



Efficacy of Sweet Flag (*Acorus calamus* L.) Rhizome Powder Against Maize Weevil (*Sitophilus zeamais* Motschulsky) in Stored Maize Under Laboratory Conditions in Rolpa, Nepal

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Abstract

Maize weevil, *Sitophilus zeamais* Motschulsky, is a destructive storage pest that reduces the quantity, quality, and seed value of stored maize in tropical and subtropical regions. Botanical materials offer a locally available alternative to synthetic storage insecticides, particularly for smallholder storage systems. A laboratory experiment was conducted in Liwang, Rolpa, Nepal, from April to June 2021 to evaluate the efficacy of sweet flag (Bojho; *Acorus calamus* L.) rhizome powder against maize weevil in stored maize grain. The experiment was arranged in a completely randomized design with five treatments and five replications. Five doses of *A. calamus* rhizome powder (2, 4, 6, 8, and 10 g per 600 g of maize grain) were tested. Mortality, grain damage, weight loss, exit holes, and weevil population were recorded and analyzed using analysis of variance; treatment means were separated using Duncan's multiple range test at the 5% probability level. *A. calamus* significantly affected all measured parameters. At 70 days after treatment, the 8 and 10 g treatments produced complete mortality, whereas mortality in the 2 g treatment was 43.04%. The lowest grain damage (0.18%), weight loss (0.01%), and number of exit holes (3.40) were recorded at 10 g per 600 g maize, while no live weevils were recorded in either the 8 or 10 g treatment at 70 days. These findings demonstrate strong relative dose performance under the tested room storage conditions. Because untreated and chemical standard controls were not included, the study does not establish absolute efficacy relative to no treatment or conventional control methods. Further controlled validation in farmer storage structures, across maize varieties, and at different grain moisture levels is recommended.

Keywords: *Acorus calamus*; Bojho; Botanical Insecticide; Maize Storage; Maize Weevil; *Sitophilus zeamais*

Introduction

Maize (*Zea mays* L.) is one of Nepal's major cereal crops and is important for food, feed and rural livelihoods. Recent national agricultural statistics show that maize occupied 940,256 ha with

production of 2,976,490 metric tonnes and average productivity of 3.17 t ha⁻¹ in 2022/23 [1]. Because a large proportion of maize is stored at household level for food, seed and feed, postharvest protection remains a practical requirement for maintaining food security and farmer income.

Stored maize is attacked by several insect pests, among which the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), is one of the most damaging. The female deposits eggs inside kernels, where the larvae feed and complete their development; emerged adults continue to damage grain. Infestation causes direct grain loss, exit holes, powdering, reduced seed viability, and deterioration of grain quality [2]. Warm storage conditions can accelerate development and contribute to rapid population growth during prolonged storage [2].

Management of stored-grain insects has often depended on fumigants and contact insecticides, including phosphine and malathion. Although these chemicals can be effective, inappropriate use raises concerns about human safety, residues, insecticide resistance, environmental contamination, and safe application in smallholder stores [3,4]. These limitations have increased interest in plant-based materials as locally adaptable alternatives for stored-grain protection.

Botanical insecticides contain diverse secondary metabolites that may act as toxicants, repellents, antifeedants, oviposition deterrents, or insect growth disruptors [4]. Sweet flag (Bojho; *Acorus calamus* L.) has demonstrated insecticidal activity against stored-product insects, including *S. zeamais* [5-7]. Its rhizomes contain asarone-type compounds, including beta-asarone and related phenylpropanoids, that have been associated with toxicity and repellency [7].

Despite these promising findings, practical dose-based evidence using simple rhizome powder under local storage conditions is still useful for farmers who may not have access to extracted oils or commercial botanical formulations. Rolpa district, where household storage of maize is common, has limited locally generated evidence on botanical protection of stored maize. Therefore, this study evaluated different doses of Bojho (*A. calamus*) rhizome powder against maize weevil under laboratory storage conditions in Rolpa, Nepal. We hypothesized that increasing doses of *A. calamus* rhizome powder would progressively reduce maize weevil survival, reproduction, and grain damage, with the highest doses providing complete population suppression by 70 days after treatment.

Materials and Methods

Study location and experimental period

The experiment was conducted under ambient room storage conditions at Liwang, Rolpa Municipality, Rolpa, Nepal, from April to June 2021. Temperature and relative humidity were neither actively controlled nor continuously recorded because of logistical and resource limitations. Consequently, the influence of environmental variation on insect development and treatment performance could not be quantified.

Preparation of *Acorus calamus* rhizome powder

Rhizomes of sweet flag were collected locally, washed with clean running water and shade-dried at room temperature for seven days. The dried rhizomes were ground using a domestic grinder and sieved to obtain a relatively uniform powder. The powder was stored in sealed plastic containers at room temperature until use.

Collection and rearing of maize weevil

Adult maize weevils were collected from infested maize grains obtained from farmer storehouses and reared in plastic jars containing maize grain. Jar openings were covered with muslin cloth to prevent insect escape while allowing aeration. After seven days of oviposition, the parental adults were removed and the grain medium was maintained until new adults emerged. Newly emerged, unsexed adults from this culture were used for the experiment; sex was not determined, so the sex ratio was neither standardized nor recorded. The available study records do not report the exact number of adults initially introduced into each experimental jar or whether dead adults were removed after each observation. These details could not be reconstructed and are acknowledged as limitations of the surviving experimental record.

Maize grain preparation

Clean and apparently uninfested grains of Manakamana-3 maize variety were collected from local farmers. The grains were cleaned and sun-dried before use. Each experimental unit consisted of 600 g maize grains placed in a plastic jar covered with muslin cloth and secured with a rubber band.

Experimental design and treatments

The experiment was arranged in a completely randomized design with five treatments and five replications, resulting in

25 experimental units. Treatments consisted of five doses of *A. calamus* rhizome powder applied to 600 g of maize grain: 2, 4, 6, 8, and 10 g per jar. The treatments were coded T1, T2, T3, T4, and T5, respectively (Table 1). Each weighed dose was added directly

to the maize in its assigned jar and mixed through the grain before the jar was covered with muslin cloth. The original record did not document mixing duration or the use of a standardized mechanical mixing procedure.

Treatment code	Dose of Bojho powder	Maize grain per jar	Approximate dose per kg grain
T1	2 g	600 g	3.33 g kg ⁻¹
T2	4 g	600 g	6.67 g kg ⁻¹
T3	6 g	600 g	10.00 g kg ⁻¹
T4	8 g	600 g	13.33 g kg ⁻¹
T5	10 g	600 g	16.67 g kg ⁻¹

Table 1: Treatment details used to evaluate *Acorus calamus* rhizome powder against maize weevil.

Data collection

Data were recorded for maize weevil mortality, grain damage, weight loss, number of exit holes and weevil population. Mortality was assessed at 10, 25, 40, 55 and 70 days after treatment (DAT). Grain damage and weight loss were assessed at 70 DAT using a 100 g grain sample from each jar. Exit holes and weevil population were recorded at 25, 40, 55 and 70 DAT.

Calculations

Maize weevil mortality (%) was calculated as: mortality = (number of dead insects/total number of insects) x 100. Grain damage (%) was calculated as: (number of damaged grains/total number of grains) x 100. Grain weight loss (%) was calculated using the count-and-weight method: $W(\%) = [(W_u \times N_d) - (W_d \times N_u)] / [W_u \times (N_d + N_u)] \times 100$, where W_u is the weight of undamaged grains, N_u is the number of undamaged grains, W_d is the weight of damaged grains, and N_d is the number of damaged grains.

Statistical analysis

The recorded data were tabulated in Microsoft Excel and analyzed using R software (version 4.0.2). One-way analysis of variance (ANOVA) was used to test treatment effects, and treatment means were separated using Duncan's multiple range test (DMRT) at the 5% probability level. Weevil population counts, which included zero observations, were square-root transformed using $\sqrt{x + 0.5}$ before ANOVA and DMRT. This transformation was used to reduce variance heterogeneity associated with nonnegative count data and zeros. Statistical tests and grouping

letters for weevil population were based on the transformed scale, whereas untransformed means are presented for biological interpretation. Other variables were analyzed on the reported scale. Statistical significance was reported as $p < 0.05$ or $p < 0.001$, as appropriate. Residual plots were inspected to verify model assumptions, and variance heterogeneity was addressed through the data transformation described above.

Results

Mortality of maize weevil

Different doses of *A. calamus* rhizome powder significantly influenced maize weevil mortality at all observation dates ($p < 0.001$). Mortality increased with dose and exposure period (Table 2; Figure 1). At 10 DAT, the highest mortality was recorded in the 10 g treatment (92%), followed by 8 g (80%), whereas the 2 g treatment produced only 20% mortality. By 25 DAT, both 8 and 10 g treatments produced complete mortality and maintained 100% mortality until 70 DAT. At 70 DAT, mortality in 2, 4 and 6 g treatments was 43.04, 57.41 and 73.05%, respectively, showing a clear dose-response pattern.

Grain damage and weight loss

Grain damage and weight loss differed significantly among the treatments at 70 DAT ($p < 0.001$). The highest grain damage was recorded in the 2 g treatment (2.78%), followed by 4 g (2.11%) and 6 g (1.18%). In contrast, 8 and 10 g treatments strongly reduced grain damage to 0.21 and 0.18%, respectively. A similar trend was

Treatment	10 DAT	25 DAT	40 DAT	55 DAT	70 DAT
2 g	20.00 ^d	33.02 ^d	35.61 ^d	36.38 ^d	43.04 ^d
4 g	36.00 ^c	45.34 ^c	46.29 ^c	52.31 ^c	57.41 ^c
6 g	56.00 ^b	57.98 ^b	64.40 ^b	67.70 ^b	73.05 ^b
8 g	80.00 ^a	100.00 ^a	100.00 ^a	100.00 ^a	100.00 ^a
10 g	92.00 ^a	100.00 ^a	100.00 ^a	100.00 ^a	100.00 ^a
LSD (0.05)	12.93	4.36	5.44	3.80	4.07
SEM (+/-)	4.38	1.48	1.84	1.29	1.38
CV (%)	17.25	4.91	5.95	4.04	4.13
F-test	***	***	***	***	***

Table 2: Effect of different doses of *Acorus calamus* rhizome powder on maize weevil mortality (%) in stored maize grains.

Note: DAT = days after treatment; SEM = standard error of the mean; CV = coefficient of variation; LSD = least significant difference; ***p < 0.001; **p < 0.01; *p < 0.05. Means within a column followed by different superscript letters differ significantly at the 5% probability level by Duncan's multiple range test (DMRT).

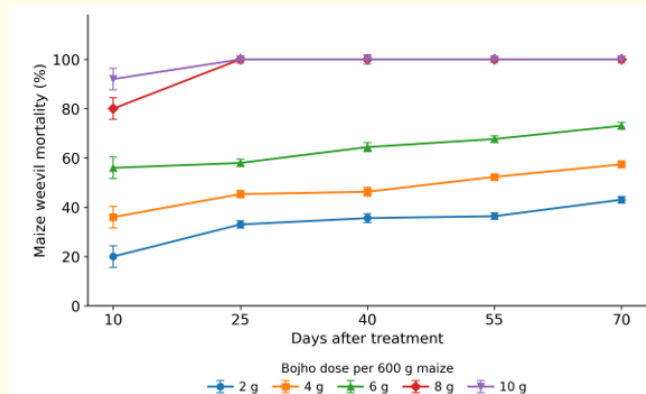


Figure 1: Mortality trend of maize weevil under different doses of *Acorus calamus* rhizome powder. Error bars represent plus or minus one pooled standard error of the mean, calculated as the square root of (error mean square/number of replications) from the analysis of variance at each observation date (r = 5).

observed for weight loss. The 2 g treatment caused 0.87% weight loss, whereas the 10 g treatment limited weight loss to 0.01% (Table 3; Figure 2).

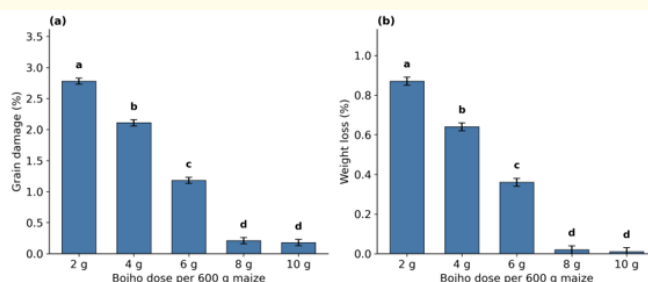


Figure 2: (a) Grain damage and (b) weight loss at 70 days after treatment under different doses of *Acorus calamus* rhizome powder. Error bars represent plus or minus one pooled standard error of the mean. Bars labelled with different letters differ significantly at the 5% probability level by Duncan's multiple range test.

Treatment	Grain damage (%)	Weight loss (%)
2 g	2.78 ^a	0.87 ^a
4 g	2.11 ^b	0.64 ^b
6 g	1.18 ^c	0.36 ^c
8 g	0.21 ^d	0.02 ^d
10 g	0.18 ^d	0.01 ^d

LSD (0.05)	0.14	0.05
SEM (+/-)	0.05	0.02
CV (%)	7.92	9.11
F-test	***	***

Table 3: Grain damage and weight loss caused by maize weevil at 70 DAT under different doses of *Acorus calamus* rhizome powder.

Note: DAT = days after treatment; SEM = standard error of the mean; CV = coefficient of variation; LSD = least significant difference; ***p < 0.001; **p < 0.01; *p < 0.05. Means within a column followed by different superscript letters differ significantly at the 5% probability level by Duncan's multiple range test (DMRT).

Exit holes and weevil population

The number of exit holes was significantly affected by dose at all observation dates. At 70 DAT, the 2 g treatment had the highest number of exit holes (52.20), followed by 4 g (39.60) and 6 g (22.20). The 8 and 10 g treatments had only 4.40 and 3.40

exit holes, respectively (Table 4). Weevil population also declined sharply as the dose increased. At 70 DAT, 2 g, 4 g and 6 g treatments supported 19.80, 13.00 and 9.60 weevils, respectively, whereas no live weevils were recorded in 8 and 10 g treatments (Table 5).

Treatment	25 DAT	40 DAT	55 DAT	70 DAT
2 g	13.20 ^a	15.20 ^a	18.00 ^a	52.20 ^a
4 g	12.20 ^a	10.40 ^b	13.00 ^b	39.60 ^b
6 g	8.20 ^b	7.80 ^c	7.80 ^c	22.20 ^c
8 g	2.60 ^c	3.00 ^d	2.60 ^d	4.40 ^d
10 g	2.60 ^c	1.60 ^d	1.60 ^d	3.40 ^d
LSD (0.05)	1.64	2.04	2.45	2.40
SEM (+/-)	0.55	0.69	0.83	0.84
CV (%)	16.00	20.38	21.63	7.75
F-test	***	***	***	***

Table 4: Exit holes caused by maize weevil under different doses of *Acorus calamus* rhizome powder.

Note: Values are numbers of exit holes per 100 g grain sample. DAT = days after treatment; SEM = standard error of the mean; CV = coefficient of variation; LSD = least significant difference; ***p < 0.001; **p < 0.01; *p < 0.05. Means within a column followed by different superscript letters differ significantly at the 5% probability level by Duncan's multiple range test (DMRT).

Treatment	25 DAT	40 DAT	55 DAT	70 DAT
2 g	34.80 ^a	31.80 ^a	29.00 ^a	19.80 ^a
4 g	21.00 ^b	21.40 ^b	13.80 ^b	13.00 ^b
6 g	12.00 ^c	10.00 ^c	11.20 ^c	9.60 ^c
8 g	0.00 ^d	0.00 ^d	0.00 ^d	0.00 ^d
10 g	0.00 ^d	0.00 ^d	0.00 ^d	0.00 ^d
LSD (0.05)	0.49	0.31	0.43	0.40

SEM (+/-)	0.16	0.10	0.15	0.14
CV (%)	11.91	7.70	11.63	11.89
F-test	***	***	***	***

Table 5: Number of maize weevils recorded under different doses of *Acorus calamus* rhizome powder. Note: Statistical separation was based on square-root-transformed [$\sqrt{x + 0.5}$] values; untransformed means are presented for biological interpretation, and DMRT letters refer to the transformed analysis. DAT = days after treatment; SEM = standard error of the mean; CV = coefficient of variation; LSD = least significant difference; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. Means within a column followed by different superscript letters differ significantly at the 5% probability level by Duncan's multiple range test (DMRT).

Treatment effects were large in magnitude across all measured parameters. Partial eta-squared values computed from the treatment and error sums of squares ranged from 0.90 to 0.99, indicating that dose accounted for more than 90% of the total variance in mortality, grain damage, weight loss, exit holes, and weevil population at every observation date.

Discussion

The results demonstrated a strong dose-dependent effect of *A. calamus* rhizome powder against maize weevil in stored maize. Higher doses, particularly 8 and 10 g per 600 g maize (approximately 13.3 and 16.7 g kg⁻¹), caused rapid mortality and prevented later population development. The difference between lower and higher doses became more evident over storage time, indicating that sufficient powder coverage and prolonged exposure are important for suppressing adult survival, oviposition, and subsequent emergence. These comparisons describe relative performance among the five doses because the experiment did not include untreated or chemical standard controls.

The 8 and 10 g treatments produced 100% mortality by 25 days after treatment. In a Nepalese laboratory study that included both untreated and chemical standard controls, Mallah., *et al.* [5] reported 80.56% mortality at 30 days with Bojho powder at 5% (w/w), complete mortality by 60 days, and complete mortality with Celphos by 30 days. Rimal., *et al.* [6] reported 100% maize-weevil mortality by six days at their highest dose of 12.5 g per 250 g grain, compared with no mortality in the untreated control. Those studies used substantially higher powder concentrations, different adult numbers, shorter or longer observation schedules, and controlled comparison groups; therefore, their numerical results are useful benchmarks but are not directly interchangeable with the present dose-response results.

At 70 days, grain damage declined from 2.78% at 2 g to 0.18% at 10 g, while weight loss declined from 0.87% to 0.01%. Neupane., *et al.* [8], using 5 g *A. calamus* powder per kg grain with an untreated control under 28.5 ± 2°C and 72 ± 5% relative humidity, reported 1.23% grain damage with *A. calamus* compared with 18.0% in the control after 120 days. Mallah., *et al.* [5] reported a final mean weight loss of 1.19% with Bojho at 5% (w/w). Differences among studies may reflect dose, storage duration, grain moisture, environmental conditions, insect density, and mixing or assessment procedures. In the present study, reduced adult survival probably limited oviposition and larval establishment, thereby reducing later emergence, exit holes, grain damage, and weight loss.

The biological activity of *A. calamus* has often been linked to asarone-type compounds. Aryal., *et al.* [7] reported that Nepalese *A. calamus* essential oil had median lethal concentrations of 2.77% and 0.23% on oil-treated maize at 10 and 16 days, respectively, and 98.75% repellency at 10% concentration after 24 hours. Essential oil and rhizome powder differ substantially in active-compound concentration and exposure route, so these values support biological plausibility rather than a direct efficacy comparison. The present study did not analyze chemical composition or mode of action; therefore, the proposed contact, fumigant, or repellent mechanisms are supported by previous literature rather than demonstrated directly.

The insecticidal activity of *A. calamus* is primarily attributed to beta-asarone and related phenylpropanoids. However, beta-asarone content varies substantially among chemotypes and geographic origins; rhizome oil from the lower Himalayan region, for example, has been reported to contain beta-asarone as the dominant constituent (83.2%) alongside alpha-asarone (9.7%) [9]. In the present study the chemical composition of the rhizome

powder was not analysed, so the active-compound profile of the locally sourced material remains unknown. Future work should characterise the essential oil composition of Nepalese accessions so that biological activity can be standardised against a documented chemotype.

From a practical perspective, the study identifies 8-10 g *A. calamus* powder per 600 g maize as the best-performing range among the tested doses. However, the study did not assess seed germination or viability, sensory properties such as odor and taste, nutritional or market quality, grain moisture and mould development, or chemical residues. These omissions are important because a treatment that suppresses insects may still affect seed use, food acceptability, or safety. Aryal, *et al.* [7] also emphasized the need for residue assessment before *A. calamus*-treated maize is recommended for food or feed. Accordingly, the present results support further controlled evaluation but not an unconditional farmer-level recommendation.

From an applied standpoint, the 8-10 g per 600 g maize range (approximately 13.3-16.7 g kg⁻¹) was the most effective under the tested conditions. For a typical 50 kg farmer storage bag this corresponds to roughly 666-833 g of dried *A. calamus* rhizome powder. Given the local availability and low cost of Bojho in Nepal, application at this rate would be logistically and economically plausible for smallholder adoption, provided that the seed-quality, sensory, and residue questions noted above are first resolved.

An important limitation is the absence of both an untreated control and a positive chemical control. Without an untreated control, natural mortality, handling effects, and background changes in damage or weight loss cannot be separated from treatment effects, and corrected mortality cannot be calculated. Without a chemical standard, efficacy cannot be benchmarked directly against conventional protection. The experiment therefore establishes a relative ranking of *A. calamus* doses rather than absolute protection or equivalence to an established control method. Interpretation is further limited by the absence of continuous temperature and relative-humidity records, the unrecoverable initial adult number and dead-insect removal procedure, and the lack of a documented standardized mixing duration. Future studies should include both controls, predefined adult density and sex handling, environmental monitoring, grain-moisture measurement, standardized mixing, germination and quality tests, and validation under farmer storage conditions.

These findings contribute to the growing body of evidence supporting botanicals as sustainable alternatives for stored-grain protection, particularly in settings where access to commercial insecticides is limited or unsafe to manage at household level. By providing dose-response data for a simple, unextracted rhizome powder under ambient storage conditions, the study helps bridge controlled laboratory efficacy trials and farmer-accessible interventions.

Conclusion

Sweet flag or Bojho (*Acorus calamus* L.) rhizome powder produced a clear dose-dependent response against maize weevil under the tested room storage conditions in Rolpa, Nepal. Among the five doses, 8 and 10 g per 600 g maize performed best, producing complete mortality by 25 days and the lowest grain damage, weight loss, exit hole counts, and live-weevil populations by 70 days. These findings identify the higher doses as promising candidates for further evaluation; however, the absence of untreated and chemical standard controls prevents conclusions about absolute efficacy or equivalence to conventional control methods. Recommendations for seed, food, or feed use should await controlled studies that document initial insect density, environmental conditions, grain moisture, standardized mixing, germination and grain quality, sensory acceptability, and residue safety under both laboratory and farmer-storage conditions. Nevertheless, field validation under actual storage conditions, including comparison with standard storage protectants and assessment of grain quality parameters, remains necessary before large-scale adoption can be recommended.

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Ethics Statement

Ethical approval was not required for this study, as it involved stored-product insects (*Sitophilus zeamais*) and did not include vertebrate animals, human participants, or endangered species.

Conflict of Interest

The authors declare that they have no conflicts of interest related to the conduct of this study or publication of its findings.

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Author Contributions

Sunil Sigdel conceptualized the study, conducted the experiment, collected and analyzed the data, and prepared the original manuscript. Astha Dhakal and Sandeep Lamichhane reviewed and edited the manuscript. All authors read and approved the final manuscript.

Data Availability

The summarized data supporting the findings are included in the tables and figures of this article. The original raw experimental data are no longer available, as the study was conducted in 2021 and only summarized records and statistical outputs were retained. Requests for additional information should be directed to the corresponding author.

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