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Mechanism of adult emergence in *Xanthopimpla pedator* (Fabricius)- a major pupal parasitoid of Tasar silkworm

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Abstract

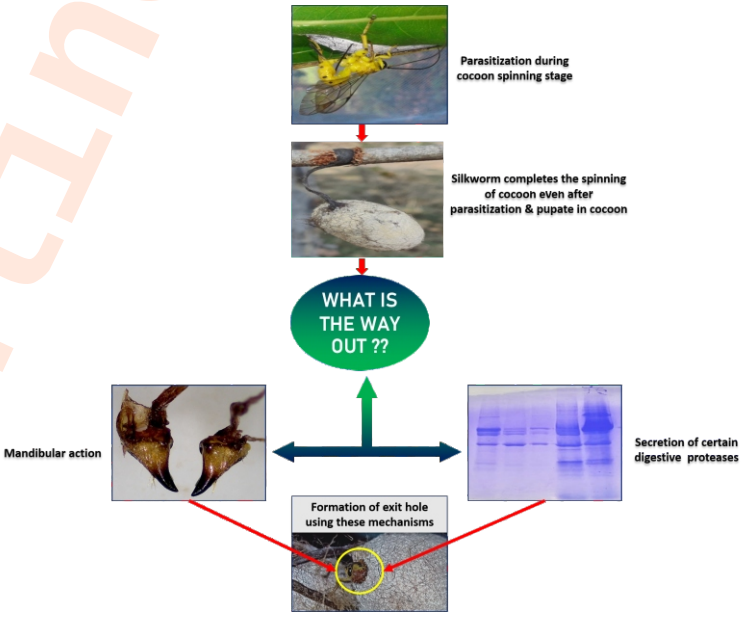
Aim: The study was conducted to elucidate the mechanism involved in adult emergence behavior of *Xanthopimpla pedator* a pupal parasitoid of tasar silkworm.

Methodology: Behavioral observations were made to study the mechanical approaches used during emergence and SDS-PAGE analysis was carried to know the possibility of proteases in the digestive secretions.

Results: The findings of the study suggest that *X. pedator* uses physical and chemical to achieve safe passage from the host pupae and cocoons. It was observed that during the process of emergence, *X. pedator* breaks host pupae by means of mandibular action (by biting the pupal shell). Further, it was noticed that to make emergence hole on host cocoon *X. pedator* found to employ digestive secretion to soften and disintegrate the silken shell, besides it also deploys mandibles to spread out the secretion on intended place of exit hole. SDS-PAGE analysis showed the presence of series of enzymes, possibly involved in cocoon softening.

Interpretation: Findings of this study forms the basis that *X. pedator*, a major pupal parasitoid of tasar silkworm, uses both physical and chemical approaches to achieve safe passage from the host pupae and protective silken cocoon.

Key words: Digestive secretion, Mandibular action, Parasitization, *Xanthopimpla pedator*



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Introduction

Tasar silkworm *Antheraea mylitta* is basically a wild sericiginous insect, which is unique to India and it is commercially reared outdoor by tribal populace of tropical India to obtain unique silk fiber, which has great demand among the silk consumers both in India and abroad (Chakraborty *et al.,* 2015). However, precontrived rearing system has made the tasar silkworm vulnerable to various predators and parasitoids. Among different pests, *X. pedator* is a chronic problem and it causes huge loss to both seed and commercial crops. Due to this, both government and private silkworm seed producing institutions are not able to meet out the farmers demand for disease free layings (dfls), and in commercial crops damage caused by this pest is affecting the market value, quality and reelability of the cocoons. *Xanthopimpla* is considered as one of the largest genera in the family of Ichneumonidae, and the species of the genus *Xanthopimpla* are endoparasitoids of the variety of Lepidopteran insects (Gomez *et al.,* 2009; Townes and Chiu, 1970; Idris and Kee, 2002). Wasps belonging to this genus are stout bodied insects with a bright-yellow-colored body, black spots, which makes this genus easily recognizable taxa among the Ichneumonidae.

Geographical distribution of the genus *Xanthopimpl*a is significantly biased towards tropical or subtropical areas and it is one of the major representatives of tropical ichneumonids in Asia (Gómez *et al.,* 2014; Townes and Chiu 1970). These endoparasitoids are known for great degree of biological adaptations (Gauld and Bolton, 1988). *Xanthopimpla* predator is a noted parasitoid of *Anthereae mylitta* with a crop loss of 35-40 %. *X. pedator* begins its life cycle in late 5th instar spinning larva of *A. mylitta* from the time of hammock formation to early cocooning stage and uses its long ovipositor to drill through silky envelope of spinning larva and deposits single egg in the abdominal segment preferably 5th to 7th day of cocoon spinning (Marepally, 2016; Aruna *et al.,* 2014; Bhatia and Yousuf, 2013; Gadad *et al.,* 2022). It chooses its hosts judiciously by selecting the early stage of spinning worms as at later stage, cocoons gets tougher and harder, which makes it difficult for ovipostion. In the course of development, despite of parasitization silkworm completes spinning and enters go into pupation stage.

Mean time the eggs hatches and grub of yellow fly starts feeding on the silkworm pupal content and complete its larval period and pupates inside the cocoon. Larval and pupal duration lasts for 20-22 days and after that the adults of *X. pedator* emerges by breaking hard shell of the cocoon (Gathalkar *et al.,* 2017). Typically, it emerges by making circular characteristic hole at the anterior region of the cocoon near peduncle (Singh *et al.,* 2010). Even though biology, stage of attack, extent of loss, alternate hosts have been well documented in several hymenopteran parasitic wasps, including the genus *Xanthopimpla*, yet our understanding of host–parasitoid relationships is generally poor, and this impediment is accentuated by a considerable deficit in the necessary knowledge on mechanism involved in adult emergence of parasitic wasps from the pupal case or cocoons. As a typical example, wide range of parasitoids infest the host insect pupae, which have a protective silken sheath and some parasitoids themselves pupate inside the silken pupal case. However, limited information is available regarding how adult parasitoids emerge from the protective sheath of the host pupae or from their own. In view of this, the present study was undertaken to elucidate the mechanism involved in breaking of silk cocoon in case of *X. pedator*.

Materials and Methods

Behavioral studies: Observations on *X. pedator* emergence was done by physical examination of parasitized cocoons from 18th day of parasitization (usually adults emerge 20-22 days after parasitization). Physical examination of cocoons mainly included hearing the sound made by the emerging adult while making emergence hole on the cocoon. Further cocoons were dissected at different durations (2, 4 and 6 hrs after hearing the sound of the adult). Thereafter, the emergence hole was observed and photographed.

Digestive secretion analysis: *Xanthopimpla pedator* parasitized cocoons were monitored regularly and when adult emergence occurred, immediately the exit hole with the digestive secretion (wet portion of the cocoon shell) was excised and transferred to 1 ml of ice-cold Tris Hcl buffer (20 mM PH 8.0). Further, the digestive secretions were thoroughly squeezed into buffer using sterile forceps and sample was centrifuged at 10000 rpm at 4 for 5 min to avoid the impurities. These samples were further subjected to SDS-PAGE analysis. Collected samples were subjected to 12 % SDS-PAGE (Laemmli, 1970). Twenty-five microliters of purified sample (protein concentration of 2 µg µl^{-1}) (Bradford, 1976) was mixed with 10 µl of 4 × sample loading buffer (125 mM Tris HCl (pH 6.8), 2.5% (w/v) sodium dodecyl sulfate (SDS), 0.1% (w/v) bromophenol blue, 25% (v/v) glycerol, 25% (v/v) β-mercaptoethanol) and heated at 100 °C for 5 min. From this, 15 µl was loaded on a 16% polyacrylamide gel. A molecular weight marker (Page Ruler, Thermo Fisher Scientific) and control (cocoon piece immersed in tris Hcl buffer) samples were included on each gel. Gels were then stained with colloidal Coomassie Blue (Bio-Rad Laboratories).

Results and Discussion

Ichneumonids are parasitoids of lepidopterans and almost emerge as adults from host pupae, and in case of silkworms another challenging task is to emerge from protective silk cocoon. Experimental results of the present study revealed that *X. pedator* employ both physical and chemical ways during the process of adult emergence. Observations on the emergence behavior divulge that breaking of host pupa was primarily mandibular action by chewing and breaking the chitinous pupal shell (cap cutting), as bite marks were observed around the emergence hole at the anterior (capital) end of the host pupa.

Fig. 1: Parasitized pupae of tasar silkworm exhibiting bite marks of *Xanthopimpla pedator.*

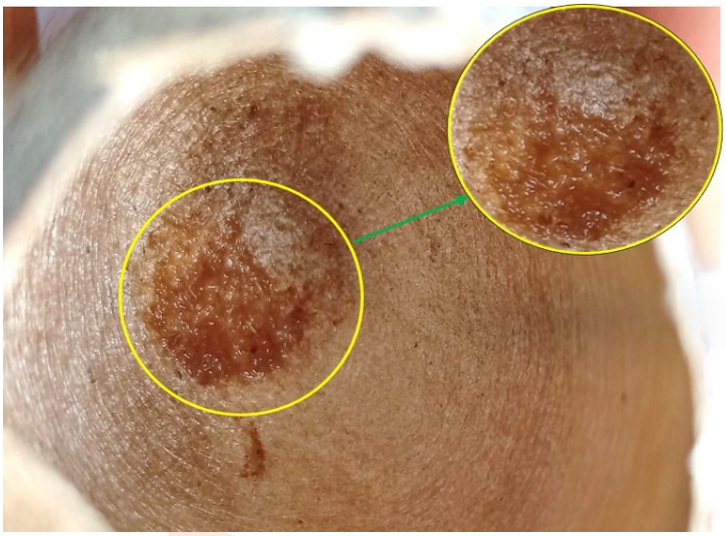

Fig. 2: Digestive secretion of *Xanthopimpla pedator* at the site of emergence hole .

However, this behavior was not consistent since in some individuals it was observed that cap cutting was very precise whereas in case of majority of the individual's emergence hole was not neat and precise as it was irregular with prominent bite marks (Fig. 1). Once after coming out of the host pupae, it has to breakthrough another barrier, *i.e.,* cocoon shell. When parasitized cocoons were observed by dissecting at different time interval based on the sound made during the emergence period it was evident that *X. pedator* used some digestive secretions to make the silken cocoon soft in order to facilitate the making of emergence hole on cocoon. (Fig. 2). Further observation was made by selecting different cocoons to check if any mechanical approach was being used for making emergence hole or it was only secretion based. It was found that after softening, the cocoon used digestive fluid. *X. pedator* individuals used their mandibles for spreading the digestive secretion externally around the edge of the hole being created for softening as well as dissociation of the host cocoon silke fibers (Fig. 3).

Overall observations on cocoon breaking mechanism revealed that once after emerging out from the host pupa, *X. pedator* secretes digestive juices at the anterior end (generally

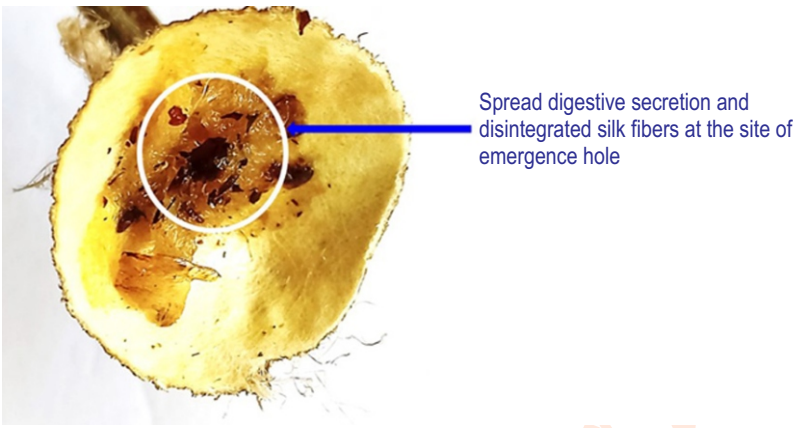

Fig. 3: Signs of mandibular action for spreading the digestive secretion and disintegrated silk fibers.

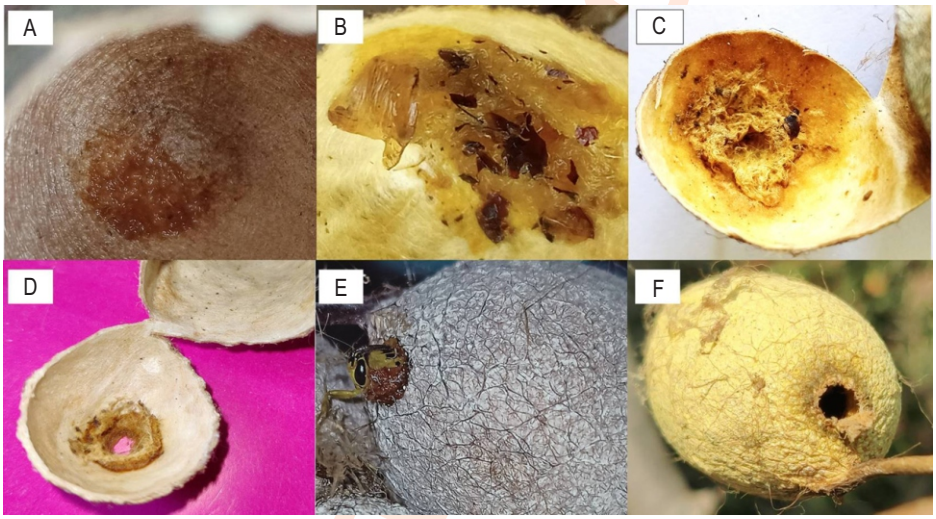

Fig. 4: Sequence in the emergence process of *Xanthopimpla pedator* from tasar silkworm cocoon. **(A-D)** Digestive secretion followed by mandibular action; **(E)** Adult emerging from tasar cocoon; **(F)** External view of emergence hole on tasar cocoon.

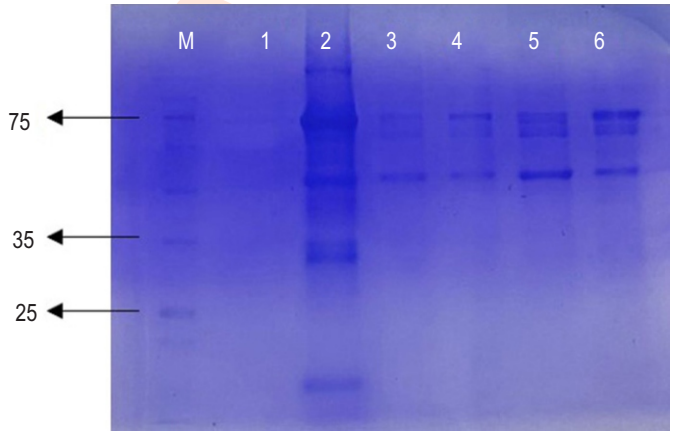

Fig. 5: SDS-PAGE profile of digestive secretion of *Xanthopimpla pedator*.

emerges from anterior end of the cocoon) of the cocoon as a part of cocoon softening in order to construct emergence hole easily. Further after digestive secretion, with the help of mandibles, it spread, the digestive secretion at the site of emergence hole. Based on the observations, it was also evident that mandibles are also deployed to disintegrate the silk fibers of the cocoon. These sequential events have been depicted in Fig. 4. Further digestive secretion was profiled using SDS-PAGE analysis suspecting the cocoonase like proteases. SDS PAGE analysis revealed the presence of promising bands between100 to 38 kDa (Fig. 5).

No bands were noticed at 26 kDa, in SDS PAGE analysis, which is actually size of cocoonase enzyme. This possibly indicates that *X. pedator* does not use cocoonase for cocoon softening. These results are in conformity with Gai *et al.* (2020) who reported that coccoonase enzyme is unique to lepidopterans. Further, it has been well documented that various sericigenous lepidopterans use cocoonase during the process of adult emergence from the cocoon (Hidetoshi *et al.,* 2005; Prasad *et al.,* 2012; Wang *et al.,* 2005; Geng *et al.,* 2014). Similar study was carried out by Shaw *et al.* (2015) who studied the emergence behavior of range of Ichneumonidae (*Trogus* subgroup) and some Chalcidoidea known to parasitize papilionid butterfly. Based on their study, they suggested that in general emergence from host pupae depends, as the existing perception has it, essentially on the action of the adult parasitoid's mandibles. However, they also suspected the possibility of using biochemicals during the emergence, as they have noticed staining around the edges of the emergence hole in some cases such as *Nymphalis polychloros* (Linnaeus) parasitized by *Hoplismenu sterrificus* (Wesmael) *Coenonympha pamphilus* (Linnaeus) parasitized by *Hoplismenus bispinatorius* (Thunberg), as well as in several Ichneumon species although it was not consistent. Zitani and Shaw (2002) and Barrantes *et al.* (2011) reported the cap cutting behavior in case of *Meteorus* spp. It is an a parasitic wasp belonging to Braconidae family and it is known to pupate inside the silke cocoon constructed by itself. In addition to this manyparasitic wasps use their mandibles to emerge out of host integument (Nakamatsu *et al.,* 2006). These findings indicate the role of mandible and digestive secretion in adult emergence in parasitic wasps.

Further findings of the study forms a base to investigate the profile of digestive secretion of *X. pedator,* which may be having certain cocoonase like proteolytic enzymes that can be explored in future for cocoon softening during the process of silk reeling.

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