Effect of Tetrahydroxy-P-Benzoquinone on reproduction performance of *Dysdercus cingulatus* (Hemiptera: Pyrrochoridae).

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Abstract:

To understand the effect of Tetrahydroxy-p-benzoquinone on reproduction performance freshly moulted 24-36 hours old adult *Dysdercus cingulatus* were separated from culture and treated with sublethal doses. Three experimental group namely Group I where both the adults male and female were treated Group II only females were treated and Group III where males were treated along control group were kept . Numbers of viable eggs laid number of eggs hatched into first instar and their further development into nymphs / adults was noted. A sterility index indicative of decreases in fecundity was calculated; with this index a value of 100 represents complete infertility.

Keywords:Tetrahydroxy-p-benzoquinone, *Dysdercus cingulatus*,sublethal doses, sterility index, fecundity, infertility.

Introduction

Now a day emphasis is given on development of insect control agents which show their sufficient efficacy against resistant and non-resistant strains of pests. Further, it is emphasized that theyshow high margin of safety for man and the environmentand also gets used in IPM (Integrated Pest Management) programmes. For identification of such suitable insecticides in IPM an array of standardized laboratory and semi-field test protocols have been developed. Thus, based on their observed lethal and sublethal effects they are categorized into different harmful and safe compounds.

Literature survey revealed that the aquacidally effective compound is obtained from quinone, naphthalenedione, anthraquinone, and mixtures of these.

Many of the secondary metabolites are studied to understand their efficacy. Quinone act as moulting and growth inhibitor and are found to interfere with chitin synthesis. Naturally occurring quinone- Plumbagin shows adverse effect on the growth and development of *D.cingulatus* .(Magdum et al 1999 ; Joshi et al 1988). Certain quinones are effective in controlling or inhibiting plant and animal growth in water include 1,4,benzoquinone (quinone), 2,5-dihydroxy, 3,6-dinitro-p-benzoquinone (nitranilic acid), 2,6-dimethoxybenzoquinone, 3-hydroxy-2-methoxy-5-methyl-p-benzoquinone (fumagatin), 2-methylbenzoquinone (toluquinone), tetrahydroxy-p-benzoquinone (tetraquinone), 2,3-methoxy-5-methyl-1,4-benzoquinone, 2,3-methoxy-5-methyl-1,4-benzoquinone, and mixtures of these(Cutler et al 2003). Ambient ultraviolet radiation degrades the aquacidal compound into harmless by-products. Such an action at extremely low concentrations offers opportunities for control over translocatable aquatic pest organisms, control or sterilization of undesired microorganism “blooms” in geographically limited regions, and similar situations.

Although the mode of action of hydroxybenzoquinone as a post-ingestive repellent is known, the mechanism behind the emetic nature in insects is still unclear. Future, research should

elucidate this mechanism. A thorough understanding in mode of action of such benzoquinone in pest species will enable more reliable use of such compounds in pest management.

There are array of parameters which are considered to understand the efficacy of compound. In standard laboratory assays, an interesting evaluatory parameter is to understand the sublethal effects of insecticide on the reproductive performance of insects. Although the toxicity of many such chemical compounds has been studied in insects, there is no evidence of studies on THBQ. Here an attempt is made to analysis the reproductive performance of said compound on *Dysdercus cingulatus*, Red cotton bug.

Cotton is the most economically important natural fiber material and man has been utilizing this for his benefits since ancient times. It is a major cash crop of India, which is often infested *by Dysdercus cingulatus*, Red cotton bug. Both nymphs and adults suck the cell sap from the leaves and tender shoots and impair the vitality of the plants. If the attack is severe, bolls open badly and the lint is of poor quality. In addition they also feed on the seeds and lower their oil content and low percentage of germination such seeds are unfit for sowing. The lint is stained by the excreta of bugs or by their body juice as they are crushed in the ginning factories so named cotton stainer. The most serious damage done is the infection of fungal spores of the genus *Nematospora* into the ball may abort and go brown because of death of seeds.

Materials and Methods

Test compound: Tetrahydroxy-1-4-benzoquinone also called Tetrahydroxy-p-benzoquinone Tetrahydroxybenzoquinone or Tetrahydroxyquinone (THBQ or THQ) is an organic compound with molecular formula $C_6O_2(OH)_4$. It is cyclohexadiene compound with four hydroxyl groups and two ketone groups in opposite (para) positions.

Test insect:

Red cotton bug, *Dysdercus cingulatus* has been used in as the test insects in present investigation. It is the major pest of cotton.

C**ollection:** Adults and nymphs *of Dysdercus cingulatus* were collected from cotton field at Chalisgaon, Maharashtra. The bugs live in colony on cotton plants hence plenty of bugs in different developmental stages was collected.

Rearing: The various nymph instars and adults collected were reared in glass jars. The culture of this pest was maintained in glass rearing jar containing a layer of moist and coarse sand. The mouth of these jars was covered with a piece of muslin cloth fixed with rubber band. All the stages were fed on the fresh healthy soaked cotton seeds .The culture was maintained at 27± 2ºC at laboratory conditions.

Treatment

Test compound THBQ was dissolved in acetone (1mg/ml). Requisite volume of test solution with the help of Hamilton microlitre syringe was then topically applied to the ventrolateral region on abdomen of 24-36 hours freshly moulted adults.The treated insects were placed back into separate jar. A control group of insects were set up with every experimental group for comparison of results. These insects were initially treated with acetone alone in equivalent amounts as the test compound solution. However no difference was observed at any stage between these acetone treated and normal (untreated) insects. In subsequent experiments therefore untreated insects were taken as controls and the same is mentioned in present investigation.

Prior to understand the reproduction performance in treated insects, LC_{50} was calculated and sublethal doses were set. Five pairs of 24-36 hours old adult male and female were kept in following four groups of for experimentation. Following are the details

Control group: this group was set as a control containing 5 pairs of insects which were untreated.

Group 1: In this group **both adult male and female** were treated and placed in jar.

Group II: In this group **only females** were treated and males were left untreated.

Group III: In this group **only males** were treated and females were left untreated.

The extent of the effect on reproduction was derived by calculating Sterility Index according to Saxena et al (1996).

Sterility Index = 100 - $\frac{Avg\ no.of\ egg\ laid\ by\ treated\ group}{Avg\ no.of\ egg\ laid\ by\ control} X \frac{Percentage\ of\ egg\ hatched}{Percentage\ of\ egg\ hatching}$ X 100

The effect of the above treatment on survival sensitivity during copulation, egg laying hatching , fecundity and survival of nymph after egg hatching was observed. The result was statistically analyzed by three-way ANOVA.

Results:

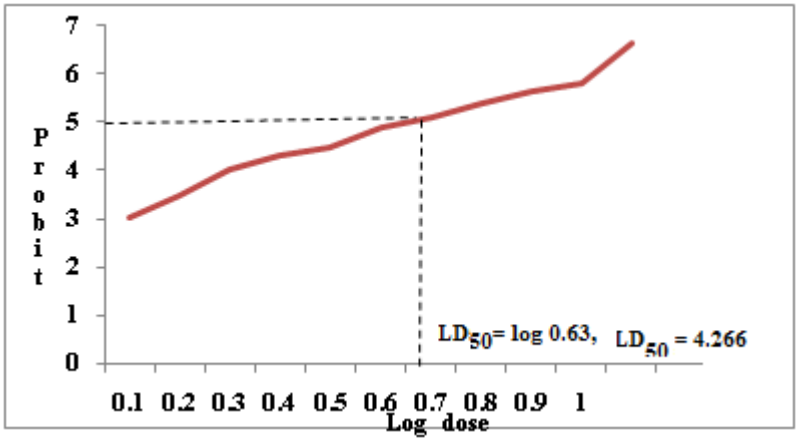
A. Estimation of the dose range and percentage of mortality

An approximate LD_{50} was initially determined as a pilot study by a so called 'staircase method' using 20 insects and increasing the doses of THBQ. Ten doses(1-10µg/ml) were chosen for determination of LD_{50} starting from no death to 100% mortality. In our study for estimation of LD_{50} of THBQ doses were given topically to ventro-lateral side of abdomen of 24-36 hours old adult *Dysdercus cingulatus* irrespective of their sexes (Table-1). The treated insects were kept with control group (without any treatment of THBQ) and observed for 7 days

Table 1: Results of the lethal doses of THBQ for the determination of LD_{50} after Topical application in *Dysdercus cingulatus* (n=20)

Dose	Log dose	% Mortality	Corrected % Mortality	Probit
Control	-	0	2.5	3.04
1	0	10	7.5	3.49
2	0.3	20	17.5	4.05
3	0.48	25	24.98	4.33
4	0.6	30	29.97	4.48
5	0.7	47	46.97	4.92
6	0.79	55	54.97	5.13
7	0.85	65	64.97	5.39
8	0.9	75	74.97	5.67
9	0.94	80	79.97	5.84
10	1	95	94.97	6.65

Fig.1. Plot of log-doses versus Probits from Table-1 for calculation of LD_{50} of THBQ administered topically in *Dysdercus cingulatus* (n=20)



In the present case the Log LD_{50} is 0.63 and LD_{50}= 4.266 µg/ml (Fig 1). The SE of LD_{50} was also calculated from the following

Approx. SE of $LD_{50} = \frac{(\text{Log } LD_{84} - \text{Log } LD_{16})}{\sqrt{2N}}$ where N is number is insects used (20 in this experiment)

Using this formula the SE of LD_{50} was obtained as 2.42.Therefore, LD_{50} of THBQ when given topically is **4.266 ± 2.42 µg/ml**, with 95% confidence interval of 2.26 – 6.68µg/ml.

B. Reproduction performance in THBQ treated *Dysdercus cingulatus*

On the basis of LD_{50}, four doses namely 3, 4, 5 and 6µg/ml of THBQ was topically applied to three groups as mentioned earlier in Materials and Methods. A control group was maintained for comparison. Result is analyzed using three way ANOVA (Table 3)

Control Group:

A control group of untreated males and females was maintained for studies on effect on reproduction. These insects showed normal mating behavior. Mating occurred on second or 3rd day of emergence in these insects.

Egg laying: Egg laying was normal and an average of 130 eggs were laid after 2-3 days of mating. These eggs were pale yellowish oval in nature. The female showed a total of three laying. The control insects survived a normal life span of 28-30 days.

Hatching and moulting: Eggs hatched to small red pin head sized nymphs after seventh day.They were seen in cluster around soaked seeds indicating normal feeding behavior. They further subsequently moulted upto adult stages by spending normal interecdysial period of 7 days.

Treated groups : Topical application of THBQ to freshly moulted (24-36 hrs) adult show remarkable changes in egg laying, hatching and moulting to next instar

3µg/ml (Table 2 -4; Fig 2, 3 and 4)

Group I: In this case copulation was observed from 2nd/3rd day of treatment and it was discontinuous. Average number of eggs laid was 35. It is observed that 20 eggs hatched and the nymphs moulted only upto IInd instar stage .These IInd instar nymph showed reluctance to feeding which resulted in flattened abdomen and finally died on 5th day

Group II: Copulation was observed on 2nd-3rd days of treatment and it was discontinuous. Average number of eggs laid by this group was 90.Out of this 55 eggs hatched and the nymph subsequently moulted upto IVth instar stage.

Group III: Copulation was observed on 2nd day and it was continuous. Average number of eggs laid by this group is 90 out of which 45 eggs hatched. The nymphs showed moulting to 3rd instar stage.

Sterility index of the above dose if compared showed that S.I of Group I is 81.82% is more as compared to S.I of Group I and Group III which are 59.09% and 50% each.

4µg/ml (Table 2 -4; Fig 2, 3 and 4)

Group I: With slight increase in dosage i.e. 4µg/ml copulation was still observed on 2nd-3rd day of treatment .Copulating insects used to get separated and thus was discontinuous. Average number of eggs laid by this group was found to be 25. It was observed that 14 eggs hatched and the nymphs moulted only to 1st instar which showed mortality on 5th day of emergence.

Group II: Copulation was observed on 2nd-3rd days even in 4µg/ml treated female group and was discontinuous type. Average number of eggs laid in this group was 67. Out of this 40 eggs hatched and moulted upto IIIrd instar stage. There was abnormal change in interecdysial period noted.

Group III: Copulation was observed on 2nd day and it was continuous. Average number of eggs laid by this group is 55 out of which 33 hatched to Ist instar and subsequently moulted to IInd instar. These IInd instar nymphs were smaller in size as compared to normal group. They showed reluctances to feeding and died on 4th day of emergence

Sterility index in 4µg/ml treated Group I was found as 87.28% while that in Group II and Group III was 70 % and 63.64% respectively.

5µg/ml (Table 2 -4; Fig 2, 3 and 4)

Group I: In 5µg/ml treated males and females; copulation was observed normal on 2nd-3rd day of treatment but was discontinuous type. Average number of eggs laid was very less i.e. 18 which were seen in 5 small groups. It was observed that 10 eggs hatched and moulted to 1st instar stage.These 1st instar survived only for 5 days and subsequently showed mortality.

Group II: In 5µg/ml treated females,copulation was observed regularly on 2nd-3rd days and it was discontinuous. Average number of eggs laid by this group was 42 in small groups.Out of this 25 eggs hatched to Ist instar on 9th day . These Ist instar moulted to 2nd instar on 9th day .

Group III: Copulation was observed on 2nd day and it was continuous. Average number of eggs laid by this group was 40 out of which 24 eggs hatched to IInd instar stage. The nymphs moulted and reached to IInd instar but survived only for 4 days.

Sterility index 5µg/ml treated Group I insect was 90.91% where as in Group II and Group III it was 78.18% and 77.28% respectively.

6µg/ml (Table 2 -4; Fig 2, 3 and 4)

Group I: With the highest dose still the copulation was observed on 2nd-3rd day of treatment and it was of discontinuous type. Average number of eggs laid was far less than control and was only 8 when both males and females were treated. These were all scattered. It is observed that only 3 eggs hatched and these nymphs showed mortality on 2nd day of emergence.

Group II: When females were treated with 6µg/ml THBQ, copulation was observed on 2nd days of treatment and it was discontinuous. Average number of eggs laid by this group was 37.Out of this, 20 eggs hatched to Ist instar stage survived for 4 days.

Group III: Copulation was observed on 2nd day when males were treated with 6µg/ml THBQ. An average of 34 eggs were laid by this group is out of which 17 eggs hatched to 1st instar stage and survived only for 3 days.

Sterility index of the above dose Group I is 97.26% is more as compared to S.I of Group II and Group III which are 84.55% and 81.82% each.

Table No 2: Average no of egg laying, hatching and moulting to different instars in THBQ treated *Dysdercus cingulatus*

LD_{50} =4.266 ± 2.42 µg/ml							
Dose	**Group**	**Average eggs laid**	**% of Eggs laid**	**Average no. of hatching**	**% of hatching**	**Nymphs reached next instar**	**Percentage of Nymphs reaching next instar**
Control		130	100	110	84.61	adult	83.78
3µg/ml	I	35	26.92	20	57.14	2nd	57.14
	II	90	69.23	55	61.11	4th	61.11
	III	60	46.15	45	75	3rd	75
4µg/ml	I	25	19.23	14	56	1st	60
	II	67	51.54	40	59.70	3rd	59.70
	III	55	42.30	33	60	2nd	45.45
5 µg/ml	I	18	13.85	10	55.55	1st	55.55
	II	42	32.30	25	59.52	2nd	59.52
	III	40	30.76	24	60	2nd	55

6 µg/ml	I	08	6.15	03	37.5	Not moulted	37.5
	II	37	28.46	20	54.05	1st	54.05
	III	34	26.15	17	50.00	1st	44.11

Table 3: Three way ANOVA

Source	DF	SS	MS	F	P
Between	4	20604.933	5151.233	15.534	0.331
Within	10	3316	331.6		
Total	14	23920.933			

Table No 4: Sterility index in THBQ treated adult groups

Dose	Groups	% of Eggs laid	% of Hatching	% of Moulting	Sterility index (SI)
Control		100	85.0	84.0	-
3µg/ml	I	26.92	57.14	57.14	81.82
	II	69.23	61.11	61.11	59.09
	III	46.15	75	75	50
4µg/ml	I	19.23	56	60	87.28
	II	51.54	59.70	59.70	70
	III	42.30	60	45.45	63.64
5µg/ml	I	13.85	55.55	55.55	90.91
	II	32.30	59.52	59.52	78.18
	III	30.76	60	55	77.28
6µg/ml	I	6.15	37.5	37.5	97.26
	II	28.46	54.05	54.05	84.55
	III	26.15	50.00	44.11	81.82

Fig 2. Impact of Tetrahydroxybenzoquinone on egg laying in *Dysdercus cingulatus*

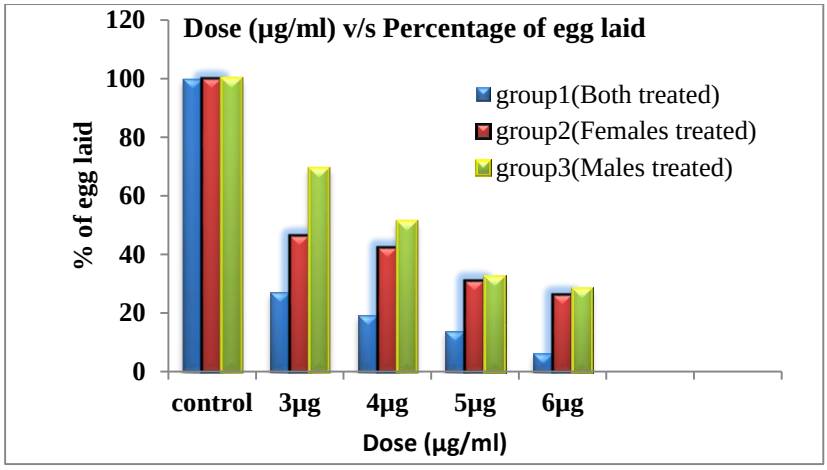


Fig 3: Impact of Tetrahydroxybenzoquinone on hatching in *Dysdercus cingulatus*

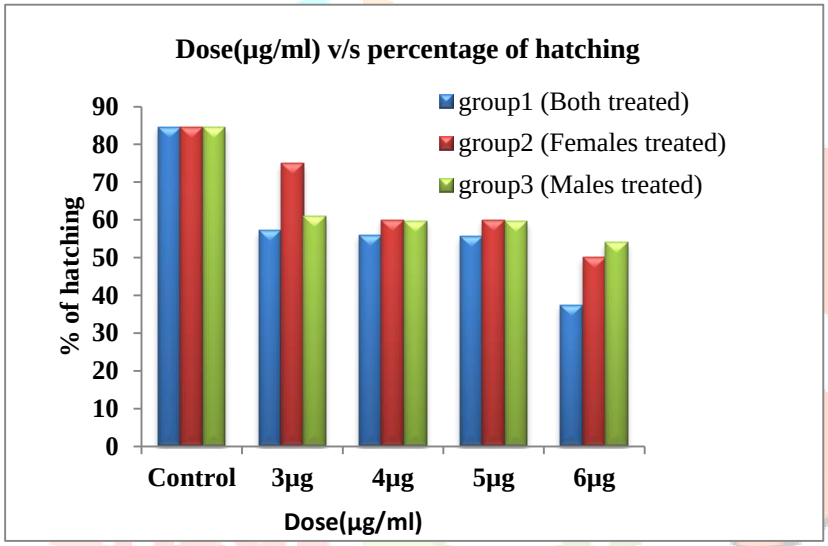
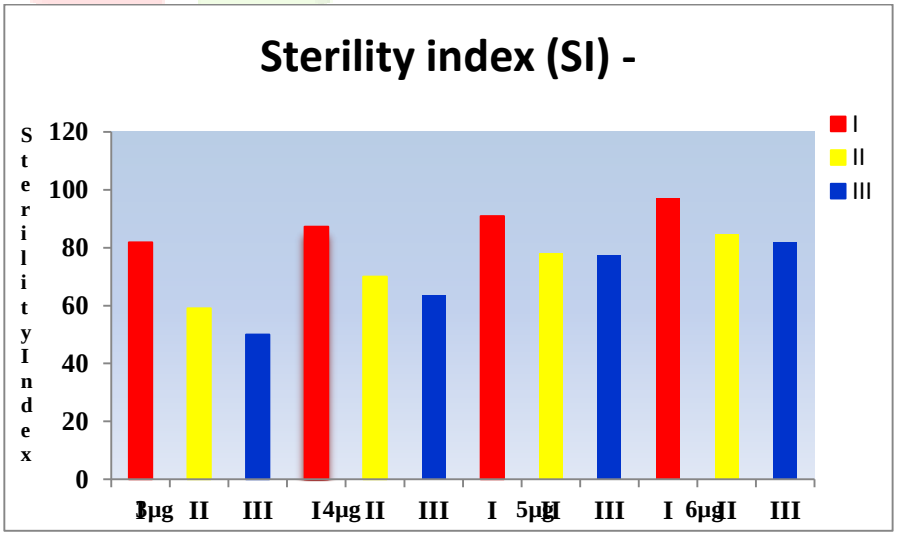


Fig 4: Sterility index in Tetrahydroxybenzoquinone treated groups of *Dysdercus cingulatus*



Discussion

Quinones encompass large number of naturally occurring compounds (Thomson 1987). They are the functional constituents of several biochemical systems. In addition they have the capacity to produce free radicals and can form adducts with cellular constituents (Ollinger and Brunmark 1991).

Tetrahydroxybenzoquinone is mainly used in hydroquinone production. The compound can be synthesized from glyoxal (A.J.Fatiadi and Sanger(1962) or from myo-inositol (phytic acid) a natural compound widely present in plants(Haiyan Chen 2009). It is also used as a polymerization inhibitor and as an intermediate in the production of a variety of substances, including rubber accelerators and oxidizing agents. Further, studies revealed that 1,4-Benzoquinone readily gets absorbed from the gastrointestinal tract and subcutaneous tissue [species not specified]. It is easily metabolized and thus gets excreted partly in unchanged form and partly as hydroquinone (IARC, 1977).Chapman et al in 1994 reported that 1,4-Benzoquinone acts as dysmorphogenic to rat embryos in an in-vitro system .Being a metabolite of benzene it is found to inhibit Fc and complement receptor-mediated phagocytosis in peritoneal macrophages in murines. It also caused marked decrease in the filamentous actin content of the macrophages. (Manning et al., 1994).

Keeping these above aspects in mind present work was undertaken with a purpose to understand the efficacy of Tetrahydroxybenzoquinone as new insect control agent.

Most of the quinine compound show morphogenetic variation and sterility in most insects. Same has been found with respect to THBQ. Sterility in *Dysdercus cingulatus* in present compound is dose dependent. It is been observed that in Group I (both treated) and Group III (Male treated) insects, mating was affected and were seen with lower fecundity. It was interesting to note that they showed copulation but females laid very few and scattered eggs which never hatched with 6µg/ml dose of THBQ.

It was observed that there was significant reduction of egg laying eggs, hatching and further development of up to final instars stage. The sterility Index increased with increase in dose of THBQ. This finding is confirmed with those obtained by (Magdum et al 2001) when the insects were treated within 2,6 dimethlybenzoquinone and menadione. Interrupted copulation, lesser egg laying and few viable eggs affects the life cycle of Dysdercus. Similar results were observed by Sharma et al in *Dysdercus cingulatus* when treated with extracts of Neem.

According to Pandey et al,2011 ,Neem Based Insecticides affects metamorphic developments, copulation and fecundity in insects. These are controlled by Juvenile Hormone by regulating the release of allotropic factor from brain. All these effects are stage specific and are dose dependent. The role of Corpora Allatum (CA) or its hormone in regulation of copulation and egg maturation has been reported earlier by (Srivastava & Tiwari (1978) and Tiwari & Srivastava(1979). It is therefore assumed that the reduction in copulation period and in egg maturation is caused by interruption of JH hormone. To have the proper understanding of effect of THBQ further detail histological, biochemical and enzymological assay is require

Conclusion

A large number of naturally occurring and synthetic quinone were evaluated for activity against the bugs, beetles, termites mosquitoes etc. Present work deals with Tetrahydroxy-p-benzoquinone which is an organic compound with formula $C_6O_2(OH)_4$. Topical application of various doses of THBQ showed that reproduction performance is affected when both the insects are treated (Group I) and when males are treated (Group III)

Result from present studies indicates that the sterility index increases with increase in dosage and it was higher in Group I (both treated)followed by Group II and III. The sterility index is directly proportional to the dosage of THBQ on adults of *Dysdercus cingulatus.*

Thus from the present studies it can be concluded that the bioactivity of Tetrahydroxy-p-benzoquinone affect the reproduction of *Dysdercus cingulatus* and induce sterility.

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