Effect of nicotine on larval behaviour and fitness in *Drosophila melanogaster*

Geeta R. Koppad and N. Shivanna

ABSTRACT

Drosophila is a simple and genetically tractable system, it has been used to unravel and understand different aspects of biology, including neurobiological studies. Nicotine is a generally occurring liquid alkaloid. This botanical is known as contact poison and it generally affects the central nervous system. It has been used as an insecticide, it stimulates nicotinic receptors and neurosecretion at the neuromuscular junction. For the present study 0.05% crude extract of tobacco (nicotine) was used to study the effects on various stages and behaviour such as, larval pupation site preference (PSP), locomotory behaviour, mating success, fecundity and productivity in *Drosophila melanogaster.* Synchronised eggs were collected and allowed to hatch, from these standard cultures first instar larvae were transferred to glass vials containing nicotine mixed media. Yeast dissolved in nicotine was added every day to larvae for feeding. Fifty first instar nicotine treated larvae were allowed till pupation in vials to study PSP, after pupation the pupa were counted and analyzed. Nicotine treated third instar larvae were used for analyzing larval locomotory behaviour. Larval patterns were taken on transparent paper placed on agar coated petri dish for five minutes and larval path lengths were measured. About thirty pairs of five day old adult flies were collected for mating success study. Mated females were transferred to separate vials to count eggs layed every day and pupa after pupation to analyze fecundity and productivity. Treated larva showed a significant difference than control in larval pupation site preference, locomotory behaviour, mating success, fecundity and productivity.

Key Words: *Drosophila melanogaster*, Larval behaviour, Fitness, Nicotine

INTRODUCTION

Drosophila exhibits 4 stages in the life cycle such as egg, larva, pupa and adult. Among these larval and adult stages shows characteristic behaviours like pupation site preference, larval locomotion, mating/courtship behaviour etc. The larval pupation site preference (PSP) is an important event in *Drosophila* preadult development, because the place selected by the larva can have decisive influence on their subsequent survival as pupae (Sameoto, and Miller, 1968). The effect of abiotic and biotic factors on PSP has been studied (Markow, 1981, Hodge *et al*., 1996, Sokolowski *et al*., 1986). It revealed that the above factor affects the PSP.

The role of larval behaviour in habitat selection, feeding, locomotory behaviour, olfactory and chemotactic responses, gravity and identification of hazardous substances has been studied in different species of *Drosophila* (Green *et al*., 1983; Sokolowski, 1997; Godoy-Herrera *et al*., 2004). Larval locomotion has been studied in different species of *Drosophila*, in relation to ethanol (Parsons, 1980), moisture and olfaction (Bala *et al*., 1998; Hussain *et al*., 2003). Mating is an important mechanism to propagate the species. Behaviours controlling the propensity to mate or remate can have large effects on fitness, because the decision to mate during a given age period can have direct effect on current and future reproduction and hence fitness (Partridge, and Andrews, 1985).

Fecundity is one of the fitness parameter that is used to assess the fitness in different species of *Drosophila* (Roff, 1992). Fecundity is the most obvious trait that influence the reproductive value of female by her genotype, body size, age and her mate as well as the effects of environmental factors (Partridge *et al*., 1986; Bounduriansky, 2001). Productivity is termed as fertility a component of fitness; it is the number of newly produced offspring from a mated pair. The productivity has been extensively studied in different species of *Drosophila* (Krishna and Hegde, 1997).

Nicotine is a natural alkaloid. It was first used as an insecticide in 1763 and still ranks as an excellent contact insecticide. As a whole symptoms of poisoning by nicotine

follow the general sequence of excitation, convulsion, paralysis and death (Matsumura, 1985). Various chemicals have been used to study different aspects of *Drosophila* development, fitness parameters etc. (Choudhary, 2003 and 2004; Srivastava *et al*., 2003; Al-Momani and Massadeh, 2005; Shivanna *et al*., 2006; Kartas and Bahceci, 2008; Gangishetti *et al*., 2009). As *Drosophila* does not encounter nicotine in their food, it can be used to test the various behavioural aspects. In view of these, the effect of nicotine on the larval PSP, larval locomotory behaviour and fitness in *Drosophila melanogaster* have been studied.

MATERIALS AND METHODS

Insect source and Rearing

Drosophila melanogaster was collected from *Drosophila* stock Centre, Department of Zoology, University of Mysore and were cultured in wheat cream agar media prepared as per the procedure described by Shivanna *et al*. 1996. Yeast was added to cultures everyday to feed the larvae and maintained at a constant temperature of 22 ± 1°C with relative humidity of 80%.

Aqueous Extract of tobacco

Leaves of tobacco (*Nicotiana tobacum*) were collected from a healthy plant. The leaves were washed and then dried under shade for 7 days. The dried leaves were kept in oven at 50° C for 2 hours to make them crisp. From this, fine powder of 100gm was weighed and soaked over night in 500ml of distilled water. This mixture was stirred on magnetic stirrer for 2 hours. Then it was filtered through cheesecloth followed by filter paper and this filtrate was evaporated under vacuum flash evaporator. The dry powder thus prepared was weighed (1 gm) and dissolved in distilled water (100 ml) to get 1% crude extract of tobacco (stock solution). The stock solution was stored in refrigerator for further use. The stock solution of 500µl of 1% nicotine was dissolved in 9.95 ml distilled water to obtain 0.05% nicotine concentration.

Larval Pupation site Preference

Synchronized eggs were obtained from the standard cultures and were allowed to hatch. About fifty, first instar larvae were transferred to glass vials containing wheat cream agar medium. 10 replicates of both control and nicotine (0.05%) treated vials were maintained at a constant temperature of 22±1° C as per the procedure described by Shivanna *et al*. (1996). Nicotine mixed yeast was added to vials everyday for feeding. The number of larvae pupated at different sites (cotton, glass wall and media), were counted and tabulated.

Larval locomotory Behaviour

The larval locomotion has been studied in *Drosophila melanogaster* in both control and nicotine treated third instar larvae. About 5 larvae were selected randomly from each of 5 replicates which contain 50 larvae. Agar was prepared and poured into petri dish. Then it was allowed to cool. Larva was placed at the centre of the petri dish containing solid agar media and another petri dish was placed on it. Transparent paper was placed on the petri dish and then larval patterns were marked on it using marker pen for 5 minutes. The larval movements like straight lines, circles, head lifts, right and left turn were recorded. The larval path lengths were measured using the procedure described by Bala *et al*. (1998).

Fitness parameters

Virgin flies were collected and kept separately in vials for 5 days. About 30 pair of flies were used to study mating success, mating period, fecundity and productivity. Mating success was studied using the procedure described by Choudhary, 2003. A total of 120 pairs were analyzed in 4 different sets such as, P = C♀ X C♂, Q = T ♀ X T♂, R = C♀ X T♂ and S = T♀ X C♂. For every set, about 30 pair of adult flies were allowed to mate in empty glass bottle. Observation was made for 4 hours. Soon after mating, males were separated and the mated females were placed separately into food vials containing wheat cream-agar medium to determine fecundity. Flies in the vials were transferred to fresh food vials everyday. This procedure was followed for 15 days. Egg count was done in each vials to analyze the fecundity of female. Eggs were allowed to hatch and dilute yeast was added to these vials till pupation. Pupal count was also recorded to analyze productivity. The data was subjected to statistical analysis (student 't' test) to find out is there any significant difference in larval locomotion and fitness parameters of control and nicotine treated larvae.

RESULTS

The control larvae prefer to pupate more on glass 89.32% and media 6.4% than treated larvae (81% on glass and 4.6% on media) and none of them on cotton. The statistical analysis revealed that pupation site preference within control and treated larvae between sites shows significant difference with G v/s M, G v/s C and M v/s C except M v/s C in control. [G- Glass, M-Media, C-Cotton] Table 1 shows the larval locomotory patterns. Treated larvae showed decrease in number of straight lines, left turns, right turns, head lifts and the distance moved compared to control, whereas the number of circles increased. Statistical analysis showed significant difference between

Table 1. Larval locomotory patterns of *D.melanogaster*.

	Left	Right	St.line	Circle	Head lift	Length
Control	24.72±5.78	23.2±6.95	46.48±11.90	3.08±2.66	6.32±3.37	22.14±5.54
Treated	22.24±6.61	23.72±6.32	32.04±12.43	3.72±2.44	3.4±2.66	16.54±4.52
't' value	1.411	0.276	4.194*	0.866	3.39*	3.92*

*P = Significant at 0.005 level

control and treated larvae in number of straight lines, head lifts and distance moved by larvae.

Mean and S.D. of fitness parameters in different crosses of *Drosophila melanogaster* are given in Table 2. Mating period was more in cross between treated flies (Q) and less in cross between control flies (P). Whereas the production of eggs and pupae formed showed contrast results than the mating period of both treated and control flies. The mating period between the treated female with control male (S) was more than the control female with treated male (R). Whereas the production of egg and formation of pupae showed contrast result than the mating period. Statistical analysis showed significant difference in mating period, number of eggs and number of pupae between treated and control larvae. The variation of mating period was found to be significant in Q v/s R and Q v/s S. Number of eggs produced significantly varied between P v/s Q, P v/s S, Q v/s R and R v/s S. The number of pupae formed was significant in all the combinations except P v/s R.

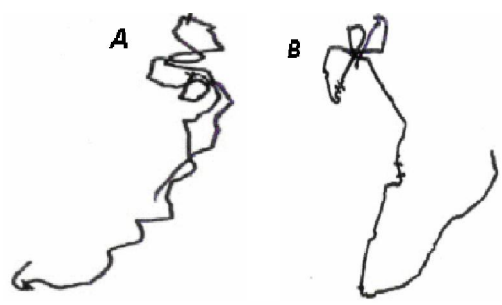

Figure 1. Larval locomotory patterns of Drosophila melanogaster

A. Control

B. Treated

DISCUSSION

The larval pupation site preference (PSP) is one of the behaviour during late third instar larva of *Drosophila*. Larvae select different substrates for their pupation and undergo further development. PSP is the percentage of larvae moved/not moved from media to pupate at different sites. It is evident from the results that the percentage of glass and media pupation was decreased in treated larvae than control. This indicates that the larval PSP is affected by the treatment of nicotine. Srivastava *et al*. (2003) observed that, some larvae failed to crawl from food on to the wall of the vials for pupation but instead attempted for pupation on or near the surface of the food medium when treated with penta co-ordinated organotin (IV). Shivanna *et al*. (2006) stated that, increase in concentration of endosulfan decreased the glass and cotton pupation of glass and cotton pupating species.

In present study, the larval and pupal abnormalities were observed. Abnormal larvae failed to pupate and deformed pupae failed to emerge into adult flies. According to Choudhary (2003), nicotine causes lethality in immature stages of *Drosophila* by inhibiting the chitin synthesis during moulting and affects the adult emergence of mutant form (yellow) of *D. melanogaster*. Study of Al-momani, and Massadeh (2005) revealed that the high concentration of heavy metals Cd, Cu, Pb and Zn affects the metamorphosis of *D. melanogaster* larvae to form the pupae. Such as reduction of adult size, including wing and abdomen. Larval locomotory behaviour was studied in both control and nicotine treated larvae (Figure 1). The larval patterns showed characteristic difference in number of straight lines, head lifts, and distance moved by larvae when compared to control. These results coincide with the findings of Wilson (1997), Choudhary (2003 and 2004) Drees and Gold (2003) for different chemicals.

Fitness parameters were analyzed in 4 different crosses (P, Q, R and S) of *D. melanogaster*. Among these the effect of nicotine on fitness parameters was more when both the sexes were treated. It was found that mating period was high in nicotine treated flies than compared to other crosses. The fecundity and productivity was less in treated females than male. These results are similar with the work on other chemicals (Borkovec, 1979; Choudhary, 2003 and 2004; Nadda *et al*., 2005; Karatas and Bahceci, 2008).

Present study revealed that, the nicotine treated larvae preferred less to pupate on glass and media and the locomotory patterns also varied compared to control. It was found that mating period was more in treated flies

Table 2. Mean ± S.D. of fitness parameters of *D.melanogaster*.

	Mating Period	Average no. of eggs	Average no. of pupae
P	18.05 ± 5.09	221.9 ± 56.60	196.5 ± 55.74
Q	23.18 ± 7.63	98.9 ± 15.11	49.7 ± 22.83
R	14.53 ± 1.88	214.7 ± 25.45	186.8 ± 28.10
S	15.6 ± 3.20	117.9 ± 32.55	88.7 ± 27.12
C = Control, T = Treated. ♀ = Female, ♂ = Male			
P = C ♀ X C ♂, Q = T ♀ & X T ♂			
R = C ♀ X T ♂, S = T ♀ X C ♂			

compared to other combinations. The fecundity and productivity of treated flies showed contrast results when compared to mating period. According to these results it was concluded that the larval PSP, larval locomotory patterns and fitness parameters were affected by nicotine.

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Geeta R. Koppad* and N. Shivanna
Dept. of Zoology, Karnatak University Dharwad-580003, Karnataka, India, *****E-mail- koppadgeeta@gmail.com