Chapter 1

Introduction

For, usually and fitly, the presence of an introduction is held to imply that there is something of consequence and importance to be introduced.

-**Arthur Machen**

Tea is a widely consumed beverage, enjoyed by approximately one-third of the global population (Khan and Mukhtar, 2013). Nowadays, it is acknowledged as one of the most widely enjoyed and cost-effective beverages, savoured in over 65 countries globally, with 4 billion cups consumed each morning (Pan et al., 2022). The pioneering use of tea as a medicinal beverage originated in China. It eventually found its place in other beverages. The hill tribes of Northeast India also embraced various approaches to consuming tea (Baruah,2015). This beverage is made from the fragile young leaves of the plant. India is the second largest producer of Tea in the world after China (Kaur et al., 2015). Tea is an economically important crop in India and employs 1.5 million skilled workers in India (https://www.ilo.org).There are three agro climatic regions cultivating tea in north Bengal which are Darjeeling, Terai, and Dooars. The average area under tea in Darjeeling, Terai, and Dooars are 18000 ha, 22000 ha, and 75000 ha respectively and the average yields are 535 kg/ha, 1800 kg/ha, and 1750 kg/ha respectively (Tea board of India, 2022).

Under the foothills of the Himalayas, the West Bengal districts of Alipurduar, Jalpaiguri, Darjeeling, and a small portion of Coochbehar and North Dinajpur are home to the tea industry of North Bengal. Technically, Bengal's whole tea-growing region has been split into three Terai, Dooars, and Darjeeling Hills are on the outskirts. The Siliguri Subdivision of the Darjeeling District and a portion of the North Dinajpur District are included in the Terai region. The Darjeeling Hills encompass the Darjeeling and Kalimpong districts, including the different subdivision. The Dooars completely encircle the districts of Jalpaiguri, Alipurduar, and a portion of Coochbehar. The largest tea-producing region in Bengal and even north India is Dooars (Peshin and Zhang, 2014).

The use of hazardous chemicals in pest control has led to several adverse consequences, with the primary concern arises from the accumulation of chemical insecticide residues and the subsequent development of pest resistance to them. Continuous innovation is imperative for agricultural success and to address these biotic stresses effectively. Given the ongoing evolution of these organisms resulting in pesticide resistance, ongoing parallel research is essential to combat them (Reddy and Chowdary, 2021).

The genesis of synthetic pesticides traces back to the revelation of DDT's insecticidal properties in 1939 by Paul Müller. In recognition of this discovery, Müller was bestowed with the Nobel Prize in 1948 for his pioneering work in uncovering the pesticidal potential of DDT (Dash et al.,2007).

In her 1962 book "Silent Spring," Rachel Carson, a marine scientist and author from the sixth century, voiced her apprehensions regarding the widespread and indiscriminate application of synthetic pesticides, particularly DDT (Dichloro-diphenyl-trichloroethane). Carson detailed how these pesticides were responsible for the deaths of numerous animal species, particularly birds, and emphasized how their usage was destabilizing ecosystems and leading to a decline in bird populations.

Since the 1800s, the increasing use of chemical pesticides has triggered numerous environmental imbalances and posed risks to human health. There's an urgent need to revamp crop protection strategies to make them both efficient and environmentally sustainable. While chemicals currently hold sway in the industry, the future lies in biological inputs. This biological approach presents the most promising solution for managing insect populations without jeopardizing other organisms or the beneficial fauna within ecosystems (Lv et al., 2011).

Integrated Pest Management (IPM) arose as a consequence of efforts to reduce dependence on chemical pesticides for pest control. Integrated Pest Management (IPM) is a holistic approach to pest management that considers the surrounding environment and the dynamics of pest populations. It utilizes a variety of suitable techniques and methods harmoniously to maintain pest populations below levels that result in economically unacceptable damage or loss (FAO, 1967).

In this context, integrated pest management has a significant role in bringing back the botanicals to mainstream agriculture. The federal IPM coordinating committee, established in 2003, played a pivotal role in shaping the primary goals and priorities of the National IPM Program. These objectives include: (i) maximizing financial benefits derived from the utilization of IPM techniques, (ii) minimizing risks associated with the use of IPM methods and pests concerning human health and the environment, and (iii) mitigating adverse environmental impacts linked to the application of IPM techniques and pests (Peshin and Zhang, 2014).

Since the 1990s, the adoption of Integrated Pest Management (IPM) programs has resulted in a notable decrease in pesticide applications, with reductions of 50–60% in cereal like rice and 19.26–50.50% in cotton cultivation. Overall, there has been a decline in pesticide usage, decreasing from 75,033 to 41,822 tonnes between the periods of 1990–1991 and 2009–2010 (http:// ppqs.gov.inIpmpest_main.htm). This reduction in pesticide use has been attributed to the implementation of IPM programs.

There is an urgent need for safe alternatives to synthetic pesticides to safeguard human and environmental health. Many currently employed synthetic pesticides are incompatible with organic agriculture practices. Moreover, addressing the escalating resistance of pests to conventional pesticides requires the development of effective and secure solutions with new modes of action. Botanical pesticides are considered effective against a diverse range of agricultural pests, and they possess the advantages of being readily biodegradable, abundant, and cost-effective (Acheuk et al., 2022).

Botanicals play a pivotal role in biological approaches due to their widespread availability, ease of preparation, and potential to generate employment in rural regions. Utilization of botanicals for pest management dates back to the Vedic era. (Koul and Walia, 2009). References to the utilization of toxic herbs for pest control can be traced back to the Rig Veda (Chopra et al., 1949). India exhibits a higher inclination towards botanicals compared to many other countries in the region. It permits the use of various materials such as pyrethrum, rotenone, neem, essential oils, ryania, and nicotine, except for sabadilla. Additionally, provisional registration of new products is allowed while toxicological and environmental data supporting full registration are collected. (Isman, 2006). The PPC (Plant Protection Code, 2019) makes mention of Azadirachtin an oxygenated triterpenoid compound obtained from the seed kernels of *Azadirachta indica* A. Juss. being effective against pink and purple mites and some caterpillars. It also mentions the aqueous extracts of certain commonly found herbs such as *Clerodendrum infortunatum* L. , *Vitex negundo* L. , *Persicaria hydropiper* (L.) Delarbre , *Senna tora* (L.)Roxb., *Xanthium strumarium* L., exhibiting promising control over most of the mite pests, insect pests, and diseases of tea.

Pesticide usage for pest control is as ancient as agriculture itself, with its origins tracing back to civilizations predating 2000 BC, including ancient China, Egypt, Greece, and India.

Approximately 4,500 years ago, the Sumerians employed sulfur dusting as a method of pest control. In ancient India, Surapala researched and suggested various botanicals and other products with biocidal qualities to manage plant diseases. The well-known "panchamula" (five plant roots) that was extensively applied during the period possesses antifungal, antiviral, antibacterial, and antifeeding effects. The use of chemical pesticides made from synthetic materials is either regulated or forbidden owing to the residual issue and consumer health concerns. prohibited or restricted. At least 500 insect and mite species are resistant to one or more pesticides (Georghiou, 1990). Botanicals have proven to be efficient in pest management and are extensively documented. Throughout history, crude plant insecticides have been used and are generally accepted in traditional and tribal civilizations around the globe (Crazywacz et al., 2005). Synthetic insecticides significantly pushed botanicals from an essential role in agriculture to a practically negligible position in the marketplace among crop protectants, as evidenced by recent history (Isman, 2006).

In recent years India has seen environmental upheavals, a return of pests, variations in sensitivity, ongoing problems with tea production, blockage of natural regulating agents, and detrimental effects, both deadly and sub-lethal, on non-target creatures, including people. (Gurusubramanian et al., 2005; Borthakur et al., 2005; Bora et al., 2007). The negative consequences have led to heightened examination of the extensive application and safeguarding of pesticides. Owing to the importing nations' severe regulations on the acceptability of the produced tea owing to pesticide residues, it has recently become a serious worry for the tea business. Concerns about the environment and public safety, changes in insect pests' sensitivity, and rising chemical costs all contribute to changes in pest control practices (Roy et al., 2015). Planters would be wise to shift away from chemical pesticides and towards ecosystem modification. Due to the extensive pesticide use and large surface area of tea leaves, tea poses a major risk for human exposure to residues. Pesticide residues may persist in tea and can be transmitted to the infusion (brew) at levels higher than the MRLs (Maximum residue level), which may pose health risks (Kumar et al., 2006). This is one of the main drawbacks of pesticide usage.

Broad-spectrum, synthetic chemical pesticides including neonicotinoids, benzimidazoles, carbamates, synthetic pyrethroids, and organophosphates are used extensively in tea growing to control pests (Chen et al., 2013). The concentration of pesticides on tea bushes typically decreases due to natural processes such as rainfall, dew, volatilization, air movement, photolysis, biodegradation, moisture, pH, and growth dilution (Bisen and Ghosh, 2000).

In the realm of Indian agriculture, the overall pesticide utilization declined from 63,406 to 63,284 metric tons between the periods 2017-2018 and 2021-2022. Conversely, biopesticide usage witnessed an increase from 7,174 to 9,321 metric tons during the same timeframe. It's noteworthy that pesticide usage in India fluctuates depending on the specific crop and geographical location. (https://ppqs.gov.in/statistical-database) (Chakraborty et al.,2023).

The continuous and unregulated application of these chemical acaricides leads to numerous adverse consequences, such as water pollution, the depletion of crucial soil microbes, a reduction in the population of biological control agents, a resurgence of pests, the emergence of pest resistance, secondary outbreaks of pests, and the presence of pesticide residues in processed tea. To solve these issues and ensure the sustainable production of tea, tea producers have looked for substitute methods of crop protection. Identification of toxins or antifeedants from plants is currently being highlighted as a possible technique to manufacture environmentally safe pesticides. (Cranham, 1966; Mobed et al., 1992; Roy et al., 2012; Hazarika et al., 2009; Roobakkumar et al.,2010; Deka et al.,2022).

The Terai and Dooars regions boast rich floristic diversity. In 2011, Ghosh and Das reported the occurrence of several poisonous and medicinal plants from North Bengal, including *Melia azedarach* L., *Vitex negundo* L., and *Urtica dioica* L., known for their pest-controlling properties. Additionally, *Melia azedarach* L. serves as a primary shade tree in tea gardens (Roy et al., 2016).

The plants are referred to as "Weed" and have consistently been neglected while also being cited as troublesome plants. Their economic significance is unclear. They mostly lower agricultural output by seeking resources including water, light, soil nutrients, and space. In addition to reducing crop quality by polluting the product, weeds can also impede harvesting. serve as hosts for agricultural illnesses or as a haven for insects that produce chemicals hazardous to humans, animals, or crop plants. There are some favourable features to take into account in addition to the previously stated adverse aspects. For example, certain weeds are edible and have therapeutic effects because they contain both macro and micro components. They play a role in preventing the loss of soil as well. Due to several chemicals that the weed plants created on their own, they had a terrible taste and odor. Certain chemicals are poisonous to insects and pests (Saha and Modi,2023). Most of the secondary metabolites produced by weed plants are of various of the secondary metabolites produced by weed plants are of a variety of chemical groups, including phenols, Terpinoids, essential oils, alkaloids, polypeptides, and many more. These substances give plants unique characteristics, such as their distinctive flavors, colors, and odors. However, on the other side, these secondary metabolites support plant defence against herbivores, insects, and microorganisms. However, the concentration of these secondary metabolites in various plants varies. Plants that are abundant in these substances can defend themselves against predators. One of these is weed vegetation. We have consistently noticed that weed vegetation is unaffected by predators, in contrast to crop vegetation, which is more sensitive to plant pathogens. Weed plants typically contain higher levels of secondary metabolites compared to crop plants. These compounds impart disagreeable tastes and odors, acting as a natural defence mechanism against predators (Rao et al.,2010; Bahadır and Öztürk, 2021). Green metal nanoparticles that were synthesised have insecticidal effect against mosquitoes, which are major disease vectors responsible for more than a million fatalities each year in tropical areas (Razaq and Shah, 2022). It was discovered that green-synthesized metal nanoparticles (NPs) made using plant extracts as capping and reducing agents are efficient insecticides and can offer a reasonably priced and environmentally friendly substitute for traditional pesticides (Salem and Fouda,2021). In impoverished countries with tropical climates where source plants are easily accessible and conventional pesticides are both expensive and harmful to users, botanical insecticides may be of enormous relevance (Isman, 2020). *Anopheles stephensi*, a mosquito that transmits malaria, *Aedes aegypti*, a mosquito that transmits dengue, and *Culex quiquefasciatus*, a mosquito that transmits filariasis are all vulnerable to photosynthesized metal nanoparticles (NPs). These NPs can act as overrides, larvicides, pesticides, adulticides, and oviposition deterrents against different mosquito species at corresponding different doses (Benelli,2016). Utilizing *Ulva lactuca* seaweed extract, the green synthesis of ZnO NPs demonstrated remarkable antibacterial efficacy, When applied at a concentration of 50 mg/mL, it effectively eradicates *Aedes aegypti* larvae in their fourth instars (Ishwarya et al., 2018). An aqueous solution of banana peel extract was employed in the biosynthesis of titanium nanoparicles (TiNPs), which had a mean diameter of 88.45 nm and an inhibitory impact on many pathogenic bacteria as well as a high death rate in three stages of house fly larvae (Hameed et al., 2019).

In 2002, the Indian Council of Agricultural Research, Government of India, began a project titled "Collection, Documentation, and

Validation of Indigenous Technical Knowledge under National Agricultural Technology Project (NATP)" intending to provide this project with indigenous technology for 38 chosen promising ITKs covering six thematic areas, including rainwater management, soil and water conservation, pest and disease management, farm implements, and horticultural crop. (Mishra, et al.,2020). Traditional Knowledge in Agriculture. Division of Agricultural Extension, ICAR, New Delhi. Pp.39). Considering the pest problem and high pesticide use in Tea, Tocklai has initiated several projects in the past or several work programs.

The tea plant is susceptible to a variety of pests, including tea mosquito bugs, red slugs, red spider mites, thrips, jassids, and others. Among these, the tea red spider mite and tea mosquito bug stand out as dominant pests in these regions, leading to significant crop losses in the tea industry annually.

In 1861, Nietner discovered *Oligonychus coffeae* on coffee plants (*Coffea arabica*) in Sri Lanka. However, the association of the red spider mite (RSM) with tea traces back to the early stages of tea production in Assam, North East India, in 1868 (Nietner 1861, Peal 1986). Wood-Mason (1884) identified it as a new species, *Tetranychus bioculatus*. Prior to Pritchard and Baker's (1955) examination of the Tetranychidae family, researchers investigating RSM in tea in Northeast India consistently labeled the pest as *T. bioculatus*. Subsequently, they recognized *T. bioculatus* as a synonym for the coffee mite and reclassified it under the name *Oligonychus* (Roy et al., 2014).

Among the aforementioned pests, tea

red spider mites are notably prominent and major contributors to crop loss annually. According to early data, the red spider mite, *Oligonychus coffeae* Nietner, (Acarina: Tetranychidae), stands out as one of the most significant pests affecting Indian tea production (Somchoudhury et al., 1995; Babu, 2010; Barua et al., 2016), responsible for causing up to 35–40% of crop loss (Sundararaju and Babu, 1999; Hazarika et al., 2009; Deka et al., 2022). In South India, the economic threshold level (ETL) of RSM in tea is reportedly 4 mites per leaf and 2–3 mites/cm^2 in North East India(Muraleedharan and Selvasundaram 2002;Muraleedharan, 2006; Banerjee, 1971). However, ETLs vary from area to region and even from field to field due to crop phenology, the cost of pesticides and manpower, weather patterns, etc.

Larvae, nymphs, and adult mites wreak havoc on the tea plant by feeding on the sap of the leaves and sporadically on the petioles. (Das,1949). Their onslaught is primarily limited to the mature foliage's top surface. Feeding causes mature leaves to become reddish bronze, and as a result, fields with RSM infestations may be seen from a distance. Drought stress can impact the delicate leaves of the tea plant as well. According to Rao (1976, 1974), when drought occurs, infestation by the red spider mite (RSM) leads to a significant loss of 340–511 kg of tea per hectare. This infestation diminishes the photosynthetic capability of the leaves, resulting in wilting due to heightened transpiration and moisture loss. Such leaves are then abscised, resulting in defoliation. The attack does not immediately affect the flush, but gradually the dry, curled -up leaves cease to function efficiently. It is difficult to estimate the extent of damage caused by RSM, but growth is certainly reduced due to reduced photosynthetic activity and excessive defoliation. Serious damage occurs to bushes with insufficient root starch reserves after pruning, especially when a severe attack is accompanied by a drought, leading to the potential death of weak bushes and young plants (Das, 1959). Light (1927) noted that RSM-induced injury often resulted in subsequent pathogen infections. Consequently, given the ongoing challenges posed by conventional pest management systems in tea cultivation, it becomes imperative to embark upon an exploration of alternative approaches, thereby necessitating a comprehensive reassessment and potential overhaul of existing strategies.

Following the comprehensive evaluation of research pertaining to botanical biopesticides for effective pest management, we have formulated the subsequent objectives:

Objectives:

- **Survey and documentation of ethnic formulation:**

Selection and Identification of plants will be done according to their insecticidal activity as previously reported (Ghosh and Das, 2011) and a survey of some selected tea garden indigenous formulations that act against insect pests.

- **Biochemical characterization of selected plants:**

The plants that acts against insect pests will be used for preparing formulation and for carrying out different quantitative and qualitative tests.

Quantitive tests like (i) chlorophyll content, (ii) protein content, (iii) total sugar content, (iv) phenol content. (v) flavonoid content will be performed in selected plants.

(i) Chlorophyll content:

Chlorophyll content will be measured using Arnon method (1949) of selected plant species.

(ii) Protein content:

Protein content will be measured using Lowry et al. method (1965).

(iii) Estimation of total sugar content:

Total sugar content will be estimated using Thimmaiah (2004) method of selected plant species.

(iv) Flavonoid content:

Flavonoid content will be estimated following the method of Atanassova et al. (2011).

(v) Phenol content:

The phenol content of the selected plant will be estimated by using Kadam et al. (2013) method with slight modification.

Different antioxidant tests would be performed to check the antioxidant property of the various plant extracts.

- **Phytochemical screening**:

Fresh samples of selected species will be dried, and extracted with methanol. Preliminary screening will be done following the method of Prakash et al., 2011.

- **Pesticidal activity:**

Pesticidal activity of formulations (including ethnic formulations) against the tea insect pests will be done in standard protocol (Roy et al., 2016).

- **Growth promoting activities of formulation:**

For growth-promoting activities, block with heavily pest-infected plants will be used for growth-promoting activities, block with heavily pest-infected plants will be used for determining their efficacy on plant growth and yield.

- **Enzymatic tests:**

Different antioxidant enzymes will be performed in both infected plant groups against positive and negative control plants sets. Following enzymatic tests may be done: Catalase, Superoxide dismutase, Glutathione peroxidas, Glutathione reductase, Peroxidase following suitable methods.

- **GC-MS analysis:**

GC-MS analysis of selected plant formulations will be done, to determine the potential bioactive compound in those formulations.

- **Molecular docking:**

Molecular docking of the potential and prominent compound against different pest and plant-related proteins will be done for the characterization of the compound found in GC-MS analysis.

- **Characterization and application of Nanoparticles:**

Selected samples showing promising results will be assayed by nanoparticle synthesis, and comparative studies will be done on botanical extracts or their formulation and nanoparticle-coupled extracts.

- ***Ex-situ* conservation of selected plants:**

Plants used in different formulations that have insecticidal activity will be conserved in the ex-situ conservatory of the Greenhouse of Molecular Cytogenetics laboratory.