Resistance Monitoring of Field Populations of *Panonychus citri* to Three Pesticides

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Abstract [**Objectives**] The paper was to investigate the development of *Panonychus citri* resistance to commonly used pesticides in major citrus producing areas in China, and to screen out new green and efficient agents for its control and prevention. [**Methods**] The resistance changes of field populations of *P. citri* to abamectin, pyridaben, and bifenazate in 12 locations across five provinces in China were compared using the leaf disc impregnation method. [**Results**] *P. citri* in the tested areas exhibited the most severe resistance to abamectin, with approximately 91.7% of the field populations showing high levels of resistance to abamectin (112.1 – 560.5 times); 50% of *P. citri* populations exhibited high levels of resistance to pyridaben (123.0 – 202.7 times), while the remaining populations showed intermediate levels of resistance (25.6 – 80.3 times); except for the Zhejiang Xiangshan 2019 and Jiangxi Yudu 2019 populations, which exhibited a medium level of resistance to bifenazate, the remaining 10 monitored populations demonstrated a high level of resistance to bifenazate (140.4 – 686.1 times). [**Conclusions**] It is advisable to discontinue the use of abamectin and bifenazate due to significant resistance observed in populations of *P. citri* monitored in 12 locations across 5 provinces. It is recommended to reduce the frequency of pyridaben use and alternate with other acaricides that have different mechanisms of action due to the varying degrees of resistance developed.

Key words *Panonychus citri*; Resistance; Pesticide; Biological activity

1 Introduction

Citrus is the most commonly cultivated fruit in China, with the largest production area and output[1]. *Panonychus citri* McGregor, also known as the citrus red mite, is a significant insect pest in citrus production, belonging to the Tetranychidae family of Acariforms[2]. The occurrence of generations each year is closely related to the temperature of that year. When the average annual temperature is around 20 ℃, approximately 20 generations in a year[3]. The species typically survives the winter as eggs on blades and branch cracks. In March, these overwintering eggs hatch in large quantities as temperatures rise to the optimal level for growth and development. This results in mass propagation, which endangers the spring shoots. The pest mite has two peak periods of occurrence in a year: April – May and September – October. During the summer months, high temperatures lead to a decline in the insect population. However, as the temperature drops in autumn, it becomes more suitable for the growth and development of *P. citri*, which can harm to autumn shoots[4].

P. citri is primarily found in several citrus producing regions in China, such as Guangxi, Guangdong, Jiangxi, Sichuan, Zhejiang, Jiangsu, Hunan, Hubei, and other provinces. The host range of this organism is quite extensive, affecting up to 112 plant species, primarily in the Brassicaceae and Rosaceae families. It has been known to cause damage to cash crops such as *Osmanthus fragrans* and *Clausena lansium*[5-6].

P. citri primarily damages citrus leaves and fruits. Grouped adult, young, nymph mites suck sap from leaves, shoots, and pericarps, causing defoliation and fruit drop. The leaves are particularly susceptible to damage, with harm occurring on both sides of the leaf blade, and damage is mainly concentrated on the two sides of the midvein and the leaf edge[7]. The leaf surface is infested with densely packed, pinhead-sized gray-white spots, or the entire leaf may become gray and tarnished, eventually falling off. This can significantly impact the strength and yield of the tree[8]. *P. citri* has two reproductive modes: sexual reproduction and parthenogenesis. A generation can be completed in about 16 d on average. The short growth and development cycle of *P. citri* results in a serious generation overlap, which poses great difficulties for its control due to its special reproductive mode[9].

Resistance of *P. citri* to pesticide has been reported since the 1980s. In 1996, Zhang Gebi *et al.*[10] observed that *P. citri* had developed a moderate level of resistance to amitraz; in 2001, Liu Chunrong *et al.*[11] found that *P. citri* in the Quzhou area exhibited a low level of resistance to propargite, with a resistance ratio ranging from 1.9 to 4.2 times; in 2001, Gao Chaoyue[12] conducted an efficacy test of different agents on *P. citri*, and discovered that the species had developed resistance to isocarbophos, an organophosphorus insecticide; Meng Hesheng *et al.*[13] reported that *P. citri*'s resistance to pyridaben increased up to 35 times after 12 generations of indoor continuous progeny selection; Liu Yonghua[14] monitored the resistance of *P. citri* populations in Beibei District, Chongqing for 2 consecutive years from 2008 to 2009. The results of his monitoring showed that the relative resistance ratio of *P. citri* to fenpropathrin and pyridaben increased by 5.1 and

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2.2 times, respectively.

Chemical application has traditionally been the most effective and direct method for controlling *P. citri*. However, due to the frequent and long-term use of acaricides, *P. citri* has developed varying degrees of resistance to commonly used acaricides in Chinese fields, making it difficult to select agents for controlling *P. citri*[15]. To ensure effective prevention and control of *P. citri* and reduce the loss of citrus production in China, it is crucial to focus on the scientific and rational use of existing acaricides to prolong their service life[16]. This is due to the gradual increase in the difficulty of developing new agents. During 2019—2020, we monitored resistance changes in 12 field populations of *P. citri* across five provinces: Guangxi, Sichuan, Hunan, Jiangxi, and Zhejiang, to determine their resistance levels to commonly used pesticides using the leaf disc impregnation method. This information provides a theoretical basis for preventing and controlling *P. citri* in major citrus producing areas and managing drug resistance.

2 Materials and methods

2.1 Materials The *P. citri* susceptible strains were reared in laboratory potted lemons that had not been exposed to acaricides for long periods of time. In 2019 and 2020, researchers collected field populations of *P. citri* from 12 locations across five provinces in China: Guangxi, Sichuan, Hunan, Jiangxi, and Zhejiang. Table 1 presents the specific collection information.

Table 1 Collection information of *Panonychus citri* from different locations in 2019—2020

Province	Location	Population	Collection date	Collected mite state	Larvae number//individual
Guangxi	Yongfu	YF19	June 2, 2019	Nymph, adult	Over 2 000
	Nanning	NN19	June 6, 2019	Nymph, adult	Over 3 000
	Yongfu	YF20	May 29, 2020	Nymph, adult	Over 3 000
	Yangshuo	YS20	July 30, 2020	Nymph, adult	Over 3 000
	Nanning	NN20	June 3, 2020	Nymph, adult	Over 4 000
Sichuan	Meishan	MS19	June 18, 2019	Nymph, adult	Over 4 000
	Meishan	MS20	June 13, 2020	Nymph, adult	Over 3 000
Zhejiang	Xiangshan	XS19	October 22, 2019	Nymph, adult	Over 2 000
	Xiangshan	XS20	October 18, 2020	Nymph, adult	Over 3 000
Jiangxi	Yudu	YD19	October 25, 2019	Nymph, adult	Over 3 000
	Yudu	YD20	September 25, 2020	Nymph, adult	Over 3 000
Hunan	Chenzhou	CZ20	September 12, 2020	Nymph, adult	Over 3 000

2.2 Reagents The study employed 1.8% abamectin EC (Adama Ltd.), 43% bifenazate SC (Shenzhen Noposion International Investment Co., Ltd.), and 15% pyridaben SC (Jiangsu Yangnong Chemical Group Co., Ltd.) as reagents.

2.3 Bioassay methods

2.3.1 Bioassay of adult mites. The leaf disc impregnation method was performed in the following steps. (i) Preparation of leaf disc. Rectangular sponges measuring 8 cm × 2 cm × 1 cm were placed individually in 9 cm Petri dishes. Citrus leaves were collected, washed, and dried before being punched into approximately 3 cm diameter discs. Three leaf discs were then placed on each sponge. Two layers of hollow filter papers measuring 3 cm × 3 cm were prepared using a puncher with a diameter of approximately 2.5 cm. The papers were then nested in leaf discs and a complete leaf disc was obtained by soaking the sponge in a petri dish filled with water. The leaf disc was designed to provide nutrients to the test insects. The sponge was spiked with water to moisturize the leaf disc and prevent female adult mites from escaping.

(ii) Inoculation of mites. Female adult *P. citri* mites were collected using a brush and placed onto leaf discs at a rate of 20 per disc, with three repetitions. After stabilizing the test insects on the leaves for about an hour, the junction of the filter paper and leaf was gently clamped with tweezers to immerse the leaf disc in the prepared solution for 5 sec. Afterwards, the leaf discs were placed on a sponge strip for air-drying naturally until there was no visible water. Finally, the leaf discs were cultured in an incubator set to T = (25 ± 1) ℃, RH = 70% ± 10%, and a photoperiod of 16 : 8 (L : D) h, and those immersed in water was set as control. The time for checking the results was determined based on the agents' speed of action. Mortality of female adult *P. citri* mites was assessed 24 h after treatment with 1.8% abamectin EC, 43% bifenazate SC, and 15% pyridaben SC. The mite's body was lightly touched with the brush tip, and if only one foot was active or completely immobile, it was considered dead. If there were very few mites that escaped from the treatment area, they were not included in the total count of mites.

2.3.2 Bioassay of mite eggs. The leaf discs were prepared using the same method as described above. Female adult *P. citri* mites were first placed onto the leaf discs and allowed to lay eggs for 24 h. The adult female mites on the leaf discs were then removed, and the number of eggs laid on each leaf disc was counted under a dissecting microscope. The leaf disc impregnation and mite culture were performed following the same procedure as that used for the female adult mites. Results were examined after 4 d of treatment for all reagents. Unhatched eggs were considered dead, and the number of hatched eggs was calculated by subtracting the number of dead eggs from the total number of egg grains on each citrus leaf.

2.4 Statistical analysis The *b*-values and their standard errors, LC_{50} values and 95% confidence limits, chi-square values, degrees of freedom, and the resistance ratio were calculated using the POLO-Plus software. The resistance ratio was calculated as follows: $RR = LC_{50}$ value of the field population/LC_{50} value of the sensitive strain.

2.5 Criteria for grading resistance levels The resistance level was assessed using Tang *et al.*'s classification criteria: sensitive ($RR \leqslant 3.0$), decreased sensitivity ($3.0 < RR \leqslant 5.0$), low resistance ($5.0 < RR \leqslant 10.0$), medium resistance ($10.0 < RR \leqslant 100.0$), and high resistance ($RR > 100.0$).

3 Results and analysis

3.1 Establishment of a baseline of relative sensitivity To evaluate the resistance of the commercial formulations used in the test more accurately, we established a baseline of the relative susceptibility of female adult mites and mite eggs of *P. citri* to three pesticides through the leaf disc impregnation method. Table 2 shows the LC_{50} values for abamectin, bifenthrin, and pyridaben against female adult mites, which were 0.131, 0.168, and 0.362 mg/L, respectively.

Table 2 Baseline of relative sensitivity of *Panonychus citri* to three pesticides

Pesticide	LC_{50} // mg/L	Slope ± SE	X^2 (df)
Abamectin	0.131 (0.095 – 0.170)	1.555 ± 0.214	0.762(3)
Pyridaben	0.362 (0.256 – 0.481)	1.387 ± 0.204	0.297(3)
Bifenazate	0.168 (0.122 – 0.218)	1.546 ± 0.213	0.864(3)

3.2 Resistance of field populations to pyridaben In 2019—2020, the resistance of female adult mites to pyridaben was monitored in 12 field populations of *P. citri* across five provinces: Guangxi, Sichuan, Jiangxi, Hunan, and Zhejiang, using the leaf disc impregnation method. The results are presented in Table 3.

Table 3 Resistance of female adult *Panonychus citri* mites to pyridaben in 2019—2020

Population	Slope ± SE	LC_{50} // mg/L	X^2 (df)	RR
YF19	0.879 ± 0.168	17.761 (9.763 – 27.688)	1.552 (3)	49.1
YF20	1.267 ± 0.207	45.467 (31.930 – 63.874)	1.408 (3)	125.6
NN19	1.672 ± 0.143	66.096 (36.980 – 105.744)	11.396 (3)	182.6
NN20	1.175 ± 0.156	73.379 (50.874 – 99.353)	0.698 (4)	202.7
YS20	1.320 ± 0.209	44.525 (28.769 – 61.055)	0.901 (3)	123.0
MS19	1.611 ± 0.258	68.934 (50.250 – 92.132)	1.574 (3)	190.4
MS20	1.276 ± 0.206	50.517 (32.862 – 69.708)	0.468 (3)	139.5
XS19	1.571 ± 0.243	9.262 (6.924 – 12.030)	0.569 (3)	25.6
XS20	1.144 ± 0.197	19.616 (11.740 – 27.806	0.458 (3)	54.2
YD19	1.281 ± 0.213	27.576 (18.617 – 37.102)	0.153 (3)	76.2
YD20	1.524 ± 0.211	28.183 (20.556 – 36.585)	0.480 (3)	77.9
CZ20	1.681 ± 0.219	29.082 (21.815 – 37.063)	1.007 (3)	80.3

In 2019, the field populations of *P. citri* in Yongfu, Guangxi (49.1 times), Yudu, Jiangxi (76.2 times), and Xiangshan, Zhejiang (25.6 times) showed intermediate levels of resistance, while those in Nanning, Guangxi (182.6 times), and Meishan, Sichuan (190.4 times) exhibited high levels of resistance to pyridaben. In 2020, three field populations of *P. citri* in Zhejiang, Jiangxi, and Hunan provinces showed moderate levels of resistance to pyridaben (54.2 – 80.3 times), while four field populations of *P. citri* in Guangxi and Sichuan provinces all showed high levels of resistance (123.0 – 202.7 times). In 2019—2020, the resistance of *P. citri* to pyridaben decreased slightly in Meishan area of Sichuan Province, but those in other areas showed an increasing trend.

In the same year, different regions showed variations in the resistance of female adult *P. citri* mites to pyridaben. The field populations of female adult *P. citri* mites in Guangxi and Sichuan showed higher resistance to pyridaben than those in Zhejiang, Jiangxi and Hunan.

3.3 Resistance of field populations to abamectin In 2019—2020, the resistance of female adult *P. citri* mites to avermectin was monitored in the main citrus-producing areas of in China. As shown in Table 4, except for the XS19 population in Zhejiang Province, which showed a moderate level of resistance to abamectin (32.4 times), the other 11 populations exhibited high level of resistance to abamectin (112.1 – 560.2 times) during the two years. The 2020 female adult mite populations exhibited significantly greater resistance to abamectin than the results obtained from the 2019 monitoring. During the same period, the populations in Zhejiang, Jiangxi, and Hunan showed lower resistance to abamectin compared to the Guangxi and Sichuan populations.

Table 4 Resistance of female adult *Panonychus citri* mites to abamectin in 2019—2020

Population	Slope ± SE	LC_{50} // mg/L	X^2 (df)	RR
YF19	1.390 ± 0.216	22.987 (17.070 – 30.826)	2.888 (3)	175.5
YF20	1.267 ± 0.207	45.467 (31.930 – 63.874)	1.729 (3)	347.1
NN19	1.067 ± 0.137	20.552 (5.472 – 49.652)	15.386 (4)	156.9
NN20	1.175 ± 0.156	73.379 (50.874 – 99.353)	0.355 (3)	560.2
YS20	1.320 ± 0.209	44.525 (28.769 – 61.055)	0.908 (3)	339.9
MS19	1.336 ± 0.242	31.486 (21.158 – 43.667)	0.837 (3)	240.4
MS20	1.276 ± 0.206	50.517 (32.862 – 69.708)	0.472 (3)	385.6
XS19	1.198 ± 0.179	4.245 (2.839 – 5.807)	1.219 (3)	32.4
XS20	1.251 ± 0.203	23.247 (15.154 – 31.896)	0.363 (3)	177.5
YD19	1.119 ± 0.164	14.688 (10.361 – 20.358)	0.175 (4)	112.1
YD20	1.459 ± 0.208	23.775 (16.716 – 31.262)	1.121 (3)	181.5
CZ20	1.389 ± 0.203	25.400 (17.774 – 33.663)	0.117 (3)	193.9

3.4 Resistance of field populations to bifenazate In 2019—2020, we monitored the field populations of *P. citri* for bifenazate resistance in 7 locations across 5 provinces of China. As shown in Table 5, among the five populations monitored in 2019, except for Xiangshan (51.6 times) and Yudu (84.7 times) populations, the remaining three populations exhibited high level of resistance to bifenazate (140.4 – 306.4 times). In 2020, all six populations monitored had high resistance to bifenazate (141.7 – 686.1 times). There was a significant difference in *P. citri* resistance among different regions in Guangxi province. The Nanning population showed a resistance of 231.2 times, while the Yongfu population showed a resistance of 686.1 times. The monitoring data from

2019—2020 indicated that *P. citri* exhibited significant resistance to bifenazate, and this resistance was increasing.

Table 5 Resistance of female adult *Panonychus citri* mites to bifenazate in 2019—2020

Population	Slope ± SE	LC_{50} // mg/L	X^2 (df)	*RR*
YF19	0.835 ±0.217	51.471 (32.529 –92.959)	3.859 (3)	306.4
YF20	1.273 ±0.199	115.271 (78.149 –156.837)	0.294 (3)	686.1
NN19	1.269 ±0.208	23.586 (15.853 –31.732)	2.911 (3)	140.4
NN20	1.232 ±0.157	38.840 (27.768 –51.796)	0.500 (3)	231.2
YS20	1.362 ±0.210	111.818 (75.936 –151.437)	2.237 (3)	665.6
MS19	1.453 ±0.256	31.542 (21.690 –43.012)	2.879 (3)	187.8
MS20	1.169 ±0.200	58.416 (37.378 –82.491)	0.774 (3)	347.7
XS19	1.465 ±0.233	8.666 (2.109 –6.145)	0.134 (3)	51.6
XS20	1.276 ±0.200	23.802 (15.829 –32.385)	0.360 (3)	141.7
YD19	1.252 ±0.218	14.236 (9.392 –19.386)	0.314 (3)	84.7
YD20	1.472 ±0.207	28.946 (20.917 –37.840)	0.720 (3)	172.3
CZ20	1.481 ±0.206	49.546 (35.276 –64.815)	0.129 (3)	294.9

4 Conclusions and discussion

This study monitored the resistance of *P. citri* field populations to three commonly used pesticides during 2019—2020. The results indicate that high levels of resistance to pyridaben, abamectin, and bifenazate have been generalized in *P. citri* field populations in 5 provinces. To improve production, it is recommended to reduce the individual use of acaricides. Monitoring the resistance of *P. citri* to common acaricides can provide insight into their development status and trends. While there may be some distinctions between the indoor bioassay and field pharmaceutical control, the results of the indoor bioassay can still serve as a useful reference for field control of *P. citri*. Additionally, it can provide a theoretical foundation for field medication.

Moderate resistance to pyridaben was observed in most of the monitored populations of *P. citri*, while high levels of resistance were found in populations from Yangshuo and Nanning in Guangxi, and Meishan in Sichuan. There have been reports of *P. citri* developing resistance to pyridaben in China. In 2011, Ding Tianbo *et al.* [17] monitored the LC_{50} of 25.5 mg/L for pyridaben in *P. citri* populations harvested from the citrus nursery garden of the China Citrus Research Institute in Beibei District, Chongqing, China, using the leaf disc impregnation method. In 2012, the same population was monitored again using the same bioassay, and it was found that the LC_{50} for pyridaben had decreased to 2.871 mg/L. Our hypothesis is that the decrease in *P. citri* resistance to pyridaben between the two years was due to the rotation of pyridaben with other agents to reduce the frequency of application. This rotation restored the susceptibility of this *P. citri* population to pyridaben. Based on our analysis of monitoring results and the history of field drug use in the monitoring area, we have determined that the high level of resistance to pyridaben in Guangxi and Meishan populations is due to over-reliance on the acaricide in the area, arbitrary increases in application dose, lack of reasonable mixing with other acaricides, and monoculture in successive years. Further verification is needed to determine whether reducing the frequency of pyridaben use in the area will also reduce the resistance of *P. citri*.

In 2019, 80% of the measured *P. citri* populations were found to have developed high levels of resistance to abamectin. By 2020, all field populations of *P. citri* in all monitored areas had developed high levels of resistance to abamectin. The potential for developing resistance increases with the long-term, large-scale, unregulated, and irrational use of any type of acaricide. The resistance of *P. citri* to abamectin remains high, and it is unlikely to change significantly in the near future. Therefore, it is essential to enhance monitoring of abamectin resistance in *P. citri* populations and closely observe any signs of resistance development.

Since its registration in China in 2008, bifenazate has been widely used due to its stable performance, efficacy, and ovicidal properties[18]. Xu Shu *et al.* [19] investigated the indoor virulence of bifenazate against *P. citri* and found that *P. citri* had developed high resistance to bifenazate. Our monitoring results showed that 60% of the monitored populations had developed high levels of resistance to bifenazate in 2019, while all monitored populations developed high levels of resistance to bifenazate in 2020. Moreover, the populations showing increased resistance to bifenazate in 2020 accounted for 100% of the monitored populations. Our hypothesis is that the high level of resistance to bifenazate in *P. citri* populations in the monitored areas was attributed to a point mutation occurred in the cytochrome b gene region of the mitochondrial genome, which led to the development of resistance due to decreased susceptibility of this target to bifenazate. The increasing trend of resistance in *P. citri* populations can also be reflected by the results of this study. Therefore, it is essential to closely observe the changes in the resistance of *P. citri* to bifenazate in the field.

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The test results also showed when an ant nest control cover was installed in the treatment of *S. invicta*, the population decline rate was 100%, the nest decline rate was 100% and the control effect was 100% at 7 d post application. Compared with the control status without control cover, the population decline rate, the nest decline rate and the control effect at 14 d post application were 71.4%, 33.3% and 33.3%, respectively, when chemical agents were applied once, and 99%, 100% and 100%, respectively, when chemical agents were applied twice. The control duration without control cover was twice as long as that with control cover, and the dose used to achieve the control effect was also twice as much. At the same time, *S. invicta* would escape and establish new nests during the control process. This indicates that the control of *S. invicta* by adding control cover can not only shorten the control time and use less medicine, but also effectively solve the problem of escape, which significantly improves the actual control effect and is economical and environmental protection compared with traditional simple application of medicine without control cover[5-7].

The results show that the control cover of *S. invicta* is a fast and effective exterminating tool to deal with *S. invicta* epidemic. Although it is not possible to use the control cover in some places because of the terrain restrictions, the vast majority of *S. invicta* nests are built in parks, green spaces, nurseries, orchards and other flat open fields[8]. The control cover is basically applicable to broad lawns and fields, and thus has broad application prospect. With the popularization and the installation tool developed subsequently, the use of control cover made from plastic is more convenient, the cost will be further reduced, so it has the value of popularization.

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