

## Disruptive effect of pyrogallol on development of *Spodoptera litura* (Fab.) larvae

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### ABSTRACT

In an endeavour to explore secondary metabolites as an important and safe means of pest management, we investigated the effects of pyrogallol, a phenolic compound, on the growth and development of *Spodoptera litura* (Fab.). It is a serious pest of a large number of economically important crops. Different concentrations viz., 1, 5, 25, 125, 625, 3125 ppm of pyrogallol were incorporated in artificial diet of larvae and the antibiosis influence was ascertained by feeding second instar larvae on this diet. The larval and total development period increased significantly with increase in concentration of the pyrogallol. Inhibitory effects of the compound were observed on per cent pupation, per cent emergence and adult longevity. All nutritional indices were also significantly affected. Thus it can be considered as a promising substance to be used as a biopesticide against the insect pest.

**Keywords:** Phenols, allelochemicals, pesticide, bioassay.

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### INTRODUCTION

In developing countries like India, feeding a rapidly growing population requires sustainable agricultural practices. Till now, pesticides have been the most popular method of controlling pest populations, increasing the agricultural crop yield to satisfy the growing population needs. But environmental concerns and the adverse effects they cause to human health and other non target organisms in addition to the problems of pest resurgence have motivated the need to decrease their use in agriculture and instead develop strategies that are safe and eco friendly. Emerging technologies such as metabolomics have provided gainful insight into the world of plant kingdom which produces thousands of metabolites. These metabolites viz., alkaloids, terpenoids and phenolics play a major role in adaptation of plants to their environment by reconfiguring their metabolism. They can be a major source for developing new pesticides as,

unlike conventional pesticides, they are biodegradable and do not persist in the environment. Among the various plant secondary metabolites, phenolics are widely gaining attention not only due to their mutualistic interactions with other animals such as pollinators and seed dispersals but also due to their role in protecting the plant from abiotic stress and herbivores (Treutter, 2010). These compounds range from simple to complex compounds and generally have an unfavourable effect on plant associated insect pests. Their insecticidal activity has been observed against a number of insects (Weissenberg *et al.*, 1997; Akhtar and Isman, 2004). *S. litura* is a total polyphagus pest capable of invading new areas (Brown and Dewhurst, 1975; Holloway, 1989). It has high reproductive potential and can lay hundreds of eggs in egg batches (Salama and Shoukry, 1972). Larvae feed gregariously and may completely devour the leaves resulting in poor growth of the plants. Moreover, the pupae are

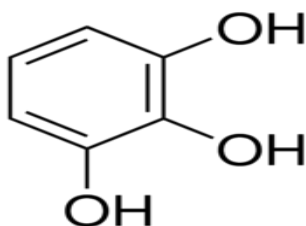
long lived, hardy and can be transported over a considerable distance. This pest has developed insecticidal resistance and therefore most of its control measures fail (Kranthi *et al.*, 2001, 2002; Ahmad *et al.*, 2007). So, the present study was undertaken to investigate the effect of pyrogallol (plant based phenol) on the growth and development of *S. litura* which would help in evaluating its potential in managing the population of *S. litura*.

## MATERIALS AND METHODS

### Test chemical

The test compound pyrogallol (1, 2, 3-Trihydroxy Benzene) having a purity of (98.5%) was obtained from Sigma Aldrich chemicals (Fig. 1).

**Figure 1.** Structure of Pyrogallol



### Rearing of *S. litura*

Rearing of *S. litura* was done under controlled conditions i.e.  $27 \pm 2^\circ$  C temperature,  $65 \pm 5\%$  humidity, L16:D18 photoperiod in the insect culture room with on castor leaves, (*Ricinus communis*) (Fab.) (Insect physiology laboratory, Guru Nanak Dev University, Amritsar, Punjab). Male and female moths were placed in oviposition jars and fed on sugar and water solution (1:4). The oviposition jars were lined with filter paper to facilitate egg laying. Once the eggs were laid in batches they were removed and placed on fresh castor leaves in petriplates. After hatching, larvae were transferred to battery jars having fresh castor leaves and were changed daily. The pupae formed were transferred to pupation jars and the adults emerged were again shifted to oviposition jars for egg laying. The second instar larvae (6-days old) were used for experiments. All the experiments were conducted at controlled conditions mentioned earlier i.e.  $27 \pm 2^\circ$  C temperature,  $65 \pm 5\%$  humidity, L16:D18 photoperiod in the Biological Oxygen Demand (BOD) incubator in the Insect Physiology Laboratory of Guru Nanak Dev University, Amritsar.

### Bioassays

To check the antibiosis effect, second instar larvae (6-days old) were fed on artificial diet incorporated with different concentrations *viz.*, 1, 5, 25, 125, 625, 3125 ppm of pyrogallol and water (control). The artificial diet was prepared according to protocol of Koul *et al.* (1997). Each experiment had six replications and there were five larvae in each replication. The various parameters studied were larval period, pupal period, total development period, per cent pupation, per cent adult emergence, per cent larval mortality and adult longevity.

### Nutritional assay

A three day nutritional assay was conducted on the second instar larvae using the same concentrations as mentioned above. 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 pyrogallol treated diets.

After three days of feeding, the larval weight, the diet left and fecal matter were weighed, kept in different containers and were then oven dried at  $60^\circ$ C for 48 hrs. They were weighed again to obtain dry weights. The dry weight readings indicated water loss under controlled conditions. The nutritional indices *viz.*, Relative growth rate (RGR), Relative consumption rate (RCR), Efficiency of conversion of ingested food (ECI), Efficiency of conversion of digested food (ECD) and Approximate digestibility (AD) were calculated from the data obtained according to the formulae proposed by Waldbauer (1968) and Koul *et al.* (2005).

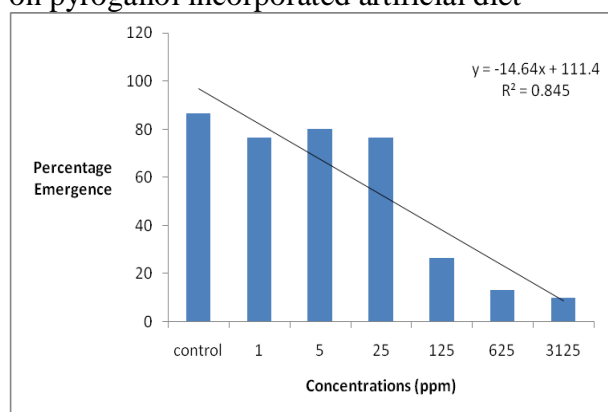
### Statistical analysis

Statistical comparisons were made between means within experiments to avoid any confounding effects from variation in methods between experiments. Data were then subjected to Analysis of Variance (ANOVA) and Tukey's test to find significant differences between the average values using Assistant and Minitab software's.

**RESULTS**

Pyrogallol significantly influenced the development of the second instar larvae of *S. litura*. The larval mortality increased with increase in pyrogallol concentration with maximum mortality (80%) noticed at 3125ppm concentration ( $F = 31.13$ ,  $P \leq 0.01$ ). The per cent adults emerged from the larvae treated with the phenolic compound decreased from 86.67 per cent in control to 10 per cent at 3125ppm thereby showing 88.4 per cent reduction at the highest concentration.

**Figure 3.** Percentage emergence of adults when second instar larvae of *S. litura* were fed on pyrogallol incorporated artificial diet



Also the longevity of adults which emerged from treated larvae decreased considerably ( $F = 7.00$ ,  $P \leq 0.01$ ) (Table 1). Similar results were obtained when *S. litura* larvae were treated with the phenolic rich extract of seed coat of red gram, *Cajanus cajan* (Linnaeus) (Bhattacharya and Chenchaiiah, 2007). Failure of I<sup>st</sup> instar larvae of *Bactrocera oleae* (Rossi) to develop into pupae at higher concentrations has been reported by Manoukas (1996). Pyrogallol has also been reported to reduce pupation and adult emergence of *Bactrocera cucurbitae* (Coquillett) larvae (Sohal and Sharma, 2011). The inhibitory effect of pyrogallol on growth of *S. litura* larvae was also apparent by a decrease in pupal weight with treatment (Table 2). Depression in pupal weight of larvae treated with pyrogallol has also been reported in *B. olea* and in *B. cucurbitae* (Manoukas, 1996; Sohal and Sharma, 2011). Both the larval period ( $F = 16.56$ ,  $P \leq 0.01$ ) and the total development period ( $F = 8.72$ ,  $P \leq 0.01$ ) of *S. litura* larvae were significantly delayed at higher concentrations of pyrogallol (Table 2).

**Table 1.** Larval mortality, Pupation and Adult longevity (% age) (means  $\pm$  S.E.) of *S.litura* when second instar larvae were fed on different concentrations of Pyrogallol

Concentrations (PPM)	Larval mortality	Pupation	Adult longevity
<b>CONTROL</b>	3.33 $\pm$ 3.33 <sup>a</sup>	96.67 $\pm$ 3.33 <sup>a</sup>	4.26 $\pm$ 0.35 <sup>ab</sup>
<b>1</b>	6.67 $\pm$ 4.22 <sup>a</sup>	93.33 $\pm$ 4.22 <sup>a</sup>	4.66 $\pm$ 0.54 <sup>a</sup>
<b>5</b>	6.67 $\pm$ 4.22 <sup>a</sup>	93.33 $\pm$ 4.22 <sup>a</sup>	4.27 $\pm$ 0.61 <sup>ab</sup>
<b>25</b>	10.00 $\pm$ 4.47 <sup>a</sup>	90.00 $\pm$ 4.47 <sup>a</sup>	3.87 $\pm$ 0.35 <sup>abc</sup>
<b>125</b>	56.67 $\pm$ 9.55	46.7 $\pm$ 11.2 <sup>b</sup>	2.16 $\pm$ 1.75 <sup>bcd</sup>
<b>625</b>	80.00 $\pm$ 10.3 <sup>b</sup>	10.3 $\pm$ 10.3 <sup>b</sup>	1.91 $\pm$ 0.89 <sup>cd</sup>
<b>3125</b>	83.33 $\pm$ 6.15 <sup>a</sup>	6.15 $\pm$ 6.15 <sup>b</sup>	1.33 $\pm$ 0.61 <sup>d</sup>
<b>F- value (df=6)</b>	31.13 <sup>**</sup>	27.57 <sup>**</sup>	7.00 <sup>**</sup>

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

Significant delay in larval period and total development period was also observed in *B. cucurbitae* when its diet was amended with another phenolic compound, phloroglucinol (Puri and Sohal, 2017). Ghumare and Mukherjee (2003) had reported that *S. litura* larvae when fed on host plants containing a

lower amount of total phenolics showed better survival. Stevenson *et al.* (1993) too had reported a dose dependent inhibition in the development of *S. litura* larvae when fed on diet containing phenolic compounds such as 3-caffeoylquinine acid, chlorogenic acid, rutin and quercetin. Similar findings were reported

by Jadhav *et al.* (2012). Delayed larval and pupal period of fall armyworm, *Spodoptera frugiperda* (J.E. Smith) after the larvae were treated with phenolic rich acetone water

extract of jabuticabeiri, *Myrciaria cauliflora* (Mart.) skin has been reported by Alves *et al.* (2014).

**Table 2.** Larval period, Pupal period, Total development period, (in days), Pupal weight (in mg) (Mean ± S.E.) of *S. litura* when second instar larvae were fed on different concentrations of Pyrogallol

Concentra-tions (ppm)	Larval Period	Pupal Period	Total Development Period	Pupal Weight
<b>Control</b>	19.13±0.45 <sup>ab</sup>	12.56±0.34 <sup>a</sup>	31.53±0.51 <sup>a</sup>	276.87±6.96 <sup>a</sup>
<b>1</b>	18.44±0.71 <sup>a</sup>	11.50±0.76 <sup>a</sup>	28.94±1.00 <sup>a</sup>	223.47±7.95 <sup>b</sup>
<b>5</b>	18.38±0.80 <sup>a</sup>	11.16±0.30 <sup>a</sup>	28.01±0.4 <sup>a</sup>	221.95±5.46 <sup>b</sup>
<b>25</b>	18.64±0.57 <sup>a</sup>	11.66±0.49	30.15±0.47 <sup>a</sup>	215.74±4.05 <sup>b</sup>
<b>12</b>	27.38±1.58 <sup>c</sup>	12.00±0.81	39.25±5.28 <sup>b</sup>	180.71±9.98 <sup>cd</sup>
<b>625</b>	33.89±2.79 <sup>d</sup>	12.00±3.79	43.83±2.17 <sup>b</sup>	172.5±17.0 <sup>d</sup>
<b>3125</b>	24.50±2.84 <sup>cd</sup>	10.50±1.50	31.33±0.33 <sup>a</sup>	211.23±9.82 <sup>bc</sup>
<b>f- value (df=6)</b>	16.56 <sup>**</sup>	0.33 <sup>ns</sup>	8.72 <sup>**</sup>	16.88 <sup>**</sup>

\*\*Significant at 1%, \*Significant at 5%, <sup>ns</sup>-Non significant. Mean followed by the same letter within the columns are not significantly different according to Tukey test at  $P \leq 0.05$ .

It has been suggested that phenolics upon ingestion reduce the nutritional quality of the diet (Felton *et al.*, 1992). Prolonged development is an adaptive mechanism on part of the insect to gain minimal amount of resources to achieve a viable size and energy for pupation and emergence (Murdoch, 1966; Campbell and Sinha, 1978; Davidowitz *et al.*, 2003). The RCR was not much affected with treatment but the RGR decreased significantly ( $F = 9.43, P \leq 0.01$ ) with treatment (Table 3).

The RGR decreased to 61 per cent over control at 3125ppm. These findings indicate that pyrogallol had a toxic effect on the larvae of *S. litura* after ingestion. Allelochemicals can influence the nutritional physiology of an insect by restricting the utilization of an otherwise balanced complement of nutrients (Beck and Reese, 1976). This was evident from a decrease in ECI ( $F = 2.48, P \leq 0.01$ ) and ECD ( $F = 10.14, P \leq 0.01$ ) with increase in concentration (Table 3).

**Table 3.** Effect on nutritional indices (Mean ± S.E.) of *S. litura* when second instar larvae were fed on different concentrations of pyrogallol

Concentration (ppm)	RGR (mg/mg/d)	RCR (mg/mg/d)	ECI (%)	ECD (%)	AD (%)
<b>Control</b>	1.412±0.083 <sup>a</sup>	14.550±0.748 <sup>a</sup>	10.32±0.957 <sup>a</sup>	12.58±1.46 <sup>a</sup>	84.838±0.985 <sup>a</sup>
<b>1ppm</b>	1.012±0.080 <sup>b</sup>	15.449±0.917 <sup>a</sup>	7.082±0.861 <sup>b</sup>	7.963±0.997 <sup>b</sup>	91.117±0.921 <sup>b</sup>
<b>5ppm</b>	1.003±0.037 <sup>b</sup>	15.099±1.38 <sup>a</sup>	7.072±0.383 <sup>b</sup>	7.695±0.446 <sup>b</sup>	92.373±0.845 <sup>cb</sup>
<b>25ppm</b>	0.920±0.077 <sup>b</sup>	16.263±0.64 <sup>a</sup>	5.950±0.436 <sup>b</sup>	6.447±0.486 <sup>b</sup>	93.129±0.541 <sup>cb</sup>
<b>125ppm</b>	0.930±0.048 <sup>b</sup>	18.336±0.554 <sup>a</sup>	5.533±0.293 <sup>b</sup>	5.922±0.373 <sup>b</sup>	94.453±0.518 <sup>c</sup>
<b>625ppm</b>	0.937±0.027 <sup>b</sup>	17.017±1.31 <sup>a</sup>	5.993±0.485 <sup>b</sup>	6.291±0.490 <sup>b</sup>	94.292±0.281 <sup>c</sup>
<b>3125ppm</b>	0.860±0.035 <sup>b</sup>	18.085±0.678 <sup>a</sup>	5.116±0.306 <sup>b</sup>	5.437±0.348 <sup>b</sup>	94.727±0.437 <sup>c</sup>
<b>f-value (df=6)</b>	9.43 <sup>**</sup>	2.48 <sup>*</sup>	8.90 <sup>**</sup>	10.14 <sup>**</sup>	25 <sup>**</sup>

\*\*Significant at 1%, \*Significant at 5%. Mean followed by the same letter within the columns are not significantly different according to Tukey test at  $P \leq 0.05$ .

ECI is a measure of an insect's ability to utilize the food that it ingests for augmenting its growth while ECD gives an idea of the overall increase or decrease of the proportion of digested food metabolized for energy. The decrease in RGR, ECD and ECI suggests higher metabolic cost of processing food containing the phenolic compound. Processing costs are associated with induction mechanisms at the level of digestion and detoxification (Lazarevic and Peric Mataruga, 2003; Silveria Ramos *et al.*, 2009). Ghumare and Mukherjee (2003) 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. Gautam *et al.* (2018) had also reported a significant decline in all the nutritional parameters when the second instar larvae of *S. litura* were fed on polyphenolic rich extracts of *Acacia nilotica* (Linnaeus). The AD of the larvae was found to increase in the treated larvae as compared to control (Table 3). The insect larvae often compensate for the detrimental digestive effect of allelochemicals by increasing their AD (Scott *et al.*, 2010). The lepidopteran larvae have an alkaline gut which promotes the oxidation of phenolics as they move through the gut. The oxidation of phenolics generates an oxidative stress in the insect thereby affecting the growth of the insect. It is evident from the findings that development and survival of the larvae were influenced significantly by pyrogallol treatment.

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