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Assessing the role of RNA in manifesting the potential of mendione as an effective insecticide against *Dysdercus cingulatus* F.

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ABSTRACT: The impact of sublethal concentrations of menadione was evaluated on the RNA content of gonads, fat body, and brain in *Dysdercus cingulatus* F. Three sublethal concentrations viz. 0.5, 0.75, and 1.0 µg were used topically using a Hamilton syringe. The test insects were dissected after the intervals of post-treatment days 2, 4, and 6. Organs of the treated insects were evaluated and compared for the RNA content with control insects. The biochemical results revealed a gradual significant decline in the RNA content of various tissues under investigation. Hence it was concluded that menadione not only hampers the protein and DNA metabolism, but it roots back to the RNA level to induce histopathological changes in organs to finally act as a reproductive inhibitor and life cycle disruptor.

KEY WORDS: Sublethal concentration, naphthoquinone, reproductive inhibitor, DNA metabolism

Menadione is a synthetic compound, which is an analogue of an effective natural pesticide, plumbagin (Magdum *et al.*, 2001). It is known to have insecticidal properties against a cotton pest, *Dysdercus cingulatus* F. (Hemiptera, Pyrrhocoridae) (Singh-Gupta *et al.*, 2015). Moreover, it is biodegradable by bacteria in soil (Li, Song and Gao, 2011) and is light-sensitive. Hence menadione may serve the purpose of protecting the crop from pests. Menadione was found to decrease the content of carbohydrates (Singh-Gupta *et al.*, 2013) and proteins in the different organs of *D. cingulatus* (Singh-Gupta and Magdum, 2019). The decline in carbohydrate content could be explained based on the antifeedant property of the plumbagin and menadione. However, the decline in the protein content cannot be stated simply. Eventually, the Nucleic acids are the master macro molecules controlling all the morphological development and metabolic activities of insects either directly or indirectly. Hence in the present work, the effect of menadione on RNA content was explored. Moreover, the effect of menadione on the gonads of *D. cingulatus* has been noted, as it is an analogue of a known chemosterilent, plumbagin (Magdum *et al.,* 2001). Menadione has produced several histological deformities (Singh-Gupta and Magdum, 2016), (Singh-Gupta *et al.,* 2014) in the gonads, and brain of *Dysdercus*, which is a reflection of a reduced amount of protein (Singh-Gupta and Magdum, 2019) and DNA content (Singh-Gupta *et al.*, 2020). The current research has been designed to find out the toxic effect of menadione on the RNA content in gonads, fat body and brain of *D. cingulatus*.

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Insects were procured from the Zoology Department, Deshbandhu College, Delhi University. The adult male and female were separated by observing the size, as males are smaller as compared to females. Four pairs of adult insects were released in each rearing jar. These insect jars contain wet moist soil forming a layer at the bottom and the mouth of these jars was closed using muslin cloth and tied with a rubber band. The insects were fed on soaked cotton seeds. The females after mating laid eggs three times during the period of 3 to 4 days. A single female laid around 70 to 80 eggs. The life cycle of *D. cingulatus* consists of 5 intermediate nymph stages before molting into final adult form. Newly molted adult *D. cingulatus* were treated topically with different sub-lethal concentrations of Menadione 0.25µg, 0.5 µg and 0.75 µg (standard solution of 1mg/ml Menadione in acetone) using a Hamilton syringe and they were dissected on different post-treatment days (PTD) viz. 2, 4 and 6 in chilled insect ringer's solution. The tissues of the treated and control males and females were separated. These tissues were homogenized separately using Eppendorf and pestle in 10 per cent chilled Buffered Saline {0.15M Sodium Chloride (NaCl) + 0.015M Sodium tri Citrate (Na_3 Citrate)}. This solution was then subjected to centrifugation at 5000 rpm for 20 minutes at cold conditions (4°C) and the supernatant was used for the estimation of RNA (Raksheskar, 2012). Later, RNA was quantified by the method of Orcinol reaction.

The results of colorimetric analysis showed universal decline in the RNA content of tissues under investigation upon treatment with different doses of Menadione. The significance of these changes was calculated by applying the T-test using the software "Minitab 14" and was noted. With the increase in the concentration of Menadione and the post treatment days, the amount of RNA declined in the tissues under investigation (Table 1).

Protein is one of the main biomolecules in an organism which plays a crucial role in regulating various metabolic pathways. Any fluctuations in the protein content are directly going to affect the functioning of an organism. Menadione has been known to induce a decline in protein content (Singh-Gupta and Magdum, 2019). The quantity of protein in an organism is a result of a delicate equilibrium between the rate of protein synthesis and degradation. Several factors may come together to influence the quantity of protein in the organs of *Dysdercus*. These factors operate at three different levels to regulate the quantity of protein in a tissue. The first level is at DNA, as Menadione is known to alter the DNA structure and bases as a result of Reactive Oxygen Species formation, which consequently leads to its degradation. So, this damage to DNA could be the probable cause of protein decrease (Jena, 2012). The second level is RNA, as the synthesis of RNA plays an important role in the process of protein synthesis. So, the inhibition of RNA synthesis at the transcription level or degradation of RNA may also affect the protein level (Lin-Quan Ge, 2009). Even any disturbance in the translation process owing to oxidative stress caused by menadione will also lead to a decline in protein content. (Kong and Li, 2010). The third level is at the protein level, where either the decrease is due to the diversion of energy when an animal is under stress (Manoharan and Subbiah, 1982; Mommnsen and Walsh, 1992) or it's due to the degradation of proteins, which could be possible through actions such as impaired incorporation of amino acids into polypeptide chains (Singh *et al.,* 1996) or formation of reactive oxygen species by Menadione.

In light of the above-discussed interrelations and the fact that menadione has led to the decline in protein and DNA (Singh-Gupta *et al.*, 2020) content of various organs, the present work has incorporated the evaluation of RNA in gonads, fat bodies, and brain. A significant decline in RNA content of tissues was in accordance with many workers like Attrib and Ravi (1980 a and b); Naqvi *et al.* (1989*,* 1993), who focused on the fact that Insect growth regulators could act as a pesticide and exhibit a moderate to high level of inhibition of both RNA and DNA. Similarly, Phillips and Loughton (1979) reported sixty percent inhibition in RNA in *Locusta migratoria* after Dimilin treatment. According to him, even protein synthesis was also inhibited. Perveen (2012) also reported the decline in the DNA and RNA content after

Table 1. RNA content (µg/ml) in the gonads/ fat body/ brain of the treated male and female *Dysdercus cingulatus* (** - Highly significant and * - significant)

Organ	Menadione (µg)	RNA (µg)±SD on PTD 2	RNA (µg)±SD on PTD 4	RNA (µg)±SD on PTD 6
Testes	Control	48.49±1.14	65.16±0.51	58.42±1.81
	0.5	47.99±0.14	63.94±0.69*	54.13±0.66*
	0.75	47.43±0.68	62.20±0.84**	50.5±1.66**
	1.0	44.19±0.79**	53.25±0.24**	50.50±1.66**
Ovary	Control	226.6±8.08	284.6±14.2	274±10.500
	0.5	219.6±4.04	279.6±2.88	267.3±14.0
	0.75	205.3±14.4*	261.6±7.5*	263±8.66
	1.0	199.3±14.6*	232.6±9.23**	231.06±9.07**
Male fat body	Control	65.04±0.41	63.3±1.15	62.70±0.28
	0.5	64.12±1.00	62.39±0.41	61.76±0.30**
	0.75	63.00±0.38**	60.34±0.60**	59.51±0.81**
	1.0	59.02±1.17**	54.11±0.27**	53.72±1.13**
Female fat body	Control	69.02±0.42	72.48±0.69	68.65±1.02
	0.5	69.02±0.42	71.54±0.78	65.62±4.89
	0.75	68.19±0.93	68.36±1.00**	64.64±0.95**
	1.0	64.41±1.06**	64.39±0.81**	63.56±0.75**
Male Brain	Control	53.67±0.88	52.43±0.75	51.42±1.91
	0.5	53.42±1.15	52.27±1.03	49.8±1.26
	0.75	51.55±1.50	51.14±0.71	49.59±1.01
	1.0	50.00±0.32**	50.49±0.40**	49.59±1.02
Female Brain	Control	53.77±1.22	52.47±0.76	52.56±1.15
	0.5	53.10±0.81	51.64±0.47	51.80±0.76
	0.75	52.26±0.00	50.14±1.34*	51.80±0.76
	1.0	48.17±1.42**	50.18±0.34*	49.43 ±0.37*

Chlofluazuron treatment in *Spodoptera litura.* Devi *et al.* (1985) also noted a decline in the content of RNA of the fat body of *Dysdercus koenigii* during the treatment of Actinomycin-D. In the present work, RNA was inhibited by the Menadione during ovaries, testes, and egg development. The decline is noted at all three levels. However, the percent decline in DNA, RNA, and protein is not the same which shows that menadione affects the protein content of gonads at different levels by affecting or degrading DNA, RNA and Protein. The disparity of DNA and RNA content decline is in accordance with Chinzei and Tojo (1972) and Premkumar *et al.* (1991) who noted a similar disparity in DNA and RNA contents when observed in *Bombyx mori* and water scorpion, respectively. These reports were also supported by Perveen (2012) that, the percentage of DNA and RNA was dissimilar after treatment with sublethal doses of Chlorfluazuron even when they were noted on the same day and at the same stage of *Spodoptera litura*. Hence, this decline in the RNA content of gonads is partially responsible for the overall decline observed in the protein content of gonads upon Mendaione exposure. Eventually, this effect was reflected in the histopathological deformities in gonads that ultimately affected the reproductive potential of *D. cingulatus* (Singh-Gupta *et al.*, 2013; Singh-Gupta and Magdum, 2016).

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