

## Review

# Preliminary studies on natural enemies of the gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae) in Yasooj, Iran

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This research was conducted in Yasooj orchards during 2007 and 2008 to identify the natural enemies and pathogenic agents of the gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae) and the following species were identify. Parasitoids: *Barchymeria intermedia* Nees (Hymenoptera: Chalcididae); *Compsilura concinnata* (Meigen), (Diptera: Tachinidae); *Exorista Larvarum* (L.) (Diptera: Tachinidae). Predator: *Allothrombium* sp. (Prostigmata : Trombidiidae) and Pathogens: *Beauveria bassiana* (Bals.), (Deutromycotina: Hyphomycetes); *Bacillus thuringiensis* Var *kurstaki*. Chemical toxicants pose destructive and mal effects as well as other side effects on living environment. It is therefore recommended to conduct more research on the effectiveness of the spore dusting of the *B. thuringiensis* in to the orchards in the spring to control pest. After confirmation of the effectiveness of this control measure in the field studies, they can be used in an IPM program of the gypsy moth in the Yasooj region.

**Keywords** : Gypsy moth, natural enemies, Yasooj.

## INTRODUCTION

Gypsy moth *Lymantria dispar* (L.), is one of the main pests for ornamental and forest trees in U.S, Asia and some parts of Europe (Cannon, 1993; Farrar and Ridgway, 1995), which is considered as a major leaf eater of fall trees in overall northern hemisphere (Elkinton and Liebhold, 1990; Fast and Regnier, 1984). Gypsy moth is the main pest for forest trees in the whole world (Brown, 1984; Gribko et al., 1998). It entered to America from Europe accidentally in 1868 or 1869 and gradually spread out in west and south of the country, rapidly covering most forests of East of America and Canada (Little, 1972). The regions of America where well covered by it and have been led to severe fatality of host trees, destruction of living environments, and heavy expenditures to fight (Kleiner et al., 1995) gypsy moth, which long lasts in the forests of Iran, was seen first in Gilan forests by (Afshar, 1937). Chemical control ever conducted against that always has resulted in ultimate failure, dangers of pollution of living environment and

demolition of natural enemies, reduction of especially, poisonings for humans and domestic animals.

Therefore, thanks to the numerous problems of chemical control, non-chemical control measures, especially biological control must be seriously studied. Many species of natural enemies on gypsy moth have ever been identified throughout the world; some of which have practically been used for biological control of the pest (Leonard 1968 and 1970) .

About 100 species of bacteria have been reported as pathogenic agents of insects (Doane, 1970), some of which have been separated from the sick Larvae of Gypsy moth. The main pathogenic bacteria in the larvae of the Gypsy moth belong to the families of Pseudomonadaceae, Lactobacillaceae, Enterobacteriaceae, Bacillaceae, and Achromobacteriaceae, (Podgwaite and Campbell, 1972). Bacteria including *Bacillus cereus*, *Streptococcus faecalis*, and *Bacillus thuringiensis* have been separated from the dead and living larvae of

Gypsy moth (Campbell and Podgwaite, 1971). *B. thuringiensis* is currently being widely used as a microbial insecticide to control hygienic, forestall, and agricultural pests (Pang, 1993). The insecticidal act of this bacterium is for production of poisoned proteinaceous crystals in sporogeny (Pang and Frankenhuyzen, 1996). Various formulations of *B. thuringiensis* Berliner have been prepared and being used in America, Canada, and a number of Asian and European countries (Farrar and Ridgway, 1995; Yendol et al., 1990). *S. faecalis* is also another major fatal agent in low population of the Larvae of Gypsy moth (Doane, 1970). To control Gypsy moth in Keif, Eukrain, virin- ENS, which is a polyhydrosis virus was treated on the pine trees (300 grams in 300 liters of water); the effects were observable on the larvae of the second age. Between 1985-1990, in Ontario state of Canada, about 204,000 hectares of areas sprayed by *B. thuringiensis*, were subject to the pest of Gypsy moth; using this microbial poison on the young larvae in spring, measuring two in a thousand which was conducted as preliminary tests against it, led to around 85 percent fatality in the population of the insect. In a study concerning the effect, size, and compression of the drops of *B. thuringiensis* on the fatality of the larvae of Gypsy moth, it was confirmed that high compressions of tiny drops (50- 150 micron) of bacillus increase efficiency of the insecticide rather than large drops (more than 150 micron) with low compression (Sadraei Najafi and Khial, 1997). More than 22 species of fungi including classifications of eggs, various ages of Larvae, and pupae of gypsy moth have been separated (Moody, 1988). A number of fungi separated from larvae and pupae include: *Beauveria bassiana* (Hajek et al., 1995). *B.bassiana* (Bals.) commercially called Boverin has been formulated in America and many European countries are used against the pest (Moody, 1988). Efficiency of pathogenic fungi including *Entomophaga maimagia* and *Paecilomyces farinosus* which have been separated from surface skin of larvae and egg mass of Gypsy moth, have been assessed (Dunbar, 1972). Most research about natural enemies of Gypsy moth is in connection with parasitoids (Weseloh, 1972). The first successive experience in using natural enemies against Gypsy moth took place in 1905, so that wide struggles conducted in these years to enter it's parasitoids from Europe and Asia to America through this, 40 species entered but 10 of which settled (Brown and Cameron, 1982; Leonard, 1974). One of the most important parasitoids of the egg of Gypsy moth is *Ooencyrtus kuvanae* (Howard) (Hym: Encyrtidae) (Brown, 1984), which currently spread throughout the Holarctic region. It was first transferred from Japan to America in 1908, then released having multiplied to 10,000 adult insect in the regions where the compression of Gypsy moth was high (Brown, 1984). Research conducted in Bulgaria confirmed that *O.kuvanae* have one or two generations in spring and five in summer; only one parasitoids grows in each egg of

the pest, and in exchange for per 20 host eggs, one parasitoid must be considered in release. *O. kuvanae*, close species to *O.masii* has been more biologically studied because it is used in a biological control against *L. dispar*. The research showed that *O. kuvanae* is multigenerational and Arrhenotokous, and the growth duration from egg to an elderly insect of these species at 27°C is 13 days. Female *O. kuvanae* spends post- mating winter in brushwoods. The efficiency of *Anastatus despairs* Buscka which is considered as a parasitoid of egg of Gypsy moth is studied in America (Bess, 1961). It had confirmed to pose a satisfactory effect on decreasing egg compression of the pest in some regions of America (Brown, 1984). The parasitism rate by it is been reported to be up to 12 percent (Tadic, 1981). The parasitoids of larvae of the pest can be *Apanteles melanoscelus* (Ratzeburg) and *A. porthetria*. Relative abundancy and efficiency of parasitoid field of pupae of Gypsy moth have been studied (Leonard, 1974). Parasitoid fields of Tachinidae family enjoy more abundancy and efficiency rather than Sarcophagidae family (Leonard, 1974). 10 species have been reported from Tachinidae family to act as parasitoids of larvae and pupae of Gypsy moth, among which, the main ones are: *Compsilura concinnata*, *Parasitigena silvestris*, *Exorista larvarum*, *Sturmia scutellata* (Leonard, 1974) In republic of Czech, *P. silvestris* and *S. scutellata* are known as the main parasitoid fields of Pupae of Gypsy moth (Leonard, 1974). The efficiency of predatory of hunter beetles called *Carabus nemoralis*, *Calosma sycophanta*, L., and *Pterostichus pensylvanicus* is subject to study. These beetles are natively American feeding from levels of larva and pupae of Gypsy moth so that the larva of these overland beetles have shown remarkable effect on decreasing Gypsy moth compressions, destroying up to 40 percent of larva and pupae population of the pest (Johnson and Reeves, 1995). Also, various species of hunter beetles belonging to Dermestidae family have been gathered from the egg masses of the pest and in some cases are nurtured artificially and released. For an illustration, *Cryptorrhopalum ruficorne* in southern regions of America first was seen and gathered feeding from the eggs of the pest in 1971 (Manson, 1977). Three species of this family, *Anthrenus verbasci* (L.), *Dermestes Lardarius* L, and *Trogoderma ornatum* (Say), have been recognized as hunters of the egg masses of Gypsy moth in America (Manson, 1977). Concerning the hunting effect of Vertebrates especially birds on the egg masses of Gypsy moth, one can refer to the research in America in 1995 in which Vertebrate hunters (birds) were distinguished to hunt 9.3 percent of the egg masses of the pest in spring and summer (Cooper and Smith, 1995). This is one of the main regulating factors of Gypsy moth population in most regions because these egg masses in regions with high compressions (more than 2000 egg mass in per hectare) are an accessible food source for winterer birds especially when Land was snow covered

(Cooper and Smith, 1995). Some species of wild ants are also introduced as one of the biological control agents of Gypsy moth particularly in Europe (Weseloh, 1994). Tiny mammals such as mouse like including *Blarina brevicauda*, *Peromyscus leucopus*, and grey squirrel called *Sciurus carolinensis* are also hunters of larvae of different ages and pupae of Gypsy moth in North America (Cook et al., 1995). Research conducted in some regions of the world show the effective role of natural enemies in decreasing the population of Gypsy moth (Manson, 1977). The research has therefore conducted with the purpose of gathering and recognizing natural enemies and pathogenic agents of Gypsy moth in Yasooj, and preliminary study of their ability in population control of the pest .

## MATERIALS AND METHODS

Mehrian region in the city of Yasooj and the suburb of the city of Sisakht were considered as two sampling centers in which certain orchards was chosen for permanent sampling. The sampling was conducted regularly once in a week or two according to facilities and vehicle .

### Sampling of different stages of Gypsy moth growth

#### A Egg

After pollution centers were recognized in the regions in question, in order to gather and define possible parasitoids of the egg, a remarkable number of egg masses were collected from different areas at different dates (From mid-July to the next April), and transferred to laboratory after the specifications of collecting location were registered, each mass was put in 35 centimeters high and 20 centimeters diameter dish. Damp cotton or narrow glass pipes containing water with spongy cap were used to secure moisture inside the dish. A cavity at the same diameter as a usual lab pipe, was made on the cap of each dish, for eventual exit of parasitoids, then a lab pipe was inverted on each of cavity around the cavities were obstructed by cotton. At the end, body of dishes was covered by black plastics .

#### B Larvae

A remarkable number of larvae were collected from each region and each growth stage in order to recognize probable parasitoids of larvae of different ages of Gypsy moth and were transferred to laboratory after the specifications of collecting location were registered. Per 50 Larvae were put in 20, 30, 75 centimeters size cartons. Then, some herbages of apple in a dish of water

were added to each carton to secure larvae food. Damp cottons or narrow glass pipes containing water with spongy cap were used to secure moisture inside cartons. At the end, to escape of existed eventual parasitoids, a textile net covered each carton. Cartons were controlled and studied once in two days.

#### C Pupae

To detect eventual parasitoids of pupae, a certain number of pupae were collected from different areas at different dates; and transferred to laboratory after the specifications of collecting location were registered. Per 10 pupae were put in a 15×20 centimeters dish. A cavity at the same diameter as a usual lab pipe was made on the cap of each dish to exit eventual parasitoids then a lab pipe was inverted on each cavity around the cavities were obstructed by cotton. At the end, the body of each dish was covered by black plastics .

### Collection method of eventual hunters from forests and orchards

Collection of the eventual hunters living at the aerial parts of the plant conducted by insect capturing net, hunters moving in the forests and orchards conducted by setting pit falls. Cylindrical glass dishes with depth of 15 and diameter of 6 centimeters were used at the pitfall and placed in the soil so that their edges were at the surface of soil. Here by hunters movement trapped in them was easier .

The pitfalls were controlled and depleted every 24 hours. The captured hunters were carried to laboratory in paper pockets together with some leaf pieces, larvae and pupae of Gypsy moth then classified and kept separately in dishes for the next day be tested.

### To recognize fungoid and bacterial pathogenic agents

During spring in 2007 and 2008 when larvae were actively feeding from leaves of host trees to the aim of recognizing fungoid pathogenic agents of Gypsy moth, larvae of different age as well as pupae which were surrounded by condensed mass of mycelium of fungi were transferred to lab and put in mild water stream shortly then dried by paper drier soaked in 10 percent solution of carax for several seconds then redried, at the end, their body surface were being sampled. Moscardin samples were cultured in pottery dishes containing PDA circumference inside a growth room with centigrade temperature 60% proportional moisture and light duration of 14 hours. After spores are revealed, preparation is made of fungus and defined, then mono-sporized by

current method transferred in to the test pipes containing culture circumference, pipe caps were closed by special cigar sheet and, the pipes observed in refrigerator, at last the moscardin generator fungus was evident. Pathogenesis of fungi separated from larvae of Gypsy moth was confirmed at 10 conidia in a mili-litter spore suspension by dipping 5 and 6 aged larvae of Gypsy moth using Bukhner funnel .

To find bacterial pathogenic agents of Gypsy moth during springs in 2007 and 2008, live and dead larvae and pupae were collected individually, put in small steel pipes, each ones complete specification was written, pipe cap closed so that no external agent could enter in, then samples promptly transferred to lab for classification and coding looking at external signs. To separate bacterium and confirm its pathogenesis, first external surface of samples were disinfected, and then washed by steel distilled water. Samples were split at the next stage using steel tools, necessary samples taken from liquids inside the body of larvae, steel in distilled water, diluted by solution and at last, cultured at the NA or BHIA circumference. After bacteria's growth, while noting colonial external signs, purification was conducted .

Each hunter arthropods or insect observed hunting or feeding from different biological stages of Gypsy moth during sampling was collected carefully, coded along with the stage in a nylon bag, and transferred to laboratory. These natural enemies were cultured in the above said growth room on different biological stages of Gypsy moth so that it was possible to study artificial mutilations of them on Gypsy moth. Making sure that each natural enemy feed from one or several biological stage of the pest, they were recognized using scientific sources and samples sent to related authorities.

## RESULTS AND DISCUSSION

The following natural enemies and pathogenic agents were collected and recognized from different growth stages of Gypsy moth (egg, larva, pupae) .

### A *Brachymeria intermedia*

Among 4350 collected male and female cocoons of Gypsy moth during summer in 2007 and 2008, 130 had been parasite. The parasitoid bee separated from pupae was detected to be *B. Intermedia*. In order to define the name and species, it was sent to the institution of pest and herbal disease, insect classification sector, and named by Dr. Ebrahimi. Average losses arising from the wasp estimated to be 3 percent at natural situations which depict low effect of the wasp on the population of Gypsy moth in the study years in Yasooj. The research has shown that the wasp was transferred to America in

1908 and has raised high losses in the population of Gypsy moth in Pennsylvania (Brown, 1984). Apparently, the wasp is estimated to be one of the main parasites of the pupae of the insect at low compressions of the Gypsy moth (Brown, 1984). The research conducted in America has proven that the wasp leave a very low effect on controlling the population of Gypsy moth in nature.



*Brachymeria intermedia* (Hym. : Chalcididae)

### B *Compsilura concinnata*

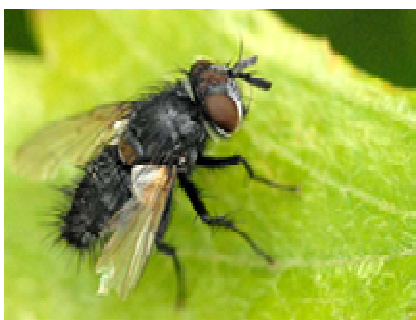
In June 1997, 250 larvae were collected from one of the apple orchards of Bahram Beigi region from the body of 8 of which parasitoid fly extracted. Samples of the fly were sent to agriculture college of Shiraz, plant protection sector and was distinguished to be *C. concinnata* (Dip: Tachinidae) (distinguisher: Dr. Ali Asghar Ahmadi). Of course, prospective struggles in 2008 to collect the fly from other parasited larvae at the same region were concluded. Therefore, thanks to a low percentage of parasitism, the fly also is concepted not to have much effect on controlling the population of larvae of Gypsy moth. The research conducted in America showed that the fly has a wide spectrum of hosts (Moody, 1988).



*Compsilura concinnata* (Dip.: Tachinidae)

### C *Exorista larvarum*

Among 1150 larvae at different ages collected in June and July 2007, 2008, a number of 25 with ages of 5 and 6 were parasited. The extracted fly from their body was called *E. larvarum* (distinguished: Dr.Ali Asghar Ahmadi). Parasitism percentage by the fly was estimated to be 2/1% in Yasooj in 2007 and 2008, which depict very low effect in these years. *E. larvarum* which is a parasitoid of the larvae of Gypsy moth had a parasitism percentage of 0% - 2/1% at the study. Although the research conducted in America in 1985 has announced low percentage, the study in Canada in 1989 announced parasitism is more than 16 percent (Leonard, 1974).



*Exorista larvarum* (Dip.: Tachinidae)

### D *Allothrombium* sp

Natural enemies at egg stage of each pest are more important than natural enemies at other growth stages of that pest because they destroy it before creating loss; therefore, they are particularly strengthened. As conducted studies of nature, marked eggs masses were controlled daily until they were settled. At these controls, 5 of all marked eggs masses were seen to be fed: After search and sampling from remains of brushwoods around egg masses, a few ticks were found usually moving on land and under rocks. The mites were distinguished having been collected and sent by Dr. Macfarlane .

It has 3/2 millimeters length, light red color, and usually exists on land when the weather is raising hot and acts on land. The feeding manner of it was studied in laboratory, mature mite inserted their oral pieces in the egg gradually sucked its contents, at the end, it turned the egg to a brown crumpled pellicle. The conducted research in Australia showed that *A. wolffi* feeds from mass eggs of *L. dispar*. Also in America, *A. wolffi* was recognized as a natural controller of egg stage of Gypsy moth. It is worth mentioning that this mite has also been collected from the eggs of butter flies in France and East Germany (Sadraei Najafi, and Khial, 1997).

### E *Beauveria bassiana*

Among 5450 high instar larvae (4, 5 and 6 instar larvae) collected during springs and summers of 2007 and 2008 compressed mass of white mycelium had grown on the body 695 ones. The fungus purified from the body of Larvae was recognized *B. bassiana* (personal correspondence with Dr. Saeid Rezaei, the PHD in herbal pathology major, science and research unit of Azad university of Tehran). Average losses raised by it in normal situations of springs / summer in 2007-2008, was 10% and 15.2% respectively, which shows a relatively remarkable effect of *B. bassiana* on Gypsy moth in Yasooj in the study years. Infection to *B. bassiana* increases in hotter regions. Well- irrigated orchards show higher parasitism percentage by *B. bassiana*. The conductor research in Ithaca showed that in nature, *B. bassiana* causes 15-20 percent fatality of larvae of Gypsy moth (Moody, 1988).

### F *Bacillus thuringiensis*

A remarkable number of 5/6 larvae were seen at apple orchards of Servak and Amir Abad Sisakht regions during springs of 2007/2008, had signs of softened and blackened. So that black liquid would spread out than larvae and pupae's body by a little touch. Also, a number of these larvae usually hanged on the trunk of trees (pine, apple, poplar) from the middle part of their bodies. The infected larvae were sent to BBA institute in Germany by biological campaign study section at the institution of herbal pests and infection research. The pathogenic agent was distinguished as *B. thuringiensis* Var *Kurstaki* by Dr. Keller Average losses raised by the bacterium at normal situations in springs of 2007/2008 were 30% and 70% respectively, which despite high effect of it on high aged larvae of Gypsy moth in Yasooj study years. About 100 bacteria are reported as pathogenic agents of insects among which, only following four bacteria are introduced bus as controllers of insects: *B. thuringiensis* (B. T), *B. papillae*, *B. lentimorbus*, and *B. spaericus* (Yendol et Al., 1990). These species have spores and are more resistant against unfavorable environmental agents rather than others. Among these four bacilli, only B.T has developed successfully being widely produced and presented in several countries as a commercial insecticide being used for crops of trees and farms and recently as a controller of larvae of butter flies in stored crops (Elkinton and Liebhold, 1990).

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