Dissipation of Flonicamid in Honeysuckle and Its Transfer during Brewing Process

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Summary

The dissipation of flonicamid in Honeysuckle and transfer pattern from Honeysuckle to its tea infusion were investigated. Flonicamid was applied on Honeysuckle crop at two dosages, 60 g of active gradient per hectare (g a.i. hm\(^{-2}\)) and 180 g a.i. hm\(^{-2}\) (recommended and triple the recommended) in Fenqiu, Henan Province in 2015 and 2016. Gas chromatography-Electron Capture Detector (GC-ECD) detection methods were developed for the analysis of flonicamid residues in honeysuckles and its infusion. The recoveries in both honeysuckles and its infusion ranged from 81.5% to 101.7% with relative standard deviations (RSDs) of 3.2-9.1%. The dissipations of flonicamid in Honeysuckle were found to follow the first order kinetics with half-life ranging between 2.8 and 3.2 d. After recommended dose pesticide application, contents of flonicamid residues were lower than theoretical maximum residue limit (tMRL). Flonicamid residues can easily transfer from Honeysuckle to its tea infusion and transfer rates of flonicamid decrease with the brewing temperature reduction or the brewing times increase. These results are helpful to establish maximum residue limit and develop guidance on the appropriate and secure use of flonicamid in Honeysuckle.

Keywords: flonicamid; Honeysuckle; dissipation; transfer; infusion
Honeysuckle (dried flower buds or open flowers of *Lonicera japonica* Thunb.) is a popular Traditional Chinese Medicine in Asia. It has been widely utilized due to its antibacterial\(^1\), antiviral, \(^2\) and antioxidant\(^3, 4\) functions in pharmacological activities. People usually use it to make tea for daily healthcare. *L. japonica* is also an important cash crop of China, which brings economic benefits to farmers. But *L. japonica* is seriously attacked by aphid from April to June every year, which cause crop loss as well as deterioration in the quality of the Honeysuckle. Flonicamid (N-cyanomethyl-4-trifluoromethylnicotinamide) is a novel systemic pesticide discovered by Ishihara Sangyo Kaisha, Ltd and has been launched on the world market, such as the USA, China, Brazil, France, Korea and Japan since 2003\(^5\). The main insecticidal mechanism of flonicamid is starvation based on the inhibition of stylet penetration to plant tissues, \(^5\) which has selective activity against hemipterous pests, such as aphids, whiteflies, and thysanopterous pests. It has no negative impact on beneficial arthropods, has a low toxicity for mammals and environment, \(^6, 7\) and is not cross-resistant to any existing class of chemicals, thus, a valuable tool for resistance management. Flonicamid has been widely used in *L. japonica* because of its good effect on killing aphids. But there is no guidance on how to use flonicamid in *L. japonica*, its residue in Honeysuckle may persist after harvest and then cause adverse effects on humans consuming them. So, it is essential to study the behaviors of flonicamid in Honeysuckle including the dissipation in field and transfer during brewing to guarantee the safety of Honeysuckle consumption.

Few reports have been made for the analysis of flonicamid in foods. Analytical
methods for flonicamid in agricultural products based on Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) extraction and LC-MS/MS analysis have been developed. 8-10) Polyclonal antibody-based enzyme-linked immunosorbent assay was also developed for the analysis of flonicamid in environmental and agricultural samples.11, 12) And methods using GC-ECD13) or Gas chromatography-Nitrogen Phosphorus Detector (GC-NPD) 14) to determine flonicamid in foods are reported. Until now, no reports were published concentrating on methods of flonicamid quantification in dried Traditional Chinese Medicines (TCMs) and tea infusion.

Some studies on dissipation behavior of flonicamid are reported. Kaiwei Shi14) studied the dissipation of flonicamid in apple in Beijing, Anhui and Shandong Province, the half-lives ranged from 7.6 d to 19 d. Xingang Liu15) studied the dissipation behavior of flonicamid in apple and cucumber in Zhejiang and Hainan Province. And some studies about the transfer patterns from tea leaves to infusion have been reported (M. Paramasivam16), deltamethrin in tea; Soon-Kil Cho17), cyhalothrin, flufenoxuron, fenitrothion, EPN, bifenthrin, difenoconazole, triflumizole, and azoxystrobin in green tea). But dissipation behaviors and transfer patterns varies from plant shapes and pesticide properties. The behavior of flonicamid in Honeysuckle could be different from the species above. To the best of our knowledge, no studies have previously been published focusing on the dissipation of flonicamid in Honeysuckle and its transfer pattern from Honeysuckle to infusion.
In addition to developing a GC-ECD method to analyze flonicamid residues in Honeysuckle and its infusion, this study was performed to observe the dissipation behavior of flonicamid in Honeysuckle and transfer pattern from Honeysuckle to its tea infusion. The study is expected to provide useful information on the dissipation of flonicamid in Honeysuckle and give useful advices to humans on how to make Honeysuckle tea. This study is also expected to provide data support to establish Maximum Residue Limit (MRL) of flonicamid in Honeysuckle and develop guidance on the appropriate and secure use of flonicamid.

• Experimental

1 Materials and Methods

1.1 Reagents, Standards, Instruments

HPLC-grade acetone was obtained from MREDA TECHNOLOGY INC (USA), analytical-grade acetonitrile, toluene, ethyl acetate and anhydrous sodium sulfate were obtained from Beijing Chemical Works Company (Beijing, China). CNWBOND NH₂ SPE Cartridge (500 mg, 6 mL) were purchased from ANPEL Laboratory Technologies Inc (Shanghai, China). Purified water was prepared by using Milli-Q water purification system (Millipore Purification Systems). Neutral alumina (200-300 mesh) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China) and activated at 550 °C for 5 h before use.

The 100 mg/L certified analytical standards of flonicamid in methanol were obtained from AccuStandard, Inc (USA). Standard stock solutions of 10 mg/L were
prepared in acetone, and the working solutions of 0.1 mg/L were prepared from the stock solution by dilutions. The stock and working solutions were stored hermetically at −20°C until analysis.

A LABOROTA 4000 rotary vacuum evaporator (Heidolph, Germany), a WH-1 vortex mixer (ShangHai Huxi Analytical Instruments, China), a 80-2 centrifuge (Jintan Medical-Equipment Company, China) and a SHB-B type water circulating multi-purpose vacuum pump (Zhengzhou Greatwall Scientific Industrial and Trade Co, Ltd.) were used. The GC-ECD system was composed of an Agilent 6890N GC and a micro-Electron Capture Detector (μ-ECD) (Agilent Co., USA). A DB-1701 fused silica capillary column (14% phenyl propyl cyanide polysiloxane as nonpolar stationary phase, 30 m length *0.32 mm i.d. and 0.25 μm film thickness; Agilent Co., USA) were used for chromatographic separation.

1.2 Field Trials

The field trials were carried out in Fengqiu, Henan Province in spring of 2015 and 2016. The experiments were designed according to ‘Guideline for Pesticide Residue Trials’ issued by the Institute of the Control of Agrochemicals, Ministry of Agriculture (ICAMA), People’s Republic of China. In the year of 2015, the experiment was conducted from May 15 to May 30 in Xiyu Village, Huangde Town, Fengqiu City, Henan Province. The daily minimum temperature was from 15 to 21 °C and the daily highest temperature was from 24 to 30 °C, wind strength is less than 4 and light rain occurred on May 21 and May 28. The area of experiment plot was 15 m². Every experiment plot contained 18 *L. japonica*. The rate of flonicamid (10%,
Water dispersible granule) application in dissipation experiments was 60 g a.i. hm\(^{-2}\) (recommended dose, T1) and 180 g a.i. hm\(^{-2}\) (triple recommended dose, T2) with one time spray. It was sprayed before honeysuckle bloom on May 15. The temperature was from 15 to 26 °C and wind strength is less than 3 that day.

In the year of 2016, the experiment was conducted from May 9 to May 24 in Dongzhonggong Village, Chengu Town, Fengqiu City, Henan Province. During the experiments, the daily minimum temperature was from 11 to 19 °C and daily highest temperature was from 16-29 °C, wind strength is less than 4, light rain occurred on May 9, May 12 and May 23 and moderate rain to light rain occurred on May 14. The area of experiment plot was 16.8 m\(^2\). Every experiment plot contained 12 *L. japonica*. Flonicamid was sprayed before honeysuckle bloom on May 9 and the rate of flonicamid is the same as that in 2015. On that day, the temperature was from 11 to 20°C, wind strength is less than 3 and a light rain occurred 3 hours after pesticide spraying.

Each treatment was designed with three replicated plots. A buffer area was maintained away from the experiment plots. Representative flower buds of *L. japonica* were collected in 0 (2 h), 1, 2, 3, 4, 5, 7, 9, 11, 13 and 15 days in 2015 after spraying of the pesticide. The fresh samples were dried in an oven at about 55°C immediately for about 24 h. All dried samples were stored at -20 °C until they had been analyzed.

### 1.3 Honeysuckle Tea Infusion Preparation

Drinking Honeysuckle tea infusion is the most common way people eat
Honeysuckle. In this study, the 0 day sample of triple recommended dose pesticide in 2015 was used to investigate the transfer pattern of flonicamid during brewing process. A 1.0 g of Honeysuckle was weighed into a 100 mL beaker to which 50 mL of water was added. After brewing for 20 min, the infusion was filtered through a funnel blocked with a cotton and cooled to room temperature. The spent Honeysuckle left after infusion were spread in a filter paper and air-dried. The infusion and the air-dried spent Honeysuckle were separately analyzed for flonicamid residues. To investigate the infusion behavior of flonicamid in Honeysuckle under different brewing temperatures, 60, 80 and 100 °C water was used to make tea infusion. And to investigate the infusion behavior of flonicamid in Honeysuckle under different brewing times, a 1.0 g of Honeysuckle was repeatedly infused in 50 mL of boiling water for 3 times, respectively to obtain 1st infusion, 2nd infusion and 3rd infusion. The Honeysuckle tea infusion and the spent Honeysuckle were analyzed by the method followed immediately.

1.4 Sample Analysis

1.4.1 Extraction and Clean-up Procedure

The Honeysuckle field samples and the spent Honeysuckle samples after making tea infusion were mechanically ground into homogeneous powder through a 24 mesh sieves (850 μm ± 23 μm). 1.0g of Honeysuckle field samples and all of the spent Honeysuckle samples were extracted with 5 mL of acetonitrile in a 10 mL centrifuge tube. The sample was shaken vigorously for 5 min using a vortex mixer then centrifuged at 3000 r/min for 15 min to separate solid and solvent. An aliquot of the
solvent was moved into a 100 mL pear-shaped flask. The extract procedure was repeated with additional 5 mL of acetonitrile. The extract was then concentrated to a few milliliters in a rotary vacuum evaporator at 40 °C. The sorbent in the NH₂ SPE cartridge was first conditioned with acetonitrile/toluene (3:1, v/v, 5 mL) prior to the addition of the sample. When the conditioning solution reached the top of sorbent, the cartridges were connected to a 100 mL pear-shaped flask; the obtained concentration described above was added to the cartridge and the container was rinsed 3 times with 1 mL of acetonitrile. The washings were placed into the cartridge. The pesticide was eluted with 15 mL of acetonitrile/toluene (3:1, v/v) and was concentrated to nearly dryness using a rotary vacuum evaporator at 40°C. Finally, the residue was dissolved in 2.0 mL acetone for GC-ECD analysis.

As for Honeysuckle tea infusion, 20 mL of tea infusion was transferred to a 50 mL centrifuge tube and added 4 g of sodium chloride, then subjected to partitioning with ethyl acetate (3 times, 15 mL, 10 mL, and 10 mL). The combined ethyl acetate layer was collected in a 100 mL pear-shaped flask and concentrated to a few milliliters using rotary vacuum evaporator at 40°C. An NH₂ cartridge was fixed to a support and filled with 4 g anhydrous sodium sulfate. The sorbent in the NH₂ SPE cartridge was first conditioned with acetonitrile/toluene (3:1, v/v, 5 mL) prior to the addition of the sample. When the conditioning solution reached the top of anhydrous sodium sulfate, the cartridges were connected to a pear-shaped flask; the obtained concentration described above was added to the cartridge and the container was rinsed 3 times with 1 mL of ethyl acetate. The washings were placed into the
cartridge, too. The pesticides were eluted with 15 mL of acetonitrile/toluene (3:1,v/v) and were concentrated to nearly dryness using a rotary vacuum evaporator at 40°C. Finally, the residue was dissolved in 2.0 mL acetone for GC-ECD analysis.

1.4.2 GC Determination

GC Determination was conducted using the Agilent 6890N GC equipped with a μ-ECD. DB-1701 fused silica capillary column was used for the quantification. Analysis was performed using oven programming at initial temperature of 120 °C for 5 min followed by a ramp rate of 15 °C/min, 190 °C for 1 min followed by 20 °C/min ramp rate, and finally, a final temperature of 260 °C with a hold time of 1 min. The temperature of the injector was set at 260 °C in splitless mode. The detector temperature was maintained at 320 °C. Nitrogen (N₂, 99.999% purity) was used as carrier gas with a flow rate of 1 mL/min. Injection volume of the sample was 1 μL.

1.4.3 Method Validation

The linearity of the method was evaluated by linear regression analysis of both the standard solution and matrix-matched calibration curves. The calibration standards (five calibration points) ranging from 0.001 to 0.5 mg/L were prepared in acetone and matrix. The calibration curves for flonicamid were obtained by plotting the peak area against the concentration of the corresponding calibration standards.

The limit of detection (LOD) of the test compounds was determined by considering a signal-to-noise ratio (S/N) of 3, whereas the limit of qualification (LOQ) was determined by considering an S/N of 10 in the matrix standard.

The recovery experiments were carried out by spiking the Honeysuckle blank...
samples (1.0 g) at three concentration levels (0.02, 0.1 and 0.2 mg/kg) and spiking the Honeysuckle tea infusion at two concentration levels (0.001 and 0.01 mg/L) in five replicate. The precision in terms of repeatability was determined by calculating % RSD associated with recovery.

2 Results and Discussions

2.1 Optimization of Sample Analysis Methods

2.1.1 Optimization of Extraction Conditions

Acetonitrile, acetone and ethyl acetate were used to extract flonicamid from dried samples to choose the best extraction solvent. The results showed that acetone extracted the most pigments and other impurities, and then ethyl acetate, acetonitrile extracted the least impurities, showing in GC-ECD analysis as baseline elevation and impurity peaks. The gas chromatograms for the extraction of honeysuckle samples are shown in Fig.1. As for extraction efficiency, acetonitrile and ethyl acetate both could extract more than 88% of flonicamid. So acetonitrile was used in the extraction step. And for Honeysuckle tea infusion, petroleum ether (60-90 °C), acetonitrile (and sodium chloride) and ethyl acetate (and sodium chloride) were investigated to choose a best solvent to extract the flonicamid from Honeysuckle tea infusion. Results showed that petroleum ether could not extract the aimed compound, and acetonitrile with sodium chloride extracted more impurities, while ethyl acetate with 4.0 g of sodium chloride could both extract the pesticide and avoid most of impurities. So ethyl acetate was used for this procedure.

2.1.2 Optimization of Clean-up Conditions
Adsorption capacity for flonicamid of two different sorbents, namely neutral alumina column and NH$_2$ SPE cartridge were compared in this study. Neutral alumina column was got by packing 4 g of activated 200-300 mesh neutral alumina between the layers of 2 g anhydrous sodium sulfate in a 25*1.1 cm i.d. glass column with Teflon stopcocks. Both the two sorbents could clean up most of impurities in Honeysuckle matrix. And 40 mL of acetonitrile could not elute the pesticide from the neutral alumina column, while 15 mL of with acetonitrile/toluene (3:1, v/v) could elute more than 99% of pesticide from NH$_2$ SPE. So the NH$_2$ SPE cartridge was used for clean-up of Honeysuckle matrix.

2.1.3 Method Validation

The linearity of the calibration curves were established with a correlation coefficient (R$^2$) of > 0.9990 in the range 0.001-0.5 mg/L. From the S/N, the LOD and LOQ were 0.01 $\mu$g/kg and 0.03 $\mu$g/kg in dried Honeysuckle samples and 0.005 $\mu$g/L and 0.016 $\mu$g/L in Honeysuckle tea infusion, respectively. The average recoveries (%) of flonicamid at 0.02, 0.1 and 0.2 mg/kg levels in Honeysuckle samples and 0.001 and 0.01 mg/L in Honeysuckle tea infusion were ranged from 81.5 to 101.7% and the corresponding RSDs were ranged from 3.2 to 9.1% (Table 1), which was within the acceptable range (recoveries, 70–120%; RSD $\leq$ 20%). 19)

2.2 Dissipation of Flonicamid in Honeysuckle

The residues of flonicamid pertaining to T1 and T2 in Honeysuckle in the year of 2015 and 2016 are presented in Table 2 and the Gas Chromatograms of standard and sample are shown in Fig. 2. The dissipation of flonicamid in Honeysuckle was fitted
into first order dissipation kinetics $C = C_0 e^{kt}$ and $t_{1/2} = \ln 2/k$, where $C$ (mg/kg) is the pesticide concentration at time $t$ (d), $C_0$ is apparent initial concentration (mg/kg), $k$ is rate constant and $t_{1/2}$ is the pesticide half-life in Honeysuckle. The dissipation regressive equations, half-lives in Honeysuckle at different application dose in the year of 2015 and 2016 are presented in Table 3. In 2015, the initial concentrations of flonicamid residues in Honeysuckle at the two application doses were 2.6 mg/kg and 10 mg/kg with half-lives of 3.0 d and 2.8 d, respectively. In 2016, the initial concentrations of flonicamid residues in Honeysuckle at the two application doses were 1.8 mg/kg and 5.0 mg/kg with half-lives of 3.2 d and 2.9 d, respectively.

The initial concentration at the two application doses in 2016 was lower than that in 2015. This may be due to the rainfall washing away part of pesticide in 3 hours after the pesticide application in 2016, while there was no rainfall in 2015. The half-lives of flonicamid in Honeysuckle ranged from 2.8 d to 3.2 d. The data in the two years had no significant difference, but was shorter than that in cucumbers (3.0-4.9 d), apples (5.1-6.1 d),\(^{15}\) and a little longer than that in cabbages (about 2.5 d).\(^{10}\) These suggest that dissipation varied with plant species, location of application, growth dilution factor and some physical and chemical factors such as light, heat, pH and moisture and so on.\(^{20}\)

China has not established a MRL for flonicamid in Honeysuckle. According to The United States Pharmacopeia\(^{21}\) and Chinese Pharmacopoeia (ChP),\(^{22}\) when the limits of a pesticide are not established, they can be calculated the limit by the formula:
Limits (mg/kg) = ADI·M/100B

Where ADI is the acceptable daily intake, as published by FAO-WHO, in mg/kg of body weight. In this study, the ADI of flonicamid is 0-0.07 mg/kg; \(^{23}\) M is body weight, in kg and set as 60 kg, usually; and B is the daily dose of the food, in kg. According to the ChP, the daily dose of Honeysuckle is 0.006-0.015 kg. So the theoretical limit calculated is 2.8-7.0 mg/kg.

In this study, to avoid the risk most, MRL of flonicamid in Honeysuckle is set as 2.8 mg/kg (tMRL). The flonicamid residues were below the tMRL value immediately after recommended dose pesticide application and in 4-5 days after triple recommended dose application in 2015 and 2016. This suggests that it is safe to collect flower buds on the day of recommended dose flonicamid application. \(L. japonica\) is a crop with multiple picking natures, where harvesting continues from May to September every year in China. The dissipation studies of flonicamid show there is no need to set pre-harvest intervals (PHIs) between the pesticide application and harvest in the experimental location and condition. However, the dissipation was related to application methods, location of application and other factors. So extra intervals should be set to ensure food safety. The advices on secure use of pesticide on \(L. japonica\) are given: Apply recommended dose of flonicamid once to control aphids, and a suitable PHI should be set. And extra application doses or times should be prevented to ensure food safety.

2.3 Transfer of Flonicamid from Honeysuckle to Its Tea Infusion

The transfer rates of flonicamid from Honeysuckle to its tea infusion under
various temperature are shown in Table 4. The transfer rates of flonicamid were
ranged from 50.2% to 90.8%. The order of transfer rates was as follows: 100°C >
80°C > 60°C, which shows that increasing brewing temperature results in increasing
transfer rate. And the transfer rates of flonicamid from Honeysuckle to its tea
infusion for three times are shown in Table 5. The transfer rates of flonicamid were
81.6%, 13.4% and 2.62%, respectively. Results show that first infusion can reserve
most part of pesticide and more times of infusion results in less transfer rates of
pesticide.

And as results shown, the transfer rates of flonicamid is high (more than 80%,
100 °C), which is related to the good water solubility (5.2 g/L, 20 °C). When
Honeysuckle that is applied triple recommended floncamid is used to make tea, even
brewing at 60 °C, the content of pesticide infusion is more than tMRL. The long
time consuming Honeysuckle tea containing high content of pesticide may pose a
threat to health. Standardizing the use of flonicamid and establishing suitable PHI
will reduce its residues in honeysuckle and then solve this problem. So, to guarantee
the safe consumption of Honeysuckle, the guidance on the usage and PHI of
flonicamid on *L. japonica* is very necessary.

3 Conclusion

A GC-ECD analysis combined with NH₂ SPE cartridge clean-up method was
established for determining flonicamid residues in Honeysuckle sample and its tea
infusion. Its short time of analysis, simple pre-treatment and good selectivity make
this method a useful tool for monitoring flonicamid in Honeysuckle. The dissipation
of flonicamid in Honeysuckle was investigated and advices on secure use of
flonicamid on *L. japonica* have been given. Flonicamid can easily transfer from
Honeysuckle to its tea infusion and the transfer are related to the brewing
temperature and times. Useful advices on safe consumption of Honeysuckle tea have
been given, too. The results of this study will be useful in developing MRLs for
flonicamid and guidance on the appropriate and secure use of flonicamid.

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Development of the 12th Five-Year Plan.

• **Conflict of Interest (COI)**

The authors declare no conflict of interest.
• References and Notes


23) WHO. Inventory of evaluations performed by the Joint Meeting on Pesticide Residues (JMPR); Available at http://apps.who.int/pesticide-residues-jmpr-database (accessed 2012).

Figures and tables

Fig. 1 Gas Chromatograms of Honeysuckle blank sample extracted by (A) acetone; (B) ethyl acetate; (C) acetonitrile
Fig. 2 Gas Chromatograms of (A) flonicamid standards dissolved in acetone (0.1 mg/L), (B) Honeysuckle field sample; 1, Flonicamid
Table 1 Recovery and RSD (%) for the determination of flonicamid in dried sample and its tea infusion (n = 5)

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Added level (mg/kg)</th>
<th>Mean recovery (%)</th>
<th>RSD (%)</th>
<th>Added level (mg/L)</th>
<th>Mean recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dried samples</td>
<td>0.02</td>
<td>92.2</td>
<td>3.2</td>
<td>0.001</td>
<td>93.9</td>
<td>9.1</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>88.0</td>
<td>3.5</td>
<td>0.01</td>
<td>101.7</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>81.5</td>
<td>6.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2 Residue concentration and dissipation rate of flonicamid in Honeysuckle after pesticide application in 2015 and 2016, [Flonicamid residues ± SD (mg/kg) (dissipation rate/%) (n = 3)]

<table>
<thead>
<tr>
<th>Days</th>
<th>Days</th>
<th>Days</th>
<th>Days</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1(^1)</td>
<td>T2(^2)</td>
<td>T1(^1)</td>
<td>T2(^2)</td>
</tr>
<tr>
<td>0.1</td>
<td>2.6±0.11(^3)(0)(^4)</td>
<td>10±1.3(0)</td>
<td>0.1</td>
<td>1.8±0.29(0)</td>
</tr>
<tr>
<td>1</td>
<td>2.3±0.44(12.7)</td>
<td>7.4±2.4(26.7)</td>
<td>1</td>
<td>1.1±0.14(41.5)</td>
</tr>
<tr>
<td>2</td>
<td>1.8±0.069(32.9)</td>
<td>4.8±0.7(51.7)</td>
<td>2</td>
<td>1.2±0.094(34.0)</td>
</tr>
<tr>
<td>3</td>
<td>1.3±0.32(49.4)</td>
<td>4.3±0.80(56.7)</td>
<td>3</td>
<td>0.84±0.12(52.3)</td>
</tr>
<tr>
<td>4</td>
<td>1.1±0.13(59.1)</td>
<td>2.9±0.20(71.7)</td>
<td>4</td>
<td>0.87±0.12(50.8)</td>
</tr>
<tr>
<td>5</td>
<td>0.94±0.17(64.3)</td>
<td>2.5±0.19(74.3)</td>
<td>6</td>
<td>0.44±0.050(74.9)</td>
</tr>
<tr>
<td>7</td>
<td>0.62±0.11(76.6)</td>
<td>1.8±0.75(82.7)</td>
<td>8</td>
<td>0.22±0.012(87.4)</td>
</tr>
<tr>
<td>9</td>
<td>0.44±0.11(83.4)</td>
<td>1.1±0.12(89.5)</td>
<td>10</td>
<td>0.19±0.013(89.1)</td>
</tr>
<tr>
<td>11</td>
<td>0.34±0.072(87.0)</td>
<td>0.82±0.26(91.8)</td>
<td>12</td>
<td>0.17±0.022(90.6)</td>
</tr>
<tr>
<td>13</td>
<td>0.14±0.013(94.6)</td>
<td>0.42±0.10(95.8)</td>
<td>15</td>
<td>0.06±0.013(96.2)</td>
</tr>
<tr>
<td>15</td>
<td>0.06±0.011(97.6)</td>
<td>0.16±0.033(98.4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) T1, recommended dose of flonicamid application

\(^2\) T2, triple recommended dose of flonicamid application

\(^3\) Concentration of flonicamid residues ± SD (mg/kg)

\(^4\) Dissipation rate of flonicamid (%), calculated by dividing initial concentration of flonicamid by the concentration those were dissipated
Table 3 Dissipation regressive equations, half-life of flonicamid in Honeysuckle

<table>
<thead>
<tr>
<th>Years</th>
<th>Treatments</th>
<th>Dissipation regressive equation</th>
<th>$R^2$</th>
<th>Half-life (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>T1$^1$</td>
<td>$C = 2.92e^{-0.232t}$</td>
<td>0.9719</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>T2$^2$</td>
<td>$C = 9.21e^{-0.247t}$</td>
<td>0.9801</td>
<td>2.8</td>
</tr>
<tr>
<td>2016</td>
<td>T1</td>
<td>$C = 1.67e^{-0.215t}$</td>
<td>0.9722</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>T2</td>
<td>$C = 5.23e^{-0.239t}$</td>
<td>0.9870</td>
<td>2.9</td>
</tr>
</tbody>
</table>

$^1$ T1, recommended dose of flonicamid application

$^2$ T2, triple recommended dose of flonicamid application
<table>
<thead>
<tr>
<th>Leaching temperature (°C)</th>
<th>Dried sample (µg)</th>
<th>Tea infusion (µg)</th>
<th>Spent Honeysuckle (µg)</th>
<th>Transfer rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>9.85±0.67</td>
<td>4.94±0.51</td>
<td>4.90±0.37</td>
<td>50.2</td>
</tr>
<tr>
<td>80</td>
<td>10.6±1.8</td>
<td>9.03±0.79</td>
<td>1.59±0.15</td>
<td>84.5</td>
</tr>
<tr>
<td>100</td>
<td>13.4±0.93</td>
<td>12.2±0.75</td>
<td>1.24±0.19</td>
<td>90.8</td>
</tr>
</tbody>
</table>
Table 5 Transfer rates from Honeysuckle to tea infusion for different leaching time (n=3)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dried sample</th>
<th>1st infusion</th>
<th>2nd infusion</th>
<th>3rd infusion</th>
<th>Spent Honeysuckle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residue content (μg)</td>
<td>13.6±1.2</td>
<td>11.1±1.1</td>
<td>1.83±0.15</td>
<td>0.35±0.025</td>
<td>0.29±0.012</td>
</tr>
<tr>
<td>Transfer rate (%)</td>
<td>-</td>
<td>81.6</td>
<td>13.4</td>
<td>2.62</td>
<td>-</td>
</tr>
</tbody>
</table>