

## Association of *Escherichia coli* with the Prevalence of Flies Population

Hassan Flayah Hassan and Marryam Alkafagi

Department of Plant Protection, College of Agriculture, University of Baghdad, Baghdad, Iraq

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### ABSTRACT

Mass releases of house fly *Musca domestica* (L) and stable fly *Stomoxys calcitrans* (L). Produced by manure piles accumulated nearby horse stables and dairy farm village in Abu-Graib provide continuous threat to inhabitants west of Baghdad. Timing of fly's mass release in association with the presence of *Escherichia coli* in manure piles was examined at these locations. Experimental result indicated that flies survived during cold period of December and January in form of larvae deep in manure piles where temperature was around 15-17°C. Once the population of fly's larvae started to increase by the second week of February, the concentration of *E. coli* was up to  $80 \times 10^6$  CFU mL<sup>-1</sup> in manure suspension. Later when larval population reached to a maximum number by the last week of April, the concentration of *E. coli* in manure sample dropped down to  $38 \times 10^2$  CFU mL<sup>-1</sup>. Similar trend was observed with the proportion of *E. coli* to general bacteria present in manure samples where the percent decreased from 89% in early season to 1.5% when maximum number of larvae was recorded. The correlation coefficient (R) between the number of larvae and coli form bacteria was = -0.73657. These results suggest the association of larval development with the consumption of *E. coli*. Thus manipulation of bacterial community in manure piles may lead to possible eradication of fly's seasonal release.

**Keywords:** *E. coli*, Flies, Manure, Prevalence, Baghdad

### 1. INTRODUCTION

The muscoid flies persist as cosmopolitan pest of human and of domestic livestock throughout much of the world, in particularly because of the ability of the immature stages to develop in variety of common organic materials such as manure, garbage and human waste (Keiding, 1974). Virtually any environment rich in organic matter, that support the growth of house fly and related muscoid maggot harbors a diverse bacterial community and becomes a suitable substrate for development of house fly and other cyclorrhapha flies (Spiller, 1964; D'Amato *et al.*, 1980; Zhao *et al.*, 1995). In spite of the fact that bacterial community in the substrates the larvae feed on varies considerably

from the bacterial community within the larval gut (Zurek *et al.*, 2000; Perotti *et al.*, 2001). In early works (Glaser, 1924; Gerberich, 1948) had reported a positive relationship between bacterial activity and house fly maggot growth, leading to the understanding that bacteria growth factors such as vitamins and sterols (Brookes, 1958) are essential for maggot growth (Chang and Wang, 1958). In recent years, the association of muscoid flies and bacteria has been observed from several perspective. The significance of bacteria for development has been examined for house fly (Schmidtman and Martin, 1992; Watson *et al.*, 1993), stable fly (Lysyk *et al.*, 1999; Talley *et al.*, 2009), face fly (Hollis *et al.*, 1985) and horn fly (Perotti *et al.*, 2001). The immature stages of these

**Corresponding Author:** Hassan Flayah Hassan, Department of Plant Protection, College of Agriculture, University of Baghdad, Baghdad, Iraq

species require bacteria during development, most likely as food source and survival can vary depending on the species of bacteria the larvae feed on. The survival is enhanced greatly in media supplemented with bacteria compared with sterile one. Another perspective that involved the digestibility of bacteria in the intestinal tract was examined in house fly (Espinoza-Fuentes and Terra, 1987), stable fly (Rochon *et al.*, 2004), blow flies (Greenberg, 1968) and black soldier fly (Qiaolin *et al.*, 2008). Persistence of *E. coli* in artificially fed larvae showed that abundance of bacteria decline in house fly larvae but remained constant in immature stable fly.

Since land applications of dairy cattle and horse manures are a common practice and have served as primary source for soil fertilizers available to farmers in Iraq, this research was initiated to understand mass release of adult flies from manure piles west of Baghdad and possible manipulation of microbial population to suppress larval development.

## 2. MATERIALS AND METHODS

Seasonal abundance and activity of flies larvae in manure piles was monitored to fecal coli form bacteria and temperatures. Between December 2012 and July 2013 approximately 40 sample/month were carried out on weekly bases from manure piles at dairy farm village and horse stables on the western site of Baghdad.

Samples for quantification of larvae and fecal coli form bacteria were obtained by taking a composite 250 mL/sample of manure substrate. Each sample was aseptically transferred to 350 mL sterilized glass and placed in Ziploc bag.

In the laboratory, the larvae were taken out of the sample, counted and raised to adult stage. The rest of manure sample was suspended in 2500 mL of PBS (PH 7.2) and placed on shaker for 30 min. A wide serial dilutions of the manure suspension ( $10^2$ ,  $10^3$  to  $10^{10}$ ) were prepared since expect number of bacteria was unknown. Dilutions were plated on two types of media, a broad spectrum nutrient agar medium and differentiating Ma Con key agar media following procedure outlined by Romero *et al.* (2006). Plates were incubated aerobically at 37°C. Greater accuracy was achieved by plating triplicates of each dilution. Colonies were counted using standard plate count

method and expressed as Colony-Forming Unit (CFU). Counts were converted to log CFU/mL.

## 3. RESULTS

During the cold period of winter, flies survived the months of December and January in form of larvae deep in manure piles where temperature was around 15-17°C. Larvae may able to survive at such temperature for a period of 70 days (Gilles *et al.*, 2005). By the spring break mid of February, larvae were encountered more frequently in manure samples and the number of larvae continued to increase up to the peak during the fourth week of April (**Fig. 1**). In contrary, the number of coli form bacteria showed slight increase early season and then started to fall down as the number of larvae increased in manure samples. The concentration of *E. coli* dropped from  $10^7$  to  $10^2$  CFU mL<sup>-1</sup> at the maximum number of larvae counted on the last week of April. After that a short increase in bacterial number was observed then a sharp reduction in both numbers of larvae and bacteria was occurred mostly due to temperature increase and drought of manure substrate. The daily field temperatures in Baghdad reached to 45°C. by the first week of July.

It seems that the reduction of bacterial number in manure sample as a result of larval number increase was generally related to coli form bacteria. The proportion of *E. coli* numbers counted on Maconkey agar to the total bacteria numbers recorded on nutrient agar decreased from 89% in early season to 1.5% when maximum number of larvae was recorded by the last week of April (**Fig. 2**).

The data indicated that the reduction in bacterial number was largely contributed by coli form bacteria although some reduction was observed in total bacterial number which may suggest the involvement of other species. The correlation coefficient between the number of larvae and concentration of *E. coli* in manure samples showed negative relationship with the value of  $r = -0.73657$  (**Fig. 3**).

The value of  $r$  indicates that 50% of larval change was related to *E. coli* number and involvement of other factors possible too, such as present of different bacteria species, temperatures and dryness of substrate. It may worth to mention that 97.8% of the larvae sampled were turned to adult house fly and 2.2% to stable fly.

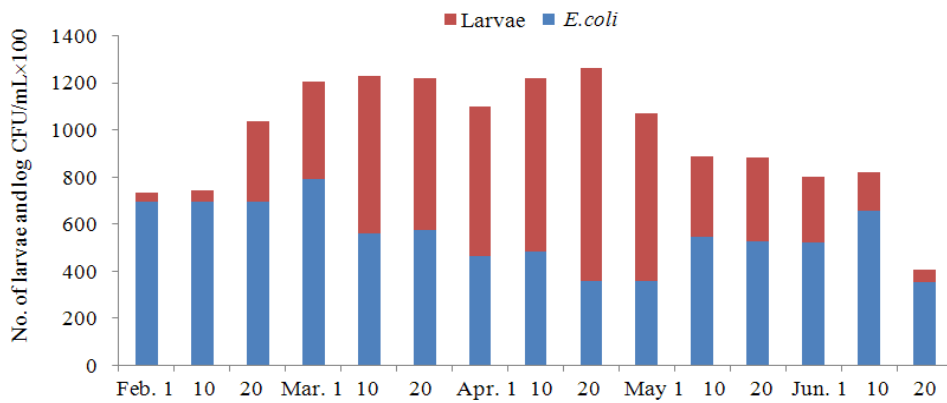


Fig. 1. Number of flies larvae and *Escherichia coli* present in manure samples west of Baghdad

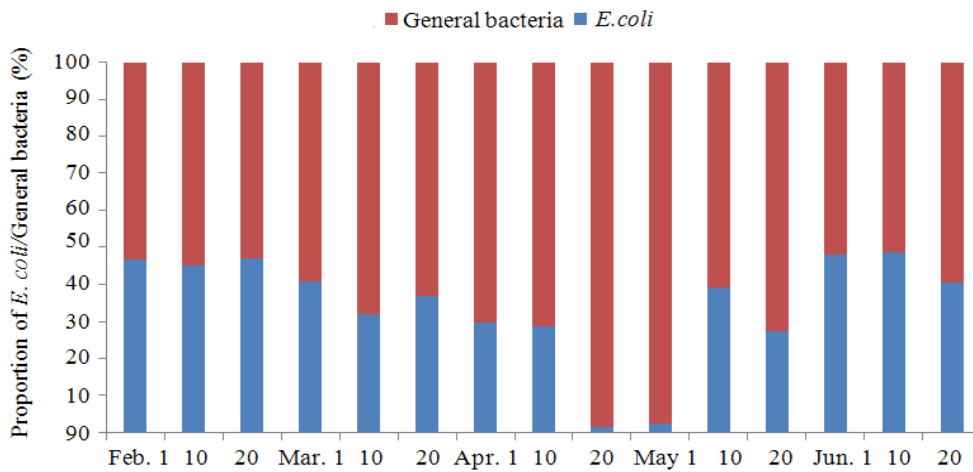


Fig. 2. Proportion of *Escherichia coli* to general bacteria present in manure suspension samples

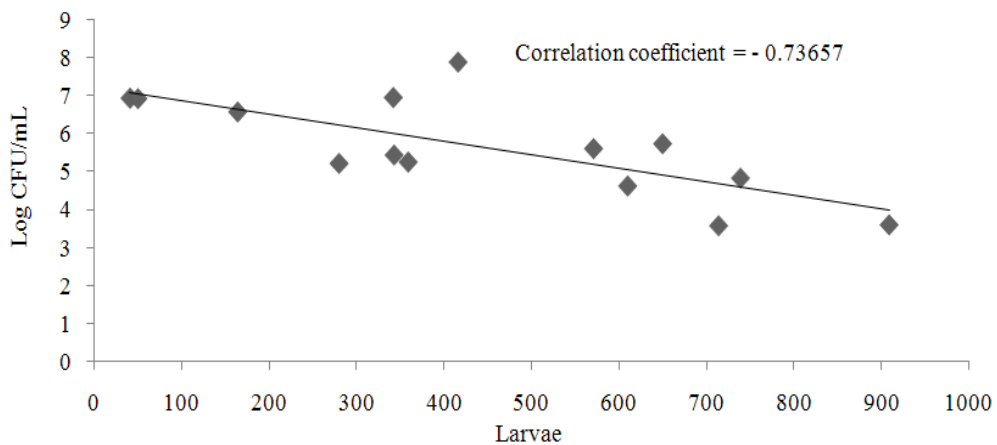


Fig. 3. Relation between number of larvae and concentration of *Escherichia coli* in manure samples

#### 4. DISCUSSION

Although several authors have demonstrated that larval development depends on live microbial community (Talley *et al.*, 2009), no study has related larval population to coliform bacteria in the field. All previous studies implied the impression of positive relation between flies and coliform bacteria. To some extent this is not correct simply because they have observed the adults rather than the larvae. The direct contact between them will reveal a negative relationship. However, this relation could be manipulated in our environment since all flies overwinter in larval stage in manure piles and thus the concentration of bacteria could be very effective factor.

#### 5. CONCLUSION

In conclusion, result indicated that consumption of coli form bacteria plays an important role in larval development. Thus the manipulation of bacterial community in manure piles may inhibits flies seasonal release.

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