INTRODUCTION

Annually, a quantity over 2 billion tonnes of grains is cultivated for the purpose of sustenance and animal feed, so constituting approximately two-thirds of the aggregate protein consumption, encompassing both direct and indirect sources (Erenstein et al., 2022; Tiwari et al., 2022). Agriculture serves as the predominant means of sustenance for around 58% of the population in India. In the crop year of 2019-20, an expected record-breaking food grain production of 295.67 million tonnes was projected. During the fiscal year 2020-21, the Government of India set a goal to achieve a food grain production of approximately 300 million tonnes (Agricultural and Processed Food Products Export Development Authority (APEDA), Union Budget 2020-21). The government of India has established a food grain production goal of 328 million tonnes for the crop year of 2022-23, spanning from July to June **(Fig. I** and **II)**. This aim represents a 4% increase compared to the previous year's record foodgrain output of 315.7 million tonnes. Despite the possible adequacy of available land resources to sustain the future global population, the Food and Agriculture Organisation (FAO) has issued a cautionary statement highlighting that a significant portion of this land is only suited for cultivating a limited range of crops. Food grains and pulses are widely consumed and commonly stored food products globally, particularly in tropical and sub-tropical climates. As a result, they play a vital role in addressing issues related to food insecurity.

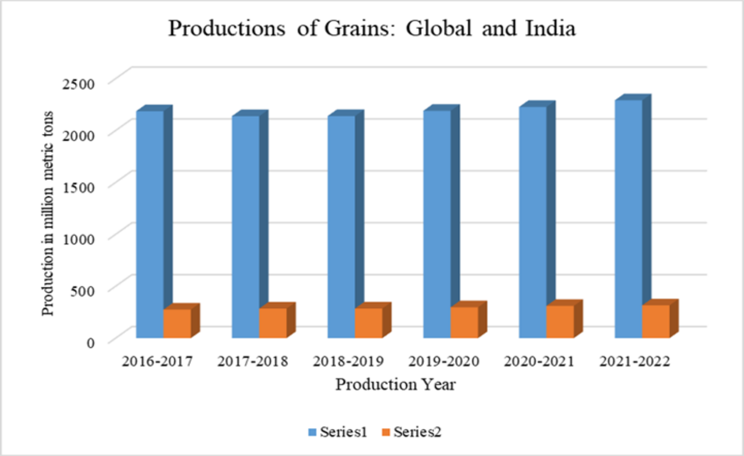

Figure I: Grain production scenario: Global and India (Statista 2023); Series 1-global production, series 2- Indian production

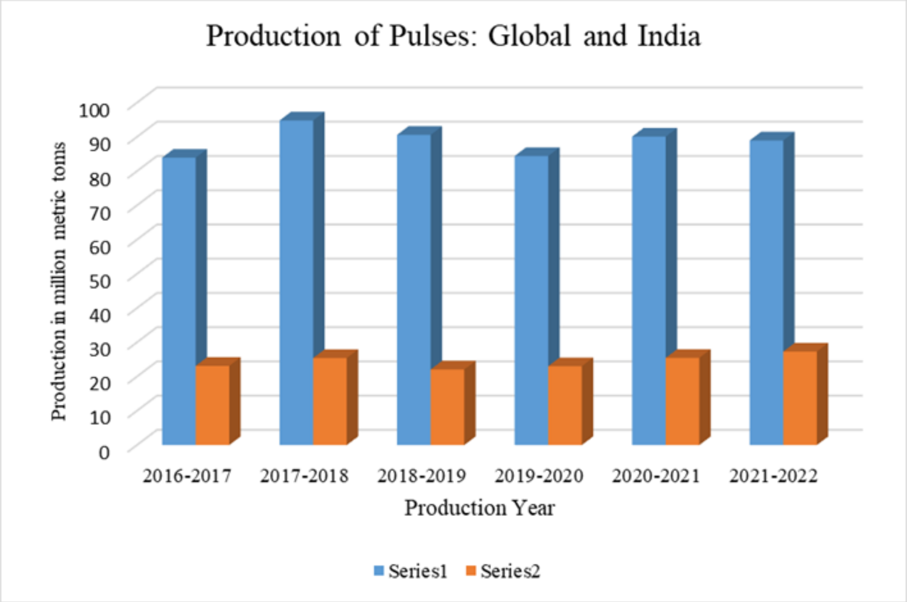

Figure II: Pulse production scenario: Global and India (Statista 2023)

According to projections by the Food and Agriculture Organisation (FAO), a significant global food shortage may arise if there is no increase in total world food production. Furthermore, the number of individuals experiencing hunger worldwide is expected to rise to a range of 702-828 million by 2021 due to various factors such as the COVID-19 pandemic, regional conflicts, natural disasters, and climate change (Fan et al., 2021; FAO et al., 2022; George et al., 2020; Janssens et al., 2020). According to the Food and Agriculture Organisation (FAO, 2011) and Shukla et al., (2019), around 33% of the global food production is lost or wasted annually throughout the food chain. This phenomenon results in economic losses amounting to an estimated 936 billion USD and contributes to approximately 8-10% of the total greenhouse gas emissions worldwide (Buzby and Hyman, 2012; Chalak et al., 2016). According to the Food and Agriculture Organisation (FAO, 2011), the majority of food loss in poor nations occurs at the initial and intermediate phases of the food supply chain. Food loss is well recognised as a significant obstacle to the advancement of food security and sustainable development in developing nations, as indicated by Abbade, (2020) and Xue et al., (2017). The enhancement of global food production is not solely reliant on raising the yield of food crops, but also on mitigating the negative effects of several factors that hinder food output. According to the United Nations' World Population Prospects report from 2017, it is projected that the global population will reach 9.1 billion by the year 2050. In order to adequately sustain this growing population, there is a need for a 70% increase in food production. In developing countries, a significant proportion of the rapidly expanding populations are currently facing varying degrees of food insecurity and limited access to food resources. In poor countries, the prevalence of hunger among children is estimated to be one in six, with a consistent upward trend in malnutrition rates observed since 2015 (World Health Statistics 2012; FAO, 2019).

A major obstacle to achieving optimal food production is pre- and post-harvest issues, which, together with insect infestation, result in significant grain loss. Before reaching the consumer, food grains must undergo several procedures after harvesting, including threshing, cleaning, drying, storage, processing, and transportation. Food losses across the post-harvest continuum have been seen to commence during the harvesting process and persist until the point of food marketing at the consumer's end. Technical challenges encompass insufficient stock management facilities, substandard packaging practises, and inadequate infrastructure, hence leading to grain loss (Hodges et al., 2011; Mesterházy et al., 2020). Based on the report published by the Food and Agriculture Organisation (FAO) of the United Nations (UN), it is estimated that approximately 1.3 billion metric tonnes of food, accounting for 33% of the total production, is lost during the post-harvest stage on a global scale. Furthermore, the report projects that if current practises persist, the projected loss is expected to reach approximately 2.1 billion metric tonnes by the year 2030.

Parfitt et al., (2010) reported that in many countries 15% of food grains are lost during or after harvest. Post-harvest grain loss in India was assessed by the FAO as 40% and post-harvest cereal loss as 30%, as recorded by the National Academy of Agricultural Sciences in the “Saving the Harvest: Reducing the Food Loss and Waste” 2019 report. According to Singh, (2010), the annual monetary losses incurred exceed Rs. 50,000 crores. Based on the estimations provided by the Associated Chambers of Commerce of India, it has been determined that a significant amount of food, valued at 92651 crore rupees, is wasted through post-harvest operations prior to its consumption by the end consumer (PIB, February 2016). According to a comprehensive nationwide study conducted by Jha et al., (2015), the findings indicate that crop losses in several categories were observed. Specifically, cereals experienced losses ranging from 3.9% to 6%, pulses from 4.3% to 6.1%, oilseeds from 2.8% to 10.1%, fruits from 5.8% to 18.1%, and vegetables from 6.9% to 13%. These losses occurred during the stages of harvesting, post-harvest activities, handling, and storage.

The occurrence of grain loss is an undeniable and regrettable reality, as stated by NITI Aayog in 2015. Warehouses frequently encounter significant losses of stored grains as a result of both biotic causes, such as insect pests, diseases, and weeds, and abiotic ones, including drought, salinity, heat, and cold (Aulakh and Regmi, 2013). These losses primarily occur owing to a lack of caution. Various types of infestation can affect grains, with insects being the predominant group due to their access to ample food resources, elevated moisture levels, and favourable temperatures (Ahmed, 1983). Considerable damage to grains is being caused by fungi, mites, birds, and rodents, according to studies conducted by Abedin et al., (2012) and Lamichhane et al., (2018). Insect pests are widely regarded as the foremost biotic agents, exerting significant influence and resulting in substantial losses estimated at roughly 30-40% (Abass et al., 2014; Kumar and Kalita, 2017; Mesterházy et al., 2020). Insects have a significant role in the degradation of stored food and agricultural commodities, resulting in annual losses estimated to range from 15% to 25% of the stored grain (Adu et al., 2014; Nayak and Solanki, 2021; Tanda et al., 2022). In comparison to other pests, insects are often regarded as the most destructive pests of stored grain commodities due to their rapid reproductive rate and short generation period. Although it is estimated that 20 species of insects (excluding psocids) out of the 100 are rated as most harmful pests and are cosmopolitan by distribution (Utono, 2013; Srivastava, and Subramanian, 2016; Singh et al., 2021; Xie et al., 2023; Yan et al., 2023).

According to Stork et al., (2018), only three orders, namely Coleoptera, Lepidoptera and Psocoptera, are recognised as pests of stored grains. Furthermore, it has been documented that certain species from the orders Hemiptera (commonly known as bugs) and Hymenoptera (specifically wasps) have been observed to have associations with stored commodities. However, it is important to note that these associations are limited to their roles as predators or parasites (Rees, 2004; O'Callaghan et al., 2005). A multitude of insect pest species are distributed throughout various regions of the globe, inhabiting areas suitable for poikilothermic organisms. The distribution of this pest species is limited to locations that are easily accessible and offer suitable food sources, as well as possessing specific biological and physical characteristics. In key crops, it has been estimated that insect pests result in output losses ranging from 15% to 20% on average. Pest insects have the potential to pose significant challenges by inflicting harm upon crops and impeding food production, exploiting cattle as hosts, and engendering adverse consequences for human well-being and health. Insects are responsible for inflicting extensive harm to agricultural and forestry commodities throughout the phases of storage and distribution. Significant quantities of stored grain are subject to deterioration due to both direct consumption and contamination of the grain caused by the presence of whole insects, insect pieces, and faecal matter (Heaps et al., 2006; Bravo et al., 2007). The persistent problem of food contamination resulting from insect pests is a perpetual cause of economic loss and apprehension. In the context of India, the incidence of post-harvest losses is mostly related to insufficient storage practises, infestation by insects as well as microbiological activity. These factors collectively result in an estimated reduction of around 10 percent in the overall quantity of food grains. The primary economic detriment resulting from insects is not solely limited to the physical matter they consume, but also encompasses the quantity of food rendered unsuitable for human consumption due to contamination from insects and their excrement. There is an estimated presence of around 600 insect species that are commonly seen in association with stored grain products. According to the International Grain Research Institute (IGMRI, 2019), there are around 100 kinds of insect pests that are responsible for causing significant economic losses in stored products.

There are two primary classifications for storage insect infestations. Primary pests have the ability to infest whole grains and possess the capacity to breach the protective seed coat and pods in order to consume the embryo, endosperm, or cotyledons. Secondary pests are known for their tendency to consume grain products or grains that have undergone prior damage caused by primary pests or as a consequence of activities such as harvesting, processing, and transportation. Primary pests, such as those that target cereals and pulses, generally exhibit a limited scope of dietary preferences. Secondary pests exhibit a broad spectrum of hosts, encompassing whole grains that have been compromised and processed goods such as flour, breakfast cereals, chocolates, and compound animal meals (Singh et al., 2014). There exists a discernible disparity in the life cycles of pests falling within these two classifications. The life cycle of a primary pest entails the deposition of eggs either within or on the external surface of the grain, subsequently leading to the growth and maturation of the pest within the grain. This progression renders the early developmental phases of the pest arduous to identify. The nomenclature "internal feeders" is commonly used to refer to main pests that complete their full life cycle within the kernel. Whereas, eggs of secondary pests are dispersed either within or in close proximity to the food, facilitating the visibility of the larvae during their development. Secondary pests are referred to be "external feeders" due to the fact that their complete life cycle occurs outside of the whole grain. The different nature of damage caused by primary pests to whole grains, as well as the distinctions in their life cycles, make pest recognition easier in comparison to secondary pests (Antary and Thalji, 2015; Nayak and Daglish, 2018; Banga et al., 2020)

Insects that cause harm to stored grains frequently gain entry from the field and subsequently establish themselves in the storage facility due to the prevailing microclimate. These insects persist throughout the processing and storage phases (Hagstrum and Phillips, 2017). Certain insect pests commence inflicting harm to crops during the ripening phase and persist throughout the storage period. Nevertheless, the primary origins of infestations consist of aged bags, storage facilities, outdated containers, cross-contamination (Perez-Mendoza et al., 2004), harvesting equipment, and other machinery (Deshwal et al., 2020; Guru et al., 2022). Several stored grain insect pests, including *T. granarium*, *R. dominica*, *S. oryzae*, *Sitotroga cerealella*, *T. castaneum*, and *C. chinensis*, have a significant detrimental impact, leading to a minimum loss of 5-10% annually. This loss is primarily attributed to the inappropriate application of insecticides (Ramzan et al., 2019). Most of the studies on stored grain pests to date have focused on strategies to reduce losses, quantity and/or quality, and any other developmental constraints occurring due to infestation; recommendation procedures for their control, which are cost effectively integrated with the production/marketing system that does not favor the development of resistance to pesticides (Dhananjayan et al., 2020); and adaptive research has also been conducted where the bio-efficacy of various plant products are evaluated (Aniwanou et al., 2020). The integration of various control techniques, known as integrated pest management, has become a focus of research on stored products. Laboratory studies on pest biology have also been intensively conducted (Babendreier et al., 2020).

A range of pest management strategies encompassing physical, mechanical, biological, and chemical approaches are accessible **(Fig. III)**. Chemical-based methods such as fumigation, grain protectants, and aerosols continue to be the predominant grain management solutions. The use of methyl bromide (CH_3Br) and phosphine (PH_3) for fumigation is a commonly employed method in numerous nations for the purpose of pest control in stored commodities (Bell, 2000). CH_3Br is a compound known for its extreme toxicity and is commonly found in the market under several trade names such as Bromogas, Celfume, Embafume, MB, MeBr, Methogas, Profume, Terrogas, and Zyto (Deb, 2020). The substance undergoes rapid dissipation in the atmosphere and poses significant risks to fumigation locations, hence causing severe impacts on the central nervous and respiratory systems. The aforementioned adverse impact persists and specifically affects the respiratory system, ocular organs, and integumentary system, hence rendering it of considerable clinical importance. Furthermore, as a result of its correlation with the loss of the ozone layer, the global prohibition of CH_3Br has been implemented (Anbar et al., 1996). The Environmental Protection Agency (EPA) proactively implemented a plan to gradually eliminate the use of CH_3Br in all countries, with the target year for completion set at 2000.

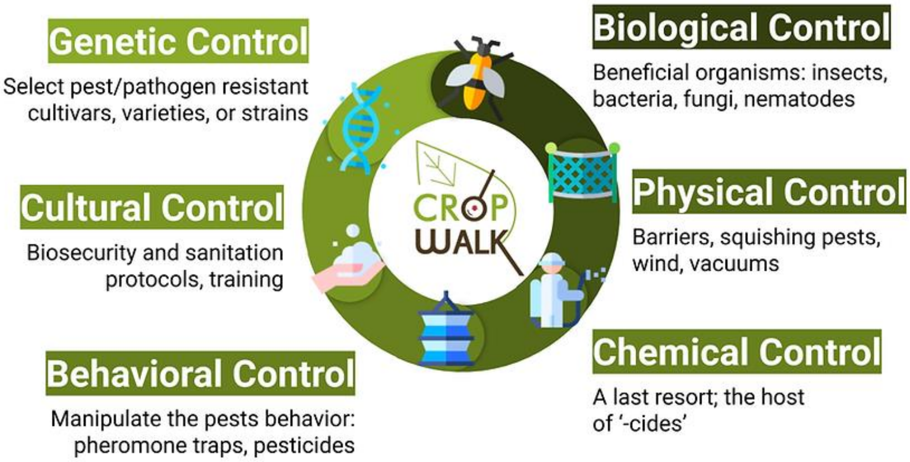

Figure III: Various methods to control Insect pest. (cropwalk)

The growing awareness of food safety and health issues has led to the emergence of green chemistry and nonchemical management practises. The management of insect infestation in stored grain mostly relies on chemical approaches involving the use of insecticides **(Fig. IV)**. These insecticides are primarily employed to eliminate specific insect species that pose a threat to the stored grain. Insecticides are classified into several chemical groups, namely carbamates, organochlorines, organophosphates, and pyrethroids, these classes are currently utilised on a significant scale (Abubakar et al., 2020; Sharma et al., 2020). Additional pesticides employed for the control of insect pests encompass chlorpyrifos methyl, primiphos methyl, methyl parathion, lindane, piperonyl butoxide, diazinon dichlorvos, pyrethrins, and malathion. Nevertheless, the widespread occurrence of insect resistance among stored grain pests has become a prevalent issue due to the frequent and excessive use, as well as the incorrect application, of pesticides and fumigants (Stejskal et al., 2021; Wakil et al., 2021). Insect resistance is an adaptive mechanism that certain pest species have evolved to ensure their survival in the presence of insecticides administered at recommended doses, which may be lethal to other members of the pest population (Skendžić et al., 2021).

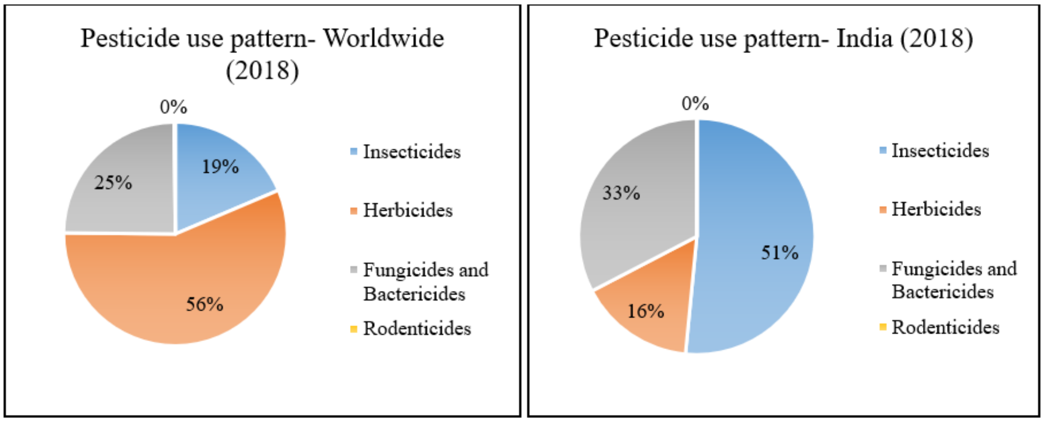

Figure IV: Pesticide use pattern: Worldwide and India (Nayak and Solanki 2021)

Insecticides are commonly used to control stored grain pests such as beetles, weevils, and moths, which can cause significant damage to stored grains. These insecticides are designed to kill or inhibit pest growth and reproduction (Ahmad et al., 2021). However, some insecticides can have unintended consequences and may have transgenerational effects on pests. Insecticide-induced transgenerational changes refer to the effects of insecticide exposure on individuals that persist across multiple generations, thereby influencing the phenotype and physiology of subsequent offspring (Brevik et al., 2018; Chirgwin et al., 2022). The mechanisms underlying insecticide-induced transgenerational changes are not fully understood but may involve epigenetic modifications. Epigenetic alterations can affect gene expression patterns without altering underlying DNA sequences. These alterations may affect various biological processes including development, metabolism, reproduction, and responses to stress or toxins. There are some potential transgenerational effects of insecticide resistance development, such as altered growth and development, behavioural changes, reproductive effects, and fitness consequences **(Fig. V)**.

Zhang et al., (2016) observed that the transgenerational effects of deltamethrin exposure on the diamondback moth (*Plutella xylostella*) showed that deltamethrin exposure had negative effects on various life history traits and increased oxidative stress in subsequent generations. Huang et al., (2021) explored the transgenerational effects of deltamethrin on the cotton bollworm. The results indicated that deltamethrin exposure resulted in reduced survival, delayed development, and decreased reproductive capacity in subsequent generations of cotton bollworm.

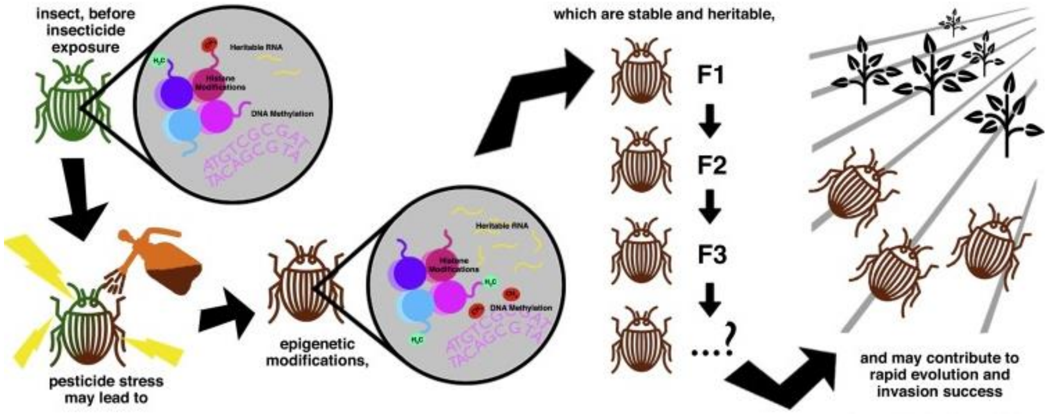

Figure V: Transgenerational effect (Brevik et al., 2018)

The transgenerational effects of insecticides on stored grain pests have been the subject of many studies because of the importance of managing these pests to protect stored food commodities. Daglish, (2008) studied the transgenerational effect of methoprene, an insect growth regulator, on the rice weevil (*Sitophilus oryzae*) and observed that methoprene exposure affected the reproductive output, developmental time, and population growth of subsequent generations of rice weevils. Nath et al., (2023) examined the transgenerational effects of phosphine, a commonly used fumigant, on fecundity and body weight in three stored-product insects: red flour beetle (*Tribolium castaneum*), lesser grain borer (*Rhyzopertha dominica*), and rice weevil (*Sitophilus oryzae*). The results showed that phosphine exposure affected the reproductive parameters and body weight of subsequent generations of these pests. Further, Rösner et al., (2020), investigated the transgenerational effects of deltamethrin on the reproductive fitness of *T. castaneum*, study found that exposure to deltamethrin affected the reproductive performance and offspring development in subsequent generations of flour beetles. Morrison et al., (2018) studied the transgenerational effects of deltamethrin on *R. dominica* and observed that deltamethrin exposure affected the development, survival, and reproductive parameters of subsequent generations of the lesser grain borer. These studies emphasize that insecticide exposure can induce transgenerational effects in insects, influencing their fitness, reproductive success, development, and population dynamics. It is important to note that specific effects can vary depending on insect species, insecticide formulation, dosage, exposure duration, and other factors.

Over the recent years, a total of 504 insect species exhibiting resistance to insecticides have been documented. Notably, there has been a consistent rise in resistance towards particular chemical compounds, with numerous species now displaying resistance to multiple classes of molecules, including DDT, malathion, pirimiphos-methyl, deltamethrin, and permethrin **(Fig. VI)** (Karaağaç, 2012; Dara, 2013 and 2016; Zhu et al., 2016; Kortbeek et al., 2019). Insects have demonstrated notable adaptability to a wide range of insecticides through the development of physiological or behavioural resistance mechanisms (Jallow et al., 2017; Nansen et al., 2016; Dara, 2017). The issue of pesticide resistance in postharvest ecosystems is a significant problem, since it poses a threat to the efficacy of grain protectants in preserving stored food stuffs (Hagstrum and Phillips, 2017; Jian 2019) and fumigants (Bajracharya et al., 2013; Nguyen et al., 2015; Nayak et al., 2020) has been well documented.

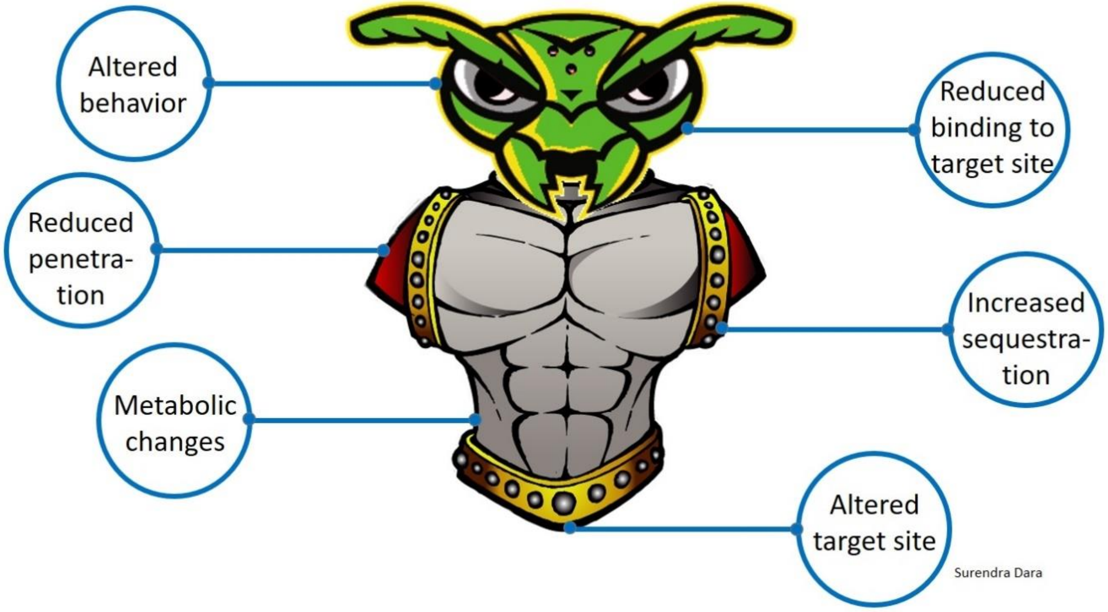

Figure VI: Insecticide induced alteration (Dara, 2017)

The progressive and evolutionary processes of pesticide resistance have led to its establishment and subsequent dissemination among insect populations. After the initial exposure to the pesticide, a period of latency ensues during which resistance genes undergo segregation and become connected with other genes that facilitate the formation of favourable conditions for resistance. Throughout the process of insecticide selection pressure, the target species exhibited a discernible augmentation in pesticide tolerance as a result of evolutionary adaptation (Nansen et al., 2016; Dara, 2017). During the subsequent phase, the emergence of pesticide resistance occurs gradually, succeeded by a period characterised by accelerated development, wherein numerous factors exert an influence on the selection of insecticide resistance. The phenomenon of pests in stored products experiencing exponential population expansion due to the rapid development of resistance poses significant challenges in terms of control and management. Detecting resistance mechanisms is a significant challenge due to their gradual emergence over extended periods of evolutionary time (Nguyen et al., 2015; Nayak et al., 2020). Several crucial aspects, including the extensive utilisation of pesticides, control measures, the manner of inheritance of resistance genes, alterations in individual fitness, and the genetic background of insects, exert an impact on resistance (Shamjana and Grace, 2021).

Numerous elements contribute to the resistance of stored grain pests to insect pests, such as biochemical, physiological, and behavioural factors.

Behavioural resistance in insects occurs when they acquire the ability to reject or avoid pesticides via the process of learning. This occurrence necessitates a stimulus, and insects that possess resistance have the capacity to perceive the hazard and subsequently refrain from eating or departing the treated region by means of locomotion or flight. According to Yu et al., (2008), these insects typically possess highly developed receptors that enable them to detect insecticides at even lower concentrations compared to insects that are vulnerable to these chemicals. Organisms employ many mechanisms to mitigate their susceptibility to pesticides as an initial line of defence (Dunlop et al., 2018; Lushchak et al., 2018). Insects have the capacity to acquire resistance to chemical substances through many methods, with one of the initial and significant ways being a behavioural reaction

(Nansen et al., 2016). In the presence of a fatal toxin, insects frequently exhibit a cessation of feeding behaviour and may opt to vacate the treated vicinity through simple locomotion (De Roode and Lefèvre, 2012; IRAC, 2022).

Biochemical factors govern resistance and stimulate the production of enzymes capable of activating and detoxifying these chemicals. Consequently, this enzymatic activity renders the target enzyme less responsive to the insecticide's effects. Target-site resistance is a prominent mechanism of resistance observed in insects to multiple kinds of pesticides. This process entails modifications or substitutions in the genetic sequences responsible for producing insecticide target proteins. These variations have a detrimental impact on the binding characteristics of poisonous chemicals. Previous studies have documented the presence of conserved target-site mutations in various genes responsible for encoding voltage-gated sodium channels in insect pests. These genes include the ryanodine receptor, acetyl cholinesterase, nicotinic receptor, and GABA receptor. These mutations have been found to confer varying levels of resistance to insecticides (Hollingworth and Dong, 2008; Yu et al., 2008). Mutations result in the stimulation of enzyme overproduction within the insect's detoxifying system. The bulk of enzymes engaged in the process of pesticide detoxification are constituents of extensive collections of genes, commonly referred to as mixed-function oxidases, esterases, and glutathione S transferases. The resistance phenomenon in insects is primarily regulated by the abundance of multifunctional oxidases, which play a crucial role in detoxification processes (Ramzan et al., 2019). The correlation between the mechanism of detoxification and multifunctional oxidases (MFOs) has been established since the discovery of multifunctional enzymes inside the cytochrome P-450 complex. Various classes of insecticides have been found to induce different types of cytochrome P-450 enzymes, each with unique catalytic capabilities. Among the several families of cytochrome P-450 that have been found in animals and plants, a total of four families (namely, families 4, 6, 9, and 18) have been the subject of investigation in the context of insects. The detoxifying activity of microsomal mixed-function oxidases (MFOs) primarily involves cytochrome P-450 oxidases, which are found in clusters of genes and belong to family 6.

Physiological mechanisms confer insect resistance through two primary means: penetration, which inhibits the transfer of insecticides, and insensitivity of nerves. The emergence of behavioural resistance is contingent upon the duration of exposure to insecticidal residues (Zhou et al., 2019). A comprehensive comprehension of resistance mechanisms exhibited by stored grain pests is of utmost significance in devising efficacious measures to mitigate the impact of insect pests that have developed resistance. The physiological classification is valuable for characterising the resistance phenotype arising from genetic alterations and facilitating targeted interventions like employing synergists to counter metabolic resistance. However, to gain a comprehensive understanding of the emergence and dissemination of resistance genes within populations, it is crucial to focus on the specific genetic changes (mutations) involved. Moreover, it plays a significant role in determining the feasibility of developing precise and responsive molecular diagnostic markers. An illustration of this concept is that a single point mutation occurring at a specific target spot can be more readily converted into a DNA-based marker compared to the up regulation of a metabolic resistance gene. In the latter scenario, the probability of establishing a diagnostic indicator utilising RNA or protein abundance is significantly higher compared to an indicator based on the specific mutation, unless there are instances of gene amplification. This is due to the limited understanding of cis- and trans-acting mutations that regulate gene expression in the majority of pests exhibiting metabolic resistance.

In spite of the considerable diversity observed in both species and chemical composition of insecticides, it is noteworthy that just three distinct processes have been identified as the primary causes of pesticide resistance **(Fig.8)**.

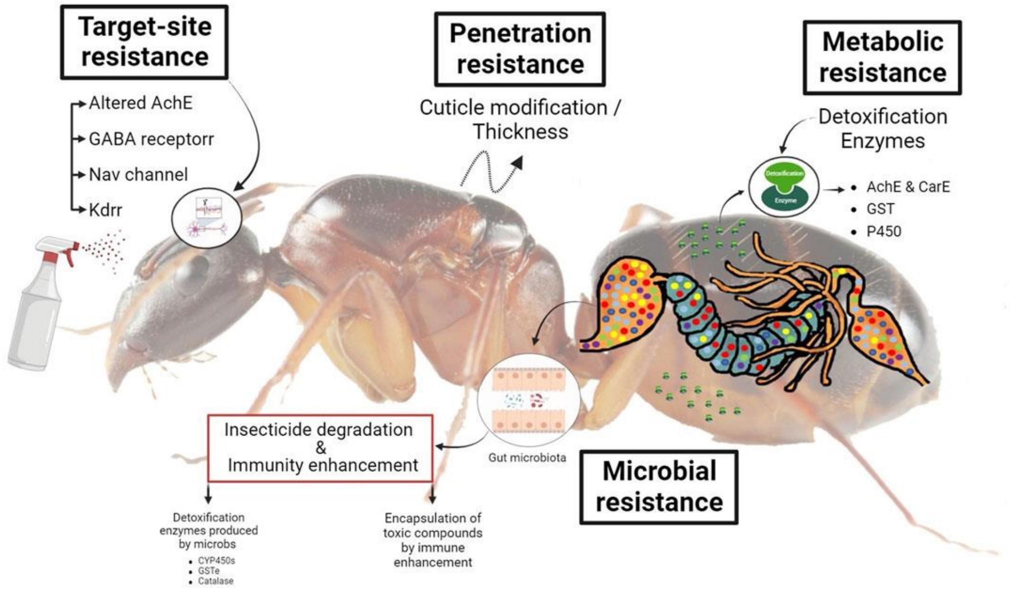

Figure VII: Mechanisms of insecticide resistance (Siddiqui et al., 2023)

1. **Target site insensitivity**, Insecticides, such as organophosphates, carbamates, and pyrethroids, induce neurotoxicity by impeding the activity of acetylcholine esterase, an enzyme closely linked to the central nervous system (Eto M. 1990, Kuhr and Dorough, 1976, Russell et al., 2004; Pang, 2014). According to Heong et al., (2015), the impact of these pesticides extends beyond their intended targets and also affects other locations within the insect nervous system, such as voltage-gated sodium channels (VGSC) and gamma aminobutyric acid (GABA) receptors. The primary focus of the DDT and pyrethroid pesticides is the voltage-gated sodium channel (VGSC) within the nervous system (Toshio,1992).
2. **Metabolic resistance**, the metabolic detoxification system is the predominant resistance mechanism observed in insects. This mechanism allows insects to expedite the degradation or sequestration of pesticides, hence minimising their toxic impact. The resistance mechanism enables insects to increase the production of enzymes, namely cytochrome P450 monooxygenases (cyp450s), carboxylesterases (cares), and glutathione S transferases (gsts), in order to counteract the harmful impacts of pesticides.

3. **Lack of penetration**, reduced penetration is also called cuticle resistance, which leads to a decrease in the amount of pesticide that reaches the insect's body, and is closely linked to insecticide resistance. Typically, contact insecticides have the ability to permeate the cuticle and effectively reach the intended site of action (Matthews et al., 2014). The cuticle is primarily comprised of two distinct constituents, namely chitin and cuticular protein. Additionally, the cuticle is organised into three functional layers, which include the outermost envelope, the protein-rich epicuticle, and the chitin-rich procuticle (Hensel et al., 2016; Kim et al., 2021). Insects gain resistance to cuticular barriers by many mechanisms, such as modifying the thickness of the cuticle, altering its composition (Chen et al., 2018; Benade, 2022), or undergoing cuticle remodelling, which is facilitated by the abundance of cuticular proteins. The upregulation of laccases and ABC transporters has been documented to play a role in the alteration of cuticle composition, hence enhancing insect resistance to insecticides within the surrounding ecosystem.

Insects primarily exhibit resistance through two key processes, namely target site resistance and metabolic resistance. Several alternative mechanisms of insect resistance are often overlooked because to their perceived limited impact on insecticide resistance. These mechanisms are regarded to provide only minor resistance to insecticides and can be controlled in conjunction with major mechanisms. The primary focus of this study pertains to the P-gp pumps, which facilitate the translocation of various metabolites and xenobiotics across cellular membranes (Hollenstein et al., 2007). In addition, new research has examined the involvement of ABC transporters in insects as a potential mechanism that regulates insect resistance. This mechanism operates by aiding the removal of insecticides and their metabolites, which are produced during phase I and II reactions (O'Donnell, 2008). In a broad sense, the process of detoxification in insects can be categorised into three distinct phases: phase I, phase II (which encompasses the activity of metabolising enzymes), and phase III (which involves the participation of transporters) (Xu et al., 2005). The primary enzymes implicated in the phase I and phase

II detoxification pathways (Xiao et al., 2018). Conversely, the principal constituents of phase III are ATP-binding cassette (abc) transporters (Ferreira et al., 2014).

The contribution of P-glycoprotein (P-gp) pumps to the phenomenon of pesticide resistance has been assessed by the examination of increased expression levels of ATP-binding cassette (abc) transporter genes across various insect species (Bariami et al., 2012; Dermauw and Van Leeuwen, 2014). Penetration resistance is an adaptive mechanism employed by certain insects to impede the effective delivery of insecticides, as it hinders the penetration of these chemical agents through the cuticle of resistant insect species. The development of penetration resistance is attributed to physicochemical changes in the cuticle structure, resulting in reduced chemical absorption. Consequently, only a limited quantity of insecticide is able to permeate these physical barriers. Nevertheless, this particular system has a limited level of insect resistance, despite its effectiveness in shielding insects from numerous forms of xenobiotics. Instead, it exerts its influence by synergistically combining with other resistance mechanisms, thereby amplifying their impacts. According to Oppenoorth and Welling, (1976) and Scott and Way, (1990), it has been proposed that detaining xenobiotics and having a limited rate of penetration could potentially allow for a longer period of time for the detoxification process.

Nevertheless, other investigations have also established a correlation between resistance and qualitative alterations in detoxification processes (Joußen et al., 2012; Riveron et al., 2013 and 2015; Zimmer et al., 2018). Merely assessing the levels of gene expression may not accurately reflect the development of resistance. Additionally, pests can acquire resistance through various mechanisms, and it is not always evident whether different populations of a particular species develop resistance through similar mechanisms. Across certain instances of target-site resistance, it has been observed that comparable, if not indistinguishable, mutations arise across diverse populations within a same species, and occasionally across distinct species as well. Resistance to pyrethroids has been linked to the presence of kdr and super kdr mutations in domain II of the voltage-gated sodium channel in a minimum of 50 arthropod species (Feyereisen et al., 2015). Several new examples have emerged, such as the G4946E mutation found in the ryanodine receptor. This mutation provides resistance to diamides and has been observed in four distinct lepidopteran species. Notably, populations of *Plutella xylostella* spanning three continents have been reported to possess this mutation (Steinbach et al., 2015; Roditakis et al., 2017; Pires Paula et al., 2021).The control of these pest insects is mainly based on the use of chemical insecticides and/or the cultivation of transgenic plants expressing insecticidal proteins (However, the efficacy of these control measures is often jeopardized by the development of resistance owing to the frequent application of insecticides and long-term exposure. Understanding the molecular mechanisms of resistance that these pest insects have developed is essential for the implementation of sustainable control methods and resistance management strategies (Le Goff and Nauen, 2021).

The utilisation of diagnostic techniques that assess the vulnerability of pest populations is of paramount importance in the selection of chemical agents. These tools enable the effective management or prevention of resistance by facilitating the identification and control of resistance incidence and propagation. Diagnostic bioassays have been extensively developed for several agricultural pests and disease vectors; however, very limited effort has been devoted to pests that infest stored grain. Resistance monitoring frequently utilises bioassays, although their suitability for high-throughput applications hinges on the ease of insect collection, storage, and laboratory cultivation. With the ongoing discovery of a growing repertoire of molecular markers associated with resistance, the use of high-throughput, expeditious, and precise molecular diagnostic platforms present a viable solution to circumvent the requirement for laborious bioassays. Resistance development is a biological process that occurs through evolution, characterised by alterations in the physiology and biochemistry of resistant strains. These changes are commonly explained in terms of toxicodynamic and toxicokinetic mechanisms. The toxicokinetic mechanisms encompass alterations in the processes of penetration, activation, metabolism, transport, and excretion, which ultimately affect the quantity of toxin that reaches the intended site. On the other hand, the toxicodynamic mechanisms involve modifications to the pesticide target site, such as structural changes, knock-out, or amplification (Tierney and Kennedy, 2013; Feyereisen et al., 2015).

The field of genomics has witnessed notable advancements, including the use of transcriptome sequencing and whole-genome sequencing, which have contributed to substantial advancements in comprehending resistance mechanisms. These mechanisms encompass metabolic resistance, penetration resistance, and knockdown resistance. The field of microbiome research, which combines entomology with microbiology, encompasses a particularly intriguing and swiftly progressing domain. This subject focuses on investigating the capacity of whole assemblages of bacteria, viruses, and fungi residing within insect hosts to mitigate the toxic effects of insecticides. Prior research has elucidated potential mechanisms of resistance in candidates, including symbiont-mediated pesticide resistance in many insect species. Additionally, these investigations have revealed the principal bacterial taxa involved in the process of adapting to detoxify xenobiotic chemicals (Dada et al., 2018; Shamjana and Grace, 2021; Wang et al., 2021).

Through the use of functional genomic technologies such as the CRISPR/Cas9 gene-editing tool, resistance mechanisms in insects should be examined not only in terms of the expression of a single gene, but also in terms of numerous gene interactions (Wei et al., 2019). A previous study suggested that there is a specific molecular mechanism underlying resistance. From 32 *D. melanogaster* genes and proteins involved in insecticide resistance, 21 genes have been identified (Zhang and Zhang, 2019).

Occasionally, a combination of processes, such as modifications to the target site and enhanced detoxification, can lead to resistance. The genetic foundation of resistance can differ among insect populations; even within populations, there may be different mechanisms of resistance. This is a crucial point to remember. To create new pesticides and adopt integrated pest management techniques that can prevent the emergence and spread of resistance, it is crucial to understand the molecular basis of resistance.

Typically, there exist variations in the feeding patterns, biological characteristics, behavioural tendencies, ecological roles, and detrimental manifestations exhibited by individual insect species. The life cycle of insects is a prominent factor in influencing their ecological interactions and the development of resistance to pesticides (Sudo et al., 2018). During the life cycle of a pest, there are variations in the manner in which it interacts with its host and the surrounding environment. The primary determinant of insecticide resistance development is the life cycle of an insect pest. Specifically, insect pests with lengthy life processes (Saulich, 2010) and a short life cycle accompanied by a high number of offspring possess the necessary characteristics for rapid resistance development (Karunamoorthi and Sabesan, 2013).

The work done in our lab in the past few years has established the mechanism of insecticide toxicity in different insect and insect cell lines. Furthermore, the successful development of primary cell lines from stored grain pests (*S. oryzae*) to test pesticide toxicity (Thakkar et al., 2020) has been well explored. However, combining the literature and laboratory data, there is a lacuna as far as the molecular mechanism for pesticide resistance of the stored grain pest *Callosobruchus chinensis* is concerned. ***Therefore, the present inventory was designed to understand the molecular mechanisms of insecticide resistance in stored grain pests (C. chinensis).***

Why *Callosobruchus Chinensis*?

Callosobruchus chinensis exhibits a cosmopolitan distribution pattern and has been observed in numerous countries as a result of the international trade of beans (Parish et al., 2017). The natural habitat of the beetle encompasses the tropical and subtropical regions of Asia, where its population has experienced significant growth as a result of the widespread production and distribution of leguminous plants. The spread of these organisms is significantly impacted by our activities, and their habitat is restricted to leguminous plants that provide ideal conditions for reproduction and larval sustenance. Both larvae and adult individuals consume legumes. The common host plants of these organisms encompass green gram, lentil, cowpea, pigeon pea, chickpea, and other pea species, although they have been observed to inhabit numerous more legume hosts (Fite and Tefera, 2022).

Seed beetles belonging to the genus *Callosobruchus* are agricultural insect pests that primarily inhabit tropical and sub-tropical regions (Southgate, 1979). These pests are known to inflict significant harm to various leguminous crops during the storage phase. The *Callosobruchus* species that are frequently observed in India include *C. maculatus* (Fabricius), *C. chinensis* (Linnaeus), and *C. analis* (Fabricius) (Raina, 1970). The infection initially originates in the field, where adult insects deposit eggs on fully developed pods. Subsequently, a secondary infestation occurs during storage after the crops have been harvested. This phenomenon is responsible for significant grain losses, occasionally amounting to as much as 99% within a six-month period (Seck, 1993). The process of oviposition takes place on the external surface of seeds, namely on the seed coat. Larvae then proceed to burrow directly into the cotyledon by penetrating the seed coat after a period of 5-6 days of incubation.

Pupation occurs within the seeds under optimal environmental circumstances characterised by a temperature range of 25-30°C and a relative humidity range of 65-70%. The emergence of sexually mature adult beetles takes place during a period of 25-35 days, with the duration varying depending on the specific host seeds (CABI, 2014a). The susceptible host seeds of *C. chinensis* have a minimum developmental time of around 22-23 days (CABI, 2014a; CABI, 2014b). In the given circumstances, adult individuals reach maturity within a 24-hour period following emergence, and exhibit an average lifespan ranging from 12 to 14 days. During this temporal span, the processes of mating and oviposition occur. Typically, multiple adults arise from a single seed, while a solitary adult beetle emerges from a seed of green or black gram (Seram et al., 2016). This unique characteristic makes it an ideal model for investigating insectcide resistance mechanisms in controlled laboratory situations.

Based on the aforementioned information, the current research aims to investigate the host preference (Chapter I) and transgenerational effects of insecticides on insect pest development (Chapter II) in order to decipher the underlying mechanism of insecticide resistance in stored grain pests (C. chinensis) using a transcriptomic approach (Chapter III).

Objectives of the study

1. Developing the Culturing conditions of the *C. chinensis* in laboratory conditions:

a. Collection and identification of *C. chinensis* from ware houses of Vadodara.

b. Studying the host preference by the insect.

2. **Transgenerational effects of insecticide on the development of *C. chinensis:***

a) Determine the lethal concentration of insecticide on the pest insect- Probit Analysis

b) Assessment of the transgenerational effect on the development parameters of *C. chinensis.*

c) Transgenerational effect of the insecticide on the repellency behaviour of *C. chinensis.*

3. **Understanding the mechanism behind insecticide resistance in stored grain pests via a transcriptomic approach**

- i. Penetration resistance
    - a. Cuticular genes
- ii. Metabolic resistance
    - a. cyp450s
    - b. esterases and gsts
    - c. abc transporter