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Computational Studies of Potential Insecticidal Limonoids from Neem Against *Spodoptera frugiperda* (J. E. Smith)

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Abstract: *Spodoptera frugiperda* (J. E. Smith) is an invasive pest which pose severe economic losses to a variety of crops. Invasion of fall armyworm (FAW) causes potential damage to staple crops which threatens the food security of India. To mitigate the pest attack, synthetic insecticide chlorantraniliprole was used by the farmers to control the attack of *S. frugiperda*. However, the development of pesticide resistance and adverse effects on health and environment are the major drawbacks in using synthetic insecticides. The lethal effects of synthetic insecticide necessitates the search for ecofriendly biopesticide from plant sources which are considered as good alternatives. *Azadirachta indica* A. Juss., is the most promising multipurpose tree species being harnessed for the synthesis of biopesticides due to its bioactive compounds which possesses antifeedant, ovicidal, larvicidal, oviposition deterrent and growth regulating activities against the insects. The objective of the present study was to evaluate and check the potency of the limonoids (Azadirachtin, Nimbin and Salannin) from *A. indica* kernels against the pest *S. frugiperda* via *in-silico* studies by targeting the ryanodine receptor of the insect in comparison with the insecticide chlorantraniliprole. Structure of ligands were retrieved from PubChem Database. The target protein sequence was downloaded from UniProtKB and modelled using SWISS-MODEL. Quality of target protein was validated using ERRAT tool prior to docking. After validation, docking was carried out using SwissDock server for all the ligands against the target. Docking results of azadirachtin, nimbin, salannin and chlorantraniliprole revealed binding energies of -7.22, -6.88, -7.08 and -7.41 Kcal/mol, respectively. In addition, the amino acid interaction also supports the binding affinity of the phytocompounds, where azadirachtin and salannin interacts with the same amino acid as that of chlorantraniliprole. From the results, it can be inferred that azadirachtin and salannin exhibited good binding affinities towards the target protein which can be used in combination with chlorantraniliprole as a formulation in integrated pest management of FAW for its relatively low toxicity to non-target organisms and biodegradability.

Keywords: *Azadirachta indica*, Limonoids, *Spodoptera frugiperda*, SwissDock, Chlorantraniliprole

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Introduction

The Fall Armyworm, *Spodoptera frugiperda* (J. E. Smith) is a destructive polyphagous insect of economically important crops distributed worldwide (Lima *et al*., 2023). It is an ecologically adaptable invasive pest due to its periodic outbreaks and feeding voracity (Song *et al*., 2023). Fall armyworm (FAW) exhibits diverse host range of over 353 plants in which maize and rice are preferred host (Ullah *et al*., 2023). The invasive pest *S. frugiperda* cause severe economic losses to variety of crops. The incursion of FAW in India was reported during May - June 2018 in maize fields at College of Agriculture, Shivamogga, Karnataka (Sharanabasappa *et al*., 2018). Karnataka ranks first in maize production amidst the major maize producing states where the yield was severely affected by the FAW which threatens the food security of India (Deshmukh *et al*., 2020).

The larval stage of *S. frugiperda* damage the crop by feeding the whorl of the plant which results in white elongated patches and release its feces in the whorl leading to substantial depletion in quality and yield. Since maize is the third most staple crop after rice and wheat in India, insect pest affecting its yield and quality requires a lot of attention (Sharanabasappa *et al*., 2018). The infestation of this insect results in 15-73% of yield losses in maize (Ullah *et al*., 2023). Therefore to mitigate the pest attack, synthetic insecticide chlorantraniliprole was used by the farmers to control the attack of *S. frugiperda*. Chlorantraniliprole works by activating the ryanodine receptor in the insect which impairs muscle function leading to paralysis and death of the insect (Lahm *et al*., 2009). However, the development of pesticide resistance is the major drawback in using synthetic insecticides to control FAW. Due to the pesticide resistance, increased dose of insecticides are sprayed to enhance the effectiveness which in turn increases crop production but adversely affects health and environment via residues in food and water (Song *et al*., 2023).

The lethal effect of synthetic insecticides necessitates the search for ecofriendly biopesticide from plant sources which are considered as good alternatives for its relatively low toxicity to non-target organisms and biodegradability (Tulashie *et al*., 2021). Extensive research work has been carried out to explore the botanical pesticides with potent insecticidal activity. It has been reported that almost 2000 plant species are well known to possess antifeedant activity (Packiam and Ignacimuthu, 2012), of which *Azadirachta indica* A. Juss. is the most promising multipurpose tree species being harnessed for the synthesis of biopesticides. Numerous phytocompounds from different parts of neem tree possess various biological activities but neem seeds are the vital source of potent phytocompounds for neem based insecticide formulations (Shannag *et al*., 2015). So far, nearly 200 phytocompounds have been reported from neem seeds (Chatterjee *et al*., 2023). The major phytocompounds of neem seeds are azadirachtin, nimbin and salannin which exhibits potent biological activities such as antifeedant, ovicidal, larvicidal, oviposition deterrent and growth regulating activities against the insects (Packiam and Ignacimuthu, 2012). Azadirachtin is the most active deterrent which inhibits the growth of larvae at less than 1 ppm in insects. Besides azadirachtin (AZA), non-AZA compounds such as nimbin and salannin also revealed antifeedant activities against insects (Bomford and Isman, 1996).

Keeping in view the importance of limonoids as insecticides, the objective of the present study was to evaluate and check the potency of the limonoids from *Azadirachta indica* kernels against the pest *Spodoptera frugiperda* via *in-silico* studies by targeting the ryanodine receptor of the insect in comparison with the synthetic insecticide chlorantraniliprole.

Materials and Methods

Preparation of target protein:

The three-dimensional structure of ryanodine

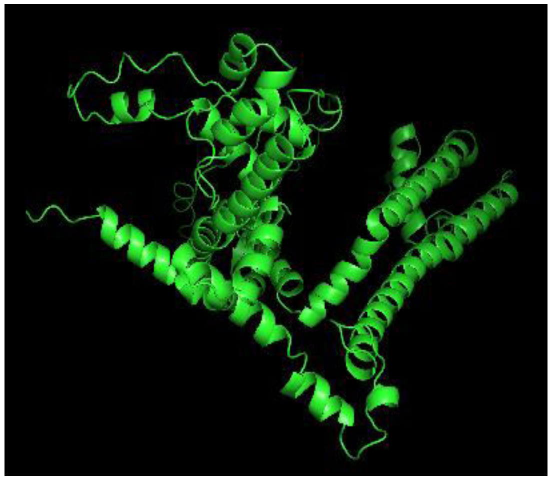


Fig. 1: Three Dimensional Structure of Ryanodine receptor of the insect *S. frugiperda.*

receptor of the insect *Spodoptera frugiperda* is not available in the Protein Data Bank (www.rcsb.org). Therefore, the 3D structure was obtained by homology modelling. The sequence of ryanodine receptor of the target insect was retrieved from UniProtKB database (www.uniprot.org) in FASTA format. Then, the sequence was submitted to SWISS-MODEL (Waterhouse *et al*., 2018) which is a fully automated protein homology modelling server that generates three dimensional structure of the target protein (Fig. 1). Further, the generated 3D structure of the target protein was validated by ERRAT online server tool for docking studies.

Preparation of Ligand:

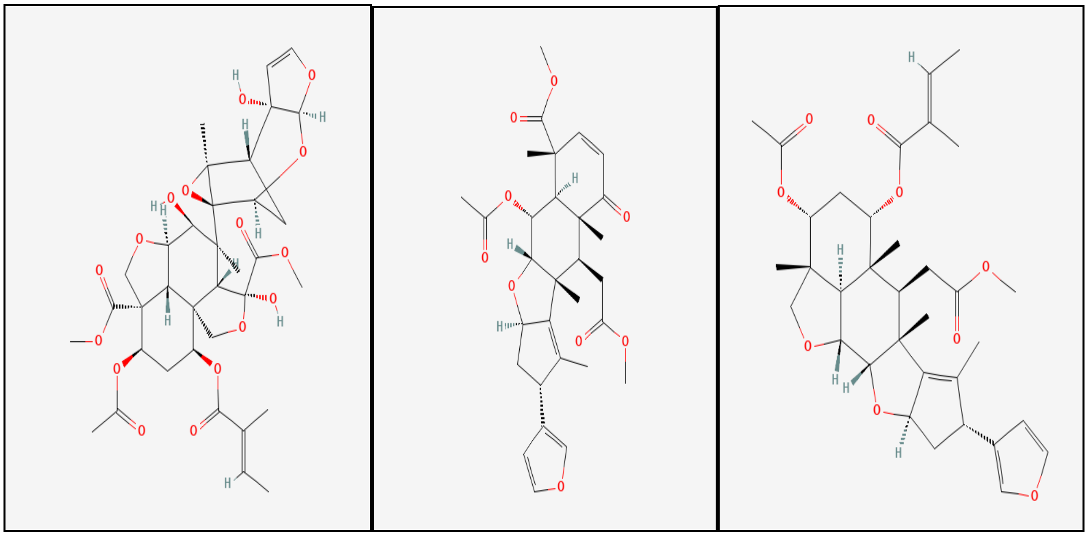
Three key limonoids such as azadirachtin, nimbin and salannin from the kernels of *Azadirachta indica* were selected for docking studies based on the reported insecticidal activity of these compounds. The structures were retrieved from PubChem Database (https://pubchem.ncbi.nlm.nih.gov/) in SDF format. Using Open babel version 2.3.1 (O'Boyle *et al*., 2011), the phytocompounds saved in SDF format was converted to MOL2 format for molecular docking. Similarly, the existing synthetic insecticide chlorantraniliprole was also retrieved and used as a standard to compare the potency of the selected phytocompounds. The structure of limonoids and standard are shown in (Figs. 2, 3).

Protein-Ligand Docking:

Molecular docking studies were performed using SwissDock web server which is based on the docking software EADock DSS. The protein and ligands were uploaded in their respective file formats for docking. After each docking run, output clusters were obtained and ranked by a specific SwissDock algorithm. It ranks the cluster based on FullFitness (FF) scoring function, where cluster 0 has the best FullFitness score which is considered to be the most favorable interaction and hence a better fit. Biovia Discovery Studio was used to visualize the docking interactions obtained from SwissDock (Grosdidier *et al*., 2011).

Results

The present study focuses on the virtual investigation of phytocompounds and chlorantraniliprole against the pest *S. frugiperda.* Prior to docking, the structure of target protein generated using SWISS-MODEL was validated using ERRAT tool to confirm the quality of the modelled structure. Overall quality of target protein was 97.02% analyzed through ERRAT (Fig. 4) which confirms that the structure is of good quality. The receptor and ligands were docked using SwissDock default parameters. Different docking poses were obtained of which



A **B** **C**

Fig. 2: Structure of limonoids (A) Azadirachtin; (B) Nimbin; and (C) Salannin present in the kernels of *Azadirachta indica.*

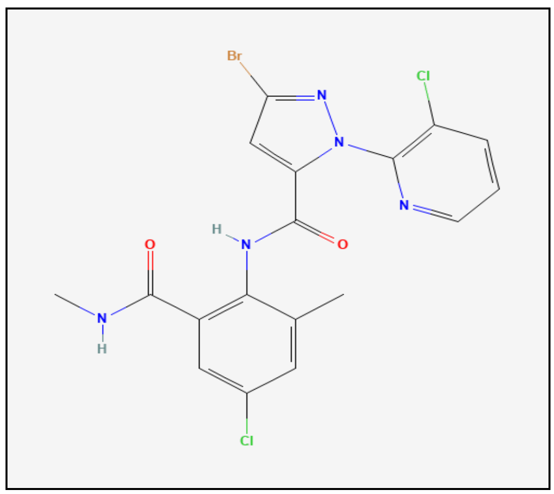


Fig. 3: Structure of Synthetic insecticide chlorantraniliprole.

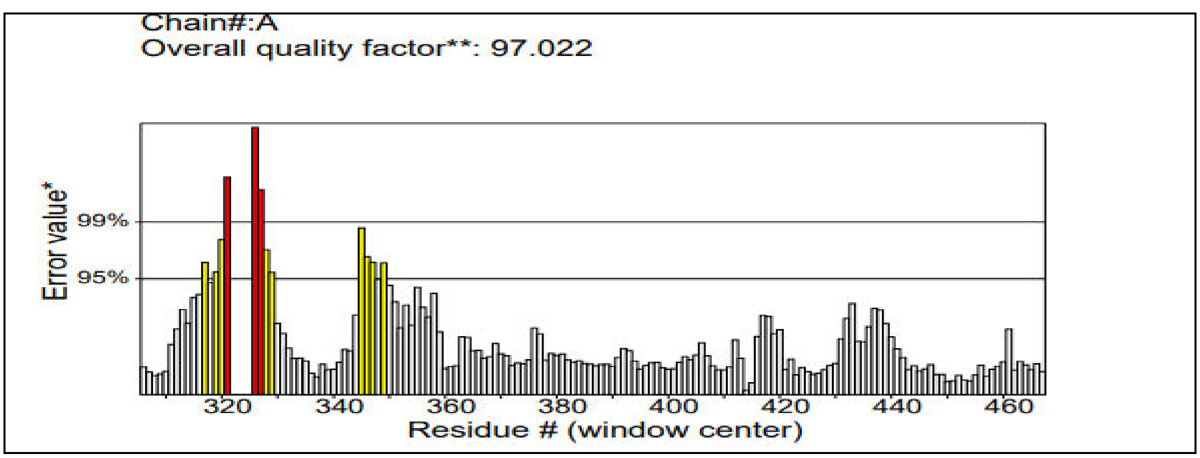


Fig. 4: ERRAT analysis of target protein.

Table 1: Docking result of ligands with the target ryanodine receptor of the insect *S. frugiperda* predicted by SwissDock

S. No.	Compound name	ΔG (Kcal/mol)	FullFitness (Kcal/mol)	Number of H-bonds	Amino acids involved in Hydrogen bond interaction
1.	Azadirachtin	-7.22	-2834.78	2	Gln 283 and Asn 288
2.	Nimbin	-6.88	-2527.11	-	-
3.	Salannin	-7.08	-2832.60	1	Gln 283
4.	Chlorantraniliprole	-7.41	-2874.53	1	Gln 283

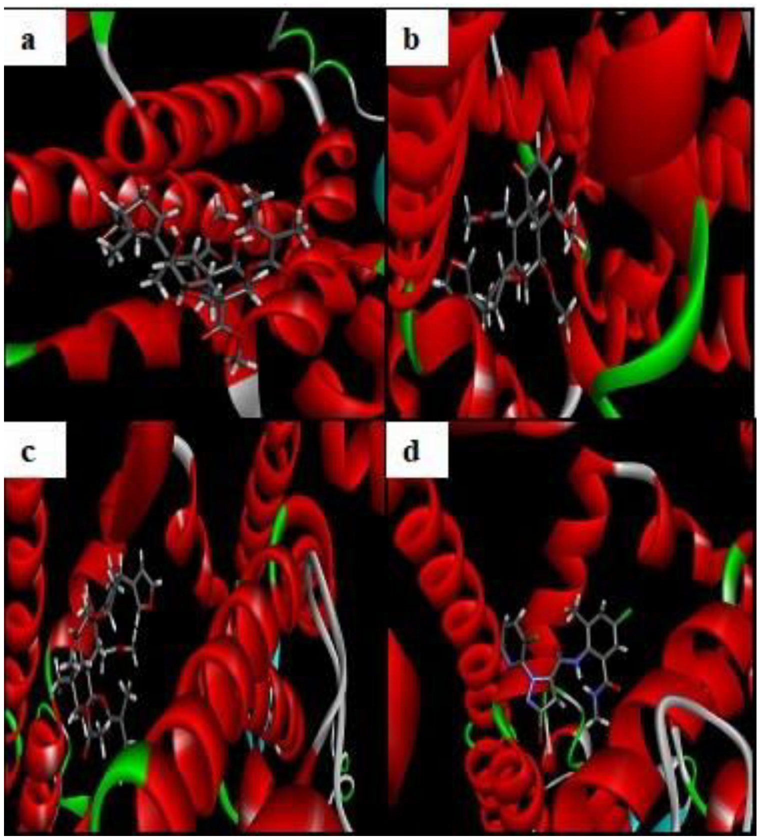


Fig. 5: Docking pose of(a) Azadirachtin; (b) Nimbin; (c) Salannin; and (d) chlorantraniliprole with the target ryanodine receptor of the insect *S. frugiperda.*

the best docked pose was screened based on lowest ΔG Kcal/mol energy. Docking results of azadirachtin, nimbin and salannin revealed binding energies of -7.22, -6.88 and -7.08 Kcal/mol followed by FullFitness scores -2834.78, -2527.11 and -2832.60 Kcal/mol, respectively. The binding energy of chlorantraniliprole was found to be -7.41 Kcal/mol with a FullFitness score of -2874.53 Kcal/mol (Table 1). The docking poses of the phytocompounds and chlorantraniliprole with the target illustrated in (Fig. 5).

Based on the binding energy and FullFitness score, chlorantraniliprole revealed higher binding affinity with the target ryanodine receptor of the

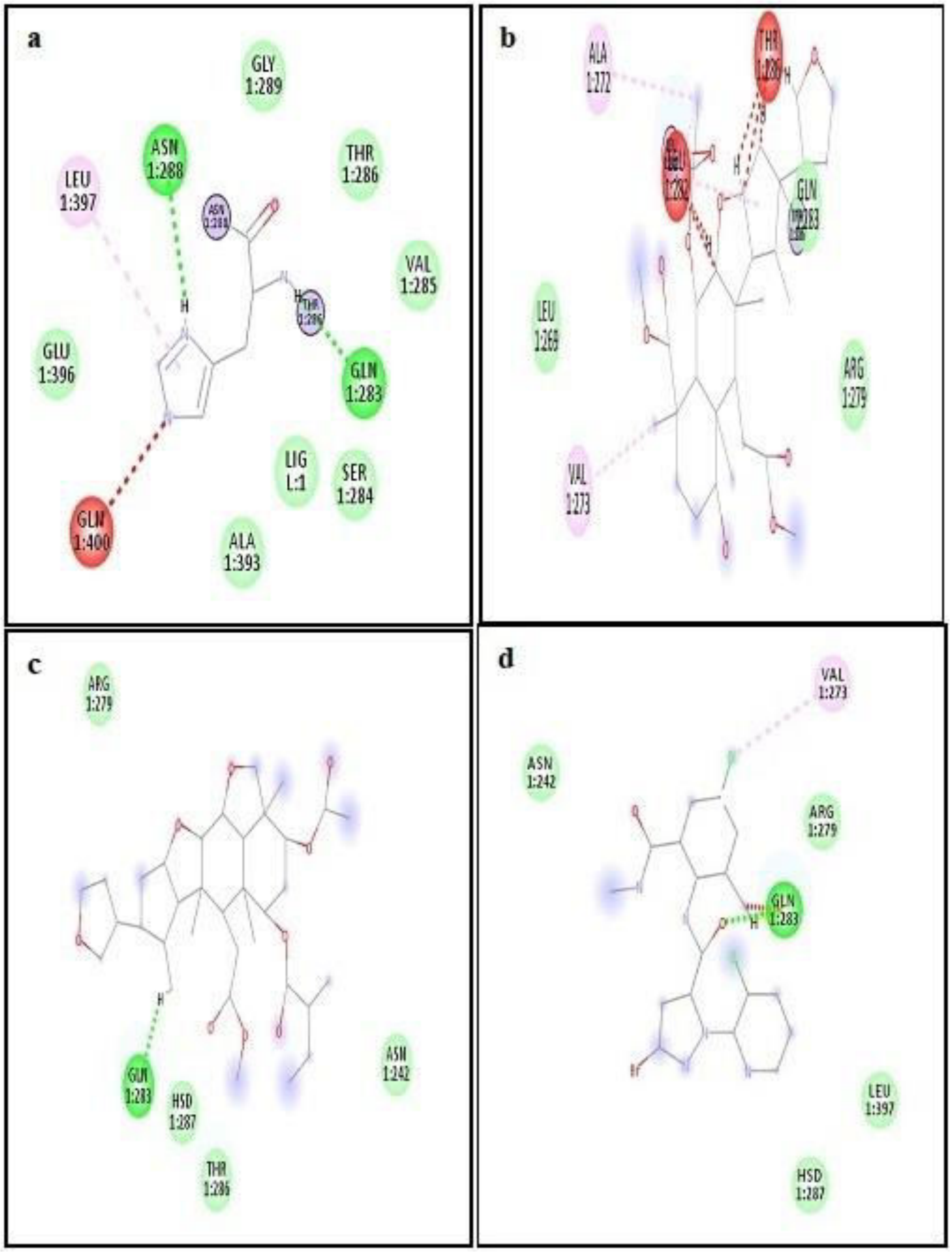


Fig. 6: Two dimensional docking interaction of (a) Azadirachtin; (b) Nimbin; (c) Salannin; and (d) chlorantraniliprole with the target ryanodine receptor of the insect *S. frugiperda.*

insect. Lower the binding energy higher the stability of the ligand to dock with the target. Similarly, azadirachtin and salannin exhibited lower binding energy which is on par with the insecticide chlorantraniliprole binding energy. In addition, the amino acid interaction also supports the binding affinity of the phytocompounds (azadirachtin and salannin) with the target.

In case of azadirachtin, a total of 2 hydrogen bonds are observed one with Gln 283 and the other with Asn 288. Whereas salannin and chlorantraniliprole were found to form one hydrogen bond with Gln 283. No hydrogen bond was observed in nimbin. The two dimensional docking interaction of the phytocompounds and chlorantraniliprole with the target using Biovia Discovery Studio are shown in Figure 6. From the docking results, it can be inferred that the azadirachtin and salannin have the potential to bind to the target ryanodine receptor and hence possesses strong insecticidal activity as that of chlorantraniliprole against the insect *S. frugiperda*.

Discussion

Agriculture is affected by the spread of invasive pests and diseases globally (Ullah *et al*., 2023). *S. frugiperda* is a destructive invasive pest in India which maintain its population throughout the year by shifting to other hosts such as sorghum, sweet corn, baby corn, cabbage, groundnut and sugarcane in the absence of maize (Sharanabasappa *et al*., 2018). Due to severe yield losses, insecticides are widely used to control the infestation of *S. frugiperda*. As per Central Insecticide Board and Registration Committee recommendation, Chlorantraniliprole 18.5 SC at a dose of 0.4 ml/l can be used for fall armyworm management in India (Kamakshi *et al*., 2023). It is a novel insecticide belongs to the group anthranilic diamide which exhibited higher efficacy against lepidopteran pests feeding on various crops (Guo *et al*., 2014). In addition, it also control pests of other orders, including Coleoptera, Diptera, Isoptera and Hemiptera (Lahm *et al*., 2009).

Chlorantraniliprole functions via a unique mode of action by activating the ryanodine receptor of the insect. The insect ryanodine receptor (RyR) is one of the largest intra-cellular calcium releasing channels which plays a critical role in muscle excitation-contraction coupling and considered to be an important target for diamide insecticides (Ren *et al*., 2022). Chlorantraniliprole exhibits relatively low toxicity against non-target organisms and environment friendly which is an excellent alternative to highly toxic conventional insecticides (He *et al*., 2019). Though Chlorantraniliprole possess higher insecticidal activity and low toxicity, several lepidopteran pests developed resistance against the insecticide (Samurkas *et al*., 2021).

Due to the development of pesticide resistance, natural products can be used for pest control. Among the natural products, neem-based pesticides exhibit remarkable activity against pests which includes growth regulation, feeding deterrence, toxicity, disruption of metamorphosis and death (Shannag *et al*., 2015). In the present study, it was observed that azadirachtin and salannin revealed greater binding affinity towards the target ryanodine receptor similar to the commercial insecticide chlorantraniliprole. The ligands (phytocompound and chlorantraniliprole) has the ability to open calcium channels in insects by binding to the ryanodine receptor which results in the release of large amount of calcium from the sarcoplasmic and endoplasmic reticulum. Thus, a significant reduction in the content of calcium triggers an imbalance in the internal environment of targeted insect leading to feeding cessation, functional muscle disorders, neurological disorders, and finally death of the insect (Ren *et al*., 2022). The effect of azadirachtin and salannin is in line with the previous studies which reported its potent insecticidal activity against various pests.

Azadirachtin is a highly oxidized tetranortriterpenoid with complex molecular structure popularly known as limonoids. It is a broad spectrum insecticide active at very low concentration and minimizes the risk of insect resistance due to its chemical complexity. In addition, azadirachtin exhibits low mammalian toxicity which falls into class U (Unlikely to present an acute hazard) of the World Health Organization (WHO) toxicity rating and also registered as a general-use pesticide in the United States with a toxicological class IV (relatively non-toxic) by Environmental Protection Agency (EPA). Azadirachtin is readily degradable, selective, non-mutagenic, and also reported as safer for non-target and beneficial organisms (Kilani-Morakchi *et al*., 2021).

A previous study on *Spodoptera frugiperda* insect cell (SF-9) treated with low concentration of azadirachtin resulted in abnormal and enlarged cells viewed under electron microscope. After 48 h of treatment inhibitory and degenerative effects were observed which includes bleebing, formation of holes, and extrusion of cytoplasmic materials leading to cell death (Reed and Majumdar, 1998). Azadirachtin is an insect growth regulator which works on two principal hormones such as 20-hydroxyecdysone (20E) and juvenile hormone (JH) which plays a critical role in the regulation of growth and development of the insect. Azadirachtin has the ability to modify or suppress hemolymph ecdysteroid and JH titers by inhibiting the secretion of morphogenetic peptide hormone (PTTH) from the brain. It also blocks cell division, protein synthesis and calcium channels (Duarte *et al*., 2020; Kilani-Morakchi *et al*., 2021) in insects. The survival of *S. frugiperda* larvae was significantly affected and proportional to the concentrations of azadirachtin (Acharya *et al*., 2023).

Though azadirachtin is the principal bioactive compound in neem, other limonoids also play an important role in the control of insect pests. The inclusion of non-AZA constituents from neem in integrated pest management helps to prolong or avoid the development of resistance in pests. In fact, there is an evidence stating that *Myzus persicae* developed resistance to pure AZA after repeated exposure but not to a neem seed extract that contained additional limonoids (Bomford and Isman, 1996). Salannin, a non-AZA limonoid showed reduction in larval growth due to reduced food intake when fed on diet containing salannin type of compounds using diet assay against neonate larvae of *H. armigera* and *S. litura*. It possesses growth inhibitory and antifeedant activity against lepidopteran pests (Koul *et al*., 2004). Earlier studies suggest that the combined effect of AZA and non-AZA constituents from neem seed extract can be effective than that of AZA alone (Bomford and Isman, 1996).

Conclusion

Molecular docking studies revealed that azadirachtin and salannin has the ability to inhibit the infestation of *Spodoptera frugiperda* alike chlorantraniliprole. To mitigate the pest attack, combination of chlorantraniliprole, azadirachtin and salannin can be used as a formulation for enhanced insecticidal activity and also reduces the potential of insect pest to develop resistance against pesticides. Emphasis has to be given on eco-friendly pest mitigating practices by using safe chemical like chlorantraniliprole and neem formulations in integrated pest management of FAW for its low toxicity and biodegradability. This formulation may exhibit strong systemic effects on the growth, development and survival of *S. frugiperda* larvae that fed on various crops and also reduces the usage of harmful chemicals which may cause residual problems in the future.

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