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Research Article

Evaluation of release methods of egg parasitoid, *Telenomus remus* against *Spodoptera litura* in tobacco crop

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ABSTRACT: *Spodoptera litura* is a major pest of tobacco crop in China. The long-term chemical application not only failed to control it but also had a severe impact on the environment. To study the inoculative field release methods of *Telenomus remus* about release factors, the egg mass of *S. litura* as the main method was chosen. Here, we aimed to explore the optimal proportion, device type, time, density and diffusion distance by highlighting the parasitic rate, rate of emergence and sex ratio of *T. remus*. We found that the highest parasitism rate was obtained 77.95, 71.68, 67.61 and 57.87% for ratio (wasps/hosts 1:10), days (within 1 day), egg age (within 0 hours), and dispersal distance (within 5 m), respectively. The highest emergence rate of *T. remus* (96.38%) was observed when the parasitized eggs were placed in transparent perforated plastic bottles. The blue and yellow sticky cards placed in the lower part of tobacco leaves had the least effect on the diffusion of *T. remus*, only stacked by 14 and 15 individuals respectively; along with the lowest feeding rate by *Nesidiocoris tenuis* (28.73%), which was observed on egg mass covered by scale layer. From the perspective of high production and low-cost technology, we conclude that these findings will establish a robust groundwork for deploying *T. remus* in heavy infested areas of *S. litura* for its management.

KEYWORDS: Biological control, field inoculation, low-cost technology, *Spodoptera litura*, *Telenomus remus*

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INTRODUCTION

China is the leading country in tobacco production and consumption globally, with the total revenue generated from the tobacco sector accounting for about 30% of the world (Wang, 2015). Tobacco is an important cash crop as well as an important source of fiscal revenue (Huang, 2008). The quality of the tobacco products determines the sales volume, and noctuid pests have a devastating effect on the quality of the tobacco leaves. The quality of the tobacco leaves is greatly reduced after being damaged by the *Spodoptera litura* (Lepidoptera: Noctuidae), which is one of the major pests of the tobacco crop resulting in lowest production, and huge economic losses to the tobacco farmers. It occurs in all major tobacco-producing areas of China (Chen *et al*., 2001). Presently, most farmers rely on chemical control to combat this pest, but due to its ill effects like increased resistance of *S. litura* to insecticides, pesticide residues, environmental pollution, there is a need to look at alternative control measures.

In 2006, the green prevention idea was first perceived to combat pests and diseases of crops to ensure plant health and environment through a variety of comprehensive control measures including conventional, physical and biological control (Yang and Chen, 2017). Biological control uses living organisms (predators and parasitoids) and their metabolites to control pests. It is reported that many countries in Latin America have achieved remarkable results in the field application of *Telenomus remus* to control the invasive pest, *Spodoptera frugiperda* (GutiErrez-Martinez *et al*., 2012; WanBin *et al*., 2019). Reports also indicated that in China, *T. remus* infests *S. litura* and *S. exigua*, which are closely related noctuid species (Goulart *et al*., 2011; Prezotti *et al*., 2004; Pomari-Fernandes *et al*., 2018; Queiroz *et al*., 2017). *Telenomus remus* (Hymenoptera: Platygastridae) is an important egg parasitoid of several noctuid pests. Under laboratory conditions, the parasitic rate of *T. remus* could reach to more than 95%. Due to its high parasitic rate and easy reproduction, *T. remus* has become one of the main biological agents to control noctuid pests (Zai-fu, 2010). *Telenomus remus* lays its eggs in the egg masses of *S. litura* and completes its later development by using the nutrients from the host (Shi *et al*., 2009).

Parasitoid and host interactions indicated that the quality of the host is highly influential for the growth and development of the parasitic wasp, their survival and sex ratio (Cingolani *et al*., 2014). The age of the host could restrict its quality due to nutrient depletion during embryonic development and the parasitoid's immature incapacity to break down the host's cuticle, leading to a lack of nutrient absorption from the host egg in later developmental phases (Strand *et al*., 1986). Report on different ages of host eggs, the age of parasitoids, and the rate of parasitism revealed that female *T. remus* aged between 1 and 10 days could infest noctuid eggs from grass-covetous sources, and the age of the parasitoid had no impact on the number of eggs parasitized or the hatching. Different ages of host eggs revealed that female wasps receiving eggs from various developmental phases separately had a notably greater number of eggs parasitized by female wasps on eggs aged 1 and 2 days compared to those from 3-day-old hosts (Queiroz *et al*., 2019; Carneiro *et al*., 2010). The functional response analysis of female wasps aged 1 and 2 days revealed an increase in parasitism rates with rising egg densities, yet these rates stabilized as the egg count hit 150 eggs (Ying *et al*., 2012). Also reported the variations in how *Trichogramma* sp. release, times, heights, and quantities influence pest control (Yu *et al*., 2022).

The use of natural enemy insects is the core link in the application of biological control and plays an important role in the development of green and sustainable agriculture (Luo *et al*., 2015). Although there are reports on the use of bio-control agents to control noctuid pests still work on inoculative field release methods of natural enemies remain limited and not well explored. In this study, the eggs of *S. litura* were used as an experimental material to explore the optimal release method of *T. remus* for controlling *S. litura* and to assess the influence of different release factors on the diffusion and parasitic effect of *T. remus*. The optimal method obtained from the experiment would lay a solid foundation for biological control of the tobacco pests, and provide a theoretical basis for achieving low-cost and highly efficient technology.

MATERIALS AND METHODS

Test site

The test plots of tobacco crop were selected in Banqiao Town, Shilin County, N: 24°41′54.79″ E: 103°16′53.30″ and Kuanzhuang Town, Fumin County, N: 25°26′57.52″ E: 102°40′29.43″, Yunnan Province (Figure 1); no chemical pesticides were applied to the test plots, and the cultivar was "Hongda"; the average daily temperature of the test plots from May to July was between 22-23 °C. On average, sunshine lasted 13 hours, monthly rainfall averaged 101 mm, and wind speeds averaged 3.85 m/s.

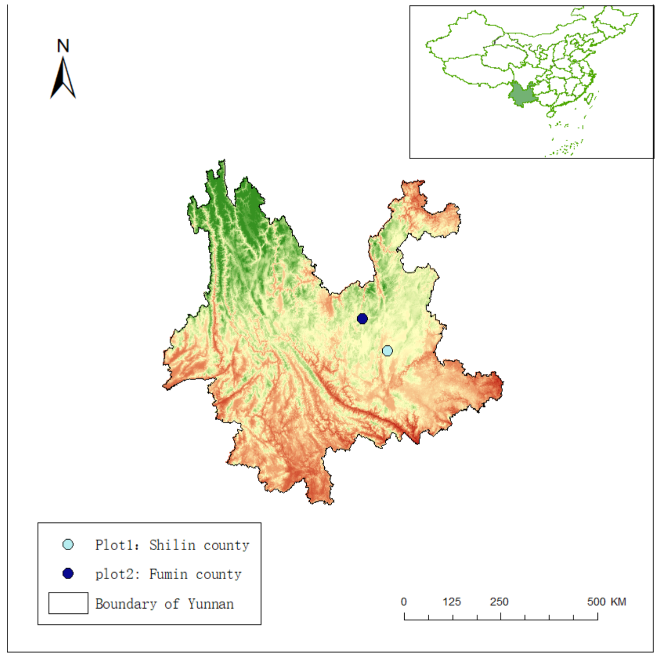

Figure 1. Study site representing the test plots of tobacco fields for the release technology of *T. remus* in two different locations in Yunnan province.

Experimental materials

Simple greenhouse, white air-permeable net, rainproof plastic film, *S. litura* egg masses, the imago of *T. remus*, the *S. litura* egg mass un-parasitized and parasitized by *T. remus*, stereoscope, soft brushes, 50 mL centrifuge tubes, markers, red string, red threads, absorbent cotton, honey water, horticultural labels, record sheets, etc.

Test methods

Different release methods

Different egg ratios of *S. litura* to *T. remus*

Unparasitized *S. litura* egg masses were glued to the tobacco leaves, and newly emerged *T. remus* were released at the centre 1m away from the tobacco leaf according to the egg ratios of 1:10, 1:30, 1:50 and 1:100; the *S. litura* egg masses were retrieved after 48 h and brought back to the indoor cultivation and observed by the stereoscope to calculate the parasitism rate, emergence rate and sex ratio of *T. remus*.

Different age eggs of *S. litura*

Unparasitized egg masses of *S. litura* of different ages at 0 h, 6 h, 12 h, 24 h and 36 h were glued to the back of the tobacco leaves, and then released the newly emerged *T. remus* at the centre 1m away from the tobacco leaf; the *S.*

litura egg masses were retrieved after 48 h and brought back to the indoor cultivation and observed by the stereoscope to calculate the parasitism rate, rate of emergence and sex ratio of *T. remus*.

Parasitization in relation to parasitoid, *T. remus* age

Unparasitized *S. litura* egg masses were glued to the tobacco leaves, and released the *T. remus* at the centre 1m away from the tobacco leaf that had emerged for one to two days; the *S. litura* egg masses were retrieved after 48 h and brought back to the indoor cultivation and observed by the stereozoom microscope to calculate the parasitism rate, emergence rate and sex ratio of *T. remus*.

The relevant formulae are given below:

Parasitism rate (%) = (E1/E2) × 100.

where, E1 = the parasitized egg masses and E2 = the total provided egg masses.

Emergence rate (%) = (F1/F2) × 100.

where, F1 = the number of emerged *T. remus* and F2 = the number of parasitized egg masses.

Sex ratio (%) = (G1/G2) × 100.

where, G1 = the number of emerged females of *T. remus* and G2 = the emerged males of *T. remus*.

Different inoculation devices and their placement

Five types of inoculation devices were used in this test: Centrifuge tubes with gauze, plastic bottles with holes and carrying handles, egg cards, 80 mesh bags, and scattering of *S. litura* egg masses. Each device was equipped with an egg mass of *S. litura* parasitized by the *T. remus* and was suspended at the top of the greenhouse, in the middle of the plant, and on the ground. The experiment was conducted in five greenhouses, with three replicates per greenhouse. After 48h of placement, the devices were brought back to the laboratory for incubation and observation by the stereoscope to calculate the emergence rate of *T. remus*. The relevant calculation formula same as above.

Study on the diffusion range of *T. remus*

The centre point of the test field was selected and extended outward in six directions to be the paste point for the *S. litura* egg masses, and the *S. litura* egg masses were pasted at 5 m, 10 m, 15 m, 20 m, 25 m and 30 m in each direction. Release of 10,000 *T. remus* at the centre of the experimental field. After 48h of placement, the devices were brought back to the laboratory for incubation and observation by the stereoscope to calculate the parasitism rate, rate of emergence and sex ratio of *T. remus*.

Study on the effect of main factors on release in the field

Effect of blue and yellow sticky cards on the diffusion of *T. remus*

Blue and yellow sticky cards were selected for the experiment and placed in the upper part of the greenhouse, the middle of the tobacco plant, and the bottom of the tobacco plant, respectively. The experiment was conducted in five greenhouses, with three replicates per greenhouse. Eight blocks of parasitized *S. litura* egg masses were pasted on the back of the tobacco leaves. After 3 days of placement, the blue and yellow sticky cards and parasitized *S. litura* egg masses were retrieved and brought back to the laboratory for incubation and observation by the stereoscope to calculate the number of *T. remus* that were captured.

Effect of feeding on *S. litura* egg by the *Nesidiocoris tenuis*

Parasitized *S. litura* egg masses with and without covered scale hairs were pasted on the back of the tobacco leaves, respectively. *N. tenuis* released at the centre of the greenhouse. After 48 h of placement, the devices were brought back to the laboratory for incubation and observation by the stereozoom microscope to calculate the feeding rate of *N. tenuis*, hatching rate of *S. litura*, emergence rate and sex ratio of *T. remus*.

The relevant formulae are given below:

Emergence rate (%) = (F1/F2) × 100.

where, F1 = the number of emerged *T. remus* and F2 = the number of parasitized egg masses.

Sex ratio (%) = (G1/G2) × 100.

where, G1 = the number of emerged females of *T. remus* and G2 = the emerged males of *T. remus*.

Feeding rate (%) = (H1/H2) × 100.

where, H1 = the number of eggs fed and H2 = the number of total eggs.

Statistical analysis

Excel, 2016 software was used for data processing, DPS software package for analysis of variance. ArcGIS software (V. 10.8) was used to draw the figure.

RESULTS

Different release methods

Effects of different ratios of *T. remus* to *S. litura* eggs on the control effect of *T. remus*

Table 1 showed that different ratios of *T. remus* to *S. litura* eggs had a significant effect on the parasitic rate of *T. remus*. With the decrease of ratio, the parasitism rate gradually decreases. The highest parasitism rate was 77.95%, when the ratio of *T. remus* to *S. litura* eggs was 1:10, and the lowest was 52.95% when the ratio of *T. remus* to *S. litura* eggs was 1:100. There was no significant difference in the parasitism rate of *T. remus* between the ratio of 1:10, 1:30 and 1:50, but the parasitism rate of 1:10, 1:30 and 1:50 were significantly higher than that of 1:100 ($P<0.05$); The ratio of *T. remus* to *S. litura* eggs had no significant effect on the emergence rate. With the decrease in ratio, the emergence rate increased first and then decreased. The highest emergence rate was 70.34%, when the ratio of *T. remus* to *S. litura* eggs was 1:50, and the lowest was 63.33% when the ratio of *T. remus* to *S. litura* eggs was 1:100; However, different ratios had no significant effect on the sex ratio of *T. remus*, which was more than 90%.

Parasitism of *T. remus* to different *S. litura* egg ages

It can be seen from Table 2 that eggs of different ages of *S. litura* have a significant impact on the parasitic rate of *T. remus*. With the increase in the egg age of *S. litura*, the parasitism rate of *T. remus* decreased gradually. The parasitism rate of *S. litura* egg masses before 12 h of egg age reached about 50%, and after the next 12 h of egg age, the parasitism rate of *S. litura* egg masses was about 25%. There was a significant difference in the emergence rate of eggs of different ages of *S. litura* to *T. remus*. When the egg age of *S. litura* was 0 h, the highest emergence rate of *T. remus* was 53.61%; When the egg age of *S. litura* was 36 h, the emergence rate of *T. remus* was significantly reduced to 10.42%. The emergence rate of *T. remus* at the age of 0-24 h was not significant, but significantly higher than that at 36 h. The sex ratio of *S. litura* eggs of different ages to *T. remus* was not significantly different. The sex ratio was above 90%.

Table 1. Effect of different ratios of *T.* ***remus*** to *S. litura* eggs on the control efficiency of *T.* ***remus***

Parasitic wasp/Hosts	Parasitized rate (%)	Emergence rate (%)	Sex ratio (%)
1:10	66.19 ± 11.76^{a}	65.34 ± 3.59^{a}	94.70 ± 2.57^{a}
1:30	63.03 ± 4.02^{a}	70.15 ± 0.37^{a}	97.32 ± 1.13^{a}
1:50	61.24 ± 5.34^{a}	70.34 ± 1.85^{a}	96.98 ± 2.09^{a}
1:100	50.28 ± 2.57^{b}	63.33 ± 2.75^{b}	97.85 ± 4.42^{a}

Table 2. Parasitism of *T.* ***remus*** to different *S. litura* egg ages

***S. litura* egg ages (h)**	Parasitized rate (%)	Emergence rate (%)	Sex ratio (%)
0	59.93 ± 7.68^{a}	49.77 ± 3.84^{a}	92.70 ± 3.57^{a}
6	47.02 ± 9.36^{a}	48.05 ± 2.19^{a}	97.38 ± 0.13^{a}
12	36.23 ± 5.49^{ab}	48.05 ± 0.33^{a}	95.98 ± 3.09^{a}
24	35.51 ± 2.13^{b}	47.40 ± 0.24^{a}	96.85 ± 4.12^{a}
36	24.73 ± 1.72^{c}	11.11 ± 0.69^{b}	94.61 ± 2.37^{a}

Parasitism of *T. remus* at different days of age on the *S. litura* egg masses

Table 3 shows that the parasitism rate of *S. litura* egg mass was significantly affected by *T. remus* at different ages, and the parasitism rate gradually decreases with the increase of age. When the age of the *T. remus* was 1 d, the parasitism rate of the *T. remus* was higher reaching more than 60%; The parasitism rate of *T. remus* was low when the wasp age was 2 d, which was 20%-30%. The emergence rate was significantly affected by different age of *T. remus*. The emergence rate was higher when the *T. remus* was 1 day, reaching up to 64.14%. The hatching rate was low at 46.40% when the of *T. remus* was 2 days. However, the sex ratio was not significantly affected by the different ages of *T. remus*.

Effect of releasing device on emergence rate of *T. remus*

It is shown in Table 4 that different releasing devices have a significant effect on the emergence rate of *T. remus*. When the egg mass of *S. litura* parasitized was placed in a porous plastic bottle, the emergence rate of *T. remus* was the highest, that reached to 95.1%. When the eggs of *S. litura* parasitized were scattered, the emergence rate of *T. remus* was the lowest, which was 32.02%. When placing three kinds of devices, namely, perforated plastic bottle, bee card and 80 mesh homemade yarn mesh bag, the emergence rate of *T. remus* was the highest when placing the devices in the middle of tobacco leaves, which could reach 96.38, 75.97 and 79.86%, respectively. The emergence rate of *T. remus* was 66.7, 72.7 and 72.2%, respectively; when the top of the greenhouse was placed with three kinds of releasing devices. While each releasing device was placed in the lower and middle parts of tobacco leaves, there was no significant difference in the emergence rate of *T. remus*, and it was significantly higher than the upper part.

Parasitism rate of *T. remus* on *S. litura* eggs with different diffusion distances

Table 5 clearly shows that the parasitism rate of *S. litura* eggs at different diffusion distances was significantly affected by *T. remus*. With the increase of the diffusion distance, the Parasitism rate of *S. litura* eggs decreases gradually. The

Table 3. Parasitism rate of *T. remus* at different days of age on the *S. litura* egg masses

The age of *T. remus* (d)	Parasitism rate (%)	Emergence rate (%)	Sex ratio (%)
1	66.48 ± 5.20^{a}	56.86 ± 7.27^{a}	93.70 ± 1.57^{a}
2	48.52 ± 1.08^{b}	45.17 ± 1.23^{b}	94.32 ± 3.13^{a}

Table 4. Effect of releasing device on emergence rate of *T.* ***remus***

T. remus **releasing device**	**Releasing device location**	**Emergence rate (%)**
Plastic bottles with holes	Upper	79.55±0.17[b]
	Middle	95.06±1.32[a]
	Lower	92.82±0.43[a]
T. remus card	Upper	48.99±1.27[c]
	Middle	71.65±4.32[b]
	Lower	72.65±2.13[b]
80 mesh self-made yarn net bag	Upper	52.06±2.16[c]
	Middle	78.5±1.36[b]
	lower	69.7±0.27[b]
Centrifugal tube with gauze	upper	74.81±1.33[b]
	middle	66.75±1.46[b]
	lower	72.19±0.72[b]
Scattered *S. litura* egg	upper	35.27±2.76[c]
	middle	43.90±3.01[c]
	lower	29.50±1.52[c]

Note: (i) The columns of emergence rate respectively represent the comparison of significant differences between the five devices and their placement positions
(ii) "Upper, middle and lower" in the releasing device location refers to that the *T. remus* release device is placed in the upper part of the greenhouse, the middle of the tobacco plant and the bottom of the tobacco plant, respectively

highest parasitism rate of *S. litura* egg mass was 57.87% when the dispersal distance of *T. remus* was 5 m. The lowest Parasitism rate of *S. litura* egg mass was 3.03%, when the dispersal distance of *T. remus* was 30 m; The *T. remus* of *S. litura* had no significant effect on the emergence rate of egg mass parasitism at different dispersal distances. The highest emergence rate of *S. litura* was 62.81% when the dispersal distance of *T. remus* was 30 m. The lowest emergence rate of *S. litura* was 57.08% when the dispersal distance of *T. remus* was 20 m. The egg mass sex ratio of *S. litura* parasitized by *T. remus* was not significantly affected, which was more than 90%; The hatching rate of *S. litura* eggs with different dispersal distances was significantly affected by *T. remus*. The hatching rate of *S. litura* increased with the increase in spreading distance. The highest hatching rate of *S. litura* was 92.76% when the dispersal distance of *T. remus* was 30 m. The lowest hatching rate of *S. litura* was 51.22% when the dispersal distance of *T. remus* was 10 m.

Study of the effect of main factors on release in the field

Effect of blue sticky card on the diffusion of T. remus

It can be seen from Table 6 that the influence of the placement height of the blue sticky card on the spread of *T. remus* was significant. The number of *T. remus* stuck showed a trend of first increasing and then decreasing according to the placement position of the blue sticky cards. On the blue sticky card, placed in the middle of the tobacco plant, the number of *T. remus* stuck was the largest, 74.04. On the blue sticky card placed in the lower part of the tobacco plant, the number of *T. remus* trapped was the least, 13.06. The number of other insects trapped on the blue sticky card placed above the tobacco plant was 92.04; the number of other insects caught on the blue sticky card placed at the lower part of the tobacco plant was 35.36.

Effect of yellow sticky card on the diffusion of T. remus

It is shown in Table 7 that the influence of the height of the yellow sticky card on the spread of *T. remus* is significantly different, and the number of *T. remus* adhered, showed a trend of first increasing and then decreasing according to the position of the yellow sticky cards. On the yellow sticky card placed in the middle of the tobacco plant, 68.53 *T. remus* were trapped the most. On the yellow sticky card placed at the lower part of the tobacco plant, the number of *T. remus* stuck was the least, 14.06; On the yellow sticky card placed above the tobacco plant, 70.07 other insects were trapped. The number of other insects trapped in the yellow sticky card placed at the lower part of the tobacco plant was 43.56.

Feeding effect on parasitized S. litura egg masses of N. tenuis with or without scale layer

It can be seen from Table 8 that the feeding rate of the parasitized *S. litura* eggs covered with scale hairs and the parasitized *S. litura* eggs not covered with scale hairs is significantly different. The egg masses of the parasitized

Table 5. Parasitism rate of *T. remus* on *S. litura* eggs with different diffusion distances

Diffusion distance (m)	Parasitism rate (%)	Emergence rate (%)	Sex ratio (%)	Hatching rate of *S. litura* (%)
5	57.55±0.32[a]	60.52±0.31[a]	93.27±4.93[a]	52.73±5.37[e]
10	45.29±0.14[ab]	61.28±1.19[a]	95.39±3.68[a]	48.49±2.73[d]
15	26.99±2.73[b]	57.75±2.73[a]	92.77±4.25[a]	66.09±2.74[c]
20	15.76±1.32[c]	53.69±3.39[a]	96.27±2.90[a]	82.52±0.17[b]
25	8.56±2.31[d]	56.79±4.32[a]	93.67±4.55[a]	87.67±2.73[ab]
30	2.95±0.18[e]	60.72±2.09[a]	92.48±3.32[a]	88.41±4.35[a]

Table 6. Effect of blue sticky card on the diffusion of *T. remus*

Blue sticky card position	other stuck insects (number)	stuck of *T. remus* (number)
Upper	88.82±3.22[a]	42.42±0.72[b]
Middle	64.65±0.27[b]	72.08±1.96[a]
lower	32.39±2.97[c]	12.83±0.23[c]

Table 7. Effect of yellow sticky card on the diffusion of *T. remus*

Yellow sticky card position	Other stuck insects (number)	Stuck *T. remus* (number)
upper	67.88±2.19[a]	41.33±4.28[b]
middle	62.44±0.28[a]	65.75±2.77[a]
lower	42.19±1.37[b]	15.08±1.59[c]

Note: "Up, middle and down" in the placement positions in Tables 6 and 7 refer to the blue and yellow sticky card positions placed in the upper part of the greenhouse, the middle of the tobacco plant and the bottom of the tobacco plant, respectively.

S. litura covered with scale hair were fed by the *N. tenuis* with the lowest feeding rate of 28.73%; the egg mass of the parasitized *S. litura* without scale hair was fed by the *N. tenuis*, which was 65.21%.

DISCUSSION

It is reported that the *T. remus* has a high parasitism rate, and more than two eggs of *T. remus* could be laid in the host eggs, but the newly hatched larvae would fight in the host eggs for survival, and only one offspring emerged at most (Wen, 1987; An *et al*., 2019). Presently, we found that the parasitic rate was higher than 70.58% when the different ratio of *T. remus* to *S. litura* eggs was 1:50. At 1:100, filial larvae hatched and the number increased, which was similar to the laboratory test results of Yonghui (Xie *et al*., 2021). It is also reported that deploying *Hippodamia variegata* in a 1:40 ratio to manage *Aphis craccivora* on cowpeas is efficient and highly colonization-friendly, effectively curbing *A. craccivora*'s infestation at the site. Additionally, due to the widespread occurrence of hyperparasitism, which hinders the growth and development of monoparasitic wasps, during parasitism by these wasps, it's advisable to decrease both the frequency and timing of pick-ups when the wasp is replicated in high numbers (Li *et al*., 2001; Jiang *et al*., 2014). From the perspective of high production and low-cost technology, 1:50 was selected as the ratio of *T. remus* to *S. litura* eggs for the field control experiment.

Table 8. Feeding on parasitized *S. litura* egg of *N. tenuis* mass with or without scale layer

Mass with or without scale layer	**Rate of feed (%)**
parasitized *S. litura* egg of *N. tenuis* mass with scale layer	24.67±4.06[b]
parasitized *S.litura* egg of *N. tenuis* mass without scale layer	57.84±7.37[a]

In the interaction between the host and the parasitic wasp, the host quality determines the growth, development, pre-adult survival and sex ratio of the parasitic wasp. If the host is not suitable, the offspring of the parasitic wasp may not develop normally (Cingolani *et al*., 2014). In this experiment, when the egg age of *S. litura* was within 12 hours, the parasitism rate of *S. litura* was higher, which could reach up to 50%; For egg parasitoids, the quality of host eggs at the advanced age is usually low (Chen *et al*., 2021). In the test of host egg age, it was found that the number of parasitic eggs on 1-day-old and 2-day-old eggs of female *T. remus* was significantly higher than that on 3-day-old eggs when they were provided with hosts at different development stages separately; When the host eggs of 1, 2 and 3 days are provided at the same time, female *T. remus* prefer to host 1 day old host eggs (Chen *et al*., 2021). It is clear from these findings that when the age of the *T. remus* was within 1 day, the vitality of the *T. remus* was higher, and the parasitism rate was higher, which reached to 71.68%. The results were similar to those reported by Penaflor and others on the highest parasitism rate of *S. litura* on the first day (Peñaflor *et al*., 2012).

With the increasingly serious occurrence of agricultural and forestry pests, the use of natural enemies for pest management has become mainstream nowadays, and the rearing and field inoculation of natural enemies has become a research hotspot. At present, the direct release method, egg card release method and jar release method are mainly used for the release of parasitic insect natural enemies at the domestic level as well as abroad. However, these methods are vulnerable to the impact of the external environment, and the eggs of parasitic natural enemies easy to be approach and eaten by other organisms, which cannot play its role much effectively; it is also testified that the presence or lack of light had no effect on the parasite rate of night moth and that some protection of the moth's eggs could increase the parasitization rate. On the one hand, the protective layer reduces the impact of rainwater on insect eggs, and on the other hand, it reduces to some extent the number of predatory insects that prey on parasitic wasp eggs (Grande *et al*., 2021). The water in the bottle will cause death, and the cost is high, so it is not easy to be promoted (Zhang *et al*., 2009). In our study, different kinds of devices, namely plastic bottles with holes, bee cards and centrifuge tubes with gauze, were selected to improve the emergence rate of *T. remus*. The method of hanging the device could reduce the feeding of parasitized *S. litura* eggs by *N. tenuis* and keep them safe from being directly exposed to sunlight and rain. Zhang *et al*. (2009) chose to release the pupae parasitized by the *N. vitripennis* on sunny or cloudy days with mesh bags to ensure the permeability of the pupae and reduce the impact of rain on the *N. vitripennis.* Xu *et al*. (2016) used discarded plastic water bottles to make release bottles to prevent and cure *Brontispa longissima*. The three devices all reduce the loss of egg mass, reduce the cost of the device and could be re-used as well.

The diffusion behaviour of natural enemy insects in the field directly affects the control effect of field release (Xu *et al*., 2016). In the process of dispersal, the parasitism rate of *T. remus* decreased with the increase of dispersal distance; The parasitism rate reached 50% within 5-10 m of the diffusion distance, and the parasitism effect in six directions had little difference. Similar to the results of Li *et al*. (2006) on the control of *Helicoverpa armigera*, the farther away the *Microplitis mediator* release point, the lower the parasitism rate of the *M. mediator* to *H. armigera*, the highest parasitism rate within 10 m near the centre of the *M. mediator* release point, and the lowest rate of damage to bud and boll, and there is no significant difference in parasitism effect between different directions. Crop height and variety will affect the spread distance of *T. remus*, thereby reducing the parasitic rate of *T. remus*.

For effective bio-control technology, it is necessary to consider the impact of other control measures and some adverse factors in the field on the spread of *T. remus* when using *T. remus* to control pests. At the same time, different coloured sticky cards have a certain lethality to the natural enemies. The placement of blue and yellow sticky cards has a certain impact on the spread of natural enemy insects. Fu *et al*. (2017), reported that yellow and blue sticky cards can trap and kill more parasitic bees, ladybirds and other natural enemy insects, while green and red sticky cards have no obvious trapping effect on natural enemy insects (Fu *et al*., 2017). However, in this study, the blue and yellow sticky cards were placed at different positions inside the greenhouse and glued with a certain number of *T. remus* egg masses*.* Because the experiment was carried out in indoor conditions, the crop ecological environment was different, the height and density of the crops were different, and the blue and yellow sticky cards were placed in different positions, sizes, quantities, shapes and positions of the *T. remus*, which led to a high number of *T. remus* emergence. It has a certain influence on the control effect of *T. remus*. However, to reduce the trapping and killing effect of the sticky cards on the natural enemies and protect biodiversity; it should be avoided to use it at the peak of the occurrence of natural enemies (Li *et al*., 2018).

Nesidiocoris tenuis can not only harm tobacco but also prey on the exposed eggs of *S. litura*. It has been found that the *N. tenuis* cannot feed on the *S. litura* egg mass covered with a scale layer (Wei *et al*., 1998). However, we found that parasitized egg mass of *S. litura* even under the cover of the scale layer has been fed by the *N. tenuis* in small numbers which is inconsistent with the result obtained by Wei *et al*. (1998). The preliminary reasons may be that the differences are caused by different experimental environments and some unknown factors. It has also been reported that the parasitization rates of egg grains in the field were 67.35% and 63.8% for *T. remus* and *T. chilonis*, respectively, and there was a competitive relationship between the two parasitical wasps (Liling *et al*., 2022).

CONCLUSION

This study showed that the highest parasitism rate reached 77.95%, 71.68%, 67.61% and 57.87% for ratio (wasps/hosts 1:10), days (within 1 day), egg age (within 0 hours), and dispersal distance (within 5 m), respectively. When the parasitized *S. litura* egg masses were placed in a transparent plastic bottle with holes, the highest emergence rate of the *T. remus* was 96.38%; While the blue and yellow sticky cards placed in the lower part of tobacco leaves had the least effect on the diffusion of *T. remus*, along with the lowest feeding rate by *N. tenuis* on egg mass covered by scale layer. From the perspective of high production and low-cost technology, we concluded that these findings will establish a robust groundwork for deploying *T. remus* in extensive areas to manage *S. litura* by providing better bio-control and serve as the basis for good release method to be used as part of integrated pest management.

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