

(1) Malathion was sprayed onto jute squares at the full (2.5%), half, and one quarter of the recommended rate. The toxicity of the resulting deposit was then assayed at intervals after treatment by confining adult weevils to the treated surface under a petridish for a pre-determined period (Plate 21).

Treated jute squares were stored at 20 C and 30 C before being tested. There were three replications in a fully randomised design.

(2) Malathion was applied at the full recommended rate of 2.5% to the outside of grain filled sacks which were then placed individually into large plastic bags into which adult grain weevils were introduced to determine their ability to enter the sacks and infest the seed. 50 adults were used for each sack.

Treated sacks were stored at 20 and 30 C as for part (1) and there were three replications in a fully randomised design.

The Behaviour of Adult Granary Weevils in the Presence of Grain Filled Sacks

Prior to setting up part (1) the behaviour of granary weevil adults was observed to determine how long they took to enter into a grain filled jute sack and how long they might have contact with the insecticide treated surface. For this purpose, a jute sack was filled with 1 kg of wheat seed and placed in a deep sided plastic box. 20 adult insects were then put on the surface of the sack and the

container was covered with a glass sheet. Insects still outside the sack were counted every 15 minutes for the first hour, then every 30 minutes for another 2 hours and then after each one hour. Within 30 minutes 4 insects had entered the sack but it took 24 hours for all 20 insects to do so. The trial was conducted at room temperature which was about 18 C during the day. During night time the container was kept in 20 C room. From these observations it was decided to confine insects on treated jute squares for 2 hours.

Insecticide Treatment

Malathion 50 and water were mixed in the proportions of 50 g/litre, 25 g/litre, 12.5 g/litre and 0 g/litre to produce the desired concentrations of 2.5%, 1.25%, 0.625% and 0.0%. Each of the 36 jute squares was sprayed both sides. Similarly the 36 jute bags were sprayed on the outside with 2.5% or 0% malathion. After drying, each treated bag was filled with 1 kg of untreated wheat seed, the open side closed with stapling and placed in a polythene bag (Plate 22).

Setting Up and Storage Environment

The experiment was commenced on 2.8.85. After treatment and on the same date, half of the jute squares and half of the jute sacks were placed in 30 C room at ambient relative humidity of 60 - 80%. The remainder were stored in 20 C room at ambient RH of 70 - 90%.

Assessment

Assessments were made after 0, 7, 14, 28, 56 and 90 days of storage. At each assessment for part (1), 20 adult granary weevils were confined beneath a petridish for 2 hours on each of 3 jute squares (3 replicates) randomly selected from each group sprayed at the 4 different rates of malathion and stored at 30 or 20 C. After 2 hours the insects were removed from the jute squares and kept in separate clean petridishes for a further 24 hours. Insects were then counted as alive or affected.

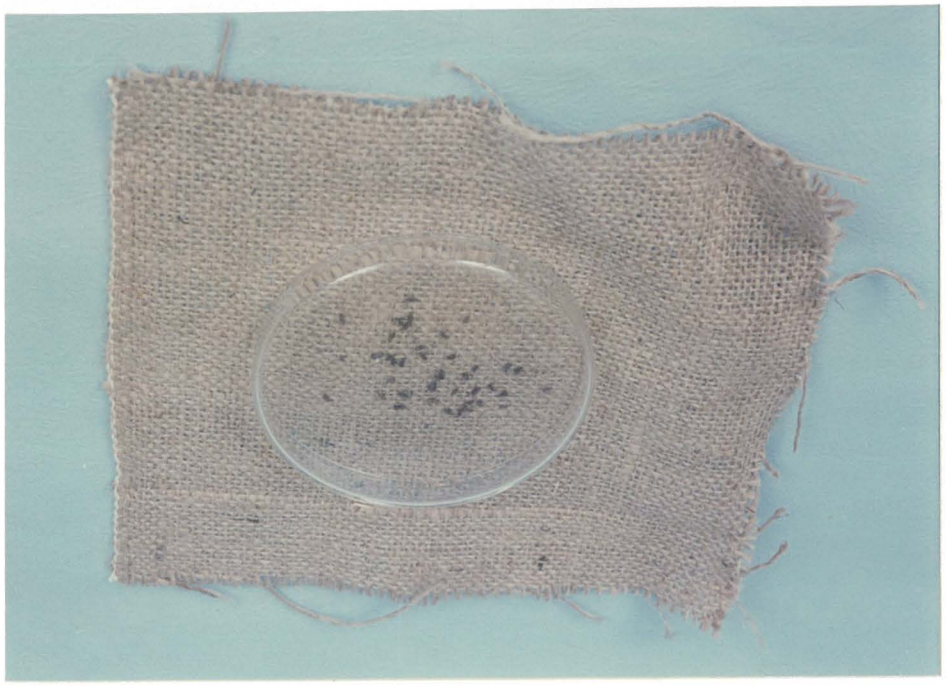


Plate 21: Granary weevil adults confined beneath petridish on jute square.

Plate 22: Jute sack with wheat seed inside polythene bag.

For part (2), on the same dates as above, 50 insects were introduced into each of 3 polythene bags (3 replicates) containing 2.5% and 0% malathion sprayed jute sacks and stored at 30 or 20 C. The polythene bags were then closed by rubber bands and stored for 56 days. During storage each polythene bag was opened once a week for a few minutes to ensure adequate aeration. After 56 days, the jute sacks were taken out from polythene bags, insects sieved out from the seed and counted as live or dead. As it was observed that no new adults emerged out from seed stored at 20 C within 56 days, all samples in 20 C room were shifted to 30 C after 56 days and then stored for another 28 days before a further count was made.

RESULTS AND DISCUSSION

A. Effectiveness of Malathion Sprayed onto Jute Squares

Numbers of granary weevil adults affected out of 20 on jute squares stored at 20 and 30 C sprayed with 4 different rates of malathion at intervals up to 90 days storage are presented in Table 5.

The results show that malathion at all three concentrations was completely effective immediately after application and also after 7 days. However, thereafter it lost its effectiveness slowly though was still about 50% effective even after 90 days. Differences between concentrations and storage temperatures were surprisingly small. Low and unexplained mortality occurred in the untreated controls from 14 days onwards.

These results seem to contradict the findings of many investigators (Parkin, 1963; Hall, 1970; Fenemore, 1984; and many others) that malathion is a short lived insecticide. It is possible that the persistence of effect in the present experiment is more apparent than real if the mortality in the untreated control from 14 days was due to decreased vigour of the test insects. However, other possible explanations are the nature of the jute sacking substrate, the rather long period of confinement used of two hours and the fact that humidity in the storage rooms dropped to 50+ RH (in 20 C) or even 50% RH (in 30 C) from time to time in the bioasssay. The significance of the results in relation to the effectiveness of whole sack treatments is discussed under B.2 below.

Table 5: Numbers of granary weevil adults affected out of 20 on jute squares at intervals after treatment

Treatments		Days after treatment					
% of concentration of malathion	Temperature	0	7	14	28	56	90
2.5	30 C	20	20	19	16	14	12
	20 C	20	20	18	14	12	10
1.25	30 C	20	20	18	16	15	13
	20 C	20	20	17	14	13	11
0.625	30 C	20	20	17	15	15	14
	20 C	20	20	17	13	10	10
0	30 C	0	0	2	4	3	4
	20 C	0	0	1	2	1	2

B. Effectiveness of Malathion Sprayed onto Jute Sacks

In the second part of the experiment the conditions of exposure of the test insects to the malathion treated surface were quite different. Polythene bags were used to confine the adult weevils to the vicinity of each wheat filled jute sack. The insects were thus free to attempt to penetrate the sack and thus come in contact with the malathion or to remain away. Therefore, the results obtained should give a good indication of the effectiveness of malathion in protecting seeds under practical storage conditions.

The results in terms of numbers of dead and live adult weevils found inside sacks at intervals after treatment are shown in Table 6 (raw data in appendix 6). The figures in the table denote the survivors of the initial number of insects introduced (50) plus any progeny produced during the 56 days post-infestation period.

1. Untreated Sacks

In untreated jute sacks approximately 660 adult weevils were present after 56 days at 30 C, an increase by a factor of about x13. At 20 C, however, when the first assessment was made after 56 days less than the number of adults initially introduced were recorded. This was clearly an effect of the lower temperature and therefore for the next two assessments the sacks were moved from 20 to 30 C after an initial 56 day period and held a further 28 days at 30 C before assessments were made. They then produced approximately 600 adult weevils, comparable to full storage at 30 C. Malathion treated sacks stored at 20 C were similarly transferred after 56 days.

2. Treated Sacks

In malathion treated sacks both at 30 C and 20 C no granary adults were detected following introduction of insects into the outer polythene bags just after spraying at 2.5% concentration. Most of the 50 adults introduced were found dead outside the sacks and a few inside.

Table 6: Numbers of live and (dead) granary weevil adults in untreated seed stored in malathion treated sacks, 56 days after infestation (mean).

Treatments			Days after treating sacks					
Temperature	Treatment		0	7		14		
30 C	No malathion		667(15) A	660(5) A		657(6) A		
	Malathion		0(50) B	267(21) C		409(26) C		
20 C	No malathion		41(5) B	42(4)	597(7)* B	41(5)	569(24)* B	
	Malathion		0(50) B	12(28)	199(20)* C	28(13)	383(22)* C	
Significance			**	**		**		
L.S.D.		1%	90.2	106.7		105.0		
		5%	62.0	73.3		72.2		

* after an additional 28 days storage in 30 C shifted from 20 C after 56 days of storage.

Values not followed by the same letter in vertical columns differ at P = 0.01.

** Significant at 1% level.

In samples where insects were introduced 7 days after spraying the sacks, 267 adults at 30 C and 12 adults at 20 C were recorded. However, 199 adults were produced in samples from 20 C when they were stored at 30 C for another 28 days. Although these numbers were significantly lower than in untreated sacks in both cases it was clear that malathion sprayed onto the sacks had lost most of its effectiveness after 7 days and some insects were able to penetrate the sacks and infest the seed. After 14 days the insect population from treated sacks was still significantly lower than untreated sacks. However, in practical terms effective control was no longer being obtained and so the experiment was discontinued.

The results of this experiment indicate that untreated wheat seed stored in sealed jute sacks can be protected from infestation by granary weevils for only a few days by application of malathion to the outside of the sacks at the comparatively high concentration of 2.5%. To keep seed free from infestation by such a treatment would require repeated spraying at intervals of not less than 7 days.

The results of the bioassay of jute squares sprayed with malathion suggested that protection should be obtained for at least a week or longer. When compared with the results of whole sack treatment however, it is apparent that the bioassay method exaggerated the insecticidal effect of the deposit, presumably because the contact time of 2 hours (and a further 24 hours before assessments were made) was too long. Evidently some insects were able to penetrate the treated sacks more rapidly than that and avoid a lethal insecticide dose so that they were able to lay at least some eggs. It would be of interest to evaluate shorter exposure times in the bioassay to see whether better correlation with the results of sack treatment could be obtained.

One may also speculate that an insecticide with known rapid contact action, such as a synthetic pyrethroid, may be more effective than malathion for external treatment of sacks for protection of enclosed grain.

CHAPTER 4

EVALUATION OF GROUND NEEM SEED AS AN INSECTICIDE

INTRODUCTION

Tropical and sub-tropical countries like Bangladesh face immense problems in preventing damage to seed by insects in storage. Environmental conditioning of storage areas which could solve the problem often is not possible for financial reasons. Regular use of insecticides is the main measure taken against insect infestation in these countries but the high cost, short persistence of some insecticides, and also the rapid development of resistance necessitate the search for new and cheaper alternative insecticides.

Recently, neem seed - local name for two botanical species, Azadirachta indica and Melia azedarach has received wide attention as a possible cheap but effective natural pesticide as an alternative to increasingly expensive and imported synthetic pesticides (Ivbijaro, 1983). Neem seed is readily available in most tropical and sub-tropical countries. This study aimed to test the effectiveness of neem seed as an insecticide against granary weevil and to assess other possible sub-lethal effects.

MATERIALS AND METHODS

MATERIALS

Seed and Insect

The wheat seed and granary weevils used in this experiment were from the same sources as those used in the earlier studies reported in this thesis.

Neem Seed

Two species of neem seed, namely, Azadirachta indica and Melia azedarach (hereafter, referred to as Azadirachta and Melia) were obtained from a reliable source in Bangladesh (Plate 23 and 24) (identification confirmed by Seed Testing Laboratory, Ministry of Agriculture and Fisheries, Palmerston North, New Zealand). Seeds were dried at 30 C and the whole seed of each species was then ground to a fine powder. The ground seeds were kept in sealed containers at 5 C

Plate 23: Neem (Azadirachta indica) seed

Plate 24: Neem (Melia azedarach) seed

until use (about one month). Ivbijaro (1983) used rates of dry ground neem seed of 0.5, 1.0 and 2.5 g/20 g maize. The middle rate of 1 g per 20 g of seed (wheat) was used in this experiment.

Experimental Containers

Containers similar to those used in the first experiment (Chapter 2) but 1/3rd the length and designed to hold 200 g of wheat seed, were used in this trial. The containers were placed in large plastic airtight drums of 25 litre capacity. Each drum contained 6 containers (one replicate).

METHODS

Experimental Design

The experiment was a randomised complete block design involving one storage environment, two species of neem seed and an untreated control treatment each with and without insects. There were 3 replicates.

Setting Up and Seed Storage Environment

Basically the same procedure as used in the first experiment (Chapter 2) was followed except that there was no drying treatment. The seed bulk was divided using a Boerner Divider to produce 18 x 200 g lots. Each lot was then placed in one of the small containers described and each treatment receiving insects infested with 50 adult weevils. No attempt was made to sex the weevils.

The experiment was commenced on 19 October, 1985 when the small containers were infested and placed in their respective large drums. A storage environment of 25 C and 80% RH was maintained in a thermostatically controlled room. Humidity was controlled by using a glycerine/water mixture of the correct proportions in each drum as employed by Hill (1965). Humidity was measured using wet and dry bulb thermometers twice a week for the duration of the trial.

Treatment with Ground Neem Seed

Treatments with neem seed were carried out 30 days after the start of the experiment (allowing insects time to lay eggs inside wheat kernels and larval stages to develop but not sufficient time for the next generation of adults to emerge).

10 g of ground neem seed was added to each 200 g of wheat seed to be treated giving an effective rate of 1 g of neem per 20 g of wheat. Containers were shaken and rotated vigorously following the addition of the ground neem to ensure thorough mixing of wheat seed and ground material.

Assessment

Seed was examined for live and dead insects present after 30, 40, 50, 60 and 90 days storage. Measurements were made of germination after 0 and 60 days storage.

MEASUREMENTS

Counting of Insects

All adult insects were sieved from infested seed and counted at each sampling time and classified as dead or alive. All dead adults were discarded but live adults were returned to their respective containers at each sampling time except after 60 days when all adults (both dead and alive) were discarded. This was done to provide some indirect measure of the effect of neem treatment on insect oviposition from the final counts at 90 days.

Germination of Seeds

Germination tests were carried out according to the standard ISTA germination method previously described to determine whether the ground neem seed had any effect on wheat seed viability.

RESULTS AND DISCUSSION

Effect of Ground Neem Seed on an Established Insect Population

Numbers of live and dead adult granary weevils recorded at intervals up to 90 days storage from the different treatments are presented in Table 7 (raw data in appendix 7).

After 30 days storage, i.e. at the time of treatment with neem seed, similar numbers of live (and of dead) adult granary weevils were recorded in all three insect treatments and slightly less than the 50 originally introduced into each container. After 40 days storage (10 days after treating with ground neem seed) numbers of adult weevils had increased by a factor of about x 9 in the untreated control by

Table 7: Mean numbers of live and (dead) granary weevil adults in different treatments up to 90 days storage (mean).

Treatment		Days of Storage					
		0	30	40	60	All adult insects removed after 60 day count	90
Untreated Control		50	41.3 (7.5)	455.7 (9.3)		959.3Aa (13.7)	1023.7Aa (23.3)
Neem seed powder	Azadirachta	50	36.9 (12.7)	327.3 (27.7)		431.6Bc (41.3)	312.7Bc (72.6)
	Melia	50	41.7 (6.3)	419.7 (16.3)		854.6Ab (23.5)	877.3Ab (39.3)
Significance			N.S.	N.S.		**	**

Values not followed by the same capital letter or small letter in vertical columns differ at P = 0.01 or 0.05 respectively.
** Significant at 1% level.

about x 6 in seeds treated with Azadirachta and by about x 8 in seeds treated with Melia. Percentage differences between numbers of dead adults for these treatments were somewhat greater but the total numbers of insects were not significantly different between treatments. After both 60 and 90 days storage, numbers of live insects in seed treated with Azadirachta were significantly different at 1% and those treated with Melia were significantly different at 5% from the untreated control.

These results are at variance with the findings of other investigators (e.g. Goyal et al., 1971; Schmutterer and Rembold, 1980; Ivbijaro, 1983) who have shown that neem seed is toxic to a range of insects. A number of possible reasons for this different response can be considered. It is possible that the grinding procedure for neem seed adopted in the present experiment resulted in decreased toxicity of the seed. Whereas other investigators used purified extracts from seeds (e.g. Butterworth and Morgan, 1968) or only ground cotyledonary tissue of seed obtained by splitting open the fruit (e.g. Ivbijaro, 1983), the present study involved grinding the whole fruit. Since the seed coat constituted a major portion of the neem seed for both species and may contain neither of the active principles, this might have diluted the insecticidal action of the ground preparation. Another factor may be that a comparatively moderate rate of ground neem seed w/w was used in this study compared with the highest application rate used by Ivbijaro (1983) in maize. Possibly for these reasons driect mortality of introduced adult insects was not observed in the present study.

However, later in the storage period, fewer live adults and greater numbers of dead adults were observed in seeds treated with ground Azadirachta seed compared to the untreated control. Intermediate values occurred for seed treated with ground Melia seed. This suggests that the seed of both species of neem had some effect on the reproductive capacity of the test insects presumably by a reduction in egg laying. Since all the live and dead insects were discarded from the insect infested treatments after 60 days storage, any further increase in the population of adults would have been dependent on the success of prior oviposition and on the ability of larvae to develop through to adulthood. When assessment was made at

90 days only 312 and 877 adults insects were recorded in seeds treated with Azadirachta and Melia respectively compared to 1023 insects in the untreated control. Thus, the emergence of adults over this period was reduced by about 70% in seeds treated with Azadirachta and by about 15% in seeds treated with Melia. In addition there were nearly three times as many dead adults in Azadirachta treated seed than in untreated.

These results suggest that Azadirachta indica does in fact have an appreciable effect, and Melia azedarach a comparatively small effect, on the reproduction of granary weevils presumably by suppressing oviposition. If an extract or ground neem seed kernel at a higher rate had been used the effect could well have been more pronounced.

Seed Quality

The percentage of normal germination of seed from all treatments after 0 and 60 days storage is presented in Table 8 (raw data in appendix 8).

The germination of seed from all treatments declined sharply after 60 days storage. The germination percentage for all three treatments without insects was similar and that of all insect infested treatments was also similar but the overall percentage for insect infested seed was considerably lower as would be expected.

This decline in germination of seeds from all treatments after 60 days of storage is obviously due to the adverse storage environment (25 C, 80% RH) used in this trial.

The results indicate that neither Azadirachta or Melia ground seed have any adverse effect on the germination capacity of wheat seed confirming similar findings by Ivbijaro (1983). The results also indicate that the destructive effect of insects on seed germinability after 60 days was about 30% and similar in all treatments. The results therefore, show that neither species of neem seed reduced insect damage to seed when added to previously infested seed as might be expected from the lack of insect control. This finding again is contradictory to those of many investigators (e.g. Ivbijaro, 1983; Gills and Lewis, 1971; Butterworth and Morgan, 1968) that neem seed is a feeding deterrent for many insects and will protect stored seed

Table 8: Percentages of normal germination of wheat seeds of all treatments up to 60 days storage (mean).

Treatments			Days of Storage	
			0	60
No Insect	No Neem		86.3	64.0 A
	Azadirachta		86.3	57.7 A
	Melia		86.3	59.3 A
Insect	No Neem		86.3	39.3 B
	Azadirachta		86.3	39.0 B
	Melia		86.3	32.7 B
Significance			N.S.	**
L.S.D.		1%		18.6
		5%		13.3

Values not followed by the same letter differ at P = 0.01
** Significant at 1% level.

from insect attack. It is possible that the antifeedant action might have been reduced through dilution during the present trial as previously discussed. Also it should be remembered that the experiment was designed to ascertain the effectiveness of neem in suppressing an already established infestation rather than as a protectant for clean seed.

The findings of the present experiment suggest that further investigation is necessary to determine exactly how neem seed can be effectively used to protect stored seed under practical conditions. The method of preparation of ground material from whole neem seed should be clearly defined and in fact whether crude ground neem seed can be effective by increasing the rate of use. It is also obviously important to ascertain which of the two botanical species commonly referred to as neem is most active insecticidally.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSION

The first experiment in the present study showed that an uncontrolled insect infestation under favourable conditions can increase exponentially and can result in rapid deterioration of seed quality. This result supports the findings of many investigators such as Eastham and McCully (1943), Eastham and Segrove (1947), Hall (1970) and Howe (1972) that development of Sitophilus granarius is favoured by seed moisture contents above 14%, (i.e. above 70% RH in storage) and an optimum temperature range of 26 C - 36 C. Under favourable conditions the whole life cycle of granary weevil may be passed in about six weeks (Howe, 1972) during which time the maximum rate of increase is 15 times (Eastham and Segrove, 1947). In the present experiment a steep increase in abnormal seedlings occurred and seeds lost any planting value within 90 days due to destruction of the seed tissues through ravenous feeding by immature larvae as well as to the egg laying activity of adults as has been previously described by Cotton and Wilbur (1974) and Howe (1972). Physical damage to seed resulting in such characteristics as discolouration, mustiness, powdering and stickiness associated with insect activity as shown by many investigators (e.g. Harein and Casas (1974); Koura and El-Halfawy (1972)) was also apparent in infested seed after 120 days storage in the present study.

Repeated drying reduced the rate of insect increase but did not eliminate the insect population. However, the results clearly show that proper drying has a beneficial effect on the retention of seed viability in storage for a comparatively longer period even under high relative humidity conditions as previously shown by Harrington (1959, 1972), Vaughan (1969), Delouche et al. (1973) and many others.

The X-ray radiography method used in this study clearly detected the presence of larvae and insect injury in infested seeds even in cases where injury was not visible externally. The X-ray photographs also confirmed that the presence of larvae and insect injury were more concentrated in the softer embryo region which is in agreement with similar findings by Golebiswaska et al. (1977).

This experiment also clearly showed that fumigation with phosphine was totally effective in eradicating an established insect population without affecting seed quality. This result was expected because fumigants are known to be able to penetrate a stack of bagged produce or bulk stored grain and kill any insect infestation present (including eggs and other immature stages inside the grains) as described by Munro (1966), Thompson (1966), Lindren and Vincent (1966), Monro (1969), Hall (1970) and Harein and Casas (1974). Phosphine also has been shown not to impair the germination of a wide variety of seed as reported by Heseltine and Thompson (1957), Lindgren et al. (1958), Strong and Lindgren (1960), Harada (1962), Beratlief and Alexandreson (1964), McGregor and White (1969), Quershi (1965), White and Jacobson (1972) and Ahmad (1976).

The present experiment also showed that malathion dust added to infested seed severely checked insect development and that when combined with drying, malathion treatment cleaned up the infestation completely. Malathion also displayed considerable residual effect. This is not entirely in agreement with the known short persistence of malathion as previously described by Watters (1959), Strong and Sbur (1960), Rowlands (1967), Hall (1970) and Fenemore (1984). However, treatment with malathion dust had no adverse effect on germination of seed which does agree with similar findings by Witt et al. (1960), Parkin (1963) and Hall (1970).

Malathion, in a second experiment, was sprayed onto the outside of storage sacks to evaluate its ability to protect enclosed seed from insect attack. The results of a bioassay method used in the study suggested that the protective effect of malathion should last at least a week or longer. However, when applied to the outside of whole sacks at the recommended dose it was found to provide only immediate protection and was inadequate after only a few days.

A simple evaluation of neem seed powder (of two botanical species - Azadirachta indica and Melia azedarach) was undertaken to determine its effect on an established infestation of granary weevil. Little or no direct mortality of adults was recorded but there was evidence of suppression of egg laying particularly with Azadirachta indica. The experiment, however,suggested that ground neem seed has potential as a pesticide as suggested by previous investigators (Attri (1975), Saradama et al. (1977), Warthen (1979), Goyal et al. (1971), Schmutterer and Rembold (1980) and Ivbijaro (1983)).

The results of the present studies re-emphasize that insects can be a major cause of damage to seeds during storage if infestation is not prevented or controlled as reported by Henderson and Christensen (1961)). Parkin (1963) has stated that, in the light of the control measures now available, such damage and loss can be due only to ignorance or negligence. However, the differential mode of action of malathion and phosphine on an established granary weevil population emphasizes that in the treatment of seed with chemicals designed to protect seed from insect attack, or to kill an insect infestation which is already present, various factors must be carefully considered. A suitable chemical in a suitable formulation must be chosen which is known to be effective against the species of insect(s) involved. Control measures applicable in one case may not apply in another as mentioned by Parkin (1963), Munro (1966) and Fenemore (1984). Correct identification of the targetted insect and technical information relating to its habits and biology are important for appropriate and judicious application of chemicals. The correct dosage and method of application must then be selected so that the chemical is used with the greatest possibility of success. The chosen treatment must also have no deleterious effect on the germination capacity of the seed during storage.

High temperature and high relative humidity ambient conditions which generally prevail in tropical and sub-tropical countries such as Bangladesh, are most detrimental to seed storage and at the same time provide very favourable environments for the development of insect populations. A general practice in such countries is to procure seed from different farmers or sources after harvest of a crop and to clean and preserve this seed in selected stores until the next sowing season for distribution to farmers. In the absence of environmentally conditioned storage facilities seeds are often repeatedly dried and a contact insecticide frequently sprayed onto storage sacks and into the storage space to protect seed from insect attack. Fumigation is carried out only when external evidence of insects is observed. The present study suggests some additional steps and modification of procedure in this system.

In Bangladesh, drying of seed is carried out as a routine operation but without any precise guideline as to what level of moisture seeds should be dried too. The present work shows that if seeds are dried when they reach a moisture content of 13%, a seed lot can maintain germinability of a 'certified' standard (80%) up to 60 days storage, but may lose germinability to levels (below 80% within 90 days. It is therefore suggested that seeds should be dried before they reach 13% moisture content to retain an acceptable standard of germination for as long as possible.

At the time of procurement of seed, there may not be any external evidence of insect infestation but it is common to find that immature stages of some insects, such as weevils, may be already present inside seed of some seed lots. The first external evidence of trouble may be when the mature insects cut holes in the seed coat through which to emerge after their development is completed. By this time extensive damage may have been caused. It is therefore often necessary to fumigate any incoming stock before it is placed in storage so that any immature stages of insects present are killed. The same procedure should be adopted for bagged or packaged seeds carried over from one season to the next because this may also provide an infestation hazard. Stocks with differing infestation potentials should be kept segregated to minimise cross-infestation and used bags should also be fumigated before filling.

Malathion is widely used in Bangladesh especially as a spray onto the structure of stores and onto storage sacks as a preventative measure against infestation. It was observed in this study that such treatment can protect seed from infestation for only a few days. It would, therefore, require repeated spraying at intervals of not less than 7 days to ensure that 'clean' seed is kept free from infestation. It would however, be preferable to apply malathion dust directly to seed to provide longer protection. In the case of sack treatment, it would be better if malathion could be replaced by some other insecticide with known rapid contact action and greater persistence, such as a synthetic pyrethroid.

It may further be suggested that use of a fumigant such as phosphine would be most appropriate in the control of established insect infestation where eggs and other juvenile stages are already present inside seeds.

It is often necessary to test new insecticides or formulations for their effectiveness and specificity. Treating whole sacks and waiting for insect infestation to develop is a slow and cumbersome method. Rapid bioassay techniques, such as that used in the present study would be an advantage.

The X-ray radiography technique can be very useful in the early detection of insect infestation before a generation has been completed and adults emerged. It can also help in the identification of the nature of internal injury to seed by a particular pest and the consequences for seed quality. Further, this technique may be of most value as a research tool to investigate the nature of injury to stored seed due to insect attack by different species.

A developing country like Bangladesh always looks for relatively cheap and new insect control methods because of the increasing cost of imported insecticides and also because of the rapid development of resistance by insects. Recently, neem seed which is readily available naturally has received some attention as a possible cheap natural pesticide. The results of the present study shows that before introducing neem seed as a pesticide further investigation is necessary to determine exactly how it may be used under practical conditions. It is also necessary to clearly identify the specificity and nature of the lethal effects of the two botanical species (Azadirachta indica and Melia azedarach) both commonly referred to as 'neem'.

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APPENDICES

APPENDIX 1: Percentage of abnormal seedlings in different treatments upto 165 days of storage (mean).

Treatments			Days of storage					
			0	30	60	90	120	165
NO INSECT	No chemical	Undried	1.0	5.0	9.8C	18.8BC	9.8AB	4.3BC
		Dried	1.0	6.8	10.0C	17.3C	11.0AB	9.3AB
	Malathion	Undried	1.0	5.0	9.8C	20.8BC	11.8AB	7.0ABC
		Dried	1.0	6.0	7.3C	15.0C	8.8B	10.AB
	Phosphine	Undried	1.0	6.8	10.C	21.3BC	15.0A	7.0ABC
		Dried	1.0	6.0	10.0C	19.3BC	110.AB	8.3ABC
INSECT	No Chemical	Undried	1.0	10.3	19.8A	25.3AB	0.0C	0.0C
		Dried	1.0	8.3	18.0AB	31.3A	12.3AB	0.0C
	Malathion	Undried	1.0	7.0	13.8ABC	20.8BC	14.0AB	5.0BC
		Dried	1.0	10.8	12.3BC	16.8C	14.3AB	8.8AB
	Phospine	Undried	1.0	10.3	13.0ABC	16.0C	12.0A	9.8AB
		Dried	1.0	10.0	10.8C	16.0C	9.0A	15.0A
Significance				NS	**	**	**	**
L.S.D		1%			6.6	6.9	6.2	8.5
		5%			4.9	5.1	4.6	6.3

Values not followed by the same letter differ at P = 0.01.
** Significant at 1% level

APPENDIX 2: Percentage of main types of abnormality in wheat seeds after 30, 60, 90, 120 and 165 days storage.

30 Days

Treatments			Types of abnormality in percent													Grand Total
			Root Abn.		Coleoptile abnormalities						Decay		Other			
			Ia	If	IIIa	IIIb	IIIc	IIId	IIIe	IIIf	Vd	Vg	VIa	VIc	VId	
No Insect	No Chemical	Undried	1	1			1					1	1			5
		dried	1		1		1					1	2	1		7
	Malathion	Undried	1		1	1						1	1			5
		dried	1		1	1						1	2			6
	Phosphine	Undried	2				1				1	1	2			7
		dried	1		1		1					1	1		1	6
Insect	No Chemical	Undried	1		2		2	1		1		1	2			10
		dried	2		1		1	1		1		1	1			9
	Malathion	Undried	1		1		1		1				1	1		7
		dried	1		2	1	1	1	1	1		1	2			11
	Phosphine	Undried	1	1	2		1			1		2	2			10
		dried	2		1	1	1			1		1	2		1	10

APPENDIX 2 continued - 60 days

Treatments			Types of abnormality in percent													Grand Total
			Root Abn.		Coleoptile abnormalities						Decay		Other			
			Ia	If	IIIa	IIIb	IIIc	IIId	IIIe	IIIf	Vd	Vg	VIa	VIc	VId	
No Insect	No Chemical	Undried	1				1	1				1	4	1		10
		dried	1		1	1			1			2	2	1		10
	Malathion	Undried	1	1	1		1				1	2	2		1	10
		dried	1		1	1		1			1	1	2			8
	Phosphine	Undried	2	1	2		1			1		2	1			10
		dried	1	1			2	1				1	3		1	10
Insect	No Chemical	Undried	1		4	2	3	1	2			3	4			20
		dried	2		3		4			1	1	2	3	1	1	18
	Malathion	Undried		1	2	1	3	1	2		1	1	2			14
		dried	1		1	2	2	1	1			2	2			12
	Phosphine	Undried	1	1	1		2					3	4	1		13
		dried	2				1	1			1	2	1		1	10

APPENDIX 2 continued - 90 days

Treatments			Types of abnormality in percent													Grand Total
			Root Abn.		Coleoptile abnormalities						Decay		Other			
			Ia	If	IIIa	IIIb	IIIc	IIId	IIIe	IIIf	Vd	Vg	VIa	VIc	VId	
Insect	No Chemical	Undried	2	2	1		2		1			5	6			19
		dried	1	1	2	1		1		1	2	3	3	1	1	17
	Malathion	Undried	1		1		1		1		3	6	8			21
		dried	2		1		2				1	5	3		1	15
	Phosphine	Undried	2	1	2	1	1	1	1			7	5	1		22
		dried	1		1			1	1	2	4	4	6			20
Insect	No Chemical	Undried	2	1	4	2	5			4		3	3		1	25
		dried	3	1	5	1	7	3	2	2		2	5	1		32
	Malathion	Undried	1		2	2	4	2	1	1	2	3	3			21
		dried	2		3	1	3	1	1	2		2	2			17
	Phosphine	Undried	1	1	2	1	2	1		1		2	4	1		16
		dried	1	1	3	1	2		2			4	1			16

APPENDIX 2 continued - 120 days

Treatments			Types of abnormality in percent													Grand Total
			Root Abn.		Coleoptile abnormalities						Decay		Other			
			Ia	If	IIIa	IIIb	IIIc	IIId	IIIe	IIIf	Vd	Vg	VIa	VIc	VId	
No Insect	No Chemical	Undried	3			1	1	1				2	2			10
		dried	2	1	1		1					1	3		1	11
	Malathion	Undried	1		1		1			1		4	3	1		12
		dried		1	1	1			1		1	1	2		1	9
	Phosphine	Undried	1	2	1		1	1		1	1	2	4	1		15
		dried	1	2	1	1	1				1	2	2			11
Insect	No Chemical	Undried														0
		dried	2		2	2	2		1			2	1			12
	Malathion	Undried	1	1	1		2		1			3	2	1	1	14
		dried	2		3	1	1		1	1	1	2	2			14
	Phosphine	Undried	2	1	2		1	1			1	1	2		1	12
		dried	1	1	2	1	2				1	1	1			9

APPENDIX 2: continued - 165 days

Treatments			Types of abnormality in percent														Grand Total
			Root Abn.		Coleoptile abnormalities						Decay		Other				
			Ia	If	IIIa	IIIb	IIIc	IIId	IIIe	IIIf	Vd	Vg	VIa	VIc	VId		
No Insect	No Chemical	Undried			1							1	2				4
		dried	1	2			1					2	3				9
	Malathion	Undried	1		1						1	1	2		1		7
		dried	2		1	1	1					2	2	.1			10
	Phosphine	Undried	1	1		1						3	1				7
		dried	1					1		1	1	2	2				8
Insect	No Chemical	Undried															0
		dried															0
	Malathion	Undried	1		1				1			1	1				5
		dried	1		2		2	1		1		1	1				9
	Phosphine	Undried	1		1	1		1			1	1	2	1	1		10
		dried	1	1	2	1	2	1	1		1	2	4				15

Key to Appendix 3 and 3a:

INS1 to INS5. = Number of live granary weevil adults after 30, 60, 90, 120 and 165 days storage respectively.

Treat = Treatment (in appendix 3)

1 = Insect, no chemical and undried

2 = Insect, no chemical and dried

3 = Insect, malathion treated and undried

4 = Insect, malathion treated and dried

5 = Insect, phosphine fumigated and undried

6 = Insect, phosphine fumigated and dried

Treatment (in appendix 3a)

1 = Insect, malathion treated and undried

2 = Insect, malathion treated and dried

3 = Insect, phosphine fumigated and undried

4 = Insect, phosphine fumigated and dried

** = Significant at 1% level

* = Significant at 5% level.

APPENDIX 3: ANalysis of variance of the experiment data on the number of live granary weevil adults of insect infested treatments after 30, 60, 120 and 165 days storage.

30 Days.

***** ANALYSIS OF VARIANCE *****

VARIATE: INS1

SOURCE OF VARIATION	DF	SS	SS%	MS	VR	
UNITS STRATUM						
TREAT	5	28569.164	99.79	5713.833	1155.607	**
RESIDUAL	12	59.333	0.21	4.944		
TOTAL	17	28628.496	100.00	1684.029		
GRAND TOTAL	17	28628.496	100.00			
GRAND MEAN		28.17				
TOTAL NUMBER OF OBSERVATIONS		18				

***** TABLES OF MEANS *****

VARIATE: INS1

GRAND MEAN 28.17

TREAT	1	2	3	4	5	6
	33.33	85.67	0.00	0.00	0.00	0.00

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	1.816

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	12	2.224	7.9

APPENDIX 3 continued - 60 days.

***** ANALYSIS OF VARIANCE *****

VARIATE: INS2

SOURCE OF VARIATION	DF	SS	SS%	MS	VR	
UNITS STRATUM						
TREAT	5	3303094	92.73	660619	30.591	**
RESIDUAL	12	259144	7.27	21595		
TOTAL	17	3562238	100.00	209543		
GRAND TOTAL	17	3562238	100.00			

GRAND MEAN	371
TOTAL NUMBER OF OBSERVATIONS	18

***** TABLES OF MEANS *****

VARIATE: INS2

GRAND MEAN 371

TREAT	1	2	3	4	5	6
	1066	861	191	106	0	0

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	120.0

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	12	147.0	39.6

APPENDIX 3 continued - 90 Days.

***** ANALYSIS OF VARIANCE *****

VARIATE: INS3

SOURCE OF VARIATION	DF	SS	SS%	MS	VR	
UNITS STRATUM						
TREAT	5	11267354	95.44	2253471	50.178	**
RESIDUAL	12	538910	4.56	44909		
TOTAL	17	11806264	100.00	694486		
GRAND TOTAL	17	11806264	100.00			

GRAND MEAN	566
TOTAL NUMBER OF OBSERVATIONS	18

***** TABLES OF MEANS *****

VARIATE: INS3

GRAND MEAN 566

TREAT	1	2	3	4	5	6
	1063	1160	41	13	0	0

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	173.0

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	12	211.9	37.4

APPENDIX 3 continued - 120 Days.

***** ANALYSIS OF VARIANCE *****

VARIATE: INS4

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREAT	5	240681440	96.76	48136288	71.660
RESIDUAL	12	8060837	3.24	671736	
TOTAL	17	248742272	100.00	14631898	
GRAND TOTAL	17	248742272	100.00		

GRAND MEAN 2469
TOTAL NUMBER OF OBSERVATIONS 18

***** TABLES OF MEANS *****

VARIATE: INS4

GRAND MEAN 2469

TREAT	1	2	3	4	5	6
	9313	5482	12	4	0	0

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	669.2

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	12	819.6	33.2

APPENDIX 3 continued - 165 Days.

***** ANALYSIS OF VARIANCE *****

VARIATE: INS5

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM	5	396275776	88.79	79255152	19.018 **
TREAT	12	50007328	11.21	4167278	
RESIDUAL	17	446283136	100.00	26251948	
TOTAL					
GRAND TOTAL	17	446283136	100.00		

GRAND MEAN 3312
TOTAL NUMBER OF OBSERVATIONS 18

***** TABLES OF MEANS *****

VARIATE: INS5

GRAND MEAN 3312

TREAT	1	2	3	4	5	6
	11082	8664	124	0	0	0

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	1666.8

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	12	2041.4	61.6

APPENDIX 3a: Analysis of variance of the experimental data on the number of live granary weevil adults in malathion and phosphine treatments after 30, 60, 90, 120 and 165 days storage.

30 days.

***** ANALYSIS OF VARIANCE *****

VARIATE: INS1

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREAT	3	0.000E 0	0.00	0.000E 0	
RESIDUAL	8	0.000E 0	0.00	0.000E 0	
TOTAL	11	0.000E 0	0.00	0.000E 0	
GRAND TOTAL	11	0.000E 0	0.00		

GRAND MEAN 0.00
TOTAL NUMBER OF OBSERVATIONS 12

***** TABLES OF MEANS *****

VARIATE: INS1

GRAND MEAN 0.00

TREAT	1	2	3	4
	0.00	0.00	0.00	0.00

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	0.000

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	8	0.000	*

APPENDIX 3a continued _ 60 days.

***** ANALYSIS OF VARIANCE *****

VARIATE: INS2

SOURCE OF VARIATION	DF	SS	SS%	MS	VR	
UNITS STRATUM						
TREAT	3	76994	63.84	25665	4.709	*
RESIDUAL	8	43604	36.16	5451		
TOTAL	11	120598	100.00	10963		
GRAND TOTAL	11	120598	100.00			

GRAND MEAN 74
TOTAL NUMBER OF OBSERVATIONS 12

***** TABLES OF MEANS *****

VARIATE: INS2

GRAND MEAN 74

TREAT	1	2	3	4
	191	106	0	0

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	60.3

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	8	73.8	99.4

APPENDIX 3a continued - 90 days.

***** ANALYSIS OF VARIANCE *****

VARIATE: INS3

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREAT	3	3362.3	32.61	1120.8	1.290
RESIDUAL	8	6948.7	67.39	868.6	
TOTAL	11	10310.9	100.00	937.4	
GRAND TOTAL	11	10310.9	100.00		

GRAND MEAN 13.6
TOTAL NUMBER OF OBSERVATIONS 12

***** TABLES OF MEANS *****

VARIATE: INS3

GRAND MEAN 13.6

TREAT	1	2	3	4
	41.0	13.3	0.0	0.0

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	24.06

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	8	29.47	217.0

APPENDIX 3a continued - 120 days.

***** ANALYSIS OF VARIANCE *****

VARIATE: INS4

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREAT	3	272.250	78.48	90.750	9.723 **
RESIDUAL	8	74.667	21.52	9.333	
TOTAL	11	346.917	100.00	31.538	
GRAND TOTAL	11	346.917	100.00		

GRAND MEAN 3.92
TOTAL NUMBER OF OBSERVATIONS 12

***** TABLES OF MEANS *****

VARIATE: INS4

GRAND MEAN 3.92

TREAT	1	2	3	4
	11.67	4.00	0.00	0.00

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	2.494

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	8	3.055	78.0

APPENDIX 3a continued - 165 days.

***** ANALYSIS OF VARIANCE *****

VARIATE: INS5

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREAT	3	34410	57.47	11470	3.604
RESIDUAL	8	25461	42.53	3183	
TOTAL	11	59871	100.00	5443	
GRAND TOTAL	11	59871	100.00		

GRAND MEAN 31
TOTAL NUMBER OF OBSERVATIONS 12

***** TABLES OF MEANS *****

VARIATE: INS5

GRAND MEAN	31			
TREAT	1	2	3	4
	124	0	0	0

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
	3
REP	46.1
SED	

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	8	56.4	182.5

Key to Appendix 4 and 5:

G1 to G5 = Normal germination percentage of seeds after 30, 60, 90, 120 and 165 days storage respectively.

AB1 to AB5 = Percentage of abnormal seedlings after 30, 60, 90, 120 and 165 days storage respectively.

Treat = Treatment

1 to 12 = Treatments as presented in Table 3 of Chapter 2 and Appendix 1. Treatment 1 is No insect, No chemical and undried, and thus continues to 12 as insects, phosphine and dried.

** = Significant at 1% level.

* = Significant at 5% level.

APPENDIX 4: Analysis of variance of the experimental data on normal germination percentage of seeds after 30, 60, 90, 120 and 165 days storage.

30 Days.

***** ANALYSIS OF VARIANCE *****

VARIATE: G1

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREAT	11	272.08	40.56	24.73	1.489
RESIDUAL	24	398.67	59.44	16.61	
TOTAL	35	670.75	100.00	19.16	
GRAND TOTAL	35	670.75	100.00		

GRAND MEAN 86.25
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: G1

GRAND MEAN 86.25

TREAT	1	2	3	4	5	6	7	8	9	10	11
	89.67	88.33	89.33	88.33	87.67	90.00	84.33	83.67	84.67	83.00	83.33

TREAT	12
	82.67

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	3.328

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	24	4.076	4.7

APPENDIX 4 continued - 60 Days.

***** ANALYSIS OF VARIANCE *****

VARIATE: G2

SOURCE OF VARIATION	DF	SS	SS%	MS	VR	
UNITS STRATUM						
TREAT	11	1423.64	74.68	129.42	6.435	**
RESIDUAL	24	482.67	25.32	20.11		
TOTAL	35	1906.31	100.00	54.47		
GRAND TOTAL	35	1906.31	100.00			

GRAND MEAN 77.64
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: G2

GRAND MEAN 77.64

TREAT	1	2	3	4	5	6	7	8	9	10	11
	82.00	80.00	80.67	85.00	80.67	82.33	61.67	69.00	74.67	76.00	78.33

TREAT	12
	81.33

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	3.662

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	24	4.485	5.8

APPENDIX 4 continued - 90 Days.

***** ANALYSIS OF VARIANCE *****

VARIATE: G3

SOURCE OF VARIATION	DF	SS	SS%	MS	VR	
UNITS STRATUM						
TREAT	11	10362.30	90.90	942.03	21.795	**
RESIDUAL	24	1037.33	9.10	43.22		
TOTAL	35	11399.64	100.00	325.70		
GRAND TOTAL	35	11399.64	100.00			
GRAND MEAN		63.7				

TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: G3

GRAND MEAN 63.7

TREAT	1	2	3	4	5	6	7	8	9	10	11
	74.0	72.7	70.3	76.3	68.0	73.0	19.0	38.0	56.7	67.3	73.0

TREAT	12
	76.0

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	5.37

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	24	6.57	10.3

APPENDIX 4 continued - 120 Days.

***** ANALYSIS OF VARIANCE *****

VARIATE: G4

SOURCE OF VARIATION	DF	SS	SS%	MS	VR	
UNITS STRATUM						
TREAT	11	20506.75	97.31	1864.25	79.049	**
RESIDUAL	24	566.00	2.69	23.58		
TOTAL	35	21072.75	100.00	602.08		
GRAND TOTAL	35	21072.75	100.00			

GRAND MEAN 59.42
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: G4

GRAND MEAN 59.42

TREAT	1	2	3	4	5	6	7	8	9	10	11
	70.67	74.00	70.67	75.00	65.00	72.67	0.00	15.67	55.00	68.00	71.67

TREAT	12
	74.67

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	3.965

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	24	4.856	8.2

APPENDIX 4 continued - 165 Days.

***** ANALYSIS OF VARIANCE *****

VARIATE: G5

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM	11	22340.3	85.10	2030.9	12.462 **
TREAT	24	3911.3	14.90	163.0	
RESIDUAL	35	26251.6	100.00	750.0	
TOTAL	35	26251.6	100.00		
GRAND TOTAL					
GRAND MEAN		33.8			
TOTAL NUMBER OF OBSERVATIONS		36			

***** TABLES OF MEANS *****

VARIATE: G5

GRAND MEAN	33.8									
TREAT	1	2	3	4	5	6	7	8	9	10	11
	17.0	67.0	16.7	67.3	23.0	66.7	0.0	0.0	9.0	52.3	40.0
TREAT	12									
	46.7									

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	10.42

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	24	12.77	37.8

APPENDIX 5: Analysis of variance of the experimental data on the percentage of abnormal seedlings after 30, 60, 90, 120 and 165 days storage.

30 Days.

***** ANALYSIS OF VARIANCE *****

VARIATE: AB1

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREAT	11	154.00	36.67	14.00	1.263
RESIDUAL	24	266.00	63.33	11.08	
TOTAL	35	420.00	100.00	12.00	
GRAND TOTAL	35	420.00	100.00		

GRAND MEAN 7.67
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: AB1

GRAND MEAN 7.67

TREAT	1	2	3	4	5	6	7	8	9	10	11
	5.00	6.67	5.00	6.00	6.67	6.00	10.33	8.33	7.00	10.67	10.33

TREAT	12
	10.00

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	2.718

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	24	3.329	43.4

APPENDIX 5 continued - 60 days.

***** ANALYSIS OF VARIANCE *****

VARIATE: AB2

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREAT	11	435.333	66.56	39.576	4.344 **
RESIDUAL	24	218.667	33.44	9.111	
TOTAL	35	654.000	100.00	18.686	
GRAND TOTAL	35	654.000	100.00		

GRAND MEAN 12.00
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: AB2

GRAND MEAN 12.00

TREAT	1	2	3	4	5	6	7	8	9	10	11
	9.67	10.00	9.67	7.33	10.00	10.00	19.67	18.00	13.67	12.33	13.00

TREAT	12
	10.67

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	2.465

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	24	3.018	25.2

APPENDIX 5 continued - 90 Days.

***** ANALYSIS OF VARIANCE *****

VARIATE: AB3

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREAT	11	710.306	75.06	64.573	6.567 **
RESIDUAL	24	236.000	24.94	9.833	
TOTAL	35	946.306	100.00	27.037	
GRAND TOTAL	35	946.306	100.00		

GRAND MEAN 19.86

TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: AB3

GRAND MEAN 19.86

TREAT	1	2	3	4	5	6	7	8	9	10	11
	18.67	17.33	20.67	15.00	21.33	19.33	25.33	31.33	20.67	16.67	16.00

TREAT	12
	16.00

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	2.560

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	24	3.136	15.8

APPENDIX 5 continued - 120 Days.

***** ANALYSIS OF VARIANCE *****

VARIATE: AB4

SOURCE OF VARIATION	DF	SS	SS%	MS	VR	
UNITS STRATUM						
TREAT	11	511.889	72.59	46.535	5.777	**
RESIDUAL	24	193.333	27.41	8.056		
TOTAL	35	705.222	100.00	20.149		
GRAND TOTAL	35	705.222	100.00			

GRAND MEAN 10.72
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: AB4

GRAND MEAN 10.72

TREAT	1	2	3	4	5	6	7	8	9	10	1:
	9.67	11.00	11.67	8.67	15.00	11.00	0.00	12.33	14.00	14.33	12.0(

TREAT	12
	9.00

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	2.317

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	24	2.838	26.5

APPENDIX 5 continued - 165 Days.

***** ANALYSIS OF VARIANCE *****

VARIATE: AB5

SOURCE OF VARIATION	DF	SS	SS%	MS	VR	
UNITS STRATUM						
TREAT	11	597.64	62.32	54.33	3.609	**
RESIDUAL	24	361.33	37.68	15.06		
TOTAL	35	958.97	100.00	27.40		
GRAND TOTAL	35	958.97	100.00			

GRAND MEAN 7.03
TOTAL NUMBER OF OBSERVATIONS 36

***** TABLES OF MEANS *****

VARIATE: AB5

GRAND MEAN 7.03

TREAT	1	2	3	4	5	6	7	8	9	10	1
	4.33	9.33	7.00	10.00	7.00	8.33	0.00	0.00	5.00	8.67	9.6

TREAT	12
	15.00

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	3.168

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	24	3.880	55.2

Key to Appendix 6:

INS1 = Number of granary weevil adults immediately after malathion treatment.

INS2 = Number of granary weevil adults 7 days after malathion treatment.

INS3 = Number of granary weevil adults 14 days after malathion treatment.

Treat = Treatments.

1 = Sacks not sprayed with malathion stored at 30 C.

2 = Sacks sprayed with malathion stored at 30 C.

3 = Sacks not sprayed with malathion stored at 20 C.

4 = Sacks sprayed with malathion stored at 20 C.

** = Significant at 1% level.

APPENDIX 6: Analysis of variance of the experimental data on the numbers of live granary weevil adults after 0, 7 and 14 days of malathion treatment.

0 Day.

***** ANALYSIS OF VARIANCE *****

VARIATE: INS1

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREAT	3	990404	99.13	330135	304.342
RESIDUAL	8	8678	0.87	1085	
TOTAL	11	999082	100.00	90826	
GRAND TOTAL	11	999082	100.00		

GRAND MEAN 179.3
TOTAL NUMBER OF OBSERVATIONS 12

***** TABLES OF MEANS *****

VARIATE: INS1

GRAND MEAN 179.3

TREAT	1	2	3	4
	676.0	0.0	41.0	0.0

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	26.89

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	8	32.94	18.4

APPENDIX 6 continued - 7 Days.

***** ANALYSIS OF VARIANCE *****

VARIATE: INS2

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREAT	3	481950	97.54	160650	105.847 **
RESIDUAL	8	12142	2.46	1518	
TOTAL	11	494092	100.00	44917	
GRAND TOTAL	11	494092	100.00		

GRAND MEAN 431
TOTAL NUMBER OF OBSERVATIONS 12

***** TABLES OF MEANS *****

VARIATE: INS2

GRAND MEAN 431

TREAT	1	2	3	4
	660	267	597	179

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	31.3

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	8	39.0	9.0

APPENDIX 6 continued - 14 Days.

***** ANALYSIS OF VARIANCE *****

VARIATE: INS3

SOURCE OF VARIATION	DF	SS	%SS	MS	VR
UNITS STRATUM					
TREAT	3	154202	92.90	51401	34.895 **
RESIDUAL	8	11784	7.10	1473	
TOTAL	11	165986	100.00	15090	
GRAND TOTAL	11	165986	100.00		

GRAND MEAN 504
TOTAL NUMBER OF OBSERVATIONS 12

***** TABLES OF MEANS *****

VARIATE: INS3

GRAND MEAN 504

TREAT	1	2	3	4
	657	107	867	383

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	31.3

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	8	38.4	7.6

Key to Appendix 7:

INS1 = Numbers of adult insects introduced initially.

INS2 = Numbers of adult insects after 30 days storage.

INS3 = Numbers of adult insects after 40 days storage.

INS4 = Numbers of adult insects after 60 days storage.

INS5 = Numbers of adult insects after 90 days storage.

Treat = Treatments

1 = Untreated control

2 = Treated with *Azadirachta indica*.

3 = Treated with *Melia azedarach*.

** = Significant at 1% level.

APPENDIX 7: Analysis of variance of the experimental data on the numbers of live granary weevil adults in neem seed trial after 0, 30, 40, 60 and 90 days storage.

0 Day.

***** ANALYSIS OF VARIANCE *****

VARIATE: INS1

SOURCE OF VARIATION	DF	SS	%SS	MS	VR
UNITS STRATUM					
TREAT	2	0.000E 0	0.00	0.000E 0	
RESIDUAL	6	0.000E 0	0.00	0.000E 0	
TOTAL	8	0.000E 0	0.00	0.000E 0	
GRAND TOTAL	8	0.000E 0	0.00		

GRAND MEAN 50.00
TOTAL NUMBER OF OBSERVATIONS 9

***** TABLES OF MEANS *****

VARIATE: INS1

GRAND MEAN 50.00

TREAT	1	2	3
	50.00	50.00	50.00

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	0.000

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	6	0.000	0.0

APPENDIX 7 continued - 30 Days.

***** ANALYSIS OF VARIANCE *****

VARIATE: INS2

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREAT	2	60.67	18.96	30.33	0.702
RESIDUAL	6	259.33	81.04	43.22	
TOTAL	8	320.00	100.00	40.00	
GRAND TOTAL	8	320.00	100.00		

GRAND MEAN 39.3
TOTAL NUMBER OF OBSERVATIONS 9

***** TABLES OF MEANS *****

VARIATE: INS2

GRAND MEAN 39.3

TREAT	1	2	3
	41.0	35.7	41.3

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	5.37

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	6	6.57	16.7

APPENDIX 7 continued - 40 Days.

***** ANALYSIS OF VARIANCE *****

VARIATE: INS3

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREAT	2	26144	65.23	13072	5.628
RESIDUAL	6	13936	34.77	2323	
TOTAL	8	40080	100.00	5010	
GRAND TOTAL	8	40080	100.00		

GRAND MEAN	400
TOTAL NUMBER OF OBSERVATIONS	9

***** TABLES OF MEANS *****

VARIATE: INS3

GRAND MEAN	400		
TREAT	1	2	3
	455	327	419

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	39.4

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	6	48.2	12.0

APPENDIX 7 continued - 60 Days.

***** ANALYSIS OF VARIANCE *****

VARIATE: INS4

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREAT	2	469372	97.04	234686	98.506 **
RESIDUAL	6	14295	2.96	2382	
TOTAL	8	483667	100.00	60458	
GRAND TOTAL	8	483667	100.00		

GRAND MEAN	748
TOTAL NUMBER OF OBSERVATIONS	9

***** TABLES OF MEANS *****

VARIATE. INS4

GRAND MEAN 748

TREAT	1	2	3
	959	431	854

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	62.9

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	6	48.8	6.5

APPENDIX 7 continued - 90 Days.

***** ANALYSIS OF VARIANCE *****

VARIATE: INS5

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM	2	846062	96.97	423031	96.11 4**
TREAT	6	26408	3.03	4401	
RESIDUAL	8	872470	100.00	109059	
TOTAL					
GRAND TOTAL	8	872470	100.00		

GRAND MEAN 737
TOTAL NUMBER OF OBSERVATIONS 9

***** TABLES OF MEANS *****

VARIATE: INS5

GRAND MEAN 737

TREAT	1	2	3
	1023	812	877

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	54.2

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	6	66.3	9.0

Key to Appendix 8:

Germ. = Normal germination percentage.

Treat = Treatment.

1 = No insect and no neem.

2 = No insect and Azadirachta.

3 = No insect and Melia.

4 = Insect and no neem.

5 = Insect and Azadirachta.

6 = Insect and Melia.

** = Significant at 1% level.

APPENDIX 8: Analysis of variance of the experimental data on normal germination of seeds in neem seed trial after 60 days storage.

***** ANALYSIS OF VARIANCE *****

VARIATE: GERM

SOURCE OF VARIATION	DF	SS	SS%	MS	VR
UNITS STRATUM					
TREAT	5	2599.33	79.49	519.87	9.302 **
RESIDUAL	12	670.67	20.51	55.89	
TOTAL	17	3270.00	100.00	192.35	
GRAND TOTAL	17	3270.00	100.00		

GRAND MEAN 48.7
TOTAL NUMBER OF OBSERVATIONS 18

***** TABLES OF MEANS *****

VARIATE: GERM

GRAND MEAN 48.7

TREAT	1	2	3	4	5	6
	64.0	57.7	59.3	39.3	39.0	32.7

***** STANDARD ERRORS OF DIFFERENCES OF MEANS *****

TABLE	TREAT
REP	3
SED	6.10

***** STRATUM STANDARD ERRORS AND COEFFICIENTS OF VARIATION *****

STRATUM	DF	SE	CV%
UNITS	12	7.48	15.4