



Research Article

Time- and Dose-dependent Toxicity of *Syngonium podophyllum* Schott (Araceae) Plant Extracts to Three Major Stored-product Pests

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Abstract

Insects that attack stored grain and pulses cause large losses of food, and the chemicals used against them are becoming less effective as the insects develop resistance. Plant extracts are one possible alternative. We tested extracts of *Syngonium podophyllum* Schott (Araceae), an ornamental plant grown widely in gardens and homes, against three of the most damaging stored-product pests: the red flour beetle *Tribolium castaneum* (Herbst), the pulse beetle *Callosobruchus chinensis* (L.) and the rice weevil *Sitophilus oryzae* (L.). Dried plant material was extracted in turn with three solvents, petroleum ether, chloroform and methanol, which draw out different groups of plant chemicals. Adult insects were exposed to four doses of each extract, in three dishes of ten insects per dose, and dead insects were counted every 6 hours for 48 hours. The petroleum ether and methanol extracts killed all three pests, whereas the chloroform extract killed none. The petroleum ether extract was the most toxic: after 24 hours, the dose needed to kill half of the insects was 0.22 mg cm⁻² for the red flour beetle and about 0.64 mg cm⁻² for the other two pests, and it was two to six times more potent than the methanol extract. Mortality kept rising with time in every case, so less extract was needed the longer the insects were exposed, and at the highest petroleum ether doses half of the insects died within about 6 to 11 hours. These results show that *S. podophyllum* contains compounds that are toxic to major storage pests, mainly in its non-polar fraction. Identifying these compounds, and testing them on stored grain under practical conditions, are the next steps.

Keywords

Botanical Insecticide, Plant Extract, Stored-product Pests, Araceae, Median Lethal Dose, Median Lethal Time, *Tribolium Castaneum*, *Sitophilus Oryzae*

1. Introduction

Grain and pulses are not safe once they are harvested. In developing countries a large share of the calories produced each year is lost after harvest, and storage is the stage at which the largest share of that loss occurs; reviews of the

grain supply chain put storage losses in developing countries at levels that scientific storage practice could reduce to one or two per cent [1]. In Bangladesh, where rice, wheat and pulses are stored on-farm in jute sacks, earthen containers

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and small metal bins, insect infestation is a routine part of that loss, and the insects responsible are the same few species that dominate warm-climate storage worldwide. Heavy reliance on agrochemicals to contain such losses is itself one of the anthropogenic pressures on the country's biological resources [2], which adds a further reason to look for materials that can be produced locally and used sparingly.

Three of those species were used in this study. The red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), is a secondary pest that cannot attack sound kernels but multiplies rapidly in flour, bran and broken grain, contaminating more than it consumes. The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), is a primary pest whose larvae develop hidden inside intact rice, wheat and maize kernels, so that infestation is often noticed only when adults emerge. The pulse beetle, *Callosobruchus chinensis* (L.) (Coleoptera: Chrysomelidae: Bruchinae), develops inside stored lentil, chickpea and other legume seeds and can ruin both the food and the seed value of a pulse stock; its reproductive performance also varies with the age of the female [3]. Because the three attack different commodities and differ in biology, a plant material that is active against all three would be more useful than one that affects a single pest.

Chemical control of these insects rests on a narrow base. Phosphine is the dominant fumigant, and resistance to it is now documented in major stored-product species across several continents, in some populations at levels that make standard treatments ineffective [4]. In stored wheat in Oklahoma, for example, eight of nine *T. castaneum* populations contained phosphine-resistant individuals, and the most resistant population was 119 times less susceptible than a laboratory strain [5]. Resistance following heavy reliance on chemicals is not confined to insect control: in the same region as the present study, tetracycline-resistant bacteria are widespread in fish ponds where antibiotics are used routinely [6]. Residual contact insecticides face parallel problems of resistance, residues in food and regulatory withdrawal. Synthetic insecticides also harm animals they are not aimed at: in Bangladesh, the carbamate carbofuran caused mortality and histopathological damage in the vital organs of the freshwater fish *Channa punctatus* [7]. This is the context in which plant-derived insecticides are studied: they are chemically diverse, are often active through several mechanisms at once, and may be produced from locally available material. Essential oils and other plant products act on several targets in the insect nervous system, generally show low mammalian toxicity and short environmental persistence, and have been slowed mainly by registration and formulation problems rather than by lack of activity [8]. In Bangladesh this line of work has a long history. Extracts of locally available species such as *Polygonum hydropiper* [9], *Urena sinuata* [10], *Melia azedarach* [11] and *Petunia integrifolia* [12] have been tested against *T. castaneum* and related pests, and neem preparations have been combined with synthetic insecticides to reduce the dose of the latter [13].

Research output on botanical insecticides has grown for four decades, yet commercial products remain few, and the gap between published activity and practical use is now openly discussed in the field [14]. Part of the problem is the quality of the screening literature itself. Many studies report that a crude extract kills insects without identifying the active constituents, without positive controls and without data that a formulator could use [15]. Careful recent work on essential oils shows how much detail matters: activity varies between pest species and between life stages of the same species, and the ranking of the materials tested changes with concentration and exposure period [16]. A screening study is therefore most useful when it reports not only whether a material is active but how activity depends on dose and on time, and how precisely each quantity has been estimated.

Syngonium podophyllum Schott (Araceae), the arrowhead vine, is native to Central and South America and is grown throughout the tropics as an ornamental, including in Bangladesh, where it is common in gardens and indoor pots. The plant contains terpenoids, flavonoids, alkaloids and saponins, and its extracts have shown antibacterial, antioxidant and cytotoxic activity [17, 18]. The same work also reported hepatotoxicity of an aqueous leaf extract in rats, so the plant cannot be assumed to be harmless to vertebrates. To our knowledge its activity against stored-product insects has not been reported. Two other ornamental aroids, *Epipremnum aureum* and *Aglaonema marantifolium*, have been screened against the same three pests using the same extraction scheme and bioassays in the same laboratory [19, 20], which allows a family-level comparison that is unusual in this literature.

A second aim of this study concerns how such data are analysed. In the standard design, dead insects are counted repeatedly in the same dishes, and a separate probit line is then fitted at each observation time. Those counts are not independent, because the same individuals are scored again and again, and the practice of treating them as independent has been criticised for decades [21]. With the narrow dose ranges typical of screening work, per-time fits also produce confidence limits that are extremely wide or cannot be computed at all, which is rarely reported. Using all observation times in a single model avoids both problems and yields the median lethal time, a quantity that matters in practice because a protectant that acts within hours is more useful than one that needs days.

The objectives of this study were therefore: (i) to determine whether petroleum ether, chloroform and methanol extracts of *S. podophyllum* are toxic to adult *T. castaneum*, *C. chinensis* and *S. oryzae*; (ii) to quantify how the lethal dose falls with exposure time and how the lethal time falls with dose, using a model appropriate for repeated counts; (iii) to compare the potency of the active extracts within each species; and (iv) to place the results alongside published values for related aroids and to state explicitly what this design can and cannot establish.

2. Materials and Methods

2.1. Study Site

The bioassays were carried out in the Crop Protection and Toxicology Laboratory, Department of Zoology, University of Rajshahi, Bangladesh, in 2017–2018.

2.2. Plant Material and Extraction

Healthy *S. podophyllum* plants were collected in the morning from the Botanical Garden, University of Rajshahi. The whole plant material was chopped into small pieces, spread on wooden trays and dried in the shade to avoid heat damage to sensitive constituents, then ground to a powder in a grinder. Powder (150 g) was extracted sequentially with three solvents in order of increasing polarity: petroleum ether, then chloroform, then methanol. For each solvent the powder was soaked in 450 mL, agitated on a shaker and filtered after 48 h, and the procedure was repeated three times. The combined filtrates were evaporated at room temperature, and the residues were transferred to labelled glass vials and stored until use. Extracting with solvents of increasing polarity separates plant constituents into broad chemical groups, so that any activity can be traced to a particular fraction. Extract yields were not recorded, and no herbarium voucher was deposited.

2.3. Test Insects

Adults of all three species came from stock cultures maintained in the same laboratory. *Tribolium castaneum* was cultured on whole-wheat flour mixed with powdered dry yeast (19:1), sterilised before use, in an incubator at 30 °C. *Callosobruchus chinensis* was cultured on lentil seeds and *S. oryzae* on wheat grain at room temperature. Cultures were checked at regular intervals and sub-cultured to maintain a continuous supply of adults of known age. Adult *T. castaneum* 3–5 days old were used in the dose–mortality tests; the age of the other two species at testing was not recorded in the source documents.

2.4. Dose–mortality Bioassays

Tribolium castaneum was tested by the residual film method. One millilitre of extract solution was delivered to a 50 mm Petri dish and spread to give a uniform film; the solvent was allowed to evaporate at room temperature, leaving a dry deposit whose mass per unit dish area defines the dose in mg cm^{-2} . Ten adults were then introduced to each dish, which was covered. *Callosobruchus chinensis* and *S. oryzae* feed differently, so for these species the extract was dissolved in 1 mL of solvent, mixed uniformly with the food medium, and the solvent was allowed to evaporate before ten adults were added. For consistency with the original laboratory records, doses for these two species are also expressed in mg cm^{-2} . That unit has no direct physical meaning in a food-based assay; it indexes relative dose within a species, and for that reason we make no comparison of absolute potency between *T. castaneum* and the other two species anywhere in this article.

Doses were selected after pilot (ad hoc) experiments. The final series were 0.203, 0.305, 0.407 and 0.509 mg cm^{-2} (petroleum ether) and 1.018, 1.273, 1.527 and 1.782 mg cm^{-2} (methanol) for *T. castaneum*; 0.509, 0.763, 1.018 and 1.273 (petroleum ether) and 1.400, 1.527, 1.655 and 1.782 (methanol) for *C. chinensis*; and 0.763, 0.891, 1.018 and 1.145 (petroleum ether) and 1.018, 1.273, 1.527 and 1.782 (methanol) for *S. oryzae*. Each dose was replicated in three dishes of ten adults, giving 120 insects per extract–species combination. Untreated controls with the same number of insects were run alongside every series; no control mortality was recorded in the source data, so the Abbott [22] correction reduced to zero and observed mortality equals corrected mortality throughout. Cumulative mortality was recorded at 6, 12, 18, 24, 30, 36, 42 and 48 h after exposure. The operational criterion used to classify an insect as dead was not specified in the source records (Section 4.7). The chloroform extract was screened at pilot doses against all three species and caused no mortality, so it was not carried through to the dose–mortality stage. The complete design is summarised in Figure 1.

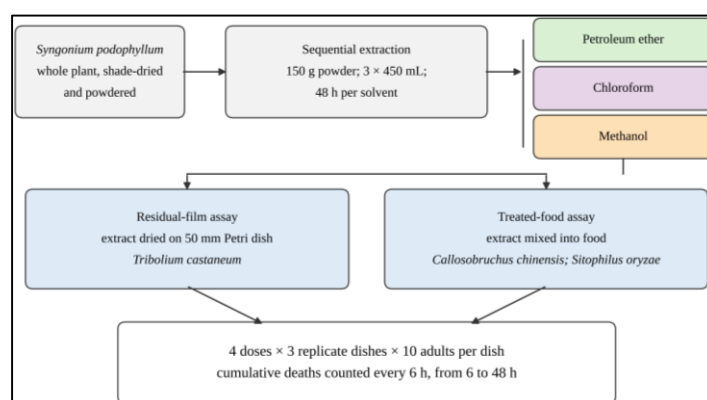


Figure 1. Design of the extraction and dose–mortality bioassays. Dried plant material was extracted sequentially in three solvents of increasing polarity. The chloroform extract was screened but caused no mortality in any species, so only the petroleum ether and methanol extracts entered the dose–mortality stage.

2.5. Statistical Analysis

The number of dead insects per dish (out of ten) at each observation time was modelled with a binomial generalized linear model with a probit link, in which the linear predictor contained the logarithm of dose and the logarithm of exposure time: $\text{probit}(p) = a + b \log_{10}(\text{dose}) + c \log_{10}(\text{time})$. Each extract–species combination was fitted separately to all 96 dish-by-time observations (12 dishes $\times$ 8 times). Because the same ten insects were scored at every time point, observations within a dish are correlated; standard errors were therefore clustered by dish, following the principle that repeated counts on the same organisms cannot be treated as independent [21]. The assumption that the dose slope does not change over time was tested by adding an interaction between log dose and log time.

Median lethal dose and median lethal time were obtained from the fitted model as $\log_{10}\text{LD}_{50}(t) = -(a + c \log_{10}t)/b$ and $\log_{10}\text{LT}_{50}(d) = -(a + b \log_{10}d)/c$. Confidence intervals (95%) were computed in two independent ways: by the delta method using the cluster-robust covariance matrix with a t critical value on 9 degrees of freedom (12 dishes minus three parameters), and by a bootstrap that resampled whole dishes with replacement within each dose (2000 resamples). The wider of the two limits is reported throughout, so that the intervals are conservative; with only three dishes per dose a bootstrap alone can understate uncertainty, which is why it is never used on its own here. Relative potency within a species was expressed as the ratio of the methanol to the petroleum ether LD_{50} , with a 95% interval computed on the logarithmic scale; the extracts were taken to differ when that interval excluded one.

Four checks were run on the analysis itself. Conventional probit models were also fitted separately at each observation time, so that our estimates can be compared with those reported by earlier screening studies that use this approach. The main model was refitted by direct maximum likelihood to confirm the estimates. Fitted and observed mortality were compared at every dose–time combination. Sensitivity to the assumed shape of the dose–response curve was examined by re-fitting every model with logit and complementary log-log links. Analyses were run in Python 3 with the statsmodels and SciPy libraries; the bootstrap seed was fixed so that results are reproducible. Replicate-level data and analysis scripts are available from the corresponding author.

3. Results

3.1. Screening and the Course of Mortality

The chloroform extract caused no mortality in any of the three species at any pilot dose, and is not considered further in this section. The petroleum ether and methanol extracts killed adults of all three species, and mortality accumulated steadily throughout the 48 h observation period rather than appearing abruptly (Figure 2). At the lowest petroleum ether dose, mortality of *T. castaneum* rose from 26.7% at 6 h to 50.0% at 24 h and 66.7% at 48 h; at the highest dose the corresponding values were 60.0%, 83.3% and 96.7%. The same pattern of separation between doses, and of continued mortality after 24 h, was present in every active combination.

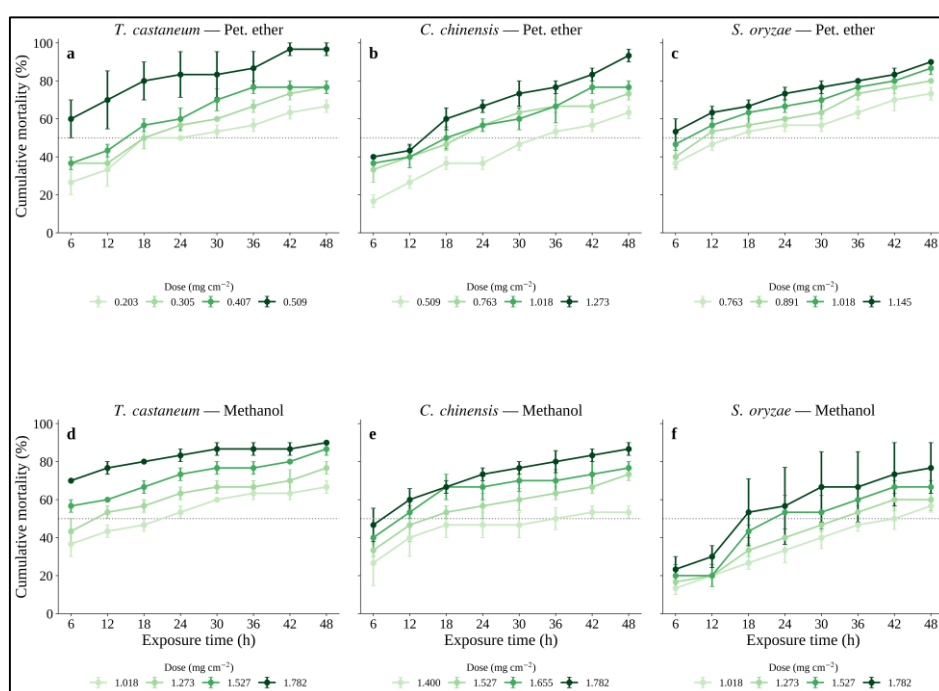


Figure 2. Cumulative mortality followed a consistent time course in every active extract–species combination. Points are means ($\pm$ SE) of three dishes of ten adults; the dotted line marks 50% mortality. (a–c) petroleum ether extract; (d–f) methanol extract. Note that doses differ between panels (see Section 2.4).

Table 1. Observed mortality (mean% $\pm$ SE of three dishes of ten adults) at 24 and 48 h. Control mortality was zero in all series, so observed and Abbott-corrected mortality are identical. Doses for *C. chinensis* and *S. oryzae* were applied to food and are not comparable with those for *T. castaneum* (Section 2.4).

Species	Extract	Dose (mg cm ⁻²)	Mortality at 24 h (%)	Mortality at 48 h (%)
<i>T. castaneum</i>	Pet. ether	0.203	50.0 $\pm$ 0.0	66.7 $\pm$ 3.3
<i>T. castaneum</i>	Pet. ether	0.305	56.7 $\pm$ 3.3	76.7 $\pm$ 3.3
<i>T. castaneum</i>	Pet. ether	0.407	60.0 $\pm$ 5.8	76.7 $\pm$ 3.3
<i>T. castaneum</i>	Pet. ether	0.509	83.3 $\pm$ 12.0	96.7 $\pm$ 3.3
<i>C. chinensis</i>	Pet. ether	0.509	36.7 $\pm$ 3.3	63.3 $\pm$ 3.3
<i>C. chinensis</i>	Pet. ether	0.763	56.7 $\pm$ 3.3	73.3 $\pm$ 3.3
<i>C. chinensis</i>	Pet. ether	1.018	56.7 $\pm$ 3.3	76.7 $\pm$ 3.3
<i>C. chinensis</i>	Pet. ether	1.273	66.7 $\pm$ 3.3	93.3 $\pm$ 3.3
<i>S. oryzae</i>	Pet. ether	0.763	56.7 $\pm$ 3.3	73.3 $\pm$ 3.3
<i>S. oryzae</i>	Pet. ether	0.891	60.0 $\pm$ 0.0	80.0 $\pm$ 0.0
<i>S. oryzae</i>	Pet. ether	1.018	66.7 $\pm$ 3.3	86.7 $\pm$ 3.3
<i>S. oryzae</i>	Pet. ether	1.145	73.3 $\pm$ 3.3	90.0 $\pm$ 0.0
<i>T. castaneum</i>	Methanol	1.018	53.3 $\pm$ 3.3	66.7 $\pm$ 3.3
<i>T. castaneum</i>	Methanol	1.273	63.3 $\pm$ 3.3	76.7 $\pm$ 3.3
<i>T. castaneum</i>	Methanol	1.527	73.3 $\pm$ 3.3	86.7 $\pm$ 3.3
<i>T. castaneum</i>	Methanol	1.782	83.3 $\pm$ 3.3	90.0 $\pm$ 0.0
<i>C. chinensis</i>	Methanol	1.400	46.7 $\pm$ 6.7	53.3 $\pm$ 3.3
<i>C. chinensis</i>	Methanol	1.527	56.7 $\pm$ 6.7	73.3 $\pm$ 3.3
<i>C. chinensis</i>	Methanol	1.655	66.7 $\pm$ 6.7	76.7 $\pm$ 3.3
<i>C. chinensis</i>	Methanol	1.782	73.3 $\pm$ 3.3	86.7 $\pm$ 3.3
<i>S. oryzae</i>	Methanol	1.018	33.3 $\pm$ 6.7	56.7 $\pm$ 3.3
<i>S. oryzae</i>	Methanol	1.273	40.0 $\pm$ 0.0	60.0 $\pm$ 5.8
<i>S. oryzae</i>	Methanol	1.527	53.3 $\pm$ 8.8	66.7 $\pm$ 3.3
<i>S. oryzae</i>	Methanol	1.782	56.7 $\pm$ 20.3	76.7 $\pm$ 13.3

3.2. Dose and Time Effects

In the time–dose–mortality models the coefficient for exposure time was positive and significant in all six extract–species combinations ($P < 0.001$; Table 2), confirming that mortality continued to accumulate after the first observation. The dose coefficient was positive and significant in five combinations ($P \leq 0.001$). The exception was the methanol extract against *S. oryzae* ($P = 0.084$), where one replicate at the highest dose had 5 of 10 insects dead at 48 h against 9 in each of the other two

replicates; that replicate was retained, and it widens the intervals reported for this combination.

The interaction between log dose and log time was not significant in any model ($P = 0.061$ – 0.624), so the assumption of a common dose slope across observation times is acceptable and the model can be summarised by a single dose slope per combination. Agreement between fitted and observed mortality was close: the largest discrepancy at any dose–time point was 13.9 percentage points, in the petroleum ether series against *T. castaneum*, and was 12 percentage points or less elsewhere. Fitted dose–mortality curves at 24 and 48 h are shown with the observed means in Figure 3.

Table 2. Coefficients of the probit time–dose–mortality model, $\text{probit}(p) = a + b \log_{10}(\text{dose}) + c \log_{10}(\text{time})$, fitted separately for each extract–species combination with dish-clustered standard errors. A positive coefficient indicates that mortality rises with the variable concerned.

Species	Extract	Dose coefficient (b)	P (dose)	Time coefficient (c)	P (time)	P (dose × time)	Largest fit gap (% points)
<i>T. castaneum</i>	Pet. ether	2.09	0.001	1.27	< 0.001	0.624	13.9
<i>C. chinensis</i>	Pet. ether	1.62	< 0.001	1.37	< 0.001	0.414	11.9
<i>S. oryzae</i>	Pet. ether	2.65	< 0.001	1.10	< 0.001	0.422	5.9
<i>T. castaneum</i>	Methanol	3.41	< 0.001	0.88	< 0.001	0.452	6.0
<i>C. chinensis</i>	Methanol	6.81	< 0.001	1.01	< 0.001	0.061	5.5
<i>S. oryzae</i>	Methanol	2.27	0.084	1.58	< 0.001	0.427	11.8

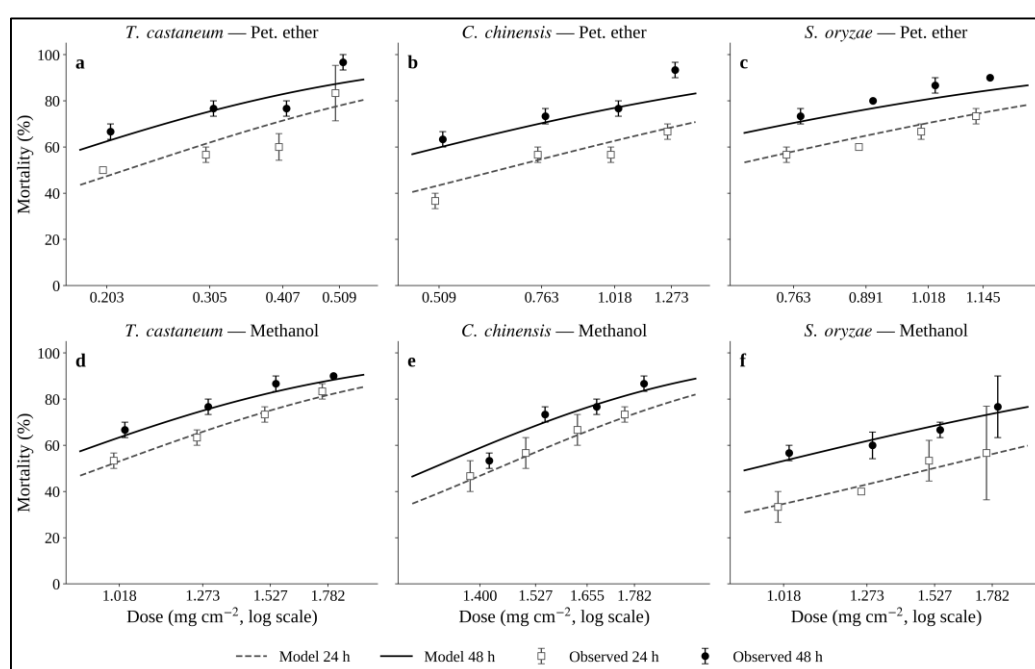


Figure 3. Mortality increased with dose and between 24 and 48 h for both active extracts. Points are observed means ($\pm$ SE) of three dishes; curves are fitted from the time–dose–mortality model. Dose is plotted on a logarithmic scale.

3.3. Median Lethal Dose and Its Decline with Exposure Time

LD_{50} fell steadily with exposure time in all six active combinations (Figure 4). For the petroleum ether extract at 24 h, the values were 0.218 mg cm^{-2} (95% CI 0.186–0.256) for *T. castaneum*, 0.644 mg cm^{-2} (95% CI 0.571–0.726) for *C. chinensis* and 0.639 mg cm^{-2} (95% CI 0.605–0.674) for *S. oryzae*; by 48 h they had fallen to 0.143, 0.358 and 0.478 mg cm^{-2} respectively (Table 3). For the methanol extract the 24 h values were 0.966, 1.439 and 1.521 mg cm^{-2} , falling to 0.807, 1.298 and 0.937 mg cm^{-2} at 48 h.

Several estimates, particularly at 48 h, lie below the lowest dose actually tested, because more than half the insects had already died at that dose by then; these are extrapolations of the fitted model and are marked as such in Table 3 and shown as dashed segments in Figure 4. Table 3 also gives the dose needed to kill 90% of the insects (LD_{90}), the figure most often used to judge practical control. In every case it lies above the highest dose tested, so a 90% kill would require doses beyond the range examined here; the closest were the methanol extract against *T. castaneum* and *C. chinensis* at 48 h, at about 1.9 and 2.0 mg cm^{-2} against a highest tested dose of 1.782 mg cm^{-2} . The widest LD_{50} interval was that for the methanol extract against *S. oryzae* at 24 h ($1.089\text{--}4.896 \text{ mg cm}^{-2}$), consistent with the non-significant dose effect for that combination.

Table 3. Median and 90% lethal doses (LD_{50} and LD_{90} , $mg\ cm^{-2}$) at 24 and 48 h from the time–dose–mortality model, with the dose slope ($\pm$ cluster-robust standard error). Confidence intervals for LD_{50} are the wider of the delta-method and dish-bootstrap limits; those for LD_{90} are delta-method limits.

Species	Extract	Time (h)	LD_{50}	95% CI	LD_{90}	95% CI	Slope $\pm$ SE	Doses tested
<i>T. castaneum</i>	Pet. ether	24	0.218	0.186–0.256	0.90 ^a	0.38–2.14	2.09 $\pm$ 0.64	0.203–0.509
<i>T. castaneum</i>	Pet. ether	48	0.143 ^a	0.078–0.215	0.59 ^a	0.32–1.06	2.09 $\pm$ 0.64	0.203–0.509
<i>C. chinensis</i>	Pet. ether	24	0.644	0.571–0.726	3.96 ^a	2.34–6.72	1.62 $\pm$ 0.21	0.509–1.273
<i>C. chinensis</i>	Pet. ether	48	0.358 ^a	0.285–0.447	2.20 ^a	1.47–3.31	1.62 $\pm$ 0.21	0.509–1.273
<i>S. oryzae</i>	Pet. ether	24	0.639 ^a	0.605–0.674	1.95 ^a	1.62–2.34	2.65 $\pm$ 0.24	0.763–1.145
<i>S. oryzae</i>	Pet. ether	48	0.478 ^a	0.426–0.537	1.46 ^a	1.27–1.67	2.65 $\pm$ 0.24	0.763–1.145
<i>T. castaneum</i>	Methanol	24	0.966 ^a	0.860–1.086	2.30 ^a	1.96–2.69	3.41 $\pm$ 0.43	1.018–1.782
<i>T. castaneum</i>	Methanol	48	0.807 ^a	0.693–0.941	1.92 ^a	1.70–2.17	3.41 $\pm$ 0.43	1.018–1.782
<i>C. chinensis</i>	Methanol	24	1.439	1.270–1.553	2.22 ^a	1.86–2.64	6.81 $\pm$ 1.65	1.400–1.782
<i>C. chinensis</i>	Methanol	48	1.298 ^a	1.084–1.461	2.00 ^a	1.75–2.29	6.81 $\pm$ 1.65	1.400–1.782
<i>S. oryzae</i>	Methanol	24	1.521	1.089–4.896	5.60 ^a	0.75–41.86	2.27 $\pm$ 1.31	1.018–1.782
<i>S. oryzae</i>	Methanol	48	0.937 ^a	0.214–1.287	3.45 ^a	0.81–14.60	2.27 $\pm$ 1.31	1.018–1.782

^a Estimate lies outside the range of doses tested and is an extrapolation. The chloroform extract caused no mortality and is not shown.

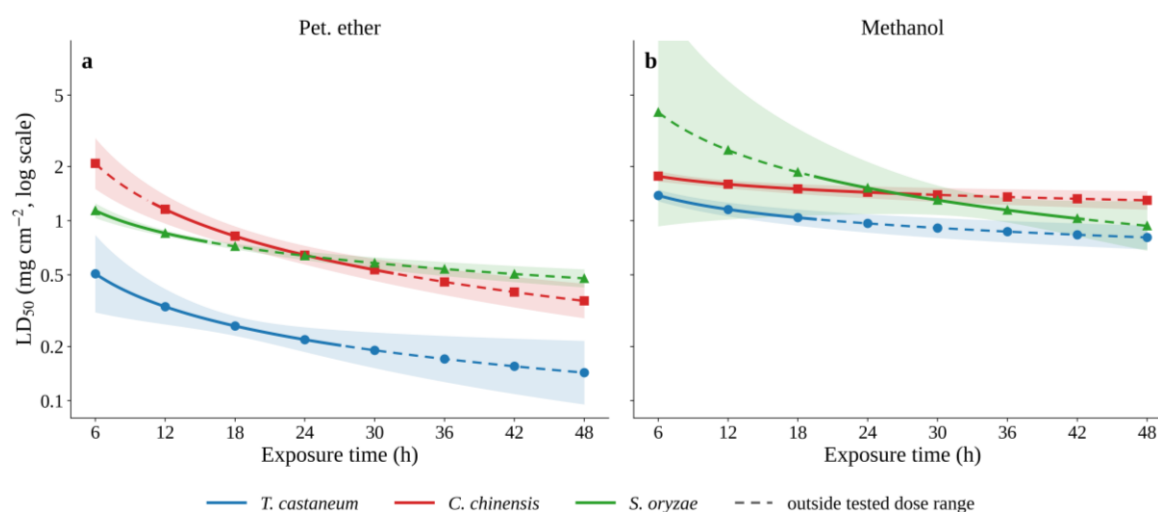


Figure 4. The dose needed to kill half the insects fell with exposure time. Lines are model estimates with 95% confidence bands; markers show the eight observation times; dashed segments lie outside the range of doses tested. (a) Petroleum ether extract; (b) methanol extract.

3.4. Dose and Time Combined

Because dose and time act together, the practical question is not the lethal dose alone but which combinations of dose and exposure reach a chosen level of control. Figure 5 shows the mortality surface predicted by the model across the tested dose range and the 48 h observation window, with contours at 50, 75 and 90% mortality. For *T. castaneum* the petroleum

ether extract reaches 50% mortality within 6 h at the highest dose tested but needs about a day at the lowest, whereas the methanol extract reaches the same level only at the upper end of its dose range. For *S. oryzae* the 90% contour is not reached anywhere within the tested surface, which is a more informative statement of the limits of activity than any single LD₅₀ value.

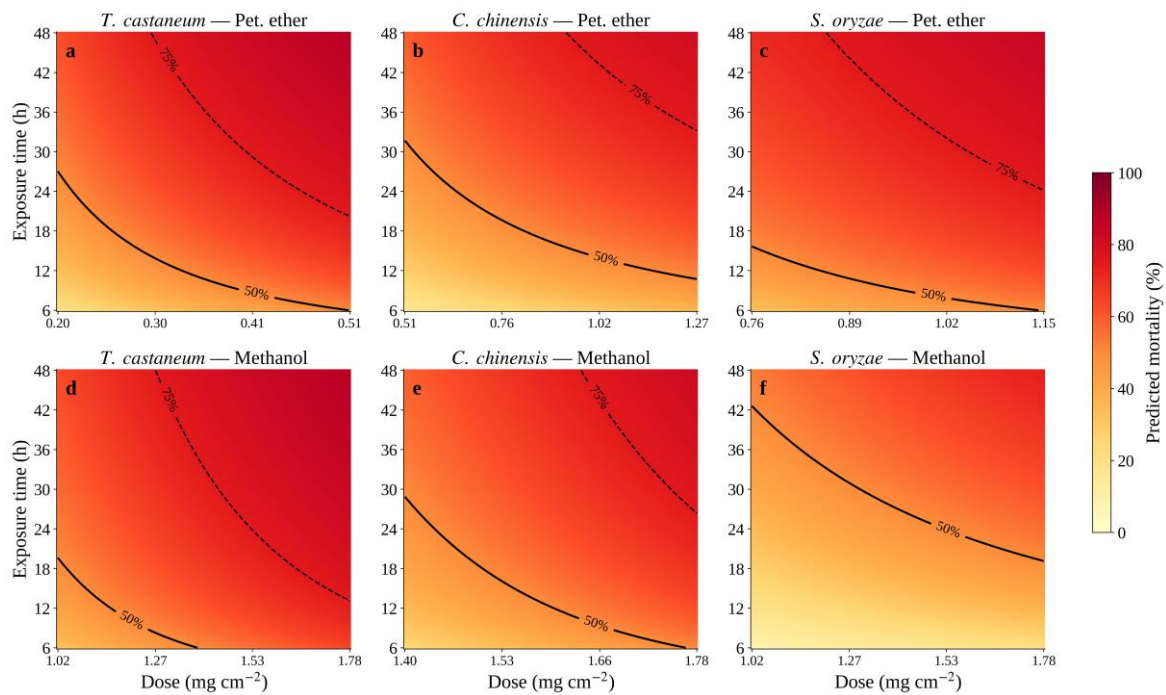


Figure 5. Predicted mortality across dose and exposure time. Colour shows mortality predicted by the fitted model; contour lines mark 50, 75 and 90% mortality. Surfaces are drawn only within the range of doses tested. (a–c) petroleum ether extract; (d–f) methanol extract.

3.5. Median Lethal Time

Expressed as time rather than dose, the same models show how quickly each dose acts (Figure 6, Table 4). For the petroleum ether extract against *T. castaneum*, LT₅₀ was 27.0 h (95% CI 19.7–37.1) at the lowest dose and 6.0 h (95% CI 2.6–13.5) at the highest. Against *C. chinensis* the values ranged from

31.7 h (95% CI 26.6–37.7) to 10.7 h (95% CI 8.4–13.6), and against *S. oryzae* from 15.7 h (95% CI 14.5–16.9) to 5.9 h (95% CI 4.7–7.4). The methanol extract acted more slowly, most obviously against *S. oryzae*, where LT₅₀ fell only from 42.6 h (95% CI 30.4–59.7) to 19.1 h (95% CI 9.2–39.7) across a near doubling of dose. Estimates shorter than 6 h or longer than 48 h lie outside the observation window and are extrapolations.

Table 4. Median lethal time (LT₅₀, h) at each dose, with observed mortality at 48 h for comparison.

Species	Extract	Dose (mg cm ⁻²)	LT ₅₀ (h)	95% CI	Observed mortality at 48 h (%)
<i>T. castaneum</i>	Pet. ether	0.203	27.0	19.7–37.1	66.7
<i>T. castaneum</i>	Pet. ether	0.305	13.9	10.3–18.6	76.7
<i>T. castaneum</i>	Pet. ether	0.407	8.6	4.9–15.3	76.7
<i>T. castaneum</i>	Pet. ether	0.509	6.0 ^a	2.6–13.5	96.7

Species	Extract	Dose (mg cm ⁻²)	LT ₅₀ (h)	95% CI	Observed mortality at 48 h (%)
<i>C. chinensis</i>	Pet. ether	0.509	31.7	26.6–37.7	63.3
<i>C. chinensis</i>	Pet. ether	0.763	19.6	17.1–22.6	73.3
<i>C. chinensis</i>	Pet. ether	1.018	14.0	11.6–16.8	76.7
<i>C. chinensis</i>	Pet. ether	1.273	10.7	8.4–13.6	93.3
<i>S. oryzae</i>	Pet. ether	0.763	15.7	14.5–16.9	73.3
<i>S. oryzae</i>	Pet. ether	0.891	10.8	9.7–12.0	80.0
<i>S. oryzae</i>	Pet. ether	1.018	7.8	6.6–9.3	86.7
<i>S. oryzae</i>	Pet. ether	1.145	5.9 ^a	4.7–7.4	90.0
<i>T. castaneum</i>	Methanol	1.018	19.6	13.0–29.7	66.7
<i>T. castaneum</i>	Methanol	1.273	8.3	6.1–11.2	76.7
<i>T. castaneum</i>	Methanol	1.527	4.1 ^a	3.1–5.5	86.7
<i>T. castaneum</i>	Methanol	1.782	2.3 ^a	1.6–3.2	90.0
<i>C. chinensis</i>	Methanol	1.400	28.9	16.0–51.7	53.3
<i>C. chinensis</i>	Methanol	1.527	16.1	10.9–23.8	73.3
<i>C. chinensis</i>	Methanol	1.655	9.3	6.6–13.3	76.7
<i>C. chinensis</i>	Methanol	1.782	5.7 ^a	3.6–9.1	86.7
<i>S. oryzae</i>	Methanol	1.018	42.6	30.4–59.7	56.7
<i>S. oryzae</i>	Methanol	1.273	31.0	24.1–39.7	60.0
<i>S. oryzae</i>	Methanol	1.527	23.9	14.7–38.7	66.7
<i>S. oryzae</i>	Methanol	1.782	19.1	9.2–39.7	76.7

^a Estimate outside the 6–48 h observation window (extrapolation).

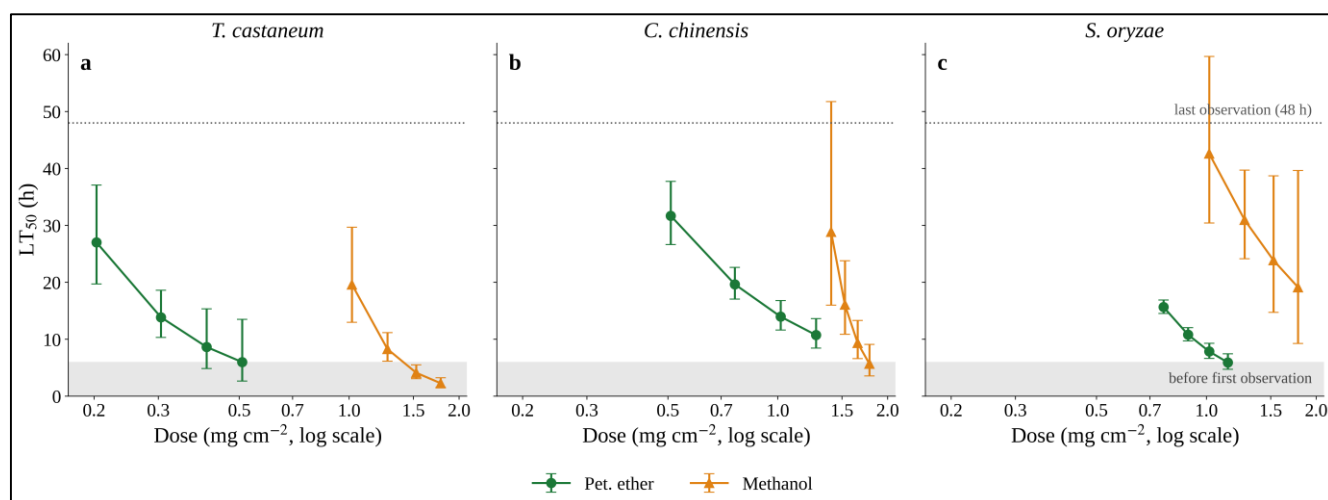


Figure 6. Higher doses killed faster. LT₅₀ ($\pm$ 95% CI) for (a) *T. castaneum*, (b) *C. chinensis* and (c) *S. oryzae*. The shaded band marks times earlier than the first observation (6 h) and the dotted line the last observation (48 h); estimates in these regions are extrapolations.

3.6. Relative Potency of the Two Active Extracts

Within every species the petroleum ether extract was the more potent of the two (Figure 7a, Table 5). The ratio of the methanol to the petroleum ether LD_{50} was 4.43-fold (95% CI 3.73–5.25) for *T. castaneum* and 2.23-fold (95% CI 1.95–2.56) for *C. chinensis* at 24 h, rising to 5.64-fold (95% CI 3.56–8.95) and 3.62-fold (95% CI 2.87–4.58) at 48 h. For *S. oryzae* the

ratio was 2.38-fold (95% CI 1.24–4.58) at 24 h; at 48 h it was 1.96-fold (95% CI 0.89–4.29) and the interval included one, so the two extracts cannot be separated at that time point for this species.

The same ranking is visible in the untransformed data, without any model. At 48 h the lowest petroleum ether dose against *T. castaneum* (0.203 mg cm⁻²) killed 66.7% of the insects, while the lowest methanol dose (1.018 mg cm⁻², five times higher) killed 66.7%.

Table 5. Relative potency of the two active extracts within each species, calculated as LD_{50} (methanol) ÷ LD_{50} (petroleum ether). A ratio above one means the petroleum ether extract is the more potent.

Species	Time (h)	LD_{50} petroleum ether	LD_{50} methanol	Potency ratio	95% CI	CI excludes 1
<i>T. castaneum</i>	24	0.218	0.966	4.43	3.73–5.25	Yes
<i>T. castaneum</i>	48	0.143	0.807	5.64	3.56–8.95	Yes
<i>C. chinensis</i>	24	0.644	1.439	2.23	1.95–2.56	Yes
<i>C. chinensis</i>	48	0.358	1.298	3.62	2.87–4.58	Yes
<i>S. oryzae</i>	24	0.639	1.521	2.38	1.24–4.58	Yes
<i>S. oryzae</i>	48	0.478	0.937	1.96	0.89–4.29	No

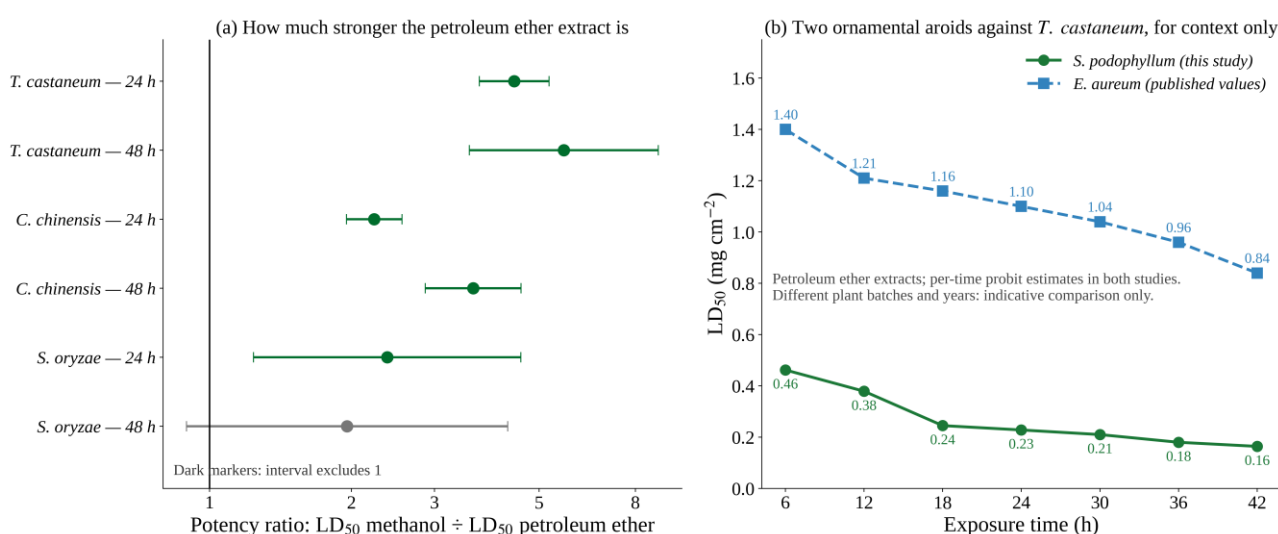


Figure 7. (a) Potency of the petroleum ether extract relative to the methanol extract, with 95% confidence intervals; values above 1 favour petroleum ether, and dark markers mark intervals that exclude 1. (b) LD_{50} at 24 h of the petroleum ether extract of *S. podophyllum* (this study) beside published values for the related aroid *Epipremnum aureum* obtained with the same bioassay design in the same laboratory. The two data sets come from different plant batches and years, so the comparison is indicative only.

3.7. Comparison with Conventional Per-time Probit Analysis

Fitting a separate probit line at each observation time, as most comparable studies do, gave point estimates close to

those from the combined model. The precision of those per-time fits, however, was poor. In 46 of the 48 fits the Fieller *g* statistic reached 0.5 or more, the point beyond which the limits become unreliable, and in 20 no finite interval existed at all, because each series used only four closely spaced doses. Those estimates are provided as Supplementary Table S1 for comparison with earlier literature, but the values in Tables 3–

5 are preferred. The main model and a direct maximum likelihood refit gave identical estimates. Replacing the probit link with a logit link changed LD₅₀ by less than 1%. A complementary log-log link, which assumes an asymmetric dose response, fitted marginally better by AIC (differences of 0.7 to 3.3 units) and raised LD₅₀ by 13 to 67%, without altering the ranking of the two extracts in any species or the direction of the time effect. Probit values are reported here for comparability with the stored-product literature, and the link dependence is noted as a limitation.

4. Discussion

4.1. Activity of the Extracts

This study provides what appears to be the first evidence that extracts of *S. podophyllum* are toxic to three of the most damaging pests of stored grain and pulses. Activity was concentrated in the non-polar petroleum ether fraction, was present but weaker in the polar methanol fraction, and was absent from the intermediate chloroform fraction. Mortality continued to rise for the full 48 h in every active series, so any single-time statement of potency understates the activity of these extracts at longer exposures and overstates it at shorter ones.

4.2. What the Solvent Pattern Suggests

Sequential extraction with solvents of increasing polarity separates plant constituents into broad chemical classes, and the pattern we observed points to at least two groups of active compounds rather than one. Toxicity in the petroleum ether fraction implicates lipophilic constituents such as terpenoids, sterols and fatty acid derivatives; activity in the methanol fraction implicates more polar material such as glycosides, phenolics and saponins, which have been reported from this species [17, 18]. The absence of mortality in the chloroform fraction is informative rather than a null result, because it indicates that the active material partitions preferentially into the least and the most polar solvents used. It is not a property of the solvent itself: chloroform extracts of *Urena sinuata* killed *T. castaneum* within 30 minutes in the same laboratory [10], so a chloroform fraction can be highly toxic when the plant supplies the right constituents. Crude extracts cannot identify the compounds responsible, and we make no claim about mode of action, although the neurotoxic targets described for plant volatiles and terpenes provide the obvious starting hypotheses [8]. What the pattern does provide is a clear direction for bioassay-guided fractionation, which should start with the petroleum ether fraction.

4.3. Species Differences and the Limits of Comparison

The three species differed in the dose required, but our de-

sign does not permit that difference to be interpreted as a difference in intrinsic susceptibility. *Tribolium castaneum* was exposed to a dried film on a dish surface, while *C. chinensis* and *S. oryzae* were exposed through treated food, and the two routes differ in how much extract an insect actually contacts or ingests. The dose unit inherited from the laboratory records compounds this, since an area-based unit is not meaningful for a food-based assay. We have therefore restricted all comparisons to extracts within a species, which the design does support. Genuine species differences are nevertheless likely, because these pests differ in feeding habit and in how they respond to the same chemical cues: *S. oryzae* and *T. castaneum* respond differently to identical food volatiles in choice tests [23]. Future work on this plant should use a common exposure route, or report doses as mass of extract per unit mass of commodity, so that species can be compared directly.

4.4. Comparison with Related Aroids and Other Botanicals

Two other ornamental aroids have been screened against the same three pests with the same extraction scheme and bioassays in the same laboratory. In *E. aureum* only the petroleum ether extract caused mortality, the chloroform and methanol extracts causing none [19]; in *A. marantifolium* the chloroform extract was likewise inactive against all three species [20]. An inactive chloroform fraction therefore recurs across three genera of Araceae, which suggests a shared distribution of active constituents in the family. The pattern is not universal among plants screened in the same laboratory with the same solvents: in *M. azedarach* the chloroform extract of fruits and leaves was likewise non-toxic but did repel *T. castaneum* [11], and in *P. integrifolia* the three solvent fractions again differed in which pest they affected [12]. Differences between solvent fractions are therefore a property of the plant rather than an artefact of the extraction scheme. *Syngonium podophyllum* differs from *E. aureum* in that its methanol extract was also toxic, so its polar fraction carries activity that the pothos apparently lacks.

The absolute values invite a comparison that must be made carefully (Figure 7b). Our 24 h LD₅₀ for the petroleum ether extract against *T. castaneum* was 0.218 mg cm⁻², against 1.10 mg cm⁻² reported for *E. aureum* [19], a roughly five-fold difference in favour of *S. podophyllum*. The two studies used different plant batches, different growing seasons and different years, and the published values come from per-time probit fits, whose precision we have shown to be limited in this kind of design. The comparison is therefore indicative of a promising difference, not a controlled test of it. The same caution applies to comparisons with the wider botanical literature, where activity varies with pest species, life stage, concentration and exposure period even for well-characterised essential oils [16]. Placed against earlier work on Bangladeshi plants, the activity reported here is of a familiar order: extracts of pithraj, *Aphan-*

amixis polystachya, are both toxic and deterrent to *T. castaneum* [24], and locally collected *P. hydropiper* reduced survival and progeny production of the same pest [9].

4.5. Why Exposure Time Matters in Practice

The most consistent finding of this study is that toxicity is a function of time as well as dose. For a protectant used in storage this is not a technical detail. Insects in a treated store are exposed continuously, so a material whose LD₅₀ halves between 24 and 48 h may be effective at doses that a short bioassay would dismiss. Conversely, a material that needs days to act gives insects time to feed, mate and oviposit before they die, so the damage is done even if mortality is eventually high. Our LT₅₀ estimates make this concrete: at the highest petroleum ether doses half of the exposed adults died within roughly 6 to 11 h, whereas the methanol extract needed 19 to 43 h against *S. oryzae* across the doses tested. For smallholder storage in Bangladesh, where stored grain is typically held for months in simple containers and where improved practice can cut losses substantially [1], speed of action and persistence would both need to be established before any practical recommendation could be made. A plant extract is also unlikely to be used alone: combinations of botanical and synthetic materials have been used to lower the dose of the synthetic component against this pest [13], and any such use would have to be planned within a resistance-management framework of the kind now recommended for fumigants [4, 5]. Neither was measured here.

4.6. Methodological Contribution

Nearly all screening studies of this kind fit a separate probit line to each observation time. Our analysis shows two problems with that practice on data of this design. The counts are not independent across times, which the literature has long recognised [21], and with four closely spaced doses the per-time fits gave unreliable or infinite confidence limits in most cases, a fact that the conventional presentation hides behind apparently precise numbers. A single model across all observation times uses the same data to estimate fewer parameters, respects the clustering of counts within dishes, and yields LT₅₀ in addition to LD₅₀. We would encourage authors of future screening studies to report intervals computed in this or a similar way, and to state when an estimate falls outside the range of doses actually tested.

4.7. Limitations

Several limitations follow from the original design and records, and they bound what may be concluded. Extract yields were not recorded and no voucher specimen was deposited, so the exact amount of plant material behind each dose and the botanical identity of the source cannot be re-verified. The extracts were not characterised chemically. No positive control,

such as a registered grain protectant, was included, so the activity cannot be placed on a scale of practical usefulness; recent work with essential oils shows how much such a control changes interpretation [16]. Only adults were exposed, so activity against eggs, larvae and pupae, and any suppression of the following generation, remain unknown; these endpoints often decide whether a material is useful as a grain protectant. Persistence, effects on seed germination and any taint of the treated commodity were not examined either. The operational criterion for scoring death was not recorded, so a small and unquantifiable misclassification of moribund insects cannot be excluded, although it would apply equally to all treatments. Absolute LD₅₀ values also depend on the assumed shape of the dose response: a complementary log-log link raised them by up to two thirds, while leaving every comparison between extracts unchanged. Doses in the food-based assays carry an area unit that is not physically meaningful. The data were generated in 2017–2018 with laboratory strains of unknown susceptibility to synthetic insecticides, and results may differ for field populations. Finally, mammalian safety cannot be assumed: an aqueous leaf extract of this species was hepatotoxic in rats [17], and nothing in our data speaks to the safety of treated food.

4.8. Recommendations for Further Work

Three steps would move this work toward practical evaluation. The first is bioassay-guided fractionation of the petroleum ether extract, with chemical identification of the active constituents. The second is a bioassay design with a common exposure route across species, doses expressed per unit mass of commodity, a registered positive control and a wider dose range, so that potency can be compared both between species and against existing materials. The third is a test of persistence on stored grain under realistic temperature and humidity, together with exposure of eggs, larvae and pupae, an assessment of progeny production in the following generation, and measurement of effects on germination and on food quality. Toxicological screening of the active fraction in a mammalian model would be required before any use on food commodities could be contemplated.

5. Conclusions

Crude extracts of the ornamental aroid *S. podophyllum* killed adults of *T. castaneum*, *C. chinensis* and *S. oryzae*. The petroleum ether extract was two to six times more potent than the methanol extract within each species, the chloroform extract was inactive, and in every active combination the lethal dose fell as exposure time lengthened, with half of the exposed adults dying within about 6 to 11 h at the highest petroleum ether doses. These results identify a common ornamental plant as a source of insecticidal material against major storage pests, and they show why screening studies should report the time

dependence of activity and the precision of their estimates rather than a single lethal dose. The next step is chemical identification of the active constituents of the non-polar fraction, followed by evaluation with positive controls under realistic storage conditions.

Abbreviations

CI	Confidence Interval
LD ₅₀	Median Lethal Dose (Dose That Kills 50% of the Insects)
LD ₉₀	Dose that Kills 90% of the Insects
LT ₅₀	Median Lethal Time (Time Taken to Kill 50% of the Insects)
Pet. Ether	Petroleum Ether
SE	Standard Error

Supplementary Material

The supplementary material can be accessed at <https://doi.org/10.11648/j.ijee.20261103.12>

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Author Contributions

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Md. Nurul Islam: Methodology, Resources, Supervision, Writing – review & editing

M. Habibur Rahman: Conceptualization, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing

Data Availability Statement

The data is available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

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