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Research Paper

Impact of carbaryl insecticides on metamorphosis of *Papilio demoleus* (l.) larvae in laboratory condition

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Abstract

Carbaryl is widely used insecticide. We administrated carbaryl on the 4th instar larvae of *Papilio demoleus* (L.) and studied developmental toxicity in laboratory condition. Carbaryl dose were 0.25, 0.5, and 1.00 mg/l as foliar spray. Larval malformation rates increased with concentration of carbaryl (R^2=0.89 and p>0.05). The skin of larvae became wrinkled and thin with yellow and brown pigmentation. They were slower in movement, loose in muscle contractions, showing hypersalivation and releasing semi-liquid frass. All metamorphosed adult evolved with wrinkled wings and paralytic flight. The result suggested that above 0.5 mg/l carbaryl was responsible for the changes (χ^2 = 0.01 - 0.1) of P. demoleus. This concentration can be considered as the safety level for the survival of the butterfly and the existing signature of malformation among both larvae and adult stage can also be used as biomonitoring purpose of the pesticide contaminated environment.

Keywords: carbaryl, *Papilio demoleus L,* malformation of larvae, metamorphosis, biomonitoring.

Introduction

Butterflies are important aspect of ecosystems for their interaction with plants as pollinators and herbivores. They are also good indicators of environmental changes as they are sensitive to habitat degradation and pollution. Near about 180,000 species of global Lepidoptera are well documented, out of which 18,000 species are butterflies[1]. *Papilio demoleus* (Lime butterfly) is one of the commonly observed tropical butterflies and their habitat is distributed from peninsular India to 7000 feet altitude in the Himalayas. It is common in agricultural lands as well as urban gardens. The body of Lime butterfly, is dark black, the fore wings are black. The whole wing is powdered by black scales. The wing span is 9.8±0.40 cm, body length is 2.9±0.16 cm and both sexes are alike [2]. The larvae consume leaves of citrus plants (family *Rutaceae*) and this is reported as an important pest of citrus causing severe damage of sapling and young stage in orchard [3]. The adults feed on a variety of floral species and they pollinate terrestrial garden and medicinal plants such as *Antigonon leptopus*, *Lantana camara*, *Catharanthus roseus, Jasminium angustifolium* and *Ixora arborea* etc [4].

Usually carbaryl, endosulfan, phosalone, triazophos and acephate like pesticides had been used to control of *Papilio demoleus* on oranges plantations. The greatest reduction of the pest population was reported after 24 hours with 0.07% endosulfan, followed by 0.2% carbaryl (87.3 and 81.9% mortality, respectively). After 72 hours, 0.08% acephate had caused the greatest reduction (95.1%), followed by endosulfan (94.6%) and carbaryl (92.5%). The toxicity of pesticides on lime butterfly is not well documented but the mode of action of carbaryle on organisms is to inhibit the activity of neurotransmitter acetylcholine and restrict the movement of organism[5,6]. The impact of carbamate

pesticides specially on insects to mammals are well reviewed[7,8,9]. Carbaryl (1-naphthyl-N-methylcarbamate) is the widely used insecticide among the carbamate group of pesticides and it controls over 100 species of insects on fruit, cotton, forests and many other crops, as well as on poultry, livestock, and pets[7,10]. Carbaryl is available in granules, dispersions and suspensions formulations. Trade names of commercial insecticides containing carbaryl include Carbamate, Tercyl, Sevin, and Nac. However, various formulations vary widely in toxicity. For example, it is categorized as toxicity class I—highly toxic (Tercyl); toxicity class II—moderately toxic (Sevin); and toxicity class III—slightly toxic. Carbaryl is reported as carcinogen, endocrine disruptor, reproductive toxicant, and developmental toxicant[11].

Applications of pesticide in agricultural fields, gardens and mosquito control also disturb the non target species of insects like butterflies and bees[12,13,14]. The Xerces Society (2008) for Invertebrate Conservation at United States reviewed the status of butterflies and bees of North America[15]. The Red List of Pollinator Insects of North America includes dozens of butterflies and bees that are facing significant threats and population declines. Gallai et al. calculated the worldwide value of pollination to agriculture. They calculated the costs using the proportion of each of 100 crops that need pollinators that would not be produced in case insect pollinators disappeared completely. The economic value of insect pollination was then €153 billion[16]. Pimentel et al. reported that pesticides indirectly cost the U.S. about $ 8.1 billion a year including losses from increased pest resistance; loss of natural pollinators (including bees and butterflies) and pest predators; crop, fish and bird losses; groundwater contamination; and harm to pets, livestock and public health[17].

The objective of the present study was to find out changes in the life cycle of a common garden butterfly (Lime butterfly- *Papilio demoleus*); with special reference to their several deformities in morphology from instars to adults after application of cabaryl pesticide in laboratory condition.

Materials and Methods

Larvae of lime butterfly (*P. demoleus*) were collected from butterfly park, Science City, Kolkata, India and reared in Jadavpur University (Kolkata, India). They were fed with the leaves of *Citrus paradise* that were collected from garden. Sevin (CAS No 63-25-2) was used as pesticide in this study which is the trade name for a widely used synthetic insecticide containing the active ingredient Carbaryl (1-naphthol N-methylcarbamate). Carbaryl belongs to the chemical class called carbamates. Sevin was dissolved in ethanol. The doses of Sevin were 0.25 mg/l, 0.5 mg/l and 1 mg/l. As the eggs and instars are never exposed to any pesticides, the test dose of carbaryl was demarcated 10% of agriculture dose mentioned by the manufacturer [18]. The instars of the butterflies were kept in different containers and divided into two groups as a control unit and other as treated population. Each dose of pesticides were sprayed on the leaves and dried in air. This individual leaf was supplied as food for the instars(3^{rd} stage). Clean leaf (washed by distilled water) was supplied for the food of control population. Both control and treated larvae were maintained in a climate chamber set at 27 ± 2° C, 65 ± 5% RH, with a photoperiod of 16:8 Light: Day. Experiment was replicated three times. Different parameters on 4^{th} stage of instars like morphological malformation (body color and skin thickness); physiology (movement, food ingestion, hyper-salivation, muscle construction) and abnormalities among adult stage (length of body, hind wings and fore wings, and flight) were recorded. Statistical significance was analyzed using the Chi-square test.

Results and Discussion

The larvae feed with 0.25 mg/l of carbaryl had shown less malformation followed by 0.5 and 1 mg/l of carbaryl concentration. The highest larval malformation was observed at 1 mg/l level. Malformed larvae were not able to form a pupal casing, and never emerged as adults. Some larvae molted into malformed larvae. Bright lime green colour of skin was observed in the control population whereas the carbaryl treated larvae became brown with yellow with brown pigmentation (Figure 1).

The malformation frequency was dependent on the test concentrations (R^2=0.89 and $p>0.05$).The skin of these larvae (95%) was more thin and wrinkled at both 0.5 and 1 mg/l carbaryl concentration whereas at 0.25 mg/l carbaryl concentration a few significant change was observed (Table 1).

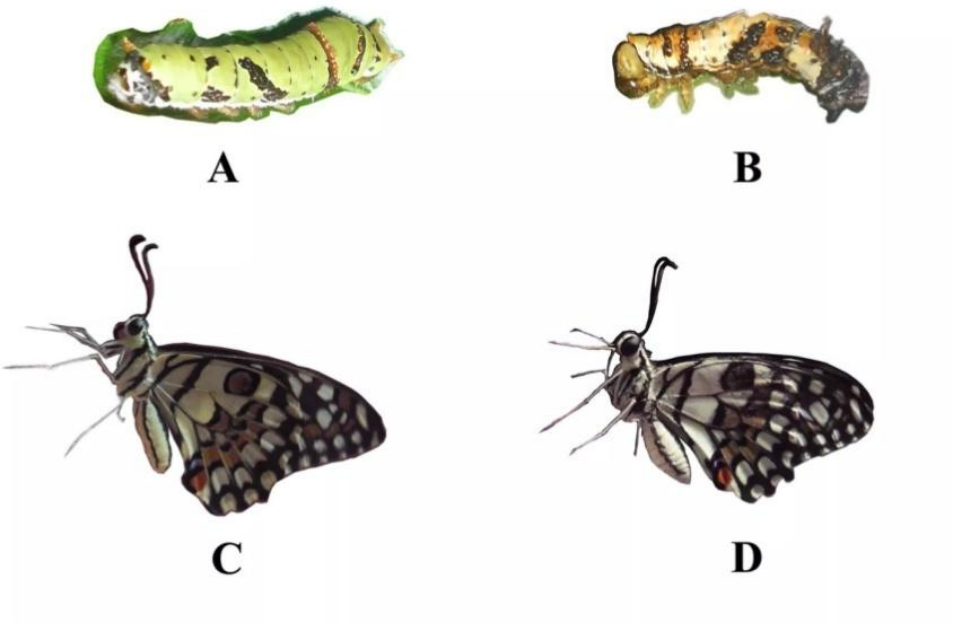

Figure 1: Malformation of larvae (observation at 4th instar stage) and adult *P. demoleus* (L.) after treatment of carbaryl in laboratory condition. A. larvae (control), B. treated larvae, C. Adult *P. demoleus* (control), D. malformed butterfly with wrinkled wings

Table 1: Malformation of carbaryl treated 4th instars larvae of *P. demoleus* (n=14)

Carbaryl (mg/l)	yellow and brown pigmentation	Wrinkled and thin skin
0	0 (0%)	0 (0%)
0.25	1 (2.4%)	1 (3.4%)
0.5	9 (66%)	13 (95%)
1	12 (88%)	13 (95 %)
df	12	
α -score	Varies from 18.40 to 25.54	
χ^2test significance level	Varies from 0.01 to 0.1	

Researchers applied different pesticides on the larvae and adult of different insect pest like lime butterfly and they showed abnormalities in morphogenetic development and abnormalities at their wings[19, 20, 21]. Saastamoinen et al. also reported the formation of plastic larvae due to local pollution of their study area[22]. Duration of the lifecycle of the test population in the present study was about 23 days which was significantly longer than the control population (14 days). Probably the carbaryl like other growth inhibitors inhibits the action of enzymes restricting the growth of larvae. Singh and Kumar administrated diofenolan in common citrus butterfly and they observed larval-larval, larval-pupal malformation and slow growth in a lab-scale study [23]. An in-vitro study also revealed the same result where pesticide inhibits the growth of cultured Sf-9 cell line of insect [24].

Five physiological events related with metamorphosis of the malformed larvae were recorded in 4th instars stage. The treated larvae were slower in their movement (R^2=0.67 and p>0.05) with the increasing treatment concentration and consumed lesser food than the control population of *P. demoleus*. Very few physiological changes were observed among the population treated with 0.25 mg/l carbaryl dose (Table 2). The salivary secretion and excretion of malformed larvae were recorded. Treated larvae produced more saliva and excrete semiliquid frass. This data was not significantly proportional with the applied carbaryl concentration. The muscle contractions were loose (R^2=0.48 and p>0.05) and above 0.5 mg/l dose treated *P. demoleus* became hypersalivate and released semiliquid frass (Table 2).

Table 2: Physiological activity of carbaryl treated 3rd instars larvae of *P. demoleus* with compare to control population (n=14)

	Applied dose of Carbaryl (mg/l)	0.25	0.5	1
Physiological activity (observation was taken at 4th instar stage of larvae)	Slow movement	4 (32%)	6 (46.5%)	9 (68.8%)
	Less feeding	0 (0%)	9 (65.5%)	12 (87.4%)
	Loose muscle construction	1 (10%)	6(47%)	9 (68.3%)
	High Saliva secretion	0 (0%)	12 (92%)	13 (94%)
	Semi liquid Excretion	0 (0%)	12 (92%)	13 (94%)
	df	12		
	α -score	Varies from 18.56 to 26.54		
	χ^2 test significance level	Varies from 0.01 to 0.1		

Molting and metamorphosis are two critical physiological events in the life of insects. All insects molt periodically in order to grow, and all but a very few go through either gradual or complete metamorphosis to become an adult. Regarding the observation of saliva secretion and excretion treated larvae produced more saliva than the control population. This data was not significantly proportional with the applied carbaryl concentration. The saliva secretion were almost same in both 0.5 and 1 mg/l carbaryl concentration (table 2).The muscle contractions were loose (R^2=0.48 and p>0.05) and above 0.5 mg/l dose treated *P. demoleus* released semiliquid frass (Table 2) than the treated population. Carbaryl is developmental toxicants [25]. Some recent reviews have identified the major neuro-active insecticide targets are acetylcholinesterase enzyme[26, 27], nicotinic acetylcholine receptor, gamma-aminobutyric acid receptor of insects and Na^+ channels [28]. Similarly in the current study the carbaryl probably inhibited the normal nerve conductions of both larvae and adults of *P. demoleus.* The both voluntary and involuntary muscle related with their movement, muscle construction and food ingestion were restricted. Salivary secretion, diarrhea and gastrointestinal problem are very common phenomenon in carbaryl toxicity [29, 30]. The behavior of saliva is one of the matrix of environmental contamination [27]. Hypersalivation is controlled by parasympathetic nerve secreting acetylcholine. A laboratory scale study reported that cholinesterase inhibitors like carbaryl and others lead to accumulation of the neurotransmitter acetylcholine and enhanced salivation in mice[31]. Carbaryl is most widely used cockroach repellent. Early study reported that the rate of excretion of male cockroach was high to remove this pesticide from their body[32]. This can be compared with the present study regarding the excreatory product of the test population (Table 2).

The adult butterfly evolved from the deformed larvae was shorter in body length with their length of fore and hind wings (Figure 1). The significant differences were observed above 0.5mg/l concentrations. All adult *P. demoleus* treated with 0.5mg/l and 1 mg/l concentrations ultimately metamorphosed with wrinkled wings and paralytic flight (Table 3 and Figure 1).

Table 3: Malformation among carbaryl treated evolved adult stages of *P. demoleus* (n=7)

Carbaryl (mg/l)	structure of wings (wrinkled)	flight (Paralytic)
0	0 (0%)	0 (0%)
0.25	0 (0%)	0 (0%)
0.5	6 (94%)	6 (94%)
1	6 (94%)	6 (94%)
df	6	
α -score	Varies from 20.40 to 30.54	
χ^2 test significance level	Varies from 0.01 to 0.1	

Previous research has been revealed that carbaryl binds with the acetylcholine esterase and form the enzyme-inhibitor complex[12] which ultimately paralysed their nuromascular junctions. The brown and yellow pigmentation of larvae of *P. demoleus,* wrinkled and paralytic wings of adult butterfly (Figure 1) are significant (P>0.05) malformation in laboratory conditions. It can be predicted that though the applied dose of carbaryl (Savin) pesticide is modarately toxic[22] to larvae, it did not kill the metamorphosed adult butterfly (Table 1). This malformation actually makes them more susceptible for their predators like birds and reptiles. This can be correlated with the migration of monarch butterfly at United State, where their populations are declined after introduction of genetically modified corn[33, 34, 35] and other butterfly species exposed to mosquito control insecticides[13, 14]. Ultimately our prediction is that the changes of pigmentation (Figure 1) of larvae, wrinkled wings and paralytic flight of adult may decline the population *P. demoleus* in natural environment.

Conclusions

The three major malformations are observed above 0.5 mg/l carbaryl concentration in laboratory condition. Above the concentration may be fatal for both larvae and adult butterfly *P. demoleus*. So the safety guideline of carbaryl in environmental media for the survival of both larvae and adult *P. demoleus* may be below the 0.5 mg/l. The existing signature of malformation among both larvae and adult stage may also be used as biomonitoring of the pesticide contaminated environment.

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