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Efficacy of Selected Acaricides against the Two-Spotted Spider Mite *Tetranychus urticae* on Strawberries in Greenhouse Production

Z. M. NIU, P. XIE and L. YU
Modern Agricultural Research Center, Shanxi Academy of Agricultural Sciences, Taiyuan, Shanxi, China

J. L. BI
University of California Cooperative Extension, Salinas, California, USA

***Abstract* – The efficacy of seven acaricides with their distinctive modes of action against the two-spotted spider mite, *Tetranychus urticae* (Koch), on strawberries was evaluated in potting and ground soil experiments in greenhouses. In the potting trial from 1 to 3 weeks post-treatment, bifenazate suppressed the adult mite numbers by 91% to 99% and the immature numbers by 98% to 100%; etoxazole and spirodiclofen reduced the immatures by 72% to 91% and 61% to 91%, respectively; pyridaben decreased the adults by 41% to 64% and the immatures by up to 67%. At 1 week post-treatment, Abamectin reduced the immatures by 59%. Up to 2 weeks post-treatment, matrine decreased numbers of immatures by 50% to 74% and beta-cypermethrin depressed the adult numbers by 61%. In the ground soil experiment from 1 to 4 weeks post-treatment, bifenazate decreased the adult numbers by 92% to 100% and the immature numbers by 98% to 99%; etoxazole reduced the adults by 62% to 87% and immatures by 91% to 97%; spirodiclofen reduced adults by 60% to 90% and immatures by 60% to 81%; pyridaben decreased the adults by 48% to 93% and immatures by 35% to 85%; abamectin suppressed adults by 43% to 84% and immatures by 45% to 75%. At 1 week post-treatment, matrine reduced the adults by 84% and immatures by 73%, while beta-cypermethrin suppressed the adults by 48% and the immatures by 61%. The potential role of these acaricides in integrated management of the two-spotted spider mite on strawberries in greenhouse production was discussed.**

***Keywords* – Tetranychus urticae, Acaricides, Strawberry.**

I. INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* (Koch), is a serious arthropod pest of strawberries and many other horticultural crops. Feeding by the two-spotted spider mite causes necrosis in young leaves and stems, which decreases photosynthesis and transpiration [1]. Large population of the spider mite can destroy whole plants, resulting in complete yield loss [2].

China is a leading strawberry production country in the world. The rapid increase in strawberry production in this country started in the 1980s. Since 2003, China has become the largest strawberry production country in the world [3]. In 2010, the total acreage was 117,280 ha while the total yield was 2,000,000 tons. Most of the production occurs in protected plastic greenhouses. In these greenhouses, strawberries are usually planted in beds on the ground. In addition to the domestic consumption, Chinese strawberries are exported to many countries such as Holland, Germany and Japan [3]. In recent years, this crop has been increasingly attacked by the two-spotted spider mite [4]-[6]. Prevention of economic losses urgently requires development of an integrated management program for this pest in the greenhouse production system.

The most widely used method for control of the two-spotted spider mite is the application of synthetic acaricides [7]. Intensive research has been carried out in recent years for evaluation of acaricides with novel modes of action. Bifenazate is a very selective acaricide that controls the spider mite, which is the first carbazate acaricide [8]. It may act on GABA-gated chloride channels but is still considered as a neural inhibitor [9]. Etoxazole is a member of the diphenyl oxazoline class of chemical insecticide, which is also developed for use on many fruit crops as an acaricide [10]. The action mode of etoxazole as an insecticide/acaricide was once argued to be moulting inhibition, but later work suggests this compound inhibit chitin biosynthesis [11]. Spirodiclofen, a tetronic acid derivative, has been commercialized as an acaricide highly effective against all relevant phytophagous mite species and because of its novel mode of action on lipid synthesis, this compound effectively controls mite populations resistant to other acaricides [12]-[14]. Pyridaben, a pyridazinone derivative, is an acaricide and insecticide for managing several species of pest mites and insects [15]. The acaricidal and insecticidal mode of action is in the mitochondrial electron transport inhibition [16]. Abamectin, which is derived from the soil micro-organisms *Streptomyces avermitilis*, belongs to the class of macrocyclic lactones [17]. This compound acts most likely on GABA and glutamate-gated chloride channels [18]. Abamectin has been widely used to control pest insects and mites in a range of agronomic, fruit, vegetable and ornamental crops [19]. Matrine is a quinolizidine alkaloid extracted from *Sophora flavescens* [20]. As a botanical pesticide, the mode of action is to paralyze the neural system, leading to death of the pest arthropod [21]. Beta-cypermethrin is one of the most widely used pyrethroid acaricides in agriculture, forestry and horticulture [22], [23]. Cypermethrin provides a wide efficacy spectrum with a relatively low environmental risk [24]. However, efficacy of these acaricides against the two-spotted spider mite on strawberries in China has not been comprehensively investigated.

In modern crop protection, acaricides should be biorational compounds: highly effective against mite pests and relatively safe to their predators, and low risk to human health and the environment [25]. In addition, the management of pest resistance requires access to a diversity of chemistries with different modes of action [26]. The wide array of modes of action of the seven acaricides as described above presents an exciting opportunity for the effective integration of many of these chemicals into pest management strategies because of their selectivity to targeted arthropod pests and relative safety to beneficial arthropods, other organisms and/or the environment. The availability of such chemical diversity should enable the development of a management strategy which minimizes the threat of acaricide resistance. The present study is initiated to test the efficacy of these seven acaricides against the two-spotted spider mite on strawberries in the greenhouse production system in China.

II. MATERIALS AND METHODS

A. *Acaricides and their concentrations*

The following acaricides were obtained from their respective manufacturers: bifenazate (Acramite 43% SC, from Chemtura, Philadelphia, Pennsylvania, USA), etoxazole (Zoom 110g/L SC, from Sumitomo Chemical, Central Ward, Tokyo, Japan), spirodiclofen (Envidor 240g/L SC, from Bayer Crop Science, Lyon, Rhône, France), pyridaben (Damanling 15% EC, from Tianjin Jingjin Pesticide, Wuqing, Tianjin, China), abamectin (Haode 1.8% EC, from Yuelian Chemical Industry, Fengxian, Shanghai, China), matrine (Kudoudou 1.3% AS, from Shanxi Dewei Biochemical, Yuncheng, Shanxi, China), beta-cypermethrin (Gaoxiaolvqingjuzhi 4.5% EC, from Tianjin Shipule Pesticide Technical, Wuqing, Tianjin, China). Acaricides applied at the label-recommended concentrations were as follows: bifenazate at 567.5 g•a.i. ha^{-1}, etoxazole at 153.3 g•a.i. ha^{-1}, spirodiclofen at 113.5 g•a.i. ha^{-1}, pyridaben at 126.8 g•a.i. ha^{-1}, abamectin at 21.3 g•a.i. ha^{-1}, matrine at 5.85 g•a.i. ha^{-1}, beta-cypermethrin at 94.6 g•a.i. ha^{-1}.

B. *Potting experiment*

Two strawberry bare-root seedlings (*Fragaria ananassa* Duch. cv. Albion) were planted in 4-L pots filled with soil. These pots were placed in a commercial plastic greenhouse in Taiyuan, Shanxi, China. Plants were watered every 2 to 3 days. The greenhouse was maintained under natural light at 28±2 C during daytime and 20±2 C at night when the experiment was conducted. The test was arranged in a randomized complete block design with eight replicates (Each pot was used as a replicate). When reached 3 to 5 trifoliate stage, plants were artificially infested with two-spotted spider mites collected from strawberries in a commercial greenhouse in Taiyuan.

All the acaricides were applied on 15 May 2013 (at two weeks after the infestation) at a volume of 35 ml per pot with a hand sprayer. The spray volume is based on the 1892.4 L per ha (200 gallons per acre) for 10,860 plants in standard commercial greenhouse production of strawberries in China. Spreader (Sijiling) was added to each acaricide solution. The control pots were left untreated.

Sampling of two-spotted spider mite adults, immatures, and eggs was initiated just before the application of the acaricides. The sampling was conducted on a weekly basis after the application and terminated in mid-June. Two middle leaflets of mid-aged trifoliates, each from a plant in each pot, were excised. These leaflets were placed into plastic zip-lock bags and transported in a cooler to the laboratory to count the mite adults, immatures, and eggs using a stereo dissecting microscope.

C. *Ground soil experiment*

The strawberry bare-root seedlings (cv. Albion) were planted in the fall of 2012 on two-row beds in a commercial plastic greenhouse in Taiyuan, Shanxi, China. Each bed was 0.85 m wide and 6.5 m long. Plants were watered every day for 20 minutes using a drip irrigation system. The greenhouse was maintained under natural light at 28±2 C during daytime and 20±2 C at night in June and July 2013 when the trials were conducted. The test was arranged in a randomized complete block design with five replicates. Each bed was used as an experimental plot. There were ca. 60 plants in each plot.

All the acaricides were applied on 5 June 2013 at a volume of 1892.4 L per ha (200 gallons per acre) with a back-pack sprayer. Spreader (Sijiling) was added to each acaricide solution. The control plots were left untreated.

Sampling of two-spotted spider mite adults, immatures, and eggs was initiated just before the application of all the acaricides. The sampling was on a weekly basis after the application and ended in early July. Eight middle leaflets of mid-aged trifoliates, each from a randomly selected plant in each plot, were excised. These leaflets were placed into plastic zip-lock bags and transported in a cooler to the laboratory to count the adults, immatures, and eggs.

D. *Statistical analyses*

Least significant difference (LSD) test in one-way randomized complete block design of ANOVA in SAS [27] was used to analyze the data and separate the means for samples from each sampling date. Before the analysis of variance, numbers of the mite adults, immatures and eggs were transformed using the formula log (y + 1) in order to normalize the data.

III. RESULTS

A. *Potting experiment*

Application of bifenazate suppressed adult two-spotted spider mite numbers by 91% to 99% ($P<0.05$) from 1 (21 May) to 3 (4 June) weeks post-treatment, compared with the untreated control, after which the adult numbers were similar ($P>0.05$) in the treatment and control plots (Fig. 1A). The bifenazate treatment reduced the immature numbers by 98% to 100% ($P<0.05$) from 1 to 3 weeks post-treatment and the effect was non-significant ($P>0.05$) thereafter (Fig. 2A). Differences in egg numbers between the bifenazate treatment and the control were not significant ($P>0.05$) on all sampling dates except 4 June, when the number was 48% fewer ($P<0.05$) in the treatment plots (Fig. 3A).

Compared with the untreated control, effect of etoxazle on adult numbers was not significant ($P>0.05$) on all the sampling dates except 28 May when the numbers were

36% fewer ($P<0.05$), and 12 June when the numbers were 10.8-fold greater ($P<0.05$) (Fig. 1B). Numbers of immatures were reduced by 72% to 91% ($P<0.05$) up to 3 weeks post-treatment and the effect of reduction was diminished ($P>0.05$) thereafter (Fig. 2B). Application of etoxazole increased egg numbers by 19% to 1.3-fold ($P<0.05$) from 21 May to 12 June sampling dates, whereas the application had no effect ($P>0.05$) on egg numbers on 28 May sampling date (Fig. 3B).

In comparison with the control, spirodiclofen had no effect on adult numbers ($P>0.05$) on all the sampling dates except 28 May, when the numbers were 59% fewer ($P<0.05$), and 12 June, when the numbers were 8.8-fold greater ($P<0.05$) (Fig. 1B). Application of spirodiclofen suppressed numbers of immatures by 61% to 91% ($P<0.05$) from 1 (21 May) to 3 (4 June) weeks post-application, whereas the effect was diminished ($P>0.05$) at 4 (12 June) weeks post-application (Fig. 2B). Spirodiclofen treatment reduced egg numbers by 39% ($P<0.05$) at 1 (21 May) week post-treatment than the control, after which the numbers were similar in the treatment as in the control ($P>0.05$) (Fig. 3B).

Compared with the control, application of pyridaben resulted in a reduction of adult numbers by 41% to 64% ($P<0.05$) from 2 (28 May) to 3 (4 June) weeks post-application (Fig. 1C), whereas the application suppressed the immature numbers by up to 67% ($P<0.05$) from 1 (21 May) to 2 (28 May) weeks post-application (Fig. 2C). Pyridaben generally had no effect ($P>0.05$) on egg numbers on all sampling dates with the exception of 4 June, when the egg numbers had decreased by 44% ($P<0.05$) (Fig. 3C).

In comparison with the untreated control, effect of abamectin on adult numbers was not significant ($P>0.05$) on all the sampling dates except 28 May when the adult numbers were 18% fewer ($P<0.05$), and 12 June when the numbers were 6.2-fold greater ($P<0.05$) (Fig. 1C). Numbers of immatures were reduced by 59% ($P<0.05$) at 1 (21 May) week post-treatment, after which the effect of reduction was generally diminished ($P>0.05$) (Fig. 2C). Numbers of eggs were decreased by 70% ($P<0.05$) at 1 (21 May) week post-treatment and the effect was non-significant ($P>0.05$) thereafter (Fig. 3C).

The adult numbers in the matrine treatment and the control were similar ($P>0.05$) on all sampling dates except 28 May, when the numbers were 59% fewer ($P<0.05$) (Fig. 1D). Numbers of immatures in the treatment were reduced by 50% to 74% ($P<0.05$) up to 2 (28 May) weeks post-treatment, after which the effect of reduction was generally disappeared ($P>0.05$) (Fig. 2D). Differences in egg numbers between the matrine treatment and control were not significant ($P>0.05$) on all sampling dates except 21 May, when the number was 50% fewer ($P<0.05$) in the treatment (Fig. 3D).

When compared with the untreated control, beta-cypermethrin treatment depressed the adult numbers by 61% ($P<0.05$) at 2 (28 May) weeks post-treatment and the effect was non-significant ($P>0.05$) on other sampling dates (Fig. 1D). Differences in immature numbers between the beta-cypermethrin treatment and control were not significant ($P>0.05$) on all sampling dates except 12 June, when the number was 2-fold greater ($P<0.05$) in the treatment (Fig. 2D). Numbers of eggs were decreased by 48% ($P<0.05$) at 1 (21 May) week post-treatment and the effect was non-significant ($P>0.05$) thereafter (Fig. 3D).

B. Ground Soil experiment

Application of bifenazate decreased adult numbers by 92% to 100% ($P<0.05$) from 1 (12 June) to 4 (2 July) weeks post-treatment in comparison with the untreated control (Fig. 4A). The immature mite numbers were decreased by 98% to 99% ($P<0.05$) by the bifenazate treatment from 1 (12 June) week post-treatment and the efficacy lasted for another 3 weeks (Fig. 5A). Bifenazate treatment had no effect ($P>0.05$) on egg numbers on all the sampling dates except 12 June, when the numbers were 38% fewer ($P<0.05$), and 2 July, when the numbers were 33% fewer ($P<0.05$) (Fig. 6A).

In comparison with the untreated control, etoxazole application reduced the adult numbers by 62% to 87% ($P<0.05$) (Fig. 4B) and suppressed immature numbers by 91% to 97% ($P<0.05$) from 1 (12 June) to 4 (2 July) weeks post-treatment (Fig. 5B). The effect of reduction on the egg numbers was non-significant ($P>0.05$) (Fig. 6B).

Treatment of spirodiclofen resulted in a reduction in numbers of adults by 60% to 90% ($P<0.05$) from 1 (12 June) to 4 (2 July) weeks post-treatment (Fig. 4B), compared with the control. Spirodiclofen application also depressed the numbers of immatures by 60% to 81% ($P<0.05$) from 1 (12 June) to 4 (2 July) weeks post-application (Fig. 5B). Egg numbers between the spirodiclofen treatment and control were similar ($P>0.05$) from 1 (12 June) to 4 (2 July) weeks post-treatment (Fig. 6B).

Adult two-spotted spider mite numbers were significantly decreased ($P<0.05$) starting from 1 week post-application (12 June) and the efficacy lasted for another 3 weeks (until 2 July) in the pyridaben treatment compared with the control, and the decrease in adult numbers ranged from 48% to 93% (Fig. 4C). Pyridaben application suppressed numbers of immatures by 35% to 85% ($P<0.05$) from 1 (12 June) to 4 (2 July) weeks post-treatment (Fig. 5C). The egg numbers in the pyridaben-treated and control plants were similar ($P>0.05$) on all the sampling dates except 12 Jun., when the egg numbers were 23% fewer ($P<0.05$) (Fig. 6C).

Abamectin application reduced the adult numbers by 43% to 84% ($P<0.05$) from 1 (12 June) to 4 (2 July) weeks post-treatment in comparison with the untreated control (Fig. 4C). Numbers of immatures were decreased by 45% to 75% ($P<0.05$) from 1 (12 June) to 3 (25 June) weeks post-treatment and the effect was non-significant ($P>0.05$) thereafter (Fig. 5C). Differences in egg numbers between the abamectin treatment and control were not significant ($P>0.05$) on all sampling dates except 12 June, when the number was 32% fewer ($P<0.05$) in the treatment. (Fig. 6C).

Numbers of adults in the matrine treatment were reduced by 84% ($P<0.05$) at 1 week post-treatment (12 June), after which the numbers increased 61% to 76% ($P<0.05$) (except 2 July sampling date, when the effect of increase was not significant (P>0.05)) (Fig. 4D). Numbers of immatures were reduced by 73% and 62% (P<0.05) at 1 (12 June) and 2 (19 June) weeks post-treatment, respectively, after which the effect of reduction was diminished (P>0.05) (Fig. 5D). Differences in egg numbers between the matrine treatment and control were not significant (P>0.05) on all of the sampling dates except 12 June, when the number was 30% fewer (P<0.05) in the treatment (Fig. 6D).

Beta-cypermethrin had no effect (P>0.05) on numbers of adult two-spotted spider mite on all sampling dates except 12 June, when the adult numbers were 48% fewer (P<0.05) and 25 June when the numbers were 83% greater (P<0.05), compared with the control (Fig. 4D). Application of beta-cypermethrin suppressed immature numbers by 61% and 18% (P<0.05) only on 1 (12 June) and 2 (19 June) weeks post-application (Fig. 5D), respectively. The egg numbers in the beta-cypermethrin treatment and control were similar (P>0.05) on all sampling dates (Fig. 6D).

IV. DISCUSSION

Our results showed that bifenazate was highly effective in killing adults and immatures of the two-spotted spider mite on strawberries (Fig. 1A, 2A, 4A, 6A) in both potting and ground soil experiments, consistent with results observed in California and Florida, USA [28]-[31]. Reference [32] reported that bifenazate was less effective on the egg stage but most nymphs that hatched from treated eggs died in a few days after hatching. Our results also indicated that bifenazate had no effect on egg numbers (Fig. 3A, 6A). The sharp decrease in nymph populations in bifenazate treatment, which took place in 2 weeks after the application (Fig. 2A, 5A), may be caused by mass mortality of immatures that hatched from the treated eggs. Previous reports have not shown any cross-resistance in the spider mites between bifenazate and other acaricides [33], [34]. In addition, predators of the two-spotted spider mite could sustain their populations on prey mites treated with bifenazate. Therefore, bifenazate can be used as an environmentally friendly acaricide for the integrated management of tetranychid mites due to its high efficacy and selectivity [32]. It can also be a valuable tool in the mite resistance management program.

It has been reported that etoxazole was very effective in killing *Panoneychus citri*, another phytophagous mite species [35]. With the strong lethal effect to phytophagous mites [36], etoxazole dramatically suppressed nymphal populations of the two-spotted spider mite in both of our potting and ground soil experiments (Fig. 2B, 5B). Etoxazole also reduced the adult numbers (Fig. 1B, 4B). However, it was reported that etoxazole was toxic to predatory mites [37], [38] and the toxicity may be due to the transovarial biotransference [39]. To minimize its adverse effect on beneficial mites, etoxizole may be used in late season as a component in the integrated spider mite management programs.

As a class of acaricide that interferes with lipid biosynthesis [40], spirodiclofen has the ability to control several phytophagous mite species on various crops [41]-[43]. This study revealed that spirodiclofen markedly depressed adult and immature numbers of the two-spotted spider mite (Fig. 1B, 2B, 4B, 5B), which may be resulted from its high toxicity to nymphs and the inhibitory effect on egg hatching of tetranychid mites [44], [45]. It was reported that spirodiclofen is safe on beneficial organisms and has a favorable environmental profile [46]. No cross-resistance of spirodiclofen to any important insecticide and acaricide was found [46]. Together with results in this study, we conclude that spirodiclofen can be an important component of the integrated spider-mite management programs on strawberries and can be a useful tool in the mite resistance management programs.

This study showed that pyridaben significantly decreased the adult and immature numbers of the two-spotted spider mite (Fig. 1C, 2C, 4C, 5C). With the action mode on mitochondrial electron transport inhibition (Kim et al., 2006), pyridaben has an acute toxicity and can effectively control pest mites [47]. However, it was reported that the persistence of pyridaben to control the phytophagous mites was undesired [48], which may be caused by the residual toxicity to predatory mites [49], [50] and/or the resistance in pest mites [51].

Results in this study indicated that abamectin effectively reduced adult and immature numbers of the two-spotted spider mite (Fig. 1C, 2C, 4C and 5C), in agreement with previous reports [52]. Although there are reports showing it has adverse effect on beneficial organisms [53], [54], abamectin could be selected for use against pest mites of economic importance in horticulture and agriculture [19] due to its rapid degradation and poor leaching potential [55].

It has been reported that matrine has a broad-spectrum activity in killing pest mites and aphids [56] but is safe to beneficial arthropods and the environment [21]. Matrine has favorable performance in the management of *Panonychus citri* on citrus [57], [58] and apple [59], [60]. In contrast, this study showed that matrine had limited efficacy on the two-spotted spider mite in either potting or ground soil experiment (Fig. 1D, 2D, 3D, 4D, 5D, 6D). The discrepancy in efficacy may be due to the difference in mite species and/or host plant species.

Beta-cypermethrin is a synthetic pyrethroid that has been used for agricultural arthropod pest control in China since 1988 [61]. In our trials, beta-cypermethrin had very limited effect on control of the two-spotted spider mite (Fig. 1D, 2D, 3D, 4D, 5D, 6D), probably due to the resistance. Many reports indicated that cypermethrin is highly toxic to predatory mites but has no appreciable toxicity to pest mites [62], [63]. High levels of resistance to this class in phytophagous mites have developed worldwide due to the intensive use over the past 30 years [64].

In summary, this study demonstrated that bifenazate, etoxazole, spirodiclofen, pyridaben and abamectin are effective in the control of the two-spotted spider mite on strawberries in the greenhouse production system in China while matrine and cypermethrin are not effective. Together with other control methods such as releases of predatory mites and use of host plant resistance [5], [65], integration of these effective acaricides would provide an excellent opportunity to manage the two-spotted spider mite and the possible resistance to any single compound in strawberry production in China.

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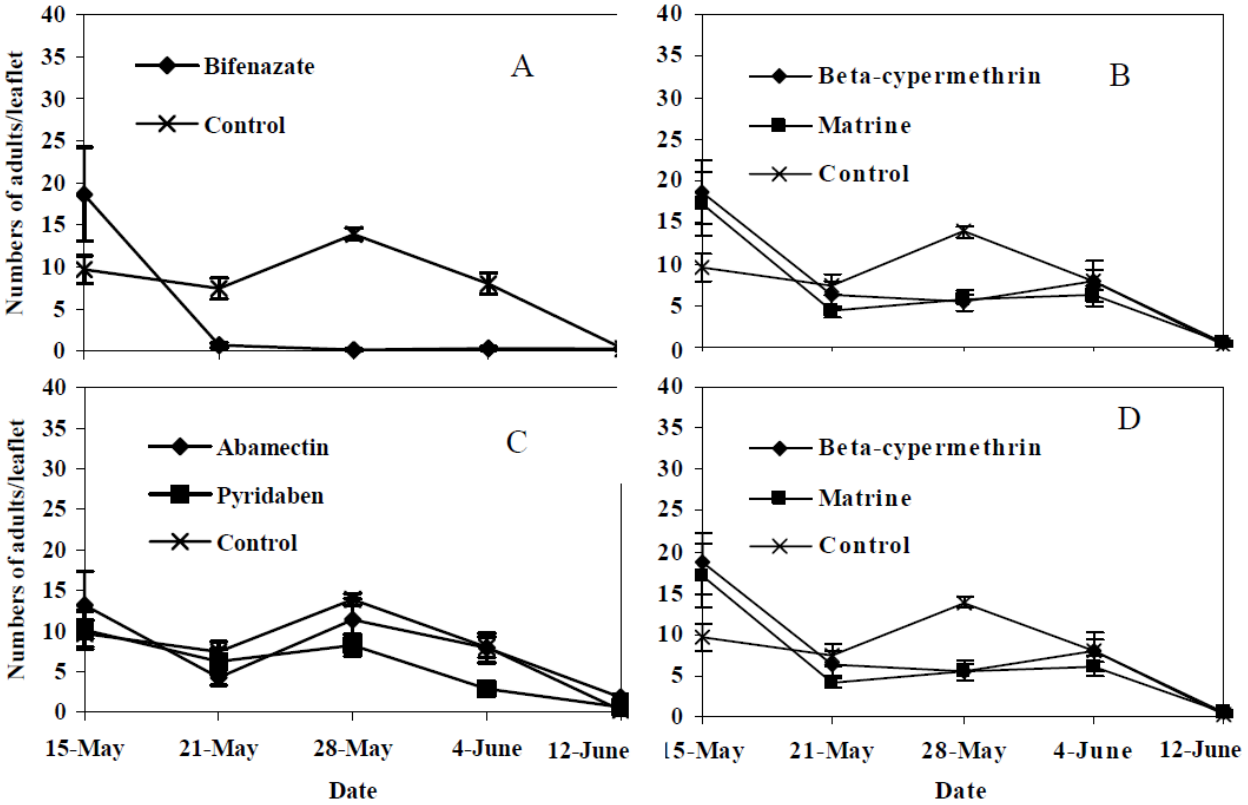

Fig. 1. Efficacy of (A) bifenazate, (B) etoxazole and spirodiclofen, (C) pyridaben and abamectin, (D) matrine and beta-cypermethrin on adult numbers of the two-spotted spider mite on strawberries in the potting experiment. Error bars represent standard errors.

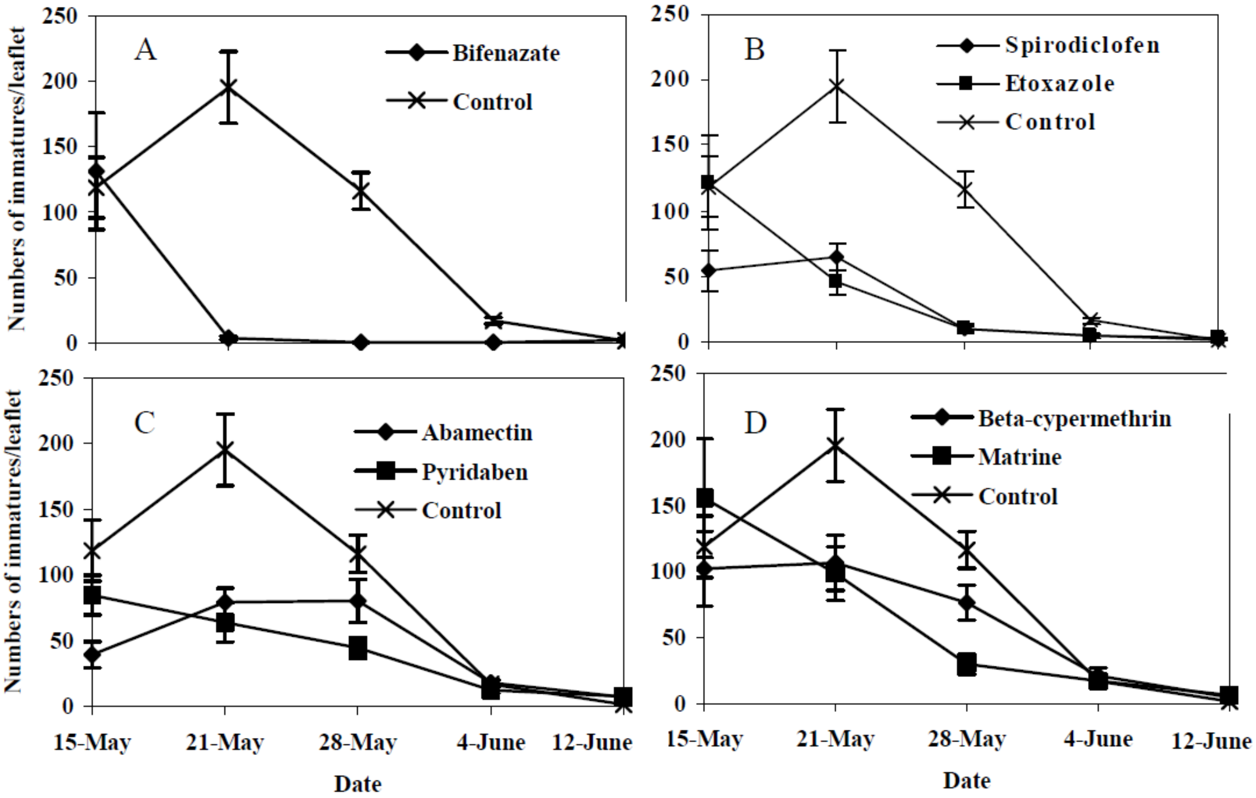

Fig. 2. Efficacy of (A) bifenazate, (B) etoxazole and spirodiclofen, (C) pyridaben and abamectin, (D) matrine and beta-cypermethrin on immature numbers of the two-spotted spider mite on strawberries in the potting experiment. Error bars represent standard errors.

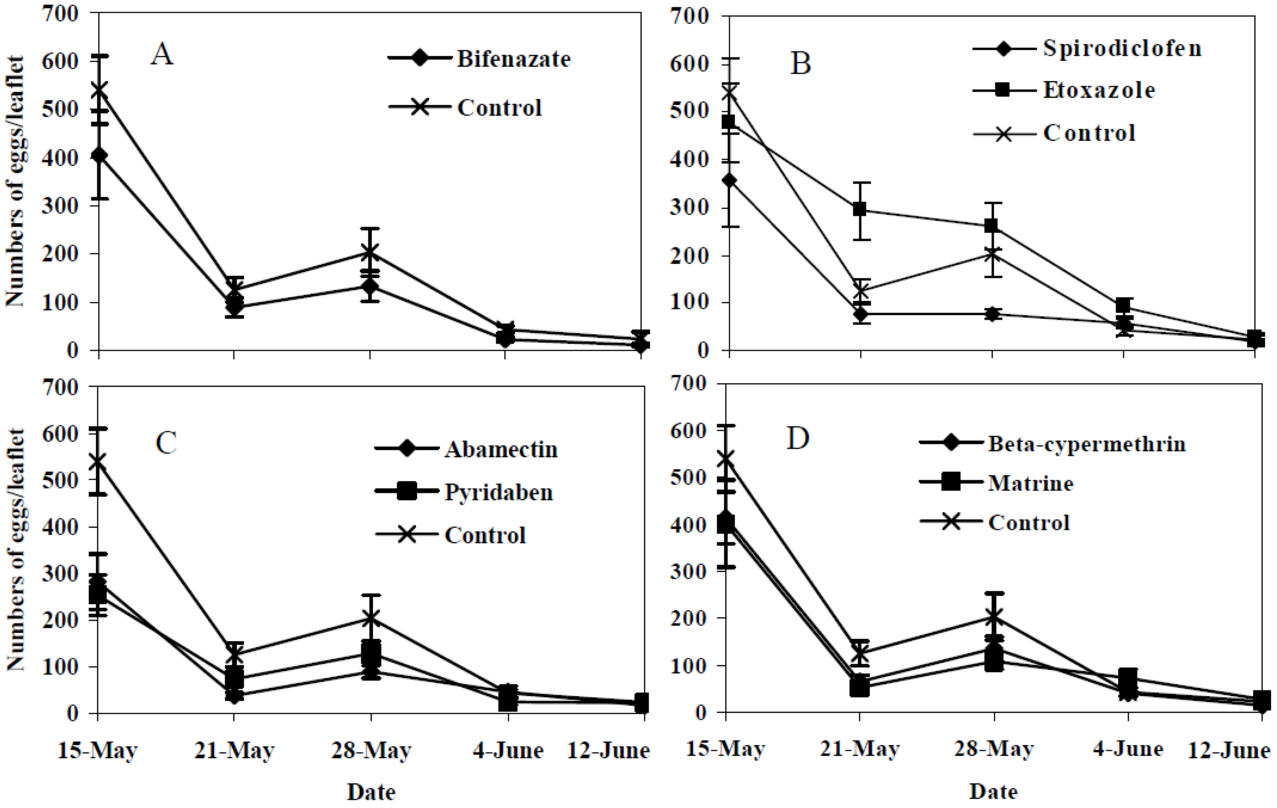

Fig.3. Efficacy of (A) bifenazate, (B) etoxazole and spirodiclofen, (C) pyridaben and abamectin, (D) matrine and beta-cypermethrin on egg numbers of the two-spotted spider mite on strawberries in the potting experiment. Error bars represent standard errors.

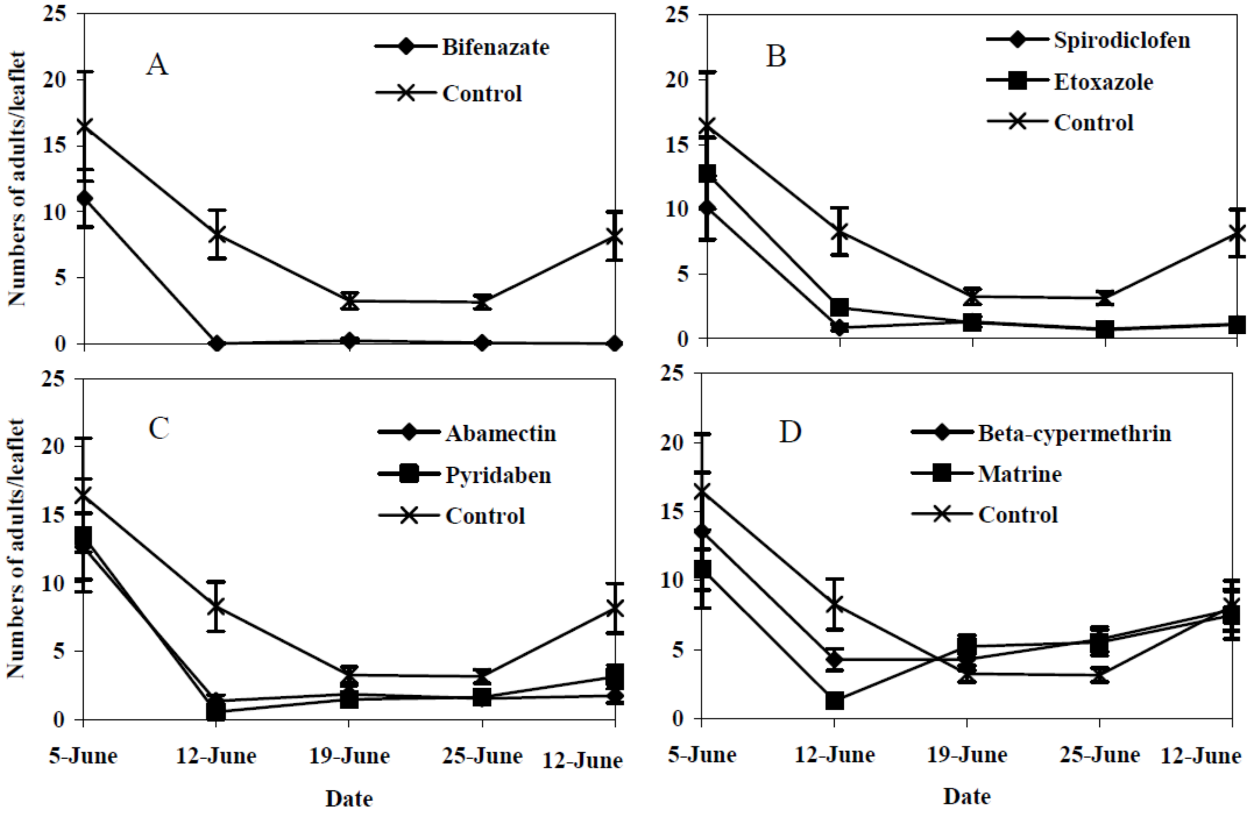

Fig. 4. Efficacy of (A) bifenazate, (B) etoxazole and spirodiclofen, (C) pyridaben and abamectin, (D) matrine and beta-cypermethrin on adult numbers of the two-spotted spider mite on strawberries in the ground soil experiment. Error bars represent standard errors.

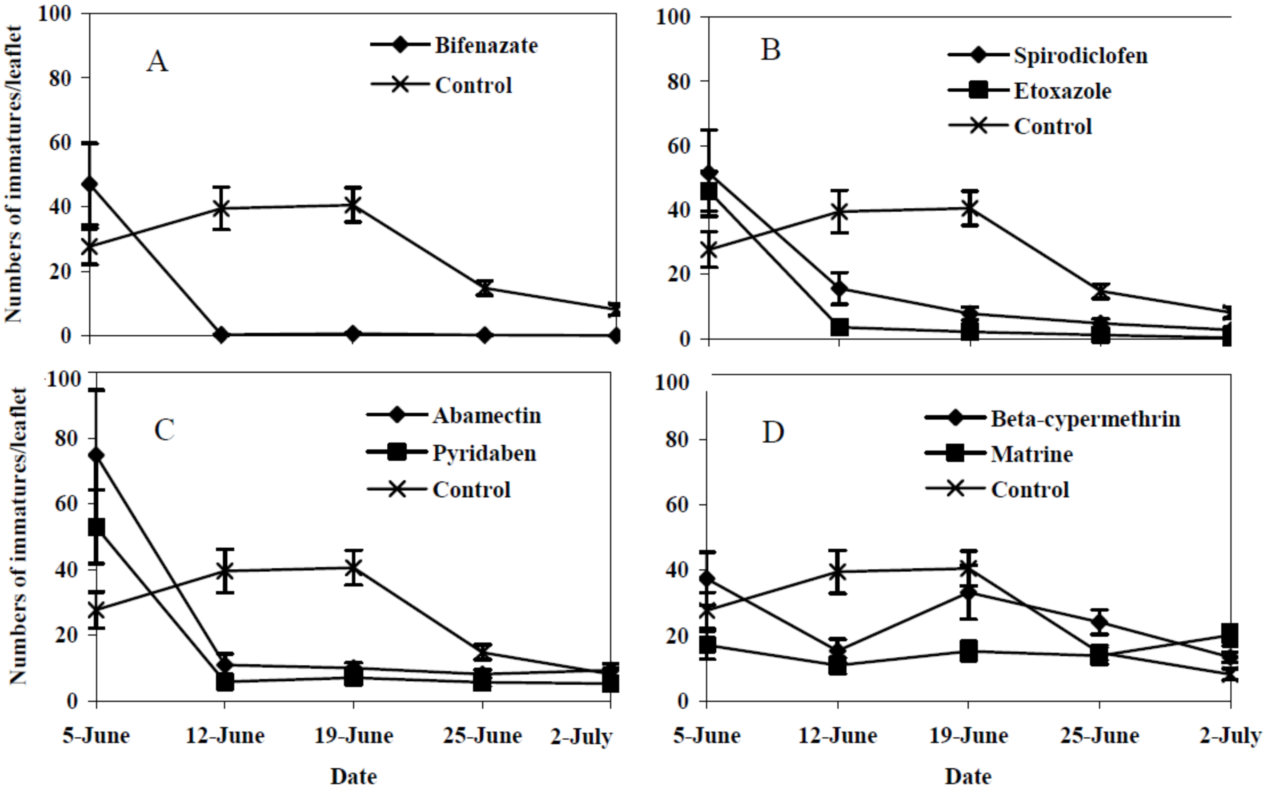

Fig. 5. Efficacy of (A) bifenazate, (B) etoxazole and spirodiclofen, (C) pyridaben and abamectin, (D) matrine and beta-cypermethrin on immature numbers of the two-spotted spider mite on strawberries in the ground soil experiment. Error bars represent standard errors.

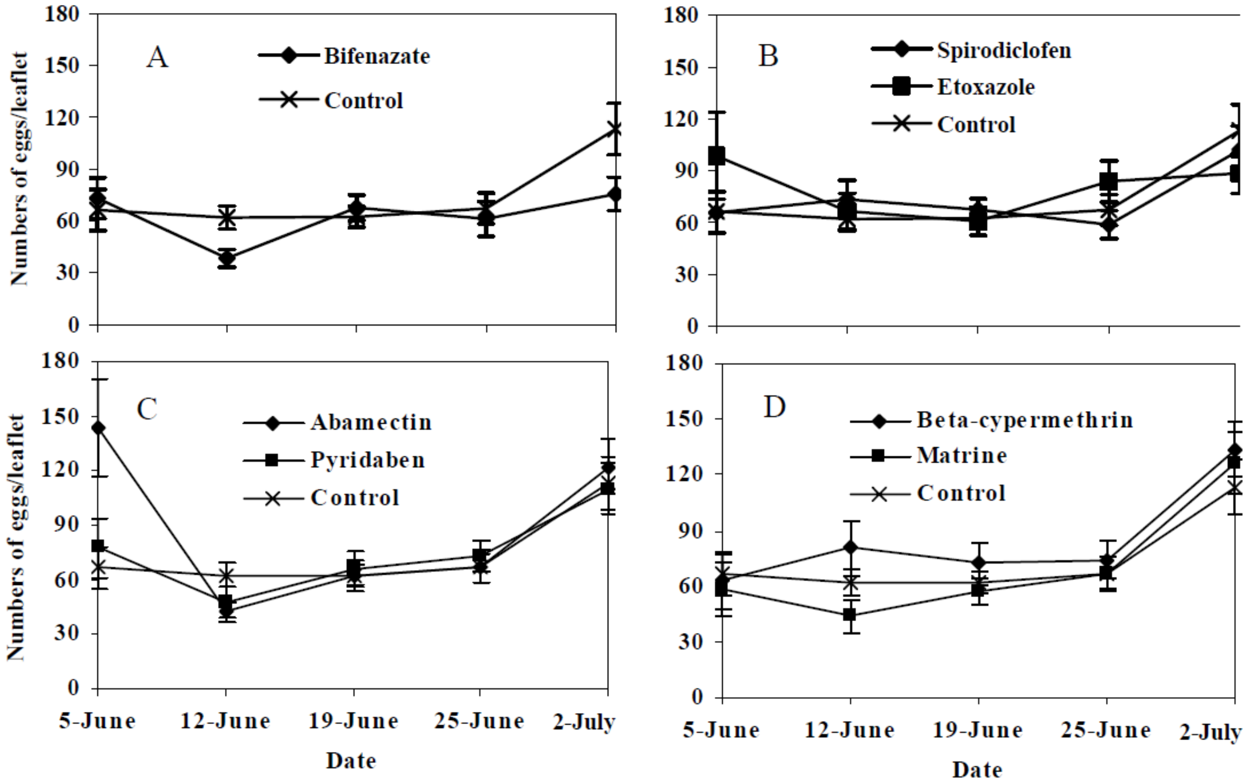

Fig.6. Efficacy of (A) bifenazate, (B) etoxazole and spirodiclofen, (C) pyridaben and abamectin, (D) matrine and beta-cypermethrin on egg numbers of two-spotted spider mite on strawberries in the ground soil experiment. Error bars represent standard errors.