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# *In vitro* detection of resistance against Insect Growth Regulator (Cyromazine) in house flies

**N Sathiyamoorthy, K Senthilvel, N Rani, K Ramya and G Ponnudurai**

**Abstract**
Adult house flies were collected from five poultry farms in different blocks of Namakkal, Tamil Nadu and reared in the laboratory. The second stage larvae obtained from the collected house flies were used for larvicide bioassay. *In vitro* trials were conducted with different concentrations of Cyromazine (1%) such as 0.025, 0.05, 0.1, 0.2 and 0.25 per cent. Twenty numbers of second stage larvae were seeded into the larval medium (100 g) incorporated with each concentration of drug and the control group with fresh water in a plastic container. The larvae in both groups were maintained at 33±1 ˚C and 60-70 per cent relative humidity with a photo period of 8:16 hours of light and dark period. The larval mortality was assessed 4 days after treatment and pupae, if any were collected and placed in a container for another 5 days to observe adult fly emergence. Data on larval mortality, abnormal pupae, intermediates and adult fly emergence were recorded. The results revealed that hundred per cent inhibition of fly emergence in three farms at the normal recommended dose (0.05 %), indicating that the fly population in these farms were susceptible to this drug and remaining two farms had shown 80-90 per cent reduction of fly emergence even at higher concentration of larvicide (0.1 %) suggesting that the population of houseflies prevalent in these farms might have developed resistance against Cyromazine.

**Keywords:** House flies, insect growth regulator, cyromazine, poultry farms, resistance

## Introduction
House fly, *Musca domestica* L. (Diptera: *Muscidae*) is a well-known pest and usually feed and breed on decaying matter, human waste and in poultry manure. Manure accumulation in the poultry farms under the cages coupled with prevailing temperature and humidity provide an ideal environment for the breeding and development of house flies. Housefly is capable to transmit many human and animal pathogens mechanically (Forster *et al.*, 2007) [4]. House fly menace is an issue of concern in this poultry belt in the recent years. High density of flies not only cause stress to birds and farmworkers, but they can also affect the egg quality, rapid erosion of the metal cages, reduced illumination of lights, degradation of paint due to vomit drops and faecal material of flies. Besides, during high fly season, they cause great annoyance to nearby human habitations which poses a serious public health problem. High reproductive potentiality of housefly, ideal climatic condition, availability of substrate for oviposition and development of larval stages help them to proliferate exponentially in a short period of time. An adult female housefly can lay between 500 and 900 eggs during its lifetime (Bay and Harris, 1988) [2] and in tropical condition they can produce up to 30 generations in a lifetime. Cyromazine is an insect growth regulator (IGR) added to the poultry feed for destruction of larvae as it acts on endocrine system of developing larvae, causing abnormal development and inhibition of fly emergence. However, indiscriminate use of insecticides and IGRs has led to development of resistance worldwide (Liu N and Yue X, 2000) [8]. Resistance is usually associated with a few genes and a limited number of mutations in each gene (Wang *et al.*, 2012) [12].

## Materials and Methods
### Selection of farms
A survey was conducted to discover the incidence of house fly menace among the poultry farms in the Namakkal district of Tamil Nadu (Reference survey article published by Sathiyamoorthy *et al.* (2018) [11]. Based on the survey, farms with continuous and / or moderate levels of fly intensity with poor response to application of insect growth regulator (IGR) was identified to carryout bioassays studies to assess the status of insect growth regulator resistance.

The farms were selected in such a way that they are located minimum of 10 kms distance apart, presuming that no migration of flies between the farms had occurred. Five private poultry farms located in Pudhupatti (Farm-I), Ponneripati (Farm-II), Moongilpatti (Farm-III), Karupattipalyam (Farm-IV) and Echavari (Farm-V) villages in Namakkal region were selected to assess status of tolerance level.

**Maintenance of housefly**

Adult flies (>100 individuals designated as parental generations) were collected from the selected five poultry farms by sweep net and introduced into the fly breeding chamber in the laboratory. These flies were reared at 33±1˚C and 60-70 per cent relative humidity and a photoperiod of 12:12 hours light. The composition of 100gm of larval medium comprises calf feed - 65 gm, Yeast-1gm and Water - 34 ml (Pinto and Prado, 2001) [9] and second stage larvae collected from larval medium was utilized for larvicide test.

**Bioassay**

The larvicide cyromazine (N-cyclopropyl-1, 3, 5-triazine-2, 4, 6-triamine) of 99.9 per cent was procured from Neospark Pvt. Ltd, India. One per cent stock solution of cyromazine was prepared and from the stock solution, five different working concentrations viz., 0.025 %, 0.05%, 0.1%, 0.2%, and 0.25% were made using clean water. Bioassay procedure on larvae was conducted as per the standard WHO (1980) [13] insecticide resistance monitoring guidelines and also the method followed by Ponnudurai *et al*. (2009) [10]. The second stage larvae obtained from laboratory colonies were used for larvicide bioassays (Fig 1). Cyromazine (1%) powdered product was dissolved in water to obtain different test concentrations of 0.025, 0.05, 0.1, 0.2 and 0.25 per cent at the time of use. Each concentration of drug was incorporated into 100 gram of larval medium in the plastic container and mixed thoroughly in the treatment group, while in the control group, fresh water was added to the medium. Twenty numbers of second stage larvae were seeded into the larval medium using soft brush and three replicates were maintained in each concentration (Fig 2). The container was then covered with a cloth to prevent intrusion of other arthropods and kept at room temperature. The larvae in treatment and control groups were maintained at 33±1˚C and 60-70 per cent RH with a photo period of 8:16 hours of light and dark period. Larval mortality was assessed 4 days after larvicide treatment and pupae, if any were collected and placed in a container for another 5 days to observe adult fly emergence. Data on larval mortality, abnormal pupae, intermediates and adult fly emergence were recorded.

**Fig 1**. Second stage house fly larvae used for cyromazine larvicide bioassay.

**Fig 2:** Larvicide bioassay carried out with Cyromazine treated medium in the laboratory.

**Result**

In the present study, the pattern of resistance against the insect growth regulator, cyromazine (1%) among the housefly populations from the poultry farms were studied with different concentrations such as 0.025, 0.05, 0.1, 0.2 and 0.25 per by *in vitro* trials. The preliminary investigation survey revealed the existence of resistance among the poultry farms against insect growth regulators. The bioassay procedure disclosed that hundred per cent inhibition of fly emergence at the concentrations of 0.025, 0.05, 0.1, 0.20 and 0.25 per cent in Farm-I, Farm-III and Farm-IV, indicating the existence of house flies susceptible to the drug cyromazine in the above three farms. Interestingly, the houseflies from Farm-II, showed 65 to 80 per cent inhibition of fly emergence at the concentrations of 0.025, 0.05 and 0.1 per cent, while 90 and 100 per cent inhibition was observed in 0.2 and 0.25 per cent respectively. Likewise, the houseflies from Farm-V exhibited 75 to 90 per cent inhibition of fly emergence at the concentrations of 0.025, 0.05 and 0.1 per cent whereas, 100 per cent inhibition was observed at concentrations of 0.2 and 0.25 per cent. The observations of the bioassay study conducted are shown in Table 1.

The highest reduction of fly emergence was observed only in the higher doses of IGR such as 0.2 and 0.25 per cent concentrations in the house fly populations of Farm-II and Farm-V. Whereas, in lower concentrations (0.025-0.1%), a moderate rate of fly emergence and more number of abnormal larviform pupae (Fig 3) were noted. It was fascinating to observe, the failure to emerge as fly from normally formed pupae and appearance of mid-way intermediaries in some treatments. (Fig 4).

In field conditions, the concentration of 0.05 per cent i.e. 473 g of cyromazine (1%) premix per ton of feed is the recommended dose to control house fly larvae in the poultry farms as feed mix or as a topical spray. The present investigation demonstrated 80 to 90 per cent reduction of fly emergence in farms II and V even at 0.1 per cent of IGR. As anticipated, no abnormal pupae were observed in all the trials, besides 100 per cent fly emergence in the control group (Fig 5).

**Fig 3:** Abnormal larviform pupae in cyromazine treated group

**Fig 4:** Fly intermediaries in cyromazine treated group

**Fig 5:** Normal pupae in control group

**Table 1:** Effect of cyromazine (IGR) on fly emergence – Larvicide bioassay

| Dose Conce. Per cent (%) | No. of larvae treated/ replicate | Farm-I | | Farm-II | | Farm-III | | Farm-IV | | Farm-V | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Inhibition of adult fly emergence | % | Inhibition of adult fly emergence (Mean ± SE) | % | Inhibition of adult fly emergence | % | Inhibition of adult fly emergence | % | Inhibition of adult fly emergence (Mean ± SE) | % |
| 0.025 | 20 | 20 | 100 | 13 ± 0.58 | 65 | 20 | 100 | 20 | 100 | 15 ± 0.67 | 75 |
| 0.05 | 20 | 20 | 100 | 15 ± 0.33 | 75 | 20 | 100 | 20 | 100 | 17 ± 0.33 | 85 |
| 0.1 | 20 | 20 | 100 | 16 ± 0.88 | 80 | 20 | 100 | 20 | 100 | 18 ± 0.33 | 90 |
| 0.2 | 20 | 20 | 100 | 18 ± 0.33 | 90 | 20 | 100 | 20 | 100 | 20 ± 0.33 | 100 |
| 0.25 | 20 | 20 | 100 | 20 ± 0.33 | 100 | 20 | 100 | 20 | 100 | 20 ± 0 | 100 |
| control | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

## Discussion

Poultry farming, an economically attractive venture in the area under study has been challenged by various threats like infectious diseases, metabolic disorders, fluctuating markets etc., House fly menace is considered to be a persistent and economically significant issue concerning the poultry farmers especially those rearing the birds by raised platform. Despite practicing different measures to control the house fly population in poultry farms, their efforts fetched insignificant results. Hence, an attempt was made to study the status of houses fly population in the study area to different control measures like insecticides, insect growth regulators etc., In the present study, the sensitivity pattern of the house flies existing in the poultry farms with high level of infestations to insect growth regulators was undertaken The bioassay of second stage larvae of house flies with cyromazine (1%) revealed hundred per cent inhibition of fly emergence in farms I, II and IV at the concentrations of 0.025, 0.05, 0.1, 0.2 and 0.25 per cent, indicating that these three farms are suspected to be susceptible/sensitive to this drug. In comparison to the filed condition, where the concentration of 0.05 per cent i.e. 473 g of cyromazine (1%) premix per ton of feed is used as feed mix or as a topical spray, the present investigation demonstrated that even 0.1 per cent concentration has shown just 80 to 90 per cent reduction of fly emergence in farms II and V suggesting that the population prevalent in these farms might have developed resistance against cyromazine. The emergence of resistant population of house flies can be attributed to the usage of Cyromazine over a period of time in those poultry farms (Khan and Akram (2017) [5].

In this study, the highest reduction of fly emergence (farms II and V) was observed at higher concentrations viz., 0.2 and 0.25 per cent might be due to earlier death of larval stage when exposed to higher concentration. Whereas, in lower concentration (0.025 – 0.1 %) a moderate rate of fly emergence and more number of abnormal pupae were observed and even the fly failed to emerge from normally formed pupae or in the midway as “intermediaries”. These findings are well comparable to the findings of Kondo and Maekawa, 1976 [6], who stated that, the larvae developing in media containing concentration of cyromazine greater than 0.8 ppm died prior to the 3rd instar. At lower concentrations larvae were able to pupate, however such larvae frequently assumed a rod-like form (larviform) and failed to emerge as adult flies (Ponnudurai *et al.*, 2009) [10].

The results of the experiment disclosed that houseflies from two out of five poultry farms exhibit resistance to cyromazine, indicating the existence of susceptible to moderately resistant fly population in the selected farms. Earlier, a study made by Pinto and Prado (2001) [9] in Brazil reported, three out of five housefly populations were cyromazine resistant in poultry farms with resistance ratio of 6.5 to 12.8. A survey on the impact of house fly resistance status in intensive animal units in UK was carried out by Learmount *et al*. (2002) [7] and observed that, all the 15 field populations analysed were found to be fully susceptible to this larvicide after 5 years of cyromazine application. While the development of resistance

to cyromazine was reported by Bloomcamp *et al.* (1987) [3], Acevedo *et al*. (2009) [1] and Khan and Akram (2017) [5]. Ponnudurai *et al*. (2009) [10] reported the development of resistance against cyromazine in house fly populations (2009) in Namakkal region. However, the existence of susceptible flies in this study might be presumed that, the resistance level declined in subsequent years when the flies were exposed with limited selection pressure to cyromazine, suggesting an unstable nature of resistance (Khan and Akram, 2017) [5].

## Conclusion

The bioassay to study sensitivity pattern of houseflies collected from poultry farms to insect growth regulator, Cyromazine revealed hundred per cent inhibition of fly emergence in three farms at normal recommended dose (0.05 %) indicating the susceptible nature of the fly population in these farms whereas the remaining two farms shown 80 to 90 per cent reduction of fly emergence even at higher concentration of 0.1 per cent suggesting the fly population prevalent in these farms might have developed resistance against cyromazine.

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