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Research Article

Preliminary studies on the activity of two photosensitizer compounds for sustainable control of aphid, *Rhopalosiphum padi* (Hemiptera: Aphididae) and its associated predator, *Chrysoperla carnea* (Neuroptera: Chrysopidae)

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ABSTRACT: The aphid, *Rhopalosiphum padi* (L.), is considered a major pest of many crops and causes huge losses in crop production by feeding or transmitting viral diseases. Insecticides were used to control this pest, but the repeated use of pesticides has led to the emergence of resistance among insects, in addition to toxic effects on animals and humans and environmental pollution. Therefore, it was necessary to develop and introduce new and environmentally safe technology for controlling insect pest populations. The photosensitizers are promising new technologies in pest control; so, the study aimed at assessing the effectiveness of two photosensitizers, Magnesium Chlorophylline (Mg-Chl) and Copper Chlorophylline (Cu-Chl), against the adult aphid, *R. padi* and its biological control agent, *Chrysoperla carnea*. Four concentrations (10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) of each compound under different exposure periods to light (0, 1, and 2 h) were evaluated on *R. padi* adults, and studied the effect of these compounds on its biological control agent, *C. carnea.* The findings showed that the mortality percentage reached 96.67% at the highest concentration of Mg-Chl (10^{-2}), while it reached 86.67% at Cu-Chl (10^{-2}) after ten days of treatment with 2 h of exposure to sunlight, but the compounds did not affect the natural enemy, *C. carnea.* Therefore, magnesium chlorophylline and copper chlorophylline could be included as part of an Integrated Pest Management (IPM) for aphid control.

KEYWORDS: *Chrysoperla carnea*, magnesium chlorophylline and copper chlorophylline, photosensitizers, *Rhopalosiphum padi*

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INTRODUCTION

The Oat aphid, *R. padi* (L.) (Hemiptera: Aphididae), is one of the most important insect pests of cereal crops worldwide. *R. padi* is also a major pest of wheat and causes both direct and indirect damage. The direct damage caused by *R. padi* is feeding on the plant bark, injecting toxins, and secreting honeydew that causes mold and thus impairs photosynthesis, leading to yield loss. Moreover, indirect damage is the transmission of several viral diseases (Parizoto *et al.,* 2013; Schliephake *et al.,* 2013). This species of aphid is responsible for significant economic losses, as yield losses due to infestation ranged from 7.5% to 18.7% (Tantawi, 1985) and reached 23% (Abd El Badeea & Alaa, 2019). Predatory insects are widespread and an important component of biological control and integrated pest management programs. One of the most important predators of aphids is *C. carnea* (El-Heneidy & Rizk, 2004). The larvae of *C. carnea* are an important predator of a variety of soft-bodied arthropods, such as aphids, whiteflies, thrips, scale insects, mites, and leafhoppers (Rugno *et al.*, 2019; Saljoqi *et al.*, 2022; Ismail *et al.*, 2023). For a variety of reasons, *C. carnea* has become more common in IPM programs in recent years. The potential advantage this important pest may have over other native or introduced foes is one of the main causes of this trend. In particular, it has a somewhat wide tolerance to a variety of pesticides, particularly when it is in the larval and cocoon stages. (Medina *et al.*, 2001; Saljoqi *et al.*, 2022; El-Dessouki & Korish, 2023).

On the other hand, the repeated use of insecticides and excessive doses have resulted in insect resistance to insecticides, harmful effects on predators, and raising serious concerns about human health and the environment (Chang *et al.*, 2010). Recently, the increasing resistance of insects to insecticides worldwide has led to the need to search for alternative methods for the control of insect pests, which are effective and safe, with the advantage of respecting the environment and natural enemies.

Photosensitizers activated by sunlight are a tool for controlling many insect species and are one of the most promising non-traditional methods to reduce the harmful effects of synthetic insecticides used to control insect pests

(Lukšiene *et al.*, 2007; Filiberti *et al.*, 2009; Ben Amor & Jori, 2000). Photosensitizers are converted into toxic photoproducts (photoactive compound) after exposure to visible light that accumulate widely in the insect body cell and lead to lethal photochemical responses and death (Lukšiene *et al.*, 2007). Photosensitizers have the ability to absorb sunlight and transfer energy to oxygen molecules. Then, they form singlet oxygen atoms and superoxide anions that damage the insect cuticle and midgut wall, causing feeding inhibition and eventual death (El-Ghobary *et al.*, 2018). Photosensitizers have a high toxicity to pests and are, therefore, an ideal tool for pest control, such as phloxine B or eosin yellow; however, Photosensitizers are non-toxic to humans (Attia, 2016). Furthermore, the photosensitizers magnesium chlorophyllin and copper chlorophyllin were more effective on *Bactrocera zonata* when exposed to sunlight for 2 h (Abd El-Naby, 2019). Copper and magnesium chlorophyllin induced midgut tissue damage and significant changes in phenoloxidase activity as a function of photosensitizing agent concentrations and caused death of *Culex pipiens* larvae via the physical damage of larval tissues and enzymes (Afify *et al.*, 2019). Also, photosensitizers, copper and magnesium chlorophyllin reduced *Bemisia tabaci* counts in tomatoes (47.1–73.3%) after 24 h of spraying (Hashem *et al.,* 2022). In addition, the Rose Bengal and Rhodamine B photosensitizers have been shown to be effective photo-insecticides against aphids, *Aphis gossypii* (EL-Shourbagy *et al.*, 2023). Photosensitizers of sodium magnesium chlorophyllin formulation, SUND-06 PS, were effective in controlling both larval and adult stages of *Franklinella occidentalis* and showing a reduction of larvae by 85% and adults by 76% (Pieterse *et al.*, 2023). *Tuta absoluta* tunnels in tomato fields were likewise decreased by the photosensitizers, magnesium, and copper chlorophyllin to 72.79% in Mg-Chl and 70.52% in Cu-Chl (Ahmed *et al.,* 2024).

Thus, the purpose of this research was to evaluate the efficacy of two photosensitizers, Copper Chlorophylline (Cu-Chl) and Magnesium Chlorophylline (Mg-Chl) against *R. padi* adults and its predator, *C. carnea* (the 2nd larval instar) under laboratory conditions.

MATERIALS AND METHODS

Insect culture

Rhopalosiphum padi

The *Rhopalosiphum padi* population was reared on wheat plants for three generations. Wheat seeds were placed in plastic pots (10 cm in diameter and 12 cm in height) between two layers of peatmoss after being soaked in water for 4 h to facilitate germination. Three days after planting, the wheat seedlings reached 2 cm, then they were artificially infested with aphids by cutting off older wheat plants, which hoisted the insect. The pots were kept at 25±2°C and 65±5% R.H., and 14L:10D. Germination took place around 3 days after planting.

Chrysoperla carnea

Chrysoperla carnea is raised using the techniques outlined by El-Arnaouty *et al.* (2001).

Photosensitizers

The photosensitizers Copper Chlorophylline (Cu-Chl) was prepared from *Stevia rebaudiana* leaf extract and Magnesium Chlorophylline (Mg-Chl) from fresh spinach leaf extract using acetone solvent based on methodologies outlined by Abbas *et al.* (2022).

Bioassay

Two photosensitizers (Mg-chl and Cu-chl) with four concentrations (10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) were evaluated to control the aphid, *R. padi.*

Evaluation of the two photosensitizers on the aphid, R. padi

Bioassays were performed on adults of *R. padi* using the leaf dip technique. The aforementioned four concentrations of the two photosensitizers (Mg-chl and Cu-chl) and water (as control) were used, either concentration or control contained 15 replicates (10 aphids/replicate). The wheat plants were dipped for 30 seconds in each concentration or water, then left to dry at room temperature before being presented to the aphid, *R. padi*. After drying, each concentration and control was divided into three groups, The first group was not exposed to sunlight (darkness), whereas the second and the third ones were exposed to sunlight outdoors for 1, and 2 h, respectively. Aphids were fed for 24 h on treated plants. These plants with aphids were exposed to sunlight outdoors and then transferred to new, fresh untreated seedlings, which were offered for aphid feeding and maintenance using Punctured Eppendorf Technique (PETT) (El-Fatih, 2014) (Figure 1), Each Eppendorf was put in plastic box, as this method allows for easy observation and counting of aphids in different treatments. All experiments were maintained at 25 ± 2°C and 65 ± 5% R.H. The number of dead aphids was noted on days 1, 3, 5, 7, and 10 after treatment to calculate mortality percentages and determine LC_{50} and LC_{90} values.

Impact of the photosensitizer on C. carnea (direct treatment)

After calculating the LC_{50} value, the 2nd larval instar of the predator was sprayed directly with the LC_{50} concentration of the tested compounds, while the control was directly sprayed with water. The control or treated larvae were divided into three groups as we mentioned previously: the first group was not exposed to light, the second one was exposed for one hour, and the last group was exposed for 2 h. Each compound and



Figure 1. Eppendorf technique (PETT).

control consisted of fifteen replicates (the replicate contained 10 larvae). After conducting treatments and exposure to light, all replicates of the experiment, whether treatment or control, were kept under the conditions mentioned previously. Daily, a sufficient quantity of *Sitotroga cerealell* eggs were provided to the larvae as food. Alive and dead larvae of *C. carnea* were recorded until pupation; also, the duration of the second and third instars was recorded. After emerged adults, ten couples (males and females) from each treatment or control were kept in a small box (7 cm in diameter and 6 cm in height) covered with black muslin to act as a location to lay eggs. The adults of *C. carnea* were fed a semi-artificial diet. Additionally, moist cotton pieces were placed on the bottom of each box to provide them with water. When the first eggs were deposited, the adult couples were transferred to new containers three times per week, and the number of eggs was counted. During the adult stage of *C. carnea*, numerous parameters were noted, including male and female longevity, pre-oviposition, oviposition, and post-oviposition periods.

Impact of the photosensitizer on C. carnea (Indirect treatment)

Wheat plants were treated with the half lethal concentration for each compound (control plants treated with water) using the immersion method and left to dry at room temperature. Fifteen replicates were made for each compound and control (each replicate contained 300 aphids). The replicates of each compound and control were divided into three groups. Aphids were placed on the treated plants and control to feed on them for 24 h. The first group was exposed to sunlight for one hour, the second for 2 h, and the third group was left in the dark. Three other groups were made as controls. After the end of the exposure period, the 2nd larval instar of *C. carnea* (ten larvae/replicate) was released to feed on the aphids that were present on the treatment or control plants. Starting from the second day, a specific suitable quantity of aphids *R. padi* was provided to larvae daily until the pupal stage. All experiments were kept under the same conditions mentioned previously. Treatment and control larvae were observed at 1, 3, 5, and 7 days after treatment to determine the percentage of mortality and recorded to calculate the duration of the 2nd and 3rd larval instars. About ten couples from treatment or control were reared individually in the same box as previously mentioned and using the same technique. During the adult stage of *C. carnea*, the laid eggs were collected daily, and fecundity was estimated; also, the longevity for male and female was recorded.

Semi-field experiment to evaluate the efficiency of the photosensitizer on C. carnea

Wheat seeds were planted in pots. The germination seedlings were infested with aphids, and five days later, the 2nd larval instar of *C. carnea* was released. The study included forty-five pots; fifteen replicates were used for each photosensitizer compound, and the rest were considered as control. In each treatment, the plants were sprayed with photosensitizer compound LC_{50}, while the control plants were sprayed with water. The control or treated pots were divided into three groups, as mentioned in previous experiments. The first group was exposed to direct sunlight for one hour, the second group for 2 h and the third group was left in the dark. Five replicates were used for each treatment or control group. Each replicate included 300 aphids and 10 larvae (the 2nd larval instar) of *C. carnea*. A glass cage was used to cover wheat seedlings to keep away any insects and prevent the predators from escaping. The top of the cages is covered with white cloth for ventilation (Figure 2). Starting from the second day after treatment, the pots are provided daily with aphid, *R. padi,* to feed the predator to the pupal stage. The number of dead larvae was recorded every alternate day starting from the first to the seventh days to calculate the mortality percentages of *C. carnea* larvae**.** This experiment was kept under the room conditions.

Statistical analysis

The data were subjected to Analysis of Variance



Figure 2. Pots planted wheat seedlings infected with aphids and sprayed with two photosensitizers compounds.

(ANOVA) using statistical software SAS statistical program software (SAS,1999) and Duncan's multiple range test (Duncan, 1955) at 5% probability level to compare the differences among treatment means. LC_{50}, LC_{90} and confidence limit values were calculated using the Ldp line computer program according to Noack & Reichmuth (1978).

RESULTS

Evaluation of the two photosensitizers on the aphids *R. padi*

The efficiency of the two photosensitizers was evaluated on the aphids, *R. padi*. The tested concentrations of magnesium chlorophyllin and copper chlorophyllin showed a significant increase in the mortality percentage of *R. padi* after treatment. Data in Figures (3-5) showed that the percentage mortality of Aphids increased significantly with increasing concentration. Treatments exposed to sunlight also had a higher effect than treatments in the dark. In the dark treatment, the mortality percentage reached 76.67% at the highest concentration of Cu-Chl (10^{-2}), while it reached 80% at the same concentration of Mg-Chl after ten days of treatment (Figure 3). For the treatments of one hour light exposure to light, the mortality percentage was 83.33% at Cu-Chl (10^{-2}) and 86.67% at Mg-Chl (10^{-2}) (Figure 4). In the 2 h exposure treatment, concentration 10^{-2} of magnesium chlorophylline recorded the highest mortality percentage (96.67%) after ten days of treatment. There was a significant interaction between treatment and 2 h light exposure on day 7 in all treatments assessed (F = 22.87, p = 0.000) (Figure 5). Furthermore, all treatments resulted in a significant increase in mortality compared with the untreated control group on all days (Figures 3-5). Generally, the magnesium chlorophylline was more effective than the copper in all concentrations after ten days from treatment.

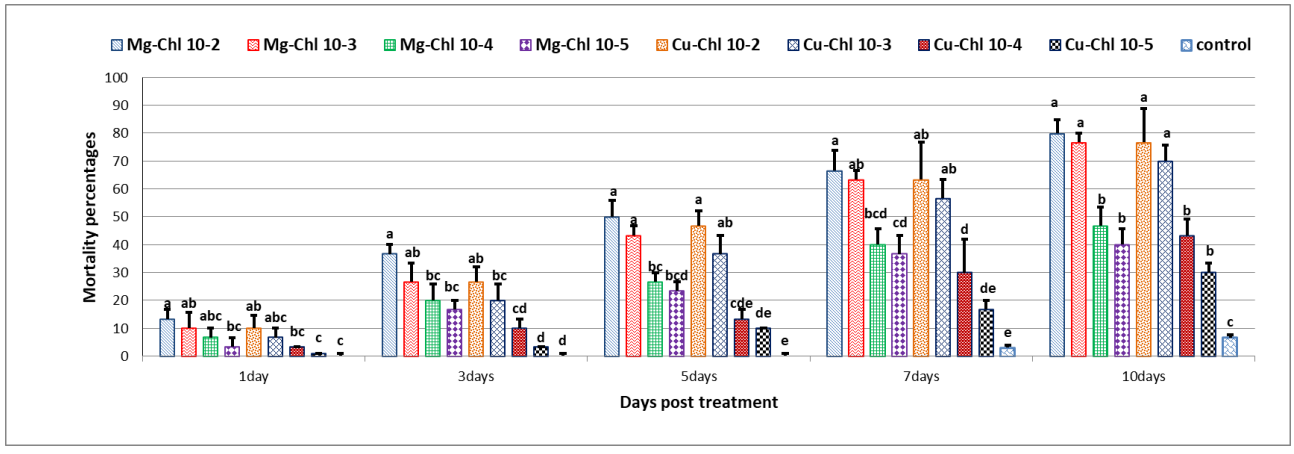


Figure 3. Mortality percentages of *R. padi* treated with magnesium and copper chlorophylline at dark.
Means followed by different letters are significantly different from each other (p<0.05).

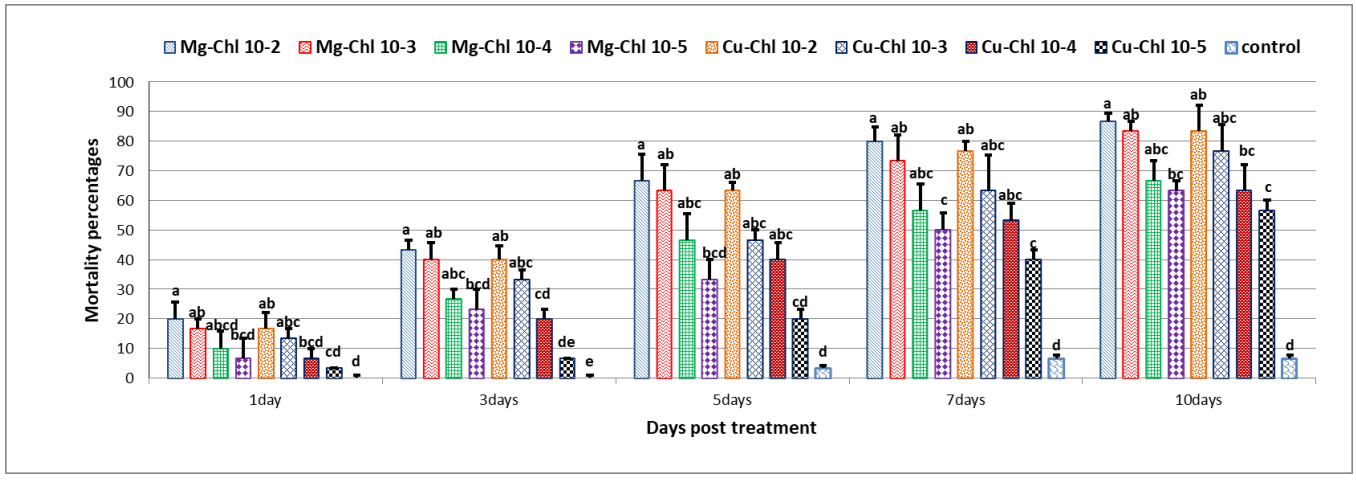


Figure 4. Mortality percentages of *R. padi* treated with magnesium and copper chlorophylline at 1 h exposure period to sunlight.
Means followed by different letters are significantly different from each other (p<0.05).

Effect of sub-lethal concentrations of the photosensitizers against the aphid, *R. padi*

Table 1 shows the LC_{50} and LC_{90} values after 3 days for aphids treated with Mg-Chl and Cu-Chl photosensitizers. Treatments in the light group had significant effects compared to those in the dark group. According to LC_{50} values, Mg-Chl at a 2 h exposure was the most effective on the aphid *R. padi,* followed by Cu-Chl at the same exposure period. The LC_{50} values of Mg-Chl were 0.15 mg/L and LC_{90} was 180.35 mg/L at the same light exposure period. The LC_{50} value of Cu-Chl was 0.32 mg/L and LC_{90} was 729.11 mg/L in the 2 h light exposure treatment.

Impact of the photosensitizer on *C. carnea* (Direct and indirect treatment)

The photosensitizers' effect of sub-lethal concentrations of the photosensitizers directly and indirectly on the mortality of *C. carnea* larvae after 0.0, 1, and 2 h of exposure to sunlight is summarized in Tables 2 and 3. Results indicated that there was a positive correlation between mortality percentages and exposure period to sunlight in all treatments. After seven days, in a two-hour exposure treatment, the high mortality percentages were recorded: 20% at magnesium chlorophylline and 14% at copper chlorophylline, while it was recorded as 4% in the control in indirect treatment. However, in direct treatment, the mortality percentages were recorded as 10, 12, and 2% for copper chlorophylline, magnesium chlorophylline, and control, respectively. Additionally, there are no significant differences between all treatments and the control. Results clearly show that the copper was generally less effective than magnesium chlorophylline on a second larval instar of *C. carnea*. The present data confirmed that the direct effect of photosensitizers on the second larval instar of *C. carnea* was less than the indirect effect.

Impact of the photosensitizer on some biological aspects of *C. carnea* (Direct and indirect treatment)

The present data in Tables 4 and 5 confirmed that there are no significant differences in the duration of the 2nd and 3rd larval instars when treating the second larval instar with copper and magnesium chlorophylline directly and indirectly at different exposure periods of light compared with the control**.** Additionally, no cumulative effects on the emerged adults from the directly treated larvae compared with the control. Data in Table 5 show an inverse proportional between the exposure period to sunlight and the average number of deposited eggs per female that emerged from the treated larvae. The emerged adults from the treated larvae

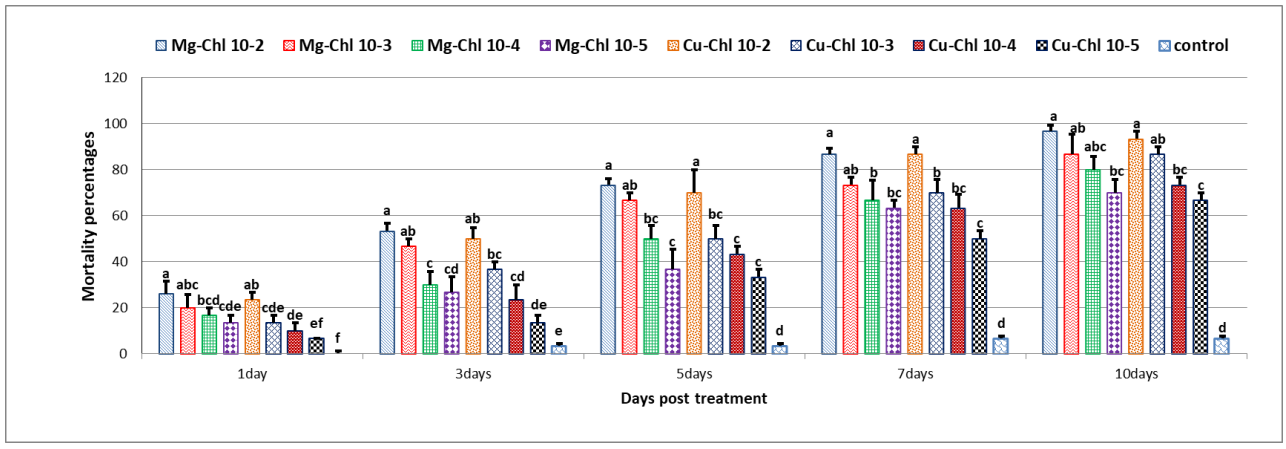


Figure 5. Mortality percentages of *R. padi* treated with magnesium and copper chlorophylline at 2 h exposure period to sunlight.
Means followed by different letters are significantly different from each other ($p<0.05$).

Table 1. Toxicity of magnesium and copper chlorophylline against the *R. padi*

Treatments	Exposure period (h)	LC_{50} (mg/L)	Confidence limits (mg/L)		LC_{90} (mg/L)	Confidence limits (mg/L)		Slope
			Lower	Upper		Lower	Upper	
Mg-Chl	0h	6.15	2.26	29.21	398405.37	17567.29	74578398.38	0.26±0.03
	1h	0.25	0.14	0.46	2686.85	433.13	42018.53	0.31±0.03
	2h	0.15	0.07	0.30	180.35	90.17	360.72	0.41±0.03
Cu-Chl	0h	8.52	5.35	16.12	337.07	124.93	1413.18	0.80±0.07
	1h	1.11	0.55	2.23	2528.05	1264.02	5056.11	0.38±0.03
	2h	0.32	0.16	0.65	729.11	364.55	1458.21	0.38±0.03

Table 2. Effect of magnesium and copper chlorophylline on larvae of *C. carnea* at different exposure periods to light (Direct treatment)

Exposure period (h)	Treatment	% Mortality of *C. carnea*			
		Days post treatment			
		1	3	5	7
0h	Cu-Chl	0	0	2	6
	Mg- Chl	0	0	4	8
	Control	0	0	0	0
	F-value	-	-	1.200	2.600
	p-value	-	-	0.335	0.115
1h	Cu-Chl	0	0	4	8
	Mg- Chl	0	0	6	10
	Control	0	0	0	0
	F-value	-	-	2.33	3.500
	p-value	-	-	0.139	0.063
2h	Cu-Chl	-	-	0.139	0.063
	Mg- Chl	0	0	6	10
	Control	0	0	8	12
	F-value	0	0	0	2
	p-value	-	-	1.733	1.235

Table 3. Effect of copper and magnesium chlorophylline on larvae of *C. carnea* after feeding treated *R. padi* at different exposure periods to light (indirect treatment)

Exposure period (h)	Treatment	% Mortality of *C. carnea*			
		Days post treatment			
		1	3	5	7
0h	Cu-Chl	0	4	8	10
	Mg- Chl	2	6	10	12
	Control	0	0	0	2
	F-value	1.000	2.333	1.909	1.759
	p-value	0.397	0.139	0.191	0.215
1h	Cu-Chl	0	6	10	12
	Mg- Chl	4	8	10	14
	Control	0	2	2	4
	F-value	2.667	0.824	1.000	0.875
	p-value	0.110	0.462	0.397	0.442
2h	Cu-Chl	2	8	12	14
	Mg- Chl	6	10	14	20
	Control	0	0	2	4
	F-value	1.400	1.556	1.938	2.130
	p-value	0.284	0.251	0.187	0.162

with copper chlorophylline at 0.0, 1 h, and 2 h of exposure to sunlight had an average of 331.6, 318.8, and 311.2 eggs/female, respectively. The emerged adults from the treated larvae with magnesium chlorophylline at the same time of exposure to sunlight had an average of 314.2, 312.2, and 306.2 eggs/female, respectively. Furthermore, there was no significant difference between the pre-oviposition and oviposition periods, longevity of male and female but there was a significant difference between the post-oviposition phase and fecundity.

A semi-field experiment to evaluate the efficiency of the photosensitizer on *Chrysoperla carnea* at pots

Evaluation of the effect of LC_{50} of magnesium and copper chlorophylline on *C. carnae* at semi-field set out in Table 6. The results indicated that the copper chlorophylline

Table 4. Effect of magnesium and copper chlorophylline on some biological aspects of *C. carnea* at different exposure periods to light (Direct treatment)

Exposure period (h)	Treatment	Duration (Days)							Fecundity
		Larval instars		Pre-Ovi-position	Oviposi-tion	Post-ovi-position	Longevity		
		2nd	3rd				Female	Male	
0h	Cu-Chl	3.48	4.36	3.0	28.0	2.2	33.2	28.8	354.6
	Mg- Chl	3.44	4.32	2.8	27.6	2.4	32.8	29	355.2
	Control	3.42	4.31	2.8	28.4	2.0	33.2	30.6	362.2
	F-value	0.077	0.085	0.500	0.063	0.150	0.016	0.531	0.152
	p-value	0.782	0.770	0.619	0.939	0.862	0.984	0.601	0.860
1h	Cu-Chl	3.44	4.32	3.2	28.0	2.2	33.4	29.2	346.8
	Mg- Chl	3.36	4.24	3.0	28.4	2.2	33.6	29.2	350.6
	Control	3.34	4.22	2.8	28.4	2.0	33.2	30.6	362.2
	F-value	0.322	0.384	1.500	0.027	0.105	0.019	0.278	0.427
	p-value	0.572	0.538	0.262	0.974	0.901	0.981	0.762	0.662
2h	Cu-Chl	3.40	4.28	3.0	27.8	2.8	33.0	29.2	347.2
	Mg- Chl	3.32	4.22	3.0	28.0	2.2	33.0	29.0	348.8
	Control	3.23	4.21	2.8	28.4	2.0	33.2	30.6	362.2
	F-value	0.335	0.424	0.286	0.047	0.765	0.005	0.213	0.374
	p-value	0.565	0.517	0.756	0.954	0.487	0.995	0.811	0.696

Table 5. Effect of copper and magnesium chlorophylline on some biological aspects of *C. carnea* at different exposure periods to light (Indirect treatments)

Exposure period (h)	Treatment	Duration (Days)							Fecundity
		Larval instars		Pre-Ovi-position	Oviposi-tion	Post-Ovi-position	Longevity		
		2nd	3rd				Female	Male	
0h	Cu-Chl	4.60	5.64	4.6	23.2ab	4.8ab	32.6	27.6	331.6b
	Mg- Chl	4.40	5.60	4.8	21.0b	5.4a	31.2	26.8	314.2c
	Control	4.41	5.55	4.2	24.8a	3.8b	32.8	29.2	352.6a
	F-value	1.500	0.082	0.824	3.050	2.649	0.444	0.627	13.740
	p-value	0.227	0.776	0.462	0.085	0.111	0.652	0.551	0.001
	LSD	---	---	---	3.366	1.530	---	---	17.208
1h	Cu-Chl	4.36	5.36	4.6	22.6	4.6ab	31.8	27.2	318.8ab
	Mg- Chl	4.44	5.44	4.6	22.2	5.2a	31.8	26.2	312.2b
	Control	4.38	5.41	4.2	24.8	3.8b	32.8	29.2	331.6a
	F-value	0.322	0.322	0.211	2.00	2.800	0.237	0.728	3.412
	p-value	0.573	0.573	0.813	0.178	0.100	0.793	0.503	0.067
	LSD	---	---	---	---	1.125	---	---	16.454
2h	Cu-Chl	4.40	5.32	4.2	22.0	4.4ab	30.6	27.8	311.2ab
	Mg- Chl	4.36	5.40	4.8	21.6	5.0a	31.6	25.2	306.2b
	Control	4.33	5.40	4.20	24.8	3.8b	32.8	29.2	331.6a
	F-value	0.082	0.336	1.13	2.34	4.35	1.15	1.26	3.98
	p-value	0.776	0.565	0.36	0.14	0.04	0.35	0.32	0.05
	LSD	-	-	-	-	1.04	-	-	20.05

was less effective than the magnesium on the second larval instar of *C. carnea* in all treatments. After 2 h of exposure to sunlight, the mortality percentages of the 2nd larval instar were 6.67 and 10 for copper and magnesium chlorophylline, respectively. However, after 1 h of exposure to sunlight, the mortality percentages were 3.33 and 6.67 for copper and magnesium, respectively. No mortality was recorded in the untreated groups. The mortality rates recorded were 3.33

Table 6. Mortality percentage of *C. carnea* after treatment with magnesium and copper

Exposure period (h)	Treatment	% Mortality of *C. carnea*			
		Days post treatment			
		1	3	5	7
0h	Cu-Chl	0	0	0	3.33
	Mg- Chl	0	0	3.33	3.33
	Control	0	0	0	0
	F-value	-	-	1.000	.600
	p-value	-	-	0.422	.579
1h	Cu-Chl	0	0	3.33	3.33
	Mg- Chl	0	0	3.33	6.67
	Control	0	0	0	0
	F-value	-	-	0.600	1.500
	p-value	-	-	0.579	0.296
2h	Cu-Chl	0	0	6.67	6.67
	Mg- Chl	0	0	10	10
	Control	0	0	0	0
	F-value	-	-	1.750	1.750
	p-value	-	-	0.252	0.252

in copper and magnesium chlorophylline at dark treatment. Moreover, there were no significant differences in the mortality percentages of the 2nd larval instar of *C. carnea* when treated with copper and magnesium chlorophylline and compared with the control under semi-field conditions. Overall, the mortality percentages of the 2nd larval instar of *C. carnea* decreased in all treatments in the semi-field experiment compared to the laboratory.

DISCUSSION

The efficacy of photodynamic sensitizers as insecticidal agents is contingent upon the presence of light and oxygen. Oxygen plays a crucial role in inducing damage across various biological systems through the generation of diverse Reactive Oxygen Species (ROS) (Paillous & Fery-Forgues, 1994). In this current study, two photosensitizers, copper chlorophyllin and magnesium chlorophyllin, were first evaluated as photoresistors to reduce the population of *R. padi* as well as the effect of these photosensitizers on its predator, *C. carnea.* The current results reveal that the mortality percentages were positively associated with an increase in the time of sun exposure and concentration of photosensitizer. The mortality rates of magnesium chlorophyllin were 80.00% in the dark and 96.67 % at 2 h of sun exposure after ten days. This result agreed with the findings of El-Shourbagy (2023), where the mortality rates increase proportionally with both the concentration of photosensitizing substances and the duration of exposure to sunlight. The mortality rate reached 96% in *Aphis gossypii* adults after 24 h of treatment when treated with Rhodamine B photosensitivity at the highest concentration and one hour of exposure to sunlight. Furthermore, El-Tayeb *et al.* (2011) found that the highest concentration (10^{-2} ml/L) of HpIX caused 83% and 96% mortality of the treated flesh fly, *Parasarcophaga argyrostoma,* after exposure to natural sunlight at irradiance of 236.5 and 1935 W/m^2, respectively. Naroz and Ibrahim (2016) found that Mg-chl (10^{-2}) and Cu-chl (10^{-2}) were more effective in reducing mealybug *Phenacoccus manihoti* populations by 83.8 and 81.0%, respectively. Our study results showed that magnesium chlorophyllin treatments were more effective than copper treatments at all concentrations. As reported in several previous studies, Abd El-Naby (2019) found that the photosensitizer magnesium chlorophyllin was more effective on *Bactrocera zonata* than copper chlorophyllin at the same concentration when exposed to sunlight for 2 h. Furthermore, Hashem *et al.* (2022) found that photosensitizers significantly reduced the whitefly, *Bemisia tabaci* population, and magnesium chlorophyllin was the best, recording a high reduction (95.16%) compared to the effectiveness of copper chlorophyllin. On the other hand, Pieterse *et al*. (2023) found that the photosensitizer formulation of sodium magnesium chlorophyllin, SUND-06 PS 0.22%, was effective in controlling both the larval and adult stages of *Franklinella occidentalis*, showing a reduction of larvae by 85% and adults by 76%. According to recent research studies, Ahmed *et al*. (2024) reported that the reduction in the number of *Tuta absoluta* tunnels in tomato fields after one day of spraying with Mg-Chl was higher than that of Cu-Chl, where the mortality rate was 72.79% and 70.52%, respectively.

In current study, the percentage mortality reached 96.67% at the highest concentration of Mg-Chl (10^{-2}), while

it was 86.67% at Cu-Chl (10^{-2}) after ten days of treatment with 2 h of exposure to sunlight. The results indicated that the mortality rate of aphids may be due to the used substance which may penetrate the surface of the plant and thus reach the aphid when sucking plant juices, or through contamination of the mouth parts during feeding. Therefore, it is recommended to use a high concentration (10^{-2} mg/L) of these photosensitizers in controlling *R. padi*. The median lethal concentrations (LC_{50}) revealed that the Mg-Chl at 2h exposure to light was more effective on the aphid, *R. padi,* followed by Cu-Chl at the same exposure. Previous studies have indicated that different insect species respond differently to photosensitizers. For example, El-Shourbagy *et al.* (2023) found that the toxicity of the photosensitivity Rhodamine B (after one hour of treatment) was higher than that of Rose Bengal on adult aphids, *A. gossypii,* according to the LC_{50} value, where its value was LC_{50} = 2.68 x 10^{-6} M. However, Abd El-Naby (2019) found that the *B. zonata* treated with magnesium chlorophyllin photosensitizer and exposed for 2 h of sunlight was more susceptible (LC_{50} = 0.00002 M) than those treated with copper chlorophyllin (LC_{50} = 0.008 M). Also, they indicated that, at dark treatment by magnesium chlorophyllin show (LC_{50} = 0.417 M) and copper chlorophyllin photosensitizer show (LC_{50} = 1908.947 M). Younis *et al*. (2021) also showed that the mortality rate reached 100% in the third larval instar of *Culex pipiens* mosquitoes after treatment with the Rose Bengal photosensitizer for 6 h; the LC_{50} of Rose Bengal was 4.9x10^{-6}.

The current results on the direct and indirect effect of sub-lethal concentrations of the magnesium and copper chlorophylline on the mortality 2nd larval instar of *C. carnea* after different exposures to sunlight indicated that the copper was less effective than magnesium chlorophylline. Similar findings were reported by Abd El-Naby (2019), Hashem *et al*. (2022) and Ahmed *et al*. (2024), who indicated that the photosensitizer magnesium chlorophyllin is better than copper chlorophyllin on *B. zonata*, *B. tabaci*, and *T. absoluta*. On the other hand, results indicated that the mortality percentage of the 2nd larval instar of *C. carnea* on the direct treatment was less than the indirect treatment, findings suggest the difference in mortality rate between direct and indirect treatment may be because in indirect treatment by spraying the aphid and presenting it to 2nd larval instar of *C. carnea*, the photosensitizer magnesium and copper chlorophylline that is used reaches the predator when feeding on this treated aphid. In pots, results show that the copper and magnesium chlorophylline had less of an effect on the 2nd larval instar of *C. carnea* than in the laboratory. This could be because the larva of *C. carnea* can hide in the natural environment, and the sprayed material does not reach it, and it can also eat a healthy aphid. Overall, the two photosensitizers, copper and magnesium chlorophylline, were more toxic against the aphid, *R. padi, than C. carnea*. There is no research available on the effect of photosensitizer on *C. carnea.* On the other hand, similar results on predators were noted by Eltaly *et al*. (2022), who examined how two phototoxins, phloxine B and eosin yellow lactone, affected mosquito larvae and their associated predators. They found that eosin was more effective than phloxine against *Anopheles pharoensis* and *C. pipiens*, while the Odonata predators were less affected by both photosensitizers.

CONCLUSION

This study is considered as the first to apply two novel photosensitizers to control the phytophagous pest, *R. padi* and its natural enemy, *C. carnea.* Our results showed that copper and magnesium chlorophyllin had a high lethal effect on aphids and were safer for the predator, *C. carnea*. Therefore, the present results suggest that copper and magnesium chlorophyllin could be included as part of Integrated Pest Management (IPM) for this insect as they are cheap, environmentally safe and without affecting the associated natural enemies. Further studies could be conducted to examine the effects of the photosensitizers, on aphids and their associated predators in the crop at field level.

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