Introduction

Science and technology have been concentrating on human needs in recent decades, especially adequate nutrition and medical treatment due to the rapid increase in the human population (Gangwar *et al.*, 2014). The fast-growing human population creates the need for the sustainable intensification of agriculture throughout the world which can be accomplished by adopting mechanisation and new technologies to close yield gaps while minimizing environmental impacts. Methods for producing food and other materials and storing them for future use have developed enormously with the continuous development of human civilization over the past 10,000 years or so (Tayade et al., 2013; Siegwart, 2015; Nayak and Daglish, 2018). To feed the world's bulging population, providing a food has become one of the most important priorities for both the developed as well as developing nations across the globe.

Approximately 11% of the 13.4 billion hectares (ha) of the earth's land is devoted to agriculture (Ausubel *et al.*, 2013; Kanianska, 2016) and despite great advances in agricultural productivity and economic well-being in much of the world over the past 50 years, food diffidence continues to be a serious issue for large sections of the human population. Agriculture has to continually adapt to rising environmental concerns in conjunction with meeting the increasing consumers demand (Pan-UK, 2003). Interestingly, the greatest episode of population growth in human history was accompanied by an increase in the per capita food supply, especially during the first half of this period. This was made possible by the 'green revolution', which resulted in a quantum jump in the productivity of major cereal crops in Asia and to a lesser extent other parts of the world. It has helped to avert mass deprivation but have contributed to the population explosion (Shahidi and Chandrasekara, 2013; Schuster and Torero, 2016; Sharma *et al.*, 2017). The agriculture sector has recorded satisfactory growth due to improved technology, irrigation, inputs and pricing policies. The environmental consequences of the green revolution have been criticized. For example, some of the fertilizer and other chemical inputs that farmers have applied to their fields have found their way into rivers, lakes, and streams, thereby causing pollution. In addition, irrigation development has in some places led to the depletion of hydrologic resources. While they do not deny these impacts, Southgate *et al.,* (2007) has pointed out that much environmental damage is mainly a consequence of misguided policies. Thus, agriculture is the largest contributor to biodiversity loss with expanding impacts due to changing consumption patterns and growing populations. During the last five decades, intensive agriculture utilizing green revolution technologies has caused tremendous damage to the natural resources that sustain it (Pimentel *et al.,* 2000; Pimentel, 2017). Fresh water, soil quality, energy and biodiversity are all being depleted, degraded and/or polluted (International Food Policy Research Institute, 2016). Along with biodiversity loss, ecosystem services also get affected.

Biodiversity refers to the variability among living things and the ecosystems they inhabit. Biodiversity is variation of life and refers to all species of plants, animals and microorganisms existing and interacting within an ecosystem (Dirzo and Raven, 2003; Samways, 2005). This biodiversity performs a variety of ecological services in an ecosystem, which support one another and work together to form a stable and sustainable ecosystem. Traditionally the determination of biodiversity was based on the number of species in an ecosystem, with ecosystems with a higher number of species being perceived as more stable and resilient. Nevertheless, biodiversity is not only about numbers in an ecosystem, but also about the particular characteristics that different species have contributed. Traits are defined as physical or behavioural characteristics that evolve in response to competitive interactions and abiotic conditions (Woodcock *et al.,* 2014). Different species have different characteristics, expressed in certain attributes, and these particular traits contribute to the health and stability of the environment. Traits will therefore influence survival, fitness and rates of resource processing, consequently influencing ecosystem services (Hoehn *et al.*, 2008; Straub *et al.*, 2008).

It is key to ensuring continued life on earth and is essential for sustainable agriculture and food production. Biodiversity is necessary to sustain vital ecosystem structures and processes, such as soil protection and health, water cycle and quality, and air quality. It also provides the genetic resources for the breeding of new, locally-adapted crop varieties. Agriculture is now known to be one of the main factors associated with biodiversity loss (Dunphy, 2019; Marques *et al.*, 2019). Several key components of biodiversity for food and agriculture at genetic, species and ecosystem levels are in decline. Many species, including pollinators, soil organisms and the natural enemies of pests, that contribute to vital ecosystem services are in decline as a consequence of the destruction and degradation of habitats, over exploitation, pollution, and other threats. In particular, land-use changes and unsustainable management practices, including over-exploitation of soil and over-reliance on pesticides and other chemicals (Kumar and Singh, 2015). Agricultural fauna is filled with valuable biodiversity that is continually influenced by farming practices. The use of crop protection products to control insects, diseases and weeds are important contributors to biodiversity loss. Such large scale human activities impact the ecology and biogeography of many organisms, including insects.

Insects have been hugely successful in terms of both species richness and abundance. Insects make up the most numerous group of organisms on earth, approximately 66 % of all animal species, and being good dispersers and exploiters of essentially all kinds of organic matter can be found almost everywhere, forming an important part of every ecosystem and are vital in our food supply chains performing important ecosystem services. Insects, as drivers of ecosystem functions play a major role in agro-ecology, the management of agricultural systems in an ecologically sound and sustainable way by encouraging ecosystem services provided by beneficial organisms (Pywell *et al.,* 2015). In terrestrial ecosystems insects play key ecological roles in diverse ecological processes such as nutrient cycling, seed dispersal, bioturbation (De Groot *et al.,* 2002; Nichols *et al.,* 2008), pollination (Gabriel *et al.,* 2006; Slade *et al.,* 2016), and pest control (Landis *et al.,* 2000; Brewer *et al.,* 2004; Bell *et al.,* 2008; Lonsdorf *et al.,* 2009). Insects are the main components in diverse ecosystems as major role players in functioning of ecosystem processes. Since insects are mostly perceived as pests or potential pests, this ecological importance of insects often goes unnoticed. The main ecological actions of insects in ecosystems are environment regeneration, pollination, predation/parasitism, and decomposition.

Insects have been predominantly perceived as competitors in the race for survival. Herbivorous insects damage 18% of world agricultural production. Despite of this damage, pests are considered to be less than 0.5 percent of the total number of known insect species. Insect pests are created through the manipulation of habitats by humans, where crops are selected for larger size, higher yields, nutritious value, and are cultivated in monocultures for maximum production (Jankielsohn, 2018). Monocultures essentially create “biological deserts” where only a limited number of species can survive. However, monocultures provide a highly beneficial environment for certain herbivorous insects to increase their population which are capable of evolving to any of the biotypes and can adapt to new situations. Due to their relatively fast life cycles and extreme selection stress, insect pests have enormous capacity to develop adaptive mechanisms to survive under the combined pressures of continued global warming and modern agricultural practices (Pinto *et al,* 2013). Natural predators are killed in the process when using pesticides, leaving minimal control over the growth of the herbivorous insect population. Ecosystem functions are further decreased by chemical fertilizers. Overuse of nitrogen fertilizer enables a few plant species to thrive, while the majority of plant species that have symbiotic relationships with insects disappear from the system (Schwägerl *et al.,* 2016). The intensification of agricultural production systems in combination with high agrochemical input in crop fields, are the primary causes for the rapid decrease of biodiversity (Robinson *et al.,* 2002) The loss of this agricultural biodiversity have both financial and social risk, as well as lasting effects on agricultural productivity, impacting food wellbeing in the long term. Continued sustainable land use in unpredictable and changing environments is only possible with conservation of maximum biodiversity (Tscharntke *et al.,* 2005). A shift to sustainable agriculture will require changes in production methods that will enhance diversity in farming systems.

Of the 39 taxonomic orders of insects, species belonging to only three orders, Coleoptera (beetles), Lepidoptera (moths) and Psocoptera (psocids) are considered as pests of stored commodities (Stork *et al.,* 2015). In addition, a few species belonging to the orders Hemiptera (bugs) and Hymenoptera (wasps) are also being reported to be associated with stored commodities, but only as predators or parasites (Thacker, 2002; Rees 2004; O'Callaghan and Gerard 2005; Heaps *et al.,* 2006; Bravo *et al,* 2007). There are thousands of species of insect pests spread across most parts of the earth where poikilothermic animals can live. That species of pest is confined to those accessible areas that provide food and other biological and physical essentials. Insect pests on an average are estimated to cause 15-20% yield losses in major crops. Pest insects may cause problems by damaging crops and food production, by parasitizing livestock, or by causing harm and human health risks. Insects cause widespread damage to agricultural and forestry products during storage and distribution. Large amounts of stored grains are damaged both by actual consumption of the grain and by contamination with whole insects, insect fragments, and faeces. The contamination of food by insect pests is a constant source of loss and concern. In India, post-harvest losses caused by unscientific storage, insects, rodents, microorganisms, etc., account for about 10 per cent of total food grains. The major economic loss caused by the insects is not always the actual material they consume, but also the amount contaminated by them and their excreta which make food unfit for human consumption. About 600 species of insects have been associated with stored grain products. Nearly 100 species of insect pests of stored products cause economic losses (IGMRI, 2019).Storage insect pests are categorized into two types viz. Primary storage pests: Internal and External feeders and secondary storage pests. Primary pests attack whole grain and are capable of to penetrate in seed coat and pod to feed on the embryo, endosperm or cotyledons. Secondary pests feed on grain products or grain that has already been damaged by the primary pests or as a result of harvesting, handling and transporting. The primary pests typically have a narrow range of food preferences such as cereals and pulses. The secondary pests, however, have a wide host range including damaged whole grains, milled products such as flour, processed and manufactured food products such as breakfast cereals, chocolates and compound animal foods (Singh *et al.*, 2014). There is a distinct difference in the life cycle of pests belonging to these two categories. The life cycle of a primary pest involves lodging of the eggs inside or on the outer coat of grain, followed by development within the grain, making the immature stages difficult to detect. Because the entire life cycle often takes place inside the kernel these primary pests are also called as internal feeders. In contrast, the eggs of secondary pests are laid in a scattered manner in or near the food source where the developing larvae can easily be seen. As the entire life cycle takes place outside the whole grain, the secondary pests are called as external feeders. Due to the major difference in life cycles, damage to whole grain by primary pests is highly distinctive in nature and pest recognition is easier compared to the secondary pests.

Most of the stored grain pests are considered as opportunists; several beetles (Coleoptera) were initially recorded under the bark of trees; several moths (Lepidoptera) were supposedly originated from dead and ripening fruits; whereas several psocids (Psocoptera) were originated from leaf litter (Rees, 2004; Chattopadhyay and Sen 2013; Chattopadhyay *et al.,* 2014a, b). A rare exception is the granary weevil *Sitophilus sp.* (Coleoptera: Curculionidae), which is the only species that has never been detected outside the storage environment (Plarre, 2010). The oldest record of storage pests associated with human beings goes back to ancient Egypt, where *Tribolium confusum Jacquelin du Val* (Coleoptera: Tenebrionidae), *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) and *Sitophilus oryzae* (Coleoptera: Curculionidae) were reported (Rees, 2004).

Over the past couple of decades, insect pest control has been mainly conducted by the application of chemical pesticides because of the low cost and efficacy, but their indiscriminate use has caused alarming problems with the emergence of insect resistance to pesticides together with secondary pest outbreaks (Mishra *et al.,* 2013). A number of insect predators and parasitic wasps attack insect pests of stored grain and can be used effectively if applied in overwhelming numbers. However, biologicals are generally not used because the US Food and Drug Administration (FDA) and food processors do not accept live insects or insect parts in raw grain. Moreover, biological agents have limited commercial availability and are cost prohibitive, except perhaps when used in organic production (Weaver and Petroff, 2004). Controlling insect pests can be very difficult because of the variety of species that can infest grain. Moreover Biological control has a limited scope in stored-grain pest management in spite of being a major part in IPM.

The development of new biotechnological approaches, with the introduction of insect-resistant transgenic Bt-plants has decreased pesticide utilization in certain key crops, such as cotton and maize, resulting into economic and environmental benefits (Gomez *et al.,* 2007; Bravo and Soberon, 2008; Kaya *et al,* 2008; Wu *et al,* 2008; Kos *et al.,* 2009; Bravo *et al.,* 2011; Soberon *et al.,* 2012; Qureshi *et al.,* 2014; Ruffner *et al.,* 2015; Peralta and Palma, 2017). The global losses due to insect pests have declined from 13.6% in post-green revolution era to 10.8% towards the beginning of this century. In India, the crop losses have declined from 23.3% in post-green revolution era to 15.7% at present (Dhaliwal *et al.,* 2015; Sangomla, 2018). But once again, insect resistance has arisen against the Bt toxins, and outbreaks of non target pests have emerged (Bravo and Soberon, 2008; Bruce *et al.,* 2009; Tabashnik *et al.,* 2013), which makes it necessary to develop innovative approaches to control selected agricultural pests. Pests and diseases are the prime factors causing low agricultural productivity, which are mostly controlled by chemical means. Despite the advantages of applying chemical pesticides in improving food quantity and quality, negative effects on human health and serious environmental problems challenge these benefits (Dhaliwal and Singh, 2000; Konradsen *et al*., 2007; Nicolopoulou-Stamati *et al.,* 2016; Chattopadhyay *et al*., 2017). In spite of various control measures against pests farmers are mainly depend on chemical control which cause consistently increase in crop loss (Dhaliwal and Koul, 2010). This is due to the misuse and overuse of insecticides that cause resistance and increase the insect pest survival rate (Douglas, 2018).

Insecticides are any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest which is harmful to stored grains. Insecticides may also be described as any physical, chemical, or biological agent that will kill an undesirable insect growth (Coleman-Jensen *et al.,* 2014). Pesticide is a generic name for a variety of agents that are classified more specifically on the basis of the pattern of use and organism killed. They are highly effective, rapid in curative action and adaptable for most situations, flexible food crop production systems to control crop pests and diseases and ensure maximum yield with relatively high market value (Damalas and Eleftherohorinos, 2011). Despite these credentials, the long and indiscriminate use of pesticides has been found ecologically unsound; associated with disadvantages like, pest resistance, outbreaks of secondary pests, adverse effects on non-target animals, objectionable pesticide residues and direct hazards to the users. The persistence of these chemical inputs in crop fields, however, enhances biodiversity and human health threats (Dicke, 2017; Ali *et al.,* 2019; Deb, 2019).

The worldwide distribution of toxic substances in the ecosystem is one of the major concerns (Stenstrom, 2013). The use of pesticides in agriculture is a part of the wider spectrum of industrial chemicals used in modern society. Modern agricultural practices reveal an increase of pesticides to meet the food demand of an increasing population which results in contamination of the environment leading to the surfaced controversy of use and abuse of pesticides. The rampant use of these chemicals, under the saying "If a little is good, a lot more will be better" has played a destruction with human and other life forms (Aktar *et al.,* 2009; EEA., 2010; Alexandratos and Bruinsma, 2012; Ullah *and* Zorriehzahra, 2015). In terms of monetary value, an average of ~36 billion US dollars is spent per year for synthetic insecticides to control agriculturally and medically important insect pests (Oliveira *et al.,* 2014; Dhaliwal *et al.,* 2015; Dhaliwal *et al.,* 2018). The problems associated with these general toxicants, including insect resistance and environmental concerns, have encouraged the development of more insect-specific insecticide screening procedures (Beckmann and Haack 2003).

Screening for novel insecticides is a slow and tedious process that could be made more efficient by using insect cells in standardized culture medium as an *in vitro* screening. Over the past decades, different industries seem to have an increasing interest in the production of *in vitro* methods for the study of insecticide effects. These approaches have recently been regarded as potential alternatives to traditional animal toxicity testing (Smagghe, 2007). They further reduce the need for time consuming and costly tests performed using animals or isolated organs (Decombel *et al.,* 2004; Watts *et al.,* 2003). The commercially available Sf9 insect cell line derived from *Spodoptera frugiperda* pupal ovarian tissue is used in *in vitro* assays to estimate the effect of different insecticides (Saito *et al.,* 2005; 2006) and to test the effect of fungal metabolites (Fornelli *et al.*, 2004) or the effect of some insect fungi that could be produced as bio pesticides (Watts *et al.,* 2003). Sf9 cell line was further used to study *Bacillus thuringiens* is toxins and to explore their mode of action (Rang *et al.,* 1999; Agrawal *et al.,* 2002; Rebek *et al.,* 2012). *In vitro* studies are becoming a useful assay method in cytotoxicity screening of insecticides, not only because they can greatly short screening time but also provide more useful information, such as insecticides' mechanisms of action (Zhang *et al.,* 2012a, b). Through cytotoxicity screening *in vitro,* some potent compounds with good insecticidal activities can be detected at cellular level.

For screening purposes, there is increasing interest in the development of *in vitro* methods to replace conventional animal toxicity tests, because mass rearing of these entire pests can be difficult and also *in vivo* bioassay can be tedious to perform. Therefore, Insect cell culture could be an alternative to insect mass rearing and its bioassay for entomopathogens and their toxins, growth regulators or various chemicals (Smagghe *et al.,* 2009). Additionally, these approaches are time and cost effective, which can be performed using animals or isolated organ (Watts *et al,* 2003; Decombel *et al.,* 2004). This being said, there are a certain number of key requirements that need thorough consideration before developing an alternative cell-based testing procedure.

In particular the following points need to be addressed:

- Dependable intra- and inter-laboratory reproducibility
- High predictive power to guarantee correct toxicity assessment decisions
- Relevance to the type of compounds that are intended to be tested
- Simplicity
- Possibility for high throughput screening (HTS) with automation
- Low cost/benefit ratio

In particular, insect cells have several qualities that suggest feasibility of large-scale production such as ambient growth conditions, low media requirements and straightforward adaptation to suspension and serum-free culture (Neermann and Wagner, 1996; Donaldson and Shuler, 1998; Lynn, 2001; Ikonomou *et al.*, 2003; Mitsuhashi and Goodwin, 2018; Rubio *et al.*, 2019). Established insect cell lines fulfil these criteria and therefore pursuing research and development by using insect cell line will be a powerful means of generating new sustainable *in vitro* technique for the screening of the insecticide directly at the cellular level. Furthermore, tests employing cell cultures can be readily automated and cell based assays can be developed that enable the elucidation of new modes of action for insecticide candidates. Insect cell cultures that have retained their arthropod specific metabolic pathways or hormonal regulation will also allow the development of screening procedures using insect specific targets.

The first true cell line from Insects was established from the pupal tissues of the moth *Antheraea eucalypti* by TDC Grace of Australia in 1962 (Grace, 1962). Grace in 1966 reported the establishment of the first mosquito cell line in the world from *Aedes aegypti* mosquitoes. Subsequent studies using isoenzyme analysis indicated this cell line as a contaminant of *Antheraea eucalypti* cell line (Greene *et al.*, 1972). From the embryonic cell lines from the cotton boll weevil *Anthonomus grandis* was established by Stiles and his team in 1992. In 1999 Iwabuchi had successfully developed the cell line from the fat body tissue of the cerambycid beetle *Xylotrechus pyrrhoderus*. Some of the efforts were also made to establish cell line from insect order Coleoptera. Successful cell line were established from 3 spices of family Scarabaeidae i.e *heteronychus arator*, *Anomala costata* and *Antitragus parvulus*, and 2 species of Chrysomelidae i.e *Diabrotica undecimpunctata* and *Leptinotarsa decemlineata* (Iwabuchi, 1999). Lepidopteran cell lines have primarily been developed to reproduce insect viruses as a bio pesticide to combat insect pests. Recently, the expression vector system of the baculovirus combined with insect cell cultures has become more effective for the expression of many heterologous proteins than other systems, such as bacterial, yeast, vertebrate viruses, etc., due to its unique characteristics (Gupta *et al.,* 2012).

This technology is also being used in the construction of recombinant baculoviruses to use as bio pesticides that offered comparatively faster killing of insect pests than by wild type baculoviruses (Fuxa *et al.,* 2002), which was further characterized and tested against cytotoxicity of solubilised crystal delta-endotoxins from different *B. thuringiensis* formulations of Destruxins, mycotoxins from *Metarhizium anisopliae,* was tested on these cell lines (Charpentier *et al,* 2002). Met was found to form a functional JH receptor with another bHLH-PAS transcription factor, steroid receptor co-activator, based on studies in *Drosophila melanogaster*, *Aedes aegypti*, and *Tribolium castaneum* (Jindra *et al.,* 2013). In Japan study was done to establish the novel cell line (TC81) from the insect *Tribolium castaneum* embryos and validate the utility of the cell line by analyzing the juvenile hormone signalling pathway (Kayukawa *et al*, 2013). The study of insect cells has made rapid progress in recent years and more than 500 cell lines from over 100 different insect species have been described worldwide. The main sources of these insect cell lines include various tissues from the insects in the orders of Lepidoptera, Diptera, Coleoptera, Blattaria, Hymenoptera, Orthoptera, Hemiptera, etc. Among them, cell lines from Diptera and Lepidoptera has the largest number (Zheng *et al,* 2013).

In the Insecta, Coleoptera has the most species and widest distribution, with over 3,60,000 reported species worldwide, accounting for approximately 1/3 of the world's known insect species. Many of these insects are herbivorous, and feed on different hosts, and thus are important pests, bringing serious losses to agriculture, forestry, and horticulture. However, in-depth studies of insect cell lines derived from Coleoptera remain deficient, and to date only fewer than 30 cell lines have been established from five families (Scarabaeidae, Chrysomelidae, Curculionidae, Cerambycidae, and Tenebrionidae) of Coleoptera (Crawford 1982; Lynn 1995; Long *et al.*, 2002; Mitsuhashi, 2003; Hoshino *et al.*, 2009; Goodman *et al.*, 2012). The successful establishment of these cell lines have provided excellent experimental materials for studies in insect pathology, insect toxicology, insecticide toxicity assay, pest control, insect virology and other research areas (Douris *et al.*, 2006; Alhag *et al.*, 2007; Li and Bonning, 2007; Soin *et al.*, 2009, Monti *et al.*, 2014; Mandrioli *et al.*, 2015).

As far as India is concerned, in 1967, the first attempt was made to establish two cell lines at the National Institute of Virology (NIV), Pune from the larval tissues of *Aedes aegypti* and *Aedes albopictus*. Further, attempts were made to establish a new embryonic cell line cultures from embryonic tissues of *Culex tritaeniorhynchus* (Athawale, 2002), a new cell line from the embryonic tissue of *Helicoverpa armigera* was established by Sudeep *et al* in 2002 and designated as NIV-HA-197. Later, Khurad and his team in 2006 developed a new cell line from ovarian tissue of a commercial variety of the silkworm and proved that new cell line is highly susceptible to BmNPV infection. A number of well characterized, Dipteran and Lepidopteran cell lines are available with NIV but the potentials of these cell lines are not yet fully exploited. In Gujarat no organization till now has dealt with study of such establishment of insect cell line but if we see national scenario there are two main organizations which are currently working for as specially insect cell line, namely GVK biosciences, Hyderabad and National Institute of Virology (NIV), Pune.

In recent years, there is renewed interest in developing new insect cell lines due to their potential application in biotechnology, virology, pathology, biochemistry, genetics and other fields of biology and medicine (Sudeep *et al.*, 2005 a and 2005 b; Li and Bonning 2007; Soin *et al.*, 2009; Monti *et al.*, 2014 and Mandrioli *et al.*, 2015).

Keeping in view the above mentioned facts the present study is an attempt to rear (Chapter I) isolate, culture and characterize insect cell line from Coleoptera stored grain pest (Chapter II) in order to provide a tool for physiological studies as well as in vitro bioassays and insecticide screening studies (Chapter III).