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All authors names and affiliations are given below, after the references.

"Plant allelochemicals as inhibitors of insect digestive enzymes: Mechanisms, insect responses and applications in sustainable pest management"

K Sowmya, S Kousalya, M Apoorva, M Mamatha, J Aruna Kumari, B Uma and KVN Sreeja

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Abstract
Insect pests rely heavily on digestive enzymes such as proteases, α-amylases, lipases and glycosidases to hydrolyse dietary macromolecules into absorbable nutrients essential for growth and reproduction. Plants, in turn, have evolved a wide array of allelochemicals that specifically inhibit these enzymes, thereby reducing nutrient utilization and impairing insect development. Proteinase inhibitors (e.g., Kunitz, Bowman-Birk, Potato I and II types), α-amylase inhibitors, lectins, flavonoids and tannins are pivotal in disrupting insect digestion. These inhibitors act through diverse mechanisms, including competitive, non-competitive, allosteric and irreversible binding to enzyme active sites. While highly effective, insects counteract these defenses via enzyme overproduction, isoform variation, detoxification pathways and symbiotic assistance, posing challenges to long-term efficacy. Recent advances such as genetic engineering, RNAi, nano-encapsulation and molecular docking provide new avenues to enhance allelochemical-based inhibitors stability, delivery and specificity. This review highlights the diversity of insect digestive enzymes, the inhibitory potential of plant allelochemicals, insect counter-adaptations and emerging strategies to exploit these natural compounds for sustainable pest management.

Keywords: Allelochemicals, enzyme inhibitors, insect digestive enzymes, detoxification, pest management.

1. Introduction
Insects depend greatly on digestive enzymes like proteases, amylases, lipases, and esterases to decompose complex food molecules into simple, absorbable nutrients vital for survival, development, and reproduction. Plants, in turn, have evolved a wide array of allelochemicals that function as natural defense agents against herbivorous insects. Among these, enzyme inhibitors play a crucial role by targeting and interfering with insect digestive processes, reducing nutrient assimilation and ultimately deterring feeding (Gatehouse, 2011; Ryan, 1990) [22, 57].

Allelochemicals, including protease inhibitors, α-amylase inhibitors, lectins, tannins, phenolics, and flavonoids, interfere directly with insect gut enzymes, hindering their normal biochemical functions. For instance, protease inhibitors block proteolytic enzymes needed for protein digestion, while α-amylase inhibitors hinder starch breakdown, leading to nutritional stress in insects (Franco *et al*., 2002; Zavala *et al*., 2004) [19, 78]. Such interactions are crucial in the ongoing coevolutionary battle between plants and insects. Inhibition mechanisms may involve direct binding to enzyme active sites, inducing structural changes, or decreasing enzyme activity. At the same time, insects respond with adaptive strategies like synthesizing detoxification enzymes, overproducing digestive enzymes, or utilizing symbiotic gut microbes.

From an application standpoint, digestive enzyme inhibitors offer significant promise in integrated pest management (IPM). They can be employed independently or alongside chemical and biological control methods, and modern biotechnological developments have further increased their effectiveness. Emerging tools like nano-encapsulation of plant-derived inhibitors are explored to improve their stability, specificity, and field applicability (Chen *et al*., 2019; Christou *et al*., 200) [9, 12].

Corresponding Author:
K Sowmya
Research Scholar Department of Entomology, College of Agriculture, Rajendranagar, PJTAU, Hyderabad, Telangana, India

However, several obstacles still hinder the large-scale use of these inhibitors. Key concerns involve their potential impact on beneficial insects, the likelihood of resistance emerging in pest populations, and regulatory issues related to environmental safety. Even so, allelochemical-based digestive enzyme inhibition is a promising and environmentally friendly strategy for advancing sustainable pest management.

2. Overview of Insect Digestive Enzymes

Insects possess a remarkably diverse and adaptable digestive system that enables them to utilize various food sources and thrive in varied ecological environments. Central to this adaptability is a suite of digestive enzymes, primarily secreted in midgut, the primary site of digestion and nutrient absorption. However, enzyme activity is also frequently observed in salivary glands and occasionally in the hindgut. These enzymes facilitate nutrient acquisition and play important roles in ecological interactions, including plant-insect chemical defenses and symbiosis. The principal categories of digestive enzymes in insects include carbohydrase's, proteases, lipases and nucleases, each tailored to degrade specific classes of macromolecules. Diet composition, gut pH, enzyme inhibitors, and microbial symbionts influence their expression and activity.

2.1 Carbohydrase's: Polysaccharide Degradation and Host Specialization

Carbohydrases are essential for breaking down complex carbohydrates such as starch, cellulose, and pectin, supporting herbivorous, sap-feeding, and detritivorous diets. These enzymes include α-amylases, cellulases, β-glucosidases, and pectinases.

α-Amylases starch digestion into maltose and other oligosaccharides, which is crucial in starch-rich diets. Cellulases and pectinases facilitate the breakdown of plant cell walls. For example, in the weevil *Eucryptorrhynchus scrobiculatus*, researchers identified 11 cellulases and 14 pectinases in the midgut, underscoring the enzyme diversity required for effective plant tissue digestion (Gao *et al*., 2020) [20].

The brown planthopper *(Nilaparvata lugens*) produces a salivary enzyme, endo-β-1,4-glucanase (NlEG1), which degrades cellulose in rice cell walls, enabling its stylet to access phloem sap for feeding (Ji *et al*., 2017) [33]. β-Glucosidases, found in species such as *Nasutitermes takasagoensis*, *Anoplophora glabripennis*, and *Myzus persicae*, catalyze the final steps of cellulose hydrolysis by converting oligosaccharides into glucose (Tokuda *et al*., 2009; Shin *et al*., 2023; Yang *et al*., 2022) [67, 62, 76].

Gut symbionts frequently contribute additional cellulolytic enzymes, especially in high-cellulose diet insects (Jian *et al*., 2025; Zhang *et al*., 2024) [34, 79], highlighting a cooperative strategy between host and microbiota.

2.2 Proteases: Protein Digestion and Nutrient Mobilization

Proteases are critical for breaking dietary proteins into peptides and free amino acids, essential for insect growth, development, and reproduction. They are highly plastic, actively respond to protein availability and environmental conditions (Lazarević *et al*., 2023) [40]. The main categories include:

2.2.1 Trypsins (serine proteases): These enzymes cleave peptide bonds after lysine or arginine residues and are common across insect orders, except for Hemiptera and Cucujiformia. Their activity peaks at alkaline pH (8-9), and they are sensitive to TLCK inhibition. Notably, trypsins are unstable under acidic conditions and are not stabilized by calcium (Shaw *et al*., 1965; Applebaum, 1985; Ward, 1975) [61, 4, 72].

2.2. Chymotrypsins: Also, serine proteases cleave after aromatic residues. These enzymes share similarities with vertebrate enzymes but are acid-labile and sensitive to TPCK and SBTI. Though less active in Lepidoptera, they are broadly distributed in insects (Christeller *et al*., 1992) [11].

2.2.3 Cysteine proteases (e.g., cathepsin B): These enzymes function optimally in acidic conditions and are common in Hemiptera and Cucujiformia, especially for digesting proteins resistant to serine proteases. For example, cathepsin B has been extensively studied in Acanthoscelides obtectus and is known to be sensitive to TLCK and TPCK (Gooding, 1969; Houseman *et al*., 1985; Wieman & Nielsen, 1988) [23, 29, 73].

Several exopeptidases assist in protein digestion

2.2.4 Aminopeptidases: Cleave N-terminal amino acids, typically exhibit alkaline pH optima (7.2-9.0) and are often membrane-bound via hydrophobic domains or GPI anchors. These are enzymes described in insects such as *Spodoptera frugiperda*, *Musca domestica*, and *Anopheles stephensi* (Ferreira & Terra, 1986) [18].

2.2.5 Dipeptidases: Less studied, hydrolyze dipeptides and contribute to intermediate digestion. Their presence in species like *Rhynchosciara americana*, *Spodoptera frugiperda*, and *Locusta migratoria* is confirmed (Hall, 1988; Ferreira *et al*., 1994) [26, 16].

2.2.6 Carboxypeptidases: Remove C-terminal amino acids, which are also involved in the final stages of peptide degradation (Terra *et al*., 1979; Ferreira & Terra, 1985) [64, 17].

2.3 Lipases: Fat Metabolism and Energy Regulation

Lipases catalyze the hydrolysis of dietary triglycerides into fatty acids and glycerol, supporting processes such as flight metabolism, energy storage, and membrane synthesis. Dietary lipids and abiotic factors like temperature and redox state influence their activity (Lazarević *et al*., 2023; Bruno *et al*., 2025) [40, 7].

In the palm weevil (*Rhynchophorus palmarum*), lipase activity in the midgut peaks at 37-45 °C and pH 6.5, demonstrating optimal performance under warm, slightly acidic conditions. These enzymes possess a Ser-His-Asp catalytic triad, exhibit broad substrate specificity, and are enhanced by reducing agents (GSH, DTT), while inhibiting oxidized glutathione (GSSG). This characterization represents a foundational understanding of lipid digestion in this economically important pest (Santana *et al*., 2017) [58].

2.4 Nucleases: Dual Roles in Digestion and Plant Defense Suppression

Nucleases, including DNases and RNases, degrade nucleic acids into nucleotides, nitrogenous bases, and sugars, serving nutritional and defensive functions. In some insects, nucleases also act as virulence factors during plant feeding. For instance, in the southern green stink bug (*Nezara viridula*), high levels of nuclease activity are detected in both salivary glands and secreted saliva. These enzymes aid digestion, suppressing host plant defense responses and neutralizing associated pathogens (Lomate *et al*., 2016) [42].

Insect digestive enzymes are essential for nutrient acquisition and ecological adaptability, enabling the breakdown of diverse dietary components. Their vital role, however, also makes them prime targets of plant allelochemicals, which can inhibit enzyme activity and disrupt digestion. This biochemical interaction forms the basis of a coevolutionary arms race, leading to the following discussion: how plant-derived compounds act as natural inhibitors of insect digestive enzymes.

3. Allelochemicals Of Plant as Enzyme Inhibitors

3.1 Protease Enzyme Inhibitors

Ryan and his colleague first proposed that digestive enzyme inhibitors act as a plant defense mechanism (Green and Ryan, 1972) [24]. Mickel and Standish reported abnormal insect larval development on soybean diets, and later, soybean trypsin inhibitors were confirmed to be toxic to *Tribolium confusum* larvae in 1947 (Lipke *et al*., 1954) [41].

Building on these foundational studies, it is now well established that protease inhibitors (PIs) are widely distributed in plants and function as key defensive compounds by inhibiting insect digestive enzymes such as serine, cysteine, and aspartate proteinases, as well as metallo-carboxypeptidases, thereby disrupting digestion, reducing nutrient absorption, and impairing insect growth and survival (Lawrence *et al*.). Among these, serine proteinase inhibitors include subfamilies such as Kunitz type, Bowman-Birk type, Potato I type, and Potato II type inhibitors (Mehrabadi *et al*., 2012) [45].

Experimental evidence across plant-insect systems strongly supports this defensive role. For instance, in *Nicotiana attenuata*, suppression of trypsin proteinase inhibitors (TPIs) promoted better growth of *Manduca sexta* larvae. At the same time, increased TPI levels restricted larval growth and delayed development by inhibiting gut proteases (Zavala *et al*., 2004) [78]. Similarly, transgenic approaches have highlighted the effectiveness of PIs in pest resistance. Rice plants expressing cowpea trypsin inhibitor (CpTI) reduced protein digestion in *Chilo suppressalis* by inhibiting the gut serine proteases, resulting in poor growth, inefficient food utilization, and higher larval mortality (Mochizuki *et al*., 1999) [47].

Another study demonstrated that transgenic rice expressing the potato PINII-2x gene inhibited midgut proteases in both *C. suppressalis* and *Cnaphalocrocis medinalis*, leading to slower growth and increased mortality (Bu *et al*., 2006) [8]. Likewise, wheat plants engineered to express BTI-CMe showed inhibition of insect gut proteases, thereby suppressing protein digestion and causing stunted growth and higher mortality in pests such as *Spodoptera frugiperda* and *Sitotroga cerealell*a (Altpeter *et al*., 1999) [3].

3.2 Alpha Amylase Inhibitors

Plant α-amylase inhibitors (α-AIs) play a critical role in defending against insect pests by disrupting starch digestion in the insect gut. These proteinaceous inhibitors target insect α-amylases, impairing nutrient acquisition and reducing growth and survival. α-AIs are found across monocot and dicot species and have demonstrated insecticidal potential *in vitro* and when expressed in transgenic plants, highlighting their biotechnological applications in pest management (Basso *et al*., 2025) [5]. In legumes, such as Phaseolus vulgaris, seeds produce two distinct α-amylase inhibitors, αAI-1, commonly found in cultivated varieties, which inhibit mammalian and *Callosobruchus* larval gut enzymes, whereas αAI-2, present in some wild accessions, specifically targets *Zabrotes subfasciatus* and is associated with resistance to the Mexican bean weevil (Morton *et al*., 2000) [48].

Further evidence comes from Achyranthes aspera, where a proteinaceous α-amylase inhibitor was purified, revealing two activity bands (AI1 and AI2) on electrophoresis. Feeding trials demonstrated high mortality of *Callosobruchus maculatu*s larvae by day five on diets containing the seed powder, and the inhibitor was shown to suppress multiple amylase isoforms from *C. maculatus*, *Tribolium confusum*, and *Helicoverpa armigera* in both gel and solution assays (Hivrale *et al*., 2011) [28]. Similarly, bioassays using artificial seeds supplemented with papaya (*Carica papaya*) α-amylase inhibitor fractions (0.5% and 1.0%) showed a marked increase in *Callosobruchus maculatus* larval mortality (up to 50%), along with reductions in insect fecundity and adult lifespan, indicating the inhibitor's potent anti-insect effects (Farias *et al*., 2007) [15].

The α-amylase inhibitor (αAI.Mol) from *Moringa oleifera* leaves disrupts digestion in insects like *Callosobruchus maculatus* and *Tribolium confusum* by inhibiting their α-amylase enzymes. This blocks starch breakdown, reduces nutrient absorption, and impairs energy acquisition, leading to slower growth, lower survival, and reduced pupation. The inhibitor is heat- and pH-stable, making it a promising bio-insecticide. (Karray *et al*.,2022) [37]. The bean α-amylase inhibitor αAI-1 affects insect digestion by inhibiting α-amylase enzymes in Hemipteran pests. Lygaeidae, Miridae, and Nabidae family species are highly susceptible; those from Cicadellidae and Membracidae show moderate susceptibility, while Pentatomidae are largely tolerant. This inhibition reduces starch digestion, impairing nutrient absorption and growth (Lüthi *et al*., 2015) [43].

The α-amylase inhibitors from beans and wheat disrupt starch digestion in stored-product insects such as *Tribolium castaneum* (red flour beetle), *Sitophilus oryzae* (rice weevil), and *Rhyzopertha dominica* (lesser grain borer). These inhibitors reduce nutrient absorption by inhibiting α-amylase in their midguts, resulting in slower growth, lower survival, and impaired development. (Pueyo *et al*., 1995) [54]. Triticale seed α-amylase inhibitors disrupt digestion in the Sunn pest (*Eurygaster integriceps*) by inhibiting salivary α-amylases. This blocks starch breakdown, reduces nutrient absorption, and impairs growth. Dose-dependent inhibition works through a mixed mechanism, affecting enzyme activity and substrate binding (Mehrabadi *et al*., 2012) [45].

3.3 Lectins

Lectins are carbohydrate-binding glycoproteins found widely in plants, especially Leguminosae, and in Graminaceous and Solanaceous species. They act as natural defense molecules with insecticidal properties, remaining stable in insect guts and binding to glycosyl residues on gut membranes, disrupting digestion and nutrient absorption.

Tobacco (ASAL): Transgenic tobacco expressing *Allium sativum* leaf lectin (ASAL) strongly resisted *Myzus persicae*. Aphid survival dropped to 16-20% after 144 hours (about 12 days) versus ~75% on control plants, indicating effective binding to gut receptors (Dutta *et al*., 2005) [14].

Wheat lectin-like gene: Systemically induced when avirulent first-instar Hessian fly larvae feed, reducing larval growth and survival by hampering feeding and nutrient uptake. It demonstrates defense beyond the feeding site (Williams *et al*., 2002) [74].

Wild Brassica: Chitin-binding lectin exhibited insecticidal activity against Brevicoryne brassicae. It disrupted gut function, interfered with digestive enzymes, and reduced nutrient absorption, leading to poor growth and higher mortality (Cole, 1994) [13].

Maize (GNA): Transgenic maize expressing snowdrop lectin (Galanthus nivalis agglutinin) impaired larval digestion and nutrient uptake in *Ostrinia furnacalis*, reducing growth, survival, and pupation (Wang *et al*., 2005) [70].

Groundnut lectins: Groundnut Leaf Lectin (GLL) and Concanavalin A (ConA) reduced growth and survival of *Helicoverpa armigera* by interfering with gut physiology and nutrient absorption, with more potent effects at higher concentrations (War *et al*., 2013) [71].

Soybean lectin: Glycine max lectin adversely affected *Bactrocera cucurbitae*. Egg hatching was unaffected, but lectin reduced larval development, pupation, and adult emergence. Digestive and detoxifying enzymes are disrupted by increased esterase activity, suppressing phosphatases, catalase, and GST activity (Singh *et al*., 2006) [63].

3.4 Flavonoids

Flavonoids, including flavones, chlorogenic acid, caffeic acid, and protocatechuic acid, act as potent plant defense compounds against Helicoverpa armigera. They disrupt larval digestion by inhibiting key enzymes such as proteases, serine proteases, and trypsin, and impair detoxification by suppressing esterases, cytochrome P450s, and glutathione S-transferases. This dual inhibition reduces nutrient assimilation, slows growth, and lowers survival. Flavones can also enhance the toxicity of Bt Cry1Ac, demonstrating their potential as synergists in integrated pest management strategies (Belete, 2018; War *et al*., 2013) [6, 71].

3.5 Tannins

Tannins from brown macroalgae significantly inhibit digestive enzymes in the cotton leafhopper (*Amrasca devastans*), including β-glycosidase, esterase, lipase, invertase, and acid phosphatase. This interference reduces nutrient digestion and metabolism. Protein analysis also showed changes in total protein levels, indicating disrupted protein synthesis. Tannins impair feeding and growth, demonstrating their potential as natural insecticides (Petchidurai *et al.*, 2023) [53].

Tannic acid negatively affects digestive enzymes in fall webworm (*Hyphantria cunea*) larvae. It reduces the activity of enzymes responsible for breaking down nutrients, limiting the insect's ability to digest food efficiently. This disruption leads to poor nutrient assimilation, slower growth, and stunted development. The effect is both concentration- and time-dependent, showing more substantial impacts with higher doses and prolonged exposure. Overall, tannic acid impairs feeding and metabolic processes in the larvae (Yuan *et al.*, 2020) [77].

4. Mechanisms of Enzyme Inhibition

4.1 Competitive Inhibition

Competitive inhibition arises when the inhibitor structurally resembles the substrate and competes for binding at the enzyme active site. Since both molecules cannot occupy the active site simultaneously, the inhibitor prevents substrate binding by forming an enzyme-inhibitor (EI) complex. Significantly, the inhibitor does not "displace" the substrate but shifts the binding equilibrium depending on relative concentrations and binding affinities.

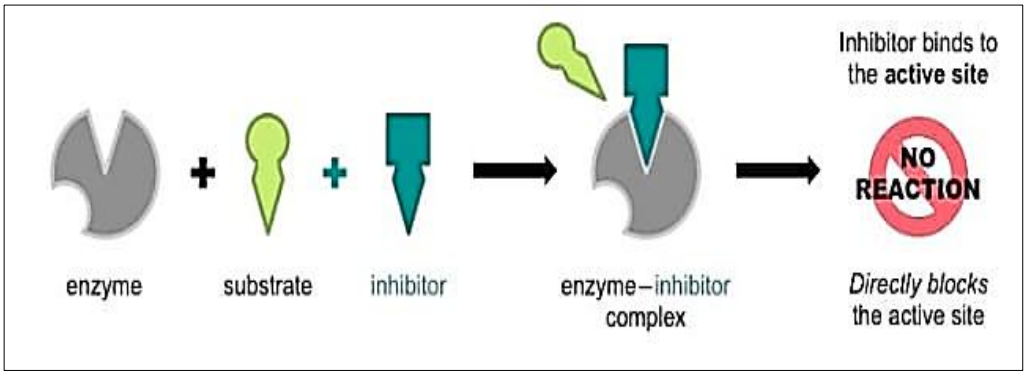

Fig 1: Mechanism of Competitive

Under competitive inhibition, the inhibitor does not change the maximum velocity of the reaction (Vmax) because high substrate concentrations can overcome its effect. However, the apparent Km (substrate concentration required to reach half of Vmax) increases, indicating a reduced apparent affinity of the enzyme for the substrate. Graphically, this results in a rightward shift of the Michaelis-Menten curve without altering the plateau corresponding to Vmax.

4.2 Allosteric Inhibition

Allosteric inhibition is mediated by binding at a regulatory site on the enzyme, distinct from the active site. Typically, a product of a metabolic pathway serves as the allosteric inhibitor, binding to the allosteric site and inducing a conformational change that reduces or blocks substrate binding at the active site. When the product concentration

decreases, the inhibitor detaches, restoring enzymatic activity as the reaction is reversible. This type of regulation enables cells to maintain metabolic balance efficiently.

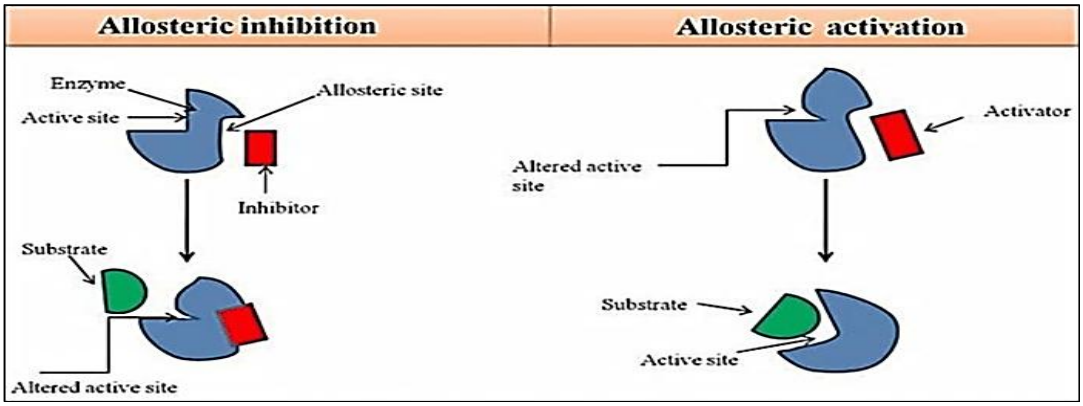

Fig 2: Mechanism of Allosteric Inhibition and Allosteric Activation

4.3 Non-competitive Inhibition

In non-competitive inhibition, the inhibitor binds at a location other than the substrate-binding site. Unlike competitive inhibitors, these molecules share no structural similarity with the substrate. The binding event can occur with the free enzyme (EI complex) or the enzyme-substrate complex (EIS complex).

The inhibitor alters enzyme conformation, diminishing catalytic efficiency without necessarily preventing substrate binding. As a result, the total number of functional enzyme molecules is reduced, usually leaving the apparent substrate affinity (Km) unaffected but decreasing Vmax. Increasing substrate concentration cannot reverse this effect because the inhibitor does not compete with the substrate for the active site. Heavy metal ions such as Ag^{+}, Pb^{2+} and Hg^{2+} are classic examples, as they interact irreversibly with sulfhydryl groups of cysteine residues, altering enzyme activity.

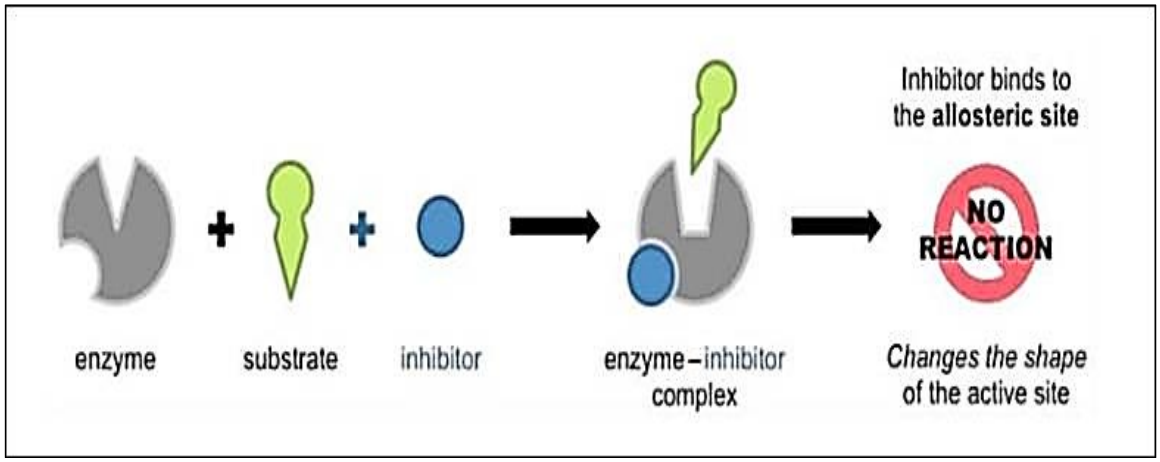

Fig 3: Mechanism of Non-competitive Inhibition

4.4 Covalent versus Non-covalent Inhibition

Enzyme inhibitors can be classified based on the nature of their interaction with the target:

4.4.1 Covalent Inhibition

Covalent inhibitors form a stable, often irreversible bond with specific residues at or near the active site. This mechanism in drug discovery, with ~30% of marketed drugs acting via covalent mechanisms, is exploited. Advantages include high specificity, prolonged duration of action, and the ability to interfere with enzyme catalytic mechanisms directly. Advances in computational chemistry and structural biology have accelerated covalent drug design strategies.

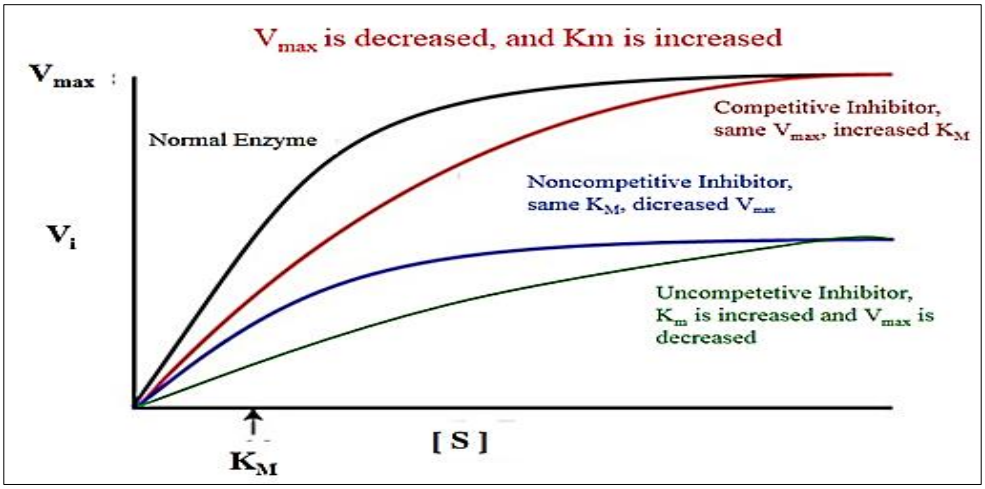

Fig 4: Kinetic Curve of Enzyme Inhibitors

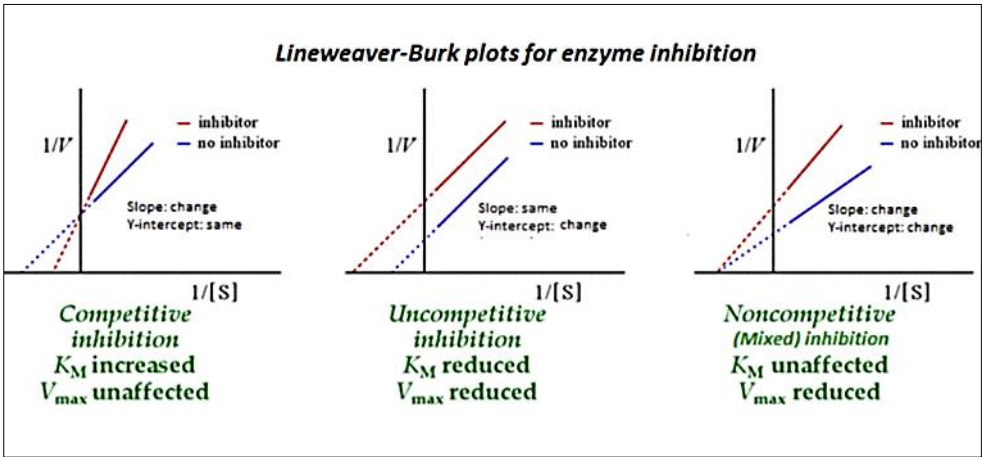

Fig 5: Kinetics of Enzyme Inhibitors

4.4.2 Non-covalent Inhibition:

Non-covalent inhibitors, in contrast, interact reversibly with their targets through hydrogen bonding, hydrophobic contacts, van der Waals forces, electrostatic interactions, or salt bridges. Their reversible nature allows continuous cycles of binding and dissociation, making them tunable in terms of potency and selectivity. Non-covalent inhibition remains the backbone of modern drug development, particularly for structure-guided optimization of substrate analogues.

5. Counter Mechanism by Insects

5.1 Symbiotic Microbes

Insects face constant exposure to plant defense chemicals (phytochemicals) and human-applied agrochemicals such as insecticides. While insects possess detoxification enzymes, symbiotic microbes within their gut and tissues provide an additional, powerful line of defense. Zhao, Lin and Guo (2022) [81] reviewed the diverse roles of these symbionts, emphasizing how they help insects counter chemical challenges and maintain survival across varied environments.

5.1.1 Symbiont-Mediated Detoxification

Many gut-associated bacteria harbour enzymatic systems capable of breaking down toxic compounds directly. These include hydrolases, oxidoreductases and transferases that metabolize phytochemicals like alkaloids and terpenoids, and agrochemicals such as organophosphates, neonicotinoids and pyrethroids. By transforming or degrading these compounds, these symbiotic bacteria help to reduce toxicity before it reaches harmful levels in the insect host.

5.1.2 Indirect Counter-Effects via Host Regulation

Symbionts also influence the host insect's physiology by modulating detoxification pathways. Microbial metabolites or signaling molecules can trigger upregulation of host detox genes, including cytochrome P450s, glutathione-S-transferases and esterases. This indirect regulation enhances the insect's natural capacity to neutralize harmful substances, showing that microbes act both as direct detoxifiers and as regulators of host defenses.

5.1.3Adaptation to Phytochemicals and Pesticides

Insects whose ability to survive on chemically defended plants or withstand pesticide exposure depends on their microbial partners. For instance, specific symbionts enable insects to feed on otherwise toxic host plants, while others degrade synthetic insecticides in the gut. These microbial contributions represent a crucial adaptive advantage, allowing insects to expand their ecological niches and persist under chemical stress.

Symbiotic microbes are central to insect counter-effect strategies against both natural and synthetic toxins. These microbes significantly enhance insect survival and

resistance by combining direct metabolic activity with regulatory influence over host detoxification. This symbiont-mediated protection underscores the ecological success of insects and presents important considerations for pest management strategies (Zhao, Lin and Guo, 2022) [81].

5.2 Detoxification Mechanism

5.2.1 Detoxification Mechanism Mediated by Gut Microbiota in Honeybees

The gut microbiota of *Apis mellifera* plays a crucial role in regulating host detoxification capacity against pesticides. The honeybees with a normal gut microbial community exhibited significantly higher expression of cytochrome P450 mono-oxygenase genes compared to microbiota-depleted or antibiotic-treated bees. Since P450 enzymes are central to phase I detoxification, their enhanced expression resulted in more efficient metabolism of pesticides such as thiacloprid, reducing toxic residue accumulation and improving survival rates.

The mechanism underlying this effect is not direct microbial degradation of pesticides but rather the indirect regulation of host detox genes through microbial signals or metabolites. The gut symbionts amplify endogenous detoxification pathways and strengthen the insect's defense against xenobiotics by influencing host gene transcription. This finding highlights that gut microbiota function as regulators of host detox systems, providing an adaptive advantage in environments with high pesticide exposure (Wu *et al*., 2020) [75].

5.3 Over-Expression OF Digestive Enzymes

5.3.1 Over-expression of Digestive Enzymes in Response to Protease Inhibitors

The study on polyphagous lepidopteran larvae highlights how insects counteract plant defense mechanisms by regulating the expression of digestive protease genes in their gut. When larvae consumed diets containing plant-derived protease inhibitors, there was a marked transcriptional adjustment in digestive enzyme genes, especially those encoding serine proteases such as trypsins and chymotrypsins. Many of these genes showed significant overexpression, enabling the larvae to maintain protein digestion despite the inhibitory pressure. This adaptive response allows insects to produce inhibitor-resistant proteases or generate such high quantities of enzymes that the inhibitors are effectively saturated and less impactful.

Therefore, the regulatory plasticity of digestive enzyme expression represents a vital counter strategy, supporting the survival and growth of polyphagous species on a chemically defended diet. The findings underscore that overexpression of digestive enzymes is not a passive process but an active, inducible mechanism that enhances insect resilience against plant protease inhibitors, contributing to their evolutionary success and broad host range (Zhao *et al.*, 2019) [82].

6. Applications in pest management

6.1 Proteases

Protease inhibitors purified from *Mimosa diplotricha* leaves showed strong activity, reducing bovine trypsin activity by ~80% and inhibiting *Chrysomya megacephala* gut proteases (IC_{50} = 28 µg/mL). It highlights its potential as a plant-derived biopesticide for pest management (Alias *et al*., 2022) [2]. Winged bean protease inhibitor (WBPI) significantly disrupted the development of *Bactrocera cucurbitae*, delaying larval, pupal, and total growth periods. It reduced pupation and adult emergence while strongly suppressing key digestive enzymes. These results suggest WBPI as a promising plant-derived tool for melon fruit fly management (Kaur *et al*., 2019) [38].

Protease inhibitors, including soybean trypsin inhibitor (STI), significantly suppressed midgut protease activities in *Plutella xylostella*, especially trypsin and chymotrypsin. This interference reduced nutrient absorption, delayed larval development, and lowered reproductive potential. The study highlights STIs and related inhibitors as promising tools for diamondback moth management (Zhao *et al*., 2012) [80].

6.2 α-Amylases

α-Amylase inhibitor (MUAI) fro*m Macrotyloma uniflorum* seeds showed strong larvicidal and moderate adulticidal effects against *Aedes aegypti*, especially in early instars. It reduced larval α-amylase activity and acted as an oviposition deterrent, though it lacked pupicidal and ovicidal effects. These findings highlight MUAI as a safe, eco-friendly biocontrol option for mosquito management (Gupta *et al*., 2011) [25].

A potent α-amylase inhibitor (αAI.Mol) from *Moringa oleifera* was purified, showing high thermal and pH stability with strong inhibition of human and insect α-amylases. It significantly reduced survival and pupation rates of *Callosobruchus maculatus* larvae. Additionally, αAI.Mol exhibited notable bactericidal activity against Gram+ and Gram- strains, highlighting its dual insecticidal and antimicrobial potential (Karry *et al*., 2022) [37]. α-Amylases are essential for starch digestion in phytophagous insects, while plants produce α-amylase inhibitors as a defensive strategy. These inhibitors disrupt insect gut α-amylases, showing entomotoxic effects naturally and when expressed in transgenic crops. Field trials confirm their potential for sustainable pest management and future commercialization (Basso *et al*., 2025) [5].

6.3 Lectins

Lectins, especially *Xerocomus chrysenteron* lectin (XCL), show strong insecticidal activity against the aphid *Myzus persicae*, significantly reducing survival, weight, development, and fecundity. XCL was far more toxic than the reference lectin ConA, highlighting its potential as a biocontrol agent. It suggests lectins could be promising alternatives to insecticides for sustainable aphid management.

Moringa oleifera seed preparations (aqueous extract, protein fraction, and lectin WSMoL) showed strong insecticidal and antinutritional effects against the poultry pest *Alphitobius diaperinus*. Treatments reduced survival, impaired digestion, and caused gut damage, with AE and PF being the most lethal. These results highlight *M. oleifera* as a potential eco-friendly tool for integrated pest management (Santos *et al*.,2025) [59]. Pea seed lectin showed strong insecticidal activity against pollen beetle larvae, causing up to 100% mortality in feeding assays. Transgenic *Brassica napus* expressing pea lectin significantly reduced larval weight and survival, with effects correlating to lectin concentration. These findings highlight pea lectin as a promising transgenic resistance factor for Brassica oilseeds (Melander *et al*., 2003) [46].

6.4 Flavonoids

Overusing chemical pesticides has caused resistance, environmental pollution and health risks, highlighting the need for safer alternatives. Flavonoids, plant-derived polyphenols, show promise as biodegradable, low-toxicity insecticides by interfering with pest feeding, growth, and development. This review summarizes their modes of action, structure-activity relationships, and challenges in applying flavonoids for crop protection (Riddick *et al*.,2024) [55]. Flavonoids act as natural defenses in plants, affecting feeding, oviposition and survival of various insect pests while being harmless to beneficials like honeybees. They can function as deterrents, stimulants, or less-toxic insecticides, offering targeted pest management.

Flavonoid-based insecticides present an environmentally friendly alternative to broad-spectrum chemicals, though field validation is needed (Pereira *et al*., 2024) [52]. Additionally, insect-resistant corn and sorghum lines are enriched with flavonoids to create biopesticides. Preliminary results indicate that flavonoids are effective against pests and pathogens, supporting sustainable and low-risk crop protection. This interdisciplinary project trains students in plant chemistry, pest resistance genetics, and eco-friendly integrated pest management (IPM) strategies (Pennsylvania State University, 2024) [50].

6.5 Tannins

Tannins and other phenolic compounds in plants can reduce insect performance by causing oxidative stress in the gut rather than directly damaging protein quality. Gypsy moth larvae showed similar susceptibility to tannin toxicity in both spring and mature leaves, with protein utilization efficiency declining from spring to summer. This research highlights oxidative stress as a key mechanism of plant defense against leaf-feeding insects and informs strategies to enhance crop resistance (University of Michigan, 2007-2011) [68].

Plant tannins are polyphenolic compounds with antimicrobial, anti-parasitic, antioxidant, and immunomodulatory properties, making them promising alternatives to in-feed antibiotics. In ruminants, tannins reduce protein degradation, control parasites, improve gut health and production efficiency. When applied correctly in monogastric animals and poultry, tannins enhance intestinal microbial balance and productivity, though efficacy varies with application and compound type (Huang *et al.,* 2017) [27].

Drifted brown seaweeds (DBSW), rich in tannins, were evaluated for insecticidal activity against the cotton leafhopper *Amrasca devastans*. *Sargassum wightii* and *Stoechospermum polypodioides* extracts showed high hydrolyzable tannins, phlorotannins and total tannins with potent oral and contact toxicity. Tannin fractions significantly reduced digestive enzymes (amylase, protease, invertase) and detoxification enzyme activities in treated insects. Treatment also lowered total body protein and altered protein profiles, as shown by SDS-PAGE analysis. These findings suggest brown macroalgae tannins can be a safe and effective alternative for managing cotton leafhopper populations (Petchidurai *et al*., 2023) [53].

7. Emerging technologies to adapt:

7.1 Protease Inhibitors (PI) in Transgenic Crop Improvement:

PI genes can improve resistance by using them specifically against insect pests in transgenic plants. A single defective gene expressed under wound-inducible or constitutive promoters of the host plant achieves this (Boulter 1993, Akbar *et al.,* 2018) [27, 1]. Cowpea trypsin inhibitor (CpTi) conferred resistance to tobacco hornworm is the first to be successful (Hilder *et al.,* 1987) [27]. Many PIs are small proteins with single inhibitory domains; some, like potato multicystatin, have multiple domains, eight tandem cystatin domains (Walsh and Strictland 1993) [27]. Expression of bean α-amylase inhibitor I in transgenic peas (*Pisum sativum*) provided complete protection against pea weevil under field conditions (Roger *et al.,* 2000) [56]. Their primary mode of action is to inhibit insect gut proteases, leading to nutrient deficiency, delayed development, reduced fecundity and mortality, through complex insect responses, including more production of proteases or compensatory feeding. However, PIs form part of a plant's multi-mechanistic defence, but their practical application in crop protection requires a deeper understanding of insect counter-defence strategies and novel inhibitor discovery.

7.2 Structural biology enzyme inhibitor interaction models:

Structural biology uses experimental techniques such as X-ray crystallography, NMR spectroscopy and Cryo-Electron Microscopy (cryo-EM) to determine the 3D structures of enzymes and inhibitors. At the same time, computational methods like molecular modelling, molecular docking and Molecular Dynamics (MD) simulations collectively provide insights into enzyme-inhibitor interactions, predicting binding modes, key residues, affinities and the stability and flexibility of complexes over time.

The crystal structure of amaranth α-amylase inhibitor (AAI) with insect (yellow meal worm) α-amylase reveals a unique knottin-like inhibition mode, offering a specific strategy for crop protection against insect pests, 3D structures of the complete amino acid sequence at 1.64 Å (Pereira *et al.,* 1999) [51]. Traditionally, researchers used X-ray crystallography; advances in cryo-EM allow them to determine high-resolution structures of flexible or heterogeneous oligomeric enzyme complexes without icosahedral symmetry. Cryo-Electron Microscopy (cryo-EM) instrumentation (especially direct electron detectors), software and image processing enabled near-atomic resolution (better than ~3-4 Å) for large macromolecular complexes (Vonck, 2017) [69]. Resolving active sites and inhibitor binding can guide the design of plant allelochemicals, protease, and amylase inhibitors against insect digestive enzymes.

7.3 Nano encapsulation of plant enzyme inhibitors

Nanoparticles enhance the efficacy of plant-derived allelochemicals, which act as digestive enzyme inhibitors. (Kannan *et al.,* 2024) [36]. Their nanoscale size (<100 nm) permits efficient penetration through insect cuticles and delivery into midgut tissues where digestive enzymes are active (Shahzad and Manzoor, 2021) [60]. By encapsulating protease, amylase, or lipase inhibitors from plants, nanoparticles improve stability, bioavailability and targeted release of these allelochemicals, ensuring prolonged interaction with gut enzymes.

Metal-based nanoparticles (e.g., AgNPs, ZnO NPs) not only interfere with cellular functions but also inhibit major key

enzymes such as acetylcholinesterase and digestive hydrolases, intensifying metabolic dysfunction. Similarly, silica nanoparticles exert their inhibitory effects physically by disrupting the gut lining, enhancing permeability and thus enhancing the inhibitory effect of encapsulated allelochemicals on digestive enzymes (Choudhary *et al*., 2022) [10]. Smart nano delivery systems (stimuli-responsive or controlled-release formulations) ensure that plant enzyme inhibitors are released precisely in the insect gut, maintaining activity over extended periods while reducing off-target effects.

Essential Oil-based Polymeric Nanoparticles (EOPNs) loaded with allelochemicals like terpenoids and phenolics inhibit midgut enzyme activity more effectively than free compounds due to higher encapsulation efficiency and slow release against pests such as *Plodia interpunctella* (Jesser *et al*., 2020) [32].

Challenges

Digestive enzyme inhibition by plant allelochemicals faces several challenges that limit its large-scale use. Insects can quickly adapt by producing alternative enzymes or changing gut conditions, reducing the effectiveness of inhibitors (Gatehouse, 2002) [21]. Some allelochemicals may also harm beneficial insects like pollinators and natural enemies, disturbing the ecological balance. High levels of crop inhibitors can lower protein digestibility and reduce the nutritional value of food and feed. Another problem is that many inhibitors are unstable in the field and lose activity under sunlight, heat, or inside the insect gut. Biosafety concerns, strict regulations and low public acceptance of genetically modified crops also slow their use. In addition, different insect groups respond differently-an inhibitor that works on caterpillars may not affect beetles or sap-sucking pests (Jongsma, 1997) [35]. Finally, relying heavily on a single inhibitor could lead to resistance, like chemical pesticides, making combining them with other pest management strategies necessary.

Conclusion

Plant allelochemicals that target insect digestive enzymes represent a powerful natural defense mechanism and a promising foundation for eco-friendly pest management. By inhibiting key enzymes such as proteases, amylases, and lipases, these compounds disrupt nutrient assimilation, impair growth and reduce pest fitness. However, the evolutionary adaptability of insects, through enzyme overproduction, isoform variation and microbial symbiosis, poses significant challenges to their long-term effectiveness. Modern biotechnological advances, including transgenic approaches, RNA interference, molecular modelling and nano-encapsulation, provide new opportunities to enhance these inhibitors stability, delivery and specificity. Integrating such strategies within sustainable pest management programs can reduce reliance on chemical pesticides, minimize ecological risks and contribute to global food security. Future research should focus on unraveling the molecular basis of insect adaptation, optimizing inhibitor formulations and evaluating their impacts on non-target organisms to realize their potential in integrated pest management fully.

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Authors Details
K Sowmya
Research Scholar Department of Entomology, College of Agriculture, Rajendranagar, PJTAU, Hyderabad, Telangana, India

S Kousalya
Research Scholar Department of Entomology, College of Agriculture, Rajendranagar, PJTAU, Hyderabad, Telangana, India

M Apoorva
Research Scholar Department of Entomology, College of Agriculture, Rajendranagar, PJTAU, Hyderabad, Telangana, India

M Mamatha
Research Scholar Department of Entomology, College of Agriculture, Rajendranagar, PJTAU, Hyderabad, Telangana, India

J Aruna Kumari
Principal Scientist Department of Biochemistry, AICRP on Forage Crops, Agricultural Research Institute, Rajendranagar, Hyderabad, Telangana, India

B Uma
Research Scholar Department of Entomology, College of Agriculture, Rajendranagar, PJTAU, Hyderabad, Telangana, India

KVN Sreeja
Research Scholar Department of Entomology, College of Agriculture, Rajendranagar, PJTAU, Hyderabad, Telangana, India