



# Predatory Efficacy of the Black Ladybird Beetle, *Stethorus* sp. against the Two-spotted Red Spider Mite, *Tetranychus urticae* Koch on Rose Plants

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## ABSTRACT

**Background:** The black ladybird beetle, *Stethorus* sp. is a generalist predator whose larvae and adult prey on all developmental stages of the two-spotted red spider mite, *Tetranychus urticae* Koch. In order to develop a biological control program of *T. urticae* in the Mekong Delta of Vietnam, the predatory efficacy of *Stethorus* sp. on *T. urticae* had been investigated.

**Methods:** The investigation was conducted under the laboratory and net-house conditions at the College of Agriculture, Can Tho University.

**Result:** Under laboratory conditions, fourth instar larvae exhibited the highest predation rate, while second instar larvae showed the lowest. Second instar larvae consumed an average of 65.63 eggs, 26.13 larvae, or 22.38 adults per day. In contrast, fourth instar larvae consumed 183.63 eggs, 88.63 larvae, or 73.63 adults per day. Adult female beetles consumed 149.38 eggs, 75.63 larvae, or 46.5 adults per day, while adult males showed lower consumption rates of 88 eggs, 62.25 larvae, or 36.13 adults per day. Under net house conditions, one pair of black ladybird beetles provided optimal control of the two-spotted red spider mites at a density of 56 mites per plant, with a control efficacy of 97.07 percent at 5 days post-release. At a red spider mite density of 168 individuals per plant, 4 and 6 pairs of black ladybird beetles demonstrated superior control efficacy of 93.02 percent and 99.54 percent respectively at 5 days post-release.

**Key words:** Black ladybird beetle *Stethorus* Sp., Predator, Prey consumed, Two-spotted spider mites *tetranychus urticae* koch.

## INTRODUCTION

The rose (*Rosa* spp.; Rosaceae) holds a prominent position in urban landscapes and the global cut flower industry (Chacón-Hernández *et al.*, 2020). However, rose cultivation faces significant challenges from pest infestations, particularly the two-spotted red spider mite, *Tetranychus urticae* Koch (Lee *et al.*, 2022). This pest causes substantial damage by feeding on plant sap through piercing-sucking mouthparts, making leaves more susceptible to secondary infections and viral diseases (Zhao *et al.*, 2023). *T. urticae* has emerged as a formidable pest in both outdoor and greenhouse environments, affecting over 1,000 plant species, including vegetables, fruit trees and ornamental flowers (Ragkou *et al.*, 2004; Li and Zhang, 2021).

The economic impact of *T. urticae* infestations is considerable, resulting in significant reductions in crop yield and quality (Rott and Ponsonby, 2000; Trivedi *et al.*, 2021). While chemical pesticides have traditionally been the primary control method (Tiftikçi *et al.*, 2020), their extensive use has raised serious concerns regarding human health, ecosystem stability, the elimination of natural enemies and the development of pesticide resistance in target populations (Assouguem *et al.*, 2022). The increasing occurrence of acaricide resistance has prompted complicated control efforts (García-Iñáñez and González-Zamora, 1999; Xu *et al.*, 2023).

These challenges have sparked growing interest in biological control agents as sustainable and environmentally friendly alternatives (Cruz-Mirallés *et al.*, 2022; Demirel

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*et al.*, 2024). Among the natural enemies of red spider mites (Moghadasi *et al.*, 2013), the black lady beetles of the genus *Stethorus* have shown particular promise. These small beetles, measuring only 1-1.5 mm in length and characterized by their black coloration with brown or yellow setae (Chazeau, 1985), are recognized worldwide as effective biological control agents of red spider mites (Roy *et al.*, 1999; Khan *et al.*, 2002).

*Stethorus* species demonstrate remarkable adaptability across various geographical and climatic regions, including both temperate and tropical zones (Biddinger *et al.*, 2009; Kundoo and Khan, 2017). Their effectiveness as predators is attributed to their rapid mobility, flight capability and high prey consumption rates (Mirza *et al.*, 2023). Additionally,

these beetles can sustain their populations during periods of prey scarcity by utilizing alternative food sources such as raisins, aphids, mealy bugs and nectar (Pemberton and Vandenberg, 1993).

While extensive research has led to the successful commercialization of certain *Stethorus* species, such as *S. punctillum* and *S. pauperculus*, for red spider mite control in various countries (Biswas *et al.*, 2007; Godhani and Shukla, 2015), such studies remain limited in Vietnam. Therefore, this study aims to evaluate the predatory efficacy of *Stethorus* sp. against *T. urticae* on rose plants, examining predation rates across different life stages and assessing its potential as a biological control agent under net house conditions. The findings could contribute significantly to the development of sustainable pest management strategies in Vietnamese agriculture, particularly in rose cultivation.

## MATERIALS AND METHODS

### Two-spotted red spider mite (*T. urticae*)

Two-spotted red spider mite were collected from the rose orchards in Can Tho area and raised on rose plants grown in biological control laboratory and net houses conditions in the Faculty of Plant Protection, College of Agriculture, Can Tho University to multiply numbers. The local potting rose variety was used in this experiment.

### Black ladybird beetles (*Stethorus* sp.)

Black ladybird beetles (*Stethorus* sp.), both in larval and adult stages, were collected simultaneously during the two-

spotted red spider mite collection process in the field. These predatory beetles were then reared through multiple generations under laboratory conditions to establish and maintain adequate population levels for experimental purposes.

### Investigation the predatory ability of black ladybird beetle against different life stage of two-spotted red spider mites on rose plants

The experiment was carried out to investigate the predatory capacity of black ladybird beetle larvae and adults against eggs, larvae and adults of the two-spotted red spider mites. The experiment followed a completely randomized design with one factor, including 4 treatments and 4 repetitions: the second instar black ladybird beetle larvae (1), the fourth instar black ladybird beetle larvae (2), male adult black ladybird beetles (3) and female adult black ladybird beetles (4).

### Experimental setup

Clean rose leaves were prepared by wrapping water-soaked cotton around the leaf petiole and placing them face down on Petri dishes lined with moistened cotton (Fig 1A). Prey items were then introduced onto the leaf surface as follows.

### Red spider mite egg stage

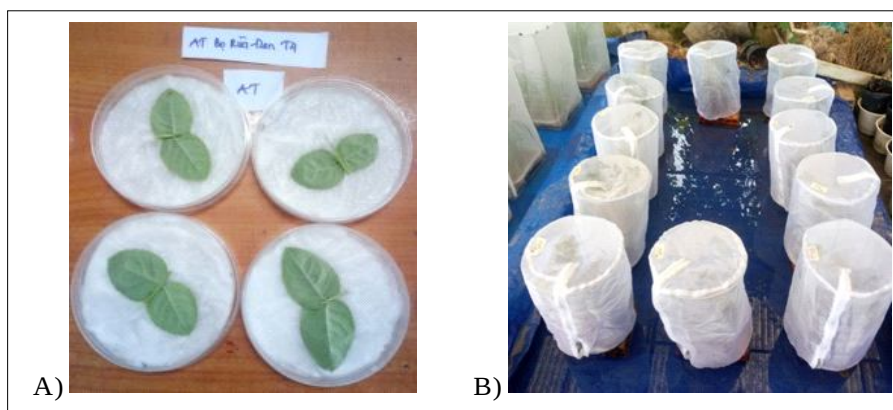
Laboratory-reared female of the two-spotted red spider mites (30 individuals/leaf) were placed on prepared rose leaves to lay eggs. After one day, all mites were removed, red spider mite eggs were counted and 200 eggs per leaf disc were retained. Then, one healthy black ladybird beetle corresponding to each treatment was introduced onto the rose leaf having red spider mite eggs as food. The Petri dish was covered with a mesh lid to prevent black ladybird beetle escape. Fresh 200 eggs of red spider mite were replaced daily until the end of the experiment.

### Red spider mite larval stage

Laboratory, reared 100 larvae of the two-spotted red spider mites were selected having uniform age and size, placed per leaf disc and allowed to settle for 24 hours before

**Table 1:** Treatments of the experiment on releasing black ladybird beetles.

| Treatment | Number of red spider mites infested leaves | Density of red spider mites/pot |
|-----------|--------------------------------------------|---------------------------------|
| 1         | 8                                          | 56                              |
| 2         | 24                                         | 168                             |
| 3         | 56                                         | 392                             |
| Control   | 56                                         | 392                             |



**Fig 1:** Clean rose leaves wrapped by water-soaked cotton (A); Rose plants were enclosed in mesh cages (B).

introducing the black ladybird beetles. One black ladybird beetle per treatment was released onto the rose leaf having red spider mite larvae and covered with a mesh lid. Fresh red spider mite larvae (100) were replaced daily until completion, of the experiment.

### Red spider mite adult stage

The procedure was similar to the larval stage experiment. The experiment was conducted with 100 red spider mite adults per leaf disc and were replaced daily.

### Observations

The monitoring criteria included counting the number of red spider mite eggs, larvae and adults consumed by the black ladybird beetles at 1 and 2 days after beetle introduction. The mean values were calculated.

### Predatory capacity of black ladybird beetle against different two spotted red spider mite

The experiment aimed to examine the predatory capacity of black ladybird beetles against different densities of red spider mites under net house conditions. The experiment followed a completely randomized design with one factor, including 3 treatments and 1 control (Table 1), with 3 repetitions.

### Experimental setup

Rose plants with uniform leaf development (approximately 80 leaves/pot) were selected. Seven adult two-spotted red spider mites were released per leaf and allowed to settle. The number of released red spider mites and treated leaves corresponded to treatments in Table 1. Plants were enclosed in mesh cages to prevent insect entry/exit (Fig 1B). One day after red spider mite release, populations were counted to ensure survival, then one pair of adult black ladybird beetles (male and female) was introduced to each prepared rose pot. The black ladybird beetles were reared from the Biological Control Laboratory (CTU).

### Observations

The monitoring criteria included counting the number of two-spotted red spider mite-infested leaves and remaining spider mites at 1, 3, 5, 7 and 14 days after releasing the black ladybird beetles. Red spider mites were counted using a hand lens. The mean values were calculated.

### Investigation of optimal density of black ladybird beetles for controlling two-spotted red spider mites

The experiment was carried out to determine the optimal density of black ladybird beetles to be released for effective control of two-spotted red spider mites under net house setting. This aims to provide a basis mass-rearing and releasing black ladybird beetles in the field. The experiment was designed as a completely randomized design with 1 factor trial with 5 treatments (1 pair of black ladybird beetles, 2 pairs of ladybird beetles, 4 pairs of ladybird beetles, 6 pairs of ladybird beetles, Control: no black ladybird beetles released), with 4 repetitions.

### Experiment setup

Uniform rose plants in terms of leaf quantity and growth were selected. Red spider mites were placed on the rose leaves in the same manner as in the previous experiment, with a total of 168 mites for each treatment (30% infested leaves). Following this, 1 pair, 2 pairs, 4 pairs and 6 pairs of adult black ladybird beetles were released according to each treatment, into rose pots that had been covered with netting and prepared with red spider mites.

### Observations

The monitoring criteria included the number of damaged leaves and the number of surviving red spider mites at 1, 3, 5, 7 and 14 days after releasing the black ladybird beetles. The two-spotted red spider mites were counted using a handheld magnifying glass.

### Data collection and analysis

The two-spotted red spider mites control rate was calculated using the Henderson-Tilton formula (Hsieh *et al.*, 2023). The mortality data and the control rate of spider mite are expressed as the mean of the replications. The percentage data was arcsin-transformed for further statistical. All data were processed by MSTATC software in subjecting to one-way analysis of variance (ANOVA). Significance of differences among means was calculated by Duncan's test.

## RESULTS AND DISCUSSION

### Predatory capacity of black ladybird beetle on different life stages of two-spotted red spider mites

The results in Table 2 demonstrate that, under laboratory conditions, both larval and adult stages of black ladybird

**Table 2:** The feeding ability of the black ladybird beetles at different stages with red spider mites.

| Black ladybird beetle stage   | Two-spotted red spider mites (avg/day) consumed |                    |                    |
|-------------------------------|-------------------------------------------------|--------------------|--------------------|
|                               | Eggs                                            | Larvae             | Adult              |
| 2 <sup>nd</sup> instar larvae | 65.63 <sup>d</sup>                              | 26.13 <sup>d</sup> | 22.38 <sup>d</sup> |
| 4 <sup>th</sup> instar larvae | 183.63 <sup>a</sup>                             | 88.63 <sup>a</sup> | 73.63 <sup>a</sup> |
| Male adult                    | 88.00 <sup>c</sup>                              | 62.25 <sup>c</sup> | 36.13 <sup>c</sup> |
| Female adult                  | 149.38 <sup>b</sup>                             | 75.63 <sup>b</sup> | 46.50 <sup>b</sup> |
| Significance level            | **                                              | **                 | **                 |

Note: Within the same column, means with the same letter are not statistically different at the 1% level (Duncan's test).

**Table 3:** Control efficacy of one pair of black ladybird beetles for two-spotted red spider mites at different densities.

| Treatments               | Two-spotted red spider mites control rate by one pair of black ladybird beetles (%) |                    |                    |                    |                    |
|--------------------------|-------------------------------------------------------------------------------------|--------------------|--------------------|--------------------|--------------------|
|                          | 1 DAR                                                                               | 3 DAR              | 5 DAR              | 7 DAR              | 14 DAR             |
| 56 red spider mites/pot  | 43.92 <sup>a</sup>                                                                  | 88.77 <sup>a</sup> | 97.07 <sup>a</sup> | 100 <sup>a</sup>   | 100 <sup>a</sup>   |
| 168 red spider mites/pot | 16.06 <sup>ab</sup>                                                                 | 16.33 <sup>b</sup> | 33.18 <sup>b</sup> | 50.85 <sup>b</sup> | 84.24 <sup>b</sup> |
| 392 red spider mites/pot | 3.95 <sup>bc</sup>                                                                  | 9.59 <sup>b</sup>  | 17.81 <sup>b</sup> | 31.67 <sup>b</sup> | 34.61 <sup>c</sup> |
| Control                  | 0.00 <sup>c</sup>                                                                   | 0.00 <sup>c</sup>  | 0.00 <sup>c</sup>  | 0.00 <sup>c</sup>  | 0.00 <sup>d</sup>  |
| Significance level       | *                                                                                   | **                 | **                 | **                 | **                 |

Notes: In the same column, means followed by the same letter are not statistically different at the (\*\*) 1% or (\*) 5% significance level according to the Duncan test. DAR: Days after releasing.

**Table 4:** Control efficacy of black ladybird beetles at different release densities against two-spotted red spider mites under net house conditions.

| Treatments         | Two-spotted red spider mites control efficacy over time after release (%) |                    |                     |                    |                    |
|--------------------|---------------------------------------------------------------------------|--------------------|---------------------|--------------------|--------------------|
|                    | 1 DAR                                                                     | 3 DAR              | 5 DAR               | 7 DAR              | 14 DAR             |
| 1 pair             | 17.12 <sup>c</sup>                                                        | 23.04 <sup>c</sup> | 39.01 <sup>c</sup>  | 73.71 <sup>c</sup> | 87.24 <sup>b</sup> |
| 2 pairs            | 38.56 <sup>b</sup>                                                        | 69.44 <sup>b</sup> | 74.69 <sup>b</sup>  | 90.56 <sup>b</sup> | 94.28 <sup>b</sup> |
| 4 pairs            | 69.42 <sup>a</sup>                                                        | 89.07 <sup>a</sup> | 93.02 <sup>ab</sup> | 100 <sup>a</sup>   | 100 <sup>a</sup>   |
| 6 pairs            | 76.86 <sup>a</sup>                                                        | 95.32 <sup>a</sup> | 99.54 <sup>a</sup>  | 100 <sup>a</sup>   | 100 <sup>a</sup>   |
| Control            | 0.00 <sup>d</sup>                                                         | 0.00 <sup>d</sup>  | 0.00 <sup>d</sup>   | 0.00 <sup>d</sup>  | 0.00 <sup>d</sup>  |
| Significance level | **                                                                        | **                 | **                  | **                 | **                 |

Note: Treatments with the same letter within the same column do not differ significantly at the 1% level (Duncan's test); DAR: Days after releasing.

beetles could prey on all developmental stages of two-spotted red spider mite. Notably, there is a significant variation in the feeding rates across different stages: Next para Second instar larvae: The fourth instar larvae, male and female larvae. Similar to other ladybird beetle species, fourth instar larvae consume the most prey to prepare for pupation. They consume an average of 183.63 eggs day<sup>-1</sup>, 88.63 larvae day<sup>-1</sup> and 73.63 adult day<sup>-1</sup> (Table 2). Adult females follow, with an average consumption of 149.38 eggs day<sup>-1</sup>, 75.63 larvae day<sup>-1</sup> and 46.5 adult day<sup>-1</sup>. The lowest prey consumption is seen at the second instar larvae, with an average of 65.63 eggs day<sup>-1</sup>, 26.13 larvae day<sup>-1</sup> and 22.38 adult day<sup>-1</sup>.

Black ladybird beetles larvae feed by sucking fluid from prey, while adults consume the entire prey. The favored stage of prey can change based on the species and growth stage of the predator (Ragkou *et al.*, 2004). Similar to the findings of Biddinger *et al.* (2009) and Sumathi *et al.* (2019), one adult female may consume 30-60 red spider mites per day depends on species of black ladybird beetles. According to Sarwar (2016), one black ladybird beetle can consume up to 75-100 red spider mites per day. Furthermore, female larvae consume more food and have a higher relative growth rate in the fourth instar larvae compared to male adults. In fact, black ladybird beetle females are known to consume more prey than males of the same species, as observed by Hull *et al.* (1977) and Chazeau (1985).

#### Control efficacy of black ladybird beetle for two-spotted red spider mites at different densities

The results in Table 3 showed that one pair of black ladybird beetles could effectively control a population of 56 two-

spotted red spider mites within 7 days, achieving 100 percent efficacy. For a population of 168 mites, efficacy is slower and lower, reaching 50.85 per cent after 7 days and 84.24 per cent after 14 days. For an initial population of 392 red spider mites, the control efficacy of one pair of black ladybird beetles is minimal, achieving only 34.61 per cent after 14 days due to the high red spider mite density, which exceeds the control capacity of one pair of black ladybird beetles. This finding is consistent with the results of Tiftikçi *et al.* (2020) who observed a decrease in control efficacy when using *Phytoseiulus persimilis* to control *T. urticae* at a low predator: prey ratio, resulting in a longer duration of control efficacy. Additionally, Opit *et al.* (2004) reported that populations of two-spotted red spider mites remained at a low level in the following weeks.

#### Control efficacy of black ladybird beetles at different release densities for two-spotted red spider mites

Table 4 shows that increasing the number of black ladybird beetles released enhances the control efficacy. The release of 4 pairs of black ladybird beetles (predator-to-prey ratios of 1: 21) provides the optimal level of control, reaching 89.07 percent efficacy by day 3 and achieving complete control by day 7, similar to the release of 6 pairs. Therefore, releasing 4 pairs of black ladybird beetles is considered optimal for controlling two-spotted red spider mites on roses or other net house plants affected by these mites. The results demonstrate that increasing the number of black ladybird beetles released enhances the efficacy of red spider mite control. The release of four or more pairs of black ladybird beetles achieved near-complete control within seven days, making these treatments effective in

reducing red spider mite populations and preventing further leaf damage. Therefore, the higher the population of black ladybird beetles, the better the ability to control two-spotted red spider mites. This is consistent with the fact that the higher the number of natural enemies that eat prey, the more prey they consume, quickly reducing the leaf damage (Chakraborty *et al.*, 2012; Akter *et al.*, 2019).

## CONCLUSION

Under laboratory conditions, the predatory capacity of black ladybird beetles against different developmental stages of two-spotted red spider mites varied. The second instar larvae of black ladybird beetles were observed to consume 65.63 eggs, 26.13 larvae and 22.38 adult mites per day. The fourth instar larvae displayed the highest predation rate, consuming 183.63 eggs, 88.63 larvae and 73.63 adults daily. Adult female ladybird beetles averaged 149.38 eggs, 75.63 larvae and 46.5 adult mites per day, while adult males consumed 88 eggs, 62.25 larvae and 36.13 adults per day. Under net house conditions, releasing a single pair of black ladybird beetles effectively controlled two-spotted red spider mite populations, reducing the percentage of infested leaves in cases of low infestation (56 two-spotted red spider mites per plant). When releasing four or six pairs of black ladybird beetles with a higher infestation level (168 mites per plant), the black ladybird beetles efficiently controlled two-spotted red spider mites in a short time, significantly reducing the proportion of two-spotted red spider mite-infested leaves. The findings of this study are promising, showing that *Stethorus* sp. exhibits significant predatory ability over two-spotted red spider mites in controlled settings. Nevertheless, further study is needed to assess the role of *Stethorus* sp. in managing two-spotted red spider mites in rose farms, particularly regarding its predatory capacity and the impact of acaricides or insecticides on these insect populations.

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## Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Conflict of interest statement

The authors declare that there is no conflict of interest.

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