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Sublethal impact of paraquat on the life span and parasitic behavior of *Diaeretiella rapae* M'Intosh

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This study focuses on assessing the impact of sublethal doses of paraquat on the survival, the emergence, the life span and the parasitic behavior of *Diaeretiella rapae* M'Intosh (Hymenoptera: Braconidae). The impact of sublethal doses was measured at room temperature using different densities of the cabbage aphid *Brevicoryne brassicae*. The results reveal that the field dose of paraquat caused 100% mortality in *D. rapae*. The percentage emergence of *D. rapae* decreased from 80.5% in the control group to 71.5% when treated with the lowest concentration of paraquat. Similarly, the life span of parasitoids that emerged from the mummy treated with paraquat also decreased significantly. Oviposition capability and ovipositor thrusting frequency of *D. rapae* also treated with sublethal dose paraquat decreased significantly along with a shortened patch residence time in the foraging area.

Keywords: Toxicity, functional response, herbicide, natural predator, biological control.

Introduction

Paraquat (the trade name for 1,1'-Dimethyl-4,4'-bipyridinium dichloride) was initially manufactured by Zeneca, (formerly Imperial Chemical Industries, ICI, United Kingdom) as a non-selective contact herbicide, defoliant, desiccant, and plant growth regulator used on more than 50 different crops. Paraquat is used in more than 100 countries.^[1] Despite its widespread use, paraquat is prohibited or restricted in many countries because of the negative effects it has on the environment and to human health. Acute and chronic paraquat poisoning, for instance, has a mortality rate as high as 80%–90%.^[2–4] Paraquat is also toxic to epithelial tissues such as skin, nails, cornea, liver, kidneys, and the lining of the gastrointestinal and respiratory tracts,^[5] and can be absorbed through skin exposure, inhalation, or ingestion.^[6]

Paraquat is also known to have a negative impact on ecological biodiversity,^[7,8] affecting the reproduction capability of birds and mammals causing teratogeny and in some cases, death. Studies have also shown that paraquat can poison beneficial soil-borne bacteria, contaminate groundwater causing toxic bioaccumulation in plants

and animals.^[9,10] By evaluating the side-effects of various insecticides, herbicides and fungicides on adults of the egg parasitoid *Telenomus remus* (Nixon), a recent study has shown that paraquat can be more harmful than glyphosate and imazethapyr.^[11]

In China, paraquat is widely applied to accelerate the defoliation and desiccation process of the stems and leaves of unwanted vegetation as well as for the clearing of wheat stubble for the cultivation of local crops such as maize, various legumes and cotton. Recently, China has seen an increased threat of paraquat on natural predators with the periodic overlapping of paraquat application and parasitoid. Several studies highlight the process of how paraquat application may impact on survival, emergence, oviposition and the foraging effect of parasitoids.^[12–17] Wang et al.^[18] have discussed the paraquat on the population dynamics of certain arthropod species. Further studies, however, are needed to further understand the sublethal effect of paraquat on *Diaeretiella rapae* M'Intosh (Hymenoptera: Braconidae) and the impact of paraquat on the parasitic activity of *D. rapae*.

Materials and methods

Insect breeding and herbicide

Insects were reared in a greenhouse at $25 \pm 3.5^\circ\text{C}$ under natural light after collection from Haobao Qing organic

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farm located in Kunming, Yunnan Province, China. Cabbage aphids, *Brevicoryne brassicae* (Linnaeus), were reared on kale (*Brassica oleracea acephala*) seedlings, and *D. rapae* were reared on the cabbage aphids. At the pupal stage, parasitized aphids were removed from the leaves and kept in petri dishes until the emergence of adults. *D. rapae* adults were stored in groups of five in glass tubes (5 × 1 cm) for 24 hours, while they were supplied with a diluted solution of honey water (80%).

The herbicide tested was Paraquat (Chemical pure, 200 mg/L aqua), manufactured by the Qingdao Hailir Pesticides and Chemicals Company in the northern coastal city of Qingdao, Shandong Province.

Toxicity of paraquat on *D. rapae*

The toxicity of paraquat on *D. rapae* was measured using the insecticide film method (IFM).^[19] Paraquat was prepared in gradients of 2 mg/L, 5 mg/L, 10 mg/L, 15 mg/L, 20 mg/L and 25 mg/L concentrations with distilled water.

The prepared gradients of paraquat solution were placed in glass test tubes (5 × 1 cm), with an amount of five drops per tube. The tubes were rolled horizontally, forming a film of the solution and allowed to dry naturally for two hours. Ten same-size adult parasitoids (five females and five males) were placed in the tubes and then covered with gauze. Fifteen replications of each treatment were prepared. All tubes were put in a cultivation box with ventilation fans at 26 ± 0.5°C, RH ranged between 60~70%, and the light: dark ratio was L14:D10. The *D. rapae* were fed with a diluted honey solution. The mortality of *D. rapae* was calculated according to Abbott's formula.^[20] The LC₅₀ and LC₂₀ values were calculated according to Finney's formula.^[21]

Impact of paraquat on *D. rapae* survival

Based on the results of the toxicity experiments (Table 1, Table 2), the 15 mg/L, 7.5 mg/L and 5 mg/L paraquat concentrations were selected as the sublethal dose treatments for the *D. rapae* survival experiment. Control treatments were conducted using distilled water. The initial film-producing process was the same as the toxicity experiment. There was no significant difference between male and female adults. Ten insects were randomly induced into each test tube and 10 replications of each treatment were prepared. The results were obtained by checking on the number of surviving parasitoids every six hours until all parasitoids were dead. To evaluate the mean longevity of the different treated individuals, a life table was used to compare the differences between paraquat-treated and control groups.^[22]

Impact of paraquat on *D. rapae* emergence

All tested mummified aphids that were parasitized by *D. rapae* were covered with gauze and dipped into a 6 mg/L

(as indicated by Table 1 for LC₂₀) paraquat solution for 10 s, then dried under natural conditions for two hours.^[23] Control treatments were dealt by treating the mummified aphids with distilled water. Every 20 treated aphids were placed into a glass tube. The tube was covered with gauze and all tubes placed in an illuminated cultivation box with ventilation fans at 26 ± 0.5°C, relative humidity (RH) with a dark ratio of L14:D10. Ten replications of each treatment were prepared. The number of emerged *D. rapae* was determined every 6 hours and the experiment was complete when there were no more emerging parasitoids. A Student's T-test was used to compare the difference of emergence rates between paraquat treated and control groups. The same competitive method used for the *D. rapae* survival experiment was used to determine the longevity of the newly emerged individuals.

Impact on parasitic behavior of *D. Rapae*

Kale seedlings were made into leaf discs (ø 6 cm, and the third instar cabbage aphids, *Brevicoryne brassicae* were placed on the kale leaf discs with density gradients of 10, 20, 50, 100 and 200 heads.^[24] The leaf discs containing the aphids were placed into petri dishes.

For the *D. rapae* treatment, a pair of *D. rapae* were selected six hours after emergence and treated with 6 mg/L (LC₂₀) paraquat solution using the IFM and fed with honey in a tube for 24 h before bioassay.^[25] Control treatments were established by treating the *D. rapae* with distilled water.

After 24 hours, each surviving female *D. rapae* was placed in a petri dish containing kale discs and aphids. Parasitic behavior of the female *D. rapae* was monitored in a room temperature between 20°C~25°C with a 2 × 40 watt lamp. The following observations were made: 1) the time when the parasitoids reached the foraging area (leaf disc with aphids); 2) the time taken to make contact with the first host; 3) the parasitoids patch residence time; 4) the time taken for the parasitoids to leave the foraging area the times that ovipositor thrusting to hosts occurred.

Following the removal of the *D. rapae* on the fifth day, the number of mummified aphids was recorded each day until no new mummified aphids were observed.

The Holling II functional response equation (Eq. 1) was used to calculate the instantaneous attacking rate of parasitoids and to fit the functional response type:^[26]

$$Na = \frac{T \cdot a' \cdot N}{1 + a' \cdot T_h \cdot N} \quad (1)$$

where Na is the number of parasitized hosts, a' is the instantaneous attacking rate, T_h is the time for the parasitoid to oviposit or parasitize on hosts, N is the host density, and T is the time cost of this experiment process. This equation can be simplified into the linear equation (Eq. 2):

$$\frac{1}{Na} = a \frac{1}{N} + b \quad (2)$$

Table 1. Toxicity of paraquat on *D. rapae* female adults.

Time	Toxicity regression equation* $y =$	R	$S. E.$	t -ratio	p	LC_{50} (mg/L) (95%CL)	LC_{20} (mg/L) (95%CL)
12h	$9.07x - 6.61$	0.9898	0.1106	12.03	0.0012	19.04 (17.14~21.15)	15.38 (12.77~18.52)
24h	$2.62x + 2.07$	0.9967	0.0378	21.406	0.0021	13.03 (9.29~18.27)	6.24 (3.47~12.77)
48h	$4.35 + 1.72$	0.9916	0.0952	13.294	0.0009	5.68 (3.63~8.89)	3.64 (1.94~6.83)

* $x = \log D$, D -concentration of pesticide used (mg/L); $y = p$ (probit, coincide with mortality according to Probability Table).

where, and. For this experiment, T was set as 1 day (24 h). Functional response data were also fitted into a Holling-III functional response model^[26] (Eq. 3) for the prey-predator system:

$$Na = \frac{a}{1 + \exp(b - cN)} \quad (3)$$

where a , b and c are all constants, N is the host density and Na is the number of parasitized hosts.

To distinguish between Type II and Type III responses, we performed a logistic regression of the proportion of host parasitized as a function of initial host density procedure.^[27,28] Since cubic models are of a high enough order to describe most curves,^[28] The following polynomial function (Eq. 4) was fitted:

$$\frac{Na}{N} = \frac{\exp(P_0 + P_1 N + P_2 N^2 + P_3 N^3)}{1 + \exp(P_0 + P_1 N + P_2 N^2 + P_3 N^3)} \quad (4)$$

where $\frac{Na}{N}$ is the probability that the prey will be hosted by a predator. As suggested by Juliano, the P -values are parameters to be estimated using maximum likelihood methods.^[28] A type II functional response occurs when the linear term (or slope of $\frac{Na}{N}$ vs. N near $N = 0$) is negative and a type III functional response occurs when the linear term is positive.^[28] Parasitoids' host foraging effect (S) was estimated according to the Holling equation^[28] (Eq. 5):

$$S = \frac{a'}{1 + a' \cdot T_h \cdot N} \quad (5)$$

Table 2. Toxicity of paraquat on *D. rapae* male adults.

Time	Toxicity regression equation $y =$	r	$S. E.$	t -ratio	p	LC_{50} (mg/L) (95% CL)	LC_{20} (mg/L) (95% CL)
12h	$9.46x - 7.00$	0.9933	0.0936	14.84	0.0007	18.54 (16.76~20.50)	15.11 (12.56~18.19)
24h	$2.49x + 2.28$	0.9979	0.0273	27.00	0.0003	12.33 (8.92~17.03)	5.67 (3.21~10.52)
48h	$4.46x + 1.72$	0.9955	0.0710	18.28	0.0004	5.42 (3.55~8.30)	3.52 (1.84~6.70)

Results

Toxicity on *D. rapae*

The relative toxicity of paraquat on *D. rapae* adults is shown as the LC_{50} and LC_{20} values in Table 1 and Table 2. Both LC_{50} and LC_{20} values decreased over time. The LC_{50} values at 12h, 24 h, and 48 h (Table 1; Table 2) were all lower than the values obtained with the commonly used concentration of paraquat (20 mg/L), as recommended by the paraquat producer. Under the field concentration, there was a 100% mortality rate for the *D. rapae* adults over the 24 h period. It can also be seen that *D. rapae* female adults had a better tolerance to paraquat than males because the LC_{50} and LC_{20} were both slightly higher for females than for males (Table 1; Table 2).

Impact on *D. rapae* survival

The survival of *D. rapae* adults under three gradients (15 mg/L, 7.5 mg/L, 5 mg/L) of sublethal paraquat is shown in Figure 1. The survival of *D. rapae* adults was significantly affected by the sublethal paraquat doses. The appearance of mortality peaks was delayed according to the decrease in paraquat concentrations. According to the life table analysis,^[22] the average longevity of *D. rapae* adults under different concentrations shortened significantly (Gehan value was 218.62, $p = 0.000$) reduced from the 131.94 ± 12.50 h in the control group to 33.84 ± 8.25 h

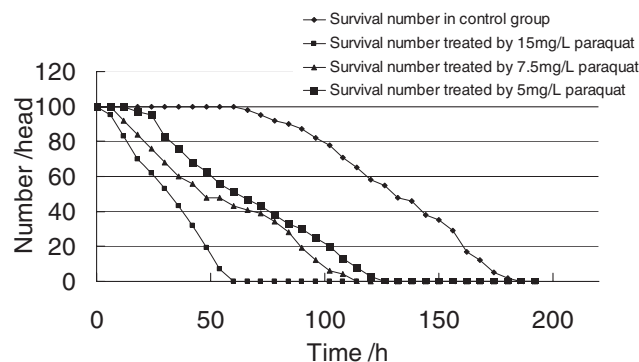


Fig. 1. The survival curves of *D. rapae* after paraquat treatment.

(15 mg/L), 57.48 ± 6.20 h (7.5 mg/L) and 68.94 ± 10.75 h (5 mg/L) in the treated groups.

Impact on *D. rapae* emergence

The percentage emergence of *D. rapae* adults significantly decreased when exposed to the LC_{20} concentration of paraquat (6 mg/L, emergence percentage 71.5%) when compared with the control group (emergence percentage 80.5%; Fig. 2; $t = 4.436$, $P = 0.000$).

There was a 2.5% mortality rate during the first 48 hours in the control group. However, the mortality rate did not increase significantly during this period when emerging from mummies treated with 6 mg/L of paraquat (mortality was 7.5% and the t value was -1.741 ($P = 0.083$)). The average life span of *D. rapae* adults emerging from treated mummies was 131 hours. They did not significantly decrease in comparison with the 154 hours of the control group (Fig. 3), while Gehan value of which was 0.399 ($P = 0.528$).

Impact on parasitic behavior of *D. rapae*

The entering time into the foraging area and the contact time with the first host were both advanced with increasing host density (Figs. 4–6). With a host density of 100 heads, the time in both the control and treated groups were stabilized. However, the influence of sublethal paraquat on both

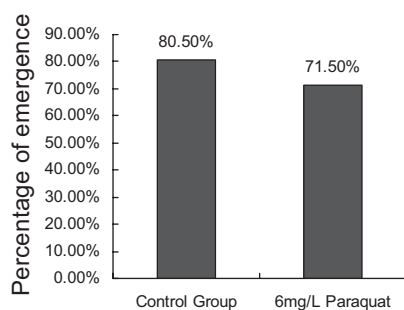


Fig. 2. The impacts on the emergence rate of *D. rapae* being treated with paraquat.

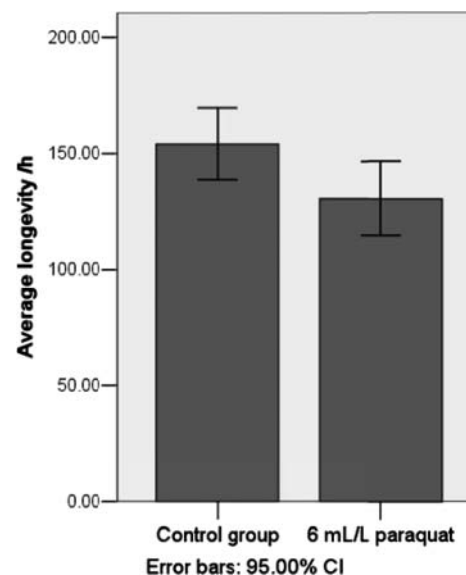


Fig. 3. The impacts on average longevity of newly emerged *D. rapae* after paraquat treatment.

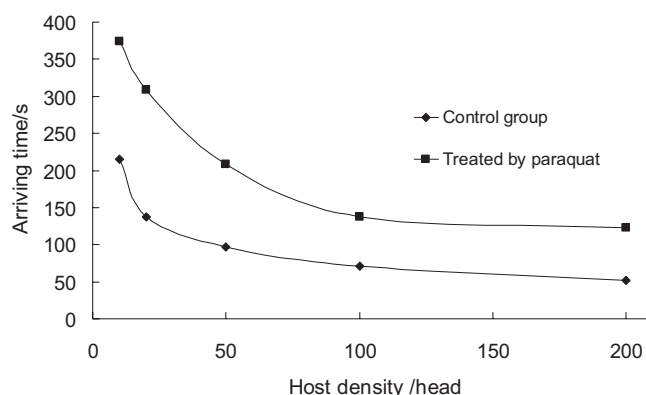


Fig. 4. The time of entering into the searching area after being treated with 6 mg/L paraquat and placed a Petri dish.

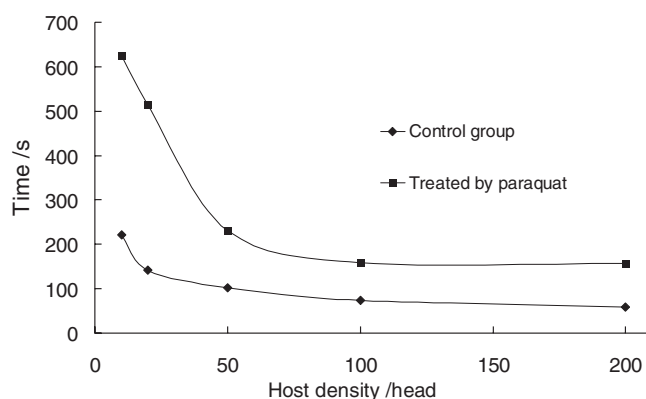


Fig. 5. The time to contact with the first host after being treated with 6 mg/L paraquat and placed a Petri dish.

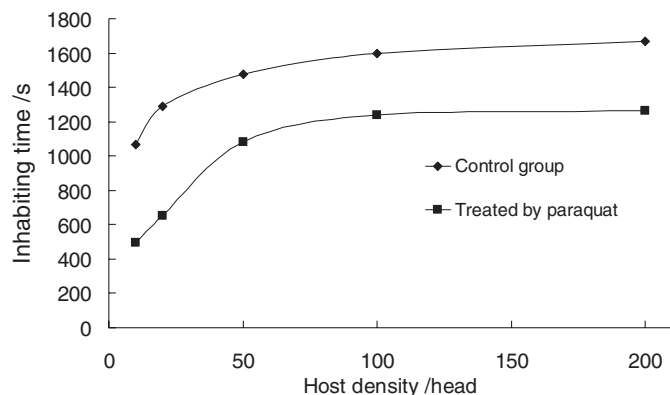


Fig. 6. The time of inhabiting the searching area treated by paraquat under different host densities.

time parameters was significant. The entering time into the foraging area was 143.1 ± 89.6 s, a time significantly delayed in comparison with the control group, the time of which was 71.1 ± 49.6 s. The patch residence time of *D. rapae* in the control group was 1668.8 ± 352.6 s which became 1263.2 ± 415.6 s after being treated with 6 mg/L paraquat.

When the *D. rapae* entered the foraging area and has made contact with the first host, the oviposition activity will become aroused. After being treated with 6 mg/L paraquat, the frequency of ovipositor thrusting to hosts decreased significantly compared with control group (Fig. 7).

The number of parasitized aphids increased with an increase in host density (Figs. 8a and b), and both curves changed from a rapid incline to a leveling off after a host density of 50 heads, based on the functional response model. All experimental data was fitted with Holling-II and Holling-III models (Table 3).

The logistic regressions reveal that the linear term was negative for the paraquat treatment group, indicating type II functional responses (Fig. 8). However, the parameters in

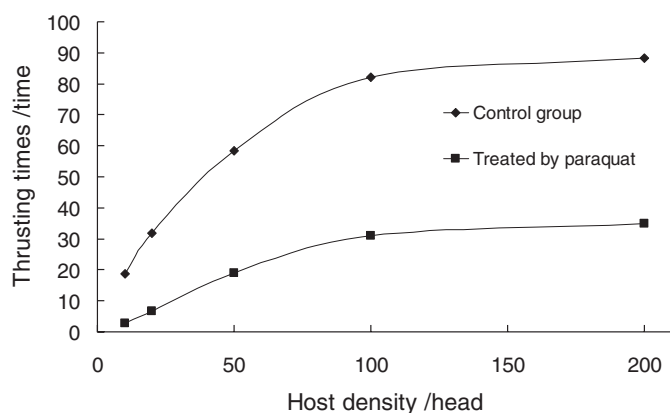
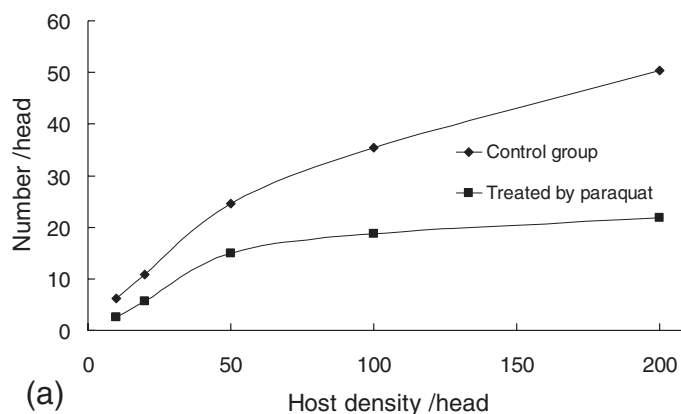
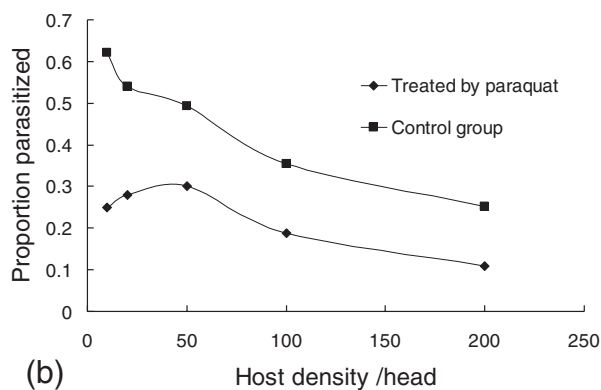


Fig. 7. The frequency of ovipositor thrusting to hosts after paraquat treatment.



(a)



(b)

Fig. 8. A. Number of parasitized hosts in paraquat and imidacloprid treated groups B. Relationship between proportion parasitized and number of host present.

the Holling-II equation changed. The instantaneous attack rates (a') decreased after the use of sublethal paraquat and the time to parasitize the hosts was prolonged compared with the control group. There was also a decrease in the maximum number of parasitized hosts.

In different host densities, the parasitic foraging effect of *D. rapae* decreased from 54.50% to 60.58% after paraquat was used (Fig. 9). This indicates that the parasitic foraging

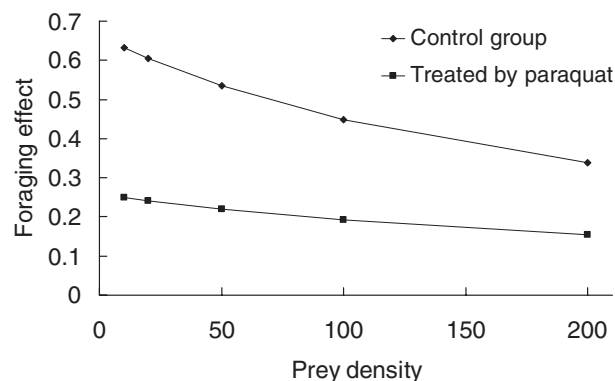


Fig. 9. The influence of paraquat on the foraging effect of *D. rapae*.

Table 3. Results of functional response in Holling model.

<i>Fitting to Holling-II Model</i>	<i>Treatments</i>	<i>Linear equation $\frac{1}{Na} = a\frac{1}{N} + b$</i>	<i>r- Correlation Coefficient</i>	<i>Disc equation $Na = \frac{T \cdot a \cdot N}{1 + a \cdot T_h \cdot N}$</i>	<i>Handling time (min)</i>	<i>Max. Parasitized hosts No. Na_{max} (head/d)</i>
Fitting to Holling-III model	Control group	$\frac{1}{Na} = 1.509\frac{1}{N} + 0.007$	0.9972	$Na = \frac{0.6625N}{1+0.0048N}$	0.22	138.02
	Treated by paraquat	$\frac{1}{Na} = 3.882\frac{1}{N} + 0.013$	0.9954	$Na = \frac{0.2576N}{1+0.0033N}$	0.39	78.06
		$Na = \frac{a}{1+\exp(b-cN)}$		r- Correlation Coefficient		
	Control group	$Na = \frac{50.45}{1+\exp(2.1906-0.0451N)}$			0.9965	
	Treated by paraquat	$Na = \frac{21.85}{1+\exp(2.0443-0.0409N)}$			0.9896	

ability of the *D. rapae* in the control group was stronger than the paraquat-treated groups.

Discussion

This study shows that paraquat is considered “high risk” to aphid parasitoids that may recolonize and oviposition on crops when emerging from mummies during the pupal emergence process. Paraquat induced a decrease in the longevity of emerged individuals when exposed to sublethal concentrations (6 mg/L).

The reduction in the life span of *D. rapae* adults after exposure to LC₂₀ paraquat is consistent with previous studies on the effects of insecticides on insect longevity.^[11,25,29,30] Premature mortality may occur when the emerging parasitoid comes out of the mummified aphid’s body and makes contact with the pesticide on the mummified aphid’s body. The emerging adults cut open the mummy with their mandibles and examine it by both walking over it and using their antennal.^[25,31] Premature mortality must be considered when assessing the effect of an insecticide on the parasitoid.^[25]

As a result of the effects of exposure to sublethal paraquat doses, the disturbed oviposition behavior of *D. rapae* adults show similar findings with previous studies reporting how chemical pesticides can disturb both sensory perception and motor functions in insect parasitoids.^[12,15–17,32]

Conclusion

It is clear that paraquat poses a high risk to aphid parasitoids such as *D. rapae*. A number of reasons account for this. Exposure to paraquat, even at sublethal doses, resulted in premature mortality of *D. rapae*, which not only affected their reproductive cycle but the oviposition capabilities and the behavior of *D. rapae* in the foraging area. Furthermore, the mummy stage of the aphids was more resilient to herbicide damage compared with the adult stage.

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