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**Research Article**

# Transgenerational effect of insecticides on zigzag ladybird beetle, *Cheilomenes sexmaculata* (Fabricius) (Coleoptera: Coccinellidae)

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**ABSTRACT:** The effect of the insecticides at sublethal doses, recommended against aphid and pod borers in cowpea ecosystem was evaluated on the survival, development and reproduction of *Cheilomenes sexmaculata* (F.) (Coleoptera: Coccinellidae) by exposing the 0-24h old grubs via residue film method. The treatments for the assay were dimethoate 30 EC, flubendiamide 20 WDG, spinosad 45 SC, thiamethoxam 25 W, all at $^{1}/_{10}^{th}$ of the recommended field dose along with the botanical component, neem oil emulsion at 0.3 per cent. Dimethoate induced complete mortality of the exposed grubs. Minimum survival at 18.33 per cent was with neem oil followed by 20 per cent in thiamethoxam in contrast to 82.50 per cent in flubendiamide, 84.17 per cent in spinosad and 94.17 per cent in absolute control. Exposure to thiamethoxam and neem oil extended the developmental time of the first and second-instar grubs. However, the mean duration of the pupa was shortened. Exposure to thiamethoxam, flubendiamide and neem oil significantly reduced the oviposition period to 47.88±3.45, 48.63±4.86 and 50±2.29 days, respectively, as against 55.88±1.15 days in control. Adult longevity was reduced drastically after exposure to thiamethoxam, neem oil, and flubendiamide. The fecundity and fertility were significantly less after exposure to all the insecticides. The hatchability of the second-generation eggs was minimal with flubendiamide (69.15%) followed by 71.10 and 71.97 per cent, respectively in thiamethoxam and neem oil as against 86.80 per cent in control. The results of the study indicated the direct mortality and indirect impact of dimethoate, thiamethoxam and flubendiamide at sublethal doses on the development and reproductive parameters of *C. sexmaculata*.

**KEYWORDS:** *Cheilomenes sexmaculata,* natural enemies, selective insecticides, sublethal effects, transgenerational effects

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## INTRODUCTION

Chemical means of pest control measures can form a realistic and practical solution to many complicated problems associated with plant health and thus can streamline crop protection practices. However, over-reliance on chemical measures with the sole aim of gaining economic benefits from crops can end up with complicated consequences. Indiscriminate use of chemical pesticides often results in control failures and has serious impacts, leading to environmental pollution and disruption of ecological balance. Unscientific use of broad-spectrum insecticides leads to pest outbreaks due to the development of pesticide resistance and the destruction of natural enemies. Biocontrol measures, even if sustainable and cost-effective, often fail to give profitable returns under high insect pest density. Integrated Pest management approach was introduced initially substituting the concept of pest control techniques with precautionary measures. Integrated Pest Management (IPM) combines cultural, biological and chemical control options to deliver effective and targeted pest management solutions that can be customized to specific climates and habitats. IPM suggests resorting to selective pesticide use in managing situations that cannot be successfully handled by other tools, and promoting integration of biological and chemical control measures. The use of selective pesticides favours the conservation of natural enemies, and the IPM programs may become more successful.

Coccinellid beetles, generally known as ladybird beetles, are the most common and efficient natural enemies in agricultural lands contributing to the natural regulation of major agricultural pests. The six-spotted zig-zag ladybird beetle, *Cheilomenes sexmaculata* (Fabricius) is an important aphidophagous predator in vegetable ecosystems. The key features of *C. sexmaculata* include its wide host range, geographic distribution, broad habitats and intense searching and feeding ability (Venkatesan *et al.*, 2006). This predator is found around the year in cowpea and lablab crop systems in association with its preferred host, *Aphis craccivora* Koch

(Joshi *et al.*, 1997) and the control against aphids provided by *C. sexmaculata* is analogous to that of insecticides such as malathion and diazinon (Bari & Sardar, 1998).

The accepted potential of *C. sexmaculata* for natural biocontrol either alone or as a component of IPM, is often undermined by the indiscriminate use of insecticides and there is a need to conserve this potent natural enemy for its effective utilization. Conservation of natural enemies in IPM programs is accomplished through either habitat manipulation or the use of selective insecticides that in turn enhance natural enemy population and hence efficient pest management. Insecticide selectivity can be attained either by reducing the exposure of natural enemies to insecticide application or by using insecticides that are harmful to the insect pests but relatively harmless to natural enemies with the ultimate goal of increasing the effectiveness of biological control. By conserving natural enemies through the use of selective insecticides, the likelihood of pest resurgence may be decreased and the number of insecticide applications can be reduced. Often investigations on natural enemies only evaluate the short-term effects, *i.e.*, lethal effects of insecticides, ignoring the long-term sub-lethal and transgenerational effects on physiology and behaviour. Selection of insecticides safer to predators like *C. sexmaculata* is recommended for which the commonly recommended pesticides effects on the life cycle and reproductive parameters of the predator to be understood for, the integration of chemical pesticides and insect predators for sustainable IPM in a crop ecosystem.

## MATERIAL AND METHODS

### Insects

The nucleus culture of *C. sexmaculata* was developed from healthy grubs and pupae of the predator collected from cowpea fields. The adults emerged were paired, and were released for egg laying on potted cowpea plants, artificially infested with aphid *Aphis craccivora* confined in rearing cages of size 30cmx30cmx30cm built of glass sides and a top and wooden frame on the bottom, at the rate of five pairs per cage. The eggs laid by *C. sexmaculata* on cowpea leaves and side glasses were collected and kept in plastic containers for hatching. The grubs at 0-24h old were used for experiments.

### Insecticides

Commercial formulations of three insecticides that are recommended against pest complex in cowpea ecosystems such as dimethoate 600ga.i/ha (Rallies India Ltd.) flubendiamide 25 g a.i/ha (Takumi 20WG, Rallis India Ltd.); spinosad 7.5 g a.i $L^{-1}$ (Taffin 45SC, Rallis India Ltd.); thiamethoxam 25 g a.i/ha (Actara 25WG, Syngenta Crop Protection Ltd.) and a botanical, neem oil 3 per cent (Sterling Farm Research and Services Private Ltd.) were evaluated at one-tenth of their recommended field doses along with untreated control (KAU, 2016).

### Bioassay

First instar grubs at 0 to 24h old were exposed to insecticides following the residue film method described by Hassan *et al.* (1985). For experimenting, 1ml of the prepared insecticide solution was pipetted out into a Petri dish. The dish (9cm) was then rotated to have a uniform thin film of insecticide solution on it. After drying, ten grubs were released into the Petri dish. Each treatment was replicated four times maintaining 30grubs/replication. Test insects were allowed to crawl in the insecticide-treated Petri dishes for an hour. After the treatment period, the surviving grubs were transferred into 30ml rearing containers for recording observations on developmental parameters, adult emergence, fecundity, fertility and adult longevity. To study the reproductive behaviour and longevity, the adults that survived in each replication were sexed by size and external genitalia (Lalithambika, 2012) and paired adults were maintained separately in Petri dishes and observed until their death.

### Data analyses

Data on survival, development and reproductive parameters were subjected to Analysis Of Variance (ANOVA) using Web Agri Statistical Package (WASP 2.0, ICAR-CCARI, India) to confirm the variability. Duncan's Multiple Range (DMRT) test was performed to determine the significant differences between treatments ($p<0.05$). The insecticides were categorized for toxicity according to the IOBC laboratory scale (Hassan *et al.*, 1994) based on their total effects (E) given by Overmeer and van Zon (1982) as: (1) harmless (<30%); (2) slightly harmful (30 - 79%); (3) moderately harmful (80 - 99%); and (4) harmful (>99%).

$$\text{Total effect (E \%)} = 100\text{-}(100\text{-Mc}).\ \frac{Rt}{Rc}$$

where, *Mc* is corrected mortality, *Rt* is the mean egg/female in treatment and *Rc* is the mean egg/female in control.

## RESULTS

### The survivability of grubs

When 0-24h old first instar grubs of *C. sexmaculata* were exposed to residue films of selected insecticides at one-tenth of the recommended field rate, the survival of grubs varied from 0 to 84.17 per cent. None of the first instar grubs survived after exposure to dimethoate. Minimum survival at 18.33 per cent was observed in neem oil followed by 20 per cent in thiamethoxam and these two did not differ statistically. Flubendiamide and spinosad treatments recorded 82.50 and 84.17 per cent survival respectively which were on par with each other and were significantly higher compared to

**Table 1.** Effect of insecticides on survival of first instar grubs of *Cheilomenes sexmaculata*

| S. No. | Insecticide | Survival of first instar grubs (%) |
|---|---|---|
| 1 | Dimethoate (60g a.i./ha) | 0.00[d] (0.052) |
| 2 | Flubendiamide (2.5ga.i./ha) | 82.50[b] (65.50) |
| 3 | Thiamethoxam (2.5g a.i./ha) | 20.00[c] (26.32) |
| 4 | Neem oil (0.3%) | 18.33[c] (24.66) |
| 5 | Spinosad (0.75g a.i./ha) | 84.17[b] (66.63) |
| 6 | Control | 94.17[a] (78.15) |
| | CD(0.05) | 7.064 |

Values in the parentheses are arc sin transformed. Figures followed by the same alphabet did not differ significantly at p =0.05.

thiamethoxam and neem oil. The survival of 94.17 per cent recorded in control was significantly higher than all other treatments (Table 1).

**Effect of insecticides on the developmental period of *Cheilomenes sexmaculata***

The mean duration of grubs in the first and second instar stages was prolonged significantly after exposure of the grubs at 0-24h old stage to neem oil and thiamethoxam. The developmental duration recorded by the first and second instar grub after exposure to spinosad and flubendiamide was comparable to that of control. However, no significant difference was observed between treatments with respect to the mean duration of third and fourth-instar grubs. At the same time, the mean pupal period of survived individuals after exposure to thiamethoxam and neem oil was significantly shortened compared to those developed after exposure of grubs to flubendiamide and spinosad.

The total developmental period from first instar to adult was prolonged significantly after exposure to neem oil and thiamethoxam with mean values of 11.13±1.46 and 11.06±1.49 days respectively as against 9.69±0.59 days in control (Table 2). At the same time, the total developmental period recorded with flubendiamide (9.99±0.64 days) and spinosad (9.84±0.61days) was on par with the control.

**Effect of insecticides on adult biology and reproduction of *Cheilomenes sexmaculata***

***Adult longevity***

Exposure of first instar grubs at 0-24h old, to thiamethoxam, flubendiamide and neem oil significantly reduced the oviposition period of *C. sexmaculata* to 47.88±3.45, 48.63±4.86 and 50.00±2.29 days respectively (Table 3), when compared to 55.88±1.15 days in control. The oviposition period in spinosad was 53.13±1.33 days and was on par with control as well as with other treatments.

The life period of males was short in thiamethoxam at 41.5±1.95 days followed by neem oil at 43.25±2.12 days and flubendiamide at 43.63±2.39 days and these three were on par with each other. Male longevity recorded in spinosad at 45.75±1.59 days was significantly higher than that of thiamethoxam but was on par with neem oil and flubendiamide and also with control with longevity of 47.25±1.41 days (Table 3).

Female longevity in flubendiamide, thiamethoxam and neem oil was 56.38±4.69, 56.50±3.53 and 59.50±2.30 days respectively which were significantly shorter compared to control at 64.38±1.15 days. The female longevity in spinosad treatment was 61.00±1.24 days, which was on par with control as well as with other insecticides.

***Fecundity***

The fecundity recorded was less with flubendiamide (1581.63±80.13 eggs) followed by thiamethoxam with 1637.13±92.10 eggs. Both these treatments were on par with neem oil recording 1727.13±51.75 eggs but were significantly lower than spinosad with 1917.00±89.43 eggs. In many instances, the eggs laid in a cluster were malformed and turned into an undifferentiated mass in neem oil treatment. Such egg masses were not counted. The fecundity in control at 2151.88±27.31 eggs was significantly higher compared to all other treatments.

***Fertility***

The fertility (offspring/female) of adult females developed from grubs exposed to insecticides was reduced significantly. The lowest fertility was in flubendiamide with 1092.25±67.02 offspring/female, followed by thiamethoxam with 1162.38±58.92 offsprings/female and neem oil with 1242.62±46.79 offspring/female and these were on par with each other. However, the fertility observed with spinosad at 1604.00±72.89 offsprings/female (Table 4) was significantly higher than with other insecticides. The fertility recorded in control with 1868±26.50 offspring/female was significantly higher than that of all other treatments.

***Egg hatchability in the second generation***

The hatchability of eggs laid by the adults developed from grubs exposed to insecticides at their first instar stage, was less in flubendiamide treatment with 69.15±3.17 per cent followed by 71.10±1.02 and 71.97±1.89 per cent respectively in thiamethoxam and neem oil and these three

**Table 2.** Effect of insecticides on pre-adult biology of *Cheilomenes sexmaculata*

| Treatment | Instar 1 (Days) | Instar 2 (Days) | Instar 3 (Days) | Instar 4 (Days) | Pupal period (Days) | Pre-adult stage (Days) |
|---|---|---|---|---|---|---|
| Flubendiamide (2.5 g a.i./ha) | 1.16±0.07$^{b}$ | 1.51±0.13$^{c}$ | 1.95±0.15$^{a}$ | 2.62±0.15$^{a}$ | 2.76±0.14$^{ab}$ | 9.99±0.64$^{b}$ |
| Thiamethoxam (2.5 g a.i./ha) | 1.44±0.21$^{a}$ | 1.95±0.35$^{a}$ | 1.97±0.29$^{a}$ | 2.78±0.30$^{a}$ | 2.92±0.33$^{a}$ | 11.06±1.49$^{a}$ |
| Neem oil (0.3%) | 1.57±0.22$^{a}$ | 1.84±0.29$^{ab}$ | 1.99±0.34$^{a}$ | 2.83±0.28$^{a}$ | 2.89±0.33$^{a}$ | 11.13±1.46$^{a}$ |
| Spinosad (0.75g a.i./ha) | 1.16±0.07$^{b}$ | 1.56±0.15$^{bc}$ | 1.93±0.15$^{a}$ | 2.58±0.10$^{a}$ | 2.61±0.15$^{b}$ | 9.84±0.61$^{b}$ |
| **Control (Water)** | 1.12±0.06$^{b}$ | 1.50±0.12$^{c}$ | 1.88±0.15$^{a}$ | 2.52±0.13$^{a}$ | 2.69±0.14$^{b}$ | 9.69±0.59$^{b}$ |
| **CD(0.05)** | 0.136 | 0.321 | NS | NS | 0.169 | 0.719 |

In vertical columns, figures followed by the same alphabets did not differ significantly by DMRT p =0.05.

**Table 3.** Effect of insecticides on adult biology of *Cheilomenes sexmaculata*

| Treatment | Pre-oviposition period (Days) | Oviposition period (Days) | Post-oviposition period (Days) | Adult longevity (Days) | |
|---|---|---|---|---|---|
| | | | | Male | Female |
| Flubendiamide (2.5 g a.i./ha) | 4.38±0.44 | 48.63±4.86$^{b}$ | 3.88±0.79 | 43.63±2.39$^{bc}$ | 56.38±4.69$^{b}$ |
| Thiamethoxam (2.5 g. ai./ha) | 4.87±0.35 | 47.88±3.45$^{b}$ | 4.25±0.53 | 41.50±1.95$^{c}$ | 56.50±3.53$^{b}$ |
| Neem oil (0.3%) | 5.38±0.47 | 50.00±2.29$^{b}$ | 3.87±0.62 | 43.25±2.12$^{bc}$ | 59.50±2.30$^{b}$ |
| Spinosad (0.75 g a.i./ha) | 4.25±0.58 | 53.13±1.33$^{ab}$ | 3.75±0.53 | 45.75±1.59$^{ab}$ | 61.00±1.24$^{ab}$ |
| Control (Water) | 4.13±0.44 | 55.88±1.15$^{a}$ | 4.38±0.44 | 47.25±1.41$^{a}$ | 64.38±1.15$^{a}$ |
| CD(0.05) | NS | 5.53 | NS | 3.52 | 5.29 |

Values are mean ±SE. Figures in vertical columns with the same alphabets did not differ significantly by DMRT p =0.05.

**Table 4.** Effect of insecticides on cumulative mortality (%) and reproductive parameters of *Cheilomenes sexmaculata*

| Treatment | Cumulative Mortality (%) | Fecundity (No. of eggs/ female) | Fertility (No. ofoff-spring/female) | Hatchability eggs hatched (%) | Total effect, E (%) (IOBC category)* |
|---|---|---|---|---|---|
| Flubendiamide (2.5 g a.i./ha) | 20.00±3.04 | 1581.63±80.13$^{c}$ | 1092.25±67.02$^{c}$ | 69.15±3.17$^{b}$ | 37.00 (2) |
| Thiamethoxam (2.5 g a.i./ha) | 82.50±3.15 | 1637.13±92.10$^{c}$ | 1162.38±58.92$^{c}$ | 71.10±1.02$^{b}$ | 85.74 (3) |
| Neem oil (0.3%) | 75.00±0.96 | 1727.13±51.75$^{bc}$ | 1242.62±46.79$^{c}$ | 71.97±1.89$^{b}$ | 84.95 (3) |
| Spinosad (0.75 g a.i./ha) | 17.49±2.50 | 1917.00±89.43$^{b}$ | 1604.00±72.89$^{b}$ | 83.67±0.31$^{a}$ | 21.26 (1) |
| Control (Water) | 6.67±3.60 | 2151.88±27.31$^{a}$ | 1868±26.50$^{a}$ | 86.80±0.29$^{a}$ | |
| CD (0.05) | 8.45 | 218.68 | 172.22 | 5.2 | |

Values are mean ±SE. Figures in vertical columns with the same alphabets did not differ significantly by DMRT p =0.05. *IOBC category based on the total effect (E%) caused by each insecticide: (1) harmless (<30%); (2) slightly harmful (30–79%); (3) moderately harmful (80–99%); and (4) harmful (>99%).

were on par with each other. In spinosad, 83.67±0.31 per cent of eggs hatched, which was significantly higher than all other insecticide treatments (Table 4) but was on par with 86.80±0.29 per cent recorded in control.

With respect to IOBC toxicity rating, spinosad was the only harmless (IOBC Category 1) insecticide among the tested insecticides. Flubendiamide was slightly harmful

(IOBC Category 2) while thiamethoxam and the botanical, neem oil was moderately harmful (IOBC Category 3) to *C. sexmaculata.*

## DISCUSSION

For efficient integration of insecticides in the IPM programme in a crop system having natural enemy biota as natural regulators, it is necessary to know the information on lethal and sub-lethal effects of insecticides on natural enemies. In the present study, the sub-lethal effects of dimethoate, thiamethoxam, neem oil, flubendiamide and spinosad on *C. sexmaculata*, an important aphidophagous predator were documented. Developmental time of *C. sexmaculata* varied to a certain extent after exposure of first instar grubs to insecticides at sublethal doses.

The studies on the evaluation of toxicity of neem oil at sub-lethal doses on *C. sexmaculata* are lacking for discussion. However, the developmental period of the spotless ladybird beetle, *Cycloneda sanguinea* Linnaeus was reported to be unaffected after exposure of second-instar grubs to neem oil even at 0.5 per cent concentration. This variation might be attributed to the stage, second-instar being less vulnerable compared to first instar grubs (da Silva & Martinez, 2004). The effect of thiamethoxam at $LC_{10}$ on coccinellid predators was documented earlier with prolongation in duration of fourth-instar grub and reduction in the pupal period of aphidophagous coccinellid, *Hippodamia variegata* (Rahmani & Bandani, 2013).

Though literature pertaining to the sub-lethal effect of flubendiamide was not available, a previous record by Nawaz *et al.* (2017) with chlorantraniliprole, an anthranilic diamide on significant prolongation of larval and pupal duration of *Harmonia axyridis* after exposure of second-instar grubs to the insecticide at $LC_{30}$ was not in line with the present finding. The variation could have been due to the different chemical and test species. The present findings with spinosad confirm with the observation of Galvan *et al.* (2005) that spinosad at 10 per cent of the field rate did not influence the developmental period of *H. axyridis*. However, according to them, a significant extension of the development period was observed after exposure to 25 and 50 per cent of the field rates. The prolongation of the developmental period upon insecticide exposure occurs as the immature stages spend more energy to detoxify the chemicals rather than diverting on growth and development (Hannig *et al.*, 2009).

Exposure of 0-24h old first instar grubs to insecticides at $^{1}/_{10}{}^{th}$ of the field recommended dose did not influence pre-oviposition and post-oviposition periods. However, flubendiamide, thiamethoxam and neem oil significantly reduced the oviposition period and adult longevity of *C. sexmaculata,* while spinosad had no significant effect. In contrast, a significant increase in the pre-oviposition period was reported by Rahmani and Bandani (2013) with thiamethoxam after exposing third-instar grubs of *H. variegata* and by Nawaz *et al.* (2017) with chlorantraniliprole, a diamide after exposing second-instar grubs of *H. axyridis* at $LC_{30}$. However, the findings of Nawaz *et al*. (2017) on the reduction in adult longevity at $LC_{30}$ confirm the present results.

The present finding on the pre-oviposition period after exposure to neem oil is in line with observations of da Silva and Martinez (2004) where neem oil even at a 5 per cent concentration had no significant influence on the pre-oviposition period of the predator, *C. sanguinea* when the second-instar grubs were exposed. However, their observation that neem oil did not influence adult longevity is a contradiction to the present finding, where the adult longevity was significantly less compared to control.

All the insecticides significantly reduced the fecundity as well as the fertility of *C. sexmaculata*. The hatchability of second-generation eggs was also significant except for spinosad. Flubendiamide caused a higher reduction in fecundity and fertility resulting in only 69.15 per cent hatching compared to control. In contrast to present findings with flubendiamide, there was a report that no reduction in fecundity and fertility was documented with coccinellid predator, *Athalia bipunctata* after exposure of fourth-instar grubs to flubendiamide even at field recommended rate (Garzon *et al.*, 2015). The earlier records on decrease or complete reduction in fecundity and fertility of *Coccinella septempunctata* with commercial neem formulations, after exposure of larvae to neemix (Banken & Stark, 1998) and after exposure of adults to Neemraj super (Regmi *et al.*, 2019) are broadly agreeable to the present findings with neem oil, where fecundity and fertility were significantly reduced.

Reduction in fecundity and fertility was observed after exposure to thiamethoxam. The observations of Rahmani and Bandani (2013) on the reduction in fecundity and fertility of *H. variegata* after exposure of later-instar grubs to sub-lethal doses of thiamethoxam agree with the present findings. On the contrary, an increase in fecundity with a reduction in the number of offspring/female was documented by Fernandez *et al.* (2016) where they exposed the last instar grubs of *C. sanguinea* to thiamethoxam at $LC_{20}$. Exposure to spinosad had no significant influence on reproductive parameters except for fecundity and offspring/female. Cole *et al.* (2010) also recorded similar findings with spinosad after exposure to first-instar larvae of *Coccinella transversalis.* While Galvan *et al.* (2005) observed a reduction in fertility with no effect on fecundity, after exposure of adults of *H. axyridis* to both

recommended and half of the recommended field rate of spinosad. Similarly, Depalo *et al.* (2017) also observed a significant reduction in fertility with no difference in fecundity of *A. bipunctata* after exposure of adults to spinosad at field recommended rate.

With the exception of spinosad, all the evaluated insecticides at sublethal concentration adversely affected the developmental and reproductive biology of *C. sexmaculata*. According to Casida and Durkin (2013), neonicotinoids exhibited a variety of lethal and sublethal effects on behaviour such as feeding, oviposition and fecundity in arthropods. Sub-lethal effects of organophosphate insecticide residues resulted in an immediate reduction in the efficiency of coccinellids in locating their prey (Singh *et al.*, 2004). The study calls for caution to be taken while including organophosphates and neonicotinoids in integrated pest management programmes involving a cropping system with *C. sexmaculata*.

## CONCLUSION

The study provides information on a measure of caution to be taken in an IPM programme where, *C. sexmaculata* is utilized as a promising biocontrol agent against the key pest, *A. craccivora* in cowpea. The results of laboratory bioassay pointed out the deleterious effect of dimethoate, thiamethoxam and flubendiamide at sublethal concentrations to *C. sexmaculata* by altering the growth and development of exposed first instar grubs and reproductive parameters of the adults. The effect was persistent to the next generation with a reduction in the hatchability of eggs. The botanical component, neem oil, which we generally recommend with the assumption of being harmless to natural enemies proved harmful to the predator. The insecticide, spinosad, with only minimal reduction in fecundity, was documented as relatively safer to *C. sexmaculata*.

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