9th BACSA INTERNATIONAL CONFERENCE "Sericulture preservation and revival – problems and prospects""SERIVIVAL"
2019, Batumi, Georgia

A Survey Possible Reasons of Non-Spinning Syndrome in Sericulture Industry and Its Incidence in Turkey

Ümran Şahan, Prof Dr, Bursa Uludağ University, Faculty of Agriculture *Department of Animal Science, Bursa- Turkey

Levent Büyükuysal'Prof ,Dr Bursa Uludağ University, Faculty of Medicine Department of Medical Pharmacology, Bursa, Turkey

Ardahan Erdemir, Agriculture Engi[nering. Kozabirlik Bursa

Introduction

Sericulture, that has national, historical, cultural and economic values for Turkey, has continued to maintain its characteristic of being a traditional production for 1500 years (Şahan, 2011). Especially Bursa was an important centre on the silk road ,

In the recent years, some problems have appeared in silkworm (Bombxy mori L.) rearing which has not been seen until now, in different cities of Turkey, as a result of pesticide applications to orchards and vegetable fields, especially when they are grown in areas close to mulberry trees. Mortality of silkworm due to pesticide poising is still regarded as a major threat to silk industry (Wu et al., 2006).

Silkworm can be exposed to pesticides in various ways. The most common route of pesticides exposure to silkworm is through contamination of mulberry leaves Apart from mortality, some pesticides, as Insect growth regulators (IGRs) are suspected to be responsible for the “non-spinning syndrome” observed in silkworm rearing farms by contaminating the mulberry leaves that fed silkworms (Kamimura, 1995; (Chitgupekar and Basavanagoud, 2014).).

Each spring, during the rearing period corresponding to IGRs treatments on orchards, IGRs also cause prolonged larval life-span’ distance from these treatments and inhibit the initiation of metamorphosis which larvae reared about several kilometers.

Bombyx mori larvaes are extremely susceptible pesticides, especially to IGRs which induces a developmental arrest in fifth instar larvae at very low doses and also cause ‘non-spinning syndrome and producing dauer larvae (Leonardi et all., 2001).They stay as permanent larvae, which continue to feed and grow but do not spin their cocoon or pupate.

The molting process in the insects is controlled by ecdysone and juvenile hormone (JH). The normal development of the insect depends on a precise adjustment of the JH concentrations at each stage (Niwa and Niwa, 2014). Insect growth regulator hormones are a group of consisting of pyriproxfen, fenoxycarp, hydroprene and methoprene. IGRs are usually synthetic versions of naturally occurring hormones and they act by maintain high the levels of juvenile hormone in the insect.

Non-spinning sendrome occurs each year in silkworm rearing farms in Iran, China, Brazil and other countries (Monconduit and Mauchamp, 1998). In recent years, cases of non-spinning sendrome have been observed in different cities throught Turkey. In additional, during the 2018 rearing season, the cases have also been observed in breeder cocoon production as well.

We have carried out a project for two years was to identify the group of IGR hormone that cause non-spinning sendrome and also the route of contamination. We selected of 14 breeder cocoon producers in two different regions (Bursa and Bolu) and analyzed their cocoon production records in years between 2016 - 2018 to determine the effect of pesticides.

Additionally, we took the hemolymph samples from the larvae and analyzed for their pesticide levels. Despite the fact that no pesticide application is known to be made in villages of breeder producers we have observed non-spinning syndrome cases.

In the result of pesticide analyze, different doses of hydroprene were found in the hemolymph of the larvae which taken of Bursa and Bolu regions.

The findings are very surprising for us because hydroprene is not registered as a pesticide in Turkey and must not be sold legally in markets in Turkey and in the EU. Therefore, there were no reported effects of hydroprene on insects and silkworm in Turkey. Unfortunately, though we do not know the exact source of it, however, we assume that it is mixed in other some pesticides or added in foliar fertilizers.

The Ministry of Agriculture have been informed accordingly the problem and the cases under assessment.

Hydroprene is primarily used to urban and stored products lepidopteron pests in the USA (Edward, 1981). Hydroprene disrupts the metamorphosis and causes different toxic effects depending on the target insect species because of the diverse roles of IGRs in insects (Wilson, 2004).

The survey research was carried out to detect the mode of action of hydroprene on silkworm that causes the non-spinning syndrome. In addition, factors effecting molting and metamorphosis on silkworm (Bombxy mori) have been discussed.

Material and Methods

In this study, cocoon production records (distributed breeder silkworm eggs (g), obtained breeder cocoon production (kg) and defective cocoon percentage (%) of a total number of fourteen breeder cocoon producers from Bursa region (eight villages) and Bolu region (six villages) were used to evaluate the effects of hydroprene. The records were taken from Kozabirlik during 2016-2018**.**

Hemolymphs collection

Hemolymphs of silkworm larvae were collected in fifth instar, fifteen larvae were selected randomly from each of the four breeders in Bursa and two in Bolu region. The hemolymphs were taken with a cut through one of the proleg and collected into Eppendorf tubes and put in refrigerator for the analysis to determine hydroprene doses. Pesticide analyzes were performed using LC-MS / MS device by the quenchers method.

The breeder cocoon producers were checked the three times in a week by the egg production specialist. During the rearing season, feeding and other conditions of larvae rearing were under standart conditions (at 75% relative humidity, 24-26 ℃ temperature) In 2018, the duration of fifth instar was also followed and recorded.

Effects of non- spinning sendrome on the larvae and cocoons were observed as follows; Giant larvae, darker color larvae, non-spinning larvae, defective cocoon, dead pupae or partial pupation and malformed pupae.

Fiqure 1. Effects of IGRs on silkworm. Larvae (Dauer) at day 15 of the fifth instar and the most of larvae died before spinning.

Fiqure 2. Non- spinning sendrome. The matured larvae in the last instar, but they died no making cocoon.

Figure 3. Cocoon spin of silkworm larvae

Fiqure 4. Normal pupa , unpupated larvae , carry out cocooning, but die inside the cocoons, malformed and normal cocoon.

Figure 5. Non-spinning sendrome. Unpupated larvae.

Results

Given that the non-spinning syndrome occurs affected the mulberry leaves contaminated by the pesticide (hydroprene), the cocoon production records for the last three years were analyzed to figures out of the exact effect of toxicity on production.

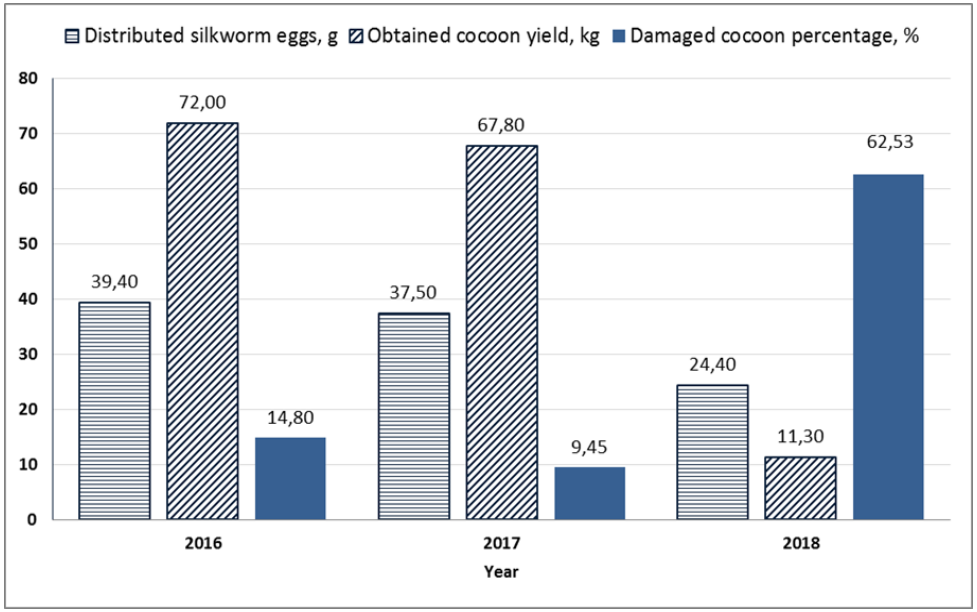


Table 1. The amount of silkworm eggs distributed to cocoon producers and the amounts of obtained breeder cocoon production in BURSA.

As can be seen in Table1, in Bursa according to amount the distributed eggs obteined breeder cocoon yield is as follows; ın 2016 and 2017, a mean of 1.80 kg of breeder cocoon was obtained from one gram of silkworm eggs, but in 2018 the cocoon yield decreased to 0.46 kg. The decrease in cocoon yield obtained in 2018 is 74 %.

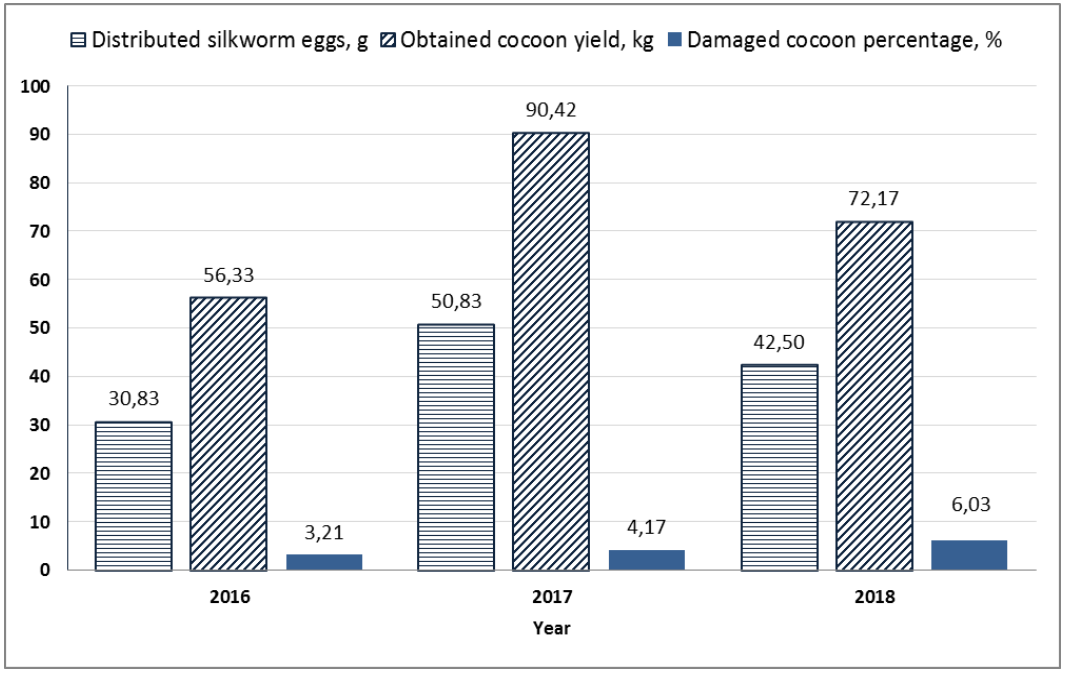
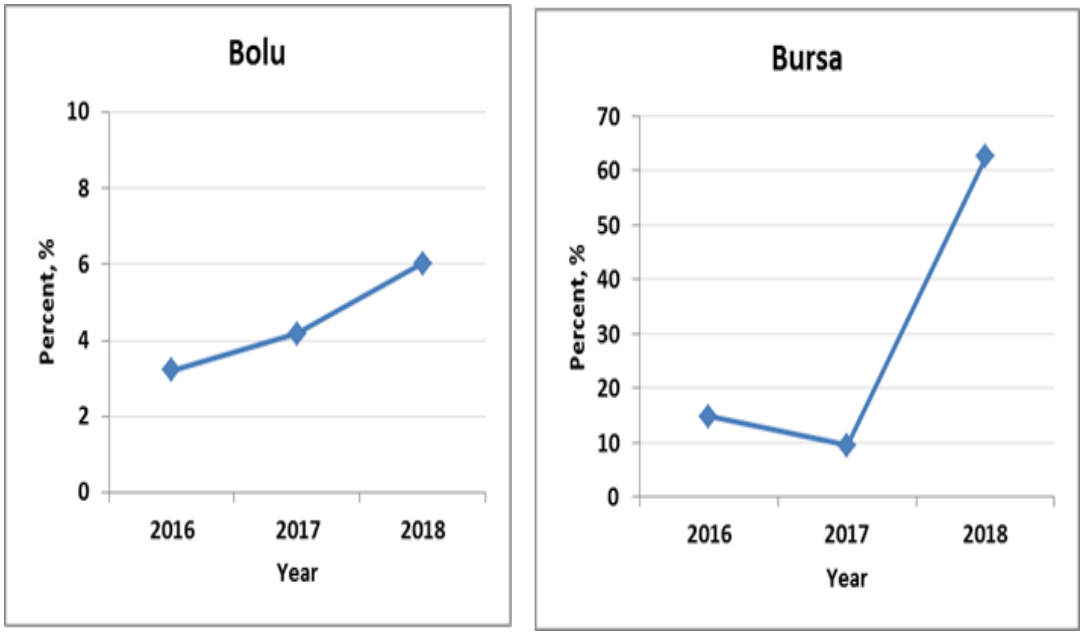


Table 2, shows the amounts of silkworm eggs distributed to cocoon producers and the amounts of obtained breeder cocoon production in BOLU.

In same years, an mean of 1.86 kg of breeder cocoon was obtained from one gram of silkworm eggs, but this amount decreased to 1.69 kg in 2018, and the decrease in cocoon yield is **9.6 %**. The loss of cocoon yield is less in the Bolu region,



Fiqure 6. While in Bursa the rate of defective cocoon rate increased from 14.80% to 69.53% in 2018, In Bolu this rate increased from 3% to 6 %.

Table 3 .The results of doses hydroprene in silkworm larvae hemolymph in Bolu and Bursa regions

Region	Producers	Detected Quality (ppm)	LOQ	LOD
	1	0,080		
BURSA	2	0,103		
	3	0,382	0,95	0,28
	4	0,325		
BOLU	1	0,003		
	2	0,007		

As shown Table 4, Hydroprene doses in hemolymph taken from producers in Bursa are significantly higher than those in Bolu. The amount of Hydroprene in the hemolymph in the Bursa and Bolu regions ranged from 0. 003 ppm to 0.382.

Discussion

IGRs are physiological processes of insects, ecdysone agonists, or molt inhibitors). IGRs application to early last instar larvae prolongs the larval stage or producer dauer larvae. They have slow action and the effects may be less noticeable than that of other insecticides.(Akai and Kobayashi (1971; Mondal and Parween, 2001)

In this study, we observed that the silkworm larvae were reaching healthy 5^{th} instar, however 5^{th} instar of larvae that lasts for 8 days under normal conditions prolonged up to 13-18 days duration and most of the larvae died before spinning or some carried out cocooning, but died inside the cocoons, as a results silkworm larvae could not making spinning cocoon and larval-pupal metamorphosis. This problem has appeared too in the native and foreign silkworm pure lines that are protected. The results were in accordance with Kuribasyashi (1988), who determined that among the silkworms affected by IGR, some grow almost normally during the larval period, but died in cocooning frames due to lack of cocooning ability.

Effects of hydroprene on stored- products insects have been published in several researches (Arbogast et al. 2000; Arthur and Dowdy 2003).

Our field observation in Turkey has indicated that cocoon yield rate decreased whereas defective cocoon rate increased when silkworms fed on mulberry leaves harvested in villages that were contaminated with hydroprene . We found especially, the doses of hemolymph in 3 villages in Bursa region were found to be more than 0.1ppm- 0.3 ppm.

In fact, hydroprene could be higher than these doses, because we don’t know when hydroprene was applied to surrounding crops. Most of the larvae in these three villages could not spinning the cocoon and some carried out cocooning, but died inside the cocoons. So, silkworm eggs could not be produced from producers in Bursa.

As a result, In 2018, according to amount distributed silkworm eggs in Bursa region the decrease in cocoon yield was %74 while the rate of defective cocoon increased %64 compared to 2016. In the Bolu region, the decrease in cocoon yield is 9.6 % and the rate of defective cocoons increased from 3% to 6%.

The results arein accordance with (Parthasarathy and Subba, 2008) who determined that even at 0.1 and 0.5 ppm, hydroprene blocks pupal metamorphosis in more than 80 % of larvae and remaining 20 % of larvae that became pupae did not survive to adulthood, but died in cocoon. In additionally, they showed that higher dosage (10 ppm) hydroprene blocked larval to pupal metamorphosis completely (100%) and most of larvae molted into supernumerary larva instar.

It has been reported by the breeder producers that during the 5th instar they fed the silkworms with mulberry leaves from other sources as their own trees do not have sufficient leaves. Fruit and vegetable production are common in the around the villages neighboring areas and different pesticides and leaf fertilizers are widely used in Bursa. Therefore, we thought that silkworms were fed with leaves that are exposed to different levels of hydroprene.

In Bolu region, the effect of hydropen leading to non- spinning syndrome was minimal. Poultry farming is very common in Bolu region therefore, crop and vegetable production and pesticides application are less than Bursa region. Thus, mulberry leaves have been less contaminated with hydroprene, nevertheless a steady increase in the rate of defective cocoon was determined.

However, it is not known when and how often the some pesticides containing hydroprene in the villages of Bolu and Bursa are applied, so when we analyze the hydroprenes in larvae, it may have been applied to the hydroprene on the crops 4-5 days ago and therefore the doses may actually be higher in the hemolymph.

Because, Parthasarathy and Subba (2008) found that hydroprene blocked larval-pupal metamorphosis when applied at 72-h after moulting into the final instar larval stage and all the larvae died during the inactive stage. In addition, they concluded that continuous feeding of hydroprene at 0.5 ppm during the final instar larval stage prolonged the larval life-span by inducing supernumerary larval, which was confirmed by our findings too.

Conclusion

Non-spinning syndrome have appeared in sericulture as a result of the pesticide applications. Pesticide residues on mulberry leaves affect the silkworm and they are susceptible to different IGRs. Application of hydroprene during the larval stages restricts and inhibits normal development and when larvae feed with leaves contaminated with hydroprene during the last stage they can not emerge as adults or they emerge as abnormal sterile adults.

Therefore, it is most important that in region where of pesticial contamination of mulberry exist, the application should be done by taking into account the length of period required for the disappearance of IGR toxicity on the silkworm. There are several articles on effects of hydroprene on stored- products insects. However, no research have been conducted on the effects of non-target insects, such as silkworm, which are fed with leaves contaminated with hydroprene. Therefore, further research is needed on where, how and how often hydroprene is used in agricultural areas and its effects on non-target insects.

Thank you attention

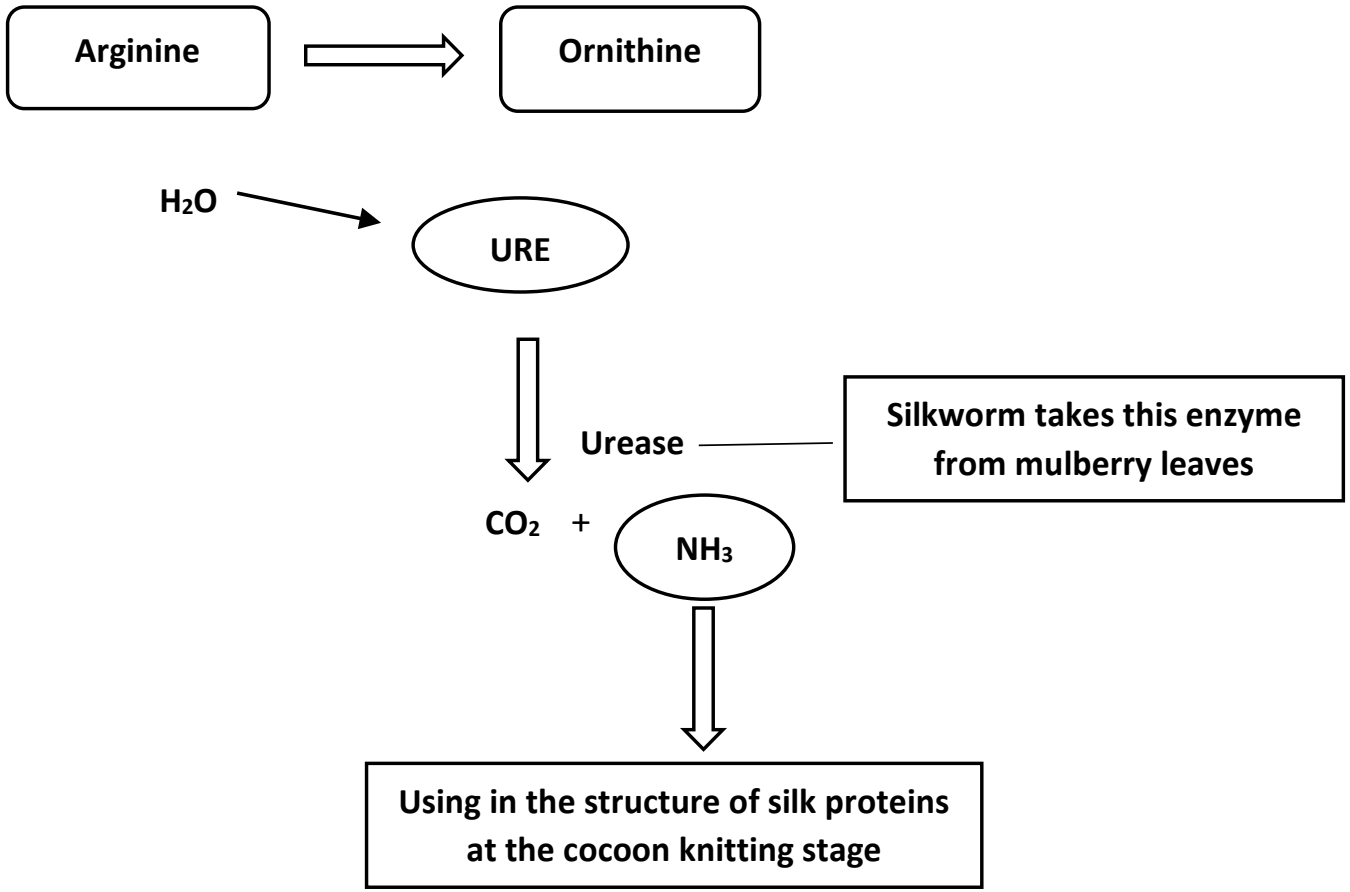


Figure 6. Urea metabolism in plants and silkworm.