

Effect of organic products (Lupine oil and Areki) against maize weevil (*Sitophilus zeamais*) on stored maize grains (*Zea mays*)

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Abstract

Humans and animals share the same environmental resources for food. Storage insect pests destroy food grains in storage. Environmentally sound pest management methods rely on cultural, physical, biological and botanical pesticides. In the present study the insecticidal activity of *Lupinus albus* (juice and powder) and *areki* (locally brewed alcoholic drink) was examined against maize weevil, *Sitophilus zeamais*. The Experiment was laid out in a completely randomized design (CRD), with three replications. Different levels (doses) of Lupine powder, Lupine juice, and areki (local areki) were used. Weevil mortality, number of holes on a random sample of maize seeds, grain weight loss and rate of germination were recorded for each treatment. According to the ANOVA, treated and untreated treatments significantly varied in weevil mortality ($F_{71, 144} = 116.15, P < 0.0001$), in number of holes ($F_{11, 24} = 631.1, P < 0.0001$), in grain weight loss ($F_{11, 24} = 1499.2, p < 0.0001$), in maize seed germination rate after treatment ($F_{11, 24} = 93.53, P < 0.0001$) and in number of offspring ($F_{71, 144} = 93.116.15, P < 0.0001$). Except the untreated check, 100% weevil mortality was achieved in just one day by all doses of areki, in three days by lupine juice and in one week by lupine powder. In all treatments, weevil mortality increased with increasing dose and with time. Except the untreated check, all treatments and their doses completely prevented weevil reproduction, holes and weight loss suggesting the power of these products against weevils. Higher level of Lupine juice affected germination of maize grain whereas local areki and Lupine powder did not have effect on seed germination. In conclusion, Lupine products and areki are ready for use for stored grain protection.

Keywords: Lupine, local alcoholic beverage areki, weevils, *Sitophilus zeamais*, maize, storage

1. Introduction

The maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) is one of the most destructive insect pests of stored cereals in tropical and sub-tropical regions [1]. Post-harvest losses due to this insect pest have become an increasingly important constraint in Africa [2]. Adult weevils attack whole grains, and larvae feed and develop within grains. The huge post-harvest loss and quality deterioration caused by this pest is a major obstacle for achieving food security in developing countries. Initial infestations of maize grain can occur in the field just before harvest and the pests get transported to the store [3]. Weevil damage of grains leads to reduced nutritional value, seed germination rate, seed weight and commercial value of the product [4].

The application of traditional products such as botanical insecticides and plant-derived pesticides to protect stored products against insect infestation is a common and an age-old practice in small-scale grain storage [5, 6, 7, 8]. The wide scale commercial use of plant extracts as insecticides began in the 1850s with the introduction of nicotine from *Nicotiana tabacum*, rotenone from *Lonchocarpus* sp., derris dust from *Derris elliptica*, pyrethrum from the flower heads of *Chrysanthemum cinerariaefolium*, *Lanthena* sp., *Capsicum* sp., and *Tagetes* sp. Some plant families may accumulate a

restricted number of anti-herbivore chemicals, so called secondary metabolites, whilst others possess a wide variety of different structural compounds.

Generally, botanical pesticides can be used as crude extracts, in development of prototypes of known active ingredients, and as sources of known active ingredients. The main advantages of botanical pesticides are that they are eco-friendly, easily bio-degradable and plant-derived natural products that are toxic to pests and they could be produced from locally available organic materials.

White lupine is a widely known annual legume, large seeded and commercially important bean in the world. It is a traditional pulse crop in the Mediterranean and the Nile valley, including Sudan and Ethiopia. In Ethiopia, it is known as Gibto, meaning something that comes from Egypt or Gibts. Smallholders in the Amhara region produce the largest share followed by Benishangul Gumuz region, which is bitter type due to its high alkaloid content [9]. In Germany, a method for extracting alkaloids from bitter lupine has been developed and researched for possible use as fertilizer or as biocide. Alkaloids extracted may have insecticidal properties that could reduce or prevent insect damage on vegetables and crops. In traditional medicine, white Lupine is used against worms, boils and skin ailments [10]. It is used to repel insects, enhance sugar tolerance in diabetics, and heal sores [11]. Also

our preliminary observations of the traditionally brewed *areki* or alcoholic beverage for storage pest protection shows unmatched efficacy. It is known that alcohol is used for the preservation of small animal specimens such as insects. That means it has the power to stop the reproduction of organisms (both micro and macro). Its cost is also notably lower than commercial pesticides. Therefore, in this study both lupine powder and *areki* were tested for their potential in storage pest management.

2. Materials and Methods

2.1 Description of the Study Area

The study was conducted in Dembecha district, which is located in West Gojam zone of the Amhara Regional State of Ethiopia on the main road of Addis Ababa to Bahir Dar (Fig. 1). The area is the second most important grain source of Ethiopia especially maize.

The altitude of the study area ranges from 1500 to 2995 meters above sea level and its annual rainfall is 1006 mm and the average minimum and maximum temperature of this district is 18°C and 24°C, respectively (Dembecha District Office of Agriculture and Rural Development, unpublished report).

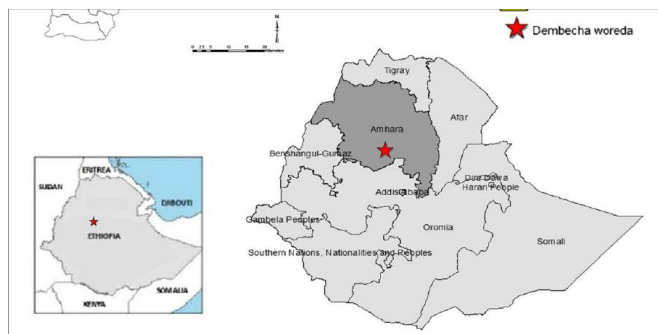


Fig 1: Location of Dembecha district in Ethiopia

2.2 Stock Culture of Weevils

Weevils were collected from traders' granary stores in town as initial stock. They were first mass reared in a separate container (tin cans covered with muslin cloth at the top). Weevils were introduced into the maize seeds and the container kept in ideal conditions for reproduction to take place. Newly emerged weevils were used for the rest of the study.

2.3 Botanical Pesticide (lupine) Preparation

Freshly harvested seeds of *Lupinus albus* and local *areki* (the traditional alcoholic drink similar to vodka) was purchased from the market at Dembecha town, northwestern Ethiopia. The seeds of lupine were washed to remove dirt and then dried in the sun immediately after cleaning. They were then ground using stone to produce powder.

To produce residue, 0.5 kg of lupine powder was mixed (and shaken) in 2 liters of purified water for 24 hr.

Clean and non-infested maize grains were obtained from trusted sources (own source) for the study. The maize grain was kept in the freezer of a refrigerator for one day to ensure freedom from any pests. After that the seed was sealed completely in a beaker to prevent any re-infestation until the seeds are ready for the study.

2.4 Study Design

The treatments (the four levels) of each product were laid out in completely randomized design (CRD) in the laboratory replicated three times.

Experiment 1. Testing the efficacy of *areki* against maize weevils (measured by w/v)

Exactly 200 g maize seeds were kept in each of 12 beakers. Treatments applied include 0, 4, 8, and 12 ml of *areki*. Each application dose was thoroughly mixed with the 200 g seeds. The experiment was laid out in CRD, replicated three times. Then 10 adult weevils were introduced into each of the 12 beakers that contained the 200 g seeds and the treatments. Data were recorded as follows. Starting from one day after introduction of the weevils, numbers of dead weevils was recorded daily for the first one week. Death was defined as no sign of movement of the weevil and stiff body. Then starting from two weeks onwards, a random sample of 20 seeds was taken from each beaker to determine the number of seeds showing signs of weevil attack or holes. At the same time, assuming reproduction continues, number of weevils emerged were counted and recorded per beaker. This was continued for three months. Then, for each beaker, the weight of the seeds was recorded and percent weight loss calculated on the basis of the original weight, which was a blanket weight of 200 g. Finally, after three months a random sample of 20 seeds was taken germination for germination test.

Weight Loss: The weight loss of maize seeds due to infestation with *S. zeamais* was determined three months after treatment. The dry weight loss was calculated as follows [12].

$$\text{Loss (\%)} = \left(\frac{\text{IDWS} - \text{DWA 3M}}{\text{IDWS}} \right) * 100$$

where IDWS stands for Initial dry weight of seeds and DWA3M for Dry weight after three months.

Number of holes: At the end of the three month observation period, the extent of weevil damage was assessed using exit-hole count as a measure of damage to grains. Grains with exit-holes were counted; the percentage holes (PH) of the weevils to the grains was calculated using the formula [13].

$$\text{PH} = \left(\frac{\text{TNGH}}{\text{TNG}} \right) * 100$$

where PH stands for percentage holes, TNGH for total number of treated grains with holes and TNG for total number of grains.

Experiment 2: Effect of lupine powder on the maize weevils

The trial was laid out in a completely randomized design (CRD) with four lupine powder treatments (0, 4, 8, 12 g w/w), replicated three times. A random sample of 200 g clean maize seeds were taken and kept in beakers. Each of the four lupine powder treatments was dusted onto the seeds and the beaker shaken to mix the powder and the maize seeds. Soon after mixing, 10 freshly emerged weevils were introduced into each beaker and their fate followed. The beakers were then covered with muslin cloth to allow air circulation. The same thing was done for each of the 12 beakers. Each

treatment was left in the laboratory bench for daily observation. Data collection was the same as experiment 1.

Experiment 3: Bioassay of lupine powder and juice toxicity against maize weevils

The contact effect of lupine juice on the adults of *S. zeamais* was investigated. The experiment was laid out in a completely randomized design (CRD) with four treatments, replicated three times. Maize grains were treated with different amounts or treatments of the residue or juice. First, 200 g maize seeds was kept in a beaker and mixed with four different lupine juice amounts, or treatments (0, 4, 8, and 12 ml per beaker). The seeds and the juice were thoroughly stirred to ensure homogeneity of the lupine juice on the treated maize grains. These treatments were replicated three times.

As soon as this is completed, 10 reared adult weevils were introduced into each of the 12 beakers and the fate of the weevils recorded. Dead weevils were counted, removed and their numbers recorded daily starting from one day after application for the next three days, and then weekly for the next one month.

Experiment 4: Germination test

The germination test was laid out in a completely randomized design by taking 20 seeds from the four treatments of each product replicated three times. Seeds were spread in Petri dishes lined with moist filter paper. The procedure was done for the different extracts (Lupines *albus* powder and juice as well as areki).

3. Results and Discussion

The results obtained in this study tend to justify the role of lupine products and areki in the storage of maize grain against degradation by storage insects. The treatments have been observed to significantly reduce the ability of maize weevils to lay eggs on the protected seeds and thus lead to a reduction in the level of damage. Therefore, according to the result of the analysis of variance, damage, i.e., grain weight loss and number of holes were significantly reduced or completely prevented by using these organic products.

3.1 Weevil Mortality

Increasing doses of lupine juice treatment significantly increased weevil mortality. That was clearly observed at 1 d after treatment (Fig. 2a). The same general pattern was observed at 2 d after treatment, after which doses did not vary in levels of mortality. Generally, higher doses meant fast knock down effect on weevils. When the powder form was used, mortality was negligible until one week (Fig. 2b). However, one week later, almost all weevils were found dead. The action of areki was fast and effective. However, there was some sign of difference in mortality between doses (Fig. 2c). All weevils treated with areki died in just one day. In all the three modes of application (juice, powder, and areki), there was no mortality in the untreated control (Fig. 2a, b, c).

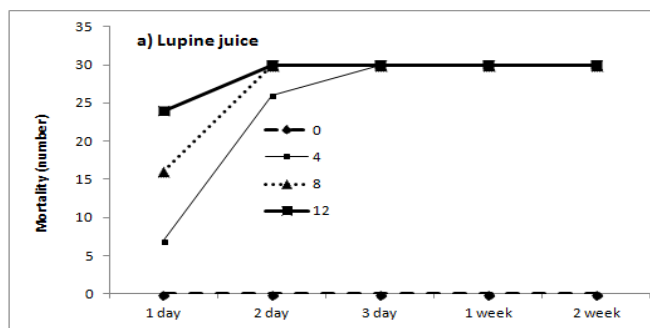


Fig 2a

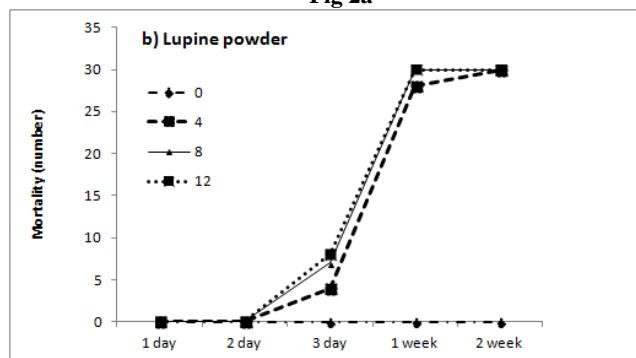


Fig 2b

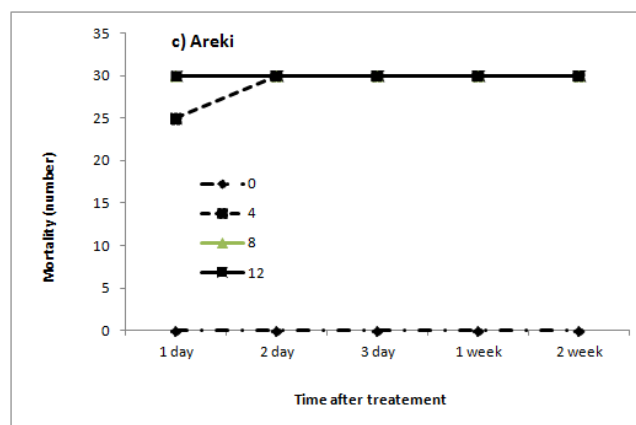


Fig 2a, b, c: Cumulative mortality of weevils at different doses and formulation of lupine and areki.

3.2 Reproduction of Weevils

According to the results of the three way factorial ANOVA, number of weevil offspring significantly varied temporally (time of data collection) and between treatment levels (doses), and their interactions ($F_{71, 144} = 116.15, P < 0.0001$). No weevil progeny were produced on maize seeds treated with all treatments, i.e., local areki, lupine juice and lupine powder (Fig. 3).

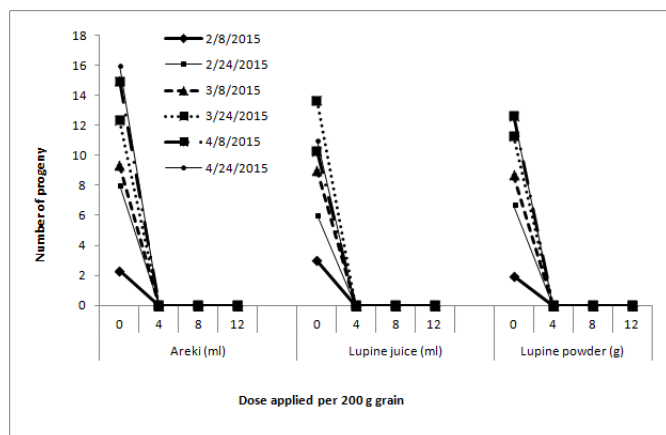


Fig 3: Effect of different doses of organic products (local areki, lupine powder and juice) on weevil reproduction at different times of sampling.

Application of the three doses of areki had completely stopped weevil reproduction regardless of dose (rate of application). That meant all doses were equally effective. Therefore, lower rates than the ones tested may be tested in the future to develop control packages that are not only technically effective but also cost-effective. In contrast, untreated seeds had progeny whose number steadily increased with time (across the 3 month period). The treated seeds might be used for consumption as well as for planting because they were not damaged by weevils.

Weevils did not reproduce on treated maize seeds because they did not find these seeds suitable for reproduction to take place. From the result, it could be concluded that treatments controlled maize weevil reproduction 100% for two months and a half. The mechanism of action may be their antifeedant or repellent nature. Antifeedants, or feeding deterrents are chemicals that inhibit feeding or those that disrupt insect feeding by rendering the treated materials unattractive or unpalatable^[14]. Because all doses or levels stopped the weevil completely, doses lower than 4 ml are suggested (for further scrutiny).

The problems posed by broad spectrum synthetic pesticides have led to the need for effective biodegradable pesticides with greater selectivity^[15]. The efficacy of the products tested in the present study indicates their potential for replacing synthetic pesticides. All three products regardless of dose prevented reproduction. That was a great leap forward.

Synthetic insecticides not only they pollute the environment but they also speed up weevil resistance to synthetic pesticides^[16]. On the other hand, pest insects have little chance of developing resistance to organic products.

Organic products are receiving more and more attention for pest control. What is needed is refining them using conventional scientific procedures. They have been with grain producers and traders for centuries. For example, Egyptian and Indian farmers used ash^[17] and leaves and seeds of neem for the control of stored grain pests^[18]. In eastern Africa, leaves of the wild shrub *Ocimum suave* and the cloves of *Eugenia aromatic* are traditionally used as stored grain protectants^[19]. In Rwanda, farmers store edible beans in a traditional closed structure and whole leaves of *Ocimum canum* are usually added to the stored foodstuff to prevent insect damage within these structures^[20].

In some South Asian countries, food grains such as rice or wheat are traditionally stored by mixing with 2% turmeric powder^[14]. The more potent, and at times commercially formulated and marketed, botanical insecticides such as pyrethrum, derris, nicotine, oil of citronella, and other plant extracts have been used for centuries^[21]. Turmeric, garlic, *Vitex negundo*, castor, *Aristolochia*, ginger, *Agave americana*, custard apple, *Datura*, *Calotropis*, *Ipomoea*, and coriander are some of the other widely used botanicals to control and repel crop pests including maize weevils^[22].

Essential oil constituents such as thymol, citronellal and α -terpineol are effective as feeding deterrents against tobacco cutworm, *Spodoptera litura*^[23]. Synergism or additive effects of a combination of monoterpenoids from essential oils have been good against *S. litura* larvae. The *H. spicigera* essential oils showed fumigant toxicity against *S. zeamais*. The mortality rate of *S. zeamais* increased with the concentration and duration of exposure to the essential oils^[24].

3.3 Number of Holes

According to the results of ANOVA, number of holes on maize seeds was significantly different between treatment levels ($F_{11, 24} = 631.1$, $P < 0.0001$). The presence of holes indicates that weevils have been feeding on grains. Number of seeds with holes were significantly different between treated and untreated maize grains ($P < 0.0001$) (Fig. 4).

After two and half months, about 70% of untreated maize grains had holes and treated ones did not have holes. Grain weight declined with increasing number of holes and, therefore, weight loss and number of holes were directly related (Pearson's correlation coefficient $r = 0.99$, $P < 0.0001$, $N = 36$). Infested maize seeds exhibit holes through which the adults emerge^[25]. Many indigenous plants, in powder form, effectively control cowpea seed beetles^[26].

3.4 Percent Grain Weight Loss

Grain weight loss significantly varied between treatment levels after two months and a half ($F_{11, 24} = 1499.2$, $P < 0.0001$). After two and half months, percent weight loss of untreated seeds was about 35%. Treated ones did not lose weight (Fig. 4). Similar results have been reported earlier. For example, weight loss of wheat was prevented by applying the powder of *A. indica* and *A. boone*^[27]. When maize weevils perforate maize grains, the weight of the grains declines. In the present experiment, the local areki and lupine products have prevented the formation of holes on seeds. This result is also supported by other researches on cowpea bruchids^[28] and common beans^[29]. Malathion treated common beans did not lose weight whereas the untreated ones did. Beans which were treated by Actellic super dust and coconut oil to prevent *Z. subfasciatus* had the lowest number of holed seeds and the highest weight of seeds as compared to the untreated ones^[29]. Increase in percent damaged bean seeds and weight loss is because of increasing bruchid number and the degradation of oils with time^[28].

Just 2% turmeric powder provided good protection to rice or wheat and reduced grain weight loss^[14]. Botanical insecticides such as pyrethrum, derris, nicotine, oil of citronella, and other plant extracts have been used for centuries^[21]. In the plant powder 99.1% mortality was recorded in *V. nugundo*, 94% in *N. speciosum*, and 96% in *A. officinarium*. Adult emergence was registered in *A. indica*

and *A. officinarum* (both 18%) followed by *G. superpa* (20%). The lowest grain weight loss was reported in *A. indica* (18.55%) and *A. officinarum* (18.56%) [30].

3.5 Percent Germination of Seeds

Similarly rate of seed germination significantly varied between treated and untreated seeds ($F_{11, 24} = 93.53$, $P < 0.0001$). Germination rates did not vary between doses of 4, 8 and 12 ml of the local areki and the lupine powder (Fig. 4). On the other hand, germination levels varied with different rates of lupine juice application. When maize weevils perforate maize grains, seed germination rate declines. Insect pests inflict their damage on stored products mainly by direct feeding [31]. Some species feed on the endosperm causing loss of weight and quality, while other species feed on the germ, resulting in poor seed germination and low viability. Local areki and lupine products prevented the formation of holes on seeds due to their insecticidal properties. In this study, the germination rate of untreated maize grain by local areki and lupine powder is lower than that of the treated one almost by 80%. Presence of *S. zeamais* in maize grains led to a reduction in germination with increasing developmental stage of the insects, from 13% at the egg stage to 93% at the adult stage [32].

This result is also supported by other researches on cowpea bruchids [28] and on common beans [29].

4. Conclusions

Maize weevils are a major factor for the loss of maize grain quality and weight in sub Saharan Africa including Ethiopia. This results in recurrent famines in the developing countries especially in Africa where most of the grains are meant for food. So, protecting our food from storage insects is a priority to ensure food security.

The finding of natural products for the control of maize weevils is advantageous for the maintenance of unpolluted environment (eco-friendly) and its non-toxicity to non-target organisms. The present research was carried out to determine the efficacy of lupine products and areki against the storage pest, *S. zeamais*.

The effect of lupine juice, lupine powder and areki on maize weevils was studied at various rates of application. The effect of these products on weevils was consistent and effective. The treatments decreased weevil reproduction, grain weight loss and grain damage (holes on grains) and increased mortality. All rates were almost equally effective. No loss of weight and perforation of holes were observed on treated maize grains. However, untreated grains sustained huge weight loss, the greatest number of offspring and holes. As the amount of lupine juice applied increased, the rate of germination was affected unlike that of lupine powder and areki, which does not have a negative impact on the rate of germination of maize grains.

Generally, lupine products and areki treatments were found to be effective against the attack of *S. zeamais*. This provides good arguments for carrying out this study on natural pesticides. Thus the tested products could serve as potential tools for the management of storage insect pests. Future efforts should focus on product optimization, packaging and marketing.

4 Conflict of interest

The authors confirm that there is no conflict of interest whatsoever.

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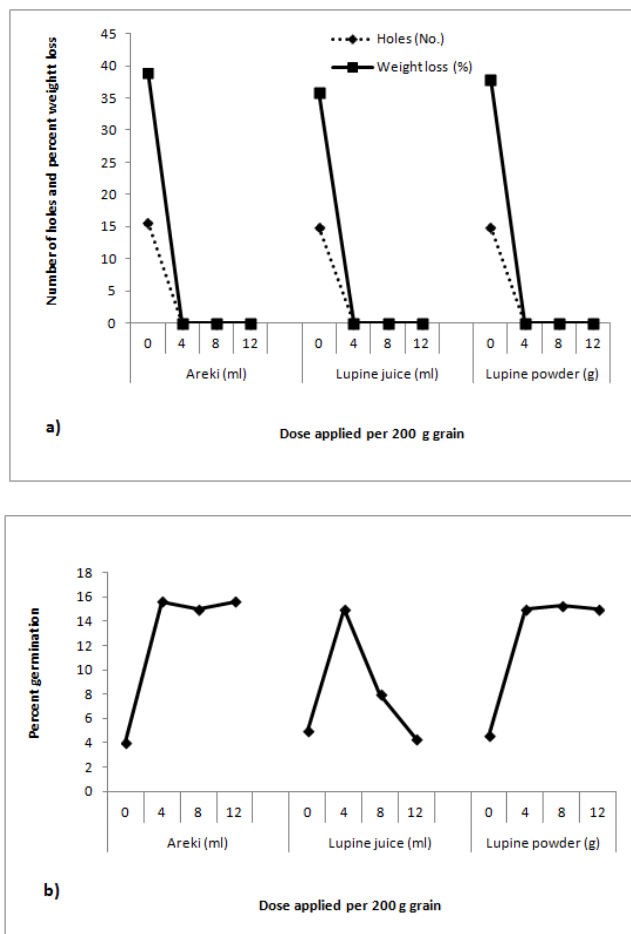


Fig 4a, b: Effect of different doses of organic products (local areki, lupine powder and juice) on number of holes, weight loss and percent germination, a) number of holes and percent weight loss, b) percent germination.

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