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Assessment of the Sensitivity and Death Rates of Adults of *Graphosoma lineatum* (Linnaeus, 1758) Fed Chlorocholine Chloride, a Plant Growth Inhibitor, Under Laboratory Conditions

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Abstract: The side effects of chlorocholine chloride, a plant growth inhibitor, on adults of *Graphosoma lineatum* were studied under laboratory conditions. In the study, 100, 250, 500, 750, 1000 and 1500 ppm of chlorocholine chloride in distilled water were applied. Chlorocholine chloride had killing effects on adults of *G. lineatum*. A 100% mortality of the male adults was observed after feeding 750, 1000 and 1500 ppm of the compound, whereas a 100% mortality of the females occurred with 1000 and 1500 ppm of the feeding compound. This study also showed that the sensitivity of the male adult insects to chlorocholine chloride was more than that of the females.

Key Words: *Graphosoma lineatum*, adults, chlorocholine chloride, sensitivity, death rates

Chlorocholine Chloride'in *Graphosoma lineatum*'un Ergin Bireylerinde Duyarlılık ve Ölüm Oranlarına Karşı Laboratuvar Koşullarında Araştırmalar

Özet: Bir bitki düzenleyici hormonu olan chlorocholine chloride'in yan etkileri laboratuvar koşullarında *Graphosoma lineatum*'un ergin bireyleri üzerinde araştırılmıştır. Bu çalışmada chlorocholine chloride'in 100, 250, 500, 750, 1000 ve 1500 ppm dozları saf su içerisinde kullanılmıştır. Chlorocholine chloride'in *G. lineatum* 'un ergin bireyleri üzerinde ölüm etkisi bulunmuştur. Erkek ergin bireylerde % 100 ölüm, 750, 1000 ve 1500 ppm dozlarında görülmüştür. Buna karşın dişi bireylerde % 100 ölüm 1000 ve 1500 ppm dozlarında bulunmuştur. Araştırma sonuçlarına bakıldığı zaman erkek bireylerin dişilerden daha duyarlı olduğu ispatlanmıştır.

Anahtar Sözcükler: *Graphosoma lineatum*, ergin bireyler, chlorocholine chloride, duyarlılık, ölüm oranı

Introduction

Plant hormones, known as plant growth regulators (PGRs), are organic natural or artificial compounds, some of which enhance plant growth while some others reduce or inhibit it. The compounds which inhibit or reduce plant growth are called "inhibitors" or "retardants". "Stimulators" are compounds or materials which induce plant growth. Both inhibitors and stimulators have used in some countries in recent years (Önder et al., 1987).

Since the effects of PGRs are well known, they are widely applied in agriculture. Our knowledge on their toxic effects on animals is limited. Therefore, many scientists have studied this subject in recent years. Some of the PGRs are, in fact, plant hormones which are most likely included in diets of herbivorous and omnivorous animals (Çelik et al., 2002). PGRs are relatively less

significant in global agriculture compared to fungicides, herbicides and insecticides. The regulatory compounds sold are barely more than 4% of total plant protection compounds (Rajala and Peltonen-Sainio, 2000). However, plant regulators may replace insecticides in the future and save our environment from pollution (Çelik et al., 2002).

Numerous plant growth inhibitors have been prepared in order to prevent plants from growing. Some of such compounds inhibit gibberellic acid biosynthesis (Arteca, 1996; Fletcher et al., 2000) through prohibition of *ent*-kaurene synthase catalysis. Among such inhibitory compounds is (2-chloroethyl) trimethylammonium chloride (Fletcher et al., 2000). This compound, C₅H₁₃Cl₂N, is one of the most effective plant growth inhibitors and is widely used (Önder et al., 1987). This

compound, commercially known as Cycocel, was sold commercially for the first time in 1959 (Önder et al., 1987; Rajala and Peltonen-Sainio, 2000), and in the late 1950s and during the 1960s it was studied and assessed by some researchers such as Tolbert (1960), Humbries et al. (1965) and Larter (1967) (Rajala and Peltonen-Sainio, 2000). This plant growth inhibitor, nowadays known commonly as chlormequat chloride and chlorocholine chloride (Önder et al., 1987; Gent and McAvoy, 2000; Rajala and Peltonen-Sainio, 2000) and commercially as Cycocel (Önder et al. 1987; Arteca, 1996; Gent and McAvoy, 2000; Rajala and Peltonen-Sainio, 2000), CCC (Önder et al., 1987; Arteca, 1996; Fletcher et al., 2000; Gent and McAvoy, 2000; Rajala and Peltonen-Sainio, 2000), Cycogan (Gent and McAvoy, 2000), Cycocel Extra, Cycocel WR62, Cycocel Cyanamid, Cycofrem, CCC-Sanac, Hermoo-Chlormequat and Metex, is available on the market as a crystallinised substance with an odour resembling that of fish (Önder et al., 1987). Considering the hygroscopic nature of the compound, it is better applied as a solution. Its acute oral LD₅₀ for male and female mice are 670 mg/kg and 1200 mg/kg, respectively, and for chickens it is 920 mg/kg. Formulations of chlorocholine chloride as 11.8%, 40%, 50% and 72.5% solutions and as a 65% wettable powder are commercially available (Önder et al., 1987).

Chlorocholine chloride is the first PGR used for controlling the stem length in cereals (Rajala and Peltonen-Sainio, 2000) and is applied to control tilt (Arteca, 1996). This substance inhibits vegetative growth, but accelerates plants' reproductive growth. It also causes shortness of the petiole and, in wheat and oat, prevents stem elongation. In pear trees, chlorocholine chloride over-induces shooting, flowering and fruiting (Önder et al., 1987).

Studies have shown that the application of chlorocholine chloride, as in soil fertilization and leaf feeding with nitrogen, enhanced ¹³⁷Cs absorption from polluted soils and its accumulation in spring wheat straw and seed (Hrynczuk and Weber, 2000). In a study, the application of CCC, twice on cotton at 25 g/ha with a 15-day interval, reduced plant height by 20%. However, the yield was enhanced after CCC application once or twice at 25 g/ha by 11.5% and 11.6%, respectively. The seed weight was also increased by CCC application (Pipolo et al., 1993). According to Martin and Worthing (1977), Cycocel protected tomato plants from *Verticillium* and the

compound was degraded by soil-dwelling micro-organisms.

The effects of PGRs on insects have already been assessed by some researchers. For example, Guarra (1970) included a PGR substance in a diet for larvae of some *Heliothis* spp. and studied its effect on their growth and reproductivity. Alanso (1971) studied the effects of gibberellic acid on the development of *Drosophila hydei*; Visscher (1982) studied the effects of plant growth hormones on growth and reproduction in some grasshoppers; and De Man et al. (1991) studied the effects of abscisic acid on vitellogenesis in *Sarcophaga bullata*. To our knowledge so far, there have been reports of the effects of PGRs on the true bugs *Dysdercus cardinalis* (Het., Pyrrhocoridae) (Carlisle et al., 1969) and *Dolycoris baccarum* (Het., Pentatomidae) (Önder et al., 1987).

Materials and Methods

Insects and laboratory conditions

Adults of the insect *G. lineatum* were obtained from Varamin Sunn Pest Research Centre and reared on parsley seeds and fed with distilled water, followed by purification over 5 generations. Adults of the sixth generation were used in the experiment. The insect rearing was done under 25 ± 2 °C, 60% ± 5 R.H. and L:D 16:8 (Voegele, 1968; Khlistovskii and Shirinyan, 1980; Karsavuran, 1992; Nakamura et al., 1996).

Chemical substance

The plant growth regulator, chemically known as (2-chloroethyl) trimethylammonium chloride, with chlorocholine chloride and chlormequat chloride as the common names, was used. This compound was obtained from Acros Organics Co., New Jersey, USA, as solid tiny white crystallinised, with 98% purity.

Experimental method and treatments

In the present study, 100, 250, 500, 750, 1000 and 1500 ppm of chlorocholine chloride solutions in distilled water were used. Distilled water was applied as the control treatment. The experiment was executed as a completely randomized design (CRD) in 4 repeats.

The experiment was performed in small opaque-white

plastic cylinders (11 cm in diameter and 10.5 cm in height). To prepare food for the insects, parsley seeds were glued on rectangular thin box paper (10.5 × 35 cm) using a mixture of water and wheat flour as the sticking agent. The paper pieces were transferred into the cylinders, after drying and losing the odours, so that the wall and floor of the cylinders were completely covered by the paper. The treatments (various concentrations of the chemical compound) were applied, by feeding, to the insects via filter papers (8 × 4 cm). To do this, 8 cm from the bottom inside the plastic container, a horizontal groove (4 × 0.1 cm) was made. Then one side of a rectangular filter paper (as long as 1 cm) was placed inside the groove and the other side of the paper was placed in a container (5 cm in diameter and 3.5 cm in height) containing one of various concentrations of the chemical compound. Water moved towards the interior part of the container via the filter paper so that the adult insects could feed on the compound ad libitum. The necks of the plastic containers were covered with a piece of 24-mesh-net. Inside each container, 10 male and/or female adults (separately), with a life expectancy of 12 h, were placed. The filter paper used in the feedings was changed daily and the remaining feeding solutions were also disposed of; then the containers were again filled up with

fresh feeding solutions. The numbers of dead adult insects were recorded up to the 15th day with 24-h intervals (Önder et al., 1987).

Results

Figure 1 shows the cumulative mortality of male and female adults of *G. lineatum* on day 10 of the experiment. In Figure 2, the final cumulative mortalities of both sexes are shown against 6 dosages of the feeding solutions over 15 days. With an increase in the concentration from 100 to 1500 ppm, the death rate of the adult insects also increased. The death of 100% of the male adults was observed after feeding 750, 1000 and 1500 ppm of the compound, whereas 100% mortality of the female adults occurred with 1000 and 1500 ppm of the feeding compound. The figure also shows higher death rates for the males than for the females, suggesting a higher sensitivity of the males to chlorocholine chloride. Both males and females did not die under the control treatment (feeding with distilled water) within the 15 days of the experiment.

The mortality trends of adult males and females of *G. lineatum* are shown in Figures 3 and 4. In all the

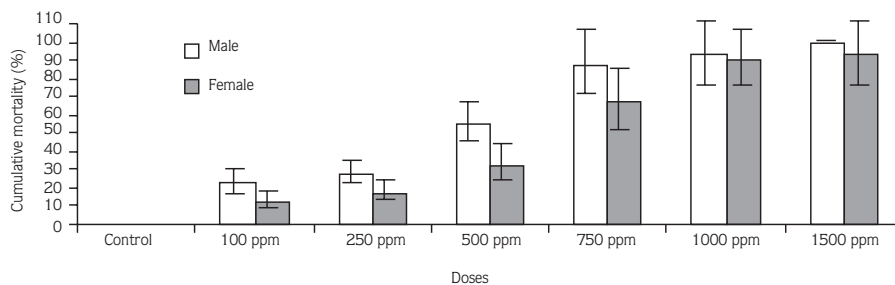


Figure 1. Cumulative mortality of male and female adults of *G. lineatum* at different concentrations of chlorocholine chloride on day 10 of the experiment.

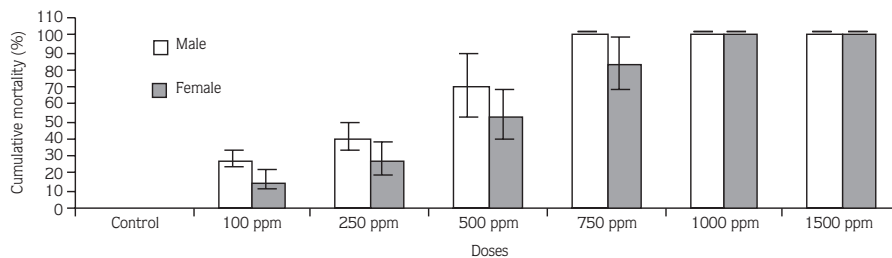


Figure 2. Cumulative mortality of male and female adults of *G. lineatum* at different concentrations of chlorocholine chloride at the end of the experiment.

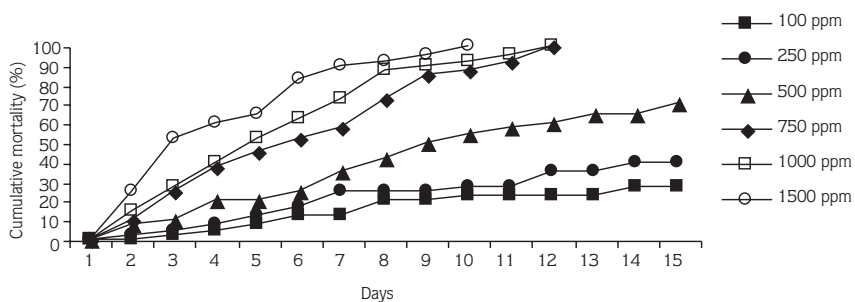


Figure 3. Cumulative mortality trends of male adults of *G. lineatum* during the 15-day period of the experiment.

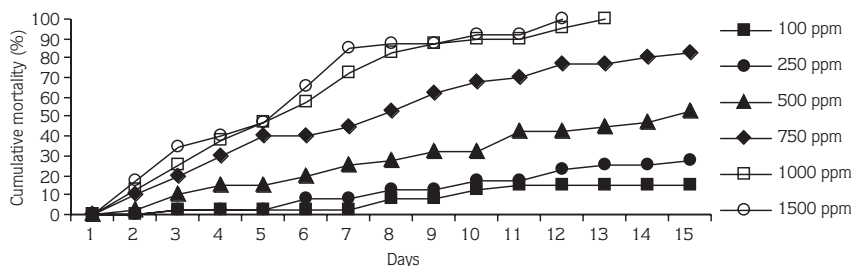


Figure 4. Cumulative mortality trends of female adults of *G. lineatum* during the 15-day period of the experiment.

treatments, no death of the adult insects was observed on the first day of the experiment. According to the results, 100 ppm chlorocholine chloride within the 2nd-10th days of the experiment caused the death of 22.5% of the males and 12.5% of the females. The total death rate over the 15-day period was 27.5% and 15% for the males and females, respectively. Some 27.5% and 17.5% of the male and female insects, respectively, died from day 2 to 10 of the experiment with a 250 ppm concentration of chlorocholine chloride. Total death rates for the males and females at the end of the 15-day period were 40% and 27.5%, respectively. A 500 ppm concentration of chlorocholine chloride caused 55% and 32.5% of the males and females, respectively, to die from day 2 to 10 of the experiment. The final death rates of males and females within the 15-day period were 70% and 52.5%, respectively. With 750 ppm of chlorocholine chloride, 87.5% and 67.5% of the males and females, respectively, died from day 2 to 10 of the experiment. The death of 100% of the male adults had occurred by day 12, whereas the death rate of the female adults on the same day was 77.5% and on day 15 it reached 82.5%. A 1000 ppm concentration of chlorocholine chloride killed 92.5% and 90% of the males and females, respectively, from day 2 to 10. At this concentration,

100% death rates of the male and female adults was observed, so that the remaining males and females died on day 12 and day 13, respectively. At 1500 ppm, 100% mortality of the males and females was observed on day 10 and day 12, respectively. The death rate for the females on day 10 was 92.5%.

Figures 3 and 4 show that the death rate of the male adults was higher than that of the females during the same period under chlorocholine chloride treatments, suggesting that the males were more sensitive to the compound. For example, at 750 ppm and on day 10, 87.5% of the males died, whereas it was 67.5% for the females. As another example, at 250 ppm of chlorocholine chloride, 27.5% of the males died on day 10, but the same death rate of the females occurred on day 15.

Discussion

The main goal of this study was to assess the mortality effects of chlorocholine chloride on adult *G. lineatum* and to compare the sensitivity of males and females to the compound. Previous research has shown that the toxic or biological effects of PGRs are different

and the dose-effect relationship is dependent on the species to which the regulators are applied (Çelik et al., 2002).

Factorial analysis of variance of the data is indicative of significance of the dose and insect sex in the death rate of *G. lineatum* adults ($P \ll 0.001$, Table 1). The effect of these factors on each other was not significant ($P = 0.1962$).

As shown in Figures 1-4, for biting-sucking insects, such as *G. lineatum*, 500 ppm and higher doses of chlorocholine chloride for the male and 750 ppm and higher doses for the female have good killing effects. Similar results were obtained by Önder et al. (1987) on *Dolycoris baccarum* and they stated that chlorocholine chloride at concentrations higher than 500 ppm caused 100% death of the males and females within 2-9 days. Accordingly, a 500 ppm dose of the compound caused 80% death of the females on the 15th day from the feeding date. They found that the sensitivity of the adult males was more than that of the adult females. Putting together the results obtained in the current study and those obtained by Önder et al. (1987), it is revealed that the sensitivity of the adult *D. baccarum* to chlorocholine chloride is more than that of the adult *G. lineatum*. This is because, for example, 100% and 80% of the adult males and females of *D. baccarum* were killed by 500 ppm of chlorocholine chloride, respectively, whereas for *G. lineatum*, 70% and 52.5% of the adult males and females died, respectively.

The direct and indirect effects of chlorocholine chloride (as commercial formulations such as Cycocel) on insects and mites have been studied by some researchers in recent years. Some studies on plants such as wheat, barley, cotton, cabbage, sorghum and some varieties of grapevine in the fields and under laboratory conditions have been performed on aphids, larvae of Lepidoptera and some other insects, and the effects of this plant growth inhibitor have been assessed. The results from

these studies are summarized as follows: the compound affected insects at all stages, even the eggs, and decreased the population of aphids and Lepidoptera (Honeyborne, 1969; Worthing, 1969; Mansour and Dimetry, 1976; Singer and Smith, 1976). Malformation or deformation and weight loss are the side effects of chlorocholine chloride (Carlisle et al., 1969; Awad and Taha, 1976). Decreasing the growth of the reproductive organs and morphological abnormalities could also occur due to feeding from this PGR (Carlisle et al., 1969; Van Emden, 1969). The compound can also prevent the insect cells from undergoing meiosis (Carlisle et al., 1969) and decrease the egg laying (Carlisle et al., 1969; Honeyborne, 1969; Prasad et al., 1977; Dreyer et al., 1984). Preventing insects from going into diapauses is another effect of chlorocholine chloride which can kill the insects, because they are exposed to cold winter weather (Bariola et al., 1976). Chlorocholine chloride also causes an increase in the amount of pectin in plant tissues, resulting in an improved resistance of the plants against pests, e.g., aphids (Honeyborne, 1969; Smith, 1969; Dreyer et al., 1984). Furthermore, it has been shown that the application of chlorocholine chloride on plants facilitates the penetration of insecticides and acaricides into the plants (Richter and Caceda, 1984). Chlorocholine chloride (Cycocel) had no effect on the parasitoid wasp, *Pimpla turionella* (Hym., Ichneumonidae) (Bogenschutz, 1984) or on the predators of aphids (Smith, 1969). Results from the application of chlorocholine chloride on the cotton boll weevil (*Anthonomus grandis*) have shown that chlorocholine chloride alone is not effective, but can be used as an auxiliary strategy to help improve the integrated pest management (IPM) of cotton pests (Pipolo et al., 1998).

Since IPM programs nowadays are the most notable methods in pest control, the integration of such PGRs into the IPM programs can complete the integrated strategy and help such programs. Accordingly, more

Table 1. ANOVA of data as factorial in CRD.

S.V.	df	SS	MS	F	P
Dose	5	47941.667	9588.333	115.06	0.0000**
Sex	1	1200	1200	14.4	0.0005**
Dose × Sex	5	650	130	1.56	0.1962 ^{ns}
Error	36	3000	83.333		

CV = 13.44%

research on the effects of PGRs to succeed on pests is a prerequisite.

The application of PGRs in the end can benefit agriculture in 2 aspects: 1) control of biting-sucking insects such as bugs and aphids and 2) crop growth regulation favours the growers (Önder et al., 1987).

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