INTRODUCTION

1. INTRODUCTION

1.1. AN OVERVIEW OF INDIAN TEA IN THE GLOBAL PERSPECTIVE

Tea, the monoculture perennial crop, is one of the cash crops that not only contributes to the Indian economy but also provides livelihood to a large group of Indian people. The initiation of tea plantation in India dates back to 1823 with the discovery of an indigenous tea variety (*Camellia assamica*) in Assam by Robert Bruce and subsequently tea plantations were established in different regions of India including Assam, Darjeeling hills, Terai and Dooars with the introduction of *Camellia sinensis* (Chinese variety) (Muraleedharan 1991; Weatherstone 1992; Sivanesan 2013). Now-a-days, *Camellia sinensis* (L.) O. Kuntze, is cultivated in the sub-Himalayan region of northern part of West Bengal (1,38,691 hectares), Assam (3,47,810 hectares), Tamil Nadu, Kerala and Karnataka (1,01,657 hectares) and other regions of northern and north-eastern part of India spreading over Sikkim, Manipur, Nagaland, Tripura, Arunachal Pradesh, Uttar Pradesh, Uttarakhand and Himachal Pradesh (31,616 hectares) as recorded on 31st March, 2022 (retrieved from www.teaboard.gov.in). Tea cultivation is done in 16 out of 28 states of India of which Assam, West Bengal, Tamil Nadu and Kerala contribute about 98% of the total production. Tripura, Himachal Pradesh, Uttarakhand, Bihar and Karnataka have traditional practice of tea plantations and Arunachal Pradesh, Manipur, Meghalaya, Mizoram, Nagaland, and Sikkim recently have entered in the tea map of India (Anonymous 2022a). In the northern part of West Bengal, tea cultivation is spread over three regions, the Darjeeling Hill (Darjeeling and Kalimpong districts), the Terai region (Darjeeling, Jalpaiguri and Uttar Dinajpur districts) and the Dooars region (Jalpaiguri, Alipurduar and some parts of Coochbehar districts). The Terai-Dooars region is extended between 26° 16' N and 20° 13' N latitudes and between 87° 59' E and 88° 53' E longitudes covering an area of 97,280 hectares. In the year 2022 (as on 31/03/2022), Tea Board of India recorded 449 large and 36,559 small tea gardens in the Dooars, Terai and Darjeeling hill regions of North Bengal covering an area of 1,38,691 ha. (retrieved from www.teaboard.gov.in).

For the last two decades India has retained the position of second largest producer of tea next to China (Table 1) accounting for about 21% of the global production (during 2021) among more than 36 countries spread over all the continents except North America (Anonymous 2022a). Tea production in India had achieved its

highest ever level during 2019 at 1,390 Million Kg in which the major tea growing states, Assam and West Bengal had major shares (82%) (Anonymous 2020a) (Table 2) (retrieved from www.teaboard.gov.in). But during the year 2020 tea production of India was declined to 1258 Million Kg (Anonymous 2021) due to nation-wide lockdown for COVID-19. In the next two years however, the production recovered to some extent with annual production of 1343 Million Kg (Anonymous 2022a) and 1340 Million Kg (retrieved from www.teaboard.gov.in) during the year 2021 and 2022, respectively. Tea productions from the northern part of West Bengal in the year 2022 were 226.37, 171.45 and 6.60 million Kg from the plantations of Dooars, Terai and Darjeeling hill regions, respectively (Table 2) (retrieved from www.teaboard.gov.in). India is the 4th largest tea exporter next to Kenya, China and Sri Lanka accounting for 10% of global share in the year 2021 (Table 1) (Anonymous 2022a) and exported 196.54 Million Kg of tea worth Rs. 5311.15 Crs. (687.9 million US$) during that period (retrieved from www.teaboard.gov.in).

1.2. A GLIMPSE OF TEA PESTS FROM DARJEELING TERAI TEA PLANTATIONS

More than a thousand species of arthropod pests have been reported from the tea plantations all over the world (Muraleedharan 2007). In India, over 300 species of these pests are known to infest the tea (Radhakrishnan et al. 2001), including about 167 species from the tea growing regions of north eastern part of India (Mukhopadhyay and Roy 2009), stretching from the Darjeeling Himalaya, Terai and Dooars regions to Assam and beyond. This huge pest infestation in this region is due to its favourable agro-climatic conditions (Barbora and Biswas 1996; Sannigrahi and Talukdar 2003). Wide varieties of arthropod pests attack almost all parts of the tea plants such as root, stem, leaves, flowers, seeds causing huge loss in crop yield (Kabir and Das 2015). Table 3 summarizes some of the common arthropod pests from the northern part of West Bengal and the parts of tea plants infested/damaged by these pests. Of these, six major pests, namely red spider mite, *Oligonychus coffeae* (Nietner) (Acarina: Tetranychidae), tea mosquito bug, *Helopeltis theivora* Waterhouse (Hemiptera: Miridae) and the larval stages of lepidopteran moths such as red slug caterpillar, *Eterusia magnifica* Butler (Lepidoptera: Zygaenidae) and looper caterpillars, *Biston* (=*Buzura*) *suppressaria* (Guenee) (Inoue 1982; Inoue 1985; Jiang et al. 2011), *Hyposidra talaca* (Walker) and *H. infixaria* Walker, (Lepidoptera: Geometridae), cause

10-55% crop loss in general (Gurusubramanian et al. 2008) in North East India including northern part of West Bengal. Planters of the tea gardens in this region are facing a lot of problems in combating the outbreaks of these sucking and defoliating pests particularly the looper caterpillars of the geometrid moths. The larval stage of different lepidopteran geometrid moths are the major tea pests and are responsible for the maximum damage of tea crop (Prasad 2016).

Table 1: Year-wise production and export share of major tea producing and exporting countries during last three years

(Anonymous 2020a; Anonymous 2021; Anonymous 2022a)

Country	Production						Export					
	Million Kg			Global Share (%)			Million Kg			Global Share (%)		
	Year			Year			Year			Year		
	2019#	2020#	2021#	2019#	2020#	2021#	2019#	2020#	2021#	2019#	2020#	2021#
China	2799	1986	3063	45	48	47	367	349	369	20	19	19
India	1390	1258	1343	23	20	21	252	210	197	13	11	10
Kenya	459	570	538	07	09	08	497*	519*	559*	26	28	29
Sri Lanka	300	278	299	05	04	05	290	263	283	15	14	15
Others	1202	1177	1212	20	19	19	499	490	521	26	28	27
Total	**6150**	**6269**	**6455**	**100**	**100**	**100**	**1904**	**1831**	**1929**	**100**	**100**	**100**

January-December * Kenya's export include the neighboring African countries produce

Table 2: State/area wise tea production (quantity in Million Kg) during 2019-2022

(Source: www.teaboard.gov.in)

State/Area	2019#	2019-20*	2020#	2020-21*	2021#	2021-22*	2022#
Assam Valley	671.44	651.43	577.88	585.51	623.79	628.17	636.31
Cachar	75.05	43.95	40.32	40.72	43.94	43.97	40.94
Assam	**716.49**	**695.38**	**618.20**	**626.23**	**667.73**	**672.14**	**677.25**
Dooars	240.25	235.63	223.66	228.42	235.75	235.00	226.37
Terai	176.08	172.41	158.89	160.89	165.41	166.58	171.45
Darjeeling	7.96	7.85	6.70	6.74	7.01	7.15	6.60
West Bengal	**424.29**	**415.89**	**389.25**	**396.05**	**408.17**	**408.73**	**404.42**
Others	**30.31**	**29.42**	**28.03**	**28.52**	**32.14**	**32.17**	**28.70**
North India	**1171.09**	**1140.69**	**1035.48**	**1050.80**	**1108.04**	**1113.04**	**1110.37**
Tamil Nadu	155.31	156.00	153.83	160.04	168.67	165.88	157.83
Kerala	59.05	59.26	63.09	66.85	61.11	60.36	67.27
Karnataka	4.63	4.86	5.13	5.34	5.24	5.12	5.02
South India	**218.99**	**220.12**	**222.05**	**232.23**	**235.02**	**231.36**	**230.12**
Total	**1390.08**	**1360.81**	**1257.53**	**1283.03**	**1343.03**	**1144.40**	**1340.49**

January-December * April-March

Table 3: Some common arthropod pests of tea plantations in the northern part of the West Bengal (Kabir and Das 2015)

Common Name	Scientific name	Order: Family	Damaging stage	Parts of tea plant affected by the pest
Cockchafer grub	*Holotrichia impressa*	Coleoptera: Scarabaeidae	Grub	Root
Life wood eating termite	*Microcerotermes obesi*	Blattodea: Termitidae	Adult	Root
Scavenging termite	*Odontotermes assamensis* *O. obesus*	Blattodea: Termitidae	Adult	Root
Red Borer	*Zeuzera coffeae*	Lepidoptera: Cossidae	Larva	Stem and branches
Stem borer	*Xyleborus fornicates*	Coleoptera: Curculionidae	Adult	Stem
Shot-hole borer	*Euwallacea fornicates*	Coleoptera: Curculionidae	Adult	Stem
Tea Thrips	*Scirtothrips dorsalis*	Thysanoptera: Thripidae	Nymphs and adults	Leaf
Tea mosquito bug	*Helopeltis theivora*	Hemiptera: Miridae	Nymphs and adults	Leaf
Tea jassids	*Empoasca flavescens*	Hemiptera: Cicadellidae	Nymphs and adults	Leaf
Aphid	*Toxoptera aurantia*	Hemiptera: Aphididae	Nymphs and adults	Leaf
Looper Caterpillar	*Biston (=Buzura) suppressaria*	Lepidoptera: Geometridae	Larvae	Leaf
Looper Caterpillar	*Hyposidra talaca*	Lepidoptera: Geometridae	Larvae	Leaf
Looper Caterpillar	*Hyposidra infixaria*	Lepidoptera: Geometridae	Larvae	Leaf
Looper Caterpillar	*Ectropis sp.*	Lepidoptera: Geometridae	Larvae	Leaf
Looper Caterpillar	*Ascotis sp.*	Lepidoptera: Geometridae	Larvae	Leaf
Looper Caterpillar	*Cleora sp.*	Lepidoptera: Geometridae	Larvae	Leaf
Red slug caterpillar	*Eterusia magnifica*	Lepidoptera: Zygaenidae	Larvae	Leaf
Bunch caterpillar	*Andraca bipunctata*	Lepidoptera: Endromidae	Larvae	Leaf
Hairy caterpillar	*Arctornis submarginata*	Lepidoptera: Erebidae	Larvae	Leaf
Tea leaf roller	*Caloptilia theivora*	Lepidoptera: Gracillariidae	Larvae	Leaf
Flush worm	*Enarmonica* (=*Cydia*) *leucostoma*	Lepidoptera: Tortricidae	Larvae	Leaf
Tea tortrix	*Homona coffearia*	Lepidoptera: Tortricidae	Larvae	Leaf
Red Spider Mite	*Oligonychus coffeae*	Acarina: Tetranychidae	Larvae, nymphs, adults	Leaf

1.2.1. Looper stage of geometrid moth: the major lepidopteran tea pest

In the Darjeeling Terai region, two congeners of *Hyposidra* belonging to the family Geometridae, *H. talaca* and *H. infixaria* along with another age-old geometrid species *Biston* (=*Buzura*) *suppressaria* form looper caterpillar complex that causes a huge damage to the tea crop as the major lepidopteran pests. In the tea plantations of Terai region, these geometrid species had probably been migrated from the adjoining forest trees (Basu Majumdar and Ghosh 2004; Das, Mukhopadhyay, Roy, et al. 2010). Though the looper stage of *Biston* (=*Buzura*) *suppressaria* was reported as a major pest in North East India long back in 1900 (Das 1965), but *Hyposidra talaca* has emerged as a dominant destructive folivores in the tea plantations of Darjeeling Terai region (Hazarika et al. 2009; Das, Mukhopadhyay, Roy, et al. 2010; Sinu et al. 2011) in recent time and has changed the entire pest dynamics in this region (Redhu and Banerjee 2011). It may be due to their shorter life cycle, absence of diapause stage during winter season, higher number of broods per year and wide range of alternate hosts (Roy et al. 2017).

1.2.2. *Hyposidra talaca*: *the* dominating folivore of tea from the Darjeeling Terai tea plantations

Black inch worm, *Hyposidra talaca* (Walker 1860) (Lepidoptera: Geometridae) is a destructive folivore of tea with a life cycle varying from 30 days (during summer: April-July) to 55 days (during winter : November-February) (Roy et al. 2017). During its larval stage, five instars have been reported; however, in some (about 8%) an additional instar was found (Das, Mukhopadhyay, and Roy 2010), probably to attain the minimum body weight required for pupation (Slansky 1982). The larvae have round, brown coloured head capsules and one pair of abdominal prolegs at the rear and one pair of claspers at the posterior end. Early larval instars are black in colour with seven white-dotted transverse lines on their body but in late instars the colour turns into dark brown with the disappearance of the white dots. Adult moths are blackish brown with diffused and wavy dark patches with a characteristic sub-marginal notch in the forewing. Male and female moths differ in size measuring 4 cm and 4.5 cm across wingspan, respectively. In males, the antennae are bi-pectinate, whereas filiform antennae are found in females (Das, Mukhopadhyay, and Roy 2010). These moths were reported from different countries of South East Asia including India, Malaysia and Thailand feeding on the forest plants and weeds (Browne 1968; Entwistle 1972;

Mathew et al. 2005; Winotai et al. 2005; Das and Mukhopadhyay 2008) and later on shifted from forest plants to the tea plantations of Darjeeling Terai and Dooars region (Basu Majumdar and Ghosh 2004; Das, Mukhopadhyay, Roy, et al. 2010), as well as the plantations in North East India having adjoining forest area (Chutia et al. 2012) and attained the status of major tea pest. It has a wide distribution (Browne 1968; Holloway 1993; Nasu et al. 2004) and has been reported from 90 plant species belonging to 28 families (Robinson et al. 2023) of which 23 host plants have been found to be infested in Terai-Dooars region (Basu Majumdar and Ghosh 2004; Basu Majumdar 2010). In the tea plantations of Terai, this defoliator feeds on the pluckable tea leaves; in the absence of which mature and maintenance leaves can also be consumed by this looper caterpillar. This major tea pest (*H. talaca*) having at least eight broods per year, attacks tea leaves throughout the year even in the winter season due to the absence of obligatory winter diapause (Das, Mukhopadhyay, and Roy 2010). Therefore, different pest management strategies are regularly employed to control this major tea pest in different tea plantations in the Terai-Dooars region of West Bengal.

1.3. A GLIMPSE OF PEST MANAGEMENT STRATEGIES EMPLOYED AGAINST *Hyposidra talaca*

The tea pests are conventionally managed by applying several rounds of synthetic pesticides in the tea plantations of India (Sannigrahi and Talukdar 2003; Gurusubramanian et al. 2008). In the Terai region most of the tea plantations manage the attack of lepidopteran pests by the 'no threshold spraying strategy' using more than two dozen varieties of synthetic, chemical pesticides viz. organophosphates, organochlorines and synthetic pyrethroids with average use of 16.75 kg ha^{-1} (Barbora and Biswas 1996; Sannigrahi and Talukdar 2003; Gurusubramanian et al. 2008). But only a few of those pesticides have been recommended by Tea Research Association, India (TRA) to control the looper complex in the tea plantations ('Plant Protection Code', Version 13, Tea Board of India) (Anonymous 2022b). Quinalphos 25 EC is used to control the early instars, whereas the late instars are controlled by emamectin benzoate 5% SG. In case of bigger caterpillars and for mixed broods, synthetic pyrethroids Deltamethrin and Bifenthrin are recommended to use in for control. Deltamethrin 10 EC is used in rainy season and flubendiamide is used in cold season (Anonymous 2020b). Despite the recommendation of the use of limited pesticides in Plant Protection Code, several other pesticides such as cypermethrin 10% EC

(pyrethroid), chlorpyrifos 50% EC (organophosphate), diflubenzuron 25% EC (benzoylurea) etc. are also used against the looper caterpillars in North East India as well as in the sub-Himalayan Terai-Dooars region of West Bengal (Gurusubramanian et al. 2008; Basu Majumdar et al. 2012; Roy et al. 2017).

According to the Insecticide Resistance Action Committee (IRAC), the different pesticides vary in their mode of action (MOA), such as organophosphates (OPs) (chlorpyrifos, malathion, parathion, quinalphos) act as acetylcholine esterase (AChE) inhibitor, organochlorines (endosulfan, dieldrin) act as GABA-gated chloride channel blockers, pyrethroids (bifenthrin, cypermethrin, deltamethrin) act as sodium channel modulators and avermectins (emamectin benzoate, abamectin) act as allosteric modulator of glutamate-gated chloride channel (GluCl) (retrieved from https://irac-online.org>moa classification) (Anonymous 2023).

Indiscriminate use of synthetic chemical pesticides to control the major defoliating tea pests has led to increasing level of tolerance against these pesticides (Roy et al. 2009; Das, Mukhopadhyay, Roy, et al. 2010; Basu Majumdar et al. 2012; Mukhopadhyay et al. 2014). In this situation, the efficacy of some potential biopesticides such as nucleopolyhedrovirus (NPV) and commercial formulations of *Bacillus thuringiensis* var. *kurstaki* (*Btk*) have been assessed in the management of tea loopers (Mukhopadhyay et al. 2007; Mukhopadhyay et al. 2010; Antony et al. 2011; Mukhopadhyay et al. 2011; Sinu et al. 2015; Dasgupta et al. 2016; Ghosh 2021a; Ghosh 2021b; Ghosh et al. 2023).

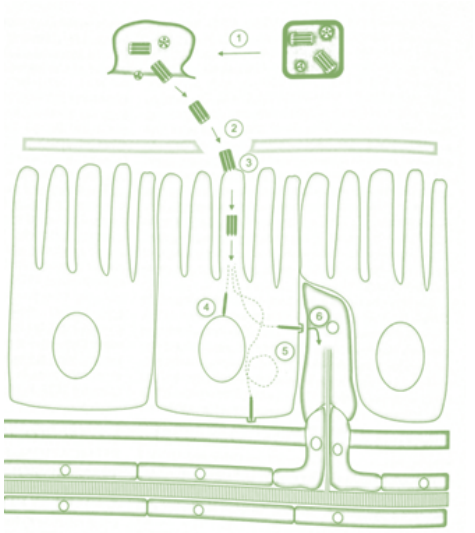
The occlusions or polyhedrals of NPVs on ingestion by the insects are dissolved in the alkaline environment of the gut juices (Pritchett et al. 1982) releasing the occlusion-derived virions (ODVs) that invade columnar epithelial cells of the mid-gut (Granados and Lawler 1981). The ODVs transport through the cytoplasm to nucleus, replicate (Russell et al. 1991; Ohkawa et al. 2010) and after assembly in the nucleus, nucleocapsids exit the nucleus, travel to the plasma membrane and bud through it as budded virions (BVs) (Harrison and Hoover 2012). These BVs found in the haemolymph of the infected larvae serve as vehicle to spread infection to other tissues in the host (Granados and Lawler 1981; Federici and Stern 1990; Long et al. 2006) (Fig. 1). The BV nucleocapsids also translocate through the cytoplasm and enter into the nucleus to replicate and exit the nucleus and bud from plasma membrane either as

BVs or enveloped within the peristromal region of the nucleus to form polyhedron coated ODVs that form OBs (Hughes 1972) (Fig. 2).

Bacillus thuringiensis (*Bt*), a widely used biopesticide is an endospore forming Gram-positive bacterium which undergoes sporulation to produce oval shaped endospore accompanied by parasporal bipyramidal or spherical crystalline inclusions. These proteinaceous crystals are composed of millions of Cry (crystal) or Cyt (cytolytic) toxin molecules (Sekar 1988) encoded by Cry genes (Schnepf et al. 1998). The mode of action of Cry toxins has been characterized in lepidopteran larvae as model (Ibrahim et al. 2010; Soberón et al. 2010). One of the most commercially relevant strain HD-1 of *Bacillus thuringiensis* var. *kurstaki* produces Cry toxins such as Cry1Aa, Cry1Ab, Cry1Ac, Cry2Aa and Cry2Ab (Jurat-Fuentes and Jackson 2012). *Bt* infection commences with the ingestion of spores and/or crystalline inclusions followed by the cessation of feeding and paralysis (Angus and Heimpel 1959). The Cry toxins bind to the receptors on the brush border membrane of the midgut epithelial cells to activate the intracellular cell-death pathway or being oligomerized insert in the membrane to form pores leading to osmotic cell lysis (Bravo et al. 1992; Zhang et al. 2006; Guo et al. 2009; Pacheco et al. 2009) (Fig. 3).

In addition to the chemical pesticides and entomopathogens, few natural enemies such as ants [*Tetraponera rufonigra* (Jerdon, 1951) (Hymenoptera)], shield bugs (Hemiptera), Lynx spider [*Oxyopes sp*. (Arachnida)] have been reported to attack *H. talaca* (Sinu et al. 2011; Roy et al. 2017).

The geometrid tea pest, *Hyposidra talaca* successfully evades these pest management strategies by eliciting their defense mechanism against the chemical pesticides and the entomopathogens used to control this pest. It makes them resistant to many of the regularly used pesticides leading to control failure. Therefore, a comprehensive knowledge of the defense system as well as the immune responses against these xenobiotics and the microorganisms is necessary to assay their efficacy as the controlling agents in the pest management program.



1. Occlusions ingested by larvae dissolve in the host midgut lumen, liberating (occlusion derived virions) ODVs
2. Translocation of ODVs through peritrophic matrix lining of the midgut epithelium
3. Binding of the ODVs to the microvilli of midgut columnar epithelium and entrance of the nucleocapsid after fusion of the ODV envelope and the membrane
4. Movement of the nucleocapsid through the cytoplasm of the epithelial cell and translocation of some to the nucleus
5. Within the nucleus nucleocapsid uncoat their DNA, express genes. Other nucleocapsids translocate to the basolateral domain of the cell and bud through the plasma membrane forming budded virions (BV)
6. Infection of the tracheoblasts with BV from midgut epithelial cell

Figure 1: A model for baculovirus primary infection of lepidopteran larvae with multiple nucleopolyhedrovirus (MNPV).

(Harrison and Hoover 2012)

1. Binding of BVs to the cell surface followed by internalization by receptor mediated endocytosis
2. Fusion between the endosomes membrane and BV envelope
3. Release of nucleocapsid into the cytoplasm which is followed by the movement through the cytoplasm and translocation to the nucleus
4. Release of DNA within the nucleus followed by early gene expression, formation of virogenic stroma (VS) accompanied by DNA replications and assembly of progeny nucleocapsid
5. Initially progeny nucleocapsids exit the nucleus and bud from the plasma membrane to form BVs
6. Later, nucleocapsids are enveloped within the peristomal region to form occlusion derived virions (ODVs)
7. Crystallization of the polyhedron around ODVs to form occlusion bodies (OBs) the surface of which is covered with the involvement of fibrillar bodies (FBs).

Figure 2: Showing secondary infection by budded virions (BVs).

(Harrison and Hoover 2012)

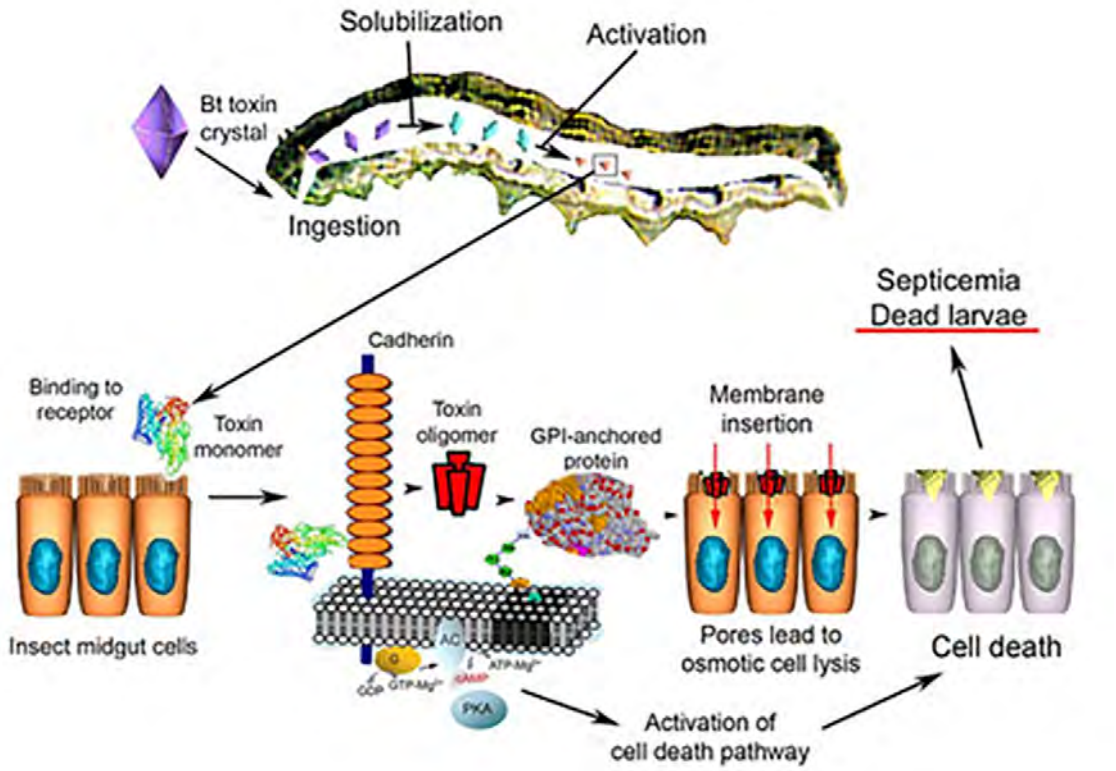


Figure 3: Mechanism of Cry toxin action.

After ingestion of toxin crystals the Cry proteins being solubilized, activated and translocate through the peritrophic membrane binds to the receptors of midgut epithelial cells. It activate the cell death pathway or being oligomerized form pores that laead to osmotic cell lysis. Disruption of the midgut epithelial barrier facilates the invatson of haemocoel by the vegetative *Bt* cells causing septicemia and larval death (Jurat-Fuentes and Jackson 2012).

1.4. DEFENSE SYSTEM OF INSECTS

Insects are the earliest and most successful group of animals accounting for more than 32 orders and nearly one million species (Narayanan 2004). Except for the sea they colonize all ecological niches where the probability for being infected by microorganisms and parasites is comparatively high. To cope with an extremely large variety of entomopathogens, insects evolved with a complex and effective innate defense system which apparently differs from the adaptive immune system of vertebrates. Insects try to resist infection or toxic effects of xenobiotics through morphological, behavioral, physiological, cellular, biochemical and molecular mechanisms (Narayanan 2004). Both haemocyte-associated molecules (cellular factors) and the haemolymph-borne molecules (humoral factors) constitute the innate immune arm of insects.

1.4.1. Cellular defense system

Cellular defense system of insects consisting of free blood cells or haemocytes, recognize a variety of foreign invaders via cell surface receptors. Haemocyte-mediated defense responses, induced by the recognition of foreign invaders, are regulated by signalling factors and effector molecules that control cell adhesion and cytotoxicity (Strand 2008).

1.4.1.1. Haemocytes

The haemolymph of insects, present in the general body cavity (haemocoel), consists of plasma and numerous blood cells or haemocytes (Jones 1962). The haemocytes circulate within the haemolymph and sometimes attach loosely to other tissues in haemocoel (Arnold 1974). The most common types of haemocytes reported from the species of the order Lepidoptera are prohaemocytes (PRs), granulocytes (GRs), plasmatocytes (PLs), spherulocytes (SPs) and oenocytoids (OEs) (Butt and Shields 1996; Lavine and Strand 2002). In the older literatures, several other names of haemocytes such as, adipohaemocytes (AHs), coagulocytes (COs), podocytes (PCs), vermicytes (VEs) have also been used in different species and orders of insects for those which are potentially one of the major haemocyte types (Jones 1962; Ribeiro and Brehélin 2006). For example, the three types of haemocytes found in *Drosophila* (Diptera), *viz.* plasmatocytes, crystal cells and lamellocytes are morphologically and functionally similar to granulocytes, oenocytoids and plasmatocytes, respectively in Lepidoptera (Lavine and Strand 2002). On the other hand, the term ‘adipohaemocyte’,

first introduced by Hollande (1911), later found to be indistinguishable from GR (Arnold 1974) and was reported as the functional stage of granulocytes formed by progressive accumulation of lipid droplets (Raina 1976). The different types of haemocytes have important roles in the protection of insects against invading microorganisms (Narayanan 2004).

Haemocytes develop from the head or dorsal mesoderm during embryogenesis (Hoffmann et al. 1979; Ratcliffe et al. 1985; Tepass et al. 1994). During larval and nymphal stages, all types of haemocytes are derived from PRs, a type of stem cell in the haematopoietic organs or by continued divisions of haemocytes already in the circulation (Akai and Sato 1971; Ratcliffe et al. 1985; Lavine and Strand 2002). Most lepidopteran larvae such as larvae of *Spodoptera frugiperda*, *Pseudoplusia includens*, *Manduca sexta, Bombyx mori* have four single-lobed haematopoietic organs located in the meso- and meta-thoracic regions, near the wing imaginal discs (Gardiner and Strand 2000; Strand 2008). The PRs differentiate into PLs in the hematopoietic organs and are released into circulation. PLs in the circulation differentiate into GRs, SPs and OEs (Beaulaton 1979). In larval stages of lepidopteran insects, the GRs and PLs together comprise more than 50% of the circulatory haemocytes and are the only haemocyte types that can adhere to the foreign surfaces and thus, are the major cellular components, involved in phagocytosis and encapsulation of pathogens (Lackie 1988; Strand and Pech 1995; Lavine and Strand 2002). SPs have been suggested to transport cuticular components (Sass et al. 1994); while OEs contain cytoplasmic phenoloxidase (PO) precursors, that likely play a role in melanization of haemolymph (Ashida and Dohke 1980; Iwama and Ashida 1986; Jiang et al. 1997). The haematopoietic organs in *Drosophila* (Diptera) are the lymph glands consisting of anterior primary lobe consisting of cortical zone, medullary zone and posterior signaling centre containing prohaemocytes, plasmatocytes, crystal cells, lamellocytes (following attack by parasitoid wasp) and several posterior secondary lobes containing pre-prohaemocytes, prohaemocytes and plasmatocytes (Lanot et al. 2001; Crozatier et al. 2004; Jung et al. 2005; Wertheim et al. 2005). Differentiated plasmatocytes are released in the haemolymph, comprising 90-95% of all mature haemocytes that function as the professional phagocytes. Crystal cells, comprising approximately 5% of the haemocytes in the circulation, synthesize the phenoloxidase (PO) cascade components (Lanot et al. 2001; Wertheim et al. 2005). In insects, the main defensive responses mediated by the

haemocytes are phagocytosis, nodulation, encapsulation and coagulation (Strand and Pech 1995; Schmidt et al. 2001; Lamprou et al. 2005; Mavrouli et al. 2005; Sideri et al. 2008).

Phagocytosis

Phagocytosis is a widely conserved defense response in which phagocytes internalize and destroy small targets (like bacteria and viruses). The haemocytes that phagocytose pathogens are plasmatocytes or granulocytes in Lepidoptera, Hemiptera and Diptera (Wittig 1965; Werner and Jones 1969; Ratcliffe and Rowley 1975; Strand 2008; Hillyer and Strand 2014). In *Drosophila* (Diptera) plasmatocytes are the professional phagocytes that engulf pathogens, dead cells and the other entities that gain entry into haemocoel (Nelson et al. 1994; Elrod-Erickson et al. 2000; Asha et al. 2003). Phagocytosis is initiated by the binding of cell surface pattern recognition molecules such as thioester containing proteins *viz.* nimrod proteins, β-integrins to the pathogen-associated molecular patterns (PAMPs) (Levashina et al. 2001; Rämet et al. 2002; Dong et al. 2006; Kurucz et al. 2007; Mamali et al. 2009).

Nodulation

Nodulation refers to binding of multiple haemocytes to the aggregation of bacteria or other particulate materials, that probably cannot be efficiently cleared by phagocytosis alone following the invasion into the haemocoel (Ratcliffe and Gagen 1977). In Lepidoptera GRs and PLs participate in nodulation, whereas lamellocytes play important role in nodulation in *Drosophila* (Diptera) (Schmidt et al. 2001; Vass and Nappi 2001). Nodulation is initiated by the GRs that interacts with bacteria and degranulate to form sticky substance entrapping large numbers of bacteria and other reactive haemocytes and a large number of PLs to form the outer sheath around the target (Gagen and Ratcliffe 1976; Ratcliffe and Rowley 1979). Nodules are melanized in some insect species but not in others (Strand and Pech 1995). The melanization pathway is activated by OEs, releasing pro-phenoloxidase (PPO) that triggers phenoloxidase (PO) cascade by the activity of serine proteases of haemolymph (Kurihara et al. 1992a).

Encapsulation

Encapsulation refers to the binding of multiple haemocytes to larger targets like parasitoids, nematodes that are too large to be phagocytosed (Salt 1963; Hall et al. 1969; Shapiro 1969). The mode of encapsulation varies in different insects. It usually occurs within 24 hrs of parasitism (Shapiro 1969) and involves the formation of multi-

layered, overlapping sheath of haemocytes (PLs and GRs) (Jones 1962; Shapiro 1969; Price and Ratcliffe 1974; Schmidt et al. 2001) that may partially or wholly become melanized, killing the enclosed parasites by asphyxiation or by toxins produced by the haemocytes (Lundgren and Jurat-Fuentes 2012). The distribution of GRs and PLs in capsules formed by some lepidopterans appears random (Wiegand et al. 2000) while in others it is highly organized, the GRs being the first cells to bind to the target, PLs attaching thereafter and finally, monolayer of GRs (Schmit and Ratcliffe 1977; Pech and Strand 1996).

Coagulation

Coagulation of insect haemolymph occurs at the site of external wounding (Gregoire 1970; Theopold et al. 2004). The role of haemolymph coagulation is to seal off wounds, prevent excessive haemolymph loss, exclude bacteria from access to the haemocoel and assist in wound repair (Gregoire 1970). The cells responsible for coagulation have been termed hyaline haemocyte (Gregoire 1970), which probably represent coagulocytes (COs) and/or granulocytes (GRs) (Rowley and Ratcliffe 1976).

1.4.2. Humoral defense system

Humoral defense is characterized by the synthesis of an array of soluble effector molecules including anti-microbial peptides (AMPs) in the fat body and subsequent secretion into the haemolymph (Bulet et al. 2004; Imler and Bulet 2005) and by a rapid activation of the complex proteolytic cascade such as phenoloxidase (PO) cascade in the haemolymph (Cerenius and Söderhäll 2004).

1.4.2.1. Anti-Microbial Peptides (AMPs)

Anti-Microbial Peptides (AMPs) are small peptides synthesized in response to infection of microorganisms by the fat body and certain blood cells and released into the haemolymph (Gillespie et al. 1997; Bulet et al. 1999; Nakatogawa et al. 2009; Yu et al. 2010; Iakovlev 2011). The AMPs produced by lepidopteran insects are lysozymes (Powning and Davidson 1973), cecropins (Hultmark et al. 1980; Boman et al. 1991) and attacins (Hultmark et al. 1983). The common features of these peptides are low molecular weight, a net positive charge at physiological pH and having amphiphilic α helices or hairpin like β-sheet or mixed structure. Some AMPs are very specific in their location and function (Hoffmann 1995), such as cecropins are located on cell membranes; other AMPs are restricted to the genital tract of the insects (Bulet et al. 1999). Another cysteine-rich antimicrobial peptide, insect defensins have been isolated

from the insects of diverse orders, namely Diptera, Coleoptera, Hymenoptera, Hemiptera, Trycoptera, Odonata, except Lepidoptera (Bulet et al. 1999; Bulet and Stocklin 2005). Simultaneous presence of a variety of AMPs acting in synergy provides insects a powerful defense against the microbial invaders (Bulet et al. 1999).

1.4.2.2. Phenoloxidase (PO) cascade

Phenoloxidases are key chemicals in the cascade of reactions that ultimately lead to sclerotization, encapsulation, melanization and wound healing. Insect contains two types of phenoloxidases, one, the laccase type enzymes (EC 1.10.3.2) which oxidize o- or p-diphenols to quinines, leading to sclerotization and tanning of cuticle (Dittmer et al. 2004; Arakane et al. 2005) and the other, tyrosinase-like enzymes (EC 1.14.18.1) which hydrolyze tyrosine and also oxidizes o-diphenols to quinines leading to melanization (Gorman et al. 2007). Phenoloxidase is found as an inactive pro-phenoloxidase (PPO) in the OEs of lepidopteran species (Iwama and Ashida 1986; Jiang et al. 1997) and as zymogen in the crystal cells of *Drosophila melanogaster* (Rizki et al. 1985; Williams 2007). These are released from the haemocytes into the plasma by spontaneous rupture upon injury and at low rate even in the absence of injury or infection (Ashida and Brey 1997). In lepidopterans, the bulk PPO appears in plasma (Saul et al. 1987; Ashida and Brey 1997), but in hemimetabolous insects, PPO appears to be mostly retained in haemocytes until microbial exposure stimulates its release (Leonard et al. 1985; Brehélin et al. 1989; Durrant et al. 1993). Though in absence of injury or infection, oenocytoid-lysis maintains an optimum level of PPO in plasma but the lysis of the PPO-producing haemocytes at the site of injury or infection could result in a high concentration of PPO at the site of the infection (Kanost and Gorman 2008). Bidla et al. (2007) reported that a signal transduction pathway stimulates crystal cell lysis in *Drosophila melanogaster*. The PPO are activated by the proteolytic cleavage at a specific site near their amino-terminus through the action of haemolymph serine proteases (Kanost and Gorman 2008).

1.4.3. Metabolic defense system

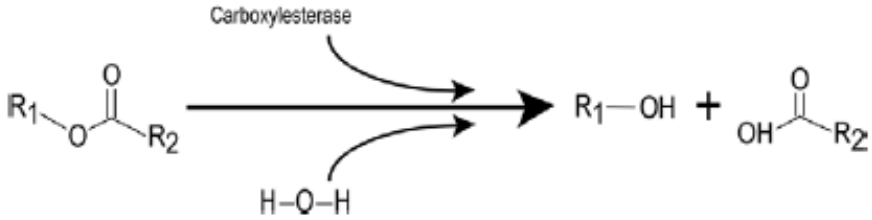
Metabolic defense is a common mechanism, based on the enzymatic systems that protect the insects by detoxifying or sequestering insecticide and/or xenobiotic molecules. The enzymes involved are those developed in insects as protection against naturally occurring plant toxins (allelochemicals), in order to overcome the potential

toxicity of the plants they feed on (Gatehouse 2002; Després et al. 2007; War et al. 2012; Heidel-Fischer and Vogel 2015). Such defense enzymes can detoxify xenobiotics into a non-toxic compound and/or into an intermediate metabolite(s) suitable for rapid elimination from the body.

Detoxification can be divided into phase I (primary) processes, consisting of hydrolysis, oxidation or reduction and phase II (secondary) processes, consisting of conjugation of phase I metabolites with endogenous compounds such as sugars, phosphate, amino acids, glutathione and their subsequent excretion from the body (Li et al. 2007). By this biotransformation the lipophilicity of the xenobiotics is reduced, so that they can be excreted from the body easily (Yu 2014). The main enzyme families involved in detoxification of the pesticides are the general esterases, oxidases and Glutathione S-transferases (GSTs).

1.4.3.1. General Esterase

Esterases are a large group of phase I enzymes that can act against a broad range of insecticides, including pyrethroids, organophosphates, carbamates (Hollingworth and Dong 2008). General esterases (GEs) catalyze the hydrolysis of the ester insecticides into their corresponding acids and alcohol compounds which increase the polarity of the insecticidal metabolites that can be excreted more readily from the insect body, as shown below:



Esterase can also sequester insecticides, therefore these toxic molecules are no longer available for interaction with their targets (Wheelock et al. 2005).

1.4.3.2. Monooxygenases

Monooxygenases (phase-I enzymes) are able to convert lipophilic compounds into polar metabolites which can be easily eliminated from the body. Cytochrome P450 (CYP) monooxygenases are microsomal or mitochondrial haem containing oxidases that catalyzes the transfer of one atom of molecular oxygen to a substrate and the

reduction of the second atom of oxygen to form water (Guengerich 2001; Feyereisen 2005), as shown in the reaction:

$$RH+O_2+NADPH+H^+ \longrightarrow ROH+H_2O+NADP^+$$

Cytochrome P450 is known to have a wide range of substrates, therefore has a greater role in the metabolism of many insecticide classes, including carbamates, organophosphates (Scott 1999; Després et al. 2007). To date more than 600 insect CYP genes have been characterized (Feyereisen 2015). Analysis of the sequences of CYP genes indicates that these fall into four major clades (Feyereisen 2006) or subclasses (Gotoh 1993) or clans (Nelson 1998). These are CYP2, CYP3, CYP4 and mitochondrial CYP clan (Feyereisen 2012).

1.4.3.3. Glutathione S-transferase

Glutathione S-transferases (GSTs) are phase-II enzymes that catalyze the conjugation of reduced glutathione (GSH) with the substrates, converting those reactive molecules into easily excretable water-soluble and non-toxic conjugates (Habig et al. 1974; Grant and Matsumura 1989; Singh et al. 2001).

$$\text{Glutathione-SH + Xenobiotic} \longrightarrow \text{Glutathione-S-Xenobiotic}$$

Insect-GSTs are primarily associated with the metabolism of organochlorines, pyrethroids (Clark and Shamaan 1984; Grant and Matsumura 1989; Ranson et al. 1997). Due to the broad range of substrates of the individual enzyme, they play an important role in developing resistance to different classes of insecticides (Panini et al. 2016). GSTs may protect the insects from the pyrethroid toxicity by sequestering the insecticide (Kostaropoulos et al. 2001). GSTs have two domains, a conserved N-terminal domain responsible for the binding of GSH and structurally diverse C-terminal domain that binds to the substrate (Danielson and Mannervik 1988).

Several studies on the defense system of the insect pests from diverse orders have been conducted. The knowledge acquired from these studies on immune status of insect pests against the chemical pesticides and entomopathogens is very important to basic insect biology in understanding the defense mechanisms and etiology of insect diseases caused by microbial infections, as well as to the applied entomology to assess the efficacy of the entomopathogens and active ingredients of the pesticides in biological and chemical control, as it depends on the immunologic defense status of insects.

Emergence of the black inch looper caterpillar of *Hyposidra talaca* as one of the major tea pests in the tea plantations of the sub-Himalayan Terai region in recent years has made the utmost necessity to study the different aspects of this pest. Few studies on bio-ecology, life cycle traits, survivorship study, consumption and utilization of food, feeding behavior, extent of damage and genome organization have been conducted (Prasad and Mukhopadhyay 2013; Das 2014; Ghosh et al. 2015; Prasad and Mukhopadhyay 2015; Dasgupta et al. 2016; Roy et al. 2017; Ghosh et al. 2019). Prasad and Mukhopadhyay (2015) also formulated an artificial diet (AD) for rearing of the larvae in the laboratory condition. However, there is no information regarding the defense system and the immune response of *Hyposidra talaca* against the challenge of chemical pesticides and entomopathogens. In this investigation the defense system of this geometrid pest along with the effect of regularly used chemical pesticides, entomopathogen-based biopesticides as well as the potential entomopathogen (NPVs) that can be developed as an effective biopesticide, on the defense system have been studied for the first time. Knowledge from this study can be helpful to optimize the pest management strategy against this major tea pest (*Hyposidra talaca*) in future.