



## Some Natural and Chemical Compounds Directly Affect Pests of Mulberry Trees and Their Side Effects on Silkworm Larvae

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**Abstract:** Mulberry trees suffer from many pest infestations. To control these pests, some natural compounds such as Techno Oil and Top 9 and some other chemicals such as Mospilan® and Chinook® were used. Mulberry trees, *Morus nigra* were treated with three different concentrations of each of Chinook, Techno Oil® and Top 9® to reduce infestation with *Icerya* sp. and *Ferrisia virgate*. A high reduction rate of more than 90% occurred when using these pesticides. On the other hand, the corrected mortality of mulberry silkworm larvae was 93.3%, 0.0% and 6.7% respectively after 8 days of treatment. When treating mulberry trees *Morus alba* with two different concentrations of each of pesticides; Mospilan and Techno Oil against whitefly, the average rate of infestation reduction was 100% and 94.94%, respectively. The corrected mortality of mulberry silkworms larvae was 100% and 0.0% after 8 days of treatment respectively. This study proved that natural compounds could be used safely instead of chemical pesticides to reduce the pest population without affecting the life of silkworm larvae or cocoon properties.

## 1 Introduction

There are three species of mulberry trees in the world, i.e., *Morus alba*, *Morus nigra* and *Morus rubra*, out of them more than 150 varieties belong to *Morus alba*. The mulberry trees differ in height and shape of leaves and thus differ in their nutritional value and their effects on mulberry silkworm larvae (Abdelmegeed 2016). The mulberry silkworms feed only on mulberry leaves, so the products of their cocoons are deeply related to the quantity and quality of mulberry leaves.

Many authors have studied how to obtain high-quality cocoons. Nikolova and Jekova (2017) and Ghosh et al (2012) studied the effect of climatic factors on different varieties of mulberry trees.

Care must be paid towards mulberry trees e.g. using suitable fertilization (Abdelmegeed 2021), pruning (Boschini 2006) and irrigation (Sudhakar et al 2018). In addition, controlling infesting pests, where the most important are the piercing-sucking insects (whiteflies and mealybugs), that cause an adverse effect on trees is of great economic importance. One of the most important of these harmful effects is sucking plant sap through their mouthparts, which leads to the yellowing of mulberry leaves. These insects also secrete the honeydew that presents on the upper surface of the leaves, causes the blocking of stomata in plant leaves, and black sooty mold fungi grow up in addition to attracting dust and ants. Piercing-sucking insects also transmit viral diseases that lead to the deformation of plant leaves (Laghari et al 2019). The roots of mulberry trees are

also infested with nematodes (da Silva et al 2021). Therefore, it is necessary to control these pests by using natural pesticides, plant extracts (Fajfer and Łochyńska 2022) or even chemical pesticides but without causing harm to silkworm larvae (Mahadeva 2018). Yeshika et al (2020a) found that dichlorvos had a high mortality rate towards mealybugs, *Maconellicoccus hirsutus*, while was very safe towards mulberry silkworm.

Most chemical pesticides used on mulberry trees are harmful to silkworms (Kordy 2014), e.g. phoxim, which is widely used as an organophosphorus pesticide in agriculture while Guo et al (2021) proved that phoxim is toxic to silkworms. Xu et al (2022) studied the effect of dinotefuran (It is a third-generation of neonicotinoid pesticide) on the metabolism of the mulberry silkworm. Chlorantraniliprole is a very toxic pesticide to mulberry silkworms (Munhoz et al 2013). Procymidone negatively affected cocoons' quality and it was also toxic to mulberry silkworms (He et al 2022).

Accordingly, the present study aimed to use some natural compounds in comparison to chemical pesticides against whiteflies and mealybugs infesting mulberry trees and examine their impacts on mulberry silkworms when fed on mulberry leaves treated with these pesticides.

## 2 Materials and Methods

All experiments were conducted during autumn season 2021, in the Laboratory of Insect Physiology, Department of Plant Protection, Fac. Agric., Ain Shams Univ., Cairo, Egypt.

### 2.1 Examining mulberry trees for pest infestation

Experiments were conducted on two types of mulberry trees (*Morus nigra* and *M. alba*) planted at the Faculty of Agriculture, Ain Shams Univ., Cairo, Egypt. Ten leaves of mulberry plant were collected from each of the ten trees of each mulberry type before applying the pesticides and were examined in the laboratory to identify the different insect species that infested both types of mulberry trees. The first type of mulberry tree (*M. nigra*) was found to be mainly infested by mealybugs (*Icerya aegyptiaca*, *I. schellarum* and *Ferrisia virgate*), while the second type (*M. alba*) was mainly infested by whiteflies (*Parabemisia myrcae* and *Aleuralva jasmine*).

### 2.2 Pesticides

Two groups of pesticides were used (at the recommended doses in addition to higher and lower concentrations) against piercing-sucking insects obtained from Starchem Industrial Chemicals, Plot 35, 6<sup>th</sup> Industrial Zone, 6<sup>th</sup> of October City, Cairo, Egypt.

The first group is a group of chemical pesticides i.e. Chinook® 35% SC {(E)-1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine} and Mospilan® 20% SP {(E)-N<sup>1</sup>-(6-chloro-3-pyridylmethyl)-N<sup>2</sup>-cyano-N<sup>1</sup>-methylacetamidine),

The second group is a group of natural pesticides i.e. Techno Oil®; it is a plant-origin product that contains a group of vegetable oils in addition to L-glutamic acid and Glutamic acid stimulates the defensive enzyme systems against insect and fungal infestation. It also affects the central nervous system in insects. Top 9® (Naphthalene acetic acid (NAA) 0.05%, Chitosan 0.1% and Natural components 99.85%).

### 2.2 Controlling mealybug species on mulberry trees (*M. nigra*)

Twenty-seven mulberry trees were treated with three pesticides; Chinook®, Techno Oil® and Top 9®. Three concentrations were used for each pesticide, Chinook (3, 4 and 5 cm<sup>3</sup> in 5 liters of water), Techno Oil (6, 8, 10 cm<sup>3</sup> in 5 liters of water) and Top9, (4, 6, 8 cm<sup>3</sup> in 5 liters of water). Each concentration was applied in three replicates on trees in addition to three trees sprayed only with water (as control). Ten leaves of mulberry were collected from each treated tree after 10, 20 and 30 days of treatment. Individuals of *Icerya* sp. and *Ferrisia virgate* were counted and the rate of reduction in infestation was calculated and compared to the numbers of insects that were counted before treatment.

### 2.3 Control of whitefly species on mulberry trees (*M. alba*)

Twelve mulberry trees were treated with two pesticides, Mospilan® and Techno Oil. Two concentrations of each pesticide were prepared and applied, Mospilan 0.5 and 0.1 g per 2 liters of water, and Techno Oil, 2.5, 4 cm<sup>3</sup> per 2 liters of water. Each concentration was sprayed on three trees (3 replicates) in addition, 3 trees were sprayed with water only (for comparison). Ten leaves of mulberry were collected from each treated tree after 10, 20 and 30 days of treatment; the nymphs of whiteflies were counted and the rate of reduction in infestation was calculated compared to the numbers of nymphs that were counted before the treatment.

To calculate the rate of reduction in the pest population, the formula of Henderson and Tilton (1955) was used as follows:

Reduction rate =  $\{1 - (Cb \times Ta / Ca \times Tb)\} \times 100$ , where

Cb = number of insects in control before treatment

Ca = number of insects in control after treatment

Tb = number of insects before treatment

Ta = number of insects after treatment

## 2.4 Toxicity of the tested materials on larvae of the mulberry silkworm

The 4<sup>th</sup> instar larvae of silkworm, *Bombyx mori*, were reared under laboratory conditions with the appropriate temperature and relative humidity. The treatments were conducted on 225 larvae, which were divided into 39 boxes; each box contained five larvae. Each concentration of the used pesticides was applied to larvae in three boxes (replicates); in addition to 6 boxes of larvae fed on untreated leaves of *M. nigra* and *M. alba* as control. The corrected mortality of treated larvae was calculated after 2 and 8 days of feeding according to the formula of Schneider-Orelli (1945) as follows: Corrected mortality =  $\{\text{number of dead larvae in treatment} - \text{number of dead larvae in control}\} / (100 - \text{number of dead larvae in control}) \times 100$

## 3 Results and Discussions

### 3.1 Control of *Icerya* sp. on mulberry trees (*M. nigra*)

Data in **Table 1** show that numbers of *Icerya* sp. were affected by different concentrations of the chemical pesticide Chinook and natural compounds, Techno oil and Top 9. The lowest numbers of *Icerya* sp. were found when trees were treated with 8 cm<sup>3</sup>/L water (0.7 individuals) followed by Tecno oil at a concentration of 10 cm<sup>3</sup>/L water (1.0 individual) and the third one was Chinook at 5 cm<sup>3</sup>/L water (2.3 individuals). Accordingly, the average rates of infestation reduction reached 94.23% in the treatment of the Chinook at 5 cm<sup>3</sup>/5L water, while reduction rates occurred when using the natural compounds (Techno Oil and Top 9) were 97.52% at 10 cm<sup>3</sup>/5L water and 98.23% at 8 cm<sup>3</sup>/5L water respectively (**Table 2**).

### 3.2 Control of *Ferrisia virgate* on mulberry trees (*M. nigra*)

As shown in **Table 3**, the numbers of *Ferrisia virgate* were also highly affected by the materials sprayed on mulberry trees (*M. nigra*). Numbers of *F. virgate* decreased, after 10, 20 and 30 days from treatment, with different concentrations of Chinook, Techno Oil and Top 9. The lowest average numbers were 0, 0, and 0.7 individuals for Techno Oil at 10 cm<sup>3</sup>/L water, followed by Top 9 at 8 cm<sup>3</sup>/L water and the last one, Chinook at 5 cm<sup>3</sup>/L water. Reduction in rates of infestation with this insect reached 92.56% by treatment with Chinook, 95.80% with Techno Oil and 96.50% with Top 9 pesticides (**Table 4**).

### 3.3 Control of white flies on mulberry trees (*M. alba*)

Mulberry trees, species *M. alba*, were infested with whiteflies more than mealybugs. The pesticide, Mospilan and the natural oil, Techno Oil, were used to control whiteflies. Whitefly numbers were affected by these pesticides when applied at each of the three concentrations. Mospilan was significantly more potent than Techno Oil where the average numbers of whitefly nymphs were 0.0 and 8.7 respectively (**Table 5**). The reduction rate in whitefly nymphs reached 100% when infested trees were treated with Mospilan at 1.0 g. /2 L water and 94.94% when applying Techno Oil at 4.0 cm<sup>3</sup> /2 L water (**Table 6**).

Most of the chemical pesticides used against mulberry pests such as whiteflies and mealybugs are effective but without a study or knowledge of their effect on silkworm larvae in terms of death rates or their productivity from pupae and eggs. Yeshika et al (2020a) reported that Buprofezin, Pymetrozine, Flonicamid, Dinotefuron, Azadirachtin and Dichlorvos were very effective against the pink mealybug, *Maconellicoccus hirsutus*. Tsagkarakis et al (2016) used extracts of *Melia azedarach* against whiteflies. Jiequn et al (2020) used chemical pesticides against *Diaghania pyloalis*.

### 3.4 Toxicity of the tested compounds on larvae of mulberry silkworms

Mulberry silkworms are very sensitive to any compound applied on mulberry trees more than mealybugs and whiteflies. Accordingly, the larvae of *Bombyx mori* were affected with chemical pesticides than natural pesticides. In **Table 7**, Techno Oil and Top 9 were too safe when applied to the leaves of mulberry and offered to larvae of mulberry silkworms to feed than Chinook. Since the corrected mortality reached 0% and 6.7% at the concentration of 10 cm<sup>3</sup> /5L water for Techno Oil and 8 cm<sup>3</sup> /5L water for Top 9 after 8 days of feeding *B. mori* larvae on treated *M. nigra* leaves, respectively.

**Table 1.** Numbers of *Icerya* sp. at pre- and post-treatments with different concentrations of Chinook, Techno Oil and Top 9 on mulberry trees (*M. nigra*)

Pesticide	Conc. cm <sup>3</sup> /5 L water	Pre-treatment count	Post-treatment counts after			Average number of post- treatment counts/10 leaves
			10 days	20 days	30 days	
Chinook	3	33	3	5	6	4.7
	4	31	3	4	6	4.3
	5	34	1	2	4	2.3
	0	29	33	30	39	34.0
Techno Oil	6	32	5	8	10	7.7
	8	31	2	3	5	3.3
	10	30	0	0	3	1.0
	0	29	33	30	39	34.0
Top 9	4	31	2	3	7	4.0
	6	30	1	2	4	2.3
	8	28	0	0	2	0.7
	0	29	33	30	39	34.0

**Table 2.** Rate of reduction in *Icerya* sp. individuals infesting mulberry trees (*M. nigra*) treated with different concentrations of Chinook, Techno Oil and Top 9

Pesticide	Conc. cm <sup>3</sup> /5 L. water	Post-treatment counts after %			Average number of post- treatment counts /10 leaves
		10 days	20 days	30 days	
Chinook	3	92.01	85.35	86.48	87.85
	4	91.50	87.53	85.61	88.17
	5	97.42	94.31	88.24	94.23
Techno Oil	6	86.27	75.83	76.76	79.62
	8	94.33	90.65	88.01	90.99
	10	100.00	100.00	92.56	97.52
Top 9	4	94.33	90.65	83.21	89.40
	6	97.07	93.56	90.08	93.57
	8	100.00	100.00	94.69	98.23

**Table 3.** Numbers of *Ferrisia virgate* in pre- and post-treatment with different concentrations of Chinook, Techno Oil and Top 9 on mulberry trees (*M. nigra*)

Pesticide	Conc. cm <sup>3</sup> /5L. water	Pre-treatment count	Post- treatment counts after			Average number of post- treatment counts/10 leaves
			10 days	20 days	30 days	
Chinook	3	15	3	3	0	2.0
	4	15	3	2	0	1.7
	5	16	2	0	0	0.7
	0	17	10	11	9	10.0
Techno Oil	6	16	0	1	4	1.7
	8	18	0	1	2	0.3
	10	15	0	0	1	0.0
	0	17	10	11	9	10.0
Top 9	4	15	2	3	5	3.3
	6	17	1	2	4	2.3
	8	18	0	0	1	0.0
	0	17	10	11	9	10.0

**Table 4.** Reduction rate of *Ferrisia virgate* on mulberry trees (*M. nigra*) treated with different concentrations of pesticide Chinook, Techno Oil and Top 9

Pesticide	Conc. cm <sup>3</sup> /5 L. water	Post- treatment counts after			Average number of post- treatment counts/10 leaves
		10 days	20 days	30 days	
Chinook	3	66	69.1	100	77.33
	4	66	79.39	100	80.73
	5	78.75	100	100	92.56
Techno Oil	6	100	90.34	52.78	81.04
	8	100	91.41	79.01	90.14
	10	100	100	87.41	95.80
Top 9	4	77.33	69.09	37.03	61.15
	6	90.00	81.82	55.56	75.79
	8	100.00	100.00	89.51	96.50

**Table 5.** Numbers of whitefly nymphs in pre- and post-treatment with different concentrations of Mospilan and Techno oil on mulberry trees (*M. alba*)

Pesticides	Conc.	Pre-treatment count	Post- treatment counts after			Average number of post- treatment counts/10 leaves
			10 days	20 days	30 days	
Mospilan g. /2 L. water	0.5	168	3	5	7	5.0
	1.0	177	0	0	0	0.0
	0	172	175	173	160	169.3
Techno Oil cm <sup>3</sup> /2 L. water	2.5	166	13	15	17	15.0
	4.0	175	6	10	10	8.7
	0	172	175	173	160	169.3

**Table 6.** Reduction rate of whitefly nymphs on mulberry trees (*M. alba*) treated with different concentrations of Mospilan and Techno Oil

Pesticides	Conc. g. /2 L. water	Post- treatment counts after			Average number of post- treatment counts/10 leaves
		10 days	20 days	30 days	
Mospilan g. /2 L. water	0.5	98.24	97.04	95.52	96.93
	1.0	100.00	100.00	100.00	100.00
Techno Oil cm <sup>3</sup> /2L. water	2.5	92.30	91.02	88.99	90.77
	4.0	96.63	94.32	93.86	94.94

**Table 7.** Corrected mortality for larvae of mulberry silkworms fed on mulberry leaves (*M. nigra*) treated with different concentrations of Chinook, Techno Oil and Top 9.

Pesticide	Conc. cm <sup>3</sup> /5L. water	Duration	
		2 days	8 days
Chinook	3	70.6	66.7
	4	100	73.3
	5	100	93.3
Techno Oil	6	19.45	0
	8	26.70	0
	10	34.06	0
Top 9	4	48.68	13.3
	6	85.38	6.7
	8	92.64	6.7

On the other hand, the corrected mortality reached 93.3% at 5 cm<sup>3</sup>/5L water for Chinook after 8 days of feeding and thus Chinook is considered very toxic to larvae of *B. mori*.

Data in **Table 8** show that Mospilan was also very toxic to larvae of mulberry silkworms, while Techno Oil is very safe for the larvae. The correct mortality was 100% at 1.0 g / 2L water for Mospilan. On the other hand, no mortality occurred when larvae of mulberry silkworms fed on leaves treated with Techno Oil.

There are pesticides, when used to control pests on mulberry trees, may affect mulberry leaves, which lead to a decrease in their nutritional value. Thus, when the larvae are fed on them, their weights and production of pupae, eggs, and weights are affected. There was no change in the death rates of the larvae (Mahadeva

2018). Kumar et al (2017) and Jyothi et al (2019) found that the residuals of pesticides in the soil under mulberry trees negatively affect the quality of cocoons. Yeshika et al (2020b) found that Flonicamid (a novel insecticide) decreased the weight of cocoons and larvae when compared with the control. Bhagyamma and Kumari (2022) found that the infestation of mulberry leaves with leaf roller, *Diaphania pulverulentalis* decreased the quality of leaves. Kumutha et al (2013) reported that Dichlorvos and Vijay neem pesticides reduced the fertility and fecundity of silkworm moths. Soliman and El Sheriff (2019) found that the quality of cocoons decreased when mulberry trees were sprayed with Cymbush® and Tracer®.

Another group of pesticides used in controlling pest infestation showed that they are highly toxic to silkworm larvae, causing high death rates such as Procymidone (He et al 2022), Dinotefuran, a neonicotinoid pesticide (Xu et al 2022), and Phoxim (an organophosphate pesticide). Guo et al (2021) were able to reduce the toxicity of Phoxim to silkworms when pretreated using  $\text{Fe}^{2+}$ ,  $\text{Cu}^{2+}$ , and  $\text{Rb}^{+}$ . Chen et al (2020) found bacteria in the gut of silkworms cause resistance against the toxic effect of organophosphate insecticides. Yeshika et al (2019) found that the Flonicamid was very toxic to silkworms. Kordy (2014) found that Vertimec and Proclaim were very toxic when applied to the 4<sup>th</sup> instar larvae of silkworms. Munhoz et al (2013) found that the high concentrations of Chlorantraniliprole were not safe but when used with low concentrations they were safe.

Therefore, breeders of silkworm larvae are advised to use safe pesticides and plant extracts that are non-toxic to the larvae and do not affect death rates or productivity, Kordy (2014) found that Takumi is very safe for silkworms. Yeshika et al (2019) found that Buprofezin, Pymetrozine, Dinotefuron, Azadirachtin and Dichlorvos pesticides were not toxic to silkworms. Jiequn et al (2020) found that Imidacloprid and Chlorfenapyr at low concentrations were nontoxic to silkworms.

There are many natural compounds that were applied to the larvae for different purposes and were safe, Mukhopadhyay and Kumar (2010) used Neem oil, Pongamia oil and Nicotine extract to control pests on mulberry trees. Tsagkarakis et al (2016) found that extracts of *Melia azedarach* were safe for silkworms. Abd El-Razek et al (2021) used natural compounds such as Salicylic acid, Henna

powder and Propolis against fungal diseases in silkworms and they were safe for the worms. Fajfer and Łochyńska (2022) used Grapefruit extract on mulberry trees, it was not toxic to silkworms but it reduced the quality of cocoons.

**Table 8.** Corrected mortality for larvae of mulberry silkworms fed on mulberry leaves (*M. alba*) treated with different concentrations of Mospilan and Techno Oil.

Pesticides	Duration Conc.	2 days	8 days
Mospilan g./2 L. water	0.5	78.26	64.57
	1.0	100.00	100.00
Techno Oil cm <sup>3</sup> /2L. water	2.5	18.8	0
	4.0	34.7	0

## 4 Conclusion

The rate of mealybugs and whiteflies infestations was reduced by treating mulberry trees with different concentrations of chemical and natural pesticides, which, in the meantime, are safe for silkworm larvae. Mospilan and Chinook proved to be effective pesticides against pests of mulberry trees, but they were not safe for silkworm larvae. On the other hand, the natural compounds, Techno oil and Top 9 proved to be effective on pests of mulberry trees and meanwhile safe for silkworm larvae.

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