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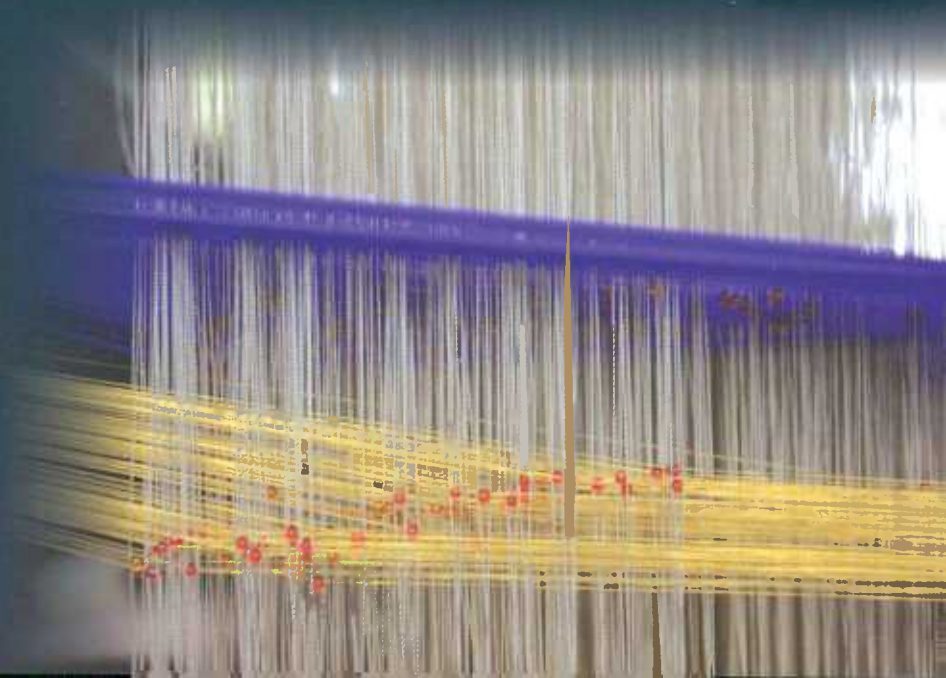
UNITED NATIONS REG. NO. 10418

ISSN 0250-3980

Volume 57 | Number 1 | 2017

SERICOLOGIA

Journal of Silkworms
A Quarterly Publication of
International Sericultural Commission



SERICOLOGIA

Journal of Silkworms

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Madiwala, Bengaluru - 560 068, INDIA
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PRINTED AT

Ramya Reprographic Pvt. Ltd
No. 16/2B, Singasandra Village
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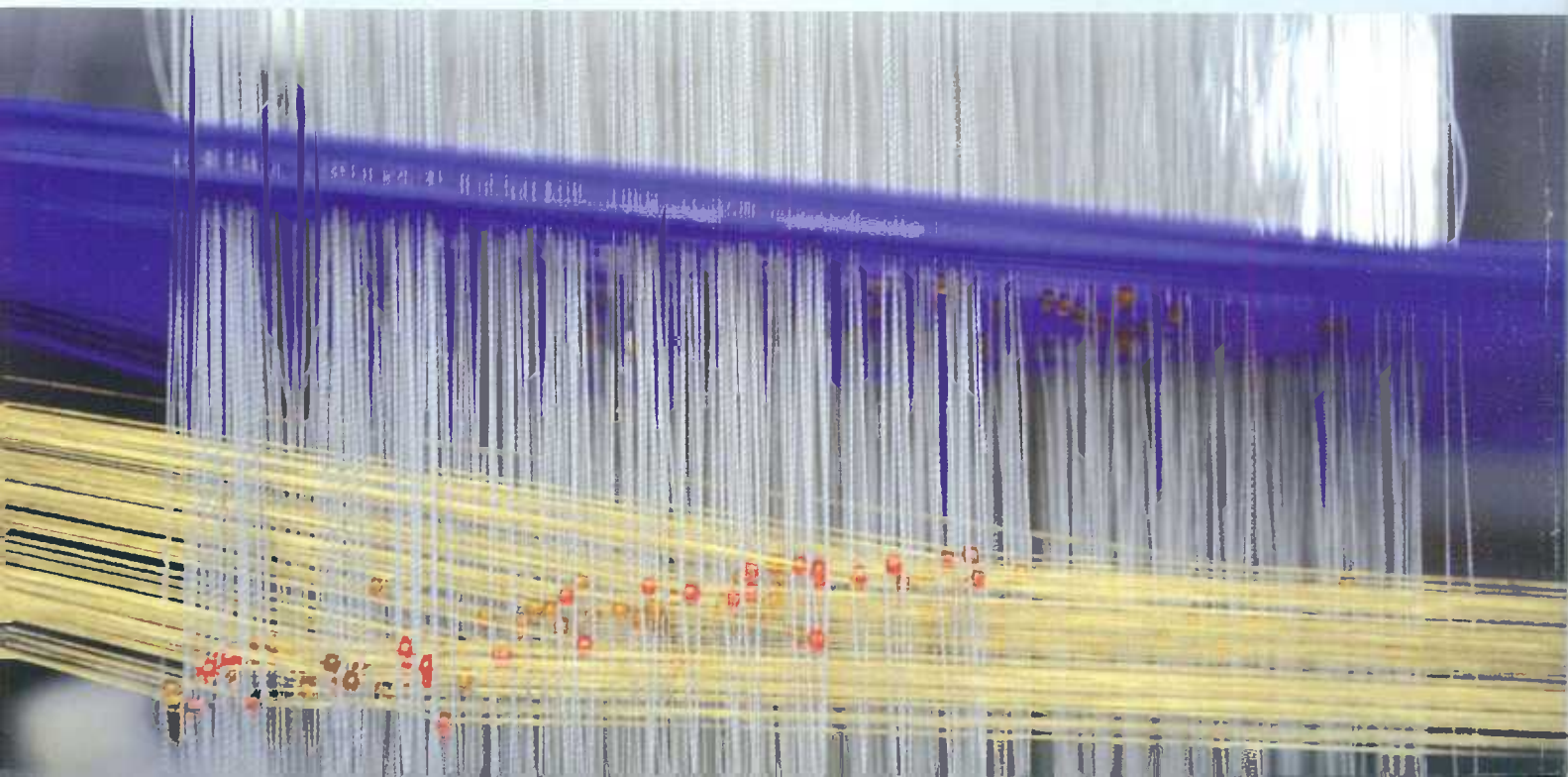
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TRANSKINGDOM RNA INTERFERENCE APPROACH TO IMPROVE RESISTANCE AGAINST GRASSERIE DISEASE IN *BOMBYX MORI* L. - A REVIEW

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ABSTRACT

Bombyx mori L. (Lepidoptera: Bombycidae) nucleopolyhedrosis virus (BmNPV) is a highly pathogenic virus encountered in the sericultural industry, often causing severe damage which hampers silk cocoon production in tropical countries. Effective management of the virus has been a challenge because of its sturdy nature and the lack of control strategies. RNA mediated silencing technology has become present day's tool of choice for induction of virus resistance in most of the higher animal systems. In this context, a new approach, known as transkingdom RNA interference (tkRNAi), was discovered where *E. coli* are engineered to transcribe short RNA (shRNA) from a plasmid known as transkingdom RNA interference plasmid (TRIP). The TRIP plasmid / vector contains gene of interest under the control of T7 promoter which efficiently produces dsRNA in *E. coli* host cells, and the vector also contains genes that are responsible for stable and efficient attachment and release of shRNA inside the target cells to bring about stable and consistent gene silencing in the respective host system. This technology is rapidly becoming an important method for analyzing gene functions and is therefore, currently the most widely used gene-silencing technique in functional genomics.

Key words: *Bombyx mori*, nucleopolyhedrosis virus, RNA interference, transkingdom RNAi.

INTRODUCTION

India is the second largest producer of silk in the world and its silk trade dates back to 15th century. Silk has been intermingled with the life and culture of Indians. On the other hand, the sericulture industry stands as the livelihood opportunity for millions owing to its unique characteristics such as high employment potential, low capital and high remuneration. The very nature of this industry with its rural based on-farm and off-farm activities and enormous employment generation potential has attracted the attention of the planners and policy makers to recognize it among one of the most appropriate avenues for socioeconomic development of a largely agrarian economy like India.

Recently, silk production has shown a remarkable

progress as its mulberry fraction alone accounts for 74.51 % (21,390 MT) of the total raw silk production of 28708 MT, followed by Eri, 16.5 % (4,726 MT), Tasar, 8.5 % (2,434 MT) and Muga, 0.55 % (158 MT). The bivoltine raw silk production achieved a record production of 4,532 MT during 2015-16 by registering 17 % growth over previous year. Similarly, vanya silk production, which includes tasar, eri and muga raw silks, has achieved 9.8 % growth during 2015-16 over 2014-15 (texmin.nic.in). The market share of Indian silk exports in the global silk trade is 4-5 % which is not significant considering the fact that India is the second largest producer of raw silk. This is because India has a large domestic market for silk goods and about 85 % of silk goods produced are sold in the domestic market (Gangopadhyaya, 2008). There are always fluctuations in production of silk due to disease incidences,

environmental uncertainties and lack of quality seed as well as large domestic consumption. But the contribution of diseases is one of the major concerns to be addressed.

History of pathology

The silkworm, *B. mori* and related species are susceptible to different kinds of diseases caused by pathogens such as viruses, bacteria, fungi, microsporidians *etc.* The most common pathogens infecting them are microsporidians *viz.*, *Nosema bombycis*, an array of viruses *viz.*, nucleopolyhedrovirus (NPV), densovirus (DNV), infectious flacherie virus (IFV) and cytoplasmic polyhedrovirus (CPV), and various bacteria.

Among the silkworm diseases which cause economic damage, it is to mention that those due to viruses inflict the most devastating effects due to their systemic infections and hence decrease crop productivity in primary infection and reduce seed quality through persistent infection. Viral epidemics are often associated with the emergence of a new form of the viral strain or a new form of vector. Four virus diseases of the silkworm are known: nuclear polyhedrosis, cytoplasmic polyhedrosis, infectious flacherie, densovirus 1 and densovirus 2.

History of sericulture has witnessed catastrophic outbreaks of polyhedrosis disease in regions of Asia and Europe where *B. mori* were reared on a large scale (Benz, 1986). The tropical climate prevailing in India favours disease outbreaks as high temperature and humidity conditions support proliferation of polyhedrosis virus. In India, the silkworm cocoon crop loss due to BmNPV (*Bombyx mori* nucleopolyhedrovirus) has been reported to an extent of 32.9-55.3 % of that due to all silkworm diseases and the most common occurrence is reported in summer (Nataraju *et al.*, 1998) (Christi and Sohaf, 1990). It is known to occur in all larval instars, more commonly in fourth and fifth instars during all seasons. Precautionary measures such as use of disinfectants, removal of diseased insects and implementation of general cleanliness and sanitation techniques as recommended (Krishnaswami, 1978) are followed by the sericulturists and researchers. In spite of adopting disease prevention measures, the incidence of

BmNPV infection during rearing at fifth instar stage is quite frequent, although the extent of the problem varies from crop to crop.

BmNPV belongs to the family of Baculoviridae and the infection pattern (life cycle) of this virus consists of two phases, *i.e.*, of occlusion-derived virus (ODV) and budded virus (BV). The ODV transmits infection from insect to insect by way of initially infecting mid-gut columnar epithelial cells, whereas, the BV is responsible for causing systemic infection within the host. Under natural conditions, NPV infects host larvae mainly via the oral route by ODV. After the ODV nucleocapsids have invaded the host cells, the viral genes begin transcription in a temporal manner to initiate primary infection. Due to the sturdy nature of the virus, it has been difficult to achieve effective control measures against this disease.

The basic information related to the cascade process involved during the infection and various genes that are involved in early and later stage of infection was unraveled due to the genome annotation of BmNPV. Among the many genes that are involved in infection, a few viral genes such as *ie-1*, a trans regulator that activates a number of late expression factors (encoded by *lef* genes), *lef-1*, a DNA primase of the replication complex (Mikhailov and Rohrmann, 2002) and *lef-3* that binds to single-stranded DNA, are critical for the viral DNA replication process and also P74, a conserved ODV structural protein, essential for oral infectivity and spreading of infection between individuals (Evans and Herman, 1997; Yao Hui Peng *et al.*, 2006; Subbaiah *et al.*, 2012). Studies conducted by Marcel *et al.* (1994) have shown that specific viral genes such as P143 and P35 are required for multiplication of AcMNPV viruses, hence lack of these two genes could be effective in controlling the infection.

The availability of a well-established method of transgenesis in *B. mori* (Royer *et al.*, 2005; Tomita and Kikuchi, 2009) led to the development of transgenic silkworm lines harboring a dsRNA-encoding transgene, targeting the essential viral gene *lef-1* (Isobe *et al.*, 2004) *ie-1* (Kanginakudru *et al.*, 2007) and P74 in a single construct so that it expresses all the three important genes involved in BmNPV infection (Subbaiah *et al.*, 2012). The development of transgenic silkworms by RNA

interference (RNAi) has shown promising results in providing >3 fold increase in virus resistance under laboratory conditions. But due to stringent regulations for transgenics in India, this technology is still far from the reach of stakeholders (Subbaiah *et al.*, 2012).

Alternative approach for disease resistance

The first report on RNA interference (RNAi) in animals detailed how an increasing number of copies of a gene in *Drosophila melanogaster* reduced rather than increasing the expression of that gene (Pal Bhadra *et al.*, 1997). In 1998, Fire and co-workers announced the discovery of RNA interference (*Caenorhabditis elegans*), which as a natural phenomenon is very intriguing, but that has become even more important as a research tool and through the years, generated a large number of highly ranked scientific publications. RNA interference (RNAi) refers to a post-transcriptional process triggered by the introduction of double-stranded RNA (dsRNA) which leads to gene silencing in a sequence-specific manner. RNAi is triggered when endogenous microRNA (miRNA), or exogenous double stranded RNA (dsRNA) or short hairpin RNA (shRNA) are processed by the dicer into short interfering RNA (siRNA) duplexes.

These processed siRNA are then bound to a large multi protein complex called RISC (RNA induced silencing complexes) wherein the double stranded siRNA is unwound into two separate strands and the sense strand is discarded. The RISC complex then locates the target mRNA using the incorporated antisense siRNA strand and typically binds to partially complementary area in the 3' untranslated regions of target mRNA and mediates gene silencing by translation repression. In the case of exogenously applied siRNA or shRNA, the RISC complex binds to perfectly complementary regions in target mRNA and cleaves them using activity of the argonaute 2 protein, thereby preventing protein production (Keates *et al.*, 2008) (Figure 1). RNA mediated silencing technology has now become the tool of choice for induction of virus resistance in animals, plants and microorganisms. RNA interference has now emerged as an important tool for the study of gene functions through silencing specific genes by degrading mRNA before it is translated.

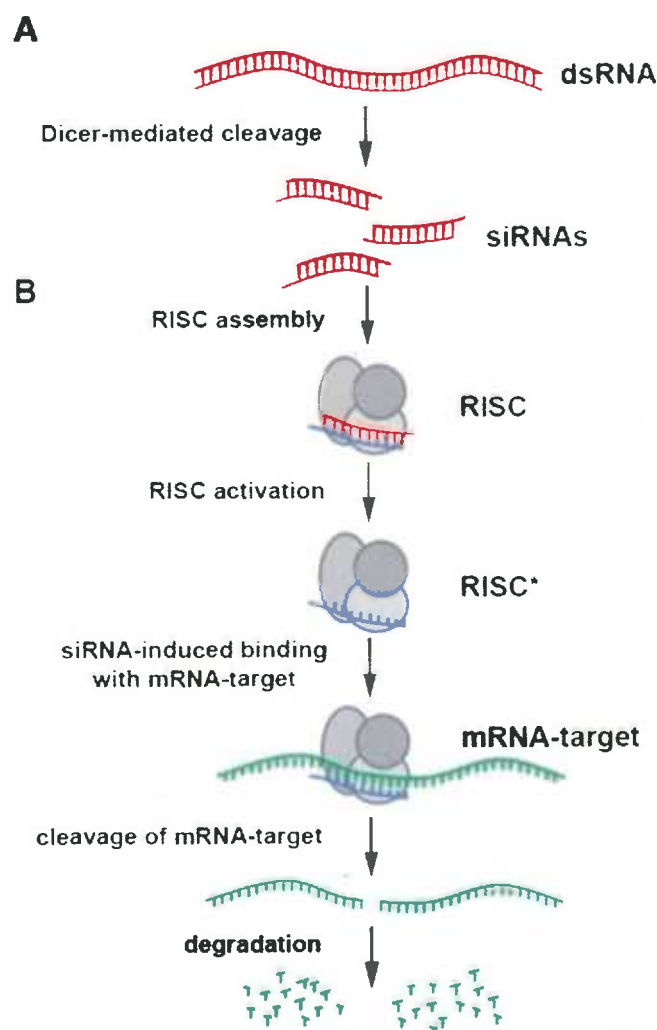


Figure1: Schematic representation of mechanism involved in RNA interference

RNAi techniques in biological research

The first report on RNA interference (RNAi) in Lepidoptera in the year 2001, at the 5th International Workshop on Molecular Biology and Genetics of the Lepidoptera generated considerable interest in the possibility of utilizing reverse genetics to investigate gene function in Lepidoptera. Since then, successful experiments related to RNAi have been carried out in a number of lepidopteran species. Lee *et al.* (2009) reported that RNAi could also work as an anti-viral response in insects. When *Drosophila* S2 cells were

infected with the flock house virus (FHV), the production of FHV-specific siRNA appeared rapidly. Decreasing amounts of FHV RNA, noticed after a few days indicated effects from RNAi (Terenius *et al.*, 2011). In *B. mori*, RNAi has been used primarily as a tool to investigate gene function at different developmental stages (Goldsmith *et al.*, 2005). Specifically, RNAi has been used to delineate a number of physiological processes in silkworm, including cocoon pigmentation (Tabunoki *et al.*, 2004), the sex pheromone biosynthetic pathway (Ohnishi *et al.*, 2006), segmentation and appendage formation (Masumoto *et al.*, 2009) and programmed cell death (Dietz *et al.*, 2007). Transgenic *B. mori* have been developed with a heat shock inducible and inheritable RNAi system to further probe gene function (Higes *et al.*, 2008). RNAi is also being used to address problems in silkworm culture. For example, transgenic *B. mori* larvae expressing dsRNA targeting a baculovirus gene, *baculoviral immediate early-1 (ie-1)*, induced strong protection against infection by the *B. mori* nucleopolyhedrovirus (Kanginakudru *et al.*, 2007). Some bee pathogens, such as *Nosema ceranae* and Israeli acute paralysis virus (IAPV) or a combination of the two, were recently implicated in world-wide losses of bee colonies. Analysis of the *N. ceranae* genome demonstrated the presence of RNA silencing machinery in this species, suggesting that RNAi can be exploited for control of infection within the host (Cornman *et al.*, 2009; Higes *et al.*, 2009). Bromenshenk *et al.* (2010) and Subbaiah *et al.* (2012) targeted several viral genes simultaneously in a single transgene construct, with the aim of installing a strong antiviral trait against the BmNPV. The dsRNA encoding transgene was shown to confer a high level of baculovirus resistance in transgenic silkworm. IAPV is a single-stranded RNA virus. IAPV infection in honeybees has been successfully repressed by exploiting the RNA interference (RNAi) pathway of the insect's innate immune response with virus-specific double stranded RNA (dsRNA). The effect of oral administration of virus-specific dsRNA was examined and resulted in an effective silencing of the virus, irrespective of the length. Interestingly, we observed that non-specific dsRNA was also efficient against IAPV (Aronstein *et al.*, 2008.)

The RNAi mechanism depends on the biology of the organism, method of dsRNA delivery, efficiency of

dsRNA up-take and relative turnover of target mRNA / protein. Further, signal amplification and systemic spread are important for robust RNAi. Extrinsic factors *viz.*, dsRNA concentration, target gene selection, transcript localization, dsRNA synthesis and route of introduction play a major role in efficient delivery of this technology (Xiang *et al.*, 2006). With all the above said criteria and concerns, the enormous potential of RNAi have met with significant challenges. The delivery of siRNA is problematic because they are negatively charged molecules and are susceptible to rapid enzymatic digestion *in vivo*, making them highly unstable. Direct delivery of dsRNA (soaking and / or feeding) is particularly popular in arthropod studies and appears to be feasible in at least some of the Hemipteran, Coleopteran, Lepidopteran and Hymenopteran insects. Though there are many published reports (Tabunoki *et al.*, 2004; Cornman *et al.*, 2009; Higes *et al.*, 2009; Bromenshenk *et al.*, 2010; Subbaiah *et al.*, 2012)) on efficient *in vitro* and *in vivo* delivery of dsRNA or shRNA, the effectiveness of the delivery still pose a challenge in many organisms. Hence, efforts are underway in *B. mori* as well as other organisms for the efficient delivery methods and new technologies for production and delivery of dsRNA.

Drawbacks of RNAi technology

As mentioned earlier, success of RNAi depends both on the biology of the organism as well as intrinsic factors *viz.*, efficiency of dsRNA up-take, relative turnover of target mRNA / protein, signal amplification and systemic spread and extrinsic factors, such as dsRNA concentration, target gene selection, transcript localization, dsRNA synthesis and route of introduction (Aronstein *et al.*, 2008). The transgenic RNAi technology is expensive, cumbersome and time consuming especially in the case of silkworms because introgression of selected genes from transgenic to all the evolving commercial varieties is a continuous process which has to be carefully monitored and also the commercially promising races have to be made transgenic which is practically difficult.

Introduction of more than one antiviral gene through a foreign vector and expressing dsRNA inside the silkworm is an induced phenomenon which does not exist naturally and hence, transgenic silkworms would certainly undergo

stress that have a negative impact on the silk production which in turn affects the productivity. The regulatory issues involved with transgenic technology is stringent and might pose a big obstacle for its promotion during field trials (Jeffrey *et al.*, 2013).

Transkingdom RNAi approach

In an effort to overcome the problem of effective delivery of dsRNA, Kruhan *et al.* (2009) first constructed the bacterial plasmid, pT7RNAi-Hly-Inv termed TRIP (trans-kingdom RNAi plasmid). The gene or the dsRNA is cloned between bacterially expressed T7 promoter and the vector contains Inv locus that codes for invasins which helps *E. coli* to enter cells that possess $\beta 1$ -integrin receptors present on epithelial cells resulting in receptor mediated endocytosis. The TRIP vector also contains HlyA gene whose product, listeriolysin O encodes a pore forming toxin that facilitates the rupture of the endosomal membrane and the subsequent release of shRNA into cell cytoplasm (Linke *et al.*, 2016). The TRIP constructs are transformed into competent *E. coli* BL21 DE3 host strain for expression of this shRNA. This new bacterially-expressed dsRNA delivery technology or Trans Kingdom RNAi (tkRNAi) approach (Keates *et al.*, 2008; Kruhan *et al.*, 2009; Xiang and Li, 2008, 2009) recently emerged as a powerful tool for the control of parasites and disease agents in mammalian systems (Figures 2 and 3). Oral and intravenous administration of *E. coli* encoding shRNA against CTNNB1 (catenin $\beta 1$) induced significant gene silencing in the intestinal epithelium and in human colon cancer xenografts in mice (Kruhan *et al.*, 2009). The tkRNAi strategy was applied for targeting the multidrug resistance mediating drug extrusion pump ABCB1 in multidrug resistant cancer cells. When treated with *E. coli* vector bearing anti-ABCB1 shRNA expression, MDR cancer cells showed 45 % less ABCB1 mRNA expression. The data provide a proof of concept that tkRNAi is suitable for modulation of classical MDR in human cancer cells (Linke *et al.*, 2016). For the first time, tkRNAi has been assessed both as a shRNA delivery vehicle to avian cells and as an antiviral vector for influenza virus. These antiviral vectors provided up to a 10000 fold reduction in viral titres shed, demonstrating *in vitro* proof of concept for using these novel anti Avian influenza virus (AIV) vectors to inhibit AIV shedding

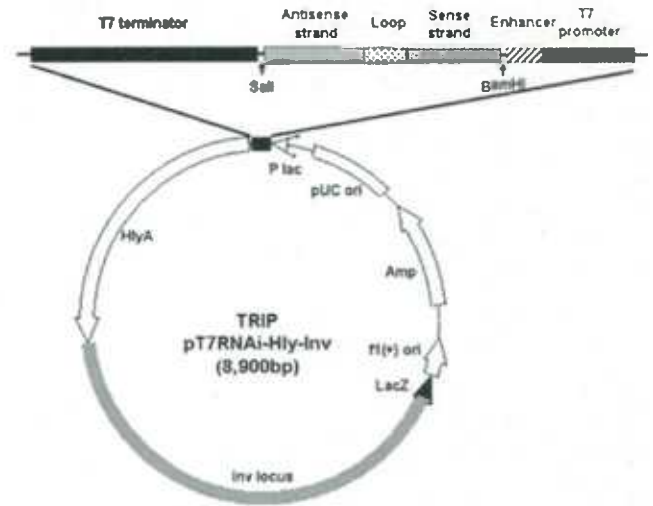


Figure 2: Structure of Transkingdom RNA interference plasmid (TRIP) and silencing sequences (Source: Xiang *et al.*, 2006, Nature Biotechnology)

(Tian *et al.*, 2009). This technology has successfully been used in nematodes (Xiang *et al.*, 2009) trophozoites (*Entamoeba histolytica*) and other organisms (Keates *et al.*, 2008) and therefore, opens up interesting perspectives for mass screening of novel gene targets and development of environmentally-safe pest control applications.

Bacteria-based RNAi is technically suitable for production of large quantities of dsRNA, and this new approach offers several advantages and has significant implications. First, this method allows the establishment of a long-term stable gene silencing system in the laboratory against genes of interests *in vitro* and *in vivo*, and enables high-throughput functional genomics screening in mammalian systems. RNAi libraries can be constructed, stored, reproduced, amplified and used with the help of *E. coli* as currently done with gene cloning. Second, this technology provides a clinically compatible way to achieve RNAi for therapeutic applications due to the proven clinical safety of nonpathogenic, engineered *E. coli* as a gene carrier, tkRNAi also eliminates the siRNA manufacture issue, and may circumvent or mitigate host interferon-like responses since siRNA is produced intracellularly (Xiang *et al.*, 2009). This technology is highly regarded as a potential therapeutic due to its high efficacy and low toxicity.

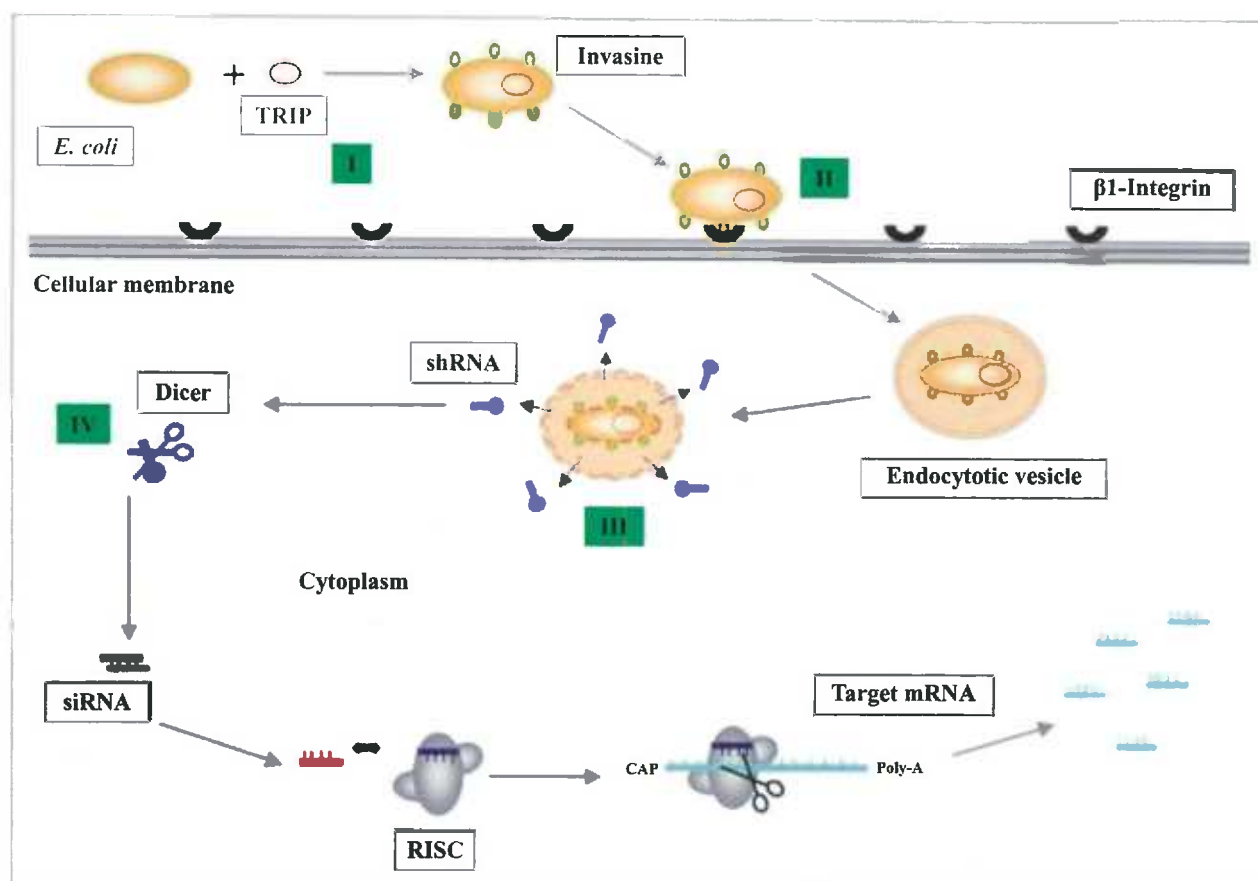


Figure 3: Schematic overview about the tkRNAi mechanism (Source: Kruhan *et al.*, 2006). I: transformation of TRIP vector into *E. coli* cells for dsRNA expression, II: attachment of invasive gene locus on to beta integrin receptors on the host cells for internalization of vector inside the cells, III: lysis of cells due to expression of listeriolysin O to release sh RNAs, IV: activation of dicer for cleaving of target genes to siRNA.

Regulatory issues in India

In some of the European countries, this technology does not come under ethical and regulatory issues but in countries like India, bio safety is a major concern when it comes to use of biotechnology in the field of agriculture with no exception for sericulture too. Therefore, in India, there are strict rules and regulations formulated by DBT (Department of Biotechnology) *i.e.*, regulations in biotechnology 1990 and 1994 which deals with genetically modified organisms (GMO's). The revised guidelines include guidelines for R&D activities on GMOs, transgenic crops, large-scale production and deliberate release of GMOs, plants, animals and products

into the environment, shipment and importation of GMOs for laboratory research. The IBSC (Institute Biosafety committee) consists of a DBT nominee and other external members; their responsibility involves to note and to approve r-DNA work, ensure adherence of r-DNA safety guidelines, prepare emergency plan according to guidelines, recommend to RCGM (Review Committee on Genetic Manipulation) about experiments with category III risks and above, and to seek RCGM's consent, inform DLC (District Level Committee) and SBCC (State Biotechnology Coordination Committee) as well as GEAC (Genetic Engineering Appraisal Committee) about the experiments needed and also act as nodal point for interaction with statutory bodies.

Advantages of TkrNAi technology

This technology involves cloning of the selected BmNPV genes into *E. coli* vector that can express dsRNA against selected BmNPV genes. Once the clones are developed, they are transformed to nonpathogenic *E. coli* host system for producing large quantities of dsRNA against NPV genes. The constructs (Anti BmNPV) developed are the key for the success of this technology. Development of efficient dsRNA construct that is able to combat the viral infection will definitely help in reducing the disease incidence to a major extent. This technology has shown that stable and cost effective RNAi can be activated *in vitro* and *in vivo* by non-pathogenic bacteria engineered for synthesis and delivery of silencing shRNA to the target cells. The technology developed is non-conjugative, nonpathogenic and environment friendly and will not involve any major regulatory issues during field trials. Since the technology involves rDNA work (recombinant DNA), prior approval from regulatory authorities is required.

Conclusion

One of the worst challenges faced by the silk industry is the frequent outbreaks of silkworm diseases and subsequent collapse of silk crops. It is proven that viral diseases are having major devastating effects due to their systemic infections leading to decreased crop productivity in first crop and reduced seed quality in subsequent generations due to persistent infection. The BmNPV is one among the most destructive pathogens in silkworm. In India, the silkworm cocoon crop loss due to NPV has been reported to an extent of 35-50 % among the total silkworm diseases.

In the above context, management of disease is a major concern during all the seasons and also throughout larval stage. The new biotechnological tool of dsRNA bacterial construct will be cost effective, less laborious and is a scientifically proven technology which can be used as a sort of probiotic during the feeding stage and thereby can control the disease occurrence even if there is an infection during early stages. The oral feeding will help in managing the spread of the diseases as well as proliferation of virus in the silkworm larvae. Hence, exploitation of this technology is expected to significantly reduce the BmNPV infection in silkworm.

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INFLUENCE OF AGRONOMIC PRACTICES ON THE INCIDENCE OF SUCKING INSECT PESTS OF MULBERRY IN KOLAR REGION (KARNATAKA, INDIA)

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ABSTRACT

In the current study, the impact of certain agronomic practices on the incidence of the sucking insect pests in mulberry crop system was documented in different seasons for three years from 2013-14 to 2015-16 at farmers' level in Kolar District in the following combinations of package of practice: a) tillage + closer spacing + organic inputs (T1), b) tillage + closer spacing + inorganic inputs (T2), c) tillage + closer spacing + organic inputs + inorganic inputs (T3), d) zero tillage + wider spacing + organic inputs (T4), e) zero tillage + wider spacing + inorganic inputs (T5), and f) zero tillage + wider spacing + organic inputs + inorganic inputs (T6). A great deal of variation in the incidence of *Pseudodendrothrips mori*, *Empoasca flavescens*, *Maconellicoccus hirsutus*, *Saissetia nigra* and *Aleurodicus dispersus* was observed among the treatments and the incidence being the highest in T4 and the least in T6 irrespective of season and year of pest monitoring. On the whole, the incidence was found decreased in the following order: T4, T2, T1, T5, T3, and T6 (*i.e.*, T4>T2>T1>T5>T3>T6). The results are discussed in the light of those existing elsewhere in other crop systems in general and mulberry in particular. The findings have a great significance on the selection of a need based pest control strategy to obviate the problem posed by the pests in question in mulberry crop system *vis-a-vis* agronomic practices employed.

Key words: Agronomic practices, mulberry gardens, pest monitoring, seasonal incidence, sucking pests.

INTRODUCTION

The silkworm, *Bombyx mori* L., is a specialist feeder having chosen to feed exclusively on mulberry (*Morus* spp.). It is reared under indoor conditions for raw silk production. Mulberry is cultivated in a wide variety of soils in different agro-climatic zones of India. Being the only host plant for the silkworm, farmers tend to provide more care to it by following diverse agronomic packages. As such, these packages have a direct bearing on the quality and quantity of the foliage produced. The foliage is available all through the year in the irrigated gardens, while it is limited mostly to rainy (July-October) and winter (November-February) seasons in the rain-fed gardens of south India. Regardless of whether mulberry is

cultivated under irrigated or rain-fed conditions, the succulent, glossy, and nutrient-rich leaves attract a host of sucking insect pests for taking shelter in the mulberry gardens for feeding and breeding, often leading to an explosive level in their populations, culminating in extensive qualitative and / or quantitative damage to foliage (Manjunath, 2004). Obviously, this in turn results in reduced cocoon and raw silk production.

The important sucking insect pests that are often encountered in the mulberry gardens of south India, including the gardens in the most prosperous sericulture belt in Karnataka, such as Kolar District, comprise 1) thrips (*Pseudodendrothrips mori* Niwa), 2) jassid (*Empoasca flavescens* Fabricius), 3) pink mealy bug

(*Maconellicoccus hirsutus* Green), 4) black scale insect (*Saissetia nigra* Nietner), 5) spiraling white fly (*Aleurodicus dispersus* Russel) and 6) papaya mealy bug (*Paracoccus marginatus* Williams & Granara de Willink). The information available on these aspects has been found sketchy and scanty as the investigations are based only on limited samples (farmers' gardens) / pests encompassing a few or all seasons of a year or so without giving due consideration for agronomic practice adopted and mulberry variety grown (Hemalatha, 2006; Balasaraswathi *et al.*, 2014; Chakraborty *et al.*, 2015).

Unlike mulberry crop system, investigations conducted in other crop systems, chiefly directed to study soil fertility management following crop fertilization with organic and inorganic manures, have led to generation of abundant useful information on the incidence and abundance of insect pests. A comprehensive and noteworthy account based on their review on these aspects has been provided by Scriber (1984) and Altieri and Nicholls (2003) who stated that soils that receive inorganic fertilizer application show elevated levels of pest incidence in crop plants, while those provided with organic inputs exhibit a great deal of resistance to pests. Even in terms of pest abundance, the soils rich in nitrogen score over those are poor in this element. A host of studies demonstrated an elevated incidence and greater congregation of pests in the chemically fertilized plants compared to those treated with organic matter (Culliney and Pimentel, 1986; Eigenbrode and Pimentel, 1988; Kajimura, 1995; Phelan *et al.*, 1995; Altieri *et al.*, 1998; Morales *et al.*, 2001; Atijegbe *et al.*, 2014). Tillage practices (Brust *et al.*, 1985; Willson and Eisley, 1992) and spacing (plant density / crop geometry) (Hill, 1989; Kulagod *et al.*, 2011) too have been reported to influence insect pest incidence.

There are clear indications that the incidence and abundance of the sucking pests vary considerably with respect to season, agronomic practice employed (spacing, nutrient input, tillage practice, irrigation, crop rotation, *etc.*), mulberry variety planted and extent of precipitation and so on. Presented herein are the findings of an investigation (field survey) conducted during 2013-14, 2014-15, and 2015-16 in Kolar region (Karnataka) on the incidence of sucking insect pests of mulberry, taking into

consideration, the agronomic practices employed by the sericulture farmers in three taluks viz., Kolar, Bangarpet and Malur, located 30-40 km apart from each other. The results emanated from the investigation are discussed in the light of those in the available literature.

MATERIALS AND METHODS

The agronomic practices followed for cultivation of mulberry gardens chosen in the current investigation included spacing (crop geometry), organic inputs, inorganic inputs and tillage operations in the following combinations:

- Gardens with tillage + closer spacing + organic inputs (T1),
- Gardens with tillage + closer spacing + inorganic inputs (T2),
- Gardens with tillage + closer spacing + organic inputs + inorganic inputs (T3),
- Gardens with zero tillage + wider spacing + organic inputs (T4),
- Gardens with zero tillage + wider spacing + inorganic inputs (T5) and
- Gardens with zero tillage + wider spacing + organic inputs + inorganic inputs (T6).

The gardens chosen for the study had drip irrigation facility and Victory-1 (V-1) mulberry variety in common. Altogether, 120 sericulture farmers' gardens drawn from twenty-seven villages falling in three taluks each with nine clusters (three / cluster) were covered for the study. These gardens were distributed equally among the treatments (T1-T6), *i.e.*, 20 / treatment. The selection of farmers' gardens was based on the fact that there was a considerable uniformity in terms of each of the agronomic components among the gardens falling in each of the treatments (T1-T6). Tillage operations were carried out employing power tiller, bullock ploughing, manual digging and tractor ploughing (in gardens with wider spacing). Crop geometry composed of closer spacing with 3' x 3' and less whereas, that under wider spacing comprised the gardens with spacing in excess of 3' x 3' that varied from 3' x 2' + 5' to (8' x 2') x 8'. Application of organic inputs composed of compost and farmyard manure (16-20 MT/ha), green manure (sunhemp, dhaincha, horsegram, *etc.*) and

mulches dumped in trenches, along with de-oiled cakes of neem / pongamia / groundnut. Chemical fertilizer application chiefly consisted of urea, apart from NPK (15:15:15, 17:17:17, or 19:19:19) applied as per the recommendations of Central Sericultural Research and Training Institute (CSRTI), Mysuru. The selected gardens under each of the treatments were monitored at monthly interval, starting from 15-20 days after pruning / shoot harvesting up to 65-70 days, for recording the incidence of *P. mori*, *E. flavescens*, *M. hirsutus*, *S. nigra* and *A. dispersus* as per the techniques / methods advocated by

CSRTI, Mysuru. The accrued data were subjected to one-way ANOVA using SPSS Package (version 21) (Sundarraaj *et al.*, 1972; Snedecor and Cochran, 1979).

RESULTS AND DISCUSSION

Results pertaining to the incidence of the sucking pests of mulberry, collected in summer, rainy, and winter seasons during 2013-14, 2014-15 and 2015-16 are presented in Tables 1-5.

Table 1: Incidence (%) of thrips (*Pseudodendrothrips mori*) in mulberry gardens subjected to different agronomical practices during 2013-14 to 2015-16 in Kolar District

Treatment	2013-14			2014-15			2015-16		
	Summer	Rainy	Winter	Summer	Rainy	Winter	Summer	Rainy	Winter
T1	7.338	4.888	6.713	7.725	6.850	7.313	9.750	5.963	5.425
	±	±	±	±	±	±	±	±	±
	0.36 ^{bc}	0.36 ^c	0.40 ^c	0.59 ^c	0.70 ^c	0.68 ^{bc}	0.37 ^{bc}	0.47 ^{bc}	0.54 ^{bc}
T2	8.538	6.000	8.150	9.725	8.850	8.813	10.75	6.963	6.425
	±	±	±	±	±	±	±	±	±
	0.36 ^b	0.39 ^b	0.48 ^b	0.59 ^b	0.70 ^b	0.68 ^{ab}	0.37 ^{ab}	0.47 ^b	0.54 ^b
T3	5.125	2.588	3.725	5.200	3.625	4.275	7.350	3.763	2.875
	±	±	±	±	±	±	±	±	±
	0.44 ^d	0.23 ^c	0.27 ^c	0.46 ^d	0.29 ^d	0.34 ^d	0.32 ^d	0.23 ^c	0.32 ^d
T4	10.54	8.000	10.00	11.73	10.85	9.288	11.49	8.413	7.925
	±	±	±	±	±	±	±	±	±
	0.36 ^c	0.39 ^a	0.44 ^a	0.59 ^a	0.70 ^a	0.40 ^a	0.55 ^a	0.28 ^a	0.32 ^a
T5	6.225	3.588	5.363	7.300	5.850	6.313	8.750	4.963	4.625
	±	±	±	±	±	±	±	±	±
	0.46 ^{cd}	0.23 ^d	0.34 ^d	0.67 ^c	0.70 ^c	0.68 ^c	0.37 ^c	0.47 ^c	0.61 ^c
T6	5.438	1.900	2.888	4.200	2.625	3.275	6.350	2.763	1.875
	±	±	±	±	±	±	±	±	±
	0.73 ^d	0.24 ^c	0.26 ^c	0.46 ^d	0.29 ^d	0.34 ^d	0.32 ^d	0.23 ^c	0.32 ^d
Mean	7.200	4.494	6.140	7.646	6.442	6.546	9.073	5.471	4.858
F-value	19.33**	52.54**	52.10**	24.13**	26.96**	19.72**	25.78**	30.22**	23.73**

T1 = Gardens with tillage + closer spacing + organic inputs;

T2 = Gardens with tillage + closer spacing + inorganic inputs;

T3 = Gardens with tillage + closer spacing + organic inputs + inorganic inputs;

T4 = Gardens with no (zero) tillage + wider spacing + organic inputs;

T5 = Gardens with no (zero) tillage + wider spacing + inorganic inputs;

T6 = Gardens with no (zero) tillage + wider spacing + organic inputs + inorganic inputs.

Data were collected at monthly interval from 20 mulberry gardens for each of the treatments and are expressed as mean ± Standard error (M ± SE).

Seasons: Summer (March-June), rainy (July-October) and winter (November-February)

Mean values followed by same superscript are not significantly different from each other; ** Indicate highly significant ($P \leq 0.01$) (one-way ANOVA).

Table 2: Incidence (%) of jassid (*Empoasca flavescens*) in mulberry gardens as impacted by different agronomical practices during 2013-14 to 2015-16 in Kolar District

Treatment	2013-14			2014-15			2015-16		
	Summer	Rainy	Winter	Summer	Rainy	Winter	Summer	Rainy	Winter
T1	5.350	4.363	5.225	5.500	4.625	5.038	7.575	4.788	4.650
	±	±	±	±	±	±	±	±	±
	0.32 ^c	0.39 ^c	0.29 ^c	0.29 ^c	0.24 ^c	0.18 ^c	0.33 ^c	0.31 ^b	0.39 ^{bc}
T2	6.350	5.363	6.225	6.500	5.625	6.038	8.575	5.788	5.650
	±	±	±	±	±	±	±	±	±
	0.32 ^b	0.39 ^b	0.29 ^b	0.29 ^b	0.24 ^b	0.18 ^b	0.33 ^b	0.31 ^b	0.39 ^b
T3	3.788	2.975	3.750	3.288	2.638	3.313	6.075	2.925	2.650
	±	±	±	±	±	±	±	±	±
	0.34 ^d	0.26 ^{de}	0.24 ^d	0.25 ^c	0.23 ^d	0.25 ^c	0.27 ^d	0.30 ^c	0.39 ^{de}
T4	8.350	7.300	8.225	8.500	7.625	8.038	10.58	7.788	7.650
	±	±	±	±	±	±	±	±	±
	0.32 ^a	0.40 ^a	0.29 ^a	0.29 ^a	0.24 ^a	0.18 ^a	0.33 ^a	0.31 ^a	0.39 ^a
T5	3.825	3.638	4.425	4.238	3.625	4.038	6.575	3.788	3.650
	±	±	±	±	±	±	±	±	±
	0.31 ^d	0.38 ^{cd}	0.27 ^d	0.26 ^d	0.24 ^d	0.18 ^d	0.33 ^d	0.31 ^d	0.39 ^{cd}
T6	2.938	2.025	2.625	2.288	1.663	2.325	5.075	2.000	1.700
	±	±	±	±	±	±	±	±	±
	0.38 ^d	0.25 ^c	0.23 ^c	0.25 ^c	0.23 ^c	0.25 ^c	0.27 ^c	0.28 ^c	0.37 ^c
Mean	5.100	4.277	5.079	5.052	4.300	4.798	7.408	4.513	4.325
F-value	36.75**	28.46**	52.66**	69.14**	81.18**	96.79**	40.43**	46.36**	31.41**

T1 = Gardens with tillage + closer spacing + organic inputs;

T2 = Gardens with tillage + closer spacing + inorganic inputs;

T3 = Gardens with tillage + closer spacing + organic inputs + inorganic inputs;

T4 = Gardens with no (zero) tillage + wider spacing + organic inputs;

T5 = Gardens with no (zero) tillage + wider spacing + inorganic inputs;

T6 = Gardens with no (zero) tillage + wider spacing + organic inputs + inorganic inputs.

Data were collected at monthly interval from 20 mulberry gardens for each of the treatments and are expressed as mean ± Standard error (M ± SE).

Seasons: Summer (March-June), rainy (July-October) and winter (November-February)

Mean values followed by same superscript are not significantly different from each other; ** Indicate highly significant ($P \leq 0.01$) (one-way ANOVA).

P. mori

The mean incidence of *P. mori* was the highest in T4 and the least in T6 in all the seasons and years of the pest monitoring. The mean values for the incidence in summer, rainy, and winter seasons varied from 10.54 ± 0.36 to 5.44 ± 0.73 , from 8.00 ± 0.23 to 1.90 ± 0.24 and from 10.00 ± 0.44 to 2.89 ± 0.26 %, respectively during 2013-14. During 2014-15, the corresponding values in summer, rainy, and winter seasons ranged from 11.73 ± 0.59 to 4.20 ± 0.46 ,

from 10.85 ± 0.70 to 2.63 ± 0.29 and from 9.29 ± 0.40 to 3.28 ± 0.34 %. The mean data for 2015-16 in summer, rainy, and winter seasons fluctuated from 11.49 ± 0.55 to 6.35 ± 0.32 , from 8.41 ± 0.28 to 2.76 ± 0.23 and from 7.93 ± 0.32 to 1.88 ± 0.32 %, respectively (Table 1).

E. flavescens

E. flavescens showed the greatest incidence in T4, while it revealed the least in T6. The results scored for

incidence of jassid in summer of 2013-14 for T4 and T6 stood at 8.35 ± 0.32 and 2.94 ± 0.38 %, respectively. In rainy and winter seasons too, similar trend was observed with the values being 7.30 ± 0.40 and 8.23 ± 0.29 % for T4, while the same for T6 were 2.00 ± 0.25 and 2.63 ± 0.23 %. During 2014-15, the mean data in summer, rainy, and winter seasons were 8.50 ± 0.29 , 7.63 ± 0.24 and 8.04 ± 0.18 % for T4 as against 2.29 ± 0.25 , 1.66 ± 0.23 and 2.33 ± 0.25 % for T6, respectively. Considering the pest incidence for 2015-16 in different seasons, the mean incidence in summer in T4 was 10.58 ± 0.33 % whereas, in T6, it was 5.08 ± 0.27 %. In rainy season, T4 showed a

value of 7.79 ± 0.31 as against 2.00 ± 0.28 % in T6 (Table 2).

S. nigra

Maximum and minimum incidence of *S. nigra* was noticed in T4 and T6, respectively. The mean incidence of the pest during 2013-14 for T4 stood at 9.03 ± 0.43 , 8.93 ± 0.50 and 9.24 ± 0.35 % in summer, rainy, and winter seasons, respectively. In T6, the corresponding values were 1.69 ± 0.29 , 1.90 ± 0.34 and 2.05 ± 0.21 %. With regard to incidence during 2014-15, while in T4, it stood at

Table 3: Incidence (%) of black scale insect (*Saissetia nigra*) in mulberry gardens exposed to various agronomical practices during 2013-14 to 2015-16 in Kolar District

Treatment	2013-14			2014-15			2015-16		
	Summer	Rainy	Winter	Summer	Rainy	Winter	Summer	Rainy	Winter
T1	5.025	4.925	5.738	5.275	3.900	4.925	6.588	4.150	3.575
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.43 ^c	0.50 ^c	0.35 ^c	0.37 ^b	0.43 ^{bc}	0.55 ^{ab}	0.68 ^{ab}	0.54 ^{ab}	0.66 ^{ab}
T2	7.025	6.925	7.488	6.088	4.738	5.263	6.813	3.775	3.075
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.43 ^b	0.50 ^b	0.35 ^b	0.36 ^b	0.46 ^{ab}	0.65 ^{ab}	0.88 ^{ab}	0.62 ^{abc}	0.57 ^{ab}
T3	2.625	2.863	3.175	2.900	2.938	3.238	5.388	2.750	2.350
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.31 ^{de}	0.35 ^{de}	0.25 ^c	0.24 ^d	0.31 ^{cd}	0.21 ^{cd}	0.28 ^{bc}	0.34 ^{bc}	0.46 ^{ab}
T4	9.025	8.925	9.238	7.088	5.738	6.263	7.788	4.650	3.975
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.43 ^a	0.50 ^a	0.35 ^a	0.36 ^a	0.46 ^a	0.65 ^a	0.88 ^a	0.66 ^a	0.60 ^a
T5	3.650	3.900	4.200	3.900	3.925	4.238	6.375	3.700	3.300
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.33 ^d	0.38 ^{cd}	0.26 ^d	0.24 ^c	0.32 ^{bc}	0.21 ^{bc}	0.28 ^{ab}	0.37 ^{abc}	0.48 ^{ab}
T6	1.688	1.900	2.050	1.900	1.988	2.263	4.488	2.288	2.050
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.29 ^c	0.34 ^c	0.21 ^f	0.24 ^c	0.30 ^d	0.20 ^d	0.29 ^c	0.26 ^c	0.44 ^b
Mean	4.840	4.906	5.315	4.525	3.871	4.365	6.240	3.552	3.054
F-value	53.92**	37.12**	82.05**	41.18**	11.65**	9.900**	3.542**	3.237*	1.850 ^{NS}

T1 = Gardens with tillage + closer spacing + organic inputs;

T2 = Gardens with tillage + closer spacing + inorganic inputs;

T3 = Gardens with tillage + closer spacing + organic inputs + inorganic inputs;

T4 = Gardens with no (zero) tillage + wider spacing + organic inputs;

T5 = Gardens with no (zero) tillage + wider spacing + inorganic inputs;

T6 = Gardens with no (zero) tillage + wider spacing + organic inputs + inorganic inputs.

Data were collected at monthly interval from 20 mulberry gardens for each of the treatments and are expressed as mean $\pm$ Standard error (M $\pm$ SE).

Seasons: Summer (March-June), rainy (July-October) and winter (November-February)

Mean values followed by same superscript are not significantly different from each other; ** Indicate highly significant ($P \leq 0.01$) (one-way ANOVA); NS: Non-significant.

Table 4: Incidence (%) of mealy bug (*Maconellicoccus hirsutus*) in mulberry gardens maintained under different agronomical practices during 2013-14 to 2015-16 in Kolar District

Treatment	2013-14			2014-15			2015-16		
	Summer	Rainy	Winter	Summer	Rainy	Winter	Summer	Rainy	Winter
T1	8.763	6.575	7.800	8.625	7.713	7.638	8.738	7.825	7.575
	±	±	±	±	±	±	±	±	±
	0.33 ^c	0.48 ^b	0.38 ^c	0.49 ^b	0.55 ^b	0.36 ^c	0.50 ^b	0.51 ^b	0.58 ^b
T2	10.76	8.575	9.300	9.625	8.713	8.638	9.738	8.825	8.575
	±	±	±	±	±	±	±	±	±
	0.33 ^b	0.48 ^a	0.38 ^b	0.49 ^{ab}	0.55 ^{ab}	0.36 ^b	0.50 ^{ab}	0.51 ^{ab}	0.58 ^{ab}
T3	5.325	3.250	4.500	5.375	4.450	4.638	5.538	4.625	4.375
	±	±	±	±	±	±	±	±	±
	0.30 ^a	0.37 ^d	0.22 ^d	0.30 ^{cd}	0.35 ^{cd}	0.32 ^c	0.33 ^{cd}	0.31 ^{cd}	0.41 ^{cd}
T4	11.76	9.575	10.30	10.63	9.713	9.638	10.74	9.825	9.575
	±	±	±	±	±	±	±	±	±
	0.33 ^a	0.48 ^a	0.38 ^a	0.49 ^a	0.55 ^a	0.36 ^c	0.50 ^a	0.51 ^a	0.58 ^a
T5	7.000	4.588	7.800	6.375	5.450	5.638	6.538	5.625	5.375
	±	±	±	±	±	±	±	±	±
	0.38 ^d	0.48 ^c	0.40 ^c	0.30 ^c	0.35 ^c	0.32 ^d	0.33 ^c	0.31 ^c	0.41 ^c
T6	4.363	2.463	3.525	4.375	3.450	3.638	4.538	3.625	3.425
	±	±	±	±	±	±	±	±	±
	0.30 ^f	0.36 ^d	0.22 ^c	0.30 ^d	0.35 ^c	0.32 ^f	0.33 ^d	0.31 ^d	0.38 ^d
Mean	7.996	5.838	7.204	7.500	6.581	6.638	7.638	6.725	6.483
F-value	80.17**	41.82**	61.11**	37.85**	29.46**	48.44**	33.74**	33.90**	24.58**

T1 = Gardens with tillage + closer spacing + organic inputs;

T2 = Gardens with tillage + closer spacing + inorganic inputs;

T3 = Gardens with tillage + closer spacing + organic inputs + inorganic inputs;

T4 = Gardens with no (zero) tillage + wider spacing + organic inputs;

T5 = Gardens with no (zero) tillage + wider spacing + inorganic inputs;

T6 = Gardens with no (zero) tillage + wider spacing + organic inputs + inorganic inputs.

Data were collected at monthly interval from 20 mulberry gardens for each of the treatments and are expressed as mean ± Standard error (M ± SE).

Seasons: Summer (March-June), rainy (July-October) and winter (November-February)

Mean values followed by same superscript are not significantly different from each other; ** Indicate highly significant ($P \leq 0.01$) (one-way ANOVA).

7.09 ± 0.36 (summer), 5.74 ± 0.46 (rainy) and 6.26 ± 0.65 % (winter) as opposed to the mean corresponding data of 1.90 ± 0.24, 1.99 ± 0.30 and 2.26 ± 0.20 % in T6. In summer, rainy, and winter seasons of 2015-16, the mean results noticed with T4 were 7.79 ± 0.88, 4.65 ± 0.66 and 3.97 ± 0.60 % as opposed to 4.49 ± 0.29, 2.29 ± 0.26 and 2.05 ± 0.44 % with T6, respectively (Table 3).

M. hirsutus

Whereas the incidence of *M. hirsutus* was the highest

in T4, it was the lowest in T6. During 2013-14, mulberry gardens with T4 showed the mealy bug incidence of 11.76 ± 0.33, 9.58 ± 0.48 and 10.30 ± 0.38 %, respectively in summer, rainy and winter seasons. As against, in T6, the corresponding values for the pest incidence for these seasons were 4.36 ± 0.30, 2.46 ± 0.36 and 3.53 ± 0.22 %. While during 2014-15, in T4, the pest incidence in summer, rainy, and winter seasons remained at 10.63 ± 0.49, 9.71 ± 0.55 and 9.64 ± 0.36 %, in T6, it was 4.38 ± 0.30, 3.45 ± 0.35 and 3.64 ± 0.32 %, respectively. The values for the pest incidence during 2015-16 in summer,

rainy, and winter seasons for T4 were 10.74 ± 0.50 , 9.83 ± 0.51 and 10.05 ± 0.50 % whereas, the same with T6 stood at 4.54 ± 0.33 , 3.63 ± 0.31 and 3.86 ± 0.29 %, respectively (Table 4).

A. dispersus

The incidence of *A. dispersus* was maximum in T4 as opposed to minimum in T6. The white fly during 2013-14 in T4 scored an incidence of 3.78 ± 0.18 , 6.91 ± 0.26 and 8.93 ± 0.27 % as against that in T6 with 1.46 ± 0.12 , 2.40 ± 0.30 and 3.00 ± 0.27 %, respectively in summer, rainy, and

winter seasons. The mean data for these seasons during 2014-15 for T4 were observed to be 3.20 ± 0.13 , 8.46 ± 0.38 and 9.10 ± 0.32 % in contrast to 1.14 ± 0.11 , 2.06 ± 0.33 and 2.81 ± 0.29 % in T6. With reference to 2015-16, whereas, T4 showed the incidence values of 3.14 ± 0.10 , 8.04 ± 0.32 and 7.90 ± 0.37 %, the results for T6 were 1.39 ± 0.09 , 1.60 ± 0.23 and 1.45 ± 0.29 %, in summer, rainy, and winter seasons, respectively (Table 5).

Statistical analysis of the mean data documented for different treatments revealed significant variations ($P \leq 0.01$) among most treatments.

Table 5: Incidence (%) of spiraling white fly (*Aleurodicus dispersus*) in mulberry gardens subjected to various agronomical practices during 2013-14 to 2015-16 in Kolar District

Treatment	2013-14			2014-15			2015-16		
	Summer	Rainy	Winter	Summer	Rainy	Winter	Summer	Rainy	Winter
T1	3.038	4.913	6.925	2.438	6.463	7.100	2.513	6.038	5.900
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.16 ^{bc}	0.26 ^c	0.27 ^c	0.10 ^b	0.38 ^c	0.32 ^c	0.11 ^c	0.32 ^c	0.37 ^c
T2	3.413	5.913	7.925	2.713	7.463	8.100	2.800	7.038	6.900
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.17 ^{ab}	0.26 ^b	0.27 ^b	0.11 ^b	0.38 ^b	0.32 ^b	0.12 ^b	0.32 ^b	0.37 ^b
T3	1.900	3.400	3.375	1.463	3.063	3.813	1.638	2.600	2.450
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.16 ^d	0.30 ^d	0.27 ^c	0.09 ^d	0.33 ^c	0.29 ^c	0.08 ^d	0.23 ^c	0.29 ^c
T4	3.775	6.913	8.925	3.200	8.463	9.100	3.138	8.038	7.900
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.18 ^a	0.26 ^a	0.27 ^a	0.13 ^a	0.38 ^a	0.32 ^a	0.10 ^a	0.32 ^a	0.37 ^a
T5	2.775	3.875	5.913	2.025	5.063	5.813	2.275	4.600	4.450
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.12 ^c	0.30 ^d	0.27 ^d	0.09 ^c	0.33 ^d	0.29 ^d	0.07 ^c	0.23 ^d	0.29 ^d
T6	1.463	2.400	3.000	1.138	2.063	2.813	1.388	1.600	1.450
	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
	0.12 ^c	0.30 ^c	0.27 ^c	0.11 ^c	0.33 ^f	0.29 ^f	0.09 ^d	0.23 ^f	0.29 ^f
Mean	2.727	4.569	6.010	2.163	5.429	6.123	2.292	4.985	4.842
F-value	33.59**	34.73**	80.30**	54.06**	49.89**	64.56**	48.56**	82.32**	58.94**

T1 = Gardens with tillage + closer spacing + organic inputs;

T2 = Gardens with tillage + closer spacing + inorganic inputs;

T3 = Gardens with tillage + closer spacing + organic inputs + inorganic inputs;

T4 = Gardens with no (zero) tillage + wider spacing + organic inputs;

T5 = Gardens with no (zero) tillage + wider spacing + inorganic inputs;

T6 = Gardens with no (zero) tillage + wider spacing + organic inputs + inorganic inputs.

Data were collected at monthly interval from 20 mulberry gardens for each of the treatments and are expressed as mean $\pm$ Standard error (M $\pm$ SE).

Seasons: Summer (March-June), rainy (July-October) and winter (November-February)

Mean values followed by same superscript are not significantly different from each other; ** Indicate highly significant ($P \leq 0.01$) (one-way ANOVA).

Perusal of the mean data indicated the following: a) there was no appreciable variation in the incidence of the respective pests over a period of three years, b) the pest incidence was the highest in the treatment involving zero tillage + wider spacing + organic inputs (T4), c) the least incidence of the pests was found in the treatment consisting of zero tillage + wider spacing + organic inputs + inorganic inputs (T6), d) the pest incidence was considerably low and at par in treatments, T3 and T6, e) the incidence of the pests was considerably more in T2 compared to T5, f) the pest incidence in T1 and T2 was appreciably more as against T5 and T6, g) the incidence of the pests was higher in T2 as against T1, while it was so in T5 as opposed to T6, and h) regardless of the year and season of pest monitoring, the incidence was found decreased in the following order: T4, T2, T1, T5, T3 and T6 (i.e., $T4 > T2 > T1 > T5 > T3 > T6$). Even the trend emerging from pooled data for different years of pest monitoring is in the same line with that for seasons.

Mulberry is chiefly cultivated as a mono-crop under the conditions prevailing in south India. As a result, a host of insect pests, including the sucking ones, show greater affinity to take shelter on this crop which by and large provides an ideal condition for the proliferation of these pest herbivores, leading to explosion of their population with concomitant loss in yield and quality of foliage. Further, a great deal of variations are observed in pest incidence and abundance *vis-à-vis* the agronomic conditions such as spacing, application of nutrient element, irrigation, method of pruning / harvesting of foliage, tillage operation, mulberry variety planted and so on. Efforts have been made by a few researchers to monitor and document the incidence and / or abundance of pests in mulberry. However, such attempts haven't given due consideration to record the impact of agronomic practices on the incidence and / or abundance of pests in mulberry. As such, our understanding about the effects of the agronomic practices employed for mulberry cultivation on the incidence and abundance of insect pests remains limited. Obviously, this has proved to be an impediment in our selection of a need based pest control strategy to keep pest incidence below the economic injury level. In the backdrop of this, nonetheless, detailed investigations are expected to assume great significance.

From the results of the current investigation extending over a period of three years from 2013-2015 and encompassing all seasons, it can be understood that agronomic practices employed at farmers' level had a significant influence on the incidence of *P. mori*, *E. flavescens*, *S. nigra*, *M. hirsutus* and *A. dirpersus*. However, it is not possible to single out the impact of an independent agronomic practice on the incidence of these sucking pests in mulberry and whatever the effect that has been recorded would undoubtedly be due to their combined action. It was noticed that the incidence of the pests was the least when the gardens were subjected to the agronomic practices such as the combination of zero tillage, wider spacing, organic inputs, and inorganic inputs (T6) as against those with other combinations of agronomic practice (T1, T2, T3, T4 and T5) where the treatment involving zero tillage, wider spacing, and organic inputs (T4) revealed the maximum incidence of these sucking pests. Interestingly, each of the pests showed almost a similar level of incidence over a period of three years with respect to a particular treatment. It is, therefore, obvious that the agronomic conditions under which the mulberry gardens were maintained under each treatment had no significant influence on perhaps the nutritional components, in addition to succulency, of foliage that supported the persistence and proliferation of the pests under consideration in the current study. The observation that the pest incidence was significantly lesser in the mulberry gardens with tillage and closer spacing receiving organic inputs (T1) falls in line with the findings of other researchers working in other crop systems (Eigenbrode and Pimentel, 1988; Altieri and Nicholls, 2003; Adilakshmi *et al.*, 2007; Zehnder *et al.*, 2007; Atijegbe *et al.*, 2013). Even the gardens with tillage and closer spacing as well as those with zero tillage and wider spacing, receiving both organic and inorganic inputs (T3 and T6), showed the reduced level of pest incidence. In contrast, the gardens with tillage and wider spacing, treated with organic nutrients (T4), revealed distinctly deviated results with higher incidence of the pests, probably due to reduced levels of soil microbial biomass and soil dehydrogenase activity as evidenced by the findings of a separate study conducted by us (unpublished data). In this regard, it is highly pertinent to record the well-established fact that the soils exhibiting higher densities of micro-fauna (microbial community) would be rich in a host of chemical

substances that improve plant health and confer resistance to it against pests and diseases (Altieri and Nicholls, 2003). In addition, in yet another study of ours, we have observed increased moisture levels of foliage in T4 that might have increased succulency / palatability of the foliage as a reflection of increased water holding capacity of soils (unpublished data) with concomitant elevation in the pest incidence. With regard to impact of tillage practices observed in the current study, it is pertinent to mention at this juncture that tillage greatly alters the physical characteristics of soil (Neher and Barbercheck, 1999; Alam *et al.*, 2014) and the matrix-supporting growth of the microbial population (Kennedy, 1999) and this may indirectly have an impact on plant resistance against pests and diseases. Planting density and spacing are also reported to influence the pest incidence in a significant way. In this regard, Hill (1989) reported that close spacing brings about effects such as colonization of natural enemies of pests and wider spacing attracts insect pests when the plant density is low. In paddy (*Oryza sativa* L.), Kulagod *et al.* (2011) have come out with the observation that close planting aggravates the pest problem.

From the foregoing account gathered from our study, it can be concluded that the agronomic practices have profound influence on the incidence of *P. mori*, *E. flavescens*, *M. hirsutus*, *S. nigra* and *A. dispersus* in mulberry gardens. The incidence varies in accordance with the combination of agronomic practices employed. However, the impact of an independent agronomic practice cannot be singled out in the present context of our study. As such, attempts if oriented in this regard would become useful in generating information to score the effect of an independent agronomic variable; so that a need based control strategy can be chosen to obviate the economic damage in mulberry crop system due to the sucking insect pests in question.

ACKNOWLEDGEMENT

The authors express their sincere gratitude to Dr.H.Jayaram, Scientist-D, Central Sericultural Research and Training Institute, Central Silk Board, Mysuru, and Dr. K. G. Manjunath, Department of Studies in Sericulture Science, University of Mysore, Mysuru, for help in statistical analysis of experimental data.

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Research Paper

SEX AND SEASON SPECIFIC PARASITIC INTERACTION BETWEEN *XANTHOPIMPLA PEDATOR* FABRICIUS (HYMENOPTERA: ICHNEUMONIDAE) AND SPINNING LARVA OF *ANTHRAEA MYLITTA* (LEPIDOPTERA: SATURNIIDAE)

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ABSTRACT

Ichneumon fly, *Xanthopimpla pedator* is an idiobiont, solitary larval-pupal endoparasitoid of *Antheraea mylitta* that deposits single egg inside spinning larva subsequently causing pupal mortality that affects seed production. Understanding on insect-host interface between ♀ *X. pedator* and *A. mylitta* is not yet complete. To explore this a bit further, four experimental rearing trials of Daba ecorace (BV) of *A. mylitta* were conducted on seven forest tree species at Forest Research Institute, Dehra Dun (Uttarakhand) during 2012 to 2014. Results indicated that ♀ ichneumon fly prefer to attack *A. mylitta* larvae during the morning (6.30-11.00 am) and evening (4.00 - 6.30 p.m.) hours by locating its host accurately, even if concealed amidst leaves. It takes 45 (±5) seconds for host selection through antennal probing, 25 (±5) seconds for choosing oviposition site and 5 (±1) seconds for depositing an egg; however, sunshine and forest tree species were found affecting its orientation pattern. β coefficient of multiple regressions indicates that length and shell thickness of ♂ cocoons and shell thickness of ♀ cocoons are strong predictors for parasitic behaviour of *X. pedator*. Cocoon shell thickness of both the sexes had negative correlation and length of ♂ cocoon had positive association with parasitic behaviour of *X. pedator*. Rearing season (DF 1, $P < 0.05$) and host plant (DF 6, $P < 0.05$) significantly affected parasitoid-host interactions. Results indicated that parasitization rate of 2.89 % of first rearing season increased to 14.52 % in second season. But, interestingly, out of all infested pupae, 85.59 % were ♂, indicating sex specific parasitic behaviour of *X. pedator*. Protandrous nature of *A. mylitta*, higher ♂ sex ratio, small sized hammock and thin shell thickness of freshly formed cocoon by ♂ spinning larvae are the main reasons to elicit sex specific parasitic behaviour of *X. pedator*.

Key words: Cocoon loss, livelihood, pest and predator, tasar seed sector, vanya silk.

INTRODUCTION

Xanthopimpla Saussure 1892 is one of the largest genera of Ichneumonidae and its species are endoparasitoid of Lepidopterans that show greater degree of biological adaptations (Gauld and Bolton, 1988; Gomez *et al.*, 2009). Ichneumon fly, *Xanthopimpla pedator* Fabricius (Hymenoptera: Ichneumonidae) and Uzi fly, *Blepharipa zebina* Walker (Diptera: Tachinidae) are the common endoparasitoids of tasar silkworm, *Antheraea mylitta* Drury (Lepidoptera: Saturniidae). In

forest, pest and predator attack causes 40-45 % cocoon loss of *A. mylitta* rearing (Jolly *et al.*, 1974; 1979).

X. pedator is an idiobiont, solitary larval-pupal endoparasitoid of *A. mylitta*. There are many *Xanthopimpla* species thriving in tropical forests and it is quite easy to recognize them by their lemon yellow colour and stout body (Figure 1d).

Ecological specialization coupled with wide range of hosts and biological characteristics makes *Xanthopimpla*,

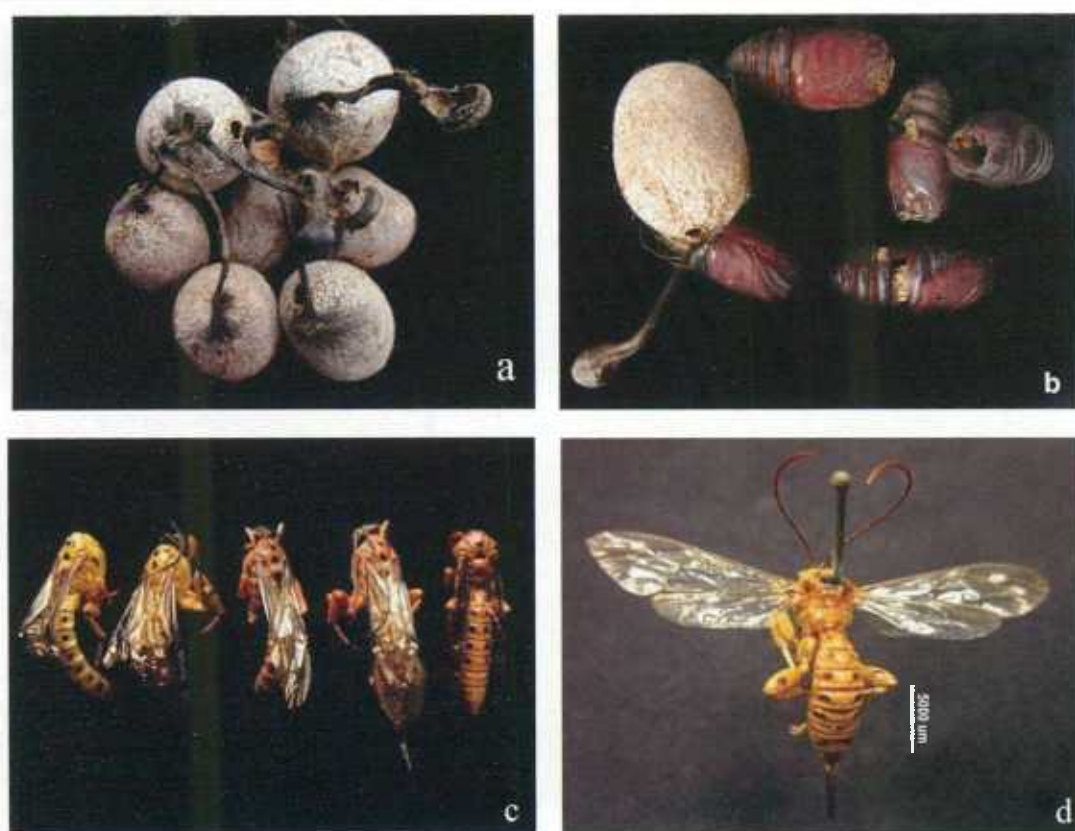


Figure 1: a) Emergence hole of *X. pedator* on host cocoon; b) Parasitized pupae ; c) Unemerged ichneumon fly; d) Adult *X. pedator*

one of the most attractive of ichneumonid groups for biological study (Gauld, 1991). It is known that *X. pedator* begins its life cycle in 5th instar, spinning larva of *A. mylitta* at the time of hammock formation (Figure 2 c) to early cocooning stage (Figure 2 d) and uses its long ovipositor to drill through the silky envelope and deposits single egg in the abdominal segment. In the course of development, silkworm transforms into pupa. After hatching, ichneumon larva feeds on the entire content of defenceless pupa (Figure 1 b) and pupates there. The parasitoid adult emerges by making a characteristic circular hole at the anterior region of the host cocoon near peduncle (Figure 1 a).

There are hundreds of published records on host-parasitoid associations between Saturniidae and Ichneumonidae, but studies specifically on *X. pedator* are scarce and complete bio-ecology of *X. pedator* is still an

enigma to sericulture scientists (Singh *et al.*, 2010). Therefore, the objectives of this study were to find out : (1) How female *X. pedator* finds and parasitizes its host?; (2) Do forest tree species influence the host selection behaviour of *X. pedator*?; (3) Does sex of the host influence parasitic behaviour of *X. pedator*? and (4) Does host cocoon characteristics influence the parasitic behaviour of *X. pedator*?

MATERIALS AND METHODS

Study area and climate

Study was conducted during 2012-2014 at New Forest, Forest Research Institute (FRI), Dehradun, Uttarakhand. Climate of New Forest is moderate due to its location at the foot of the Himalayas. May to early June is the hottest month with mean temperature of 36.20°C and maximum

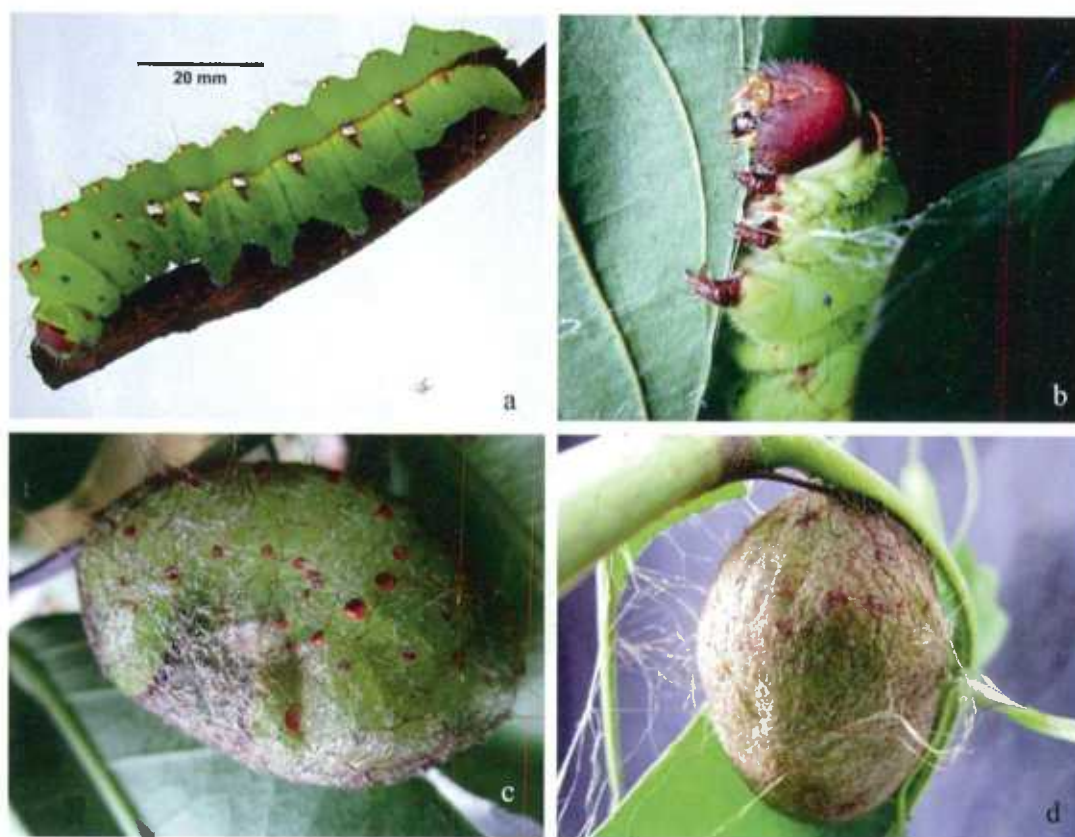


Figure 2: a) 5th instar larva of *A. mylitta*; b) Spinning larva of *A. mylitta*; c) Cocoon hammock of *A. mylitta*; d) Newly formed cocoon of *A. mylitta*

of 42°C. Winter starts in November and continues up to February with mean daily minimum and maximum temperature of 6.1 and 19.1°C, respectively. The average annual precipitation at the site was 2123.8 mm.

Forest host tree species and test insect

Seven forest tree species were evaluated to assess parasitic behaviour of *X. pedator*. Identification of forest tree species was carried out by Systematic Botany Division, FRI, Dehradun, and voucher specimen was deposited. Bivoltine (BV) Daba ecorace of *A. mylitta* was utilized because of its high exploitation potential.

Forest based outdoor rearing and grainage operation of *A. mylitta*

Outdoor rearing of 300 newly hatched larvae of *A.*

mylitta was conducted on 5-6 year old trees of seven forestry host plants in six replications. Seventy-five DFLs of *A. mylitta* was procured from Central Silk Board, Govt. of India, Surguja, Chhattisgarh. First crop was taken in July-August and second crop during September-November following the integrated rearing technology of Mathur *et al.* (1998). Cocoons were harvested after completion of pupation and kept in different insect cages (21" L x 21" W x 30" H) to carry out grainage operation.

Host selection and oviposition behaviour of *X. pedator* on different forestry host plants

When fifth instar larvae of *A. mylitta* started spinning the cocoons, a host of adult flies of *X. pedator* appear in the near vicinity. Visual observations on host selection and oviposition behaviour of *X. pedator* and response of spinning *A. mylitta* to attack of *X. pedator* during both the

rearing seasons were made. Observations on host selection and oviposition behaviour of *X. pedator* were recorded from all the replicates of spinning larvae of seven treatments and based on three visits of *X. pedator* on every replicate.

Identification of the parasitoid

Dead adult ichneumon flies found in parasitized pupae of *A. mylitta* were identified by comparing with the specimen available at National Forest Insect Collection (NFIC), Forest Research Institute, Dehradun, India. The specimens were identified (Figures 1c and 1d) by tallying its taxonomic features with the insect kept in cabinet number 15.1 (Hymenoptera: Ichneumonidae) having accession number 4463, named as *Xanthopimpla pedator* Fabricius (Hymenoptera: Ichneumonidae) and the voucher specimen of the insect was submitted to NFIC.

Variables measured

Data on number of cocoons harvested and rate of parasitism of *X. pedator* from all the replicates were recorded. To assess the parasitic behaviour of *X. pedator*, all the cocoons wherein the moths failed to emerge, from all the replicates were separated and cut open. Infested pupae were sexed out into male and female by observing the shape of ventral genital aperture above the anal opening on ninth pupal segment. All the pupae of non-diapausing cocoons were dissected to find out ichneumon infestation, whereas in diapausing lot, it was not required, because all the parasitized pupae were found completely consumed by *X. pedator* leaving only intact pupal exoskeleton filled by dried excreta of the parasitoid larvae. Biometric study of the infested cocoons of both the sexes was carried out by using Mahr Digital Callipers 16 EX to assess the effect of size, shape and shell thickness of cocoon on parasitic behaviour *X. pedator*.

Statistical design and data analysis

Per cent mortality data of ♂ and ♀ pupae required angular transformation to normalize the data by using angular transformation table of Fisher and Yates (1963). Two-way completely RBD factorial design was used to test the significance through factorial ANOVA by using

STATISTICA 10 software. Rearing seasons and host plants were treated as the fixed effects, where, pupal mortality, cocoon length, diameter and shell thickness of male and female cocoons served as dependent variables. Level of significance was fixed at $P=0.05$. Post HOC test was carried out by using Tukey's HSD test to compare the homogeneous pairs of means. Multiple regression analysis was carried out to examine the kind of existing relationship between biometric parameters of the cocoons (independent variables) and rate of parasitism in male and female pupae (dependent variable) and to find out the potential predictor(s) for pupal parasitization.

RESULTS

Host selection behaviour of *X. pedator*

Female ichneumon parasitoids were noticed to appear in the rearing field from mid July and continued up to November till hardening of the host cocoons, but parasitoid population was larger during second rearing season of September-November. The parasitoids preferred to attack the spinning larvae or freshly formed cocoons in the morning (6.30-11.00 am) and evening hours (4.00 - 6.30 p.m.) only. The female parasitoids accurately located their host, even when they were concealed. Time spent in larva selection through antennal probing lasted for $45 (\pm 5)$ seconds, and time spent in choosing the oviposition site after the fly aligned on spinning worm lasted for $25 (\pm 5)$ seconds, and actual time taken to deposit an egg inside the larval body was $5 (\pm 1)$ seconds. In most of the cases, after oviposition, the fly immediately started searching for another host. In a particular case, during second rearing season, it was found that a fly remained in the rearing field for $45 (\pm 10)$ minutes and visited nine silkworm larvae on different forestry host plants. In non-diapausing cocoons of the first rearing season, the life cycle of *X. pedator* from the date of oviposition to adult emergence lasted for $15 (\pm 1)$ days, however, in diapausing replicates, it took $17 (\pm 1)$ days.

Effect of rearing seasons and host plants on parasitic behaviour of *X. pedator*

Null hypothesis (H_0) for no difference on effect of rearing seasons and host plants on parasitic rate of *X.*

Table 1: ANOVA for effect of rearing seasons and host plants on parasitization rate of ♂ and ♀ pupae

Parameter	Source	DF	F value	P- value
Parasitization of ♂ pupae (%)	Rearing season	1	999.53	0.000
	Host plant	6	16.84	0.000
	Rearing season x host plant	6	2.06	0.067*
Parasitization of ♀ pupae (%)	Rearing season	1	103.13	0.000
	Host plant	6	3.45	0.00393
	Rearing season x host plant	6	0.75	0.060*

*Non-significant at p=0.05

Table 2: Effect of rearing seasons and host plants on parasitic behaviour of *X. pedator*

Rearing season	Host plant	Harvested cocoons (No.)	Mortality in ♂ pupae			Mortality in ♀ pupae		
			No.	Infestation* (%)	Proportion (%) in total infested pupae	No.	Infestation* (%)	Proportion (%) in total infested pupae
I Crop	<i>T. alata</i>	177±2.45 ^h	13	6.14 ± 0.70 ^a	76.47	4	2.89 ± 0.92 ^{ab}	23.53
	<i>T. tomentosa</i>	162±9.73 ^{ab}	19	8.10 ± 0.46 ^{ab}	82.61	4	2.58 ± 1.19 ^a	17.39
	<i>T. arjuna</i>	153±9.19 ^{fgh}	21	8.63 ± 0.48 ^{ab}	80.77	5	3.26 ± 1.08 ^{ab}	19.23
	<i>L. speciosa</i>	138±5.06 ^{cig}	23	9.58 ± 0.30 ^{ab}	82.14	5	4.00 ± 0.80 ^{abcd}	17.86
	<i>T. bellirica</i>	117±4.82 ^{cd}	20	9.74 ± 0.40 ^b	86.96	3	2.56 ± 1.15 ^a	13.04
	<i>L. tomentosa</i>	87±3.61 ^{bc}	14	11.34 ± 0.59 ^b	77.78	4	4.91 ± 1.56 ^{abcd}	22.22
	<i>T. chebula</i>	60±1.97 ^{ab}	16	10.04 ± 0.45 ^b	80.00	4	3.96 ± 1.26 ^{bce}	20.00
	Total / Average	894	126	-	81.29	29	-	18.71
II Crop	<i>T. alata</i>	153±9.32 ^{gh}	88	18.14 ± 0.81 ^c	88.89	11	6.31 ± 0.41 ^{abcde}	11.11
	<i>T. tomentosa</i>	138±5.37 ^{efg}	85	18.21 ± 0.64 ^c	86.73	13	7.21 ± 0.42 ^{bcd}	13.27
	<i>T. arjuna</i>	129±6.47 ^{ef}	88	19.81 ± 0.92 ^{cd}	85.44	15	8.01 ± 0.53 ^{cdef}	14.56
	<i>L. speciosa</i>	123±5.81 ^{def}	105	22.21 ± 0.35 ^{de}	88.24	14	7.91 ± 0.53 ^{cdef}	11.76
	<i>T. bellirica</i>	96±3.18 ^{cd}	99	24.54 ± 0.63 ^e	88.39	13	8.51 ± 0.56 ^{def}	11.61
	<i>L. tomentosa</i>	60±3.34 ^{ab}	41	24.38 ± 1.70 ^e	82.00	9	11.22 ± 0.96 ^f	18.00
	<i>T. chebula</i>	39±1.03 ^a	51	22.17 ± 0.56 ^{de}	82.26	11	10.10 ± 0.64 ^{ef}	17.74
	Total / Average	738	557	-	86.63	86	-	13.37
Combined for I and II crop	<i>T. alata</i>	165±7.88 ^a	101	12.14 ± 1.88 ^e	87.07	15	4.60 ± 0.71 ^a	12.93
	<i>T. tomentosa</i>	150±6.4 ^{bc}	104	13.15 ± 1.57 ^e	85.95	17	4.89 ± 0.92 ^a	14.05
	<i>T. arjuna</i>	141±6.15 ^{ab}	109	14.22 ± 1.76 ^{gh}	84.50	20	5.64 ± 0.92 ^{ab}	15.50
	<i>L. speciosa</i>	130.5±4.31 ^a	128	15.89 ± 1.92 ^h	87.07	19	5.95 ± 0.75 ^{ab}	12.93
	<i>T. bellirica</i>	106.5±4.19 ^f	119	17.14 ± 2.26 ^h	88.15	16	5.53 ± 1.08 ^{ab}	11.85
	<i>L. tomentosa</i>	73.5±4.69 ^e	55	17.86 ± 2.14 ^f	80.88	13	8.07 ± 1.29	19.12
	<i>T. chebula</i>	49.5±3.38 ^d	67	16.10 ± 1.86 ^f	81.71	15	7.03 ± 1.14 ^{ab}	18.29
	Grand total / Average	816	683	-	85.59	115	-	14.41

(1) Significant differences within every variable are indicated by different superscripts, whereas, same superscript within every variable represents homogeneous groups (Tukey's HSD test at alpha = 0.05).

(2) * Mortality % shown in the table is Angular transformed values of original infestation rate; the mortality rate (%) values in the row against 'Total' are original ones.

Table 3: Analysis of variance for effect of rearing seasons and host plants on biometric parameters of male and female cocoons

Parameter	Source	DF	F value	P- value
Length of male cocoon (mm)	Rearing season	1	5929.5	0.00000
	Host plant	6	596.6	0.00000
	Rearing season x host plant	6	5.1	0.00012
Length of female cocoon (mm)	Rearing season	1	5929.5	0.00000
	Host plant	6	596.6	0.00000
	Rearing season x host plant	6	5.1	0.00017
Diameter of male cocoon (mm)	Rearing season	1	6951.2	0.00000
	Host plant	6	632	0.00000
	Rearing season x host plant	6	5.5	0.00011
Diameter of female cocoon (mm)	Rearing season	1	6951.2	0.00000
	Host plant	6	632	0.00000
	Rearing season x host plant	6	5.5	0.00014
Shell thickness of male cocoon (mm)	Rearing season	1	213.82	0.00000
	Host plant	6	221.69	0.00000
	Rearing season x host plant	6	1.91	0.0807*
Shell thickness of female cocoon (mm)	Rearing season	1	213.82	0.00000
	Host plant	6	221.69	0.00000
	Rearing season x host plant	6	1.91	0.0905*

*Non-significant at $p=0.05$

pedator was rejected by ANOVA. Analysis of variance (Table 1) indicates that rearing season (DF 1, $P<0.05$) and host plant (DF 6, $P<0.05$) significantly affected rate of parasitism of ♂ and ♀ pupae; however, their interactions were not significant. Overall parasitic rate of 2.89 % in first crop rearing season increased to 14.52 % in second season. Tukey's HSD test indicated that ♂ and ♀ pupal mortality differed among groups (Table 2).

Effect of host plants on biometric parameter of the cocoons and its influence on parasitic behaviour of *X. pedator*

Results indicated that length and diameter of ♂ and ♀ cocoons were significantly affected by rearing seasons (DF 1, $P<0.05$), host plants (DF 6, $P<0.05$) and their interactions (DF 6, $P<0.05$) (Table 3). Similarly, shell thickness was also significantly affected by rearing seasons (DF 1, $P<0.05$) and host plants (DF 6, $P<0.05$), but effect of their interactions were not significant (DF 6, $P>0.05$). Length, diameter and shell thickness of ♂ and ♀ cocoons have shown an increase in the second rearing

season on all the tested forestry host plants. Further, all the biometric variables of ♀ cocoons were higher than those of ♂ cocoons in both the rearing seasons. *Terminalia alata* produced the best quality of cocoons in terms of length, diameter and shell thickness, followed by *T. tomentosa*, *T. Arjuna* and *L. speciosa*. Cocoon biometric parameters differed significantly on different host plants in both the rearing seasons (Table 4).

Relationship between biometric parameters of cocoons and parasitic behaviour of *X. pedator*

β coefficient (Tables 5 and 6) revealed that the length and shell thickness of ♂ cocoon, and shell thickness of ♀ cocoon are strong predictors for pupal parasitization. Scatter plot indicated that cocoon shell thickness of both the sexes is negatively associated with pupal parasitization rate (Figures 3 a and 3 b); however, length of ♂ cocoon was having positive association (Figure 4). Further, diameter of male and female cocoons, and length of female cocoons was found to have positive association with pupal parasitization rate, but were not significant.

Table 4: Biometric assessment of male and female cocoons infested by *X. pedator* (mean $\pm$ SE)

Rearing season	Host plant	Male cocoon			Female cocoon		
		Length (mm)	Diameter (mm)	Shell thickness (mm)	Length (mm)	Diameter (mm)	Shell thickness (mm)
First	<i>T. alata</i>	36.02 $\pm$ 0.17 ^d	22.11 $\pm$ 0.06 ^d	0.413 $\pm$ 0.014 ^{fe}	42.38 $\pm$ 0.20 ^d	26.02 $\pm$ 0.08 ^{de}	0.486 $\pm$ 0.017 ^{fg}
	<i>T. tomentosa</i>	34.92 $\pm$ 0.09 ^c	21.27 $\pm$ 0.07 ^c	0.354 $\pm$ 0.007 ^{de}	41.08 $\pm$ 0.11 ^c	25.02 $\pm$ 0.09 ^c	0.416 $\pm$ 0.008 ^{de}
	<i>T. arjuna</i>	33.28 $\pm$ 0.14 ^b	20.01 $\pm$ 0.11 ^b	0.319 $\pm$ 0.003 ^{cd}	39.16 $\pm$ 0.17 ^b	23.54 $\pm$ 0.13 ^c	0.376 $\pm$ 0.004 ^{cd}
	<i>L. speciosa</i>	32.53 $\pm$ 0.07 ^b	19.35 $\pm$ 0.04 ^b	0.283 $\pm$ 0.005 ^{bc}	38.27 $\pm$ 0.08 ^b	22.76 $\pm$ 0.04 ^b	0.333 $\pm$ 0.005 ^{bc}
	<i>T. bellirica</i>	31.27 $\pm$ 0.12 ^a	19.02 $\pm$ 0.03 ^{ab}	0.278 $\pm$ 0.002 ^{ab}	36.78 $\pm$ 0.14 ^a	22.38 $\pm$ 0.04 ^{ab}	0.315 $\pm$ 0.002 ^{ab}
	<i>L. tomentosa</i>	30.58 $\pm$ 0.08 ^a	18.66 $\pm$ 0.05 ^a	0.235 $\pm$ 0.002 ^a	35.98 $\pm$ 0.09 ^a	21.95 $\pm$ 0.06 ^a	0.276 $\pm$ 0.003 ^a
	<i>T. chebula</i>	31.71 $\pm$ 0.26 ^a	18.00 $\pm$ 0.14 ^a	0.193 $\pm$ 0.008 ^a	33.78 $\pm$ 0.30 ^a	21.18 $\pm$ 0.16 ^a	0.227 $\pm$ 0.009 ^a
Second	<i>T. alata</i>	43.40 $\pm$ 0.20 ^h	26.64 $\pm$ 0.08 ^g	0.497 $\pm$ 0.017 ^g	51.06 $\pm$ 0.24 ^g	31.34 $\pm$ 0.09 ^f	0.585 $\pm$ 0.020 ^c
	<i>T. tomentosa</i>	42.07 $\pm$ 0.11 ^g	25.62 $\pm$ 0.09 ^g	0.436 $\pm$ 0.008 ^f	49.49 $\pm$ 0.13 ^f	30.14 $\pm$ 0.11 ^f	0.501 $\pm$ 0.009 ^g
	<i>T. arjuna</i>	40.10 $\pm$ 0.17 ^f	24.11 $\pm$ 0.14 ^h	0.385 $\pm$ 0.004 ^{ef}	47.18 $\pm$ 0.20 ^f	28.36 $\pm$ 0.16 ^e	0.452 $\pm$ 0.004 ^{ef}
	<i>L. speciosa</i>	39.19 $\pm$ 0.09 ^f	23.31 $\pm$ 0.05 ^g	0.341 $\pm$ 0.006 ^d	46.11 $\pm$ 0.10 ^e	27.43 $\pm$ 0.05 ^g	0.402 $\pm$ 0.007 ^d
	<i>T. bellirica</i>	37.67 $\pm$ 0.15 ^e	22.92 $\pm$ 0.04 ^{fg}	0.322 $\pm$ 0.002 ^d	44.32 $\pm$ 0.17 ^e	26.96 $\pm$ 0.05 ^{fg}	0.379 $\pm$ 0.003 ^d
	<i>L. tomentosa</i>	36.85 $\pm$ 0.09 ^d	22.48 $\pm$ 0.06 ^{ef}	0.283 $\pm$ 0.003 ^{bc}	43.35 $\pm$ 0.11 ^d	26.44 $\pm$ 0.07 ^{ef}	0.333 $\pm$ 0.003 ^{bc}
	<i>T. chebula</i>	34.59 $\pm$ 0.31 ^c	21.69 $\pm$ 0.17 ^{cd}	0.233 $\pm$ 0.009 ^a	40.70 $\pm$ 0.36 ^c	25.52 $\pm$ 0.20 ^{cd}	0.274 $\pm$ 0.011 ^a
Combined for first and second rearing season	<i>T. alata</i>	39.71 $\pm$ 1.12 ^f	24.38 $\pm$ 0.68 ^h	0.455 $\pm$ 0.017 ^g	46.72 $\pm$ 1.32 ^c	28.68 $\pm$ 0.81 ^e	0.536 $\pm$ 0.019 ^e
	<i>T. tomentosa</i>	38.49 $\pm$ 1.08 ^f	23.44 $\pm$ 0.66 ^h	0.389 $\pm$ 0.012 ^{ef}	45.29 $\pm$ 1.27 ^c	27.58 $\pm$ 0.78 ^e	0.459 $\pm$ 0.014 ^d
	<i>T. arjuna</i>	36.69 $\pm$ 1.03 ^d	22.06 $\pm$ 0.62 ^d	0.352 $\pm$ 0.010 ^d	43.17 $\pm$ 1.22 ^d	25.95 $\pm$ 0.73 ^d	0.414 $\pm$ 0.012 ^d
	<i>L. speciosa</i>	35.85 $\pm$ 1.01 ^c	21.33 $\pm$ 0.60 ^c	0.312 $\pm$ 0.009 ^b	42.19 $\pm$ 1.18 ^d	25.09 $\pm$ 0.70 ^d	0.367 $\pm$ 0.011 ^b
	<i>T. bellirica</i>	34.46 $\pm$ 0.97 ^c	20.98 $\pm$ 0.59 ^b	0.296 $\pm$ 0.008 ^b	40.55 $\pm$ 1.14 ^c	24.67 $\pm$ 0.69 ^c	0.347 $\pm$ 0.010 ^b
	<i>L. tomentosa</i>	33.71 $\pm$ 0.95 ^b	20.57 $\pm$ 0.58 ^b	0.259 $\pm$ 0.007 ^a	39.66 $\pm$ 1.11 ^b	24.19 $\pm$ 0.68 ^c	0.306 $\pm$ 0.009 ^b
	<i>T. chebula</i>	31.65 $\pm$ 0.91 ^a	19.84 $\pm$ 0.57 ^b	0.213 $\pm$ 0.008 ^a	37.24 $\pm$ 1.07 ^a	23.35 $\pm$ 0.67 ^c	0.251 $\pm$ 0.010 ^a

(3) Significant differences within variable are indicated by different superscripts; whereas, same superscript within every variable represents homogeneous groups (Tukey's HSD test at $\alpha = 0.05$).

Table 5: Multiple regression summaries for dependent variable: Parasitization of male pupae (%)

R = .92567246; R² = .85686950; Adjusted R² = .85150210; F (3, 80) = 159.64; p < 0.0000; Std. Error of estimate: 2.5871 N = 84

Predictor (N=84)	Beta coefficient	Std. Err.	b	Std. Err.	t (80)	p-value
Intercept			-47.295	2.990985	-15.8127	0.000000
Length of male cocoon (mm)	1.46775	0.341478	2.314	0.538256	4.2982	0.000048
Diameter male cocoon (mm)	0.26809	0.328497	0.707	0.866650	0.8161	0.416868*
Shell thickness of male cocoon (mm)	-1.37138	0.080292	-109.836	6.430743	-17.0798	0.000000

*Non-significant at p=0.05

Table 6: Multiple regression summaries for dependent variable: Parasitization of female pupae (%)

R = .76004600; R² = .57766992; Adjusted R² = .56183254; F(3,80)=36.475; p < .00000, Std. Error of estimate: 2.3161

Predictors (N=84)	Beta coefficient	Std. Err.	b	Std. Err.	t(80)	p-value
Intercept			-19.9531	2.677646	-7.45172	0.00000
Length of female cocoon (mm)	0.62106	0.586575	0.4337	0.409588	1.05878	0.29288*
Diameter female cocoon (mm)	0.79412	0.564275	0.9281	0.659480	1.40733	0.16320*
Shell thickness of female cocoon (mm)	-1.18968	0.137922	-42.2099	4.893493	-8.62573	0.00000

*Non-significant at p=0.05

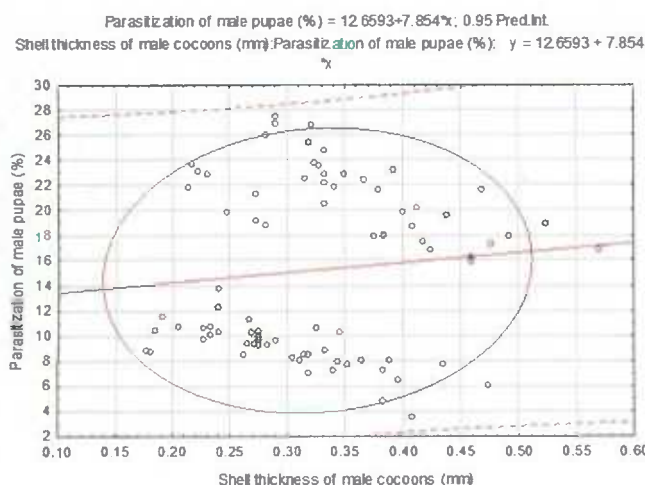


Figure 3 a: Scatter plot for parasitization of male pupae against shell thickness of ♂ cocoons

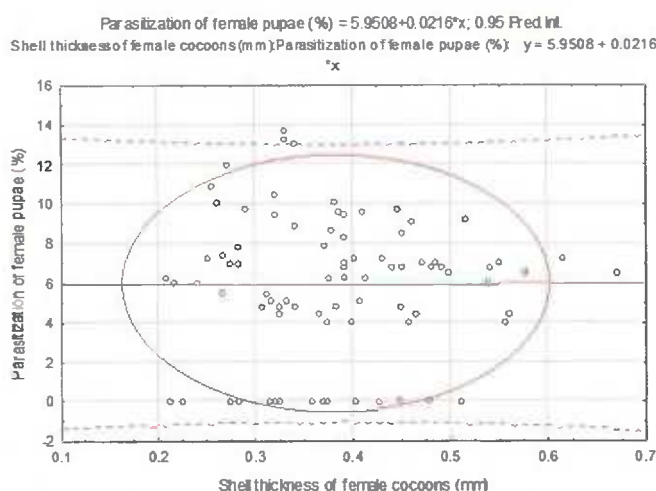


Figure 3 b: Scatter plot for parasitization of female pupae against shell thickness of ♀ cocoons

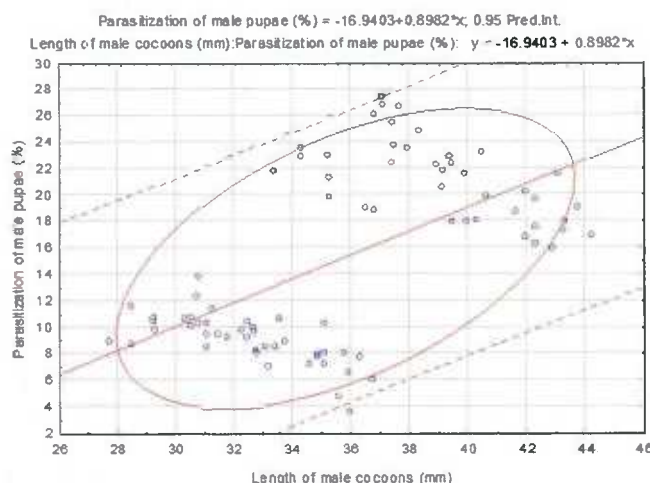


Figure 4: Scatter plot for parasitization of male pupae against length of ♂ cocoons (mm)

DISCUSSION

Host selection behaviour of *X. pedator*

Biological success of any parasitoid is determined by its capacity to locate its host first in the ecosystems and then, in its microhabitat and finally parasitizing the targeted host (Mattiacci *et al.*, 1999; Hausmann *et al.*, 2005). These events happen in sequence by responding to a succession of cues (Vinson, 1976) in five steps viz., host habitat location, host location, host acceptance, host identification and finally host discrimination (Vinson, 1975; 1976; 1981).

X. pedator finds its host habitat by responding to environmental clues, such as temperature, shade, humidity and vegetation (Weseloh, 1972) and then locates its host in microhabitat by using special searching and finding proficiency and finally parasitize it by using specialized attacking mechanisms (Xiaoyi and Zhongqi, 2008) and this series of action is accomplished through synchronised combination of five behavioural cues viz., olfaction (semiochemicals or info-chemicals), vision (chromatic aberration of host covers), contact stimuli (characters of host covers), hearing (vibrational sounding) and infrared induction (differences in environmental temperatures) (Xiaoyi and Zhongqi, 2008) in hierarchical manner.

According to Hou and Yan (1997), semiochemicals that originate from hosts and damaged plant tissues are the most common cues for hymenopterous parasitoid to find out their Lepidopteran hosts. Since, *X. pedator* parasitizes non-feeding spinning larvae of *A. mylitta* inside the cocoon hammock (Figures 2 b and 2 c) or spun up larvae of *A. mylitta* inside newly formed cocoon (Figure 2 d), the volatile semiochemicals or allelochemicals (allomones, kairomones or synomones) from damaged host plant's leaves cannot be a reliable cue for *X. pedator* to locate their host. In my opinion, *X. pedator* orients towards stimuli produced by spinning larvae of *A. mylitta*; because, silk gland of *A. mylitta* is a typical exocrine gland that secretes large amount of silk proteins (sericin and fibroin) during cocoon spinning. Since, *X. pedator* is attracted only towards spinning larvae; it is obvious that the olfactory stimuli produced by the silk gland of spinning larvae mediate the host selection behaviour of *X. pedator*. Vinson

et al. (1975) and Weseloh (1981) have also mentioned in their research communication that many parasitoids locate their hosts in response to their host's pheromone. For example, mandibular gland secretion of *Ephestia kuehniella* that contains a pheromone is used by its parasitoid, *Venturia canescens* (Hymenoptera: Ichneumonidae) for host location (Corbet, 1971).

Secondly, *X. pedator* analyses vision information to discriminate between feeding and spinning larvae (Figure 2 a and 2 b) both differing in colour contrasts, and inserts its ovipositors into spinning larvae only avoiding super-parasitism. *Pimpla turionellae* (Hymenoptera: Ichneumonidae) also discriminate blue bands of different reflectance intensities from yellow bands and insert their ovipositors significantly more often into the area of blue bands (Fischer *et al.*, 2004). Similarly, *Pimpla instigator* Fabricius (Hymenoptera: Ichneumonidae) can also discriminate between blue and yellow host plant models (Schmidt *et al.*, 1993). Vision as a component of the host location, has also been demonstrated by Askew (1961) and Richerson and Borden (1972).

Third, *X. pedator*, being a niche-specific parasitoid, has acquired contact response ability to differentiate between smooth surface of fifth instar feeding larvae and rough surface of newly formed hammock of the spinning larvae of *A. mylitta* to locate its concealed host. Xiaoyi and Zhongqi (2008) have also demonstrated the function of contact stimuli (character and hardness of the host covers) as an influential cue in host selection behaviour of ichneumon parasitoids. Schmidt *et al.* (1993) evaluated contact and vision responses of *P. instigator* and had noticed that surface texture of host's cylindrical model significantly affected the frequency of punctures made by wasp's ovipositors.

Fourth, vibrational sounding is an important cue for host location in many hymenopterous parasitoids. This mechanism is used as a kind of echolocation, wherein self-produced vibrations are transmitted onto the substrate and perceived by subgenual organ in the tibiae (Kroder, 2006). Wackers *et al.* (1998) established that ichneumon wasps transmit their self-produced vibrations via antennae onto the substrate covering of the host and analyse the reflected signals to detect the position of host

for accurate oviposition. Our observations revealed that *X. pedator* while selecting the spinning larvae, applies antennal probing onto the cocoon hammock, lasting for 45 seconds. Based on the behaviour of other hymenopterous ichneumon flies, it can be construed that *X. pedator* produces vibrational sounding and analyses the reflected signals by receptor organ of tibia to detect the position of host for accurate oviposition; however, there is no specific study on this aspect of host finding mechanism of *X. pedator*. Secondly, how many spinning larvae are parasitized by a single *X. pedator* fly in its reproductive phase of life is also not known.

Season specific parasitic behaviour of *X. pedator*

The extent of pupal loss in *A. mylitta* caused by *X. pedator* varies from season to season and place to place (Singh *et al.*, 2010). It is known that season, insect voltinism and vegetation settings of a particular season influence the kind, abundance and arrival timings of different insect enemies (Altieri *et al.*, 1993). Results indicate that in the first rearing season of July-August, parasitization rate was just 2.89 % that rose to 14.52 %, registering five-fold increase during second rearing season of October-November. Higher pace of larval development due to prevalence of high temperature regime during first rearing season is the main reason for low parasitic behaviour of *X. pedator*; whereas, in the second rearing season, temperature remaining favourable for effective host location of spinning larvae of *A. mylitta* by *X. pedator* is attributed for increased parasitic activity.

Sex specific parasitic behaviour of *X. pedator*

Observation on sex-wise pupal mortality indicated an interesting fact about male specific parasitic behaviour of ichneumon fly, which was confirmed by high proportion (85.59 %) of male infested pupae reported out of total parasitisation. It means, *X. pedator* apply sex specific host finding mechanism to choose between male and female spinning larvae of *A. mylitta*. In bisexual insect species, male emerges prior to female (Fitton and Rotheray, 1982); that means, growth and development of male *A. mylitta* larvae is faster than female and become available first for parasitization by *X. pedator*. The second possible reason for sex specific parasitic behaviour of *X. pedator* may be

attributed to a male biased sex ratio (60:40) of *A. mylitta*.

Effect of cocoon biometric parameters on parasitic behaviour of *X. pedator*

Klomp and Teerink (1962) concluded that hymenopterous parasitoids use their antennae to explore size, shape and surface texture of the prospective host to elicit oviposition response. Price (1970) observed that ichneumonid, *Pleolophus indistinctus* would only lay egg in a sawfly cocoon, if it could stand on the cocoon with all six legs and reach its apex with the antennae. It indicates that size of cocoon matters in parasitic behaviour of ichneumonid. Biometric study of cocoons revealed that the length and diameter of female cocoons is always more than that of the male cocoons. Based on the observations of Price (1970), it can be inferred that owing to larger size of female cocoon hammock, *X. pedator* might have found it difficult to stand on all six legs and reach the apex of the cocoon with the antennae; hence, female spinning larvae were normally not preferred for oviposition. In contrast, smaller size of male cocoon is better suited for the above mentioned type of oviposition behaviour of *X. pedator*.

Results also indicated that shell thickness of cocoon in both the sexes were negatively associated with the parasitization rate of *X. pedator* (Tables 5 and 6). This may be one of the reasons for the parasitoid selecting the male host pupae for oviposition. It indicates that cocoon having less shell thickness is preferred more in host selection. Results show that male host cocoons characterized by thin shell facilitates easier emergence of adult ichneumon fly, in comparison to incidence of dead *X. pedator* in diapausing cocoons, which were found to be female pupae. It indicates that *X. pedator* finds it difficult to make desired size of exit hole in thick shelled female cocoon.

Sex specific parasitic behaviour of *X. pedator* may in turn affect the sex ratio in *A. mylitta* that ultimately reduce coupling percentage and seed production. Velide and Bhagavanulu (2012) suggested that parasitization rate of *X. pedator* can be reduced by keeping cocoons under captivity, but owing to the wild nature of *A. mylitta* and associated large coverage area during final instar; all the spinning larvae or newly formed cocoons cannot be kept under captivity.

ACKNOWLEDGEMENT

The author wishes to place on record, his special thanks to Mr. Raman Nautiyal for his help in selection of statistical tool, data analysis and insightful comments on their interpretation. He also thanks Dr. Mukhtar Ahmad, Mr. R. S. Bhandari and Dr. Sudhir Singh for their suggestions in preparation of manuscript, and Mr. Nirmal Ram, for providing weather data of the study site. He also extends his heartiest thanks to the anonymous reviewer for upgrading the quality of the manuscript and also improving the clarity.

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MORPHOLOGY AND PATHOGENICITY OF A NEW MICROSPORIDIAN ISOLATED FROM SILKWORM (*BOMBYX MORI* L.) CROPS OF PURE MYSORE SEED AREA OF KARNATAKA, INDIA

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ABSTRACT

Different species of microsporidia cause microsporidiosis in silkworm, *Bombyx mori* L. A number of microsporidia have been isolated from silkworm and mulberry pests by many. Recently, a new microsporidian infection was reported in Pure Mysore (multivoltine) silkworm rearings of Mysore seed area, Karnataka, India. In the present study, the authors collected this microsporidian infected samples from the field, isolated and purified spores, and studied their morphological characters, pathogenicity and transmission in four successive generations in silkworm. Scanning Electron Microscope study revealed that the purified spores of the new microsporidian are ovo-cylindrical in shape and measured $4.62 \pm 0.34 \mu\text{m}$ in length and $2.05 \pm 0.24 \mu\text{m}$ in width with 1:0.44, length : width ratio. In contrast, the spores of *Nosema bombycis*, the common microsporidian are oval in shape having $3.80 \pm 0.01 \mu\text{m}$ length, $2.60 \pm 0.01 \mu\text{m}$ width and 1:0.68, length : width ratio. At the inoculation doses of 1×10^4 and 1×10^5 spores/ml, with the new microsporidian, the larval mortality recorded during rearing was 2.33 to 6.00 %. At subsequent high inoculation doses (1×10^6 to 1×10^8 spores/ml), 10.33 to 70.00 % mortality was observed and the cocoon parameters of silkworm were adversely affected by the infection. Studies also indicated that the transovarian infection by the new microsporidian in four successive generations had impact on larval mortality and cocoon characters of silkworm. The results are discussed in the light of the studies carried out.

Key words: New microsporidian, morphology, pathogenicity, silkworm.

INTRODUCTION

Nosema bombycis is the common microsporidian which is known to cause pebrine disease in silkworm. The destructive effect of the disease and its cause was first clarified by Louis Pasteur (1870). In the recent past, many microsporidia have been isolated from silkworm which are morphologically different from the standard strain, *N. bombycis*, exhibiting varying degree of pathogenicity in silkworm (Tanaka *et al.*, 1972; Fujiwara, 1980, 1984 a, b, 1985; Fang *et al.*, 1991). From India also, three microsporidia designated as NIK-2r (*Nosema* sp., Mysore), NIK-3h (*Nosema* sp. M11, Hassan) and NIK-4m (*Nosema* sp. M12, Mysore) were isolated from

silkworm and were found to be different from *N. bombycis* (Ananthalakshmi *et al.*, 1994) in their morphology, pathogenicity and transmission rate. Kishore *et al.* (1994) have also reported that certain species of *Catopsilia* which are frequent visitors / inhabitants of the fields around mulberry gardens carry microsporidian spores which after testing were found to be infective to silkworms. Later, Samson *et al.* (1999 a, b) provided information on certain butterflies harbouring microsporidia that are infective to silkworm. Still another new microsporidian (NIK-5hm) which cause pebrine disease in the silkworm was isolated by Selvakumar *et al.* (2009 a) who studied its pathogenicity and rate of horizontal and vertical transmission (Selvakumar *et al.*,

2009 b, 2010). Of late, Sharma *et al.* (2014) isolated six microsporidia from the silkworm, *Bombyx mori* and studied their pathogenicity and transmission rate and reported them as different from *N. bombycis*. Recently, there was a report on the incidence of microsporidian infection in Mysore seed area and an initial survey was conducted during July, 2014. Samples (281 nos.) from different places of Mysuru seed area (Basic Seed Farm rearings, P3, P2 and P1 Grainages, Govt. Cocoon markets and farmers of different TSCs) were collected and tested. Two samples revealed the microsporidian infection. Later, scientists from Central Sericultural Research and Training Institute (CSRTI), Mysuru, Karnataka State Sericulture Research and Development Institute (KSSRDI), Bengaluru, National Silkworm Seed Organization (NSSO), Bengaluru and Silkworm Seed Technology laboratory (SSTL), Bengaluru again conducted crop assessment during September, 2014 for pebrine incidence and tested 3,339 samples and found microsporidian infection in 44 samples. Microsporidian spores observed in the above samples were same and appeared to be having similar morphological characters. Morphologically, this new microsporidian is different from *N. bombycis*. Hence, in the present study, the pathogenicity and nature of transmission of this new microsporidian were studied covering four successive generations.

MATERIALS AND METHODS

Isolation and purification of the new microsporidian spores

Samples of diseased silkworms were collected from the Pure Mysore seed area (Kunigal and Magadi) of Karnataka where incidence of microsporidiosis was reported. Tissue homogenates containing spores of the new microsporidian were prepared and filtered using wet layer of absorbent cotton and subsequently centrifuged at 3,000 rpm for 5 minutes. The centrifugation process was repeated 2-3 times by adding distilled water to the sediment. The sediment obtained was suspended in minimal volume of distilled water and brought to the Silkworm Pathology Laboratory of CSRTI, Mysuru and purified by percoll gradient centrifugation (Sato and Watanabe, 1980). The sediment was suspended in

distilled water and thoroughly mixed on cyclomixer thus giving the stock suspension of microsporidian spores. Stock inoculum was suitably diluted and spore concentration was quantified by following standard haemocytometer count method (Cantwell, 1970). The desired concentrations were prepared from the stock suspension.

Morphological characterization of the spores

Purified spores of microsporidian were subjected to morphological characterization following the standard method as described by Fujiwara (1980) and compared with the spores of the known strain, *N. bombycis*. To understand the behaviour of the spores, wet mount spores were also observed under phase contrast microscope. To determine the spore size, the spores were immobilized on microslide coated with mineral oil droplet. A drop of mineral oil was placed on a slide and a cover slip with small drop ($< 5 \mu\text{l}$) of spore suspension of microsporidian was applied on top of the oil. Water having a better affinity for glass, spreads out on the surface of the cover slip, leaving spores individually trapped in "holes" in the oil for measurement. The prepared slides were observed under phase contrast microscope at $600\times$ magnification. Fifty spores of each microsporidia were measured for their length and width following the standard micrometry method (Fujiwara, 1980).

Electron microscopic study was conducted at Electron Microscopy Unit, CSRTI, Mysuru, India. The purified spores of the microsporidian were air dried at room temperature and transferred onto double stick cellophane tape pasted on copper stubs used for mounting specimen for scanning electron microscope. The mounted stubs were coated with about 20 nm gold in sputter coater (EMS-550) and viewed under JEOL-100 CX-II electron microscope fitted with an ASID-4D scanning attachment (Tokyo, Japan 20 kV). The spores were photographed at different magnifications and compared its shape, size and texture with that of *N. bombycis* spores.

Pathogenicity of the microsporidian to silkworm

Inoculum samples of different concentrations (1×10^8 , 1×10^7 , 1×10^6 , 1×10^5 and 1×10^4 spores/ml) were prepared from the quantified stock of microsporidian by

serial dilution. Freshly moulted III instar (after II moult) larvae of Pure Mysore race, on day zero were per orally inoculated with the above doses of spore suspension smeared on to the mulberry leaves fed to them. The larvae were allowed to feed on the treated leaves for six hours. Each inoculum concentration formed a treatment. For each treatment, three replications of 100 larvae were maintained. Different sets of larvae were inoculated separately with different concentrations. Yet another set was maintained without inoculation which served as control batch. After six hours, the larvae were fed with normal leaves and reared till cocooning. Observations on the mortality due to the microsporidian infection were recorded daily. The dead larvae were homogenized and examined for the presence of microsporidian infection under phase contrast microscope. The data on the pupation rate, cocoon weight, shell weight and shell percentage were recorded and statistically analyzed. The results were compared with that of the control batch.

Impact of transovarian transmission of the microsporidian on survivability and cocoon parameters of silkworm

To study the impact of the microsporidian infection through transovarian transmission on silkworm survivability and cocoon characters, the infected mother moths after egg laying were individually homogenized and the homogenates were filtered, centrifuged and the sediments were observed under phase contract microscope and recorded the number of spores/field. Based on the number of spores *viz.*, 2, 20 and >300 spores/field, the eggs laid by the concerned female moths were categorised and brushed separately for next generation. The larvae were reared separately under optimum rearing conditions up to cocoon spinning. During the rearing, mortality due to the microsporidian infection was confirmed by homogenizing the dead larvae in 0.6 % K₂CO₃ and examining under phase contrast microscope. The mortality and the data on cocoon weight, shell weight and shell percentage were recorded. Based on the number of spores *viz.*, 2, 20 and >300 spores/field, in the infected female moths, the eggs laid by them were collected and brushed for 2nd generation. Data were recorded as in the 1st generation. Similarly, the experiment was continued for 3rd and 4th successive generations and

data were collected. The results were compared with that of the control batch.

RESULTS

The observations recorded on the morphological characteristics of the isolated new microsporidian spores and standard strain of *N. bombycis* spores are presented in Table 1. The spores isolated and purified from the infected silkworms of Mysore seed area, Karnataka were ovoid-cylindrical in shape measuring $4.62 \pm 0.34 \mu\text{m}$ in length, $2.05 \pm 0.24 \mu\text{m}$ in width with 1:0.44, length: width ratio. In contrast, the spores of *N. bombycis* were oval in shape measuring $3.80 \pm 0.01 \mu\text{m}$ in length, $2.60 \pm 0.01 \mu\text{m}$ in width with 1:0.68, length: width ratio. The wet mounts of isolated new microsporidian spores exhibited the characteristic Brownian movement and high refractive index similar to that of *N. bombycis* spores. The Scanning Electron Microscopy (SEM) of the isolated new microsporidian and *N. bombycis* spores revealed a smooth texture (Figures 1 and 2).

The impact of the new microsporidian infection on larval mortality and cocoon parameters when inoculated at different concentrations is furnished in Table 2. Results show that among treatments of different inoculation doses, there were significant variations in larval mortality and pupation rate. The mortality during larval stage increased from 2.33 to 70.00 % as the pathogen concentration increased from 1×10^4 to 1×10^8 spores/ml. The cocoon parameters *viz.*, cocoon weight, shell weight and shell percentage also showed significant differences according to the spore concentration of the inoculum.

The effect of transovarian transmission of new microsporidian with varying intensity of infection (1-2 spores, 20 spores and >300 spores/field in mother moths)

Table 1: Morphological characterization of a new microsporidian and *Nosema bombycis* spores

Microsporidian spore	Shape	Length (μm)	Width (μm)	Length : width ratio
New microsporidian	Ovo-cylindrical	4.62 ± 0.34	2.05 ± 0.24	1:0.44
<i>Nosema bombycis</i>	Oval	3.80 ± 0.01	2.60 ± 0.01	1:0.68

Table 2: Effect of different concentrations of new microsporidian on larval survivability and cocoon parameters of Pure Mysore race of silkworm, *Bombyx mori*

Treatment (spores/ml)	No. of larvae inoculated after II moult	No. of diseased larvae during rearing			Pupation (%)	Cocoon weight (g)	Shell weight (g)	Shell %
		Pebrine	Others	Total				
1×10^4	100	02.33	12.00	14.30	85.67	1.12	0.134	11.96
1×10^5	100	06.00	12.33	18.33	81.67	1.12	0.132	11.79
1×10^6	100	10.33	12.00	22.33	77.67	1.12	0.129	11.52
1×10^7	100	23.33	11.33	34.67	66.33	1.11	0.127	11.47
1×10^8	100	70.00	11.33	81.33	18.67	1.07	0.116	10.84
Control (Without any inoculation)	100	0.00	11.00	11.00	89.00	1.13	0.138	12.24
	CD at 5 %	0.949	0.9139	1.171	1.171	0.009	0.001	0.088

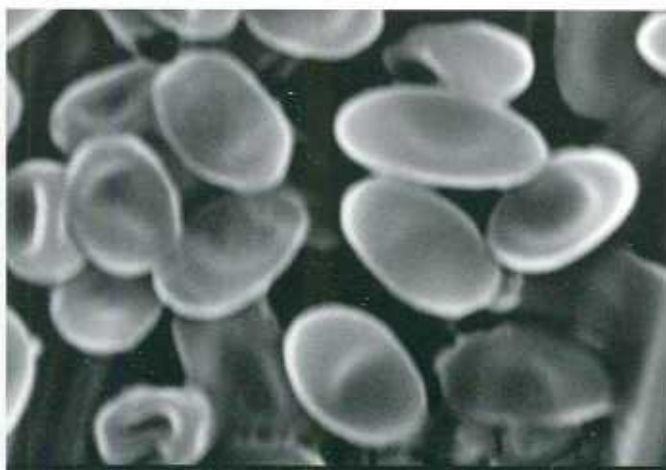


Figure 1: New microsporidian spores

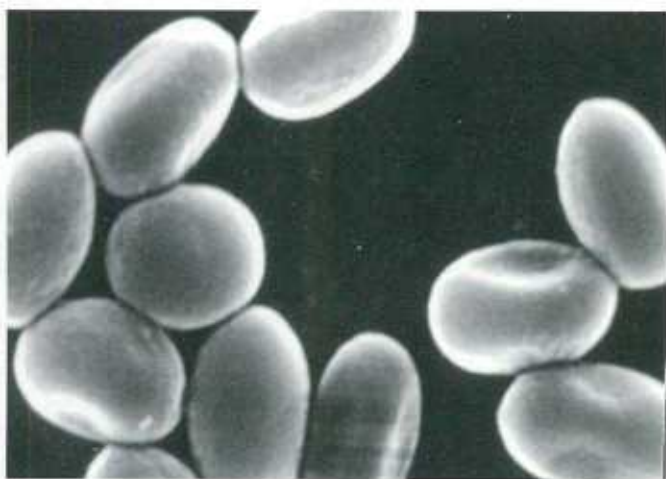


Figure 2: *Nosema bombycis* spores

on larval and cocoon parameters in four successive generations are furnished in Table 3. There was a significant increase in larval mortality due to microsporidiosis from 1st generation to 4th generation in all the intensity of infections. In 1-2 spores/field level of intensity, larval mortality was 3.33 % in 1st generation and 13 % in 4th generation. In 20 spores/field intensity, from 1st to 4th generation, the mortality has shown an increase from 6 to 18 %. In the case of higher intensity (>300 spores/field), mortality rate increased from 66 % in 1st generation to 77.67 % in 4th generation. The mortality due to flacherie was also high in microsporidian infected batches when compared to control. The cocoon parameters viz., cocoon weight, shell weight and shell percentage were also significantly affected in microsporidian infected batches compared to control batch wherein 1.12 g of cocoon weight, 0.136 g of shell weight and 12.14 % of shell were recorded. These cocoon parameters were significantly decreased to 1.08 g, 0.121g and 11.20 % , respectively from 1st to 4th generation in 1-2 spores/field intensity. In the batches having 20 spores/field, the cocoon weight was 1.06 g, shell weight, 0.119 g and shell %, 11.19. In the batches with higher intensity of infection i.e., >300 spores/field, the cocoon parameters were decreased to the minimum of 0.99 g of cocoon weight, 0.105 g of shell weight and 10.63 % of shell in the 4th generation.

Table 3: Effect of transovarian transmission of new microsporidian on larval survivability and cocoon parameters of Pure Mysore race (multivoltine) in four successive generations

Treatment*	Generation	No. of larvae kept	% of larval mortality due to			Pupation (%)	Cocoon weight (g)	Shell weight (g)	Shell %
			Micro-sporidiosis	Others	Total				
1-2 spores/field	1 st	100	03.33	13.33	16.67	83.33	1.12	0.130	11.61
	2 nd	100	07.00	15.33	22.33	77.67	1.11	0.127	11.41
	3 rd	100	11.00	16.00	27.67	72.33	1.10	0.124	11.24
	4 th	100	13.00	16.00	29.00	71.00	1.08	0.121	11.20
20 spores/field	1 st	100	06.00	11.00	17.00	83.00	1.10	0.126	11.45
	2 nd	100	12.00	14.00	26.00	74.00	1.09	0.124	11.38
	3 rd	100	15.00	16.00	31.00	69.99	1.08	0.122	11.29
	4 th	100	18.00	15.33	33.33	66.67	1.06	0.119	11.19
>300 spores/field	1 st	100	66.00	14.67	80.67	19.33	1.05	0.116	11.05
	2 nd	100	70.00	14.33	86.33	13.67	1.03	0.114	10.96
	3 rd	100	74.00	15.00	89.00	11.00	1.02	0.111	10.85
	4 th	100	77.67	16.33	94.00	6.00	0.99	0.105	10.63
Control (Without any inoculation)	1 st	100	0.00	09.67	9.67	90.33	1.13	0.138	12.21
	2 nd	100	0.00	10.33	10.33	89.67	1.13	0.137	12.10
	3 rd	100	0.00	11.00	11.00	89.00	1.13	0.136	12.00
	4 th	100	0.00	11.00	11.00	90.00	1.12	0.136	12.14
CD at 5%			2.28	2.13	2.88	2.88	0.02	0.001	0.15

* Intensity of infection in the mother moth

DISCUSSION

Microsporidiosis is a chronic disease of silkworm caused by a virulent microsporidian, *N. bombycis* Naegeli. Apart from *N. bombycis*, several other strains and species belonging to microsporidia have been isolated from silkworm (Fujiwara, 1984 a, b; Ananthalakshmi *et al.*, 1994). Some of these have been reported to be less virulent to silkworm and cause mild infection that often does not kill them and yet others have been reported to be highly virulent to silkworm.

In the present study, the spores of the isolated new microsporidian from Pure Mysore silkworm crops of Mysore seed area are different from that of *N. bombycis* in

shape, the former possessing ovo-cylindrical whereas, the latter with oval spores. It is more elongated than the *N. bombycis* spores and the length - width ratio is also different. It is clear from the light microscopic and electron microscopic studies that the microsporidian is conspicuously different from *N. bombycis* and can easily be distinguished from the latter just by a glance of the spore itself. The pathogenicity studies and its effect on larval and cocoon parameters suggest that the new microsporidian is virulent and pathogenic to the silkworm, *B. mori*. When silkworms were inoculated with a lower dose of 1×10^4 spores/ml after II moult (III instar), only 2.33 % mortality was observed during larval stage with 85.67 % pupation. As the dose increased, the larval mortality also increased from 2.33 to 70 % (1×10^8

spores/ml) simultaneously bringing down the cocoon parameters to a significantly inferior level. The new microsporidian is virulent and caused chronic disease at low concentration, whereas, it was highly virulent and produced acute disease at higher concentrations. Jameson (1922) and Ghosh (1944) have reported that pebrine infected silkworms spin poor quality silk cocoons. The infection is transmitted to the progeny by means of transovarial transmission. In the progenies infected transovarially, with the isolated microsporidian having 1-2 spores/ ml, 3.33 % mortality was recorded in 1st generation which gradually increased to 13 % in 4th generation. As the spore intensity increased from 1 to >300 spores/field, the larval mortality also increased significantly. The mortality rate increased from 1st to 4th generation which is a clear indication that this microsporidian will become highly virulent and the spore load will increase from generation to generation.

The present study assumes significance as authors have come across incidence of a new microsporidian infection which is different from *N. bombycis*, in all stages of silkworm in Pure Mysore seed area of Karnataka, India. This microsporidian is virulent, pathogenic and adversely affect the silkworm crop at higher intensity. The infection transmits to the progeny by means of transovarial transmission. The present study also indicate that the constant elimination of the new microsporidian through mother moth examination at each generation and using disease free layings at Basic Seed Farms will effectively prevent any economic loss at subsequent levels of seed rearings as the new microsporidian is virulent and cause adverse impact on the economic parameters of cocoons when the spore load increases.

ACKNOWLEDGEMENT

The authors are thankful to the staff of Pure Mysore seed area, Department of Sericulture, Karnataka for their help extended during the survey period and collection of samples.

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Research Paper

PRODUCTION OF BULKY RAW SILK YARN USING COCOON ENTANGLEMENT METHOD DURING THE PROCESS OF REELING

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ABSTRACT

During the process of reeling, cocoon filaments are combined to form raw silk yarn in the reeling machine. The cocoons are set to move slowly during the unwinding of silk from them. If the speed of movement of cocoons is increased by incorporation of a suitable device, it is possible that entanglement of filaments take place resulting in a bulky silk yarn. Central Silk Technological Research Institute (CSTRI), Bengaluru, India has developed a mechanism to rotate the inner vessel of reeling machine keeping the outer vessel constant, taking care that water won't be spilled during the rotation of the vessel in the reeling process. Due to this, the cocoon filaments entangle and also get false twisted thus making the end product, a very strong raw silk yarn. When the reeling was done with different number of cocoons per end, it revealed that as more number of cocoons are reeled per end, the average denier and size deviation are significantly influenced at 1 % level. The bulky yarn produced using cocoon entanglement reeling machine has exclusive end uses.

Key words: Bulky yarn, cocoon entanglement, raw silk quality, reeling basin.

INTRODUCTION

Silk is a highly valued textile fibre of animal origin, used almost entirely for the production of high quality textiles. Silk reeling industry in India is a small scale industry with limited capacity to cater to the needs of the customers for a wide range of products (Sonwalkar, 1993). Presently, variations in the fabrics are brought about through creative designs, varying fabric geometry and other weaving parameters. From the reeling sector, it was thought to develop a bulky yarn in the process of reeling itself, with small attachments fitted to the reeling basin so that cocoon filaments during the process of reeling will be entangled and bulky effects could be introduced in the yarn structure during the process of reeling.

Development of new varieties of raw silk always has demand in the silk sector for production of different varieties of fabrics. During the process of reeling, cocoons

are combined to form raw silk yarn in the reeling machine wherein they move slowly while the silk filaments keep unwinding (Lakshmipathaiah *et al.*, 2000). The possibility of increasing the speed of movement of cocoons by incorporating a suitable device was thought of, so that entanglement in filaments will take place resulting in the production of a differently structured bulky silk yarn (Masaaki Okajima *et al.*, 2011).

The bulky silk yarn will have more porosity so that varying appearance of fabrics can be produced. The production of bulky yarn will enable the reelers get better earnings, as the fabrics produced out of these yarns will have different appearance and niche market. These yarns are used in the making of suitings, heavy weight silk fabrics and sarees with better drape. In order to cater to the needs of present generation of customers who wants a variety of products at moderate price with different appearance, these developments would be of much help. In India, apart from raw silk, only dupion silk is produced,

and hence, there is scope that modified structured bulky silk yarn would facilitate the weavers to go for different variety of fabrics.

In the present study, a suitable gadget to entangle the cocoons during the process of reeling was developed and the process parameters and quality of raw silk produced were studied in comparison with the yarn produced with same number of cocoons in multiend reeling using the standardized speeds of inner vessel and reel. In order to test the novelty of bulky yarns produced by cocoon entanglement method as against regular multiend reeled silk, the reeling performance and quality of raw silk were assessed by varying the number of cocoons reeled per end.

MATERIALS AND METHODS

Development of gadget to create cocoon entanglement

Usually, the required number of cocoons are combined during the process of reeling, vertical to the axis and wound on to the reels of the reeling machine. During the process of reeling, the cocoon filaments unwind smoothly based on the process parameters adopted in the reeling process. However, it was thought to increase the speed of movement of the cocoons during reeling by rotating the vessel so that cocoon filaments are entangled and the resultant bulky yarn is produced. The number of cocoons maintained in the process of reeling decides the denier of

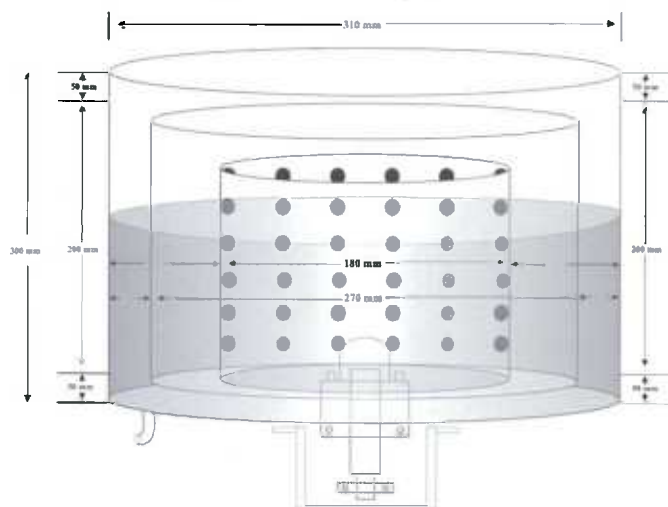


Figure 1: The line diagram of the vessels used for making the cocoon entanglement during reeling process

raw silk reeled. The bulky yarn thus produced is wound on to the reels, which are later re-reeled to convert into standard sized skeins for further processing. The line diagram of the vessels used for making the cocoon entanglement during reeling process is shown in Figure 1. The vessels and the reels are rotated by separate motors. The speed of the vessel and reels can be controlled separately depending on the requirement. Two different diameter vessels have been developed to reel medium and coarse raw silk. Figures 2 and 3 depict photographs of the concept machine developed for the production of bulky yarn through cocoon entanglement.



Figure 2: The concept machine developed at CSTRI for the production of bulky yarn by cocoon entanglement method



Figure 3: Reeling trials for the production of bulky yarn by cocoon entanglement method

Raw material: The commercial cocoons of bivoltine hybrid (CSR2 x CSR4) reared in Karnataka, during November 2015 were used in three replications.

Drying conditions: The cocoons were dried in Batch type hot air drier following the temperature pattern of 110-100-85-70-55°C for a period of 5 hours. The degree of drying achieved was 39–41 % for the cocoons.

Cooking conditions: The hot air dried cocoons were cooked using two-pan following the temperature profiles and durations viz., retting at 70°C for 30 seconds, high temperature permeation at 96°C for 120 seconds, low temperature permeation at 70°C for 60 seconds, cooking at 98°C for 120 seconds, stop steam for 20 seconds and adjustment from 98 ~ 70°C for 120 seconds by pouring cold water. The cooked cocoons were brushed manually at 80°C using paddy husk brush.

Reeling conditions: Two hundred good cocoons picked from the brushed cocoons were selected for reeling on multiend reeling machine, maintaining fixed number of cocoons to a reeling end i.e., 20, 30 and 40 cocoons per end. Another sample of 200 good cocoons were reeled on newly developed cocoon entanglement bulky silk reeling machine by maintaining fixed number of cocoons to a reeling end i.e., 20, 30 and 40 cocoons per end separately. The temperature of reeling bath was maintained at 45°C and reeling speed at 120 m/min on both the machines. The reeling characteristics viz., renditta, raw silk recovery and waste percentage on silk weight were recorded.

Silk quality testing: The raw silk after reeling was assessed for selected quality characteristics. Among the eleven characters used for grading of raw silk, four characters, viz., average denier, size deviation, tenacity and elongation were determined. The testing equipment, viz., winding machine, wrap reel and serigraph tester were used for the study.

Data: The reeling characteristics viz., renditta, raw silk percentage, raw silk recovery percentage and waste % on silk weight were recorded while reeling the cocoons using different number of cocoons per end. The data on quality parameters viz., average denier, size deviation, tenacity and elongation were noted after testing raw silk reeled

with different number of cocoons per end and different reeling devices (Bureau of Indian standards IS 15090 Part 1 to 11, 2002). The data thus obtained were analyzed statistically using SPSS package.

RESULTS AND DISCUSSION

The analysis of variance results and mean results of reeling and quality characteristics of bivoltine cocoons reeled with different number of cocoons per end viz., 20, 30 and 40 cocoons, on multiend reeling machine and cocoon entanglement reeling machine are presented in Tables 1 and 2.

Effect of number of cocoons reeled on multiend and cocoon entanglement reeling machine on reeling and quality characteristics

Renditta

Table 1 shows that renditta is not significantly influenced by variations in the number of cocoons reeled and reeling machines. However, the renditta seems to slightly increase when heavy denier raw silk was reeled on both the machines (Table 2), which may be attributed to droppings of cocoons at higher speed and in turn, re-brushing of dropped cocoons increased the renditta.

Raw silk recovery percentage

Table 1 shows that raw silk recovery is also not significantly influenced by the number of cocoons reeled and different reeling machines. However, the raw silk recovery has shown a slightly decreasing trend as the number of cocoons reeled increased (Table 2) on both the machines.

Waste per cent on silk weight

Table 1 shows that waste (%) on silk weight is not significantly influenced by the number of cocoons reeled and different reeling machines.

Average size of raw silk

Average denier of raw silk reeled using 20 and 30

Table 1: Analysis of variance results of influence of number of cocoons reeled on multiend and cocoon entanglement reeling machine on reeling and quality characteristics

Characteristic / No. of cocoons	T-value	Degree of freedom	Significant (2-tailed)	Mean Difference
Renditta				
20	-0.632	8	0.545	-0.0400
30	-0.452	8	0.663	-0.0600
40	-0.730	8	0.486	-0.0400
Raw silk recovery				
20	0.619	8	0.553	0.4272
30	0.449	8	0.665	0.6446
40	0.752	8	0.473	0.42046
Waste (%) on silk weight				
20	-0.920	8	0.385	-0.4000
30	-1.076	8	0.313	-0.4800
40	-2.491	8	0.037	-1.000
Average size (Denier)				
20	0.779	8	0.459	2.824
30	1.129	8	0.292	5.516
40	3.709	8	0.006**	22.634
Size deviation				
20	-13.24	8	0.000**	-2.011
30	-5.667	8	0.000**	-7.543
40	-3.578	8	0.007**	-1.012
Tenacity				
20	1.892	8	0.095	0.222
30	1.011	8	0.342	0.160
40	1.764	8	0.116	0.1257
Elongation				
20	0.574	8	0.582	0.800
30	1.074	8	0.314	1.400
40	0.453	8	0.663	0.400

** - Significant at 1 % level

Table 2: Reeling and quality characteristics of number of cocoons reeled on multiend and cocoon entanglement reeling machine

Particulars	Group	Mean	Std. Deviation
Renditta			
20	MRM	6.52	0.084
	CEM	6.56	0.114
30	MRM	6.54	0.207
	CEM	6.60	0.212
40	MRM	6.74	0.114
	CEM	6.78	0.045
Raw silk recovery (%)			
20	MRM	71.35	0.919
	CEM	70.92	1.238
30	MRM	71.18	2.248
	CEM	70.53	2.292
40	MRM	69.02	1.163
	CEM	68.60	0.457
Waste (%) on silk weight			
20	MRM	16.46	0.723
	CEM	16.86	0.650
30	MRM	16.38	0.876
	CEM	16.86	0.477
40	MRM	17.46	0.532
	CEM	18.46	0.723
Average size (Denier)			
20	MRM	58.31	4.592
	CEM	55.49	6.683
30	MRM	91.72	7.343
	CEM	86.20	8.093
40	MRM	133.72	8.656
	CEM	111.08	10.551
Size deviation			
20	MRM	4.66	0.110
	CEM	6.67	0.321
30	MRM	7.31	0.255
	CEM	8.07	0.154
40	MRM	8.66	0.078
	CEM	9.67	0.628
Tenacity (g/d)			
20	MRM	3.66	0.246
	CEM	3.44	0.090
30	MRM	3.62	0.115
	CEM	3.46	0.335
40	MRM	2.56	0.155
	CEM	2.44	0.035
Elongation (%)			
20	MRM	19.80	1.304
	CEM	19.00	2.828
30	MRM	18.60	1.517
	CEM	17.20	2.490
40	MRM	17.20	1.924
	CEM	16.80	0.447

Data are mean of 5 observations; MRM - Multiend reeling machine; CEM - Cocoon entanglement machine



Figure 4: a) Bulky raw silk yarn reeled by cocoon entanglement method b) Multiend machine-reeled raw silk yarn, using equal number of cocoons.

cocoons per end did not show significant difference in the different number of cocoons reeled (Table 1). However, average denier of raw silk reeled using 40 cocoons per end showed significant difference at 1% level, because while reeling more number of cocoons, maintaining uniform denier is quite difficult. Further, in cocoon entanglement reeling machine, since the cocoons are rotating, it is quite difficult to observe the number of cocoons being reeled and only by feeling the thickness of the thread the cocoons have to be fed in the present experiment (Table 2).

Size deviation of raw silk

Table 1 shows that size deviation of raw silk is significantly influenced by the number of cocoons reeled and different reeling machines. From Table 2, it could be inferred that as the number of cocoons increased in reeling, the size deviation also increased on both the machines, because maintenance of cocoons with higher number per end is difficult for the reeler.

Tenacity and elongation of raw silk

Tenacity and elongation of raw silk did not show significant differences while reeling different number of cocoons on both the reeling machines (Table 1). The tenacity of bivoltine raw silk reeled on cocoon entanglement machine showed a decreasing trend as the number of cocoons increased in the present study (Table 2).

Conclusion

Thus the study reveals that using the newly developed cocoon entanglement reeling machine, different denier raw silk can be produced by rotating the cocoons during reeling. The studies conducted on reeling different number of cocoons per end reveal that as more number of cocoons are reeled per end, the average denier and size deviation are significantly influenced at 1% level. The results imply the need for fine tuning of the machine to produce uniform quality cocoon entangled raw silk (Figure 4).

ACKNOWLEDGEMENT

Thanks are due to the staff of Mulberry Silk Reeling Division, Central Silk Technological Research Institute, Central Silk Board, Bengaluru, India for their cooperation while conducting the study.

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IDENTIFICATION OF TARGET TISSUE(S) OF BmIFV INFECTION IN THE SUSCEPTIBLE / TOLERANT BREEDS OF SILKWORM, *BOMBYX MORI* L. THROUGH IMMUNODIFFUSION TEST

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ABSTRACT

Among the silkworm diseases, infectious flacherie caused by the virus BmIFV (*Bombyx mori* infectious flacherie virus) is highly prevalent. In India, this disease was first reported by Sato (1992) and later, many have initiated the work on BmIFV. However, there are only a few reports on the susceptibility status of different silkworm breeds / hybrids to BmIFV and only a handful of breeds susceptible to this pathogen have been identified. BmIFV infected larvae exhibit atypical morphological symptoms but not many studies have been conducted so far on the symptomatology of this disease and target tissues during the progressive infection in silkworm. Hence, in the present study, attempts were made to identify BmIFV infected tissue(s) during its progressive infection in the identified susceptible (CSR2) and tolerant (5N) breeds of silkworm through serological test. Immediately after III moult, newly ecdysed 4th instar larvae were per orally inoculated with an infective dose of BmIFV and larvae were reared up to 12 days of post inoculation (DPI). The control batches were also maintained without any inoculation. From both treated and control batches of CSR2 and 5N breeds, different tissues viz., haemolymph, midgut, fat body, silk gland, gonads and malpighian tubules were collected daily (0 to 12 DPI) and conducted Agarose gel immunodiffusion test using BmIFV antibody. The results indicated that BmIFV infected only midgut tissue but none other where the precipitin line was formed between central antibody well and antigen well having midgut homogenate. Both the breeds were infected with BmIFV, however, mortality due to BmIFV was more in CSR2 (susceptible) compared to 5N (tolerant) breed. However, in the case of CSR2, the first signs of infection were recorded on 4 DPI and the infection progressed up to 12 DPI. In 5N breed also, BmIFV infected only midgut tissue but not haemolymph, fat body, silk gland, gonad and malpighian tubules. But in this breed, the primary symptoms of infection were recorded on 6 DPI and infection continued up to 12 DPI. Manifestations of the course of infection are discussed.

Key words: *Bombyx mori* L., BmIFV, infected tissue, susceptible/tolerant breed.

INTRODUCTION

The main challenge before the sericulture industry is always the crop loss due to occurrence of silkworm diseases. As mulberry silkworm, *Bombyx mori* L. has been domesticated for over 4,000 years, the continuous rearing of silkworm has resulted in loss of certain wild characters which has incidentally made it susceptible to infection by an array of pathogens leading to diseases and crop losses. Among viral diseases of silkworm, infectious

flacherie disease is caused by a picornavirus, *Bombyx mori* infectious flacherie virus (BmIFV). BmIFV was first reported in Japan by Yamazaki *et al.* (1960) and later, several immunoassays viz., precipitin test (Sekijima, 1971), immunodiffusion test (Seki and Sekijima, 1976; Shimizu, 1982), neutralization test (Kurusu *et al.*, 1975), hemagglutination test (Suto and Kawase, 1971), latex agglutination test (Shimizu *et al.*, 1983), fluorescent antibody test (Inoue and Ayuzawa, 1972) and enzyme-linked immunosorbent assay (ELISA) (Shimizu, 1982)

were used to identify/detect this pathogen in silkworm. In India, Sato (1992) and Sivaprasad *et al.* (1993) reported the prevalence of non-occluded viral diseases viz., infectious flacherie and denisonucleosis in different sericultural areas of southern districts of Karnataka. Later, the prevalence of infectious flacherie disease was also reported in northern districts of Karnataka (Sanakal *et al.*, 1996). Nataraju and Datta (1999) developed a textile dye based-dipstick immunodiagnostic kit for the detection of infectious flacherie disease during silkworm rearing. Subsequently, different silkworm breeds were screened for their comparative susceptibility to BmIFV (Hemanth Kumar, 2002; Mamatha, 2002; Mary Flora *et al.*, 2002). BmIFV infected larvae exhibit atypical morphological symptoms but not many studies have been conducted so far on the symptomatology of this during the progressive infection in silkworm. In this direction, Mamatha and Balavenkatasubbaiah (2008 a) identified productive breeds of silkworm susceptible and tolerant to BmIFV and studied the comparative progressive morphological changes in the identified susceptible/tolerant breeds infected with BmIFV (Mamatha and Balavenkatasubbaiah, 2008 b). However, there is scanty information on identification BmIFV target tissue(s) in the silkworm during the progressive infection and comparative account on variation in the susceptible and tolerant breeds. Hence, the present study explored to identify BmIFV infected tissue(s) during the progressive infection in the susceptible/ tolerant breeds of silkworm through serological diagnosis.

MATERIALS AND METHODS

Identification of breeds of silkworm susceptible and tolerant to BmIFV

Earlier, Mamatha and Balavenkatasubbaiah (2008 a) screened fifteen productive bivoltine pure breeds of silkworm viz., CSR2, CSR4, CSR6, CSR16, CSR17, CSR26, S1, S5, 5HT, 8HT, D13, D15, 5N and 61N and JPN8 available from germplasm stock of Central Sericultural Research and Training Institute (CSRTI), Mysore against BmIFV and identified CSR2 and 5N as the most susceptible and tolerant breeds, respectively to BmIFV. These two breeds formed the experimental material for the present study.

Identification of BmIFV infected tissues in the susceptible/tolerant breeds

CSR2 and 5N breeds were brushed *en masse* and reared following the standard rearing method up to III instar. Immediately after III moult, one hundred, newly ecdysed 4th instar larvae were per orally inoculated with an infective dose of BmIFV at $10^{-7.255}$ dilution of stock inoculum, by smearing on mulberry leaf @ 1 ml/100 sq.cm leaf/100 larvae. The control batches were maintained without any inoculation. The larvae were reared up to 12 DPI and collected the samples for further investigation.

From both treated and control batches of CSR2 and 5N breeds, different tissues viz., haemolymph, midgut, fat body, silk gland, gonads and malpighian tubules were collected daily from 0 to 12 DPI, by the following methods.

Larval prolegs were cut and haemolymph was collected in Eppendorf tube containing a speck of phenyl thiourea and stored at 5°C for further use. The silkworm larvae were then dissected and midgut, fat body, silk gland, gonad and malpighian tubule were collected and homogenized separately in sterilized distilled water using mortar and pestle to obtain 1 % tissue homogenate (1 g tissue in 100 ml of water). These homogenates were stored at 5°C for further use.

Agarose gel immunodiffusion test

The test was performed by following the procedure described by Ouchterlony (1964) and Nataraju (1995). 1.0% agarose solution was prepared by melting agarose in PBS (0.01M potassium phosphate buffer of pH 7.0 containing 1% sodium azide and 0.85 % NaCl). The temperature of molten agarose was brought down to 55°C and poured onto a 24 × 74 mm glass micro slide (pre-coated with a thin film of 0.2 % agarose in distilled water) to form a layer of agarose gel of 3 mm thickness. The gel was allowed to solidify and then kept in a humid chamber at room temperature for 1 to 2 h. Seven wells of 3 mm diameter were made in the gel, one in the centre and the remaining six wells at hexagonal angles using a gel puncher.

The test was performed by filling the central well with 20 μ l of antibody of BmIFV (received from the Silkworm Pathology section, CSRTI, Mysore) and the surrounding wells with 20 μ l of different samples *viz.*, haemolymph, 1% homogenates each of midgut, fat body, silk gland, gonad and malpighian tubule. The gel plate was then incubated at $26 \pm 1^\circ\text{C}$ for 48 h in a humid chamber and observed for precipitation line between central antibody well and the surrounding antigen wells (Figure 1). The results were recorded as positive (+) for BmIFV infection and negative (-) for no infection (Figure 2). This was done for samples from 0 to 12 DPI for both the breeds.

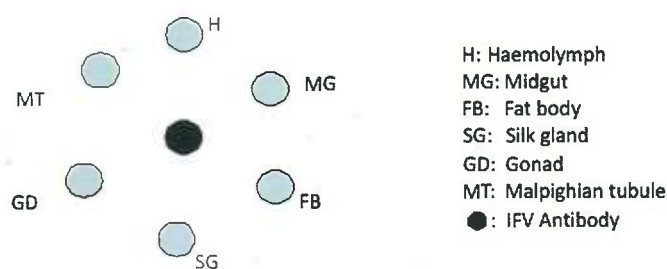


Figure 1: Agarose gel diffusion test - Placement of different tissue homogenates in the wells



Figure 2: Agarose gel diffusion test - Formation of precipitin line indicates BmIFV positive result

RESULTS

Results of the Agarose gel diffusion test indicated that there was no formation of precipitin line between central antibody well and the surrounding antigen well in control batches of both the breeds (Figure 3). In the case of CSR2 treated batch, the precipitin line was formed between

central antibody well and 2nd surrounding antigen well (having midgut homogenate) thereby revealing that BmIFV infected only midgut tissue but not haemolymph, fat body, silk gland, gonads and malpighian tubules. The signs of infection were first recorded on 4 DPI, it being noticed as progressing till 12 DPI, only in the midgut tissue (Table 1, Figures 3 and 4).

Similarly, in 5N breed also, BmIFV infected only the midgut tissue where precipitin line was formed between central antibody well and 2nd surrounding antigen well (having midgut homogenate). But in this breed, the infection was first recorded on 6 DPI which continued up to 12 DPI, only in the midgut tissue (Table 1, Figures 3 and 4).

DISCUSSION

Results of the Agarose gel diffusion test indicated that BmIFV infected only the midgut tissue in both the breeds. However, CSR2 breed exhibited symptoms two days earlier *i.e.*, on 4 DPI than that by 5N which displayed the symptoms of infection since 6 DPI onwards. Similar observations were also recorded by Inoue and Ayuzawa (1972) who probed the distribution of an infectious flacherie virus in the silkworm, *B. mori* with the fluorescent antibody technique. Specific fluorescence was observed in the cytoplasm of the midgut cells, but not in the smears of silk gland, malpighian tubules, trachea, brain ganglion, ovary, testis, blood cells, integument and fat body. In the silkworm, epithelium of larval midgut is the sole target of cytoplasmic polyhedrosis virus, densovirus and infectious flacherie virus. After infection by these viruses, epithelial cells of the midgut show various cytopathic changes characteristic to respective viruses, and general outcome of the infected cells is either to be discharged into midgut lumen (Inoue, 1974) or to be sequestered into neighboring cells (Matsui, 1973). These processes of infection and resulting cytopathic events are altered depending upon the different physiological status of the midgut epithelium (Choi *et al.*, 1989).

The results indicated that during the course of BmIFV infection, the virus infected and multiplied in both the susceptible (CSR2) and tolerant breeds. However, in

Table 1: Detection of BmIFV infection in different tissues during its progressive infection in CSR2 and 5N breeds of silkworm

Tissue	Detection of BmIFV infection on different DPI												
	0	1	2	3	4	5	6	7	8	9	10	11	12
Haemolymph													
CSR2	+	+	+	+	-	-	+	-	-	-	-	+	+
5N	+	+	+	+	+	+	+	+	+	+	+	+	+
Midgut													
CSR2	+	+	+	+	+	+	+	+	+	+	+	+	+
5N	+	+	+	+	+	+	+	+	+	+	+	+	+
Fat body													
CSR2	+	+	+	+	+	+	+	+	+	+	+	+	+
5N	+	+	+	+	+	+	+	-	-	+	-	-	-
Silk gland													
CSR2	+	+	+	+	+	+	+	+	+	+	+	+	+
5N	+	+	-	-	-	+	+	+	+	-	-	-	-
Gonad													
CSR2	+	+	+	+	+	+	+	+	+	+	+	+	-
5N	-	-	-	-	-	+	+	+	+	-	-	-	-
Malpighian tubule													
CSR2	+	+	+	+	+	+	+	-	-	-	-	-	-
5N	-	+	-	-	-	-	+	+	+	+	-	-	+

+ = Infection of BmIFV; - = No infection of BmIFV

CSR2, the number of infected goblet cells increased continuously while in the resistant breed, the infected goblet cells were less in number as the infection progressed. This may be due to the discharging of infected cells into gut lumen and the regeneration of new goblet cells from the *nidi* cells, allowing physiological repair. The mechanism of regeneration of midgut epithelial cells differed in different regions of the midgut. The regeneration of the goblet cells in place of infected cells helps in prolonging the life of infected larvae. The tolerance level depends on the regenerative capacity of midgut. When the micro-organism invades the midgut, there is an increased mitotic activity in the regenerative *nidi* layer and the infected cells are normally extruded into

the gut lumen. The midgut cells formed subsequently may be free from infection. Thus the susceptibility of silkworm to BmIFV is related to the ability of regeneration of midgut cells by the host (Nataraju *et al.*, 2005). This is the basis for the differences observed in the larval mortality between susceptible and tolerant breeds of silkworm, due to this pathogen.

ACKNOWLEDGEMENT

First author wishes to express her thanks to the University Grants Commission, New Delhi for providing Rajiv Gandhi National Fellowship to carry out her

DPI

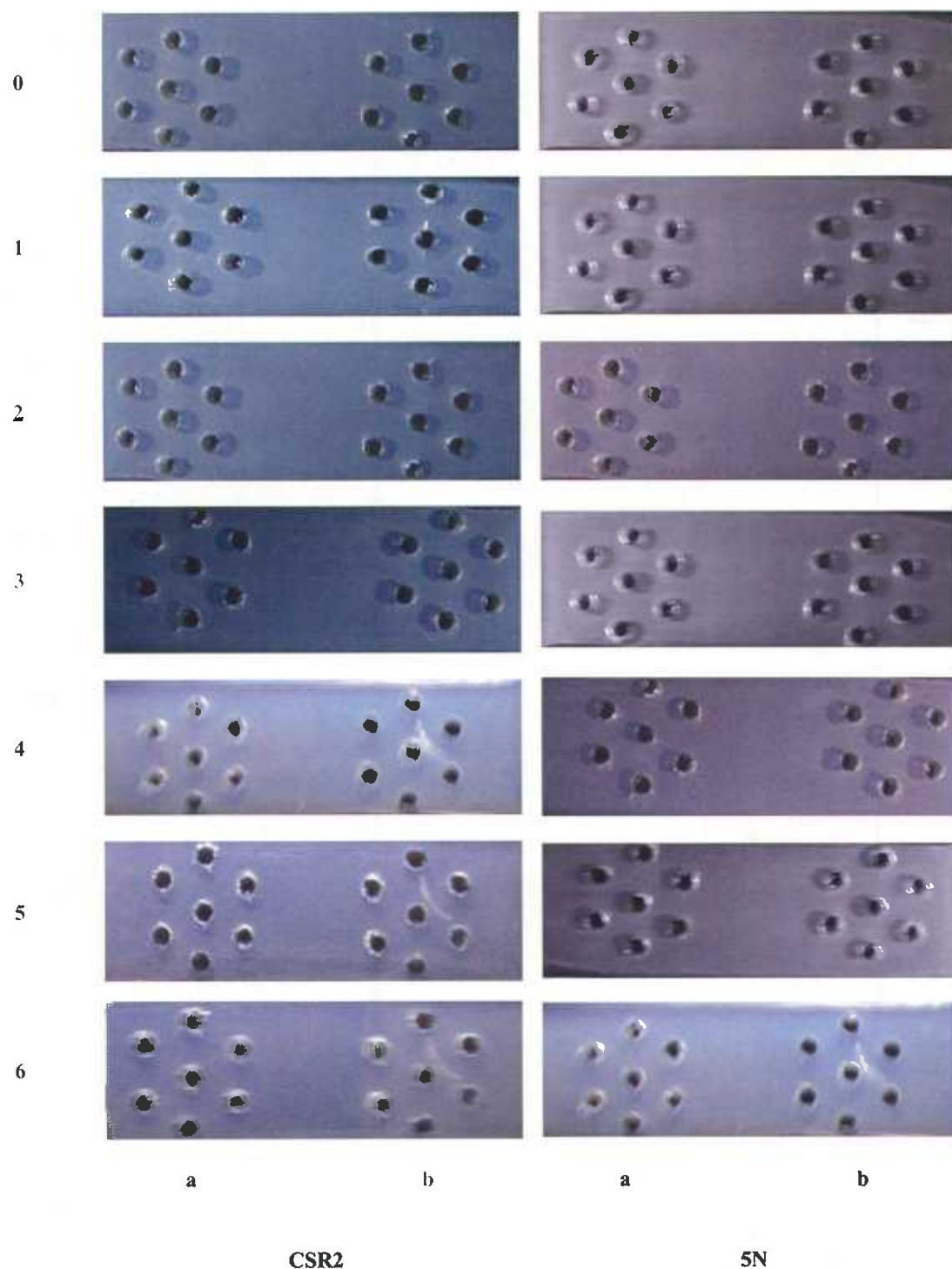
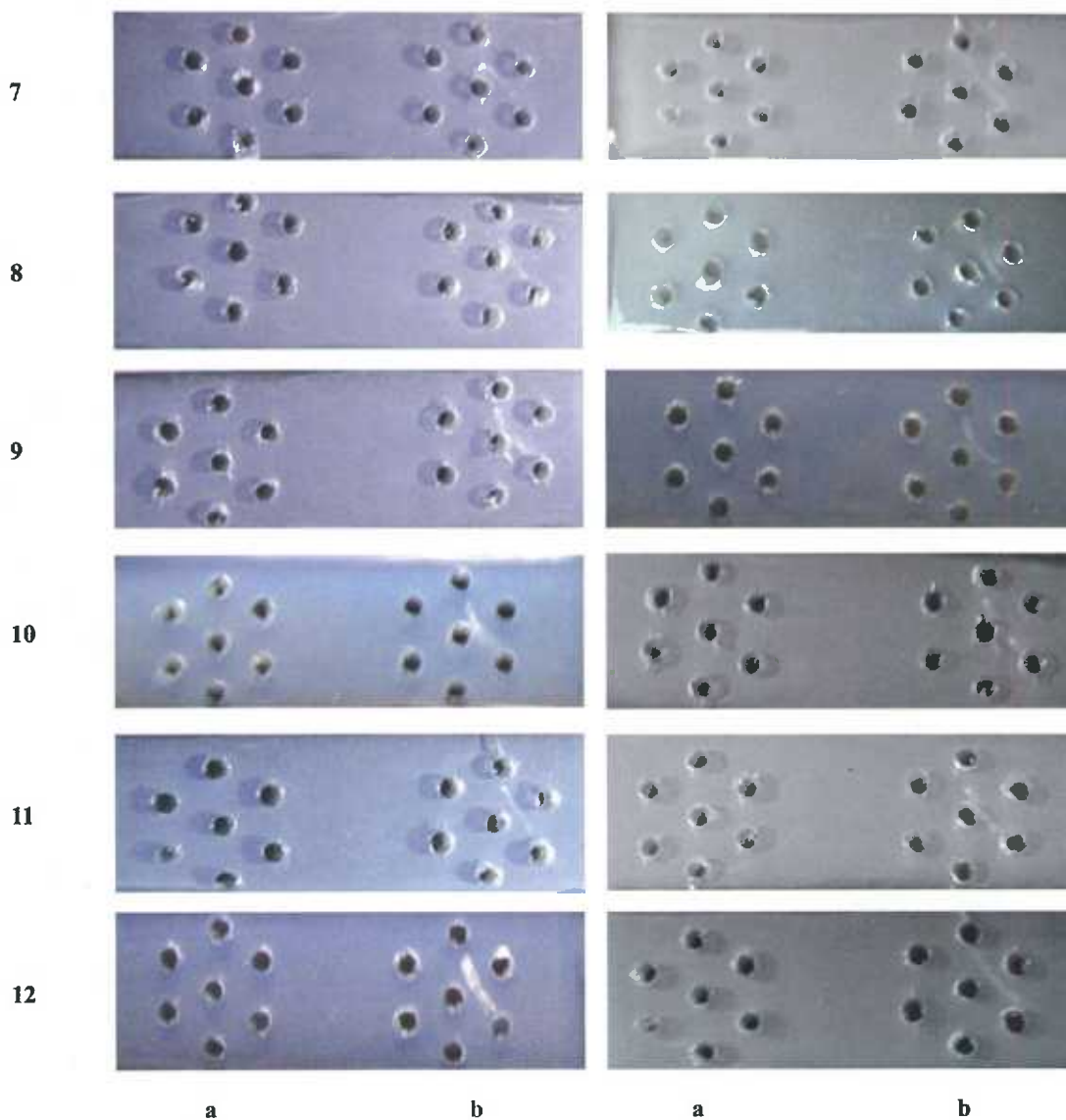


Figure 3: Agarose gel immunodiffusion test for the detection of BmIFV in different tissues of CSR2 and 5N breeds from 0 to 6 DPI: a – Control; b – Treated; DPI- Days post inoculation

DPI



CSR2

5N

Figure 4: Agarose gel immunodiffusion test for the detection of BmIFV in different tissues of CSR2 and 5N breeds from 7 to 12 DPI: a – Control; b – Treated; DPI- Days post inoculation

research work. She is also thankful to the University of Mysore, Mysore for permitting her to carry out this work and CSRTI, Mysore for providing laboratory facilities and encouragement.

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HOLISTIC APPROACH FOR EFFECTIVE MANAGEMENT OF SILKWORM UZI FLY, *EXORISTA BOMBYCIS* (LOUIS) (DIPTERA: TACHINIDAE)

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ABSTRACT

In an effort to increase the efficiency of management of the silkworm uzi fly, *Exorista bombycis* (Louis) (Diptera: Tachinidae), the existing non-chemical IPM (integrated pest management) package comprising of components, such as rearing of silkworms under nylon net enclosure, use of yellow liquid trap and release of an ecto-pupal parasitoid viz., *Nesolynx thymus* (Girault) (Hymenoptera: Eulophidae) was implemented along with a newly added strategy of cultural control i.e., keeping silkworm litter and other rearing residue packed in plastic bags for two weeks before disposal. This holistic management practice was carried out for a period of one year by involving all the sericulturists of the experimental village, covering 783 crops of 94,965 disease free layings (DFLs), which could drastically reduce the uzi fly infestation from 10.7 to 1.14 % with sustained effect throughout the year.

Key words: Disease free layings, *Exorista bombycis*, IPM, silkworm, uzi fly.

INTRODUCTION

The Indian uzi fly, *Exorista bombycis* (Louis) (Diptera: Tachinidae) is a primary endoparasitoid of mulberry silkworm, *Bombyx mori* L. It causes considerable damage to silkworm cocoon crop in the traditional silk producing states of south India, to the tune of 10 to 15 % of cocoon yield loss i.e., about 104 kg cocoons/ha/year (Siddappaji, 1985; Prakash *et al.*, 2013). Various control measures, such as rearing of silkworms under nylon net enclosure, use of yellow liquid trap, release of an ecto-pupal parasitoid viz., *Nesolynx thymus* (Girault) (Hymenoptera: Eulophidae), application of ovicidal formulation/uzicide *etc.* are advocated under the IPM (integrated pest management) package of uzi fly (Narayanaswamy and Devaiah, 1998; Dandin and Giridhar, 2010).

During a routine program of survey to assess the uzi incidence, it was noticed that once the crop is done with, several hundreds of uzi pupae are left out in the litter/rearing residue in each rearing house. It is a common practice that the sericulturists throw and dump the same in

front of their rearing houses which leads to a rise in the population of uzi flies in their village. So, in order to curb this and in turn the incidence itself, a study was undertaken by implementing the already existing IPM package along with one more additional control strategy of packing and keeping the litter in plastic bags for 2 weeks followed by its disposal.

MATERIALS AND METHODS

A village, namely Chikkaharohalli in Srirangapatna Taluk of Mandya district, Karnataka comprising of 167 sericulturists was selected for the study. The work was carried out in co-ordination with the Department of Sericulture, Govt. of Karnataka, Srirangapatna. At the outset, a bench mark survey was conducted to know the initial level of uzi fly incidence.

Package implemented

- The study was conducted at farmers' condition wherein the windows and main entrance of the rearing houses were already affixed with nylon net.

- Placement of uzi traps on each window base both outside and inside the rearing house throughout the rearing (Figure 1). Traps were prepared by dissolving one uzi tablet in one litre water and the resulting yellow solution which attracts both the sexes of uzi fly was placed in a white plastic tray (size 22 x 17 cm).
- Release of *N. thymus*, an ecto-pupal parasitoid during fifth instar @ 2 pouches/100 DFLs (Figure 2). From one pouch containing 50 ml of parasitized pupae of housefly (about 2,000 pupae by number), approximately 10,000 to 12,000 parasitoids emerge (@ about 10 parasitoids per pupae on an average).
- After the completion of silkworm rearing, while removing the rearing residue, the shoot portion was separated and only the silkworm litter and left out leaf material were collected and packed tightly in plastic bags (Figure 3). These bags were kept outside the rearing house for 2 weeks so as to kill the uzi fly pupae/adults present inside. This was practiced consistently for one year.

The above package was implemented with all the 167 farmers in the village covering 94,965 DFLs with a release of 1,932 pouches of *N. thymus* and the impact was monitored through regular visits.

Parameters recorded

- No. of uzi flies trapped: The number of uzi flies that were caught in the uzi trap solution kept both inside and outside the rearing house was counted on daily basis and summed up for each crop.
- Per cent uzi fly incidence: A total of 50 worms were randomly selected from 10 different positions on the rearing bed and those with the presence of black scar caused due to uzi fly attack were counted.
- Per cent parasitization rate: One week after the release of parasitoids, 50 uzi fly pupae were randomly collected inside the rearing house and observed for emergence of uzi fly and the per cent parasitization rate was calculated by applying the following formula:

$$\% \text{ Parasitization rate} = \frac{A}{B} \times 100$$

Where, A = No. of pupae from which uzi fly didn't emerge
B = Qty of pupae collected *i.e.*, 50

- Per cent successful parasitization rate: From the above randomly collected uzi fly pupae, the pupae with exit holes indicating the emergence of parasitoids were counted and the following formula was applied:

$$\% \text{ Successful parasitization} = \frac{C \times 100}{D}$$

Where, C = No. of parasitized pupae with emergence holes
D = No. of parasitized pupae kept for parasitoid emergence

- No. of uzi fly pupae/kg of litter: After the silkworm rearing was over, the mulberry twigs were separated and left out unfed leaf and litter material was considered for recording this parameter. Number of uzi fly pupae available in ten samples, each weighing 1 kg was counted.

RESULTS AND DISCUSSION

Although several workers have evolved various control measures of cultural, physical, mechanical/exclusion, chemical, biological, genetic and quarantine nature, uzi fly incidence is still a big menace in the traditional silk growing states of south India. Presently, majority of silkworm rearers are adopting IPM against uzi fly and the package is restricted only to exclusion method and placement of uzi trap, with a very few implementing biological control. However, in the village selected for the study, it was observed that the high population as well as high incidence of uzi fly was mainly due to improper management of rearing residue.

In the present study, a non-chemical uzi fly management package comprising of components, such as placement of uzi trap in the rearing house, release of bio-control agent, *N. thymus* @ 2 pouches /100 silkworm layings and keeping the silkworm litter packed for a period of 15 days was implemented with all the 167 farmers in the



Uzi fly laying egg on silkworm



Symptoms of uzi fly attack



Adult uzi flies caught in uzi trap

Figure 1: Uzi fly, *Exorista bombycis* (Louis), the endoparasitoid of mulberry silkworm



N. thymus parasitizing uzi pupa



N. thymus developing inside uzi fly pupa



N. thymus pouch

Figure 2: *Nesolynx thymus*, the biological control agent of uzi fly, *E. bombycis*



Uzi fly pupae in rearing residue



Farmers' practice of dumping rearing residue in front of the rearing house



Litter packed in plastic bags

Figure 3: Silkworm rearing residue and its effective disposal

village covering 783 crops (94,965 DFLs). A total of 1,932 pouches of *N. thymus* were released during the study period. The impact of the package on the number of flies trapped, parasitization rate and successful parasitization rate of uzi fly pupa with *N. thymus*, per cent uzi incidence *etc.*, were recorded and following results were obtained (Table 1):

i) No. of uzi flies trapped

A total of 25,219 uzi flies were trapped in 783 crops with an average of 36.5 flies/ crop, which ranged from 10.6 to 82.3. It is also evident from the data that number of flies trapped per crop during first eight months of study period *viz.*, rainy and winter months, was more as compared to

Table 1: Month-wise details of IPM program against silkworm uzi fly, *Exorista bombycis*

Month	No. of silkworm crops	DFLs	Uzi flies trapped (No.)	Uzi flies trapped per crop (average No.)	<i>N. thymus</i> released (No. of pouches)	Parasitization rate (%)	Successful parasitization rate	<i>N. t.</i> adults recovered per successfully parasitized pupa	No. of pupae /kg litter	Uzi fly incidence (%)
July	77	10890	4136	53.7	232	55.60	47.60	78.72	5.18	2.2
August	37	4735	3046	82.3	101	61.08	51.28	46.63	5.10	1.6
September	45	6355	3267	72.6	128	54.33	45.33	52.69	4.26	1.9
October	84	10110	3955	47.1	208	58.62	43.70	41.88	4.44	1.4
November	56	7325	2153	38.5	145	51.83	39.86	82.46	3.88	1.1
December	69	7440	1434	20.8	150	49.70	37.50	49.27	2.15	1.0
January	114	13500	1933	16.9	264	50.46	36.88	64.38	3.12	1.1
February	96	11625	2507	26.1	236	50.18	35.35	58.62	1.78	0.93
March	53	6350	1023	19.3	108	40.66	30.10	74.74	1.92	0.72
April	45	4705	629	13.9	115	38.70	30.20	44.82	2.44	0.13
May	107	11930	1136	10.6	245	39.56	29.58	50.28	1.06	0.43
Total/Average	783	94965	25219	36.5	1932	50.07	38.85	58.59	3.21	1.14
S.D.				24.56		7.58	7.44	14.39	1.43	0.61

Note: Bench mark uzi fly incidence during June: 10.73 %

last four summer months (Table 1).

ii) Parasitization and successful parasitization rate

The parasitization rate of uzi fly pupa with *N. thymus* ranged from 38.7 to 61.08 % with an average of 50.07 %. However, the average successful parasitization rate was 38.85 % with a range of 29.58 to 51.28 %. Further, the number of parasitoids emerged from parasitized pupa ranged from 41.88 to 82.46 with an average of 58.59. It appears that high mortality rate noticed may be due to partial development of parasitoid inside the host pupa. When the parasitized puparia were dissected out and examined, some of them revealed the presence of fully developed *N. thymus* which failed to emerge. Similar observations were also made by Malhotra (2007) in the case of *Tetrastichus howardii* (Hymenoptera: Eulophidae) on house fly pupae.

iii) No. of uzi fly pupae per kg litter

It was observed that the uzi fly pupae available in one kg of rearing residue ranged from 1.06 to 5.18 with an

average of 3.21. It was observed that uzi fly pupae available in the rearing residue were more during rainy season compared to other months.

iv) Per cent uzi fly incidence

In the present study, the bench mark survey indicated an uzi fly incidence of 10.75 % in the month of June which was drastically reduced to 2.2 % within a month's period on implementation of uzi fly management package. A similar survey conducted by Kumar *et al.* (1993) had revealed that, uzi fly incidence was maximum during rainy season (12.5 %) followed by winter (11.8 %) and summer seasons (9.47 %) in Karnataka. Further, due to implementation of IPM involving one more additional strategy of cultural control, the average uzi fly incidence could be reduced to 1.14 % with the range being 0.13 to 2.2 %.

Conclusion

Based on the findings of the present study, it is suggested that the eco-friendly, cost effective and highly

efficient non chemical package for uzi fly management should be implemented on war foot basis in all the sericultural villages with the participation of all farmers in the area to obtain effective control of the pest.

ACKNOWLEDGEMENT

The authors are thankful to Dr. A. M. Babu, Scientist-D, Research Extension Centre sub-unit, Central Silk Board, Avinashi (Tamil Nadu) and Dr. S. H. Divya, Former Junior Research Fellow, Central Sericultural Research and Training Institute, Mysuru for providing a few important photographs in support of the study.

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APPLICATION OF TOTAL QUALITY MANAGEMENT (TQM) CONCEPTS FOR BUSINESS IMPROVEMENT IN A SILK DYEING UNIT AT KANCHEEPURAM

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ABSTRACT

The present day's markets are characterised by extremely high competition and ever-changing customer demands. Hence, the most important merit of a product needs to be its unquestionable quality that sustains the business. In order to ensure the quality factor, many tools / techniques are in place today, the latest being the application of total quality management (TQM) principles. In this context, to ensure a production environment that delivers quality products, the total quality management concepts were applied for improving the business in a silk dyeing unit. The elements of TQM, such as employee participation and development, cause and effect diagram *etc.* were applied for the tangible/intangible benefits. The study revealed significant improvement in business performance parameters, such as production figures showing a drastic increase from 50 to 100 kg/day, enhanced quality of dyeing using CSTRI method of dyeing compared to commercial method, reduction in processing and delivery time by 24 hours, 5-50 % of cost savings, reduction in employees' grievances and increased profit, higher levels of customer satisfaction, team development and a higher rating in employee/ employer satisfaction from 3 (good) to 4 (very good).

Key words: Cost savings, production, silk dyeing, total quality management.

INTRODUCTION

Total Quality Management is a philosophy that involves everyone in an organization to be in a continuous effort to improve quality and achieve customer satisfaction. Six basic concepts of TQM are i) A committed and involved management to provide long term top to bottom organizational support, b) an unwavering focus on the customer, both internally and externally, c) effective involvement and utilization of the entire work force, d) continuous improvement of the business and production process, treating suppliers as partners and f) establishment of performance measures for the process. The elements of TQM are customer driven quality, employee participation, Fishbone (Cause and effect) diagram, process capability *etc.*

The implementation of TQM provides tangible

benefits, such as productivity improvement, better product quality, reduced costs, reduced employee grievances and increased profitability. Intangible benefits are team work, enhancement of job interest, improvement in human relation and work area morale, participation culture, customer satisfaction, enhanced problem solving capacity and so on (Shridhara, 2009). A central principle of TQM is that mistakes are made by people, but most of them are caused or permitted by faulty systems and processes. It means that the root cause of such mistakes can be identified and eliminated and repetition can be prevented by changing the process (Gilbert, 1992). TQM engages all divisions, departments and levels of the organization. TQM companies are focused on the systematic management of data in all processes and practices to eliminate waste and pursue continuous improvement. The goal is to deliver the highest value for the customer at the lowest cost while achieving sustained profit and economic

stability for the company (Nicoleta Isac, 2010). TQM encompasses not only the product, service and process improvement, but those relating to costs and productivity and to people involvement and development (Dale *et al.*, 2007). Syduzzaman *et al.* (2016) reported that after implementing the proposed TQM framework in apparel manufacturing processes, especially in sewing, the production has been improved as total alters and rejections have been considerably reduced.

Culture plays a significant role on the issues of transferability of management. By adopting these Japanese management practices, the Indian textile industry can improve quality, reduce costs, increase throughput, increase flexibility, improve responsiveness, reduce product development time and improve capability to broaden the product range (Lee, 2011). Companies and managers try to deliberate every one of the organization through customer happiness with products performance, and implements effective production procedures that minimize waste of raw materials, eliminate rework and invent valuable manufacturing tools (Parul, 2015). Berry (1991) defined the TQM process as a total corporate focus on meeting customer's needs and significantly reducing costs. TQM has been described by many authors and encompass elements such as management commitment, employee's involvement, supplier partnership, continuous improvement *etc.* TQM is a way of managing an enterprise towards achieving business excellence (Dahlgaard *et al.*, 1998).

The analysis of the application of the TQM strategy revealed that the implementation of the methodology for the total quality management increased the efficiency of the centre. Textile companies that use the services of the center have got reduced the time needed for the preparation of work orders by 20 % and increased utilization of fabrics by 5 %, and the productivity by 50 %. The service center helped in achieving a quick and effective response to market demands and the textile enterprises significantly reduced the production costs and time of production (Elizabeta Mitreva and Nako Taskov, 2014).

For example, color strength (K/S) is the most important parameter to test the quality measurement of a

sample in terms of depth of the color dyed fabric (Baumen *et al.*, 1987). The colour strength (K/S) of a dye is a measure of its ability to impart colour to other materials. This property is characterized by the absorption in the visible region of the spectrum and can be expressed as a colour strength value. Such kind of management concepts (TQM) for better business performance could not be traced in small scale dyeing units. Therefore, this study aims to focus on the possibility of implementing total quality management for a small scale dyeing unit located at Kancheepuram for the better business improvements. The results are expected in terms of improvements in production, cut down in cycle time, comparative outlook on quality of dyeing by commercial method of dyeing (CMD) and CSTRI method of dyeing (CSD), development of team work, cost savings, profit and better customer and employee, and employer satisfaction levels.

MATERIALS AND METHODS

Silk (18/20 denier of 2 ply) was purchased from TANSILK, Kancheepuram. Acid dye (Nitonol yellow GL Spl) supplied by Vasanth Dyes and Chemicals Pvt. Ltd., Bengaluru was used. Chemicals such as acetic acid and Glauber's salt (Na_2SO_4) were procured from Chennai. The commercial methods of dyeing of silk materials were studied at local silk yarn dyeing unit, Kancheepuram from the month of June to November 2016 and data for the production for the month of May 16 was considered from company's document information. K/S value and absorbance / dye concentration (g/l) were measured using UV-Vis spectrophotometer. Evaluation of production, cut down in cycle time, cost savings, profit and feedback of customer, employee and employer have been carried out based on logical and total quality management concepts. Degumming and dyeing recipe of CMD and CSD of silk materials are presented in Table 1.

Analysis

Figure 1 depicts Ishikawa Diagram (cause and effect diagram) indicating factors affecting various business performance parameters. Employer, who is not aware of the reasons that hinder unit progress were exposed to these concepts and possibility of implementing suitable

Table 1: Recipe for degumming and dyeing - commercial (CMD) and CSTRI (CSD) methods

Degumming recipe			Dyeing recipe		
Recipe	CMD	CSD	Recipe	CMD	CSD
M:L	1:8.5	1:30	M:L	1:20	1:30
Soap	2 litres (10 gpl)	5 gpl	Dye	4 % (owm)	4 % (owm)
Soda	3.2 gpl	1 gpl	Glauber's salt	Nil	10 % (owm)
pH	-	9.5-10.5	Acetic Acid	3 % (owm)	4-6 % (40 % str.)
			pH	-	4-6

owm - on weight of material

solutions instantly. By way of bringing in a hike in the salary of experienced and skilled workers, there has been an indirect impact on boosting the morale, interest and motivational levels of workers. Incidentally, there was better understanding among employees, between employee and employer ensuring efficient team work. Work allocation for employees has been changed from specific work to multi-skilled work so that job pending is avoided due to an employee. Time and manpower were utilized to the optimum in order to eliminate waiting time

and also to achieve better quality of dyeing and increased production. It was made a point that the materials usage such as dye powder, soap, acetic acid and wood used for boiler for dyeing of silk materials were of precise measurements so that it reduced wastages and increased cost savings. The savings per day was to the tune of, dye powder: 5 %, soap: 5 %, acetic acid: 5 % and wood for boiler: 50 %.

The process mapping of dyeing showed that dyeing tubs that were kept idle were also put into use effectively in parallel, for the production. The shed used for drying of silk was relocated. These changes contributed towards reduced process time, increased production (Figure 2), avoiding of waiting time, unnecessary work of employees and cost savings which are impacts of TQM (Shridhara, 2009). The existing methods of working when replaced with proper schedule of planning, employees' utilization to the best and reduced waiting time, all put together has resulted in higher production and delivery time reduction by 24 hours. The comparative analysis indicated that CSTRI method of dyeing of silk is better than commercial

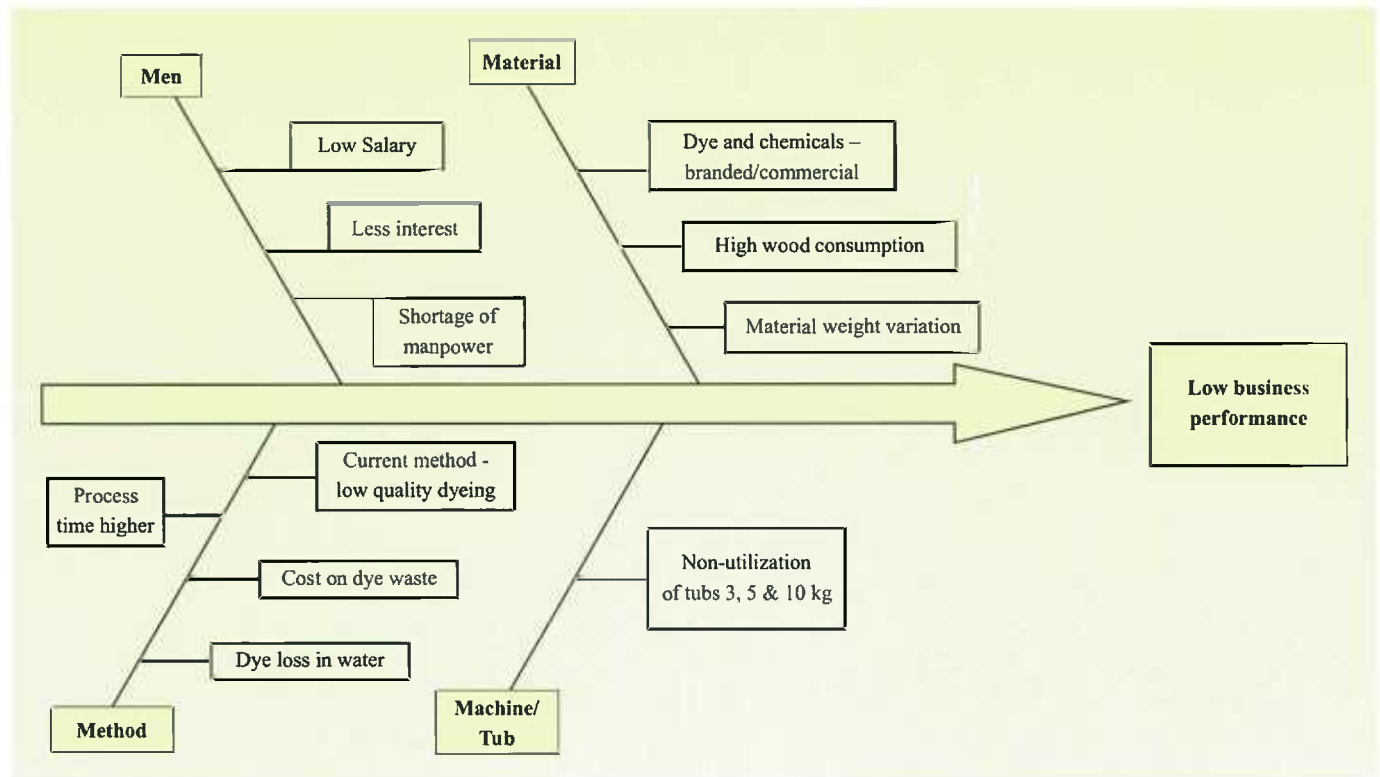


Figure 1: Cause and effect (Ishikawa) diagram on business performance parameters of silk dyeing unit
 (googleweblight.com/i?u=http://www.project-management-skills.com/fishbone)

Table 2: Comparison of CMD and CSD method of dyeing of silk materials

Parameter	CMD	CSD
Dyeing time (except preparation and washing)	20 min.	45 min.
Acetic Acid during dyeing	Not added	Added
Acetic Acid after dyeing	Done	Not done
Glauber's salt, Na ₂ SO ₄	Not added	Added
Temperature	Upto boil	85-90°C
Production	High	Low
Quality	Good	Very good
Dye waste after dyeing in tub water	High	Low
Amount spent on waste water treatment	High	Low
Amount spent on dye for same shade %	High	Low
Wood and steam consumption	High	Low

method of dyeing (Table 2) in terms of reduced dye waste, cost savings on dye and dye waste water treatment and mainly for better quality of dyeing on silk materials which was implemented for the customers, on demand.

From Figure 3, it can be observed that after washing of silk materials, K/S of all samples, such as CSTR method of dyeing-Branded dye [CSD (BD)], commercial method of dyeing-Branded dye [CMD (BD)] and commercial method of dyeing-commercial dye [CMD (CD)] reduce. The difference was low for CSD (BD) followed by CMD (BD), whereas, it was higher for CMD (CD). It means that dye loss is higher for CMD (CD) *i.e.*, comparatively more amount of dye goes as waste in this case.

The darker dye waste water of CMD for which estimated loss was up to 45 % before acid wash, whereas, it was 5 % in CSD (from dye calibration curve of absorbance v/s dye concentration, g/l). The reason for CMD to be darker may be due to non-addition of acetic acid and Glauber's salt during dyeing. But, they are added during dyeing of silk in CSD method that enhances fixation of dye molecules and reduces waste. The cost of dye that goes into waste water is expected to be ₹2,94,840/ per annum (dye rate @ 750 /kg) for CMD v/s 32375/- for

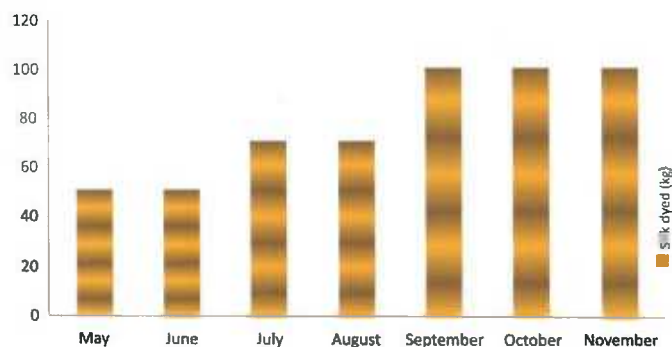
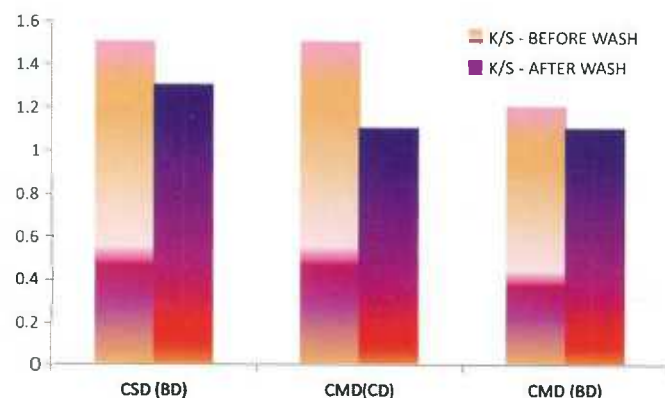


Figure 2: Month-wise (year 2016) production of dyed silk material in the dyeing unit



CSD (BD) - CSTR method (Branded dye)

CMD (CD) - Commercial method (Commercial dye)

CMD (BD) - Commercial method (Branded dye)

Figure 3: Comparison of dye uptake (K/S) of dyed silk samples using brand and non-brand dyes by different methods

CSD method of dyeing of silk. The difference in amount, *i.e.*, ₹2,62,103 per annum can be saved using CSD method of dyeing. Dye waste water treatment cost is 6/litre/day for commercial method (CMD) and consumes > 4 h. It can be reduced to 1/litre/day using CSD method of dyeing with less than 4 hours and a detailed study is need for this purpose. Feedback from various people with satisfaction levels (Figure 4) shows that, after the study of implementing TQM concepts, satisfaction level of employee, customer, employer and team development has been increased from rating 3 (good) to 4 (very good) indicating that reduced employee grievances, team work and customer satisfaction are the benefits of TQM (Shridhara, 2009).

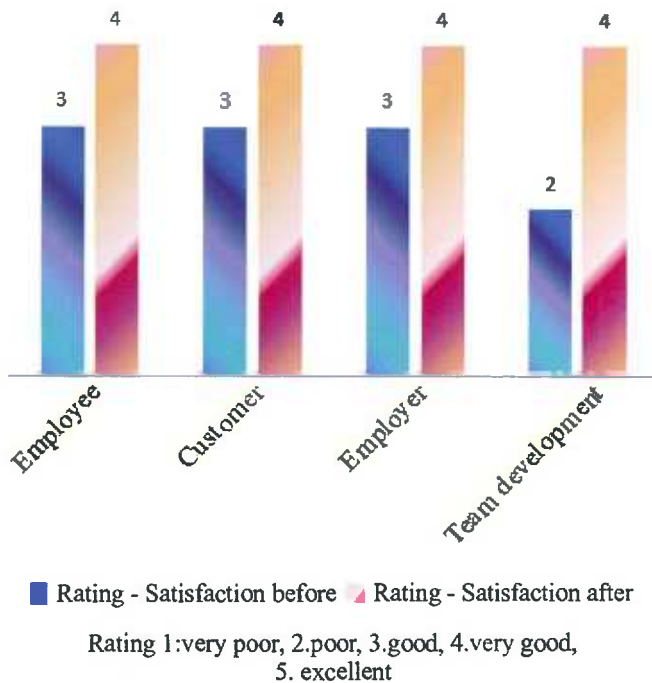


Figure 4: Feedback before and after implementation of TQM concepts

Conclusion

The impact on implementation of total quality concepts in the business scenario of a silk dyeing unit at Kancheepuram was analysed. The attempt brought in key changes in both the tangible and intangible parameters. The overall positive changes realised could raise employees' motivational levels and instil efficient team work. The production figures witnessed a 100 % improvement simultaneously reducing the process and delivery time by 24 hours and increasing cost savings of materials up to a significant level. CSTRI method (CSD) of dyeing was test verified as better than commercial method (CMD) in terms of better quality of dyeing, cost savings on dye and waste water treatment cost.

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Executive Committee Meeting of ISC held at Australia



The Executive Committee (EC) Meeting of the International Sericultural Commission (ISC) was held at the Deakin University campus, Geelong, Melbourne during 20th to 21st February 2017. The EC delegates have also visited the various labs of Deakin University to enlighten on the research being undertaken in diverse areas. Apart from approving the activities proposed for the year 2017, the following were the major decisions taken in the Meeting:

- 1) The 25th International Congress of ISC on Sericulture and Silk Industry scheduled during 2019 at Japan would be held at Tsukuba, which is about 70 km from Tokyo.
- 2) The Executive Committee approved to introduce Award of Excellence in sericulture and silk industry under the four categories as per

details given below:

The award shall be given to scientists, professionals and other stakeholders associated with activities related to silkworm host plant and all silkworms and all silks including mulberry, non-mulberry, spiders, bees, hornets, and the other silk insects. Only individual persons shall be considered for the award. Any specific invention, research, action or any other efforts of the persons/group resulted/which can result significant impact to the specified areas of silk and related industries shall be considered for the award. The contribution shall have field applicability and contribute to the welfare of the silk industry in any manner. Louis Pasteur Awardees are excluded from applying for this award. The Award shall be announced once in three year and one person shall apply for one award area. The award shall commence during the year 2019.

The Award shall consist of a specially designed medal and a certificate of Excellency signed by the Secretary General, ISC. The awardees shall be provided with financial support for attending the Congress/Conference to receive the award.

Category	Areas
ISC Award of Excellence in Silkworm Host Plant Development	Host plant development for mulberry, tasar, eri and muga sericulture – agronomy, breeding, pest and disease management, molecular biology, cultivation, tissue culture, machinery development, <i>etc.</i>
ISC Award of Excellence in Silkworm Development	Silkworm breeding, silkworm protection, physiology, silkworm rearing practices, seed production, extension, machinery development, <i>etc.</i>
ISC Award of Excellence in Post cocoon and Post yarn Technology	All activities in post cocoon and post yarn development, machinery development, extension, fashion industry, marketing, promotion, <i>etc.</i>
ISC Award of Excellence in Silkworm and Silk in Non-Textile Industry	Use of silkworm and silk in frontier areas of research including biotechnology, biomaterial, pharmaceutical, by-product utilisation, <i>etc.</i>

- 3) The Executive Committee approved to organize the 25th Governing Council Meeting (Conference) of

ISC in India during February / March 2018.

International Training on "Post Cocoon Technology" conducted at Bengaluru during 16th to 26th April 2017

A 10 days training on "Post Cocoon Technology" for the candidates nominated from ISC Member Countries was conducted at Central Silk Technological Research Institute, Bengaluru (CSTRI), which is the premier R&D Institute on Post Cocoon Technology in India. Under this programme, the entire expenses of trainees like: international and local travel, food, accommodation, training cost, *etc.* were met by International Sericultural Commission.

18 candidates from 8 Countries were nominated for the training programme. The participating countries were; Bangladesh, Egypt, Ghana, Iran, Kenya, Madagascar, Romania and Thailand. Apart from the classroom and practical oriented training on the various aspects of Post Cocoon Technology, the trainees were also taken to fields, industry units and other facilities of Silk Industry in and around Bengaluru.

The training programme was inaugurated by Dr.H.Nagesh Prabhu, Secretary General of ISC at 10:30hrs on 17th April 2017 in the Auditorium of Central Silk Technological Research Institute, Bengaluru, India. The Certificates for the trainees were distributed by the Hon'ble Chairman, Central Silk Board, Ministry of Textiles, Govt. of India in the valedictory function held at the HQs of Central Silk Board on 25th April 2017.



International Training on "Post Cocoon Technology" conducted at Bengaluru during 16th to 26th April 2017





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