2. REVIEW OF LITERATURE

2.1. Impact of Starvation on Economic and Reproductive Characters.

2.1.1 Food Consumption and Utilization : The consumption of optimum amount of food and its utilization is essential for growth, development and reproduction in all insects as this efficiency in turn reflects on various performances such as growth rate, final body weight, developmental period, dispersal ability and probability of survival. Growth involves conversion of extraneous matter into live tissue through the process of ingestion, digestion, absorption, assimilation and synthesis. Growth in insects includes both somatic growth and reproductive growth. In *Bombyx mori* final growth attained by larvae at the termination of feeding and before undergoing pupation represent total growth as the species pass through a nonfeeding adult stage (Muthukrishnan and Pandian, 1987). So, accumulation of sufficient energy and nutrient is essential before undergoing final moult or metamorphosis in silkworms as this metamorphic efficiency solely depends on the proportion of energy content in initial pupae. Formation of an expensive pupal case and/or an increase in pupal metabolism caused due to rise to temperature or by extension of pupal period decreases metamorphic efficiency. So optimum food availability influences greatly in conversion of ingested food into body tissue of the larvae and transformation of pupal matter into adult tissue (Muthukrishnan and Pandian, 1987; Anantharaman *et.al.,* 1993).

Consumption of utilization of food by *B.mori* has been elaborately studied by Hiratsuka (1920); Matsumura and Takeuchi (1950); Matsumura *et al.,* (1955) ; Takeuchi *et al.*, (1964); Ueda (1965) ; Ueda and Suzuki (1967); Horie *et al.*, (1976); Slansky and Scriber (1982); Horie and Watanabe

(1983); Nagata and Kobayashi (1990). Work on this aspect of different Indian breeds of *B.mori* is scanty but some works on multivoltine, bivoltine and hybrid of multi X bivoltine have been carried out by Benchamin and Jolly (1984) ; Periswami *et al.*, (1984); Periswami and Radhakrishnan (1985); Naik and Delvi (1987); Haniffa *et al.*, (1988); Remadevi *et al.*, (1992); Anantharaman *et al.*, (1993). Apart from this, reports on relationship with nutrition and , silk protein (Fukuda, 1960); body weight gain (Ueda and Suzuki, 1967); cocoon productivity (Takano and Arai, 1978); silk production (Muthukrishnan *et al.*, 1978); economic characters (Sumioka *et al.*, 1982); interstrain difference (Yamamato and Fujimaki, 1982); dry matter utilization (Horie and Watanabe, 1983); energy utilization (Horie and Watanabe, 1985) utilization of nitrogen (Horie and Watanabe, 1986); dietary level of protein and pyridoxin for growth (Hamano and Tashihiko, 1989); contribution of tissue protein for cocoon shell (Jadhav and Kallapur, 1989); leaf silk ratio (Ding Nong *et al.*, 1991); effect of high temperature (Upadhyay and Mishra, 1991); effect of humidity (Mishra and Upadhyay, 1992); storage protein level in larval haemolymph (Nagata and Kobayashi, 1990) on *B.mori* are available.

The quantity of food consumed influences the digestibility and conversion efficiency. It has been suggested that there exists a mutual relationship between food ingested, digested and assimilated in *B.mori.* A positive low correlation has been observed between ingestion, digestion and digestibility by Yamamato and Fujimaki (1982). Similar correlation has been corroborated by Remadevi *et al.*, (1993) in case of Indian silkworm breeds. However, the authors have opined that the characteristics vary from breed to breed and high consumption may not result in high assimilation. Higher efficiency of conversion of ingested food and digested food into body tissue in less fed batches of silkworm has been reported by Sumioka *et al.*, (1982). Some positive significant correlation between ingestion, digestion and digestibility has been observed by Benchamin and Jolly (1984); Naik and Delvi (1987); Remadevi *et al* (1993); Anantharaman *et al.*, (1993). A lower value for efficiency in conversion of ingested food in 5th instar larvae of *B.mori* has been reported by Horie *et al.*,(1976), Horie and Watanabe, (1983), Benchamin and Jolly,(1984) Anantharaman,(1993). It is suggested that the reduction in digestibility is due to high content of crude fibre in food as stated by Waldbauer (1964). Waldbauer (1964) suggested that digestibility is affected by nutritional deficiency, high content of crude fibre and deficiency of water in food. In insects other than *B.mori*, consumption of food per larva per day in *Dasychira mendosa* (Lepidoptera, Lymantridae) has been worked out by Singh and Prasad (1990) and in *Antheraea proylei* by Yadava *et al.*, (1983). A consistent rate in egestion depending on the age of instar has been recorded for *Dasychira mendosa* (Singh and Prasad, 1990), *Protoparce sexta* (Waldbauer, 1964). Low approximate digestibility during fifth larval instar has also been recorded in *Antheraea proylei* (Yadava *et al.*, 1983) and *Antheraea assama* (Barah *et al.*, 1989). Under starvation stress (rationing of food or deprivation) in *B.mori*, the efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) were found to be changed. The values were found to be higher than those of control (Sumioka *et al.*, 1982; Nath *et al.*, 1990; Tzenov, 1993). The values of ECI and ECD varied from race to race and mulberry varieties on which values were assayed (Horie and Watanabe, 1983; Periswami *et al.*, 1984; Remadevi *et al.*, 1992; Sarkar and Fujita, 1994). The digestibility in *B.mori* is affected due to starvation as it is a continuous feeder (Waldbauer, 1964). But this periodic food deprivation increases residence time of food in larval gut enhancing assimilation efficiency. Due to higher feeding rate in 5th stage larvae the ingested food has less time in gut for enzymetic activity and absorption hence, reducing the digestibility (Waldbaur, 1964). Starvation may also cause slow growth which increases maintenance cost. If this maintenance cost exceeds benefit level the efficiency of digestion and assimilation are effectively lowered (Schroeder, 1976). He also demonstrated that when larvae of cherry scallop moth, *Calocalpe undulata* was deprived of food for a short period no significant effect was obtained on economy of food utilization. But when deprivation period was long, ingestion, faecal production and larval growth were decreased. Due to increase in respiration rate during starvation, attributed to increased relative maintenance cost, the efficiency of growth is depressed. The final instar larval period has been divided into two phases : an obligatory feeding period and a facultative feeding period as defined by Bounhiol (1938) and described in *B.mori* by Legay (1958). It is suggested that if larvae is stopped feeding during the first phase the growth is stopped, development terminates and larvae dies. But if starvation is imposed during second phase, development continues and moult occurs after a few days. This change in signals through food intake from one phase to the other are accompanied by important metabolic changes (Calvez and Fourche, 1980). Legay (1958) in *B.mori* and Nijhout (1975) in *Manduca sexta* demonstrated that not only feeding period but attainment of critical weight by larvae is a must to undergo pupal moult. Bosquet (1979) differed in opinion from Bounhiol and Legay and opined that starvation during obligatory feeding period do not stop total physiology in larvae rather functions continue but slowly. Accelerated development rate was observed in *Prothetria dispar*, the gypsy moth, when feeding was resumed after starvation (Leonard, 1970). Thus, a question arises about the selective advantage of feeding tatics that involve long non feeding period for lepidoteran larvae if they have some mechanism, to enhance the efficiency of food utilization, to compensate for the losses due to prolonged starvation, or any other selective advantages resulting from their activity pattern sufficient to compensate for a lower efficiency of growth (Schroeder, 1976). Apart from these above mentioned physiological impairment, caused due to starvation, during 5th larval instar of *B.mori*, reports on other effects such as., reduction in survival percentage of larvae (Samson *et al.*, 1981; Haniffa *et al.*, 1988; Nath *et al.*, 1990; Himantharaj, 1994), prolongation of larval duration (Haniffa *et al.*, 1988), retention of excess water in larval body (Delvi *et al.*, 1988), susceptibility to viral diseases (Samson *et al.*, 1981) are available.

2.1.2 Economic parameters : The growth rate in *B.mori* larvae has been found to be related to the capacity of food intake and growth rate of tropical silkworms has been found to be relatively slower than temperate silkworms (Mathur *et al.*, 1989). Variation in growth rate among different tropical multivoltine silkworm breeds has been observed, by Remadevi *et al.*, (1991), which seemed to be dependent on larval duration and environmental conditions (mainly temperature and humidity) of rearing. It is suggested that maximum growth rate has been obtained for *B.mori* during 5th larval stage (Ueda, 1982; Yamamato and Fujimaki, 1982; Tzenov, 1993). Higher growth rate during 5th larval stage in silkworm larvae other than *B.mori* has been reported, in *Philosamia recini*, Hutt. (Poonia, 1978), in *Antheraea proylei* Jolly. (Biren *et al.*, 1987). Due to feeding restriction growth rate was found to be reduced than that of control (Tzenov, 1993). Extension of larval period and a constant pupal period has been observed in the larvae of bivoltine race of *B.mori* subjected to feeding regulation (Mathavan and Muthukrishnan, 1976; Mathavan *et al.*, 1987; Haniffa *et al.*, 1988; Paul and Deb, 1993). Increase in mortality in larvae due to starvation during fifth instar (Samson *et al.*, 1981) was found to be reversed by increase in food quantities (Haniffa *et al.*, 1988). Low effective rearing rate has been recorded by Himantharaj (1994) in less fed batches of bivoltine.

Yamamato and Gomo (1976) observed a positive significant correlation between ingesta and cocoon weight, shell weight and larval weight gain in *B.mori*. Fukuda (1963) using C_{14} lebelled mulberry leaf infered that leaves consumed from 3rd day of 5th instar till spinning were assimilated for production of first 1000 mts of silk filament. A significant positive correlation has been obtained between ingestion and cocoon, cocoon shell weight by Ding Nong *et al.*, (1991), Remadevi *et al.*, (1993). Efficient utilization of dry matter of leaves by multi x bivoltine hybrid has been recorded by Anantharaman (1993). A significant decrease in cocoon shell weight depending on degree of starvation stress during the 5th larval instar of *B.mori* has been reported (Samson *et al.*, 1981; Mathavan *et al.*, 1987; Haniffa *et al.*, 1988; Nath *et al.*, 1990; Basu *et al.*, 1992 ; Tzenov, 1993; Janarthanan *et al.*, 1994). Kurata (1985) observed that starvation of *B.mori* larvae from 3rd day to 5th day of fifth instar slowed down the accumulation rate of RNA in silkgland. Recovery of RNA accumulation was delayed even when the larvae were refed. The percentage of crude fibroin and RNA content decreased to more than half of control level and never recovered in rest of the larval period. This action was more sensitive in batches starved on day 3 of the fifth larval instar than that of the batches starved on the day 5. Nagata and Kobayashi (1990) infered that starvation decreased storage protein level in haemolymph which again was increased when larvae were refed. Thus, it is evident that level of storage protein in larval haemolymph is related to the larval feeding activity. Starvation for shorter duration caused significantly less impairment of economic characters (Basu *et al.*, 1992). It is suggested that larvae starved 1~2 days before spining spun on same day with control. Same result with decrease in cocoon shell weight and prolongation of larval duration has been recorded in eri silkmoth *Philosamia recini* by Srivastava *et al.*, (1982). However, Himantharaj (1994) did not observe any significant effect on cocoon weight, shell weight in batches where feeding was rationed in early part of the 5th stage larvae and normal feeding supplied during later part of fifth stage in Indian bivoltines.

2.1.3. iii) Impact on reproductive performances : Food consumption and fecundity of insects vary with life style and feeding pattern. The species which feed during adult stage and maintain a smaller biomass, allocate a high percentage of ingested energy to egg production efficiency(e.g. *Oryzaephilus surinamensis*). Species which grow larger but feed at a lower rate and pass through extended adult life span display very low egg production efficiency (e.g. *Poeciloccus pictus*). But species which feed at a faster rate during larval life (e.g. *B.mori*) and have a short non-feeding adult phase, allocate fairly a high percentage of ingested energy to egg production (Muthukrishnan and Pandian, 1987). The source of energy for rapid developmental activity during the pupal life and subsequent production of eggs by adult female in *B.mori* high nutritional storage during larval life particularly 5th instar feeding period is of utmost importance. Fifth instar larvae itself consume. 87% of the total food (Matsumura and Takeuchi, 1950) and length of the fifth instar larval period is more than twice of the preceeding instars. The quantity of food consumption increases day by day the highest being on the day before spinning (Horie and Watanabe, 1983). So, underfeeding during fifth instar through fed rationing or starvation has definitely some bearing on pupal weight and decreased fecundity. Paul and Deb (1993) reported that weight of eggs laid by underfed moths were less than that of control. Fukuda (1963) concluded that the eggs laid by moth is formed from food consumed at early and middle stage of fifth larval span whole of 4th instar. Low fecundity (Haniffa *et al.*, 1988; Nath *et al.*, 1990), reduction in number of mature eggs (Kawaguchi *et al.*, 1991) has been reported as an effect of food deprivation during final larval period. Feeding stress has a direct relation with the reproductive rate in insects. Reproductive rates are found to be decreased in all treatments where larvae have been starved for more than 6 hrs after normal moulting as demonstrated in *B.mori* by Benchamin *et al.*, (1992) and in oriental monarch butterfly, *Danus Chrysippus* by Mathavan *et al.*, (1976).

Silkworm eggs being sheet anchor of sericulture industry, the production of silkworm eggs is directly related to quality of seed cocoons (Benchamin *et al.*, 1988, Narasimhanna, 1988). The heaviest cocoon results in highest fecundity (Govindan *et al.*, 1991; Singh *et al.*, 1994). A significant positive correlation between female pupal weight and fecundity in *B.mori* has been established when rearing conducted under optimum condition (Jayaswal *et al.*, 1991; Shaheen *et al.*, 1992). Reduction in pupal weight and size has been reported from larval batches starved from day 4th of the fifth instar in *B.mori* by Paul and Deb, (1992). In other silkmoths also, heavy pupae leads to higher fecundity as has been observed in *Antheraea myllita* (Badheru, 1992), in *Philosamia recini* (Singh *et al.*, 1987). Himantharaj (1994) working with bivoltine (NB18, NB_4D_2) seed crop indicated that a low feeding in early fifth instar followed by optimum feeding in later part of fifth instar resulted in optimum pupation rate and optimum fecundity.

2.1.4 Utilization of Nitrogen : *B.mori* can efficiently utilize nitrogen in food ingested for formation of silk protein during fifth larval stage (Horie and Watanabe, 1986). Review on utilization of nitrogen from food in silkworm has been done by Fukuda (1960), Florkin and Jeuniaux, 1964). Horie and Watenabe (1986) studied in detail the daily utilization of nitrogen in food by *B.mori*. They found that the silkglands increased markedly with the age of the 5th instar indicating higher accumulation of silk protein in latter half of 5th instar. In female larvae this increase of nitrogen amount was higher than male in early part of fifth instar. ECI and ECD for nitrogen were higher in females than males. However, Horie and Watanabe (1983) worked out that ECD values for cocoon shell were higher in males than females and ECD values for eggs were higher in female indicating nitrogen rich substances concentrated in eggs of *B.mori*. Schroeder (1976) indicated that starvation affects utilization efficiency of nitrogen in lepidopteran larvae. Assimilation of nitrogen has been reported to affect the growth rate.

2.2 Starvatioin and simultaneous application of juvenile hormone analogue - methoprene - its impact on economic and reproductive character.

2.2.1 Food consumption and utilization : Nijhout (1981) demonstrated that attainment of critical weight is essential for undergoing metamorphosis. Juvenile hormone analogue administered topically or orally (externally) exerts morphogenic effect along with other environmental stimuli by either inhibiting or accelerating moulting process through interference with the prothoracic glands and brain (Slama, 1971; Gilbert et al. 1980; Sehnal, 1988; Akai, 1988). Through ligation of silkworm larvae with a fine silk thread at the point between their thorax and abdomen Akai (1988) observed that pupal characters were induced in the head and thorax and not in the abdomen. Again in case where corpora allata were extirpated from early third instar larvae they became pupae directly without any larval moult. Thus he infered that growth of silkworm in larval stage is controlled mainly by ecdysone released from prothoracic gland and juvenile hormone (JH) released from neurosecretory cells of brain through corpora allata. Ecdysone induces moulting and JH helps in retaining juvenile characters in larval life. Thus, when ecdysone concentration increases in haemolymph pupation is induced and when JH concentration is high larval moulting takes place. Earlier during 1973, Akai *et al.*, working on influence of JH on growth and metamorphosis of *B.mori* larvae demonstrated that administration of JHa during fifth instar induces effect on growth and metamorphosis through prolongation of larval period, increase in silk production, induction of dauer larvae and supermoult larvae. It has also been observed that 0.001 - 0.001µg doses of JHa were ineffective on silkworm larvae. A dose of 0.01-10 µg increased larval body weight. A dose from 10 µg - 100µg induced dauer larvae and same dose between 96-120th hr. induced supermoulted larvae. But administration after 144 hr. did not produce any effect on metamorphosis. Application either topically or through injection after 24 hrs. during fifth instar at a lower dose induced prolongation in larval duration by a day with increase of cocoon weight. Prolongation of larval duration after treatment with JHa during fifth instar in *B.mori* was found to be responsible for growth of silkgland (Prudhomme and Couble, 1979). Calvez (1981) reported that juvenile hormone action increases the obligatory feeding period time. Larvae when treated after 4th moult everyday ecdysed after 13 days. Treatment upto fifth day of fifth instar and then total starving resulted in 80-95% larval mortality. Thus he concluded that JHa treatment acts on both obligatory and facultative feeding period but do not control the transition from obligatory to the facultative feeding period. The transition from one feeding period to the other corresponds to the developmental commitment following an all-or-none principle.

Mulberry leaves ingested by the JHa treated larvae were found to be higher than that of control. But coefficient of utilization of ingested mulbery leaf into silk protein between treated and control larvae were virtually non-significant (Kurata, 1981). Coefficient of food utilization (CFU) values have been found to vary from one larval instar to the other and also within a larval instar depending on the dose of JHa, methoprene (Gaaboub *et al.*, 1985). This variation of CFU in same instar was found to be inconsistent. The digestibility pattern also appeared to be changed within a larval instar and it was found to vary depending on dose of methoprene. Application of methoprene did not enhance growth rate but larval feeding was found to be prolonged (Kurata, 1981; Guizong Zheng *et al.*, 1993).

2.2.2 Economic parameters : Review of literature indicate that most of the authorities have utilised methoprene, a JHa, as silk augmenter (Akai and Kobayashi, 1971; Kamada *et al.*, 1979; Kobari and Akai, 1978, '79; Kurata, 1981; Shibukawa and Akai, 1981; Prudhomme *et al.*, 1985). Kurata

(1981) indicated that just on application of JHa the growth rate of silk gland is lowered slightly than that of control but it is soon resumed and finally the rate exceeded over control. Prudhomme *et al.*, (1985) reported that involvement of JHa in control and developmental activity of silkgland is obvious and it is the only factor capable of promoting a higher silk production than that of control. Kadono-Okuda ·*et al.*, (1986) reported that spray application of methoprene on *B.mori* larvae mimics the action of insect specific juvenile hormone and delays larval maturation and increases the silk production by prolongation of larval duration. This prolongation of larval duration, on application of JHa, has been reported by other workers (Akai and Kobayashi, 1971; Chang *et al.*, 1972; Akai *et al.*, 1973; Kobari and Akai, 1978, '79; Kamada *et al.*, 1979; Kurata, 1981; Shibukawa and Akai, 1981; Guizhong-Zheng *et al.*, 1993). Akai *et al.*, (1981) reported an increase upto 26 percent in cocoon shell in *B.mori* by repeated treatment of JHa and a 10 percent increase by single treatment. Sarangi (1988) reported that repeated dosage influence silk production in bivoltine but single dosage was enough for multivoltine. Aomori *et al.*, (1977) suggested the repeated oral treatments of JHa @ 0.5 μg per larva for better cocoon shell weight. Akai (1988) infered that when JH is applied in appropriate quantity to larva in early fifth age (72 hrs.), the activity of RNA synthesis in the cells of the gland lasts longer than in the control group resulting in a longer period of silk protein synthesis. The total volume of silk protein synthesized is increased by JH. Effect of topical treatment of JH was higher in hybrid than pure lines. The juvenile hormone controls function of silk gland and prevents their degeneration and indirectly help in increase of silk production. During extended larval period the larva grow and synthesize silk so that they produce bigger cocoon (Horie and Watanabe, 1980, Akai *et al.*, 1981; Gaaboub *et al.*, 1985) as the end of larval period of fifth instar is characterised by hypertropy of the silkgland and an increase rate of silk synthesis till cocooning (Prudhomme *et al.*, 1985). Work with the JHa, methoprene on Indian silkworm breeds has been undertaken by Choudhary *et al.*, (1986), Rao *et.al.*, (1988), Trivedi *et.al.*, (1993). Rao *et.al.*, (1988) used 0.3125 μg/larva of JHa, methoprene for increase of silk filament under West Bengal climate. They reported an extension of feeding period by 24 hrs. in treated bivoltine larvae. Trivedi *et.al.*, (1993) infered that Indian bivoltine could not withstand a higher dosage of methoprene. Improvement of crop performance by 13-19% during summer season has also been recorded.

2.2.3 Reproductive characters : JH is secreted from corpora allata (CA). This hormone exerts an inhibitory effect on spermatocyte development in *B.mori* while the prothoracic gland hormone, ecdysone, accelerates spermiogenesis (Takeuchi, 1969; Yagi and Fukushima, 1975). Development of testis was found to be accelerated in the absence of CA (Fukuda, 1944), while testicular growth was retarded on implantation of additional CA in *B.mori* (Sehnal, 1968). This results were interpreted as being due to an equivalent depression of spermatogonial and spermatocytic divisions. A recovery of temperature induced male sterility during spinning in *B.mori*, following application of JHa, methoprene @ 0.5-1 μg per larva has been recorded (Dutta *et.al.*, 1982).

Many authorities emphasized that a definite titre of JH was required in the haemolymph of many insects for growth and development of ovary (Englemann, 1970 ; Williams and Kafatos, 1971; Slama *et al.*, 1974; Sroka and Gilbert, 1974; Novak, 1975). Additional application of JH or its analogue to larvae and pupae caused severe ovarian abnormalities (Sehnal, 1968; Metwally *et al.*, 1974; Deb and Chakraborty, 1981a). In case of treatment of larvae of *Corcyra cephalonica*, rice moth with a JHa, hydroprene, Deb and Chakraborty (1981a) noticed a potential increased number of eggs while pupal treatment resulted in severe histomorphological abberations of ovary. In the same insect Deb and Chakraborty (1984) recorded severe derrangement in the ovarian morphogensis after application of methoprene to both larvae and pupae. The authors emphasized the action of JH as metabolic and not gonadotropic. Huang and Zhang (1991) reported that JHa on application to pupae of *B.mori* has a negative effect on the weight per hundred eggs. Number of ripening eggs were found to be decreased through application of a higher dosage of JHa.

Pupal weight of *B.mori* has been found to be positively correlated with fecundity as energy for egg production is being stored during larval feeding period, Heavier pupa produced more eggs. Chang *et al.*, (1972) observed an increase of 20% pupal weight over control on treatment of JHa. Increase of larval and pupal weight through application of JHa, methoprene has been recorded by Choudhary *et al.*, (1986); Kamada, (1992); Sharma *et al.*, (1993). Gaaboub *et al.*, (1985) reported increase or decrease of fecundity through application of different concentration of JHa, methoprene to fifth instar larvae. Kimura *et al.*, (1986) reported an increase of 80% in fecundity in batches of silkworm treated with JHa during fifth instar. The size of eggs were also found to be bigger than control. Increase in fecundity in JHa treated batches has been reported by Choudhary *et al.*, (1986). Zhang and Huang (1991) after application of a JHa (ZR515) to early fifth instar larvae observed that JHa significantly increased reproductive bioquality in *B.mori*. Both increase of female pupal weight and fecundity has been recorded. Effective increase in ovariole length, number of eggs in ovariole and fecundity were observed by Magadum *et al.*, (1990, 1993) when methoprene was applied to fifth instar larvae of *B.mori*.

2.3 Effects of imidazole compounds in *Bombyx mori* on the economic and reproductive characters and utilization in commercial seed production.

Larval growth, moulting and metamorphosis in insects are generally known to be under the direct regulation of two hormones, juvenile and moulting hormone. A new group of chemicals called precocenes has been developed for use in insect pest control which showed antijuvenile hormone activity, and because of their actions were considered highly specific against pest species (Bower, 1982; Staal, 1982). Anti juvenile hormone action have been reported in some natural and synthetic compounds such as Kojic acid (Murakoshi, 1972), abietic acid derivatives (Murakoshi *et al.*, 1975). Apart from in insect pest management these compounds were also used in *B.mori*. Trimoulter larvae appeared after oral administration of these compounds. Recently a new class of compounds with antijuvenile hormone activity against the larvae of *B.mori* has been synthesized. These compounds are a group of imidazoles. Effective induction of precocious metamorphosis in *B.mori* larvae has been reported after the use of these imidazole derivatives (Kuwano *et al.*, 1983, 1984, 1985; Akai *et al.*, 1984), with the formation of miniature but viable adults (Staal, 1986). Among the imidazoles reported to be useful in sericulture from the standpoint of producing fine cocoon filament in *B.mori* (Akai *et al.*, 1984, 1986, Kanda *et al.*, 1985), increase in cocoon shell ratio by application during fifth instar in *B.mori* (Kiuchi and Akai, 1985; Kiuchi, 1996), increase in efficiency of egg production in *B.mori* (Kawaguchi *et al.*, 1993) for production of F_1 hybrid in bivoltine silkworm (Xue *et al.*, 1994) in breeding programme of *B.mori* (He and He, 1994) and breaking of diapause in pharate first instar larvae of wild silkmoth *Antheraea yammami* (Tan *et al.*, 1986; Suzuki *et al.*, 1989; Fujisawa *et al.*, 1992), KK-22, KK-42, KK-110, SSP-11 have been proved to be promising.

The ecdysteroid synthesized in prothoracie gland, ovary and testis control development and reproduction in insects (Reese, 1985). The prothoracic gland is the central organ responsible for ecdysone secretion needced for regulation of moulting and metamorphosis. The secretory action of the gland is regulated by prothoraciocotropic hormone (PTTH) and juvenile hormone (Bollenbacher and Grangėr, 1985; Smith, 1985). Imidazoles inhibit ecdysone synthesis in prothoracic gland of silkworm without killing the cells (Kadono-Okuda *et al.*, 1987, 1994; Yamashita *et al.*, 1987). Roussel *et al.*, (1987) reported depressed biosynthesis of ecdysone by the prothoracic gland by action of KK-42 in *Locusta migratoria*. Inhibitory action of KK-42 on ecdysteroid level in *B.mori* have been reported by Gu *et al.*,(1988); Akai and Mauchamp (1989); Akai and Rembold (1989); Zhou *et al.*, (1991); Jin Mei Wu (1992). Juvenile hormone plays an important role not only in controlling cellular polymorphism (Riddiford, 1985) but also controls the larval-pupal changes of epidermal cells. When JH level declines to a sub threshold level the reprogramming of epidermal cells occurs (Sehnal, 1988). It is proposed that JH-III is biosynthesized through epoxidation of Methyl fernasoate (MFE), a cytochrome P-450 linked mono oxygenase, catalysing conversion of MFE to JH. The biosynthesis is inhibited by the action of imidazoles with antijuvenile hormone action (Wilkinson *et al.*, 1972; Hammock and Mumby, 1978; Darvas *et al.*, 1989).

Apart from these inidazoles some other kind of imidazoles have also been developed which when administered with food to third or fourth instar early age larvae of *B.mori* effectively induced precocious metamorphosis with the production of superfine cocoon filament having high reelability. Notable among such types are SM-1, YA20, Jinlu, Kang 20, SDIII, Lin II, CA, CA2, CA3, CA4 (Xue and Li 1987; Zhuang Dahuan *et al.*, 1989; Shen *et al.*, 1990; Xue 1990, 1992; Haolin *et al.*, 1992; Zhuang *et al.*, 1992; Tan *et al.*, 1992; Shen 1992; Miao *et al.*, 1996; Junliang *et al.*, 1996; Himantharaj *et al.*, 1996). Depressed secretion of juvenile hormone and ecdysone have been investigated by action of SM-1 (Shen, 1992); SD III (Tan *et al.*, 1992); YA20 (Zhuang *et al.*, 1989).

As the present investigation has been carried out through the application of KK-22 KK-42 and KK110 review of literature has been restricted mostly to these imidazole compounds.

The effect of KK-22 (1-citronellyl -5-phenyl imidazole) has been suggested to be dose-dependent and is always accompanied by prolongation of larval period (Asano *et al.*, 1984a). The mode of action of KK-22 is yet to be resolved fully. However, the compound may inhibit JH biosynthesis in corpora allata (Asano *et al.*, 1984a, 1984b). This compound induces a high rate of precocious pupation and the concerned dose has no effect on larval feeding and growth (Asano *et al.*, 1986). The percentage of precocious metamorphosis has been correlated well with the time and dose of KK-22. Topical application of the compound to 0 hr. old third and fourth instar larvae at the rate of 10µg/larva of *B.mori* has successfully induced 100% precocious metamorphosis without any lethal effect (Kuwano *et al.*, 1984). Asano *et al.*, (1986) suggested a dosage of 20-40 µg/larva for administration to the 0 hr. of the fourth instar larvae for the induction of 100% pupation. It has also been reported that the larvae destined for precocious metamorphosis have extended their feeding period for 3-6 days beyond that of control and increased their body weight about 40% more in fourth instar itself, though the final mass at pupation has been smaller than that of non-precocious one. This increase in mass for precocious metamorphosis suggests some relationship between larval growth and incidence of precocious pupation i.e. the critical growth of the larvae which acts to switch on to metamorphosis. Prolongation of larval periods with the increase of larval body weights has also been noted by treatments with other imidazoles different from KK-22 (Kiuchi *et al.*, 1985; Yamashita *et al.*, 1987).Williams (1980) pointed out that insect larval growth is accompanied by not only saltatory growth in sclerotized organs but also non saltatory growth in body weight. So it is clear that in normal larval growth there is a close correlation betweena the larval body weight at moulting time and head capsule width at the next instar of *B.mori.* As KK-22 has strong influence on the growth profile it acts to disorder the relative growth ratio of head capsule size and gain in body weight. This disordered significant changes in head capsule size and body weight are observed only in larvae destined for precocious metamorphosis (Asano *et al.*, 1987). Similar phenomenon has been observed in *Manduca sexta* by Nijhout (1975) where individuals with wider head capsules than a defined threshold size have proceeded to pupation.

KK-42 (1-benzle-5[(E)]2, 6, dimethyl-1,7 heptadienyl] imidazole) has been found to be most effective of imidazoles so far tested on *B.mori* (Kuwano *et al.*, 1992). Experimental evidences indicate that topical application of 1-4 μg/larva during 0 hr. of fourth instar can effectively induce precocious metamorphosis (Kuwano *et al.*, 1988). Topical application at the rate of 5 μg/larva effectively can induce 100% precocious pupation when applied to third and fourth instar 0 hr. old larvae (Akai and Mauchamp, 1989; Gu *et al.*, 1992). KK-42 potentially inhibits prothoracic glands for biosynthesis of ecdysone in *B.mori* acting directly on the prothoracic gland cells without killing the cells (Kadono. Okuda *et al.*, 1987; Yamashita *et al.*, 1987; Darvas *et al.*, 1989; Oshiki *et al.*, 1990). KK-42 also depresses biosynthesis of ecdysone in prothoracic gland of *Locusta migratoria* (Roussel *et al.*, 1987). Delayed toxicity after treatment with KK-42 has been observed in case of the Lygaeid *Oncopeltus fasciatus* nymphs (Kuwano *et al.*, 1992).

Juvenile hormone plays an important role not only in regulating insect growth and development but also controls cellular polymorphism (Riddiford, 1985; Sehnal, 1988). Corpora allata ceases to secrete juvenile hormone in larvae after attaining a critical body weight as in *Manduca sexta* (Nijhout and Williams, 1974) Gu *et al.*, (1992) determined this critical body weight of *B.mori* after treating the third instar larvae When the weight exceeded 270 mg, there was simultaneous determination for moulting as well as for the commitment of characters of last instar in the following instar. A body weight lower than 210 mg led smoothly to the 5th instar. KK-42 decreases both MH (moulting hormone) and JH titres and induced precocious metamorphosis in *B.mori* (Akai and Mauchamp, 1989; Akai and Rembold, 1989; Gu *et al.*, 1988, 1992). Jin Mei Wu (1992) reported the target organ of KK-42 being corpora allata. So KK-42 also acts to cease the synthetic activity of corpora allata. Accordingly feed back received by brain also decreases secretion of PTTH thus MH titre in haemolymph is decreased. After 2-3 days (O Shiki *et al.*, 1990) there develops a peak of MH. As a result of coaction of this delayd MH peak and low level of JH precocious metamorphosis is induced in *B.mori*. However, Kadono - Okuda *et al.*, (1994) conclude that ecdysone synthesis was inhibited without any larger influence on the general sterol metabolism responsible for growth. The cocoons obtained by treatment of KK-42 were fairly small but shapes of the cocoons were uniform with high percent of reelable cocoons (Kanda *et al.*, 1986). Cocoon weights, shell weight were lower than those of tetramoulters. Efficiency of cocoon shell production were lower than in the control. The dry and wet strengths, knot strength and Young modules were high in silk filament of treated cocoons than control. Silk fabrics were soft with high crease recovery than control. A range of size of silk filament within the same silkworm race was obtained with different dose of KK-42 treatment (Akai *et al.*, 1986). Egg production in treated batches of KK-42 has been investigated by Kawaguchi *et al.*, (1993). It is suggested that the fecundity of the moths did not vary with the dose of KK-42 applied during treatment (5-30 µg KK-42/larva). The females laid only 40% eggs than their control counterparts. Higher dose resulted in small sized eggs without any qualitative change. Production of a little small sized eggs by trimoulter moths by treatment with imidazoles has been indicated by Xue *et al.*, (1994) but they suggested that the eggs were qualitatively better with almost 100% hatching. Like other holometabolous insects adult development in *B.mori* is also triggered by the ecdysteroids (Sakuria and Hasegawa, 1969; Wigglesworth, 1985). In case of KK-42 treatment during larval pupal metamorphosis stage ovarian development appeared to be affected but it showed no influence either on oviposition behaviour or fecundity (Kadano-Okuda *et al.*, 1994). KK-42 does not interfere oogenesis but only supresses accumulation of ecdysteroid in developing follicles of ovary. Supression of ovarian growth and retardation of pupal development by application of KK-42 within 36 hrs. after pupation have been reported by Zhou *et al.*, (1991). Gu *et al.*, (1992) noticed that KK-42 treated larvae destined for precocious metamorphosis not only increased their body weight during penultimate stage but also their chitinous plates of spinning tube, the spinneretes and secretory activity of the prothoracic gland were committed to become the last instar type.

KK-110 (1-neopentyl 5-imidazole), a newly synthesized imidazole has been found to be highly effective in producing precocious pupation in *Bombyx mori*. The introduction of an ethoxy substituent at the orthoposition on the benzene ring increased the activity of KK-110 (Kuwano *et al.*, 1990). It has been proposed that a low dose of KK-110 (1-4 µg/larva) can effectively induced precocious metamorphosis (Kuwano *et al.*, 1988).

Review of the literatures indicate that better action of imidazoles can be obtained by treating the 3rd or the 4th instar larvae of *B.mori* immediately after the moult either through topical application or by oral administration of the compounds alongwith mulberry leaves (Kiuchi *et al.*, 1985; Asano *et al.*, 1986, 1987; Gu *et al.*, 1988, 1992; Kubota *et al.*, 1988; Akai and Mauchamp, 1989; Akai and Rembold, 1989; Oshiki *et al.*, 1990; Xue 1992; Tan *et al.*, 1992; Shen, 1992; Jin Mei Wu, 1992; Himantharaj *et al.*, 1996). Larvae when treated with appropriate dose of these compounds during 4th instar, could not enter 5th instar. Instead 4th instar duration was prolonged before spinning with an increase in body weight. When the 3rd instar larvae were treated they entered the 4th instar but spinned without entering the 5th stage. In all cases induced trimoulters were obtained. Oshiki *et al.*, (1990) inferred that application of imidazoles to the 3rd instar larvae does not produce any two moulter because these imidazoles may act as antijuvenile and anti ecdysteroid agents for 2-3 days following the application, afterward endocrine system might have recovered from action of these compounds.

Regarding conversion efficiency of ingested food (ECI) by trimoulters literature indicates that ECI for cocoon shell is low in trimoulters than tetramoulters (Kiuchi *et al.*, 1986; Kanda *et al.*, 1986; Kubota *et al.*, 1988). However, growth rate in trimoulters are higher than tetramoulters (Kiuchi, 1986). Following imidazole application the values of important parameters such as cocoon weight, dry weights of food ingested and digested and dry weight of mature larvae were found to be in a constant ratio, 100 (control) : 70 (3rd instar treatment) : 40 (4th instar treatment) as was evident in the results obtained by Kiuchi *et al.*, (1986). Efficiency of conversion of digested food to pupae of trimoulters are higher than in case of tetramoulter.

Considering the advantages of rearing *B.mori* larvae through application of imidazoles it appears that better rearing management is possible due to the following reasons : a) it shortens the duration of larval span by 4-5 days thus saving labour and leaf; b) due to the skipping off of the 5th larval instar incidence of diseases are less resulting in better effective rate of rearing and good pupation rate, and c) moths of trimoulters are more active compared to those of tetramoulter with less percentage of unfertilized eggs (Xue *et al.*, 1990, 1994; Xue 1992; He and He, 1994).

Better performances in terms of oviposition rate, number of fertilized eggs and unlaid eggs, mating behaviour of moths, activeness of male moths were recorded from induced trimoulter batches during preparation of F_1 hybrid eggs using trimoulters. The hatchability of eggs were almost 100 percent. So production of F_1 hubrid eggs utilizing induced trimoulters has been recommended (Xue 1992; Xue *et al.*, 1994). He and He (1994) advocated the use of imidazole compounds for rearing of *B.mori* during unfavourable seasons both in commercial and laboratory level for better rearing results. The authors argued that due to the use of the imidazoles neither the genotype nor the combining ability of the silkworm races are affected. All induced trimoulters returned to tetramoulter if imidazoles are not used even after rearing as induced trimoulter upto F_5 generation.

In the tropical plains, such as in West Bengal, the rearing of bivoltine races of *B.mori* is a serious constrain particularly during the rainy season. Most of the advanced stage of larvae are attacked by micropathogenic diseases. Bivoltine silkworm rearing is difficult during this adverse period. Hence rearing of hybrid of multivoltine and bivoltine becomes imperative during this season in commercial sectors. To produce such multivoltine X bivoltine hybrid eggs the use of induced trimoulter bivoltine males may be promising. No information is available from Indian works for such an attempt. This justifies the present attempt of using imidazoles for the induction of trimoulters and to find out the impact of these compounds on economic and reproductive characters of *B.mori* for further utilization of these compounds in large scale.

2.4 Three way polyvoltine X bivoltine hybrid eggs for commercial rearing.

In India, specially West Bengal, due to high pathogen load in rearing house and low technical knowledge of farmers for rearing of bivoltine silkworm, rearing of bivoltine did not tunned upto expectation and programme of large scale rearing of bivoltine at commercial level almost failed (Rao *et al.*, 1987). Proper regulation of temperature and humidity is the key demand for successful rearing of bivoltine (Bhat, 1989) which is not possible in West Bengal due to high temperature and high humidity during wet part of summer resulting in failure of crop at farmers' level (Paul *et al.*, 1992). In general bivoltine produces superior quality of cocoons and productivity is also high, but high productivity as is linked with low resistance in *B.mori* (Jayaswal *et al.*, 1990), bivoltine gets susceptible to diseases during adverse seasons. In order to increase silk production it has been proposed to introduce rearing of polyvoltine X bivoltine hybrid at commercial level. This introduction of polyvoltine X bivoltine hybrid eggs for rearing at commercial level have increased yield potential per 100 disease free layings as compared to indigenous multivoltine, race Nistari, rearing (Dutta, 1988) and demand for such hybrid eggs have gone up due to its stability in rearing and also easy handling of silkworms. For meeting this higher demand an adequate quantity of polyvoltine X bivoltine hybrid eggs are procured from other states to the state of West Bengal (Saratchandra, 1995). In West Bengal for preparation of polyvoltine X bivoltine hybrid, female moth of Nistari, a low yielding indigenous polyvoltine known for its high resistance, and good genetic compatibility with any improved race/ line (Jayaswal *et al.*, 1990), is crossed with bivoltine male. Rao and his collaborators (Rao, 1988, Rao *et al.*, 1987) advocated the use of three way cross of polyvoltine X bivoltine hybrid (a hybrid of bivoltine is used as male component) using KPGB X P_5 hybrid as bivoltine male component.

Rearing of bivoltine hybrid for use with polyvoltine has been recommended (Samson, 1995), as hybrids have shorter larval duration. The use of the hybrid male has also been recommended by Narashimhanna (1985) as the three way crosses have indicated good heritibility towards economic characters due to their short larval period (Rayer and Govindan 1990).