Simulation-Based Assessment of the Ladybird Beetle, *Cheilomenes sexmaculata* (Fabricius), for Biological Control of Cotton Aphid, *Aphis gossypii* Glover, in the Selu Region of Parbhani District, Maharashtra

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Abstract

Cotton aphid, *Aphis gossypii* Glover is a frequent sucking insect of cotton in the semi-arid Marathwada region. Honeydew production and sooty-mould development, coupled with rapid colony growth can decrease plant vigour and promote repeated insecticide applications, which can negatively affect natural enemies and resistance management. Potential of the indigenous zigzag ladybird beetle, Cheilomenes sexmaculata (Fabricius) as augmentative biological-control agent in a field scenario was assessed in the present study, which was parameterised for the Selu region of Parbhani district, Maharashtra. A literature-calibrated simulation followed a randomized complete block design with five treatments and four replications: an untreated control; single releases of 100 and 200 second- or third-instar larvae per 35 m² plot; two releases of 200 larvae per plot, seven days apart; and a selective-aphicide reference check. The density of the aphids after release was affected by treatment and observation date, as well as the interaction between them. The repeated-release treatment resulted in 80.8-89.4% corrected suppression 7-21 days after release, with the greatest predator abundance, a low leaf-curling and sooty-mould score, and a modelled seed-cotton yield of 2.06 t ha-1. It had a slightly better illustrative benefit-cost ratio than the selective chemical check. Results suggest that early, repeated release of C. sexmaculata can offer a more persistent aphid management program than a single release in conjunction with the failure to eliminate native natural enemies and the lack of use of disruptive insecticides. The simulation numerical evidence used in this study should be validated with field results for multiple locations and seasons before an operational release rate is recommended for the Selu region of Parbhani district.

Keywords: Augmentative biological control; Cotton; Coccinellid; Predator-prey ratio; Marathwada; Integrated pest management; Simulation modelling.

1. Introduction

Cotton, *Gossypium hirsutum L.*, is one of the major rain fed commercial crop of Maharashtra and is of particular significance in Marathwada region where the performance of crop is largely dependent on the monsoon rainfall and its distribution. In the context of the major crops of the region with deep to medium black soil, the rainfall is around 500-1100 mm with a considerable inter-annual variability, cotton is one of the major crops identified by Vasantrao Naik Marathwada Krishi Vidyapeeth (VNMKV). Finally, Parbhani district is home to an old-established cotton research station, thus making it a suitable district for assessment of locally relevant pest-management options (VNMKV, 2026a, 2026b).

The cotton or melon aphid, Aphis gossypii Glover (Hemiptera: Aphididae), is an important early- and mid-season sucking pest of cotton due to the ability of colonies to grow quickly on tender leaves and shoots. Phloem sap is removed by nymphs and adults resulting in leaf curling, chlorosis, loss of vigour and delay of plant development. Honeydew laid on the foliage surface promotes growth of the sooty-mould and hinders photosynthesis, and can soil open lint. Aphids also have remarkable reproductive plasticity – they can have parthenogenetic multiplication which means that colonies can rapidly respond to good host and weather conditions (Blackman & Eastop, 2000; van Emden & Harrington, 2017).

Systemic or contact insecticides have been commonly used to control aphid outbreaks. Chemical intervention will give immediate suppression, but repeated or inopportunely timed applications can result in development of resistant populations, decrease populations of natural enemies, promote resurgence of secondary-pest populations and increase production costs. For cotton systems these are especially important, as broad spectrum sprays targeting one pest can kill predators and parasitoids that control aphids, whiteflies, jassids and other soft-bodied arthropods. Thus, compatibility between selective chemistry and transgenic cotton and biological control is a crucial prerequisite in integrated pest management (Dhillon & Sharma, 2009; Naranjo, 2011; Romeis et al., 2008).

One of the best known aphid predators are the ladybird beetles (Coleoptera: Coccinellidae). Larvae and adults are highly active in plant canopies, feeding on considerable amounts of prey and exhibiting numerical response to patches of aphids. Cheilomenes sexmaculata (Fabricius) is known as zig zag or six spotted ladybird beetle and is well-distributed in South and Southeast Asian countries and found in field, vegetable and horticultural crops. Its wide prey spectrum, relatively short generation time, genetic variability between Indian populations and ability to be multiplied in the laboratory has led to studies on prey suitability, feeding potential, functional response and life-table performance (Omkar & Bind, 2004; Pervez & Omkar, 2005; Abbas et al., 2020; Rakshith et al., 2025). Laboratory studies have shown that C. sexmaculata can develop and reproduce on a variety of aphids, and that generally the later larval instars and adults consume the most. Studies on A. gossypii have shown that it is a density-dependent forager and able to remove significant amounts of prey, as well as non-consumptive effects on aphid behavior and reproduction (Venkanna et al., 2021a, 2021b). However, lab usage is not directly convertible to field usage. Dispersing released beetles, rain may cause bugs to fall, ants may be resident in aphid colonies, prey may be concealed in the canopy, and pesticides may affect predator survival. These factors render local field validation essential.

A literature-based field simulation for the Selu region of Parbhani district was developed and analysed in the present study. The primary hypothesis was that repeated release of C. sexmaculata in the early stages would help to maintain a more favorable predator-prey ratio and suppress A. gossypii for a longer period than a single release. Modelling of crop injury, seed-cotton yield and treatment economics were also analyzed. The simulation was intended to be an open transparent, reproducible evaluation of the proposed biological-control strategy, and is not observations already gathered from fields in the Selu region.

1.1 Target pest and predator

Fig. 1. Field infestation of cotton aphid, *Aphis gossypii* Glover, on the underside of a cotton leaf in the Selu region of Parbhani district.

Source: Author's field photograph, Selu region, Parbhani district, Maharashtra.

Fig. 2. Adult zigzag ladybird beetle, *Cheilomenes sexmaculata* (Fabricius), observed on a cotton leaf in the Selu region of Parbhani district.

Source: GPS-tagged field photograph captured by the author at Selu, Maharashtra, on 11 August 2025.

2. Review of Literature

2.1 Biology and damage potential of *Aphis gossypii*

Aphis gossypii is very polyphagous and is quite variable with respect to colour, size, host association and seasonal biology. Usually on cotton, colonisation is started on tender foliage and apterous females are parthenogenetic and produce overlapping generations. Host tissue is succulent, and weather conditions allow the host to be fed on continuously, population growth is favoured. Winged morph production can be stimulated by crowding, poor host quality or even disturbance by predators, which aids dispersal to other plants and fields (Blackman & Eastop, 2000; van Emden & Harrington, 2017).

There is direct and indirect damage. Ongoing sap removal decreases resources for assimilation and salivary influences can affect plant physiology. Heavy colonies result in curling and crinkling of the foliage; black sooty mould grows on the honeydew that is present. This leads to decrease in effective leaf area, which can be very detrimental during square and boll development. Economic injury is influenced by crop stage, water status, variety, natural enemy pressure and the length of infestation, not just on one aphid count.

2.2 Biology and prey use by *Cheilomenes sexmaculata*

C. *sexmaculata* has a life cycle of egg, four larval instars, pupa and adult. Eggs are deposited in clusters near the colony of the prey. The newly hatched larvae are relatively small while the third and fourth instar larvae are more mobile and can be handled. Adults also are highly aphidophagous and will continue to live on other prey or supplemental foods if aphids are not present. Development, survival, adult longevity and fecundity are influenced by prey quality and a prey species that is easily consumed may not be as suitable for reproduction (Omkar & Bind, 2004; Singh et al., 2008).

Age-stage, two-sex life-table studies have confirmed that accounting for the effects of variable developmental rates and both sexes is important when assessing the mass-rearing potential. Positive population-growth parameters were obtained on various prey by Abbas et al. (2020) and Safeer et al. (2024) reported that the prey identity influenced chemotaxis, development and reproductive output. This suggests that locally available aphid prey may be used to maintain colonies, but also warns that laboratory performance may vary as a result of changing greenhouse or field conditions.

2.3 Feeding potential and functional response

Daily consumption, attack rate, handling time and functional response are used to describe predation efficiency. The type-II functional response is the most common reported for aphidophagous coccinellids and is defined by an increase in prey consumed with increasing prey density that is eventually constrained by handling and digestion. Pervez and Omkar (2005) showed that logistic analysis was a helpful tool in determining the shape of functional-response curve and in estimating biologically meaningful parameters.

Venkanna et al. (2021a) reported high density-dependent foraging by larvae and adults of C. sexmaculata feeding on cotton aphid. Likewise, laboratory studies show that the fourth instar larvae and adults consume a great number of prey throughout their life cycle (Guru Pirasanna Pandi et al., 2012; Venkanna et al., 2020). These measurements give a rational basis for the choice of release stages; however, consumption in a confined arena would be expected to be higher than in the field due to the increased dispersion of prey and the increased time spent searching, dispersing and avoiding bad weather by predators.

2.4 Consumptive and non-consumptive biological-control effects

Biological control does not just apply to prey killed. Aphid settlement, movement, development and reproduction may be affected by predator cues, interrupted feeding and unsuccessful attacks. Venkanna et al. (2021b) demonstrated that C. sexmaculata can cause non-consumptive stress to A. gossypii. These could slow colony expansion or promote dispersal in a field crop, but also alate dispersal could result in re-distribution of infestation. To make a complete assessment, aphid morphs, predator numbers and colony recovery after release should therefore be recorded, not just a single post-release count.

The performance of coccinellids is also set in a world of predators. Suppression may be aided by other predators such as lacewings, syrphids, spiders and other ladybirds; intraguild predation and competition may reduce the effectiveness of any single predator. Complex habitat may serve as both prey and predator refuges. The net effect of augmentative release thus will be a function of the indigenous assemblage of natural enemies and the presence of other prey and floral resources that will influence the persistence of the natural enemies (Obrycki et al., 2009; Snyder, 2009).

2.5 Field constraints and compatibility with IPM

The dispersal is a big constraint of augmentative ladybird releases. Adults may immediately emerge from a treated plot, especially if prey is scarce or canopy is poor. The advanced larvae are not immediately as dispersive as the adults, and may offer more consistent short-range contact with aphid colonies. Repeated release can make up for mortality and dispersal, but cost must be balanced against longer suppression and/or greater yield.

Chemical compatibility is also a factor. Although selective aphicides are likely to spare more predators than a broad spectrum organophosphate, carbamate or pyrethroid treatment, even a selective product can cause sublethal effects depending on route and timing of exposure. Bt cotton is generally tolerant to most natural enemies, but depending on the general spray programme in the crop, natural enemy survival may be affected (Dhillon & Sharma, 2009; Naranjo, 2011). Thus, conservation biological control will involve avoiding spraying during the release season and avoiding spraying when thresholds are not reached and retention of unsprayed refuges where possible.

2.6 Research gap and rationale for Selu, Parbhani district

It has been established from the literature that C. sexmaculata is able to feed on A. gossypii and is able to feed and complete its life cycle on aphid prey. But, there is not much information available to know the release rate, frequency and persistence of such a predator in rain-fed cotton, where climatic role of monsoon is unpredictable in the Selu region of Parbhani district. Aphid development and maintenance of predators may be affected by the black soils, intermittent rains and warm kharif weather in the district. A locally designed experiment is needed to distinguish effect of rapid knockdown from effect of sustained control, and to determine the economic competitiveness of a repeated release vs a selective chemical check.

This gap was addressed in the study by correlating entomological counts with injury, yield and economics. Additionally, weather and resident natural enemies were included so that one could not isolate the treatment effects. The numerical data set was obtained from a literature calibrated simulation, so the results are explicit modelled expectations and an analytical basis for the subsequent field validation.

3. Materials and Methods

3.1 Study area

The field scenario was parameterised using a research farm or a randomly selected, uniform cotton field in the Selu sub-district (Selu region) of Parbhani district, Maharashtra, India. Parbhani lies between 18°45′ and 20°10′ N latitude and 76°13′ and 77°39′ E longitude. The area of Marathwada is mainly under black basaltic soil, with monsoonal dominated rainfall regime and high spatial and temporal variability. Cotton is one of the important crops in the region and Cotton research infrastructures are already in place which are relevant for local evaluation of pest-management (District Administration Parbhani, 2026; VNMKV, 2026a, 2026b).

Table 1. Kharif climate profile used to parameterise the Selu-Parbhani field scenario

Month	Mean minimum (°C)	Mean maximum (°C)	Rainfall (mm)	Relevance to the field scenario
June	23.6	36.5	156.5	Crop establishment; first monsoon flush
July	22.5	32.0	222.0	Rapid vegetative growth and early sucking-pest colonisation
August	22.0	31.0	235.0	Dense canopy; rainfall may dislodge aphids and predators
September	21.5	32.0	180.0	Square/boll development; aphid resurgence may occur
October	18.0	32.0	75.0	Declining rainfall and maturing crop

Source: VNMKV (2026b); monthly values are taken from the university's official climate profile.

3.2 Experimental crop, pest and predator

The simulated crop was a locally recommended cultivar or hybrid of Gossypium hirsutum crop under uniform agronomic management which was sown during normal kharif sowing window. The desired pest was A. gossypii and the biological control was C. sexmaculata. No broad-spectrum insecticide was used during the release and observation time. Other pest-management measures were considered to be uniformly applied as needed to avoid confounding crop loss.

The colony of predators was assumed to be reared in an insectary in host plants infested with aphids, and had to be passed through at least 2 generations before being released. Egg batches were taken every day, and larvae were raised in well-ventilated cages in which plenty of prey was provided. Larvae of the second or third instar were chosen as these are strong enough to be moved, but not as likely to quickly fly long distances as adults. The rearing and release procedure was based on the published biology and cotton-aphid rearing techniques (Venkanna et al., 2020; Venkanna & Suroshe, 2023). It was assumed that the larvae of these flies were leaving at the cooler time of the morning or late afternoon to land directly on aphid-infested plants.

3.3 Experimental design and treatments

A randomized complete block design having five treatments and four replications produced 20 plots for the simulation. Gross plot size was set at about 35 m^2 and buffer rows between plots were specified to reduce movement of predators between treatments. To ensure similar initial infestation, pretreatment aphid counts were used. The first release was after a uniform population of aphids was established and the second release in T4 was 7 days later. The predator-release treatments used 100 or 200 larvae per 35 m^2 plot. With four replications, the complete predator requirement was 2,800 larvae: 400 for T2, 800 for T3 and 1,600 for the two T4 releases.

Table 2. Treatment structure used in the simulation

Code	Treatment category	Operational definition
T1	Untreated control	No intentional predator release or aphicide
T2	Low single release	100 second- or third-instar larvae per 35 m^2 plot at day 0 (approximately 2,857 larvae ha-1)
T3	High single release	200 second- or third-instar larvae per 35 m^2 plot at day 0 (approximately 5,714 larvae ha-1)
T4	Repeated release	200 larvae per 35 m^2 plot at day 0 and again at 7 DAR (approximately 5,714 larvae ha-1 per release)
T5	Reference check	One selective aphicide treatment at the locally recommended label/advisory rate

Source: Authors' experimental design informed by C. sexmaculata biology and predation studies (Abbas et al., 2020; Venkanna et al., 2020, 2021a); release rates are experimental treatments, not recommendations. Release quantities are expressed per 35 m^2 experimental plot to keep insectary production and field application operationally feasible.

3.4 Sampling procedure and response variables

The sampling protocol called for random 10 plants per plot from the center rows of the plots. A standard count was made on three leaves from the top, middle and bottom of the canopy for one fully expanded leaf per plant. When possible, aphid nymphs and adults were separated and combined. C. sexmaculata eggs, larvae, pupae and adults were counted on the whole sampled plant. Observations were arranged just prior to release, and at 1, 3, 7, 10, 14 and 21 days after the initial release (DAR).

Crop injury was scored ordinally from 0-4 for leaf curling and sooty mould with 0 being no visible symptoms and 4 being severe, widely-spaced symptoms. The harvest variables were bolls per plant, mean boll weight and seed-cotton yield per plot on a net-plot basis (kg ha-1). Treatment costs were predator production, predator release and the application of aphicide. Because of the absence of an official market price for cotton, one illustrative price was used to calculate gross return, net return and benefit-cost ratio, and to be able to compare treatments.

Table 3. Core variables included in the analytical dataset

Dataset domain	Variables	Purpose
Identification	Site, block, plot, treatment, plant, date, crop stage and DAR	Defines the repeated-measures structure
Pest	Nymphs, adults, total aphids per three leaves and winged morphs	Primary biological-control response
Predator	Eggs, larvae, pupae and adults per plant; predator-prey ratio	Establishment and persistence
Crop injury	Leaf-curling and sooty-mould scores (0-4)	Functional crop protection
Yield	Bolls plant-1, boll weight and seed-cotton yield	Agronomic outcome
Weather/economics	Temperature, relative humidity, rainfall, costs and returns	Context and feasibility

Source: Authors' data framework based on randomized-block agricultural experiments and repeated-measures analysis (Gomez & Gomez, 1984; Piepho et al., 2003, 2004).

3.5 Data provenance and simulation design

A reproducible data set was created, calibrated with literature values, to assess the treatment structure without providing synthetic values as field observations. Treatment trajectories were parameterised based on the direction and approximate magnitude of the responses reported in laboratory and field-oriented studies including increases in aphid abundance in the untreated crop, increases in predator abundance after treatment with the selective aphicide, stronger and more persistent decreases in predator abundance after multiple releases, and temporary decreases in predator abundance after chemical exposure. Random plot and observation level variation was introduced with a fixed seed (20260804), 4 replications and biologically constrained, non-negative values.

There were 140 plot-date records (five treatments × four replications × seven dates) in the analytical dataset. A field execution using the same protocol would leave plant-level observations and generate about 1400 plant-date observations pre-plot level. The weather values were not considered as the observations of an agricultural season but a plausible kharif sequence parameterised from Selu-Parbhani climate profile. All tables and figures use the word 'simulated' to denote the values.

3.6 Statistical analysis

Aphid and predator counts were presented as means ± SE. One-way analysis of variance was used to assess the pretreatment homogeneity. Square-root transformation [$\sqrt{(x + 0.5)}$] was used to analyse repeated observations; the treatment, date and treatment × date terms were accounted for, with plot identity. The model structure was similar to that used in previous mixed-model designs for randomized experiments with repeated measures (Piepho et al., 2003, 2004). The results were analysed by treatment means at $P \leq 0.05$ following the examination of the residual assumptions.

Aphid reduction was corrected by the Henderson-Tilton equation: Reduction (%) = $100 \times [1 - (Ta \times Cb)/(Tb \times Ca)]$, where Tb and Ta are the numbers of aphids per plant before and after release, respectively, and Cb and Ca are the numbers of aphids per plant in untreated controls before and after release, respectively. Randomized-block ANOVA was used to analyse crop injury and yield. Descriptive analysis of relationships between predator abundance, aphid density and weather variables was performed using Pearson correlations. The statistical outcomes measure the simulated treatment scenario, and do not determine the field efficacy.

4. Results

Results are shown for the simulated data set, calibrated with the literature. The numerical patterns were designed to simulate the biologically realistic treatment responses in the Selu-Parbhani field scenario; these are not directly observed from a completed field trial but are modelled outcomes.

4.1 Pretreatment comparability

Table 4. Pretreatment aphid density

Treatment	Description	Aphids per three leaves (mean ± SE)
T1	Untreated control	24.69 ± 0.43
T2	Single release: 100 larvae per 35 m² plot	24.86 ± 0.84
T3	Single release: 200 larvae per 35 m² plot	25.73 ± 0.41
T4	Two releases: 200 larvae per 35 m² plot at 0 and 7 DAR	24.37 ± 0.74
T5	Selective aphicide reference check	23.89 ± 0.76

Source: Authors' analysis of the literature-calibrated simulated dataset; response directions were informed by Omkar and Bind (2004), Abbas et al. (2020) and Venkanna et al. (2021a, 2021b).

The individual plot densities before treatment were between 21.6 and 26.8 aphids/3 leaves. Means of treatments were not statistically different ($F = 1.06$, $P = 0.411$) and the divergence was shown to be dependent on treatment and time, and not an imposed baseline imbalance. For a real trial, this test should be complemented by analysis of spatial gradients and – if needed – use the pretreatment count as a covariate.

4.2 Aphid population dynamics after release

Table 5. Temporal aphid density following predator release or reference treatment

Trt.	0 DAR	1 DAR	3 DAR	7 DAR	10 DAR	14 DAR	21 DAR
T1	24.7 ± 0.4	27.4 ± 0.6	34.0 ± 0.5	43.4 ± 0.9	48.5 ± 0.6	58.7 ± 1.7	62.4 ± 1.0
T2	24.9 ± 0.8	22.6 ± 0.3	17.8 ± 0.5	14.4 ± 0.2	16.1 ± 0.6	19.1 ± 0.4	22.0 ± 0.8
T3	25.7 ± 0.4	21.2 ± 0.3	15.3 ± 0.4	9.3 ± 0.4	10.7 ± 0.2	13.2 ± 0.5	15.4 ± 0.4
T4	24.4 ± 0.7	19.6 ± 0.8	13.7 ± 0.1	8.2 ± 0.2	5.6 ± 0.3	6.1 ± 0.2	7.0 ± 0.3
T5	23.9 ± 0.8	13.7 ± 0.4	7.0 ± 0.3	3.9 ± 0.4	5.3 ± 0.2	6.5 ± 0.3	10.5 ± 0.2

Source: Authors' analysis of the literature-calibrated simulated dataset; biological calibration was based on Omkar and Bind (2004), Abbas et al. (2020) and Venkanna et al. (2021a, 2021b).

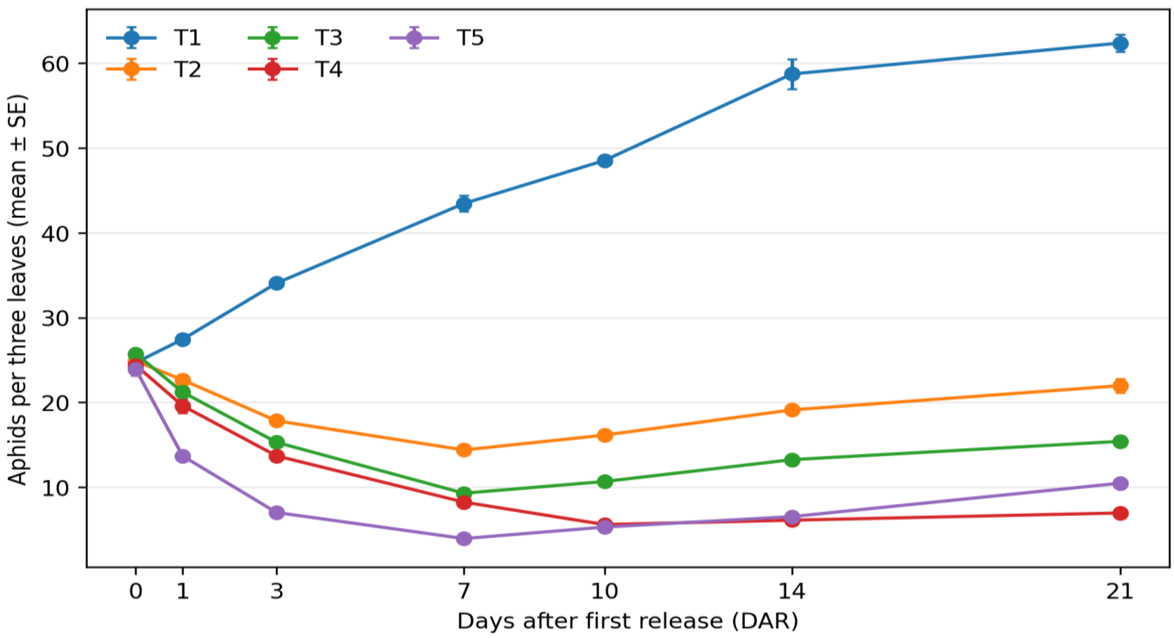

Fig. 4. Simulated aphid population trajectories under the five treatments.

Source: Authors' analysis of the literature-calibrated simulated dataset; error bars represent SE of four plots.

leaves at 21 DAR. The aphid population was depressed with one low release but started to rebound after 10 DAR. The high single release resulted in greater and more lasting suppression, while the upward trend of 14-21 DAR showed decreasing effects of predators. With the repeated release, the lowest counts for biological treatment were following the second release, holding about 5-7 aphids/three leaves between 10 and 21 DAR. The reference aphicide was the first to decrease and then gradually increased.

Ecologically this is a feasible pattern. Rapid mortality may occur as a result of chemical check, whereas encounter and handling of prey is required for a coccinellid release. Larvae and emerging adults, however, can cause pressure for several days once established. The second release in T4 was to replace the first cohort as it would be dispersing, pupating or dying out. Laboratory functional response studies indicate that consumption is density dependent, however it will plateau, thus early release prior to reaching extremely high aphid densities is likely more efficient than attempting to control a severe outbreak (Pervez & Omkar, 2005; Venkanna et al., 2021a).

Table 6. Repeated-observation analysis of simulated aphid counts

Source	Sum of squares	df	F	P
Plot	197.954	19	933.75	<0.001
Observation date	24.308	6	363.10	<0.001
Treatment × date	145.225	28	464.84	<0.001
Residual	1.004	90		

Source: Authors' mixed-model analysis of square-root-transformed simulated counts following Piepho et al. (2003, 2004); plot was included as the repeated subject.

The analysis showed that there was a significant observation-date effect and a significant treatment × date interaction ($P < 0.001$), as is usually found when different treatments start at similar densities but then spread differently afterwards. The interaction is more informative than the overall treatment mean, as it indicates that efficacy varied over time. All future field reporting should then include the entire temporal profile and not just an average of the post-treatment response.

4.3 Corrected aphid reduction

Table 7. Corrected reduction in aphid density relative to the untreated control

DAR	T2 (%)	T3 (%)	T4 (%)	T5 (%)
3	48.0	56.9	59.2	78.7
7	67.1	79.5	80.8	90.6
10	67.0	78.9	88.3	88.7
14	67.7	78.3	89.4	88.5
21	65.0	76.3	88.7	82.6

Source: Authors' calculations from the simulated dataset using the Henderson-Tilton (1955) equation.

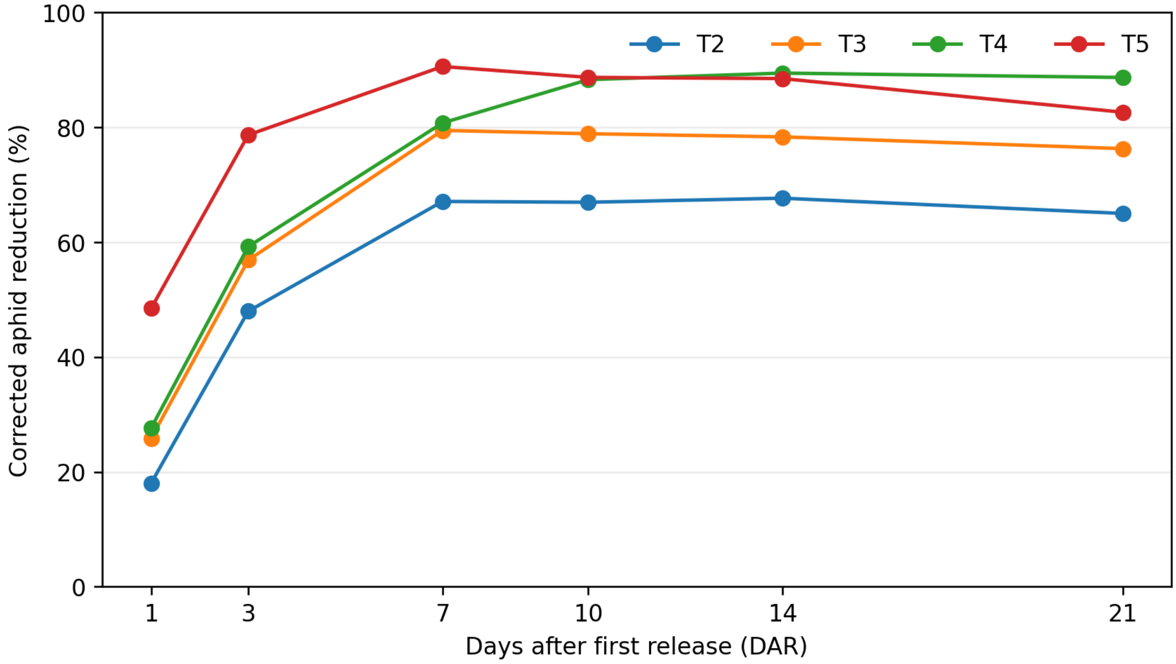

Fig. 5. Corrected aphid reduction through 21 days after first release.

Source: Authors' calculations from the literature-calibrated simulated dataset.

The repeated-release treatment reduced to 80.9% at 7 DAR, but was near or equal to 88% at 10, 14, 18 and 21 DAR. The high single release began at about 77-81% reduction for the same length of time, while the low single release dropped to about 65% at 21 DAR. The percentage of reference check was greater than 85% from 7 through 14 DAR with a decreasing trend as the aphid population increased. So the key benefit of the repeated predator release was not the most rapid initial effect, it was its maintaining effectiveness after the first week. Field validation should be used to determine if this stability will hold when movement among plots happens. Adult coccinellids are very mobile and even larvae can move around in the canopy. The use of buffer rows and adequate size plots decreases but does not eliminate spillover. Future estimates of retention could be improved by using mark-release-recapture techniques or protein marking. In absence of such data, low predation counts could be due to prey avoidance, pupation and/or dispersal or predation mortality.

4.4 Predator establishment and prey association

Table 8. Simulated establishment of *Cheilomenes sexmaculata*

Trt.	0 DAR	1 DAR	3 DAR	7 DAR	10 DAR	14 DAR	21 DAR
T1	0.35	0.42	0.53	0.58	0.70	0.82	0.86
T2	0.39	0.85	1.25	1.56	1.28	0.97	0.72
T3	0.34	1.27	1.74	2.02	1.88	1.43	1.00
T4	0.38	1.40	2.11	2.32	2.74	2.62	1.84
T5	0.33	0.10	0.09	0.11	0.22	0.29	0.43

Source: Authors' analysis of the literature-calibrated simulated dataset; values represent all life stages of C. sexmaculata per sampled plant.

The abundance of predators was higher after release in T2-T4 and was the highest in T4 after the second release. The reference chemical check, however, exhibited a temporary decrease, which could be a direct or indirect exposure. There was a negative association between predator and aphid abundance for predator-release and untreated treatment-date means ($r = -0.64$, $P = 0.000$). The association is descriptive, both variables also varied with time and treatment and causality should be tested with the experimental contrast, not only the correlation.

The model's prediction of the predator feeding pattern is consistent with the biological expectation that later instar larvae and adults can feed heavily on aphids, and the quality of the prey influences prey survival and reproduction. Appearance of eggs/young larvae after release would be particularly significant as it would suggest that local reproduction had occurred, and not just that the released individuals were temporarily present. It is therefore necessary that released larval stages and newly produced eggs, pupae and adults be separated whenever possible in an actual study.

4.5 Crop injury and seed-cotton yield

Table 9. Simulated crop injury and yield response

Trt.	Leaf curl (0-4)	Sooty mould (0-4)	Bolls plant-1	Boll wt. (g)	Yield (kg ha-1)
T1	2.86 ± 0.03	2.51 ± 0.03	27.6 ± 0.3	4.17 ± 0.02	1542 ± 16
T2	1.77 ± 0.07	1.59 ± 0.02	30.9 ± 0.4	4.27 ± 0.02	1754 ± 20
T3	1.34 ± 0.06	0.84 ± 0.08	34.4 ± 0.3	4.45 ± 0.05	1867 ± 14
T4	0.70 ± 0.02	0.40 ± 0.05	37.2 ± 0.1	4.60 ± 0.03	2060 ± 16
T5	0.90 ± 0.02	0.51 ± 0.04	36.4 ± 0.4	4.56 ± 0.04	1980 ± 31

Source: Authors' analysis of the literature-calibrated simulated dataset; injury scores and yields are modelled values.

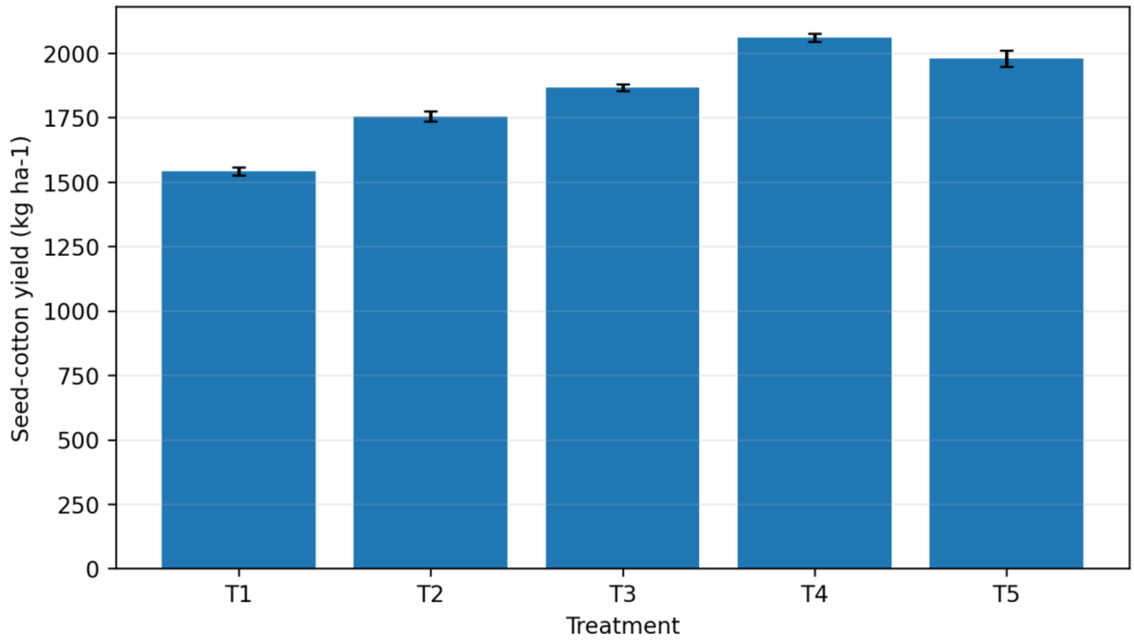

Fig. 6. Simulated seed-cotton yield under the five treatments.

Source: Authors' analysis of the literature-calibrated simulated dataset; error bars represent SE of four plots.

There were differences in simulated yield between treatments ($F = 98.3$, $P < 0.001$). The mean yield at T4 was the highest, followed by T5 and T3. The untreated control suffered from the greatest injury and produced the lowest number of bolls and yield. The estimated increase in yield of T4 over T1 was about 519 kg ha-1. This type of response is reasonable to expect if the aphids are suppressed during the time of assimilating leaf area in the reproductive growth stage, although it may be more or less depending on the timing of the infestation, drought, and other pests.

The evaluation of biological-control experiments should not be based on reduced insect populations. A treatment that reduces aphids temporarily, but does not have a positive effect on plant condition or yield may not be agronomically worthwhile. Natural enemies, on the other hand, can keep an outbreak from happening without noticeably reducing the number of insects at each check. Such incorporation of the arguments of injury, yield and economy will therefore yield a more defensible assessment than abundance alone.

4.6 Economic analysis

Table 10. Comparative treatment economics

Trt.	Yield kg ha-1	Treatment cost (₹ ha-1)	Total cost (₹ ha-1)	Gross return (₹ ha-1)	Net return (₹ ha-1)	B:C
T1	1542	0	65,000	107,911	42,911	1.66
T2	1754	1,800	66,800	122,783	55,983	1.84
T3	1867	2,800	67,800	130,668	62,868	1.93
T4	2060	4,000	69,000	144,221	75,221	2.09
T5	1980	3,000	68,000	138,574	70,574	2.04

Source: Authors' economic calculations based on simulated yield, an assumed farm-gate price of ₹70 kg-1 and a base cultivation cost of ₹65,000 ha-1; the values are analytical assumptions, not observed prices or costs.

The repeated release produced the greatest simulated net return and slightly more than 2.0 benefit-cost ratio. The selective check also proved to be economically favourable, with the untreated control having the lowest return without spending anything. A key practical message is that a more expensive biological treatment may be economically viable if it affords adequate yield and minimizes the need for re-sprays. However, the actual predator cost is dependent on the scale of mass rearing, transport, labour required for release and local availability. These costs should be recorded and included in the costing of a future trial and not on the catalogue price.

An economic interpretation should also account for other benefits that may not be fully captured by the B:C ratio for a single season, such as conservation of other natural enemies, reduced exposure of workers, avoidance of residues and slower selection of resistance. These benefits do not make an ineffective treatment worthwhile, but they may have an impact on adoption where the financial return is the same for biological and chemical treatment.

4.7 Weather associations and local interpretation

Table 11. Simulated associations among aphids, predators and weather

Relationship	r	P	Interpretation
Predator abundance vs. aphid density (T1-T4 treatment-date means)	-0.64	<0.001	Negative association consistent with biological suppression
Mean temperature vs. untreated aphids	0.83	0.021	Positive trend in the simulated sequence
Relative humidity vs. untreated aphids	-0.48	0.270	Negative trend as the sequence became drier
Rainfall vs. untreated aphids	-0.04	0.933	Weak/unstable with only seven dates

Source: Authors' descriptive analysis of the simulated dataset; the local climate context was derived from VNMKV (2026b).

Predator release is a dynamic situation in the Selu-Parbhani monsoon context. Aphids can be physically removed from plants by heavy rainfall and the small larvae may be reduced by dry periods when the plants are not physiologically active or irrigated. Development is more likely to be accelerated by high temperature, however if canopy conditions are stressful then handling and searching periods may be reduced. Thus, the impact of weather is likely to be nonlinear and stage specific. Seven observation dates are not sufficient to give a reliable weather modelling; multi-season weekly data are better.

An additional field covariate should be ant attendance as ants have been known to protect aphid colonies from coccinellids in exchange for honeydew. Likewise, if other prey is available, ladybirds may stay where aphid numbers are low. The differences in same nominal release rate may be explained by these mechanisms, manifesting as differences between villages or seasons. Consequently, local recommendations are to be based on replicated environments and not on a single research-farm trial.

5. Discussion

5.1 Biological efficacy and consistency of suppression

Table 12. Relationship between modelled outcomes and published evidence

Evidence domain	Published finding	Implication for Selu-Parbhani trial	Selected source
Prey suitability/life table	Positive population growth of *C. sexmaculata* on several aphids	Supports mass rearing, but prey-specific performance varies	Abbas et al. (2020); Safeer et al. (2024)
Functional response	Consumption rises with aphid density and then approaches a plateau	Releases should begin before very high aphid densities	Pervez & Omkar (2005); Venkanna et al. (2021a)
Non-consumptive effects	Predator presence can alter aphid stress and reproduction	Counts may underestimate total predator effect	Venkanna et al. (2021b)
Field persistence	Dispersal and variable conditions reduce laboratory-predicted efficacy	Repeated releases may be more stable than one release	Obrycki et al. (2009); Snyder (2009)
IPM compatibility	Natural enemies perform best when disruptive sprays are avoided	Use selective products only when thresholds justify intervention	Dhillon & Sharma (2009); Naranjo (2011)

Source: Authors' synthesis of peer-reviewed studies cited in the table.

The best 'modelling' performance of T4 demonstrates a general ecological principle and not the direct copying of a published experiment: the pressure of predators needs to be high enough and long enough to interrupt the growth of the aphid population. C. sexmaculata has been reported to have favourable life-table performance on several aphid prey, remove large numbers of aphids and generally exhibit type-II functional response (Omkar & Bind, 2004; Pervez & Omkar, 2005; Abbas et al., 2020; Venkanna et al., 2021a). The second release in the current simulation was made to replace the predator cohort as it was depleted by the dispersal, pupation or mortality of the first cohort. The most important benefit was not the most rapid initial reduction, but the continued suppression following the first week.

However, the selective aphicide was still used as an essential reference treatment, as biological control methods should not just be compared with an untreated control. It resulted in rapid initial effect followed by a gradual recovery of aphids, and reduced predators. This contrast is in line with the challenge to balance immediate pest reduction with conservation of cotton's natural enemies in IPM (Dhillon & Sharma, 2009; Naranjo, 2011; Romeis et al., 2008). Repeated release, therefore, is proposed as a hypothesis for stable suppression, with the final operational rate left to the field experiments.

5.2 Predator establishment and compatibility with integrated pest management

The modelled treatment response was focused on predator establishment. Repeated-release treatment had the highest abundance of C. sexmaculata at 7 DAR and the predator numbers temporarily decreased in the selective-aphicide check. Biological suppression of aphids was indicated by a negative association between predator and aphid abundance, but this association cannot be distinguished from changes in the abundance of each through time. It is important to differentiate between released larvae and locally produced eggs, pupae and adults, as the latter are better evidence of establishment than the former.

The field processes expected to influence the performance of C. sexmaculata were simplified in the simulation. Predators and parasites may be held or drawn away by other prey, ants may protect aphid colonies, heavy rain may blow small stages away, and adults and advanced larvae are capable of dispersion. Control may be further modified by habitat complexity and interactions with lacewings, syrphids, spiders and other coccinellids (Obrycki et al., 2009; Snyder, 2009). These mechanisms account for the inability to convert laboratory feeding rates to a field release recommendation.

Predator is most likely to be effective when released early in aphid colony development when insecticides are not disruptive or used, and when the natural enemies were already present in the IPM programme. Biological control may be insufficient to stop aphid pressure, under which conditions selective chemistry may be necessary, but treatment timing should be chosen to minimise the risk of attacking released larvae and adults. The use of C. sexmaculata is thus intended as a method of better ecological regulation and to minimize unnecessary sprays, not to replace all chemical measures.

5.3 Agronomic, economic and local implications

The simulated decrease in leaf curling and sooty mould, as well as the increase in boll number and seed-cotton yield, illustrate the importance of assessing crop protection in biological-control research. The simulated yield was highest after repeated-release treatment and lowest in the untreated control, with the highest and lowest injury respectively. The direction of response is biologically probable if the aphids are suppressed and the assimilating leaf area is protected during the reproductive development, but the magnitude of yield gain in the field will be further influenced by timing of the infestation, water stress, cultivar and other pests.

The economic analysis indicated that the repeated release is competitive even if the direct cost of treatment is larger if it does not cost additional sprays and protects adequate yield. The calculation did not value reduced pesticide exposure, conservation of other natural enemies, avoiding residues or delaying pesticide resistance. These broader advantages can help in the adoption decision if there is potential for similar benefit from biological or chemical control, but do not replace proper pest control. The actual cost of the predator production, labour requirements, and season specific cotton prices have to be observed, however, for a final recommendation.

The Selu region of Parbhani district offers a relevant and practical test environment. Due to the monsoon pattern, black soils, and rainfed cotton production in the district, significant variability of plant growth and aphid buildup and predator persistence may exist between sowing dates and locations. Therefore, conclusions need to be drawn from replicated research and farmer fields in and around Selu over more than one kharif season and not based on one environment.

5.4 Limitations and field-validation priorities

The greatest constraint is that the numerical data set is simulated. Biological plausibility is enhanced by calibration to published evidence, but cannot reproduce full variability of a field trial with the monsoon season. The model addresses one crop, one district, one release-stage strategy and predator dispersal, ant attendance, alternative prey, pesticide residues and naturally occurring coccinellids are simplified. Therefore, estimated efficacy, yield protection and economics are preliminary.

Field validation should be carried out in two stages. First, the 100- and 200-larvae-per-plot release rates should be tested in a uniform cotton field in the Selu region to determine whether they can be applied evenly and whether second- and third-instar larvae remain on cotton plants for 24-72 h. Retention counts at 6 h, 1 d and 3 d would distinguish immediate dispersal from subsequent mortality or pupation. A second efficacy test should be conducted at the Selu site and at least two additional farmer fields representing different sowing dates and/or moisture regimes over two kharif seasons.

The principle efficacy criterion should be a decrease in the cumulative aphid pressure, (aphid-days), and not a favourable aphid count on one date. Yield, insecticide avoidance and released predators recovery should be considered co-primary practical outcomes. All release batches must be screened for species identification, larval stage, transport survival, and recent feeding history. Release shall not take place just prior to predicted heavy rainfall and records shall be kept of all pesticide exposure in and around the trial.

Raw data, metadata, analysis code and protocol deviance should be stored in a recognized repository at the plant level. A transparent, data-based data record will enable the same tables and figures to be produced based on field observations and will prevent the format of the report from clouding the reporting of uncertainty of the evidence.

Table 13. Minimum evidence required before a district-level release recommendation

Validation component	Minimum requirement	Decision criterion
Pilot retention	One site; 5 treatments × 4 blocks; retention counts at 6 h, 1 d and 3 d	At least 60% of released larvae detected or accounted for at 24 h
Seasonal efficacy	Weekly aphid/predator counts through the principal infestation window	Repeated release lowers cumulative aphid-days relative to T1 and T2
Multi-location stability	One research or farmer field in Selu plus two additional farmer fields for two kharif seasons	Direction of treatment response is consistent across environments
IPM compatibility	Document every insecticide exposure and resident natural enemy	No avoidable broad-spectrum spray during the release window
Economic validation	Use observed predator, labour, spray and market costs	Net return is competitive with the selective chemical reference
Data transparency	Archive raw plant-level data, metadata and analysis script	All reported values are reproducible from original observations

Source: Authors' validation framework derived from field-biological-control principles (Obrycki et al., 2009; Snyder, 2009; Venkanna et al., 2021a).

6. Conclusion

The literature calibrated simulation suggests that C. sexmaculata holds a potential for suppressing A. gossypii in cotton grown in the Selu region of Parbhani district, provided that the predator pressure is created early and continued using repeated release. Of the modelled strategies, two releases of 200 larvae per 35 m² plot, spaced seven days apart resulted in the most consistent aphid reduction, the least crop-injury rating, the greater seed-cotton yield and the best illustrative net return. The selective aphicide resulted in more rapid initial knockdown, aphid resurgence and temporarily reduced predator numbers.

These findings are indicative of a field testable release strategy and not an immediate farmer recommendation. Replicated multi-location and multi-season trials are necessary for operational use to confirm predator retention, cumulative suppression of aphids, crop protection and treatment economics under Selu-Parbhani conditions. In integrated pest management, use of C. sexmaculata is best situated as a tool that will conserve ecological regulation, delay or prevent needless spraying and complement threshold-based selective chemistry.

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