# ASSESSMENT OF POSTHARVEST PEST MANAGEMENT PRACTICES IN NEPAL AND EFFICACY OF HYPOXIA FOR CONTROLLING *SITOPHILUS ORYZAE L.* (COLEOPTERA: CURCULIONIDAE)

by

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*This work is dedicated to my grandparents.*

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# ABSTRACT

Farmers in Nepal lose about a third of their harvested grain due to postharvest handling and storage. This has led to food insecurity and economic losses. Despite the importance of postharvest, the grain storage system in Nepal relies on traditional storage structures like bamboo granaries. The incidence of storage pests is reported up to 100% in these structures. To minimize the storage loss, farmers use different grain protection methods including toxic chemicals. Multiple cases of pesticides-related poisoning and deaths have been caused by misuse and overuse of pesticides. To push safer, chemical-free alternatives like hermetic storage it is important to understand current pest challenges and management practices in Nepal. Adaptation of chemical-free pest management strategies like hermetic storage largely depends upon basic and applied laboratory research findings. Determining the baseline adult mortality under various hypoxia levels and subsequent insect emergence will help determine the effectiveness of a low oxygen environment in controlling *Sitophilus oryzae* (L.). This would increase our understanding of hermetic storage technology and help improve its application to both farmers and commercial users and serve as a possible substitute to traditional or chemical pest control methods. In chapter one, I report the result of the survey conducted in Nepal to understand i) current post-harvest storage practices and (ii) assessment of the best delivery approach for storage innovations. In chapter 2, I report the result from laboratory experiment conducted to understand the lethality of hypoxia at 5% oxygen level and below against *Sitophilus oryzae* (L.), which is a major storage pest reported by farmers in Nepal.

# CHAPTER 1. REVIEW OF LITERATURE

## 1.1 Food security in Nepal

The population of Nepal is expected to reach 35.32 million on the year 2040, and meeting the food requirement for this increasing population is going to be a big challenge (Bista et al., 2013; WPR, 2021). The rural population of Nepal directly depends on the agriculture sector (Tamang et al., 2014). Agriculture is a vital source of income for household, education and health needs. Developing agriculture is fundamental for food security, poverty alleviation, and sustainable development (WPR, 2021).

Even though Nepal is primarily an agrarian country with 60% of people involved in agriculture, an estimated 4.6 million people are food insecure (USAID, 2019). The agriculture of Nepal is mostly centered around major cereal crops such as rice, maize, and wheat, and legume crops such as lentils, chickpeas, and beans (Gairhe et al., 2018; Shrestha et al., 2011; Tripathi et al., 2018). The domestic production of grain is not enough to meet the national need. Nepal imported 66,352 MT of rice, 42,243 MT of maize, and 1,119 MT of legumes in 2012 (WFP, 2012). On average, 20-30% of the grain produced is lost in the post-harvest period and the majority of these losses occur during storage. Reducing post-harvest losses would be an integral part of strategies to addressing hunger and food security. The gap in production and import opens the possibility of growth of the agriculture sector by improving production and postharvest practices.

## 1.2 Storage practices and losses Nepal

In Nepal, farmers plant their major crops in the monsoon season from June to July and harvest in September to December (Manandhar et al., 2011; Paudel, 2016). After the harvest, threshing, transporting from field to storage house, winnowing, drying, storing, and milling are done manually (Sharma et al., 2009; Tripathi et al., 2018). In Nepal, farmers have limited access to knowledge and technology for long-term seed and food grain storage systems (Subedi et al., 2009). They rely on traditional storage structures such as *bhakari* (rolled bamboo or straw mat), bamboo basket, wooden basket, earthen clay pot, and heaping of grain in circular structure over the ground. These storage structures are culturally acceptable, cheap, and easily available but are not always effective in protecting grain from pests during short or long-term storage.

Traditional storage structures prevent grain from spillage and safe from rain and sun; they facilitate the flow of ambient air and moisture (Manandhar et al., 2018). Shivakoti and Manandhar (2000) identified insects and diseases as the principal cause of postharvest loss. These losses are exacerbated when grains are stored in traditional structures like bamboo granaries. Studies prior to the 2000s reported postharvest losses in the cereal of about 15-20% and these losses were more severe in flat lands compared to hills and mountains (KC, 1992; Pradhan and Manandhar, 1992). Based on the study of seasonal variation in stored grain, damages were most severe in May when the temperature is hot and humid (Manandhar and Mainali, 2000). These environments during storage promoted the growth of molds such as *Penicillin* and *Aspergillus,* causing a loss of up to 5% (Manandhar and Batsa, 2000). Furthermore, storage loss was significantly influenced by time of harvest, altitude, occupation of household head, and structures for storing grains (Paneru and Paudel, 2018). Because of a lack of proper storage structures and to avoid storage losses, farmers often sell their grain right after harvest at a low price (Nainabasti and Bai, 2009). Minimizing storage losses would allow farmers to sell grain when the prices are high and to avoid having to buy grains from the market at double or triple prices after a few months.

## 1.3 Most important storage pests

Pests (particularly insects) are the major source of storage loss in Nepal. There are nearly a dozen of economically important storage insect pests in Nepal. Among them are rice/ maize weevil (*Sitophilus* spp*),* Angoumois grain moth (*Sitotroga cerealella,* Oliver), Indian meal moth (*Ploida interpunctella,* Huber), Lesser grain borer *(Rhyzopertha dominica* F.), Khapra beetle (*Trogoderma granarium,* Everts), red rust flour beetle (*Tribolium castaneum,* Herbst) and legume weevil (*Callosobruchus spp*.). They can either damage grain internally by laying eggs inside the grain and spend major portion of their life inside the kernel or externally by feeding and spending their life cycle on the surface of grains and grain particles. These pests usually have a very high reproductive rate and can damage stored grain from 5 to 100% within one season (Manandhar et al., 2018; Subedi et al., 2009).

Various studies have reported pest incidence in Nepal. GC (2006) found that maize stored in local structures was heavily infested by *S. cerealella,* which caused losses of 15-30%. *Sitophilus* spp*, T. castaneum, R. dominica*, and *S cerealella* are present in maize stored in traditional storage structures like the bamboo basket, hanged in the rope with husk and raised and piled structures

(Manandhar and Shrestha, 2000). The incidence of infestation of *S. oryzae* leads to severe loss in quantity and quality in stored wheat. The damage worsened when the grain moisture content, relative humidity, and temperature were above 12%, 70%, and 27 ° C, respectively (Khanal et al., 2021). There was a 100% incidence of *Sitophilus* weevils in grains stored in the Surkhet and Chitwan districts of Nepal (Bhusal and Khanal, 2018). It is reported to be present in all regions of Nepal and damages the range of stored grains (Subedi et al., 2009).

### 1.4 *Sitophilus oryzae* (L.)

*Sitophilus oryzae* (L.) is a worldwidely distributed pest and is invasive in nature. It is believed to have originated in India and spread around the world through commerce (Koehler, 2012; Romano et al., 2016). It feeds on cereal and cereal products and prefers whole grains such as wheat, rice, corn, and barley. It can also be found feeding on beans, nuts, processed cereals, spaghetti, pasta, cassava, pet food, and decorative Indian corn (Mason and McDonough, 2012). It is extremely attracted to freshly harvested grains for carrying out metabolic activities and the continuity of generations (Phillips et al., 1993).

The females of *S. oryzae* oviposit their fertilized eggs inside of a grain. Eggs are white, , ovoid to pear-shaped, and are laid in aggregation (Bhargava et al., 2007). Female weevils then carefully seal their eggs to hide the oviposition puncture (Smith, 1986). The eggs hatch after six to seven days and the larval stage begins. The larval stage of *S. oryzae* has four instars. It is not free-living and develops completely inside the grain (Soderstrom, 1960). The larvae gain sustenance by consuming the endosperm of the whole grain (Hansen et al., 2004; Longstaff, 1981; Soderstrom, 1960). Molting occurs soon after, and the larvae then enter the pupal stage. The pupal stage is considered the dormant stage in the lifecycle of *S. oryzae*. In the experiment by Soderstrom (1960) the pupation rate was maximized at or above 25 days after oviposition with only a few pupating before then. The pupal form lasts for one to two weeks. During this, *S. oryzae* undergoes numerous changes in form. They grow wings and legs and develop sexual organs. Mobility, feeding, and respiration are all highly suppressed in this stage, which makes them highly resistant to many control methods (Howe, 1973; Punj and Verma, 1970). After the pupal stage is complete, adult *S. oryzae* emerge from their grains.

Males and females of *S. oryzae* become sexually mature around 42 hours after emerging from the pupal stage and start laying eggs. The females can lay around 300- 600 eggs in their lifespan of three to six months. Adult *S. oryzae* feed by boring into grain to consume the endosperm and reduces the carbohydrate content (Bello et al., 2001; CABI, 2019). The damage causes loss in quantity, loss of nutritive value and germination, and contamination by mites and fungus which makes it undesirable to consume and leads to a loss in the market value (Pittendrigh et al., 1997; Subedi et al., 2009).

## 1.5 Management of *Sitophilus oryzae* (L.)

*Sitophilus oryzae* (L.) is universally regarded as one of the most destructive primary pests of stored cereals. They are carried from field to storage area and can also go from old to newly stored grain (CABI, 2019). Primarily, proper cleaning of the storage area minimizes the infestations in storage areas. Various other strategies of pest management have been used to control *S. oryzae* which are explained below.

### 1.5.1 Chemical control

The most widely used method for the control of *S. oryzae* is fumigation with phosphine (Kim et al., 2019; Nguyen et al., 2015). Phosphine is popular across the globe as it is relatively low priced, easy to apply and has a rapid killing mechanism. *S. oryzae* can also be controlled by using the mixture of insecticides such as organophosphorus compounds, fenitrothion, and perimorphs-methyl (CABI, 2019). Even though fumigation of various compounds can control *S. oryzae* for a certain period it does not provide protection against reinfestation (Abd El-Aziz, 2011; CABI, 2019). The dormant stage with low physiological activity like the pupal stage of *S. oryzae* has comparatively low susceptibility to the fumigants (CABI, 2019). The use of insecticides is also associated with health problems for applicators and consumers, and environmental challenges (Dubey et al., 2008; Mbata and Phillips, 2001; Roller and Baumgartner, 2016). Using more and more of these pesticides would have more effect on consumers and increase the resistance of these insects to chemicals (Afful et al., 2018; Chen et al., 2015; Nayak et al., 2020). *S. oryzae* is developing resistance to pesticides.

### 1.5.2 Manipulation of temperature

The temperature of 25°C to 33°C is considered the ideal for the growth and development of stored pests (Abd El-Aziz, 2011). Research has found *S. oryzae* are the least cold-tolerant, hence freezing a storage area is the most effective method of temperature manipulation (Mason and McDonough, 2012). The adult emergence is seized at 15°C, and metamorphoses is completely suppressed at 10°C (Nakakita and Ikenaga, 1997). Alteration of temperature is a good alternative to fumigation in developed countries to avoid storage pest damage (Mason and Strait, 2020). However, it might be challenging to use this method for smallholder farmers in developing countries where access to appropriate technologies and energy are limited.

### 1.5.3 Botanical control

Plant extracts have been used for a long time to repel and kill insect pests during grain storage. In fact, it is the earliest method of protecting different types of grains including cereals from *S. oryzae.* Extracts from different plants like basil, tabasco pepper, neem, and ginger are traditionally used to control *S. oryzae*.  The use of *Acorus calamus* (L.) kills 98.33% of adults and prevents grain damage (Khanal et al., 2021). Ethanol extracts from leaves of *Psidium guajava* (L.) *are* also a good alternative to pesticides with a mean repellency of 70.33% (Akhtar et al., 2013). Ethanol extracts from *Melia azdarach* (L.)*, Myrtus communis* (L.), and *Mentha longifolia* (L.) kill 61.2%, 48.20%, and 47.40% of adult weevils (Saljoqi et al., 2006). Botanicals are a good alternative to synthetic pesticides as they are biodegradable and environment friendly. Furthermore, the less toxic nature of some botanicals makes it safer for mammals (Rajashekar et al., 2012).

The use of botanicals is not completely free from limitations. There can be economic uncertainties associated with the seasonal seed production and the short life span of botanical trees. The instability of active ingredients and rapid degradation, when exposed to direct sunlight might also cause a problem. Additionally, most botanicals are not widely available and some are expensive compared to conventional pesticides (Rajashekar et al., 2012).

## 1.6 Hermetic control

Hermetic storage is a promising chemical-free method to storage pest management. It uses sealed, airtight containers to control the moisture and living organisms (e.g., insects) in stored dry agricultural commodities (Baributsa et al., 2010; Kharel et al., 2019; Murdock and Baoua, 2014). This method of storage restricts the exchange of gases between internal and external environments and stored commodities, maintaining the initial level of moisture while controlling pests by depriving them of oxygen (Baributsa et al., 2010; Murdock et al., 2012). Hermetic storage provides a safe, organic, and sustainable storage and is effective for different grains and seeds specially in hot and humid climates (Baoua et al., 2014; Martin et al., 2015; Williams et al., 2014).

With increasing awareness of the negative effect of pesticides among the public, hermetic storage methods like Purdue Improved Crop Storage (PICS) bags are getting more popular and widely adopted. PICS bags provide an affordable and flexible storage option to small holders farmers and minimizes the loss caused by *S.oryzae.* Wheat stored in a PICS bag had a minimal loss and similar to non-infested grain (Martin et al., 2015). PICS bags preserve grain by creating a hermetic condition where the insect pest uses the oxygen available and stops additional air from getting inside. Murdock et al (2012) explained that the reduced oxygen level leads to the halting of larval feeding, which in turn stops the development of the insect. Insects cannot complete their lifecycle and do not develop into adults. Lowering oxygen level to 2% caused complete mortality of eggs, larvae, pupae, and adults within fifteen days of exposure (Kharel et al., 2019).

The study of the effect of hypoxia on the acoustic activity of storage insects was done to find insect response to reduced oxygen environment (Njoroge et al., 2019, 2017). This study found that exposure of *S. oryzae* to hypoxia below 5% caused their acoustic death i.e. acoustically silent. This means the rate of burst and impulse produced by insect activity was below the threshold level of causing damage. However, being acoustically silent doesn’t always mean the biological mortality of *S. oryzae*. Repeating the experiment with hypoxia levels of 1%, 3% and 5% with a time factor would give an insight into the effect of hypoxia on the mortality of *S. oryzae*, it’s resurgence, and assessment of progeny following the treatment at the above-mentioned oxygen level.

## 1.7 Research objectives

The overall goal of this thesis was to (1) understand the status of postharvest storage practices and flow of information to farmers in Bagmati province of Nepal, and (2) estimate the lethality of hypoxia against major pest, *Sitophilus oryzae* (L.). The specific objectives are as mentioned below:

**Objective 1:** To assess the postharvest storage practices and delivery of new storage innovations in Nepal.

Sub-objective 1: To understand current storage management practices among farmers in two districts of Bagmati province, Nepal.

Sub-objective 2: To evaluate the factors that influence farmers' decision to use an insecticide in grain storage.

Sub-objective 3: To assess how farmers obtain information, and how extension services disseminate information on postharvest technologies.

**Objective 2**: To estimate the lethality of hypoxia in *Sitophilus oryzae* L. at levels of hypoxia below 5%.

Sub-objective 1: To assess the effect of hypoxia below 5% on the biological mortality of *S. oryzae.*

Sub-objective 2: To assess progeny development following exposure to hypoxia below 5%.

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# CHAPTER 2. ON FARM GRAIN STORAGE AND INFORMATION DELIVERY TO FARMERS IN BAGMATI PROVINCE, NEPAL

## 2.1 Introduction

Nepal has an agrarian-based economy that contributes one-third to the country's GDP and more than 60% of the total population are engaged in agriculture (Sharma, 2000). The most cultivated staple crops in Nepal include rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), and maize (*Zea mays* L.) (Devkota et al., 2018; B. Shrestha et al., 2011; Tripathi et al., 2018). Agriculture is mostly rain-fed; thus, farmers produce a major portion of their crop during the rainy season; from June to September (Manandhar et al., 2011). Most postharvest operations, including threshing, transportation (from field to house), winnowing, drying, storage, and milling, are done manually (Sharma et al., 2009; Tripathi et al., 2018). Storage losses range from 5.0 to 20% for several cereal crops and are mostly caused by insects and rodents (Ganesh, 2001; Manandhar and Mainali, 2001; Paneru et al., 2018; Subedi et al., 2009b). These storage losses are exacerbated by prevailing hot and humid conditions during the monsoon season (Boxall and Gillett, 1982).

Farmers use several approaches to address storage losses including traditional methods and pesticides (Ganesh, 2001; Sharma et al., 2013). Traditional storage methods and their variants include plant and plant derivatives, indigenous materials (e.g *Thangros* i.e.vertical/or horizontal poles to store maize with sheaths), and bamboo granaries (Ganesh, 2001; Manandhar and Mainali, 2001). Challenges with traditional granaries include their inability to protect grain from insect and disease attacks during storage (Shivakoti and Manandhar, 2001). Insecticides have shown to be effective at mitigating insect-caused storage losses. When available, insecticides are used by farmers to prevent storage losses because they tend to be affordable (Obeng-Ofori et al., 2015). However, misuse and overuse may have adverse human health consequences, even death (Sharma et al., 2013). Incidences of pesticide poisoning have been reported in Nepal, particularly in the Dhading district where 20 people were hospitalized and a family of six died after consuming food treated with pesticides (Rathore, 2015).

Studies comparing the efficacy of various storage technologies reported better performance of improved storage techniques (e.g., metal bins) compared to traditional structures in reducing insect damage and weight loss, as well as in preserving seed viability and germination (Devkota et al., 2018; Ganesh, 2001; Sharma and Tiwari, 2020). Several efforts were made to promote the use of storage methods to reduce postharvest losses including botanicals, metal bins, granaries, synthetic insecticides, and traditional storage structures plastered with mud, cement, or plastics (Ganesh, 2001; Manandhar and Mainali, 2001). Despite these efforts, the adoption of improved storage methods at the farm level has been minimal due to technical and institutional constraints such as a heavy focus on crop pre-production and limited training on postharvest technology generation and dissemination (Adhikari, 2001).

Extension agents play a vital role in building awareness and increasing the adoption of new agricultural technologies (Suvedi et al., 2017). The role of extension services in Nepal is similar to that of many other developing countries. Extension agents take information from government research agencies to farmers in collaboration with local extension advisory units. This top-down extension approach often lacks understanding of the on-the-ground situation and fails to consider farmers' needs; hence missing opportunities to improve its efficiency (Dhital, 2017; FAO, 2017). In today's time of rapid agricultural transformation requires that agricultural extension adapt and transform itself to meet the needs of its clientele. Inefficient, underfunded, and understaffed extension services with weak delivery systems can only result in low adoption of innovations and technologies (Agbarevo, 2013; Birner and Anderson, 2007). In Nepal, government extension services capacity (personnel, infrastructure, funding, etc.) are inadequate to meet the extension demands of farmers and private sectors (Babu and Sah, 2019).

With the introduction of improved storage technologies in Nepal, there was a need to assess the status of postharvest practices and the flow of information. Therefore, the objectives of this study were to: (i) understand current storage management practices among farmers, (ii) evaluate the factor that influences farmers' decision to use insecticides during grain storage, and (iii) assess how farmers obtain and extension services disseminate information on postharvest technologies. Results would be useful to government and development partners interested in disseminating improved storage technologies to reduce grain losses and health risks associated with chemical use.

## 2.2 Methods

### 2.2.1 Site selection

The study was conducted in December 2018 in two ecologically and socioeconomically diverse districts in the Bagmati Province of Nepal: Chitwan, and Dhading. The agricultural characteristics of the Chitwan and Dhading districts are markedly different. Farmers in Chitwan, a lowland area, practice commercial farming and have easy access to infrastructures (e.g., irrigation) and technologies (Piya et al., 2012). Access to year-round irrigation allows these farmers to harvest rice twice a year- Nov/Dec and Jun/Jul (Tripathi et al., 2018). Legumes such as beans (*Phaseolus vulgaris* L.) and lentils ( *Lens culinaris* Medik.) are grown in between rice seasons (Pokhrel and Pokhrel, 2013). Dhading district, in contrast, is midland, where most of the villages are in a rural setting and rice is grown in the wetland areas. Maize is the major cereal grown in unirrigated lands in both districts, and usually intercropped with soybean (*Glycine max* L. Merr) and cowpea (*Vigna unguiculata* L. Walp) (Gharti et al., 2014). The survey was implemented in six Village Development Committees (VDCs), three in each district (Figure 2-1). Data was collected during the transition from VDCs to new administrative units “Gaupalika” or “rural municipality” created under the recent political reforms in Nepal. The targeted VDCs were Mahadevsthan, Sankosh, and Pida in Dhading and Patihani, Jagatpur, and Sukranagar in Chitwan. These VDCs were selected based on crop production and issues related to excessive use of pesticides (Shrestha et al., 2010).

### 2.2.2 Sampling and Data Collection

Survey data was collected with two questionnaires: one for farmers and the other for extension agents. Both questionnaires were semi-structured with open and closed-end questions. The questionnaire for farmers focused on understanding farmers’ demographics, storage challenges of major cereal and legume crops, storage loss mitigation approaches and limitations, and source of agriculture information. The questionnaire for extension agents focused on identifying their role and approaches in disseminating new postharvest technologies and potential challenges. Both questionnaires (farmers and extension agents) were uploaded into the KOBO toolbox (https://www.kobotoolbox.org) and answers were recorded using Android tablets. In each of the VDCs, 40 farmers were randomly selected from the farmers’ list provided by the local nongovernment organizations (NGOs). Interviews were conducted at the houses of farmers. Prior to each interview, a brief introduction of the study was provided, and oral consent was requested from the participant. If a farmer did not agree to participate, the interview was discontinued. The lists of extension agents were obtained from farmers' groups and cooperatives. The interviews were conducted in their offices or agrovet shops. Agrovet refers to aginput store that sells supplies such as fertilizers, seeds, insecticides, animal feed, and veterinary supplies. 241 farmers (121 and 120 respondents in Chitwan and Dhading districts, respectively) and 81 extension agents (40 from Chitwan and 41 from Dhading) were interviewed.

### 2.2.3 Data analysis

Raw data from the Kobo toolbox was downloaded and cleaned before analysis for congruity. Some choices on questions were removed because no response was recorded, new categories were created based on the responses obtained, and several choices were combined into one category. After cleaning, the data was analyzed with SPSS 26.0 (IBM Corp., 1026, New York, United States). Cross tabulations were constructed, and the data was summarized with the descriptive statistics. Correlation tests were used to ascertain the relationships between variables. Factors that influenced farmers' decision to use pesticides were evaluated by performing a logistic regression analysis in R v 3.5.3. Likelihood ratio (L.R.) test statistics (Hosmer and Lemeshow, 2000) was used to assess fitness of logistic regression models. Boxplots were used to conceptualize the data on the quantity of grain stored.

## 2.3 Result

### 2.3.1 Demographic characteristics of farmers and extension agents

Most of the respondents were female (66.4%), married (95.9%), and had basic to high school education (73.0%) (Table 2-1). About three-fifths of the respondents were 41 years or older. Among the respondents, 90% had farming as their main economic activity, 88.4% had ten years or more of farming experience, 95% had cellphones, and only 45% had a radio in their house. Contrary to farmers, most extension workers were males (80.2%). The portion of extension agents with a college or university degree was higher (85.2%); however, Chitwan had more extension agents (65.0%) with a university degree than Dhading (41.5%) (Table 2-1). The majority of extension agents (86.4%) had five years or more of experience in their position (Table 2-1). Among all extension agents, 95% played a role in introducing new technologies to farmers in the last five years and 64.2% had introduced postharvest technologies (Table 2-1).

### 2.3.2 Source of information and training approaches

Though 41.5% of farmers were in contact with extension agents, only 12.0% of farmers depend on them as their primary source of information (Table 2-2). Among farmers who were in contact with extension agents, 60% were being supported by agricultural cooperatives, farmers' groups, and NGOs' extension services, while the rest were in contact with government extension (Table 2-2). The majority of extension agents (70.4%) noted that training/demonstration were the current methods used to make farmers aware of new technologies (Table 2-2).

However, training/demonstration dropped to only 42.0% when extension agents were asked about effective approaches to make farmers aware of new technologies. The village was the most predominant platform used by extension agents (69.1%) to train farmers in both districts. Extension agents went traveled to farmers' villages and trained them mostly in community halls. Extension agents in Chitwan relied on government agencies (65.0 %) as their source of new technologies, while in Dhading it was only 32%. Extension agents in Chitwan provided information to farmers on where to buy new technologies (80.0%); while in Dhading extension agents (53.7%) collected money and bought technologies for farmers (Table 2-2).

### 2.3.3 Grain and seed storage, and pest challenges

The proportion of farmers storing grain varied by crop and district. The proportion of farmers storing crop in both districts were 97.4 % for maize, 93.0% for soybean, and 75% for cowpea in Dhading; while in Chitwan it was 88.6% for lentils, 76.4% for beans, and 58.9% for rice. Quantity of cereal grains stored by farmers varied by crop and was significantly higher for rice than maize (p=0.0001). Quantity stored ranged from 5 to 2,800 kg for rice (median 1,150 kg) and 80 to 2,050 kg for maize (median 537.5 kg) (Figure 2-2). The quantity of legumes stored was smaller and significantly different (p=0.0006) among crops. They varied from 5 to 600 kg for lentils (median 35 kg), 0.75 kg to 825 kg for beans (median 14.5 kg), 2.5 to 200 kg for soybean (median 26.5 kg), and 2 to 105 kg cowpea (median 17.5 kg) (Figure 2-2). A few additional legumes were stored by a limited number of farmers including Mung beans (*Vigna radiata* L.), Black gram (*Vigna mungo* L.), Pea (*Pisum sativum* L.). About half of farmers who stored kept their grain for more than nine months and they primarily stored for home consumption (Table 2-3).

Granaries and woven bags were mostly used to store rice (67.3% of farmers), while maize was stored by piling it on the ground in a circular raised structure (68.4% of farmers). Granaries were significantly likely to be used by farmers storing rice (Pearson correlation coefficient r=44.5%, p=0.000). Insecticides were mostly applied to grain stored in granaries and woven bags. Hermetic storage technologies (HSTs) were predominantly used to store legume crops: beans (75%), lentils (88.7%), and cowpea (64.5%) (Table 2-3). Farmers indicated that the benefit of using HSTs included effectiveness in protecting grain (93.5%), not using chemicals (34.8%), and easy to use (15.9%). Among farmers who did not use HSTs (n=108), the main reasons were lack of awareness (77.0%), unavailability (16.4%), and high price (6.7%).

Insect damage was the major storage challenge for cereals but less for legumes. About two-thirds of farmers reported no damage on most stored legumes, except cowpea. Farmers identified several pests of stored cereals including *Sitophilus oryzae* (L.) (91.3%), *Corcyra cephalonica*, (Stainton) (89.2%), *Rhyzopertha dominica* (Fab.) (1.2%), *Plodia interpunctella* (Hübner) (0.8%), *Oryzaephilus surinamensis* (L.)  (0.8%), and *Sitotroga cerealella* (Oliver) (0.4%). Farmers with infested cereals were more likely to have *S. oryzae* (Pearson correlation coefficient r=60.4%, p=0.000) and/or rice moth (Pearson correlation coefficient r=53.1%, p=0.000) as the major pests. Stored rice infested by *S. oryzae* was significantly likely to be attacked by *C. cephalonica* as well (Pearson correlation coefficient r=79.4%, p=0.000). Legume crops were mostly infested by bean *Bruchus* spp. and *Callosobruchus* spp. (Table 2-4).

Seed storage was practiced by most farmers (77.2%, n=241) in both districts (Table 2-5). Farmers who did not store seed (n=55) purchased it from agrovet shops (60.8%), farmers' groups/cooperatives (35.3%), or community seed banks (3.9%). Among farmers who stored seeds, 71.4% had insect damage. Farmers used a variety of seed storage methods including hermetic methods (23.8%), botanicals (17.5%), pesticides (11.6%), ash (11.6%), and drying (2.6%). A third of farmers (31.2%) did not protect their seeds during storage.

### 2.3.4 Grain protection and farmers' decision to use insecticides during storage

Among farmers using pesticides to protect cereals during storage (n=89), 98% of them applied chemicals on rice. Most farmers storing maize (68.4%) did nothing to protect their grains. Ninety-six percent of farmers who used pesticides to store grain obtained them from agrovet shops (Table 2-6). Farmers used different types of pesticides to control insects on cereals: 83.2% for Aluminum Phosphide, 9.0% for Dichlorvos (DDVP), 3.4% for Malathion, 2.3% for Methyl parathion. Furthermore, 4.5% of farmers used rodenticides outside grain storage containers to avoid infestation by rodents (Table 2-6). It should be noted that a small proportion of farmers (1.1%) used a fungicide (Mancozeb) on stored cereal grains. Pesticides were significantly likely to be used by farmers in Chitwan (Pearson correlation coefficient r=53.8%, p=0.000). Only a small number of farmers (n=7) used pesticides (Aluminum Phosphide, Malathion, and Methyl parathion) to protect legumes against stored grain pests. Farmers preferred pesticides for several reasons, the major one being efficacy (61.1%). The majority of farmers (63.3%), among those applying pesticides, noted that there were no issues with chemical use.

Among farmers using hermetic containers to store legumes, the majority used them to protect beans (59.7%) and lentils (70.4%). Most farmers storing soybean (65.1%) did not protect their commodity. About three-fourths of farmers were aware of HSTs but only 12.4% were trained on the use. A little over half of the farmers (56.8%) had used HSTs including plastic drums, PICS bags, SuperGrainbags™, and metal silos. Plastic drums were the most used HSTs in Chitwan (86.6% of farmers), and farmers purchased them from agrovet shops. Most farmers in Dhading noted that flexible HSTs including PICS bags (47.3% of farmers) and SuperGrainbags™ (29.1% of farmers) were obtained through donations from NGOs and relief interventions after the 2015 earthquake (Table 4). Farmers using HSTs (n=108) indicated that the benefits included effectiveness (93.5%), chemical-free (34.8%), and ease to use (15.9%). Farmers who were not using HSTs (n=104) gave several reasons including lack of awareness (77.0%), unavailability (16.4%), high price (6.7%), lack of training on how to use (2.9%), and not effective (1.0%). Farmers (n=137) mentioned food security was the main motivation to use HSTs and rarely used insecticide or fumigation inside them (Table 2-7).

To evaluate factors that influence farmers' decision to use pesticides for grain storage, we considered "district", "contact with extension agents", "storage container", "storage duration", "storage location", "gender", and "insect damage" as independent variables in the logistic regression model (Likelihood Ratio test p<0.001). The decision to apply pesticides in grain was affected by the crop stored, storage method, and the incidence of insect damage. Farmers who stored rice (Odds Ratio=8.5) were more likely to use insecticides than those storing maize. Similarly, farmers who stored in granaries (Odds Ratio=1.0) were more likely to use insecticides than those who stored in woven bags. Additionally, farmers experiencing insect damage (Odds Ratio=9.8) were more likely to use pesticides (Table 2-8)

## 2.4 Discussion

### 2.4.1 Source of information and training approaches

Most farmers in both districts relied on their personal experience or that of their peers for agricultural information, just like those in the hilly and terai or lowland districts of Nepal (Devkota and Phuyal, 2018; Manandhar et al., 2011). This is because most of these farmers had limited contact with extension agents and years of accumulated experience in farming. In addition, extension services at the district level in Nepal have challenges in addressing the needs of farmers due to limited staffing, funding, and resources (Babu and Sah, 2019; Suvedi and McNamara, 2012). The void created by the inefficiencies of government extension services is filled by non-public service providers such as agricultural projects, NGOs, farmers' cooperatives, and the private sector (i.e., input dealers). There was an increase in perception among extension agents that media and phones would be effective ways to reach farmers with new information. With increased access to radios and cellphones among farmers, there is an opportunity to explore the use of mass media and digital solutions in reaching farmers with information on new knowledge or innovations (Baributsa et al., 2014; Devkota and Phuyal, 2018; FAO, 2017; Suvedi and McNamara, 2012).

Awareness of new technologies was created through training/demonstrations and house visits by most extension agents. These findings are in agreement with research that showed training and visits were still the most widely used approaches for creating awareness among farmers on agricultural innovations in Nepal (Dhital, 2017). However, it appears that these methods are not yielding the expected outcome as most farmers relied on themselves for agricultural information. On-the-job training of extension agents could help improve the process-oriented competencies for disseminating postharvest technologies and develop skills for personal interactions with farmers and other stakeholders (Martin and Bin Sajilan, 1988). Such approaches have been used to improve the capacity of extension agents in scaling up postharvest storage technologies to millions of smallholder farmers in countries across sub-Sahara Africa (Baributsa et al., 2014; Baributsa and Ignacio, 2020).

### 2.4.2 Cereal and seed storage, and pest challenges

Rice, an essential staple diet in Nepal, is grown by family farms to meet their household needs for consumption as well as for income generation (Ghimire et al., 2015; Nainabasti and Bai, 2009). This in part explains why rice was stored in larger quantities compared to other crops. Overall, in both districts, crop production was strongly focused on cereals followed by legumes (Gharti et al., 2014; Subedi et al., 2009; Tripathi et al., 2018). In Dhading, maize is mainly intercropped with soybean, while in Chitwan rice is grown together with beans. The storage duration of grains for six months or more in Nepal is in congruence with findings from other developing countries in Sub-Saharan Africa and Latin America (Díaz-Valderrama et al., 2020; Njoroge et al., 2019). Though farmers in Chitwan who had access to year-round irrigation grew rice twice a year, they preferred to store fine rice (grown in the main season) for more than nine months because of its organoleptic traits (Tripathi et al., 2018). Maize, on the other hand, a vital animal feed, was usually shredded and mixed with rice bran and given to lactating animals (Osti, 2019). Legume crops were produced in smaller quantities and were mostly used for home consumption. However, in recent years, farmers have increased the production and commercialization of legume crops such as lentils due to higher market demands (Gharti et al., 2014).

Farmers in Dhading and Chitwan used a variety of storage methods but preferred traditional granaries and woven bags; just like farmers in other developing countries (Bajracharya et al., 2007; Díaz-Valderrama et al., 2020; Manandhar et al., 2018). Maize is stored by piling up cobs on the floor/wooden platform or hanging on vertical poles and ropes inside the house (Manandhar et al., 2018). This practice was common all over Nepal (Paneru et al., 2018). Because legumes such as cowpea and soybean were usually stored in small amounts, farmers often used small and portable containers such as woven bags and plastic containers (Manandhar et al., 2018).

Grain stored in traditional structures and woven bags has higher losses due to pests. Traditional granaries are conducive to pests' development that led to grain damage and loss of germination (Bhandari et al., 2017; Khatri et al., 2019). Insects caused the most damage followed by the rodents, as found in another study in Nepal (Shivakoti and Manandhar, 2001). Insect damage increased with the duration of storage with severe losses observed when grain was stored for more than six months, particularly during summer months (Ransom, 2000). Other studies in Nepal have also reported on storage pests identified during this survey(Boxall and Gillett, 1982; Regmi et al., 2012; Shivakoti and Manandhar, 2001). Insect pests can cause losses ranging from 15% to more than 75.0% on maize and rice stored in traditional granaries without insecticides (Bajracharya et al., 2007; Subedi et al., 2009). To protect grain from  pests during storage, farmers applied various types of insecticides (Ghimire and GC, 2018). Affordable technologies such as hermetic bags will help reduce these losses and hence improve food security and increase the earnings of smallholder farmers (Devkota et al., 2018; Tripathi et al., 2018).

Most farmers in both districts stored seeds for planting in the subsequent cropping seasons. Insect damage was quite common during seed storage. Farmers in Chitwan used hermetic containers (e.g., plastic containers and metal bins) to preserve their seed, while those in Dhading mostly used ash and botanicals. Pesticide use for seed storage was low in both districts. Botanicals such as oil of *Melia azdarach* (L.) and *Acorus calamus* (L.) powder have been used to protect and store seed because they have shown to be as effective as malathion in controlling insects (Manandhar and Mainali, 2001). Farmers purchased seed (mostly those who did not store) from agrovet shops but community-based seed production by agricultural cooperatives is increasing in both districts in Nepal (Joshi et al., 2012; Kshetri, 2010).

### 2.4.3 Crop protection and farmers' decision to use insecticides during storage

The distribution of pesticides in Nepal is done primarily through agrovet shops and to a lesser extent cooperatives (Ghimire and GC, 2018; Shrestha et al., 2010). These pesticides are imported mostly from India and China. Pesticides are not regulated and are easily available over the counter. Our results support findings that pesticide use was higher in the lowlands (Chitwan compared with Dhading) (Ghimire and GC, 2018). Farmers used a variety of pesticides to control insects and rodents during storage. Most of these pesticides were misused resulting in food poisoning, environmental pollution, and sometimes death (Rathore, 2015; Sharma et al., 2013; Shrestha and Neupane, 2002). Pesticide poisonings often result from incidental and occupational exposures; this may be why only a few farmers reported pesticides as hazardous (Sharma et al., 2013). Inadequate awareness and training on the safe and efficient application of pesticides have led to poisoning (Khanal and Singh, 2016). A limited number of farmers used a fungicide (i.e., Mancozeb) on stored products. Other farmers used pesticides (i.e. Dichlorvos) that are no longer recommended for use on stored grain by the Plant Protection Directorate of Nepal (Shrestha and Neupane, 2002).

Farmers' decision to apply insecticides on stored commodities varied by crop, storage methods, and level of infestation. Farmers were more likely to treat their grain with insecticides if they stored rice, used traditional granaries, and incurred insect damage during storage. Similar findings were reported in Peru were farmers experiencing insect problems while storing grains were more likely to apply insecticides (Díaz-Valderrama et al., 2020). Because rice is the major cereal for food security, farmers were more likely to protect it from pests during storage. In addition, as reported in Sub-Sahara Africa, farmers who stored longer, tended to use pesticides (Obeng-Ofori et al., 2015). Clearly, farmers in Bagmati province (particularly Chitwan) preferred pesticides to protect stored commodities, just like farmers in other countries, because they are effective (Sharifzadeh et al., 2018). Finding safer alternatives to insecticides will help reduce their negative effects on human health and the environment (Khanal and Singh, 2016; Sharma et al., 2013).

Hermetic storage methods are viable chemical-free alternatives to traditional methods and pesticides use for grain storage among smallholder farmers (Baributsa and Ignacio, 2020; Bhandari et al., 2017). They effectively control pests of stored products, maintain seed quality, enhance food security, and increase earnings of smallholder farmers (Baributsa and Njoroge, 2020; Bhandari et al., 2017; Khatri et al., 2019; Murdock and Baoua, 2014). Though farmers reported not having issues while using hermetic storage methods, some of the challenges associated with rigid containers such as silos and drums are cost, efficacy when not fully filled, and scalability (Abass et al., 2018; Baributsa and Ignacio, 2020; Walker et al., 2018). Training and capacity building on the use of these rigid containers may help to address some of these issues.

Flexible hermetic containers (e.g., PICS bags and SuperGrainbags™) are often attractive alternatives to airtight rigid storage containers among smallholder farmers. A recent study conducted in Chitwan, Nepal showed that PICS bags and SuperGrainbags™ were effective at preserving stored maize (Khatri et al., 2019; Sharma and Tiwari, 2020). The use of hermetic bags has been scaled-up to millions of smallholder farmers in Africa (Baributsa and Ignacio, 2020). Though there are suppliers of both PICS and SuperGrainbags™ in Nepal, the use of these technologies among farmers remains low due mostly to limited awareness and unavailability. Similar adoption constraints were observed in sub-Saharan Africa (Moussa et al., 2014). Only a small proportion of farmers complained about the price, which is about 250 Nepalese Rupees (about $2.50) for a 50kg hermetic bag. Farmers who produce enough to store seldom complain about the price of hermetic bags because they are affordable (Baributsa and Ignacio, 2020). Creating awareness and developing a sustainable supply chain of hermetic bags will increase adoption among smallholder farmers in rural areas of Nepal.

## 2.5 Conclusion

This study found that rice is the most stored grain in Bagmati Province, Nepal. Cereal and legume crops were mostly stored for consumption. Insect pests were the major sources of losses during storage. Traditional storage structures such as granaries offered little or no protection to grain during storage unless pesticides were applied. Because most farmers stored grain for nine months or more, they often apply pesticides, which are often highly toxic or prohibited. These practices have resulted in food poisoning and sometimes loss of human lives. Hermetic bags and other cost-effective hermetic technologies provide alternatives to pesticides and traditional storage methods. Disseminating hermetic bags to store rice would significantly impact food security, safety, and income of farmers in Nepal; given its importance in the production system (most stored), storage challenges, and high pesticide application. Targeted interventions to build the capacity of extension agents in postharvest management would help scale up these hermetic technologies among smallholder farmers.

## 2.6 Recommendations

Storage interventions should be tailored on the need of farmers, districts, crop stored, current storage methods and pest management approaches. This study focused just on storage approaches but understanding whether there is a need for drying and moisture management is equally important to address the problem of minimizing storage loss at the farmers' level. Rice was the major crop stored by farmers and they reported storage loss which was the most important factor that influenced the use of insecticides. Focusing extension efforts on rice would help address the current storage problems and help minimize storage losses, increase income, and decrease food insecurity. This study suggested that farmers mostly get pesticides and agricultural technologies mostly from the agrovet. Making hermetic technologies (e.g., PICS and SuperGrainbags™) easily available in the local agrovet shops would increase the chances of adoption. Providing training to agrovet owners on the advantages and use of hermetic bags would help improve their communications with farmers. We found that extension agents from NGOs and cooperatives were working actively to disseminate agricultural technology in addition to government extension workers. There is need for collaboration among cooperative, NGOs, and government extension services when introducing new postharvest technology to farmers. Though training and demonstration were the basic extension approaches, media and particularly mobile phones had potential to improve communication with farmers. There is a need to assess the effectiveness of these new extension approaches in reaching farmers with new information.

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Table 2-1. Demographics of farmers and extension agents in Chitwan and Dhading districts, Nepal

| Variables | | Categories | Chitwan | Dhading | Overall |
|---|---|---|---|---|---|
| Farmers (%) | | | (n=121) | (n=120) | (n=241) |
| | Gender | Female | 67.8 | 65.0 | 66.4 |
| | | Male | 32.2 | 35.0 | 33.6 |
| | Age | 18- 30 years | 8.3 | 15.8 | 12.0 |
| | | 31- 14 years | 28.1 | 28.4 | 28.2 |
| | | 41 -50 years | 26.4 | 18.3 | 22.4 |
| | | >50 years | 37.2 | 37.5 | 37.3 |
| | Education level | None | 9.9 | 21.7 | 15.8 |
| | | Basic Literacy | 18.2 | 34.1 | 26.1 |
| | | Primary school | 18.2 | 20.0 | 19.1 |
| | | High school | 38.0 | 17.5 | 27.8 |
| | | College/ Tertiary | 7.4 | 5.0 | 6.2 |
| | | University | 8.3 | 1.7 | 5.0 |
| | Years in activity | < 5 years | 5.0 | 0.8 | 2.9 |
| | | 5-10 years | 5.0 | 12.5 | 8.7 |
| | | >10 years | 90.0 | 86.7 | 88.4 |
| Extension agents (%) | | | (n=40) | (n=41) | (n=81) |
| | Gender | Male | 85.0 | 75.6 | 80.2 |
| | | Female | 15.0 | 24.4 | 19.8 |
| | Education level | Basic Literacy | Na | 2.4 | 1.2 |
| | | Primary school | 2.5 | 2.4 | 2.5 |
| | | High school | 10.0 | 12.2 | 11.1 |
| | | College/ Tertiary | 22.5 | 41.5 | 32.1 |
| | | University | 65.0 | 41.5 | 53.1 |
| | Years in current position | <5 years | 7.5 | 19.5 | 13.6 |
| | | 5- 10 years | 37.5 | 43.9 | 40.7 |
| | | >10 years | 55.0 | 36.6 | 45.7 |
| | | | (n=33) | (n=33) | (n=66) |
| | Postharvest technology introduced | Drying | 18.1 | 6.1 | 12.1 |
| | | Storage | 75.8 | 87.8 | 81.8 |
| | | Moisture measurement | 6.1 | 6.1 | 6.1 |

Table 2-2. Source of information for farmers and dissemination of new technologies by extension agents in Chitwan and Dhading districts, Nepal

| Variables | | Categories | Chitwan | Dhading | Overall |
|---|---|---|---|---|---|
| Farmers | Source of information | | (n=121) | (n=120) | (n=241) |
| | | Personal experience | 50.4 | 60.8 | 55.6 |
| | | Other farmers | 19.8 | 17.5 | 18.7 |
| | | Extension agents | 10.7 | 13.3 | 12.0 |
| | | Agrovet agents | 16.5 | 5.8 | 11.2 |
| | | Media | 2.5 | 2.5 | 2.5 |
| | Contact with extension | | (n=41) | (n=59) | (n=100) |
| | | Government | 41.5 | 39.0 | 40.0 |
| | | Farmers' cooperative | 51.2 | 16.9 | 31.0 |
| | | NGOs | 7.31 | 44.1 | 29.0 |
| Extension agents (%) | | | (n=40) | (n=41) | (n=81) |
| | Affiliation | Government | 65.0 | 36.6 | 50.6 |
| | | NGO | 12.5 | 36.6 | 24.7 |
| | | Community leader | 7.5 | 14.6 | 11.1 |
| | | Agrovet shop | 2.5 | 12.2 | 7.4 |
| | | Farmers' cooperative | 12.5 | Na | 6.2 |
| | Current extension approaches | Training/demonstration | 77.5 | 63.4 | 70.4 |
| | | Door visit | 15.0 | 24.4 | 19.8 |
| | | Media | 7.5 | 4.9 | 6.2 |
| | | Phone | 0.0 | 3.7 | 3.7 |
| | Potential effective extension approaches | Training/demonstration | 32.5 | 51.2 | 42.0 |
| | | Flyers/ brochures | 25.0 | 9.8 | 17.3 |
| | | Phone | 15.0 | 7.3 | 11.1 |
| | | Media | 27.5 | 31.7 | 29.6 |
| | Training platform | Village | 55.0 | 82.9 | 69.1 |
| | | Field days | 32.5 | 7.3 | 19.8 |
| | | Markets | 5.0 | 4.9 | 4.9 |
| | | Farmers' Cooperatives | 7.5 | 2.4 | 4.9 |
| | | Media | 0.0 | 2.4 | 1.2 |
| | Source of new technologies | Government agencies | 65.0 | 31.7 | 48.1 |
| | | Development agencies | 7.5 | 48.8 | 28.4 |
| | | Shops/markets | 25.0 | 19.5 | 22.2 |
| | | Farmers' cooperatives | 2.5 | 0.0 | 1.3 |
| | Facilitation of new technology | Provide information on where to buy | 80.0 | 46.3 | 63.0 |
| | | Buy for farmers | 20.0 | 53.7 | 37.0 |

Table 2-3. Storage practices, challenges, and protection methods for cereals and legumes among farmers in Chitwan and Dhading districts, Nepal

| Variables | Categories | Rice | Maize | Beans | Lentils | Soybean | Cowpea |
|---|---|---|---|---|---|---|---|
| | | n=202 | n= 38 | n=72 | n=44 | n=43 | n=31 |
| Reason for storage | Consumption | 99.0 | 73.7 | 69.4 | 97.7 | 86.0 | 93.6 |
| | Sell | 1.0 | 2.6 | 30.6 | 2.3 | 14.0 | 6.4 |
| | Animal feed | 0.0 | 23.7 | 0.0 | 0.0 | 0.0 | 0.0 |
| Storage Duration | < 3 months | 11.9 | 21.1 | 31.9 | 34.1 | 34.8 | 19.4 |
| | 3-6 months | 12.4 | 10.5 | 1.4 | 6.8 | 9.3 | 25.8 |
| | 6-9 months | 7.4 | 5.3 | 8.3 | 9.1 | 4.7 | 3.2 |
| | >9 months | 68.3 | 63.1 | 58.4 | 50.0 | 51.2 | 51.6 |
| Storage options | Granaries [a] | 67.3 | 7.9 | 2.8 | 4.5 | 4.6 | 3.2 |
| | Woven bags | 31.2 | 18.4 | 22.2 | 6.8 | 60.5 | 32.3 |
| | Hermetic | 1.0 | 5.3 | 75.0 | 88.7 | 34.9 | 64.5 |
| | Hanging/piling | 0.5 | 68.4 | 0.0 | 0.0 | 0.0 | 0.0 |
| Storage challenges | Insects | 49.7 | 52.2 | 25.0 | 36.4 | 16.3 | 64.5 |
| | Rodents | 42.4 | 43.3 | 2.8 | 2.3 | 13.9 | 0.0 |
| | Decay/mold | 7.9 | 4.5 | 0.0 | 0.0 | 0.0 | 0.0 |
| | No damage | 0.0 | 0.0 | 72.2 | 61.3 | 69.8 | 35.5 |
| Primary method of protection | Chemicals [b] | 40.6 | 5.3 | 0.0 | 4.6 | 0.0 | 9.7 |
| | Botanicals[c] | 18.3 | 18.4 | 13.9 | 6.8 | 18.6 | 22.6 |
| | Hermetic | 3.5 | 7.9 | 59.7 | 70.4 | 16.3 | 22.6 |
| | Do nothing | 33.2 | 68.4 | 26.4 | 11.4 | 65.1 | 41.9 |
| | Others | 4.4 | 0.0 | 0.0 | 6.8 | 0.0 | 3.2 |

[a] Granaries refers to traditional storage structure of rolled bamboo mat, or rolled paddy straw mat

[b] Chemicals refers to synthetic pesticides

[c] Botanicals refers to plant and plant derivatives

Table 2-4. Major insect species in cereal and grain stored in Chitwan and Dhading district of Nepal

| Types of Grain | Insect Species | % Respondents |
|---|---|---|
| Cereal stored<br>(n=202) | *Sitophilus oryzae* (L.) | 91.3 |
| | *Corcyra cephalonica* (Stainton) | 89.2 |
| | *Ryzopertha dominica* (Fab.) | 1.2 |
| | *Oryzaephilus surinamensis* (L.) | 0.8 |
| | *Ploida interpunctella* (Hübner) | 0.8 |
| | *Sitotroga cereallela* (Oliver) | 0.4 |
| Legume stored<br>(n=86) | *Bruchus spp.* | 51.2 |
| | *Callosobruchus maculates* (L.) | 25.6 |
| | *Sitotroga cereallela* (Oliver) | 15.1 |
| | *Callosobruchus chinensis* (L.) | 9.3 |
| | *Sitophilus granarius* (L.) | 2.3 |
| | *Ryzopertha dominica* (Fab.) | 2.3 |

Table 2-5. Practices of seed storage by farmers in two districts; Chitwan and Dhading

| | Variables | Chitwan (n=121) | Dhading (n=120) | Overall (n=241) |
|---|---|---|---|---|
| Seed storage | Yes | 72.7 | 81.7 | 77.2 |
| | No | 27.3 | 18.3 | 22.8 |
| Place for buying seed | | (n=32) | (n=19) | (n=51) |
| | Agroshop | 53.1 | 73.7 | 60.8 |
| | Agriculture cooperative | 37.5 | 26.3 | 33.3 |
| | Community seed bank | 6.3 | 0.0 | 3.9 |
| | Farmers group | 3.1 | 0.0 | 2.0 |
| Insect damage in storage | | (n=121) | (n=120) | (n=241) |
| | Yes | 47.1 | 76.7 | 61.8 |
| | No | 52.9 | 23.3 | 38.2 |
| Method of preserving seed | | (n=103) | (n=110) | (n=213) |
| | Do nothing | 33.0 | 34.5 | 33.8 |
| | Hermetic | 41.7 | 6.4 | 23.5 |
| | Botanicals | 12.6 | 19.1 | 16.0 |
| | Pesticides | 11.7 | 12.7 | 12.2 |
| | Ash | 1.0 | 21.8 | 11.7 |
| | Drying | 0 | 5.5 | 2.8 |

Table 2-6. Information on pesticides and hermetic storage technologies (HSTs) in Chitwan and Dhading districts, Nepal

| | Variables | Categories | Chitwan | Dhading | Overall |
|---|---|---|---|---|---|
| | | | (n=57) | (n= 32) | (n=89) |
| Pesticides (%) | Pesticides used to protect cereals [a] | Aluminum Phosphide | 98.3 | 56.3 | 83.2 |
| | | Dichlorvos (DDVP) | 3.5 | 18.8 | 9.0 |
| | | Malathion | 1.8 | 9.4 | 3.4 |
| | | Mancozeb | 0 | 9.4 | 1.2 |
| | | Methyl parathion | 0 | 3.1 | 2.2 |
| | | Rodenticides | 0 | 6.3 | 4.5 |
| | Source of pesticides | Agrovet shops | 96.5 | 93.9 | 95.6 |
| | | Farmers' cooperatives | 3.5 | 6.1 | 4.4 |
| | Advantages of pesticides | Effective | 57.9 | 66.8 | 61.1 |
| | | Easy to use | 22.8 | 12.1 | 18.9 |
| | | Don't know | 7.0 | 15.1 | 10.0 |
| | | Locally available | 12.3 | 3.0 | 8.9 |
| | | Low price | 0 | 3.0 | 1.1 |
| | Disadvantages of pesticides | None | 68.4 | 54.5 | 63.3 |
| | | Hazardous | 17.6 | 27.3 | 21.1 |
| | | Not effective | 7.0 | 15.2 | 10.0 |
| | | Lack of knowledge | 7.0 | 3.0 | 5.6 |
| Hermetic Storage Technologies (HSTs) (%) | | | (n=82) | (n=55) | (n=137) |
| | Source of HSTs | Agrovet shops | 100 | 16.3 | 66.3 |
| | | Donation from NGO | 0 | 76.3 | 30.7 |
| | | Government agencies | 0 | 7.4 | 3.0 |
| | HSTs used to protect grains | Plastic drum [b] | 86.6 | 18.2 | 59.1 |
| | | PICS Bag | 1.2 | 47.3 | 19.7 |
| | | SuperGrainbags™ | 2.4 | 29.1 | 13.1 |
| | | Metal Silos | 9.8 | 5.4 | 8.1 |

[a] The total is more than 100% because some farmers used more than one pesticide

[b] Commonly available plastic barrels in market

Table 2-7. Motivation, challenges, and information about HST in two districts; Chitwan and Dhading

|  | Variables | Chitwan | Dhading | Overall |
|---|---|---|---|---|
| Reason for using HST |  | (n=81) | (n=56) | (n=137) |
|  | Food security | 86.4 | 80.4 | 83.9 |
|  | Income | 1.2 | 0 | 0.7 |
|  | Seed storage | 12.4 | 19.6 | 15.4 |
| Challenges to use HST |  | (n=50) | (n=49) | (n=99) |
|  | Not aware | 86.0 | 75.5 | 80.8 |
|  | Alternatives are cheaper | 6.0 | 8.2 | 7.1 |
|  | Not effective | 4.0 | 0 | 2.0 |
|  | Lack of training | 2.0 | 16.3 | 9.1 |
|  | High price | 2.0 | 0 | 1.0 |
| Fumigation in using HST |  | (n=82) | (n=55) | (n=137) |
|  | Yes | 9.8 | 16.4 | 12.4 |
|  | No | 90.3 | 83.6 | 87.6 |
| Source of HST information |  | (n=82) | (n=97) | (n=179) |
|  | Friends and family | 79.3 | 45.4 | 60.9 |
|  | Extension agents | 8.5 | 40.2 | 25.7 |
|  | Cooperatives | 1.2 | 9.3 | 5.6 |
|  | Agro dealers | 4.9 | 4.1 | 4.4 |
|  | Media | 2.4 | 1.0 | 1.7 |
|  | Others | 3.7 | 0.0 | 1.7 |
| Received training on HST |  | (n=82) | (n=55) | (n=137) |
|  | Yes | 0.0 | 30.9 | 12.4 |
|  | No | 100 | 69.1 | 87.6 |
| Source of training |  | (n=0) | (n=17) | (n=17) |
|  | Village demonstration | 0.0 | 64.8 | 64.7 |
|  | Farmer’s group | 0.0 | 17.6 | 17.6 |
|  | Agriculture office | 0.0 | 17.6 | 17.6 |

Table 2-8. Factors influencing farmers' decision to use insecticides to protect grain during storage in Chitwan and Dhading districts, Nepal.

| Variables | Categories | OR[a] | 95% CI[b] | P value | L.R. test[c] |
|---|---|---|---|---|---|
| District | Chitwan | 1.0 | (referent) | | $X^2 = 43.844$<br>$df = 6$<br>$p = 0.000$<br>$LogLik = 7.9388)$ |
| | Dhading | 0.7 | (0.4, 1.5) | 0.4 | |
| Stored crop[d] | Maize | 1.0 | (referent) | | |
| | Rice | 8.5 | (1.4, 100.7) | 0.04 | |
| Storage containers[e] | Granaries | 1.0 | (referent) | | |
| | Raised and piled up structure[f] | 0.5 | (0.05, 5.37) | 0.56 | |
| | Woven bags | 0.4 | (0.21, 0.79) | 0.009 | |
| Storage duration[g] | Less than six months | 1.0 | (referent) | | |
| | More than six months | 1.6 | (0.84, 3.34) | 0.15 | |
| Insect damage | No | 1.0 | (referent) | | |
| | Yes | 9.8 | (1.7, 183.4) | 0.03 | |

[a] OR= odds ratio.

[b] CI = confidence interval.

[c] L.R.= Likelihood Ratio test; $X^2$= Chi-square value; $df$= degrees of freedom; $p$= probability value; $LogLik$= model's log likelihood

[d] Wheat was excluded because of low percentage value

[e] Hermetic storage was excluded because of low value

[f] Traditional maize storage: maize cobs are tied together using their husks and then piled up

[g] Storage durations were grouped into less than six months and more than six months

Source:Approximate GPS locations were collected from Google maps and mapped using ArcGis-10.6developed by ESRI

Figure 2-1. Map of Nepal showing area where the study was conducted. Each dot represents a Village Development Committee (VDC) surveyed in Chitwan or Dhading districts in Bagmati province.

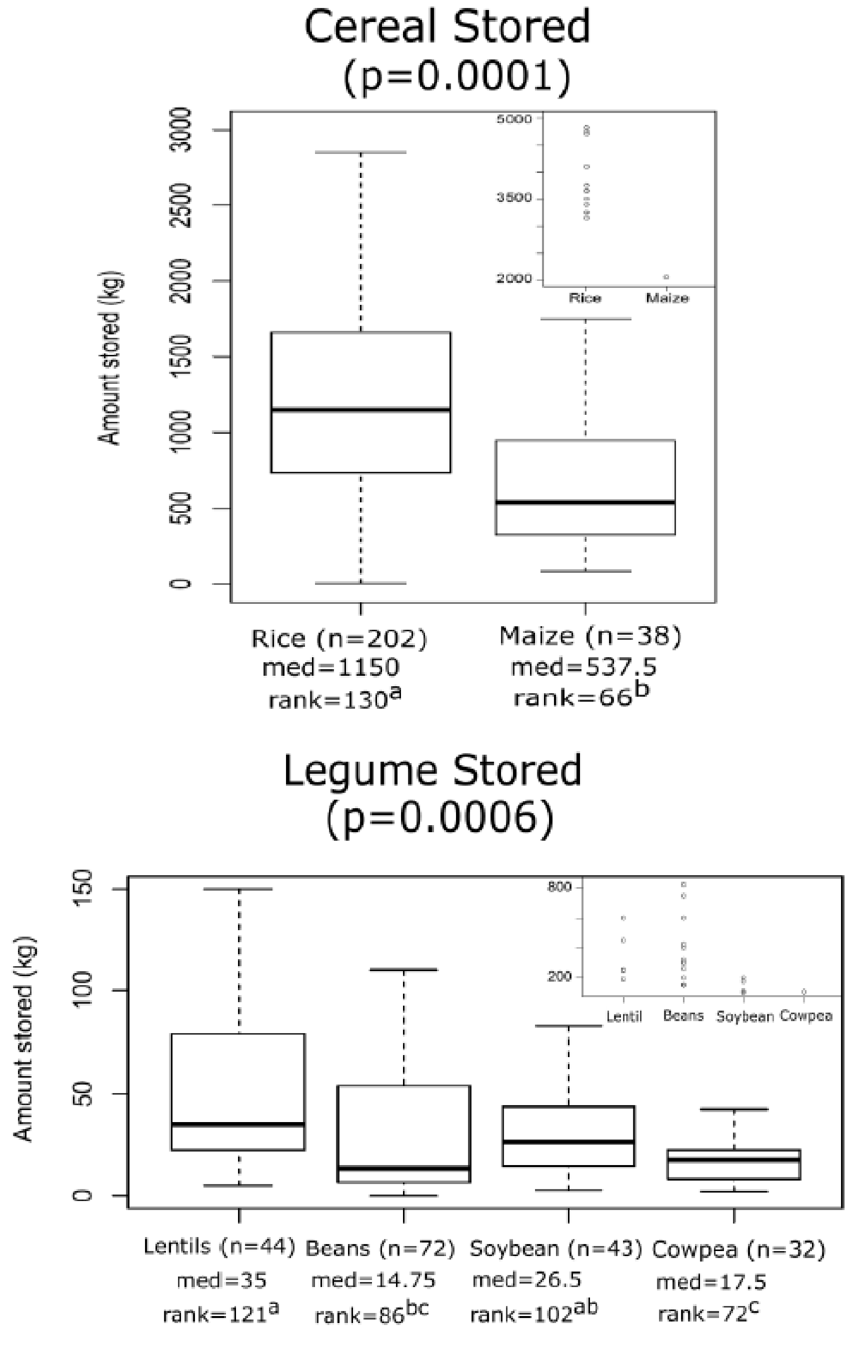

Figure 2-2. Boxplot showing the quantity of cereals (upper) and legumes (lower) stored by farmers in Bagmati province, Nepal. Smaller graph embedded in the main plot contains the outliers of each dataset, i.e., data points greater than the 75th percentile value plus 1.5 times the interquartile range.

# CHAPTER 3. ESTIMATION OF LETHALITY OF *Sitophilus oryzae* L. ADULTS UNDER HYPOXIC CONDITIONS

## 3.1 Introduction

*Sitophilus oryzae* (Linneaus) is a destructive and widespread primary pest of stored cereals and legumes. It destroys a varieties of grains and processed food stuff including rice, wheat, barley, corn, sorghum, rye, buckwheat, cereals, spaghetti, nuts, and birdseeds (Martin et al., 2015; Mason and McDonough, 2012). The damage caused by *S. oryzae* is mostly due to feeding activities by adults and grubs (Hasan et al., 2017; Longstaff, 1981). Infestation of *S. oryzae* also attracts secondary pests that influence the internal temperature and humidity of infested grains and enhances the vulnerability of grains to pathogens (Stejskal et al., 2015). Thus, damage of *S. oryzae* result in loss of quantity and quality leading to reduction in market value (Baoua et al., 2016; Tubbs et al., 2016; Williams et al., 2014).

Pest control of *S. oryzae* is mostly dependent on phosphine fumigants, methyl bromide, organophosphorus, and pyrethroid insecticides (Abd El-Salam, 2010; Hossain et al., 2014; Nayak et al., 2020; Ribeiro et al., 2002). The usage of chemical pesticides comes with many challenges such as ineffectiveness to dormant insect stages, pesticide poisoning to applicators and consumers, environment pollution, and the development of insect resistance (Afful et al., 2018; CABI, 2019; Chen et al., 2015; Dubey et al., 2008). Widely used fumigant methyl bromide and aluminum phosphide are being banned due to their high toxicity and depletion of the ozone layer (Mbata and Phillips, 2001; Roller and Baumgartner, 2016). This explains the need to develop environmentally friendly and safer storage pest management alternatives to pesticides (Sousa et al., 2003).

Since the early 1980s, modified atmospheres have been investigated as possible alternative protection methods to traditional fumigants against stored-product insect pests (Fleurat-Lessard, 1990; Ofuya and Reichmuth, 2001). Hermetic storage is one of the modified atmospheres that works by depleting oxygen ($O_2$) in storage closure through natural processes by respiration of insects and other biological activities leading to insect mortality (Adler et al., 2000a; Hoback and Stanley, 2000; Martin et al., 2015b; Navarro, 2006). Low oxygen leads to the cessation of feeding that arrests growth and development, hence limiting damage (Murdock et al., 2012).

Studies on the effect of low-oxygen environments on postharvest insect pests are not new (Hashem et al., 2012; Mbata and Phillips, 2001; Navarro, 2012; Navarro et al., 1985). Studies have been conducted to assess the timing of insect mortality under hypoxia and carboxia (Gunasekaran and Rajendran, 2005; Ofuya and Reichmuth, 2001; Soderstrom, 1960). Brandl et al. (1983) found that a modified atmosphere with 8% $O_2$, 60% $CO_2$, and 30% $N_2$ killed 100% of fourth instar larvae of *Ephestia cautella* (Walker) within three days and 95 % in *Amyelois transitella* (Walker). Banks and Annis (1990) found that internal seed feeders such as *Callasobruchus* spp. can be killed by a combination of 60% $CO_2$ and 8% $O_2$.The combination of 40% $CO_2$ and 2% $O_2$ proved to control *Calandra granaria* (L.) within 17 days (Bailey, 1955). The mixture of 15 % of $O_2$ with 36% of $CO_2$ provided a lethal punch to *Tribolium castaneum* (Herbst) and larvae of *Ploida interpunctella* (Hubner) (Harein and Press, 1968). Exposing the pupae of *P. interpuctella* to 80% of $CO_2$ and 20% $N_2$ at 32.2°C killed 100% of adults (Sauer and Shelton, 2002). Lindgren and Vincent (1970) reported that adults of *S. oryzae* were most susceptible to $N_2$, $CO_2$ and Helium followed by larvae, eggs, and pupae. Storing grain in elevated $CO_2$ levels of 40%, 60%, and 80% not only avoided the seed damage and restricted adult emergence but also preserved the seed quality (Shekar et al., 2018). These studies explain the effectiveness of modified storage and lay the foundation for using it as an alternative to pesticides.

Low oxygen levels in airtight hermetic containers have proven to be effective in controlling *S. oryzae* by arresting the population growth and maintaining stored product quality (Martin et al., 2015b). Murdock et al. (2012) explained that low oxygen limits the supply of water. Storage pests rely on metabolic water for survival and with depleted oxygen, they cannot produce the amount of water required for metabolic activities. Kharel et al. (2019) reported that eggs and larvae are more vulnerable compared to pupae and adults. Lowering the oxygen level to 2% caused complete mortality in 3 days for eggs, seven days for young larvae, 10 days for old larvae and pupae, and 15 days for adults. In the nutshell, hypoxia suppresses insect development and controls all life stages of the stored pests.

Oxygen levels below 5% suppress rice weevil activities and adult emergence (Njoroge et al., 2019c, 2017). Exposure of *S. oryzae* to hypoxia below 5%, caused their “acoustical death” within five days (Njoroge et al., 2018). But being “acoustically dead” did not mean adult weevils were biological dead. It simply meant that the rate of bursts had fallen below the infestation level (Mankin et al., 2008). It is important to evaluate when insects are biologically dead under hypoxia conditions. This experiment was implemented to assess (i) the effect of hypoxia levels at 5% and below on the biological mortality of adult rice weevils, and (ii) progeny development following exposure to the above-mentioned oxygen levels.

## 3.2 Materials and methods

### 3.2.1 Insect rearing

Insects used in this trial were reared in Conviron insect growth chamber (Model CMP4030; CONVIRON., MB, Canada). The wheat variety AG 1189 (Alumni Seed Co. Romney, IN) stored at -18 ± 1°C for disinfestation was used in the experiment. Before the colony was set up, wheat was thawed for 24 hours at 20 ± 1°C. *S. oryzae* were taken out of an existing colony using a vacuum aspirator and then transferred to one-liter jars containing wheat. Insects were allowed to breed for 48 hours and then removed using a vacuum aspirator. After removing the adult, grain was held in a growth chamber at 25 ± 1°C and 40 ± 5% RH until adult emergence. Newly emerged *S. oryzae* adults (3-5 days) were used for the experiment. A no. 10 sieve was used to remove adults from grain. Ten adults were transferred to each 30 ml container (Wheaton Glass Sample bottle, CP Lab Safety, CA).

### 3.2.2 Experimental Setup and Design

Three oxygen levels (1, 3, and 5%) were each maintained for six different time periods. The different time periods were: 48, 72, 96, 120, 144, and 168 h for 1%; 120, 144, 168, 192, 216, and 240 h for 3%; and 168, 192, 216, 240, 264, and 288 h for 5%. The exposure periods for each oxygen level were set based on preliminary experiments that assessed when insect mortality begins for *S. oryzae* under each hypoxia level. All of these treatment and the control were replicated thrice.

The experiment was conducted at a room temperature of 20 ± 1°C. Clear polycarbonate vacuum chambers (41.9 cm × 34.5 cm × 38 cm) with 35 liters capacity (Bel-Art - SP Science ware, NJ) were used to expose adults of *S. oryzae* to different levels of hypoxia. Ten recently emerged adults of *S. oryzae* were put in a 30 ml container that had 10 ± 0.50 grams of wheat. To ensure gas exchange when placed in hypoxia chambers, each container was covered with a perforated lid with small holes. Five containers of each treatment were introduced in a vacuum chamber and replicated three times (three vacuum chambers, total n=15). The control was kept under a normoxic (20 – 21%) environment and replicated only once for each treatment. Each run of the experiment consisted of one oxygen level and all the periods, given the limited number of vacuum chambers. During the experiment, each chamber had two treatments (same oxygen level at two different times) inside it. For example, a vacuum chamber at 1% oxygen level had five containers held for 48 h (T1R1) and the other five containers kept in for 72 h (T2R1) (Figure 3-1). A second vacuum chamber at 1% had five containers held for 96 h (T3R1) and the other five containers held for 120 h (T4R1). A third vacuum chamber at 1% had five containers held for 144 h (T5R1) and the other five containers held for 168 h (T6R1). Each chamber with these treatments was replicated three times (total nine chambers) (Figure 3-1). This run of the experiment was repeated twice for increasing the accuracy and minimizing the error.

### 3.2.3 Hypoxia Treatment

The level of hypoxia in the chamber was created by removing the air from the chambers by adding nitrogen gas (Airgas, Kokomo, IN) till the targeted oxygen level was achieved. An Oxysense 5250i oxygen reader device (Industrial Physics, Devens, MA) was used to measure oxygen level inside the chamber through fluorescent yellow Oxydots sticked to the interior of the chamber. Oxygen level was maintained within ± 1.00% of the required oxygen levels. During the time of the experiment, each chamber had two treatments inside it. For maintaining the desired oxygen level once samples were removed, nitrogen was pumped back to the desired oxygen level within five minutes.

### 3.2.4 Data Collection

#### *Temperature and RH*

A USB data logger (Lascar, Erie, PA) was kept inside each of the nine vacuum chambers to monitor temperature and relative humidity (Figure 3-1). Two data loggers were kept outside of the chambers to monitor the room's ambient atmosphere. The temperature and RH inside and outside the chamber were recorded every 30 min for the duration of each treatment.

#### *Adult Mortality*

After treatments were taken out from hypoxia chambers, each container was then emptied on the white paper to assess whether the *S. oryzae* adults were alive or dead. An adult *S. oryzae* adult that started moving immediately once exposed to normoxia was recorded as alive and kept in a separate container. Immobile adults were touched using forceps. If they showed any movement following the touching they were recorded as alive. Lastly, *S. oryzae* adults that didn't respond were kept further in the growth chamber 25±1 °C and 40 ±5% RH for 24 hours when they were assessed again to determine whether or not they were alive. This was done to ensure that insects were dead and not in hypoxic stress.

#### ***Adult emergence***

To assess progeny from insect exposed to different treatments, grains in the five- 30 ml container were combined in 450 ml containers (Wheaton Glass Sample bottle, CP Lab Safety, CA). Hence, there were three containers for each treatment including the control. The four 450 ml containers for each treatment were incubated in Caron insect growth chambers (Model 6025 -1, 115 VAC, Caron Growth chambers, OH) at 25 ± 1°C and 40 ± 5% RH for 45 d. After 45 d, adult emergence was recorded for each treatment (three replications along with control).

### 3.2.5 Statistical analysis

Temperature and relative humidity data from all hypoxia chambers were imported to Excel. The daily average was calculated by averaging the data of every 30 minutes taken from data loggers. The daily averages were then plotted to excel for 1%, 3%, and 5% based on each treatment and control. Tukey's test was conducted to compare the means of RH within the treatments and control.

Mortality from each treatment replicate (container) was assessed and recorded in Microsoft Excel. As each container had just 10 insects, all *S. oryzae* adult in the five containers were added to make the sample size normal i.e., 50 adults. A generalized linear model (Pearson's test and likelihood ratio) was conducted to check the fitness of the model (Johnson, 2016). After that, the R package " Ecotox" was used to calculate the lethal time (Hlina, 2020). For adult emergence, the count was transformed using a squared rooted transformation to better fit the data in a linear model. Next, Tukey's test was conducted to compare means of adult emergences for each oxygen level. Means were separated using Bonferroni adjustments at a 95% confidence level.

## 3.3 Results

### 3.3.1 Temperature and Relative humidity

The temperature was 20 ± 0.5°C throughout the experiment regardless of oxygen level. The average RH ± standard deviation were 33.4 ± 4.1%, 36.6 ± 1.7%, and 47 ± 1.8% for 1, 3, and 5% of hypoxia levels, respectively (Figure 3-2). As each hypoxia chamber had two treatments inside them (Figure 3-1), the data of RH of these two treatments are overlapped. The control had an average RH ± standard deviation of 51.5 ± 1.9%. Relative humidity was significantly different between the treatments and control in each hypoxia level ($p<0.05$). However, no significant difference was observed among treatments within each hypoxia level.

### 3.3.2 Lethal Time

The average mortality of *S. oryzae* across different treatments (exposure time within each oxygen level) is shown in Table 3-1. There were significant differences between treatments and control within each hypoxia level. At 1% oxygen level, maximum average mortality of 100% in 120 hours of exposure. At 3%, the average mortality of 100% was attained in 264 hours of exposure. The average mortality was low at 5% of hypoxia and only 21% was achieved even after exposure to 288 hours.

The analysis of the lethal time of adult *S.oryzae* exposed to hypoxia at 1, 3, and 5% is shown in Table 3-2. The $LT_{50}$ of *S. oryazae* exposed to hypoxia at 1% is 69.7 (65.8 – 73.3) h and $LT_{99}$ is 120.6 (112.8- 131.3) h and are significantly different ($p < 0.05$). Data shows that 100% of weevils' mortality was achieved within the duration of the exposure to hypoxia for 192 h. Subsequently, the $LT_{50}$ for 3% of hypoxia is 179.0 (169.8 -187.1) h and is significantly different ($p <0.05$) than $LT_{99}$ 269 (256.1- 287.2) h. Furthermore, at 5% of hypoxia, $LT_{50}$ for adult rice weevil is 417.2 (363.3 - 541.5) h and $LT_{99}$ is 691.4 (575.8 – 925.6) h. The relation between the probability of adult mortality when exposed to a different time at 1, 3, and 5% oxygen levels at different times are shown in Figure 3-3. Though the slope is low for these hypoxia levels in Table 3-2, the graph strongly supports our assumption of higher adult mortality with the increase in time of treatment. Figure 3-3 shows that most of the data points in 1% and 3%, are within the confidence levels and well distributed throughout the graph. In contrast, in 5%, most of the data points are centered around the starting point in the curve as 100% mortality wasn't attained during the experiment.

This skewed distribution of mortality points is a plausible reason for the widened confidence level limits that were obtained in LT analysis.

### 3.3.3 Adult emergence

There was no adult emergence on grains exposed to 1% and 3% oxygen levels. In 5%, however, there were some emergences from the grains that were used in the treatment. Figure 3-4, shows the difference in the square root of the number of adult emergences in treatments and controls along with the trend lines. Adult emergence at 5% oxygen level was much lower compared to control. The trend line shows a slight increase in adult emergence with longer exposure time, though it was minimal. The Tukey's test comparing means at 95% didn't show any significant difference in adult emergence among the treatments.

## 3.4 Discussion

The use of modified storage is often associated with low RH (Navarro, 1978). In contrary to the study of Kharel et al. (2019), we found RH inside the chamber was affected by the level of hypoxia. The average RH was lowest at 1%, followed by 3% and 5%. Even though there was a slight increase in RH over time, we found that the addition of nitrogen sharply decreased the RH. Maintaining the oxygen level at 1% required frequent pumping of nitrogen gas compared to 3% and 5%. A combination of *S. oryzae* activity such as low metabolic water production with the regular addition of nitrogen gas might be the reason for much significant gap between treatment and control at 1% of hypoxia. Further experimental tests should be conducted to estimate the relationship of RH with insect activity and what influences the addition of nitrogen to the hypoxia chamber may have on RH, insect mortality, and grain EMC.

The mortality of *S. oryzae* followed the trend of cessation of insect acoustic activity previously observed at 1% oxygen level (Njoroge et al., 2019). The low adult mortality in the first two days of the experiment is corroborated by the high insect sound burst rate observed previously by Njoroge et al. (2019) study. By the fourth day, *S. oryzae* mortality at 1% hypoxia level increased to 90%, and the result from acoustic activities with a threshold level below 0.002 bursts/sec. This finding is substantiated by previous studies that showed a hypoxia level of 1% caused a rapid mortality of storage pests (Adler et al., 2000; Navarro, 2012). Following exposure to hypoxic treatments, no adults of *S. oryzae* recovered 24 hours after being kept in a normoxic environment. This suggests that the immobility of *S. oryzae* was an indicator of the actual insect mortality. This result further strengthens the use of hypoxia as the modified storage technique to protect grain against *S. oryzae* damages.

At 3% oxygen level, insect mortality did not follow the trend of cessation of insect acoustic activity reported by Njoroge et al. (2019). This prior study suggested a gradual decline in the rate of bursts in the first three days of exposure to hypoxia, and reaching a threshold below 0.002 bursts/sec by the fourth day. In contrast, it took ten days to reach above 90% *S. oryzae* mortality. The time period of the rate of burst impulse only took six days to get below the threshold level, during that time period only 15.67% of *S. oryzae* were dead and was not markedly different from control. The movement of *S. oryzae* wasn't detected from the visual observation after the fourth day of exposure but the weevils were still alive and respiring. This further explains the need of understanding the timeframe needed to expose the insects to hypoxia. If the insects aren't dead and are just immobile, if exposed to normoxic conditions, there is a high likelihood of insects feeding and breeding again (Kharel et al., 2019).

At 5 % oxygen level, the mortality of insects was significantly lower (Table 3-1). Although 100 % mortality of *S. oryzae* was not attained in 5 % of hypoxia, the mean time frame of cessation of acoustic activities was achieved between fourth and fifth days (Njoroge et al., 2019). Even though this study found 21% of adult mortality within twelve days of exposure, calculation of the lethal time recommends that *S. oryzae* need to be exposed to 5% oxygen level for about 29 days to achieve 99 % adult mortality. This finding provides good insight in terms of controlling *S. oryzae* in grain storage under hermetic conditions. The oxygen level of 5 % and below is attainable in hermetic bags like Purdue Improved Crop Storage (PICS) bags (Baoua et al., 2014; Tubbs et al., 2016). Oxygen level below 5 % achieved in hermetic bags can effectively eliminate pest infestation if maintained for several months (Baoua et al., 2014; Njoroge et al., 2014). In these circumstances, hermetic storage produces similar effects as controlled atmospheres on storage pests (Njoroge et al., 2019). The present study provides a vital rationale for storing grain in hermetic storage conditions for a minimum of 45 days to achieve adult *S. oryzae* control.

Our result on adult emergence substantiates with previous findings that show oxygen levels below 5% can suppress adult emergence of pests in stored products (Kharel et al., 2019; Njoroge et al., 2018; Yan et al., 2016). In our study, no adults emerged within 45 days post-treatment following the exposure to 1% and 3% oxygen levels. The 5% of hypoxia had some adult emergence but it was significantly lower compared to control. The 5% hypoxic condition slows down the overall population growth of pests by reducing oviposition, progeny development, and longevity of insects (Azzam et al., 2010; Cheng et al., 2013; Yan et al., 2016). Carli et al.( 2010) found that there was an inhibition of offsprings development of *S. oryzae* when exposed to $CO_2$ for 30 days. This opens the possibility of extending the time frame beyond 12 days to achieve complete mortality post-treatment. Further work needs to be done to understand factors that inhibit adult emergence in prolonged exposure to hypoxia.

Modified or controlled atmosphere are potential alternatives to conventional chemical fumigants (Cao et al., 2019; Feston et al., 2020). Modified storage structures are advantageous in many ways as they do not leave any harmful residues in treated products, are safe for the environment, have fungistatic effects on grain by reduction of product respiration, and have a low risk of developing insect resistance (Adler, 1997). Furthermore, the mammalian $LD_{50}$ for the commonly used fumigant aluminum phosphide is 11.5 mg/kg and methyl bromide is 104- 214 mg/kg while for nitrogen is 5000mg/kg (Dupont, 2010; EPA, 2000; Extension Toxicology Network, 1996). This highlights the level of worker safety when using modified storage compared to chemical pesticides.

In conclusion, exposing *S. oryzae* adults to 3±1% or below oxygen level for 11 days was found to lead to 100% mortality. Even though 5% hypoxia didn’t achieve 100% mortality of adults, it suppressed adult emergence following the treatment.

### 3.5 Recommendations

Adaptation of chemical-free alternatives largely depends on laboratory and applied research findings. This research focused on understanding the time frame of adult mortality when exposed to hypoxia. The 1%, 3%, and 5% of hypoxia controlling 99% of adults requires a time frame of five, eleven, and twenty-eight days respectively. The hypoxia level in this research was maintained with nitrogen gas. Estimating the total volume of nitrogen gas required to maintain hypoxia at each level of oxygen would give an insight into understanding the economics of using hypoxia as treatment. The economic analysis and further research would strengthen the adaptation of hypoxia for the management of *S. oryzae*. In addition, future research should look into exposure of different life stages of *S. oryzae* to optimize hypoxia teatments. This would help promote the use of hypoxia as an effective modified storage technology to control all stages of *S. oryzae* and prevent insect damage on grains.

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Table 3-1. Average mortality of *S. oryzae* adults exposed to 1, 3, 5% oxygen levels at different time periods. Each treatment (combination of oxygen level and time period) has 5 samples replicated 3 times (n=15); except for the control which had only 5 samples (n=5). The whole set of experiments was repeated twice leading to a total sample, n=30 and n=10 in control. Means within rows with the same letter are not significantly different by Tukey's test with Bonferroni adjustments (p<0.05)

| | **Time** | **48hrs** | **72hrs** | **96hrs** | **120hrs** | **144hrs** | **168hrs** | **Control** |
|---|---|---|---|---|---|---|---|---|
| 1% | Mortality (%) | 20.33a | 46.67b | 89.33c | 100c | 100c | 100c | 2.83a |
| | **Time** | **144hrs** | **168hrs** | **192hrs** | **216hrs** | **240hrs** | **264hrs** | **Control** |
| 3% | Mortality (%) | 15.67ab | 30.33bc | 42.67c | 75.33d | 97.67e | 100e | 1.17a |
| | **Time** | **168hrs** | **192hrs** | **216hrs** | **240hrs** | **264hrs** | **288hrs** | **Control** |
| 5% | Mortality (%) | 4.33a | 7.67a | 8.67ab | 14.67bc | 16.33bc | 21.00c | 1.00c |

Table 3-2. Time mortality regression for *S. oryzae* adults exposed to different level of hypoxia levels.

| % Hypoxia | LT_Value[a] | N | Mortality (h) | LCL[b] | UCL[c] | $\chi 2$[d] | DF[e] | slope |
|---|---|---|---|---|---|---|---|---|
| 1% | 25 | 1600 | 54.9 | 49.4 | 59.3 | 102.8 | 34 | 0.046 |
| | 50 | 1600 | 69.7 | 65.9 | 73.3 | | | |
| | 99 | 1600 | 120.6 | 112.9 | 131.3 | | | |
| 3% | 25 | 1600 | 164.2 | 155.3 | 171.3 | 169.5 | 34 | 0.029 |
| | 50 | 1600 | 187.8 | 181.6 | 193.7 | | | |
| | 99 | 1600 | 269.0 | 256.1 | 287.2 | | | |
| 5% | 25 | 1600 | 298.3 | 279.3 | 333.2 | 49.7 | 34 | 0.0076 |
| | 50 | 1600 | 386.6 | 352.2 | 474.8 | | | |
| | 99 | 1600 | 691.4 | 575.8 | 925.6 | | | |

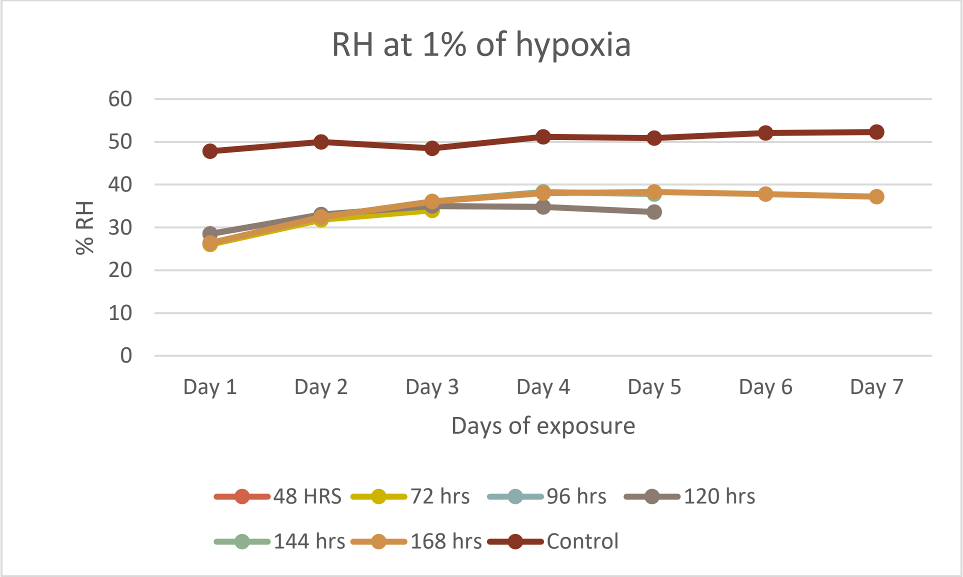
[a] Lethal time value

[b] Lower confidence level, [c] Upper confidence level

[d] Chi square value

[e] Degree of freedom

Figure 3-1: Experimental setup design for 1, 3, and 5% of hypoxia



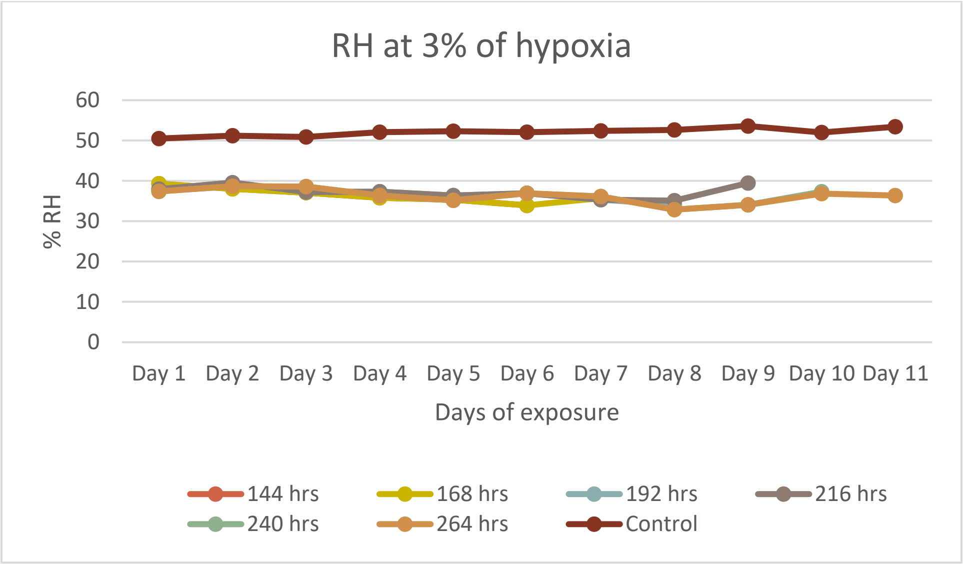
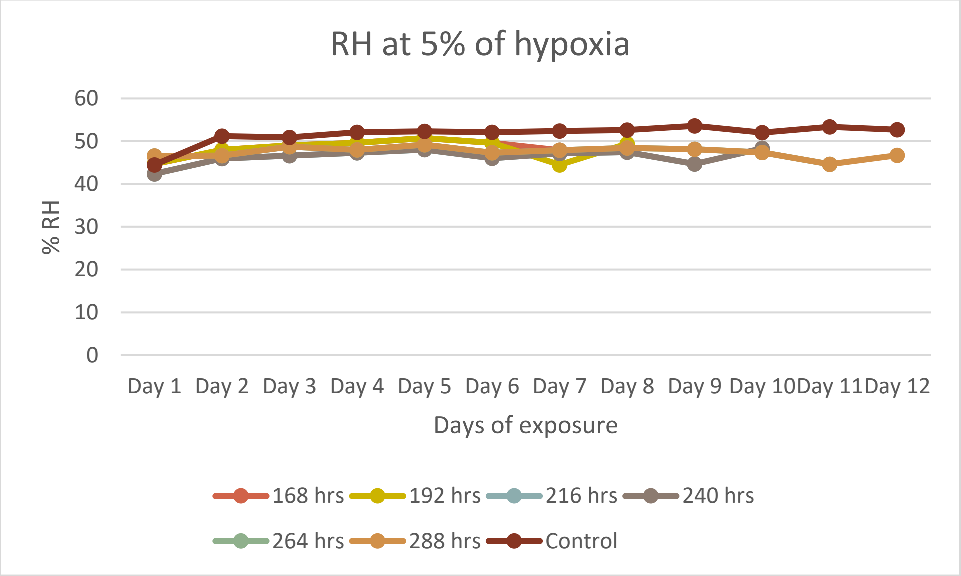


Figure 3-2. Average Relative Humidity across each replication of six treatments with respect to days of exposure.

Figure 3-2 continued



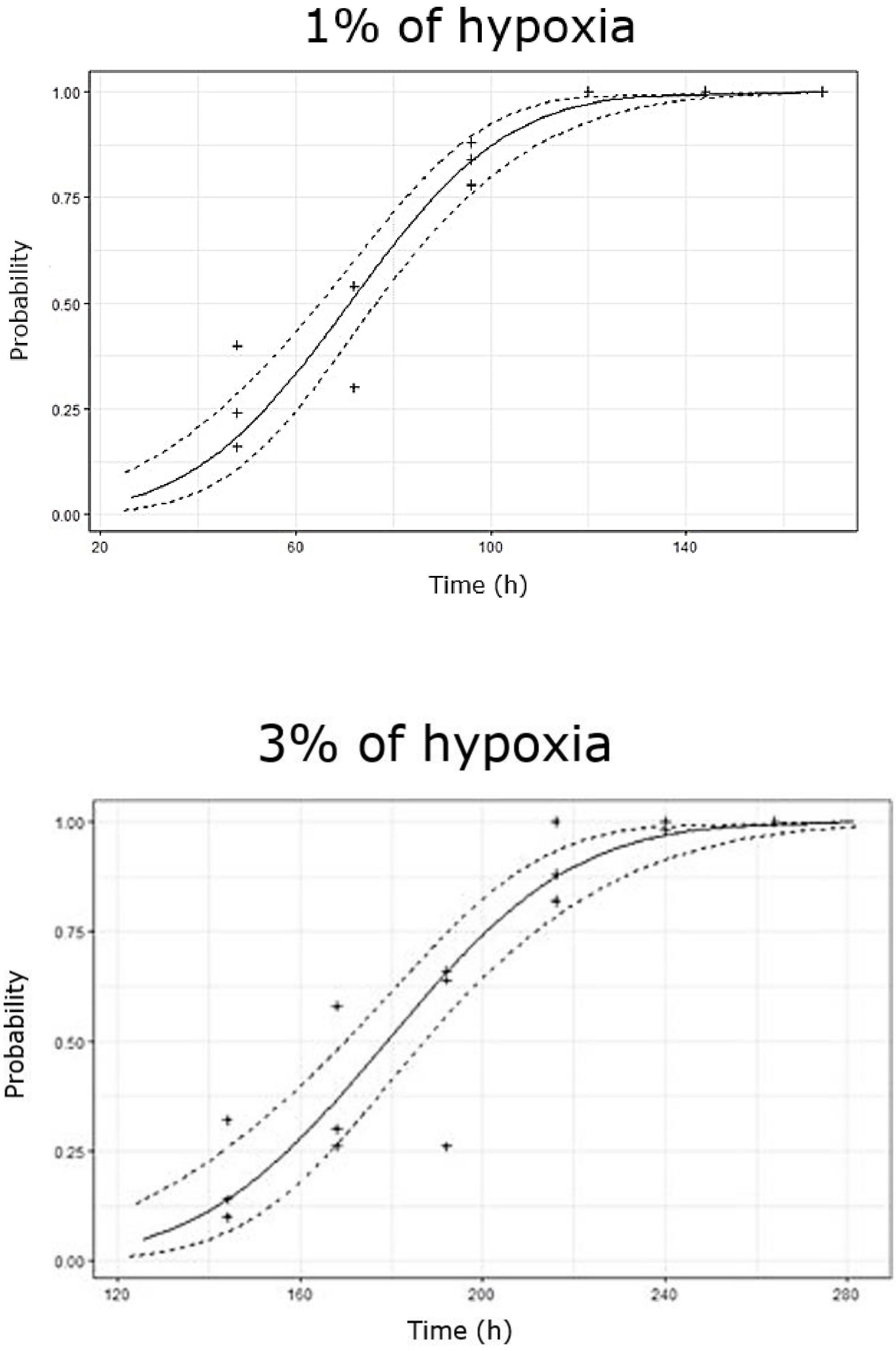
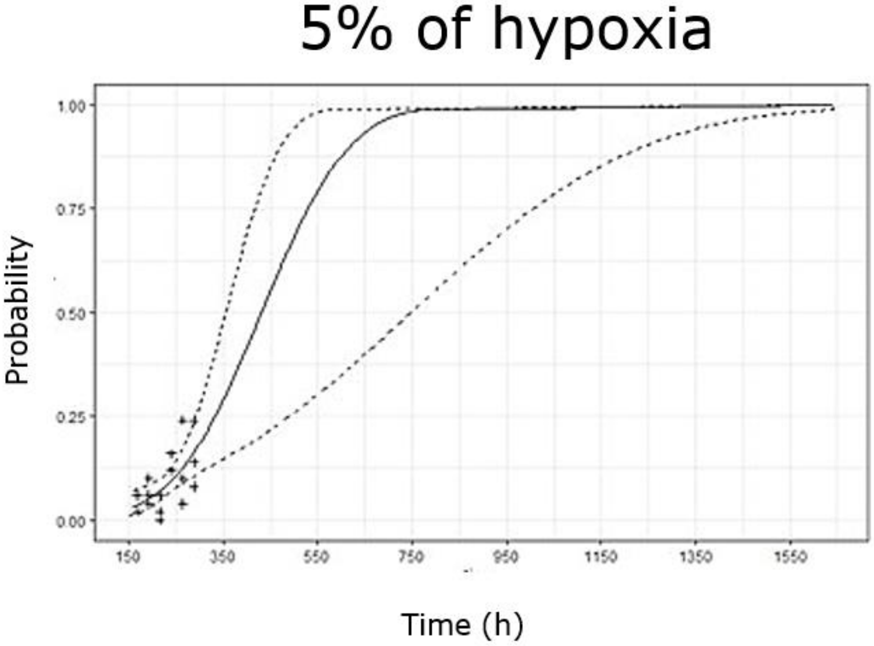


Figure 3-3. Probability of mortality of *S. oryzae* with respect to time (Hours) in 1%, 3% and 5% of hypoxia. The dotted line represents the upper and lower level of 95% confidence interval of regression analysis.

Figure 3-3 continued



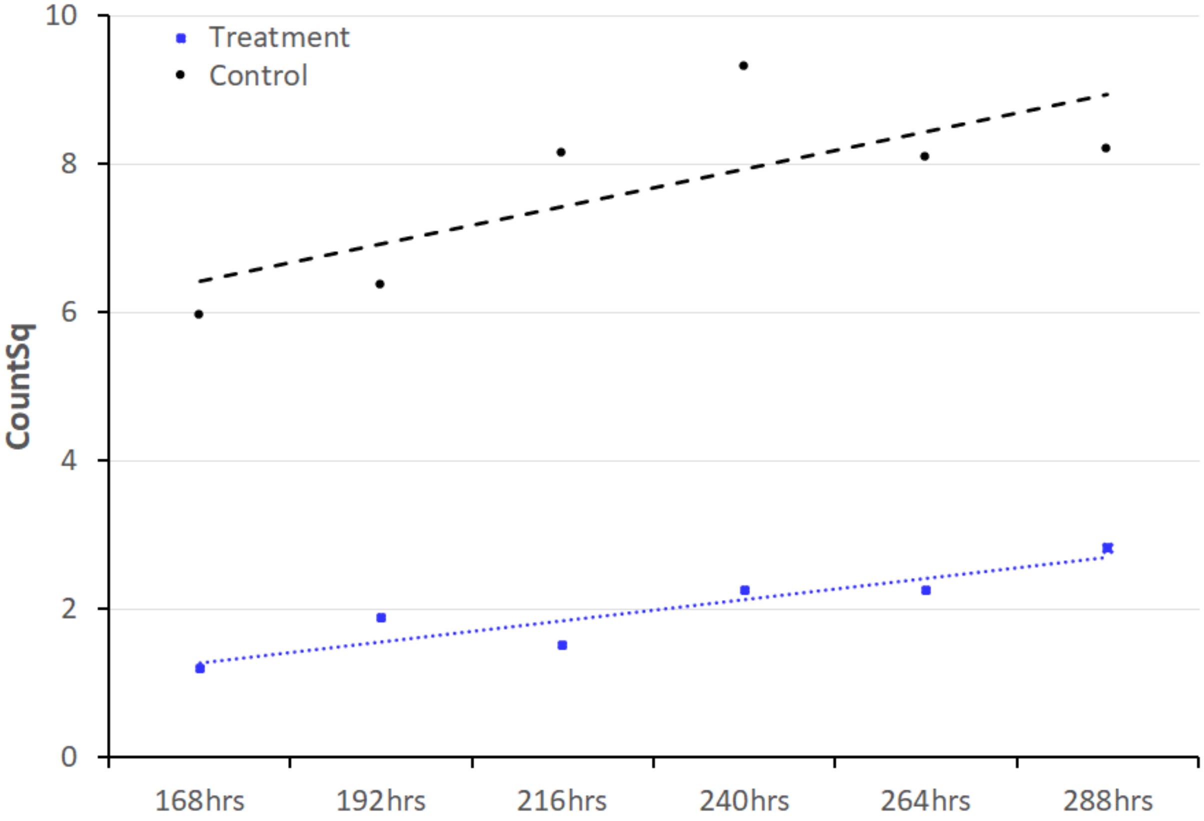


Figure 3-4 Insect emergence post treatment in 5% of hypoxia after exposure of treated grains to 25±1 °C and 40 ±5 % for forty-five days. Countsq refers to square rooted value of number of adult emergences in the experiment

# CHAPTER 4. CONCLUSIONS

This study examined current post-harvest pest management practices in Nepal and laid the foundation for providing a safer alternative to pesticides to farmers in Nepal. In my first objective, I found that rice and lentil were the most stored cereal and legume crops in the Bagmati province of Nepal. Farmers mostly stored their grains for home consumption in traditional grain storage systems like granaries for more than nine months. Farmers reported insects were the major sources of losses in stored grain among which the *Sitophilus oryzae* (L.) and *Corcyra cephalonica* (Stainton) were the most notorious in cereals, whereas *Bruchus* spp. and *Callosobruchus* spp. were the most notorious pest in legumes. The use of the chemical fumigant phosphine was the most common method to control pests on stored grain. Farmers mostly relied on themselves or neighbors for information and had minimal contact with extension agents for post-harvest information. My second objective found that *S. oryzae* adults were highly susceptible to oxygen levels below 5%. Median (50%) levels of mortality were achieved by exposing weevils to 3, 8, and 16 days at hypoxia levels of 1%, 3%, and 5%, respectively, and higher mortality can be attained with longer storage periods. Storing grain killed 50% of weevils. Storing grain in these optimal hypoxia levels minimized the *S. oryzae* population growth and damage. I would like to conduct further research to figure out the relation of RH, pumping of dry gas, grain moisture content, and insect activity. There was a sharp decline in RH right after the nitrogen was pumped to the chamber, research on insect respiration and metabolic water production immediately after pumping nitrogen would be useful. Furthermore, this study showed the suppression of adult emergence. Experimenting to understand, the effect of hypoxia on mating, oviposition, oviposition plug, and effect of hypoxia in different stages of the life cycle of *S. oryzae* would be effective. These laboratory findings would strengthen the adaptation of hypoxia to control *S. oryzae.* Providing storage technologies that reduce the oxygen level in stored grain can help reduce losses due to insect damage, pesticide use, improve food security and can potentially increase farmer’s income in Nepal.

## APPENDIX A. QUESTIONAIRE OF SURVEY

# Survey on postharvest storage technology in Nepal

Objectives: To determine the current post-harvest storage techniques being used and to find a practical approach to deliver innovative storage technology to farmers in Nepal.

District……………….
Village development community (VDC)…………………
Ward number…………………
Geographical Coordinate of the village: (automatic data)

**PERSONAL DATA**

**1.** Name of the enumerator: …………………….
**2.** Contact number of enumerator………….
**3.** Code of the respondent: …………………………………
**4.** Date of interview: ………Time of interview: from…………………to …………………….
**5.** Gender of the respondent: 1. Male 0. Female
**6.** Age of respondent:
  - 18 to 30 years
  - 31 to 40 years
  - 41 to 50 years
  - Older than 50 years

**7.** Marital status
  - Single
  - Married
  - Widower
  - Divorced/Separated

**8.** Size of the household (total number of people- Adults and all children)
**9.** Level of Education
  - None
  - Adult literacy school
  - Primary school
  - High School
  - Tertiary (College/polytechnic/University)

**10.** What is your primary/main economic activity?
  - Agriculture
  - Trade/commerce/business
  - Full-time employee (teacher, etc.)
  - Others (specify): …………………...

**11.** How many years of experience do you have in your activity?
  - Less than 5 years
  - Between 5 and 10 years
  - More than 10 years

**12.** Do you have contact with extension or research agents?
- Yes
- No

**13.** If yes, which research/extension do you have contact with?
- Government extension/research
- NGOs/Projects extension
- Farmers’ group extension
- Other (Specify) : ………………

**14.** Are you a member of an association/farmers’ group?
- Yes
- No

**15.** Do you have a radio?
- Yes
- No

**16.** Do you have a cellphone?
- Yes
- No

**17.** If yes, what is the main use of your cellphone? *(Select one)*
- Calls
- Sending messages
- Watch videos
- Listen to radio stations
- Take photos
- Other (specify): ……………

**18.** What are the limitations of using your cellphone?
- Lack of electricity
- Lack of network
- Do not know how to use the cellphone
- Other (specify): ………

## SEED SOURCING

**19.** Do you store seed?
- Yes
- No

**20.** If you don’t store your seed, where do you get the seeds from?
- Relatives/friends/Neighbors
- Local market
- Agro dealer/distributor
- Other (specify)……………

**21.** Have you had any losses due to insects during seed storage?
- Yes
- No

**22.** How to you preserve your seeds during storage?

- Pesticides
- Ash
- Botanicals
- Hermetic
- Nothing
- Others, specify…………

## GRAIN STORAGE AND CHALLENGES

**23.** Do you store grain?

- Yes
- No

If yes, continue.

If not Go to Q78

## For Cereal

**24.** What is the most important cereal crop do you produce?

- Rice
- Maize
- Wheat
- Finger millets
- Others (specify) …………………

**25.** What is the most important cereal crop you store?

- Rice
- Maize
- Wheat
- Finger millets
- Others (specify) …………………

**26.** Who makes the decision to store the most important cereal grain?

- Man
- Woman
- Myself (if not married)
-

**27.** Who makes the decision to sell the most important cereal grain?

- Man
- Woman
- Myself (if not married)

**28.** What is the main reason to store main cereal grain? Select one.

- Home consumption
- To sell
- Animal feed

**29.** If 18. Yes. Total quantity of main cereal produced and stored 2017- 2018

| Year | Produced (kg) | Stored (kg) |
|---|---|---|
| – 2017 | | |
| – 2018 | – | – …….<br>– ……… |

**30.** What is the storage duration?

- Less than 3months
- 3-6months
- 6-9months
- More than 9months
- Other (specify)………………

**31.** What containers do you use to store grain after harvest?

- Jute bags
- Woven bags
- Mud silos
- Granaries
- Other – specify…………….

**32.** Where do you store your grain after harvest?

- Outside the house
- Inside house
- Others (specify) …………………

**33.** Which challenges do you encounter during crop storage? (Check all applicable)

- Insect damage
- Decay/mold damage
- Rodent damage
- Theft
- Ineffective insecticides
- Others (specify) …………………

**34.** Please specify two major insect pests of stored cereal crop in Q25:

- ………………..

**35.** What is your primary method for grain protection for main cereal crop in Q25?
- Do nothing
- Chemical pesticides
- Natural products (Extract of plants)
- Hermetic/ airtight methods
- Others (specify) …………………

**36.** Do you use the chemical products for grain storage for main cereal crop in Q25?
- Yes
- No

**37.** If you don't use chemicals, what is the main reason for not using chemicals? Select one
- No attacks of insects
- Not available
- Too expensive
- Not effective
- Toxic/harmful to health
- Others (specify): ………………, go to next section

**38.** If you use chemicals, where do you get the chemicals?
- Agro-dealers/Shops
- Farmers' Markets
- NGOs
- Others (specify) ……………

**39.** If you use chemicals, Do you need a second treatment?
- No
- After 3 Months
- After 6 Months
- After 9 months

**40.** What is the main reason you prefer to use chemicals? Select one
- Easy to use
- Availability
- Efficacy
- Low price
- Cheap laborers,
- Others, specify………………

**41.** Who applies the insecticides to treat the stored crop?
- Myself
- Hired Labor
- Crop protection agent/Consultants
- Other, specify……….

**42.** What are the challenges in the use of chemicals?

- Not effective
- Cost
- Hazardous
- Lack of knowledge on use
- No problem

## For Legumes

**43.** What is the most important legume crop you produce? Select one

- Cowpea
- Mung beans
- Beans
- Lentils
- Soybean
- Chickpea
- Others (specify) …………………

**44.** What is the most important legume crop you store?

- Rice
- Maize
- Wheat
- Finger millets
- Others (specify) …………………
-

**45.** Who makes the decision to store the most important legume grain?

- Man
- Woman
- Myself (if not married)

**46.** Who makes the decision to sell the most important legume grain?

- Man
- Woman
- Myself (if not married)

**47.** What is the main reason to store main legume grain? Select one.

- Home consumption
- Animal feed
- To sell

**48.** If 18. Yes. Total quantity of main legume produced and stored 2017- 2018

| Year | Produced (kg) | Stored (kg) |
|---|---|---|
| – 2017 | | |
| – 2018 | – | – …….<br>– ……… |

**49.** What is the storage duration?

- Less than 3months
- 3-6months
- 6-9months
- More than 9months
- Other (specify)………………

**50.** What containers do you use to store legume grain after harvest?

a. Jute bags
b. Mud silos
c. Granaries
d. Woven bags
e. Other – specify……………...

**51.** Where do you store your grain after harvest?

- Outside the house
- Inside house
- Others (specify) …………………

**52.** Which challenges do you encounter during crop storage? (Check all applicable)

- Insect damage
- Decay/mold damage
- Rodent damage
- Theft
- Ineffective insecticides
- Others (specify) …………………
-

**53.** Please specify two major insect pests of stored legume crop in Q44:

- ………………..
- ………………..

**54.** What is your primary method for grain protection for main legume crop in Q44?

- Do nothing
- Chemical pesticides
- Natural products (Extract of plants)
- Hermetic/ airtight methods
- Others (specify) …………………

**55.** Do you use the chemical products for grain storage for main legume crop in Q44?

- Yes
- No
-

**56.** If you don't use chemicals, what is the main reason for not using chemicals? Select one

- No attacks of insects
- Not available
- Too expensive
- Not effective
- Toxic/harmful to health
- Others (specify): ………………, go to next section

If you use chemicals, where do you get the chemicals?

- Agro-dealers/Shops
- Farmers' Markets
- NGOs
- Others (specify) ……………

If you use chemicals, Do you need a second treatment?

- No
- After 3 Months
- After 6 Months
- After 9 months

**57.** What is the main reason you prefer to use chemicals? Select one

- Easy to use
- Availability
- Efficacy
- Low price
- Cheap laborers,
- Others, specify………………

**58.** Who applies the insecticides to treat the stored legume crop?

- Myself
- Hired Labor
- Crop protection agent/Consultants
- Other, specify……….
-

**59.** What are the challenges in the use of chemicals?

- Not effective
- Cost
- Hazardous
- Lack of knowledge on use
- No problem

**HERMETIC STORAGE USE**

**60.** Have you heard of hermetic storage technologies (HST)? (These are airtight storage methods that control insect infestation without the use of chemicals).

- Yes
- No

**61.** If yes, which HSTs have you heard of? (check all applicable)

- PICS bags
- Grain Pro
- Super Bag
- ZeroFly
- Metal silos
- Plastic Drums
- Jerri cans
- Others (specify)……………

**62.** Where did you hear of the HST in Q62 method you are currently using?

- Radio
- TV
- Newspaper
- Extension agents
- Agro dealers
- Leaflets, pamphlets and brochures
- Farmers/friends/relatives
- Others (specify)……………………………………..

**63.** Have you used hermetic storage technologies (HST)?

- Yes
- No ,

**64.** if you have used HST, which HST method do you use the most?

- PICS bags
- Grain Pro
- Super Bag
- ZeroFly
- Metal silos
- Plastic Drums
- Jerri cans
- Others (specify):…………

**65.** What do you use it for?

- Crop Storage
- Water harvesting
- Others, specify…………

**66.** Where did you get your HST?

- From donation, NGOs, relief funds
- Bought myself
- Extension agent/Government agency
- Family members

**67.** Did you receive training on use of HST?

- Yes
- No

**68.** If yes, who provided the trainings?

- Extension agent
- Farmers/neighbors
- Agro dealer (shop or market)

**69.** Where did you receive the training on how to use the HST in Q62?

- Village demonstration
- Market demonstration
- Field days
- Media (TV, radios, flyers, videos)
- Others (specify)……………………….

**70.** What are the benefits of using HST in Q62?

- Effective - Better protection of grain
- No use of insecticides
- Price premium: Higher prices for the grain store without insecticides
- Cheaper than other storage methods
- Ease of use
- Others (specify)………………………………………

**71.** What is the main reason for using HST in Q62?, Select one

- Food security/store for home consumption
- Income/ store and sell later
- Others (specify)………………………………………

**72.** Do you add insecticide or fumigate grain during storage in HST in Q62?

- Yes
- No

**73.** Do you encounter any challenges while using HST in Q62?

- Yes
- No

**74.** If yes, what are the challenges in the use HST in Q62?

- Not available nearby
- Lack of training on their use
- Not available at harvest
- High prices
- Not effective
- Others, specify………..

**75.** If not using HST, what is the main reason for not using HST?

- Not aware
- Cost/Expensive/high price
- Not available
- Don't know how to use HST
- Not effective
- Alternatives are cheaper (e.g insecticides)
- Other, please specify………..

**76.** If not, why don't you store?

- Not enough production
- Problem of insects
- Good price of the crop at harvest
- Sell to meet the needs of the household
- Others (specify) …………… go to next section

**At this point, demonstrate the use of the PICS bag, tell about**

**77.** Would you buy a PICS bag?

- Yes
- No

**78.** Would you be willing to pay S/7 – 10 for a 100kg PICS bag?

- Yes
- No

**79.** Which legume crop would you use the PICS bag for?

- Cowpea
- Mung beans
- Beans
- Lentils
- Soybean
- Chickpea
- Others (specify) …………………

**80.** Which cereal crop would you use the PICS bag for?

- Rice
- Maize
- Wheat
- Finger millets
- Others (specify