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Growth regulatory effect of hydroquinone on the larvae of *Spodoptera litura* (Fabricius)

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Abstract
The present study was aimed at investigating the effect of the plant compound hydroquinone on the growth and development of the second instar larvae of tobacco caterpillar, *Spodoptera litura* (Fabricius), a polyphagous noctuid with high reproductive potential and ability to migrate long distances as adults. The effect was ascertained by feeding second instar larvae on artificial diet incorporated with different concentrations (1ppm, 5ppm, 25ppm, 125ppm, 625ppm, 3125ppm) of hydroquinone and water as control. The present findings revealed that the performance of *S. litura* larvae is related to the content of hydroquinone with higher concentrations exhibiting a deterrent effect on its growth and development. The toxicity of the compound was also evident from the various aberrations observed in the larvae of *S. litura*. Thus our study suggest that hydroquinone can be used as an alternate ecofriendly, biodegradable and safe alternative to conventional pesticides.

Keywords: Bioassay, Biopesticide, Hydroquinone, *Spodoptera litura*

Introduction
The common cutworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) is an important polyphagous insect-pest having huge potential to invade new areas due to its ability to adapt to new climatic and ecological situations [1, 2]. The adult moths have a flight range of 1.5 km during a period of 4h overnight, aiding dispersion and oviposition on a large number of different hosts [3]. It attacks a wide range of economically important crops throughout the year [4]. Its young larvae feed voraciously on leaves and on maturity can eat almost every plant part. The usage of various insecticides like methyl-parathion, and other organophosphates have failed to keep its population within limits, rather there are reports regarding the development of resistance and cross resistance in many cases [5, 6]. Moreover, the unjudicial use of synthetic pesticides has resulted in their bioaccumulation and biomagnifications which has drastically altered the natural balance of the ecosystem resulting not only in faster evolution of resistant forms of pest but also has harmed non-target organisms. According to the international "Insecticide Resistance Action Committee" (IRAC), there are currently more than 550 insect pest species, resistant against most current insecticide groups, implying a high demand for novel insecticide agents.

Plants are a rich source of biologically active compounds like phenolics, terpenoids, and alkaloids [7]. Most of these compounds primarily defend the plant from insect pests. These compounds represent a large resource which is mostly untapped for exploration as pesticides. Phenolic compounds are a group of secondary metabolites produced through the shikimic acid and malonic acid pathways in plant tissues, exhibiting broad structural heterogenous group of nearly 10,000 individual compounds and consisting of a hydroxyl group (-OH) bound directly to an aromatic hydrocarbon group (6-carbon ring) and are the most stable plant products. A number of phenolic compounds have been implicated as enzymatic and metabolic inhibitors in insects [8, 9]. Hydroquinone (Benzene-1, 4-diol) is a naturally occurring plant secondary metabolite found in castorea, *Roldana barba-johannis*, as an active toxin in *Agaricus hondensis* mushroom and is also a chief constituent of the natural product propolis. The scanning of literature has revealed that this simple phenol has not been explored much for its activity against insects. Therefore the present study was aimed at investigating the influence of hydroquinone on the growth and development of second instar larvae of *S. litura*.

Materials and Methods

Test chemical

To study the effect of plant phenolic compound, the test compound Hydroquinone (Benzene-1, 4–diol), having purity of (99%) was obtained from Sigma Aldrich chemicals.

Test organism

The study was carried out on 6 days-old larvae of *S. litura* obtained from the culture maintained in the Insect Physiology Laboratory of the Department of Zoology, Guru Nanak Dev University, Amritsar. All the ethical concerns were also cautiously adhered while performing the experiments.

Culturing of *S. litura*

The *S. litura* of was reared on castor leaves, *Ricinus communis* (Linnaeus) in the battery jars covered with muslin cloth under controlled conditions i:e 27±2 ºC temperature, 65±5% humidity, L16:D18 photoperiod in the insect culture room in the Department of Zoology, Guru Nanak Dev University, Amritsar. After the moths were identified they were placed in oviposition jars having a cotton swab soaked with solution of sugar and water (1:4) as food. The oviposition jars were lined with filter paper to facilitate egg laying. Egg batches were removed and were kept on fresh castor leaves, in the petriplates. The newly hatched larvae were then transferred to battery jars with fresh castor leaves in it. The castor leaves were changed daily. The pupae formed were transferred to pupation jars with 2–3 cm layer of moist sand covered with filter paper. The adult moths which emerged were shifted to oviposition jars for egg laying.

Insect bioassay

The antibiosis influence of hydroquinone was ascertained by feeding second instar larvae (6 days old) on artificial diet incorporated with different concentrations (1ppm, 5ppm, 25ppm, 125ppm, 625ppm, 3125ppm) of hydroquinone and water (control). The stock solution of 15625ppm was prepared in distilled water (60ml) by adding 937.5mg of the test compound and the experimental concentrations were prepared from the stock. A broad range of these concentrations were taken to ascertain the LC_{50} concentration of the hydroquinone. The artificial diet was prepared according to protocol of Koul *et al.* [10] and observations were recorded daily for the various developmental parameters such as larval period, pupal period, total development period, per cent adult emergence, per cent larval mortality, per cent female, adult longevity and aberrations. Each experiment had six replications and there were five larvae in each replication and the experiment was repeated twice.

Nutritional assay

The nutritional assay was conducted for a period of three days with 5 second instar larvae in each of the 6 replicates taken for each concentration of the hydroquinone. The newly molted second instar larvae starved for six hours were weighed for recording their initial weight and were then released in sterilized plastic containers. The larvae were allowed to feed for 72h on weighed quantity of control and hydroquinone treated diets. After three days of feeding, the larval weight, the diet left and faecal matter were weighed, kept in different containers and were then oven dried at 60°C for 48 hours. They were weighed again to obtain the dry weights. The dry weight readings indicated water loss under controlled conditions.

From the data obtained the following nutritional indices were calculated on dry weight basis after 3 days of feeding as proposed by Waldbauer and Koul *et al*. [11, 12].

$$RGR = \frac{\text{Change in larval dry weight/day}}{\text{Starting larval dry weight}}$$

$$RCR = \frac{\text{Change in diet dry weight/day}}{\text{Starting larval dry weight}}$$

$$ECI = \frac{\text{Dry weight gain of insect}}{\text{Dry weight of food ingested}} \times 100$$

$$ECD = \frac{\text{Dry weight gain of insect}}{\text{Dry weight of food ingested - Dry weight of frass}} \times 100$$

$$AD = \frac{\text{Dry weight gain of insect - Dry weight of frass}}{\text{Dry weight of food ingested}} \times 100$$

Where RGR = Relative growth rate, RCR = Relative consumption rate, ECI = Efficiency of conversion of ingested food, ECD = Efficiency of conversion of digested food, AD = Approximate digestibility

Statistical analysis

Statistical comparisons were made between means within experiments to avoid any confounding effects from variation in methods between experiments. Data were then subjected to the analysis of variance (ANOVA) and Tukey's test to find significant differences between the average values using ASSISTAT and MINITAB softwares.

Results and Discussion

Bioassays conducted with the phenolic compound, hydroquinone revealed an antibiosis influence on the development of the second instar larvae of *S. litura*. It was found to have a significant effect on per cent larval mortality and per cent adult emergence (Table 1). When the second instar larvae were fed on hydroquinone incorporated artificial diet the larval mortality increased from 0% in control to a maximum of 36.67% at the highest concentration of 3125ppm. The adult emergence was reduced significantly when the larvae were fed on diet incorporated with various concentrations of hydroquinone. The LC_{50} concentration of the hydroquinone was found to be 2685ppm. The per cent females emerged showed a constant decrease when the concentration of hydroquinone was increased from 25ppm to 3125ppm. At 3125ppm the per cent female emergence decreased by 81.25% when compared with control. Longevity of adults declined when larvae were treated with different concentrations of hydroquinone compared to control. It decreased to 67.14% of the control at the highest concentration of 3125ppm. Insecticidal activities of hydroquinone derivatives isolated from methanol extracted from aerial part of *R. barba-johannis* have also been reported against the fall armyworm, *Spodoptera frugiperda* (J.E Smith) [13]. Increasing concentration of hydroquinone from 0.1% to 1% also adversely affected the survival, pupation, larval weight and pupal weight of olive fruit fly, *Dacus olea* (Gmelin) [14]. A significant impact of phenolics such as quercetin, chlorogenic acid and rutin on the development and mortality of neonate larvae of *S. litura* on interspecific derivatives obtained from crossing wild species of *Arachis kempffmercedoi* with susceptible variety of *Arachis hypogaea*. (L.) [15].

Table 1: Larval mortality, Adult emergence, Female emergence (%age) (Means± S. E.) of *S. litura* when second instar larvae were fed on different concentrations (ppm) of hydroquinone

Concentrations	Larval mortality	Adult Emergence	Female emergence
Control	0.00±0.00^{a}	96.67±3.33^{a}	53.33±6.67^{a}
1	6.67± 6.67^{ab}	76.67±6.15^{ab}	40.00±7.30
5	20.00± 5.16^{abc}	40.00±8.94^{c}	26.67±6.67^{bc}
25	26.67±4.22^{bc}	63.33±9.55^{abc}	30.00±4.47^{abc}
125	30.00±8.56^{bc}	56.67±9.55^{bc}	20.00±5.16^{bc}
625	23.33±6.15^{abc}	56.67±6.15^{bc}	16.67±6.15^{bc}
3125	36.67±6.15^{c}	46.67±8.43^{bc}	10.00±4.47^{c}
F-Value (df=6)	4.95**	6.09**	6.19**

**Significant at 1%. Means followed by the same letter within the columns are not significantly different according to Tukey's test at $P \leq 0.05$

Significant effect of hydroquinone were also noticed on larval, pupal and total development period when the second instar larvae were fed on amended diet when compared with control. The larval period showed an increase with increase in concentration. It extended by 15.95 days at the highest concentration (3125ppm) as compared to that of the larvae reared on control diet. The total development period increased from 32 days in control to 46 days at 3125ppm concentration thereby showing a 46% increase relative to control. The pupal period too was increased significantly when the second instar larvae were fed on diet incorporated with different concentrations of the hydroquinone (Table 2). No significant effect of hydroquinone was observed on pupal weight. The observations recorded for larval period and total development period revealed an increase with increase in concentration of hydroquinone. Phenolic compounds present in the extract obtained from seed coat of red gram were reported to suppress the growth and development of *S. litura* [16]. The compounds identified in the extract were hydroquinone, chlorogenic acid, gallic acid and syringic acid. Other researchers have also shown a negative relationship between the levels of phenols in plants, artificial diet and the performance of *S. litura*. An inhibition in the development of *S. litura* larvae when the neonate larvae were fed on diet containing phenolic compounds such as 3-caffeoylquinine acid, chlorogenic acid, rutin and quercetin [17]. The inhibition of larval growth by these compounds was also dose related. Better performance of *S. litura* larvae on host plants was also noticed with lower amount of total phenolics [18, 19].

Table 2: Larval period, Pupal period, Total development period, Adult longevity (in days) and Pupal weight (in mg) (Means ± S.E.) of *S. litura* when second instar larvae were fed on different concentrations (ppm) of hydroquinone

Concentrations	Larval period	Pupal period	Total development period	Pupal weight	Adult longevity
Control	19.06±0.57^{a}	12.97±0.29^{ab}	32.09±0.51^{a}	261.50±8.33	4.26±0.35^{a}
1	26.02±0.77^{bc}	11.30±0.70^{b}	36.68±0.73^{abc}	240.36±4.94	3.211±0.63^{ab}
5	25.07±0.74^{bc}	14.66±0.98^{a}	38.20±0.97^{bc}	230.19±8.69	3.860±1.18^{ab}
25	23.12±1.10^{ac}	13.29±0.27^{ab}	36.19±1.52^{ac}	253.51±7.11	3.430±0.46^{ab}
125	26.07±0.80^{bc}	13.13±0.32^{ab}	39.15±0.94^{bc}	235.46±13.10	3.526±0.86^{ab}
625	27.70±1.25^{b}	13.69±0.23^{a}	41.23±1.05^{b}	229.88±14.4	3.526±0.45^{ab}
3125	35.01±1.39^{d}	13.71±0.40^{a}	46.83±1.23^{d}	268.01±12.00	2.866±0.50^{b}
F-Value (df=6)	24.10**	3.76**	19.57**	2.24^{ns}	2.54*

**Significant at 1%, *Significant at 5%, ^{ns}Non significant. Means followed by the same letter within the columns are not significantly different according to Tukey's test at $P \leq 0.05$

Varied effects of different hydroquinone concentration incorporated in artificial diet were observed on nutritional indices of *S. litura* larvae. The nutritional indices viz. RGR and RCR were significantly less at higher concentrations than at lower concentrations. RGR and RCR decreased significantly in the larvae fed on hydroquinone incorporated diet. The decrease was considerably more at higher concentrations of 625 and 3125ppm. RGR decreased to a maximum of 49.64% at 3125ppm. Maximum decline in RCR (36.97%) was observed at 625ppm when compared with control. On the other hand, ECI and ECD increased at concentrations ranging from 25 to 625ppm but decreased notably at the highest concentration as compared to control. AD showed a non-significant increase after treatment compared to control. The decrease in consumption rate accounts for the decrease in growth rate of *S. litura*. The presence of allelochemicals can restrict the utilization of an otherwise balanced complement of nutrients and may affect dietary balance. ECI and ECD increased at lower concentrations but decreased significantly at the highest concentration of 3125ppm (Table 3). ECI measures the insect's ability to utilize the food that it ingests for growth while change in ECD indicates the overall increase or decrease of the proportion of digested food metabolized for energy. The decline in ECI suggested that the amount of food that was being converted into biomass was less and most of it was being utilized for generating energy. The decrease in ECD indicates diversion of energy from producing biomass into detoxification of the compounds [20]. The low value of AD in the treated larvae indicated poor digestibility ability of the insect. Ghumare and Mukherjee [19] had also attributed low levels of ECI in *S. litura* larvae fed on mint and cotton leaves to higher levels of phenolics in them as compared to other host plants. The toxicity of the compound was also evident from the various aberrations observed in the larvae of *S. litura*. These abnormalities were in the form of half emerged adults, adults with crumpled wings, wrinkled pupae and pupal head bulged out (Fig. 1).

Table 3: Effect on RGR, RCR, (mg/mg/d) ECI, ECD, AD (%) (Means±S. E.) of *S. litura* when second instar larvae were fed on different concentrations (ppm) of hydroquinone

Concentrations	RGR	RCR	ECI	ECD	AD
Control	1.41±0.08^{a}	14.55±0.74^{a}	10.32±0.95^{abc}	12.58±1.46^{a}	84.84±0.98
1	1.12±0.12^{ab}	12.83±0.84^{a}	9.37±1.64^{abc}	11.53±2.95^{a}	88.02±3.71
5	1.0±0.03^{ab}	13.26±0.39^{a}	8.38±0.31^{bc}	8.90±0.47^{a}	88.71±0.57
25	1.38±0.15^{a}	13.14±1.04^{a}	13.14±1.04^{ab}	12.10±1.24^{a}	86.56±1.76
125	1.37±0.09^{a}	11.88±0.47	13.73±1.44^{a}	16.21±1.38^{a}	79.59±3.05
625	0.93±0.06^{b}	9.17±0.70	12.40±1.29^{ab}	16.71±3.75^{a}	88.12±1.48
3125	0.71±0.04^{b}	11.36±1.07	7.39±0.551^{c}	8.63±0.85^{a}	87.73±1.76
F-Value (df=6)	7.48**	4.75**	4.79**	2.41*	2.18^{ns}

**Significant at 1%, *Significant at 5% ^{ns}Non significant. Means followed by the same letter within the columns are not significantly different according to Tukey's test at $P \leq 0.05$.

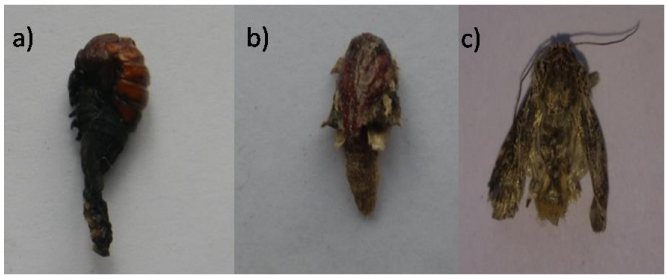

Fig 1: Aberrations in pupae and adults of *S. litura* a) Bulged out head pupa b) Partially emerged adult and c) Adult with deformed wings

The growth of an organism depends on its ability to convert food into body substance through the process of ingestion, absorption, assimilation and synthesis and requires energy. A decrease in food intake is compensated by a delay in pupation. The insect larvae needs to achieve appropriate weight essential for it to pupate, therefore prolonged development is an adaptive character of an insect to acquire minimum energy reserve required for pupation and emergence [21, 22]. A diet rich in nutrients helps the insect to pass over the non-feeding pupal and comparatively less active feeding adult period. The present findings showed a decrease in pupal weight of the treated larvae but the decrease was non-significant compared to control. However prolonged feeding of the larvae on treated diet to achieve the desirable weight adversely affected the pupation, adult emergence and survival of the adults which decreased significantly at higher concentrations. Phenolics slow down the passage of food through the insect's alimentary canal, reduce its digestibility and inhibit food uptake by the herbivorous insects [23]. The present findings clearly revealed that the performance of *S. litura* larvae is related to the content of hydroquinone in its diet. Higher concentrations had a deterrent effect on the development of *S. litura* larvae.

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Compliance with ethical standards
Ethical approval
This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest
The authors declare that they have no conflict of interest.

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