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Abstract

he presnt work was carried out to evaluate the toxic and latent effects of Chromafenozide on the eggs, larval and adult stages of Chrysoperla carnea (Stephens) under laboratory conditions. Results indicate that the LC_{20} and LC_{50} values of Chromafenozide for 1-2 days old adults were 58.40 and 135.75 ppm, respectively. Highly reduction of adults (males and females) longevity, mean number of eggs/female/day and percentage of eggs hatchability were observed. Application on eggs resulted from treated adults represented LC₅₀ value 62.24 ppm, while eggs from untreated adults gave 93.34ppm. The results show the direct effect of Chromafenozide on incubation periods and percentage of hatchability, as well as, the latent effect on larval duration, and cocoons duration were also highly significant affected and a reduction in adult emergence from both egg treatments with increase in malformation by 43.7 and 27.8% compared to 0% in control and high affected by treatment, when reared on prey; Pectinophora gossypiella (Saund.) eggs. Also, the biochemical study shows clear reduction in total protein of larvae reared on eggs treated with Chromafenozide reduced the total protein content to -22.53% compared with the control larvae. The phenoloxidase which necessary in melanin production during cuticle content, it decreased to -46.04% in the treatment. Which due to changes in N-acetylglucos-amine reached to -26.91%, and the changes in chitinase decreased to -4.68% in treatment compared with control.

Key words: chromafenozide, Virtu (5% SC), predator *C. carnea*, preys *P. gossypiella*.

INTRODUCTION

Family Chrysopidae insects are predators of many species of arthropods, and play an important role in the natural biological control of several crop pests. *Chrysoperla carnea* (Stephens) (green lacewing) is an important natural enemy, (Chrysopidae: Neuroptera) (Tauber *et al.*, 2000). This predator has been observed associated with a wide prey range including aphid nymphs, eggs and neonate larvae of Lepidopteran insects such as, *Pectinophora gossypiella, Earias insulana* (Boisd.) and *Spodoptera littoralis* (Boisd.), scale insects, whiteflies, mites and other soft bodied insects.

Biological control agents such as predators are usually more sensitive to pesticides than the target pests. The adverse impact of insecticides on predators can be decreased /controlled through timing of insecticide application, choice of insecticide and dosage (Galven *et al.* 2005). Selective insecticides can minimize the likelihood of development of resistance in pest (Hassan *et al.* 1985). The use of selective chemicals is an important strategy within pest management programs, since it reduces the population of the phytophagous insects without significantly affecting the natural enemies.

Using pesticides for controlling insect pests resulted in many of side effects such as; pollution in air, water and soil, increase pesticide residues in agriculture crops and their products to residue toxicity on crops, in addition, pest resistance to various classes of pesticides. The excessive use of pesticides, particularly those with long residual effect, has caused several harms to natural balance between pests and their natural enemies (Amr and Marei, 2001).

Insect growth regulators fall into three categories based on mode of action: 1) juvenile hormone mimics, or analogs, (agonists); 2) ecdysone antagonists; and 3) chitin synthesis inhibitors. Juvenile hormone analogs arrest development and cause insects to remain in an immature stage, preventing them from completing their life cycle. Ecdysone antagonists disrupt molting by inhibiting metabolism of the molting hormone, ecdysone. Chitin synthesis inhibitors interfere with enzymes that stimulate the synthesis and formation of chitin, which is an essential component of the exoskeleton. Without chitin, insect pests die in the immature stage or mature into sterile male or female adults, as well as, produce maleformation in adult stages.

Chitinases are glycosyl hydrolases have the ability to degrade chitin directly to low molecular weight chitooligomers, which serve a broad range of industrial, agricultural (Yuli *et al.* 2004). Chitinases have been receiving an increased attention due to their role in the biocontrol of harmful insects (Mendonsa *et al.* 1996). *N*-Acetylglucosamine is the monomeric unit of the polymer chitin, which forms the outer coverings of <u>insects</u>. Phenoloxidase is an important enzyme in the immune system that is effective in the melanin pathway (Soderhall and Cerenius, 1998) and plays a key role in melanin production during cuticle sclerotization at external wound sites and during defense responses, i.e., nodulation (Mason, 1955; Ratcliffe *et al.*, 1984 and Cerenius *et al.*, 2008).

The present investigation was carried out to study the toxicity and latent effect of Chromafenozide compound on the different stages of the *Chrysoperla carnea*.

MATERIALS AND METHODS

- Insects used as predator:

- Chrysoperla carnea:

The adults of *C. carnea* used in this experiment were reared in Trichogramma mass production unit, Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt, and reared for one generation at bollworms department, Plant Protection Research Institute on *P. gossypiella* eggs and larvae for using in the experiment.

- Insects used as prey:

-Pectinophora gossypiella:

The eggs of PBW used in these experiments were reared in the laboratory on semi-artificial diet according to Rashad and Ammer (1985).

-Insecticides used: IGR

- **Common name: chromafenozide** is a novel dibenzoylhydrazine and is categorized to be an insect hormone ecdysone moulting hormone agonists.

Trade name: Virtu (5% SC), and used at the rate of 40 cm³/Fedan.

-Toxicological studies:

To study the activity of Chromafenozide, against newly emergence adults of *C. Carnea*, serial concentrations ranged from 200, 100, 50, 25 and 21.5 ppm of for Chromafenozide were freshly prepared from the stock solution of the commercial compound (1 gm/ 1 liter water). The LC₂₀ and LC₅₀ were estimated by Finney (1971).

-Treatment of C. carnea adult:

Ninety pairs of *C. carnea* from the stock culture were collected and divided to three groups each group 30 pairs of male and female adults. Each group divided to 10 pairs of *C. carnea* adult was used in three replicates in glass cages under the previously mentioned rearing condition. The first group, fed on piece of cotton wool previously soaked only on LC₂₀ (58.40 ppm), and the second group fed only on LC₅₀ (135.73ppm) of chromafenozide, for adults feeding and changed after 2 days, by a piece of cotton wool soaked in the semi arteficial diet containing (2 yeast extract: 1 fructose: 1 distilled water) provided once a day on sticky tape with help of fine brush Hassan (2014). While, the last group fed on the original diet only as control. Each cage was examined daily where the pre-oviposition, oviposition, post-oviposition periods and number of egg laid/ female and longevity of males and females for compound were recorded.

-Treatment of *C. carnea* eggs:

The eggs resulted from untreated and treated adults with LC_{50} ; sequence treated by the same concentrations (400, 200,100, 50 and 25 ppm) of Chromafonozide.

The strips of muslin cloths with attached eggs of *C. carnea* were dipped into the aforementioned selected concentrations of chromafenozide. The control eggs were dipped into water only. The strips were left for 1-2 hr until drying, kept at the rearing condition and examined daily until eggs hatched. LC_{20} , LC_{50} and LC_{90} were determined and recorded.

-Latent effect of chromafenozide on C. carnea:

Newly hatched larvae of *C. carnea* resulted from eggs treated with the dose of LC_{20} and LC_{50} of chromafenozide were kept, individually, in glass vials stoppered with cotton-wool and fed on eggs of PBW. The vials were examined daily until the cocoons were formed. Larval and cocoon durations, percentage of adult emergence and malformed were estimated. All studies were carried out at 26 ± 1 °C and 65-70 % R. H.

-Effect of recommended and LC50 treatment on C. carnea larvae:

Under the same laboratory conditions, newly hatched larvae resulted from untreated eggs of *C. carnea* were kept individually in glass vials Stoppard with cotton-wool and fed on eggs of PBW treated with Recommended and LC₅₀ dose of chromafenozide. The vials were examined daily until the cocoons were formed, larval and cocoon durations and malformed for each group were estimated. As well as percentage of adult emergence and malformed were also estimated.

The recorded data values were statistically analyzed with one-way analysis of variance (ANOVA) (P < 0.05 %) (Snedecor, 1952) and Duncan's multiple range test of means (Duncan, 1955) were used.

-Biochemical studies:

-Determination of enzyme activity:

Determination of chitinase activity was prepared according to Bade and Stinson (1981) and the reaction mixture of enzyme assay according to Ishaaya and Casido (1974). Determination of N-acetyl- glucosamine by the sensitive method of Waterhouse *et al.* (1961). Phenoloxidase activity was determined according to modification of Ishaaya (1971) and Total proteins were determined by the method of Bradford (1976).

-Statistical analysis:

One way analysis of variance (ANOVA) and Duncan's multiple range tests of means were used Duncan's, (1955).

RESULTS AND DISCUSSIONS

Effect of Chromafenozide on reproductive, fecundity and longevity of treated *Chrysoperla carnea* adults:

Treated adults of *C. carnea* with LC_{20} and LC_{50} values of Chromafenozide were 58.40 and 135.75 ppm, respectively, (Table 1).

Ovipositonal period:

Table (1) showed that the ovipostion period of *C. carnea* females treated with LC_{20} (58.40 ppm) and LC_{50} (135.75 ppm) were slightly shorter than those untreated. These periods were 12.86 and 10.3 days/ female, respectively, compared with 16.6 days in untreated.

Female fecundity:

The average number of eggs laid/ female and percentage of hatchability decreased when the predator females fed on chromafenozide compared to untreated one. The mean numbers of deposited eggs were 121.0 and 80.6 eggs/female when treated with LC_{20} and LC_{50} , respectively, compared to 168.3 eggs/female in untreated. On the other hand, the hatchability was significantly high decrease up to 53% when female treated with LC_{50} and 68% with LC_{20} , Table (1). From this data it can be concluded that; when this compound was applied to both sexes (females & males) of insect predator, BPUs induced a variety of effects on reproduction; it caused a decrease in fecundity, fertility and/or hatchability to half time than contol.

Theses resulted agreed with (Apperson *et al.*, 1978; Medina *et al.*, 2002 and 2003a). They recorded that used (IGR) diflubenzuron caused decrease in total eggs laid and hatchability in the lacewing *C. carnea* and in the larvae survival. Medina *et al.*, (2003b) recorded that the three modern insecticides, pyriproxfen, spinosad and tebufenozid have highly effect on reproduction of *C. carnea*.

	Conc.	Pre- ovipostion	Ovipostio n period	Post- ovipostion	Total no. of eggs/೪/day	Mean no. of eggs/೪/ Day	% Hatchability	% sterility	Adult longevity	
Treatment	Ppm								ŶŶ	ರೆರೆ
Check		2.3ª±0.2	16.6ª±0.9	2.9ª±0.3	168.3ª±8.6	10.7ª	82ª	-	21.8°±1.6	16.9ª
LC ₂₀	58.40	4.2 ^b ±1.1	12.86 ^b ±1.3	3.3ª±0.6	121.0 ^b ±3.3	10.6ª	68 ^b	44.97	20.6 ^{ab} ±3.2	14.3 ^b
LC ₅₀	135.75	3.5ª±1.2	10.3°±1.6	3.0ª±0.1	80.6°±9.8	6.9 ^b	53°	71.37	16.8 ^b ±2.1	10.16 ^c
LSD		1.52	1.52	0.69	26.36	3.42	5.37		2.379	1.65

 Table 1. Reproductive, fecundity and longevity of treated *Chrysoperla carnea* adults with chromafenozide

-Effect of chromafenozide on C. carnea adult longevity

The predator female and male longevities were slightly shortened when the adult treated by LC_{50} data in Table (1) which recorded the differences between control

and treated adults longevity period. These periods were 14.3 and 10.16 days/ male and 20.6 and 16.8 days/ female treated with LC_{20} and LC_{50} , respectively, compared with 16.9 & 21.8 days in untreated male and female.

-Effect of chromafenozide on eggs stage

Data in Table (2), show that the eggs incubation period and different immature stages of *C. carnea* when sequence treated eggs resulted from females treated with LC_{50} values (62.24) (E1) and eggs resulted from untreated females and sequence treated by LC_{50} (92.34) of chromafenozide (E2) compared with untreated eggs (check eggs).

Data in Table (2) recorded that the egg resulted from female treated with chromafenozide and sequence treated (E1) the more affected and susceptibility to chromafenozide (LC_{50} value estimated by 62.24ppm, on contrast this value increased to 92.34 ppm when treated eggs resulted from untreated females (E2).

-Egg stage:

Data in Table (2) indicated that incubation period affected by repeated the treatment with LC_{50} of chromafenozide; this average increased significantly to 6.3 days/ eggs (E1) after treated with the LC_{50} and 5.4 days in (E2) compared to 3.4 days in untreated eggs.

					%	Cocoon stage		Total	%	
Treatment	Conc. Ppm	Incubation period in days	% Hatchability	Larvae duration in days 1 st - 3 rd instar	% Pupation for larvae	Duration in days	Malfor mation %	al Immature stage	› Adult Emergence	% Malformation
Eggs(1)	62.24	6.3	84	18.3 ^b ±2.1	75	9.8 ^b ±0.9	10	28.1	57	43.7
Eggs(2)	92.34	5.4± 0.3	51	16.4°±1.9	81	8.2 ^b ±0.4	6	24.6	63	27.8
Eggs(3) check	-	3.4±0.2	98	10.3±1.2	96	6.3ª±2.1		16.6	98	0.0

Table 2. Immature stages resulted from treated eggs

- 1- Eggs resulted from females treated by LC₅₀ values (62.24) and sequence treated by LC₅₀ of chromafenozide (E1)
- 2- Eggs resulted from untreated females and sequence treated by LC₅₀ (92.34) of chromafenozide (E2)
- 3- Untreated eggs

-Durations of immature stages of *C. carnea* resulted from (E1&E2) treated with LC_{50} of chromafenozide:

-Larval stage:

Table (2) demonstrates that the average larval duration resulted from treated or sequence treated eggs with LC_{50} varied from 1^{st} instar to 3^{rd} instar larvae when fed on *P. gossypiella* eggs.

The larval duration period significantly increased in case of treated eggs compared to untreated eggs, Table (2). The duration of larval stage averaged 18.3 days when larvae hatched from eggs treated by LC_{50} (62.34) of chromafenozide (E1) and 16.4 days when larvae resulted from (E2) treated eggs by LC_{50} (92.24) and reared on *P. gossypiella*, compared to 10.3 days/larvae resulted from eggs check.

These data indicate that the eggs treated with LC_{50} prolonged the duration of larval instars approximately from 1.6 to 1.8 times than control.

Cocoon:

The duration of cocoon increased when resulted from (E1) or (E2) than control, Table (2) showed that, pupal duration were 9.8 and 8.2 days resulted from treated eggs (E1 & E2) respectively, compared with 6.3 days for untreated eggs with larvae fed on PBW. The total immature stage, from 1st instar larvae to adults emergence were 28.1 days in (E1) and 24.6 days in (E2) compared to 16.6 days in control.

In this respect, Vinuela et al. (2001) found that 13 different pesticides induced only slight reduction in percent of egg-hatch in C. carnea. Shaalan and Kandil (2010) observed that Radiant 12 % shortened the life span and a reduction in fecundity of predatory C. carnea. In contrast, Dutton et al. (2003) also found that developmental period of C. carnea lasted longer when larvae were fed upon Btcontaminated S. littoralis larvae. Ali et al. (2015) found that Neem and Datura was less toxic than confidor against first, second, and third instar larvae of C. carnea as well as pupae and adults are also affected by confider. Zia et al. (2017) spinosad and flubendiamide are relatively safe. Imidachloprid was safest for the adults causing 10 to 40% mortality. Chloropyrifos is the most injurious insecticide used causing highest mortality 33-100% of C. carnea larvae and adults. Also, Bhojani et al., (2018) assessed the relative toxicity against the green lacewing, Chrysoperla zastrowi sillemi in the laboratory. buprofezin 25 SC at 0.05% and fipronil 5 SC at 0.02% were categorized as slightly harmful. While assessing relative toxicity through food contamination technique against the adults of C. zastrowi, buprofezin 25 SC at 0.05% was categorized as harmless.

Percentage of Adult emergence:

Data in Table (2) show the percentages of adult emergence from the pupae resulted from treated eggs (E1&E2) when reared on PBW eggs decrease to 57 and 63 % compared to 98% in the control. Generally, treating (1-2 day old) eggs of *C. carnea* with LC₅₀ compound related to chitin synthesis inhibitor and /or moulting hormone agonists reflected high effects on immature stage and the adult stage and reduced fecundity and hatchability in comparison with control.

Percentages of larvae and cocoons malformed:

Data in Table (2) and (Fig. 1, 2&3) show the late effect of chromafenozide on larvae, cocoons and adults malformed percentages when resulted from eggs treated with LC_{50} . The percentages increased with the treated eggs resulted from adult treated to 43.7 % and decreased approximately to half time 27.8% when treated eggs resulted from adult untreated compared with 0% in control.



Fig. 1. Effect of LC50 on 3rd instar larvae of C. carnea



Fig. 2. malformed adults resulted from eggs resulted from adult treated and sequence treated by LC₅₀ of chromofenozide with LC₅₀ (62.24ppm).



Fig. 3. Malformed adults resulted from eggs treated with LC₅₀ (92.3 4 ppm)

Duration and feeding capacity of *C. carnea* when fed on *P. gossypiella* eggs treated with recommend and half recommend compound of chromafenozid:

Data in Table (3) demonstrates that the average larval duration from 1st to 3rd instar larvae increased significantly when fed on treated *P. gossypiella* eggs with recommended and half recommended concentrations of chromafenozide compared with untreated eggs. The duration of larval instar averaged 21.6 and 19.3 days when larvae fed on *P. gossypiella* eggs treated with recommended and half recommended concentrations of chromafenozide, respectively, compared to 10.7 days/larvae in control. These data indicate that feeding *C. carnea* on eggs treated with recommend and half recommend prolonged in the duration of larval instar approximately 2 time than control at the same time, decreased the feeding capacity to 223 and 254 treated eggs/ larvae of *C. carnea*, respectively, compared to 385 eggs in control. Medina *et. al.* (2003b) recorded the effect of three modern insecticides, pyriproxfen, spinosad and tebufenozid on toxicity, hatchability eggs and survival of *Chrysoperla carnea*.

Malformation larvae:

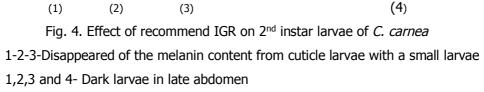
As shown in Table (3) and Fig. (3), malformed percentage appeared in larvae resulted when fed on treated eggs with recommend and half recommended concentrations, the high percent estimated by 35 % when larvae fed on treated eggs with recommended chromafenozid and the lowest percentage of malformation recorded by 21.3% when larvae feed on treated eggs with half recommended. Generally, chromafenozid treatments caused very small larvae and dark larvae in thorax and late abdomen and darken larvae after mortality.

		Initial number	%	Larval stage			Pupal stage		
Tested Compound	Treated PBW Eggs		Mortality In 1-3 instar larvae	Duration from 1-3 instar larvae	Feeding capacity	% Malformed	Duration	% Mortality & dead	Total immature stage
	Recommended	75	58ª	21.6ª	223	35.0ª	13.8ª	16.3	35.4ª
chromafenozide	Half- recommend	75	35.3⁵	19.3 ^b	254	21.3 ^b	9.5⁵	12.3	28.8 ^b
	Control	30	2 ^c	10.7 ^c	385	-	6.8 ^c	1.3	17.5°
	LSD		2.40	1.41		2.66	1.67		1.95

Table 3. Duration and feeding capacity of *C. carnea* when fed on *P. gossypiella* treated with chromafenozide

Malformed *C. Carnea* larvae after feeding on *P. gossypiella* eggs treated with recommend of chromafenozide treated .





Cocoon stage:

Data recorded in Table (3) and Fig. (4) show that duration of cocoon increased significantly when larvae feed on eggs treated with recommend and half recommended compound it lasted 13.8 days and 9.5 days, respectively, compared to 6.8 days when fed on untreated eggs of PBW, The total immature stage, from 1st instar larvae until emergence of adults were 35.4 and 28.8 days when fed on PBW eggs treated with two concentration, respectively, compared to 17.5 days in control.

-Biochemical studies:

Some important enzymes such as total protein, phenoloxidase, chitinase and N-acetylglucoseamine which plays a key role in development and molting processes in body of *C. carnea*;. Recorded in Table (4) showed the effect of Chromafenozide on some biochemical analyzes of *C. carnea*, it cussed changes in the total contents of protein and phenoloxidase when the 2nd instar larvae fed on treated eggs of PBW with

recommended concentration of Chromafenozide. Treatment with Chromafenozide reduced the total protein content to -22.53% compared with the control larvae. While the phenoloxidase which very necessary in melanin content during cuticle production high decreased to -46.04 % in the treatment Fig 4 (1, 2 &3). Assar *et al* (2012) found that after treated *S. littoralis* with the IGR, the activity of phenoloxidas decreased significantly. Sabry and Khedr (2014) found that molting hormone agonist decreased phenoloxidase activity comparing to control larvae of *S. littoralis* as follows: methoxyfenozide (-20.91%) and tebufenozide (-3.84%).

Treatment	Treted	Control	%	
			Changes	
Total protein	14.10 ^b ±0.38	18.20ª±0.53	-22.53	
(mg/g.b.wt)				
Phenoloxidase	2745.89 ^b ±53.31	5088.89 ^a ±66.93	-46.04	
(O.D. unit x10 ³ /min/g.b.wt)				
Chitinase	1211.00ª±12.43	1271.56°±24.49	-4.68	
(µg NAGA/min/g.b.wt)				
N-acetylglucosamine	476.00 ^b ±7.21	651.33°±12.14	-26.91	
(µg NAGA/g.b.wt)				

Table 4. Effect of Chromafenozide on some biochemical analyzed of C. carnea.

Data in Table (4) showed the changes in chitinase decreased to -4.68% in treatment compared by control. Whereas, changes in N-acetylglucosamine reached to -26.91% compared with control. Theses data can be indicate that, when PBW eggs treated with Chromafenozide and directly fed the predator larvae on the treated PBW eggs, some malformed appeared on predator instars larvae, this may be due to molting hormone agonist decreased chitin's by -4.68% and N-acetylglucosamine to -26.91%.

Sabry and Khedr (2014) showed reduction in chitinase activity (-3.82%) when treated the 4^{th} instar larvae of *S. littoralis*.

CONCLUSION

In conclusion, we demonstrate in this study the direct and indirect effect of Chromafenozide on some biological and biochemical parameters of the predator *C. carnea*.

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التأثير المتاخر لمركب Chromafonozide علي إنتاجية وبعض الخصائص البيولوجية لأسد المن

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تم در إسة تأثير مركب من مجمو عات مانعات التغذية Chromafonozide السام و المتأخر على للبيض، اليرقات والحشرة الكاملة لاسد المن. دلت النتائج ان قيم LC50, LC20 للحشرة الكاملة عمر 1–2 يوم كانت 75,135و 85,45 جزء في المليون على التوالي. أظهرت النتائج مدة عمــر الحشرة الكاملة قد قصر جدا للإناث والذكور، تم متابعة متوسط عدد البيض/فراشة/يوم ونسبه فقـس البيض. ايضا انخفاض عالى في عدد البيض الموضوع وادى هذا الانخفاض الى انخفاض معنوى في متوسط عدد البيض لكل انثى في اليوم و% الفقس. من ناحية اخرى البيض الناتج من معاملة الحشرة الكاملة تمت معاملتة مرة اخرى بقيمة LC50 (62,24) بينما زادت هذه القيمة الـــى 34,93 عنــدما عومل البيض الناتج من الحشرات الكاملة الغير معاملة. اظهرت النتائج التأثير المباشر للمركب على فترة حضانه البيض و% الفقس كما التأثير المتأخر على فترة تطور اليرقات وفترة الطـور العــذرى تأثرت ايضا معنويا وحدث إنخفاض في خروج الحشرة الكاملة من كل من معاملات البيض مع زيادة في التشوهات بقيم 43٫7، 27٫8 % مقارنة بالكنترول وتأثير عالى في المعاملة عند تربيتهـ ا علــي بيض دودة اللوز القرنفلية. ايضا أظهرت الدراسات البيوكيميائية ان تربية يرقات أسد المن على بيض معامل بمركب Chromafonozide تسبب في خفض البروتين الكلي الي 53_22- % مقارنــة بيرقات الغير معاملة. أما الفينول اكسيديز الذي يعتبر هام في انتاج الميلانين اثناء تكوين الكيوتيكــل انخفض الى 46,04 - % في المعاملة. بناءا على التغيرات في N-acetylglucoseamine وصلت الى 26,91 - % والتغيرات في الكيتينيز انخفض الى 4,68 - % في المعاملة مقارنة بالغير. معامل.

كلمات مفتاحية: كروموفينوزيد – مفترس اسد المن – دودة اللوز القرنفلية.