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Evaluation of different concentration of Bojho (*Acorous calamus*) against maize weevil *Sitophilus zeamais* (Motschulsky, 1955) and wheat weevil *Sitophilus granaries* (*Linnaeus,* 1758)

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Abstract
To evaluate the efficiency of different concentrations of Bojho (*Acorous calamus)* on maize and wheat weevil (*Sitophilus* spp.), a lab experiment was conducted from 13, February 2019 to 19, February 2019 at the entomology lab. The design setup was Completely Randomized Design (CRD) with 5 treatments including control whereas each treatment was replicated 5 times. Grains were first adjusted to 14% moisture and placed in 50 jars with 15 cm height x 8 cm diameter. 250 grams of grains were placed in each jar with 25 jars containing wheat grains and another 25 containing maize grains. Grains were then treated with different concentrations of Bojho (5, 7.5, 10, 12.5g per kg of grains) and control to evaluate its efficiency against weevil. Powdered botanical extract Bojho was thoroughly mixed with grains in each jar. Mortality rate and weight loss were recorded at an interval of 24 hrs for 6 days. 12.5g Bojho/kg grains have shown the highest weevil mortality rate and lowest weight loss of grains in both maize and wheat.

Keywords: Biopesticides, Bojho, *Zea mays,* storage pest, *Acorous*, *Sitophilus zeamais*

Introduction
Cereal pests may infest the corn grain during storage and transport. One among them is the maize weevil (*Sitophilus zeamais* Motschulsky), a ¼ inch long, reddish-brown to black snout weevil. This pest damages the harvested maize by making holes and feeds the inner starch which causes weight loss and reduced the quality [1].

In maize and wheat, the attack may start in the mature crop when the moisture content (MC) of the grain has fallen to 18-20%. Subsequent infestations in-store result from the transfer of infested grain into a store or from the pest flying into storage facilities, probably attracted by the odor of the stored grain. The average loss by *S. zeamais* is estimated at 20-80% in tropical countries [2]. The chewing damage caused by the insect brings about increased respiration in the cereal (hot spots), which promotes the evolution of heat and moisture and in turn provides favorable living conditions for molds leading to the production of aflatoxin. Subsequently, at very high moisture levels, bacterial growth is favored which ultimately gives rise to depreciation and finally total loss. Controlling stored pests is not an easy job although synthetic chemicals are available for use. Effective pest control is no longer a matter of the heavy application of pesticides, partly because of the rising cost of petroleum-derived products but largely because excessive use of pesticide promotes faster evolution of the resistant form of pests.

Among these is the use of botanical pesticides with low mammalian toxicity and can effectively prevent and/or suppress insect pests especially in storage. The use of botanical pesticides to protect plants from pests is very promising because of several distinct advantages. Pesticidal plants are generally much safer than conventionally used synthetic pesticides. Pesticidal plants have been in nature as its component for millions of years without any ill or adverse effect on the ecosystem. Besides, plant-based pesticides are renewable in nature and cheaper. Also, some plants have more than one chemical as an active principle responsible for their biological properties. These may be either for one particular biological effect or may have diverse ecological effects. The chances of developing quick resistance to different chemicals are highly unlikely.

Insect pests commonly attack stored grains like maize and wheat. They cause severe damage to the commodity resulting in losses in weight, seed viability, and nutritive quality of foodstuffs. A considerable amount of grain weight losses including a loss in quality have been observed in the storehouse. The appropriate and safe weevil management practices are lacking in storehouses in Nepal. However, the use of celphos (aluminum phosphide) is common practice all over Nepal. The current pesticide management practices deteriorate the health and environment and caused many complex health problems [3]. Hence, alternative and safe methods of weevil management have been realized for their integrated management. Nepal is rich in biodiversity especially with a diverse family group of plant materials. These plant materials have the potentiality to repel the storage pest from the storehouse [4]. The common plants and their parts such as *Acorus calamus* (Bojho) have been used to minimize the infestation of storage pests. This plant is known for its insecticidal property against storage insect pests thus, this study was conducted. In general, the loss (pre and post-harvest) due to pests has been estimated to be 15-20% [5] and in times of epidemics, the figure may exceed. Similarly, KC, G (1992) mentioned that grain storage losses in Nepal ranges from 15-30% annually [6]. This research aimed to study the efficacy and potentiality of the *(Acorous calamus*) to repel the storage grain pest.

Materials and Methods

This study focused on the effectivity of powdered, *Acorus calamus* (Bojho) as protectants of stored maize grains against attack by *sitophilus spp*. Effectively was based on weight loss, repellency, anti-oviposition/growth inhibitory and adult mortality tests under laboratory conditions. The experiment was conducted at the Entomology Laboratory,

Fresh leaves and rhizome of *Acorus calamus* (Bojho) were gathered and brought immediately to the laboratory. Leaves were air-dried in the laboratory until crispy. The dried leaves were pulverized using a micro pulverizer and were sieved through a 0.5 mm size mesh to obtain uniform particle size. The resulting powders were kept separately in glass containers with a screw cap and stored at room temperature before use. More than enough weevils (*sitophilus spp)* were gathered and brought immediately to the laboratory and provide them a suitable environment before use in our treatment.

Mass rearing of Sitophilus spp

The material for research such as maize seed and wheat seed (local variety) was selected. 15 cm high x 8 cm diameter plastic jars, were thoroughly cleaned and dried in sun for 2 to 3 hours to ensure the absence of insects, mites or disease-causing microorganisms. The sealed plastic jars were allowed to equilibrate to the ambient temperature before they were opened to avoid excessive loss or gain of moisture. Corn and wheat grains were adjusted to 14% moisture content (MC) before use. 250 grams maize and wheat grains of both local varieties were placed in each plastic jar and the temperature and R.H. of the lab were maintained for weevil culture. Male and female adult weevils were introduced in each jar. After the introduction of the Weevil in the medium, the top of the jar was covered airtight. The jars were arranged 6 cm apart in a laboratory table. Although daily observation was made for the mortality rate.

Adult mortality test

Two hundred fifty grams of maize and wheat grains each of local which variety were adjusted to 14% moisture content, Adult (male and female) weevil in the number of 20 were introduced in plastic jar of 15cm high * 8 cm diameter and kept for 24 hours. It was then mixed with different doses of powdered Bojho on the very next evening and kept it gently on the laboratory table. The plastic jars were air tightly covered. The adult mortality was monitored or observed daily at an interval of 24 hours. The percentage of adult mortality was determined by counting the number of dead insects divided by the total number of insects introduced multiplied by 100.

Weight loss test

Two hundred fifty grams of maize and wheat grains each of local which variety were adjusted to 14% moisture content, Adult (male and female) weevil in several 20 were introduced in plastic jar of 15cm high * 8 cm diameter and kept for 24 hours. It was then mixed with different doses of powdered Bojho on the very next evening and kept it gently on the laboratory table. The plastic jars were air tightly covered. The weight loss was measured by the weighing machine. At the first weight of each jar was also measured along with adult mortality tests in the interval of 24 hours. As forty (40) identical jars were used in the experiment, the weight of an empty jar was reduced from the weighing machine before weighing each jar. The combined weight of grains and Bojho doses were used in data analysis. Change in weight due to a change in some weevils was neglected. The loss of weight in every treatment was carried out in each interval and calculation was made out by calculating average weight loss in each treatment.

Treatments combination

Table 1: The treatment combination of the experiment

S. N	Name of grain	Dose per 250 g
1.	Maize	Control
2.	Maize	5 g
3.	Maize	7.5 g
4.	Maize	10 g
5.	Maize	12.5 g
6.	Wheat	Control
7.	Wheat	5 g
8.	Wheat	7.5 g
9.	Wheat	10 g
10.	Wheat	12.5 g

Layout and Randomization

Twenty newly emerged adults of *Sitophilus spp.* were introduced in each treatment. The experiment was conducted on the laboratory condition and the mortality rate of adult weevil and weight loss test was determined. The experiment was laid out into Complete Randomized Design (CRD) with five treatments each repeated five times in each crop variety.

Data Recordings

Daily data were recorded on the mortality rate of maize weevil at the interval of 24 hours for 7 days but 100% mortality was observed on the 5th day of the treatment on wheat and 6th day of the treatment on maize. Weight loss was recorded on the 7th day of treatment.

Analysis of data

The collected data were entered in an Excel sheet and analyzed with the help of R-package and SPSS was used for making graphs. The obtained results were interpreted with the help of table, figures and related literature

Results

The results show the effectiveness of different doses of *Acorus calamus* such as T1(5g/250g grains), T2 (7.5g/250g), T3 (10g/250g), T4 (12.5g/250g) and control against weevil (*Sitophilus spp.*) in wheat and maize respectively. Analysis of variation (ANOVA) revealed significant differences (P>0.05) in the mean percent mortality of weevil in both wheat and maize.

Table 2: Mortality rate (%) of wheat weevil

Treatments	1 DAS	2 DAS	3 DAS	4 DAS	5 DAS	6 DAS	Weight loss
T0 (0 g/250 gm grains)	0 c	0 e	0 e	0 e	0 e	0 e	13.46 a
T1 (5 g/250 gm grains)	2 c	9 d	21 d	35 d	53 d	63 d	8.66 b
T2 (7.5 g/250 gm grains)	6 b	18 c	34 c	53 c	73 c	82 c	5.82 c
T3 (10 g/250 gm grains)	8 b	25 b	45 b	66 b	86 b	89 b	1.52 d
T4 (12.5 g/250 gm grains)	12 a	33 a	56 a	82 a	100 a	100 a	0.08 e
Grand Mean (GM)	5.6	17	31.2	47.2	62.4	66.8	5.91
SEM (±)	5.5	21.5	35.5	54	56	9	1.19
Test of sig.	***	***	***	***	***	***	***
LSD $_{0.05}$	3.09	6.11	7.86	9.69	9.87	3.95	1.43
CV (%)	41.87	27.27	19.09	15.56	11.99	4.49	18.46

After one day of application of *Acorus calamus*, the highest mortality rate was observed from the concentration 12.5g *Acorus* powder (12%) followed by 10g *Acorus* powder (8%), 7.5g *Acorus* powder (6%), 5*g Acorus* powder (2%) whereas no mortality was observed in control. The mortality rate from 7.5g *Acorus* powder was statistically at par with 10g *Acorus* powder and the mortality rate from 5g *Acorus* powder was statistically at par with 0g *Acorus* powder. Similarly, in two DAS, the highest mortality rate was observed from the 12.5g *Acorus* powder (33%) followed by 10g *Acorus* powder (25%), 7.5g *Acorus* powder (18%), 5*g Acorus* powder (9%) whereas no mortality was observed in control. In three DAS, the highest mortality rate was observed from the 12.5g *Acorus* powder (56%) followed by 10g *Acorus* powder (45%), 7.5g *Acorus* powder (34%), 5*g Acorus* powder (21%) whereas no mortality was observed in control. In four DAS, the highest mortality rate was observed from the 12.5g *Acorus* powder (82%) followed by 10g *Acorus* powder (66%), 7.5g *Acorus* powder (53%), 5*g Acorus* powder (35%) whereas no mortality was observed in control. In five DAS, a 100% mortality rate was observed from the 12.5g *Acorus* powder followed by 10g *Acorus* powder (86%), 7.5g *Acorus* powder (73%), 5*g Acorus* powder (53%) whereas no mortality was observed in control. In six DAS, the mortality rate was observed as 89% from 10g *Acorus* powder, 34% from 7.5g *Acorus* powder, 21% from 5*g Acorus* powder and no mortality was observed in control.

Table 3: Mortality rate (%) of maize weevil

Treatments	1 DAS	2 DAS	3 DAS	4 DAS	5 DAS	6 DAS	Weight loss
Control	0 d	0 e	0 e	0 e	0 e	0 e	13.46 a
5 g Bojho powder	2 d	9 d	19 d	33 d	50 d	63 d	8.66 b
7.5 g Bojho powder/250 g	5 c	16 c	31 c	46 c	66 c	82 c	5.82 c
10 g Bojho powder/250 g	8 b	22 b	37 b	56 b	75 b	89 b	1.52 d
12.5 g Bojho powder/250 g	12 a	29 a	49 a	74 a	93 a	100 a	0.08 e
Grand Mean	5.4	15.2	27.2	41.8	56.8	66.8	5.944
SEM (±)	4.5	9.5	9.5	14	17	9	1.07
Test of sig.	***	***	***	***	***	***	***
LSD $_{0.05}$	2.79	4.07	4.07	4.93	5.44	3.96	1.36
CV (%)	39.28	20.27	11.33	8.95	7.25	4.49	17.40

After one day of application of *Acorus calamus*, the highest mortality rate was observed from the concentration 12.5g *Acorus* powder (12%) followed by 10g *Acorus* powder (8%), 7.5g *Acorus* powder (5%), 5*g Acorus* powder (2%) whereas no mortality was observed in control. The mortality rate from 5g *Acorus* powder was statistically at par with 0g *Acorus* powder. Similarly, in two DAS, the highest mortality rate was observed from the 12.5g *Acorus* powder (29%) followed by 10g *Acorus* powder (22%), 7.5g *Acorus* powder (16%), 5*g Acorus* powder (9%) whereas no mortality was observed in control. In three DAS, the highest mortality rate was observed from the 12.5g *Acorus* powder (49%) followed by 10g *Acorus* powder (37%), 7.5g *Acorus* powder (31%), 5*g Acorus* powder (19%) whereas no mortality was observed in control. In four DAS, the highest mortality rate was observed from the 12.5g *Acorus* powder (74%) followed by 10g *Acorus* powder (56%), 7.5g *Acorus* powder (46%), 5*g Acorus* powder (33%) whereas no mortality was observed in control. In five DAS, the highest mortality rate was observed from the 12.5g *Acorus* powder (93%) followed by 10g *Acorus* powder (75%), 7.5g *Acorus* powder (66%), 5*g Acorus* powder (50%) whereas no mortality was observed in control. In six DAS, a 100% mortality rate was observed from the 12.5g *Acorus* powder followed by 10g *Acorus* powder (89%), 7.5g *Acorus* powder (82%), 5*g Acorus* powder (63%) whereas no mortality was observed in control as in Table 2 and 3.

Discussions

The toxic action of *Acorus calamus* in both maize and wheat was found highly significant at the rate of 12.5g/250 grams of maize and wheat in comparison with other concentrations i.e. 10g/250 gram grain, 7.5g/250 gram grain, and 5g/250 gram grain. The increase in adult mortality increased with ascending exposure days for *Acorus calamus.* This result also supported by Tiwari *et al.*, (2018), Who found the toxicity effect of plant material on stored grain pest, *Acorus calamus* powder caused 100% mortality in weevil adults within 6 days after treatment [7]. The result also showed that *Acorus calamus* powder exhibited a very high level insecticidal and/or antifeedant properties resulting in a very high *Stilophilus spp.* Mortality. There is no doubt that many plant secondary metabolites are toxic to insects. Results from the present study indicated that there is great potential in using botanical plant extract as maize and wheat weevil protectants against *Stilophilus spp.* Different plant materials significantly influenced the final weight of maize. The final weight of maize grain treated with bojo was highest (8.18 kg) followed by that of maize treated with acetallic super (8.04kg) than that of maize treated with common salt (7.49kg), Timur (7.46kg) and aluminum phosphate (7.40kg). [8] G.C. (2006) also found Acorus calamus (50gm/kg) was most effective against maize weevil with the least damage for the nine months [8]. This result also follows the findings of Paneru and Thapa (2018), with the high efficacy of Acorus calamus rhizome dust against weevils with the lowest grain damage in the storehouse [9]. The insecticidal activity of *A. calamus* could be ascribed to the presence of its active constituents, such as sequestrine ketones, trans- or alphaasarone (2,4,5-trimethoxy-1-propenylbenzene) and beta-asarone (cis-isome) [10, 11]. Asian varieties of *A. calamus* contain varying amounts of beta-asarone and cause sedative effects when ingested, and also possess anti-gonadal activity against some insect species [12, 13]. Monoterpene hydrocarbons, also an active component of sweet flag, are competitive inhibitors of acetylcholinesterases, which are neurotoxic to insects and plant diseases like Rice Blast [14, 15]. A similar result was also proposed by Panthee (1997) and suggested that *A. calamus* treated maize seed produce lower weevil population compared with other treatments such as neem, Malabar nut tree, mugwort treated seeds [16, 17].

In the laboratory, research was conducted and the mortality rate of weevils, as well as weight loss to the application of various concentrations of Bojho, is observed. Four different concentrations of Acorus calamus i.e. 5g/250 g of grain, 7.5g/250 g of grain, 10g/250 g of grain, 12.5g/250 g of grain along with control is applied on plastic jars containing 20 weevils each and 250 g of wheat and maize respectively and repeated for five times. After the application of different concentrations of Bojho, no. of dead and alive weevils were counted daily at 24-hour interval until 100% mortality was observed.

Based on mortality of weevil by different concentrations of Bojho in wheat, 100% mortality rate was observed from the 12.5g *Acorus* powder followed by 10g *Acorus* powder (86%), 7.5g *Acorus* powder (73%), 5*g Acorus* powder (53%) and no mortality was observed in control after 5 days of treatment whereas, in maize, 100% mortality rate was observed from the 12.5g *Acorus* powder followed by 10g *Acorus* powder (89%), 7.5g *Acorus* powder (82%), 5*g Acorus* powder (63%) and no mortality was observed in control after 6 days of treatment.

Based on weight loss caused by weevil after different concentrations of Bojho in wheat, lowest weight loss was observed in the concentration of 12.5 g/250 g grain i.e 0.08% and highest weight loss was observed in control i.e 13.46% after six days of treatment whereas, in maize, lowest weight loss was observed in the concentration of 12.5 g/250 g grain i.e 0.08% and highest weight loss was observed in control i.e 13.46% after six days of treatment.

The result on preference study shows that the concentration of 12.5 g of Bojho powder per kg grain is the most effective dose than other concentrations i.e 10 g/250 g, 7.5 g/250 g and 5 g/250 g against *Sitophilus spp.*

Conclusion

This experiment concludes that 12.5 g Bojho powder per kg of grain seems to be most effective for weevil than other concentrations i.e. 5g/250 g of grain, 7.5g/250 g of grain, 10g/250 g of grain used in the experiment in storage condition. So farmers are recommended to treat maize with 12.5 g Bojho powder per kg of grain during storage than other concentrations used in the experiment. This result also proved that other concentrations are also significantly effective against *Sitophilus spp.* so special care should be given while storing maize and wheat grains. Researchers who are intended to research storage pest especially in *Sitophilus spp.* are recommended to find the most suitable storing structure for storing maize and wheat grain, not only this they should recommend the proper management of the *Sitophilus spp.*

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