Screening of insecticides against the mulberry fruit gall midge *Cotarina* sp.

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Abstract. In recent years, a kind of mulberry pests which were known as the mulberry gall midge *Cotarina* sp. in different mulberry planting areas occurred popularly in China. This study aims to screening high efficacy and low toxicity insecticides for controlling *Cotarina* sp.. The current study can help understand integrated pest management (IPM) of *Cotarina* sp. by scientific and reasonable insecticide use. Field experiment was carried out to investigate eight insecticides, treated with 3 concentration gradients. The result shows that Imidacloprid, Thiamethoxam, Cyromazine, Bifenthrin and Cypermethrin·Profenofos had high control effect on the mulberry gall midge. Their pesticide residues were all less than Chinese Standard GB2763-2016, which means that they were feasible to control this pest. This paper shows that, to control *Cotarina* sp. in fruit mulberry production, 10% Imidacloprid WP with 2000~3000 dilution and 25% Thiamethoxam WDG with 1500~2500 dilution and 80% Cyromazine WDG with 1500~2000 dilution are the best. 2.5% Bifenthrin EW with 1000~1500 dilution and 440g/l Cypermethrin·Profenofos EC with 1000~2000 dilution should be used by selection. However, Bifenthrin or Cypermethrin·Profenofos cannot be used in mulberry field for both fruits and leaves, so as to avoid causing silkworm poisoning.

1 Introduction

Cotarina sp. (Diptera: Nematocera: Cecidomyiidae) is the main pest on mulberry^[1], known as mulberry fruit gall midge, first discovered in the 1980s, and distributed in the Guangzhong area of Shaanxi in China^[2]. The pest is parasitic in the small fruit of mulberry, resulting in malformation of mulberry fruit and abnormal development of mulberry seeds^[1-4]. Mulberry fruit gall midge and mulberry fruit sclerotiniosis are commonly known as "one disease and one worm"^[5]. Zhisong Xia (2005) reported that the mulberry fruit gall midge made serious damages in Sichuan-Shaanxi area. The damage rate of mulberry fruit was as high as 90%^[6]. In recent years, some scholars have reported the occurrence of *Cotarina* sp. in Chongqing- Sichuan and other area^[7-9]. It is mainly about the disease area, biological characteristics, cultivation techniques, etc. However, few studies have been done on the control of mulberry fruit gall midge. Therefore, this study is carried out screening of high-efficiency and low-toxicity insecticides for controlling *Cotarina* sp., providing the theory basis for resolving the problem of the pest in scientific, proper, economical and effective ways.

2 Materials and Methods

2.1 Experimental materials

The experiment was carried out at the Ganning Base of the Chongqing Three Gorges Academy of Agricultural Sciences. The experimental site is 325 m above sea level. Flat terrain, uniform fertility and consistent field management was choiced for experiment. The test soil was sandy loam soil, with thickness equal to or higher than 1.0 m.

The mulberry variety used in this experiment was hongguo no.2 with planting density of 9000 plants /hm².

2.2 Experimental treatments

In the spring of 2017-2018, insecticides were selected for testing listed in table 1. The first and second spraying were carried out in peak flower stage and late flower stage. Field experiment was carried out to investigate eight insecticides, each of which treated with 3 concentration gradients. Each concentration was sprayed forty-nine trees. Control was sprayed by clean water. The shape of the plot was square with basically the same area, around which guardrows established. The insecticides were sprayed evenly and thoroughly on the mulberry trees by using electric sprayers after the dew dried up in the morning. The total number of mulberry fruits and wormy mulberry fruits was investigated when the color of mulberry fruit began to turn red. Investigated the number of parasites by picking wormy mulberry fruits, then calculated wormy fruit rate and correction control effect. Maximum headage refers to the largest number of larvae in 20 wormy fruits. Average headage

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means the average number of larvae in each mulberry fruit during one month of continuous investigation. The following is the calculation formula^[10]: Wormy fruit rate = Total number of mulberry fruits / Number of wormy mulberry fruits $\times$ 100%; Correction control effect

= (Wormy fruit rate in control group - Wormy fruit rate in treatment group) / Wormy fruit rate in control group $\times$ 100%.

Table 1. Insecticides information.

Year	Insecticides	Content/ Formulation	Manufacturer	Type
2017/2018	Imidacloprid	10% WP	Shandong Jiacheng crop science Co. Ltd	Neonicotinoids
2017/2018	Thiamethoxam	25%WDG	Jiangsu Changqing agrochemical Co. Ltd	Neonicotinoids
2017/2018	Cyromazine	80% WDG	Guangdong Zhongxun agricultural science Co. Ltd	Insect growth regulators
2018	Chlorfenapyr	8% ME	Guangxi pastoral biochemistry Co. Ltd	Novel pyrrole compounds
2018	Bifenthrin	2.5% EW	Chengdu kelilong biochemical Co. Ltd	Pyrethroids
2018	Cypermethrin·P rofenofos	440g/L EC	Shandong Jiacheng crop science Co. Ltd	Compound pesticide
2017	Pymetrozine	50% WP	Jiangxi Weiniu crop science Co. Ltd	Pyridine
2017	Abamectin	1.8% EC	Yifan biotechnology group Co. Ltd.	Macrocyclic lactones

2.3 Data analysis

Statistical analysis of data was performed by using Microsoft Office 2010 and IBM SPSS 16.0. One-Way ANOVA was used to conduct difference analysis, and the data was expressed $\bar{x} \pm s$ (n=3).

3 Results and analysis

3.1 Field trials control efficacy of various insecticides against *Cotarina* sp.

Field trials control efficiency of five insecticides were tested in spring 2017. The results showed that they had certain control effect on *Cotarina* sp.. The control efficacy of 10% imidacloprid WP, 25% Thiamethoxam WDG and 80% Cyromazine WDG was significantly higher than that of control group (shown in table 2). 10% imidacloprid WP and 25% Thiamethoxam WDG, of which the correction control effects were more than 97%, no significant difference between different concentrations, significantly higher than that of other insecticides, gained the best control effects against *Cotarina* sp.. 80% Cyromazine WDG was significant difference between 1500 times solution and 2000~2500 times solution. The correction control effect of 1500x

was 86.70%, gained the second field trials control efficacy. 50% Pymetrozine WP gained poor controlling effect. The 1.8% Abamectin EC's trials control efficiency was less than 20%, gained the worst insecticide for controlling of *Cotarina* sp..

Field trials control efficiency of six insecticides were tested in spring 2018 on the basis of 2017. The result shows that the control efficacy of different insecticides was different, which had certain control effect on *Cotarina* sp. (shown in table 3). In general, the control efficiency increased with the dosage increased. 10% imidacloprid WP and 25% Thiamethoxam WDG, the correction control effects more than 99%, no significant difference between different concentrations, gained the best control effects against *Cotarina* sp.. 80% Cyromazine WDG gained the second effective, more than 95%, which was significantly improved compared with the 2017's test. 8% Chlorfenapyr ME, 2.5% Bifenthrin EW and 440 g/l Cypermethrin·Profenofos EC had certain control effect on *Cotarina* sp.. The correction control effects of 8% Chlorfenapyr ME with 1500~2000 dilution was more than 91%. The correction control effects of 2.5% Bifenthrin EW with 1000~500 dilution was more than 93%. The correction control effects of 440 g/l Cypermethrin·Profenofos EC was more than 84%. There was significant difference between different concentrations.

Table 2. Control effect of different insecticides dosages on *Cotarina* sp. in 2017

Fungicides	Dilution times	Total number of mulberry fruits	Number of wormy mulberry fruits	Wormy fruit rate/%	Correction control effect/%	Maximum headage /heads	Average headage /heads
Abamectin	3000	432.00±34.27n	364.67±29.17c	84.40±0.23e	10.18±0.24e	316.00±14.73d	10.53±0.49d
	3500	471.33±23.88n	424.00±23.50cd	89.92±0.47f	4.31±0.50f	511.33±26.03e	17.04±0.87e
	4000	434.67±14.95n	398.00±14.29c	91.56±0.14fg	2.57±0.15fg	653.33±48.26f	21.78±1.61f
Imidacloprid	2000	470.67±31.06n	4.00±0.58a	0.84±0.08a	99.10±0.09a	4.67±1.20a	0.16±0.04a
	2500	464.67±45.06n	6.00±0.58a	1.30±0.13a	98.61±0.14a	17.00±1.73a	0.57±0.06a

	3000	502.33±13.34n	11.00±1.15a	2.18±0.18a	97.68±0.19a	29.33±2.33a	0.98±0.08a
	2000	504.33±8.01n	396.33±11.20c	78.67±3.18d	16.28±3.38d	486.67±9.39e	16.22±0.31e
Pymetrozine	2500	484.33±43.18n	383.67±43.21c	78.86±2.24d	16.08±2.39d	514.33±5.70e	17.14±0.19e
	3000	485.00±43.25n	406.67±34.80cd	83.90±0.30e	10.72±0.32e	523.33±6.94e	17.44±0.23e
	1500	523.67±26.41n	5.0±0.58a	0.95±0.066a	98.99±0.07a	1.67±0.33a	0.06±0.01a
Thiamethoxam	2000	511.67±38.35n	9.33±0.88a	1.82±0.053a	98.06±0.06a	8.33±1.20a	0.28±0.04a
	2500	532.00±22.34n	13.33±0.88a	2.50±0.60a	97.34±0.06a	12.33±1.76a	0.41±0.06a
	1500	537.67±24.01n	67.00±4.93ab	12.50±1.05b	86.70±1.11b	102.00±4.93b	3.40±0.16b
Cyromazine	2000	519.00±31.34n	90.33±13.30b	17.31±1.89c	81.58±2.01c	144.67±3.48b	4.82±0.12b
	2500	487.33±27.39n	98.33±2.40b	20.25±0.62c	78.45±0.66c	228.33±4.98c	7.61±0.17c
CK		502.33±59.30n	472.67±58.37d	93.97±0.54g	0g	519.67±40.07e	17.32±1.34e

Values followed by different letters at the same column indicate significant difference ($P<0.05$), and those followed by the same letters indicate no significant difference ($P\geq0.05$), the same as below.

Table 3. Control effect of different insecticides dosages on *Cotarina* sp. in 2018

Fungicides	Dilution times	Total number of mulberry fruits	Number of wormy mulberry fruits	Wormy fruit rate/%	Correction control effect/%	Maximum headage /heads	Average headage /heads
	2000	503.33±45.27n	3.33±2.40a	0.59±0.40ab	99.36±0.44ab	2	0.1
Imidacloprid	2500	470.00±29.60n	1.33±1.33a	0.32±0.32a	99.66±0.34a	2	0.1
	3000	461.33±7.42n	0a	0a	100a	2	0.1
	1500	492.67±43.67n	3.33±1.20a	0.68±0.26ab	99.26±0.28ab	1	0.05
Thiamethoxam	2000	473.33±76.34n	1.00±1.00a	0.17±0.17a	99.81±0.18a	4	0.2
	2500	468.00±62.68n	2.67±2.67a	0.68±0.68ab	99.25±0.75ab	3	0.15
	1500	571.33±25.98n	19.67±2.33ab	3.43±0.32abc	96.26±0.35abc	40	2
Cyromazine	2000	551.33±36.45n	22.33±2.85ab	4.15±0.81bc	95.47±0.88abc	41	2.05
	2500	481.00±27.57n	13.33±2.40ab	2.82±0.60abc	96.92±0.66abc	39	1.95
	1000	496.67±35.41n	115.00±12.17e	23.05±0.85g	74.85±0.93g	22	1.1
Chlorfenapyr	1500	466.67±62.50n	15.33±5.04ab	3.17±0.70abc	96.54±0.77abc	40	2
	2000	501.00±25.70n	41.33±11.20bc	8.17±2.14de	91.09±2.34 de	45	2.25
	1000	560.67±10.97n	16.67±1.20ab	2.97±0.21abc	96.76±0.23abc	2	0.1
Bifenthrin	1500	500.00±24.58n	29.33±7.97ab	5.83±1.51cd	93.63±1.65cd	8	0.4
	2000	518.00±28.38n	121.67±5.70e	23.51±0.38g	74.34±0.42g	86	4.3
	1000	546.67±96.09n	58.33±16.05cd	10.33±0.98e	88.72±1.07e	49	2.45
Cypermethrin	1500	535.33±34.07n	77.00±14.57d	14.49±2.89f	84.18±3.16f	61	3.05
·Profenofos	2000	502.00±45.43n	75.00±16.37d	14.57±2.15f	84.09±2.34f	86	4.3
CK		511.33±23.50n	468.67±23.14f	91.63±0.83h	0 h	1650	82.5

Table 4. Measurement of the content of residual insecticide in the treated mature mulberry fruits

Residue definition	Dilution times	Maximum residue limit(mg/kg)	Detection limit	Detection result
Imidacloprid	2000	0.05	5.50 ug/kg	not detected
Thiamethoxam	1500	0.1	no	0.048 mg/kg
Cyromazine	1500	0.5	0.02 mg/kg	not detected
Bifenthrin	1500	1	no	0.039 mg/kg
Cypermethrin	1000	0.07	no	0.032 mg/kg
·Profenofos		0.05	0.04 mg/kg	not detected

At present, there is no specific maximum residue limit for mulberry in the national standard for food safety^[11]. Therefore, the food with the minimum residue limit was selected as the reference among grains, oils, vegetables, fruits, sugar and beverages in this experiment. The residue of imidacloprid has reference to fresh maize. The residue of thiamethoxam has reference to sugarcane. The residue of cyromazine has reference to string-pea. The residue of bifenthrin and cypermethrin have reference to strawberries. The residue of profenofos has reference to apple.

3.2 Residual analysis

Mulberry residue detection was carried out after 35 days of insecticide application by picking mulberry fruits in the high concentration area of the five insecticides which had better control effect (shown in table 4). The result shows that no residue was detected in the sample sprayed with imidacloprid 2000 times solution. The residual amount of the sample sprayed with thiamethoxam 1500 times solution was only 0.048 mg/kg, and no residue was detected in the sample sprayed with cyromazine 1500 times solution. The residual amount of the sample sprayed with bifenthrin was only 0.039 mg/kg. In the sample sprayed with Cypermethrin·Profenofos, the residual amount of cypermethrin was only 0.032 mg/kg, and profenofos was not detected. The pesticide residues in this study were significantly less than Chinese Standard GB2763-2016.

4 Conclusions and discussion

There are two kinds of chemical control for mulberry gall mosquitoes: First, soil medication^[3]; Second, tree spraying^[1,12-14]. It has been reported that the control effect of tree spraying is better than that of soil medication^[1]. Therefore, tree spraying method was used in this study. However, tree spraying method mainly used 40% dimethoate emulsion 1000 times solution^[1,12-14] and 10% imidacloprid 2000 times solution^[12-14]. Among them, only the control effect of 40% dimethoate emulsion has been reported to control *Cotarina* sp.^[1], while the effect of 10% imidacloprid has been rarely reported. Therefore, 10% imidacloprid WP 2000~3000 times solution was used in this study to verify its control effect on *Cotarina* sp.. Also, pesticide residues was analyzed, but no residue was detected in the sample. According to the results of chemical control and residue analysis, it is feasible to select the concentration of 10% imidacloprid WP 2000~3000 times.

Among 7 kinds of insecticides selected in this study, 4 kinds of insecticides had good control effect on *Cotarina* sp.. Among the new insecticides in this study, imidacloprid and thiamethoxam had the best control effects against *Cotarina* sp.. Cyromazine, chlorfenapyr, cifenthrin and cypermethrin·profenofos had good control effects, which were closely related to its unique chemical structure and novel mechanism. Imidacloprid and thiamethoxam, mainly act on the central nervous system

of insects, the inhibitors of nicotinic acetylcholine receptors, keeps nerve impulses going, disrupting the normal transmission of nervous system signals and killing insects, are neonicotinoids^[15]. Cyromazine, an insect molting hormone analogue, the characteristics of strong selectivity, low toxicity, friendly environment and resistance to pests, is insect growth regulators. If used properly, it has little impact on the natural enemies of pests^[16]. In this experiment, its control effect was inferior to imidacloprid and thiamethoxam, which may be related to its internal absorption and conduction, still need to further research. Chlorfenapyr, mainly obstructs the respiration of insects through the de-coupling of oxidative phosphorylation, making the insects unable to produce energy and leading to paralysis of the body. So that they cannot carry out normal physiological activities and die, own the characteristics of wide insecticidal spectrum, high control efficiency and long duration, is a novel pyrrole compound^[17]. However, the ripening time of mulberry fruit in the test area was 5 days later than other areas. It can be further studied that whether this was caused by application of remains of chlorfenapyr. Bifenthrin is a pyrethroid insecticide. Cypermethrin·profenofos is a compound of pesticides. They all contain pyrethroids, due to pyrethroid pesticides have strong toxicity and extremely long residual effect on silkworm^[18]. Bifenthrin or Cypermethrin·Profenofos cannot be used in mulberry field for both fruits and leaves, so as to avoid causing silkworm poisoning.

Imidacloprid, Thiamethoxam, Cyromazine, Bifenthrin and Cypermethrin·Profenofos which can be recommended as novel insecticides had high control effect on *Cotarina* sp.. To control *Cotarina* sp. in fruit mulberry production, 10% Imidacloprid WP with 2000~3000 dilution and 25% Thiamethoxam WDG with 1500~2500 dilution and 80% Cyromazine WDG with 1500~2000 dilution are the best. 2.5% Bifenthrin EW with 1000~1500 dilution and 440g/l Cypermethrin·Profenofos EC with 1000~2000 dilution should be used by selection.

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