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Effect of Pesticide Residue in Soil on Silkworm, *Bombyx Mori* L- Survey Analysis

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Abstract

Silkworm larval mortality specifically during spinning stage leading to non-spinning with specific morphological symptoms was frequently complained by sericulturists in Karnataka, India during 2015. Survey was conducted and information collected through questionnaire from the identified farmers who faced the problem of non-spinning in both traditional and non-traditional areas of Karnataka. Survey results indicate that the problem is specific to the silkworm crop of those farmers' who shifted from other crops of agriculture/ horticulture/ olericulture/ floriculture to Sericulture. Silkworm rearing performance of the batches fed with these leaves confirmed that the pesticide sprayed to the crops previous to mulberry, remain in the soil and when mulberry plantation is taken up in these gardens, the pesticide is absorbed by the roots of mulberry plants and transported to the leaves. Silkworms that feed on these mulberry leaves, grow and ripen normally but during spinning stage, larvae die with external symptoms like regurgitation, body shrinkage, rectal protrusion later become hook shaped leading to non-spinning or partial metamorphosis into pupa and death or spin flimsy cocoons. Larval mortality ranged from a minimum of twenty five percent to a maximum of hundred percent. The problem was noticed from the first harvest of leaves and lasted for a maximum period of 36 months. Cocoon crop loss depends on the concentration, duration and type of pesticides used previously for other crops.

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Introduction

Mulberry silkworm, *Bombyx mori* L., an economically important Lepidopteran insect is domesticated for its lustrous silk in many countries, especially in South East Asian countries like India. Karnataka state is the highest producer of mulberry silk in India. Silkworm rearing is conducted throughout the year because of the salubrious environment available. Farmer's shift from other crops to sericulture or vice versa depends on the cocoon price. Prophylactic methods like general disinfection, bed disinfection and maintenance of hygiene during rearing are practiced by sericulturists to manage diseases in silkworm.

During 2015, sericulturists in Karnataka, first complained about the problem during spinning stage where the larvae remain healthy till the day of spinning and ripen normally, but when allowed to spin cocoons, the larvae show varied external symptoms like regurgitation of the gut juice, body shrinkage, swollen integument, hook shape body, rectal protrusion, leading

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to non-spinning or partial metamorphosis into pupa and death or spin flimsy cocoons. In some of the rearings, larval mortality was observed during 3rd and 4th instars with external symptoms of vomiting of the gut juice, larval body shrinkage, further the larvae become hook shaped leading to death. Microscopic examination of these larval samples and bioassay of the larval homogenate did not reveal the presence of any pathogen.

There are reports on the toxic effects of various pesticides to silkworm by direct food intake or *per os* method and contact method (Cappellozza and Burlini, 1992; Wu *et al*., 2004; Ye and Lu, 1991; Munhoz *et al*., 2013). Park *et al*. (2007) indicated that spray drift of insect growth regulator pesticides cause silkworm non-spinning. Deformities in silkworm body due to effect of sublethal dosage of some insecticides have been reported (Naseema and Shivanandappa, 2003).

Preliminary investigation and circumstantial evidence revealed that the above symptoms observed in the silkworm rearings are specific to the cocoon crops where the farmers have shifted from other crops to sericulture. Considering the seriousness of the problem and to ascertain the factor causing silkworm death during spinning stage leading to either complete or partial crop loss, a detailed survey was conducted during 2015-16 in both traditional and non-traditional sericulture areas of Karnataka state in India.

Material and Methods

Based on the complaint received on silkworm mortality on the day of spinning, total of fifty five farmers who have shifted from other crops to sericulture, spread over seven districts of Karnataka, India, that includes five districts in traditional sericulture belt- Ramanagara, Chickaballapura, Kolar, and Tumkur (Mysore seed area) and Bangalore rural and non-traditional sericulture districts like Ballary, Dharwad, Belgavi were included in the survey. Area of survey was identified based on the purposive sampling procedure. Relevant information on various demographic characteristics like agronomical practices followed for mulberry, agriculture/horticulture crops grown earlier to mulberry, pesticides used for the previous crops, silkworm rearing methodologies, disinfection practices and hygiene maintenance etc., were obtained through a structured questionnaire designed for the study.

The farmers were grouped into low, medium and high income based on mean standard deviation of scores of quantum of cocoon harvested. These variables in the model were quantitatively measured based on scoring technique of crop losses with specific external symptom, the type of agricultural, horticultural/ olericulture crops cultivated prior to mulberry plantation, use of pesticides before mulberry etc. The average output of cocoons was recorded in terms of kg per 100 dfls.

Results and Discussion

Survey was conducted in traditional and non-traditional sericulture areas of Karnataka, India, to ascertain the reasons for the crop loss during silkworm spinning stage with specific symptoms. The selected traditional sericulture districts of Karnataka include Ramanagara, Chickaballapura, Kolar, and Tumkur (Pure Mysore seed area), Bangalore rural and non-traditional sericulture districts include Ballary, Dharwad and Belagavi (Fig. 1).

Survey analysis revealed that, the identified fifty five sericulturists followed all the general practices like pruning, manuring, irrigation, mulching etc., for mulberry cultivation and silkworm rearing practices like egg incubation, general disinfection, maintenance of ideal environmental conditions, hygiene, etc. However, silkworm cocoon non-spinning with the above indicated external symptoms is seen in all the races, irrespective of the type of rearing house either separate or rearing cum dwelling, room disinfection conducted and bed disinfectants used. It was not specific to any season and was observed throughout the year (Table 1).

However, the common factor in all the fifty five identified

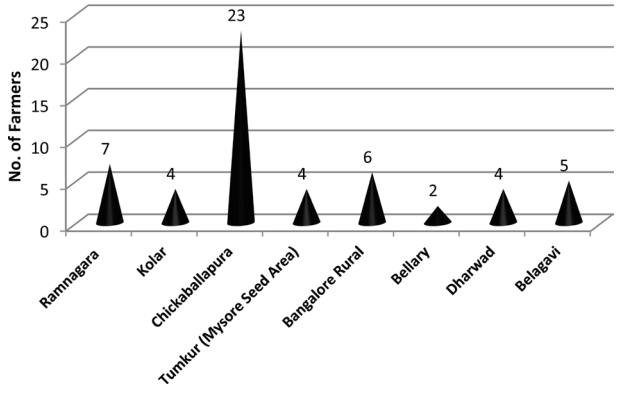

Fig. 1. Survey details of the farmers with crop loss during spinning stage due to pesticide residue in soil in different districts of Karnataka

Table 1. General sericulture practices followed by selected farmers who shifted from other crops to sericulture

Mulberry cultivation/ Rearing practices		Technology Adoption (%)
Type of irrigation	Drip	87
	Furrow	13
Mulberry variety- Victory-1		100
Use of Chemical fertilizer		100
Use of FYM		92
Trenching/ Mulching practiced		10
Bed waste used directly as fertilizer		39
Use of Tank silt		28
Type of rearing house	Separate	94.87
	Rearing cum dwelling	5.18
Exhaust openings in rearing house		78
Race	Cross Breed	76.92
	Bivoltine Hybrid	15.38
	Pure Mysore	7.69
Source of chawki	Own Chawki	17.94
	Purchased Chawki	82.05
Disinfection of Rearing house and appliances		100
Use of bed disinfectant		93
Use of lime		100
Use of Phytoecdysone for uniform spinning		0
Crops earlier to mulberry (%)	Vegetables/Horticulture Crops	91
	Cotton	5
	Groundnut	2
	Onion	2

farmers is that the land was used previously to grow other crops of agriculture, horticulture, olericulture, floriculture and had then shifted to sericulture. Pesticides were sprayed to other crops either heavily or profusely and mulberry was transplanted in the same garden after uprooting the previous crop.

The pesticides sprayed to the earlier crops remain in the soil and are taken up by the mulberry plants through roots and transported to the leaves. When silkworms are fed with the pesticide containing leaf harvested from these newly established mulberry garden, the larvae exhibit the above symptoms during spinning stage. The larvae are healthy, ripen normally but when allowed for spinning, they die with various external symptoms *i.e.* the larvae regurgitate, body shrinks and become hook shaped and die (Fig. 2a to 2c) or some larvae spin only the outer cocoon layer (Fig. 2d) or half metamorphose into pupa and die (Fig. 2e). However, two rearers lost the crop during 3rd /4th instar itself in the 1st rearing (Fig. 2f) with external symptom of vomiting of the gut juice and in their subsequent rearings, the larvae ripened normally but showed the above symptoms during spinning stage (Fig 2c and 2d). This indicates that if the pesticide concentration is high in soil the larval mortality with sudden knock down and vomiting can be expected during silkworm rearing period only.

Details of the sample farmers selected from different districts have been enlisted to give a bird's eye view of the crops grown earlier to mulberry, duration of the pesticides used and its effect on the silkworm crop performance (Table 2). The crop loss pattern of 55 farmers was monitored vis-à-vis the age of the mulberry garden. The same is grouped into six groups with class interval of six months duration.

During the first six months of establishment of the new mulberry garden the plants were allowed to grow and mulberry leaf was not utilized for silkworm rearing. From 7-12 months of plantation all the selected 55 farmers lost crop, but quantum of cocoon crop loss ranged from 25% to complete 100%. Further, with the increase in the age of the mulberry garden i.e. from 13 to 18 months of establishment of the garden, only 41 farmers lost the crop out of the fifty five selected and the problem stopped in 14 farmers. The quantum of crop loss also reduced gradually. In the duration of 19 to 24 months, 36 rearers lost the crop, with the improvement in the cocoon harvest and the problem stopped in 5 of them. In the duration of 25 to 30 months 7 rearers lost the crop and stopped in 29 of the farmers. Finally in the duration of 31 to 36 months out of the total fifty five sericulturists the problem remained in only 3 with partial crop loss (Table 3). Hence, it can be concluded that the effect of the pesticide residue in soil on silkworm depends on the type, concentration and duration of the pesticide used earlier.

Among the fifty five selected sericulturists about 15% were aware of the deleterious effect of pesticide residue on silkworm mortality during rearing, but were unable to relate it to the non-spinning problem. About 12% of the sericulturists had adopted phyto-remedial measures for soil amelioration. However, it was conducted only once or twice before the plantation of mulberry and was done unsystematically. About 5% of the farmers were of the opinion that if the mulberry leaf from the pesticide residue problem garden is fed till IV moult and leaf from the normal

Table 2. Details of the sample farmers, their crop pattern and experience with pesticide residue in soil

Sl. No	Farmers Name and address	Crops grown earlier to mulberry	Duration of pesticide used to the previous crop (months)	Duration of mortality/ non-spinning observed from the 1st silkworm crop (months)	Larval Mortality during spinning stage as observed in the 1st rearing (%)
1	Krishnappa, Kanakapura taluk, Ramnagara Dist.	Tomato	3	6	60
2	Umesh, Magadi Taluk, Ramnagara Dist.	Tomato, Cabbage, Capsicum,	72	30	100
3	Ramesh, Magadi Taluk, Ramnagara Dist.	Tomato, Cabbage.	36	24	60
4	Nagraj, Magadi Taluk, Ramnagara Dist.	Tomato, Brinjal, Bitter guard	24	30	30
5	Vasanthkumar, Jangamakote, Sidlagatta. C. Ballapura Dist.	Tomato, Cabbage,	36	48	40
6	Anand, Jangamakote, Sidlagatta, C. Ballapura Dist.	Tomato, leafy green vegetables	48	6	70
7	Shivanna, Jangamakote, Sidlagatta, C. Ballapura Dist.	Tomato, leafy green vegetables	48	6	50
8	Marappa, Chokkahalli, Nandi, C. Ballapura Dist.	Cabbage, Watermelon	36	20	80
9	Mahesh, Dharwad Tq & Dist	Cotton, wheat, Sugarcane	96	20	80
10	Hiremani, Benkal Hobli, Hospet Tq., Ballary Dist	Onion, Cotton	96	24	60

Table 3. Details of the duration of pesticide effect and extent of crop loss among the selected fifty five sericulturists

Duration range of pesticide effect from the initiation of mulberry garden (months)	Cocoon Crop loss experienced by the farmers from the initiation of mulberry plantation (%)				
	Upto 25	Upto 50	Upto 75	Upto 100	Total farmers
1-6	Allowed the mulberry plants to establish during this period				
7-12	4	8	19	24	55
13-18	6	12	20	3	41
19-24	7	19	8	2	36
25-30	3	2	2	0	7
31-36	1	2	0	0	3

garden is fed in the V instar non-spinning could be avoided. The same was practiced by them until rectification of the soil (Table 4).

During V instar, mulberry leaf requirement by the larvae is high. Hence, if there is shortage of leaf, the general practice among sericulturists is to purchase leaf from the available mulberry garden. If normal garden leaf is fed to silkworms upto IV moult and pesticide residue problem garden leaf is fed during V instar even then silkworms are normal till spinning ripen normally but non-spinning with the above symptoms are seen. This shows that feeding during V instar is crucial to avoid crop loss during spinning stage due to pesticide residue in soil.

Table 4. Sericulturists awareness on the effect of pesticide residue in soil on silkworm and its management

Details of the use of pesticides, farmers awareness and its management in sericulture	Awareness %
Sprayed Pesticide to other crops previous to mulberry	100
Farmers' awareness on the effect of pesticide residue in soil to silkworm (%)	15
Farmers adopting phytoremedial measures to reduce effect of pesticide residue in soil on silkworm (%)	12
Farmers adopting rectification method during silkworm rearing (use of pesticide problem leaf till IV instar and normal leaf during V instar)	05

During the survey it was found that various pesticides, fungicides and herbicides are used by the farmers for other crops. The list of pesticides commonly used in Karnataka, India is given in Table 5. Majority of the agriculturists are unaware of the deleterious effect of the pesticides being sprayed and mainly depend on the information provided by the pesticide vendor/ neighbour/ media/ department official/ sales executive with little or no knowledge on the pesticides being used and are only interested in getting high crop yield.

Pesticide the main pollutant is causing havoc, affecting economically beneficial insects like silkworm, honey bee and other beneficial pest bio control agents. Under the safety evaluation

Table 5. List of commonly used pesticides for various crops earlier to mulberry plantation

Sl. No.	Brand name	Main Chemical Ingredient	Crops grown earlier to mulberry
1	Agni	Tricyclazole	
2	Bullet, Dimete	Phorate	
3	Confidor	Imidachloprid	
4	Conserve	Spinosad	
5	Coragen	Chlorantraniliprole	
6	Corazole EC	Hexaconazole	
7	Furadan	Carbofuran	
8	Gramacile	Propiconazole	
9	Karate, Judo	Lamda cyhalothrin	
10	Kavach, Jatayu	Chlorothalonil	
11	Larva	Emamestin Benzoate	
12	Lancer Gold	Acephate+ Imidachloprid	Tomato, Cabbage, Leafy green vegetables, Cotton, Watermelon, Capsicum, Beet root, Onion, Brinjal, Bitter guard, Green chilli, Pulses, Brinjal.
13	Mancozeb	1,2-Ethanedicarbamic acid + Tetrathion	
14	Magnum	Choropyriphos + Cypermethrin	
15	Monocil	Monocrotophos	
16	Nuvan, Starchlor	Dichlorovos	
17	Pendal	Pendimethalin	
18	Regent	Fipronil	
19	Ride	Acetamiprid	
20	Ridomil	N-(2,6-Dimethylphenyl)-N-(Methoxyacetyl) Alanine Methyl ester	
21	Rogor, Tafgor	Dimethoate	
22	Shikari	Deltamethrin 1% + Triazophos	
23	Solomon	Imidacloprid+Beta Cyfluthrin	
24	Starthene	Acephate	
25	Tagmil	Metalaxyl+ Mancozeb	

Fig. 2. (a) Enmass larval mortality during spinning stage on the mountage (b) An enlarged view of the dead larvae on the mountage with hook shape, shrunken body and rectal protrusion. (c) An enlarged view of the ripened but dead larvae with hook shape, shrunken and swollen body. (d) Formation of flimsy cocoon by ripe larvae, shrinkage and death. (e) Formation of naked pupae and death. (f) Larval mortality during rearing with specific symptom of vomiting of gut juice, body shrinkage and become hook shaped when pesticide residual concentration is high in soil.

of pesticides effect of organophosphorous and organochlorine compounds on silkworm by food intake method and contact method has been reported. Kuribayashi (1981) reported the ovicidal action of some pesticides treated during V larval instar. The reasons attributed for the silkworm non-spinning in the present study corroborates with the results of Munhoz *et al.* (2013) that *per os* feeding of chloranthraniliprole contaminated mulberry leaf due to spray drift cause larval morality during rearing and during spinning stage. Park *et al*. (2007) predicted that molinate an insect growth regulator caused non-spinning syndrome due to spray drift to sericulture gardens.

Topozzada *et al.* (1968) reported that contact or oral uptake of selected pesticides caused considerable cytopathological changes in the midgut epithelia of *Spodoptera littoralis* and the external and cytological symptoms appeared faster when the larvae were poisoned orally. Kuwana *et al.* (1967) have clearly bifurcated the toxic symptoms in pesticide treated silkworm *B. mori* like slight excitement: raising the head at regular intervals, swinging: swinging of the anterior half of the body, vomiting: vomiting of digestive juice, lying on the side: inelasticity of the body and lying on the side, body shortening: shortening of the body due to loss of digestive juice and hook shape, muscle contraction due to contraction the body shrinks, paralysis: legs lose clasping power and effect of nervous system. Action of pesticide is either on the secretion of the hormone or on the nervous system where both factors are crucial to attain spinning stage and complete the spinning process. According to Matsumura (1975) based on half-lives pesticides can be divided into: non persistent-less than 30 days; moderately persistent - 30 to 100 days; and persistent - greater than 100 days and half-life values vary considerably depending on environmental conditions. Sudden knock down of the insect by pesticides either by direct contact or spray drift is known. However, this is the first report on the effect of pesticide residue in soil on non-spinning syndrome in silkworm.

To reduce the pesticide concentration the soil, as an immediate measure the farmers are being recommended to follow the general cultural practices like Phyto-remedial measures for early absorption of pesticides from the soil, high organic manure application for early

break down of pesticides and increased irrigation for early leaching from the root zone. During silkworm rearing as practised by the farmers, it is recommended to feed leaf from the pesticide affected garden upto IV instar and normal mulberry garden during V instar upto spinning and thus avoid complete cocoon crop loss due to pesticide residue in soil (Table 5).

However, detailed work on the identification of pesticides causing non-spinning, pesticide uptake by the mulberry plant and recommendations for early degradation of pesticides in the soil and soil amelioration methods are some of the virgin field for study and is the need of the hour. Studies are ongoing in this direction.

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