

Research Article

Repellent and Attractant Activity of *Syngonium Podophyllum* Schott (Araceae) Plant Extracts Against Three Stored-product Pests: Species-specific Responses of *Tribolium Castaneum*, *Callosobruchus Chinensis*, and *Sitophilus Oryzae*

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Abstract

Insects that live in stored grain and pulses are usually controlled with chemicals, but plant extracts that keep insects away, known as repellents, are a possible alternative. Most studies of plant repellents test only one kind of insect and ask only whether it is driven away. We asked whether the same plant extract could push one pest away while drawing another one in. Extracts of the ornamental plant *Syngonium podophyllum* Schott (Araceae) were prepared with three solvents, petroleum ether, chloroform and methanol, and offered to three major stored-product pests: the red flour beetle *Tribolium castaneum* (Herbst), the pulse beetle *Callosobruchus chinensis* (L.) and the rice weevil *Sitophilus oryzae* (L.). In each test dish, one half was treated with extract and the other half was left untreated; ten adult insects were released in the centre and counted on each side every hour for five hours, at five doses with three dishes per dose. The red flour beetle and the pulse beetle avoided all three extracts, and did so in every one of the 90 dishes in which they were tested; average repellency was about 55% for petroleum ether, 44 to 51% for chloroform and 22 to 25% for methanol. The rice weevil behaved differently: it was repelled only weakly and inconsistently by petroleum ether, was not affected by methanol, and was attracted to the chloroform extract, settling on the treated side in all 15 of its dishes. The chloroform extract, which killed none of the three pests in toxicity tests, was therefore one of the most active materials in changing their behaviour. These results show that one plant extract can have opposite effects on pests that share the same store. A treatment that protects pulses or flour could draw rice weevils toward stored cereals, while the attractive fraction could be developed as a bait for weevil traps.

Keywords

Repellency, Attraction, Insect Behaviour, Botanical Repellent, Plant Extract, Araceae, Stored-product Pests, *Sitophilus Oryzae*

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1. Introduction

Storage is the stage at which the largest avoidable share of post-harvest grain loss occurs in developing countries [1]. Protecting stored grain does not always require killing insects. A material that keeps pests away from a commodity can reduce infestation, oviposition and the contamination that follows, and repellents are therefore a recognised component of behaviourally based management in storage [2]. Interest in plant-derived repellents has grown as the chemical options have narrowed: resistance to phosphine is now documented in major storage pests on several continents [3], including field populations of *T. castaneum* [4], while commercial development of botanicals has lagged far behind the volume of published screening work [5].

Most of that screening work shares a design. A plant extract is applied to half of a filter-paper disc, insects are released at the centre, and the proportion settling on the untreated half is converted to a percent repellency value [6, 7]. The method is simple and reproducible, and recent studies continue to use it alongside olfactometers to rank essential oils and extracts against storage pests [8, 9]. Screening studies in this field are often criticised for reporting activity without the detail needed to use it [10], and two habits in particular limit what they can tell us. First, most studies test one pest species, usually *T. castaneum*, so a material is declared repellent on the basis of one insect's behaviour. Second, negative values are treated as failure rather than as information, although the same measurement scale records attraction as readily as repellency.

There is good reason to expect species to differ. The three pests used here occupy different niches in a store. *Tribolium castaneum* (Coleoptera: Tenebrionidae) is a secondary pest that exploits flour and damaged grain, *C. chinensis* (Coleoptera: Chrysomelidae: Bruchinae) develops inside pulse seeds and its reproductive performance varies with female age [11], and *S. oryzae* (Coleoptera: Curculionidae) bores into intact cereal kernels. Their chemical ecology differs accordingly. *Sitophilus oryzae* locates host grain by olfaction and is attracted to specific grain volatiles: adults respond positively to nonanal, hexanal and 1-octen-3-ol released by stored rice, and nonanal has been proposed as a monitoring lure for the species [12]. Volatile profiles also change as infestation proceeds, which is being explored for early detection of the same pest [13]. A plant extract rich in volatile constituents is therefore as likely to resemble a host cue for one species as an irritant for another.

Reliance on synthetic chemicals also carries costs beyond resistance in insects. In Bangladesh the carbamate insecticide carbofuran caused mortality and organ damage in the freshwater fish *Channa punctatus* [14], heavy agrochemical use is one of the pressures on the country's biodiversity [15], and antibiotic-resistant bacteria are now widespread in fish ponds of the Rajshahi region where antibiotics are used routinely [16]. Locally available plant materials have therefore been screened against storage pests for many years in Bangladesh, including

Polygonum hydropiper [17], *Urena sinuata* [18], *Melia azedarach* [19] and *Petunia integrifolia* [20], sometimes in combination with reduced doses of synthetic insecticides [21]. *Synгонium podophyllum* Schott (Araceae), the arrowhead vine, is a common ornamental whose tissues contain terpenoids, flavonoids, alkaloids and saponins [22, 23], and two related ornamental aroids have been screened against the same three pests with the same methods [24, 25].

If an extract can attract one pest while repelling another, both the risk and the opportunity are practical. The risk is that a botanical applied to protect pulses or flour could concentrate weevils on treated cereals stored nearby. The opportunity is that an attractive extract is a possible lure: attraction is the basis of monitoring traps and of push–pull schemes, in which pests are driven from the commodity and drawn to a trap [2].

In this study we used the same three extracts of *S. podophyllum* whose toxicity is reported in a companion article (Hoque et al., manuscript under review), and asked three questions. Does the plant repel these pests, and at what dose? Does the response differ between species and between solvent fractions? And is behavioural activity linked to toxicity, or independent of it?

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 Extracts

Healthy *S. podophyllum* plants were collected from the Botanical Garden, University of Rajshahi. The whole plant material was chopped, dried in the shade and ground to powder. Powder (150 g) was extracted sequentially with petroleum ether, then chloroform, then methanol, three times with 450 mL of each solvent and 48 h of agitation per cycle, and the filtrates were evaporated at room temperature. Extracting with solvents of increasing polarity separates plant constituents into broad chemical groups, so that behavioural activity can be traced to a particular fraction. The extracts were the same preparations used in the companion toxicity study.

2.3. Insects

Adults came from stock cultures of the same laboratory: *T. castaneum* reared on whole-wheat flour with powdered yeast (19:1) at 30 °C, *C. chinensis* on lentil seeds and *S. oryzae* on wheat grain at room temperature.

2.4. Two-choice Arenas

For *T. castaneum*, a measured volume of extract solution was applied to one half of a 9 cm Whatman No. 40 filter-paper disc and air-dried for 10 min; the treated half-disc was joined edge to edge with an untreated half-disc using adhesive tape and placed in a Petri dish (Figure 1a). For *C. chinensis* and *S. oryzae*, which orient to food rather than to a bare surface, a 9 cm Petri dish was divided into three sections with two narrow sticks; treated food was placed on one side, untreated food on the other, and the central strip was left empty (Figure 1b). Five doses were prepared by serial dilution: 0.1571, 0.0785, 0.0392, 0.0196 and 0.0098 mg cm⁻². The area unit refers to the treated half-disc and is inherited from the original laboratory records;

in the food arenas the extract was applied to food rather than to a defined surface, so for those two species the values index relative dose within a species and are not physically comparable with the filter-paper assay. The age and sex of the adults used were not recorded. Ten adults were released at the centre of each dish, and dish orientation was changed between replicates to cancel any directional stimulus in the room. Each dose was replicated in three dishes. The number of insects on the untreated side (N_c) was counted after 1 h and hourly thereafter to 5 h, and percent repellency was calculated as $PR = (N_c - 5) \times 20$ [7, 26], so that positive values indicate repellency, negative values attraction, and zero an even distribution. An arena treated with solvent on both halves was not recorded; the implications are examined in Section 4.5.

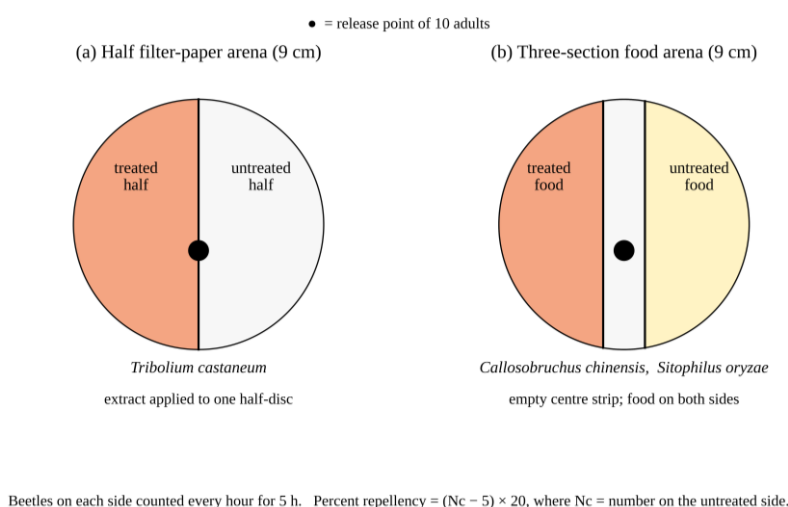


Figure 1. Two-choice arenas. (a) Half filter-paper arena used for *T. castaneum*; (b) three-section food arena used for *C. chinensis* and *S. oryzae*. Ten adults were released at the centre of each dish and counted on each side every hour for 5 h.

2.5. Statistical Analysis

The same ten insects were counted five times in each dish, so hourly counts within a dish are not independent [27]. The five hourly values of a dish were therefore averaged, giving one value per dish and 15 dishes (5 doses $\times$ 3 replicates) per extract and species, and the dish was treated as the unit of replication throughout. Mean repellency was compared with zero by one-sample *t*-test, and, as an independent check, the proportion of insects on the untreated side was modelled with a binomial generalized estimating equation with an exchangeable correlation structure within dish; a logit intercept different from zero indicates departure from an even 50:50 split. Because nine extract–species combinations were tested, *P* values were adjusted by the Benjamini–Hochberg false discovery rate and an effect is called clear only when $q < 0.05$. Two further tests were applied to guard against the assumptions of the main analysis. A two-sided sign test asked whether the direction of the response was consistent across dishes without assuming any distribution. Because pooling the five doses treats

them as replicates, the analysis was repeated with the five dose means as the units of replication ($n = 5$), which is the conservative alternative if dose has an effect of its own.

Dose responses were examined by regressing dish means on $\log_{10}(\text{dose})$ and by one-way analysis of variance across the five doses; the time course was examined with a generalized estimating equation containing hour, and by comparing the first hour with hours 2 to 5 within dishes. Differences among species and extracts were tested by two-way analysis of variance on dish means with $\log_{10}(\text{dose})$ as a covariate, followed by Tukey tests. Mean values were assigned to repellency classes 0 to V [6]. For each combination we also computed the smallest difference from zero that the design could detect ($\alpha = 0.05$, power = 0.8), so that null results can be read correctly. As sensitivity checks the analysis was repeated after dropping the highest dose, and the lowest dose was analysed on its own. Analyses used Python 3 (statsmodels, SciPy) with a fixed random seed; data and scripts are available from the corresponding author.

3. Results

3.1. Direction and Consistency of the Response

The two smaller-bodied pests avoided every extract, and did so in every dish (Figure 2). For *T. castaneum*, mean repellency was 54.9% (95% CI 49.7 to 60.2; $q < 0.001$) with the petroleum ether extract, 44.3% (95% CI 37.9 to 50.7; $q < 0.001$) with the chloroform extract and 24.5% (95% CI 21.2 to 27.9; $q < 0.001$) with the methanol extract. The corresponding values for *C. chinensis* were 56.0% (95% CI 51.0 to 61.0; $q < 0.001$), 50.9% (95% CI 45.3 to 56.6; $q < 0.001$) and 22.4% (95% CI 15.8 to 29.0; $q < 0.001$). In all six of these combinations, 15 of 15 dishes gave positive repellency (sign test $P = 0.00006$ for each), so the direction of the response did not depend on dose, on replicate or on the individual dish. Repeating

the analysis with the five dose means as units gave the same conclusion for all six ($P = 0.00001$ – 0.0024).
Sitophilus oryzae responded in the opposite direction to the chloroform extract. Mean repellency was -16.3% (95% CI -22.0 to -10.6 ; $q < 0.001$), and all 15 dishes were negative, meaning that more weevils settled on the treated side than on the untreated side in every dish (sign test $P = 0.0002$; analysis on dose means $P = 0.002$). The petroleum ether extract repelled this species weakly (10.4% (95% CI 2.7 to 18.1 ; $q = 0.013$), positive in 10 of 15 dishes), but this was the one result that did not survive the stricter checks: the sign test gave $P = 0.092$ and the analysis on dose means $P = 0.116$, so we treat it as suggestive rather than established. The methanol extract had no detectable effect (3.5% (95% CI -4.0 to 10.9 ; $q = 0.334$)). The generalized estimating equations reached the same conclusion as the *t*-tests in all nine combinations.

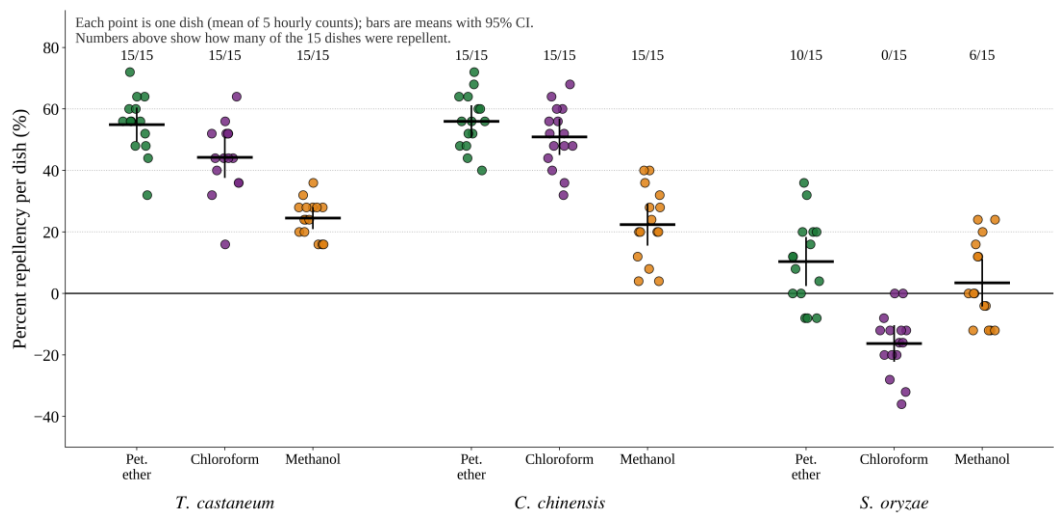


Figure 2. Every dish is shown. Percent repellency of individual dishes (mean of five hourly counts), with the group mean and its 95% confidence interval. Points above zero indicate avoidance of the treated side and points below zero indicate preference for it. The numbers above each group give how many of the 15 dishes were repellent.

Table 1. Behavioural response of each species to each extract. Values are means of 15 dishes (5 doses $\times$ 3 replicates), each dish being the mean of five hourly counts. Classes follow McDonald et al. [6]; negative means indicate attraction. "Smallest detectable effect" is the difference from zero this design could detect ($\alpha = 0.05$, power = 0.8). The final column gives the outcome of the companion toxicity study for the same extract and species.

Species	Extract	Mean PR $\pm$ SE (%)	95% CI (%)	Class	Dishes repellent	q	Smallest detectable effect (%)	Toxicity (companion study)
<i>T. castaneum</i>	Pet. ether	54.9 $\pm$ 2.5	49.7 to 60.2	III	15/15	< 0.001	25	toxic
<i>T. castaneum</i>	Chloroform	44.3 $\pm$ 3.0	37.9 to 50.7	III	15/15	< 0.001	30	no mortality
<i>T. castaneum</i>	Methanol	24.5 $\pm$ 1.6	21.2 to 27.9	II	15/15	< 0.001	14	toxic
<i>C. chinensis</i>	Pet. ether	56.0 $\pm$ 2.3	51.0 to 61.0	III	15/15	< 0.001	19	toxic
<i>C. chinensis</i>	Chloroform	50.9 $\pm$ 2.6	45.3 to 56.6	III	15/15	< 0.001	26	no mortality
<i>C. chinensis</i>	Methanol	22.4 $\pm$ 3.1	15.8 to 29.0	II	15/15	< 0.001	29	toxic

Species	Extract	Mean PR $\pm$ SE (%)	95% CI (%)	Class	Dishes repellent	q	Smallest detectable effect (%)	Toxicity (companion study)
<i>S. oryzae</i>	Pet. ether	10.4 $\pm$ 3.6	2.7 to 18.1	I	10/15	0.013	27	toxic
<i>S. oryzae</i>	Chloroform	-16.3 $\pm$ 2.7	-22.0 to -10.6	Attractant	0/15	< 0.001	27	no mortality
<i>S. oryzae</i>	Methanol	3.5 $\pm$ 3.5	-4.0 to 10.9	I	6/15	0.334	33	toxic

3.2. Effect Size, and Behaviour Compared with Toxicity

Ranked by effect size (Figure 3a), the petroleum ether extract was the most repellent material for both *T. castaneum* and *C. chinensis*, reaching class III, and the chloroform extract followed closely, also in class III for both species. The methanol extract was consistently the weakest, in class II. For *S.*

oryzae the order was reversed at the negative end of the scale, with the chloroform extract the most active material in behavioural terms.

Behavioural activity and toxicity were not aligned (Figure 3b). In the companion study the chloroform extract caused no mortality in any of the three species at any dose, yet here it repelled two species strongly and attracted the third. Conversely the methanol extract was toxic to all three species but was the weakest repellent. The fractions responsible for killing these pests and for moving them are therefore not the same.

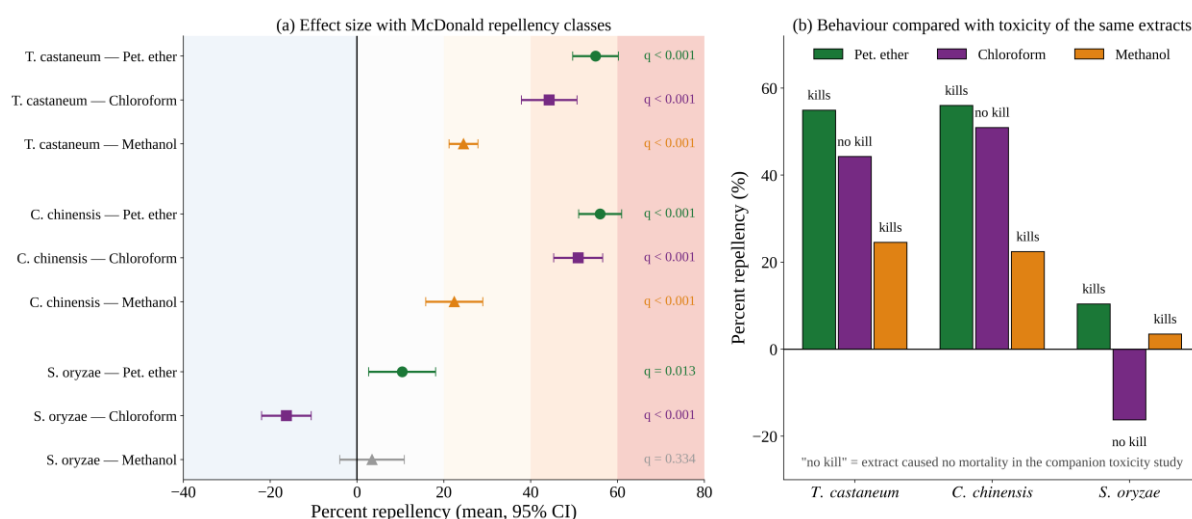


Figure 3. (a) Effect size for each species and extract, with 95% confidence intervals, plotted against the McDonald repellency classes (shaded bands); grey markers did not survive false-discovery correction. (b) The same behavioural means annotated with the outcome of the companion toxicity study, showing that the chloroform extract moved insects without killing them.

Table 2. Mean percent repellency at each dose, with the repellency class in brackets (A = attraction). Each value is the mean of three dishes. Doses in mg cm^{-2} .

Species	Extract	0.0098	0.0196	0.0392	0.0785	0.1571
<i>T. castaneum</i>	Pet. ether	54.7 (III)	49.3 (III)	52.0 (III)	57.3 (III)	61.3 (IV)
<i>T. castaneum</i>	Chloroform	44.0 (III)	42.7 (III)	42.7 (III)	37.3 (II)	54.7 (III)
<i>T. castaneum</i>	Methanol	24.0 (II)	20.0 (I)	22.7 (II)	25.3 (II)	30.7 (II)
<i>C. chinensis</i>	Pet. ether	53.3 (III)	46.7 (III)	62.7 (IV)	54.7 (III)	62.7 (IV)
<i>C. chinensis</i>	Chloroform	42.7 (III)	49.3 (III)	50.7 (III)	53.3 (III)	58.7 (III)

Species	Extract	0.0098	0.0196	0.0392	0.0785	0.1571
<i>C. chinensis</i>	Methanol	17.3 (I)	20.0 (I)	14.7 (I)	28.0 (II)	32.0 (II)
<i>S. oryzae</i>	Pet. ether	-1.3 (A)	4.0 (I)	10.7 (I)	9.3 (I)	29.3 (II)
<i>S. oryzae</i>	Chloroform	-21.3 (A)	-16.0 (A)	-21.3 (A)	-13.3 (A)	-9.3 (A)
<i>S. oryzae</i>	Methanol	-5.3 (A)	2.7 (I)	0.0 (0)	2.7 (I)	17.3 (I)

3.3. Dose Response

Repellency was already present at the lowest dose tested for both *T. castaneum* and *C. chinensis* (Figure 4, Table 2). At 0.0098 mg cm⁻², sixteen times weaker than the highest dose, mean repellency was 54.7% for *T. castaneum* with the petroleum ether extract ($P = 0.011$) and 53.3% for *C. chinensis* ($P = 0.004$). Regression on log dose was accordingly shallow and mostly non-significant for these two species, and analysis of

variance across doses detected no differences (Table 3). The lowest effective dose therefore lies below the range tested, and the assay was saturated across most of it.

Sitophilus oryzae behaved differently in this respect too. With the petroleum ether extract, repellency rose from -1.3% at the lowest dose to 29.3% at the highest, giving the clearest dose response in the study (slope 22.1 percentage points per tenfold increase in dose, $P = 0.004$; analysis of variance across doses $P = 0.043$). Attraction to the chloroform extract was present at every dose and was strongest at the lowest (-21.3%).

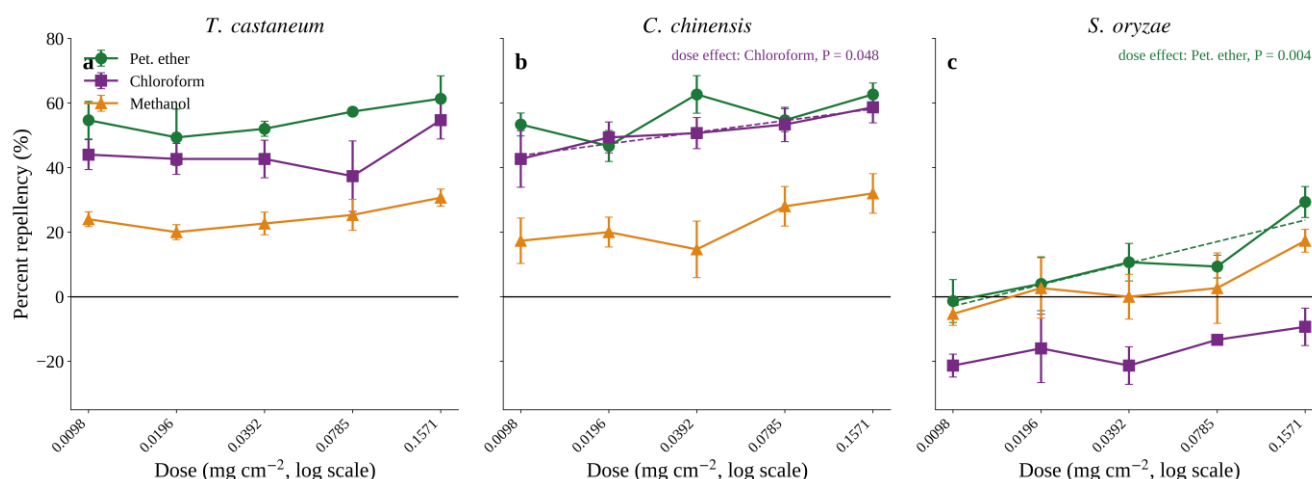


Figure 4. Dose response of each species. Points are means ($\pm$ SE) of three dishes at each dose; dashed lines show fitted log-dose regressions where the slope was significant. Repellency in *T. castaneum* and *C. chinensis* was already near its maximum at the lowest dose, whereas *S. oryzae* showed a clear dose response to the petroleum ether extract.

Table 3. Dose and time effects, and a sensitivity check. The dose slope is in percentage points of repellency per tenfold increase in dose; the hour slope is in percentage points per hour. The last two columns give the mean and its P value when the highest dose is removed from the analysis.

Species	Extract	Dose slope	P (dose slope)	P (ANOVA across doses)	Hour slope	P (hour)	Mean without top dose (%)	P
<i>T. castaneum</i>	Pet. ether	7.1	0.231	0.640	-1.6	0.129	53.3	< 0.001
<i>T. castaneum</i>	Chloroform	5.3	0.469	0.513	+1.3	0.236	41.7	< 0.001
<i>T. castaneum</i>	Methanol	6.2	0.089	0.283	+1.5	0.192	23.0	< 0.001
<i>C. chinensis</i>	Pet. ether	8.9	0.104	0.114	+0.0	1.000	54.3	< 0.001
<i>C. chinensis</i>	Chloroform	11.9	0.048	0.461	+0.1	0.889	49.0	< 0.001

Species	Extract	Dose slope	P (dose slope)	P (ANOVA across doses)	Hour slope	P (hour)	Mean without top dose (%)	P
<i>C. chinensis</i>	Methanol	12.4	0.085	0.366	+1.2	0.446	20.0	< 0.001
<i>S. oryzae</i>	Pet. ether	22.1	0.004	0.043	+2.5	0.119	5.7	0.090
<i>S. oryzae</i>	Chloroform	8.9	0.164	0.611	-1.1	0.649	-18.0	< 0.001
<i>S. oryzae</i>	Methanol	15.0	0.061	0.345	+1.6	0.355	0.0	1.000

3.4. Stability over the Observation Period

Responses were stable within the 5 h of observation (Figure 5). The hour term was not significant in any of the nine combinations ($P = 0.119$ – 1.000), and comparing the first hour with

hours 2 to 5 within dishes gave no significant change either ($P = 0.098$ – 1.000). Avoidance in *T. castaneum* and *C. chinensis* was therefore established within the first hour and maintained, and attraction of *S. oryzae* to the chloroform extract persisted throughout.

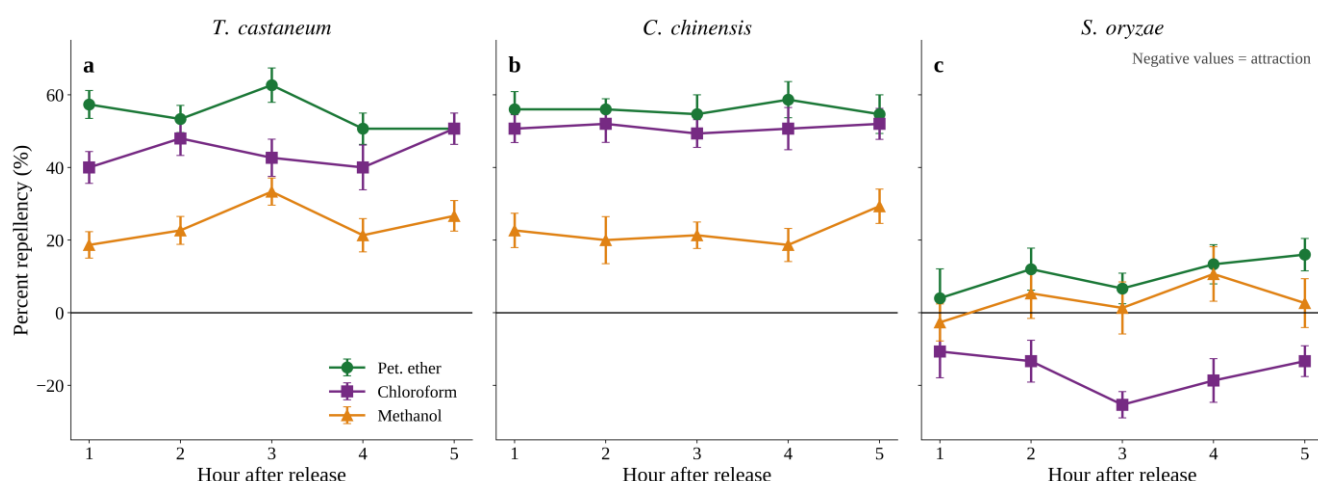


Figure 5. Responses were stable over the observation period. Mean percent repellency ($\pm$ SE of dishes, doses pooled) at each hour after release. Values above zero indicate avoidance and below zero attraction.

3.5. Differences Among Species and Extracts

In the two-way analysis of variance on dish means, repellency differed among species ($F_{2,125} = 288.5$, $P < 0.001$) and among extracts ($F_{2,125} = 66.2$, $P < 0.001$), with a significant interaction ($F_{4,125} = 26.0$, $P < 0.001$) and a positive effect of

log dose ($F_{1,125} = 30.1$, $P < 0.001$). In Tukey comparisons, *S. oryzae* differed from both other species for every extract ($P < 0.001$), whereas *T. castaneum* and *C. chinensis* never differed from each other. Within *T. castaneum* all three extracts differed; within *C. chinensis* petroleum ether and chloroform were indistinguishable and both exceeded methanol; within *S. oryzae* the chloroform extract differed from the other two.

Table 4. Two-way analysis of variance of dish-mean repellency (type II sums of squares) with $\log_{10}$ dose as a covariate.

Source	df	Sum of squares	F	P
Species	2	55491.1	288.45	< 0.001
Extract	2	12739.1	66.22	< 0.001
Species $\times$ extract	4	9984.5	25.95	< 0.001

Source	df	Sum of squares	F	P
log ₁₀ dose	1	2894.9	30.10	< 0.001
Residual	125	12023.5		

3.6. Sensitivity Checks

Removing the highest dose from the analysis changed no conclusion (Table 3): repellency remained clear for *T. castaneum* and *C. chinensis* with all three extracts, attraction of *S. oryzae* to the chloroform extract remained (−18.0%, *P* < 0.001), and the weak repellency of *S. oryzae* by the petroleum

ether extract became non-significant, as expected once the only dose with a strong response is discarded. Analysing the lowest dose alone gave the same directions throughout. Taken together with the sign tests and the dose-mean analysis (Section 3.1), eight of the nine conclusions are unchanged under every alternative treatment of the data; the exception is the weak repellency of *S. oryzae* by the petroleum ether extract, which is reported here as suggestive only.

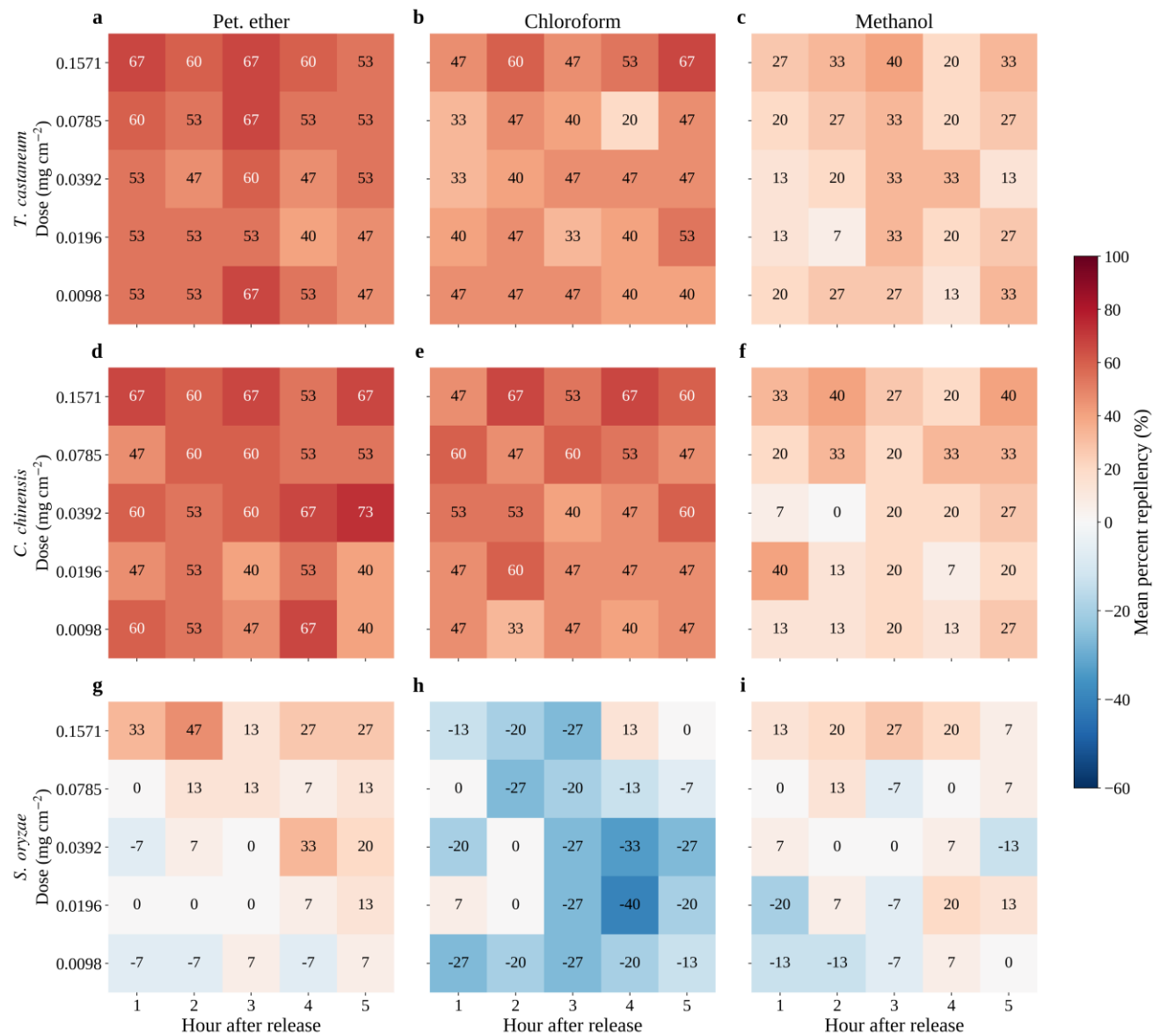


Figure 6. Mean percent repellency of three dishes at every combination of dose and hour, for each species and extract. Red indicates avoidance of the treated side and blue preference for it. The pattern is uniform within each panel, which is why neither dose nor hour explains much variation for the two repelled species.

4. Discussion

4.1. One Extract, Opposite Responses

The central result is that the same three extracts moved three storage pests in different directions. *Tribolium castaneum* and *C. chinensis* avoided all of them, in all 90 dishes in which they were tested. *Sitophilus oryzae* was barely repelled by the petroleum ether extract, was unaffected by the methanol extract, and settled preferentially on the side treated with the chloroform extract in all 15 of its dishes. Consistency at this level is unusual in repellency work, where means are often reported without the spread behind them, and it is the strongest evidence we can offer that the direction of the response is a property of the species rather than of a particular dish or dose.

The arena cannot explain the difference. *Callosobruchus chinensis* and *S. oryzae* were tested in the same food arena, and the two species responded in opposite directions, while *C. chinensis* behaved like *T. castaneum*, which was tested on filter paper. If the arena were driving the result, the two food-arena species would have grouped together, and they did not.

4.2. Why a Weevil Might be Attracted

Attraction to a plant extract is biologically plausible for *S. oryzae*. The species locates host grain by olfaction, and adults are attracted to specific volatiles released by stored rice, including nonanal, hexanal and 1-octen-3-ol, with nonanal proposed as a monitoring lure [12]. Volatile profiles of infested grain are themselves being developed as early-detection indicators for this pest [13]. Plant volatiles and terpenes act on several targets in insects, including the nervous system [28], and aldehydes and short-chain alcohols of the kind that dominate such profiles are common constituents of plant extracts, and the chloroform fraction of plant material would be expected to carry medium-polarity volatiles of this type. Earlier work already showed that *S. oryzae* and *T. castaneum* respond differently to identical food volatiles [29], and our result extends that difference from food odours to a non-host plant extract. Two behavioural mechanisms could produce the same counts. The weevils may be orienting toward the extract from a distance, or they may simply be arrested once they encounter the treated side and move less. A static count of where insects sit cannot separate orientation from arrestment, and an olfactometer or a video-tracked arena would be needed to do so. Without chemical analysis of the fraction, the mechanism also remains unidentified; characterising the constituents of the chloroform fraction is the obvious next step.

4.3. Repellency and Toxicity are Separate Properties

The chloroform extract was behaviourally the second most active material in this study but killed none of the three species in the companion toxicity work, while the methanol extract

killed all three and repelled least. The same dissociation appears in the two other ornamental aroids screened in this laboratory: in *Epipremnum aureum* the chloroform extract caused no mortality yet repelled *T. castaneum* and *C. chinensis*, and in *Aglaonema marantifolium* the chloroform extract was non-toxic but repellent to *C. chinensis* [24, 25]. A non-toxic but behaviourally active chloroform fraction therefore recurs across three genera of Araceae, and it is not confined to that family: chloroform extracts of *Urena sinuata* repelled *T. castaneum* [18], and the chloroform extract of *Melia azedarach* was non-toxic yet repellent to the same beetle [19]. For screening practice the implication is direct: a fraction dismissed on a mortality test may be the most useful one behaviourally, and studies that report only mortality will miss it.

4.4. What the Result Means in a Store

Two practical readings follow, one cautionary and one constructive. The cautionary reading is that a botanical repellent developed on one pest may redistribute another. In a small-holder store in Bangladesh, pulses, flour and paddy are often kept side by side, and a treatment that drives pulse beetles and flour beetles away from treated sacks could increase weevil pressure on untreated cereals nearby. Screening programmes that test a single species cannot detect this, and we would encourage authors to report negative repellency values rather than treating them as failures.

The constructive reading is that an attractive fraction is a resource. Behaviourally based management of storage pests uses attractants for monitoring, mass trapping and attract-and-kill, and combines repellents and attractants in push-pull schemes; the main constraints identified for these tactics are competition with the abundant food odours already present in a facility and the difficulty of finding species-specific cues [2]. A plant fraction that attracts *S. oryzae* against a background of food in the arena, as ours did, is worth testing as a trap bait. That would require formulation, release-rate measurement and validation in traps, none of which this study provides.

4.5. Limitations

Several limitations bound the conclusions. First, an arena with solvent applied to both halves was not recorded, so a contribution of residual solvent odour to the response cannot be formally excluded; the treated paper was dried for only 10 min. Three features of the data argue against solvent alone driving the result: the three solvents produced different magnitudes of response in the same species, the direction reversed between species for the same solvent, and a genuine dose response was detected in one combination. A solvent artefact would be expected to act in the same direction in every species and to be independent of dose. Even so, a solvent control is the first thing a repetition of this work should include.

Second, repellency was near maximal at the lowest dose tested for the two repelled species, so the assay was saturated

and the lowest effective dose was not established; a series extending below 0.0098 mg cm⁻² is needed. Third, observations lasted 5 h, which says nothing about persistence over the weeks or months that a protectant would have to work, and nothing about whether the response habituates. Fourth, the extracts were not characterised chemically, the plant was not vouchered and extract yields were not recorded, so the active constituents cannot be named and the doses cannot be related back to a mass of plant material. Fifth, the assay records where insects settle, not how they got there, so preference and arrestment cannot be separated, and the dose unit is not physically comparable between the filter-paper and food arenas. The age and sex of the test insects were not recorded, and sex-specific responses are known in storage pests. Finally, with three dishes per dose the design could detect differences of roughly 14 to 33 percentage points from zero depending on the combination (Table 1), so the null result for the methanol extract against *S. oryzae* means the absence of a large effect, not the absence of any effect.

4.6. Recommendations for Further Work

The priorities are, first, a repetition with solvent-only controls, a wider and lower dose range and longer observation; second, chemical characterisation of the chloroform fraction with electroantennography and olfactometry to identify the constituents that attract *S. oryzae*; third, an evaluation of the attractive fraction as a trap bait against grain-baited controls; and fourth, tests of the repellent fractions on treated commodities, measuring infestation, oviposition and progeny production rather than settling position alone, since short-term avoidance in a dish does not by itself demonstrate protection of stored grain.

5. Conclusions

Plant extracts of *S. podophyllum* repelled *T. castaneum* and *C. chinensis* consistently and at doses well below those needed for toxicity, while the chloroform extract attracted *S. oryzae* in every dish tested; repellency of that species by the petroleum ether extract was weak and not robust. Behavioural activity was independent of toxicity: the fraction that moved insects most was the one that killed none. Screening studies of botanical repellents should therefore test more than one pest species, should report attraction as an outcome in its own right, and should treat an attractive fraction as a lead for trapping rather than as a negative result.

Abbreviations

ANOVA	Analysis of Variance
CI	Confidence Interval
GEE	Generalized Estimating Equation
PR	Percent Repellency
Pet. ether	Petroleum Ether

SE Standard Error

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Author Contributions

Nazmul Hoque: Data curation, Formal Analysis, Investigation, Writing – original draft

Tandid Kamal: Investigation, 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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