EFFECTIVENESS OF BOTANICAL PREPARATIONS FOR THE CONTROL OF RICE WEEVIL (*SITOPHILUS ORYZAE*) DURING RICE SEED STORAGE AND THEIR IMPACT ON THE RICE SEED VIABILITY.

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Errata

Page vi:	LIST OF TABLES: Change x to ix-x LIST OF FIGURES: Change x-xi to x
Page 3, line 3	delete "such as Malathion", move (Hill, 1987) to after "... in the environment".
Page 4, item 3:	Change botanical to botanicals, and delete "extracts".
Page 5, lines7 and 8:	Add Linnaeus to *O. sativa* and Steudel to *O. glaberrima* as authorities i.e. *Oryza sativa* (Linnaeus) and *Oryza glaberrima* (Steudel).
Page 10, line 6:	Change *Rhyzopertha domonica (Fabricicus*) to *Rhyzopertha dominica (Fabricius).*
Page 14, line 3-5:	Change sentence to read "Fumigation is still one of the most effective methods for disinfesting stored food, feedstuff and other agricultural commodities from insect infestation but gives no lasting protection".

Page 17,para 3, line 10:Add "abrasive" in front of powders and dusts.

Page 17 bottom line:	Change poses to possess.
Page 36 4.2.3:	Change "Effect of storage time on weevil mortality" to "Effect of storage time of treated rice seed on adult weevil mortality"

Alieu Mortuwah Sartie.

Abstract

Food security and the maintenance of seed quality from harvest to planting are key issues for peasant farmers. In Sierra Leone, up to 28% of rice seed can be damaged by rice weevil in the six months storage period. The use of chemical insecticides to control this insect is not practical for traditional farmers. Some tribes use pepper powder (*Capsicum spp.*) as a seed protectant. In this study, I have compared the effects of neem (*Azadirachta indica*) oil, neem powder, pepper (*Capsicum frutescens* cv. "Habanero") powder and lentil (*Lens culinaris* cv. "Raja") powder on the survival of adult rice weevil (*Sitophilus oryzae*) and weevil offspring during rice (*Oryza spp.*) seed storage, and on the germination of the rice. Treatment of stored rice with neem oil, neem powder and lentil powder gave some protection from rice weevil damage. Neem oil at the rate of 0.005ml/kg rice seed effectively controlled weevil damage without reducing the seed germination. Lentil and neem powders at the rate of 0.02g/kg rice seed gave effective protection against rice weevil damage with no reduction in viability of the seeds. Pepper powder did not kill adult rice weevil. Neem oil reduced the development of weevil offspring in rice seed, but the powders of neem, lentil and pepper did not. Low relative humidity of 42.5% in seed storage environment and a reduction in seed moisture content below 10% enhanced the mortality of adult rice weevils on rice seed.

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"Ask, and you will receive; seek, and you will find; knock, and the door will be opened to you. For everyone who asks will receive, and anyone who seeks will find, and the door will be opened to those who knock".

Matthew 7: 7- 8 Good News Bible.

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DEDICATION

This work is dedicated to my father, Mr. Moinina Sartie and my mother, Mrs. Mattu Jeneba Sartie for the many tribulations they went through to put me on the academic ladder. I pray that they will live long to enjoy the fruit of their labour.

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INTRODUCTION

1.1 Importance of rice

Rice (*Oryza spp.*) is one of the most important food crops of the world, and is produced on all continents. In-fact, it is the most important food for mankind (Way and Bowling, 1991). It was an important food even before the dawn of written history (Adair, 1972). However, a comparatively small amount of rice moves in world trade because a high percentage of the world rice crop is consumed in the countries where it is produced. Annual world rice production amounts to approximately 563 million metric tons (t) grown on roughly 150 million ha (FAO, 1998). The average annual amount of rice in world trade is just over 28 million t (FAO, 1998), less than 5% of the total world production.

The area of rice production is second only to the area devoted to wheat, but in terms of importance as a food crop, rice provides more calories per hectare than any other cereal crop (McDonald and Copeland, 1997). The world rice crop area is only about 58% as much as that for wheat. However, the average yield of rice is much higher than for wheat, so that the total world production of rice is only about 6% less than for wheat. Over 95% of the world production of rice is used for human food (Adair, 1972).

Rice is the principal cereal food in Asia and some countries in Africa and Latin America. It is used as a food in all countries in the world (Adair, 1972). Rice is the staple food for more than half the world's population, mostly in Asia countries (Alam, 1970). Although it is the staple food, or fast becoming so, in most West African countries, scarcely any are self-sufficient in rice. Demand is increasing faster than production, mainly due to use by an expanding urban population and ease of cooking and storage (WARDA, 1994).

In Sierra Leone, rice is the staple food grown by more than 95% of rural families. However, domestic rice production fell from 508,000t in 1989-91 to 411,000t in 1998 (FAO, 1998). Imported rice is estimated to represent an average of 20% of total consumption (120,000t in 1991) and the last year of rice export was 1953. Rice importation has been on the increase since 1980 from 62,119t to 243,200t in 1995 (FAO/GIEWS, 2000). This trend is continuing. The area under rice in 1999 was restricted mainly by shortages of rice seeds. In some cases, the quality of seeds was also very poor (FAO/GIEWS, 2000).

Dichter (1976) estimated that in sub-Saharan regions of Africa, losses of food grains during storage at farm or village level can amount to 25-40% of the harvested crops. This wastage of food is unacceptable when so many people in Africa remain hungry.

Rice cultivation is being promoted by national and international organisations but increased production currently comes from expansion of the cultivated area and not from higher yields per unit area. Rice yields in West Africa are well below the world average of 3.7 t/ha, due to a combination of production economics, limited research impact and the complexities of the rice growing environment (WARDA, 1994).

1.2 Control problem in insect pests

Insect pests are a serious limiting factor in rice production which must be protected and increased to foster human health and world peace and stability (Cogburn, 1991). The rice weevil, (*Sitophilus oryzae*) is the most destructive pest of stored grain in the world (Bhuiyah *et al.*, 1990), and is one of the most serious pests of stored rice, especially in the tropics where it causes tremendous losses (Grist and Lever, 1969). It causes about 5–28 % storage loss of seed rice in northern Sierra Leone (Sartie, unpublished, 1998). Adult weevils feed mainly on the endosperm, reducing the carbohydrate content and the larvae feed preferentially on the germ of the grain, thus removing a large percentage of the protein and vitamins (Bello et al., 2001).

The use of pesticides to control rice weevil has not been very successful as most farmers are too poor to pay the costs of these chemicals, and insects readily develop resistance to the chemicals (Champ, 1968; Lale, 1995; Arthur, 1996)). In addition, the chemicals are hazardous to their users (Ecobichon *et al.*, 1990) and may also leave harmful residues in food commodities (Lale, 1995). The long-term cost of using insecticides is inestimable when the possible effects of pollution are considered. Pollution poses a problem to human health since some pesticides, such as Malathion, persist in the environment and can be accumulated in our tissues with multiple, unpleasant and even lethal consequences (Hill, 1987). For this reason, some pesticides such as DDT have been banned from many countries. When an insecticide is first used it may be very effective but, over the years, becomes less so if insects become resistant to it. Repeated use of single chemical on one target pest increases the likelihood of resistance developing. With the present rate of use, many insect pests may before long become resistant to all known pesticides (Kumar, 1984). Management of resistance is one of the most pressing problems of modern pest control (Kumar, 1984). According to the WHO (1976), resistance is probably the biggest single obstacle in the struggle against vector-borne diseases and is mainly responsible for preventing successful malaria eradication in many countries. According to Georghiou and Taylor (1977), the number of species of insects and acarines in which resistant strains have been reported has increased from 1 in 1908 to 364 in 1975.

Pesticides may also kill non-target species. The use of pesticides in the attempted control of cocoa mirids has resulted in killing of a number of key stone parasites and predators of the pest. This has probably contributed to the emergence of a new pest of cocoa, the shield bug *Bathy coeha* which is reported to cause an 18% loss in the yield of cocoa in the Eastern and Brong-Ahafo regions of Ghana (Owusu-Manu, 1971).

As a result of these problems with synthetic chemicals, there has been a renewed interest in the use of natural plant products in the protection of stored agricultural products against weevils. Most of these natural products, especially the edible ones, are relatively safe, cheap and readily available in the tropics (Lale, 1995) and also in temperate regions. The use of natural plant products has been reported successful in the control of rice weevil in stored maize and wheat (Su, 1977; Ivbijaro, 1983b; Abdel-Kawy and Gharib, 1992), but their role in controlling weevil during rice seed storage is not clear.

1.3 Objectives

The objectives of this research were;

1. To assess the effectiveness of using the natural plant products like neem oil, neem powder, lentil seed powder and pepper powder to control adult rice weevil during rice seed storage.
2. To determine the effect of the botanicals on the development of rice weevil offspring.
3. To determine the effect of these botanical extracts on the viability of the rice seed.

2

LITERATURE REVIEW

2.1 Rice

2.1.1 Origin and Species

Rice is a grass (Gramineae) belonging to the genus *Oryza* (Linnaeus.). It has been cultivated for countless ages, but its origin is still not clear. Botanists base their evidence of the origin of rice largely on the habitats of the wild species, and they presumed that the cultivated species have developed from certain of the wild rices (Grist, 1986). About twenty-five species of rice are distributed through tropical and subtropical regions of Asia, Africa, Central and South America and Australia (Grist, 1986). There are only two cultivated species, *O. sativa* which is native to South and Southeast Asia, and *O. glaberrima* which originated in West Africa (WARDA, 1994; Rao and Jackson, 1995). Morphologically there are only small differences between these species, mainly in ligule size and glume pubescence, but *O. glaberrima* always has a red pericarp (Grist, 1986). *O. glaberrima* also lacks secondary branches in the panicle (WARDA, 1994). The most commonly cultivated is *O. sativa*. *O. glaberrima* is cultivated in pure or mixed stands with *O. sativa*. There are three ecogeographic races of rice within *Oryza sativa* that exist today: indica (tropical and subtropical cultivars), japonica (temperate cultivars) and javanica (cultivars native to Indonesia) (Chang and Li, 1991; McDonald and Copeland, 1997). The cultivated forms of *Oryza* are almost invariably annuals. However, when moisture and temperature are suitable some varieties may produce further growth after the first harvest. Should a second crop be reaped from such growth it is termed a ratoon crop (Grist and Lever, 1969).

The crop is grown over a wide range of climatic, soil and water conditions, from wet tropical to regions of semi-arid, warm-temperate climate; in heavy clays or poor sandy soils; on dry land or in swamp with water that may be 450 to 600 cm deep; in fresh or brackish water. The thousands of varieties of rice that exist account for this cosmopolitan nature, for it is true to say that a variety may be found to suit almost any condition, provided that the plant is subject to abundant sunshine and given water sufficient for the requirements of the particular variety (Grist, 1986).

2.1.2 Structure and composition of rice seed

The rice seed is a caryopsis or one seeded dry indehiscent fruit. It is composed of the hull (lemma and palea), caryopsis coat (pericarp, seed coat and nucellus), endosperm and embryo. The seed is 7-10mm long and 3.5-4.0mm wide and 2.0mm thick with an embryo that is extremely small relative to other cereal seeds (Mcdonald and Copeland, 1997). The proportions of the principal parts of a matured rice kernel are: germ (3.5%), pericarp (1.5%), aleurone (4-6%) and endosperm (89-94%) (Sauer, 1992). Rice is comparatively high in caloric value and niacin, and is comparatively low in protein, although rice protein has a fairly good balance of the essential amino acids (Adair, 1972). The composition of rice is influenced by variety and environment (McCall, *et al.*, 1953). Among cereals, rice has a comparatively high content of essential amino acids and fairly low content of glutamic acid and some other nonessential amino acids. The nutritional level of rice is comparatively high among cereals and other grain and root crops (Adair, 1972).

2.1.3 Rice seed storage

2.1.3.1 Storage conditions

Rice seed exhibits orthodox storage behaviour and will maintain viability for long periods under cool dry storage. It is generally stored in the hull, and the silica-rich hull (consisting of lemma, palea, and sterile lemmas) helps protect the caryopsis by deterring granary insect penetration and gaseous exchange. However, the hull adds to the grain's hygroscopic properties (IRRI, 1988). Rough rice (lemma and palea and rice caryopsis) can be safely stored at moisture contents below 14%, with 12% being recommended (McDonald and Copeland, 1997). About 70% of the world's rice is produced and consumed in the humid areas of the tropics and subtropics. For practical purposes, maintaining a room at 20°C and 60% RH will provide a life expectancy of three years or slightly longer for seed initially dried to 12% moisture content (IRRI, 1988). Rice seed life span can go as far as over 28 years if dried below 11% moisture content and stored at 2°C (Chang, 1991), but this longevity varies within varieties (Sikder, 1988). Moisture contents commonly accepted for "safe" storage of rough rice are 13% for less than six months, and 12% for longer-term storage (Schroeder and Calderwood, 1972). The ideal seed moisture equilibrium for storage of rice is maintained when rice seed is dried to moisture content between 14% and 12% and stored at 25°C and a relative humidity of 60% to 75% (Agrawal, 1986). For safe storage of rice, the seed should be healthy, mature, of high viability and low moisture content, and ideally be stored under low humidity and temperature conditions (Alam, 1970). The moisture content of the seed increases as the RH increases, and as the seed moisture content increases, the germination percentage decreases (Alam, 1970), due to greater colonization by storage fungi (Pratima, 1998).

Although temperature has little effect on the moisture content of the seeds at a given relative humidity, it does have a decided effect on the rate of deterioration of the seed (Alam, 1970). Cogburn *et al.* (1983) stated that higher temperature during storage causes moisture migration, and recondensation at the surface of the rice bed creating a zone of wet rice. The combination of heat and moisture produces ideal conditions for fungi growth, and results in discoloured grains. Pratima (1998) reported that the development of fungi on rice seed during storage is greatest at 20-30°C and least at 40°C and 15°C, but infection varies with rice varieties. Among the storage fungi, *Penicillium spp.* is active at 15-25°C, while *Aspergillus spp.* being thermophilic, is active at 40°C (Pratima, 1998). The grain must be adequately cooled during storage to prevent mold growth and insect activity. Although there is no one optimum storage temperature, mold growth is inhibited below 21°C (for rice at 13% moisture content), and insect activity is considerably reduced at temperature below 16°C (Cogburn, 1991).

2.1.3.2 Storage facilities

Storage facilities take many forms, ranging from piles of unprotected grain on the ground, underground pits or containers, and piles of bagged grain, to storage bins of many sizes, shapes and types of construction. Major classifications are farm storage, bin sites, and elevators (Sauer, 1992). In Sierra Leone, rice seed is generally stored in jute bags and wooden boxes (Sartie, 1998). Storage structures are classified as "flat" or "vertical" depending upon their height in relation to the width or diameter. A commercial bulk storage system designed for long-term safe storage of rough rice must (1) provide proper aeration to prevent spontaneous heating, and (2) maintain the rice grain at low moisture content to protect it from fungus and insects (Wang and Luh, 1991). The spontaneous generation of heat is of serious consequences in bulk storage, since rice has a porous, granular structure and low thermal conductivity. If no convective currents are present, the grain mass practically insulates itself (Wang and Luh, 1991). Thus, respiration could lead to heat-damaged grains if proper care is not taken.

2.1.4 Effects of exogenous compounds on rice seed germination.

Fumigation of crop seeds with chemicals during storage reduces weevil infestation, but also reduces the quality of the seeds. Poor germination and seedling abnormalities in stored seeds fumigated with phosphine and methyl bromide at varying doses and exposure periods have been reported in green gram (*Vigna radiata*) (Gupta and Kashyap, 1995), in cotton (Khanna *et al*., 1992), in wheat (Khanna and Yadav, 1987; Scudamore and Goodship, 1992) and in rice (Krishnasamy and Seshu, 1990). Treatment of rice seed with herbicides such as Butachlor-2.4-D and Thiobencarb reduces root and shoot growth respectively, leading to abnormal germination of the seeds (Mabbayad and Moody, 1992).

There are reports in the literature of plant extracts having promotory and toxic effects on the germination of rice seed. Prasad and Srivastava (1991) reported that extracts from certain weeds like goat weed (*Ageratum conyzoides* L.), barnyard millet (*Echinochloa crus-galli* L.), dayflower (*Commelina benghalensis* L.), burweed (*Xanthium strumarium* L.), white sedge (*Lantana camara* L.), and nut grass (*Cyperus rotundus* L.) not only reduce and delay germination of rice seed, but can also increase the development of abnormal seedlings during germination. The inhibitory effect of root and shoot extracts of browntop millet (*Brachiaria ramose),* smallflower umbrellaplant (*Cyperus difformis),* rice flatsedge *(Cyperus iria),* nut grass (*Cyperus rotundus),* blinding-tree (*Excoecaria agallocha),* lesser Fimbristylis (*Fimbristylis miliacea),* water primrose (*Ludwigia parviflora),* pickerel weed (*Monochoria vaginalis),* torpedo grass (*Panicum repens),* and bulrushes (*Scirpus spp.*) on seed germination in rice is also reported (Sobhana *et al*., 1990; Rajangam and Arumugam, 1999). In contrast, Jeeva and Ramabadran (1995), report an increase in germination and a reduction in seedling abnormalities for rice seeds infested with rice weevil, and treated with paradise flower (*Caesalpinia pulcherrima)* and moonflower (*Ipomoea crassicaulis)* extracts. Rajangam, (2000), reported that extracts from leaves of *Suaeda maritime* (Dum) increased plumule and radicle length of rice seed during germination, and he suggested that these effects might be due to the presence of phenolic acids. In treating rice seed with extracts of glory-tree (*Clerodendrum viscosum*) against a seedborne pathogen, *Curvularia lunata*, Parimelazhagan and Francis (1999), observed significantly higher seed germination rates and a greater increase in root and shoot lengths of the treated seeds. Neem treatment has been reported to have no effect on rice seed germination, and can in fact promote seedling vigour (Kareem *et al*., 1989). Mehta *et al*. (1999) also reported an increase in germination and seedling growth of rice seed treated with plant extracts. The use of pepper (*Capsicum frutescens*) to control insect pests in stored wheat had no effect on the seed germination and vigour (Lertsilpmongkol *et al*., 1997). Pal and Basu (1993) also reported that the treatment of high vigour (harvest-fresh) wheat seed with red chilli pepper (*Capsicum frutescens* L.) powder at 0.05% wt/wt significantly slowed down the deterioration of seeds in storage. This suggests that the use of pepper does not negatively affect the viability of treated seed.

2.2 Pests of rice

2.2.1 Insect pests

Insects are the most successful group within the animal kingdom; over 80% of all living animals are insects. They are man's chief competitors on earth as they eat his crops and some of his other possessions, and also transmit diseases to him and his domesticated animals (Kumar, 1984). Many insect species infest rice. Lamb (1974), in listing the pest of tropical crops, stated that no less than 4098 species of insects have been recorded on rice alone. Grist and Lever (1969), recorded over 800 insect species that attack standing and stored rice. Some 30 species of insects have been found to infest stored rice and rice products, but only a few of these are a serious menace to rice in good condition (U.S. Dept. Agric., 1968). Stored grain insect pests can cause reductions in weight, quality, commercial value and seed viability. Seventy-five percent of these insects are coleopterans (Vinuela *et al*., 1993) and the most damaging species of storage insects are in the genera *Sitophilus* and *Tribolium* (Marsans, 1987; Khan and Selman, 1988; Pinto *et al.*, 1997). Insect damage in stored grains and pulses may amount to 10-40% in countries where modern storage technologies have not been introduced (Shaaya *et al.*, 1997). According to Pathak and Dhaliwal (1981), these pests account for rice losses of 24% while Cramer (1967) reports 35%. The most destructive insect pests of rough rice are the lesser grain borer, *Rhyzopertha domonica* (Fabricicus); the rice weevil, *Sitophilus oryzae* (Linnaeus); and the Angoumois grain moth, *Sitotroga cerealella* (Olivier). The lesser grain borer and the Angoumois grain moth are capable of entering the endosperm through the hull, but the rice weevil attacks only grains with broken hulls or with hulls that failed to close properly after blooming (Schroeder and Calderwood, 1972). Weight losses greater than 10% in rice stored only one year have been attributed to insect pests. The importance of rice insect pests can be grasped by the fact that US$910 million were spent annually during 1988 and 1989 in attempts to control their activities worldwide with insecticides (Woodburn, 1990). Furthermore, the value of insecticides applied to rice (both in the field and in storage) in 1988 was 15% of the total world usage of insecticides (Woodburn, 1990).

2.2.1.1 Rice weevil

The rice weevil, *Sitophilus oryzae* L. belongs to the sub-family Calandrinae, and family Curculionidae. It is characterised by the elongation of the front parts of the head into a snout or proboscis, the tip of which bears the mouthparts. This structure is utilised for both feeding and to facilitate oviposition (Cogburn, 1991). The adult is brownish black in colour and has a dull appearance. Larvae are 3.5-4.0mm in length, often with a shiny appearance while most adults have four, more or less clearly defined, large reddish patches (spots) on the elytra (outer hard cover of wing). It has achieved economically important pest status as it causes a high loss of cereal seeds, and is one of the most important pests of post-harvest cereals in storage (Stephensons, 1983). Champ and Dyte (1976) stated that the rice weevil is the most important storage pest of raw cereals in the world. The species not only occurs throughout the tropical and warm temperate areas of the world, but also extends into certain cooler areas but is unsuccessful in regions with very high summer temperatures (Longstaff, 1981).

The rice weevil attacks all grains, offals, macaroni, tapioca, caked flour and meal (but not when free-flowing), mixed feed and vetch seed, but it is unable to breed in paddy with an intact husk, even when the moisture content is high (Grist and Lever, 1969). It is also reported that the rice weevil does not attack grains with intact hulls (Breese, 1960; Cogburn *et al.*1983). It has been found to also attack split peas and hard grain products like pasta but can only breed in whole grain cereals (Longstaff, 1981). It attacks almost any stored cereal seed, the only criterion being that an individual seed being large enough to allow complete development of a larva (Scott, 1984). The female chews a cavity in the grain into which one egg is deposited, then seals the cavity before going to the next grain. The number of eggs recorded per female laid over a period of several weeks varies from 300 to 576 (Grist and Lever, 1969; Hill, 1987). The larvae spend all their life inside the grain, eating their way close to the surface in preparation for pupation. Adults emerge inside the grain and eat through the remaining thin layer to escape and mate. The life cycle takes about one month at 26°C (Scott, 1984) (Plate 1). The adult weevils can live 4 to 5 months in the tropics but do not survive temperate winters. Bhuiyah *et al.* (1990) reported that the life span of rice weevil varies from 143 to 150 days for male and from 148 to 155 days for female, and the adult longevity also varies from 114 to 115 days for male and from 119 to 120 days for female. The number of generations varies from five to seven per year (Grist and Lever, 1969).

Both the larva and adult are destructive; the former feeds within the rice grain, reducing it to powder, or hollowing out the endosperm. The rate of feeding, however, depends on temperature, humidity, the type of food product and the pest density (Stephensons, 1983). The oviposition rate of rice weevil is dependent on grain characteristics and variety. Ewer (1945) observed that weevils lay eggs more rapidly in larger grains than smaller ones, and Russell (1968) in studying the effect of rice variety on oviposition, reported that soft grain varieties received 2-6 times as many eggs as hard grain varieties. The daily oviposition rate of rice weevil at 25°C increases with relative humidity. The critical point is at about 60% relative humidity below which egg laying declines rapidly and mortality is high (Howe, 1952). The range of relative humidity suitable for rice weevil development is between 60 to 100% (Tsai and Chang, 1935), and that below 60% relative humidity would result in the death of adult rice weevils and also prevents the development of larva populations (Khan *et al.*, 1993), but Reddy (1950) concluded that egg laying could also occur at relative humidity as low as 52%. Khan (1948) determined that 50% relative humidity irrespective of temperature was unfavourable for larvae development and that a temperature of 15°C was fatal for this insect. Nakakita and Ikenaga (1997) also reported that the population increase of rice weevil would be completely suppressed at 15°C. The optimum conditions are 27-31°C with humidities above 60% relative humidity (Hill, 1987). Mathleen (1938) and Moutia (1942) reported that rice weevils can develop in maize grains having moisture content as low as 8.5%, but did not mention the relative humidity under which this would occur. However, Davidson (1940), Harris (1943) and Birch (1945) have all recorded 10% moisture content as the minimum requirement for rice weevil development. Khan *et al.*, 1993 suggested that the range of seed moisture content for breeding of rice weevil is restricted to10%-16%.

Weevils can fly and infest the nearby field crops (Kirk, 1965) so that harvested and unthreshed paddy can be carried into store with developing insects within it. Weevils can also infest newly harvested grains placed in already infested farmers bins, warehouse or terminal elevators (Bhuiyah, *et al.*, 1990). Bhuiyah *et al.* (1990), reported that the infestation of cereals by weevils begins in the field, but did not indicate the type of cereal or the weevil species. Longstaff (1981), however, stated that rice weevil rarely attacks field crops since the conditions are unfavourable for its development.

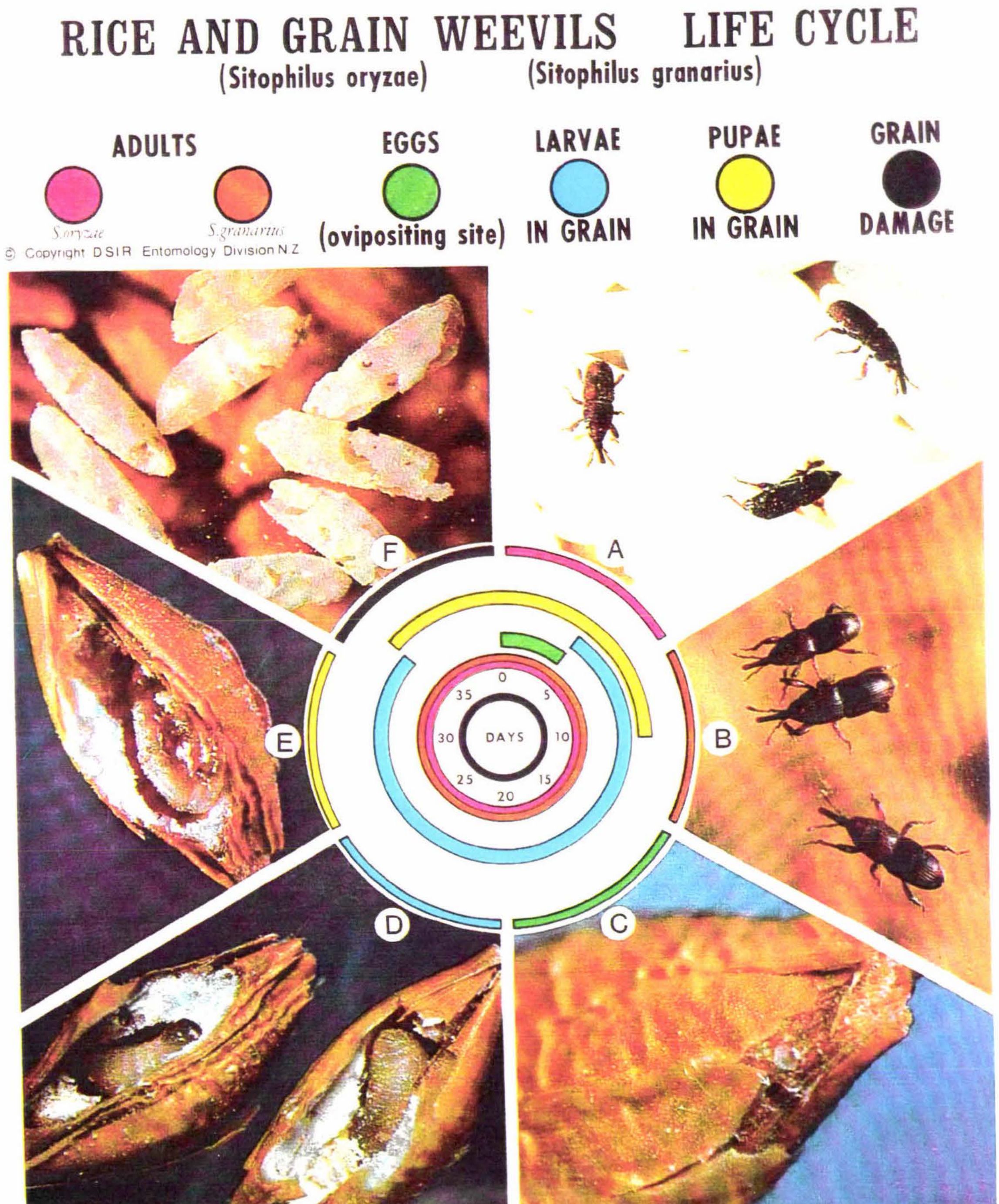


Plate 1: Life cycle of rice weevil (*Sitophilus oryzae*) (Charles, 1976).

2.3 Storage pest control

2.3.1 Chemical insecticides

The control of insect pests on stored products has been primarily through the use of fumigants and residual chemical insecticides to augment the more obvious approach of hygiene (Brooker *et al.*, 1992; Adane *et al.*, 1996). Fumigation is still one of the most effective methods for the protection of stored food, feedstuff and other agricultural commodities from insect infestation. Lately, however, the number of fumigants for insect control has decreased drastically as problems of insect resistance have intensified and as mounting social pressures against the use of toxic chemicals have limited the introduction of new compounds. At present, methyl bromide and phosphine are the widely used fumigants. Methyl bromide has been identified as a major contributor to ozone depletion (WMO, 1995), which casts doubts on its future use in insect control (Shaaya *et al.*, 1997). There have been repeated indications that certain insects including the rice weevil have developed resistance to phosphine (Nakakita and Winks, 1981; Mills, 1983; Tyler *et al.*, 1983). Many insects and mites are capable of tolerating virtually all pesticides available for their control as a result of cross and multiple resistance (Metcalf, 1980). In addition many of the gaseous and liquid insecticides pose possible health hazards (to warm-blooded animals) and a risk of environmental contamination. Thus, there is an urgent need to develop safer alternatives that can replace toxic fumigants, yet are simple and convenient to use (Shaaya *et al.*, 1997).

The excessive use of conventional chemical insecticides has resulted in a number of serious problems in addition to resistance to chemical insecticides. These are elimination of economically beneficial insects, environmental contamination, toxicity to humans and wildlife and higher costs of grain production (Khan and Selman, 1989). Nakakita and Ikenaga (1997) suggest that conventional chemicals, either grain protectants or fumigants, have been or will be restricted globally for use in the control of stored product insects because of problems related to the persistence of toxic residues in food grains, the development of insect resistance and adverse environmental impact. Today, the use of synthetic insecticides for the protection of stored crop products has declined significantly in developing countries due mainly to high cost and the drastic reduction or complete withdrawal of subsidy by governments (Lale, 1995).

2.3.2 Plant extracts (botanicals)

Plants are a rich source of compounds that have insecticidal activity (Arnason *et al.*, 1989). Plant materials with insecticidal properties have been used for generations throughout Africa, Asia and the Americas (Belmain *et al.*, 1999). Prior to the discovery of the organochlorine and organophospate insecticides in the late 1930s and early 1940, botanical insecticides were important products for pest management in industrial countries (Isman, 1997). The importation of plant material or derivatives thereof for use as insecticides represented a considerable enterprise: for example, over 6700 U.S. tons of *Derris elliptica* roots was imported into USA from Southeast Asia in 1947, but this had decreased to 1500 tons by 1963 (Wink, 1993) assuming a steady decline over the years rather than a sudden drop. This reflects the extent to which botanicals have been replaced by synthetic insecticides. At best, botanical insecticides presently constitute 1% of the world insecticide market, but annual sales growth in the range of 10-15% is possible (Isman, 1997). The impact of botanicals will perhaps be most noticeable in the home – and – garden sector, where they might conceivably achieve as much as a 25% market share within five years (Isman, 1997). Botanical treatments are particularly relevant for small-scale subsistence farmers during post-harvest storage of their commodities (Dales, 1996).

The use of locally available plant materials for stored-product protection is a common practice, and has more potential in the subsistence and traditional farm storage conditions often found in developing and underdeveloped countries (Poswal and Apka, 1991). In these countries, the cost of developing or registering new pesticides and their availability is a cumbersome or costly process. Therefore, botanicals may play a significant role in stored-product protection (Weaver and Subramanyam, 2000).

Many plants have insecticidal properties based on compounds of relatively low toxicity to mammals. These may be good candidates for large-scale use because they present lower toxic risks. Also, if developed as pure compounds or in very simple mixtures, they may result in a residue that is neither highly complicated nor difficult to quantify, which is an important consideration for evaluation of the safety of the treated commodity for consumers. Admixtures of actual plant tissues may also pose lower risk to consumers because most of the material can be physically removed before use (Weaver and Subramanyam, 2000). These products are also safe because their use on harvested commodities may not result in environmental contamination, because treated postharvest commodities are contained in enclosed structures. In the case of sowing treated seeds in the field, there has been no report in the literature of any hazardous effect on the environment.

Plants are a vast storehouse of potentially useful products, and indeed, many laboratories worldwide have screened thousands of species of higher plants not only in search of pharmaceuticals, but also for pest control products. This is illustrated by the studies of Arnason *et al.* (1989) and Van Beek and Breteler (1993) which have pointed to numerous plant species possessing potential pest controlling properties under laboratory conditions. However, the step from the laboratory to the field eliminates many contenders, even when judged only on their efficacy under realistic field conditions (Isman, 1997).

The use of plant parts and derivatives, for example wood-ash, to control insect pests of stored products and backyard vegetables, as well as mosquitoes, has been long standing practice in Africa agricultural culture (Owusu, 2001). Unfortunately, most of these methods, though effective, have not been fully ultilized. Plants, such as neem, both leaves and seeds, (Saxena, 1986; Lowery and Isman, 1993), as well as others like gum (*Eucalyptus spp.*), peppermint (*Menthabpiperitia spp.*), sage (*Salvia sclarea*), bay laurel (*Pimenta racemosa*) (Shaaya *et al.*, 1991; Owusu, 1993; Talukder and Howse, 1993; Weaver *et al.*, 1995), have been successfully used to control insect pests (Owusu, 2001). Plant products used as protectants of stored agricultural commodities are normally obtained from leaves, roots, fruits and seeds, and to lesser extent from bark and stems (Dupriez and De Leener, 1989). Seeds and fruits, however, appear to be the commonest and perhaps the most important sources of natural chemicals for the management of these pests (Su *et al.*, 1972a, b; Taylor, 1975; GTZ, 1980; Ivbijaro, 1983a, b; Franke, 1985;

Ivbijaro and Agbaje, 1986; Lale, 1991, 1992a, b). Currently, it is impracticable to consider using admixtures in large bulks of grains because of the large amounts of plant material required, therefore, only extremely active materials should be grown for large-scale use (Weaver and Subramanyam, 2000).

Irrespective of the part of plant from which they are obtained, powders, oils and aqueous solutions are the three main formulations of plant products used for the protection of stored commodities (Lale, 1995).

Powders are prepared by harvesting the appropriate plant part which is then sun-dried and pulverised by means of pestle and mortar, an electric kitchen mill or coffee blender. The use of plant products in the form of powders in the control of stored product insect is perhaps the most convenient: the powders are easy to apply, the commodities remain clean compared to those treated with fixed plant oils, and the moisture content of the food grain is not increased as it is if aqueous solutions are used (Lale, 1995). Powders of various species have been used successfully for the control of different species of stored product Coleoptera like cowpea bruchid (*Callosobruchus maculates*), rice weevil, rust-red flour beetle (*Tribolium castaneum*) and maize weevil (*Sitophilus zeamais*) (Jotwani and Scircar, 1965; Taylor, 1975; Ivbijaro and Agbaje, 1986; Lale, 1992a,b, 1993). Powders or dusts kill insects by destroying the water-proofing properties of their cuticles. This permits a lethal rate of water loss from the insect body (Kumar, 1984). The application rates range generally from less than 1g/kg of grain to 20g/kg of grain: the amount of powder does not usually constitute more than 2% of the weight of grain (Subramaniam, 1949; Jacobson, 1975; Jilani and Su, 1983; Mishra *et al.*, 1984; Stoll, 1988).

Oils are usually extracted from flowers, fruits, seeds or roots by means of organic solvents and used very much like emulsifiable concentrates. Both fatty and essential oils are used in the control of stored products against Coleoptera (Lale, 1995). Plant oils can play an important role in stored-grain protection and reduce the need for, and risk associated with, the use of insecticides (Shaaya *et al.*, 1997). Many plants and their extracts are known to poses insecticidal activities, which are frequently present in the essential oil fraction

(Brattsten, 1983; Schmidt *et al.*, 1991; Shaaya *et al.*, 1991). Most of the essential oil constituents are monoterpenoids, which are secondary plant chemicals and considered to be of little metabolic importance (Shaaya *et al.*, 1997). The use of plant oils in storage insect pest control is an ancient practice (Qi and Burkholder, 1981) and it is done in many countries in Asia and Africa.

Oils are often used in the control of stored product insects because of their relatively high efficacy against all stages in the life-cycle of the insects (Lale, 1995). This method is convenient and inexpensive for the protection of stored seeds in households and on small farms (Shaaya *et al.*, 1997). Fatty oils, however, reduce the cleanliness and visual appeal of treated commodities, and some plant oils, especially non-edible oils such as neem seed oil, have odours and tastes that may taint commodities intended for human consumption. There are concerns of oil rancidity or effects on germination potential when larger amounts are deployed to control longer-lived species (Qi and Burkholder, 1981; Don Pedro, 1989). They do not detrimentally affect seed germination (Jacobson, 1975; Singh *et al.*, 1978; Sighamony *et al.*, 1986; Lale, 1987) except when used in higher concentrations (Qi and Burkholder, 1981). Application rates of plant oils are usually given in ml/kg of grain, with effective rates typically being 1 to 10 ml per kg of grain (Lale, 1995).

2.3.2.1 Neem products

The neem tree, *Azadirachta indica* A. Juss (Meliaceae) is native to India and Pakistan, and grows widely throughout Indo-Malaysia, tropical Africa and the Caribbean (Schmutterer, 1990; Ascher, 1993). It is a haven for many beneficial organisms including honeybees, wasps, spiders, birds, bats, mycorrhizae, and soil-inhabiting collembola, pauropods, mites, millipides and earthworms (Saxena, 1999). The neem tree has many medicinal uses. Notable among these are its use as an antiseptic and diuretic (Sclar, 1994). It has been used to cure many illnesses from diabetes to syphilis, and is widely relied upon by herbalists in its native habitat (Jacobson, 1989; Koul, 1990). The use of neem as a source for natural insecticide was discovered approximately 30 years ago (Ascher, 1993). Neem is particularly valuable in averting losses caused by crop and stored-product pests and is effective against a wide variety of noxious crop pests and pathogens (Saxena, 1989;

Ascher, 1993; Schmutterer, 1990; 1995), yet it is relatively safe to non-target organisms, including humans (Jacobson, 1995; Schmutterer, 1995) or any warm-blooded animals (Schmutterer, 1988). Every part of neem is bitter in taste; the bitterness is due to the presence of an array of complex, bioactive compounds called 'limonoids' or 'triterpenes' (Saxena, 1999). Azadirachtin is a compound classified as a limonoid and is the major active constituent in neem. Azadirachtin is well known for its antifeedant, toxic and growth regulating effects on insects (Xie, 1995). Schmutterer, (1988) suggests azadirachtin can be regarded as an anti-hormonal active compound.

Neem extracts have been shown to act on various insects in the following ways (Anon, 1992):

-disrupting or inhibiting the development of eggs, larvae, or pupae;
-blocking the moulting of larvae or nymphs;
-disrupting mating and sexual communications;
-repelling larvae and adults;
-detering females from laying eggs;
-sterilising adults;
-poisoning larvae and adults;
-detering feeding;
-blocking the ability to swallow;
-interfering with metamorphosis at various stages;
-inhibiting the formation of chitin.

2.3.2.1.1 Neem oil

Studies of neem oil from around the world show it controls insects by intervening at several stages of their life (The Tribune, 2000). Oil and powder obtained from neem seeds have been reported to provide sustained protection to stored grains such as wheat and maize (Jotwani and Scircar, 1965; Ivbijaro, 1983a, 1983b; Makanjuola. 1989; Ogunwolu and Idowu, 1994; Lale and Ajayi, 1996; Ogunwolu and Odunlami, 1996). The treatment of cowpea seeds with neem seed oil at rates of 1.0%, 1.5% or 2.0% wt/wt reduced seed damage by cowpea bruchid from over 25% in control to less than 10% (1.0% wt/wt neem oil application rate) and less than 5% (1.5% or 2.0% wt/wt) respectively (Lale and Mustapha, 2000).

2.3.2.1.2 Neem powder

Wheat grain is protected against the rice weevil, the lesser grain borer, *Rhizoperta dominica* (Fabr.), and the khapra beetle, *Trogoderma granarium* (Ev.), for nearly a year when mixed with powdered neem seed kernel at a rate of 0.01g to 0.02g/kg seeds (Jotwani and Scircar, 1965). Neem seed powder had been found to be very effective not only as a contact insecticide, but also as a repellent. The repellency increases with the concentration of the powder in the product, and Ignatowicz and Wesolowska (1996) noted 5% concentration, by weight, as the most effective against stored product pests. As a contact insecticide, a 5% concentration of neem seed powder proved very effective against adult lesser grain borers, on wheat seeds (Patel *et al.*, 1993).

2.3.2.2 Pepper

The hot pepper (*Capsicum frutescens*) is one of the five domesticated species of peppers. Hot peppers originated in South America, and today they are grown all round the world. They are a staple in most African countries, including Sierra Leone, where several varieties are cultivated for local use. There are many varieties of hot peppers, growing large and bushy, bearing fruits of interesting colours and shapes and with varying degrees of hotness. They are commonly known as capsicum or chilli pepper in some parts of the world. In Sierra Leone, the varieties grown only have local names, but some are similar in appearance to Habenero. Habenero is more widely cultivated than any other variety, and it is one of the hottest. The fruit of the Habanero variety has a smoky flavour and is usually red or yellow in colour (http://www.home.earthlink.net/~redbudfarm/Hot.html). Habaneros have the highest concentration of capsaicin, the active ingredient in pepper. They are also the most dangerous in terms of burns. It is particularly dangerous when it comes into contact with sensitive parts like the eyes.

Black pepper (*Piper nigrum*) is another species of pepper, but it is not grown in Sierra Leone. The active ingredient in black pepper is piperine.

The use of pepper as a traditional storage protectant for cereals, has been reported (Sartie, 1998; Hell *et al*., 2000), but the varieties used were not stated. Pepper extracts have been used to protect wheat against rice weevil and lesser grain borer over a period of 60 days (Sighamony *et al*., 1986). Some farmers use whole dried fruits while others use ground fruits and seeds in their storage systems. The mode of action of pepper whether as an insecticide or repellent is not clear. However, there are some reports in the literature that indicate pepper is an insecticide. Ground black pepper and its 95% ethanol crude extract were highly toxic to the rice weevil when they were used to surface treat wheat subsequently infested with insects (Su, 1977). Both adult mortality and progeny development are affected by increase in concentration of pepper extract. Su (1977), observed a very high adult mortality with no F1 progeny development, and a high adult mortality with very little F1 progeny development on wheat treated with black pepper at concentrations of 0.5% wt/wt and 0.25% wt/wt respectively.

2.3.2.3 Lentil

Legumes contain a wide range of phytotoxins, including non-protein amino acids, protein poisons (e.g. phytohaemagglutinins), alkaloids and terpenoids (Harborne *et al*., 1971; Bell, 1978) and many toxic phytochemicals (Holloway, 1986). The function of many of these secondary plant substances appears to be insecticidal (Janzen, 1978; Harborne, 1982), and it is probable that the majority of rice weevil strains are susceptible to them and thus are unable to infest most legumes (Holloway, 1986). Weevil mortality on legumes however, depends on the strain of the weevil and also on the type of legume. Pea protein has been found to have insecticide properties (Elden, 2000; Morton *et al*.,2000). Previous studies have shown that rice weevil raised on a chestnut and acorn diet died quickly after eating split peas (Coombs, *et al*., 1977; Holloway, 1986). Chander *et al*. (1992), investigating the breeding potential of some cereal pests in pulses, reported that the majority of adults of rust-red flour beetle, lesser grain borer and rice weevil survived a 14-day exposure period on all pulses except lentils. Bodnaryk, *et al*. (1997) reported that certain lentils are also a good source of insecticidal activity. In contrast, in a study of the mortality of rice weevil bred on yellow split-pea, adzuki bean (*Vigna angularis*), brown lentil, cowpea (*V. unguiculata*), green gram (*V.radiata*) and soyabean, Holloway and Mackness (1988), observed that soyabean was more toxic than the other five legumes. Holloway and Mackness (1988), report that some populations of rice weevil have evolved the ability to survive on normally toxic legumes, suggesting that the insects overcame all of the toxins in the legumes.

2.3.3 Others

2.3.3.1 Modified atmosphere.

This essentially involves reducing oxygen level or increasing carbon dioxide or nitrogen level in airtight storage. Carbon dioxide and nitrogen have been proposed for long-term storage of grains. Under the dry (10% grain moisture) storage conditions of wheat in Australia, practical application of CO_2 in bulk grain stored in large warehouses has not been completely successful mainly due to difficulties of rendering warehouses airtight and differential susceptibilities of various insect species, and developmental stages to CO_2 as a fumigant (De Lima, 1990). However, it has been shown that in a CO_2 atmosphere of 25%, the use of phosphine in low concentrations (50 ml/l) provides a more rapid kill of several insect species including confused flour beetle, *Tribolium confusum*, khapra beetle, grain weevil, and lesser grain borer (Desmarchelier and Wohlgemuth, 1984). Commercial nitrogen application also faces difficulty, mainly because almost 99% nitrogen atmospheres need to be maintained which is expensive (De Lima, 1990).

Banks and Sharp (1979), demonstrated the use of carbon dioxide added as dry ice as a means of disinfestation of bagged wheat and rye enclosed in a PVC membrane. They discovered that maintaining carbon dioxide at 60% could control some species of stored product pests including lesser grain borer and rust-red flour beetle, with complete mortality of adult insects. The early stages (pupa) of the lesser grain borer and parasitoids like *Anisopteromalus calandrae* and *Choetospila elegans* however, survived the treatment. The rice weevil, however, is the most tolerant to controlled atmosphere (Calderon and Barkai-Golan, 1990). Press and Harein (1967) observed complete mortality of the stored pest red-rust flour beetle (*Tribolium castaneum)* on peanut stored in hermetically sealed jars.

Oxygen depletion is also a key factor in reducing insect population (De Lima, 1990), but insects are only killed when oxygen levels drop to below 2% (Bailey, 1965). In large-scale airtight storage, the drop in oxygen levels is slow (De Lima, 1984) and does not inhibit insect development when populations are at low levels (De Lima, 1990).

In studying the use of low temperature storage to control insect pests, Nakakita and Ikenaga (1997) found that the population increase of rice weevil was completely suppressed at 15°C.

2.3.3.2 Resistant varieties

Several factors are known to produce resistance in stored grains against infestation by storage insects (Mbata, 1992). These factors include seed-coat characteristics (Nwanze and Horber, 1976), seed size (Nwanze *et al.*, 1975), hardness of seed (Nwanze and Horber, 1975) and presence of compounds which inhibit oviposition or/and development of insects on the seeds (Mbata, 1987; Throne, *et al.*, 2000). Physical features of the pericarp and seed, along with biochemical components of these structures that elicit behavioural responses of stored-product insects (Baker and Loschiavo, 1987), affect the degree to which insects can utilise this cereal fruit for growth and development. Singh *et al.* (1974) and Ram and Singh (1996) found that rice weevil preferred to oviposit on varieties with large grains, whereas McGaughey *et al* (1990) found that grain size was not useful for evaluating resistance against either rice weevil or lesser grain borer. Tightness of the hull is a major resistance mechanism against stored grain insect attack on rough rice, and harvesting methods that minimise damage to rough rice can eliminate or reduce subsequent insect damage during storage (Juliano, 1981). Breese (1960) demonstrated that rice weevil could not attack rice grains with intact hulls, and concluded that the degree of insect infestation in stored paddy is dependent upon the number of grains with broken or damaged hulls. Russell (1968) and Cohen and Russell (1970) found that the infestation of rice grains by *Sitophilus* species and Angoumois grain moth, *Sitotroga cerealella* was related to the number of gaping hulls or broken palea and lemma. Throne, *et al.* (2000) then concluded that there are at least two hull characters that impart insect resistance during storage, and both can be used in rice breeding programmes.

Most resistance grain varieties have compounds which are markedly different within a range of host species in the way the affect specific pests (Mbata, 1992). The substances conferring resistance may be secondary plant compounds such as alkaloids, rotenoids, polysaccharides and saponins (Johnson, 1981; Birch *et al.*, 1985; Nash *et al.*, 1986) which play antibiotic roles by being antimetabolic or toxic towards the pests (Gatehouse *et al.*, 1979).

2.3.3.3 Transgenics

Transgenic technology is a relatively new method of crop protection, which can generate new plants and "super seeds" with value-added traits (Baker and Kramer, 1996; Estruch *et al.*, 1997; Gatehouse and Gatehouse, 1998). The production of transgenic seed can provide better solutions to farmers' production problems (Davis, 1994). Genes from microorganisms, plants, and animals are being expressed in new varieties that, as a result, are more resistant to insect and fungal pests than the parent variety. These recombinant transgenes encode novel proteins detrimental to critical insect life processes in the endocrine, nervous, skeletal, and digestive systems and include α-amylase inhibitors, digestive system toxins, anti-nutritional proteins, hormone-metabolizing enzymes, neurotoxins, carbohydrate-binding proteins, chitinases, and proteinase inhibitors (Throne, *et al.*, 2000).

New rice varieties have resulted in dramatic increases in yield, but the acreage planted with rice varieties resistant to insects represents only a small fraction of the total rice production. Furthermore, these varieties are bred for resistance only to insects affecting crop production such as brown planthopper, *Niliparvata lugens* (Stahl) (Heinrichs, *et al.*, 1985; Rao, *et al.*, 1998; Lee, *et al.*, 1999). The identification of resistant genes in rice against field pests and diseases has been reported (Taura, *et al.*, 1992; Naqvi, *et al.*, 1995; Zheng, *et al.*, 1995; Sebastian, *et al.*, 1996; Yoshimura, *et al.*, 1998; YongHee, *et al.*, 1999). Transgenic seeds are very stable during storage (Pen, 1996), but currently, resistance to attack by insects during storage is not generally considered during breeding programmes or evaluated prior to release of commercial varieties. No breeding lines for insects attacking stored rice, however, are yet available, and no varieties resistant to store-grain insects have been released. Transgenic crops currently are not used for control of stored-product insect pests (Throne, *et al.*, 2000).

3

EXPERIMENT ONE

MATERIALS AND METHODS

3.1 Weevil culture

Rice weevil (*Sitophilus oryzae)* strains were obtained from wheat stored in a silo at a Mt. Maunganui Flour mill, New Zealand, and were cultured in glass jars on commercially available brown rice under controlled temperature (27±1°C) and relative humidity (70±5%), with 12 hours light cycle. Weevil identification was confirmed by the New Zealand Plant Protection Centre (Appendix I).

3.2 Plant materials

The botanicals used (Plate 2) to treat the rice seed were:

1. dried pepper fruits (*Capsicum frutescens* cv. "Habanero"), obtained from Kaitaia Fire, Northland, New Zealand,
2. neem seeds (*Azadirachta indica*), obtained from Australia (Neeming, Australia Pty Ltd),
3. neem oil (Azatin xl) with 3% azadirachtin (active ingredient), obtained from Yates New Zealand Limited, Auckland, New Zealand and
4. lentil seeds (*Lens culinaris* cv. "Raja"), obtained from Crop and Food Research, Lincoln, New Zealand.

Plate 2: Botanicals (before and after grinding) and rice varieties used in this study.

3.3 Assessment of viability of botanicals

3.3.1 Neem seed

Initial viability of the neem seed was determined using the method described by Eeswara *et al* (1998) where viability was assessed using radicle emergence. Three replicates of 20 seeds were placed in 15 x 10 cm plastic boxes each containing a thick germination towel (Seed Germination Kimpak Paper, Seedouro Equiupment Company, Illinois, USA). Benlate solution (0.1%) prepared with 30 ml distilled water was added to saturate each germination towel before placing the seeds in each box. Boxes were covered with aluminium foil and kept under dark conditions at 25°C. The number of germinated seeds was counted on 7, 11 and 14 days after the start of the germination test. The seeds were considered as viable and removed when the length of the radicle was more than 5 mm.

3.3.2 Lentil seed

The initial viability of the lentil seeds was determined using the between papper method (ISTA, 1999). Four replicates of 50 seeds were placed between two sheets of germination paper (Anchor Paper, USA) and germinated at 20°C. The number of germinated seeds was counted 5 and 10 days after the start of germination. A seedling was considered normal if the root and shoot systems were well developed and it had two cotyledoms.

3.4 Rice varieties

Two rice varieties, Doongara and Amaroo, from the 2000 harvest were used. These were obtained from the Yanco Agricultural Institute, Department of Agriculture, New South Wales, Australia.

The 1000-grain weight of these varieties was determined by hand counting of 1000 grains in four replications of each variety and then weighed on a beam balance (Mettler PE 3600 Delta Range, Switzerland).

The initial seed moisture content of the varieties was assessed using the high constant oven method at 130°C (±2°C) for two hours (ISTA, 1999). Four replicates of five grams seed of each variety were used. The rice seeds were ground for moisture content assessment using a laboratory mill (Tecator, Sweden).

The initial viability of the varieties was determined using four replicates of 100 seeds of each variety. The seeds were dusted with Thiram 80W (Nufarm Ltd, Auckland, New Zealand). The seeds were germinated on top of blue blotter paper (Anchor Paper, USA) at 25°C for 14 days (ISTA, 1999). Counting of normal, abnormal and dead seeds was done 7 and 14 days after the start of the germination test. A seedling was considered normal when the root and shoot systems were well developed, abnormal when the primary and seminal roots were stunted, retarded or missing, and/or the primary leaves deformed, damaged or missing, and dead when there was no sign of seedling development during the 14 days of the germination test.

3.5 Bioassay

3.5.1 Weevil experiment

The neem seeds, pepper fruits and lentil seeds were initially ground into powder in a laboratory mill (Tecator Cyclotec 1092) and then further ground in a coffee grinder (Braun type 4041) to produce fine powder. The powders were mixed with each rice variety at the rates of 0.005, 0.01 and 0.02g/kg rice seed, and the oil at 0.005, 0.01 and 0.02ml/kg rice seed. The mixing was done in bulk in an open bowl and the materials thoroughly stirred with a dessert-spoon (10 mls) for about 2 minutes. The bowl was cleaned with alcohol (70%) between each botanical preparation to prevent cross contamination. Twenty grams of each of the treated rice variety, together with controls (untreated rice seeds) were placed in plastic jars (60ml) with perforated lids to allow gaseous exchange but to prevent escape of weevils. The jars were then split by treatment into four randomised complete blocks (Plate 3). At 1 day, 4 weeks, 8 weeks, 16 weeks and 24 weeks after treatment, twenty unsexed weevils aged between four and six weeks were introduced into each of the jars containing either treated or untreated rice seeds. The experiment, for the first few weeks, was conducted under the same conditions (temperature of 27±1°C and relative humidity of 70±5%) used to rear the weevils but later on transferred to the main laboratory because the insect rearing room was undergoing renovation. The temperature and relative humidity levels in the main laboratory were different (temperature of 26±3°C and relative humidity of 40±10%) from those in the insect rearing room.

The numbers of dead and living weevils in each jar were counted seven days after each time of weevil introduction. This involved shaking each jar for one minute to activate the weevils and then pouring the contents of the jar into a scoop. The raised walls of the scoop were smeared with Fluon to prevent the escape of live weevils. Counting of dead and living weevils was done by hand (Plate 4). A weevil was considered dead if it did not show any sign of movement within a period of three minutes.

After removing the adult weevils, the jars were kept under the same storage conditions for another five weeks to allow offspring to develop. The weevil offspring in each jar were then counted as described above and recorded.

Plate 3: Layout of experiment.

Plate 4: Counting of dead and live weevils.

3.5.2 Seed viability experiment

The same procedure and replication described for the weevil experiment above, was used for the seed viability experiment except that no weevils were introduced into the jars. A germination experiment of the treated and control seeds was performed at 1 day, 4 weeks, 8 weeks, 16 weeks and 24 weeks after treatment. Germination was done as described above in section 3.4 except for each treatment 100 seeds were taken from each jar and 50 were dusted with Thiram and 50 were left undusted.

3.5.3 Seed moisture content test

Five grams of seed of each rice variety (untreated) was placed in a 60ml plastic jar with a perforated lid, replicated four times in the same way as the treated rice seeds, and stored together with the viability experiment. Seed moisture was assessed as described in section 3.4 at each time the germination experiment was carried out, that is, at each storage time.

3.5.4 Relative humidity

The relative humidity in the storage room was monitored at one day, four, eight, 16 and 24 weeks after the start of the experiment using a dry and wet bulb thermometer (zeal, England) and a psychometric chart.

3.5.5 Data analysis

Data analysis was performed using GENSTAT (Genstat 5 committee, 1995). The data were subjected to analysis of variance (ANOVA), examining the effects of rice variety, botanical treatment and rate of application, and the length of time after treatment, as well as the interactions between these effects. Prior to analysis the data was transformed in the following ways: The weevil mortality and seed germination data (percentage of dead weevils, percentages of normal, abnormal and dead seeds) were first transformed using the inverse sine (angular) transformation in order to deal with the heterogeneous variance across the range of data, a typical problem with percentage data. The offspring data (counts) were transformed using natural logarithms, also to stabilise the variation. Both the transformed and back-transformed means (transformed means converted back to original units, e.g. percentage) are presented in the results. The least significant differences (LSD) shown are for comparing the transformed means only. Note that the inverse sine transformation produces values (angles) between 0 and 90, and that 86 and 90 correspond to percentages which have been rounded up to (the nearest whole number) 100%.

4

RESULTS

4.1 Preliminary assessment of botanicals and rice

4.1.1 Viability

The initial viability of neem and lentil seeds were 64% and 92% respectively, and 98% and 97% for Doongara and Amaroo rice varieties respectively. The other 36% of neem seeds were dead.

4.1.2 Seed moisture content

The moisture content of the rice seed on receipt was 11% for Doongara and 12% for Amaroo.

4.1.3 1000 grain weight

Morphologically the grains are quite distinct. The grains of Doongara were long and slender with a thousand grain weight of 26 grams whilst those of Amaroo are medium and bold with a thousand grain weight of 27 grams.

4.2 Weevil mortality

4.2.1 Effect of botanicals on weevil mortality

The botanicals most effective in killing weevils were neem oil (98% effective on average), lentil powder (82%) and neem powder (81%). Although pepper had a 62% kill, this was significantly lower than that found in the control (70%). There were significant interactions between botanical application rate and weevil death, which qualify the means. Neem oil was effective at all rates compared to control in contrast to lentil which was effective at 0.01g/kg and 0.02g/kg only. Neem powder was only effective at 0.02g/kg. Pepper was not significantly different ($p \leq 0.05$) to the control at 0.02g/kg but promoted weevil survival at the two lowest rates used (0.005g/kg and 0.01g/kg) (Table 1).

Table 1: Percentage weevil mortality at each botanical treatment rate.

Rate (wt/wt)	Mortality (%)			
	Lentil powder	Neem Oil	Neem Powder	Pepper powder
0.005	*60*[1] (75[2])	*80* (97)	*59* (74)	*51* (60)
0.01	*66* (83)	*89* (100)	*61* (76)	*51* (61)
0.02	*69* (87)	*90* (100)	*72* (91)	*53* (65)
Untreated Control: *57* (70 %)	LSD (5% level, d.f.=410) = *4.7*[3]			

[1]Transformed means (first numbers in each column).

[2]Back-transformed means presented in brackets and rounded to nearest whole number, expressed as percentage mortality.

[3]LSD may be used to compare any two (transformed) means in this table.

4.2.2 Effect of rice variety on weevil mortality

The rice varieties used had differing effects on the ability of the botanicals to control rice weevil. Lentil and neem powders were more effective on Doongara, but neem oil was equally effective on both cultivars. Again there were interactions between application rates, botanicals and rice variety. Lentil powder was effective on Doongara at all application rates but was only effective on Amaroo at the two highest application rates (0.01g/kg and 0.02g/kg). Neem powder was effective on Doongara at both 0.01 and 0.02g/kg seed, but was only effective on Amaroo at 0.02g/kg seed. Neem oil was effective at all rates irrespective of variety. Pepper powder promoted weevil survival on Amaroo at 0.005g/kg but had no significant ($p \leq 0.05$) effect on weevil mortality at 0.01g/kg or 0.02g/kg nor at any application rate on Doongara (Table 2).

Table 2: Percentage weevil mortality at different treatment rates in each rice variety.

Rate (wt/wt)	Treatment	Mortality (%)	
		Rice variety	
		Amaroo	Doongara
-	Untreated Control	*53*[1] (65[2])	*60* (75)
0.005	Lentil	*52* (62)	*68* (86)
	Neem oil	*83* (99)	*77* (95)
	Neem powder	*56* (69)	*63* (79)
	Pepper	*45* (49)	*57* (71)
0.01	Lentil	*60* (75)	*71* (90)
	Neem oil	*88* (100)	*90* (100)
	Neem powder	*52* (63)	*69* (88)
	Pepper	*48* (55)	*55* (70)
0.02	Lentil	*65* (82)	*73* (92)
	Neem oil	*90* (100)	*90* (100)
	Neem powder	*71* (90)	*73* (92)
	Pepper	*51* (61)	*56* (68)
LSD (5% level, df=410) = *6.7*[3]			

[1]Transformed means (first numbers in each column).

[2]Back-transformed means presented in brackets and rounded to nearest whole number, expressed as percentage mortality.

[3]LSD for comparing transformed means.

4.2.3 Effect of storage time on weevil mortality

The death of rice weevils in stored rice seeds treated with botanicals increased with the storage period. Weevil mortality was significantly lower ($p \leq 0.05$) at one day after seed treatment than at any other storage period except for neem oil. Neem oil gave high mortality at all rates and storage times except at the lowest application rate (0.005ml/kg) where mortality was reduced to 89% at four weeks after seed treatment.

One day after treating the rice, only the neem oil treatments, lentil powder at the two highest rates and highest neem powder rate gave significantly higher mortality than the control. Pepper at all application rates significantly increased the survival of the weevils one day after treatment. At four weeks after treating the rice, all botanicals except pepper gave significantly higher mortality than the control. The mortality figures at eight weeks were either unchanged or lower than after four weeks, but the control mortality increased to 68%. Only the neem oil treatment gave significantly higher mortality than the control at eight weeks. By 24 weeks, there was no difference in weevil mortality between the control and any treatments (Table 3).

Table 3: The effect of botanical rate and time after application on weevil mortality.

Rate (wt/wt)/ (v/wt)	Treatment	Mortality (%)				
		1 day	4 weeks	8 weeks	16 weeks	24 weeks
-	Untreated control	*22*[1] (14[2])	*44* (48)	*56* (68)	*79* (96)	*84* (99)
0.005	Lentil	*22* (13)	*63* (79)	*55* (68)	*79* (97)	*81* (98)
	Neem oil	*90* (100)	*71* (89)	*74* (92)	*79* (97)	*87* (100)
	Neem powder	*14* (6)	*61* (77)	*59* (74)	*75* (94)	*86* (100)
	Pepper	*8* (2)	*42* (44)	*48* (55)	*71* (89)	*86* (100)
0.01	Lentil	*33* (29)	*67* (84)	*59* (73)	*85* (99)	*85* (99)
	Neem oil	*90* (100)	*90* (100)	*86* (99)	*90* (100)	*90* (100)
	Neem powder	*31* (26)	*59* (74)	*50* (58)	*77* (95)	*88* (100)
	Pepper	*10* (3)	*49* (57)	*39* (39)	*76* (94)	*83* (98)
0.02	Lentil	*33* (29)	*73* (91)	*65* (82)	*84* (99)	*90* (100)
	Neem oil	*90* (100)	*90* (100)	*90* (100)	*90* (100)	*90* (100)
	Neem powder	*52* (61)	*77* (95)	*60* (75)	*82* (98)	*90* (100)
	Pepper	*11* (3)	*51* (61)	*40* (42)	*80* (97)	*85* (99)
LSD (5% level, df=410) = *10.6*[3]						

[1]Transformed means (first numbers in each column).

[2]Back-transformed means presented in brackets and rounded to nearest whole number, expressed as percentage mortality.

[3]LSD for comparing transformed means.

4.2.4 Effect of botanicals on weevil offspring development

There were more weevil offspring in Amaroo than in Doongara. The emergence of the rice weevil offspring was completely prevented by neem oil in both rice varieties, but the powders of neem, lentil and pepper gave no significant ($P \leq 0.05$) effect on weevil offspring development (Table 4). There was no development of weevil offspring at 16 and 24 weeks in both control and treatment.

The emergence of weevil offspring was not significantly affected by the rate and time of application of the botanicals.

Table 4: Mean weevil offspring emergence from seeds of rice varieties treated with botanicals.

Treatment	Offspring counts per 20 weevils	
	Amaroo	Doongara
Untreated Control	*0.60*[1] (1.32[2])	*-0.33* (0.22)
Lentil	*1.03* (2.30)	*-0.39* (0.19)
Neem powder	*0.23* (0.76)	*-0.52* (0.09)
Neem oil	- (0)	- (0)
Pepper	*0.97* (2.15)	*-0.36* (0.19)
LSD (5% level, df= 185) = *0.35*[3]		
LSD (5% level, df= 185) = *0.58*[4]		

[1]Transformed means from analysis of the logarithms of the data with a small constant (0.5) added (first numbers in each column).

[2]Back-transformed means.

[3]LSD for comparing transformed means of neem, lentil and pepper powders.

[4]LSD for comparing transformed means of treatments (except neem oil) versus control.

4.3 Effect of botanicals on rice seed viability

Over the 24 week storage period there was no significant ($p \leq 0.05$) decline in the germination of the untreated (control) seed of either rice variety. Pepper, neem powder and lentil had no effect on germination at any application rate or at any time of storage. Germination of rice seed treated with these powders remained above 90% throughout the storage experiment (Figure 1). Neem oil at 0.005ml/kg seeds did not significantly affect germination except at four and 24 weeks where germination dropped to 90% and 93% respectively, but germination levels were affected at the other rates of neem oil (Figure 2). At a 0.01ml/kg neem oil application rate, normal germination was significantly lower than the control germination except at 24 weeks where it recovered to 95%. At a 0.02ml/kg neem oil application rate, normal germination was significantly reduced to 47% 1 day after treatment (Figure 2). It remained at this level after four weeks however, it recovered to 79% by 24 weeks. At a 0.02ml/kg neem oil application rate, abnormal germination (seedlings whose primary and seminal roots were retarded or missing) was high (47%) 1 day and four weeks after treatment. However, by eight weeks abnormal germination reduced to 35% and further down to 16% by 24 weeks. Abnormal germination remained low (below 7%) at 0.005ml/kg neem oil application rate (Figure 2). Percentage of dead seed remained low (2 - 7%) throughout the experiment.

Figure 1: Percent germination of rice seed from time of treatment

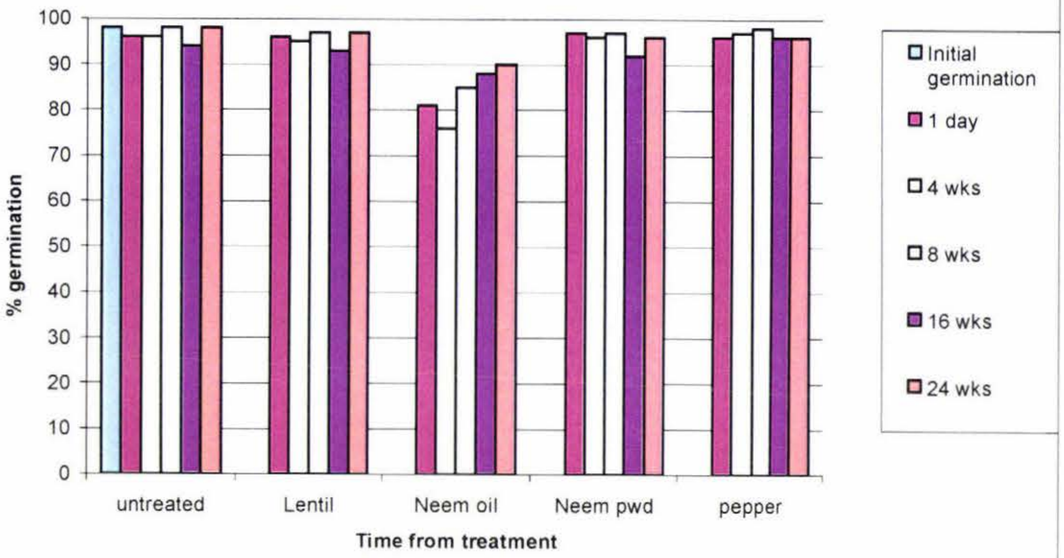
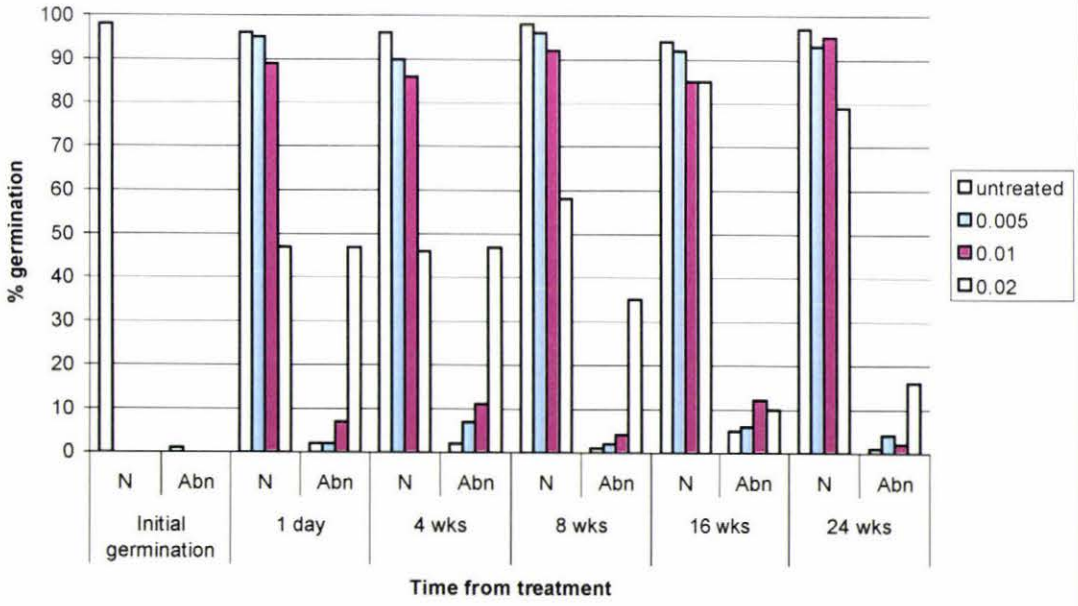


Figure 2: Effect of neem oil concentration (ml/kg seed) on normal (N) and abnormal (Abn) germination of rice seed.

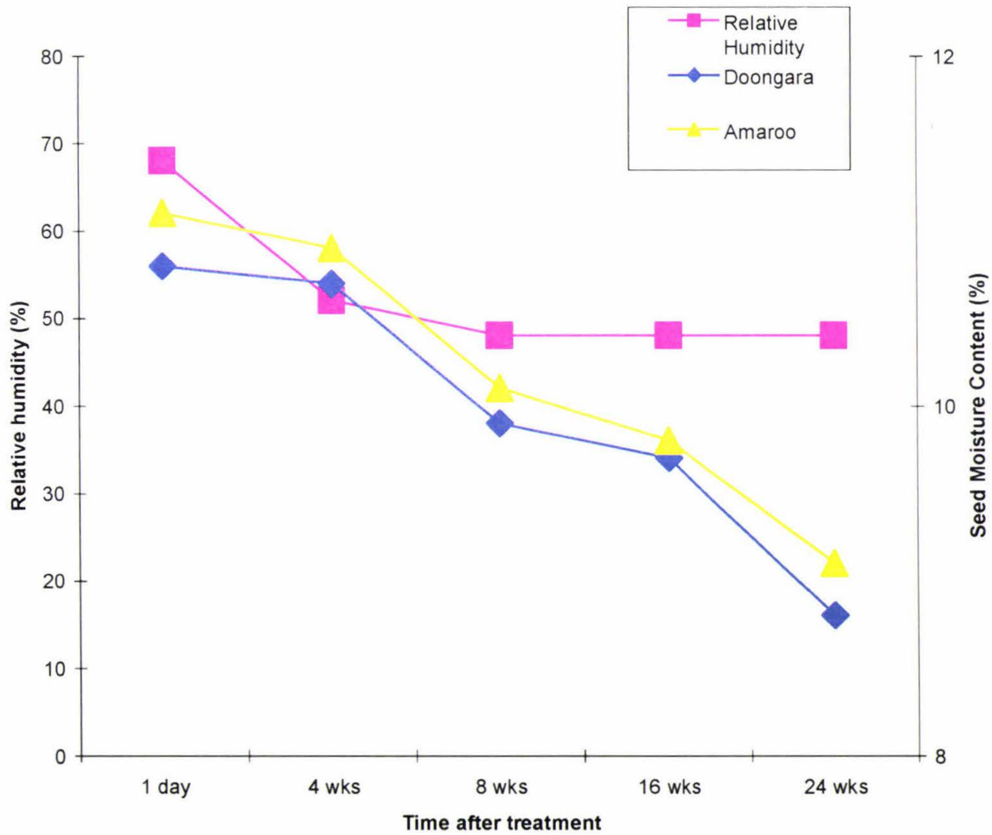


Thiram treatment had no significant effect on the germination of rice seed in either cultivar at any botanical treatment rate.

4.4 Relative humidity changes during storage

The relative humidity of the storage environment dropped down from an initial level of 68% at the start of the experiment to 48% at eight weeks and remained at this level through to 24 weeks (Figure 3). This reduction in relative humidity was due to the change in storage location during the first four weeks as mentioned in section 3.5.1 above. In response to this lower relative humidity, the seed moisture content of both varieties dropped from 11-12% to below 10% for the remainder of the storage period.

Figure 3: Variation in relative humidity and seed moisture content of Doongara and Amaroo seeds during seed storage.



5

DISCUSSION

5.1 Initial viability of botanicals and rice seed

The germination percentage of neem and lentil seeds before grinding into powders indicated that the seeds were viable and that their compounds were active enough to indicate any effect on the rice seeds and against the weevils. The high initial germination of the rice cultivars indicated that the seeds were viable before the start of the experiment so that any decline in their viability during and after experiment would be attributed to the effects of the botanicals or the storage environment.

5.2 Effect of botanicals on rice weevil

Neem oil, neem powder and lentil powder exhibited insecticidal properties against rice weevil. Weevil mortality increased with increasing concentration of the botanical treatments, except for neem oil where weevil mortality was not significantly different at lowest and highest concentrations. Weevil mortality was very high at all neem oil rates, indicating that the use of neem oil doses above 0.005ml/kg rice seed is not warranted. This also suggests that lower concentrations of neem oil below 0.005ml/kg rice seed may control rice weevil, but the time interval for this research did not allow this to be evaluated. There are reports (Sighamony *et al.*, 1986) of the use of plant oils (edible oils) at lower doses (less than 0.02 ml/kg seed) for an effective control of rice weevil on wheat, but these oils were solvent (acetone) extracted to increase concentration of the active ingredient. This process however, is beyond the reach of traditional farmers because it is not only complicated but also cost-prohibitive. The botanical powders were most effective against weevils at four weeks. The low mortality of weevils on the first day after treatment with botanical powders may indicate that the extracts take some time to affect both seeds and weevils. Contrary to previous reports (Su, 1977 and Sighamony *et al.*, 1986), pepper did not kill the weevils, but these authors used acetone extracts of black pepper, rather than powder of hot pepper to kill the weevils. The active ingredient in black pepper is piperine (Su, 1977; Sumathikutty *et al*, 1981; Verzele *et al*, 1989; Koul and Aruna, 1993; Singh *et* *al*, 1994; Bajad *et el*, 2001), different from that in Habaneros, and the black pepper may also have different mode of action against rice weevil. Traditional farmers do use hot pepper to control weevil in their stored grains (Sartie, 1998, unpublished), but it is not clear whether this success is a varietal effect or as a result of differences in the mode of action of pepper. The mode of action of the pepper may be a repellent (driving away the insects) rather than insecticidal (killing the insects). In this experiment repellency was not assessed.

The death of weevils was higher on Doongara than on Amaroo, and this suggests that Doongara may be resistant to rice weevil or Amaroo susceptible. The former seems more likely given that the hulls of Doongara are tightly closed and intact whereas those of Amaroo are less tight and not properly closed together. Several authors (Breese, 1960; Russell, 1968; Cohen and Russell, 1970; Juliano, 1981) have suggested that the hull morphology observed in Doongara may be responsible for this cultivar being resistant to weevils. The seeds of Doongara are smaller, slender and harder than Amaroo with a low glycaemic index (Ricegrowers Co-op Technical Information, Unpublished). The glycaemic index gives an indication of how quickly the carbohydrate in food is converted to glucose for the body to use. These characteristics (size, shape and hardness) may also contribute to its being resistant to weevil (Singh *et al*., 1974; Nwanze and Horber, 1975; Ram and Singh, 1996), although McGaughey *et al.* (1990) reported that seed size did not confer resistance to rice weevil in wheat, they observed that the most susceptible and the least susceptible grain classes of wheat had very large kernels, that is, no relationship between seed size and resistance. The contribution of the low glycaemic index to resistance to weevils is not clear. The botanical powders at lower doses were effective in killing the weevils in Doongara, but only did so in Amaroo when they were applied at higher doses. This suggests that the use of botanical powders in combination with varietal resistance in rice can be a more successful method of weevil control.

Weevil mortality in both treated and untreated seeds at eight, 16 and 24 weeks of storage was very high. This high mortality could be attributed to the reduction in the relative humidity of the storage environment, and the subsequent reduction in the seed moisture content at those times. The low relative humidity environment may cause the weevils to dehydrate, and the low seed moisture content can render the seeds too hard or dry for the weevils to feed on. Dehydration and starvation may be the reasons for the high mortality of the weevils. There is no report in the literature on the effect of low relative humidity and low seed moisture content on the survival of rice weevil on rice seed. However, high weevil mortality on maize at low relative humidity (below 60%), and at seed moisture content below 10% has been reported by Tsai and Chang, (1935) and Khan *et al*, (1993) respectively, but some other authors have argued that rice weevils can survive on maize seeds with moisture content as low as 8.5% (Mathleen, 1938 and Moutia, 1942), but do not state the level of relative humidity at which this equilibrium moisture content was reached. The reduction in relative humidity and seed moisture content at the beginning of eight weeks may have caused weevil death, and this would have confounded the data from this time on. Therefore, no firm conclusion can be drawn on the effect of these botanical preparations on weevil mortality from eight to 24 weeks of storage.

Interestingly, in this work, the weevils cultured on brown rice however, remained alive even though they were all in the same environment with the experiment. This is an indication that at low relative humidity rice weevil fails to survive on hulled rice but can survive on brown rice (dehulled) in storage. The hull may become too hard and dry for the weevils to burrow through under low relative humidity which may cause them to starve. The moisture content of the brown rice however, was not determined, and so it is not clear whether the survival of the weevils on the brown rice was related to high or low seed moisture content.

Weevil offspring development was completely prevented by neem oil. This was probably because of the high mortality of the adults with little or no chance to mate and reproduce, or that the neem oil was more effective in reducing egg- laying and adult emergence. This is in agreement with literature evidence that neem oils have relatively high efficacy against virtually all stages in the life cycle of the insects (Abdel-Kawy, 1992; Lale, 1995; Cardet *et al*, 1998), but in this work the effect of neem oil on the developmental stages (egg, larval and pupal) of the rice weevil was not monitored. The powders of neem, lentil and pepper had no effect on rice weevil offspring development. The low weevil offspring development in Doongara may be as a result of its seed morphology. There are no cracks to allow the weevils to easily burrow holes and deposit eggs in the grain endosperm. It may also be that the grains are too small or too hard for the weevils to burrow holes and lay eggs.

5.3 Effect of botanicals on rice seed viability

Pepper, neem powder and lentil did not affect germination of the rice varieties throughout the storage period. Prativa and Basu (1993) conducted an experiment with wheat and concluded that pepper powder and neem leaf powder had no deteriorative effect on the viability of seeds in storage, and this was confirmed in this experiment. The neem oil at lowest rate (0.005ml/kg seeds) had no detrimental effect on seed germination. Although statistically there was a significant reduction in germination at four and 24 weeks mainly as a result of the large degree of freedom involved (Table 8), this decline is unlikely to be practically important because actual germination remained high (above 90%). This is consistent with the observations of Jacobson, (1975); Singh *et al.*, (1978); Sighamony *et al.*, (1986) and Lale, (1987) who found that plant oils do not detrimentally affect seed germination, but they used edible oils from groundnut, clove and cedarwood. However, at higher rate of 0.02ml/kg seed, normal germination was significantly reduced. Again this is in agreement with findings of Singh *et al* (1980), and Qi and Burkholder (1981). They observed that treatment of wheat with edible oils at 1ml/kg of wheat, 82-90% of wheat remained capable of germination, but this was reduced to 42-33% at 5ml/kg wheat and to 28% at 10ml/kg of wheat grain. The mechanism by which neem oil at higher rates affects germination is not understood, but Sinniah *et al* (1983) reported that treatment of seed with neem oil during storage caused fungal contamination, mostly *Aspergillus* species and consequently reduced germination. However, in this experiment, there was no difference in germination of seeds treated with neem oil and dusted with thiram compared to seeds treated with neem oil alone. This and the relative absence of fungal growth on seedlings, suggest that *Aspergillus* is not the cause of the reduced germination in this experiment. The recovery of normal germination at 24 weeks of seeds treated with neem oil is an indication that the neem oil toxicity reduces with increase in storage period. Abnormal germination, the cause of the reduced normal germination was very high at the one day and four week times of treatment application, but reduced later on with increase in storage period. This suggests that the neem oil did not affect the embryo of the treated seeds and that its deleterious effect reduces with storage period. This could be related to the instability of the active ingredient (azadirachtin A) in neem oil. Several factors including, environmental, chemical and formulation auxiliaries have been reported to affect the stability and performance of the active ingredients in neem (Dragon, 1988; Parmar and Dureja, 1994), and Kumar and Parmar (2000) reported that the half-life of the active ingredient in neem could be enhanced by storing the concentrates at lower temperatures ($\leq 10^{o}C$). In this experiment, higher temperatures above $20^{o}C$ were used, and this may have caused the degradation in the bioactivity of the neem oil on rice seed germination as the storage period advanced hence the recovery of normal germination at 24 weeks of storage.

6

EXPERIMENT TWO

MONITORING OF CHANGES IN ENVIRONMENTAL FACTORS AND THEIR EFFECT ON THE SURVIVAL OF ADULT RICE WEEVIL.

6.1 Introduction

Experiment one was planned to be conducted under conditions of 70±5% relative humidity and at temperature of 27±1°C. The experiment was set up in the insect rearing room where the air conditioning was controlled by a humidifier (SteaMaster Electrode-humidifier, Germany) to maintain the relative humidity at 70±5%. However, the experiment had to be transferred from the insect rearing room to the main laboratory when the former was undergoing renovation. In the main laboratory, there was no humidifier and conditions were quite different from what was proposed for the experiment. The relative humidity was below the stipulated level. The death of the weevils was unexpectedly high in the control from eight weeks to 24 weeks of storage (Table 3). The reason for this high mortality of the weevils was not clear, but suggestions in the literature attributed high natural weevil mortality to reduction in relative humidity to below 60% and seed moisture content to below 10% (Tsai and Chang, 1935: Khan *et al*, 1993). Experiment two was therefore set up to investigate the reasons for the high mortality of the weevils in the control.

The specific objectives included;

1. To monitor the relative humidity and temperature of the main laboratory and of the insect rearing room for 16 weeks.
2. To compare weevil mortality on treated (lentil seed powder) and untreated rice seeds in the main laboratory and in the insect rearing room at 8 and 16 weeks of seed storage.
3. To assess the viability of treated and untreated seeds of the rice varieties at 8 and 16 weeks stored in the main laboratory and in the insect rearing room.

4. To determine the effects (if any) of weevil infestation and lentil treatment on the seed moisture content of rice.

6.2 Materials and Methods

6.2.1 Weevil culture

The same strain of rice weevil used in experiment one was used in this experiment. During experiment one, the weevils were cultured on brown rice in glass jars and kept by the experiment in the main laboratory. The cultured weevils however, remained alive and active on the brown rice as opposed to the high weevil mortality on the hulled rice in the experiment. The culture was then transferred to the insect rearing room (after renovation) and kept under conditions described in Section 3.1.

6.2.2 Plant materials

The same lentil seed lot used in experiment one was used for this experiment. They were kept in water impermeable sealed plastic bucket and stored at 5°C until required for experiment two. The germination was assessed prior to experiment two using the germination method described in Section 3.3.2. The powder was prepared using the procedure described in Section 3.5.1.

6.2.3 Rice varieties

The Doongara and Amaroo seed lots used in experiment one were used in this experiment. The seeds were stored in water impermeable sealed plastic buckets at 5°C until required for experiment two. The germination and seed moisture content of each lot was assessed prior to experiment two using the methods described in Section 3.4.

6.2.4 Bioassay

6.2.4.1 Weevil experiment

The lentil seed powder was mixed with each rice variety at the rate of 0.02g/kg rice seed following procedures used in Section 3.5.1. Experiment one demonstrated lentil powder at this rate (0.02g/kg rice seed) was effective in controlling rice weevil in stored Amaroo and Doongara rice (Table 1). Twenty unsexed weevils aged between four and six weeks were introduced into each of the jars containing either treated or untreated rice seeds at eight and 16 weeks after treatment. The treatments were replicated in four randomised complete blocks in each of the two experimental environments. Counting of dead and living weevils in each jar was done using procedure described in Section 3.4.1. However, in this experiment weevil offspring development was not assessed because of time limitation in this study period.

6.2.4.2 Seed viability experiment

Seed viability was assessed using procedure described in Section 3.4.2, except that germination was only evaluated at 8 weeks and 16 weeks after treatment. Experiment one demonstrated that Thriam had no effect on germination compared to undusted seed, therefore, no non-Thiram treatment was included, and for each experiment 50 seeds from each jar were dusted with Thiram 80W.

6.2.4.3 Seed moisture content test

The procedure described in Section 3.4.3 was used to determine the seed moisture content at the different storage times. The moisture content of treated and untreated seeds after weevils' removal was also assessed using the procedure outlined in section 3.4.3. This was to determine any change in seed moisture as a result of both the botanical treatment and the inclusion of the weevils.

6.2.4.4 Temperature and Relative Humidity readings

The temperature and relative humidity readings were done using temperature and humidity data loggers (Gemini Data Loggers Tinytag Plus, U.K. LTD., West Sussex). One data logger was placed with the experiment in the laboratory and another one in the insect rearing room. Readings were recorded at 15 minute intervals from the start to the end of each experiment.

6.2.4.5 Data analysis

Data were analysed using same procedure in Section 3.4.4 except that no transformation of the data was required because there was no heterogenous variation across the range of data.

This may be due to their being a greater spread of mortality and germination rate in this experiment. T-test was used to analyse the seed moisture content data.

6.3 Results

6.3.1 Assessment of rice and lentil seeds prior to experiment

6.3.1.1 Viability

The seed viability of Doongara, Amaroo and lentil were 98%, 96% and 91% respectively indicating no significant ($p \leq 0.05$) change from the viabilities of these seed lots on receipt (Table 9, Appendix II).

6.3.1.2 Seed moisture content

The seed moisture was 11% for Doongara and 12% for Amaroo. There was also no significant change in seed moisture content of the rice varieties from time of receipt to the start of experiment two.

6.3.2 Relative humidity and temperature in main laboratory and in the insect rearing room

6.3.2.1 Relative humidity

The average relative humidity was higher in the insect rearing room (66.6%) than in the main laboratory (42.5%) as shown in figure 4. Although the relative humidity in the insect rearing room was controlled, there were still unusual variations as a result of mechanical problems of the humidifier, and this indicates the difficulties involved in controlling relative humidity in a room.

6.3.2.2 Temperature

The temperature of the insect rearing room throughout the experiment ranged from 27.8°C to 31°C with a mean of 29.5°C and from 23°C to 30.9°C with a mean of 26.1°C in the main laboratory respectively (Figure 5).

Figure 4: Relative Humidity in the main laboratory and in the insect rearing room.

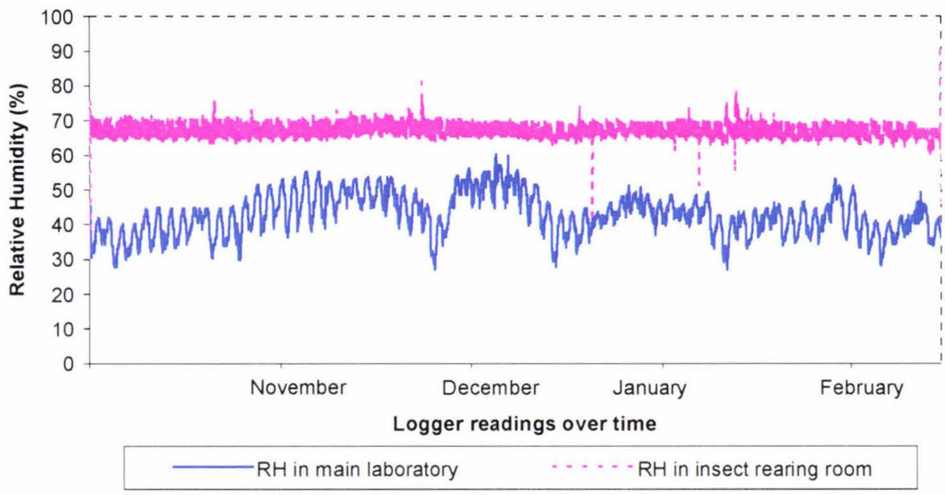
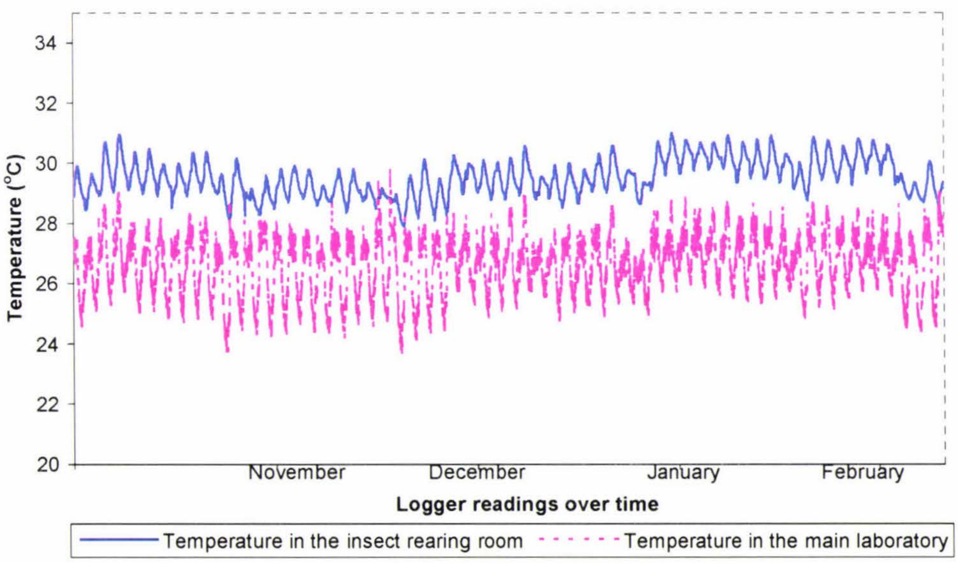


Figure 5: Temperature in the main laboratory and in the insect rearing room.



6.3.3 Weevil mortality

The percentage mortality of weevils was significantly higher ($p \leq 0.05$) in the main laboratory (75%) than in the insect rearing (14%) for both rice cultivars. Weevil mortality was higher on treated seeds (56%) than on untreated control seeds (33%). The overall weevil mortality was higher on Doongara (51%) than on Amaroo (38%), but to compare this in the storage environments, there was no significant difference in weevil mortality on Doongara and on Amaroo in the insect rearing room (Table 5).

Table 5. Percentage weevil mortality on rice variety in storage environments.

Storage environments	Amaroo	Doongara
Main lab	65.0	85.0
Rearing room	10.3	17.1
LSD (5% level, df = 28.64) = 7.80		

The percentage mortality of weevils on untreated seeds (control) was significantly higher ($p \leq 0.05$) at 16 weeks than at 8 weeks in the main laboratory but not in the insect rearing room, but on treated seeds weevil mortality was higher at 16 weeks than at eight weeks only in the insect rearing room (Table 6).

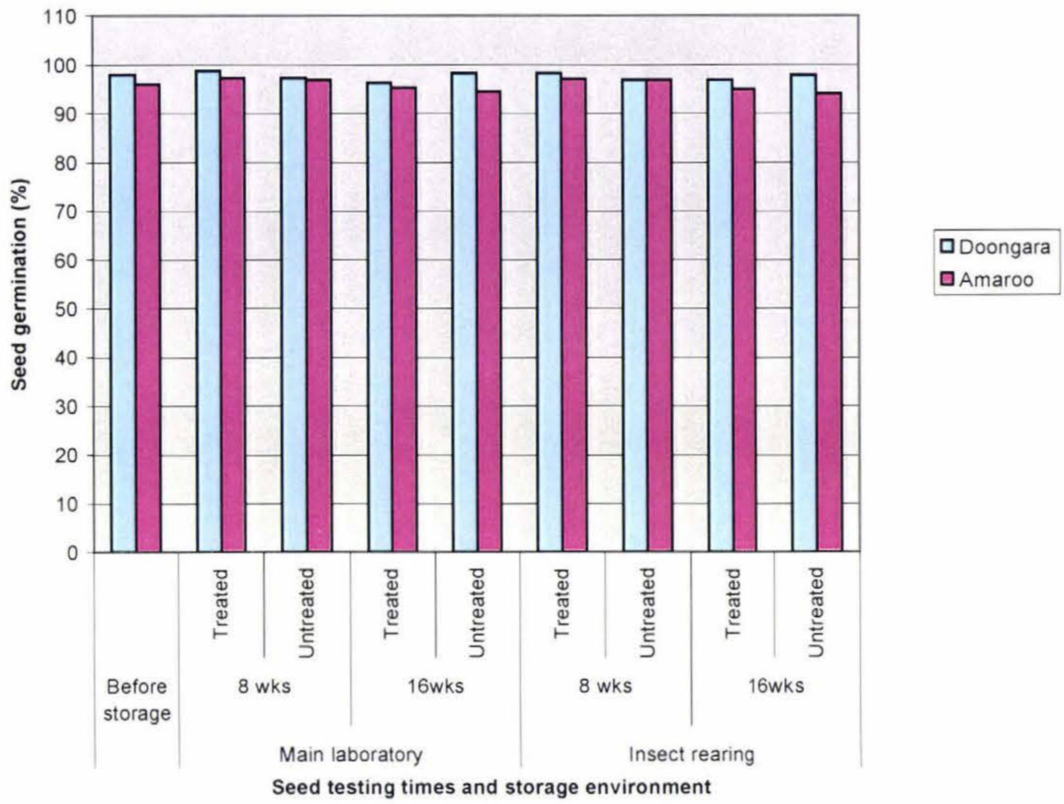
Table 6. Percentage weevil mortality in the main laboratory and in the insect rearing room from time of seed treatment.

Storage environments	Control		Treated	
	8 wks	16 wks	8 wks	16 wks
Main lab	50.0 %	71.9 %	88.1 %	90.0 %
Rearing room	1.3 %	11.3 %	10.0 %	33.7 %
LSD (5% level, df=45.93) =11.41				

6.3.4 Rice seed viability

The viability of rice seeds remained high (above 90%) throughout the experiment with no significant difference ($p \leq 0.05$) between their germination at the beginning and end of the experiment (Table 9, Appendix II). The percentage germination of Doongara and Amaroo either treated or untreated were not significantly different in either of the storage environments (Figure 6).

Figure 6: Percent germination of rice seed from start to end of storage in main laboratory and in insect rearing room.



6.3.5 Seed moisture content

In seed without weevil infestation, the seed moisture content of Amaroo and Doongara were not significantly different ($p \leq 0.05$) within each storage environment throughout the storage period (Table 10, Appendix III). The seed moisture content of both rice varieties was lower in the main laboratory than in the insect rearing room. In the insect rearing room, the seed moisture content of Amaroo and Doongara was not significantly different ($p \leq 0.05$) from their moisture contents at the start of experiment, but in the main laboratory the moisture content of both rice varieties was significantly lower than their moisture content before the experiment (Figure 7).

The moisture content of treated and untreated seeds of both rice varieties after weevils were removed was significantly lower ($p \leq 0.05$) in the main laboratory than in the insect rearing room (Table 11, Appendix III). The moisture contents of treated and untreated seeds of Doongara and Amaroo after removing the weevils were lower than their moisture content before the experiment only in the main laboratory, but were not different in the insect rearing room (Figures 8).

The moisture content of treated and untreated seeds of Doongara and Amaroo after weevils were removed was not different from the moisture content of the seeds of the varieties without weevil infestation in any of the storage environment.

Figure 7: Seed moisture content of Amaroo and Doongara rice varieties without weevil infestation stored at different times in main laboratory and in insect rearing room .

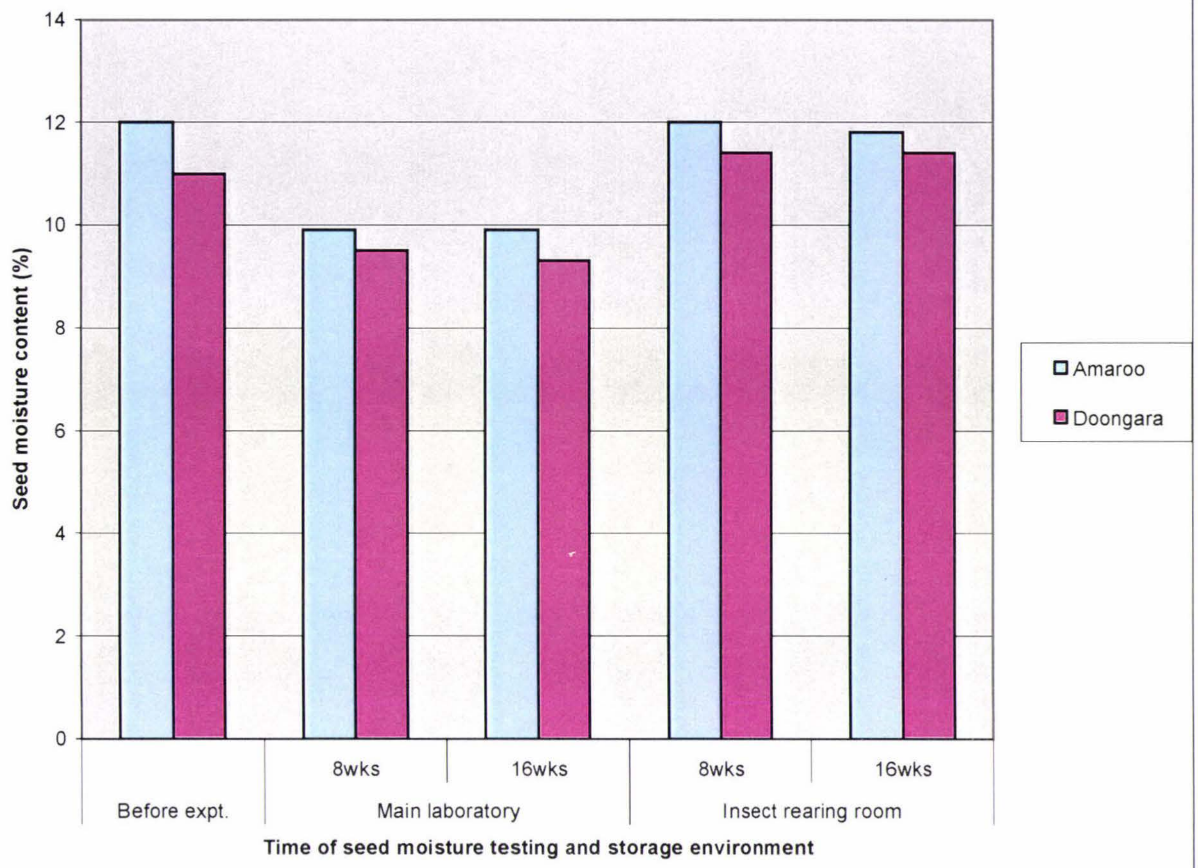
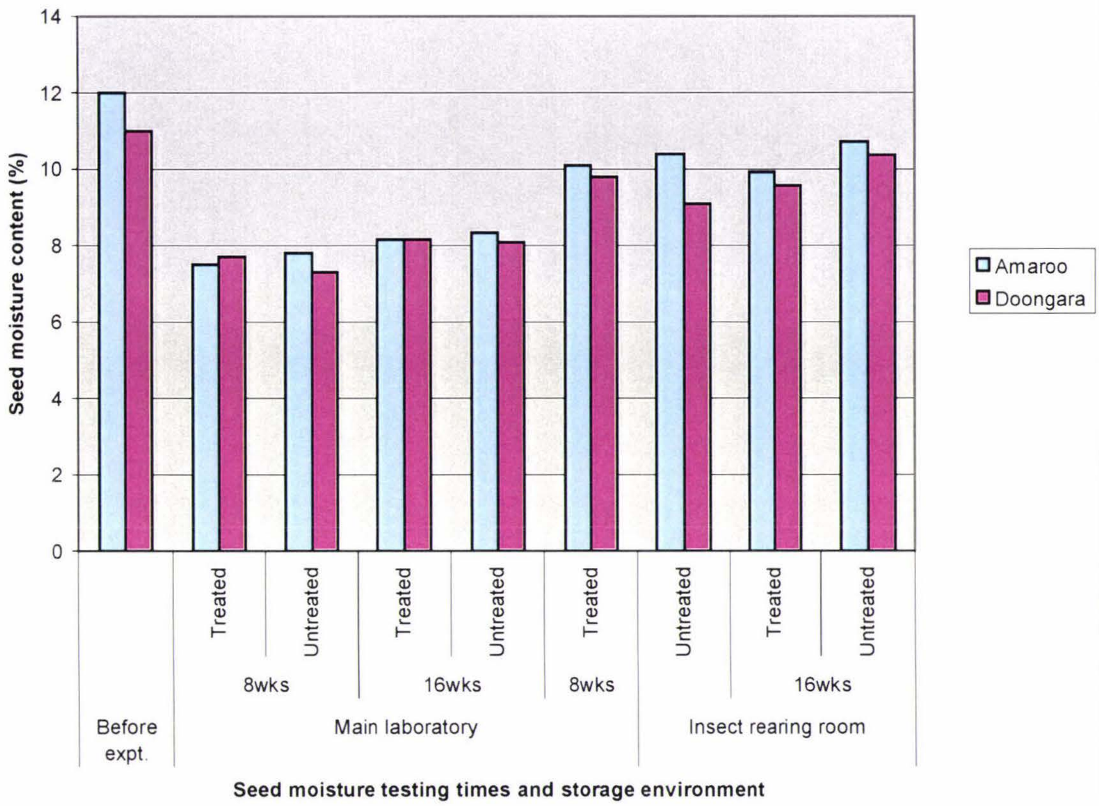


Figure 8: Seed moisture content of treated and untreated rice varieties after weevils were removed.



6.4 Discussion

6.4.1 Assessment of rice and lentil seeds before storage

The seeds of Doongara, Amaroo and lentil used remained viable between experiment one and two. The moisture content of the rice varieties did not change from the initial level at time of receipt. This indicates that these seeds at their moisture content of receipt can be stored for at least nine months at 5°C without deterioration.

6.4.2 Relative humidity and temperature in main laboratory and in insect rearing room

Relative humidity and temperatures of the storage environment are the most important factors affecting seed quality during storage. Of these, relative humidity is the most important (Arvier, 1983). The relative humidity affects seed moisture content because seeds are hygroscopic and tend to absorb moisture from a wet atmosphere and loose moisture to a dry atmosphere. The equilibrium seed moisture content is associated with the relative humidity of the seed storage environment. This experiment confirmed that relative humidity and temperature were lower in the main laboratory than in the insect rearing room, and that the findings in experiment one are confounded by the change of storage environment. This low humidity resulted in a reduction in seed moisture content of the rice varieties as the seeds lost moisture to the storage environment to maintain equilibrium moisture content with the environment. This low relative humidity in the main laboratory may also have caused the weevils to dehydrate and this could be part of the reasons for the high weevil mortality in the main laboratory.

6.4.3 Seed moisture content

The seed moisture content of the rice varieties stored without weevils in the insect rearing room did not change from the initial moisture contents whereas those stored in the main laboratory had significantly reduced moisture content both at eight weeks and 16 weeks storage times. This indicates that at 29.5°C and 67% relative humidity the equilibrium moisture content of the rice seed is 11% and 12 % for Doongara and Amaroo respectively. This is in agreement with Arvier (1983) and Agrawal (1986) who listed similar seed moisture content (11.7%) and relative humidity (65%) for maintaining rice seed moisture equilibrium during storage, but at a lower temperature (25°C). The moisture contents of Doongara and Amaroo were not significantly different at the start of the experiment and this was maintained throughout the experiment, suggesting seeds of these varieties have similar equilibrium seed moisture content irrespective of their physical characteristics.

The lentil powder and weevils did not change the moisture content of the rice seed which means that treatment of rice seed with lentil powder has no effect on the moisture content of the seed, and also the existence of weevil on rice seed does not affect the moisture content of the seed.

6.4.4 Weevil mortality

Weevil mortality was higher in the main laboratory than in the insect rearing room on both rice varieties and this confirms the result of experiment one. This means that the low relative humidity (42.5%) and low seed moisture content (below 10%) in the main laboratory were responsible for the high mortality of the rice weevils. This supports the observations made earlier by Tsai and Chang (1935), Davison (1940), Harris (1943), Birch (1945), Hill (1987) and Khan *et al.* (1993) that low relative humidity (below 60%) and low seed moisture content (below 10%) deter the survival of adult rice weevils.

Weevil mortality was higher on treated seeds than on untreated seeds of both rice varieties. This confirms the result of experiment one that lentil powder is an insecticide against rice weevil.

The percentage mortality of weevils was higher on Doongara than on Amaroo in the main laboratory, but in the insect rearing room there was no difference in weevil mortality on these varieties. It is therefore difficult to say that Doongara is more resistant to rice weevil than Amaroo as observed in experiment one. Further research therefore needs to be done to monitor the number of eggs laid and developed on Doongara and Amaroo in addition to mortality of adult weevils, before any valid conclusion can be drawn on the resistant status of these rice varieties to rice weevil.

6.4.5 Rice seed viability

The seed viability of both varieties remained high throughout the experiment, and this confirms the findings of experiment one that treatment of rice seed with lentil seed powder has no effect on rice seed viability. Over 90% of the seeds of both rice varieties germinated at the end of the storage period.

7

GENERAL CONCLUSIONS

7.1 Effect of botanical preparations on rice weevil and on rice seed viability

Treatment of stored rice with neem oil, neem powder and lentil powder can give some protection from weevil damage. Pepper powder can not kill adult rice weevil. Dusting of seed rice with powders of lentil and neem at the rate of 0.02g/kg seed can give effective protection against rice weevil damage with no reduction in viability of the seeds. Treatment of rice seed with neem oil at the rate of 0.005ml/kg seed can effectively control weevil damage without reducing the seed germination. Pepper, lentil and neem powders do not reduce rice seed viability in storage up to 24 weeks. Neem oil can reduce the development of weevil offspring in rice seed. Powders of neem, lentil and pepper have little or no effect on the reproductive activity of rice weevil. Relative humidity and seed moisture content greatly affect the survival of adult rice weevil. Low relative humidity of 42.5% and seed moisture content of less than 10% enhance the mortality of adult rice weevils on rice seed. Storing rice seed at a low relative humidity of 42% can be a control measure against rice weevil, but this is not practically possible with traditional farmers in Sierra Leone where relative humidity is about 70% on average for most part of the year. The equilibrium moisture content of rice at 11-12% can be maintained if the seeds are stored at a relative humidity of 66.6% and a temperature of 29.5°C.

7.2 Practical utility

The result of this research can be very useful more to low-income subsistence rice farmers. The farmers will have the practical advantage of being able to use locally available material to protect stored rice destined for household or small-scale use.

7.3 Future research

1. This experiment is confounded by change in relative humidity hence needs to be further investigated in order to know how long the botanicals remain bioactive.

2. The production of abnormal seedlings was observed with the use of neem oil at a higher dose, but this effect declined with increase in storage period of the treated seeds. That is, the treated seeds recovered normal germination as they stayed longer in storage, but the mechanism of this process is not known. It will therefore be necessary to determine the mechanism by which neem oil at higher rate affects seed germination.

3. Pepper did not kill weevil in this experiment, but traditional farmers are using it to control this storage pest. It will be necessary to know if pepper is a repellent rather than an insecticide against rice weevil, or if the insecticidal property of pepper is a varietal effect.

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Appendices

Appendix I. Confirmation of rice weevil used in experiments by AgriQuality NZ.

AgriQuality New Zealand Limited
Huarangi Aotearoa

New Zealand Plant Protection Centre

DIAGNOSTIC REPLY FORM 131 Boundary Road, Lynfield 1007 P O Box 41, Auckland 1015

AgriQuality Assurance Services

Test Costs	$90.00	**NZPPC Reference:**	**18345**
Other Costs			
Total	$90.00	**Client Reference :**	0125 - 5988
NOTE: DO NOT PAY ON THIS AMOUNT GST AND ADJUSTMENTS WILL BE INCLUDED IN THE INVOICE		**Charge/Analysis Code:**	5540 - 07 - 1129

November 16, 1999

Allen Sartie
c/o Craig McGill
Seed Technology Centre
Massey University
PALMERSTON NORTH

Dear Allen

Re: Insect identification

Genitalia of adult male weevil specimens were dissected out and identified as **rice weevils, *Sitophilus oryzae* (Coleoptera: Curculionidae)**. This species is well established in New Zealand and can be found on many stored products.

If you have any further queries or would like additional information please do not hesitate to contact me.

Signed: M.G. Bullians Mark Bullians (Entomologist)
Ph (09) 627 2525

DISCLAIMER : The information in this report is based upon an examination of the sample supplied and the best information available to the writer at the time of examination. In the absence of knowledge of specific growing conditions or other conditions pertaining to the sample and in the absence of control over how the information is to be applied, any action taken in reliance of this report is the sole decision of the user of the information and is taken at his or her own risk. Accordingly NZPPC disclaims any liability arising out of any use of this information or in respect of any actions taken in reliance upon this information.

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Appendix II. Germination of rice seed during experiments one and two presented in graphical forms in main text including statistical analysis.

Table 7: Data used in Figure 1 in main text - Percent germination of rice seed from time of treatment.

Treatments	1 day	4 weeks	8 weeks	16 weeks	24 weeks
Untreated	96	96	98	94	98
Lentil pwd.	96	95	97	93	97
Neem pwd.	97	96	97	92	96
Pepper pwd.	96	97	98	96	96
Neem oil	81	76	85	88	90
Initial germination	98				
LSD (5 % level, df = 411) = 3.5					

Table 8: Data used in Figure 2 in main text - Effect of neem oil concentration (ml/kg seed) on normal (N) and abnormal (Abn) germination of rice seed.

Rate (ml/kg)	1 day		4 weeks		8 weeks		16 weeks		24 weeks	
	N	Abn	N	Abn	N	Abn	N	Abn	N	Abn
Untreated	96	2	96	2	98	1	94	5	97	1
0.005	95	2	90	7	96	2	92	6	93	4
0.01	89	7	86	11	92	4	85	12	95	2
0.02	47	47	46	47	58	35	85	10	79	16
Initial germination	N=98 Abn=1									
LSD (5 % level, df=411) = 2.5										

Table 9: Data used in Figure 6 in main text - Percent germination of rice seed from start to end of storage in main laboratory and in insect rearing room.

Rice variety	Initial germ	Main laboratory				Insect rearing room			
		8 weeks		16 weeks		8 weeks		16 weeks	
		Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
Amaroo	96	97	97	95	95	97	97	95	94
Doongara	98	99	97	98	98	98	97	97	98
LSD (5 % level, df = 49) = 3.0									

Appendix III. Seed moisture content of Amaroo and Doongara rice varieties during experiment two, presented in graphical form in main text.

Table 10: Data used in Figure 7 in main text - Seed moisture content (%) of Amaroo and Doongara without weevil infestation.

Rice varieties	Storage time	Main laboratory (±SE)	Insect rearing room (±SE)	SE(diff)	t_{cal}
Amaroo	8 weeks	10.0 ± 0.12	12.0 ± 0.23	0.259	8.301
	16 weeks	10.0 ± 0.21	12.0 ± 0.00	0.210	9.143
Doongara	8 weeks	10.0 ± 0.00	11.0 ± 0.32	0.320	5.938
	16 weeks	9.0 ± 0.19	11.0 ± 0.21	0.283	7.597

df = 6, P=0.05, t_{tab}=2.447

Table 11: Data used in Figure 8 in main text - Seed moisture content (%) of Amaroo and Doongara after weevils were removed.

Rice varieties	Treatment	Storage time	Main laboratory (±SE)	Insect rearing room (±SE)	SE(diff)	t_{cal}
Amaroo	Treated	8 weeks	8.0 ± 0.23	10.0 ± 0.39	0.453	5.739
		16 weeks	8.0 ± 0.18	10.0 ± 0.34	0.385	4.545
	Control	8 weeks	8.0 ± 0.18	10.0 ± 0.15	0.234	11.453
		16 weeks	8.0 ± 0.19	11.0 ± 0.19	0.269	8.810
Doongara	Treated	8 weeks	8.0 ± 0.34	10.0 ± 0.15	0.372	5.726
		16 weeks	8.0 ± 0.19	10.0 ± 0.25	0.314	4.682
	Control	8 weeks	7.0 ± 0.19	9.0 ± 0.23	0.298	5.939
		16 weeks	8.0 ± 0.18	10.0 ± 0.19	0.262	8.779

df = 6, P=0.05, t_{tab}=2.447

Appendix IV. Initial seed moisture content of Amaroo and Doongara rice varieties.

Table 12: Seed moisture content of (%) Amaroo and Doongara rice seeds before experiment one.

Amaroo (±SE)	Doongara (±SE)	SE(diff)	t_{cal}	df	t_{tab}
11.0 ± 0.033	11.0 ± 0.39	0.511	0.361	6	2.447

P=0.05

Table 13: Seed moisture content (%) of Amaroo and Doongara before experiment two.

Amaroo (±SE)	Doongara (±SE)	SE(diff)	t_{cal}	df	t_{tab}
12.0 ± 0.23	11.0 ± 0.23	0.325	0.831	6	2.447

P=0.05