

Original Research

Efficacy of Various Plant Extracts and Synergism Against Domestic Species of Rice Weevil *Sitophilus Oryzae* (Curculionidae: Coleoptera)

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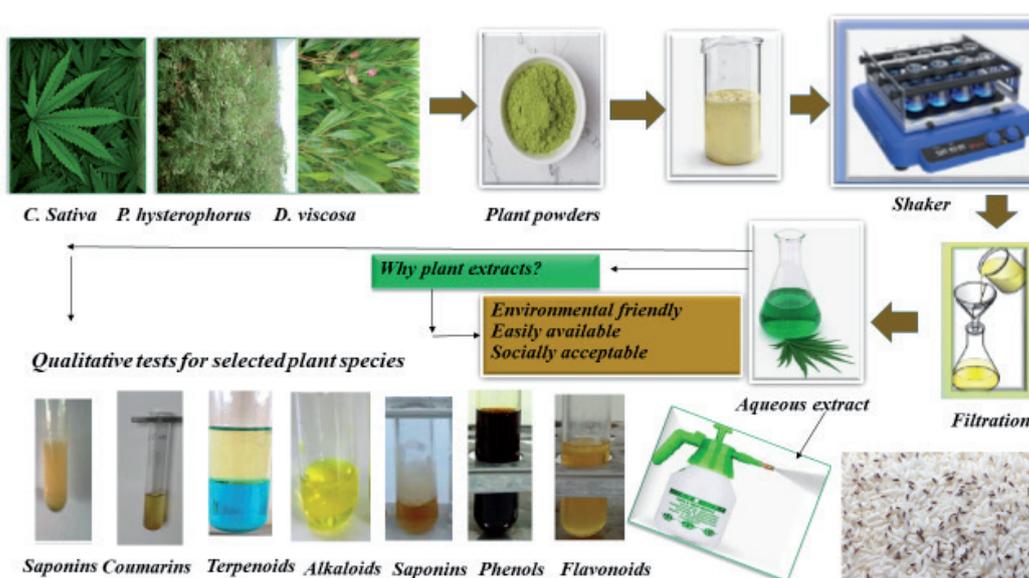
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Abstract

Despite being successful in preventing pests from entering stored goods, residual synthetic pesticides have had a negative impact on the environment. However, the prohibitive cost of synthetic pesticides and the risk of target pests developing insecticide resistance have prompted researchers to develop alternative management strategies. Since plant-based pesticides are biodegradable and less dangerous for organisms other than the target species, they are a useful alternative to chemical pesticides. The aim of the present study was to investigate the effectiveness of plant extract in preventing the rice weevil *Sitophilus oryzae* (Linnaeus) (Coleoptera: Curculionidae) from infesting stored-products. For toxicity and synergism against *S. oryzae*, aqueous extracts of *Cannabis sativa* L., *Dodonaea viscosa* L., and *Parthenium hysterophorus* L., were evaluated at different concentrations. *D. viscosa*

and *P. hysterophorus* extract concentrations were 0.25, 0.5, 1.0, 2.0, and 3.0%, and *C. sativa* extract concentrations were 0.62, 1.25, 2.5, 5.0, and 7.5%. A completely random design with three replications was used, with distilled water serving as the control. The experimental setting was kept at $27 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ R.H. The findings showed that *D. viscosa*, *P. hysterophorus*, and *C. sativa* caused 97%, 90%, and 83% of mortalities at the highest doses, respectively. At highest concentrations, the mortalities found for binary combinations of *D. viscosa* and *P. hysterophorus*, *D. viscosa* and *C. sativa*, and *P. hysterophorus* and *C. sativa*, respectively, were 80%, 77%, and 70%. When used in combination, the effects of *D. viscosa*, *P. hysterophorus*, and *C. sativa* produce 100% mortalities, indicating synergism. In single applications for all treatments, plant extracts were the most toxic. However, when used in combination, *S. oryzae* is completely killed. Phytochemical screening test all the compounds were detected but saponins and coumarins were higher in *D. viscosa* and *P. hysterophorus*. As a result, mixing plant extract greatly increases the effectiveness of reducing *S. oryzae* in storage facilities. Small-scale farmers may benefit from the study's findings since they may offer them a grain storage approach that is both practical and affordable.

Keywords: bio rational, Integrated Pest Management, phytochemicals plant extracts, synergism and toxicity



Introduction

An estimated 600 species of store grain pests have been identified so far which attack and cause a significant quantitative and qualitative losses to these valuable agricultural commodities [1]. The rate of infestation is high in tropical region (20–30%, most likely due to suitable weather conditions that favor their growth and reproduction) than temperate zone (5–10%) [2]. One such detrimental pest of rice crop commonly found in Pakistan is the rice weevil *Sitophilus oryzae* L. (Coleoptera: Curculionidae). The most damaging insect pest of rice in stored in Pakistan is the rice weevil. In rice, the adult female weevil lays eggs from which the larvae feed and mature into the adult stage [3]. In addition to rice, this pest also lives on sorghum, wheat, corn, oats, rye, barley, rice, and dry beans [4].

S. oryzae attacks milled and stored rice seeds, using them to produce significant financial losses. The carbohydrates in rice seeds are consumed by both adults and larvae also causing weight loss and deterioration [5]. The stored seeds may also sustain up to 100% harm in a loss of control [6]. *S. oryzae* increases both the temperature and humidity of damaged seeds, which promotes the growth of secondary pests and creates the most ideal conditions for pathogens and further infestation [7].

The most widely used fumigants for controlling stored grain insect pests are methyl bromide, phosphine, and sulfuryl fluoride. These fumigants provided effective control of grain pest insects in storage buildings quickly [8]. However, using artificial fumigants as pesticides has resulted in various health and environmental issues. Insect resistance development is another factor [9].

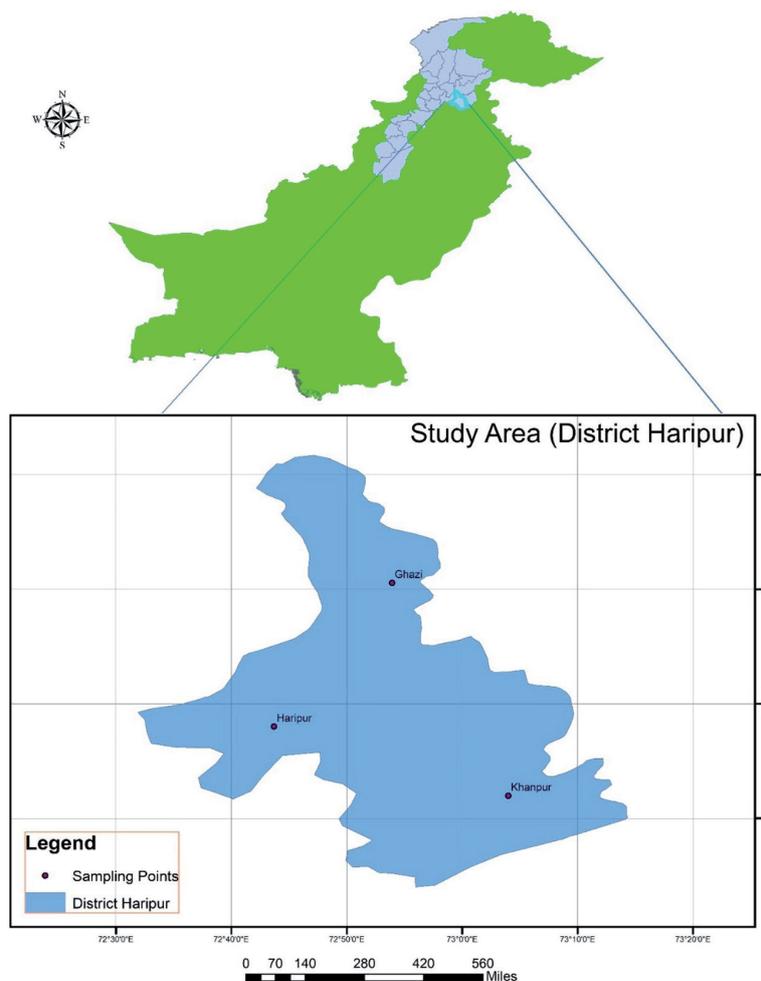


Fig. 1. Location where plant species from Haripur-Pakistan were harvested in 2022.

Table 1. Three different plant species and their tissues used in bioassays.

Plant species	Family	Parts used	Concentrations
<i>D. viscosa</i>	Sapindaceae	Leave	0.25, 0.50, 1.00, 2.00 and 3.00%
<i>P. hysterophorus</i>	Asteraceae	Leave	0.25, 0.50, 1.00, 2.00 and 5.00%
<i>C. sativa</i>	Cannabaceae	Leave	0.62, 1.25, 2.50, 5.00 and 7.50 %

The plant has an active ingredient that serves as defense against insect pests. There are many techniques, such as those that are poisonous, attractive, antifeedant, and repulsive [10]. Effective components found in plants, such as saponins, alkaloids, flavonoids, triterpenoids, and glycosides, can have an insecticidal impact, according to [11]. Medicinal herbs are typically the source of prospective bioinsecticide-producing plants [12]. Because of their plentiful supply of secondary metabolites like alkaloid, saponin, tannin, and flavonoid, most medicinal plants from the Zingiberaceae family [13].

Dodonaea is a genus of flowering plants that includes up to 70 species. It belongs to the Sapindaceae family, is used in numerous studies. Infrequently used plant parts in the creation of common biological

insecticides included stems, leaves, and fruits. *Dodonaea* species exhibit weedicide, pesticide, and insecticide activity in addition to their allelopathic effects [14]. The crude extracts of *D. viscosa* also contain several phytochemicals, including as terpenoids, saponins, and tannins [15].

Parthenium hysterophorus L. (Asteraceae) is an invasive weed with the common names chatakchandani and gajarghas [16, 17]. Sesquiterpene lactones, which are present in all plant sections, are known to be toxic [18, 19]. *Parthenium* is a highly productive weed that causes challenging problems with money, health, and the environment. It is also known to supply allelochemicals that prevent meadow weeds and other plants from germinating and growing [20].

Plant with flowers essentially a dioecious plant that is seasonal, *Cannabis sativa* produces cannabinoids, a secondary metabolite found mostly in the plant's glandular trichomes [21]. Cannabinoids, which are formed from geranyl diphosphate (GPP) and olivetolic acid, are among at least 120 unique secondary metabolites, or C21 terpenophenolic constituents [21]. *Cannabis* has more than 400 compounds, but if they're not present in large amounts, the aqueous extracts can operate as an effective insecticide [22]. Numerous volatile substances, including esters, ketones, and terpenes, ooze from the leaf glands to provide the plant's pungent odor [23].

Synthetic pesticides have been used carelessly for a long time, causing major issues such direct toxicity to people, fish, and beneficial insects. Most small holding godown owners and farmers choose alternate techniques of disease and insect control due to the unstable supply and high costs of conventional pesticides. Numerous plants have demonstrated promise in reducing insect pests without causing any negative side effects. Plant-based products are readily available, easily produced, and socially and environmentally acceptable. These plants may provide a fresh approach to the control of insect pests. The comparative lack of effectiveness of botanicals was a significant issue. Exploiting the synergistic effects of these biological when utilized as compounds can be one of the methods to increase the potency of these botanical choices.

The combinatorial effect of plant extracts against *S. oryzae* has rarely been investigated so far. Here, we report the synergistic effects of mixtures of plant extracts against *S. oryzae* for which LC_{50} of each material both alone and in combination was obtained. We believe that the botanical combinations will offer an alternate method for reducing the use of insecticides against insect pests.

Materials and Methods

Collections of Insect

S. oryzae adults were obtained from grain godowns in the district of Haripur in Pakistan. Adults that had been collected were brought to the entomology lab at the University of Haripur in Khyber Pakhtunkhwa, Pakistan [24].

Established of Stock Culture

After being placed in a plastic bag, the rice was autoclaved at 121°C for 30 minutes to sterilize it and, if necessary, destroy any stored grain insects. Each glass jar was filled with roughly half a kilogram of rice, covered with muslin, and secured with a rubber band after sterilization. The growing chamber in which the rearing jars were housed was maintained at 28°C + 20°C and 60+5% R.H. 10. *S. oryzae* pairs

were introduced to the rearing jar and where they were allowed to mate and oviposit for 48 hours before being moved to the next rearing jar and so on. Adult *S. oryzae* started emerging from the rice in the jars after 28 days. For experimental purpose, adult *S. oryzae* of an similar age and size were collected by sieving the jar at regular intervals [25].

Collection of Plant Species

As indicated in Fig. 1, many different plant materials, including leaves from *Dodonaea viscosa*, *Parthenium hysterophorus*, and *Cannabis sativa*, were collected from various sites throughout the district of Haripur in Pakistan. Additionally, several formulations of each plant were chosen for the study. Table 1 shows the bioassays conducted with untreated seeds of a commonly grown rice cultivar in Pakistan.

Plant Powder Preparation

The obtained plant parts were washed with distilled water and left to shade dry for a month at the ambient temperature, before grinding them into powder. The powders were obtained by grinding the dried plant tissues in a kitchen grinder and sieved with a sieve with a mesh size 60. To prevent quality loss, the powders so obtained were stored in air tight polythene bags at room temperature [26].

Preparation of Aqueous Extract

Each plant parts contained a 14 g fine powder that had been shade-dried, and the mixture was shaken for an hour at 140 rpm and 37°C, followed by its filtration with filter paper (Whatman No. 1). For experimental application, the 10% stock solution prepared was later on diluted as per the need [27].

Concentration of Plants Aqueous Extracts

To assess the effect of plant extracts, on newly emerging beetles, stock solutions of various plant extracts were diluted and applied at concentrations of 1%, 2%, 3%, 4%, 5%, 6%, 7%, and 8%. Using a mist sprayer, Petri dishes (9 cm diameter) were sprayed thoroughly from all directions and allowed to air dry for 30 minutes. A camel hair brush was used to cage ten pairs of adult beetles that were the same size. After treatment, the number of adult beetles that are dead were counted, and the percentage of deaths is converted for each concentration after a 24-hour exposure period [28]. The LC_{50} of three plant species, namely *D. viscosa* (1%), *P. hysterophorus* (1%) and *C. sativa* (2.5%), were calculate after determining the corrected mortalities of *S. oryzae*. Based on these LC_{50} values, five alternative concentrations for every plant species were made.

Plant Aqueous Extract Adulticidal Bioassay

The three plant species' extracts' lethality to *S. oryzae* were assessed. Distilled water was utilized as a standard and was used to dilute each plant species concentration (10%) to obtain five varied concentrations, including *C. sativa* (0.62, 1.25, 2.5, 5.0, and 7.5%), *D. viscosa* (0.25, 0.5, 1.0, 2.0, and 3.0%) and *P. hysterophorus* (0.25, 0.5, 1.0, 2.0, and 3.0%). With the aid of a mist sprayer, Petri dishes (9 cm in diameter) were fully sprayed from all angles until runoff, and they were then allowed to air dry for 30 minutes. A camel hair brush was used to cage ten equally sized adult beetle couples. Twenty-four hours of post-treatment to each concentration, mortality counts of adult beetles were taken and converted to a percentage mortality. Experiments were repeated with three replicates each [28].

Binary Combination of Plant Extracts Utilized in the Adulticides Bioassay

Each mixture was created with five different concentrations. These tests were performed to investigate the synergistic action produced by five concentrations, including i.e., + *D. viscosa* + *P. hysterophorus* (0.25 + 0.25, 0.5 + 0.5, 1.0 + 1.0, 2.0 + 2.0 and 3.0 + 3.0), *P. hysterophorus* + *C. sativa* (0.25 + 0.62, 0.5 + 1.25, 1.0 + 2.5, 2.0 + 5.0 and 3.0 + 7.5), *C. sativa* + *D. viscosa* (0.62 + 0.25, 1.25 + 0.5, 2.5 + 1.0, 5.0 + 2.0 and 7.5 + 3.0) and *D. viscosa* + *P. hysterophorus* and *C. sativa* (0.25 + 0.62 + 0.25, 0.5 + 1.25 + 0.5, 1.0 + 2.5 + 1.0, 2.0 + 5.0 + 2.0 and 3.0+7.5+3.0). The only treatment used on the control was distilled water. After 24 hours, *S. oryzae* mortality was examined. The Synergism for the combined formulation were calculated using CompuSyn.

Qualitative Assessment of Phytochemicals

To determine whether there were any phyto-constituents present or absent, phytochemical screening procedures were carried out i.e. Alkaloids, coumarins, flavonoids, polyphenol, saponins, tannins, terpenoids were in *D. viscosa*, *P. hysterophorus* and *C. sativa* extracts using standard methods [29].

Terpenoids

2 ml extract was added to of acetic anhydride and H_2SO_4 , Formation of a blue and green ring indicated the presence of terpenoids [30].

Tannins

2 ml of the extract was added a few drop of 1% lead acetate. A yellowish precipitate indicated the presence of tannins [31].

Saponins

20 ml distilled water and 5 ml of plant extract in a graduated cyclinder was agitated for 15 min. Foam formation confirms the presence of saponins [32].

Coumarins

3 ml of 10% NaOH was added to 2 ml of aqueous extract formation of yellow color indicates the presence of coumarins [33].

Flavonoids

A 3 ml extract solution was mixed with two ml of dilute sodium hydroxide (NaOH), which gave the solution a yellow hue. After treating with 1 ml of 5 N hydrochloric acid (HCl), the solution became colourless, indicating the presence of flavonoids [34].

Phenols

2 ml of distilled water, 1 ml of plant tissue extract, and a few drops of 10% ferric chloride ($FeCl_3$) solution were combined. The presence of phenol is indicated by the development of a green or blue tint [35].

Alkaloids

All plant extracts were separately treated in dilute hydrochloric acid and filtered. The Mayer's reagent (Potassium Mercuric Iodide) was added to the filtrates. The presence of alkaloids was revealed by the yellow precipitate [34, 35].

Data Analysis

To find the dose effect responsiveness and compatibility index of the multiple combinations employed in the experiment, results were analyzed using "compusyn" [36].

Results

Phytochemical Tests

Powdered leaves of *D. viscosa*, *P. hysterophorus* and *C. sativa* were given a precise chemical and reagent treatments. The modified plant powder underwent additional light microscopy analysis. All three plant powders were treated with dilute sodium hydroxide (NaOH), which produced a yellow colour confirming the flavonoids. Likewise, when treated with 1% lead acetate, a dark blue to black colour indicated tannin. The emergence of a green or blue tint after adding a few drops of 10% ferric chloride ($FeCl_3$) showed the existence of phenols. The creation of foam after 15 minutes of stirring in a graduated cylinder with

Table 2. Preliminary screening of secondary metabolites.

S. No	Phytochemical	<i>D. viscosa</i>	<i>P. hysterophorus</i>	<i>C. sativa</i>
1	Phenol	+	+	+
2	Tannins	+	+	+
3	Terpenoids	+	+	+
4	Saponins	+++	++	+
5	Flavonoids	+	+	+
6	Alkaloids	+	+	+
7	Coumarins	++	++	+

Note: ++high presence, +moderate presence, -absence.

Table 3. Contact mortality of *S. oryzae* exposed during 24 hours to five different concentrations of *D. viscosa*, *C. sativa*, and *P. hysterophorus* in 2022.

Plant species	Concentrations%	Effect/ Mortalities	m, slope	Dm, LC ₅₀	r, radius
<i>D. viscosa</i>	0.25	20.00	1.80017 +/- 0.27839	0.62758	0.96595
	0.5	40.00			
	1.0	60.00			
	2.0	83.00			
	3.0	97.00			
<i>C. sativa</i>	0.62	27.00	0.98348 +/- 0.14513	2.06599	0.96885
	1.25	37.00			
	2.50	47.00			
	0.50	67.00			
	7.50	83.00			
<i>P. hysterophorus</i>	0.25	30.00	1.04386 +/- 0.27028	0.67898	0.91245
	0.50	43.00			
	1.00	53.00			
	2.00	63.00			
	3.00	90.00			

Note: Insect mortality as a result of concentration, m, is slope, Dm, LC₅₀, and r, radius, is the effect.

5 ml of extract and 20 ml of distilled water indicated the presence of saponins. On the other hand, when treated with dilute hydrochloric acid and filtered, the filtrate after reacting with Mayer's reagent (Potassium Mercuric Iodide) produced a yellow precipitate which confirmed alkaloids in the plant extracts. When 2 ml of the plant extracts from each plant were put to 2 ml of acetic anhydride and a concentration of H₂SO₄, Mayer's reagent (Potassium Mercuric Iodide), the production of a yellow coloured precipitate showed the presence of alkaloids. Formation of blue green ring by adding 2 ml acetic anhydride and concentrated H₂SO₄ to 2 ml of extracts indicates terpenoids. When NaOH was added to 2 ml of aqueous extract, a yellow colour developed,

signifying the presence of coumarins. *D. viscosa* and *P. hysterophorus* exhibited maximum phytochemicals as compared to *C. sativa* as shown in Table 2.

Toxicity of Plant Species Extracts

S. oryzae was treated with the botanical insecticides *D. viscosa*, *P. hysterophorus*, and *C. sativa*. At 24 hours after treatment, the median lethal concentration (LC₅₀) was calculated to determine the contact toxicity of plant extracts.

From (Table 3 and Fig. 2(a,b,c)). It was obvious that there were five different *D. viscosa* concentrations. The model was a good fit, according to the value of r (0.96).

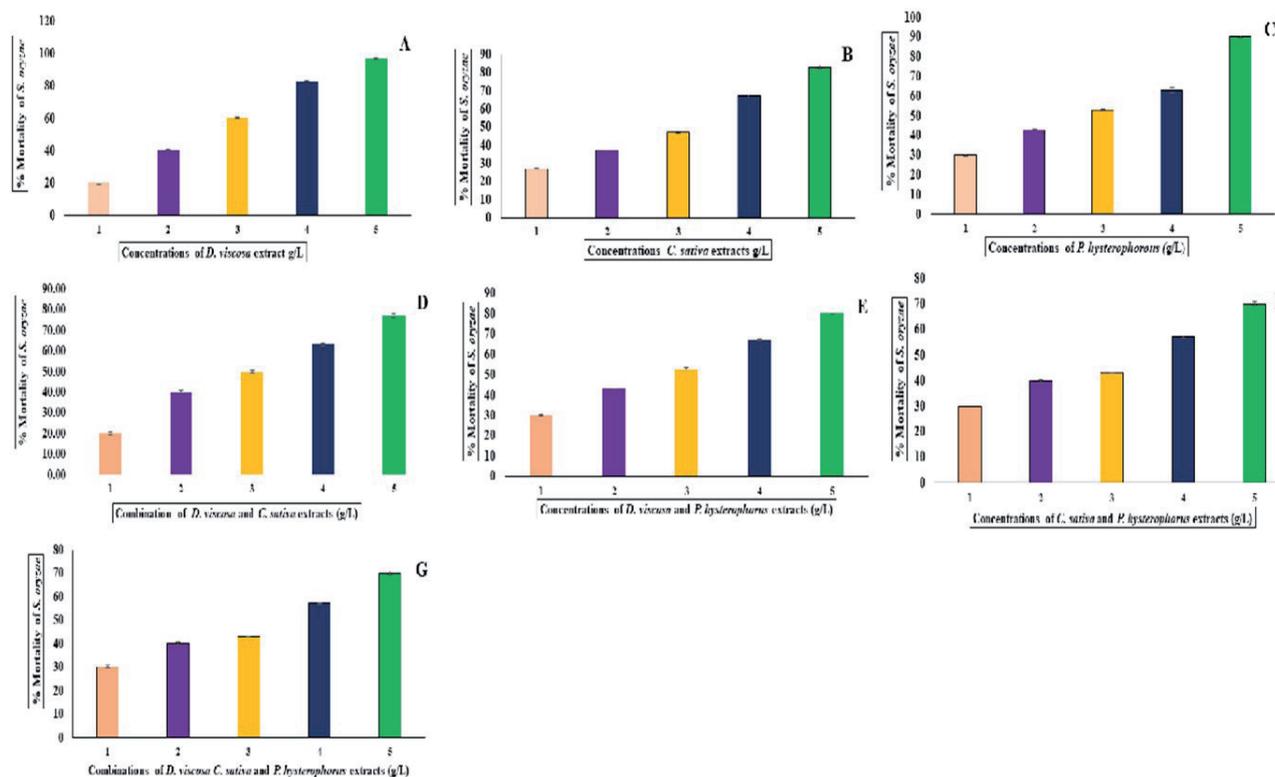


Fig. 2. Mean percent of *S. oryzae* mortality when given various doses of *D. viscosa*. a) *C. sativa*; b) *P. hysterophorus*; c) *viscosa* + *C. sativa*; d) *D. viscosa* + *P. hysterophorus*; e) *C. sativa* + *P. hysterophorus*; f) and *D. viscosa* + *P. hysterophorus* + *C. sativa*; g) after 24 hours' exposure period.

LC₅₀ (Dm) value was 0.62%. Mortality rates ranged from 20% at the lowest dose (0.25) to 97% at the highest concentration (3.0%). In *C. sativa* the value of *r* (0.96) indicate that the model was a good fit as shown in when five different concentration of *C. sativa* were used. The value of LC₅₀ value (Dm) was observed 2.06%. Minimum concentration of *C. sativa* 0.62% when used it gave 27% mortality and highest concentration used (7.5%) showed 83% death rate. The adults of *S. oryzae* showed significant toxicity toward the plant extracts under study. Concentration influenced the extracts' toxicity for the *P. hysterophorus* plant species that was tested. The model fit the data well, as indicated by the value of *r* (0.91). LC₅₀ value was 0.67% (Dm). After 24 hours of exposure contact mortality of *S. oryzae* treated with five different concentrations of each plant species, the lowest concentration used (0.25%) caused 30% mortality and the maximum concentration used (3.0%) gave 90% mortality.

The result in (Table 4 and Fig. 2(d,e,f)) showed that when binary combinations of selected plant extracts when used it gave promising death rate to *S. oryzae*. *D. viscosa* + *C. sativa* shows that a number of combination effects, depending on the concentration of both ingredients, revealed antagonistic effects. When combined with *C. sativa* (0.62%), *D. viscosa* (0.25%) is shown to be antagonistic. However, the subsequent higher level (5.0% *D. viscosa* mixed with 7.5% *C. sativa*) resulted in a significant mortality while continuing showing strong

antagonism. Results revealed that various combinations of plant extracts exhibited a number of antagonistic effects, subject to the different concentration of both component, exhibited. Antagonism was also observed when *P. hysterophorus* (0.25%) was combined with *D. viscosa* (0.25%) giving a mortality rate of 30% in *S. oryzae* adults. A subsequent, high concentration, 5.0% *D. viscosa* mixed with 5.0% *P. hysterophorus*, caused a high mortality rate of 80%, still demonstrated a strong antagonistic effects. According to the results, different binary combinations of (*P. hysterophorus* + *C. sativa*) demonstrated antagonistic effects depending on the concentration of both components. Both at lower i.e. (0.25% + 0.62%) and higher concentrations i.e. (5.0% + 7.50%) exhibited high mortalities i.e. 30% and 70% to *S. oryzae* after 24 hours' exposure period.

Depending on the concentration of the ingredients, the effects of the triadic combination range from synergism to antagonism. When the three plants *D. viscosa*, *P. hysterophorus*, and *C. sativa* were used combined, synergism occurs at higher concentrations. At this concentration, the effect size appears to be substantial. However, at greater concentrations, the combination instigated a maximum mortality (100%), still demonstrating synergism, and making it a desirable option for the control of *S. oryzae* as shown in (Table 5 and Fig. 2g). Contact mortality of *S. oryzae* exposed for 24 hours and treated with five different concentrations of *D. viscosa*, *P. hysterophorus*, and *C. sativa*.

Table 4. *S. oryzae* contact mortality after five different combinations of concentrations of *P. hysterothorus*, *D. viscosa* and *C. sativa* after 24 hours during 2022.

Plant species	Effect/ Mortalities	CI	
<i>D. viscosa</i> + <i>C. sativa</i>			
0.25 + 0.62	0.20	2.08911	Antagonism
0.50 + 1.25	0.40	2.70759	Antagonism
1.00 + 2.50	0.50	2.80349	Antagonism
2.00 + 5.00	0.63	3.77986	Strong Antagonism
5.00 + 7.50	0.77	3.50567	Strong Antagonism
<i>D. viscosa</i> + <i>P. hysterothorus</i>	Effect/ Mortalities	CI	
0.25 + 0.25	30.00	1.466	Moderate Antagonism
0.50 + 0.50	43.00	1.896	Moderate Antagonism
1.00 + 1.00	53.00	2.803	Antagonism
2.00 + 2.00	67.00	3.649	Strong Antagonism
5.00 + 5.00	80.00	3.383	Strong Antagonism
<i>C. sativa</i> + <i>P. hysterothorus</i>	Effect/ Mortalities	CI	
0.25 + 0.62	30.00	1.539	Moderate Antagonism
0.50 + 1.25	40.00	1.599	Moderate Antagonism
1.00 + 2.50	43.00	3.541	Strong Antagonism
2.00 + 5.00	57.00	4.065	Strong Antagonism
5.00 + 7.50	70.00	3.496	Strong Antagonism

Note: Insect mortality is the result, while CI stands for combination index. CompuSyn software was used to investigate synergistic effects. *, $p < 0.05$.

Discussion

In order to reduce the usage of synthetic pesticides, it has been thoroughly studied a comparison between plant extracts and pesticides, enhance their toxicity, make the environment safer and minimize pesticide resistance [37]. We looked at how toxic and effective plant extracts were against *S. oryzae*. Our research revealed that the combined action of plant extracts had an impact on *S. oryzae* mortalities.

The findings of [34] that plant extraction mortalities increase as concentration increases agree with the findings of our study. [15] stated that all portions of the *D. viscosa* plant's aqueous extract contained saponins, tannins, flavonoids and terpenoids. According to our findings, the presence of several phytochemicals may be the cause of the maximum toxicity in *D. viscosa*. Our findings were supported by the findings of [38] which showed that *Callosobruchus maculatus* adults died at a rate of 100% when *D. viscosa*'s unsap fraction, 1.0%, was used. The similar results of [39] were also supported by our most recent research, which showed that *D. viscosa* was the plant extract that killed *S. oryzae* most quickly. [40] stated that the use of *Trigonella foenum-graecum*

extracts topically significantly reduced the susceptibility of *Tribolium castaneum* and *Acanthoscelides obtectus*.

Mortality ranged from 27% at the lowest dose (0.62) to 83% at the highest exposure (7.5%). According to [41] Cannabis sativa leaf extracts are poisonous to insect pests at all life stages. There are at least 120 separate secondary metabolites, or cannabinoids, that are generated from geranyl diphosphate (GPP) and olivetolic acid [21]. The danger three cannabis terpenoids, limonene, linalool, and pinene, are sold as pesticides [42]. Our study's findings were consistent with those of [43] who claimed that *P. hysterothorus* caused the greatest number of deaths when used against *T. castaneum* at 3% concentrations. According to an antifeedent bioassay, lactone is around 2.25 times more powerful than parthenin against *Spodoptera litura* larvae of 6th instar and pyrazoline adduct is the most effective pesticide against *Callosobruchus maculatus* adults, a pest that attacks storage grain [44]. Parthenin and other phenolic chemicals found in *P. hysterothorus*, which have insecticidal characteristics, are accountable for the toxicity [19]. Parthenin is the substance that makes *P. hysterothorus* effective. Parthenin, the main sesquiterpene lactone, exhibits a wide range of biological

Table 5. Mortality of *S. oryzae* in contact bioassay after 24 hours of exposure to a triple combination of *D. viscosa*, *P. hysterothorus*, and *C. sativa* at five various doses in 2022.

<i>C. sativa</i> + <i>D. viscosa</i> + <i>P. hysterothorus</i>	Effect/Mortalities	CI	
0.62 + 0.25 + 0.25	40.00	1.485	Moderate antagonism
1.25 + 0.50 + 0.50	53.00	1.947	Moderate antagonism
2.50 + 1.00 + 1.00	70.00	2.161	Strong antagonism
5.00 + 2.00 + 2.00	87.00	1.945	Moderate antagonism
7.50 + 5.00 + 5.00	100.00	0.470	Synergism

Note: The effect is insect mortality and CI is combination index. Synergistic effects were analyzed with CompuSyn software. *, $p < 0.05$

characteristics, including cytotoxicity, anticancer, allergy, antibacterial, antifeedant, phytotoxic, and insecticidal effects [45, 46]. These plants' interference with the primary metabolic, behavioral biochemical and physiological elements of insects is what cause their elevated mortality rates. Additionally, their high obsession can bring about by damaging their nerve cells, insects kill [47] as it was suggested at higher amounts of the extracts.

When combined with *C. sativa* (0.62%), *D. viscosa* (0.25%) is shown to be antagonistic. However, the subsequent greater concentration (5.0% *D. viscosa* mixed with 7.5% *C. sativa*) resulted in a high mortality while still displaying a strong antagonistic relationship. Tetrahydrocannabinol (THC), a cannabinoid with the highest psychoactive effect, may be responsible for the potential antagonistic effect. The cannabinoids may interact with one another to provide either supplementary or contradictory effects [48]. Cannabidiol (CBD), for instance, counteracts some of the effects of THC [49]. The next higher dosage, however, caused a high mortality of 70% while still displaying strong antagonistic effects (5.0% *P. hysterothorus* mixed with *C. sativa* 7.5%). Our findings supported the conclusions of [50, 51]. Comparing the insecticidal performance of individual components to combinations, the insecticidal efficacy of each component improves by about seven times. In addition to lowering the amount of synthetic pesticides used and improving the application's efficacy, utilizing such a control method partially resolves the issue of pests' insecticide resistance [51, 52]. The results of [53] demonstrating synergism between synthetic insecticides and extracts of *Dictyota dichotoma*, *Caulerpa scalpelliformis* and *Rhizophora apiculata* against *Aedes aegypti* are consistent with the results of our investigation. Although the precise mechanism of synergism is not entirely understood, prior research has demonstrated that bioactive substances and synthetic insecticides will inhibit the acetylcholine esterase (AChE) activity of *S. oryzae* [54, 55]. Due to the damage of the midgut epithelial cells' strength caused by particular enzymes known as synergism insects stop feeding and eventually perish [16]. Combining

botanicals may result in long-lasting, low-cost bio-pesticides [56]. The usage of natural insecticides could be beneficial an efficient strategy for reducing resistance because conventional pesticides are frequently used [57].

Conclusion

According to the findings of the current study, three plant species *D. viscosa*, *P. hysterothorus*, and *C. sativa* cause the greatest number of deaths at various concentrations. *S. oryzae* mortalities also increased with higher plant species concentrations in each of the examined plant extracts. *S. oryzae* experienced maximum mortalities of 97, 90, and 83% when extracts of *D. viscosa*, *P. hysterothorus*, and *C. sativa* were used in the highest concentrations. When employed in binary combinations of *D. viscosa* + *C. sativa*, *C. sativa* + *P. hysterothorus* and *P. hysterothorus* + *D. viscosa* at the highest concentration, mortalities decreased by 77, 80, and 70%, respectively, and antagonism was seen. When combined at their highest concentration, extracts of *D. viscosa*, *P. hysterothorus*, and *C. sativa* achieve highest mortalities of 100% and exhibit synergism. Mostly the phytochemicals were also detected in selected plant extracts. Because plant aqueous extract is environmentally favorable, both socially and financially acceptable and more research is needed to examine the stated possibility of using plant species' extracts to tackle *S. oryzae*. The selected plant materials could be further studied for commercialization purpose. Quantitative phytochemical screening and GC-MS analysis of the selected plant extracts is needed to find the exact compounds responsible for toxicity and synergistic effect against tested insect.

Novelty of the Study

The examined plant material has considerable synergistic potential and has never been tested against *S. oryzae* which is important for minimizing the toxic

effect of synthetic pesticides and providing safe control of the species.

Conflicts of Interest

The authors declare no conflict of interest

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