Asian Journal of Chemistry; Vol. 24, No. 9 (2012), 3829-3832

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Effect of Three Pesticides on Chlorogenic Acid Concentration of *Lonicera japonica* Thunb

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(*Received*: 20 May 2011; *Accepted*: 11 April 2012) AJC-11249

Lonicera japonica Thunb flowers are widely used in Chinese medicine as an antipyretic. However, production of this medicinal plant is commonly affected by diseases such as powdery mildew in Feng Qiu area of China. A field study was conducted in 2005 and 2006 to investigate the influence of two insecticides (imidacloprid and acetamiprid) and one fungicide (triadimefon) on concentration of chlorogenic acid in flowers of *Lonicera japonic*. The *Lonicera japonic* was sprayed with imidacloprid and triadimefon at 1x and 3x field recommend rate and sprayed with acetamiprid at 1x and 5x field recommend rate at flowering stage. Results showed that the 1x and 5x field recommended rate of acetamiprid increased the concentration of chlorogenic acid ($p < 0.05$) in 2005 and 2006, while imidacloprid and triadimefon in 2005 had no significant effects ($p > 0.05$) on chlorogenic acid concentration. But 1x and 3x field recommended rate of triadimefon had significant effects ($p < 0.01$) on the chlorogenic acid concentration and decreased the concentration in 2006.

Key Words: Acetamiprid, Chlorogenic acid, Imidacloprid, *Lonicera japonica*, Triadimefon.

INTRODUCTION

With the increase of cultivation areas and quantities of different species of cultivated herbs, the damage due to diseases and insect pests has become more and more serious in recent years. Diseases and insect pests decrease the yield and quality of herbs and cause enormous economic loss[1]. The damage of *Grylloalpa orientali* is very common and serious on cultivation of Gastrodia elata in the GAP plantation base in Guizhou province of China[2]. *Dolycoris baccarum* and *Gryllotalpa unispina* Sausure is the main pests in the cultivation of *Trollius chinensis* Bunge[3]. Indeed, many species of pests can be found in one species of herb. For example, there are 49 species of pests on cultivation of *Radix isatidis*[4]. Disease is another threat to the quality and yield of herbs. There are 7 kinds of main diseases in the cultivation of *Astragalus membranaceu*., among them powdery mildew and root rot disease can reduce the yield greatly[4].

Lonicera japonica, as an antipyretic, is widely used Chinese medicine. It was recorded in a medical book (Zhou Hou Bei Ji Fang) dated back about 1700 years ago in Jin Dynasty of China[5]. Flower is the medicinal part of the plant. This plant has shown a wide spectrum of biological and pharmacological activities such as antibacterial, antiviral, antioxidant and inhibition of the platelet activating factor[6]. Chlorogenic acid is the main target ingredient of *Lonicera japonica* (China Pharmacopoeia, 2005).

Feng Qiu county of He Nan province is one of the biggest production areas of lonicera. Powdery mildew, which causes leaf powdery appearance and browning, reduced fall colour intensity and early leaf senescence, is the major disease that affects the local lonicera plantation. Aphids, a group of small, sap-sucking insects which are serious pests of agricultural crops around the world[7], causing chlorosis and necrosis upon infestation, is the major insect pest that affects the yield and quality of lonicera. The visible damage symptoms of Aphids feeding are distinct white, yellow, purple, or reddish-purple longitudinal streaks at times, with severe leaf roll in fully expanded leaves and the prevention of unrolling of developing leaves[8].

Pesticide control is considered to be the best method for the control of diseases and insect pests on herbs in China. Imidacloprid (1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine) and acetamiprid ((E)-N1-[(6-chloro-3-pyridyl)-methyl]-N2-cyano-N1-methylacetamidine) have good effect on controlling Aphids on lonicera and commonly used by local growers. Triadimefon (1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)-2-butanone) is also commonly used to control powdery mildews. But the safety of pesticides and their effects on the yield, especially on the concentration of activity components of herbs were not studied.

This study aimed at investigating the effect of pesticides on chlorogenic acid concentration of *Lonicera japonica* Thunb.

EXPERIMENTAL

Imidacloprid (IUPAC name: 1-((6-Chloro-3-pyridinyl)-methyl)-4,5-dihydro-N-nitro-imidazol-2-amine, 10 % WP) was obtained from Huan-yuan Agricultural Biochemistry Co. acetamiprid (IUPAC name: (E)-N1-[(6-chloro-3-pyridyl)-methyl]-N2-cyano-N1-methylacetamidine, 3 % EC) was obtained from Daiso Co. and triadimenfon (IUPAC name: 1-(4-Chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)-2-butanone, 20 % EC) was obtained from Jian-nong Agro-chemical Co. All standard samples of pesticides were purchased from Sigma. Chlorogenic acid was purchased from National Institute for the control of pharmaceutical and biological products (Beijing, China).

Acetonitrile (HPLC-grade) were produced by Beijing chemical plant (Beijing, China).

High-performance liquid chromatograph (Waters 600 controller, USA), with a WatersTM 486 tunable UV absorbance detector. The HPLC column was an Extend-C_{18} (5 μm particle size, 250 mm × 4.6 mm i.d.) from Agilent. Hand-held compression spray can have 1.5 L volume (Hua-you, China).

Experimental design: The experiment was carried out in a *Lonicera japonica* field at fengqiu county, He Nan province of China. The *Lonicera japonica* was planted in 2001. The experiment treatment was imposed in May of 2005 and 2006. Two insecticides (imidacloprid, acetamiprid) and one fungicide (triadimefon) were selected for this study. These pesticides were frequently used by local producers in this region.

Fungicide triadimefon was applied at three rates (0, 1x and 3x of the recommended concentration) and insecticide acetamiprid was applied at two rates (0 and 5x of the recommended concentration) in 2005. In 2006, insecticide imidacloprid and fungicide triadimefon were applied at three rates (0, 1x and 3x of the recommended concentration) and insecticide acetamiprid was applied at three rates (0, 1x and 5x of the recommended concentration). The experiment was a randomized complete plot design and carried out at flowering stage of *Lonicera japonica*. Each treatment has three replications. The experiment plot was 10 m^2. There was a border strip between two plots.

Pesticides preparation and spray: Pesticides were applied at recommended rates and multiple recommended rates in water, according to the dosages of plots. Pesticides were equably sprayed by Hand-held compression spray to avoid pesticides mutual interferers among the plots.

Sample collection: In 2005 test, flowers were collected at 6:00 of 1st, 2nd, 3rd, 5th, 7th, 10th, 13th day after application of pesticides, while flowers were collected at 6:00 of 1st, 2nd, 3rd, 5th, 6th, 7th, 9th, 10th, 12th days after application of pesticides in 2006 test. Picked flowers were dried in drying-house and then reserved at -20 ºC in refrigerator.

Determination of chlorogenic acid: The concentrations of chlorogenic acid of the flowers of *Lonicera japonica* were determined according to 2005 edition of China Pharmacopoeia method.

Precisely sampling the samples 0.25 g into 50 mL capacity bottle, ultrasonic dissolving in appropriate 50 % methanol, added 50 % methanol to scale, shook up. Extracts were passed through a 0.45 μm filter (Millex-HV, Millipore).

The HPLC analyses were carried out on a Waters (600 controller, USA) high-performance liquid chromatograph, with a WatersTM 486 tunable absorbance detector. The HPLC column was an Extend-C_{18} (5 μm particle size, 300 mm × 4.6 mm i.d.) from Agilent. The oven temperature was set at 25 ºC and a volume of 5 μL of solution was injected through the RP-C_{18} column for analytical HPLC. The flow rate was 1.0 mL min^{-1} and the mobile phase consisted of acetonitrile: 0.4 % phosphoric acid (13:87, v/v), data were recorded on a computer with the Millenium32 software from Waters, chromatograms were acquired at 327 nm.

RESULTS AND DISCUSSION

The ANOVA Statistical Analysis and LSD-t test were carried out to find differences between the treatments.

Imidacloprid: Table-1 presents the concentration of chlorogenic acid in flowers of *Lonicera japonic*, treated by imidacloprid and the blank control in 2006. By ANOVA statistical analysis, we can find out that both of the concentrations of chlorogenic acid in flowers of lonicera applied with 1x and 3x field recommended rate of imidacloprid had no significant differences ($p > 0.05$), compared with blank test.

Acetamiprid: Table-2 presents the concentration of chlorogenic acid in flowers of *Lonicera japonic*, treated by acetamiprid and the blank control group in 2005. By ANOVA statistical analysis, we can find out that the concentrations of chlorogenic acid in flowers of lonicera applied with 5x field recommended rate of imidacloprid were increased and had significant differences ($p < 0.01$), compared with blank test.

Table-3 presents the concentration of chlorogenic acid in flowers of *Lonicera japonic*, treated by acetamiprid and the

TABLE-1
CONCENTRATION OF CHLOROGENIC ACID OF FLOWERS IN DIFFERENT DAY AFTER TREATMENT BY IMIDACLOPRID IN 2006 (mg kg^{-1})

Treatment	Days after lonicera treated by pesticide (day)									
	0	1	2	3	5	6	7	9	10	12
CK	3.12	3.12	3.34	3.34	3.15	2.85	3.13	3.03	3.25	3.00
IMX Ⅰ	3.50	3.28	3.89	3.73	3.97	3.11	3.97	3.51	3.68	3.60
IMX Ⅱ	3.37	3.42	3.65	3.66	3.53	1.92	3.70	3.44	3.36	3.68
IMXⅢ	3.42	3.08	3.56	3.08	3.12	2.94	3.28	3.13	3.18	3.39
IM3X Ⅰ	3.50	3.59	3.93	3.89	3.98	2.38	3.93	3.67	3.70	3.55
IM3X Ⅱ	3.31	3.38	3.85	3.72	3.75	2.53	3.84	3.54	3.51	3.76
IM3XⅢ	3.14	3.20	3.56	3.08	3.08	2.35	3.21	3.36	3.18	3.45

IM: Imidacloprid; X: the recommended dose; 3X: three times the recommended dose; Ⅰ, Ⅱ, Ⅲ: 3 replications; CK: blank control.

TABLE-2
CONCENTRATION OF CHLOROGENIC ACID OF FLOWERS IN DIFFERENT DAY AFTER TREATMENT BY ACETAMIPRID IN 2005 (mg kg^{-1})

Treatment	Days after lonicera treated by pesticide (day)							
	0	1	2	3	5	7	10	13
CK	2.82	3.11	3.06	2.78	2.99	2.86	2.91	3.26
AC5X	3.5	4.51	3.94	3.42	3.71	3.58	3.89	3.67

AC: Acetamiprid; 5X: five times the recommended dose; CK: blank control.

TABLE-3
CONCENTRATION OF CHLOROGENIC ACID OF FLOWERS IN DIFFERENT DAY AFTER TREATMENT BY ACETAMIPRID IN 2006 (mg kg^{-1})

Treatment	Days after lonicera treated by pesticide (day)									
	0	1	2	3	5	6	7	9	10	12
CK	3.12	3.12	3.34	3.34	3.15	2.85	3.13	3.03	3.25	3.00
ACX Ⅰ	2.61	2.96	3.24	3.06	3.03	2.67	2.84	2.80	2.28	2.70
ACX Ⅱ	3.08	3.27	3.79	3.41	3.07	3.42	3.41	3.32	3.36	3.06
ACXⅢ	3.17	3.50	3.18	3.52	3.14	3.57	3.49	3.43	3.28	2.93
AC5X Ⅰ	3.23	3.12	3.12	3.55	3.46	3.49	3.56	3.35	3.32	3.32
AC5X Ⅱ	3.72	3.20	3.24	3.48	3.41	3.53	3.46	2.99	3.41	3.75
AC5XⅢ	3.52	3.39	3.81	3.78	3.62	3.58	3.58	3.37	3.19	3.75

AC: Acetamiprid; X: the recommended dose; 5X: five times the recommended dose; Ⅰ, Ⅱ, Ⅲ: 3 replications; CK: blank control.

TABLE-4
CONCENTRATION OF CHLOROGENIC ACID OF FLOWERS IN DIFFERENT DAY AFTER TREATMENT BY TRIADIMEFON IN 2005 (mg kg^{-1})

Treatment	Days after lonicera treated by pesticide (day)							
	0	1	2	3	5	7	10	13
CK	2.82	3.11	3.06	2.78	2.99	2.86	2.91	3.26
TRX	2.82	2.83	3.11	2.76	2.93	2.39	2.65	2.86
TR3X	3.14	3.22	3.15	2.66	2.83	2.76	2.78	3.21

TR: Triadimefon; 3X: three times the recommended dose; CK: blank control.

TABLE-5
CONCENTRATION OF CHLOROGENIC ACID OF FLOWERS IN DIFFERENT DAY AFTER TREATMENT BY TRIADIMEFON IN 2006 (mg kg^{-1})

Treatment	Days after lonicera treated by pesticide (day)									
	0	1	2	3	5	6	7	9	10	12
CK	3.12	3.12	3.34	3.34	3.15	2.85	3.13	3.03	3.25	3.00
TRX Ⅰ	3.75	3.56	3.74	3.82	3.27	3.11	3.27	3.19	3.28	3.37
TRX Ⅱ	2.07	2.53	2.31	2.16	2.28	1.92	2.02	2.16	2.84	2.09
TRXⅢ	2.91	2.33	2.71	2.68	2.46	2.94	2.46	2.42	2.19	3.35
TR3X Ⅰ	2.29	2.77	2.53	2.97	1.93	2.38	2.18	2.13	2.62	2.15
TR3X Ⅱ	2.88	2.96	3.01	2.57	2.40	2.53	2.12	2.22	2.20	2.08
TR3XⅢ	2.99	2.96	2.80	3.05	2.68	2.35	3.10	2.19	2.56	3.18

TR: Triadimefon; X: the recommended dose; 3X: three times the recommended dose; Ⅰ, Ⅱ, Ⅲ: 3 replications; CK: blank control.

blank control group in 2006. By ANOVA statistical analysis and LSD-t test, we can find out that both of the concentrations of chlorogenic acid in flowers of lonicera applied with 1x and 5x field recommended rate were increased and had significant differences ($p < 0.05$ and $p < 0.01$, respectively), compared with blank test.

Triadimefon: Table-4 presents the concentration of chlorogenic acid in flowers of *lonicera japonic*, treated by triadimefon and the blank control group in 2005. By ANOVA statistical analysis, we can find out that both of the concentrations of chlorogenic acid in flowers of lonicera applied with 1x and 3x field recommended rate had no significant differences ($p > 0.05$), compared with blank test.

Table-5 presents the concentration of chlorogenic acid in flowers of *Lonicera japonic*, treated by triadimefon and the blank control group in 2006. By ANOVA statistical analysis and LSD-t test, we can find out that the concentrations of chlorogenic acid in flowers of lonicera applied with 1x and 3x field recommended rate of triadimefon were decreased and had significant differences ($p < 0.01$), compared with blank test.

Secondary metabolites, such as phenylpropanoids, flavonoids, terpenoids, steroidal saponins and alkaloids, are organic compounds that are not directly involved in the normal growth, development or reproduction of organisms. Plants produce secondary metabolites against fungi[9], bacteria, viruses[10], herbivores[11] and other stress[12]. Plants also produce them as colourful pigments to attract insects for pollination[13].

The secondary metabolites content in the plant is determined by genetic and environmental factors[14]. In the wild,

plants are constantly interacting with external environmental factors, such as climate, temperature[15], fertilizer[16], CO_2, water[17], insect pest[18], pathological attack[19] and chemicals, *etc*.

Pesticides are routinely used to protect plants in agricultural and ecological studies that investigate plant secondary metabolism, although the impact of exposure to agrochemicals on the concentrations of secondary metabolites in plant tissues is largely unknown[20]. Some pesticides can influence the activity of enzymes[21], cell cycle[22], gas exchange and floral development[23] and other metabolic effects[24]. At least, pesticides will have effect on the plant height[25], growth regulation[26], the concentrations of main nutrition compositions and crop yield[27].

Pesticides also influence the concentration of secondary metabolites of plant, such as the application of dexon on Datura innoxia Mill, have resulted in the suppression of alkaloids content[28]. Application of methomyl increased proanthocyanidin concentrations in mature cotton leaves[29].

From the results of this experiment, we can find out that both of the concentrations of chlorogenic acid in flowers of lonicera applied with 1x and 3x field recommended rate of imidacloprid had no significant differences ($p > 0.05$), compared with blank test. So we can use the recommended concentration of imidacloprid to control aphids in the cultivation of *Lonicera japonica*. 1x and 5x field recommended rate of acetamiprid increased the concentration of chlorogenic acid. So we advise the recommended dose of acetamiprid to control aphids. The results of triadimefon in two years are not consistent. May be the reason is that other factors, such as the climate, have bigger effect than acetamiprid at that time.

Chlorogenic acid is a compound of phenylpropaniods, which were derived from cinnamic acid pathway and cinnamic acid was derived from shikimate pathway, which produces the important branch point intermediate, chorismate.

Chorismate is then the biosynthetic precursor for anthranilate (oaminobenzoic acid), prephenate, isoprephenate and various of other aromatic compounds[30]. It is assumed that up to 60 % or more of the ultimate plant mass (dry weight) is represented by molecules once shuffled through the shikimate pathway[31]. The possible reasons for the effect of pesticides on concentration of chlorogenic acid were as follow: pesticides inhibit or promote the biological activity of the related enzymes of shikimate pathway which will generate chlorogenic acid; pesticides inhibit or promote the biological activity of the related enzymes of shikimate pathway which will not generate chlorogenic acid. Such as pesticides inhibit or promote the transformation of chorismate into other aromatic compounds; pesticides may be have reaction with some intermediates of shikimate pathway; chlorogenic acid is the product of esterification reaction between quinic acid and caffeic acid[32]. Pesticides may be influence the progresses of the reaction; Some insecticides may impact plant gas exchange processes, photosynthesis and alter plant growth and development[23]. In certain circumstances and conditions, plants generate a certain proportion of primary and secondary metabolites. But pesticides would impact on plant physiology and lead to the change of the concentrations of primary and secondary metabolites.

ACKNOWLEDGEMENTS

This paper was supported by National S & T Major Special Project on Major New Drug Innovation (Item Number: 2009ZX09502-027).

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