https://doi.org/10.48047/AFJBS.6.13.2024. 3117-3135

African Journal of Biological Sciences

Structure based in-silico analysis and toxicity study of *Pongamia pinnata* (Linn.) seed extracts against stored grain pest *Callosobruchus chinensis* (Linn.)

Sasanka Sekhar Ghosh[1], Lupamudra Borah[1], Dipanjan Dey[2], Suraj Chetri[3*]

[1]Department of Zoology, Cotton University, Guwahati, Assam, India
[2]Department of Zoology, Tihu College, Nalbari, Assam, India
[3*]Department of Zoology, Assam down town University, Sankar Madhab Path, Gandhinagar, Panikhaiti,Guwahati, Assam, India, Pin 781026
Corresponding Author: Suraj Chetri
Corresponding author email: surajc30@gmail.com

Article History

Volume 6, Issue 13, 2024

Received: 18June 2024

Accepted: 02July 2024

doi:***10.48047/AFJBS.6.13.2024.*** 3117-3135

Abstract:
Callosobruchus chinensis is a stored grain pest of legumes that is known to cause huge economic losses each year. As the adverse effects of chemical control strategies became more evident, alternative plant resources for pest control have become the key for sustainable pest control. The present study focuses on the use of *Pongamia pinnata* seed extracts as a natural controlling agent for the pest. An *in-silico* study was designed to evaluate the effect of bioactive phytochemicals of *P. pinnata* seeds against enzymes of the target pest. The results of the *in-silico* study were then correlated with direct toxicity tests and other parameters of insect life cycle. The results of the initial *in-silico* analysis with six bioactive phytochemicals of *P. pinnata* shows significant values for energy of interactions with the target enzymes of *C. chinensis* with highest mean rerank score of and -73.28672±7.857 for pongamol with glutathione s transferase and -88.1554±5.399 for pongapin with alpha amylase, thus indicating effectiveness of *P. pinnata* in natural control of the target pest. The initial results were confirmed with results of direct toxicity tests on *C. chinensis* with graded concentrations (2%, 4%, 6%, 8%, 10% and 12%) of *P. pinnata* seed extracts. Results show an overall mean percentage mortality of 61.9±23.51. The Probit analysis data indicates a LC_{50} (% conc) value of 4.16 for adult insects. Further analysis of the effects of the plant extract on the oviposition and emergence of adult insects after fertilizations shows significant outcome. Treatments with graded concentrations of *P. pinnata* seed extracts show the overall effect on oviposition at an average of 21.44±9.60 and the effect on the mean percent emergence of adult at 60.6±11.68. From the results thus obtained from the study it can be ascertained that the seed extracts of *P. pinnata* shows significant effects as a natural control product of stored grain pest *C. chinensis*
Keywords: *Callosobruchus chinensis*, molecular modelling, *In-silico*, mortality, oviposition, biopesticide, phytochemicals, *Pongamia pinnata*

Introduction:

Callosobrachus chinensis is a common stored grain pest of legumes. Commonly known as adzuki bean weevil, chinese bruchid, cowpea bruchid or pulse beetle. It is cosmopolitan in distribution however its natural ranges lie in topical Asia (Olajire *et al.*, 2011). The pest causes both quantitative and qualitative damage to stored products (Dhaliwal *et al.*, 2015). Different geographical strains of this species are known which show slight morphological differences (Utida, 1972).

The life cycle of pulse beetle is completed in about 29-32 days. The pest develops about 7-8 generations annually (Hosamani *et al.*, 2018). Infestation begins before harvest as the female lay eggs on the surface of legume pods. The larvae after emerging from the eggs bore inside the pod and feed on them. More than one larva may be present on the same pod. The larvae undergo developmental changes inside the pod. When they reach the 1st larval instar they pupate inside the pods. When adults emerge from the pupa, they come out of the pods and fly away. They then mate and the female lays her eggs on another batch of legume thus beginning a new infestation (Varma and Anandi, 2010). Infestation reaches its zenith in July-August.

Legumes are one of the important components of diet. They rich is dietary benefits especially for those who favor plant-based diet (Polak *et al.*, 2015). But every year large quantities of legume crops are destroyed in storage by the pulse beetles (Rawat and Srivastav, 2011). In order to reduce damage various methods are followed such as periodical exposure to sun, drying the crops to minimum kernel moisture levels (~7%), storing pods in polyethene bags and dipping such bags in 10% Malathion solution and fumigation with aluminium phosphide. But chemical pesticides are known to cause several health disorders and detrimental environmental effects (Nicolopoulou-Stamati *et al.*, 2016). Chemical pesticides are the primary choice of farmers but most of them are unaware of the harmful effects of the pesticides or their proper handling methods (Rijal *et al,* 2018). Farmers select chemical pesticides mostly based on financial criteria and easy accessibility (Sharifzadeh *et al.,* 2018) Studies on alternative pest control practices have found that botanical insecticides are best suited for production and postharvest protection of food products as they pose very little threat to the environment and human health (Isman, 2006). The use of natural biopesticides provide several benefits over traditional pest management practices (Mishra *et al.,* 2018).

This study aims to identify bioactive phytochemicals that can be used in the control of *C. chinensis* with the use of virtual screening and docking techniques. Computer based techniques are used as they provide quick and precise comprehension of the effect of the chosen treatment on the target pest. For this, important enzymes of the insect body are targeted whose behavioral alterations would cause serious implications in the biology of the organism. The results of the *in-silico* analysis were confirmed through direct toxicity tests. Two more parameters, oviposition of eggs and emergence of adults were added to obtain a more detailed result conclusion on the success of *Pongamia pinnata* seed extracts in the natural control of legume pest *C. chinensis.*

Materials and Methods:

The adult insects were collected and a culture was maintained on seeds of green gram (*Vigna radiata*) at room temperature for the period of study. The insects were kept in plastic containers (12cm×8cm), tightly sealed with muslin cloth at room temperature (18-32^{0}C) and relative humidity 70±5%. Seeds of *P. pinnata* were collected, properly washed and then dried at room temperature. The dried seeds were grinded and a methanolic extract was prepared by Soxhlet extraction. The crude extract thus prepared was then stored for further use. A preliminary phytochemical analysis was performed for detection of active phytochemicals such as alkaloids, glycosides, tarpenoids, saponins, flavonins, phytosterols, phenolic compounds, steroids etc. in the extract (Kotake, 1994; Kotake *et al.,* 1995; Sofowara & Odebivi, 1978; Trease and Evans, 1996).

In Silico analysis:

The target enzymes were selected based on their biological activity in the body to the target organism. Glutathione S Transferase is an enzyme that that is responsible for the detoxification of endogenous xenobiotics in insects (Eaton and Bammler, 1999) and alpha amylase is an enzyme that is important for digestion in insects as it hydrolyses polysaccharides such as starch and glycogen. The molecular structures of the selected enzymes, Alpha amylase (1VIW) and Glutathione S Transferase (1GNW) (Fig.1) were downloaded from RCBS PDB database (https://www.rcsb.org/). The ligands were selected based on the preliminary phytochemical screening and available literature (Al Muqarrabun *et al.,* 2013; Chopade *et al.,* 2018). The structures of the selected ligands, β-Sitosterol, Karanjin, Pongaglabrone, Pongamol, Pongapin and Sigmasterol (Fig 2), were downloaded from ZINC database (https://zinc.docking.org/).

The molecular interactions between the ligands and target receptors were predicted by molecular docking using a software, Molegro Virtual Docker (MVD 2010.4.0.0) for Windows. The energy of interactions obtained by the docking analysis are displayed as scoring functions using energy parameters such as Van der Waals forces, H-bond energy, E-Inter total, E-Inter (protein-ligand) etc. (Ruyck *et al*., 2016). The scores are compiled and then plotted in a graph. The receptor-ligand interactions were visualized using BIOVIA Discovery Studio Visualizer 2021. The interacting molecules were displayed in both 3D and 2D formats for analysis.

***Direct toxicity test*:**

Direct toxicity tests were performed to establish the results of *in-silico* analysis. For the purpose different concentrations viz. 2%, 4%, 6%, 8%, 10%, 12% of the crude plant extract was prepared in acetone which is considered as good mid polar solvent dissolving chemicals with broad polarity range (Altemimi *et al.*, 2017). The insects were immobilized and 20 microliter solution of extracts were applied on the dorsal surface of the insect using a micropipette. Three replicas and a control were maintained for each treatment each replica with 20 insects. A positive control group was also maintained where Malathion 50% EC was used. The whole experiment is performed in completely randomized design.

The insects were examined hourly and insect mortalities were recorded at 24, 48 and 72 hours after treatment (HAT) (Zafar *et al.*, 2018). Then the observed data were subjected to Probit analysis (Finney, 1942).

***Evaluation of additional parameters*:**

The seeds were mixed with different concentration of plant extract and 5 gm seeds were placed in each mating chamber. One pair of newly emerged insects were introduced in each chamber and allowed to mate. Three replicas along with a control and a positive control set were maintained. To study the effect of plant extract on oviposition, after 8 days of releasing insects, adults were removed and number of eggs laid is counted (Kalita, 2016). To study effect on emergence of adult the mating chambers with eggs are kept undisturbed for 15-20 days. Then the % of adult emergence is calculated using following formula:

$$\text{\% of adult emergence} = \frac{Number\ of\ adults\ emerged}{number\ of\ eggs\ laid} \times 100$$

***Data Analysis*:**

The analysis of variance (ANOVA) was performed using R software (R Core Team (2020). Significance between the various parameters taken the study were calculated in two ways, i.e. in between the different doses & control group and in between treated plant extract & control group. The probit analysis was done using the Henry simplified table and following the protocol of Finney (1971). The table & graph preparation and SD value measurement were done using Microsoft excel, 2016.

Results and Discussion:

In order to evaluate the efficacy of *P. pinnata* seed extracts in the natural control of *C. chinensis*, the present study was performed in two stages. At first the effect of bioactive phytochemicals present in *P. pinnata* seeds against target enzymes of the pest were analyzed using *in-silico* techniques. The initial study was followed by direct toxicity tests to obtain a comprehensive result.

Result of *in silico* analysis: The interaction of the bioactive phytochemicals present in *P. pinnata* seeds with the target enzymes of *C. chinensis* was evaluated through structure based virtual screening and molecular docking techniques. A docking software was used generate potential binding orientations with the target receptors. The energy of candidate interactions was tabulated with negative rerack scores signifying more stable interactions. The results thus obtained indicated that all the phytochemicals used in the study showed positive interactions with the target enzymes (Fig 3). The best interaction with the enzyme glutathione s-transferase was shown by Pongamol (ZINC100003085) with a mean rerank score of -73.28672±7.857. On the other hand, the best interaction with the enzyme alpha amylase was shown by Pongapin (ZINC14677195) with a mean rerank score of -88.1554±5.399. On visual analysis of the interactions of pongamol and pongapin with glutathione s transferase and alpha amylase respectively, we observe a number of significant molecular interactions with the amino acids of target enzymes (Fig 4 and Fig 5). The observations thus indicate positive interactions in both enzyme targets. Thus, it can be concluded that the bioactive phytochemicals of *P. pinnata* seeds are capable of obstructing the normal physiological activities of the target enzymes and thus can be used in the natural control of the target pest species.

Result of direct toxicity test and additional parameters: The results of the initial *in silico* analysis were confirmed by conducting direct toxicity tests with test extracts of *P. pinnata* seeds in different concentrations. The results of the test clearly indicate a dose dependent increase in the toxicity of the test extract against the target (Fig 6). The study indicates an overall mean percentage mortality of 61.9±23.51 (Table 1). The probit analysis of the data showed a LC_{50} value of 4.16 against adult *C. chinensis* (Table 2).

To obtain a comprehensive picture of the effect of *P. pinnata* seed extracts on the target insect, additional parameters such as the egg laying capacity and emergence of adult insects from the laid eggs were studied. The results showed that the effect of test extract on the insect was dose dependent (Fig 7 and Fig 8). When treated with different concentrations of extract the change in oviposition showed an average value of 21.44±9.60 (Table 3). Similarly, the total number of adults that emerged after treatment showed a mean percentage of 60.6±11.68 (Table 4). This indicates that the test extracts showed significant effect on both oviposition and emergence of adult insects. This in association with the results of *in silico* analysis clearly indicates that *P. pinnata* seed extracts can be used as insecticides to control stored grain pest *C. chinensis*.

The result from the present study ascertain the potential of *P. pinnata* in controlling the *C. chinensis* to a great extent. The in-silico analysis reveals the possible interaction between ligands of selected plants with the active site of two important enzymes viz. glutathione s transferase and alpha amylase of *C. chinensis* (Figure1). Salinas et al., (1996) reported that glutathione s transferase plays important role in removing the toxic xenobiotic compounds from insect body. Enayati et al., (2005) also reported its role in intracellular transport, protection against oxidative stress and production of hormones. Da (2018) stated that alpha amylase enzyme involved in carbohydrate metabolism in insects. Thus, for a pesticide to be effective, it must be able to overpower the functioning of these two enzymes as these enzymes control various important functions in their normal physiology. The interaction between the ligands of selected plant and enzymes were depicted in terms of mean rerank score and interaction between ligands and selected enzymes were shown in 2D and 3D format (Figure 4&5). Pongamol shows highest rerank score of -73.28672±7.857 with enzymes glutathione s transferase and pongapin shows highest rerank score of -88.1554±5.399 with enzyme alpha amylase (Figure 3). Thomson & Christian (2006) stated that negative rerank value signifies stable interaction between receptor and ligands. The rerank score of present study strongly suggest the interaction between ligands and enzyme of interest and its potential in controlling the *C. chinensis*. Similar in silico approach for identifying potential biopesticide against insect pest was reported by Yadav et al., (2015), Srinivasan et al., (2015), Shintu et al., (2020) against *Helicoverpa armigera*, *Anophele stephensi* and *Anophele gambiae* respectively.

The web lab results on the mortality of adult *C. chinensis* in present study shows that the insect mortality increases with the increase in the concentration of doses (Figure 6). The P-value in ANOVA shows less than 0.001 signifying a relationship between adult mortality and concentration of doses at 99% confidence level. Dose dependent adult mortality was also reported by Bindu et al., (2015) and revealed that application of *Hydrocotyl asiatica* shows highest mortality on *C. chinensis.* The overall mean percentage mortality was found to be

61.9±23.51 which is significant ($p<0.001$) over the control treatment (0.55±0.85) (Table 1). The finding is in line with the works of several authors. Mortality of *C. chinensis* was reported by Jachuk et al., (2010) & Mathur et al., (1985) and found turmeric, black pepper, neem and clove fruit effective in controlling the insect. Botanicals like *C. longa* and *A. indica* most effective in controlling C. chinensis (Zafar et al., 2018).

The LC_{50} value of *P. pinnata* in the present study was found to be 4.16% (Table 2). Jadhav & Pardeshi (2017) revealed that LD_{50} value of fungal extract from leaf of *Jatropha curcus* against *C. chinensis* was 42.32mg/kg in methanol and 64.76mg/kg in ethyl acetate. The LC_{50} value for petroleum ether extract of *Leucas lavandulifolia* was found to be 2.309% at 72 hours after treatment as reported by Murasing et al., (2018).

The oviposition by *C. chinensis* was found to be dose dependent (Figure 7) and overall average oviposition was found to be 21.44±9.60 in treatment group which is significant ($p<0.001$) over control group (66.94±1.83) (Table 3). Similar works has been done by Singh, (2011), Zafar, (2018), Kalita et al., (2002) and found that different plant extracts are effective in reduction of fecundity of *C. chinensis*. Similarly, reduction in number of eggs laid in seeds treated by powder, ash and oil from *Vitex negundo*, *Eucalyptus globules*, *Ipomoea sepiaria*, *Azadirachta indica*, *Carthamus tinctorius*, *Sesamum indicum* and *Acacia arabica* was reported by Rahman & Talukdar (2006); Mishra et al., (2015).

The total number of adult *C. chinensis* emerged was also found to be dose dependent (Figure 8) and overall mean percentage of emergence of adult was found to be 60.6±11.68 in treatment group which is significant ($p<0.001$) over the control group (77.32±7.11) (Table 4). The results obtained were closely similar with the works done by several other authors. Miah et al., (1993); Rahman & Talukdar (2006) reported reduced adult emergence in seeds treated with *Vitex negundo* leaf powder. The reduced adult emergence rate of *C. chinensis* in seeds treated with sesamum oil, soybean oil, mustard oil, neem oil etc. was reported by Verma et al., (1983), Bhatnagar et al., (2001).

Figures and Table legends:

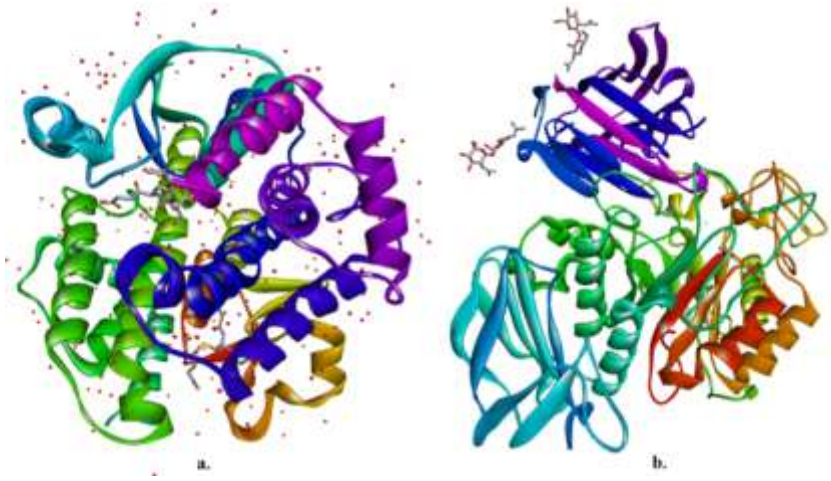


Figure 1: Structures of target enzymes, a. Glutathione S Transferase and b. Alpha amylase

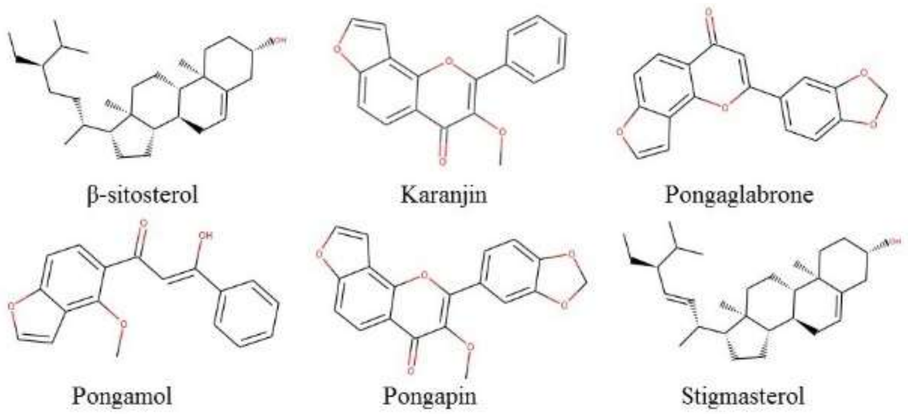


Figure 2: Structure of ligands present in the seeds of *P. pinnata*

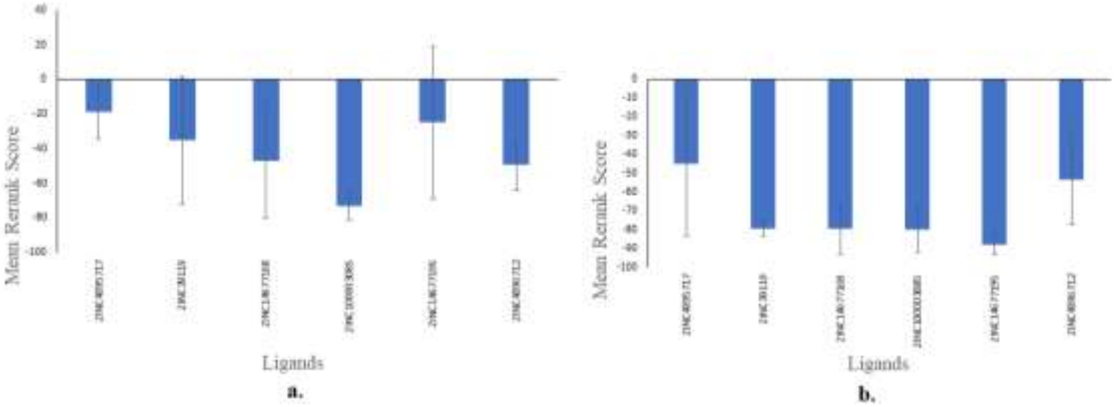


Figure 3: Graph showing rerank scores of ligands of *P. pinnata* against a. Glutathione S Transferase and b. Alpha amylase

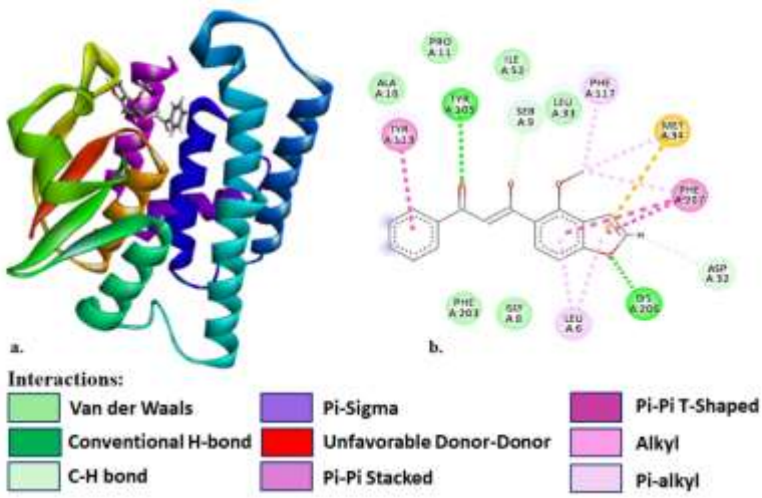


Figure 4: Interaction of Pongamol with Glutathione S Transferase, a. 3D image and b. 2D image

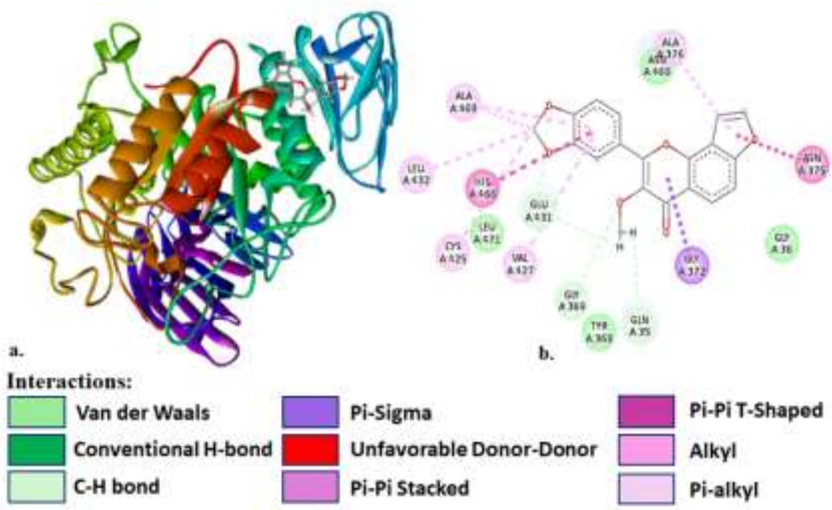
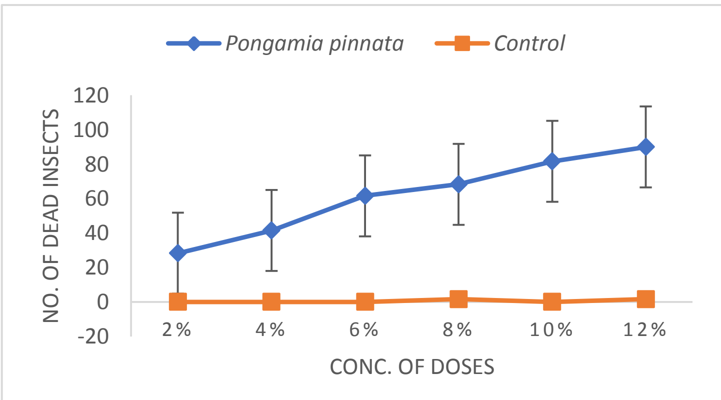


Figure 5: Interaction of Pongapin with Alpha Amylase, a. 3D image and b. 2D image



Sasanka Sekhar Ghosh /Afr.J.Bio.Sc. 6(13)(2024).3117-3135

Figure 6: Graph showing mortality of adult *C. chinensis* when treated with test extract.

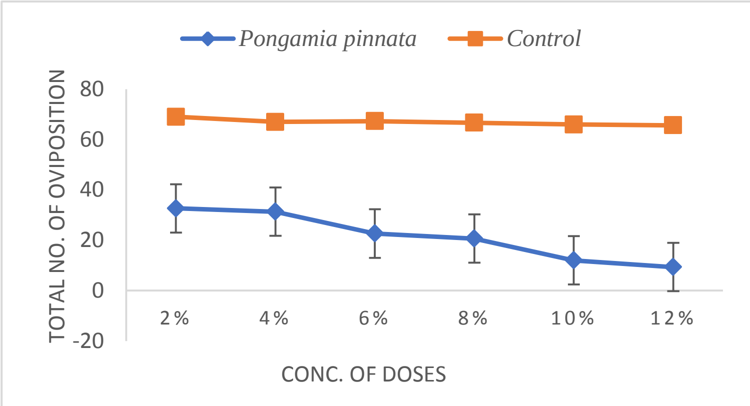


Figure 7: Graph showing effect of test extract on oviposition of *C. chinensis*

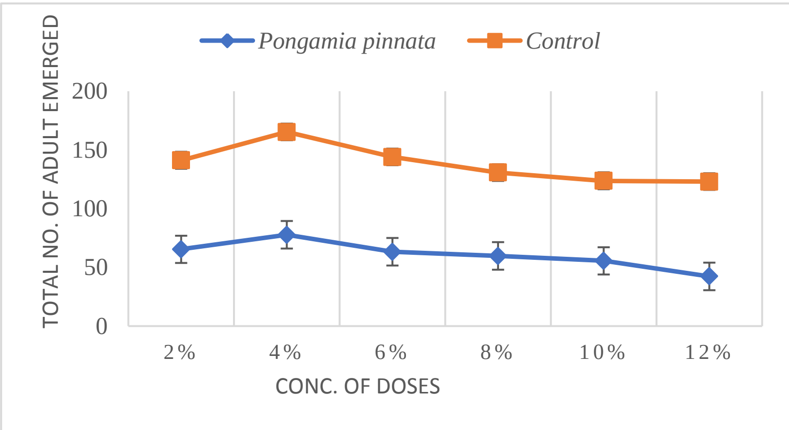


Figure 8: Graph showing effect of test extract on emergence of adults in *C. chinensis*

Table 1. Mortality of adult *C. chinensis* when treated with different concentrations of *P. pinnata*

Sl. No.	**Plant Extract**	**Total no. of Dead Insects**						**Overall mean percentage mortality**
		Dose						
		2%	**4%**	**6%**	**8%**	**10%**	**12%**	

1	*Pongamia pinnata*	5.66±1.15	8.33±1.52	12.33±2.3	13.66±1.52	16.33±1.52	18±1	61.9±23.51
2	Control	0±0	0±0	0±0	0.33±0.57	0±0	0.33±0.57	0.55±0.85

Dose wise (ANOVA, F=48.63, df=6, p<0.001)

Plant extract wise (ANOVA, F=15.35, df=9, p<0.001)

Table 2. Probit analysis data for LC_{50} determination of adult *C. chinensis*

Sl. No.	**Plant extract**	**Regression Equation**	**Significance value**	**F in regression**	**LC_{50} (% Conc.)**
1	*Pongamia pinnata*	y=2.32x+3.56	0.00126424		4.16

Table 3. Total number of eggs laid by *C. chinensis* when treated with different concentrations of *P. Pinnata*

Sl. No.	**Plant Extract**	**Total no. of eggs laid**						**Overall average oviposition**
		Dose						
		2%	**4%**	**6%**	**8%**	**10%**	**12%**	
1	*Pongamia pinnata*	32.66±4.72	31.33±3.51	22.66±4.72	20.66±3.78	12±2.64	9.33±1.52	21.44±9.60
2	Control	69±1	67±5	67.33±4.72	66.66±5.50	66±7	65.66±6.11	66.94±1.83

Dose wise (ANOVA, F=98.41, df=6, p<0.001)

Plant extract wise (ANOVA, F=76.12, df=9, p<0.001)

Table 4. Total number of *C. chinensis* emerged when treated with different concentration of *P. pinnata*

Sl. No.	**Plant Extract**	**Total no. of adult emerged**	**Overall mean**
		Dose	

		2%	**4%**	**6%**	**8%**	**10%**	**12%**	**percentage of EOA**
1	*Pongamia pinnata*	21.33±3.51	24.33±3.05	14.33±5.85	12.33±2.51	6.66±1.52	4±1	60.6±11.68
2	Control	52.33±3.05	58.66±3.51	54.33±3.51	47.33±3.21	45±2.64	53±5.56	77.32±7.11

Dose wise (ANOVA, F=81.68, df=6, p<0.001)

Plant extract wise (ANOVA, F=87.73, df=9, p<0.001)

Conclusions:

The present study concludes that the seed extracts of *P. pinnata* have high insecticidal efficacy against the pest species *C. chinensis* as indicated by the results of *in-silico* analysis and direct toxicity test. Treatment with the seed extracts also showed significant effects on the oviposition and emergence of adults in the target pest species. Thus, *P. pinnata* can be effectively used an alternative plant resource in the natural control of *C. chinensis.*

Acknowledgements - The authors are thankful to Dr. Akalesh Kumar Verma, Department of Zoology, Cotton University, for providing access to the Molegro Virtual Docker software for the *in silico* work.

References:

Al Muqarrabun, L.M.R., Ahmat, N., Ruzaina, S.A.S., Ismail N.H., & Sahidin, I., (2013). Medicinal uses, phytochemistry and pharmacology of *Pongamia pinnata* (L.) Pierre: A review. *Journal of Ethnopharmacology*, 150, 395–420

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., & Lightfoot, D. A. (2017). Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. *Plants (Basel, Switzerland)*, *6*(4), 42. https://doi.org/10.3390/plants6040042

Bhatnagar, A., Bhadauria, N. S., and Jakhmola, S. S. (2001) Efficacy of vegetable oils against pulse beetle, *Callosobruchus maculatus* in cowpea. *Indian Journal of Entomology*. **63**(3): 237-239.

Bindu, V.R., Ganga, S., Dayanandan, S. (2015). Mortality effects of some medicinal plants on the pulse beetle *Callosobruchus chinensis* (Coleoptera: Bruchidae). *Journal of Biofertilisers & Biopesticides.* 6(1):55-61.

Chopade V.V., Tankar A.N., Pande V.V., Tekade A.R., Gowekar N.M., Bhandari S.R., Khandake S.N., (2008), *Pongamia pinnata*: Phytochemical constituents, traditional uses and pharmacological properties: A review. *International Journal of Green Pharmacy*, 72-75 (Citation imcomplete)

Da, L.J.L. (2018). The amylases of insects. *International Journal of Insect Science*. 10:1-14.

Dhaliwal, G.S., Jindal, V & Bharathi, M. (2015) Crop Losses due to insect pests: Global and Indian Scenario. *Indian Journal of Entomology*, 77. 165. 10.5958/0974-8172.2015.00033.4.

Eaton, D.L. and Bammler, T.K., (1999), Concise review of the glutathione S-transferases and their significance to toxicology. *Toxicological Sciences*, 49 (2): 156–64

Enayati, A.A., Ranson, H., Hemingway, J. (2005). Insect glutathione transferase and insecticide resistance. *Insect Molecular Biology*. 14(1):3-8.

Hosamani, G.B., Jagginavar, S.B., and Karabhantanal, S.S., (2018). Biology of pulse beetle *Callosobruchus chinensis* on different pulses. *Journal of Entomology and Zoology Studies,* 6(4): 1898-1900

Isman, M.B., (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entomology,* 51:45–66

Jachuk, M., Prakash, A. and Rao, J. (2010) Evaluation of certain plant products as grain protectants against pulse beetle in stored black gram. *Journal of Applied Zoological Research*. **21**(1): 61-62.

Jadhav, P.N. and Pardeshi, A.B. (2017). Insecticidal activity of endophytic fungal extract of Jatropha curcas against *Callosobruchus chinensis*. *Bioscience Discovery*. 8(3). 556-562.

Kalita, J., Gogoi, P., Bhattacharya, P. R. and Handique, R. (2002) A study on effect of traditional storage protectants on oviposition of *Callosobruchus chinensis* and their damage to stored pulses. *Journal of Applied Zoological Research*.**13** (2&3): 251-254.

Mathur, Y. K., Shanker, K and Ram, S. (1985). Evaluation of some grain protectants against *Callosobruchus chinensis* on black gram. *Bull. Grain Tech*. **23**(3): 253-259

Miah, M. R. U, Elias, M., Torofder, G. S., Islam, B. N., Sardar, M. A. and Karim. M.A. (1993). Evaluation of local plant material against the pulse beetle (*Callosobruchus chinensis* Linn.) on chickpea. *Bangladesh. Journal of Zoology*. **21**(2): 151-153.

Mishra S K, Macedo M L R, Panda S K, Panigrahi J. (2015). Bruchid pest management in pulses: past practices, present status and use of modern breeding tools for development of resistant varieties. *Annals of Applied Biology*. 5(3). 113-116

Mishra, R.K., Bohra, A., Kamaal. .N, Kumar, K., Gandhi. K., Sujayanand, G.K., Saabale, P.R., Satheesh, N.S.J., Sarma, B.K., Kumar, D., Mishra, M., Srivastava, D.K., and Singh, N.P., (2028), Utilization of biopesticides as sustainable solutions for management of pests in legume crops: achievements and prospects. *Egyptian Journal of Biological Pest Control*, 28:3

Murasing, C., Das, P., Satish, K. and Hazarika, L.K. (2018). Laboratory evaluation of *Leucas lavadulifolia* leaf extracts against pulse beetle in stored green gram seed (*Vigna radiate*). *International Journal of Pure & Applied Bioscience*. 6(5). 732-737.

Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., and Hens, L.,(2016). Chemical Pesticides and Human Health: The Urgent Need for a New Concept in Agriculture. *Frontiers in Public Health*, 4: 148.

Olajire, A. G., John C. M., Graham, J. H. (2010). Legume type and temperature effects on the toxicity of insecticide to the genus *Callosobruchus (*Coleoptera: Bruchidae). *Journal of Stored Products Research*, Volume 47, Issue 1, 2011, Pages 8-12, ISSN 0022-474X, https://doi.org/10.1016/j.jspr.2010.08.001.

Polak, R., Phillips, E. M., & Campbell, A. (2015). Legumes: Health Benefits and Culinary Approaches to Increase Intake. *Clinical diabetes: a publication of the American Diabetes Association*, 33(4), 198–205. https://doi.org/10.2337/diaclin.33.4.198

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rahman, A. and Talukdar, F. A. (2006) Bioefficacy of some plant derivatives that protect grain against the pulse beetle, *Callosobruchus maculatus*. *Journal of Insect Science*. **6**(3): 1-10.

Rawat, S., and Srivastava, M. (2011). Evaluation of qualitative and quantitative losses caused by *Callosobruchus chinensis* in some pulses. *Journal of Entomological Research.* 35(2): 117-120

Rijal, J.P., Regmi, R., Ghimire, R., Puri, K.D., Gyawaly, S. and Poudel, S. (2018). Farmers Knowledge on Pesticide Safety and Pest Management Practices: A Case Study of Vegetable Growers in Chitwan, Nepal. *Agriculture.* 2018, 8: 16

Ruyck, J., Brysbeart, G., Blossey, R., and Lensink, M.F. (2016). Molecular docking as a popular tool in drug design and in silico travel. *Advances and Applications in Bioinformatics and Chemistry*. 9:1-11

Salinas, A.E., Wong, M.G. (1999). Glutathione S-transferases-a review. *Current Medicinal Chemistry*. 6(4):279-310.

Sharifzadeh, M.S., Abdollahzadeh, G., Damalas, C.A., and Rezaei, R. (2018). Farmers Criteria for Pesticide Selection and Use in the Pest Control Process. *Agriculture* 2018, 8, 24

Shintu, M.I., Bello, U.R., Safiyan, S. (2020). Molecular docking and ADMET properties of Citrus sinensis phytochemicals on insecticide resistance Anopheles gambiae: An in-silico analysis. *Journal of Entomology and Zoology Studies*. 8(5):1030-1036.

Singh, Ravinder. (2010). Evaluation of some plant products for their oviposition deterrent properties against the *Callosobruchus maculatus* (F.) on Chik pea seeds. Journal of Agricultural Technology 2011 Vol. 7(5): 1363-1367.

Srinivasan, R., Natarajan, D., Shivakumar, M.S., Vinuchakkaravarthy, T., Velmurugan, D. (2015). Bioassay guided isolation of mosquito larvicidal compound from acetone leaf extract of *Elaeagnus indica* Servett Bull and its *in-silico* study. *Industrial Crops and Products*. 76:394-401.

Thomsen, R., Christensen, M.H. (2006). MolDock: A New Technique for High-Accuracy Molecular Docking. *Journal of Medicinal Chemistry*. 49:3315-3321.

Utida, S. (1972). Density dependent polymorphism in the adult of *Callosobruchus maculatus* (Coleoptera, Bruchidae), Journal of Stored Products Research. 8(2): 111-125.

Varma, S., Anadi, P. Biology of Pulse Beetle (*Callosobruchus chinensis* Linn., Coleoptera: Bruchidae) and Their Management Through Botanicals on Stored Mung Grains in Allahabad Region. *Legume Research: International. Journal.* 2010, 33 (1): 38–41.

Verma, S. P., Singh, B. and Singh, Y. P. (1983) Studies on comparative efficacy of certain grain protectants against *Sitotroga cerealella. Buletein of.Grain Technology*. **21**(1): 37-42.

Yadav, R.P., Ibrahim, K.S., Gurusubramanian, G., Kumar, N.S. (2015). *In silico* docking studies of non-azadirachtin limonoids against ecdysone receptor of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). *Medicinal Chemistry Research.* 24(6):2621-2631.

Zafar, U., Rashid, M.M. and Shah, M. (2018). Entomotoxicity of plant powders against pulse beetle (*Callosobruchus chinensis*) on stored mung bean (*Vigna radiata*). *Journal of Entomology and Zoology Studies.* 6(1). 1637-1641.