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Research Article

An *in silico* approach to assess the effectiveness of custard apple (*Annona reticulata*) in management of stored grain pests

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ABSTRACT: *Sitophilus oryzae* and *Callosobruchus chinensis* are the predominant insects commonly found in stored grains resulting in considerable economic losses and posing significant threat to food safety. Management of these stored grain pests with plant-derived alternatives is imperative in the current context. An *in silico* investigation was conducted to identify effective phytochemicals in custard apple, *Annona reticulata* against these insect pests. Our findings affirm that the phytochemicals, namely dopamine, salsolinol, sitosterol, liriodenine, reticulin, coclaurine, and anonain, present in *A. reticulata*, possess the capability to disrupt the normal functioning of the Glutathione S Transferase protein in *S. oryzae* and *C. chinensis* by obstructing its active site. *In vitro* studies also validated the potential of *A. reticulata* extract in management of *S. oryzae* and *C. chinensis*, indicating its suitability as an alternate source of bioinsecticide.

KEYWORDS: Botanical, molecular docking, phytochemical, storage pest

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INTRODUCTION

Rice, scientifically known as *Oryza sativa*, is often referred to as the "grain of life" and holds significant cultural importance as a staple food for Asians. It ranks as the second most widely consumed cereal grain globally, after wheat, and is a crucial food crop in India. With approximately three billion people, almost half of the world's population, relying on rice as a dietary staple, its significance continues to grow. In addition to rice, pulses, a category of leguminous crops, play a vital role in human nutrition, providing rich sources of protein, vitamins, minerals, and essential components for daily diets (Vijayalakshmi *et. al*., 2021). India cultivates major pulses like green beans, chickpeas, Bengal gram, black matpe, pigeon pea, red kidney bean, black-eyed pea, white pea, and lentils. The country dedicates an annual cultivation area of 24-26 million hectares, producing 17-19 million tonnes of pulses (Chandel, 2017). Pulses serve as an alternative source of animal protein for the population and contribute to nitrogen fixation, enhancing soil fertility (Gayathri, 2014). Notably, pulses contain nearly three times more protein than cereals, with grain legumes providing up to 33% of the required dietary protein nitrogen for humans (Vance, 2001; Kosar & Srivastava, 2016).

Sixty per cent of the total food grain production in India is conventionally stored, while forty per cent is distributed to traders and government agencies. In the storage process, ten per cent of the food crops are lost due to insect damage. Hence, safeguarding crops is imperative and integral to contemporary agricultural practices. Notably, during storage, the rice weevil, *S. oryzae*, poses a significant threat to rice grains, causing substantial damage (Prasad *et al.*, 2008). While *C. chinensis* emerges as a major pest affecting stored pulses (Ahmed *et al.*, 2014).

To address the challenges associated with managing stored grain pests, synthetic insecticides may be employed, but these can bring about various undesirable side effects (Valsala & Das, 2015). To tackle these issues, an alternative approach needs to be developed for controlling pests affecting stored grains. In lieu of chemical insecticides, many countries have adopted the use of plant-based insecticides. These alternatives are user-friendly, biodegradable, readily available, cost-effective, less hazardous, and environmentally friendly (Ahad *et al.*, 2012). Secondary metabolites found in plants, such as alkaloids, phenols, glycosides, terpenoids, and tannins, play a crucial role in the defence mechanisms of plants and have physiological and behavioural effects on insects (Anandhabhairavi *et al.*, 2022). These plant secondary metabolites serve various functions, such as repellents, toxins, insect growth inhibitors, oviposition deterrents, and

antifeedants to protect against herbivores at different stages of growth. This defensive mechanism against insects is vital (Koul *et al.*, 2000).

The current research underscores the quest for an efficient botanical solution for controlling significant stored grain pests. Conducting wet lab experiments to screen each plant against the target pests is impractical due to the substantial time involved. To expedite the search for potential phytochemicals, an *in silico* approach was employed to assess the efficacy of compounds derived from custard apple (*A. reticulata*) against *S. oryzae* L. and *C. chinensis* L. The findings from the *in silico* analysis were further confirmed through wet lab analysis.

MATERIALS AND METHODS

In silico analysis to determine the efficacy of the selected plant extract

In the initial phase of this study, *in silico* techniques were employed to assess the efficacy of a chosen plant extract in managing the designated pest. The analysis focused on utilising key enzymes of the target pests as receptors, with a specific emphasis on the Glutathione S transferase protein. This protein belongs to a metabolic isoenzyme family and plays a role in catalysing the conjugation of Glutathione to a diverse range of exogenous substrates, rendering the compounds water-soluble and facilitating detoxification (Eaton & Bammler, 1999). The enzyme's targets encompass a broad spectrum of toxins, including pesticides. Inhibiting the functionality of this enzyme disrupts the insect's natural detoxification process. A positive outcome, signified by a favourable binding affinity between the selected phytochemicals and the chosen enzyme, can offer valuable insights into the potential use of these phytochemicals for controlling the designated pests.

Receptor and ligand preparation

The molecular configurations of the Glutathione S Transferase receptor (enzyme) (1GNW) (Figure 1) were obtained by downloading them from the PDB database (https://www.rcsb.org/). The structures of the ligands (phytochemicals) (Figure 1) found in the extracts of the *A. reticulata* plant were acquired from the Zinc database (https://zinc.docking.org). The selection of ligands for this study was based on choices made from previously documented literature.

Molecular docking

To explore potential molecular interactions between the receptors (enzymes) and ligand molecules (phytochemicals), the docking process utilised Molegro Virtual Docker (MVD 2010.4.0.0) for Windows. Rerank scores were considered in the analysis of receptor-ligand interactions. These scores incorporate various energy parameters, including E-Inter total, E-Inter (protein-ligand), Steric, Van der Waals, H-Bond energy, E-Intra (tors, ligand atoms), among others.

Receptor-ligand interactions visualisation

In terms of visualisation, BIOVIA Discovery Studio Visualizer 2021 was employed. Developed by Dassault Systèmes BIOVIA in 2021, this program is utilised for the viewing, sharing, and analysis of proteins and small molecules.

Collection and maintenance of stock culture of *S. oryzae* L. and *C. chinensis* L.

Adult weevils of *S. oryzae* L. and *C. chinensis* L. were collected from different storage facilities as well as from various grocery shops in the Guwahati area. Identification was carried out using a key book (GRDC, 2019). The weevils

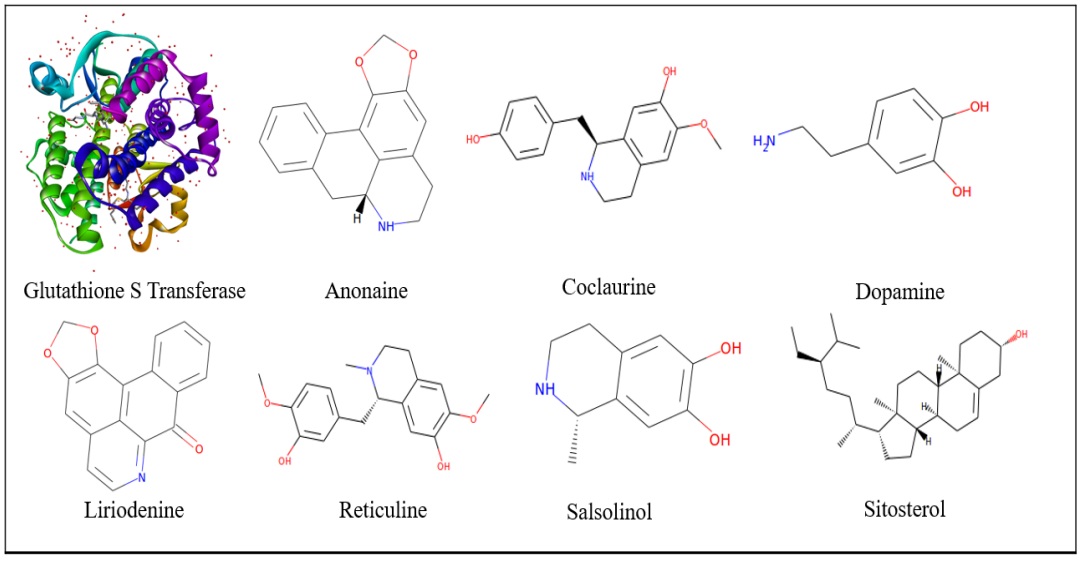


Figure 1. Structures of the receptor (enzyme) in the target pest and phytochemicals (ligands) present in *A. reticulata*.

were cultured on healthy, sterilised rice grains and green gram continuously throughout the entire study duration. The grains, together with the insects, were placed in plastic containers (12cm×8cm) covered with muslin cloth and tightly sealed with rubber bands to ensure proper ventilation. These containers were stored at room temperature (18-32ºC) and a relative humidity of 70±5%. To sustain the culture over an extended period, newly emerged adult insects were regularly transferred to new plastic jars with fresh, uncontaminated food, allowing them to mate and lay eggs. This process was repeated until a sufficiently large population of insects was obtained, ensuring an ample number of cultures for the entire study period. Newly emerged insects were utilised for the experiments.

Collection of seeds (Rice and Green Gram)

Healthy and uninfected seeds of rice (*O. sativa*) and green gram (*Vigna radiata*) were collected from local farmers. To make the seeds free from any hidden infestation, they were sundried or heated in an oven at 60ºC for 30-40 min (Chauhan & Ghaffar, 2002). Afterwards, these seeds were kept in a closed jar at room temperature, and later on, the seeds were used for experimental purposes.

Collection of plants and extract preparation

Annona reticulata was collected from the nearby areas of Guwahati city. For the identification of the plants, the key book (Kanjilal *et. al*., 1934-1940) has been used, and further confirmation was done in the Department of Botany, Assam Downtown University, Guwahati.

For the preparation of plant extract, leaves were washed with distilled water and air dried in the shade at room temperature until they became fully dry. Thereafter, the leaves were ground into fine powder using a grinder. For the preparation of methanolic extracts, the maceration process was used. Then the crude extracts were preserved in sealed air air-tight Falcon tubes in a refrigerator for further use.

Repellency test

A repellency test was conducted as per the method described by Talukdar and Howse (1993). The results computed were expressed as Percentage Repellency (PR%) by the following formula:

$$PR\% = \frac{A - B}{A} \times 100$$

where, A = Average number of insects present in the control portion, B = Average number of insects present in the treated portion.

The positive (+) values represent repellency, and negative (-) values signify attractancy.

As per the scales described by McDonald *et. al.* (1970), average data were then categorised according to the classes of 0, I, II, III, IV and V, having per cent repellency rate of >0.01 to 0.1, 0.1 to 20, 20.1 to 40. 40.1 to 60, 60.1 to 80, and 80.1 to 100, respectively.

Direct toxicity on the mortality of insect pests

The direct toxicity test was carried out based on the procedures outlined by Ogunleye and Adefemi (2007), with slight adjustments, employing direct topical application. Six various concentration levels—4%, 6%, 8%, 10%, and 12%—were selected for the investigation. Insects were examined daily, and those exhibiting no response or movement upon gentle touch were deemed deceased. Insect mortalities were documented at 24, 48, and 72 Hours After Treatment (HAT), as per the methodology outlined by Zafar *et al.* (2018).

Evaluation of the efficacy of plant extracts as grain protectants

The experiment was conducted employing a completely randomised design. In each treatment, 50g of seeds were meticulously blended with varying concentrations (10% and 12%) of the chosen plant extracts. The mixed seeds were then arranged in a petri dish, creating three replicas. Ten pairs of newly emerged beetles were introduced and permitted to mate and infest at their discretion. The assessment of the selected plant extract's effectiveness was gauged by the percentage of weight loss and seed damage after a storage period of 90 days, following the approach outlined by Kalita (2018).

Percentage weight loss:

To calculate the percentage weight loss, an electric balance was used to weigh 200 randomly selected healthy seeds and 200 randomly selected mixed seeds from each treated replica. For the replicas, the average weight was taken, and the percentage weight loss was determined, following Barthakur (1992).

$$\text{Per cent weight loss} = \frac{\text{weight of 200 healthy seeds} - \text{weight of 200 mixed seed}}{\text{weight of 200 healthy seed}} \times 100$$

Percentage of damaged seeds

To calculate the percentage of damaged seeds, a total of 200 seeds were chosen randomly from each replication, and the number of damaged seeds was recorded for each replication. For the three replicas, the average was taken, and the percentage of damaged seeds was determined, following Barthakur (1992).

$$\text{Per cent damaged seed} = \frac{\text{Number of damaged seed}}{\text{Total number of seed}} \times 100$$

Statistical analysis

Statistical analysis was performed with data analysis in the Microsoft Excel software 2021 for Windows. Mean and standard deviation were calculated to record the results.

RESULTS

In silico analysis

The molecular docking result reveals that all the ligands taken in the present study show negative rerank scores with the enzyme Glutathione S Transferase. Anonain shows the highest rerank score (-57.55±5.53) followed by Dopamine (-56.53±5.69), Liriodenine (-56.37±7.94), Salsolinol-54.54±1.96), Sitosterol-47.6±7.39), and Reticulin (-33.53±23.65) (Table 1). The graphical representation of the rerank score is shown in Figure 2. The negative rerank score signifies a good interaction between phytochemicals and with active sites of the selected protein. Thus, it indicates that the selected phytochemicals used in the present study have the potential to inhibit the normal enzyme activity.

The 3D and 2D interactions of selected phytochemicals with the protein show different types of interactions like Van der Waals, H-Bond, C-H Bond, Non-covalent bonds like Pi-Alkyl bond, Pi-pi T-shaped, Pi- Sulphur bond, *etc*. The top three 3D and 2D representations of interaction between selected phytochemicals, namely, Anonain, Dopamine and Liriodenine with the active site of Glutathione S Transferase protein are shown in Figure 3.

Table 1. Mean rerank score of phytochemicals present in *A. reticulata* with Glutathione S Transferase protein

Ligand	Zinc ID	Mean rerank score
Dopamine	ZINC33882	-56.53±5.69
Salsolinol	ZINC4027288	-54.54±1.96
Sitosterol	ZINC4095717	-47.6±7.39
Liriodenine	ZINC6256	-56.37±7.94
Reticulin	ZINC895630	-33.53±23.65
Coclaurine	ZINC896120	-55.72±10.40
Anonain	ZINC898396	-57.55±5.53

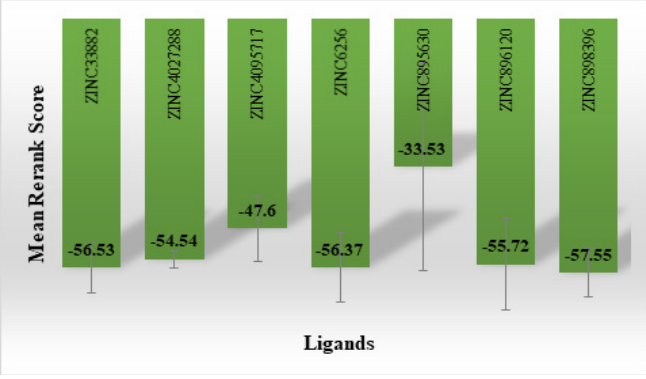


Figure 2. Graph showing the rerank score of ligands of *A. reticulata* with Glutathione S Transferase Protein (Data obtained from Molegro Virtual Docker, MVD 2010.4.0.0).

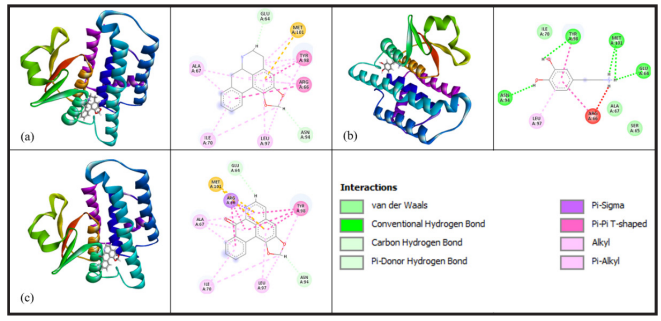


Figure 3. 3D and 2D representations of
(a) Interaction of Anonain with the active site of Glutathione S Transferase Protein,
(b) Interaction of Dopamine with the active site of Glutathione S Transferase Protein,
(c) Interaction of Liriodenine with the active site of Glutathione S Transferase Protein.

Repellency Test

The repellency result reveals that the plant extract of *A. reticulata* has a significant degree of repellency against *S. oryzae* and *C. chinensis*. For experimental purposes, two different doses of varying concentration, *viz*. 10% and 12% were used, and in both, the concentration target pest shows repellency of Class V, *i.e.*, >80.1 to 100%. At 10% concentration of test extract, the insect *S. oryzae* shows repellency to *A. reticulata* (87.67%) and at 12% concentration (89.74%) over the control. On the other hand, at 10% concentration of test extract, the insect *C. chinensis* showed repellency to *A. reticulata* (86.56%) and at 12% concentration (80.81%) over the control (Table 2).

Direct toxicity test on the mortality of adults of *S. oryzae* and *C. chinensis*

The result of the direct toxicity test reveals a varying degree of mortality against insect pests in different concentrations. In case of *S. oryzae*, at 4% concentration, *A. reticulata* shows 2±2 number of dead insects followed by 1.33±2.30 at 6%, 4.66±3.05 at 8%, 4±2 at 10% and 8±2 at 12% concentration. In case of *C. chinensis,* at 4% concentration, *A. reticulata* shows 12.66 ±3.05number of dead insects, followed by 13.33±3.05 at 6%, 16.66±1.15 at 8%, 18±2 at 10% and 18.66±1.15at 12% concentration (Table 3).

Per cent weight loss and damaged seeds

The result revealed that the percentage of weight loss of rice at 10% and 12% concentration was 8% and 2.28%, respectively. However, in green gram was recorded as 0.67 and 2.6% (Table 4). The percentage of damaged seeds of rice at 10% and 12% concentration was 8.55 and 5.66%, and in green gram, 0.33 and 0.16% (Table 5).

Table 2. Repellency test

Plant extracts	Insect pests	Doses (%)	Repellency rate (%)						Mean repellency (%)	Repellency class
			0.5 HAT	1 HAT	1.5 HAT	2 HAT	2.5 HAT	3 HAT		
A. reticulata	*S. oryzae*	10	82.33	94.73	88.88	88.88	82.35	88.88	87.67	V
		12	88.88	94.73	88.88	94.73	82.35	88.88	89.74	V
	C. chinensis	10	88.88	88.88	88.88	75.00	88.88	88.88	86.56	V
		12	88.88	88.88	75.00	57.14	100	75.00	80.81	V
N=20, HAT: Hours After Treatment										

Table 3. Mortality of test insects when treated with different concentrations of selected plant extract

Plant extract	Insect	Total no. of dead insects				
		Dose/ concentration				
		4%	6%	8%	10%	12%
Annona reticulata	*S. oryzae*	2±2	1.33±2.30	4.66±3.05	4±2	8±2
	C. chinensis	12.66±3.05	13.33±3.05	16.66±1.15	18±2	18.66±1.15
Control		0±0	0±0	0.05±0.57	0±0	0±0

Table 4. Per cent weight loss of seeds when treated with different concentrations of *A. reticulata* extract

Grain	Per cent weight loss of seeds (%)	
	Dose/concentration	
	10%	12%
Rice	8.0	2.28
Green gram	0.67	2.6
Control (Rice)	10.85	10.85
Control (Green gram)	5.0	5.0
N=300		

Table 5. Per cent of damaged seeds when treated with different concentrations of *A. reticulata* extract

Name of Grain	Per cent of damaged seeds	
	Dose/ concentration	
	10%	12%
Rice	8.55	5.66
Green gram	0.33	0.16
Control (Rice)	91.88	91.88
Control (Green gram)	96.0	96.0
N=300		

DISCUSSION

The molecular docking analysis illustrated the interactions between ligands and receptor molecules. This analysis identifies potential binding sites using a cavity detection algorithm. In this study, the rerank score was considered to assess the binding efficiency between ligands and receptor molecules. The selected ligands (phytochemicals) exhibited negative results, indicating a favourable interaction between the ligand and receptor molecule. Among the seven phytochemicals examined, Anonain demonstrated the highest rerank score, followed by Dopamine, Liriodenine, Salsolinol, Sitosterol, and Reticulin. The rerank score, a combination of MolDock score and Lennard-Jones approximations to steric energy, suggests stable interactions between receptors and ligands (Thomsen & Christensen, 2006). Thus, the rerank score of phytochemicals derived from the plant extract of *A. reticulata* with Glutathione S Transferase implies that the plant extract could be an effective control agent against *S. oryzae* and *C. chinensis*. The visualisation of interactions revealed various types, such as Van der Waals, C-H Bond, H-Bond, and non-covalent bonds like Pi-pi T-shaped, Pi-Alkyl bond, etc. Similar *in silico* work by Mejia *et al.* (2018) demonstrated that phytochemicals isolated from Calceolaria exhibited good binding potential to ecdysone receptors in insects. Another study by Shintu *et al.* (2020) revealed the strong binding potential of the agERG protein with phytochemicals isolated from *Citrus sinensis*. Nascimanto *et al.* (2011) also found that phytochemicals from *Ricinus communis* plants are potent alpha-amylase inhibitors.

The repellent qualities of the chosen plant extract were evaluated against *S. oryzae* and *C. chinensis*, revealing varying degrees of repellency towards the tested insects. This finding is promising, indicating that the application of this extract could effectively deter insect pests from stored grain. *A. reticulata* displayed the highest repellency, reaching 89.74% against *S. oryzae* and 86.56% against C. chinensis. These

results align closely with the research conducted by earlier scholars. Akhtar *et al.* (2013) investigated the management of rice weevil, *S. oryzae*, using plant extracts for their repellent effects, highlighting *Psidium guajava* as the most effective and *Azadirachta indica* as the least effective. Jayakumar *et al.* (2017) focused on the control of adult rice weevil, *S. oryzae*, through repellent activity, with camphor showing the highest repellency among plant oils on the Proportion Index (EPI). Soujanya *et al.* (2016) explored the efficacy of plant extracts against the rice weevil, revealing strong repellency from *Adathoda vasica, Lantana camera*, and *Vitex negundo*. Madhavi *et al.* (2022) investigated the control of stored grain pest pulse beetle using various botanical plants, with sweet flag extract exhibiting the maximum repellency at 90.07%, surpassing Clove (80.13%) and Black pepper (83.51%), indicating potent repellent effects against grain pests. Jain *et al.* (2006) explored the use of various plant extracts against the pulse beetle, *C. chinensis,* in green gram for repellency, reporting values ranging from 52-80% repellency against *C. chinensis* at the concentration of 1ml/100 g seeds.

Promising outcomes were obtained in evaluating the lethal impact of a specific plant extract on adult stages of *S. oryzae* and *C. chinensis*. *A. reticulata* demonstrated the highest mortality at a 12% concentration against *S. oryzae*, while for *C. chinensis*, it exhibited relatively higher mortality rates across all concentrations compared to *S. oryzae*. The mortality effect of the chosen plant extract was significantly superior to the control in both cases, suggesting that the selected plant extract serves as a viable alternative to chemical pesticides. Various scholars have reported the efficacy of different plant extracts in controlling *S. oryzae*, showcasing varying degrees of potential for control. Sheeba *et al.* (2001) investigated the management of the rice weevil, *S. oryzae*, using *Beauveria bassiana*, revealing higher mortality rates up to 75.8% at elevated doses (7.6 log conidia/ml). Saljoqi *et al.* (2006) studied the effects of certain plant extracts against the rice weevil in stored wheat grains, highlighting bakain drupes as the most effective, causing the highest mortality (61.2%) of the insect. Abdullahi and Majeed (2010) reported that *P. pinnata* leaf extract induced 73.1% mortality against *C. chinensis* after 48 hours at a 20mg/g concentration. Ekeh *et al.* (2013) also discovered an 89.3% mortality of *Callosobruchus* sp. after 4 days when treated with *O. gratissimum* leaf.

The outcomes concerning the percentage of weight loss in grains and damaged seeds resulting from the application of *A. reticulata* confirm that the phytochemicals present in *A. reticulata* protect the grains from insect infestation and minimise damage. Pawara *et al.* (2019) investigated an eco-friendly approach for controlling the pulse beetle *C. chinensis* on *Vigna radiata*, noting the effectiveness of castor oil (5ml/kg), sesame oil (5ml/kg), and Karanj oil (5ml/kg) in reducing weight loss caused by the pulse beetle. Chayengia *et al.* (2010) explored the management of *S. oryzae* using various plant products, finding that *Polygonum* sp. effectively reduced grain weight loss (5.01%) after 35 days of exposure. In the realm of managing insect pests, Yankanchi and Gadache (2010) investigated the control of rice weevil, *S. oryzae*, using leaf extracts of locally available plants. They reported that leaf extracts of *Clerodendrum inerme* and *Withania somnifera* could be employed for protecting stored wheat from *S. oryzae* infestation. Padmasri *et. al.* (2017) studied the management of rice weevil, *S. oryzae*, in maize using botanical seed treatments, highlighting that *Acorus calamus* rhizome powder, when applied to seeds, exhibited lower weight loss. Examining the control of infestation in mung bean under storage conditions, Kumar *et al.* (2017) tested various plant oils against pulse beetles, *C. chinensis*. Neem oil at 2.5ml/kg seeds was found to be more effective than other treatments, resulting in lower seed damage and adult emergence compared to the untreated control against *C. chinensis*. Ahad *et al.* (2012) conducted a study on the control of pulse beetles, *C. chinensis*, through screening and the biological activity of indigenous plant extracts. They reported that *Polygonum hydropiper* reduced seed damage by 9.33%, while *A. reticulata* caused 2% damage to seeds.

CONCLUSION

Sitophilus oryzae and *C. chinensis* represent major destructive insect pests that emerge during storage. Opting for organic control methods is an ideal and environmentally friendly approach for sustainable agriculture. Our *in silico* investigation substantiates that Dopamine, Salsolinol, Sitosterol, Liriodenine, Reticulin, Coclaurine, and Anonain, found in *A. reticulata*, possess the potential to impede the normal functioning of Glutathione S Transferase protein—an essential protein in *S. oryzae* and *C. chinensis*. Furthermore, wet lab analysis affirms that the phytochemicals present in *A. reticulata* have the capacity to repel insects, induce direct toxicity leading to insect mortality, and mitigate weight loss while minimising damage to stored grains. Therefore, the extract of *A. reticulata* at very low concentration holds promising source of bioinsecticide.

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