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## **EFFICACY OF *CLERODENDRUM INFORTUNATUM* AND *CHROMOLAENA ODORATA* ON CARBOHYDRATE CONTENT IN HAEMOLYMPH OF THE SIXTH INSTAR LARVAE OF *ORTHAGA EXVINACEA* HAMPSON**

**Jagadeesh G. Nambiar<sup>\*1</sup>, K. R. Ranjini<sup>2</sup>**

<sup>\*1</sup> Department of Zoology, Malabar Christian College, Calicut, India

<sup>2</sup> Associate Professor, Department of Zoology, Malabar Christian College, Calicut, India

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### **Abstract:**

*Orthaga exvinacea* is one of the major pests of mango crop and the caterpillars defoliate the leaves and thereby reduce the crop yield. Use of synthetic insecticide is the quick method for the control this pest but its uncontrolled usage has resulted in serious lethal effects on non-target organisms and environmental pollution. Botanical insecticides are very effective, safe and ecologically acceptable. In the present study, the impact of methanolic leaf extracts of *Clerodendrum infortunatum* and *Chromolaena odorata* on carbohydrate concentration in the haemolymph of sixth instar larvae of *O. exvinacea* was studied under laboratory conditions. The different concentrations (1% to 5%) of each botanical treated mango leaves were fed to the sixth instar. After 48 hours, larvae were sacrificed to collect haemolymph and the quantitative estimation of carbohydrate has been done. The results showed that there was some noticeable decrease in the amount of carbohydrate in the treated larvae when compared to control. The decrease in level of carbohydrate concentration was correlated with the increase in concentration of botanicals. Among the botanicals tested *C. odorata* possessed more efficacy than that of *C. infortunatum* and this experiment reveals the potency of both botanicals to be used as natural biopesticides against this pest.

**Keywords:** *Orthaga Exvinacea*; *Clerodendrum Infortunatum*; *Chromolaena Odorata*; Haemolymph; Carbohydrate.

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### **1. Introduction**

The mango leaf-webber, *Orthaga exvinacea* Hampson is one of the major pests of mango crop. In larval stage, they defoliate the leaves and thereby reduce the crop yield but in adult stage they do not cause any damage to the crop. The infestation by this caterpillar directly affects flowering of the plant and thereby reduce the fruit formation. The infestations of *O. exvinacea* were reported in different agro-climatic zones of India, especially in Andhra Pradesh, Uttar Pradesh, Uttaranchal

and Kerala [1]. Earlier, it was considered as minor pest of mango but since last few years, due to its severe infestation and extent of damage caused, it has been reported as a major pest of mango in Kerala [2]. In general, the synthetic insecticides are considered to be the quick method for the control of insect pests. But the use of conventional insecticides in pest management programs around the world has resulted in environmental pollution, lethal effects on non-target organisms, pest resistance to insecticides and pest resurgence.

Botanical insecticides have been reported as attractive alternatives to synthetic chemical insecticides for pest management. The adverse effects of plant derived phytochemicals on insects are in several ways, lethal toxicity, growth retardation, oviposition deterrence, feeding inhibition, suppression of calling behaviour and reduction of fecundity and fertility [3,4,5,6,7] and such a wide variety of effects of botanicals provide as potential alternatives to the use of synthetic chemical insecticides [8].

*Clerodendrum infortunatum* and *Chromolaena odorata* are locally available weeds which had proved their insecticidal property by many researchers. The insecticidal activity of *Clerodendrum* spp. against a number of pests has already been reported by many scientists [9, 10]. Furthermore, the impact of *C. infortunatum* against *Oryctes rhinoceros* [11, 12] and *Helopeltis theivora* [13] were also studied. Bioefficacy of *C. infortunatum* on the fat body of *Oryctes rhinoceros* causes extensive changes like reduction in the lobes of fat body and their derangement together with the disintegration of cell membrane, shrunken and scattered nucleus [14]. Similarly, many workers have proved the insecticidal activity of *C. odorata* and it was reported that *C. odorata* leaves mixed with soil in sweet potato beds before planting reduces weevil infestation [15]. Methanolic extracts of *C. odorata* leaves caused disruption of oocyte development and vitellogenesis in *Oryctes rhinoceros* [12]. These effects revealed the potency of both botanicals to be used as natural biopesticides for the control of the pest, *O.exvinacea*.

Carbohydrates are the major source of energy in living organisms and their breakdown facilitates the liberation of the energy required for many biological activities. The different forms of carbohydrates serve for the storage of energy and its usage is directly related to the physiological work of an organism. Insect's haemolymph is characterized by the presence of the non-reducing disaccharide trehalose and also have the unique feature like very high concentration of trehalose compared with that in vertebrates and many other invertebrates. Especially the lepidopteran insects maintain relatively higher amounts of blood sugar when compared with other insects [16, 17]. Insects have to spend energy for their biological needs, and if they are under starved condition, they can live on reserves accumulated in periods of food abundance but under stress condition, intensive utilization of reserve energy lead to mortality of insect. Hence the quantitative estimation of carbohydrate in haemolymph is an inevitable biochemical parameter to study the physiological condition of insect under stress condition. This investigation deals with the studies on the effect of methanolic leaf extracts of *C. infortunatum* and *C. odorata* on carbohydrate concentration in the haemolymph of sixth instar larvae of *Orthaga exvinacea*.

## 2. Materials and Methods

### 2.1. Rearing of *Orthaga exvinacea*

The pupae and larvae of *O. exvinacea* were collected from the field, reared and maintained in laboratory conditions. The larvae were reared in plastic troughs covered with muslin clothes and kept inside rearing cages. Fresh mango leaves were given till the pupation of the larvae. Adult moths emerged were sorted out for their sexes and kept in plastic jars and fed with 50% honey. When the eggs hatched, young larvae were fed with fresh tender mango leaves. Laboratory reared sixth instar larvae were used for the experiment.

### 2.2. Preparation of Leaf Extracts

Fresh leaves of both *C. infortunatum* and *C. odorata* were collected from the field, washed and shade dried. These dried leaves were ground into fine powder with an electric mixer grinder and sieved through a muslin cloth. This powder was used for preparing solvent extracts. 50 gm of leaf powder was extracted using 500 ml methanol in Soxhlet apparatus at 70<sup>0</sup>-80°C temperature. The extract was allowed to evaporate in a pre-weighed petridish in an oven at 50<sup>0</sup>-60°C. After complete evaporation of solvent, 10% stock solution was prepared from the weighed extract using methanol. From this stock different desirable concentrations of both botanicals (1%, 2%, 3%, 4% and 5%) were prepared.

### 2.3. Bioassay

Haemolymph samples were collected from the larvae by amputating its thoracic legs and immediately transferred into pre-chilled tubes which were kept among ice cubes to prevent melanization. After protein precipitation of the sample by using 80 % ethanol, the supernatant was poured in to petriplate and was kept in oven for evaporating the water content. After complete evaporation, remaining white powder was dissolved in 1 ml of distilled water, centrifuged again and the estimation of carbohydrate content was carried by Anthrone method [18].

## 3. Results

The treatment with botanicals has influenced the carbohydrate concentration in haemolymph of the larvae. The carbohydrate content in haemolymph of control larvae was found to be 14.62 mg/ml and it reduced from 12.75 mg/ml to 3.31 mg/ml with increasing concentrations of *C. infortunatum* from 1% to 5% (Table I). Initially 12.79% of reduction in carbohydrate content was noticed at 1% treatment and it gradually increased with increasing the concentration of treatment and reached 77.36% at 5% of treatment (Table I). Similarly a drastic decline in carbohydrate content was observed in the case of *C. odorata* treatment (Table I). The carbohydrate concentration of haemolymph was reduced from 10.34 mg/ml to 1.18 mg/ml with the increase in the treatment concentration from 1% to 5% and the maximum of 91.93% reduction in carbohydrate content was noticed at 5% and the minimum of 29.27% reduction was observed at 1% treatment (Table I). Among the botanicals tested, *C. odorata* showed greater efficacy in reducing carbohydrate concentration in haemolymph than *C. infortunatum* (Figure I).

Table 1: Efficacy of leaf extracts of *Clerodendrum infortunatum* and *Chromolaena odorata* on carbohydrate concentration in haemolymph of sixth instar larvae of *Orthaga exvinacea*.

Botanicals	Treatments	Concentration of carbohydrate		Percentage reduction of carbohydrate from control
		mg/ ml	mg/ larva	
Control		14.62±0.105	1.83±0.013	
<i>C. infortunatum</i>	1%	12.75±0.132	1.59±0.016	12.79%
	2%	10.81±0.074	1.35±0.009	26.06%
	3%	7.60±0.281	0.95±0.035	48.02%
	4%	4.54±0.181	0.57±0.023	68.95%
	5%	3.31±0.031	0.41±0.004	77.36%
F value		4225.16	4218.87	
<i>C. odorata</i>	1%	10.34±0.076	1.29±0.010	29.27%
	2%	6.74±0.156	1.03±0.022	53.90%
	3%	8.28±0.175	0.84±0.020	43.36%
	4%	3.88±0.140	0.48±0.017	73.46%
	5%	1.18±0.073	0.15±0.010	91.93%
F value		7067.89	7003.41	

The values are expressed as means of five replicates for each concentration with standard deviation (Mean ± standard deviation). All values were found to be highly significant ( $P < 0.01$ ).

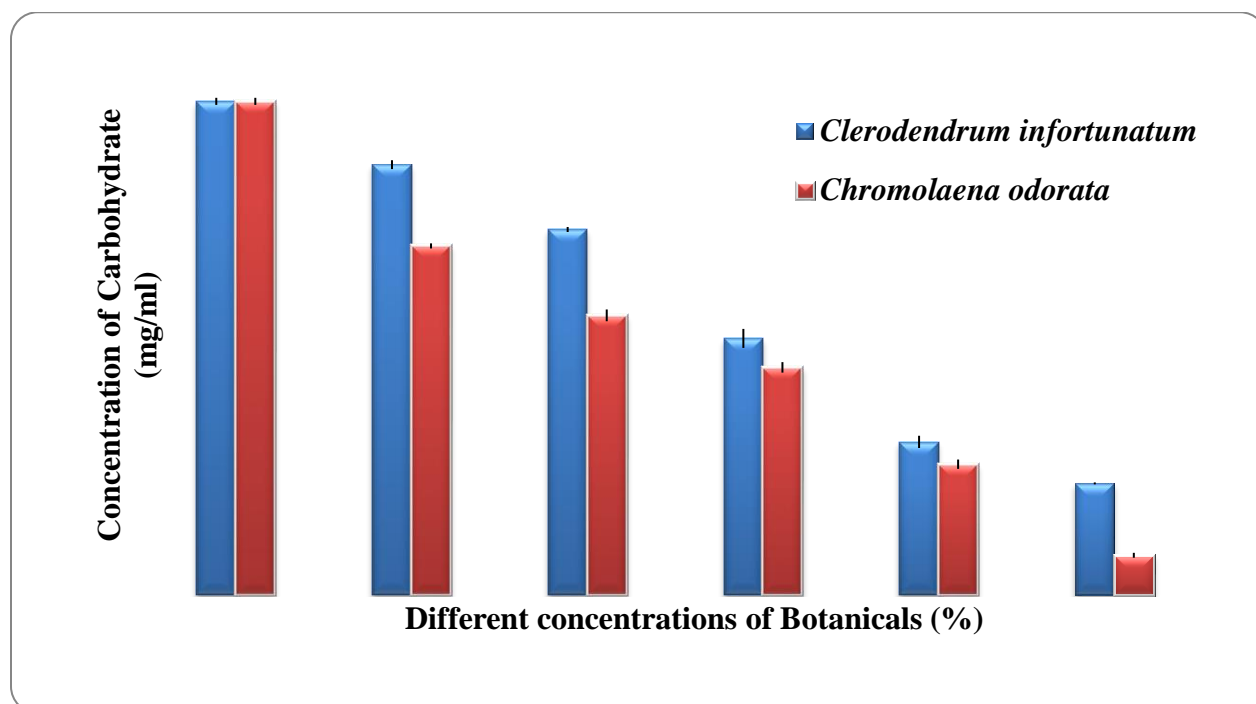


Figure 1: Graph showing the reduction of carbohydrate concentration in the haemolymph with increase in concentrations of botanicals.

#### 4. Discussion

The results of quantitative studies on carbohydrate concentration in haemolymph revealed the potency of both leaf extracts for decreasing the carbohydrate content in the larvae. Several comparable research findings were reported with similar results. The significant reduction in carbohydrate content of *S. litura* larvae was noticed after the larvae were treated with *Calendula officinalis* extracts [19]. The reduction in carbohydrate content in haemolymph of *Plodia interpunctella* was reported due to the toxic impacts of two volatile oils from leaves and stems of *Piper cubeba* and *Salvia officinalis* and three fixed oils from *Rumex dentatus* and *Trigonella foenumgraecum* seeds and *Acacia nilotica* leaves [20]. Similar reduction in carbohydrate content in haemolymph was reported in the larvae of *Crocidolomia binotalis* treated with extracts from *Lippia nodiflora*, *Vitex negundo* and *Strychnos nuxvomica* [21]. The effect of sub lethal concentrations of two volatile oils and three fixed oils on *Plodia interpunctella* larvae showed that all oil treatments declined the level of haemolymph carbohydrate content in larvae [22, 23]. The petroleum ether extract of *Ammi majus* and *Apium graveolens* fed to 6<sup>th</sup> instar larvae of *Agrotis ipsilon* produced greatly reduced carbohydrate concentration in haemolymph of the larvae [24]. The secondary metabolites in leaf extracts were responsible for the antifeedant activity that may lead to serious digestive disorders and reduction in the carbohydrate content in haemolymph of the larvae. Similar results were found in *Spodoptera litura* larvae treated with neem extract and it was reported that mode of action of phytoextracts can block the alimentary canal and it lead to the inhibition of feeding thereby lowering the glucose level in larvae [25]. Severe decrease in carbohydrate content was observed in *Crysoperla carnea* female at higher concentration of neem oil treatment and it may be due to the antifeedant property expressed by the phytochemicals present in it [26]. According to Sak et al. (2006) [27] the glycogen levels in juveniles and adults of *Pimpala turionellae* tend to decrease significantly when exposed to cypermethrin.

Under stress condition, the higher energy demand may increase the catabolic activities of nutrients to meet the energy loss. To overcome the toxic condition through detoxification process, intensive utilization of the carbohydrate reserves in both haemolymph and fat body may be the reason for decrease in carbohydrate content in haemolymph. According to Sharma et al. (2011) [28] the significant reduction in glucose content was observed in mosquito larvae when treated with *A. indica* and suggested that it may be due to the utilization of stored glucose in tissues as a result of insecticidal stress. It was reported that under stress conditions, the absorbed nutrients get catabolized to overcome the high energy demand [29] and finally, it may lead to the mortality of insect due to decreased energy metabolism [30]. The phytochemicals can directly or indirectly cause metabolic disorders in insects by creating biochemical changes that may lead to inactive or lethal state of insect. The present study suggests that the active principles present in both botanicals might be the cause for the reduction of carbohydrate content in the treated larvae. Hence higher concentration of methanolic leaf extracts of these two plants can be used as insecticides for the management of *Orthaga exvinacea*.

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\*Corresponding author.

E-mail address: jagan\_lkd2007@ yahoo.co.in