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Research Article

Efficacy of *Prosopis* spp. botanicals as sustainable protectants against adzuki bean weevil in stored grains

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ABSTRACT: Global interest in botanical biopesticides has surged in recent decades, driven by the need to reduce the environmental and health impacts of chemical pesticides. *Prosopis* species, notably *P. juliflora* and *P. cineraria*, are promising due to their rich phytochemical profiles. Traditionally used for medicinal purposes, these plants also exhibit allelopathic and insect-repellent effects. This study evaluates the efficacy of *P. juliflora* and *P. cineraria* leaf combinations in the management of *Callosobruchus chinensis* in a laboratory condition. Formulations of aqueous extracts, ether extracts, and aqueous suspensions at 1%, 2.5%, 5%, and 10% concentrations, alongside smoke treatments, were tested. Adult mortality ranged from 22.22% to 77.78%, with the highest mortality (77.78%) observed in the 10% aqueous extract. Formulations at 5% and 10%, and the 2.5% ether extract, achieved over 50% mortality, significantly outperforming the control (22.03%). Smoke treatments also showed notable effects. The results of the study highlight the calibre of *Prosopis*-based biopesticides as eco-sustainable IPM alternatives that are affordable and eco-friendly. By leveraging local *Prosopis* species, this approach could empower farmers to protect crops while minimising environmental harm, aligning with global sustainability goals.

KEYWORDS: Adjuki bean weevil, botanicals, *Callosobruchus*, storage pests, pulse beetle

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INTRODUCTION

The past couple of decades have seen a rising trend in research in the field of potential botanicals. Many nations across the globe have changed their policies regarding the use and commercialisation of these products to popularise their use and safeguard the environment against ill effects of chemical pesticides. Compared to developed countries, developing and underdeveloped countries face the brunt of pesticidal toxicity more due to lenient food security laws, making these countries better candidates for the use of biopesticides. (Isman, 2008).

The plants belonging to the genus *Prosopis* have been used in traditional medicine for the treatment of cancer, diabetes, inflammation, and as antimicrobial agents in our country (Asati *et al.*, 2022). The leaf and bark extracts were used in European countries for the treatment of oral ulcers, bronchitis, dermatitis, urinary infection, and as an anti-helminth (Pasiecznik, 1999).

Due to the existence of tannins, quinones, flavonoids, terpenes, phenolic components, and alkaloids, the *Prosopis* extracts exhibit remarkable activities against infection caused by helminths, protozoans, parasites, and bacteria, and act as analgesics, antioxidants, anti-ulcer, anti-pustule, and probiotics. It is also effective as a larvicide in malaria control (Henciya *et al.*, 2017).

In one research done in 2012 to assess the alkaloid content of different parts of *Prosopis juliflora*, it was found that leaves and pods (fruit) contained about 12 alkaloids (julifloridine, prosopine, prosopinine, and prosafrinine), whereas the flowers had only 4, mainly Juliprosopine (Singh & Verma, 2012).

Along with piperidine alkaloids, shikimic acid metabolites are also responsible for the allelopathic properties of *P. juliflora* (de Brito Damasceno *et al.,* 2018).

Prosopis cineraria and *P. juliflora,* along with two other species, were reported by Sharifi-Rad *et al.* (2019) to have the greatest potential as established by studies of numerous species for anti-plasmodial, anti-microbial, antioxidant, anti-cancer, cardio-protective, and analgesic properties, along with plant growth inhibitor effects. Neurotoxic effects were observed with leaves of *P. juliflora* when screened against Wistar rats, cattle, and goats.

Mesquite pods (fruit) had adverse effects on sexual function in female and male rats. Phenolic compounds reported in *Prosopis* plants are anthocyanins, flavonoids, apigenin, luteolin, quercetin, and their derivatives (Sharifi-Rad *et al.,* 2019). Extracts from *P. juliflora* seed had caused significant mortality of *Spodoptera litura* larvae (Kumarasamy *et al.,* 2018). *Prosopis juliflora,* along with Neem oil, was used to control third instar larvae of Khapra beetle, *Trogoderma granarium* and was found to enhance the bio-effectiveness of Neem Oil as a botanical synergist by Kumari *et al.* (2017). Earlier, Singh *et al.* (2012) attributed the glycosidic compounds present in *P. juliflora,* which might be the reason for the enhanced toxicity of neem oil. So, the present study was taken up to evaluate the efficacy of *Prosopis* spp. against adzuki bean weevil, a stored grain pest.

MATERIALS AND METHODS

A laboratory assay was designed to adjudge the effectiveness of combinations of two plants of the genus *Prosopis*, *viz*. *P. juliflora* and *Prosopis cineraria* on the adult mortality of the pest insect, *Callosobruchus chinensis*. Combinations of *P. juliflora* leaf + *P. cineraria* leaf and *P. juliflora* bark and *P. cineraria* fruit were formulated as aqueous extracts, ether extracts, and aqueous suspensions. The reason for using this particular combination was the excellent results obtained after testing various permutations and combinations of the different plant parts of these two. Different dose concentrations of 1%, 2.5%, 5%, and 10% of the formulations were applied to the test insects. Besides, the aspect of adult mortality was also studied by treating the pest with the smoke produced by the combination of the two plants. This study was done in the Department of Entomology, Dungar College, Bikaner, Rajasthan, India, under these phases:

Culturing and maintenance of pest culture

The test pest chosen for the assay was *Callosobruchus chinensis* Linn. A pure line culture was raised from its single pair. Sexes can be easily differentiated as the insect exhibits distinct and clear sexual dimorphism. For insect rearing, the cleaned and disinfested (60^0c °C for 4 h) seeds of moth bean *Phaseolus aconitifolius* Jacq., green gram *Vigna unguiculata* Linn., and cowpea *Vigna radiata* were used, which were placed in glass containers covered with muslin cloth to allow insects to breathe. The jars were set aside in a Biological Oxygen Demand incubator, kept at 28±2°C temperature and 70% relative humidity. 5 pairs of insects were inoculated per 100 g of all three pulses. Three replicas of each were taken.

Preparation of botanical formulations

The plants used in the test were collected from the vast college campus. The plant parts were picked from the tree, washed, and shade-dried for 14-15 days, ground, and kept in air-tight containers for later use. The combination of *P. juliflora* leaf + *P. cineraria* leaf and *P. juliflora* bark + *P. cineraria* fruit in powdered form was employed as liquid extracts, powder suspension, and as smoke.

(i) Aqueous extract – A measured quantity of 1 g powdered plant material was loaded into a thimble and extracted in 50 ml of distilled water by boiling until the volume was concentrated to 10 ml, giving a 10% stock solution. Working solutions of 1%, 2.5%, and 5 % were obtained by serial dilution with distilled water.

(ii) Ether extract – 1 g of dried, powdered plant material was placed in a thimble and subjected to Soxhlet extraction using petroleum ether. The extract obtained was concentrated and adjusted to a final volume of 10 ml, yielding a 10% stock solution. From this stock, working solutions of 1%, 2.5% and 5% were prepared through dilution. Fresh ether extracts were prepared prior to each application to minimise solvent evaporation and prevent changes in concentration.

(iii) Powder suspension – The powdered plant material was weighed accordingly to obtain concentrations of 1%, 2.5% 5% and 10%, and suspensions were prepared by mixing with distilled water.

(iv) Smoke treatment – For the smoke treatment, 10 g of powdered plant material was placed in an incineration flask connected via a tube to a fumigation chamber of 10 L capacity. Upon heating, the plant material was incinerated, and the resulting smoke was allowed to fill the chamber for 10 min. A beaker containing host grains along with 50 adult insects, covered with muslin cloth, was positioned in the lower section of the fumigation chamber.

Experimental design

Four treatments were evaluated: aqueous extract, ether extract, powder suspension, and smoke. For the suspension and extract treatments, 5 g of host grains were treated with 1 ml of plant preparation at concentrations of 1%, 2.5% 5% and 10%. Five pairs of *Callosobruchus chinensis* adults were released into each treatment set, and all treatments were replicated three times. For the smoke treatment, 10 g of powdered plant material was incinerated in a flask connected to a 10 L fumigation chamber. A beaker containing 5 g of grains and 50 adult insects was placed inside the chamber and exposed to the smoke for 10 min. The insects were observed for mortality after 24 h and 48 h. The smoke treatment experiment was also replicated three times.

Observations recorded

- *Adult mortality (%)* – The number of surviving insects was recorded for three consecutive days after treatment, and per cent mortality was calculated.

- *Smoke-induced mortality (%)* – Insects exposed to smoke were assessed for mortality after 24 and 48 h.

Statistical analysis

Data on adult mortality were analysed using Analysis of Variance (ANOVA) in SPSS. Mean separation was performed using the critical difference test at the 5% probability level.

RESULTS

The percent adult mortality of *C. chinensis* under different treatments has been presented in Tables 1-3 and the result of ANOVA has been presented in Table 4.

Adult mortality (%) – During the present study, the mean per cent adult mortality was observed to be 22.03% in control sets. The mortality of *C. chinensis* ranged from 22.22 to 77.78% in various sets treated with a combination of the two test plants. The maximum adult mortality (77.78%) was recorded in treatments with 10% aqueous extract prepared from the combined leaf material of the two plants. Mortality levels exceeding 50% were also observed in treatments with 5% and 10% formulations, as well as with the 2.5% ether extract, all of which were significantly higher than the control. Statistically significant mortality level above 50% was observed in general in all the experimental sets treated with 5% and 10% formulations.

Smoke-induced mortality (%) – The results of the smoke treatment of different parts of the two plants on the mortality of *C. chinensis* have been presented in Table 3. The smoke of the combination of *P. juliflora* leaf and *P. cineraria* leaf after 48 h resulted in more than 50% adult mortality.

a. R Squared = 0.557 (Adjusted R Squared = 0.537)
Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares

Table 1. Adult mortality (%) in *C. chinensis* under treatment with *P. juliflora* leaf and *P. cineraria* leaf

	Control	Aqueous extract				Ether extract				Aqueous suspension			
		1%	2.50%	5%	10%	1%	2.50%	5%	10%	1%	2.50%	5%	10%
Mean	22.03	22.22	38.89	61.11	77.78	38.89	55.56	55.56	61.11	27.78	38.89	55.56	66.67
SD±	0.00	9.62	19.24	19.24	19.25	9.62	9.62	9.62	9.62	9.62	9.62	9.62	16.67

Table 2. Adult mortality (%) in *C. chinensis* under treatment with *P. juliflora* bark and *P. cineraria* fruit

	Control	Aqueous extract				Ether extract				Aqueous suspension			
		1%	2.50%	5%	10%	1%	2.50%	5%	10%	1%	2.50%	5%	10%
Mean	22.03	38.89	50.00	61.11	72.22	27.78	44.44	50.00	61.11	33.33	44.44	55.56	61.11
SD±	0.00	9.62	16.67	9.62	19.24	9.62	9.62	16.67	9.62	0.00	9.62	19.25	9.62

Table 3. Adult mortality (%) in *C. chinensis* by smoke treatment with combinations of *P. juliflora* and *P. cineraria*

Treatment type		Duration of treatment	
		24 h	48 h
Control	Mean	11	4
	S.D.±	2.2	0.3
***P. juliflora* leaf + *P. cineraria* leaf**	Mean	40	56
	S.D.±	8.2	7.8
***P. juliflora* bark + *P. cineraria* fruit**	Mean	46	62
	S.D±.	5.2	4.9

Table 4. ANOVA for Adult mortality (%) under formulations employing combinations of *P. juliflora* and *P. cineraria*

Source	Type III Sum of Squares	Df	Mean Square	F	Significance
Treatment	21154.113	7	3022.016	21.365	0.000
Extract	535.100	2	267.550	1.891	0.153
Concentration	27161.534	3	9063.845	64.007	0.000
Error	38898.685	275	141.450		

The error term is Mean Square (Error) = 188.630

a. Uses Harmonic Mean Sample Size = 72.000
b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type 1 error levels are not guaranteed.
c. Alpha = 0.05

DISCUSSION

The current study demonstrates the efficacy of a combination of *P. juliflora* leaf, *P. cineraria* leaf, and *P. juliflora* bark + *P. cineraria* fruit formulations as biopesticides against *C. chinensis*, a primary pest of stored pulses. The observed adult mortality rates, ranging from 22.22% to 77.78%, with the 10% aqueous extract achieving the highest mortality (77.78%), highlight the potential of these botanicals as efficient green pest control methods. The significant mortality in 5% and 10% formulations and the 2.5% ether extract (all exceeding 50%) compared to the control (22.03%) underscores the bioactivity of plants of the *Prosopis* genus, particularly in combination. These findings give impetus to the growing research in the field of botanical pesticides, where rising environmental concerns and pesticide resistance demand sustainable pest management approaches (Regnault-Roger *et al.,* 2012).

In their study, Ketoh *et al*. (2006) investigated the bioactivity of essential oil derived from *Cymbopogon schoenanthus* and its principal compound, piperitone, against different life stages of *C. maculatus*. The results showed that piperitone was more toxic to adults, with an LC_{50} value of 1.6 µl/l, compared with 2.7 µl/l for the crude oil.

According to Mbaiguinam *et al*. (2006), solvent-extracted seed oils (methylene chloride) from *Azadirachta indica*, *Ricinus communis*, *Thevetia nerifolia*, *Balanites aegyptiaca*, *Moringa oleifera*, and *Khaya senegalensis* exhibited strong insecticidal effects by reducing adult survival, with *T. nerifolia* being the most potent.

Fumigant bioactivity of *Artemisia sieberi* essential oil on stored product insects, *C. maculatus, Tribolium castaneum* and *Sitophilus oryzae,* was tested by Negahban *et al.* (2007). Absolute mortality was obtained at a concentration of 37 µl/l after 24 h. LC_{50} values were 1.45 µl/l for *C. chinensis*, 3.86 µl/l for *S. oryzae* and 16.67 µl/l for *T. castaneum.*

Nagahban and Moharramipour (2007) studied the toxic effects of fumes of three essential oils from *Eucalyptus intertexa*, *E. sargentii,* and *E. camaldulensis* against stored grain pests*C. maculatus*, *S. oryzae,* and *T. castaneum*. Adult insects aged 1-7 days exhibited higher mortality with increasing doses (37-926 µl/l air) and longer exposure times (3-24 h). For the essential oils tested, LC_{50} values ranged between 2.55 and 3.97 µl/l air in *C. maculatus*, 6.93–12.91 µl/l air in *S. oryzae*, and 11.59-33.5 µl/l air in *T. castaneum*.

The effectiveness of *Prosopis* plant parts combinations is in agreement with studies on their phytochemical constituents, which include tannins, phenolic compounds, flavonoids, and alkaloids, which have been reported by de Brito Damasceno *et al.* (2020) for insect-repellence and toxicity. War *et al.* (2013) attributed that the better performance of the aqueous extract might be an indicative of water-soluble phenolics such as quercetin and gallic acid playing a key role in disrupting *C. chinensis* physiology, probably by inhibiting digestive enzymes or neural functions. Combinations of *P. juliflora* and *P. cineraria* leaves may enhance this effect through synergistic interactions, as observed by Kumari *et al.* (2017 wherein they combined the *Prosopis* extracts with neem oil against Khapra beetle *T. granarium*.

Much recent research in the field of biopesticides has used multiple plant parts and formulations (aqueous extracts, ether extracts, powder suspensions, and smoke), where varied application methods have been found to maximise the efficiency.

Sileem *et al.* (2020) investigated the fumigant potential of essential oils derived from garlic (*Allium sativum* L.), chilli pepper (*Capsicum annuum* L.), and nigella (*Nigella sativa* L.) on *Tribolium castaneum*, targeting both adults and 20-day-old larvae. The smoke treatment in the present study probably released volatile carbonyls and phenolic acids, as observed by Ruto *et al.* (2018), when they used *Prosopis* smoke for the preservation of food. The fumigant effect thus holds promise, predominantly for *C. chinensis* management in storage conditions, where gaseous penetration is critical (Pavela & Benelli, 2016).

Since *C. chinensis* is a major pest causing 20–30% losses in stored pulses in tropical regions (Rajendran, 2020), the findings of the study hold significance. Several botanicals have been used against this pest, yet research which have dealt with *Prosopis* combinations is lacking. Ukatu *et al.* (2023) observed that *A. indica* leaf extracts achieved 65% mortality in *C. chinensis* at 5% concentration, lower than the 77.78% observed here with 10% aqueous extract of the combination of leaves of the two plants under study. A fumigant assay with *Lantana camara* essential oils reported an LC50 of 130.3 µl/l in *C. chinensis* (Rajashekar *et al.,* 2014). The better results of aqueous extracts over ether extracts in this study may reflect the better stability of polar compounds in storage conditions, in contrast to unstable terpenes prone to evaporation in ether extracts. (Isman & Grieneisen, 2014). The moderate efficacy of the aqueous suspension could be due to contact toxicity through ingestion of bioactive residues, as a similar study reported for *P. juliflora leaf* powders against

T. castaneum (Pugazhvendan *et al.,* 2009). In a similar work, Vijayalakshmi *et al.* (2021) observed that a combination of *P. cineraria* leaf and bark reduced *C. chinensis* oviposition by 50%. These works suggest that combining plant parts may diversify the phytochemical profile, reducing the risk of pest resistance, a common issue with single-compound botanicals (Miresmailli & Isman, 2014). The leaf combinations in the present assay possibly benefited from such diversity, though specific compounds were not identified.

The practical implications of the present study for arid regions are huge, where *Prosopis* species are abundant and easily identifiable. Local communities can use these plants to protect pulses, reducing dependence on expensive and unsafe chemicals like methyl bromide (Fields & White, 2002). The affordability of the treatments will also not burn a hole in the pocket, unlike synthetic fumigants, which require specialised and costly equipment and the need for experts (Subramanyam & Hagstrum, 2012).

Some botanicals function by preventing the adult from laying eggs, as observed by Gupta & Kumar (2020, where *P. juliflora* extracts were found to reduce *C. chinensis* oviposition by 40%. However, the controlled environment followed in the study (28 ± 2°C, 70% RH) generally doesn't reflect storage conditions, where humidity and temperature fluctuations could degrade the plant extracts (Shaaya & Kostyukovsky, 2006).

That said, there is a scope of future research in exploring the active compounds in *P. juliflora* and *P. cineraria* combinations using GC-MS to identify synergistic pairs. Combining the extracts of *Prosopis* with other plant extracts, such as *Ocimum basilicum*, which reduced *C. chinensis* populations by 70% (Kéïta *et al.,* 2001), could enhance IPM strategies.

CONCLUSION

It is concluded from the present study that *P. juliflora* and *P. cineraria* combinations offer an effective yet sustainable solution for eco-friendly management of *C. chinensis.* The aqueous extracts and smoke treatments of the combinations show particular promise. The approach of harnessing the local flora gives a boost to pest management strategies that aim to align with the SDG goals. Further research on phytochemical synergy, ecological safety, and field applications will solidify *Prosopis*-based botanicals as biopesticides of futuristic and eco-friendly agriculture.

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