# Effect of tetra hydroxyl-ρ-benzoquinone on growth and metamorphosis of *Spodoptera litura* Fabr. (Lepidoptera: Noctuidae) larvae

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## ABSTRACT

Secondary metabolites of plant are useful in defensive mechanism. These includes vast array of chemicals like phenols alkaloids, quinines, etc. Quinones are heterocyclic compounds consisting of prototypical member of the class includes 1,4-benzoquinone or cyclohexadienedione, 1,2-benzoquinone (*ortho*-quinone), 1,4-naphthoquinone(*para*-quinone) and 9,10-anthraquinone. 1,4-benzoquinone or cyclohexadienedione is often called quinone and are found to either stimulate or inhibit the feeding in insects. Of these, tetrahydroxy-1,4-benzoquinone, also called tetrahydroxy-*p*-benzoquinone, was tested for its effects on growth and metamorphosis of *Spodoptera litura* larva. Fifth instar larvae were topically treated with various concentrations of acetone solutions of tetrahydroxy -*p*-benzoquinone to test the efficacy of the compound. It was interesting to note that the treated group showed reduced relative feeding. Also interference in moulting process, ecdysial failure and blockage of normal adult emergence were the important morphogenetic abnormalities observed which resulted in the formation of larval-pupal intermediates, abnormal pupae, and deformed adults. These deformed adults were further observed for development and reproduction. Moreover dose dependent effects were observed in growth and metamorphosis of *Spodoptera litura* larvae which were compared with the control group of same age. Although several classes of chemical have been found to interfere with moulting and metamorphosis in insects, tetra hydroxy *p*-quinone is found to be one of the potent insect growth inhibitor.

**Keywords:** tetra hydroxyl-ρ-quinone, anti-feedant, insect growth regulator, morphogenetic abnormalities, ecdysial failure

## INTRODUCTION

In nature different plant species are accepted or rejected as food by various phytophagous insects. This totally depends on the presence of certain chemicals which are known as secondary metabolites of plants. These secondary metabolites include phenols, alkaloids, quinines, etc and are found to either stimulate or inhibit the feeding in insects. More commonly these are defined as botanical pesticides which are now emerging as one of the prime means to protect crops and their products .Also they are useful in protecting the environment from pesticide pollution as can degrade more rapidly than chemical pesticides, and are, therefore, considered relatively environment friendly. Further studies have provided information that they are less likely to kill beneficial pests than synthetic pesticides with longer environmental retention [1].



pISSN 2319-1244, eISSN 2279-0160

In recent years many feeding deterrents in the plants are investigated which can be used as protectants against many of the insects. Most of the botanical pesticides generally degrade within few days and sometimes within a few hours, these pesticides needs to be applied more frequently. More frequent application coupled with higher costs of production makes botanicals more expensive to use than conventional pesticides. In spite of wide recognition that many plants possess pesticidal properties, only a handful of pest control products obtained from plants (pyrethrum, neem and rotenone) are in use because commercialization of botanicals is hindered by several issues [2]. Moreover, in a country like ours, where agriculture is an important source of living but where farmers have only limited means, we urgently need to look for alternatives to chemical knowledge of compounds of plant origin which could be used in insect pest management. It will also help the poor farmers to compete with insect pest. Thus, alternative strategies for development of new types of pesticides which are often effective against a limited number of specific target species, and also are biodegradable into nontoxic products and are suitable for use in integrated pest management programs are required.

In this context isolation of different quinones from plants and micro-organisms are carried out ambitiously. These quinones are heterocyclic compounds and are named with a prefix that indicates the parent aromatic hydrocarbon. They commonly includes benzoquinone, naphthoquinone , anthraquinone or their derivatives. All these are oxidized derivatives of aromatic compounds and are often made from reactive aromatic compounds with electron-donating substituents such as phenols and catechols, which increase the nucleophilicity of the ring and contribute to the large redox potential needed to break aromaticity.

Hydroxybenzoquinone (formula: $C_6H_4O_3$) is one of the several organic compounds that can be viewed as derivatives of a benzoquinone through replacement of one hydrogen atom (H) by an hydroxyl group (-OH). The number of hydroxyl group helps in the nomenclature of the compound. Synthetic organic chemists encouraged by the potential applications of quinones have devised a plethora of synthetic strategies which led to the explosion of articles reporting newer and interesting benzoquinone derivative. One such derivative is tetrahydroxy-1,4-benzoquinone. It is also called tetrahydroxy-*p*-benzoquinone, tetrahydoxybenzoquinone or tetrahydroxyquinone (Tetrahydroxybenzoquinone, THQ), with molecular formula $C_6O_2(OH)_4$. Its molecular structure consists of a cyclohexadiene ring with four hydroxyl groups and two ketone groups in opposite (*para*) position. According to Khalafy et al. [3], hydroxyls adjacent to the ketone groups often exhibit intramolecular hydrogen bonding, which affects their redox properties and their biochemical properties. The hydroxyquinones include many biologically and industrially important compounds, and are a building block of many medicinal drugs. These act as antifeedents.

Thus here an attempt is made to study effect of tetrahydroxybenzoquinone on *Spodoptera litura* (Fabr.), one of the most destructive and widely distributed Lepidopteran pests of many important crops. The larva feed on a wide range of plants and has been recorded mostly from dicotyledonous plant families. It is a major pest of tomato, sugar beet, legumes, cotton, maize, soybean, tobacco, pepper, potato, onion, sunflower, citrus, etc. Through the insect's powerful mandible, it could destroy and damage all the stages of crops by cutting the stem and consume the whole seedlings, chewing the leaves in the open field causing irregular holes and boring the developing heads with lace of larval excrements. If not controlled, the insect can reduce yield to as high as 50 % to 100 %. A control measure for this pest is expensive and unsuccessful because the pests are fecund and polyphagous. Moreover it has developed resistance against insecticides. The most successful crop protection recommendations include monitoring of pest populations, implementation of integrated pest management with conservation and augmentation of parasites and predators, the use of sex pheromone traps and hormone inhibitors, and the use of transgenic Bt crops [4-6]. The purpose of this present research was to (1) determine the efficacy of Tetrahydroxybenzoquinone as chemical alternatives to control fifth instar larva of *Spodoptera litura* and (2) to evaluate the toxicity of this alternative. The benefits would cut down expense and get rid of the toxic residue problem in environment. Moreover according to Kim et al. [7] larvae of 4-5th instar have high tolerance to chemical pesticides. Thus these were chosen for experimentation.

## MATERIALS AND METHODS

### Collection and identification and rearing of *Spodoptera litura*

Initially insect's larvae were collected from the Vani region, Nashik and reared. On adult emergences these were identified using the standard keys and used for rearing. The *S. litura* test insects were the progenies that have undergone one generation rearing in laboratory conditions were later used for experimentation.

### Specific characteristics of *Spodoptera litura*

Adult moths measure between 15-20 mm (0.59-0.79 inches) in length and have a wingspan of 30-38 mm (1.18-1.5 inches). Forewings are gray to reddish-brown, with a complex pattern of creamy streaks and paler lines along the veins. Hind wings are grayish-white with grayish-brown margins. Males have a blue-grey band from the upper corner (apex) to the inner margin of each forewing. Females lay 1000 to 1500 eggs covered with body hair scales on the ventral surface of castor leaf. Eggs hatch to first larval stage after 8 days of laying. The larvae pass through six instars in 15-23 days at 25-26°C. Larvae were pale green with bright yellow stripes along the back and the sides. These later on transformed to brown coloured mature larvae with three thin yellow, longitudinal lines: one on the top or dorsal side and one each lateral side. A row of black dots runs along each lateral side, and a row of dark triangles decorate each side of the middle, dorsal line. Young larvae or caterpillars are a translucent green with a dark thorax. They are smooth-skinned. First to third instar larvae feed in groups, leaving the opposite epidermis of the leaf intact. Later, the fourth and fifth instar larvae disperse and spend the day in the ground under the host plant, feeding at night and early in the morning. Thus feeding is initially by skeletonizing, or leaving the outline of the leave veins on the plant. As growth continues, caterpillars/larvae eat entire leaves, and even flowers and fruits. The pupal period is spent in earthen cells in the soil and lasts about 11-13 days at 25°C. Longevity of adults is about 4-10 days, being reduced by high temperature and low humidity. Thus, the life cycle can be completed in about 5 weeks. The Caterpillar burrows into the soil several centimeters and there pupates without a cocoon. While pupating, it produces large amounts of fluid. Attempts to allow pupation in captivity within an empty glass jar have resulted in drowning.

### Rearing of *Spodoptera litura*

Three pairs of adult insect were kept in a large glass rearing chamber of 2 × 1 feet dimension for the growth of the culture. This glass rearing chamber was laid with soil base of 2 and 1/5 inches and was covered with cotton cloth. Castor leafs, *Ricinus communis* were kept in the chamber to support laying as the female moth lays egg on ventral surface of leaves. All experiments were conducted in a growth chambers with 15 × 15 cm under the same conditions. Larvae were closely monitored and freshly moulted 24-36 hours old fifth instar larvae were used to set up the experiment.

### Test material

Efficacy of acetone solutions of Tetrahydroxybenzoquinone on growth and metamorphosis of lepidopteran insect *Spodoptera litura* were conducted in the laboratory of the PG Department of

Entomology, KTHM College, Nashik. The temperature of 28 ± 2° C, 12 : 12 L : D photoperiod and 70 ± 5 % relative humidity during the experiments was maintained in the laboratory.

## Acute toxicity

Acute toxicity (short term effects) of tetra-hydroxybenzoquinone was tested against fifth instar larva of the *Spodoptera litura*. The efficacy of sublethal doses ($LD_{50}$) was determined for Tetra-hydroxybenzoquinone using Reed and Muench method [8].

## Growth and metamorphosis

Percentage of larval mortality was calculated and corrected by Abbott's formula [9] as given below:

$$\text{Abbott's corrected mortality in \%} = \frac{\text{\% mortality in treated - \% mortality in control}}{\text{100- \% mortality in control}} \times 100$$

## Statistical analysis

Data were analyzed using completely randomized design using the different concentrations of Tetrahydroxybenzoquinone. Mean values were adjusted by Duncan's Multiple Range test [10]. For mortality tests, original data were corrected by Abbott's formula, transformed into percentage values and then data were analyzed by probit analysis [8]. One-way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) test was performed.

## RESULTS AND DISCUSSION

Three replicates of ten fifth instar larvae were used for each treatment. Each replica in its container were fed with castor leaves for the entire experiment. Thus, a total of thirty larvae were used per treatment per experiment. Observations were made from 24 hrs till the emergence of adult and their survival. During the post treated period the larvae showed behavioral changes such as sluggishness, reduced feeding and covering themselves under mud before the onset of pupation. Other than behavioral changes some morphological changes were observed such as reddening of the ventral side of the insect with darkening of dorsal side. Mucus from body surface decreased considerably, pupation was seen at normal but emergence of adult delayed than the normal time noted for the controlled larvae.

Acute toxicity of tetra-hydroxybenzoquinone was tested against fifth instar larva of the *Spodoptera litura*. The $LD_{50}$ value ($LD_{50} = 5.001\mu g$ for fifth instar larvae) of the compound was calculated according to method described by Reed and Munech [8]. Larval mortality after treatment due to toxicity of acetone solution of tetra-hydroxybenzoquinone on *Spodoptera litura* was monitored till pupation and further up to adult emergence. Mortality percentages were directly proportional to the dosages and also with time after treatment (Table 1). For mortality tests, original data was corrected by Abbott's formula, transformed into percentage values and then data were analyzed by probit analysis.

Insecticidal activity of acetone solution of Tetrahydroxybenzoquinone on *S. litura* was studied at different concentrations and the results are presented in table 2. Insecticidal activity was calculated on basis of larval mortality after treatment. High larval mortality normally indicates potential insecticidal activity of Tetrahydroxybenzoquinone. In the present study it was observed the along with effect on growth and metamorphosis there was cannibalism observed with increase in dosage in treated 5th instar larvae. Data pertaining to the insecticidal activity clearly revealed that maximum insecticidal activity was recorded with higher dosage of Tetrahydroxybenzoquinone i.e. 6 to 10 μg as compared to control. One-way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) test showed statistical significance ($p<0.001$) compared to control (Figure 1). The improvement in insecticidal activity requires a reasonable combination of substituents in the parent structure, which provides some hints for further investigation on structure modification [12].

Table 1. Acute toxicity of tetra-hydroxybenzoquinone against fifth instar larva of the *Spodoptera litura* ($LD_{50}$ = 5.001μg for fifth instar larvae).

| Dose (μg) | $Log_{10}$ Conc. | Number of insects | % mortality | % survival of 5th instar to pupa | | % Corrected mortality | Probit values |
|---|---|---|---|---|---|---|---|
| | | | | Normal | Abnormal | | |
| Control | 0.0 | 30 | 10 | 90 | 0 | 0 | 0 |
| 2.0 | 0.3010 | 30 | 20 | 80 | 0 | 11 | 3.7 |
| 4.0 | 0.6012 | 30 | 50 | 50 | 0 | 44 | 4.85 |
| 6.0 | 0.7782 | 30 | 67 | 22 | 11 | 63 | 5.33 |
| 8.0 | 0.9031 | 30 | 80 | 0 | 20 | 78 | 5.77 |
| 10.0 | 1.0000 | 30 | 90 | 0 | 10 | 89 | 6.23 |

Table 2. Effect of Tetrahydroxybenzoquinone on growth and metamorphosis on the 5th instar larvae of *Spodoptera litura*.

| Dose (μg) | Number of insects | % mortality | % survival 5th instar to pupa | | Pupation period in days | % of adult emergence | |
|---|---|---|---|---|---|---|---|
| | | | Normal | Abnormal | | Female | Male |
| Control | 30 | 10 | 90 | 0 | 10-13 | 47 | 43 |
| 2.0 | 30 | 20 | 80 | 0 | 12-15 | 47 | 33 |
| 4.0 | 30 | 50 | 50 | 0 | 13-17 | 33 | 17 |
| 6.0 | 30 | 67 | 22 | 11 | 15-18 | 23 | 10 |
| 8.0 | 30 | 80 | 0 | 20 | 18-21 | - | - |
| 10.0 | 30 | 90 | 0 | 10 | 18-25 | - | - |

After treatment within 24 hrs their activities like feeding and crawling decreased considerably in all the treated groups. Later feeding on castor leaves stopped within 72hrs of treatment and then there was increase in cannibalism observed in higher doses of treated larvae (6, 8 and 10 μg). Some of the larvae after treatment with Tetrahydroxybenzoquione wrapped themselves from mud and others showed loss of mucus from their external surface, their ventral surface started reddening. This could be the probable reason of shrinkage in the larvae. Also the effect of treatment on feeding could have added ill effects on growth and metamorphosis. These larvae showed shrinking and died at 96 hrs, other larvae which went underground started pupating, the color and size of these pupae were normal till 72 hours of treatment with 2 and 4μg of tetrahydroxybenzoquinone. The emergence of adult was delayed from 10 to 13 day in controlled to 17th day after treatment. Adult obtained were normal but the percentage of male emergence was less as compared to females. Same holds true even with 6μg of tetrahydroxybenzoquinone except for the fact that these larvae became sluggish within 4-5hrs of treatment. Loss of mucus from body surface, reddening of ventral surface and pigmentation of dorsal surface was observed after 24hrs of treatment. 11% of females emerged after 18th day of treatment (10 µg/larvae) showed deformity in one of its wing (Figure 2b). Rest of the pupae remained in dormant condition.

With still higher dose of 8 µg/larvae feeding was detoriated still more and cannibalism was shown by 3 larvae. Loss of mucus from body surface, reddening of ventral surface and pigmentation of dorsal surface was observed from 24 hrs of post treated period. 67% of treated larvae shrinked and died before 72hrs and the remaining 30% survived larvae pupated normally at 92 hrs whereas the others remained in intermediate stage of larvae and pupae, and then died (Figure 2c). Two adult emerged with deformity on 17th day, other pupae remained dormant. With the highest dose of 10µg of tetrahydroxybenzoquinone/larvae, all the observations were same as those found in 8 µg/larvae till 72 hrs. Only 90% of treated larvae showed cannibalisms and only 10% of the treated group survived. Of these survivals one of the larvae pupated on 6th day of treatment. This pupa was darkly pigmented and its size reduced from 1.9 to 1.4 cm in length.

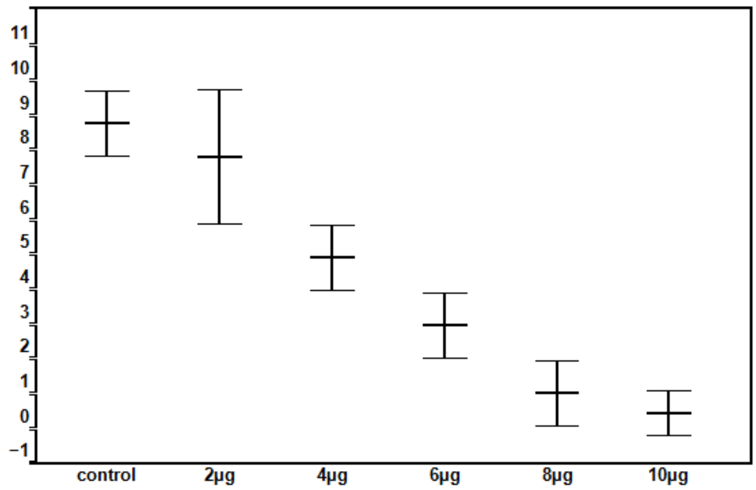


Figure 1. One way randomized ANOVA Analysis of adult emergence in tetrahydroxybenzoquinone treated fifth instar *Spodoptera litura* larvae.


Figure 2. Morphological changes in larvae and adult in treated Tetrahydroxybenzoquinone. a: Shrinked larvae from 4.6 cm to 3.2cm and 2.5cm; b: Adult emerged with deformity; c: Intermediate stage of larvae and pupae; d: Larval size in control and treated.

The approximate growth and metamorphosis of fifth instar *S. litura* larvae on the tetrahydroxybenzoquinone treated fifth instar larvae was found to be significant effect ($F$ = 27.88; df = 5; $p < 0.001$) and it was interesting to note that the significant difference was noted at higher doses. Thus the data clearly show that *S. litura* performed differently in growth and metamorphosis of fifth instar larval and pupal development and survival, pupal size, and fecundity of emerged adults when treated with Tetrahydroxybenzoquinone [Figure 2a-d]. As secondary metabolites, quinones frequently possess antibiotic and cytotoxic activities, in addition to acting sometimes as pathogens. Several plants and animals, especially insects, use quinonoid substances for defense, often with spectacular results. On the macromolecular level, quinone moieties have a key role in the plant kingdom in lignin biosynthesis; the biosynthesis of melanoproteins exemplifies the reactions of *o*-quinones in the animal kingdom. In insects, cross-linking of structural proteins through quinones and quinone moieties result in the construction of the sclerotized exoskeleton. Probably this cross linking is affected with the topical treatment of tetrahydroxybenzoquinone which is why there are some morphological changes observed such as reddening of the ventral side of the insect with darkening of dorsal side. For this further studies with reference to chitin synthesis need to be carried out.

All forms of life maintain a reducing environment within their cells. This reducing environment is preserved by enzymes that maintain the reduced state through a constant input of metabolic energy. In chemical terms, oxidative stress is a large rise (becoming less negative) in the cellular reduction potential, or a large decrease in the reducing capacity of the cellular redox couples, such as glutathione. The effects of oxidative stress depend upon the size of these changes, with a cell being able to overcome small perturbations and regain its original state. However, more severe oxidative stress can cause cell death and even moderate oxidation can trigger apoptosis, while more intense stresses may cause necrosis. A particularly destructive aspect of oxidative stress is the production of reactive oxygen species, which include free radicals and peroxides. Some of the less reactive of these species (such as superoxide) can be converted by oxido-reduction reactions with transition metals or other redox cycling compounds (including quinones) into more aggressive radical species that can cause extensive cellular damage. The major portion of effects is inflicted by damage on DNA. Most of these oxygen-derived species are produced at a low level by normal aerobic metabolism and the damage they cause to cells is constantly repaired. However, under the severe levels of oxidative stress that cause necrosis, the damage causes ATP depletion, preventing controlled apoptotic death and causing the cell to simply fall apart. Probably this could be the cause of insecticidal activity on growth and metamorphosis of $5^{th}$ instar larvae. Thus, toxicity can arise as a result of use of quinones as well as by the metabolism of other drugs and various environmental toxins or dietary constituents. Cytotoxicity associated with exposure to quinones is due to redox cycling or due to the subsequent development of “oxidative stress” and/or to their interaction with cellular nucleophiles, such as protein and non-protein sulfhydryls. Oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system’s ability to readily detoxify the reactive intermediates or easily repair the resulting damage.

Therefore from present studies the efficacy of Tetrahydroxybenzoquinone as chemical alternatives to control fifth instar larva of *Spodoptera litura* can be determined. Still further investigations are required to develop the compound as potent compound useful in IPM.

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