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Insect Cell Lines: A Paradigm Shift in Insect Toxicology

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The continuous growth of the global population has increased the demand for improving both the quantity and quality of food production. Insecticides have proven to be one of the most effective tools for controlling pests and protecting crops. Studies indicate that insecticides can prevent crop losses ranging from 21% to 94% in a given year (Scott and Buchon, 2019). Therefore, the development of new insecticides has been an essential and ongoing requirement for effective pest management.

However, discovering and commercializing new crop protection compounds is a complex, time-consuming, and expensive process. A major challenge in this process is the evaluation of insecticidal activity and understanding the mode of action of potential compounds. Traditional testing methods, which rely on whole insects or mammalian models, are often costly, labor intensive and time intensive. As a result, there is an increasing need to develop alternative models that can complement or replace conventional approaches, reducing both cost and effort while improving efficiency (He *et al.*, 2023)

Advances in modern life sciences, particularly in molecular and cell biology, have enabled researchers to study the effects of insecticides at the cellular level. Over the past few decades, numerous insect cell lines have been successfully established from various insect species, tissues, and orders. These cell lines are not only widely used for protein expression but also serve as valuable tools in toxicological research and pesticide development (Battu *et al.*, 2023).

Insect cell lines are increasingly used as screening models for identifying and evaluating new insecticidal compounds. They also play a significant role in studying the mechanisms of action of chemical insecticides under controlled in vitro conditions. Compared to traditional models, insect cell-based systems offer several advantages, including lower cost, ease of maintenance, high reproducibility and suitability for high-throughput screening (Arunkarthick *et al.*, 2017). Additionally, their use minimizes ethical concerns associated with animal testing. As a complementary approach to conventional toxicological methods, insect cell-based models provide a more detailed and comprehensive understanding of insecticide effects.

What is Insect Cell Line?

Cultured cells derived from insects are known as insect cell lines. The isolation of cells from insect tissues and their subsequent and successful growth in artificial culture is referred as cell culture. Insect cell lines can be derived from a variety of insect tissues, including larval midgut, embryonic tissues, adult ovaries and larval ventral nerve cords (Reid *et al.*, 2016).

History of Insect Cell Lines

Science has functioned as a relentless engine for human progress, systematically dismantling biological mysteries to prepare society for the future. This journey into the microscopic world began in earnest with the invention of the microscope, but it was Robert Hooke's 1665 discovery of cells that provided the fundamental blueprint for modern biology. Following this revelation, the scientific community shifted its focus toward isolating these living units and attempting to grow them in vitro outside of a living organism under strictly controlled laboratory conditions (Battu *et al.*, 2023). The late nineteenth and early twentieth centuries marked the birth of cell culture, moving from Wilhelm Roux's 1885 saline buffered chick embryo cultures to Ross Harrison's 1907 "hanging drop technique," which successfully sustained frog embryonic tissues in their own lymph (Jedrzejczak-Silicka, 2017).

The specific discipline of insect cell culture followed a similar path of trial and refinement. The first major milestone occurred in 1915 when Goldschmidt cultured follicle cells from the gonads of *Hyalophora cecropia* pupae, using the hemolymph of the same insect as the primary medium (Goldshmidt and Wilhelm, 1915). By 1935, William Trager advanced the field significantly by successfully culturing baculoviruses within primary ovarian cells of the silkworm, *Bombyx mori* (Trager, 1935). However, these early attempts relied on very simple media, until the mid-twentieth century that the "recipe" for keeping these cells alive became sophisticated. Silver Wyatt expanded the media of Trager, basic 6-ingredient formula to a complex 34-ingredient mixture of salts, sugars, and amino acids (Wyatt, 1956). Shortly after, Thomas Grace perfected this by adding vitamins and balancing the critical ionic ratios of sodium to potassium and calcium to magnesium. Grace's Medium proved so effective at establishing stable cell lines like cell lines from ovarian tissues of *Bombyx mori*, *Antheraea eucalypti* and larval tissues of *Aedes aegypti* (Grace, 1966; Grace 1967).

However, with the development of much advanced cell cultures, the focus has shifted toward using these cells as for creating complex medical products, such as vaccines and recombinant proteins. Since its development, the baculovirus expression system (BEVS) has been extensively used for the production of recombinant proteins in insect cells. This system has found significant application in vaccine manufacturing, beginning with the world's first HIV candidate vaccine to several vaccines currently under development, produced using cultured high five cells derived from *Trichoplusia ni* (Puente Massaguer *et al.*, 2021). More recently, BEVS has also been utilized in the production of vaccines against COVID-19, targeting the SARS-CoV-2 virus and its variants such as Omicron, using cultured Sf9 cells derived from *Spodoptera frugiperda* (van Oosten *et al.*, 2021).

Disadvantages of *In Vivo* (Whole Insect) Toxicology

1. Black box matrics - end-point metrics like LD50 only confirm mortality failing to isolate how or why a toxin works.
2. The rearing bottleneck- maintaining large scale laboratory colonies demands immense space, food and labor, while life cycle studies take weeks.
3. Biological noise- individual insect variations in age, health and genetics create unavoidable statistical variances.

Source of Insect Cell Lines

There are more than one million insect species present on Earth (Arunkarthick *et al.*, 2017), however, according to Expasy-Cellosaurus records, only around 1200 cell lines have been established to date from over 170 species representing eight orders, Blattaria, Coleoptera, Diptera, Hymenoptera, Homoptera, Hemiptera, Lepidoptera, and Orthoptera (Zhang *et al.*, 2007). As depicted in Figure 2, more than half of the available insect cell lines have been derived from Lepidoptera, which constitutes a major group of agricultural pests. Lepidopteran insects alone have contributed over 600 reported cell lines, originating from families such as Noctuidae, Tortricidae, Sphingidae, and 16 additional families. Diptera represents the second largest source of insect cell lines, accounting for approximately 40 percent of the total. In contrast, only a limited number of cell lines have been established from other orders, including Coleoptera, Blattaria, Hymenoptera, Homoptera, Orthoptera, and

Hemiptera. Among these, the fewest cell lines have been reported from Homoptera (He *et al.*, 2023).

From a tissue perspective, insect cell lines are derived from various tissues and organs at different developmental stages. A significant milestone in insect tissue culture was the first successful establishment of a cell line from embryos of *Trichoplusia ni* (Hink *et al.*, 1970). In general, most insect cell lines have been developed from embryos (45.8%), neonate larvae and ovaries (Fig. 3). Among these, undifferentiated embryonic tissues are the most preferred starting material, contributing to nearly half of all cell lines, followed by larvae (23%) and ovaries (15.4%). In recent years, cell lines have also been established from specialized tissues such as the central nervous system and midgut, which are important for studying pesticide targets, absorption, metabolism, and discovery (He *et al.*, 2023).

Several midgut-derived cell lines have been established from different insect species, including BCIRL-HzMG8 (Pringle *et al.*, 2003) and RP-HzGUT-AW1 (Goodman *et al.*, 2004) from *Helicoverpa zea*, BPH22 from *Poekilocerus pictus* (Kharat *et al.*, 2010), BTI-TnMG1 from *T. ni* (Granados *et al.*, 1986), and SfMG1-0611 and SfMG-0617 from *S. frugiperda* (Zhou *et al.*, 2020). These midgut cell lines exhibit variable characteristics compared to in vivo systems, particularly in their responses to Bt Cry proteins and viral infections. Different cell lines with their pictures are shown in Fig. 4.

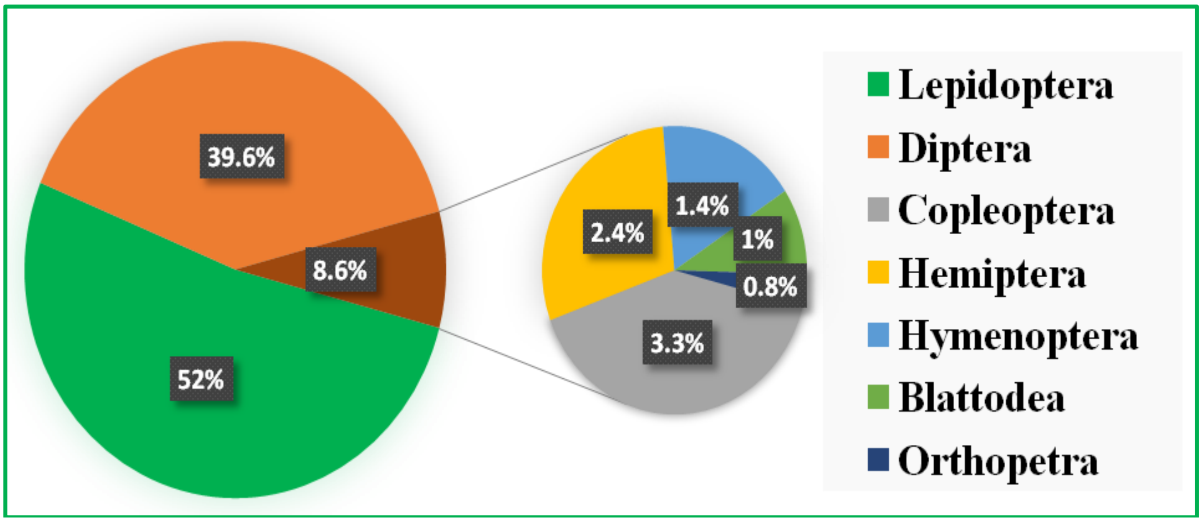


Fig. 1: The proportion of different orders contributing to established insect cell lines

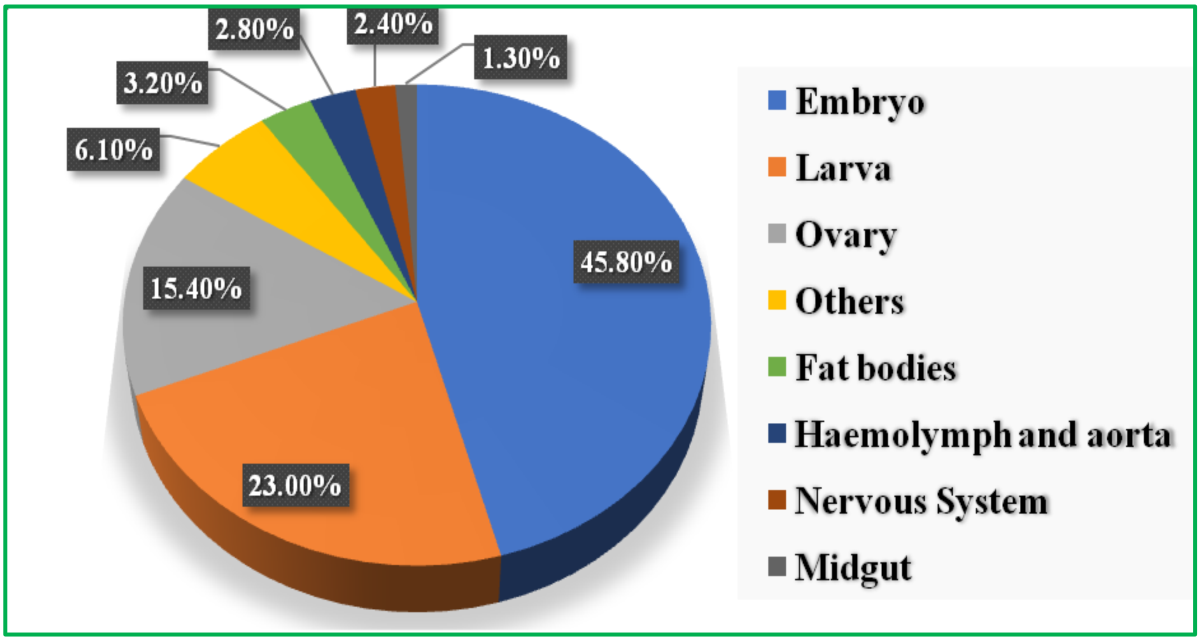


Fig. 2: The proportion of different tissues in establishing in insect cell lines.

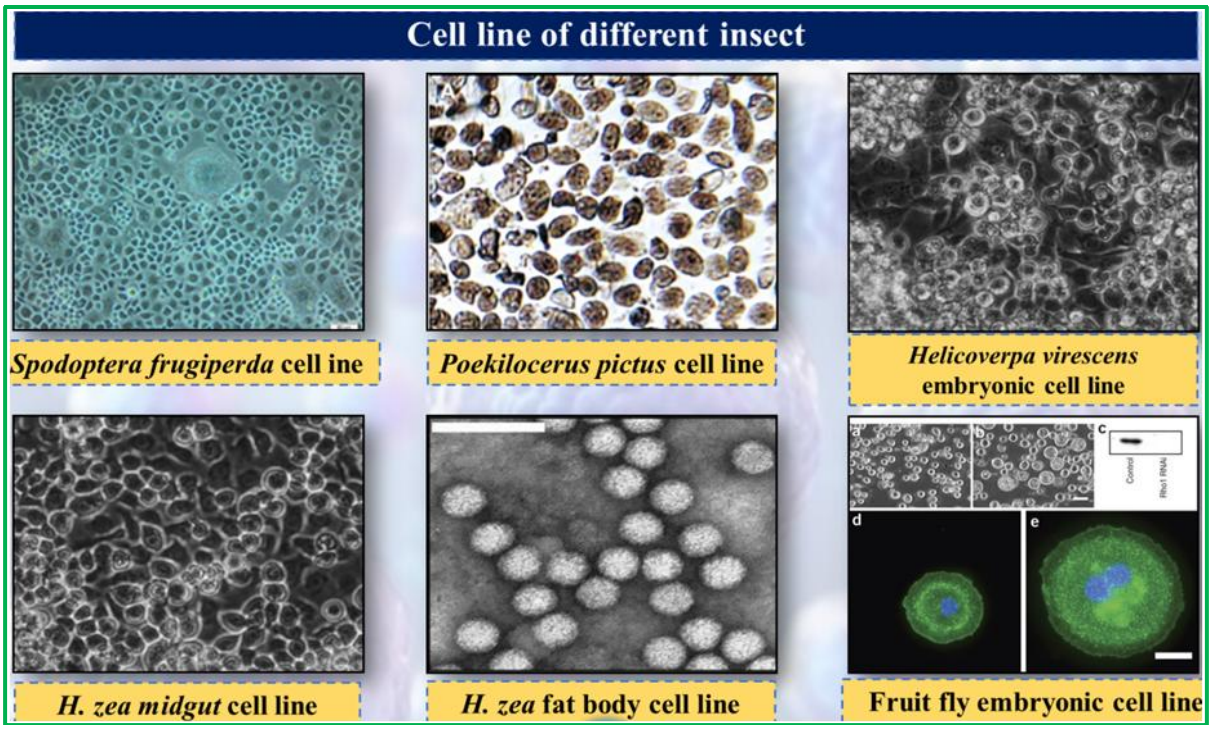


Fig. 3: Pictures of different insect cell lines isolated from various insects

Verification of Insect Cell Lines

To verify the origin and function of insect cell lines, researchers use biochemical and molecular techniques such as western blotting, immunofluorescence, radiolabeled ligand assays, and PCR. Goodman developed multiple cell lines from different insect tissues (nerve cord, midgut, ovaries, and embryos), which were initially characterized based on morphology, showing diverse shapes and network like growth in neural cells. PCR techniques like ISSR PCR helped distinguish cell lines from different species (Goodman *et al.*, 2004). Various insect lines with their source and applications are tabulated in Table 1 and Table 2 represents the **origin, medium and PDT of insect cell lines.**

Table 1: Various insect cell lines

Sr. No.	Name of Insect Cell Line	Source	Applications	References
1.	FPMI-CF-1	*Choristoneura fumiferana* (midguts)	Expression of recombinant proteins using baculovirus vectors	Hink *et al.* (1991)
2.	BTI-EAA	*Estigmene acrea* (larval hemocytes)	For recombinant protein production	Hink *et al.* (1991)
3.	IBL-SLO-1A	*Spodoptera litura*	Useful in studies like replication of *S. litura* nuclear polyhedrosis virus *in-vitro*	Shih *et al.* (1997)
4.	LPC-Aa98-19	*Anacridium aegyptium*	Suitable for virus multiplication and manipulation	Hernandez-Crespo *et al.* (2000)
5.	NIV-HA-197	*Helicoverpa armigera* (embryonic tissue)	Application in the mass production of baculovirus as a bioinsecticide	Sudeep *et al.* (2002)
6.	Sf21 cells	*Spodoptera frugiperda* (pupal ovarian tissue)	Research on baculoviruses and their use for producing recombinant proteins	Chen *et al.* (2005)
7.	RAE25	*Rhipicephalus appendiculatus*	Useful tool in defining the complex nature of the host vector and pathogen relationship	Bell-Sakyi *et al.* (2007)
8.	High Five cells	*Trichoplusia ni* (cabbage looper ovarian cells)	Recombinant protein expression using baculovirus or transfection	Zhang *et al.* (2008)
9.	TI-1	*Thysanoplusia intermixta*	Studies of insect pathogenic viruses and baculovirus expression vector system	Hashiyama *et al.* (2011)
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.	KLBIQ-Chsu-I	*Chilo suppressalis*	Production of insect virus expression vector	Liu *et al.* (2015)

12.	AW1	ventral nerve cord of *H. zea* larvae	For screening and revealing the mechanisms of neuroactive insecticides	Ren *et al.* (2019)
13.	TN-5B1-4	*Trichoplusia ni*	Research on pesticide mechanisms	Nuringtyas *et al.* (2014)
14.	Spex cell line	*Spodoptera exigua*	Research on pesticide mechanisms	

Table 2: Origin, Medium and PDT of Insect Cell Lines

Order	Species	Common Name	Cell Line	Derived Tissue	Cell Type	Primary Medium	Subculture Medium
Hemiptera	*Nilaparvata lugens*	Brown planthopper	NARO-NL72	Embryos	Adherent	MGM-464 + 20% FBS	MGM-450 + 10% FBS
Coleoptera	*Sitophilus oryzae*	Rice weevil	NARO-Sior-2	Pupae	Adherent	MGM-450 + 20% FBS	Grace + 10% FBS
Hymenoptera	*Allantus luctifer*	Buckwheat sawfly	NARO-Allu-F1	Fat body (larva)	Weakly adherent	MGM-450 + 30% FBS	MGM-450 + 10% FBS
Hymenoptera	*Trichogramma* sp.	Egg parasitoid	NAROTricho-14	Pupae	Adherent	MGM-450 + 30% FBS	MGM-450 + 10% FBS
Diptera	*Culicoides oxystoma*	Biting midges	NAROCuOx-11	Embryos	Adherent	MX30	IPL-41 + 10% FBS
Diptera	*Culicoides oxystoma*	Biting midges	NAROCuOx-101	Embryos	Adherent	MX30	IPL-41 + 10% FBS
Lepidoptera	*Spodoptera litura*	Cotton leafworm	NARO-SL55-3	Malpighian tubules	Adherent	MGM-464 + 20% FBS	MGM-450 + 10% FBS
Lepidoptera	*Spodoptera litura*	Cotton leafworm	NARO-SL63	Larval fat bodies	Adherent	MGM-464 + 20% FBS	Grace + 10% FBS
Lepidoptera	*Spodoptera litura*	Cotton leafworm	NARO-SL68-2	Larval fat bodies	Adherent	MGM-464 + 20% FBS	Grace + 10% FBS
Lepidoptera	*Mythimna separata*	Northern armyworm	NAROMs11	Injured larval tissues	Adherent	MGM-464 + 20% FBS	Grace + 10% FBS
Lepidoptera	*Mythimna separata*	Northern armyworm	NAROMs12A	Injured larval tissues	Weakly adherent	MGM-464 + 20% FBS	Grace + 10% FBS
Lepidoptera	*Mythimna separata*	Northern armyworm	NAROMs14B	Injured larval tissues	Weakly adherent	MGM-464 + 20% FBS	Grace + 10% FBS

Source: Watanabe *et al.* (2025)

Types of Cell Cultures

1. Monolayer (Adherent) Cultures

Cells grow attached to a surface and are anchor dependent. They allow easy observation under a microscope and are widely used for cytology and continuous product studies. However, growth is limited by surface area and requires tissue culture treated vessels. Cells are separated using enzymatic or mechanical methods (Arunkarthick *et al.*, 2017).

2. Suspension Cultures

Cells grow freely in a liquid medium (anchorage independent) with agitation. They are easy to scale up, require no dissociation and are suitable for bulk protein production. Growth depends on cell concentration and regular monitoring is needed (Willems and Jorissen, 2004).

Insect Cell Cultures

Insect cell culturing media is being used commercially for its expediency and in fact it has become an important component of modern biotechnology. Therefore, improved media are needed that strongly support the cell growth.

1. Conventional Media

In order for the insect cells to flourish in vitro, they require an environment that promotes their proliferation outside of their original tissue. Consequently, early insect cell cultures were maintained in a basic mixture of vitamins, amino acids, carbohydrates and salts supplemented with a poorly defined biological fluid, such as serum or hemolymph, known as conventional media (Schlaeger, 1996). Moreover, for the growth of insect cell lines, serum rapidly became the favored supplement (Hink, 1970). Table 3 represents different serum media.

2. Serum Free Media

By replacing the serum with appropriate nutritional and hormonal compositions, serum-free medium (SFM) avoids the problems associated with utilizing animal sera. Many primary cultures and cell lines, including recombinant protein generating lines, numerous hybridoma cell lines, the insect lines Sf9 and Sf21 and cell lines that act as viral hosts (VERO, MDCK, MDBK), and others, have serum free medium formulations (Li *et al.*, 2021). One of the most significant advantages of employing serum free media is the flexibility to make the medium to specific cell types by selecting the right mix of growth factors. Serum has a variety of well-known drawbacks, including high cost, mycoplasma or contamination risk, low batch to batch repeatability, poorly defined composition and challenges in downstream processing. Table 4 various represents serum free media.

Table 3: Serum Media

S. No.	Media	Formulation	References
1.	ISFM medium	Prepared based on IPL-41, ultrafiltered yeastolate (4g) and a complex lipid emulsion were added	Inlow *et al.* (1989)
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)
3.	Sf 900 medium	It's a medium with low protein content	Weiss *et al.* (1992)
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)

Table 4: Serum Free Media

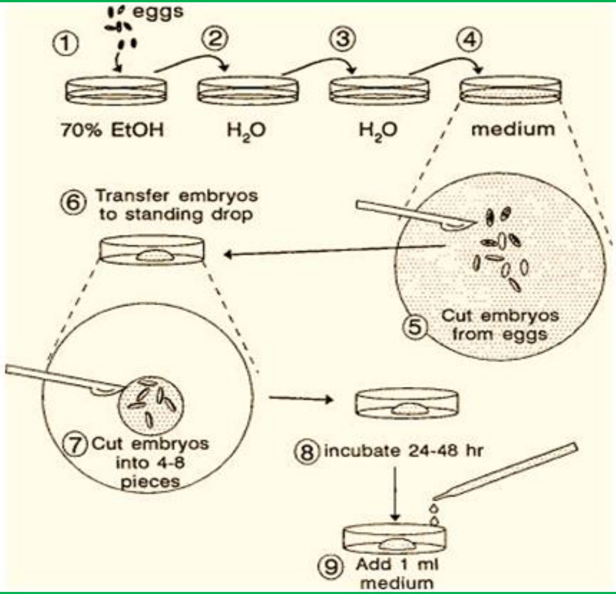
Manufacturer	Medium	Insect cell lines
Biological Industries	BIOINSECT-1	Sf9, Tn5
Sigma-Aldrich	EX-CELL™ 420	Sf9, Sf21
Sigma-Aldrich	EX-CELL™ 405	Tn5
GENTAUR	MED-10002	Sf9, Sf21, Tn368, Tn5
Allele Biotech	SFICM (Sapphire™)	Sf9, Sf21, Tn5

Source: Chan and Reid, 2016

Maintainance of Insect Cell Cultures

Most primary cell cultures typically survive for less than two months, however, this duration is adequate for many experimental purposes, including viral propagation in cultured cells and studying the role of immunocytes in immune responses to different stimuli (Smagghe *et al.*, 2009). During this period, cell morphology (shape and structure) should be regularly monitored every 2–3 days using an inverted microscope. Additionally, proper records must be maintained, including the date, culture name, type, volume and specific source of the culture medium used. Regular microscopic observation of cells during handling is essential to detect early signs of contamination and prevent its spread to other cultures in the laboratory, while also ensuring that the cells remain healthy. Indicators such as increased granularity around the nucleus, detachment of cells from the substrate and the presence of cytoplasmic vacuoles may suggest contamination, cellular aging (senescence) and toxic substances in the medium or the need to replace the culture medium. Therefore, the use of modern laminar flow hoods along with proper aseptic techniques is generally sufficient to maintain clean cultures, reducing the need for routine use of antibiotics and antimycotic agents (Battu *et al.*, 2023).

Subculturing or passaging, involves removing old (spent) medium, adding fresh medium, and transferring cells from an old culture vessel to a new one to support continued growth (Goodman *et al.*, 2001). When cells fully cover the available surface or in suspension cultures, exceed the capacity of medium, their growth slows down or stops. Therefore, maintaining optimal cell density by regularly providing fresh medium is essential for continuous proliferation. Many established insect cell lines are widely used in molecular biology, virology, industrial biotechnology and insect pest management. Fig. 5 represents the process of development of insect cell lines.



Culture media for maintaining cell culture lines are combinations of **amino acids (L-glutamine), salts, vitamins, carbohydrates (glucose/galactose)** and hormones

pH is maintained by different **buffers** *viz*., CO_2/sodium bicarbonate, phosphate and **HEPES** (N-[2-hydroxyethyl] piperazine-NO-[2-ethanesulfonic acid]) as the most commonly used

Insect cell lines are usually maintained at **26-28 °C**

Newly established insect cell lines are characterized using the usual parameters of **morphology, growth curve, species specificity, karyology** *etc*.

Fig. 4: Process of development of insect cell lines

Applications in Insect Toxicology

1. High Throughput Screening

There is an increasing shift toward **in vitro screening methods** using insect cell cultures as alternatives to traditional **in vivo insect assays.** Conventional insect-based tests are reliable but have major limitations like; they require large numbers of insects with uniform physiological conditions are time-consuming and demand significant labor and resources. To address this, researchers are using **high-throughput screening (HTS)** models based on insect cell lines, commonly combined with the **MTT assay (**thiazolyl blue tetrazolium bromide assay), which measures cell survival and proliferation. For instance, the two insect cell lines AW1 and SL2 were applied as activity screening models for discovering the lead compound among synthetic pyrazole carboxamides and furanone analogs, respectively (Ren *at al*., 2018; Ren *et al*., 2019). These in vitro methods are faster, as cell cultures grow within days and they reduce costs and workload compared to rearing insects. They also offer better experimental control, sensitivity and simpler data readouts. However, despite these advantages, **in vitro models cannot fully replace in vivo testing**. Some compounds that appear active in cell based assays may fail in real insects (false positives). Therefore, in vitro methods are mainly used for **initial large scale screening** and promising candidates are later validated using traditional insect-based assays.

2. Cell Death Modalities and Signaling Pathways

Insect cell based models are not only useful for screening insecticides but also help in understanding **how these chemicals work at the cellular level**. Insecticidal compounds can disturb normal cell functions (both structurally and biochemically), which eventually leads to **cell death**. There are three main types of cell death: **necrosis, apoptosis and autophagy.** These occur depending on different stimuli and signaling pathways. Studying these processes helps researchers identify the **target sites of insecticides,** such as specific genes, proteins or cellular pathways.

a.) **Necrosis:** Necrosis is a type of cell death that occurs when cells are damaged due to external factors like chemicals or physical stress. It is generally considered an uncontrolled and accidental form of cell death. Certain insecticides, such as methomyl, can cause necrosis in insect cells. This leads to DNA damage, including, formation of micronuclei, chromosome abnormalities and sister chromatid exchanges. Studies on insect cell lines (like Sf9, SL-1 and S2 cells) have shown that chemicals like rotenone cause necrosis with clear features such as, breakdown of the cell membrane, destruction of cell organelles and irregular DNA degradation (not the organized "ladder pattern" seen in apoptosis) (Sun *et al.*, 2021).

b.) Apoptosis: Apoptosis is a specific form of programmed cell death (PCD) regulated by gene networks and triggered by multiple stimuli (George *et al.*, 2000). It plays a vital role in both mammals and insects, with increasing importance in pest management. Plant derived pesticides such as neochamaejasmin A and isochamaejasmin induce apoptosis in Sf9 and AW1 insect cells, confirmed by morphological changes and activation of caspase-3/9 (Ren *et al.*, 2019; Ren *et al.*, 2021). Spinosad causes mitochondrial dysfunction via increased ROS, mPTP opening and MMP collapse, leading to cytochrome C release and apoptosis, along with oxidative stress and DNA damage (Yang *et al.*, 2017; Xu *et al.*, 2018). Azadirachtin and camptothecin also induce apoptosis in insect cells with characteristic features like DNA laddering and chromatin condensation (Zhang *et al.*, 2017; Haung *et al.*, 2013). These compounds act through different apoptotic pathways and time scales, often accompanied by cell cycle arrest at G1 or G2 phases (Huang *et al.*, 2011; Ren *et al.*, 2019).

c.) Autophagy: Autophagy, also known as type II programmed cell death (PCD), is a highly regulated process that removes stressed or damaged cells and prevents the accumulation of cytosolic proteins and organelles through a conserved catabolic pathway (Huett *et al.*, 2010). It plays a significant role in various developmental and physiological functions, including cell survival, cell death, metabolism and innate immunity. In holometabolous insects such as silkworms, honeybees and fruit flies, autophagy often occurring alongside apoptosis, is widely involved in the degradation of tissues and organs during metamorphosis (Tettamanti *et al.*, 2011). However, excessive activation of autophagy, particularly due to exposure to xenobiotics like insecticides, can also lead to normal cell death. Avermectin, commonly used as a systemic insecticide against sucking pests, has been shown to exert cytotoxic effects on Sf9 cells by inducing DNA damage and programmed cell death (Li *et al.*, 2021). It enhances the expression of autophagy related proteins such as Beclin1 and LC3-II, while reducing the level of p62. LC3 is a key marker of autophagy and exists in two forms: LC3-I and LC3-II.

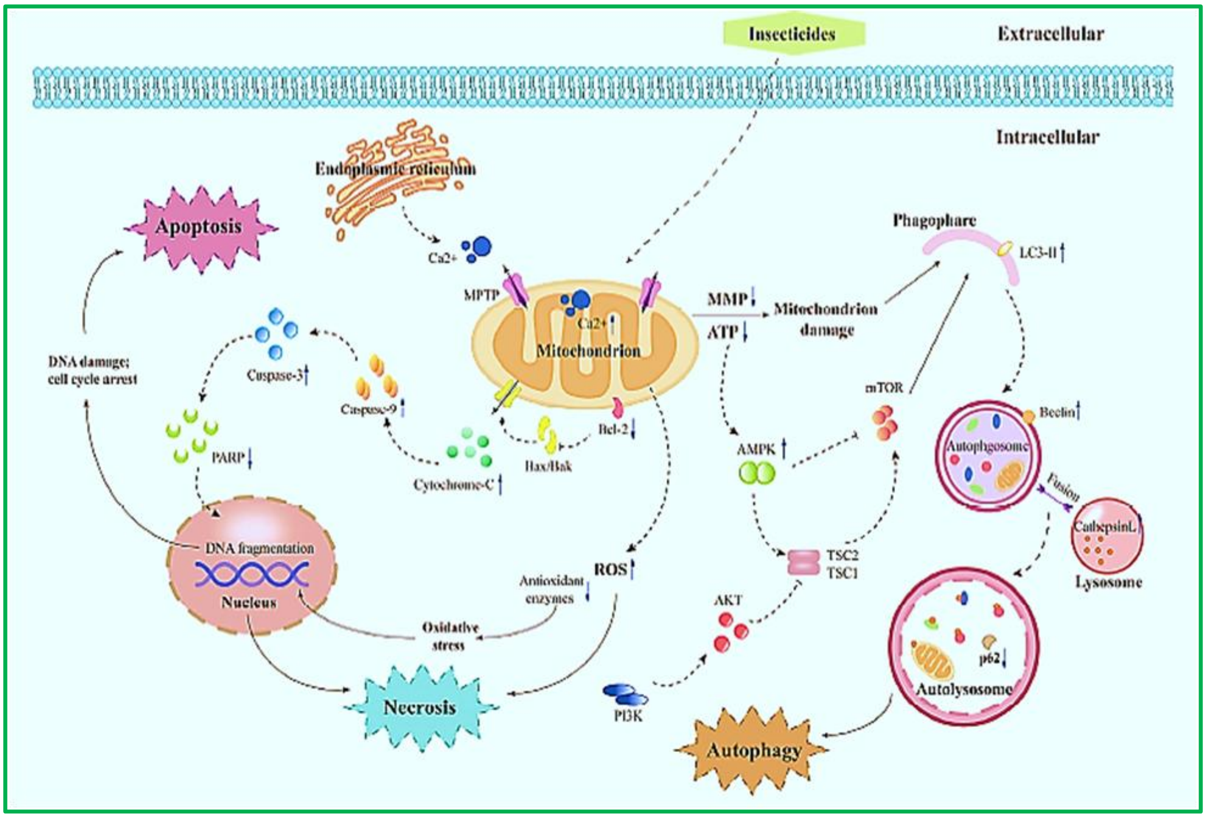


Fig. 5: Potential cell death mode and signal pathway in insect cell lines after insecticide treatment

During autophagy, LC3 is transported to autophagosomal membranes and converted from LC3-I to LC3-II. Beclin1 plays an essential role in the initiation of autophagy by facilitating the nucleation of autophagosomal membranes. Its association with pre autophagosomal structures makes it critical for the onset and progression of autophagy (Ren *et al.*, 2022). P62 contains both an LC3 interacting region and an ubiquitin binding domain, allowing it to serve as a selective receptor that links ubiquitinated substrates to the autophagic machinery for degradation within autolysosomes. Also, p62 itself is also degraded during the autophagic process (Ren *et al.*, 2022).

3. Ion Channels

Most commercial insecticides, whether natural or synthetic, act on a limited number of targets, with ion channels in the insect nervous system being the most important (Sattelle *et al.*, 2018). Ion channels are membrane proteins that form pores, allowing ions to move across membranes along their electrochemical gradients. They are present not only in the cell membrane but also in intracellular organelles such as mitochondria, endoplasmic reticulum and nucleus. Beyond ion transport, they play key roles in physiological processes like nerve impulse transmission, cell excitability, apoptosis and cell proliferation (Restrepo-Angulo *et al.*, 2010). Ion channels are classified based on properties such as gating mechanisms and biophysical characteristics. The primary insecticide targets include voltage gated sodium and potassium channels, calcium channels (ryanodine receptors), chloride channels (GABA and GluCl receptors) and nicotinic acetylcholine receptors (Raymond-Delpech *et al.*, 2005). Studies using insect cell systems along with voltage and patch clamp techniques have significantly improved understanding of the mode of action of neuroactive insecticides.

a.) **Potassium channels:** Wang (Wang *et al.*, 2006; Wang *et al.*, 2006) studied the impact of cyhalothrin on the transient outward potassium current (IA) and delayed rectifier potassium current (IK) in isolated central neurons of *Helicoverpa armigera* using patch clamp techniques. The outcomes demonstrated that cyhalothrin has neurotoxic effects on the nervous system through the regulation of activation potentials and inactivation state of IA channels.

b.) **Sodium channels:** Patch-clamp analyses of cockroach dorsal unpaired median (DUM) neurons have exhibited that DCJW, a decarbomethoxylated derivative, could block voltage sensitive sodium channels in the insect. DCJW causes a dose related restraint of the peak inward sodium current. The activity of DCJW does not affect sodium channel activation and shows no effect on either reversal potential or voltage dependence of both steady state inactivation and sodium conductance (Song *et al.*, 2006).

c.) **Chloride channels:** Research on native GABARs on Drosophila cell lines has proven that fipronil works specifically on ionotropic GABARs, while current studies on cockroach neurons have proved that fipronil also acts on GluCls. These novel outcomes suggest that GluCls and ionotropic GABARs may share certain parallels in binding sites for fipronil (Raymond *et al.*, 2002).

d.) **Calcium channels:** Ryanodine receptors (RyRs) are calcium channels that regulate Ca^{2+} release from intracellular stores located in the sarcoplasmic reticulum. To discover effective insecticides targeting ryanodine receptors, (Liu *et al.*, 2016) synthesized a series of novel anthranilic diamide analogs containing N substituted phenylpyrazole.

4. Vector virus relationship

Insect cell culture has become an essential tool to study the complex interactions of plant viruses and their insect vectors. The in vitro cell cultures of several insect vectors of plant viruses including leafhoppers, planthoppers, aphids, thrips and whiteflies have been successfully employed to understand the functions of viral proteins and receptor mediated endocytosis into vector cells (Ghosh *et al.*, 2020). The insect cell baculovirus expression vector system (IC-BEVS) is widely recognized as a cost-effective and efficient platform for protein production (Rao, 2003). Baculoviruses are DNA viruses and represent one of the most important groups infecting arthropods (Miller and Lu, 1997). They have been reported to infect over 600 insect species across several orders, including Lepidoptera, Hymenoptera, Diptera, and Coleoptera, with a predominant association in lepidopterans (Martignoni and Iwai, 1986). These viruses serve as powerful tools for recombinant protein expression in insect cell systems. Baculoviruses are obligate parasites with a restricted host range and are harmless to vertebrates, which makes the baculovirus expression system highly suitable for vaccine and therapeutic development (Felberbaum, 2015). Through BEVS, both heterologous proteins and virus like particles (VLPs) can be efficiently produced in vitro. At present, this system is also being applied in the production of VLP based COVID-19 vaccines (Sacks, 2021). The mechanism of BEVS is illustrated diagrammatically in Fig. 8.

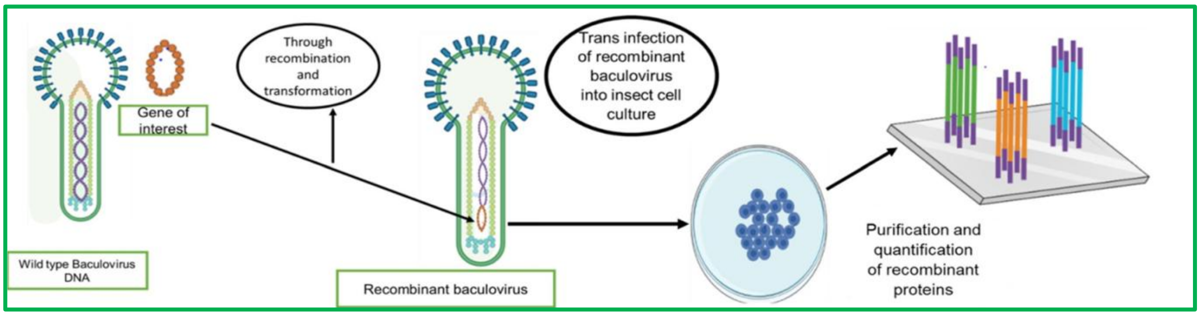


Fig. 6: A diagrammatic description of the mechanism in BEVS

Challenges and Limitations

1. **Establishment of selective targeting cell lines-** most of the isolated cells might only survive for several days to months and later these cells might not be suitable for the aim. Hence, developing techniques of cell culturing is crucial for the continuous establishment of selective cell lines.
2. **Insecticidal activity differences *in-vitro* and *in-vivo*-** the active results of *in-vitro* may be completely distinct from those of *in-vivo*. *In-vitro* cell-based models suited for HTS generate many 'hits' but lack relevant whole organism physiology to further validate the findings.
3. **The evaluation models and methods-** the MTT model is not always the best to assess cytotoxicity because it strictly relates to cell metabolic activity. Cells with low metabolic activity (*e.g.*, lymphocytes) must be used in high numbers.

Future Thrust

- ✓ Develop new cell lines by expanding the range of insect pest species.
- ✓ Integrate with genomic and proteomic approaches by combining cell line studies with omics technologies for deeper insights.
- ✓ Conduct more field trials based on insect cell line toxicological data.

Conclusion

Insect cell lines are crucial in evaluating the activity of candidate insecticides to discover their toxicity. The cell based studies increase efficiency and reduces 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 HTS 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. 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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