

CHAPTER 1

1.0 INTRODUCTION

1.1 Indian agriculture- History & New Trends

Agriculture has been the mainstay of the Indian economy since history and it continues to be the same for long. In a recent report, highlights of Indian agriculture was portrayed, which cited that 17% of the world's total population is supported by our nation (Pandey, 2009). Unfortunately, the current yield is unable to meet the demand of the burgeoning population (Ali & Gupta, 2012). One added difficulty to this is the climatic variation, which often leads to poor yield. All these “factors” collectively questions the food security of the world. In a recent survey the aching fact of hunger prevalence in India was illustrated with 842 million in 2012-13 (FAO, 2013). This constrains demand for awareness and proper management of grains throughout its journey from harvesting in the fields until it reaches its end-users. These demands are indeed complex and challenging but need to be addressed.

Agriculture is undoubtedly the largest supporter of livelihood majorly in rural areas (Ahluwalia, 1978). Hence, the basic focus of masses is to increase the yield which could be made possible with the development of technology. By the end of 19 century, concept of sustainable utilization of the existing commodities was budding. Sustainable agriculture assures the food security, rural development and implementation of environmentally sound technologies, which are dubbed to be the holistic approach towards this substantial challenge.

Low productivity is an additional limitation faced and soon realized by the central government who has initiated major programmes to fill the lacunae. Department of Agriculture Cooperation & Farmers Welfare, Headquarters at New Delhi, planned and implemented numerous schemes namely ATMA, AGMARKNET, DBT in Agriculture, Pradhanmantri Krishi Sinchayee Yojna, Plant Quarantine Clearance, etc. to address these issues.

1.2 Major food grains: Harvesting & Post harvesting threats

As different initiatives have struck country's agrarian economy, population engaged in agricultural activities (> 60%) could boost the yield. A major share of agriculture comes from the cultivation of cereal grains like wheat, rice, barley, oats, sorghum etc., which constitutes the staple food of our country. Surprisingly, India, being a cosmopolitan nation, depicts regional preference for the grains which depends on the type cultivated in that locality (Chakravarti, 1974). However, the diet chart is dominated by rice and wheat and are categorised as the most favoured cereal in our nation (Gupta, 1980).

Perhaps, the importance of these grains has created interest among the masses about its cultivation process. Starting from harvesting followed by grain processing and finally its storage in warehouses are possibly the major steps. The two prevalent seasons of our country is Kharif season (July to October) and Rabi season (November to May) which harbours rice and wheat cultivation respectively. Though harvesting is circumscribed to abiotic factors, its demand continues throughout the year. To meet this demand, more than 70% of the food grains are stored. However, challenges do not end here. Grains in the storage call for an additional level of attention in the warehouses. It can be explained since storehouses harbour humid environment, copious food resources and many more (Driscoll et al., 2000). Moreover, increased shelf life increases the concern for healthy maintenance of the grains adding greatly to the difficulty (Hertog, et al., 2014).

Grain loss is an unfortunate fact which cannot be denied (NITI Aayog, 2015). More often than not, warehouses experience a heavy loss of stored grains due to biotic (insect-pests, diseases, weeds), abiotic (drought, salinity, heat, cold, etc.) factors and majorly due to lack of caution (Aulakh & Regmi, 2013). Grains are prone to different types of infestation and the list is dominated by insects due to abundant food resources, high moisture content and suitable temperature (Ahmed, 1983). Among the less concerned agents, fungi, mites, birds and rodents are causing considerable damage to the grains. This is to worry that 10-40% grain deterioration is reported every year in storage

(Sharon et al., 2014). In his work, Sharon cited that major damage is inflicted to wheat putting food security of the world in question.

1.3 *Triticum aestivum*

As westernization grasped our nation, processed wheat food has spellbound the masses. Bread wheat commonly known to scientific community as *Triticum aestivum* (Figure 1) is favoured across the globe due to its wide usage. With time and popularization, its consumption is expanded beyond the primary producer countries like China, India, Russia, United States of America, France, etc. and reaches almost every corner of the orb (FAO, 2014). Factual image nominates wheat as the world's most preferred staple food with 767 million tonnes produced in 2019. According to recent surveys, India stood as the second-largest producer of Wheat and holds 22nd rank in productivity. This is the acme of Indian scenario and without a doubt increases our concern for its future maintenance.



Figure 1: Wheat cultivation in the fields of Waghodia, Vadodara

With regard to low productivity, several culprits viz. climatic conditions, seed quality, irrigation has been found out (Abbas et al., 2005). As research community relentlessly uncovered the problems of low productivity, the government is attending the issues with improved seed quality and better irrigation system (NITI Aayog, 2015). According to the United States

Department of Agriculture forecast, India will experience an improved production of the cereal in the coming years (USDA, 2019). This could be aided by the implementation of ideal growing conditions to boost the yield.

After looking into the various aspects of wheat, it is needless to quote the dietary importance of the grain. However, it would be incomplete rather unfair without it, which is worth mentioning. Being a staple food, it constitutes the most important source of carbohydrate in a majority of countries predominantly in the least developed ones. 13% of their total weight is water. Moreover, their high demand is due to the presence of essential nutrients including proteins, fats, dietary fibres along with several B vitamins, and minerals in high quantities. Hence, this nutritionally rich cereal is largely recommended to children and adults to meet their daily dietary needs. Given that, the damage incurred by the insect pests would be a substantial loss in the less developed countries and the reason for malnutrition too.

1.4 *Tribolium castaneum*- The model beetle

The pest community often make its entry in top-rated journals for being a nuisance to wheat and its products. Of the standard models, *Tribolium castaneum* (Herbst, 1797) has won the tag of “cataclysmic pest” as early as 1930s (Good, 1933). Referring age-old textbooks like “IMMS” places it taxonomically in the largest order Coleoptera and family Tenebrionidae. Popular as Red flour beetle (Figure 2), they follow holometabolous life trend, which is largely characterised by the pupal stage. However, the beetle is broadly considered as a pest due to the insatiable feeding habit of larvae and adults which was made possible due to their characteristic chewing type of mouthparts.

The beetle is cosmopolitan in nature and known to exist in almost all geographical locations possible due to its ability to withstand extreme conditions (Bergerson & Wool, 1988). Rimi et al. (2017) are of the view that they are a common inhabitant of warehouses, mills etc. and can survive on a wide range of stored grains including cracked grains of rice, dried fruit, cornmeal, barley flour and oatmeal apart from the wheat flour. The feeding

stages viz., adults and larvae are the primary pest of wheat flour and other milled products whereas a secondary pest of wheat grains, not capable of attacking undamaged whole grains (Leonard LeCato, 1975). Regardless of their choice, they primarily bore through the grains and destroy the endosperm and leave the goods for no use.

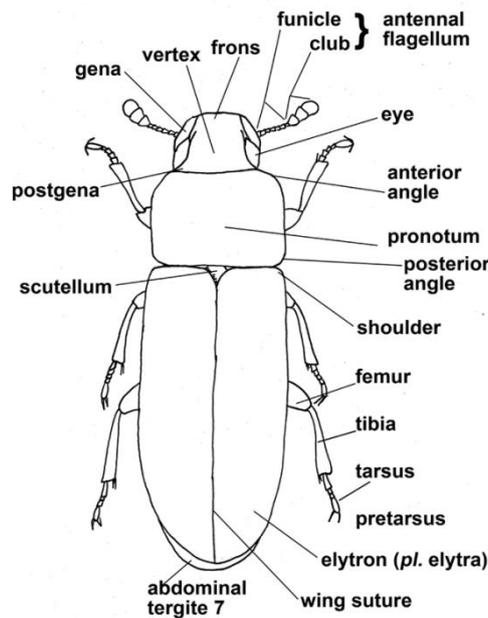


Figure 2: External morphology of *Tribolium castaneum*; Donitz et al., 2013

Though science strictly criticizes the repetition of work, still *T. castaneum* and its new victim grains has always been the talk of the town. It is due to continuous expansion of its host range. This keeps the flame enlighten within the research community which recently has led to the discovery of 233 commodities including flour, cracked wheat, wheat bran, maize bran, nuts, beans, barley, corn, cornmeal, spices, millets, oats, rice, and many more as the targets of the pest (Hagstrum, 2017). Therefore, man-pest conflict intensifies leading to a complete chaos. One major criterion which could be the possible reason behind this long list of host grains is “resistance”, which is aided by the qualities like sexual selection, easy adaptation to new environments, sustaining extreme conditions and rapid dispersal to colonise new food patches (Arnaud et al., 2005) (Fedina & Lewis, 2008).

The infestation of the grains intensifies with time due to the high reproductive potential of the beetle. This is supported by the work of Ajayi & Rahman

(2006), which quotes that approximately 40% devastation of wheat flour by the pest. Additionally, characteristic nibbling of food grains causes serious economic damage due to the loss in market price and nutritional efficiency (Shafique et al., 2006). Apart from the economic damages, its contamination also proposes major health concerns. Flour beetle is known to secrete toxic quinone's which turn the flour greyish and hence reduces its aesthetic and nutritive values (Ladisch et al., 1967). Moreover, the carcinogenic attribute of the quinone's is affirmed thus poses a serious health risk to human (El-Mofty et al., 1989). Hence, the management strategies may vary but adopted worldwide to fight against the notorious pest.

1.5 Current management strategies and their drawbacks

The journey of man-insect conflict started with the chemical pesticides. The very use of chemicals has gained popularity due to its rapid action which led to the "Era of pesticides" in the 60s. However, the continuity has left our environment polluted with toxic material rendering many health problems. "Endosulfan disaster in Kerala" is one such incidence which destroyed the lives of thousands and even after decade now glimpse of the havoc are seen in masses in the form of abnormalities. These issues led to the development of eco-friendly strategies like integrated pest management (IPM).

Use of fumigation, grain protectant, and aerosols, which heavily relies on chemicals, still dominates the list of grain management strategies. Among all, fumigation through methyl bromide (CH_3Br) and phosphine (PH_3) is widely practiced in most of the countries to manage stored product pests (Bell, 2000). A lesser familiar strategy is physical control where heat treatment, grain chilling, etc. is encouraged but there effect decreases without sanitation.

To begin with, CH_3Br , a highly toxic substance commercialise in the trade names of Bromogas, Celfume, Embafume, MB, MeBr, Methogas, Profume, Terrogas, and Zyto. It dissipates rapidly into the atmosphere and highly hazardous for the fumigation site thus severely affects the central nervous system and respiratory system. The negative effect continues and target lungs, eyes, and skin, hence are of high clinical significance. Moreover due to its

association in ozone layer depletion, CH₃Br is banned worldwide (Anbar et al., 1996). The initiative was taken by EPA and scheduled a phase-out of CH₃Br from all the nations by the year 2000.

Evaluating another candidate, PH₃, a colourless gas with a distasteful odour of fish or garlic, is widely used as a gas fumigant. It is highly toxic, dangerous and flammable. Moreover, it has already triggered much negative influence on the environment which includes pest resistance towards PH₃, secondary pest outbreak, environmental pollution and negative health concern to the non-target animals including humans (Benhalima et al., 2004).

Apart from the two, Carbonyl sulfide (OCS) and Methyl iodide (CH₃I) is also employed as a fumigant (Fields & White, 2002). According to the US EPA listing, all these fumigants pose serious health threats and emerged as a possible human carcinogen. The factual image claims the ban of synthetic pesticides for increasing incidence of pest resistance, growing labour cost and lethal after-effect on the users (Okonkwo & Okoye, 2015). As the most reliable control measures are forced to be delisted from the category, the pressure to replace them with the best alternatives has taken the research community by storm.

With the increase in literacy level and awareness, increasing public concern over the level of pesticide residues in food has been sensed. Food safety is receiving increased attention worldwide as the links between food and health are increasingly recognized. Improving food safety is an essential element of improving food security, which exists when populations have access to sufficient and healthy foods. At the same time, as food trade expands throughout the world, food safety has become a shared concern among developed and developing nations.

1.6 Pesticidal plants and new paradigms in research

Replacement of these conventional fumigants with an eco-friendly alternative seemed an intelligent move to control the pest which has inclined global research towards the efficacy of pesticidal plants. In the race of finding a better insecticidal candidate, different plant varieties were tested and

fortunately, they emerged as a potential solution towards the challenge (Okwute, 2012). In a survey, existence of 2, 50, 000 plant species across the globe were come to knowledge (Mamedov, 2012). Surprisingly, only 10% of the total species existed has been examined for chemical composition and their possible connection with human health or as an insecticide. Hence, there is a serious need and enormous scope as well to explore the area. Moreover, the success achieved until date would inspire to design and conduct further research in the same.

In the late 1990s, serious attention has been given towards the identification of novel plant products as chemotherapeutics and plant protectants. Due to the non-toxic and biodegradable nature, they emerged as a potential candidate for IPM (Mishra & Dubey, 1994). Work of Yang & Tang (1988) has shown that the botanicals used for controlling the insect pest have a strong connection with the medicinal plants. This reduces the uncertainty associated with human health for consuming plant treated commodities. Scientific thrust does not end here and one such work (Lale, 1992) have demonstrated the pest control potential of many tropical plants. Based on the previous reports, use of plant parts which are easy to collect and handle ensures positive potential.

Plants are well known for a wide range of essential oils (Sasidharan et al., 2011). EOs contain a plethora of organic compounds that are relatively non-toxic for the environment and can be a potent alternative for the synthetic pesticides (Isman B., 2000). The views were also supported by previous studies where EOs of *Ricinus communis* seeds and *Datura stramonium* extracts were found effective in controlling the red flour beetles (Abbasipour et al., 2011) (Babarinde et al., 2014). Moreover, problems like resistant development in pests can be minimised using EOs. This is due to the fact that multiple volatile groups present in EOs would work in synergy hence the chances to grow resistance against a single compound evades (Langeveld et al., 2014).

Though EOs could finally make their way to market still they are limited to a small number of mosquito repellents. No report has established commercial success of EOs until date. This is due to factors like high costs and availability

of other competing products in the market. Regardless of their efficiencies, these limitations barred its application in the agricultural field and warehouses. These issues need to be addressed by searching other plant variants which can be useful in tackling the above-mentioned challenges.

The factual scenario of biopesticides in India signifies only 2.89% of the overall insecticides available commercially. It was anticipated to experience a growth rate of about 2.3% in the coming years (Thakore, 2006). According to Das (2014), nineteen biopesticides, registered under the insecticides act, (1968) dominates Indian market. Though slow but the increasing trend of EOs marks the very change in society which gradually accepts the EOs for the safety and betterment.

1.7 *Artemisia annua*- Properties and uniqueness

One such variety is genus *Artemisia*, the widely distributed genera of the Asteraceae family. It is extensively used for its medicinal properties in Asian countries. *Artemisia* is native to southeast countries and commonly called as Qinghaosu. The variety becomes popular for containing a wide range of EOs and secondary metabolites. As reviewed by Janssen et al. (1987), the toxic effect of the genus against pathogens could be garnered. Moreover, their use in human diets and animal fodder reported no worry. Among other highlights, research conducted by various groups (Liu et al., 2006) (Negahban et al., 2007) (Abdelgaleil et al., 2008) (Gonzalez-Coloma et al., 2012) has testified the insecticidal and antifeedant activities of the genus. These conducive traits have pushed it far in the race of “potential insecticidal plant”.

Artemisia has been the subject of research interest for decades which is reflected in the wide range of studies conducted across the globe (Bora & Sharma, 2011). The review throws light in the fact that species of *Artemisia* possess either pharmacological or insecticidal properties. This demands further research in the same field to explore other species for their holistic benefits.

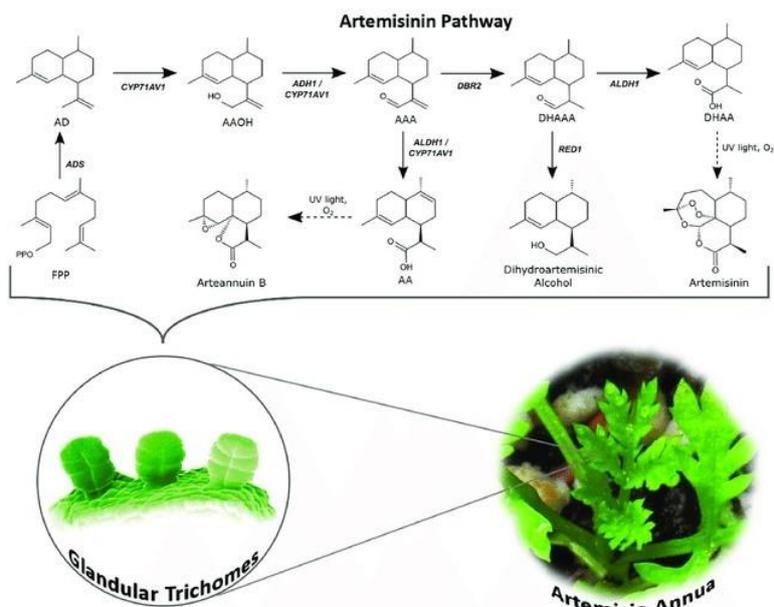


Figure 3: Artemisinin in the glandular trichomes of *A. annua*; Ikram et al., 2017

One of a kind is *Artemisia annua*, commonly known as sweet wormwood, sweet sagewort or annual wormwood (Chinese: qinghao). The species is an aromatic annual plant that grows up to 2 m in height. It is native to Southeast Asia especially China but now naturalized throughout the globe including India where it is cultivated in Kashmir valley for the production of Artemisinin (Dangash, 2017). The plant was introduced by CSIR-CIMAP in the early 1980s, from the Royal Botanic Gardens, Kew, UK. Artemisinin

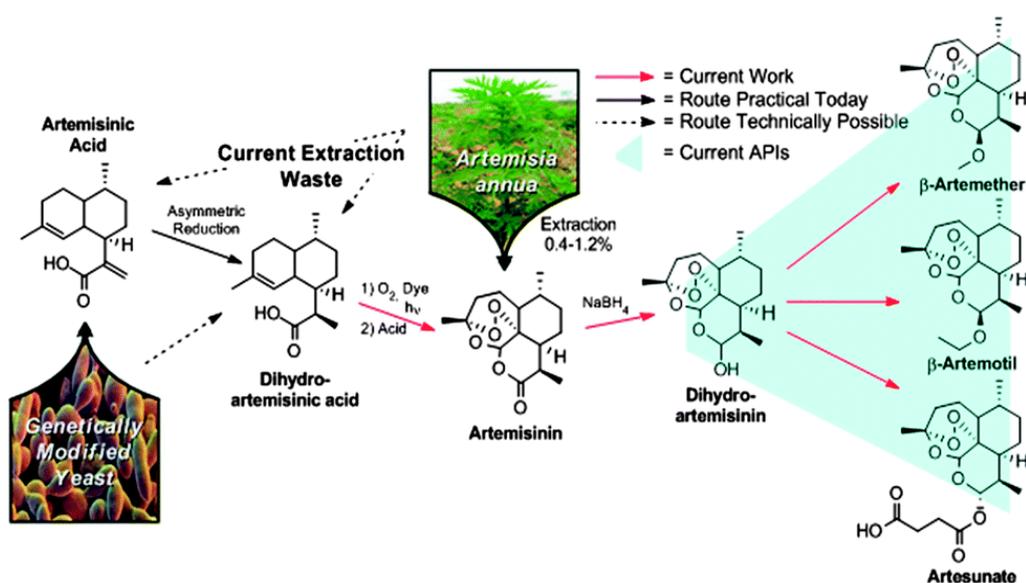


Figure 4: Artemisinin and Semi-synthetic Artemisinin; Gilmore et al., 2014

(Figure 3), which is of considerable research interest, is found in leaves and flowers in extremely high concentration. Additional reports on the possible presence of Artemisinin in glandular trichomes are a boon for the scientific community (Duke & Paul, 1993).

Artemisia annua, the sole producer of Artemisinin is mainly studied for its efficacy against the malarial parasites, *Plasmodium*. Artemisinin is a sesquiterpene lactone characterised by a unique endoperoxide bridge which is believed to be the reason for its anti-malarial properties. Though the species has joined the bandwagon lately but considerable work is seen in no time. Artemisinin and Artemisinin-based combination therapy (ACTs) has emerged as the most efficient antimalarial drug available in the market against MDR strains (Klayman, 1985) (Eastman & Fidock, 2009). The species is highly popular among the folk medicines. World Health Organization (WHO) shows high interest with artemisinin and its chemical derivatives (Figure 4). Moreover, *A. annua* is included in the official Pharmacopoeia of China and the drug directories of India, Japan, and Vietnam.

1.8 Essential oils and stored grain pests

EOs are volatile, natural plant-based compound marked by its strong odour and clear appearance. They are produced as secondary metabolites by aromatic plants localised in temperate to warm countries where they are cited as a vital representative of the traditional pharmacopoeia. All plant organs i.e. buds, flowers, leaves, stems, seeds, fruits, roots, and bark can be used for the production of EOs where it remains deposited deep into plant cells like cavities, epidermal cells or glandular trichomes. EOs production is believed to be a part of the plants defence mechanism in response to pests and pathogens (Rattan, 2010).

The extraction of EOs from different plant parts is majorly done by steam or hydro-distillation. Though modern technologies like supercritical carbon dioxide, microwave has replaced the conventional ones due to great-efficiency but still they prevail. Moreover, selection of the extraction process depends solely on the final product as differences in chemical profile and quantity is

obvious with different soil types and climatic conditions (Masotti et al., 2003). The chemical composition of the EOs also varies in the number of molecules as well as in their stereochemistry based on the type of extraction finalised. Hence, extraction of EOs with the same chemical composition requires a scientist to follow the same protocol. Fortunately, employing the most hyphenated technique of GC-MS has made chemotyping possible which has brought ease in the process of commercialization.

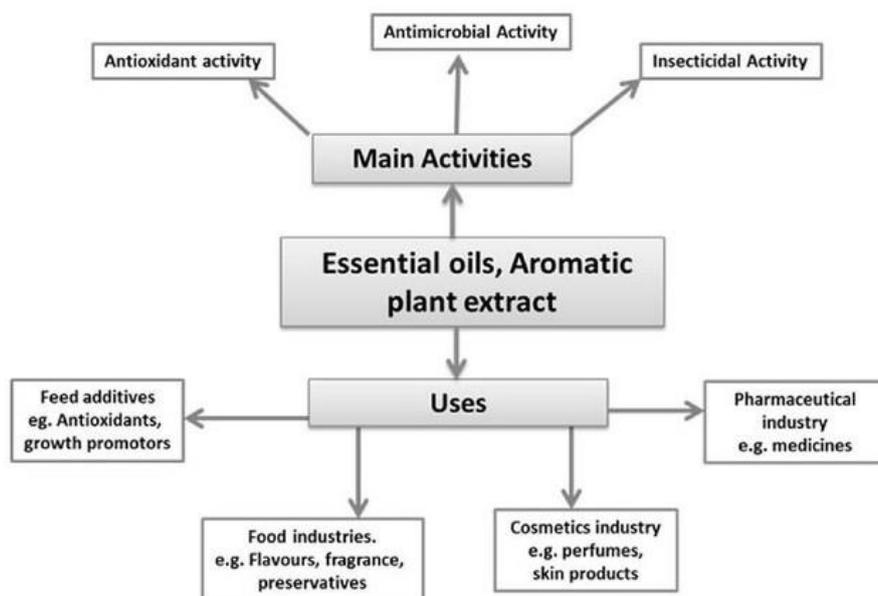


Figure 5: Major activities and uses of essential oils; Pandey et al., 2017

The use of EOs is widespread (Figure 5) due to antimicrobial, analgesic, sedative, and anti-inflammatory properties (Bakkali et al., 2008). Reichling et al. (2009) has testified the escalation in the use of EOs in Pharmaceuticals and food industries due to the above mentioned traits. It is interesting to know the efficiency of EOs in retaining organoleptic properties of the foodstuffs (Dorman & Deans, 2000). The safe status portrayed by the food industries has made their acceptance easy among mankind and open new ventures for multipurpose use.

The composition of EOs includes a wide range of components including aldehyde, phenolics, sesquiterpene, terpene, etc. Previous studies have claimed the anti-microbial nature of aldehyde groups reported from plant EOs (Moleyar & Narasimham, 1986). Other players like eugenol, thymol, and

carvacrol, belonging to the phenolic group, are known to possess anti-microbial and antifungal properties (Abbaszadeh et al., 2014). Well known chemical groups like sesquiterpene and terpene works wonder against a wide range of microbes (Dahham et al., 2015). Hence, one can harness the literature for identifying bioactivities of the EOs of an unknown plant, if chemical composition is unveiled.

One major criterion that needs attention while using EOs is their persistence as an insecticide. Various researches with EOs showed repellency against stored-grains pest for several days. This could be due to the persistence of EOs in the treated sets. Taking a few concerns into account, i.e. (i) efficacy of EOs & (ii) damage caused by *T. castaneum*, controlling the pest is of utmost importance with an eco-friendly method to reduce the economic burden in developing countries like India.

Regardless of numerous studies, EOs are only screened for their pesticidal activities but systematic studies of their mode of action along with the safety profile yet need more detailed evaluation. This intensifies the urge to bioprospect the insecticidal properties of EOs in vivo and in vitro systems. This enables the recommendation of their practical application in the warehouses against post-harvest deterioration and hence enhances the shelf life of different food commodities.

1.9 Plant compounds and Biomolecular crosstalk

As discussed in the preceding section, certainty of compound mediated toxicity inflicted in the mode of action is rarely evaluated. EOs or their purified compounds occasioned in structural symptoms point towards a broad range of neurotoxic mode of action. Neuroexcitation in the form of convulsion, hyperactivity, seizures, tremors, and ultimate paralysis was prominent in pest animals due to neuromuscular fatigue and energy exhaustion. Neuroinhibition, on the other hand, can be ascertained by immobility followed by paralysis due to decreased respiratory capacity leading to death. Numerous biological signals are responsible for maintaining the precision in synaptic transmission hence would play a major role in neural communication too.

Insect pests are known to feed on a wide range of plants which produces moderate to highly toxic substances. The substances attack multiple molecular targets including proteins like ion channels, receptors, enzymes, etc. and biomembranes to nucleic acids (Rattan, 2010). Secondary metabolites interfere with the vital components of signalling mechanism. This in turn affects the normal course of functioning of crucial enzymes and signalling of nervous system (Kostyukovsky et al., 2002) (Priestley et al., 2003). Moreover, repellent and antifeedant effects which are prominent in plant-based insecticidal treatments ascertain that insect physiology gets affected in multiple receptor sites. EOs mainly seen to affect the biochemical processes, hence disrupt the normal course of endocrine lineage. The conclusion drawn based on the preceding works highlighted that biomolecules of neural and endocrine pathways are primarily targeted by EOs (Figure 6).

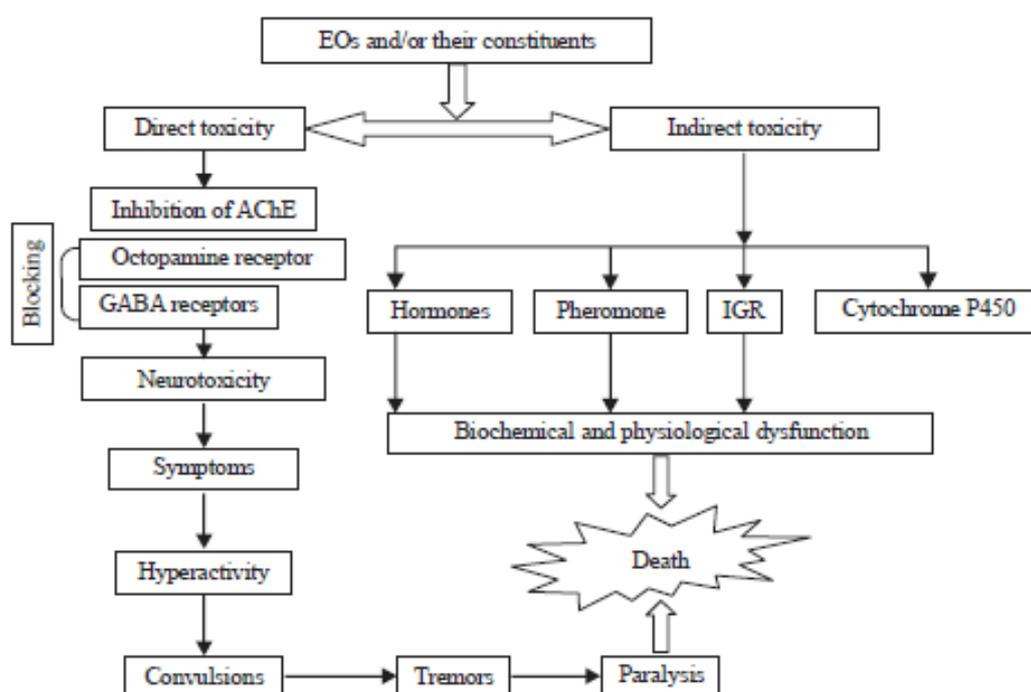


Figure 6: Proposed mechanism of EOs-induced toxicity to insects; Mossa, 2016

Detoxifying enzymes like P450, Glutathione-S-transferase, and esterase are the warriors of insect gut which facilitate the detoxification process by eliminating lethal components out of their system. In cases where insecticidal components display lethal effect is mediated chiefly by hindering the normal course of these enzymes. A few studies (Evans, 1984), (Bloomquist, 2001),

(Matsuda et al., 2001) with synthetic insecticides are cited to understand the mechanism of action by targeting various channels. However, a few studies (Mills et al., 2004) (Enan, 2005) (Olmedo et al., 2015) with EOs showed toxicity by affecting anti-oxidant pathways. Taking these works into account, unveiling the biomolecular targets of *T. castaneum* affected by EOs of *A. annua* would add new dimension in pest management science.

Though my introduction majorly revolved round the pest and pesticidal plant but it would be unfair to skip the nutritional properties of the grain. It is so expanded that a whole chapter can be made to discuss it in length and breadth. However, focusing primarily into the nutritional damages caused by the pest helped to concise it to the minimum.

1.10 Nutritional resources

Wheat is high in nutritional properties which include proteins, carbohydrates, minerals, fibres, and essential vitamins. 90% of the total wheat weight is carbohydrate which includes starch. Fibre content in the whole grain constitutes about 10-12% of the dry weight. The main fibre present is Arabinoxylan which constitutes about 70% of total fibre content. Protein comprises about 7-22% of the wheat's dry weight and gluten dominates the protein content with about 80% of the total weight. Vitamins and minerals are present in high quantities. The type and amount of minerals depend on the type of soil in which it has grown. However, the list is dominated by selenium, manganese, phosphorus, copper and folate and many other vital to good health.

The wheat kernel contains vital nutrients that lay profound metabolic effects in the human system. Three major sections (Figure 7) constitute a grain where endosperm occupies 83% of the total weight. This region contains a major proportion of carbohydrate, proteins and vitamins. Whereas, bran (14.5% of total weight) and germ (2.5% of total weight), two nutritionally important parts of the grain majorly contain important minerals, vitamins, fibres, etc. While whole grain passes through the phases of milling and refining, bran and germ get removed. Hence wheat flour is relatively poor in minerals and vitamins compared to wheat grain.

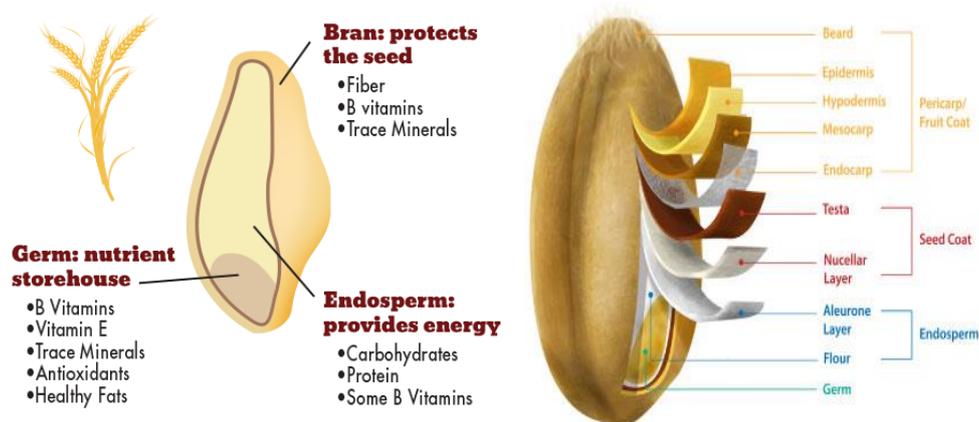


Figure 7: Anatomy of Wheat Grain

Insect infestation, which is discussed earlier in the chapter, severely affects the nutritious contents of the grain. Padín et al., (2002) in his work has testified the damage of wheat grains by stored grains pests like *Tribolium castaneum*, *Sitophilus oryzae* and *Acanthoscelides obtectus*. It severely damages the wheat grains chiefly the inner mass leaving a nutrition deficient grain coat. Moreover, decrease in protein content and increase in uric acid content beyond tolerance is marked in the grains infested with various pest (Jood & Kapoor, 1993). This increase the concern for better management practices for the grains in storage. To address these issues, modern practices including Nuclear magnetic resonance (NMR) and Ultrasound-based techniques are in use (Salimi et al., 2018).

1.11 Rationale of the study

Reports on EOs emphasize employing the mixture of secondary metabolites than a single compound as the former would be deterrent against the pest for the longer duration. This could be reasoned by the very presence of different physical properties in a mixture which enhances the persistence of defence. Whilst the first decade of twenty-first century was engrossed in unveiling the involvement of 1, 8- cineole isolated from *A. annua* on *T. castaneum* management, it was strongly believed that further insight into the efficacy of the EOs from the species and molecular mechanism therein would immensely benefit the field of stored pest management.

1.12 Aim of the work

The current piece of work was undertaken to elucidate the effectiveness of *Artemisia annua* against *Tribolium casteneum* and the mechanism succours in bringing about successful pest management. Hence the aim of the work was “to evaluate the efficacy of Essential oils of *Artemisia annua* against *Tribolium casteneum* (Herbst, 1797)”.

Objectives of the study

To fulfil the aim following objectives were undertaken.

1. Study of the biology of model organism, *Tribolium casteneum* (Herbst, 1797) in laboratory conditions.
2. Extraction, fractionation, and identification of chemical compositions of essential oils of *Artemisia annua*.
3. Identification of lethal doses (LD₅₀ & LD₉₀) and repellency of the crude extracts against *Tribolium casteneum* employing different bioassays.
4. Understanding the differential tolerance in control and major lethal groups using different enzyme biomarkers.
5. Assessment of nutritional properties of insect free & insect infested wheat grain and wheat flour.

“Agriculture is locomotive of our economy and a prosperous rural economy based on agriculture will ultimately make the nation prosperous.”

–Sardar Vallabhbhai Patel