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RESEARCH ARTICLE

# Evaluation of sweet flag (*Acorus calamus* L.) rhizome for its chemical properties and their effect on growth and seed longevity in field bean (*Lablab purpureus* L.)

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## Abstract

Sweet flag (*Acorus calamus* L.), known for its pesticidal and seed-quality–enhancing properties due to its essential oil richness. The study quantified sweet flag rhizome compounds and assessed their effectiveness in improving field bean (var. HA-5) under field and lab conditions. Essential oil was extracted by hydro-distillation and quantified using gas chromatography. A foliar spray experiment was conducted using a randomized complete block design to assess the effects of sweet flag rhizome powder, rhizome oil, and a chemical check on crop growth, pest and disease incidence. A nine-month storage study was conducted under laboratory conditions to evaluate seed storability. The essential oil yield aveaged 0.29 % and was dominated by β-asarone. Results indicated that moderate concentrations of sweet flag rhizome powder and oil significantly improved seed emergence, plant growth, and plant stand, while also reducing pest and disease incidence. Seed treatment with rhizome powder at 10 g/kg consistently maintained higher seed quality during storage. Sweet flag rhizome–based treatments demonstrated strong potential as eco-friendly options for enhancing field performance and seed storability.

**Keywords:** β-asarone; essential oil; field bean; foliar spray; seed storage; sweet flag

## Introduction

Pulses play a crucial role in sustainable agriculture by providing plant-based protein and improving soil fertility through biological nitrogen fixation, and field bean (*Lablab purpureus* L.), a widely cultivated and multipurpose legume in India, is particularly suitable for evaluating eco-friendly botanical interventions (1). Field bean (*Lablab purpureus* L.) is among the oldest cultivated vegetable legumes, grown widely across India, Africa and Southeast Asia. Originating in India (2,3), it is valued as a multipurpose crop used as a pulse, vegetable, green manure, fodder and cover crop for soil conservation. Its adaptability to diverse agro-ecologies and its role in improving soil structure and nitrogen status make it an important crop for small and marginal farmers in peninsular India.

India is the world's largest producer of pulses, contributing around 25% of global production and 32% of the total cultivated area (4). Karnataka is a major pulse-growing state and accounts for nearly 80% of the national field bean area, cultivating 0.52 lakh ha with a production of 0.28 lakh tonnes (5). Nutritionally, field bean is notable for its high protein content (18.3–31.6%) and balanced amino acid profile, including lysine (6.1%), along with essential minerals and vitamins (6). These attributes highlight its importance in vegetarian diets and rural nutrition. Seed quality is the most critical input for successful crop establishment. In pulses, storage losses of 30–40% occur due to bruchid infestation, fungal contamination and biochemical deterioration under ambient storage (7). Field bean (*Lablab purpureus* L.) is particularly vulnerable to storage pests and fungi due to its relatively soft seed coat, high protein content and the practice of prolonged on-farm storage under warm and humid conditions, which favour rapid bruchid multiplication and fungal colonization compared to several other pulse crops. Low-cost, eco-friendly technologies are therefore required to maintain seed viability and vigour during storage, particularly in rainfed regions where seed replacement rates are low.

Botanicals, especially sweet flag (*Acorus calamus* L.) rhizome, have gained attention as natural seed protectants. Sweet flag is a monocotyledonous perennial with aromatic rhizomes rich in essential oils such as β-asarone, α-asarone, methyl eugenol and camphor (8). These bioactive constituents exert fumigant and contact toxicity against storage insects, disrupt cellular membranes, and inhibit fungal growth through phenylpropanoid-mediated antimicrobial activity, thereby reducing seed infestation and deterioration (9). Accordingly, emphasis has been placed on the agricultural and seed-protective relevance of sweet flag rather than its medicinal applications (10). Recent agricultural research indicates that sweet flag powders and oils can protect stored seeds, reduce infestation and enhance germination and seedling vigour in several pulse crops. Integrating this traditional knowledge with scientific evaluation provides opportunities to develop cost-effective, eco-safe seed enhancement technologies for smallholders in South India, where access to synthetic chemicals is limited. Considering the extensive cultivation of field bean in Karnataka under both rainfed and irrigated systems and the increasing interest in botanical-based seed enhancement, there is a strong need to scientifically evaluate sweet flag rhizome for improving crop performance and seed storability. Despite its traditional use and reported bioactivity, empirical evidence on concentration-dependent effects of sweet flag on field bean performance and storability remains limited. The objectives are quantifying the chemical profile of sweet flag rhizome essential oil, evaluate the influence of foliar-applied rhizome powder and oil on growth and pest incidence in field bean and assess the effect of rhizome-based seed treatments on storability and physiological quality.

## Materials and Methods

Dried sweet flag (*Acorus calamus* L.) rhizomes were procured from local market and botanically authenticated prior to extraction. Essential oil extraction and quantification were carried out at CSIR–CIMAP, Allalasandra, Yelahanka, Bengaluru, located in the Eastern Dry Zone (Zone–5) of Karnataka, during June–September 2024. The experiment was conducted in a completely randomized design (CRD) with one sample of rhizome and three replications, using 500 g of rhizomes per replication.

### Essential oil extraction (Hydro-distillation)

Dried rhizomes were chopped uniformly into small pieces to ensure consistent particle size, and subjected to hydro-distillation. Essential oil was extracted using a Clevenger apparatus connected to a 5000 mL round-bottom flask. A 1:5 w/v ratio (500 g rhizome: 2500 mL distilled water) was maintained. Distillation was carried out for 240 minutes after the onset of boiling. The extracted oil was dried using anhydrous sodium sulfate ($Na_2SO_4$) prior to chemical analysis (Fig.S1.)

### Gas chromatographic analysis

GC analysis was performed using an Agilent 7890B Gas Chromatograph with a DB-5 capillary column. A 1.0 µL aliquot of essential oil was injected in split mode (1:35). The oven program began at 60°C, increased at 3°C/min, and held at 240°C for 2 min. Hydrogen served as the carrier gas at 1 mL/min. The split ratio was 1:35. Injector and FID detector temperatures were 250°C and 280°C, respectively. Quantitative analysis was carried out using GC-FID, while compound identification was confirmed by GC–MS based on mass spectral matching and retention indices. Essential oil constituents were identified based on retention time, elution order, and Relative Retention Index (RRI), calculated using a homologous series of n-alkanes ranging from $C_8$ to $C_{25}$ under identical operating conditions. Six major compounds were identified: β-asarone, α-asarone, Methyl isoeugenol-Z, Methyl isoeugenol-E, Methyleugenol, Camphor. Chromatograms generated by the GC system were used for identifying and quantifying compounds. Peak areas were used to calculate the relative percentage composition of each essential oil component (CIMAP) (Fig.S2.). The relative composition of individual constituents was expressed as percentage peak area contribution with respect to the total chromatographic area.

Geographically, the experimental sites were located in the Eastern Dry Zone (Zone-5) of Karnataka. The field experiment was conducted during *rabi* 2024–25 (December 2024–April 2025) at the Research Institute on Organic Farming (RIOF), University of Agricultural Sciences, GKVK, Bengaluru (13°05′ N, 77°34′ E; 930 m MSL). The study was laid out in a randomized complete block design (RCBD) with eight treatments and three replications, using the field bean variety Hebbal Avare-5 (HA-5). The soil was red sandy clay loam (Alfisols), neutral in reaction (pH 6.42), medium in available nitrogen (291.2 kg $ha^{-1}$), phosphorus ($P_2O_5$, 26.24 kg $ha^{-1}$), potassium ($K_2O$, 228.3 kg $ha^{-1}$) and organic carbon content (0.64%). Seasonal environmental conditions such as temperature and relative humidity remained within the optimum range for field bean growth.

### Crop Management

Foliar treatments were imposed at vegetative (30–35 DAS) and anthesis (45–50 DAS) stages (Fig.1.): $T_1$ (control), $T_2$–$T_4$ (sweet flag rhizome powder at 5, 10, 15 g $L^{-1}$), $T_5$–$T_7$ (sweet flag rhizome oil at 2.5, 5, 7.5 mL $L^{-1}$) and $T_8$ (spinosad at 0.04 mL $L^{-1}$). Spray solutions were prepared and applied uniformly using a knapsack sprayer. The field was ploughed, harrowed and leveled, FYM was applied and seeds were sown in late December 2024 at 60 × 30 cm spacing in 3.0 × 3.0 m gross plots (2.4 × 1.8 m net). Irrigation was provided at pre-sowing, pre-flowering and pod development stages; thinning (14 DAS), weeding, inter-cultivation and neem oil spray (5 mL $L^{-1}$) were carried out as needed. Harvesting was completed in 3–5 pickings when pods turned yellow, after which tagged plants were recorded, pods dried, threshed and seeds shade-dried to 9% moisture.


**Fig. 1.** General view of experiment and foliar spray application (vegetative and anthesis stage) with sweet flag rhizome powder, oil and spinosad in field bean crop.

## Sampling and data collection

The experimental data were collected by randomly selecting and tagging five plants from the net plot area of each treatment and replication. These plants were used to record crop growth, physiological, seed yield, and seed quality parameters, and the mean value was computed for all biometric observations. Field emergence was assessed on the 10th day after sowing by counting the number of seedlings that emerged in each plot, and the percentage was calculated using the formula:

Field emergence (%) = (Number of seeds emerged / Total number of seeds sown) × 100.

Plant stand was recorded on the 20th day after sowing by counting all healthy plants established in each plot. Plant height was measured at 30 and 60 days after sowing and at maturity by measuring the tagged plants from ground level to the tip of the main shoot, and the average height was expressed in centimeters. The number of branches per plant was counted at maturity from the five selected plants, and the mean value was recorded. Days to 50% flowering were noted when half of the plants in each plot produced at least one fully opened flower. Days to maturity were recorded when about 75–80% of the pods turned yellowish or dry, indicating physiological maturity.

Pest infestation was recorded from 30 DAS to harvest by counting plants showing visible pest presence, and the percentage was calculated using the formula:

Pest infestation (%) = (Number of infected plants / Total number of plants observed) × 100.

Disease incidence was also assessed from 30 DAS to harvest by identifying plants showing symptoms of fungal, bacterial, or viral infections. The percentage was calculated using:

Disease incidence (%) = (Number of diseased plants / Total number of plants observed) × 100.

Pod damage was recorded at harvest by selecting ten plants per plot and counting the total number of pods along with the number of damaged pods. The percentage was computed using:

Pod damage (%) = (Total number of damaged pods / Total number of pods per plant) × 100.

## Storage study design

Storage studies were conducted in the Department of Seed Science and Technology, UAS, GKVK, Bengaluru, under controlled laboratory conditions following a Completely Randomized Design (CRD) with eight treatments and three replications (Fig.S3.). Field bean seeds (variety HA-5: Hebbal Avare-5) were treated with sweet flag rhizome powder, sweet flag rhizome oil and Spinosad and stored in cotton cloth bags under ambient laboratory conditions maintained at 19–29 °C with relative humidity ranging from 60–85% for a total duration of nine months with trimonthly observations. Treatment details were as defined earlier and all treated seeds were evaluated periodically under CRD for seed quality parameters.

## Seed quality parameters and assessment methods

Observations on seed quality parameters were recorded at three-month intervals from each treatment and replication during the nine-month storage study. Seed moisture content (%) was determined using the high-constant-temperature oven method as per ISTA. For each treatment, 5 g of seeds from three replications were dried in aluminium boxes at 130 ± 2°C for 1 hr, cooled in desiccators for 30 min and the moisture percentage was calculated on a dry-weight basis using the formula:

$$\text{Seed moisture content (\%)} = \frac{M_2 - M_3}{M_2 - M_1} \times 100$$

Where

$M_1$= Weight of the empty container with lid

$M_2$= Weight of the container with lid and seeds before drying

$M_3$ = Weight of the container with lid and seeds after drying

Seed germination (%) was evaluated according to ISTA (2021) using 50 seeds per replication placed on moist paper towels and incubated at 25 ± 1°C and 90% RH, with first and final counts taken on the 4th and 10th day, respectively, and expressed as normal seedling percentage.

Seed infection (%) was assessed by placing 50 seeds from each treatment in three replications on moist paper towels arranged in the between-paper method and incubated in a germination chamber at 25 ± 1°C and 90% RH.

Seed infection was recorded on the 10th day, and the percentage was calculated as:

Seed infection (%) = (Number of infected seeds / Total number of seeds tested) × 100.

Seedling length (cm) was measured on the 10th day by randomly selecting ten normal seedlings per replication and recording the total length from the tip of the primary root to the tip of the apical shoot. Seedling dry weight (mg) was obtained by drying the same ten seedlings at 80°C for 24 hr, cooling in a desiccator for 45 min and weighing on an electronic balance.

SVI-I = Germination (%) × Seedling length (cm), SVI-II = Germination (%) × Seedling dry weight (mg), both expressed as whole numbers.

## Results and Discussion

### Essential oil yield from sweet flag Rhizomes

The extraction of essential oil from dried sweet flag rhizomes resulted in an oil yield of 1.43 mL from 500 g of rhizome material, corresponding to 0.29% (v/w). The observed yield value are presented as recorded measurements and no causal interpretation is included in this section (Table 1).

#### Chemical composition of essential oil

The chemical composition of sweet flag essential oil is presented in Table 1. β-asarone accounted for the highest proportion of the oil (85.21%), followed by methyl isoeugenol -Z (3.55%) and α-asarone (1.63%), while methyl isoeugenol-E (0.21%), methyleugenol (0.09%) and camphor (0.08%) were present in minor proportions. All constituents were quantified based on relative peak area percentages (Table 1 and Fig.2.)

**Table 1.** Quantification of sweet flag rhizome for its chemical properties

| Essential oils | Mean (%) |
|---|---|
| Beta-asarone | 85.21 |
| Alpha-asarone | 1.63 |
| Methyl isoeugenol-Z | 3.55 |
| Methyl isoeugenol-E | 0.21 |
| Methyleugenol | 0.09 |
| Camphor | 0.08 |
| Mean | 15.13 |

#### Growth performance

Foliar application of sweet flag rhizome powder and oil influenced emergence, plant stand and vegetative growth parameters of field bean as presented in Table 2 and Fig.3. Field emergence differed significantly among treatments, with sweet flag rhizome powder at 10 g $L^{-1}$ recording 88.0% emergence, which was significantly higher than the control (81.9%) as per the CD values (Table 2). A similar trend was observed for plant stand, where the same treatment recorded 84.9%, showing a significant increase over the untreated control. Plant height varied significantly across treatments at 30 DAS, 60 DAS and at maturity, with foliar application of sweet flag rhizome oil at 5 mL $L^{-1}$ consistently recording the highest values at all stages, as indicated by significant CD values in Table 2. Number of branches per plant also differed significantly, with the maximum branching (5.49 branches $plant^{-1}$) observed under sweet flag rhizome oil at 2.5 mL $L^{-1}$ compared to the control. Phenological parameters showed significant variation among treatments, with earlier flowering (44.5 days) and maturity (95.7 days) recorded in sweet flag–treated plots, whereas the control exhibited delayed flowering and maturity as per the significance levels reported in Table 2.

#### Pest and disease incidence

Significant reductions in pest infestation, disease incidence and pod damage were recorded under sweet flag rhizome–based foliar sprays when compared to the untreated control (Table 3. And Fig.4.). Among the treatments, sweet flag oil at 2.5 ml $L^{-1}$ ($T_5$) recorded the lowest pest infestation (3.43%) and lowest disease incidence (1.32%), indicating strong protective action against insect and pathogen attack. Sweet flag oil applied at 5 ml $L^{-1}$ ($T_6$) resulted in the least pod damage (15.4%) over the control.

### Seed quality performance

Initial seed quality parameters were recorded before treatments were applied, as shown in Table 4.

#### Moisture content

Seed moisture content varied significantly among treatments during storage (Table 5). Sweet flag rhizome powder at 10 g $kg^{-1}$ maintained lower moisture content at both observation points, recording 8.68% at 0 MAS and 11.65% at 9 MAS, which was significantly lower than the untreated control (12.90% at 9 MAS) as per CD values.

#### Germination

Germination percentage showed significant treatment differences throughout the storage period (Table 5 and Fig.5.). Seeds treated with sweet flag rhizome powder at 10 g $kg^{-1}$ recorded 99.5% germination at 0 MAS and 79.9% at 9 MAS, which were significantly higher than the control at the corresponding storage intervals.

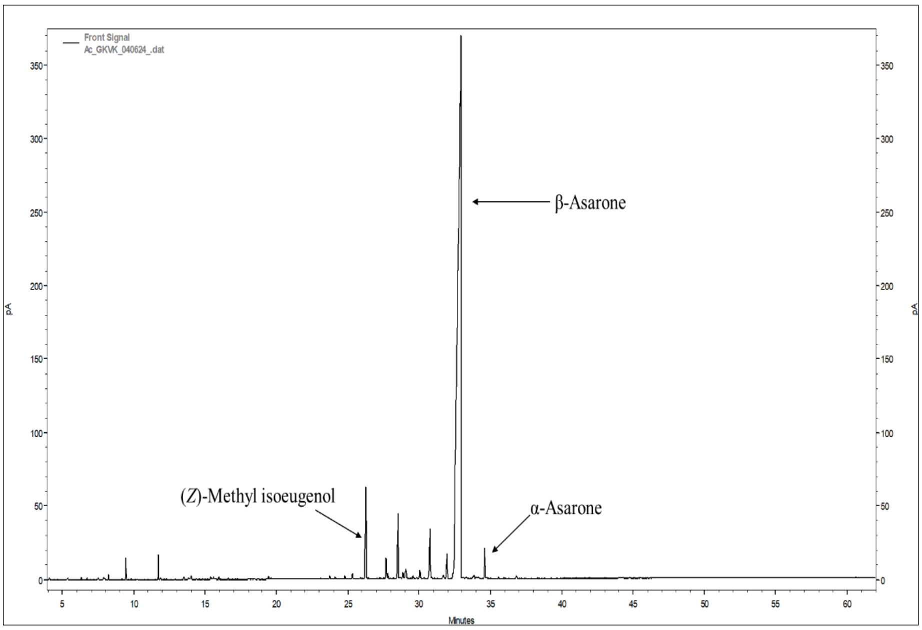


**Fig. 2.** GC Chromatogram for quantification of the chemical components of the essential oil in sweet flag rhizome.

**Table 2.** Effect of sweet flag rhizome foliar spray on growth performance, pest and disease incidence in field bean

| Treatments | Field emergence (%) | Plant stand (No.) | Plant height (cm) | | | Number of branches/ plant | Days to 50 % flowering | Days to maturity |
|---|---|---|---|---|---|---|---|---|
| | | | 30 DAS | 60 DAS | Maturity | | | |
| T1: Control | 81.9 | 80 | 16.1 | 49.4 | 50.3 | 3.29 | 46.9 | 101.3 |
| T2: Sweet flag rhizome powder at 5 g/l | 83.4 | 80.4 | 17.6 | 62.9 | 64.7 | 4.9 | 46.5 | 98.5 |
| T3: Sweet flag rhizome powder at 10 g/l | 88 | 84.9 | 18.7 | 64.2 | 66.4 | 5.3 | 46.7 | 96.3 |
| T4: Sweet flag rhizome powder at 15 g/l | 84.1 | 82 | 19.2 | 52.4 | 54.5 | 5.11 | 44.9 | 97.1 |
| T5: Sweet flag rhizome oil at 2.5 ml/l | 87.7 | 82.9 | 18.6 | 63.4 | 64.4 | 5.49 | 44.5 | 95.7 |
| T6: Sweet flag rhizome oil at 5 ml/l | 82.8 | 81 | 19.6 | 66.3 | 69.1 | 5.39 | 44.9 | 95.9 |
| T7: Sweet flag rhizome oil at 7.5 ml/l | 81.9 | 80.1 | 16.5 | 65 | 67.4 | 4.19 | 45.8 | 98.9 |
| T8: Spinosad at 0.04 ml/l | 84.9 | 82.9 | 18.5 | 59.4 | 63.1 | 4.49 | 45.9 | 97.8 |
| Mean | 84.3 | 81.8 | 18.1 | 60.4 | 62.5 | 4.77 | 45.8 | 97.7 |
| S.Em.± | 2.25 | 2.32 | 0.43 | 1.95 | 1.99 | 0.14 | 1.25 | 2.61 |
| CD (P=0.05) | 6.84 | 7.04 | 1.32 | 5.91 | 6.05 | 0.42 | 3.8 | 7.91 |
| CV (%) | 8.02 | 8.52 | 7.19 | 9.67 | 9.57 | 8.79 | 8.22 | 8.01 |
| Significance | NS | NS | S | S | S | S | NS | NS |

CD (P = 0.05): Critical Difference at 5% probability level; treatment means differing by more than the CD value are considered statistically significant.

NS: Non-significant; differences among treatment means were not statistically significant at P = 0.05.

CV (%): Coefficient of variation, indicating the extent of variability in relation to the mean.

**Seedling traits**

Seedling length and seedling dry weight differed significantly among treatments (Table 6). The same treatment recorded higher seedling length (54.6 cm at 0 MAS and 33.8 cm at 9 MAS) and greater seedling dry weight (109.3 mg at 0 MAS and 84.6 mg at 9 MAS) compared to the control, as indicated by CD values.

**Vigour indices**

Vigour indices showed significant variation among treatments during storage (Table 7). Sweet flag rhizome powder at 10 g $kg^{-1}$ recorded higher vigour index-I values of 5433 at 0 MAS and 2701 at 9 MAS, and vigour index-II values of 10875 at 0 MAS and 6760 at 9 MAS, which were significantly higher than those of the untreated control.

**Seed infection**

Seed infection percentage differed significantly across treatments (Table 7). The lowest infection was recorded under sweet flag rhizome powder at 10 g $kg^{-1}$, with 0% at 0 MAS and 11.00% at 9 MAS, compared to 21.97% seed infection in the untreated control at 9 MAS.

## Discussion

### Essential oil yield from sweet flag Rhizomes

The observed differences in oil yield suggest that raw material condition and environmental factors significantly influence extraction efficiency. Variability in moisture content, rhizome maturity and agro-climatic origin likely alters essential oil biosynthesis, thereby affecting yield consistency. The

**$T_1$: Control**

**$T_6$: Sweet flag rhizome oil at 5 ml/l** **$T_5$: Sweet flag rhizome oil at 2.5 ml/l** **$T_5$: Sweet flag rhizome oil at 2.5 ml/l**

**Fig. 3.** Effect of sweet flag rhizome foliar spray on plant height, number of branches and days to 50 % flowering in field bean.

**Table 3.** Effect of sweet flag rhizome foliar spray on growth performance, pest and disease incidence in field bean

| Treatments | Pest infestation (%) | Disease incidence (%) | Pod damage (%) |
|---|---|---|---|
| T1: Control | 10.84 | 5.25 | 26 |
| T2: Sweet flag rhizome powder at 5 g/l | 5.68 | 2.52 | 17.3 |
| T3: Sweet flag rhizome powder at 10 g/l | 4.73 | 1.43 | 15.8 |
| T4: Sweet flag rhizome powder at 15 g/l | 7.13 | 2.64 | 18 |
| T5: Sweet flag rhizome oil at 2.5 ml/l | 3.43 | 1.32 | 14.6 |
| T6: Sweet flag rhizome oil at 5 ml/l | 4.67 | 1.39 | 15.4 |
| T7: Sweet flag rhizome oil at 7.5 ml/l | 5.22 | 1.51 | 18.5 |
| T8: Spinosad at 0.04 ml/l | 5.88 | 2.58 | 16.4 |
| Mean | 5.95 | 2.33 | 17.8 |
| S.Em.± | 0.17 | 0.06 | 0.47 |
| CD (P=0.05) | 0.5 | 0.19 | 1.43 |
| CV (%) | 8.35 | 8.05 | 7.99 |
| Significance | S | S | S |

CD (P = 0.05): Critical Difference at 5% probability level; treatment means differing by more than the CD value are considered statistically significant.

CV (%): Coefficient of variation, indicating the extent of variability in relation to the mean.

dominance of β-asarone underscores the chemotypic identity of Indian *Acorus calamus* and its suitability for pesticidal and seed-quality enhancement applications. The strong antifungal activity reported in earlier studies (11,12) complements its insecticidal performance, demonstrating its value as a broad-spectrum bioactive compound.

## Growth performance

The results clearly demonstrate that foliar application of sweet flag rhizome powder and oil significantly enhances plant growth, phenological development and crop protection in field bean. Treatments $T_3$, $T_5$ and $T_6$ performed particularly well, reflecting the influence of bioactive-rich botanicals on both physiological and defensive processes. These growth-promoting effects may be attributed to the antifungal, insecticidal, antimicrobial and antioxidant potential of β-asarone, methyl isoeugenol (Z/E) and methyleugenol, which reduce early-season pathogen load, suppress insect stress and enhance physiological vigour (13,14). The results corroborate earlier findings where botanical foliar sprays improved vegetative growth and phenology in crops such as black gram, sesame, rice and chickpea (15,16,17), confirming the beneficial role of bioactive-rich botanicals in growth enhancement.

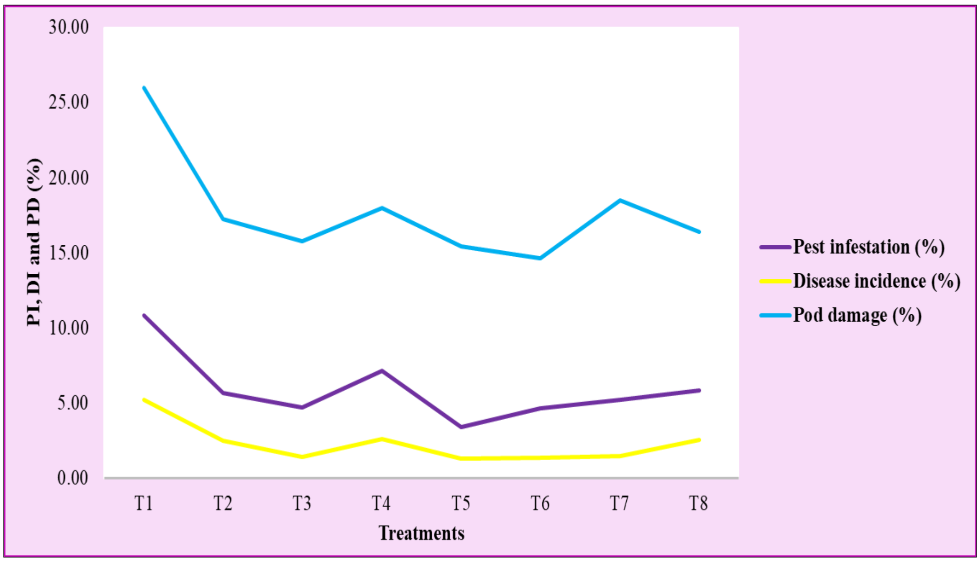


**Fig. 4.** Effect of sweet flag rhizome foliar spray on pest infestation (%), disease incidence (%) and pod damage (%) in field bean.

**Legend**

$T_1$: Control
$T_2$: Sweet flag rhizome powder at 5 g/l
$T_3$: Sweet flag rhizome powder at 10 g/l
$T_4$: Sweet flag rhizome powder at 15 g/l
$T_5$: Sweet flag rhizome oil at 2.5 ml/l
$T_6$: Sweet flag rhizome oil at 5 ml/l
$T_7$: Sweet flag rhizome oil at 7.5 ml/l
$T_8$: Spinosad at 0.04 ml/l
PI: Pest infestation
DI: Disease incidence
PD: Pod damage

**Table 4.** Initial seed quality parameters of field bean used for storage studies

| SI.No. | Quality parameters | Initial value |
|---|---|---|
| 1 | Seed moisture content (%) | 9.05 |
| 2 | Seed germination (%) | 96.2 |
| 3 | Speed of germination | 16.1 |
| 4 | Seed infection (%) | 2.16 |
| 5 | Shoot length (cm) | 23.1 |
| 6 | Root length (cm) | 18.2 |
| 7 | Seedling length (cm) | 41.3 |
| 8 | Seedling dry weight (mg) | 96.2 |
| 9 | Seedling vigour index-I | 3973 |
| 10 | Seedling vigour index-II | 9254 |
| 11 | Catalase activity (μmol $H_2O_2$ /min/g) | 159.7 |
| 12 | Peroxidase activity (μmol/min/g) | 0.28 |
| 13 | SOD activity (Units/min/g) | 1.10 |
| 14 | TDH activity activity (Optical Density at 520 nm) | 0.692 |
| 15 | Total protien content (mg/g) | 94.15 |
| 16 | Electric conductivity (μS/cm/g) | 130.7 |

## Pest and disease incidence

The results of the present investigation confirm the strong pesticidal, antimicrobial and protective roles of sweet flag rhizome–based formulations in field bean. Treatments containing sweet flag rhizome oil, particularly $T_5$ (2.5 ml $L^{-1}$) and $T_6$ (5 ml $L^{-1}$), consistently reduced pest infestation, disease incidence and pod damage by a wide margin compared to the untreated control. These protective effects are likely due to the strong antimicrobial and pesticidal activities of β-asarone, methyl isoeugenol and methyleugenol, which suppress key pathogens like *Magnaporthe grisea* and *Colletotrichum orbiculare* and reduce insect pressure (13,14). These results are consistent with previous reports where botanicals such as neem, turmeric and pulse sprout extracts effectively reduced insect and disease incidence in legumes and cereals (18,19,20). Conversely, untreated plots showed the highest pest infestation (10.84%), disease incidence (5.25%) and pod damage (26.0%), aligning with observations in chickpea and mung bean where the absence of botanical treatments led to high biotic stress and reduced crop performance (21,22). Overall, sweet flag foliar sprays markedly enhanced crop protection, reduced biotic stress and supported improved field performance of field bean.

## Seed quality performance

Sweet flag rhizome powder at 10 g $kg^{-1}$ ($T_3$) consistently demonstrated superior performance across all seed quality parameters during storage. The improved viability, vigour and storability can be directly attributed to the bioactive phenylpropanoids that protects membranes from lipid peroxidation, stabilizes cellular integrity and enhances metabolic activation during germination. These compounds also support reserve mobilization by maintaining enzymatic efficiency under ageing stress. The results corroborate earlier findings of (23,24), reported that Acorus calamus-based botanicals improve seed physiology, promote healthy seedling establishment and maintain membrane stability during storage.

Sweet flag rhizome powder at 10 g $kg^{-1}$ ($T_3$) was also the most effective treatment in suppressing seed infection and insect-mediated deterioration, recording 0% infection at 0 MAS and only 11.00% at 9 MAS compared with 21.97% in the control. The marked reduction in infection and infestation is attributed to the antimicrobial and insecticidal properties of β-asarone, methyl isoeugenol and other volatile constituents that disrupt fungal cell membranes, inhibit ergosterol biosynthesis and exhibit fumigant toxicity against bruchids. These properties minimized fungal invasion, insect damage

**Table 5.** Effect of sweet flag rhizome treatment on seed moisture content (%) and seed germination (%) in field bean during storage.

| Treatments | Seed moisture content (%) | | | | Seed germination (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 MAS | 3 MAS | 6 MAS | 9 MAS | 0 MAS | 3 MAS | 6 MAS | 9 MAS |
| T1: Control | 9.05 | 9.73 | 11.31 | 12.9 | 96.2 | 94.2 | 85 | 68.7 |
| T2: Sweet flag rhizome powder at 5 g/kg | 8.96 | 9.61 | 10.62 | 12.19 | 97.4 | 96.3 | 86.1 | 74.8 |
| T3: Sweet flag rhizome powder at 10 g/kg | 8.68 | 9.27 | 10.11 | 11.65 | 99.5 | 99 | 90 | 79.9 |
| T4: Sweet flag rhizome powder at 15 g/kg | 8.87 | 9.55 | 10.57 | 12.14 | 98 | 96.9 | 87.1 | 72.4 |
| T5: Sweet flag rhizome oil at 2.5 ml/kg | 8.76 | 9.35 | 10.24 | 11.79 | 99.1 | 98.2 | 89.3 | 79.2 |
| T6: Sweet flag rhizome oil at 5 ml/kg | 8.82 | 9.42 | 10.4 | 11.95 | 98.6 | 97.9 | 88.5 | 76.9 |
| T7: Sweet flag rhizome oil at 7.5 ml/kg | 9.05 | 9.67 | 10.73 | 12.31 | 96.8 | 96 | 86.7 | 71.3 |
| T8: Spinosad at 0.04 ml/kg | 8.82 | 9.48 | 10.46 | 12.04 | 98 | 97.4 | 87.9 | 74.7 |
| Mean | 8.88 | 9.51 | 10.56 | 12.12 | 97.9 | 97 | 87.6 | 74.7 |
| S.Em.± | 0.09 | 0.1 | 0.11 | 0.13 | 1.04 | 0.98 | 0.93 | 0.8 |
| CD (P=0.01) | 0.27 | 0.3 | 0.32 | 0.38 | 3 | 2.82 | 2.68 | 2.3 |
| CV (%) | 3.93 | 4.01 | 3.92 | 4.06 | 3.92 | 3.72 | 3.92 | 3.95 |
| Significance | S | S | S | S | S | S | S | S |

CD (P = 0.05): Critical Difference at 5% probability level; treatment means differing by more than the CD value are considered statistically significant.

CV (%): Coefficient of variation, indicating the extent of variability in relation to the mean.

MAS: Months after storage

S: Significance

**$T_1$: Control**

**$T_2$: Control**

**$T_3$: Sweet flag rhizome powder at 10 g/kg**

**$T_3$: Sweet flag rhizome powder at 10 g/kg**

**Fig. 5.** Effect of sweet flag rhizome seed treatment on seed germination, seed infection and seedling length in field bean at 9 months after storage.

and secondary microbial colonization, thereby slowing deterioration. The relatively better storability observed with rhizome powder compared to oil may be due to the gradual and sustained release of bioactive compounds from the powdered matrix, along with its ability to form a physical coating on the seed surface that restricts moisture uptake and insect movement. In contrast, essential oils, being more volatile, may dissipate more rapidly during prolonged storage, resulting in comparatively reduced long-term protective effects. Similar pest suppression effects of *Acorus calamus* have been reported earlier (25,26), supporting its effectiveness as a botanical seed protectant.

**Table 6.** Effect of sweet flag rhizome treatment on seedling length (cm) and seedling dry weight (mg) in field bean during storage.

| Treatments | Seedling length (cm) | | | | Seedling dry weight (mg) | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 MAS | 3 MAS | 6 MAS | 9 MAS | 0 MAS | 3 MAS | 6 MAS | 9 MAS |
| T1: Control | 41.3 | 38.7 | 31.2 | 21.1 | 96.2 | 94 | 85 | 77.2 |
| T2: Sweet flag rhizome powder at 5 g/kg | 44.7 | 43.4 | 36 | 30 | 99.6 | 97.2 | 88.5 | 80.4 |
| T3: Sweet flag rhizome powder at 10 g/kg | 54.6 | 52.9 | 44.2 | 33.8 | 109.3 | 102 | 92.7 | 84.6 |
| T4: Sweet flag rhizome powder at 15 g/kg | 44.6 | 43.6 | 37 | 30.2 | 99.2 | 97.3 | 89 | 81.7 |
| T5: Sweet flag rhizome oil at 2.5 ml/kg | 54.2 | 52 | 43.6 | 33.1 | 102.2 | 101.1 | 92.2 | 83.9 |
| T6: Sweet flag rhizome oil at 5 ml/kg | 45.7 | 44.4 | 37.4 | 26.5 | 101.7 | 99.5 | 91.5 | 83.6 |
| T7: Sweet flag rhizome oil at 7.5 ml/kg | 42.4 | 41.4 | 34.1 | 26.2 | 97.3 | 95.3 | 87 | 79.1 |
| T8: Spinosad at 0.04 ml/kg | 48.6 | 47.4 | 40.2 | 31.1 | 100.1 | 98 | 90 | 82.5 |
| Mean | 47 | 45.5 | 38 | 29 | 100.7 | 98.1 | 89.5 | 81.6 |
| S.Em.± | 0.5 | 0.49 | 0.41 | 0.31 | 1.07 | 1.05 | 0.98 | 0.96 |
| CD (P=0.01) | 1.45 | 1.4 | 1.17 | 0.89 | 3.07 | 3.02 | 2.82 | 2.75 |
| CV (%) | 3.95 | 3.94 | 3.94 | 3.92 | 3.9 | 3.91 | 3.93 | 3.93 |
| Significance | S | S | S | S | S | S | S | S |

CD (P = 0.05): Critical Difference at 5% probability level; treatment means differing by more than the CD value are considered statistically significant.

CV (%): Coefficient of variation, indicating the extent of variability in relation to the mean.

MAS: Months after storage

S: Significance

**Table 7.** Effect of sweet flag rhizome treatment on in field bean during storage seedling vigour index-I, seedling vigour index-I and seed infection (%)

| Treatments | Seedling vigour index-I | | | | Seedling vigour index-II | | | | Seed infection (%) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 MAS | 3 MAS | 6 MAS | 9 MAS | 0 MAS | 3 MAS | 6 MAS | 9 MAS | 0 MAS | 3 MAS | 6 MAS | 9 MAS |
| T1: Control | 3973 | 3646 | 2652 | 1450 | 9254 | 8855 | 7225 | 5304 | 2.16 | 3.97 | 9.97 | 21.97 |
| T2: Sweet flag rhizome powder at 5 g/kg | 4355 | 4179 | 3100 | 2244 | 9704 | 9360 | 7620 | 6014 | 1.47 | 2.96 | 6.93 | 16.93 |
| T3: Sweet flag rhizome powder at 10 g/kg | 5433 | 5237 | 3978 | 2701 | 10875 | 10098 | 8343 | 6760 | 0 | 0 | 5 | 11 |
| T4: Sweet flag rhizome powder at 15 g/kg | 4371 | 4225 | 3223 | 2186 | 9722 | 9428 | 7752 | 5915 | 1.86 | 3.03 | 7.03 | 15.87 |
| T5: Sweet flag rhizome oil at 2.5 ml/kg | 5371 | 5106 | 3893 | 2622 | 10128 | 9928 | 8233 | 6645 | 0 | 1.03 | 4.97 | 11.97 |
| T6: Sweet flag rhizome oil at 5 ml/kg | 4504 | 4347 | 3310 | 2038 | 10024 | 9741 | 8098 | 6429 | 0.1 | 1.96 | 6.03 | 12.83 |
| T7: Sweet flag rhizome oil at 7.5 ml/kg | 4104 | 3974 | 2956 | 1868 | 9417 | 9149 | 7543 | 5640 | 2.05 | 2.98 | 7.96 | 18.9 |
| T8: Spinosad at 0.04 ml/kg | 4763 | 4617 | 3534 | 2323 | 9810 | 9545 | 7911 | 6163 | 0.09 | 1.97 | 5.97 | 14.8 |
| Mean | 4609 | 4416 | 3331 | 2179 | 9867 | 9513 | 7841 | 6109 | 0.97 | 2.24 | 6.73 | 15.53 |
| S.Em.± | 49.53 | 47.36 | 35.74 | 23.42 | 104.48 | 101.47 | 83.74 | 65.61 | 0.01 | 0.03 | 0.09 | 0.2 |
| CD (P=0.01) | 142.35 | 136.12 | 102.73 | 67.33 | 300.28 | 291.64 | 240.67 | 188.55 | 0.04 | 0.08 | 0.26 | 0.56 |
| CV (%) | 3.95 | 3.94 | 3.95 | 3.95 | 3.9 | 3.93 | 3.93 | 3.95 | 4.95 | 4.76 | 4.86 | 4.65 |
| Significance | S | S | S | S | S | S | S | S | S | S | S | S |

CD (P = 0.05): Critical Difference at 5% probability level; treatment means differing by more than the CD value are considered statistically significant.

CV (%): Coefficient of variation, indicating the extent of variability in relation to the mean.

MAS: Months after storage

S: Significance

In contrast, untreated seeds showed progressive increases in moisture, infection and vigour loss due to bruchid infestation, seed coat perforation and accelerated ageing, as previously observed (27,28). Thus, sweet flag rhizome powder at 10 g $kg^{-1}$ consistently proved superior in mitigating insect and pathogen damage, maintaining seed health and ensuring better storability of field bean seeds.

## Conclusion

The combined findings from the three experiments demonstrate that sweet flag (*Acorus calamus*) rhizome and its essential oil possess measurable pesticidal, physiological and seed-protective effects. Foliar application improved plant growth, advanced flowering and reduced pest and disease incidence, which is consistent with the bioactive demonstrate that the sweet flag rhizome strong pesticidal, physiological and seed-protective potential of sweet flag (*Acorus calamus*) rhizome and its essential oil. Foliar application of sweet flag significantly enhanced growth, flowering behaviour and reduced pest and disease incidence in field bean, primarily due to the bioactive compounds β-asarone, α-asarone and methyl isoeugenol. Chemical profiling confirmed β-asarone as the dominant constituent (85.21%), validating its role in antifungal, insecticidal and antioxidant activities that contribute to improved plant and seed performance. During storage, sweet flag rhizome powder at 10 g $kg^{-1}$ consistently maintained lower moisture content, higher germination, superior vigour and minimal seed infection, outperforming synthetic treatments. Overall, sweet flag rhizome represents an effective, eco-friendly and multifunctional botanical input for crop protection, growth enhancement and long-term seed preservation.

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## Authors' contributions

MB carried out experimental work, including data collection and laboratory analysis, and contributed to data interpretation and manuscript preparation. PBV assisted in conducting experiments, data collection, and analysis and interpretation of results. SR performed molecular genetic studies, participated in sequence alignment, and drafted the manuscript. OT & UR contributed to the design of the study and performed the statistical analysis. P conceived the study and participated in its design and coordination. VSP provided technical guidance and assisted in the experimental procedures for the quantification of sweet flag (*Acorus calamus*) rhizome oil at CIMAP.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

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**Declaration of generative AI and AI-assisted technologies in the writing process**

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