Bioefficacy of *Rhynocoris longifrons* (Stål) (Heteroptera: Reduviidae) against multiple cotton pests under screen house and field conditions

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*Rhynocoris longifrons* (Hemiptera: Reduviidae) is a generalist predator of many cotton insect pests. The hiding behaviour of this predator, which is one of the key factors of predation success, was investigated under screen house conditions. Moreover, we evaluated its biocontrol potential against *Aphis gossypii* (Hemiptera: Aphididae), *Dysdercus cingulatus* (Hemiptera: Pyrrhocoridae), *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae), and *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) under screen house and field conditions. Results showed that *R. longifrons* life stages preferred to hide under small pebbles in the screen house tests. All the *R. longifrons* life stages showed a biocontrol potential against the four insect pests under screen house conditions. However, their biocontrol potential had not varied in relation to day and night hours. Augmentative releases of *R. longifrons* were carried out for two seasons such as South-west monsoon, 2011 and post-monsoon, 2012. The augmentative release of *R. longifrons* reduced significantly insect pests on cotton. In fact, the release of this predator in cotton fields was capable to reduce the population of *H. armigera* (50%), *P. solenopsis* (28%), *D. cingulatus* (18.8%), and *A. gossypii* (11.8%) during the rain fed condition (south-west monsoon season). During irrigated condition (post-monsoon season), populations of *D. cingulatus* were reduced by 26%, than *P. solenopsis* (20.6%), and *A. gossypii* (16.8%). Except ants, no negative impact was reported on other natural enemies present in the cotton field. Significantly higher crop yield and cost benefit ratio was observed in the predator release plots indicating that *R. longifrons* can be used in an integrated pest management program for multiple cotton pests.

Cotton (*Gossypium hirsutum* L., Malvaceae), is an important cash crop throughout the world. However, its production is severely hampered by several abiotic and biotic factors, such as insect attacks that lead to significant yield reduction. *Aphis gossypii* Glover (Hemiptera: Aphididae)¹, *Dysdercus cingulatus* (Fab.) (Hemiptera: Pyrrhocoridae)², *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae)³, and *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae)⁴ are considered as economically important pests of cotton. Conventional synthetic chemical insecticides are typically extensively used causing significant side effects including pesticide resistance⁵ as well as having many ecological and biological impacts⁶.

Members of Reduviidae are abundant predators of many economically important insect pests⁷–⁹. Reduviids are common in cotton agro-ecosystems¹⁰,¹¹. However, they often fail to colonize fields to provide effective control of the pests. In such a situation, augmentative biological control can be an important approach to protect the cotton. Augmentative biological control is practiced worldwide with more than 150 species of natural enemies now commercially available¹². However, generalist predators, particularly predatory bugs, have been largely ignored for augmentative biological control of cotton pests¹³,¹⁴.

A number of researches investigated the impact of augmentative release of various reduviids against a wide variety of insect pest's world-wide¹⁵–²⁰. However, in cotton growing regions of India, biocontrol potential of reduviids have not fully explored. Native reduviid predator species have shown good predation against many insect pests²¹–²⁵. One of the most important genera of Reduviidae, as well as widely present in many agro-ecosystems is *Rhynocoris* Hahn (Hemiptera: Reduviidae)²⁶. Specifically, *Rhynocoris longifrons* (Stål) is a general predator of

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Many insect pests in cotton fields such as: Hemiptera [A. gossypii, D. cingulatus, P. solenopsis22, Clavigralla gibbosa Spinola23 and, Nezara viridula Linnaeus24]; and Lepidoptera [H. armigera and, Spodoptera litura Fab.24].

Many reduviid predators possess either morphological adaptive characters25 or behavioural adaptive features26 including hiding27 to successfully capture and feed the prey's. Shelter provisioning with pieces of clay pots and stones in cotton field enhanced reduviids population and increased cotton production21. To date, no systematic study has been made of the feeding potential of these predatory R. longifrons in relation to day and night hours. Therefore, we conducted a series of studies to assess their possible integration in an augmentative biological control program for cotton pests in Tamil Nadu, India. The specific objectives of this study were to: 1) understand R. longifrons hiding behaviour under open field conditions, 2) evaluate their biocontrol potential in relation to day and night hours, 3) test their efficacy in an augmentative release program under field conditions for two seasons, and 4) estimate the cost benefit ratio and percent avoidable loss.

Results
Hiding behaviour of the predator under screen house. The hiding behaviour of R. longifrons revealed that the percentage of predator adults hiding under pebbles was higher at 6 a.m. (\(F = 42.53; df = 3; P < 0.000\)) and 10.30 a.m. (\(F = 8.60; df = 3; P < 0.05\)) than other hiding places (Fig. 1). As day light increased, the predator moved under the plants (\(F = 41.60; df = 3; P < 0.005\)) and then moved either under pebbles (\(F = 8.63; df = 3; P < 0.05\)) or under the fallen leaves (\(F = 8.62; df = 3; P < 0.05\)) for hiding. Fifth-instar reduviids preferred to hide under pebbles (\(F = 42.63; df = 3; P < 0.0005\)), whereas fourth-instar (\(F = 8.60; df = 3; P < 0.05\)) predator first hid under fallen leaves and latter moved into pebbles, again left the place and returned to the fallen leaves (\(F = 8.61; df = 3; P < 0.05\)) to hide (Table 1).

Biocontrol potential of R. longifrons in relation to day and night hours. Rhyncorhis longifrons life stages significantly consumed more P. solenopsis (\(F = 32.563, df = 1, p < 0.000\)) and A. gossypii adults (\(F = 6.696, df = 1, p < 0.05\)), D. cingulatus nymphs (\(F = 49.439, df = 1, p < 0.000\)), and H. armigera larvae (\(F = 40.119, df = 1, p < 0.05\)) compared to the control category. No significant differences in the predation rate of R. longifrons life stages was recorded on A. gossypii (\(F = 1.022, df = 23, p > 0.05\)), D. cingulatus (\(F = 1.410, df = 23, p > 0.05\)), P. solenopsis (\(F = 0.794, df = 23, p > 0.05\)), and H. armigera (\(F = 1.092, df = 23, p > 0.05\)) during dawn hours. Similarly, there was also no much significant difference in the activity of R. longifrons life stages on D. cingulatus (\(F = 0.667, df = 23, p > 0.05\)), P. solenopsis (\(F = 0.426, df = 23, p > 0.05\)), H. armigera (\(F = 2.037, df = 23, \)

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**Table 1.** Hiding location selection (%) of R. longifrons life stages released under screen house conditions (n = 10). Percentage followed by different letters within the same line is significantly different at 0.05 level as determined by the SNK test.

| Predator life stages | Observed percentage of predator life stages in different hiding areas (Mean ± SE) |
|----------------------|----------------------------------------------------------------------------------|
|                      | In plants                          | Under the pebbles       | Under the fallen leaves | Other objects |
| Fourth instar        | 5.0 ± 1.9c                         | 40.0 ± 3.0b             | 50.0 ± 7.0a             | 5.0 ± 1.9c    |
| Fifth instar         | 17.5 ± 2.5b                        | 52.5 ± 4.5a             | 15.0 ± 2.9c             | 12.5 ± 2.5d   |
| Adults (male + female)| 35.0 ± 9.5b                        | 45.0 ± 3.5a             | 15.0 ± 3.0c             | 5.0 ± 2.0d    |

**Figure 1.** Hiding area of R. longifrons fourth and fifth stadium nymphs and adult (male and female) (%) released from morning 6 a.m., 8.30 a.m., 11.00 a.m., and to 3.30 p.m. and observation made in 2-hours intervals under screen house conditions.
and *P. solenopsis* (50%), was significantly reduced after release of life stage during post-monsoon season (F = 16.8%). *A. gossypii* (20.6%) and *D. cingulatus* (10%) populations of *P. solenopsis* against *A. gossypii* was significantly consumed more preys by predator third-instar (F = 1.081, df = 47, p = 0.05) had significant reduction of *A. gossypii* population (F = 89.9, p = 0.000). During irrigated condition, a low *R. Longifrons*- *P. solenopsis* (F = 23, p = 0.05) predator releases. Similarly, during post-monsoon season, populations of *D. cingulatus* (F = 299, p = 0.05) and *H. armigera* (F = 299, p = 0.05) releases of the *P. solenopsis* (F = 32.147, df = 299, p = 0.000) populations were recorded respectively during the first and third releases of *R. longifrons* life stages. A significantly lower incidence of *A. gossypii* population (F = 1.098, df = 299, p = 0.05) and *A. gossypii* (F = 1.098, df = 23, p ≥ 0.05) during the dusk hours. A similar trend was observed when compared predation rate between dawn and dusk hours of *R. longifrons* life stages on the four preys species (Table 2). When we considered total predation rate (dawn and dusk hours), no significant difference of predation rate was observed between *R. longifrons* life stage when feeding on *D. cingulatus* (F = 1.422, df = 47, p ≥ 0.05). *P. solenopsis* (F = 1.081, df = 47, p ≥ 0.05) and *H. armigera* (F = 0.839, df = 47, p ≥ 0.05). However, *A. gossypii* was significantly consumed more preys by predator third-instar (F = 3.017, df = 47, p ≤ 0.05).

### Bioefficacy of the predators under cotton field conditions.

In the cotton fields, when the total number of insect pests was considered, the most predominant insect pests were *A. gossypii* (81%), *P. solenopsis* (10%), *D. cingulatus* (6%), and *H. armigera* (3%), during rain fed condition. However, during irrigated condition, the predominant insect pest was *P. solenopsis* (60.2%), *A. gossypii* (32.8%) and *D. cingulatus* (8.2%). Therefore, we mainly concentrated on these pests throughout our observations. A significant reduction in *A. gossypii* population was observed in *R. Longifrons*-released plots compared to control after the first (F = 58.571, df = 299, p ≤ 0.000) and second (F = 22.415, df = 299, p ≤ 0.000) predator releases during south-west monsoon (Table 3). Yet, during the post-monsoon season a significant reduction in *A. gossypii* population was observed in *R. Longifrons*-released plots after the first (F = 22.761, df = 299, p ≤ 0.0005) and third (F = 5.596, df = 299, p ≤ 0.05) predator releases. During the rain fed condition, significant reduction of *D. cingulatus* (F = 6.951, df = 249, p ≤ 0.05) and *P. solenopsis* (F = 32.147, df = 299, p ≤ 0.0005) populations were recorded respectively during the first and third releases of *R. longifrons* life stages. A significantly lower incidence of *H. armigera* (F = 19.930, df = 299, p ≤ 0.000) was reported only during the first release of *R. longifrons* life stages. During the irrigated condition, a low *D. cingulatus* population appeared in cotton fields after the first release of *R. longifrons* life stages (Table 3). However, no significant reduction of *D. cingulatus* population (F = 0.000, df = 299, p ≥ 0.05) was observed in *R. Longifrons*-treated plots compared with the control. Similarly, during post-monsoon season no significant reduction of *P. solenopsis* population was observed after the first (F = 0.020, df = 299, p ≥ 0.05), second (F = 0.000, df = 299, p ≥ 0.05) and, third (F = 1.198, df = 299, p ≥ 0.05) predator releases of the predator life stages. When we take in account the total mean population after all three-predator releases, there was a significant reduction of *A. gossypii* (F = 41.908, df = 899, p ≤ 0.000), *P. solenopsis* (F = 4.949, df = 899, p ≤ 0.05), and *H. armigera* (F = 12.734, df = 899, p ≤ 0.000) during south-west monsoon. While, only *A. gossypii* population was significantly reduced after release of *R. longifrons* life stage during post-monsoon season (F = 12.167, df = 899, p ≤ 0.05).

In general, the release of *R. Longifrons* in cotton fields was capable to reduce the populations of *H. armigera* (50%), *P. solenopsis* (28%), *D. cingulatus* (18.8%), *A. gossypii* (11.8%) during south-west monsoon season (Fig. 2). However, during post-monsoon season, populations of *D. cingulatus* were reduced by 26%, followed *P. solenopsis* (20.6%) and *A. gossypii* (16.8%).

### Effect on natural enemies’ populations.

Other natural enemies like ants, coccinellids, wasps, other reduviids, and spiders were observed in the cotton fields. During the rainy season, the populations of reduviids (F = 13.223, df = 299, p ≤ 0.000), spiders (F = 20.926, df = 299, p ≤ 0.000), and coccinellids (F = 4.015, df = 299, p ≤ 0.05) had significantly increased in *R. Longifrons*-treated plots from the first and second releases respectively compared to control.

### Table 2. Biocontrol potential of *R. longifrons* third, fourth and fifth stadium and adult (male and female) against *A. gossypii*, *D. cingulatus*, *H. armigera* and *P. solenopsis* in relation to dawn and dusk hours (Mean ± SE). Percentages followed by different letters within the same line are significantly different at 0.05 level as determined by the Student-Newman-Keuls test.

| Pest species | Stage of predator | Predator releasing time (mean ± SE) | Total mean | Anova between dawn and dusk releases |
|--------------|-------------------|------------------------------------|------------|-------------------------------------|
|              |                   | Dawn hours                         | Dusk hours |                                    |
| *A. gossypii*| Third instar      | 0.83 ± 0.31a                       | 0.50 ± 0.34a| 0.67 ± 0.22b                       | Df = 1 |
|              | Fourth instar     | 0.25 ± 0.17a                       | 0.50 ± 0.31a| 0.38 ± 0.18ab                      |       |
|              | Fifth instar      | 0.28 ± 0.18a                       | 0.00 ± 0.00a| 0.14 ± 0.09a                       | F = 0.316 |
|              | Adult             | 0.05 ± 0.05a                       | 0.11 ± 0.11a| 0.08 ± 0.06a                       | F = 0.575 |
| *D. cingulatus*| Third instar    | 0.25 ± 0.17a                       | 0.33 ± 0.10a| 0.29 ± 0.09a                       |       |
|              | Fourth instar     | 0.17 ± 0.10a                       | 0.25 ± 0.11a| 0.21 ± 0.07a                       | Df = 1 |
|              | Fifth instar      | 0.33 ± 0.08a                       | 0.44 ± 0.14a| 0.39 ± 0.08a                       | F = 0.216 |
|              | Adult             | 0.49 ± 0.9a                        | 0.72 ± 0.10a| 0.61 ± 0.11a                       | F = 0.145 |
| *H. armigera* | Third instar      | 0.41 ± 0.15a                       | 0.67 ± 0.16a| 0.54 ± 0.11a                       |       |
|              | Fourth instar     | 0.50 ± 0.18a                       | 1.08 ± 0.35a| 0.79 ± 0.21a                       | Df = 1 |
|              | Fifth instar      | 0.55 ± 0.14a                       | 0.38 ± 0.13a| 0.47 ± 0.09a                       | F = 0.013 |
|              | Adult             | 0.88 ± 0.13a                       | 0.49 ± 0.20a| 0.68 ± 0.13a                       | F = 0.909 |
| *P. solenopsis*| Third instar     | 0.91 ± 0.15a                       | 1.00 ± 0.22a| 0.96 ± 0.13a                       |       |
|              | Fourth instar     | 1.00 ± 0.28a                       | 0.75 ± 0.17a| 0.87 ± 0.13a                       | Df = 1 |
|              | Fifth instar      | 0.65 ± 0.26a                       | 0.61 ± 0.23a| 0.63 ± 0.17a                       | F = 0.028 |
|              | Adult             | 0.82 ± 0.25a                       | 0.82 ± 0.32a| 0.82 ± 0.19a                       | P = 0.867 |
recorded in the South-west monsoon season (14.5%) than in the post-monsoon season (4.9%) (Table 5).

A higher PAL was compared with the predator-released field. Similarly, the cost benefit ratio was higher in the predator-release plots (837.0 and 753.4 kg/Hectare) for the reduviids population (F = 14.764, df = 899, p ≤ 0.05) compared with the control plots during south-west monsoon. It is the same trends observed after the second release of *R. longifrons* (F = 299, p ≤ 0.05) and wasps (F = 4.388, df = 299, p ≤ 0.05) in post-monsoon season where significant reduction of ants (F = 4.983, df = 299, p ≤ 0.05) and wasps (F = 4.388, df = 299, p ≤ 0.05) populations was observed after the third release of *R. longifrons* life stages. Similar trend was also observed during south-west monsoon season where significant reduction of ants (F = 4.983, df = 299, p ≤ 0.05) and wasps (F = 4.388, df = 299, p ≤ 0.05) populations was observed after the second release of *R. longifrons* life stages.

When we considered the total mean population after the three releases, there were significant increasing in the population of coccinellids (F = 8.171, df = 899, p ≤ 0.05) compared with the control plots during south-west monsoon. It is the same trends observed for the reduviids populations (F = 14.764, df = 899, p ≤ 0.000) during post-monsoon season (Table 4). However, a significant reduction of ants (F = 4.056, df = 299, p ≤ 0.05) and wasps (F = 5.279, df = 299, p ≤ 0.05) populations was observed after the second release of *R. longifrons* life stages. Similar trend was also observed during post-monsoon season where significant reduction of ants (F = 4.983, df = 299, p ≤ 0.05) and wasps (F = 4.388, df = 299, p ≤ 0.05) populations was observed after the third release of *R. longifrons* life stages.

Cotton production, Cost Benefit Ratio (CBR) and Percent avoidable loss. Cotton production was higher in the predator-release plots ($837.0 and 753.4 kg/Hectare) for south-west monsoon and post-monsoon seasons respectively. Similarly, the cost benefit ratio was higher in the predator-release treatment (1:1.28) than in the control treatment (1:1.17) during the south-west monsoon season, as well as post-monsoon (Table 5). Cost of cultivation was less in the control (≈ 4872.0 (73.08 US Dollar) and 26652.0 (399.78 US Dollar) for South-west monsoon and post-monsoon seasons respectively) compared with the predator-released field (≈ 29096.0 (436.44 US Dollar) and 28134.0 (422.01 US Dollar), for South-west monsoon and post-monsoon seasons respectively). A higher PAL was recorded in the South-west monsoon season (14.5%) than in the post-monsoon season (4.9%) (Table 5).

### Table 3. Effect of augmentative releases of *Rhynocoris longifrons* life stages on the four predominant pest in the cotton fields during South-west monsoon (S-WM, July to September 2011) and Post-monsoon season (P-M, December to February 2011–12) at Virudhunagar and Tuticorin districts respectively. Means followed by different letters in a column for each release and season or the total mean population are significantly different (SNK test, P < 0.05).

| Number of releases | Seasons     | Field treatment | Predominant pests (mean number/plant ± SE) |
|--------------------|-------------|----------------|---------------------------------------------|
|                    |             |                | *A. gossypii* | *D. cingulatus* | *P. solenopsis* | *H. armigera* |
| First release      | S-WM        | Control        | 112.81 ± 5.74a | 1.13 ± 0.18a | 6.63 ± 0.82a | 0.19 ± 0.04a |
|                    |             | *R. longifrons* release | 73.49 ± 6.37b | 2.00 ± 0.39a | 1.56 ± 0.33b | 0.00 ± 0.00b |
|                    | P-M         | Control        | 74.79 ± 3.81a | -            | 12.71 ± 2.09a | - |
|                    |             | *R. longifrons* release | 51.54 ± 3.14b | -            | 7.25 ± 0.76a | - |
| Second release     | S-WM        | Control        | 98.35 ± 5.62a | 0.15 ± 0.04a | 0.96 ± 0.21a | 0.01 ± 0.00a |
|                    |             | *R. longifrons* release | 72.52 ± 3.16b | 0.11 ± 0.03a | 0.45 ± 0.11a | 0.01 ± 0.00a |
|                    | P-M         | Control        | 46.19 ± 2.55a | -            | 8.86 ± 1.11a | - |
|                    |             | *R. longifrons* release | 46.64 ± 2.15a | -            | 7.85 ± 0.82a | - |
| Third release      | S-WM        | Control        | 78.77 ± 3.15a | 0.17 ± 0.04a | 7.65 ± 1.47a | - |
|                    |             | *R. longifrons* release | 68.13 ± 2.92a | 0.05 ± 0.02b | 7.93 ± 1.51a | - |
|                    | P-M         | Control        | 36.85 ± 1.93a | 0.17 ± 0.07a | 9.09 ± 0.91a | - |
|                    |             | *R. longifrons* release | 30.25 ± 1.81b | 0.08 ± 0.05a | 9.38 ± 0.84a | - |
| Total mean population after release | S-WM | Control | 96.64 ± 2.94a | 0.72 ± 0.13a | 5.08 ± 0.58a | 0.06 ± 0.01a |
|                    |             | *R. longifrons* release | 71.38 ± 2.55b | 0.48 ± 0.06a | 3.31 ± 0.54b | 0.00 ± 0.00b |
|                    | P-M         | Control        | 52.61 ± 1.81a | 0.06 ± 0.02a | 10.22 ± 0.85a | - |
|                    |             | *R. longifrons* release | 42.81 ± 1.48b | 0.03 ± 0.01a | 8.16 ± 0.47a | - |

![Figure 2](image-url)  
Augustinative releases of *R. longifrons* on the natural enemies population (number/plant) under rainfed condition. Plots without reduviid predator (A) and Plots without reduviid predator (B).

![Table 3](image-url)  
Effect of augmentative releases of *Rhynocoris longifrons* life stages on the four predominant pest in the cotton fields during South-west monsoon (S-WM, July to September 2011) and Post-monsoon season (P-M, December to February 2011–12) at Virudhunagar and Tuticorin districts respectively. Means followed by different letters in a column for each release and season or the total mean population are significantly different (SNK test, P < 0.05).
Reduviids are distributed in cotton agro-ecosystems worldwide and considered as important biocontrol agents\(^9,41,42\). Hence, use of reduviid predators to manage cotton pests can be one of the effective components of IPM programme, thus basic information is needed about hiding behavior, predatory potential, and other factors. The present study clearly shows that *R. longifrons* hide under different objects and, the preference for an object is function of the time of the day. The reduviid predator *Rhynocoris fuscipes* (Fabricius) also showed similar hiding behavior\(^31\). This hiding behavior is an advantageous in the framework of an IPM because it allows this predator to better surprise the prey it captures. Moreover, it allows a better escaping from their natural enemies such spiders and wasps\(^31–33\). Therefore, we can conclude that *R. longifrons* nymphs and adults can survive and adapt in the cotton agro-ecosystem after its release.

Our results showed that, feeding potential of *R. longifrons* life stages on *P. solenopsis* and *A. gossypii* adults, *D. cingulatus* nymphs, and *H. armigera* larvae had not varied in relation to day and night hours. This result was surprising because some authors suggested that reduviid predators are either nocturnal or diurnal\(^34\). For example, Lira et al.\(^35\) found immature Harpactorini assassin bug feeding on a scorpion during the night time only. In contrast, *Sycanus* sp. and *Scipinia* sp. life stages were more active in the morning (6.30 to 10.0 a.m.)\(^36\). Our results imply that *R. longifrons* life stages can be released at any time of the day and we recommend the release of the predator third-stadium for *A. gossypii* control in screen house.

The predominance of *A. gossypii* and *P. solenopsis* populations in cotton fields respectively during south-west and post-monsoon seasons is not surprising. In fact, it is known that the population dynamics of aphids and mealybugs can be affected by seasonal changes in weather conditions\(^37\). Moreover, Wang et al.\(^38\) showed that high rainfall was unfavourable for serious infestation by *P. solenopsis*. Our study showed that, augmentative releases of *R. longifrons* significantly reduced the number of insect pests in the cotton fields. The first release of this predator in cotton fields was capable to reduce *A. gossypii* and *P. solenopsis* populations during south-west and post monsoon compared with the control. Similarly, the field release of some reduviid predators such as *Pristhesancus plagipennis* Walker\(^17,39\), *Platynemis laevicollis* Distant\(^40\) and *Rhynocoris marginatus* (Fab.)\(^19,20\) were successful in reducing various pests in coconut palm, cotton and groundnut fields. Our results showed that *R. longifrons* can be an effective predator of multiple preys, which is consistent with previous results for generalist predators\(^41,42\).

Further, no adverse interaction between *R. longifrons* and indigenous predators like coccinellids, spiders and, other reduviids were observed. This situation suggests reduviid predators do not interfere with other natural enemies. Consequently, reduviid predator may be an ecofriendly protection for cotton pest populations and are likely to be highly detrimental to their biological control. Reduviids can be utilized as part of a multiple species release program as suggested for *Dephastus catalinae* (Horn) (Coleoptera: Coccinellidae)\(^43\). However, Symonds et al.\(^44\) reported that at natural ecosystems, change of biotic and abiotic variables is common and that under such circumstance, it was difficult to predict the interspecific and intraspecific interactions between released generalist predators, and the predators dwelled in the eco-system. The decrease of ants in *R. longifrons* treated plots could be explained among other factors, by the decrease of aphids and mealybugs populations, which bribe them with their honeydew excretion. In fact, it is known that *P. solenopsis*\(^45\) and, *A. gossypii*\(^46\) present a mutualism association with ants.
Comparatively of the cotton production (1585 kg/ha) at Tamil Nadu of India, the yield recorded during our study in cotton fields during south-west monsoon (2068 kg/ha) and post-monsoon season (1862 kg/ha) reveal that release of reduviids reduced impact of insect pests thus enhance cotton production. Similar trends were also observed when *P. plagipennis* was release in cotton fields\(^\text{37,39}\). Although the cost benefit ratio was low (1:1.28 or 1:1.24) comparatively to entomopathogenic insecticides such as HaNPV (1:3.50) or Bt (1:1.23) used for cotton protection\(^\text{47,48}\), it had been higher than those of control highlighting the benefit of integrating this predator in cotton pest management. Furthermore, the small-scale mass production technology for *R. longifrons* developed by Sahayaraj and Ravi\(^\text{49}\) is available for an augmentative biological control program. In view of these encouraging results, it would be interesting in the framework of a biocontrol of cotton pests program to integrate this predator in other agro-climatic zones in Tamil Nadu as well as in other states in India.

**Conclusion**

Based on the observed hiding behaviour of *R. longifrons*, we conclude that this predator can survive and adapt to the cotton agro-ecosystem after its release. In the framework of an IPM program, *R. longifrons* life stages can be released at any time of the day. Augmentative releases of this predator reduced significantly the number of insect pests in the cotton fields. *R. longifrons* increased the cotton production and enhanced the cost benefit ratio increased in predator released cotton field. It is concluded that reduviid predators can be integrated into cotton integrated pest management program.

**Methods**

**Collection and maintenance of insects.** Life stages of *R. longifrons* were collected from cotton fields (2 females, 1 male, 5 nymphs) and scrub jungle (3 females, 2 males, and 8 nymphs) bordering agro-ecosystems (8.7038° N, 77°0.8625° E) in Tirunelveli districts of Tamil Nadu, India. They were maintained on a factitious host, fourth and fifth instar *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) under laboratory conditions at 30 ± 2 °C, 61 ± 5% relative humidity (RH) and photoperiod of 11 L:13 D hr in 1 L transparent plastic containers (15 cm diameter × 8 cm height) as described by Sahayaraj et al. (2002). *Corcyra cephalonica* eggs were purchased from the Agriculture Office, Palayamkottai, and maintained in 2 L plastic troughs (20 cm diameter × 10 cm height) on crushed wheat and groundnuts. For conducting various studies, *R. longifrons* life stages, third (26.8 ± 0.02 mg), fourth (35.4 ± 0.03 mg), fifth (54.8 ± 0.07 mg) nymphal instars, and adults (male = 67.8 ± 0.08 mg, female = 76.8 ± 0.09 mg) were used.

Adults and immatures of the *A. gossypii*, *P. solenopsis*, *D. cingulatus*, and eggs and larvae of *H. armigera* were collected from cotton and maintained on their natural host plant under the above mentioned laboratory conditions. Life stages of *D. cingulatus* were maintained on wet cotton seeds. Both *A. gossypii* and *P. solenopsis* life stages were maintained on cotton seedlings (5–10 days old) which were established in small plastic tubs (10 cm height × 8 cm upper diameter × 6 cm lower diameter). On each seedling 40–50 *D. cingulatus* nymphs or adults were accommodated. *H. armigera* larvae were maintained individually in plastic containers (8 × 6.5 cm) with a mixture of healthy cotton flowers, flower buds and young leaves.

**Hiding behaviour of the predator inside the screen houses.** To record the hiding places of the predator when it was released in cotton during an augmentative pest management programme, the methodology described by Tomson et al.\(^\text{31}\) was used. Observations of hiding behaviour of *R. longifrons* were carried out in a screen house (12 m length × 7.8 m width) using 30 day-old cotton plants (variety: MCU-5). The plot consisted of 52 plants (4 column × 13 rows) with inter-plant spacing of 0.60 m and inter-row distances of 0.75 m, oriented east to west. Before releasing the different life stages of the *R. longifrons*, the cotton plants, fallen leaves, pebbles, and other objects found in the plots were checked thoroughly to confirm the absence of any reduviids. Then, 10 fourth

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**Table 5.** Augmentative release of *R. longifrons* on cost benefit ratio analysis under irrigated and rainfed cotton cultivation.

| Expenses (in US dollar) | Field conditions | 
|-------------------------|-----------------|
|                         | Irrigated condition | Rain fed condition |
| Plough                  | Treatment | Control | Treatment | Control |
| 54.07                   | 54.07     | 36.06   | 36.06     |         |
| Sowing                  | 29.68     | 25.23   | 25.23     |         |
| Manure                  | 84.28     | 45.05   | 45.05     |         |
| Harvesting              | 55.46     | 54.07   | 54.07     |         |
| Transportation          | —         | 58.05   | 58.05     |         |
| Weeding                 | 86.51     | 90.11   | 90.11     |         |
| Cost of cultivation in *R. longifrons* | 21.07 |         | 21.07 |         |
| Total expenses          | 331.07    | 329.64  | 308.56    |         |
| Total Income            | 424.61    | 410.57  | 388.95    |         |
| Cotton production (Kg/h) | 837.0   | 753.35  | 716.30    |         |
| Cost Benefit Ratio (CBR) | 1.28      | 1.24    | 1.26      |         |
| The percent avoidable loss (%) | 14.5     | 4.9     |           |         |

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**Table 5.** Augmentative release of *R. longifrons* on cost benefit ratio analysis under irrigated and rainfed cotton cultivation.
PAL was also calculated according to the method proposed by Krishnaiah. Cotton in each plot was harvested. It was then cleaned, weighed and sold in the local market. The cost benefit ratio and percent avoidable loss were also presented.

**Biocontrol potential of R. longifrons in relation to day and night hours.** Modified methodology of Tomson et al. was followed for bioefficacy experiments. For the experiments, cotton seeds of MCU-5 variety were sown in cement pots (36 × 30 × 22 cm) and maintained in screen house at St. Xavier's College, Palayamkottai. The bioefficacy of R. longifrons life stages (third, fourth and fifth nymphal instars and adults) was tested against preferred life stages of A. gossypii (4 adults/predator), D. cingulatus (second and third nymphal instars, 2 of each instar/predator), H. armigera (second and third instar larva, 2 of each instar/predator) and P. solenopsis (4 adults/predator). The predatory potential of R. longifrons was evaluated between 6 a.m. and 6 p.m. (day experiments) and between 6 p.m. and 6 a.m. (night experiments). Pests were released on the 25–30-day-old cotton plants covered by a nylon net at 6 a.m. for day or 6 p.m for night experiments respectively. One hour after pests release, 24-hour pre-starved R. longifrons (third, fourth and fifth nymphal instars, and adults - 2/plant) were introduced separately on the infested cotton plants. Six replications were used for each pest and predator life stage for both experiments. Three replications without predator were established as control. After 12 h, the number of live and dead prey in each plant was counted and predation rate of the R. longifrons life stages was calculated.

**Experimental design for the augmentative biocontrol potential evaluations.** The augmentative biocontrol potential was evaluated during rain fed condition (South-west monsoon) 2011 (July to September) in the cotton field at Kothankulam (9.4692° N, 77.6046° E), in the Virudhunagar district, Tamil Nadu and also in irrigated condition (post-monsoon season) 2011–2012 (December to February) at K. Duraisamiyapuram (E 77° 35′, N 22.16′), in the Tuticorin district of Tamil Nadu. The methodology described by Tomson et al. with slight modifications was used for these evaluations. For weed control and fertilization farmers’ standard cultural practices were used to grow cotton (SVPR4 cultivar). Two treatments were evaluated, a cotton field into which R. longifrons was released and a control field, free of pesticides and predators. Unplanted buffer zone (2 m) was established between treatment and control plots. The treatment plots were arranged in randomized complete block design with five replications (10 plots total with a size of 10 m × 5 m each). To identify the predominant pests and predators in experimental cotton fields, we examined in each field 8–10 leaves of 10-randomly selected plants from two days before predators release. R. longifrons nymphal instars (50 individuals each) were released individually during 6–8 a.m. in each experimental cotton fields 40, 55 and 70 days after the seedling emergence (ASE). In addition, three cards (3 × 3 cm) containing each 50 eggs of R. longifrons were also placed onto the twigs of a plant in each cotton field. In total, a mixture of 900 R. longifrons (150 eggs, 250 nymphal instars × 3 releases = 900/plot) were released in each plot. Three days after the predators’ release, 10 randomly selected cotton plants in each experimental cotton field were visually examined for the presence of A. gossypii, D. cingulatus, P. solenopsis and H. armigera. The number of each species of predators encountered was also recorded per plant.

**Cost Benefit Ratio (CBR) and Percent Avoidable loss.** At the completion of the growing seasons, the cotton in each plot was harvested. It was then cleaned, weighed and sold in the local market. The cost benefit ratio (CBR) was calculated based on the income per hectare thus generated. In addition, the percent of avoidable loss (PAL) was also calculated according to the method proposed by Krishnaiha.

**Statistical analysis.** Before variance analysis, the data normality was tested using Levene’s test. Data on the percentage of predator hiding under various objects in relation to life stages was subjected to arcsine transformation, while the total number of prey consumed by a predator in 24 h, numbers of different insect pests and natural enemies present at each release (first, second and third), as well as their total mean populations were log-transformed to homogenize the variances before being subjected to variance analysis using IBM SPSS Statistics version 25 software package. Significant differences between the means were separated using the Student Newman Keuls test (p ≤ 0.05). Original means are presented in tables and figures.

Received: 10 September 2019; Accepted: 24 January 2020; Published online: 20 April 2020

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**Acknowledgements**

Authors are thankful to the Council for Scientific and Industrial Research, Govt. of India (37/1043/08/EMR II), for funding to Sahayaraj. Authors are gratefully than the Authorities of St. Xavier’s College, Palayamkottai for providing the necessary laboratory and field facilities.

**Author contributions**

K.S. and S.K. designed the work and analyses the data, S.K. executed the work and made rough version, K.S. and L.Y.L.E. corrected first and second version of the work.

**Competing interests**

The authors declare no competing interests.

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