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Original Article

Solanum incanum Leaf Powder Exhibits High Insecticidal Efficacy Against *Sitophilus zeamais* in Stored Maize Seeds: Implications for Sustainable Pest Management

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Abstract

Introduction

The maize weevil, *Sitophilus zeamais* Motschulsky, represents one of the most economically devastating storage pests affecting cereal seeds in tropical regions. However, dependence on synthetic insecticides poses environmental, health, and economic constraints that limit their sustainable use in smallholder farming systems. This study evaluated the insecticidal efficacy of 10 botanical powders against *S. zeamais* in stored maize seeds over 56 days, examining mortality rates, dose-response relationships, temporal efficacy dynamics, and seed protection capacity.

Methods

Laboratory bioassays conducted at Sokoine University of Agriculture employed a completely randomised design with botanical treatments (*Cajanus cajan*, *Azadirachta indica*, *Eucalyptus globulus*, *Cymbopogon citratus*, *Lantana camara*, *Ricinus communis*, *Ocimum basilicum*, *Hyptis suaveolens*, and *Solanum incanum* leaves and fruits) at four concentrations (5, 10, 15, and 20 g per 200 g maize seeds) compared against Actellic Super Dust® (pirimiphos-methyl 16 g/kg + permethrin 3 g/kg) and untreated controls.

Results

Two-way factorial analysis of variance (ANOVA) revealed significant main effects for botanical type ($p < 0.001$) and concentration ($p < 0.001$), with a significant interaction ($p = 0.019$) indicating differential dose-response patterns among species. *Solanum incanum* leaf powder exhibited the strongest efficacy, achieving 62.52-84.09% mortality across concentrations compared with 100% for the synthetic standard. Two-way repeated-measures ANOVA with Greenhouse-Geisser correction demonstrated significant effects for treatment ($p < 0.001$), storage interval ($p < 0.001$), and their interaction ($p < 0.001$), confirming that temporal dynamics varied among botanical classes. *Solanum incanum* retained 61% of its initial 14-day efficacy at 56 days, demonstrating superior temporal stability attributed to the environmental persistence of glycoalkaloid compounds. In contrast, terpenoid-based *E. globulus* lost 84% of initial efficacy over the same period, reflecting volatile compound dissipation. *Solanum incanum* leaf powder provided seed protection statistically equivalent to synthetic standards at 56 days (0.25% versus 3.55% weight loss in untreated controls). The significant treatment × storage interval interaction for weight loss ($p < 0.001$) indicates that protective capacity varies differentially across time.

Conclusion

These findings establish *S. incanum* as a promising candidate for botanical insecticide development, offering smallholder farmers an accessible, locally available alternative for integrated pest management in stored maize seed systems. The widespread distribution of this species throughout tropical Africa, combined with simple processing requirements aligned with traditional practices, positions *S. incanum* as a practical solution for resource-constrained households where synthetic insecticides remain economically prohibitive.

Categories: Organic agriculture, Seed technology, Field & Horticultural crops sustainable production

Keywords: botanical insecticides, postharvest losses, sitophilus zeamais, solanum incanum, stored seed protection

Introduction

Maize (*Zea mays* L.) occupies a position of paramount importance in global food systems, serving as the foundational staple upon which the food security of billions depends. Together with rice and wheat, maize provides at least 30% of dietary calories for more than 4.5 billion people across 94 developing countries, while simultaneously fulfilling critical functions as livestock feed and industrial raw material [1,2]. The significance of this crop is particularly pronounced in sub-Saharan Africa, where maize constitutes a critical staple food for over 300 million people, providing more than 25% of daily caloric intake in eastern and southern Africa and exceeding 50% in countries such as Lesotho, Malawi, and Zambia [1,2]. In Tanzania, maize dominates the agricultural landscape, with over 50% of farmers engaged in its cultivation and smallholders responsible for approximately 80-85% of national production. For these farming households, maize fulfils multiple essential functions: it provides the primary source of household nutrition, generates income through market sales, and supplies the farm-saved seed upon which agricultural continuity depends. However, the realisation of maize's potential contribution to food security is severely constrained by postharvest losses attributable to storage pests, which represent a persistent threat to both grain quantity and seed quality [3,4].

Among storage pests affecting cereals, the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), represents the most economically devastating species in tropical and subtropical regions, inflicting quantitative losses ranging from 14% to 36% depending on storage duration, environmental conditions, and population density [5]. The destructive capacity of *S. zeamais* derives from its cryptic feeding behaviour, wherein females oviposit directly into grain kernels and larvae develop internally, consuming the nutrient-rich endosperm while concealed within the seed coat [6,7]. This internal feeding strategy renders early infestation detection exceedingly difficult and compromises both nutritional quality and germination potential of affected seeds. Female *S. zeamais* may lay up to 150 eggs under favourable conditions, although typical mean fecundity in laboratory cultures for maize is 67 ± 3 eggs per female [8]. Under traditional storage conditions prevalent in smallholder systems, the magnitude of destruction escalates dramatically, with 41-80% of harvested grain lost within three to six months of storage, representing a substantial drain on household resources and threatening the agricultural sustainability of future cropping cycles.

Contemporary management of storage pests relies predominantly on synthetic insecticides, including organophosphates and pyrethroids, which achieve mortality rates approaching 100% under controlled conditions [9,10]. Despite this efficacy, the sustainability of chemical-based pest management is increasingly questioned on multiple grounds. Widespread and often injudicious application has generated substantial concerns regarding environmental contamination, residue accumulation in food products, and the documented emergence of insecticide resistance in *S. zeamais* populations across multiple geographic regions [11-13]. Furthermore, synthetic insecticides remain economically inaccessible for many smallholder farmers in sub-Saharan Africa; pesticide-related health costs alone can represent 45-83% of total household pesticide expenditures, substantially increasing the true cost of chemical pest management beyond the direct purchase price [14]. These converging limitations, environmental, resistance-related, and economic, have catalysed growing scientific and policy interest in alternative approaches to storage pest management.

Botanical insecticides have emerged as particularly promising alternatives, offering a constellation of attributes aligned with sustainable agriculture principles: biodegradability that minimises environmental persistence, reduced mammalian toxicity that enhances user safety, and compatibility with organic production systems increasingly valued in regional and international markets [15,16]. The accessibility of botanical insecticides renders them especially appropriate for smallholder farming systems, where indigenous plant species with documented insecticidal properties are often locally available and can be processed using traditional methods requiring no external inputs or specialised equipment [17,18]. The complex phytochemical composition of botanical materials, comprising multiple bioactive compounds with diverse modes of action, may reduce the likelihood of resistance development compared to single-compound synthetic insecticides, offering potential for more durable pest management solutions [19,20]. The insecticidal activity of botanical materials derives from diverse secondary metabolites, including alkaloids, terpenoids, phenolic compounds, and essential oil constituents, that exert toxic effects through multiple mechanisms encompassing neurotoxicity, antifeedant activity, growth regulation disruption, and reproductive impairment [21,22].

Previous investigations have demonstrated encouraging results with various plant species as seed protectants against *S. zeamais*. Nukenine et al. [23] reported that *Plectranthus glandulosus* achieved 100% mortality at 20-40 g/kg after 16-32 days exposure, with LC50 values of 8.6 g/kg. Ogendo et al. [24] documented 82.7-93.7% mortality with *Lantana camara* and *Tephrosia vogelii* after 21 days of exposure. Recent studies have demonstrated that several medicinal plants exhibit strong insecticidal activity against *S. zeamais*, with some extracts achieving high adult mortality and complete inhibition of F1 progeny emergence [23,25]. Essential oils derived from *Eucalyptus* species have exhibited significant fumigant and contact toxicity, with 1,8-cineole identified as a principal bioactive constituent [26,27]. Neem-based products (*Azadirachta indica*) have received extensive attention due to the growth-disrupting properties of azadirachtin; Erenso and Berhe [28] reported that neem seed powder achieved 82% mortality while leaf powder achieved 77.75% mortality at 3% w/w concentration, demonstrating that plant part selection significantly influences insecticidal efficacy. Despite these advances, translation of laboratory efficacy into consistent field-level performance has remained challenging, with considerable variability reported across botanical species and application contexts [6,15].

Despite accumulating evidence supporting the potential of botanical protectants, the translation of research findings into practical recommendations for smallholder systems is impeded by several critical knowledge gaps. First, systematic comparative evaluations examining multiple botanical species under homogeneous experimental conditions remain limited, precluding direct comparison of efficacy across species and preventing establishment of reliable hierarchies to guide protectant selection. Second, temporal dynamics of botanical insecticide efficacy over extended storage periods exceeding 42 days have been inadequately documented, despite practical relevance for smallholder storage systems where seeds are typically retained for 4-8 months before planting. Third, dose-response relationships have not been consistently characterised across diverse botanical species, preventing identification of species-specific concentration thresholds that maximise pest mortality while minimising material costs. Fourth, *Solanum incanum* L. (Solanaceae), despite its widespread distribution throughout tropical Africa and documented content of bioactive glycoalkaloids [29], has received minimal scientific attention as a potential seed protectant. The Solanaceae family produces steroidal glycoalkaloids, including solasonine and solamargine, which demonstrate insecticidal activity through multiple mechanisms [21,30]. Ethnobotanical surveys in Masasi District reveal that traditional farmers preferentially employ *S. incanum* leaves for pest deterrence in storage systems, representing indigenous knowledge that warrants systematic scientific validation.

The powder formulation approach adopted in this investigation reflects deliberate methodological choices aligned with smallholder realities. Powder preparations require only shade drying and mechanical grinding, processes achievable with locally available materials and traditional techniques. Unlike solvent extraction or steam distillation, powder preparation preserves heat-sensitive compounds through ambient temperature processing while eliminating dependence on external inputs, specialised equipment, or technical expertise. This formulation strategy maximises the probability of farmer adoption by aligning research methodology with existing practices rather than introducing unfamiliar technologies requiring behavioural change or capital investment.

Based on the documented phytochemical properties of glycoalkaloids and their putatively greater environmental stability relative to volatile terpenoids, we hypothesised that *S. incanum* leaf powder would demonstrate superior and more persistent insecticidal activity compared to essential oil-based botanicals. This study evaluated the insecticidal efficacy of 10 botanical powders against *S. zeamais* in stored maize seeds over a 56-day storage period. This study makes three novel contributions to the botanical insecticide literature: (i) it provides the first systematic comparative evaluation of *S. incanum* against multiple botanical alternatives under standardised conditions; (ii) it extends mortality assessment to 56 days, substantially beyond the 21-42-day periods typical of previous studies; and (iii) it characterises interval-specific rather than cumulative mortality, enabling detection of temporal efficacy patterns that cumulative measurements obscure. The specific aims were to (i) assess mortality rates relative to a synthetic insecticide control and untreated baseline; (ii) determine dose-response relationships across four concentrations (5, 10, 15, and 20 g per 200 g maize seeds); (iii) evaluate temporal efficacy dynamics over the extended storage period; and (iv) assess seed protection capacity through interval-specific weight loss measurements. These objectives collectively address the critical knowledge gaps constraining evidence-based recommendations for integrating botanical seed protectants into sustainable maize storage systems in sub-Saharan Africa.

Materials And Methods

Study area and experimental site

The field component of this investigation was situated in Masasi District, Mtwara Region, in the southeastern quadrant of Tanzania (10.73° S, 38.81° E) (Figure *1*). This district occupies a position within the coastal agro-ecological zone, characterised by a unimodal rainfall pattern delivering approximately 910 mm annually, with mean temperatures averaging 27.5°C. The selection of Masasi District as the study site was predicated upon several converging factors: maize constitutes the principal staple crop cultivated by the predominantly smallholder farming population; postharvest storage losses attributable to *S. zeamais* infestation represent a persistent and economically significant challenge; and the district harbours considerable botanical diversity offering opportunities for sustainable pest management innovation.

Agricultural production within the district is dominated by smallholder subsistence systems, with farming households cultivating and preserving an indigenous maize landrace locally designated 'Katumbili' for dual purposes of household consumption and seed retention for subsequent planting seasons. Traditional storage methodologies, including the suspension of maize cobs above indoor cooking hearths to exploit smoke-derived pest deterrence, remain prevalent throughout rural communities, yet have demonstrated diminishing effectiveness against increasingly problematic weevil populations. The ethnobotanical heritage of Masasi District encompasses documented utilisation of various plant species for pest management purposes, with *A. indica*, *Eucalyptus globulus*, *Cymbopogon citratus*, and *L. camara* featuring prominently in indigenous practice. Of particular relevance to this investigation, *S. incanum* L. (Solanaceae), known locally as 'Ndulele' and commonly referred to as sodom apple, has been employed by traditional farmers as a pest deterrent in storage systems, representing indigenous knowledge warranting systematic scientific validation.

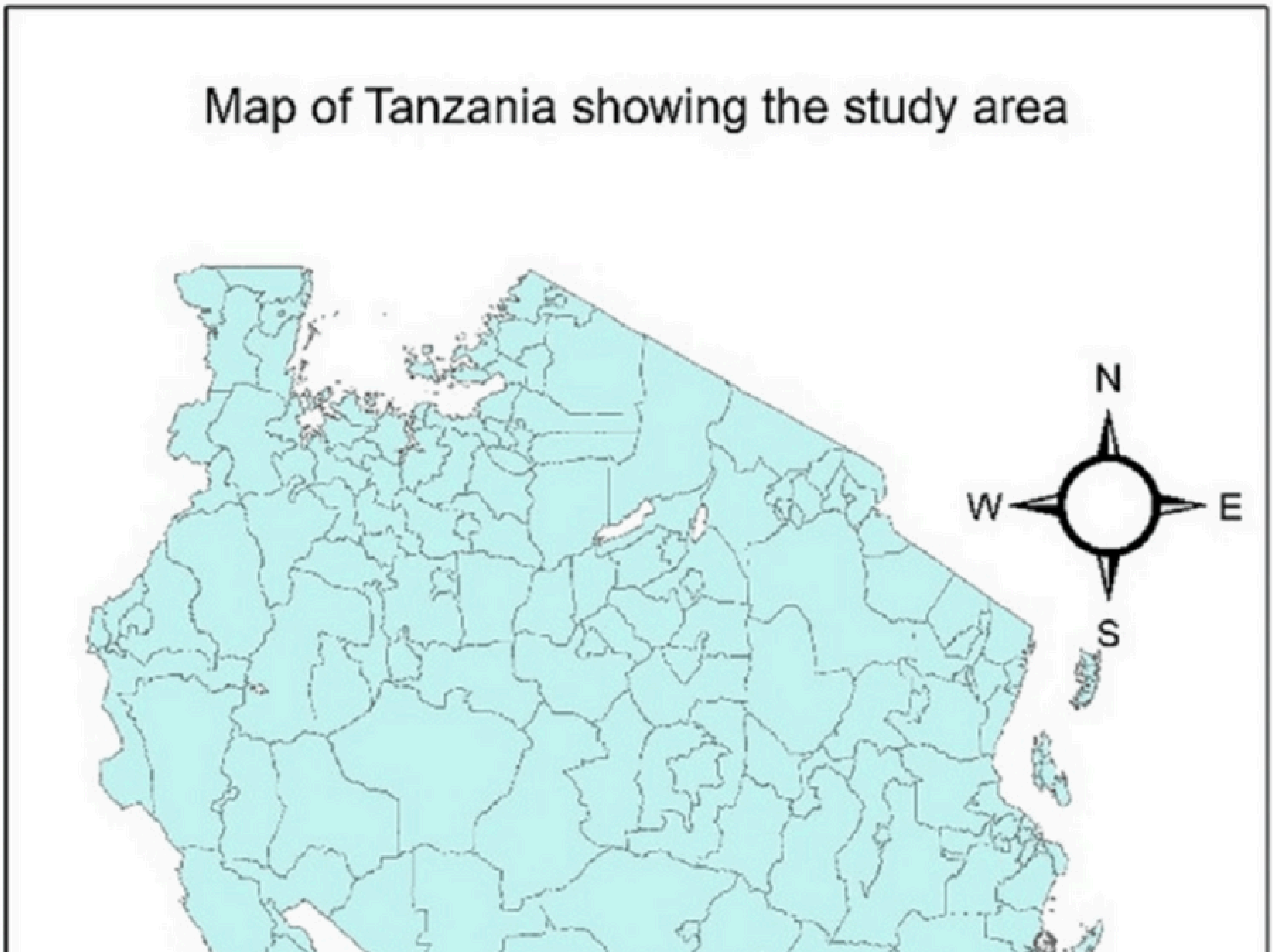
Map of Tanzania showing the study area
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FIGURE 1: Geographic location of Masasi District (10.73° S, 38.81° E), Mtwara Region, southeastern Tanzania, showing the study area where plant materials were collected and S. zeamais-infested maize seeds were sourced.

Laboratory bioassays were executed at the Entomology Laboratory, Institute of Pest Management, Sokoine University of Agriculture, located in Morogoro (6.8278° S, 37.6591° E). This facility provided controlled environmental conditions essential for standardised insecticidal efficacy assessment. Ambient laboratory parameters were maintained at 26.5 ± 2°C and 83 ± 2% relative humidity throughout the 56-day experimental period, with a 12:12-hour light/dark photoperiod regime. These conditions approximate the temperature and humidity ranges encountered in traditional smallholder storage environments within tropical East Africa, thereby enhancing the translational relevance of laboratory findings to practical field applications.

Collection, identification, and rearing of experimental insects

Experimental insects were obtained from naturally infested maize stocks to ensure biological relevance to field populations encountered in smallholder storage systems. Approximately 50 kg of weevil-infested maize seeds was procured from a local market in Masasi District, Tanzania, providing a source population from which *S. zeamais* specimens were isolated to establish laboratory colonies. Taxonomic verification was conducted at the Entomology Laboratory, Department of Crop Science and Horticulture, Sokoine University of Agriculture, following standardised entomological protocols [25]. Adult weevils were immobilised through cold exposure at -4°C for 15 minutes to facilitate safe handling during morphological examination. Individual specimens were scrutinised under a stereomicroscope at 10-40× magnification (Figure *2*), with identification based on diagnostic morphological characteristics specific to *S. zeamais*: an elongated, slender, and gently curved rostrum; geniculate antennae with distinct segmentation; four distinctive reddish-brown spots on the elytra; densely distributed coarse punctures on the pronotum with scattered fine setae; and a clearly visible scutellum that distinguishes this species from the morphologically similar *Sitophilus oryzae* [25].

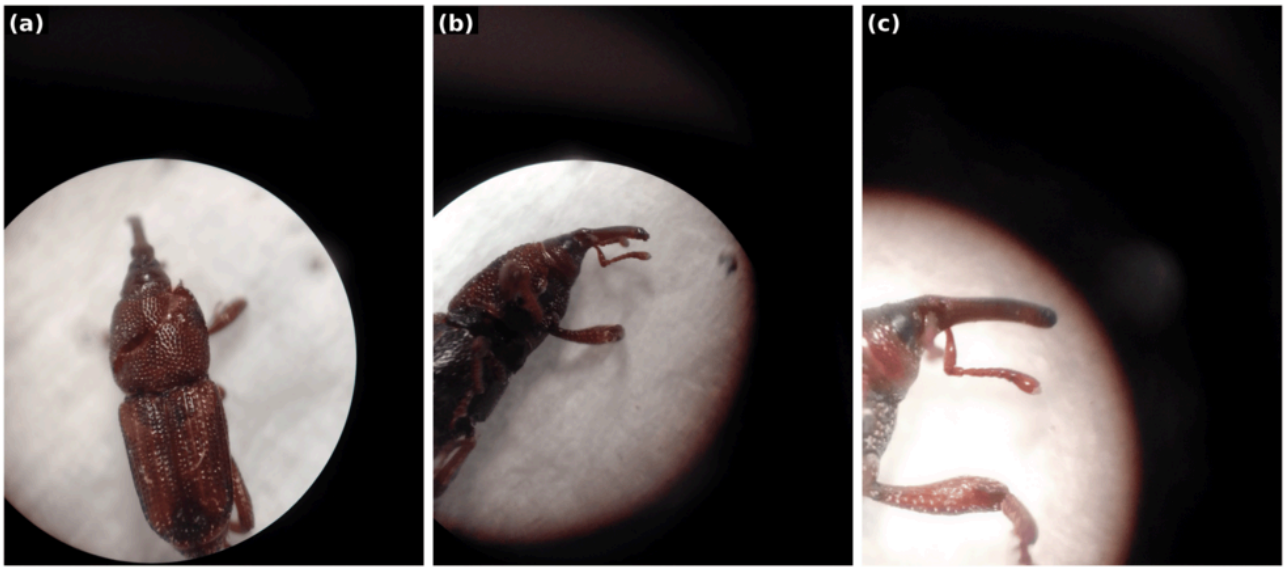

FIGURE 2: Morphological identification of Sitophilus zeamais Motschulsky under a stereomicroscope (10–40× magnification). (a) Dorsal habitus showing an elongated body, a coarsely punctured pronotum, and four distinctive reddish-brown spots on the elytra, characteristic of S. zeamais; (b) lateral view of the head region showing an elongated, gently curved rostrum and geniculate antennae with distinct segmentation; (c) close-up of rostrum morphology and the antennal insertion point.

Laboratory colonies were established following standardised protocols for culturing stored-product Coleoptera [24]. Eight hundred unsexed adults were introduced into two 15-litre plastic containers, each provisioned with 5 kg of clean, insecticide-free maize seeds as the rearing substrate. The utilisation of unsexed adults conforms to established practice, as *S. zeamais* exhibits minimal sexual dimorphism in insecticide susceptibility, and natural sex ratios approximating 1:1 are maintained when random samples are drawn from laboratory cultures [31]. Containers were covered with muslin cloth to ensure adequate aeration while preventing insect escape. Cultures were maintained under ambient laboratory conditions of 26.5 ± 2°C, 83 ± 2% relative humidity, and a 12:12-hour light/dark photoperiod throughout the rearing period. Adult weevils were permitted to mate and oviposit undisturbed for two months to ensure completion of at least one full developmental cycle. Following this period, newly emerged adults were separated by sieving through a 2-mm mesh and collected for experimental use, ensuring that all insects employed in bioassays were of known age and physiological status.

Collection, identification, drying, and processing of plant materials

Nine botanical species (Table *1*) were selected for evaluation based on documented phytochemical profiles containing bioactive compounds with established efficacy against storage pests, prioritising species demonstrating greater than 50% pest mortality in published literature while ensuring local availability for sustainable sourcing within the study area. Plant materials were collected on 14 December 2024 from agricultural fields and natural vegetation throughout Masasi District, Tanzania, adhering to sustainable harvesting practices that minimised ecological impact. Only mature, healthy plant parts exhibiting no visible signs of disease, herbivore damage, or senescence were harvested using sterilised cutting tools. Harvested specimens were immediately placed in labelled, sterile polypropylene bags with comprehensive collection metadata recorded, including geographic coordinates, habitat description, phenological stage, and collector identification.

It is important to note that environmental stress conditions, including drought, temperature extremes, herbivory, and nutrient limitations, can significantly influence secondary metabolite biosynthesis and accumulation in plants, potentially affecting the insecticidal potency of botanical materials [15,20]. All nine botanical species evaluated in this study were collected from naturally occurring wild populations and agricultural fields in Masasi District, Tanzania, rather than from cultivated or experimentally managed plantings under controlled agronomic conditions. As such, the plants were subjected to the prevailing natural environmental conditions of the coastal agro-ecological zone, characterised by a unimodal rainfall pattern delivering approximately 910 mm annually and mean temperatures averaging 27.5°C. Collection was conducted on 14 December 2024, corresponding to the early onset of the rainy season, a period when soil moisture availability was increasing following the preceding dry season. The phenological growth stage at collection for each species has been documented in Table *1*, ranging from vegetative pre-anthesis to reproductive fruiting stages, providing context for the physiological state and likely secondary metabolite profiles of the collected materials at the time of harvest. While the natural field conditions under which these plants grew may introduce variability in phytochemical composition compared to greenhouse-cultivated specimens, this collection strategy was deliberately adopted to reflect the conditions under which smallholder farmers would realistically source botanical materials for pest management applications, thereby enhancing the translational relevance of the findings to practical field use.

Botanical identification integrated ethnobotanical knowledge obtained through consultation with local farmers with formal taxonomic verification conducted by trained botanists. Fresh specimens were examined for diagnostic morphological characteristics including leaf arrangement patterns, venation architecture, and branching morphology, with identifications verified against East African botanical keys and the World Flora Online database. Voucher specimens were prepared following standardised herbarium protocols and deposited at the Muhimbili University of Health and Allied Sciences (MUHAS) herbarium, Dar es Salaam, for permanent archival and future reference.

Plant materials were shade-dried at ambient temperature (25 ± 2°C) for 14 days in a well-ventilated environment protected from direct sunlight, thereby preserving heat-sensitive bioactive compounds including essential oils, alkaloids, and glycoalkaloids that might otherwise degrade under elevated temperatures [15,21]. It is important to clarify that this study employed dried powder formulations rather than solvent-based extracts; consequently, no cold storage, refrigeration, or cryogenic preservation was applied during any stage of material preparation or storage. This methodological choice was deliberate: the powder formulation approach was selected to align with smallholder farmer practices in rural communities where cold chain facilities and specialised extraction equipment are unavailable, thereby maximising the practical applicability and adoption potential of the findings. Following drying, materials were processed into fine powder using an electric ceramic grinder (Model YL-112M-2, Yongkang Jinli Industry and Trade Co., Ltd., China). Ground materials were subsequently passed through standardised sieves (U.S. Standard Testing Sieve Series) with 0.5 mm and 1.0 mm mesh apertures to obtain uniform particle sizes between 0.5 and 1.0 mm, thereby maximising surface area available for contact toxicity while ensuring consistent dosimetry across experimental units. Processed powders were sealed in airtight polyethylene bags and stored at room temperature in darkness until required for bioassay use.

Common Name	Local Name	Family	Part Used	Phenological Stage
Pigeon pea	Mbaazi	Fabaceae	Leaves	Vegetative (pre-anthesis)
Neem tree	Mwarobaini	Meliaceae	Leaves	Reproductive (anthesis)
Eucalyptus	Mkalutusi	Myrtaceae	Leaves	Vegetative (pre-anthesis)
Lemon grass	Mchaichai	Poaceae	Leaves	Vegetative (regrowth)
Lantana	Lantana	Verbenaceae	Leaves	Vegetative (pre-anthesis)
Castor plant	Mbono	Euphorbiaceae	Leaves	Reproductive (anthesis)
Sweet basil	Kifumbasi kidogo	Lamiaceae	Leaves	Vegetative (pre-anthesis)
Bush mint	Kifumbasi kikubwa	Lamiaceae	Leaves	Vegetative (pre-anthesis)
Sodom apple	Ndulele	Solanaceae	Leaves	Reproductive (fruiting)
Sodom apple	Ndulele	Solanaceae	Fruits	Reproductive (mature fruits)

TABLE 1: Botanical identity, taxonomic classification, plant parts used, and phenological growth stages at the time of collection (14 December 2024) of plant species evaluated for insecticidal activity against Sitophilus zeamais in stored maize seeds.

Experimental procedure for botanical insecticide efficacy testing

Forty kilograms of local maize (*Z. mays* var. Katumbili) were harvested from Masasi District, Tanzania, with selection criteria ensuring seeds had no prior exposure to synthetic insecticides or botanical treatments. Seeds were subjected to phytosanitary treatment at −5°C for 72 hours to eliminate any pre-existing infestation while preserving seed viability, following established protocols for stored-product insect bioassays [23,25]. The absence of surviving insects was verified through visual inspection followed by 14-day incubation at 27°C to detect any emergence from potentially surviving eggs or larvae. Sterilised seeds were subsequently acclimatised under laboratory conditions (26.5 ± 2°C and 83 ± 2% relative humidity) for 48 hours prior to experimental use to equilibrate moisture content with ambient conditions.

The experimental design incorporated three treatment categories: an untreated negative control providing baseline reference for natural mortality and seed damage in the absence of intervention; a synthetic positive control establishing the efficacy benchmark against which botanical performance was evaluated; and botanical treatments representing the primary focus of investigation. The synthetic positive control consisted of Actellic Super Dust (pirimiphos-methyl 16 g/kg + permethrin 3 g/kg; Syngenta, distributed by Twiga Chemical Industries Ltd., Kenya), applied at 0.111 g per 200 g maize seeds (equivalent to 0.556 g/kg grain) as derived from manufacturer label recommendations of 50-60 g product per 90 kg grain. This application rate delivers approximately 8.9 mg pirimiphos-methyl and 1.67 mg permethrin per kilogram of treated grain.

Botanical powders were evaluated at four concentration levels: 5, 10, 15, and 20 g per 200 g maize seeds, corresponding to 2.5%, 5%, 7.5%, and 10% weight/weight ratios, respectively. These dosages align with established protocols for dose-response screening of botanical insecticides against *S. zeamais* in stored-grain bioassays [24]. For each experimental unit, botanical powder or synthetic agent was thoroughly mixed with 200 g of sterilised maize seeds in 500-ml plastic jars for 5 minutes to ensure uniform distribution and complete coverage of seed surfaces. The 200 g grain quantity per jar was selected to simulate typical household storage volumes while maintaining pest densities comparable to those encountered in farmer-managed storage systems. Jars were covered with muslin cloth secured with rubber bands to permit adequate gas exchange while preventing insect escape [25].

Each experimental unit was infested with 20 unsexed adult *S. zeamais*, following standard protocols for stored-grain Coleoptera bioassays [24]. Use of unsexed adults is standard practice because *S. zeamais* exhibits limited sex-specific differences in susceptibility, and natural sex ratios (approximately 1:1) are maintained when adults are drawn from laboratory cultures [31]. All treatment combinations were replicated four times, with the same experimental units evaluated repeatedly at each assessment time point constituting a repeated-measures design. The experiment comprised 168 experimental units in total: 160 units for botanical treatments (10 botanicals × 4 concentrations × 4 replicates), 4 units for the synthetic positive control, and 4 units for the untreated negative control. Accordingly, 3,360 adult insects were introduced across all experimental units (168 jars × 20 insects per jar). Following infestation, jars were covered with muslin cloth and incubated at 26.5 ± 2°C and 83 ± 2% relative humidity under ambient laboratory light conditions to simulate tropical smallholder storage environments.

Experimental design and replication

A completely randomised design was employed to assess the insecticidal efficacy of botanical treatments against *S. zeamais* [32]. The study encompassed 12 treatment groups: 10 botanical powders (*A. indica*, *Cajanus cajan*, *C. citratus*, *E. globulus*, *Hyptis suaveolens*, *L. camara*, *Ocimum basilicum*, *Ricinus communis*, and *S. incanum* leaves and fruits), one synthetic insecticide positive control (Actellic Super Dust®), and one untreated negative control. Botanical treatments were tested across four concentration levels (5, 10, 15, and 20 g per 200 g maize seeds), while synthetic and untreated controls were applied at single levels. Each treatment-concentration combination was replicated four times, yielding 168 experimental units that were arranged randomly within the laboratory space to minimise positional effects arising from spatial heterogeneity in temperature, humidity, or light exposure [33].

Data collection

The same experimental units were assessed repeatedly at 14, 28, 42, and 56 days post-treatment, constituting a repeated-measures design that enabled characterisation of temporal efficacy dynamics across the storage period. The 14-day assessment interval was selected to capture temporal patterns in mortality, residual insecticidal activity, and population dynamics across at least one complete generation of *S. zeamais*, consistent with established protocols for botanical insecticide evaluation [23-25]. At each assessment, mortality was recorded by two independent observers blinded to treatment identity to minimise observer bias. Jars were sieved through a 2-mm mesh to separate live adults, dead adults, frass, and grain. Mortality determination employed the probe-response method, wherein insects failing to exhibit coordinated movement after gentle prodding with a fine brush were classified as dead [34].

Because the same jars containing the same surviving insects were evaluated at each interval, with mortality recorded sequentially over time, the dataset constitutes a repeated-measures structure requiring appropriate analytical treatment. Dead insects were removed at each assessment to prevent confounding of subsequent mortality counts, while live insects were returned to their original jars, ensuring that each jar remained an independent experimental unit throughout the 56-day experimental period. Mortality percentage was calculated as follows: Mortality (%) = (Number of dead weevils/Total weevils) × 100. Control mortality remained below 10% throughout the experimental period; therefore, Abbott's correction was not applied, consistent with World Health Organization recommendations that control mortality below 10% (with values below 5% preferred) does not necessitate mathematical adjustment [35].

Seed weight loss was quantified at each 14-day interval using an analytical balance with 0.01 g precision. Weight loss values represented interval-specific losses calculated independently for each assessment period, not cumulative losses from experiment initiation, following the methodology of Suleiman et al. [36]: Weight loss (%) = [(W1 − W2)/W1] × 100, where W1 represents the seed weight at the beginning of the interval and W2 represents the weight at interval conclusion. This interval-specific approach enables detection of temporal patterns in feeding damage that cumulative measurements would obscure.

Data analysis

Statistical analyses were conducted using analysis of variance (ANOVA) frameworks appropriate to the experimental objectives and data structure. Three separate analytical approaches were employed corresponding to distinct research questions. Dose-response relationships among botanical treatments were examined using two-way factorial ANOVA with botanical type (10 levels) and concentration (4 levels: 5, 10, 15, and 20 g per 200 g maize seeds) as fixed factors, based on 160 observations (10 botanicals × 4 concentrations × 4 replicates). This analysis excluded synthetic and untreated controls to focus specifically on concentration-dependent effects within the botanical treatment category.

Temporal dynamics of mortality were analysed using two-way repeated-measures ANOVA, with treatment (12 levels: 10 botanicals, synthetic control, and untreated control) as the between-subjects factor and storage duration (four levels: 14, 28, 42, and 56 days) as the within-subjects factor, based on 192 observations (12 treatments × 4 time points × 4 replicates). The repeated-measures framework was necessitated by the non-independence of observations across time arising from repeated assessment of the same experimental units. Sphericity was evaluated using Mauchly's test prior to interpretation of within-subjects effects; where the sphericity assumption was violated ($p < 0.05$), degrees of freedom were adjusted using the Greenhouse-Geisser correction when epsilon was less than 0.75, or the Huynh-Feldt correction when epsilon exceeded 0.75, following established recommendations for repeated-measures designs [37]. Interval-specific mortality values at each time point represented deaths occurring within each discrete 14-day period rather than cumulative mortality from experiment initiation.

Seed weight loss was similarly analysed using two-way repeated-measures ANOVA, with treatment (12 levels) as the between-subjects factor and storage interval (four levels: 0-14, 14-28, 28-42, and 42-56 days) as the within-subjects factor, based on 192 observations. Weight loss values represented interval-specific measurements calculated independently at each assessment period. Sphericity was assessed and corrected as described above. Mortality proportions were arcsine square-root transformed [arcsin(√p)] prior to analysis to stabilise variance and improve approximation to normality, following established conventions for proportional data in entomological bioassays [38]. Variance homogeneity for between-subjects effects was evaluated using Levene's test. Where variances were homogeneous, Tukey's Honestly Significant Difference (HSD) test was applied for pairwise comparisons among treatment means; where Levene's test indicated heterogeneity ($p < 0.05$), Games-Howell comparisons were employed to maintain valid Type I error rates. All analyses were performed using R version 4.4.1 [39], with statistical significance determined at $\alpha = 0.05$.

Results And Discussion

This investigation evaluated the insecticidal efficacy of 10 botanical treatments and a synthetic insecticide control against *S. zeamais* in stored maize seeds, examining three principal parameters: dose-response relationships across four concentrations (5, 10, 15, and 20 g per 200 g maize seeds), temporal dynamics of weevil mortality across four storage intervals (0-14, 14-28, 28-42, and 42-56 days), and seed protection capacity as quantified by interval-specific weight loss.

Dose-response relationships in botanical insecticide efficacy

Mortality exhibited progressive increases with concentration across the majority of treatments, although the magnitude of response varied considerably among botanical species (Figure 3). Two-way factorial analysis of variance revealed highly significant main effects for botanical type ($F_{9,120} = 149.53$, $p < 0.001$) and concentration ($F_{3,120} = 39.58$, $p < 0.001$), with a significant interaction effect ($F_{27,120} = 1.68$, $p = 0.019$), indicating that dose-response slopes differed among botanical treatments (Table 2).

Solanum incanum leaf powder exhibited the strongest dose-response relationship among all botanical treatments evaluated, with mean mortality (±SEM) increasing from 62.52 ± 6.24% at 5 g per 200 g seeds to 84.09 ± 4.17% at 20 g per 200 g seeds, representing a 21.57 percentage-point increase across the concentration range. Post-hoc analysis (Tukey's HSD, $\alpha = 0.05$) positioned *S. incanum* within a distinct high-efficacy statistical grouping (Group 'a') at all concentrations tested. Notably, even the lowest concentration evaluated (5 g per 200 g seeds, equivalent to 2.5% w/w) achieved mortality exceeding 60%, suggesting that *S. incanum* delivers substantial efficacy at economically favourable application rates.

Cajanus cajan demonstrated the steepest proportional dose-response among intermediate-efficacy treatments, with mortality rising from 23.45 ± 2.46% at 5 g per 200 g seeds to 58.85 ± 3.38% at 20 g per 200 g seeds, a 35.4 percentage-point increase, representing a 2.5-fold enhancement across the concentration range. *Eucalyptus globulus* exhibited comparable response patterns, with mortality increasing from 29.75 ± 5.18% to 44.43 ± 5.92% across the concentration range. Both species occupied intermediate statistical groupings (Group 'b' and 'c'), demonstrating moderate but concentration-responsive efficacy.

In contrast, several botanical treatments exhibited consistently low efficacy irrespective of concentration. *L. camara*, *O. basilicum*, *C. citratus*, and *H. suaveolens* achieved maximum mortalities below 20% even at the highest concentration tested (20 g per 200 g seeds) and were consistently assigned to the lowest efficacy statistical groups (Groups 'd' and 'e'). The failure of these treatments to demonstrate meaningful dose-response relationships indicates limited practical potential regardless of application rate, suggesting that their bioactive compound profiles lack sufficient contact toxicity against *S. zeamais* adults under the experimental conditions employed.

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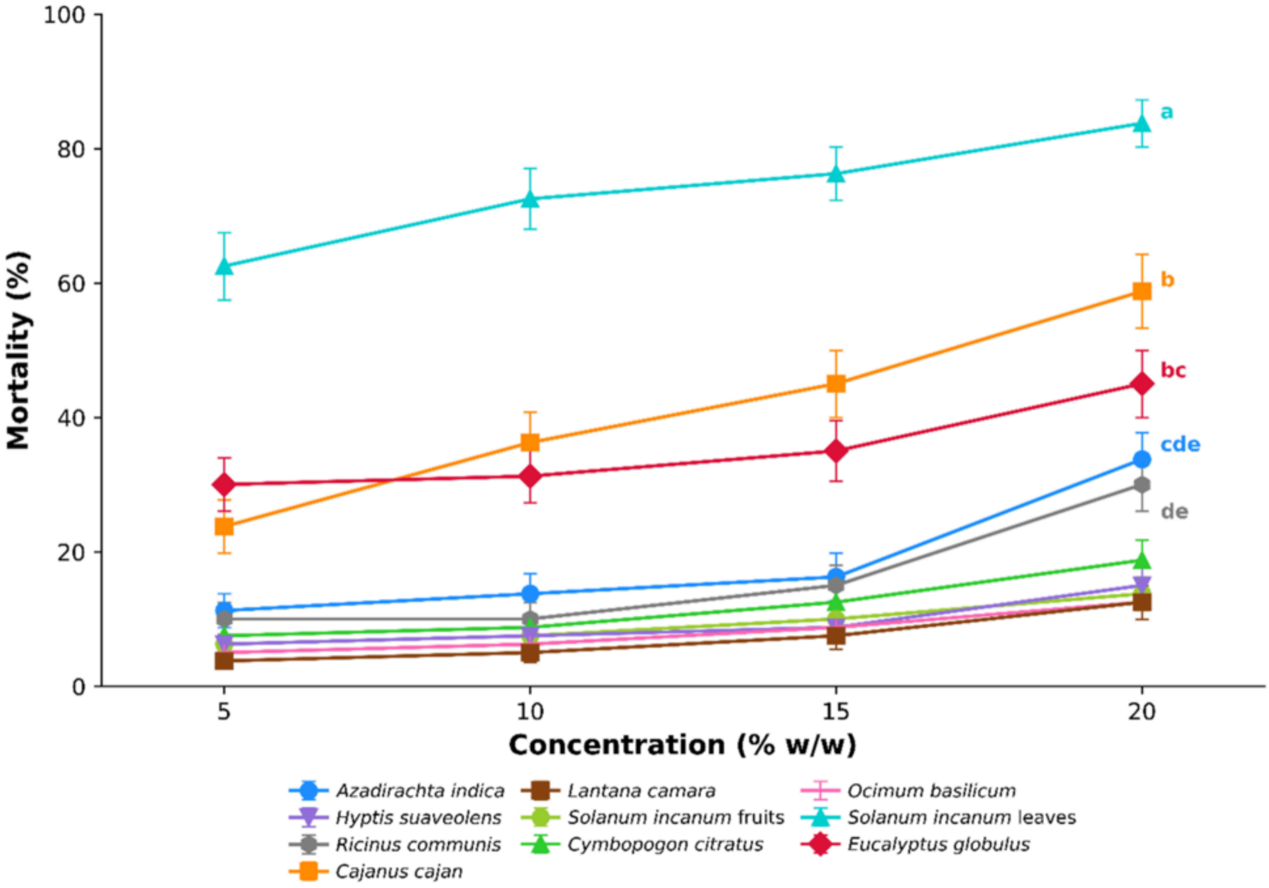

FIGURE 3: Dose-response relationships of S. zeamais adult mortality (%) to 10 botanical powder treatments at four concentration levels (5, 10, 15, and 20 g per 200 g of maize seeds). Data are presented as mean values ± SEM (n = 4 replicates per treatment-concentration combination). A two-way factorial ANOVA revealed highly significant main effects of botanical treatment ($p < 0.001$) and concentration ($p < 0.001$), as well as a significant interaction effect ($p = 0.019$), indicating differential dose-response patterns among botanical species. Treatment means sharing the same letter within each concentration level are not significantly different (Tukey's HSD, α = 0.05).

ANOVA, Analysis of Variance; HSD, Honestly Significant Difference; SEM, Standard Error of the Mean

Source of Variation	df	Sum of Squares	Mean Square	F-value	p-value
Botanical treatment	9	191,452.36	21,272.48	149.53	<0.001***
Concentration	3	16,891.06	5,630.35	39.58	<0.001***
Botanical × Concentration	27	6,453.61	239.02	1.68	0.019*
Residual	120	17,069.37	142.24	—	—
Total	159	231,866.40	—	—	—

TABLE 2: Two-way factorial ANOVA examining the effects of botanical treatment and concentration on S. zeamais adult mortality (%). The analysis evaluated 10 botanical powders across four concentration levels (5, 10, 15, and 20 g per 200 g maize seeds) in a completely randomised factorial design (n = 4 replicates per treatment combination; N = 160 observations).

***p < 0.001; *p < 0.05.
ANOVA, Analysis of Variance

This investigation provides the first systematic comparative evaluation of 10 botanical powders against *S. zeamais* under controlled laboratory conditions, addressing four interconnected objectives: mortality assessment relative to synthetic standards, dose-response characterisation across multiple concentrations, temporal efficacy dynamics over an extended 56-day storage period, and seed protection capacity as quantified through interval-specific weight loss. The principal finding establishes that *S. incanum* leaf powder achieved exceptional interval-specific mortality during the initial 0-14-day assessment period (88.75%), approaching synthetic insecticide standards (100%), while maintaining substantial insecticidal activity across all subsequent assessment intervals and providing seed protection statistically equivalent to chemical controls during the final storage interval (0.25% versus 3.55% interval-specific weight loss in untreated controls at 42-56 days).

These findings provide robust support for our hypothesis that *S. incanum* leaf powder would demonstrate superior and more persistent insecticidal activity compared to essential oil-based botanicals. The temporal stability data offer particularly compelling evidence: *Solanum incanum* maintained substantial interval-specific mortality across all four assessment intervals (0-14, 14-28, 28-42, and 42-56 days), whereas *E. globulus*, representative of terpenoid-based treatments, exhibited progressively diminishing interval-specific mortality with minimal insecticidal activity during the final assessment interval. This differential persistence aligns with the hypothesised greater environmental stability of glycoalkaloid compounds relative to volatile terpenoids [21,40]. The highly significant treatment × storage interval interaction ($p < 0.001$) further underscores that temporal dynamics vary fundamentally among botanical classes, validating the importance of compound chemistry in determining practical storage protection periods.

Dose-response relationships and species-specific concentration

Our analysis revealed significant main effects for botanical type ($p < 0.001$) and concentration ($p < 0.001$), with a significant interaction ($p = 0.019$), demonstrating that dose-response patterns differ substantially among botanical species. This interaction carries important practical implications: optimal application rates cannot be universally prescribed across all botanical treatments; rather, concentration recommendations must be species-specific to maximise efficacy while minimising material costs and potential phytotoxic effects.

Solanum incanum leaf powder exhibited the strongest absolute dose-response relationship among all treatments evaluated, with mortality increasing from 62.52% at 5 g per 200 g seeds to 84.09% at 20 g per 200 g seeds. Notably, even the lowest concentration tested exceeded 60% mortality, suggesting that *S. incanum* achieves substantial efficacy at economically favourable application rates that minimise material requirements without sacrificing protective capacity. This finding contrasts markedly with several other treatments, including *L. camara*, *O. basilicum*, and *H. suaveolens*, which failed to exceed 20% mortality even at maximum concentrations, indicating limited practical potential regardless of application rate.

Cajanus cajan demonstrated the steepest proportional dose-response among intermediate-efficacy treatments, with mortality rising from 23.45% to 58.85% across the concentration range, a 2.5-fold enhancement that suggests higher application rates may be warranted for this species, particularly given its widespread availability as a food crop throughout Tanzania. In contrast, *A. indica* showed a relatively shallow dose-response (10.75-34.72%), consistent with reports that azadirachtin's primary mode of action involves growth regulation and antifeedant effects rather than acute contact toxicity [15].

Temporal dynamics of *S. zeamais* mortality in response to botanical treatments

Storage duration exerted a significant effect on treatment efficacy, with overall mean mortality across all treatments declining from 35.05% during the initial 0-14-day interval to 13.56% during the final 42-56-day interval, representing an approximately 61% reduction in mean efficacy over the experimental period (Figure *4* and Table *3*). Two-way repeated-measures ANOVA, with treatment as the between-subjects factor and storage duration as the within-subjects factor, revealed highly significant effects across all components of the model. Mauchly's test indicated violation of the sphericity assumption for the main effect of storage duration ($p < 0.001$) and the treatment × storage duration interaction ($p < 0.001$); consequently, degrees of freedom were corrected using the Greenhouse-Geisser estimate ($\varepsilon = 0.638$ for storage duration; $\varepsilon = 0.612$ for the interaction term).

The between-subjects effect of treatment was highly significant ($F11,36 = 231.42$, $p < 0.001$), confirming substantial differences in overall mortality among botanical treatments across the experimental period. Within-subjects analysis revealed significant effects for storage duration ($F1.91,68.94 = 65.23$, $p < 0.001$) and the treatment × storage duration interaction ($F21.04,68.94 = 3.14$, $p < 0.001$), indicating that efficacy deterioration rates differed substantially among treatments across the experimental timeline.

Solanum incanum leaf powder demonstrated exceptional and sustained efficacy across all storage intervals, achieving mean interval-specific mortalities of 88.75% (0-14 days), 81.56% (14-28 days), 70.53% (28-42 days), and 54.36% (42-56 days). This treatment consistently approached the performance of the synthetic standard, which maintained 100% mortality throughout the experimental period under laboratory conditions. At 56 days post-treatment, *S. incanum* leaf powder retained 61% of its initial 14-day activity (calculated as 54.36/88.75 × 100), demonstrating superior temporal stability compared to all other botanical treatments evaluated.

Cajanus cajan provided consistent mid-range efficacy throughout the storage period, with interval-specific mortalities of 42.92% (0-14 days), 44.59% (14-28 days), 45.17% (28-42 days), and 30.96% (42-56 days). This treatment exhibited relatively stable performance through 42 days, retaining 72% of initial activity at the final assessment (30.96/42.92 × 100), suggesting moderate persistence of bioactive compounds under the experimental storage conditions. The stable efficacy profile through the first three intervals, followed by decline only in the final interval, distinguishes *C. cajan* from treatments exhibiting progressive deterioration throughout the experimental period.

Eucalyptus globulus achieved initial interval-specific mortalities of 48.11% (0-14 days) and 48.10% (14-28 days), maintaining stable efficacy through the first month of storage. However, mortality declined to 37.07% during the 28-42-day interval and dropped markedly to 7.83% during the final 42-56-day interval, representing an 84% reduction in efficacy over the experimental period (calculated as [48.11 − 7.83]/48.11 × 100). This pronounced decline in the final interval likely reflects volatilisation of eucalyptol (1,8-cineole) and other monoterpenes responsible for insecticidal activity, compounds characterised by high vapour pressures that promote rapid dissipation under ambient storage conditions.

Post-hoc analysis (Tukey's HSD, α = 0.05) clustered treatments into five statistical groupings based on overall efficacy: Group 'a' encompassed *S. incanum* leaves and the synthetic control, exhibiting highest efficacy (54-100% mortality at 56 days); Group 'b' encompassed *C. cajan* and *E. globulus*, demonstrating moderate efficacy (8-48% mortality); Group 'c' encompassed *A. indica* and *R. communis*, showing low-moderate efficacy (4-25% mortality); Groups 'cd' through 'de' encompassed treatments with low efficacy (3-17% mortality); and Group 'e' represented the untreated control baseline (0-3% mortality).

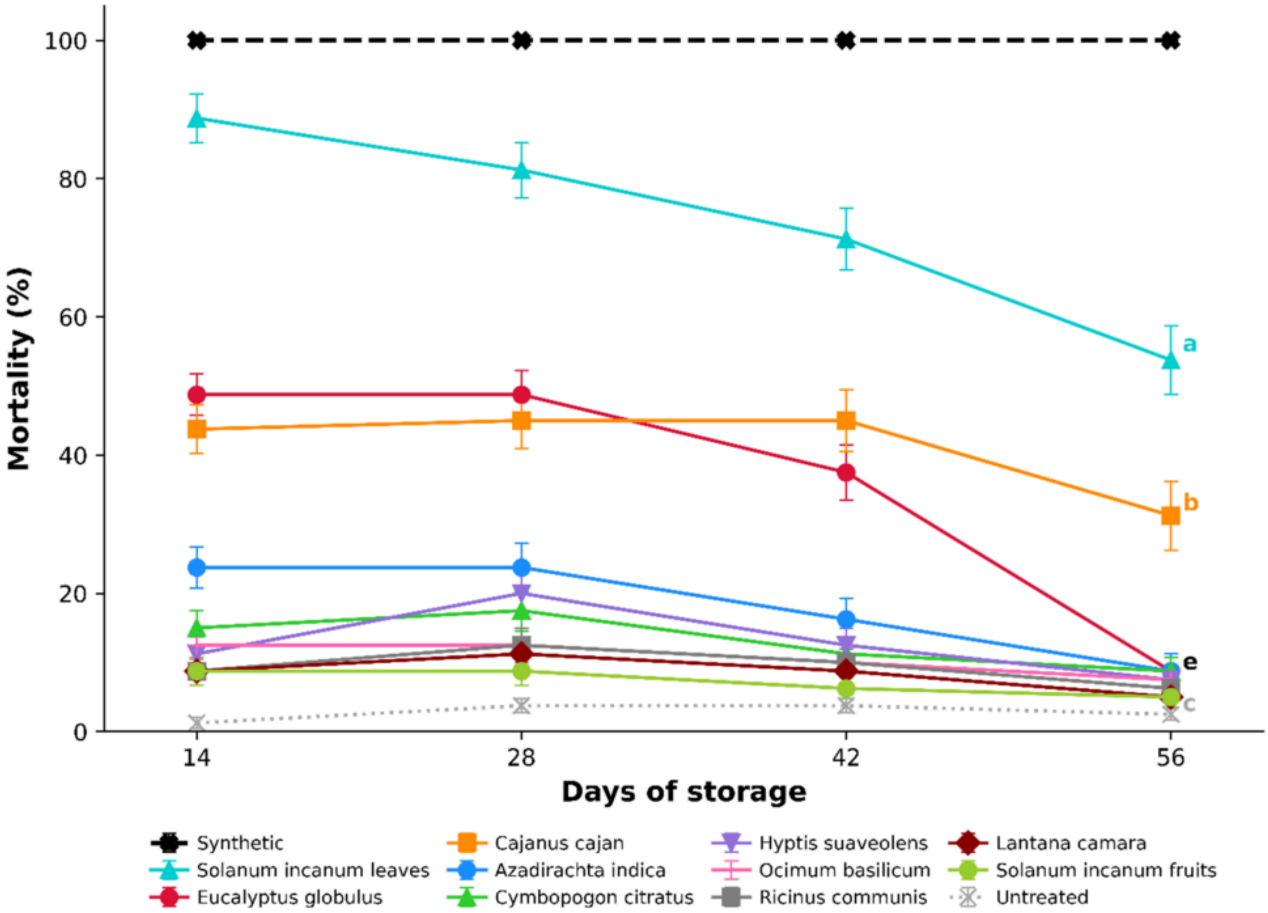

FIGURE 4: Temporal dynamics of interval-specific mortality (%) of S. zeamais adults exposed to 10 botanical powders, a synthetic pesticide control (Actellic Super Dust®), and untreated control across four storage intervals (0–14, 14–28, 28–42, and 42–56 days). Mortality values represent the percentage of deaths occurring within each discrete 14-day interval rather than cumulative mortality from the experiment's initiation. Data points represent mean mortality ± SEM (n = 4 replicates per treatment). Two-way repeated-measures ANOVA with Greenhouse-Geisser correction revealed highly significant effects for treatment (p < 0.001), storage interval (p < 0.001), and their interaction (p < 0.001). Lowercase letters denote significant differences among treatments at the 42–56-day interval (Tukey's HSD, α = 0.05). Solanum incanum leaves exhibited the highest sustained efficacy among botanical treatments, maintaining substantial interval-specific mortality throughout the experimental period.

***p < 0.001.

ANOVA, Analysis of Variance; HSD, Honestly Significant Difference; SEM, Standard Error of the Mean

Source of Variation	df	Sum of Squares	Mean Square	F-value	p-value
Between-Subjects Effects					
Treatment	11	277,483.64	25,225.79	231.42	<0.001***
Error (between)	36	3,924.00	109	—	—
Within-Subjects Effects (GG corrected)					
Storage duration	1.91	21,330.71	11,152.21	65.23	<0.001***
Treatment × Storage duration	21.04	11,308.83	537.49	3.14	<0.001***
Error (within)	68.94	11,772.00	170.77	—	—

TABLE 3: Two-way repeated-measures ANOVA examining the effects of botanical treatment (between-subjects factor) and storage interval (within-subjects factor) on S. zeamais adult interval-specific mortality (%). The analysis evaluated 12 treatment groups (10 botanical powders, synthetic insecticide positive control, and untreated negative control) across four storage intervals (0–14, 14–28, 28–42, and 42–56 days post-treatment). Mortality values represented interval-specific deaths occurring within each discrete 14-day period rather than cumulative mortality from the experiment's initiation. Experimental units (n = 48 jars: 12 treatments × 4 replicates) were assessed repeatedly at each time point, yielding N = 192 total observations. Mauchly's test indicated violation of sphericity (p < 0.001); Greenhouse-Geisser corrections were applied (ε = 0.638).

***p < 0.001.
ANOVA, Analysis of Variance

Temporal efficacy dynamics and phytochemical stability

The 56-day assessment period represents a critical methodological extension beyond most previous botanical insecticide studies, which typically evaluate efficacy over 21-42 days and thereby fail to capture the efficacy dynamics relevant to the 4-8-month storage periods characteristic of smallholder systems. This extended timeline revealed substantial differences in residual activity that would not have been apparent in shorter trials, directly addressing our objective regarding temporal efficacy dynamics. The interval-specific mortality approach adopted in this study enabled detection of temporal patterns in treatment lethality across discrete 14-day periods, revealing whether treatments caused rapid initial mortality that subsequently diminished or sustained mortality throughout the storage period.

The consistent clustering of *S. incanum* leaves in statistical Group 'a' alongside the synthetic control throughout all assessment intervals confirms that this botanical can deliver performance approaching conventional chemical standards under controlled conditions. *Solanum incanum* leaf powder maintained substantial interval-specific mortality throughout the experimental period, retaining 61% of its initial 14-day activity at 56 days (calculated as 54.36/88.75 × 100). The practical significance of these findings depends on storage requirements: for short-to-medium term storage (8-10 weeks), *S. incanum* may provide adequate protection without reapplication, whereas extended storage would benefit from reapplication or integration with complementary management strategies.

The contrasting temporal profiles of glycoalkaloid-based versus terpenoid-based botanicals provide mechanistic insight into the determinants of residual activity. *Eucalyptus globulus* achieved comparable initial interval-specific mortality to *C. cajan* during the 0-14-day interval but exhibited markedly reduced mortality during subsequent intervals, with minimal insecticidal activity observed during the 42-56-day interval, representing an 84% loss of initial efficacy. This pattern of declining interval-specific efficacy aligns with the known volatility of 1,8-cineole and other monoterpenes responsible for eucalyptus insecticidal activity [26,40]. Ngamo Tinkeu et al. [41] similarly reported that essential oil-based insecticides against *S. zeamais* rarely maintain efficacy beyond 21 days without reformulation. In contrast, the relative stability of *S. incanum* interval-specific mortality across all assessment periods is consistent with the non-volatile nature of steroidal glycoalkaloids, which remain biologically active under ambient storage conditions [29].

Interval-specific seed weight loss across storage duration and insecticidal treatments

Weight loss was quantified as interval-specific loss at each 14-day assessment period, representing the percentage of seed mass lost during each discrete interval rather than cumulative loss from experiment initiation. Two-way repeated-measures ANOVA, with treatment as the between-subjects factor and storage interval as the within-subjects factor, revealed significant effects across all model components (Table 4). Mauchly's test indicated violation of the sphericity assumption for the main effect of storage interval ($p < 0.001$) and the treatment × storage interval interaction ($p < 0.001$); consequently, Greenhouse-Geisser corrections were applied ($\varepsilon = 0.687$ for storage interval; $\varepsilon = 0.659$ for the interaction term).

Source of Variation	df	Sum of Squares	Mean Square	F-statistic	Pr (>F)
Treatment	11	295.343	26.849	28.36	<0.001***
Storage duration	3	144.547	48.182	50.89	<0.001***
Treatment × Storage duration	33	154.474	4.681	4.94	<0.001***
Residuals	518	490.475	0.947	–	–

TABLE 4: Two-way repeated-measures ANOVA examining the effects of botanical treatment (between-subjects factor) and storage duration (within-subjects factor) on interval-specific percentage weight loss of maize seeds over the 56-day experimental period. Mauchly's test indicated violation of the sphericity assumption for the main effect of storage interval ($p < 0.001$) and the treatment × storage interval interaction ($p < 0.001$); consequently, Greenhouse-Geisser corrections were applied ($\varepsilon = 0.687$ for storage interval; $\varepsilon = 0.659$ for the interaction term).

***$p < 0.001$.

The between-subjects effect of treatment on interval-specific weight loss was highly significant ($F_{11,36} = 28.36$, $p < 0.001$), confirming that botanical treatments differed substantially in their capacity to prevent feeding damage. Within-subjects analysis revealed significant effects for storage interval ($F_{2.06,74.18} = 50.89$, $p < 0.001$) and the treatment × storage interval interaction ($F_{22.66,74.18} = 4.94$, $p < 0.001$), indicating that protective capacity varied differentially among treatments across the experimental timeline.

Untreated controls exhibited substantially higher interval-specific weight loss compared to all treated samples across all assessment periods, demonstrating the protective value of both botanical and synthetic interventions. Interval-specific weight loss in untreated controls peaked at 5.64% during the 14-28-day interval and subsequently declined to 3.55%

during the 42-56-day interval. This temporal decline is consistent with reduced weevil feeding intensity during later storage periods, attributable to population aging, resource depletion, and density-dependent mortality effects within the untreated experimental units. The synthetic insecticide control (Actellic Super Dust®) provided complete seed protection throughout the experimental period, with interval-specific weight loss values of 0% from 28 days onward (Table *5* and Figure *5*).

Among botanical treatments, *S. incanum* leaf powder exhibited superior protective efficacy, with consistently low interval-specific weight losses of 0.83% (0-14 days), 1.42% (14-28 days), 0.33% (28-42 days), and 0.25% (42-56 days). During the final two assessment intervals, *S. incanum* achieved seed protection statistically equivalent to the synthetic standard (Tukey's HSD, $\alpha = 0.05$). The progressive decline in interval-specific weight loss observed across storage periods reflects diminishing feeding pressure as weevil populations declined due to treatment-induced mortality, thereby demonstrating the translation of insecticidal efficacy into tangible seed protection.

Eucalyptus globulus demonstrated comparable protective capacity during early storage intervals, with weight loss values ranging from 0.25% to 1.25% across the first three assessment periods. However, protective efficacy diminished during the final interval, corresponding to the marked decline in mortality observed at 56 days post-treatment. *Cajanus cajan* provided moderate seed protection with interval-specific weight losses ranging from 0.25% to 2.00% across the experimental period, consistent with its intermediate mortality efficacy. The significant treatment × storage interval interaction confirms that protective capacity is dynamically linked to residual insecticidal activity, with treatments exhibiting sustained mortality, particularly *S. incanum* leaf powder, maintaining low weight loss throughout the storage period, whereas treatments with declining efficacy exhibited correspondingly increased weight loss as surviving weevil populations resumed feeding activity.

Treatment	0–14 days		14–28 days		28–42 days		42–56 days	
	n	Weight loss (%) ± SEM	n	Weight loss (%) ± SEM	n	Weight loss (%) ± SEM	n	Weight loss (%) ± SEM
Azadiracht a indica	12	1.33 ± 0.225 ab	12	1.43 ± 0.194 cd	12	0.51 ± 0.234 bcd	12	1.36 ± 0.228 b
Cajanus cajan	12	0.83 ± 0.166 ab	12	2.00 ± 0.246 bcd	12	0.25 ± 0.179 cd	12	0.93 ± 0.293 b
Cymbopog on citratus	12	0.58 ± 0.193 b	12	3.01 ± 0.302 b	12	1.44 ± 0.152 bc	12	1.45 ± 0.236 b
Eucalyptus globulus	12	1.25 ± 0.218 ab	12	1.26 ± 0.131 d	12	0.25 ± 0.131 cd	12	1.27 ± 0.830 b
Hyptis suaveolens	12	1.50 ± 0.151 a	12	2.35 ± 0.287 bcd	12	1.70 ± 0.314 b	12	1.29 ± 0.340 b
Lantana camara	12	0.58 ± 0.193 b	12	2.51 ± 0.229 bc	12	0.76 ± 0.310 bcd	12	1.36 ± 0.366 b
Ocimum basilicum	12	0.92 ± 0.229 ab	12	2.09 ± 0.313 bcd	12	1.44 ± 0.405 bc	12	1.71 ± 0.448 b
Ricinus communis	12	0.58 ± 0.193 b	12	2.09 ± 0.228 bcd	12	0.68 ± 0.228 bcd	12	0.93 ± 0.292 b
Solanum incanum fruits	12	0.75 ± 0.250 ab	12	2.68 ± 0.310 b	12	1.44 ± 0.361 bc	12	0.94 ± 0.153 b
Solanum incanum leaves	12	0.83 ± 0.166 ab	12	1.42 ± 0.193 cd	12	0.33 ± 0.222 cd	12	0.25 ± 0.179 b
Actellic Super Dust ®	11	1.00 ± 0.000 ab	11	0.00 ± 0.000 e	10	0.00 ± 0.000 d	10	0.00 ± 0.000 b
Untreated control	11	1.00 ± 0.000 ab	11	5.64 ± 0.152 a	11	3.48 ± 0.447 a	11	3.55 ± 0.578 a

TABLE 5: Mean interval-specific percentage weight loss (± SEM) of maize seeds treated with 10 botanical powders, a synthetic insecticide (Actellic Super Dust®), and an untreated control across four successive 14-day storage intervals (0–14, 14–28, 28–42, and 42–56 days). Values represent interval-specific losses calculated independently for each assessment period, following the methodology of Suleiman et al. [36]. Superscript letters denote statistical groupings within each storage interval based on Tukey's HSD test (α = 0.05); treatments sharing the same letter within a column are not significantly different.

HSD, Honestly Significant Difference; SEM, Standard Error of the Mean

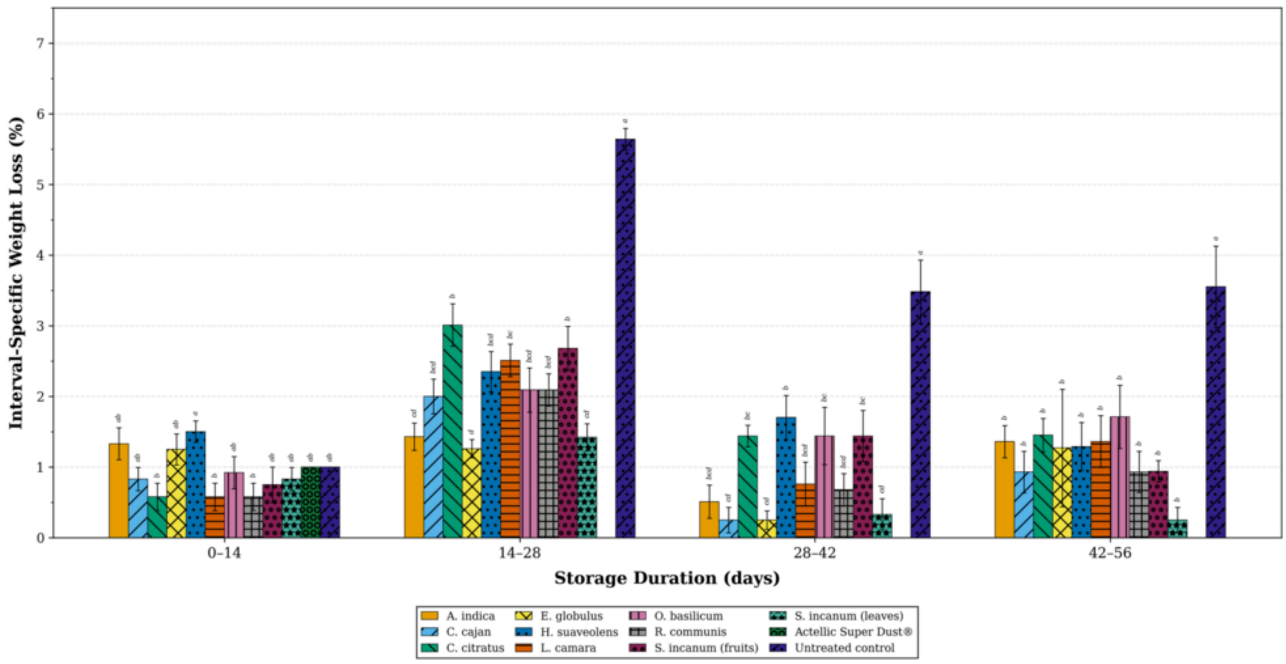

FIGURE 5: Mean interval-specific percentage weight loss (± SEM) of maize seeds treated with botanical powders across four successive 14-day storage intervals (0–14, 14–28, 28–42, and 42–56 days after treatment). Two-way repeated-measures ANOVA revealed highly significant effects for treatment (F11,36 = 28.36, p < 0.001), storage duration (F2.06,74.18 = 50.89, p < 0.001), and treatment × storage duration interaction (F22.66,74.18 = 4.94, p < 0.001). Greenhouse-Geisser corrections were applied (ε = 0.687 for storage interval; ε = 0.659 for the interaction). Superscript letters denote statistical groupings within each storage interval (Tukey's HSD, α = 0.05); treatments sharing the same letter are not significantly different. Untreated controls exhibited the highest weight loss across all intervals, peaking at 5.64% during the 14–28-day interval and declining to 3.55% during the 42–56-day interval. Solanum incanum leaf powder and Actellic Super Dust® provided consistently superior seed protection, with S. incanum achieving statistical equivalence with the synthetic standard during the final two assessment intervals.

ANOVA, Analysis of Variance; HSD, Honestly Significant Difference; SEM, Standard Error of the Mean

Seed protection capacity and translation of mortality to practical outcomes

Weight loss measurements provide a direct indicator of seed protection capacity, translating mortality data into practical outcomes of immediate relevance for seed security. The significant treatment effect ($p < 0.001$) and treatment × storage interval interaction ($p < 0.001$) demonstrate that botanical treatments differ not only in their capacity to kill weevils but also in their ability to prevent feeding damage over extended periods, a distinction of critical importance given that seed quality degradation accumulates progressively even at sublethal pest densities.

Solanum incanum leaf powder achieved seed protection statistically equivalent to the synthetic standard during the final two assessment intervals (28-42 and 42-56 days), with interval-specific weight losses of 0.33% and 0.25%, respectively. This equivalence is particularly noteworthy given that mortality rates remained below synthetic levels; it suggests that sublethal effects including antifeedant activity and behavioural disruption may contribute to seed protection beyond what mortality rates alone would predict. Solanaceae glycoalkaloids are known to exert antifeedant effects through bitter taste compounds and digestive enzyme inhibition [21], which may reduce consumption even among surviving individuals, thereby providing a dual mechanism of seed protection encompassing both direct mortality and feeding suppression.

Comparative performance of low- and intermediate-efficacy botanical treatments

While the primary focus of this investigation centres on the exceptional performance of *S. incanum* leaf powder, a comprehensive interpretation of the results requires systematic discussion of the differential performance observed across all botanical treatments evaluated, as several species demonstrated limited efficacy that merits explanation in the context of their known phytochemical properties and modes of action.

Four botanical treatments, *L. camara*, *O. basilicum*, *C. citratus*, and *H. suaveolens*, consistently achieved low mortality (<20%) even at the highest concentration tested (20 g per 200 g seeds), failing to demonstrate meaningful dose-response relationships across the entire concentration range. These findings contrast with reports of moderate to high insecticidal activity for these species when applied as essential oil extracts or fumigants. Ogendo et al. [24] documented 82.7-93.7% mortality with *L. camara* and *T. vogelii* after 21 days of exposure; however, their study employed aqueous extracts rather than dry powders, which likely facilitated greater bioavailability of volatile terpenoid constituents. The low efficacy of these species in the present study can be attributed to the fundamental limitations of the powder formulation for volatile compound-dependent plants: the primary bioactive constituents of *L. camara* (lantadenes), *O. basilicum* (linalool, eugenol), *C. citratus* (citral, geraniol), and *H. suaveolens* (1,8-cineole, β-caryophyllene) are characterised by high vapour pressures that promote rapid dissipation under ambient conditions [16,26]. In the dried powder formulation employed in this study, the shade-drying process, although conducted at ambient temperature to minimise thermal degradation, would have inevitably reduced the concentration of these volatile compounds relative to fresh plant material or liquid extract preparations. Furthermore, the contact toxicity mechanism required for powder-based delivery may be inherently less effective for species whose primary mode of action operates through fumigant or vapour-phase activity [16,22]. These findings suggest that the insecticidal potential of *L. camara*, *O. basilicum*, *C. citratus*, and *H. suaveolens* against *S. zeamais* may be better realised through alternative delivery systems, including essential oil extraction and encapsulation, rather than the simple powder formulations evaluated here.

Azadirachta indica exhibited a relatively shallow dose-response relationship, with mortality increasing from 10.75% at 5 g per 200 g seeds to only 34.72% at 20 g per 200 g seeds, representing moderate but substantially lower efficacy than *S. incanum* leaf powder across all concentrations. This performance is consistent with the established pharmacological profile of azadirachtin, the principal bioactive compound in neem, which exerts its insecticidal effects primarily through growth regulation disruption, ecdysis inhibition, antifeedant activity, and reproductive impairment rather than acute contact toxicity [15,28]. Erenso and Berhe [28] reported that neem seed powder achieved 82% mortality while leaf powder achieved 77.75% mortality at 3% w/w concentration; however, their experimental protocol involved a 28-day assessment period with naturally infested grain, conditions that may have captured the cumulative effects of antifeedant-induced starvation and growth regulation disruption more effectively than the interval-specific mortality measurements employed in the present study. The sublethal modes of action characteristic of azadirachtin, while potentially contributing to long-term population suppression through reduced fecundity and disrupted larval development, manifest less prominently in short-term adult mortality assessments, suggesting that the true protective value of *A. indica* powder may be underestimated by mortality-based efficacy metrics alone.

The stark contrast between *S. incanum* leaf powder (62.52-84.09% mortality) and fruit powder (9.55-13.32% mortality) warrants particular attention, as this 5-7-fold efficacy differential between plant organs carries important implications for both the understanding of bioactive compound distribution and practical extension recommendations. Leaves of

Solanaceae species typically accumulate substantially higher concentrations of steroidal glycoalkaloids, including solasonine and solamargine, compared to reproductive tissues, as these defensive compounds serve a primary ecological function in protecting photosynthetically active tissues from herbivory [21,29]. The distribution pattern of glycoalkaloids within *S. incanum* is consistent with the optimal defence theory, which predicts that constitutive chemical defences are concentrated in tissues of highest fitness value to the plant [29,30]. From a practical perspective, this finding carries critical implications for extension services and farmer recommendations: botanical insecticide formulations based on *S. incanum* should exclusively specify leaf material, and clear guidance must be provided to avoid inadvertent inclusion of fruit material, which would substantially dilute efficacy. Fortunately, leaf harvesting represents a sustainable approach, as it permits regrowth without destroying the parent plant, thereby enabling repeated harvesting from established *S. incanum* populations.

Ricinus communis demonstrated low-to-moderate efficacy, occupying an intermediate position between the low-efficacy volatile oil-based treatments and the moderately effective *C. cajan* and *E. globulus*. The bioactive constituents of castor, principally ricin and ricinine in seed tissue and ricinoleic acid derivatives in leaves, have documented insecticidal properties [22]; however, the leaf powder formulation employed in this study may not have delivered sufficient concentrations of these compounds to achieve meaningful mortality against adult *S. zeamais*, which possesses a heavily sclerotised exoskeleton that may limit penetration of contact-acting toxicants.

Temporal patterns in seed weight loss and their ecological interpretation

The temporal dynamics of interval-specific seed weight loss merit further discussion, as the patterns observed provide insight into both treatment efficacy and pest population ecology within the experimental system. Untreated controls exhibited peak interval-specific weight loss of 5.64% during the 14-28-day interval, which subsequently declined to 4.33% (28-42 days) and 3.55% (42-56 days). This declining trajectory in untreated controls is attributable to the interplay of several ecological factors: progressive population attrition through natural mortality and senescence of the original adult cohort; density-dependent resource competition as the finite seed substrate became increasingly depleted and degraded; and the accumulation of metabolic waste products (frass) that may have impeded further feeding activity within the confined experimental units [6,7]. The observation that weight loss did not accelerate in untreated controls despite ongoing infestation suggests that the carrying capacity of the 200 g grain substrate was approached during the mid-storage period, a finding consistent with the known population dynamics of *S. zeamais* in confined storage systems [8].

The progressive decline in interval-specific weight loss observed for *S. incanum* leaf powder-treated samples across storage intervals (0.83%, 1.42%, 0.33%, and 0.25% for the 0-14, 14-28, 28-42, and 42-56-day intervals, respectively) demonstrates the practical translation of sustained insecticidal efficacy into tangible seed protection. The transient increase in weight loss during the 14-28-day interval likely reflects a brief period of compensatory feeding by surviving weevils before the cumulative mortality effects of continued glycoalkaloid exposure reduced the remaining pest population to levels insufficient to cause measurable damage. The near-complete elimination of feeding damage during the final two intervals (0.33% and 0.25%), approaching the 0% weight loss achieved by the synthetic standard from 28 days onward, indicates that *S. incanum* leaf powder provides functionally equivalent seed protection to Actellic Super Dust® during extended storage, despite achieving lower absolute mortality rates. This observation suggests that the sublethal antifeedant effects of glycoalkaloid compounds [21,22] may provide an additional layer of protection beyond direct mortality, reducing consumption even among the fraction of the weevil population that survives exposure.

The significant treatment × storage interval interaction for weight loss ($p < 0.001$) merits emphasis, as it demonstrates that the temporal relationship between treatment and seed protection is not uniform across botanical classes. Treatments with sustained mortality, particularly *S. incanum* leaf powder and to a lesser extent *C. cajan*, maintained low and stable weight loss across all intervals, whereas treatments exhibiting declining mortality, notably *E. globulus*, showed corresponding increases in weight loss during later intervals as residual insecticidal activity dissipated and surviving weevil populations resumed active feeding. This dynamic coupling between insecticidal persistence and seed protection underscores the practical importance of selecting botanical protectants with sustained rather than merely high initial efficacy, particularly for smallholder storage systems where re-application at regular intervals may not be feasible.

Mechanistic basis for *S. incanum* superiority

Solanum incanum is reported to contain steroidal glycoalkaloids, including solamargine and solasonine, which exert insecticidal effects through multiple complementary mechanisms: acetylcholinesterase inhibition disrupting neuromuscular function, membrane cholesterol complexation causing cellular lysis, and mitochondrial respiration impairment reducing metabolic energy availability [21,22,42]. This multi-target mode of action may explain both the high initial mortality and sustained efficacy observed throughout the 56-day experimental period, as insects would need to develop resistance across multiple physiological systems simultaneously, a substantially higher evolutionary barrier than resistance to single-target synthetic compounds.

The stark contrast between leaf powder (62.52-84.09% mortality) and fruit powder (9.55-13.32% mortality) provides indirect evidence for glycoalkaloid involvement, as leaves typically accumulate substantially higher concentrations of these defensive compounds compared to reproductive tissues [29]. This 5-7-fold efficacy difference between plant organs carries important practical implications: botanical insecticide development should prioritise leaf material over fruits for *S. incanum*-based formulations, and extension recommendations should clearly specify leaf harvesting to avoid efficacy reduction from inadvertent inclusion of fruit material.

We did not conduct phytochemical assays on the materials used in this study; thus, the proposed mechanisms remain hypothetical and are inferred from the established literature on Solanaceae bioactive compounds. Targeted phytochemical profiling of the leaf powders employed here is recommended to confirm the presence and concentrations of solasonine, solamargine, and other candidate compounds before inferring causal mechanisms with greater confidence.

Implications for sustainable pest management in smallholder systems

These findings carry substantial implications for postharvest management strategies in sub-Saharan Africa, where synthetic insecticides remain economically inaccessible for many smallholder farmers and where pesticide-related health costs can represent 45-83% of household pesticide expenditures [14]. The demonstrated efficacy of *S. incanum* leaf powder offers a potentially transformative, locally available alternative for Masasi District and similar agro-ecological zones throughout tropical Africa.

The widespread distribution of *S. incanum* throughout tropical Africa, including Tanzania where it grows as a common weed in agricultural landscapes and fallow fields, enhances adoption potential within existing farming systems without requiring external inputs, specialised knowledge, or cash expenditure [43]. The simple processing requirements, shade drying and grinding, align with traditional farmer practices and require no specialised equipment beyond that available in most rural households. The economic implications are substantial: synthetic insecticides such as Actellic Super retail at 36,900-49,200 TZS per kilogram in Tanzanian markets, whereas preparation of *S. incanum* leaf powder requires only labour inputs for harvesting, drying, and grinding. Given that postharvest grain losses impose an estimated USD 4 billion annual burden on African farming households [5], integrating low-cost botanical protectants may offer a practical and affordable means of reducing losses and enhancing seed security for smallholder farmers cultivating maize for both consumption and farm-saved seed retention.

Study limitations and future research directions

Several limitations of this study warrant acknowledgement and suggest priorities for future investigation. First, laboratory bioassays conducted under ambient laboratory conditions (26.5 ± 2°C and 83 ± 2% RH) may not fully replicate the environmental variability encountered in traditional seed storage facilities; field validation under actual smallholder storage conditions is essential before practical recommendations can be confidently advanced. Second, the study evaluated botanical powders without examining potential effects on seed germination or seedling vigour, parameters essential for practical applicability where treated seeds will be planted in subsequent seasons. Third, the single-generation assessment over 56 days did not capture potential effects on *S. zeamais* reproduction, oviposition behaviour, and progeny development across multiple generations. Fourth, the absence of phytochemical characterisation prevents direct correlation of observed efficacy with specific bioactive compound concentrations.

Future research should prioritise (i) comprehensive phytochemical characterisation of *S. incanum* leaf extracts to identify and quantify glycoalkaloids responsible for insecticidal activity; (ii) germination and seedling vigour tests to ensure treated seed acceptability for planting; (iii) on-farm participatory validation trials under realistic storage conditions with variable temperature, humidity, and pest pressure; (iv) assessment of sublethal effects on pest reproduction and F1 generation development; and (v) investigation of potential resistance development in *S. zeamais* populations following repeated botanical exposure.

Conclusions

This study systematically evaluated 10 botanical powders against *S. zeamais* in stored maize seeds over 56 days, addressing mortality assessment, dose-response characterisation, temporal efficacy dynamics, and seed protection capacity. The principal finding is that *S. incanum* leaf powder exhibits exceptionally high insecticidal efficacy, achieving 88.75% interval-specific mortality during the initial 0-14-day assessment period and statistical placement in Group 'a' alongside the synthetic standard Actellic Super Dust® (Tukey's HSD, $\alpha = 0.05$) throughout the experimental period, with 54.36% mortality maintained at 56 days compared to 100% for the synthetic standard and 2.75% for untreated controls ($p < 0.001$). Dose-response analysis revealed significant main effects for botanical type ($p < 0.001$) and concentration ($p < 0.001$), with a significant interaction ($p = 0.019$), demonstrating differential response patterns among species; *S. incanum* leaf powder exhibited the strongest absolute dose-response, with mortality ranging from 62.52% at 5 g per 200 g seeds to 84.09% at 20 g per 200 g seeds, demonstrating substantial efficacy even at the lowest economically favourable application rate. Temporal efficacy dynamics differentiated botanical classes based on compound chemistry, as the significant treatment × storage duration interaction ($p < 0.001$) confirmed that *S. incanum* retained approximately 61% of its initial bioactivity after 56 days, attributable to the environmental persistence of glycoalkaloid compounds, whereas the terpenoid-based *E. globulus* lost approximately 84% of its initial efficacy over the same period, supporting the hypothesis that glycoalkaloid-based botanicals offer superior persistence compared to essential oil-based treatments. Seed protection assessment confirmed that *S. incanum* leaf powder provided protection statistically equivalent to synthetic standards during the final assessment intervals, with interval-specific weight loss of 0.25% at 42-56 days versus 3.55% in untreated controls ($p < 0.001$ for the treatment × storage interval interaction), indicating that protective capacity is dynamically linked to residual insecticidal activity.

These findings carry substantial implications for postharvest management in sub-Saharan Africa, where postharvest grain losses impose an estimated USD 4 billion annual burden on farming households and where synthetic insecticides remain economically prohibitive for many smallholder farmers, with pesticide-related health costs representing 45-83% of household pesticide expenditures. The widespread distribution of *S. incanum* throughout tropical Africa, combined with simple processing requirements (shade drying and grinding) aligned with traditional farmer practices and negligible input costs relative to synthetic insecticides (36,900-49,200 TZS/kg), positions this botanical as a uniquely accessible and affordable alternative for resource-constrained households. Within the broader framework of integrated pest management, *S. incanum* leaf powder should be considered as a valuable component of multi-strategy approaches combining botanical protectants with improved storage structures and cultural practices. Notwithstanding these promising findings, critical knowledge gaps remain that must be addressed before practical field recommendations can be advanced, including: effects on seed germination and seedling vigour essential for seeds intended for planting; phytochemical characterisation to confirm glycoalkaloid involvement and optimise formulations; on-farm participatory validation trials under realistic smallholder storage conditions with variable temperature, humidity, and pest pressure; and assessment of sublethal effects on *S. zeamais* reproduction and F1 progeny development across multiple generations. *Solanum incanum* leaf powder warrants prioritised consideration as a candidate for botanical insecticide development targeting stored seed protection in sub-Saharan Africa, and the demonstrated convergence of high efficacy, temporal stability, seed protection capacity, accessibility, and economic advantages collectively support accelerated field validation of *S. incanum* formulations as components of integrated pest management programmes for smallholder maize seed storage systems.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

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Disclosures

Plant subjects: The study involves use of specimen, the voucher no Not yet assigned by the MUHAS Herbarium. was submitted to Yes. Voucher specimens were prepared following standardized herbarium protocols and deposited at the Muhimbili University of Health and Allied Sciences (MUHAS) herbarium, Dar es Salaam-Tanzania, for permanent archival and future reference. herbarium. **Human subjects:** All authors have confirmed that this study did not involve human participants or tissue. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** This study was funded by the Agroecology Hub in Tanzania in collaboration with Swissaid (T). **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

Data Availability Statements

The datasets (and/or code) supporting this study are available from the corresponding author upon reasonable request.

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