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Insect cell line: A paradigm shift in insect toxicology

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Abstract
Advances in molecular and cell biology have enabled more detailed investigations of insect physiology at the cellular level. During recent decades, research on insect cell culture has expanded significantly, leading to the development of thousands of cell lines from various insect species and tissues. These cell lines have become valuable tools in insect science, especially for pest management, where they help to evaluate insecticide activity and investigate toxicological mechanisms. Advances in technology have further improved the use of insect cell lines, making insecticide research more efficient and cost-effective compared to traditional whole-insect studies. Although these models provide deeper insights into insecticide mechanisms, challenges remain particularly in translating *in-vitro* results to *in-vivo* effectiveness. Overall, recent progress highlights the potential of insect cell line-based models to enhance development of newer molecules of insecticides to develop effective pest management strategies.

Keywords: Insect cell lines, Sf9 cells, *in-vitro* bioassay, pesticide screening, high-throughput screening

Introduction

Rising global population demands higher food production, in spite of availability of total land for agriculture is decreasing at the same time insect pests cause up to 45% annual crop losses (Battu *et al*., 2023) [3]. Insecticides are highly effective, preventing 21-94% of potential yield loss caused by insects (He *et al*., 2023) [13], making the development of new compounds continually essential. However, discovering and validating new insecticides is increasingly complex and expensive, largely due to time-consuming *in-vivo* testing. Therefore, developing alternative models to supplement or replace traditional insect and mammalian systems offers a practical way to reduce costs and improve research efficiency.

The cost of discovering and developing a new crop-protection active ingredient has risen steadily, increasing from $152 million in 1995 to $184 million in 2000, $256 million in 2005-08, $286 million in 2010-14 and $301 million in 2014-19. These data indicate an average cost increase of about 5.7% every five years. Conversely, the cost of chemistry-related development has gradually declined from 2005-08 through 2014-19, largely due to technological advances such as structural activity relationship (SAR) modeling and molecular docking approaches. In recent years, overall toxicology costs particularly mammalian toxicology have also decreased, showing a 7.3% reduction per five-year interval (Anonymous, 2024) [1].

Advances in molecular and cell biology have enabled more detailed investigations of insect physiology at the cellular level. Over recent decades, extensive efforts have led to the development of thousands of insect cell lines derived from diverse species and tissue types (Table 1). Beyond their established use in protein expression, these cell lines have become valuable tools in pest management research, particularly for screening candidate compounds and studying insecticide mechanisms *in-vitro*. Insect cell-based models offer several advantages, including low cost, ease of culture, high reproducibility, suitability for high-throughput screening and the absence of major ethical concerns. As complementary systems to traditional *in-vivo* toxicological models, they provide a broader and more detailed understanding of insecticide toxicology.

In simple words, the insect cell lines are cultured cells derived from insects. The isolation of cells from insect tissues and their subsequent and successful growth in artificial culture situation is referred as cell culture. Insect cell lines can be derived from a variety of insect tissues, including larval midguts, embryonic tissues, adult ovaries and larval ventral nerve cords (Reid *et al*., 2016) [28].

Table 1: Timeline for insect cell line development

Sr. No.	Name of Scientist and Year	Contribution
1.	Hooke, 1665	Discovery of cells, which marked a major turning point in biological research
2.	Harrison, 1906 [11]	Pioneered modern cell culture by successfully growing frog embryonic cells in its own lymph which later became famous as the "hanging drop technique."
3.	Goldschmidt, 1915 [8]	First documented *in-vitro* culture of insect cells using follicle cells from male pupae of *Hyalophora cecropia* in own haemolymph
4.	Grace, 1962 [9]	Established the first long-term insect cell lines from ovarian tissues of *Bombyx mori* and *Antheraea eucalypti*
5.	Grace, 1966 [10]	Developed cell lines from larval tissues of *Aedes aegypti*; Grace culture medium remains widely used today in insect cell culture
6.	Vaughn *et al*., 1977 [36]	Developed *Spodoptera frugiperda* cell lines as primary models for *Autographa californica* nucleopolyhedrovirus (AcMNPV) replication
7.	Grandos *et al*., 1994	"Hi-Five" cell line from *Trichoplusia ni* embryos noted for strong recombinant protein production

8.	Nagata *et al*., 1997 [26]	First successful multiplication of a tospovirus in thrips cell culture
9.	Ma *et al*., 2014	Established the first continuous planthopper cell line for studying rice stripe virus-insect interactions
10.	Watanabe *et al*., 2020 [37]	First report of establishment of cell lines derived from *Cydia kurokoi* (NARO-Cyku), *Cephonodes hylas* (NARO-Cehy), *Haritalodes basipunctalis* (NARO-Haba) and *Theretra oldenlandiae* (NARO-Thol) to screen and validate chemical reagents involved in pesticide development.

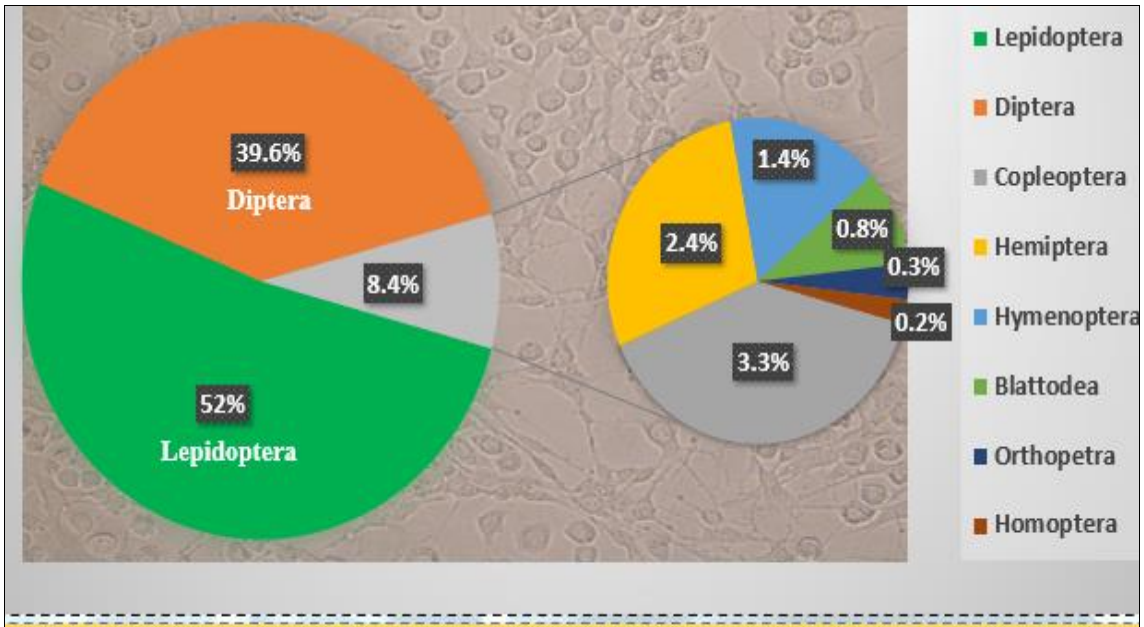
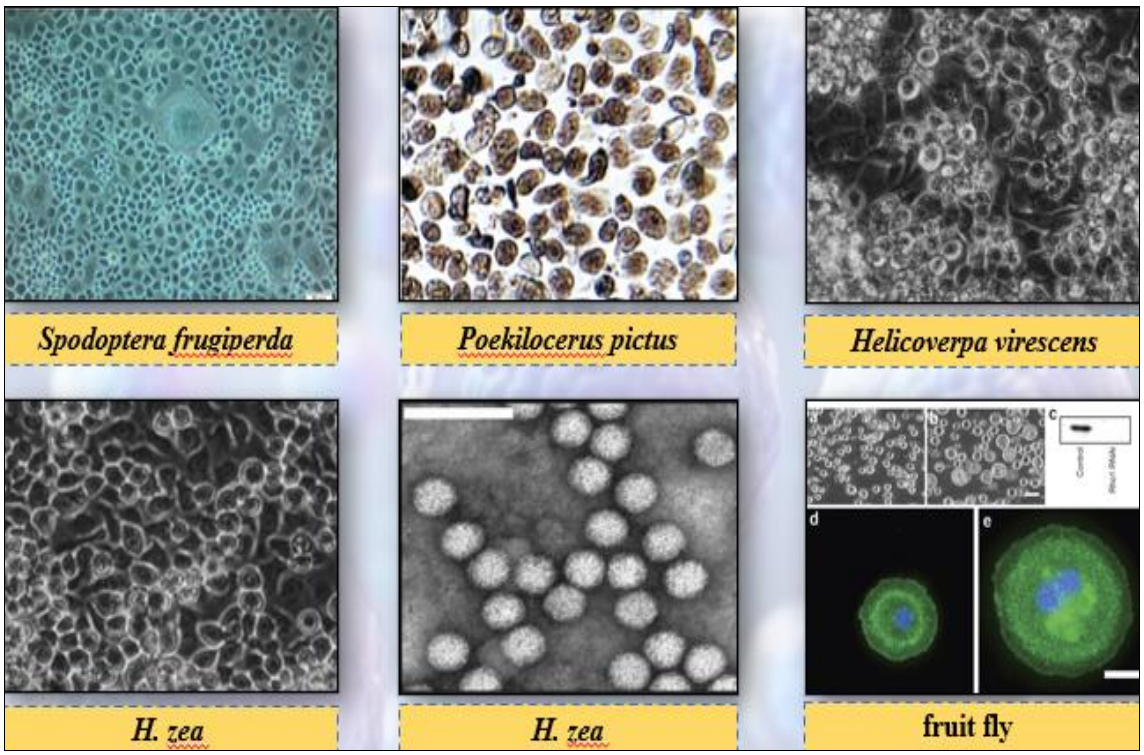


Fig. 1: The proportion of different orders contributing to established insect cell lines

In terms of tissue origin, the majority of insect cell lines have been derived from embryos, larvae and ovaries. Particularly, the undifferentiated embryonic tissues are preferred for primary culture because they yield higher success rate in establishing continuous cell lines. Statistical data indicated that nearly 50% of insect cell lines originated from embryonic tissues, followed by larval tissues (23%) and ovarian tissues (15.4%) (He *et al*., 2023). In recent years, cell lines derived from more specialized tissues-such as the central nervous system and midgut-have also been developed. These tissues are especially valuable because they play key roles in pesticide target interaction, absorption and metabolism, making them important tools for insecticide research and discovery.

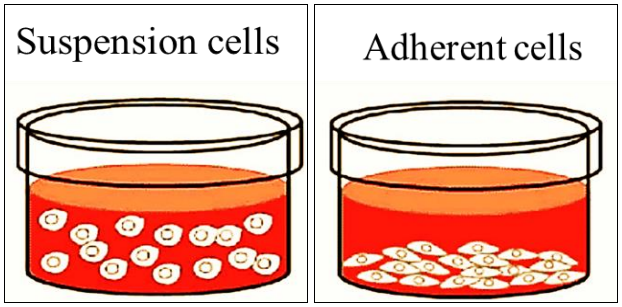


Cell lines of different insects

Table 2: List of significant insect cell lines and their applications

Sr. No.	Name of insect cell line	Source	Applications	Reference
1.	FPMI-CF-1	*Choristoneura fumiferana* midguts	Expression of recombinant proteins using baculovirus vectors	Hink *et al.* (1991) [16]
2.	BTI-EAA	*Estigmene acrea* larval hemocytes	Recombinant protein production	Hink *et al.* (1991) [16]
3.	IBL-SLO-1A	*Spodoptera litura* pupal ovaries	Replication of *S. litura* nuclear polyhedrosis virus *in-vitro*	Shih *et al.* (1997) [33]
4.	LPC-Aa98-19	*Anacridium aegyptium* embryonic cells	Virus multiplication and manipulation	Hernandez-Crespo *et al.* (2000) [14]
5.	NIV-HA-197	*Helicoverpa armigera* embryonic tissue	Mass production of baculovirus as a bioinsecticide	Sudeep *et al.* (2002) [34]
6.	Sf21 cells	*Spodoptera frugiperda* pupal ovarian tissue	Research on baculoviruses and their use for producing recombinant proteins	Chen *et al.* (2005) [6]
7.	RAE25	*Rhipicephalus appendiculatus* embryonic cells	Defining the complex nature of the host vector and pathogen relationship	Bell-Sakyi *et al.* (2007) [5]
8.	High Five cells	*Trichoplusia ni* cabbage looper ovarian cells	Recombinant protein expression using baculovirus or transfection	Zhang *et al.* (2008) [43]
9.	TI-1	*Thysanoplusia intermixta* larval and pupal tissue	Insect pathogenic viruses and baculovirus expression vector system	Hashiyama *et al.* (2011) [12]
10.	Sf9 cells	*Spodoptera frugiperda* pupal ovarian tissue	Recombinant protein production using baculovirus and in evaluation of host-virus interaction	Ma *et al.* (2014)
11.	TN-5B1-4	*Trichoplusia ni* larval tissue	Research on pesticide mechanisms	Nuringtyas *et al.* (2014) [27]
12.	Spex cell line	*Spodoptera exigua* larval tissue	Research on pesticide mechanisms	
13.	KLBIQ-Chsu-I	*Chilo suppressalis* larval fat bodies	Production of insect virus expression vector	Liu *et al.* (2015) [22]
14.	AW1	*H. zea* larval ventral nerve cord	Screening & revealing the mechanisms of neuroactive insecticides	Ren *et al.* (2019) [30]

Types of insect cell line



There are mainly two types of insect cell cultures developed *viz.*, Monolayer cultures and Suspension cultures.

1. **Monolayer cultures:** Monolayer or adherent cell cultures are cell lines that are developed while attached to a substrate only.

2. **Suspension cultures:** Suspension culture is an anchorage-independent culture where multiplication of small aggregates of cells or single cells takes place that suspended in a liquid medium.

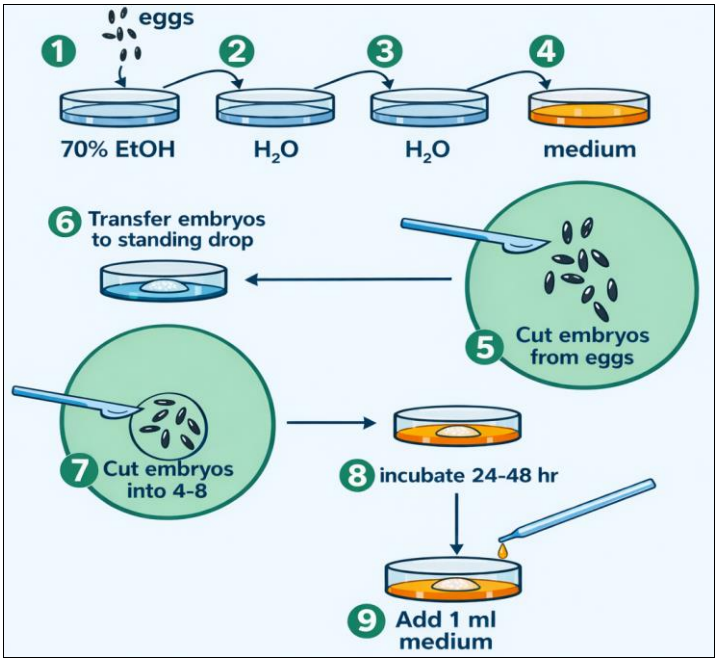


Fig 2: Stepwise procedure for establishment and maintenance of insect cell lines

Establishment and maintenance of insect cell lines

Culture media used for maintaining insect cell lines consist of complex formulations containing amino acids (including L-glutamine), inorganic salts, vitamins, growth factors, carbohydrates such as glucose or galactose, metabolic precursors, trace elements and hormones.

The pH of the medium is regulated using buffering systems, most commonly CO_2/sodium bicarbonate, phosphate buffers, or HEPES [N-(2-hydroxyethyl)-piperazine-N'-(2-ethanesulfonic acid)]. The stepwise procedure for establishment of insect cell lines has been given in Fig. 2.

Insect cell lines are typically maintained at 26-28°C and newly established lines are characterized through assessments of morphology, growth kinetics, species specificity and karyological profiles.

Different types of insect cell culture media

Insect cell culture is now widely utilized in commercial applications due to its efficiency and has become an essential tool in candidate compound screening and modern biotechnology. Consequently, there is a growing need for optimized culture media that can robustly support insect cell growth. The primary types of insect cell culture media are listed below.

(1) Conventional media			
Sr. No.	**Media**	**Formulation**	**Reference**
1.	Grace medium	Medium consisted of 21 amino acids, 4 organic acids (Krebs cycle intermediates), 10 vitamins, 2 antibiotics, 6 salts, 3 sugars and insect plasm	Grace (1962) [9]
2.	MM insect culture medium	Medium consisted of only 6 salts, yeastolate, fetal bovine serum (FBS), lactalbumin hydrolysate, glucose and antibiotics	Mitsuhashi (1964) [25]
3.	Hink's TNM-MH medium	Medium consisted of lactalbumin, yeastolate as source of vitamins (B-complex) and hemolymph with heat-inactivated fetal bovine serum	Hink (1970) [15]
4.	BML-TC/10	Medium consisted of glucose (only hexose sugar), fetal bovine serum and tryptose extract	Schlaeger (1996) [32]

(2) Serum media			
Sr. No.	**Media**	**Formulation**	**Reference**
1.	ISFM medium	Prepared based on IPL-41, ultrafiltered yeastolate (4g) and a complex lipid emulsion were added	Inlow *et al.* (1989) [19]
2.	Ex-Cell-400 medium	It's a semi-defined medium with a protein concentration of 15 mg/ml or less	Belisle *et al*. (1992) [4]
3.	Sf 900 medium	It's a medium with low protein content	Weiss *et al*. (1992) [39]
4.	ExCeU 401 medium	It's a protein-free medium that allows for higher cell density and higher yields of expressed recombinant proteins	Schlaeger (1996) [32]

(3) Serum-free media			
Sr. No.	**Medium**	**Insect cell lines**	**Manufacturer**
1.	BIOINSECT-1	Sf9, Tn5	Biological Industries
2.	EX-CELL™ 420	Sf9, Sf21	Sigma-Aldrich
3.	EX-CELL™ 405	Tn5	Sigma-Aldrich
4.	MED-10002	Sf9, Sf21, Tn368, Tn5	GENTAUR
5.	SFICM (Sapphire™)	Sf9, Sf21, Tn5	Allele Biotech

Applications in insect toxicology

Insect cell lines provide powerful *in-vitro* platforms for insect toxicology studies, enabling detailed analysis of cellular metabolic processes, signal transduction pathways and stress responses. These systems facilitate high-throughput screening of insecticidal targets, elucidation of toxicant modes of action and investigation of virus transmission mechanisms in insect vectors. Their reliability and experimental precision significantly enhance the efficiency and accuracy of insecticide evaluation.

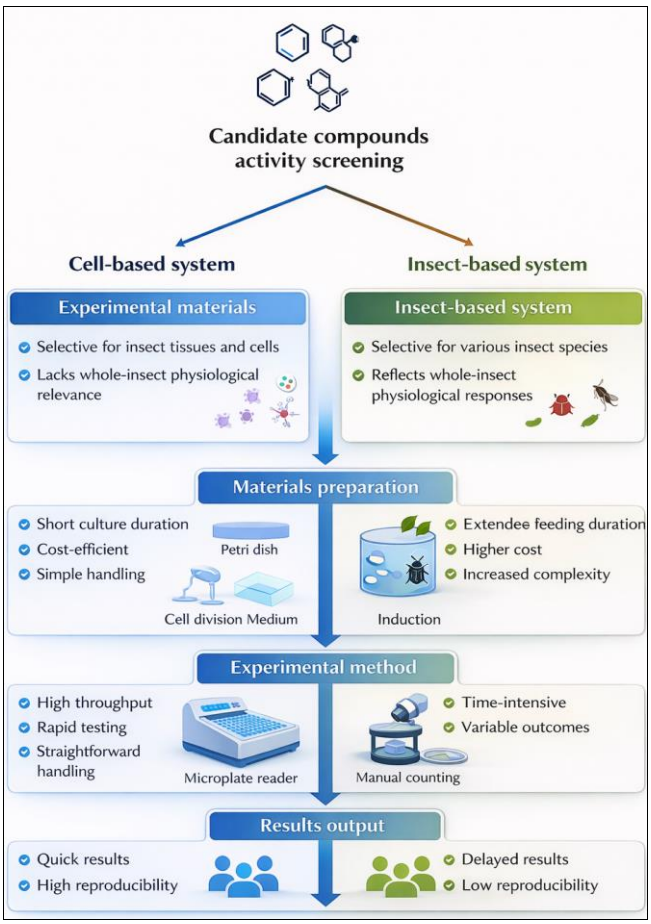
1. Cytotoxicity mechanisms

Drosophila melanogaster is widely used as a model organism in genetic research and its Schneider S2 cell line has become an important tool in pest management studies. The impact of azadirachtin on the viability of S2 cells was investigated and found the compound induced cytotoxicity in a dose- and time-dependent manner. Azadirachtin exerts toxic effects on *Drosophila* cells and the associated increase in intracellular Ca^{2+} levels may serve as a key early event leading to apoptosis in S2 cell lines (Xu *et al*. 2016) [40].

Sf9 cells derived from *S. frugiperda* are widely used to investigate cytotoxicity and the signaling pathways involved in programmed cell death. Key regulators of autophagy *viz*, Beclin-1, LC3 and p62 play central roles in this process. Yang *et al*. (2017) [41] demonstrated that spinosad exposure induces autophagy in Sf9 cells in a time-dependent manner. This autophagic response was evidenced by upregulation of Beclin-1, enhanced conversion of LC3 to its lipidated autophagosome-associated form (LC3-II) and increased degradation of p62, collectively indicating activation of the autophagic flux.

Several insect cell lines, including TN-5B1-4 from *Trichoplusia ni*, AW1 from *Heliothis zea* and Spex from *Spodoptera exigua* are commonly used to investigate the toxicological mechanisms of insecticides (Huang *et al*., 2010, Ren *et al*., 2021, Nuringtyas *et al*., 2014 [18, 27, 31],). The AW1 cell line derived from the ventral nerve cord of *H. zea* larvae was particularly valuable for studying neuroactive insecticides and elucidating their modes of action (Ren *et al*., 2019) [30]. As research advances, it is anticipated that additional cell lines from specialized insect tissues will be developed to support the study of specific toxicological pathways.

2. High-Throughput Screening (HTS)



Comparison of insect cell line based *in-vitro* & insects *in-vivo* systems for insecticide toxicity tests

Efforts to enhance screening efficiency have led to growing interest in developing in-*vitro* methods that can serve as alternatives to conventional whole-insect bioassays. While assays using live insects provide dependable results, they require large numbers of physiologically similar individuals and prolonged observation periods, making them both time-consuming and labour-intensive. Consequently, insect cell line-based assays, especially when combined with high-throughput screening (HTS) platforms, have gained prominence in insecticide discovery. Among these systems, the integration of insect cell lines with the thiazolyl blue tetrazolium bromide (MTT) assay remains the most widely employed approach for evaluating cell viability and proliferation.

Advantages of HTS

- In terms of testing speed, *in-vitro* assays offer a significant advantage because insect cells can generate new populations within a few days, whereas rearing or hatching insects requires substantially more time. This results in a shorter overall testing cycle.
- Rearing insects also demands greater manpower and resources compared with cell culture. Although establishing cell-based systems involves high initial costs, they ultimately provide better long-term economic efficiency.
- *In-vitro* assays generally offer higher accuracy, sensitivity and better-controlled experimental conditions. Emerging technologies such as infrared beam-break systems and automated video analysis are beginning to replace manual observations used in insect bioassays, particularly for species that exhibit immobility or defensive "feigning death" behaviours. In contrast, most cell-based assays rely on standardized colorimetric methods that can be applied uniformly across cell types (He *et al*., 2023).

Cell-based models are typically used for preliminary high-throughput screening, after which promising hits are validated using whole-insect assays. This HTS approach is now widely applied for screening and purifying natural and synthetic compounds. For example, the AW1 cell line of *H. zea* and the SL2 cell line of *S. litura* have been used to identify lead compounds among synthetic pyrazole carboxamides and furanone analogs, respectively (Ren *et al*., 2018) [29].

3. Cell death modalities and signaling pathways

Insect cell-based assays not only facilitate rapid and precise screening of candidate insecticides but also provide effective platforms for elucidating their cellular mechanisms of action. These systems help to characterize major cell-death pathways *viz*, necrosis, apoptosis and autophagy which are differentially activated depending on the specific stimuli and signalling processes involved.

Necrosis typically occurs in cells subjected to physical or chemical injury and represents an uncontrolled, accidental form of cell death characterized by specific cellular and molecular alterations. Studies have shown that rotenone exposure to *S. litura* SL1 and *S. frugiperda* Sf9 cell lines induces marked time- and dose-dependent cytotoxicity. This inhibition of cell viability and proliferation was associated with hallmark necrotic features, including plasma membrane disruption, organelle breakdown and irregular DNA fragmentation (Sun *et al*., 2021) [35].

Apoptosis is a fundamental programmed cell death (PCD) pathway conserved across both mammals and insects, initiated by diverse stimuli and regulated through complex gene networks. Natural product insecticides are well-studied inducers of apoptotic responses in insect cell lines. For example, spinosad triggers mitochondrial dysfunction in Sf9 cells by elevating reactive oxygen species (ROS), promoting mitochondrial permeability transition pore (mPTP) opening and causing mitochondrial membrane potential (MMP) loss, ultimately leading to cytochrome c release and apoptosis. Similarly, azadirachtin induces pronounced apoptotic activity in Sf9 and SL-1 cells, characterized by early-stage apoptotic features (Zhang *et al*., 2017) [41].

Autophagy is essential for various developmental and physiological functions, including cell death, survival, innate immunity and metabolism. In holometabolous insects such as silkworms, honeybees, lepidopteran larvae and fruit flies, autophagy often accompanies apoptosis to remove obsolete tissues during metamorphosis. Studies by Li *et al*. (2021) and Yang *et al*. (2017) [41] demonstrated that both avermectin and spinosad trigger autophagy in Sf9 cells *via* the AMPK/mTOR signaling pathway. Specifically, spinosad increases ROS production and disrupts mitochondrial membrane potential, leading to mitochondrial dysfunction, cytochrome c release and subsequent autophagy in *S. frugiperda* Sf9 cells.

4. Ion channels & mode of action (MoA)

Most widely used insecticides whether natural or synthetic exert their effects by targeting ion channels in the insect nervous system. These ion channels are membrane proteins that enable ions to move across the membrane along their electrochemical gradients. Key neural targets include voltage-gated sodium and potassium channels, ryanodine (calcium) receptors, chloride channels such as GABA and GluCl receptors and nicotinic acetylcholine receptors. Research using insect cell systems, combined with voltage-clamp and patch-clamp electrophysiology, has greatly advanced our understanding of how these neuroactive insecticides function.

The whole-cell patch-clamp analysis of neurons isolated from the thoracic ganglia of the American cockroach (*Periplaneta americana*) was used to evaluate the effects of indoxacarb and its metabolite DCJW on voltage-gated Na^+ channels. The results demonstrated that the two compounds differentially inhibited type-I and type-II Na^+ currents due to their distinct voltage-dependent effects on channel inactivation. Notably, DCJW produced an essentially irreversible blockage of Na^+ currents, which likely underlies its high insecticidal potency (Zhao *et al*. 2005) [44].

Liu *et al*. (2016) [23] examined central neurons of *Helicoverpa armigera* to identify insecticidal compounds targeting ryanodine receptors (RyRs), with particular emphasis on novel anthranilic diamides, including chlorantraniliprole and its structural analogs. Calcium-imaging assays showed that compound 12i increased intracellular Ca^{2+} levels in a concentration-dependent manner. Furthermore, selective activation of RyRs completely blocked the Ca^{2+} release induced by both chlorantraniliprole and 12i, indicating that RyRs in the central neurons of *H. armigera* larvae are likely the primary targets of these compounds.

5. Insect vector-virus relationship

Insect cell culture has become an important system for examining the complex interactions between plant viruses and their insect vectors. *In-vitro* cultures of key insect vector species such as leafhoppers, planthoppers, aphids, thrips and whiteflies have been effectively used to investigate viral protein functions and the mechanisms of receptor-mediated endocytosis in vector cells (Ghosh *et al*., 2020) [7].

Insect vector cell cultures have provided valuable insights into the intracellular processes underlying plant virus infection. In insect cell lines inoculated with Rice Gall Dwarf Virus (RGDV), virus-associated microtubules form within viroplasms and interact with viral proteins Pns7, Pns12 and the core protein P5, facilitating both intracellular transport of the virus and its release from infected cells (Wei *et al*., 2009). Similarly, Jia *et al*. (2012) [20, 38] demonstrated that primary cultured cells from the white-backed planthopper infected with Southern rice black-streaked dwarf virus (SRBSDV) developed distinct viroplasms containing the nonstructural protein P9-1, viral RNA and viral particles components, essential for viroplasm formation and successful viral replication.

Challenges and limitations

Despite advancements, several challenges hinder the full potential of insect cell lines in insecticide research. The establishment of stable and selective cell lines is critical, as many primary cells exhibit limited survival and may become unsuitable for long term studies. *In-vitro* insecticidal assays frequently produce results that do not correlate with *in-vivo* responses due to the absence of whole-organism physiological interactions. Furthermore, commonly used cytotoxicity assays such as MTT rely solely on metabolic activity, which can lead to inaccurate assessments, particularly for cell types with inherently low metabolic rates, thereby requiring higher cell numbers for reliable evaluation.

Conclusion

Insect cell lines are crucial in evaluating the activity of candidate insecticides to discover their toxicity. These cell-based studies increase efficiency and reduce the cost involved in insecticide screening, providing a global and in-depth perspective to reveal and verify the mode of action of insecticides. Insect cell lines are valuable tools in toxicology as they offer ethical, practical and scientific advantages. Applications of insect cell lines in High-Throughput Screening for revealing mode of action, defining cell death modalities and their relevant signaling pathways, cell lines of insect vector for examining insect-virus interactions are useful in developing innovative management methods. Cell lines of insect vector for examining insect-virus interactions are useful in developing innovative management methods. Insect cell line-based models promote the quick screening and thereby promote the development of novel insecticides for benefit of the farming community.

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