The common housefly (Musca domestica) is found throughout the world, and acts as a vector of human and animal diseases causing organisms besides a reservoir of several pathogens, i.e., bacteria, viruses, protozoan cysts, and helminth eggs. It has been recorded that the emergence of rhino-conjunctivitis in humans is specifically due to sensitization to the adult housefly. Cyromazine is an insect growth regulator commonly used to control immature house flies in poultry farms. Cyromazine is formulated as premix (1%), which is added to poultry feed; it is also formulated as a water-soluble granule and a soluble powder (50%) for topical application to manure containing fly larvae. Cyromazine produces irreversible morphophysiological changes, which culminate in the death of insects. The effect varies according to developmental stages. Morphogenic aberrations develop at the prepupal stage and deformations may be observed in the pupal stage, which result from interference with chitin synthesis.

Azadirachtin, a biologically active compound in neem has been promoted as a new insecticide that is considered more eco-friendly than synthetic insecticides. Azadirachtin does not kill the insects immediately. Instead it repels as well as disrupts their growth and reproduction. It is structurally similar to insect growth hormones called "ecdysones," which control the process of metamorphosis as the insects pass from larva to pupa to adult. Neem formulations can be standardized and used as best insecticides for fly control in poultry farming operations, because of the absence of resistance development, lack of residues, environmental safety, ready availability and cost effectiveness.

Hence, this study was designed with an aim of assessing the efficacy of neem products i.e, neem seed and neem seed oil for their larvicidal and pupicidal activities on house fly in comparison with cyromazine in chicken layers.

**MATERIAL AND METHODS**

The present study was conducted at the experimental poultry shed of Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Namakkal, in layer chickens. Ready to lay pullets were bought from commercial poultry farm in Namakkal.

**EXPERIMENTAL BIRDS AND ADOPTION PERIOD:** One hundred and ninety two layer pullets were purchased at the age of twenty weeks. The birds were maintained for three weeks in the layer shed with standard diet for adoption and for allowing development of house flies in the shed, before initializing the

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A study was conducted to evaluate the larvicidal and pupicidal efficacies of neem in comparision with cyromazine in poultry since fly menace is a serious problem in modern poultry industry. One hundred and ninety two layer birds of twenty four weeks were randomly divided into eight groups of twelve each with two replicates and the study was conducted for a period of six weeks. Cyromazine (1%) and powdered neem seed were mixed in the feed at the dose rate of 500 gm and 1 Kg per tonne of feed, respectively and fed throughout the experimental period and neem oil (5%) was sprayed on litter twice a week until the completion of the study in the treatment groups. Various combinations of these treatments were also used in addition to individual treatments. Pupicidal activity was observed on 5th day by in vitro method after spraying neem oil on 3rd day. All the feed incorporated treatments possessed good larvicidal activity on 14th, 28th and 42nd day. Mere spraying of neem oil possessed good larvicidal effect only on 28th and 42nd day. Pupicidal activity was significant in neem oil sprayed group compared to that of control on 5th day of treatment. It may be concluded that dietary inclusion of neem seed and neem oil spray in litter material showed good larvicidal activity and it was very economical to the poultry farmers. The efficacy of neem products was comparable with that of cyromazine and their combinations were synergistic.
### Table. Experimental Design.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of Birds</th>
<th>Feeding protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>Control</td>
<td>24</td>
<td>Fed only standard diet</td>
</tr>
<tr>
<td>T2</td>
<td>Cyromazine (1%) (500 gm per tonne of feed)</td>
<td>24</td>
<td>Through feed throughout the study period</td>
</tr>
<tr>
<td>T3</td>
<td>Neem seed (1 kg per tonne of feed)</td>
<td>24</td>
<td>Through feed throughout the study period</td>
</tr>
<tr>
<td>T4</td>
<td>Neem oil spray (5% v/v)</td>
<td>24</td>
<td>Topical spray on litter material (twice a week)</td>
</tr>
<tr>
<td>T5</td>
<td>Cyromazine (500 gm per tonne of feed) + Neem seed (1 kg per tonne of feed)</td>
<td>24</td>
<td>Through feed throughout the study period</td>
</tr>
<tr>
<td>T6</td>
<td>Cyromazine (500 gm per tonne of feed) + Neem oil spray (5% v/v)</td>
<td>24</td>
<td>Cyromazine - through feed Neem oil - spray on litter material</td>
</tr>
<tr>
<td>T7</td>
<td>Neem seed (1 kg per tonne of feed) + Neem Oil Spray (5% v/v)</td>
<td>24</td>
<td>Neem seed - through feed Neem oil - spray on litter material</td>
</tr>
<tr>
<td>T8</td>
<td>Cyromazine (500 gm per tonne of feed) + Neem seed (1 kg per tonne of feed) + Neem oil Spray (5% v/v)</td>
<td>24</td>
<td>Cyromazine and Neem seed - through feed Neem oil - spray on litter material</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>192</td>
<td></td>
</tr>
</tbody>
</table>

### Table-1. Mean ± S.E. fly larval count in poultry layer manure as influenced by dietary inclusion of neem seed, cyromazine and spraying of neem oil on litter material

<table>
<thead>
<tr>
<th>Days</th>
<th>0</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>14</th>
<th>28</th>
<th>42</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>78.16 ± 11.41</td>
<td>82.66 ± 6.02</td>
<td>89.66 ± 7.30</td>
<td>81.83 ± 7.40</td>
<td>71.00 ± 9.87</td>
<td>91.00 ± 6.61</td>
<td>79.16 ± 9.55</td>
</tr>
<tr>
<td>T2</td>
<td>77.16 ± 10.89</td>
<td>78.16 ± 9.75</td>
<td>73.50 ± 8.74</td>
<td>70.66 ± 10.69</td>
<td>35.00 ± 5.29</td>
<td>14.33 ± 2.48</td>
<td>6.00 ± 1.06</td>
</tr>
<tr>
<td>T3</td>
<td>81.50 ± 6.60</td>
<td>78.66 ± 6.50</td>
<td>83.00 ± 10.81</td>
<td>68.50 ± 9.58</td>
<td>31.66 ± 4.94</td>
<td>12.66 ± 1.68</td>
<td>5.00 ± 0.96</td>
</tr>
<tr>
<td>T4</td>
<td>76.33 ± 9.20</td>
<td>85.00 ± 8.68</td>
<td>82.83 ± 6.83</td>
<td>82.16 ± 5.55</td>
<td>51.66 ± 9.65</td>
<td>32.33 ± 3.44</td>
<td>31.83 ± 5.31</td>
</tr>
<tr>
<td>T5</td>
<td>84.66 ± 6.70</td>
<td>68.66 ± 5.79</td>
<td>78.83 ± 6.89</td>
<td>67.50 ± 8.12</td>
<td>32.83 ± 5.31</td>
<td>12.66 ± 1.80</td>
<td>3.16 ± 0.47</td>
</tr>
<tr>
<td>T6</td>
<td>82.66 ± 6.66</td>
<td>78.66 ± 6.60</td>
<td>89.33 ± 8.82</td>
<td>74.83 ± 6.87</td>
<td>43.50 ± 6.39</td>
<td>13.33 ± 1.85</td>
<td>4.00 ± 1.39</td>
</tr>
<tr>
<td>T7</td>
<td>79.16 ± 9.55</td>
<td>74.00 ± 7.20</td>
<td>90.50 ± 8.70</td>
<td>66.66 ± 4.64</td>
<td>28.66 ± 5.17</td>
<td>11.33 ± 1.85</td>
<td>3.00 ± 1.43</td>
</tr>
<tr>
<td>T8</td>
<td>84.66 ± 7.67</td>
<td>81.00 ± 5.31</td>
<td>68.00 ± 10.84</td>
<td>63.00 ± 9.58</td>
<td>22.66 ± 5.32</td>
<td>8.00 ± 1.77</td>
<td>1.5 ± 1.56</td>
</tr>
</tbody>
</table>

n=12; Mean values with in column bearing different superscripts differ significantly (P ≤ 0.05)

### Table-2. Mean ± S. E. pupal count as influenced by spraying of neem oil on litter material by in vitro method.

<table>
<thead>
<tr>
<th>Groups</th>
<th>5th Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28.66 ± 0.49</td>
</tr>
<tr>
<td>Treatment</td>
<td>10.66 ± 0.88</td>
</tr>
</tbody>
</table>

n=30 Mean values with in column bearing different superscripts differ significantly (P ≤ 0.05)

**MAINTENANCE OF BIRDS AND THE EXPERIMENT:** The birds were reared in cages under standard managemental practices and the experiment was conducted from twenty three weeks to twenty nine weeks of layers’ age. The birds were fed with standard layer ration without insect growth regulator obtained from feed manufacturing technology unit, Veterinary College and Research Institute, Namakkal. The birds had access to ad libitum feed and water throughout the study. The experiment was approved by the IAEC (Institutional Animal Ethical Committee) of Veterinary College and Research Institute, Namakkal (Approval no: IAEC/19/ 2012).
DRUGS AND CHEMICALS: Cyromazine was obtained as gratis from M/s. Nutricon Company, Chennai. Dried neem seeds were obtained as gratis from a farmer who is maintaining his organic layer farm. Neem oil was obtained from local market in Namakkal. Soft soap was used as emulsifier.

PILOT STUDY: A pilot study was conducted with different concentrations of neem oil such as 2.5, 5, 10 and 15% to find out optimal larvicidal activity and based on that 5% neem oil was chosen for the study.

ADMINISTRATION AND DOSE OF DRUGS:

CYROMAZINE: Cyromazine (1%) was administered through feed to the treatment groups as mentioned in the experimental design at the dose rate of 500 gm per tonne of feed.

NEEM SEED: Dried neem seeds were powdered using a laboratory blender, kept in airtight container in a cool and dry place, and was administered through feed to the treatment groups as mentioned in the experimental design at the dose rate of 1 kg per tonne of feed.

NEEM OIL: An emulsion of 5 per cent neem oil in 10 per cent soap solution was prepared and sprayed on the litter material of the treatment groups as mentioned in the experimental design. Ten per cent soap solution was used as an emulsifying agent for neem oil.

METHODOLOGY: Eight groups of layer birds of 12 each in two replicates were divided in wire cages arranged in two rows. Feed was provided in linear feeder and it was partitioned by using cardboards for each group to prevent the mixing of feed into other treatment. The faecal material below each group was partitioned by using thick cardboard and fixed tightly to prevent mixing of litter material as well as prevent larval migration. The trial was conducted for six weeks and subjected to different treatments as tabulated below. 1% cyromazine and neem seed were administered through feed to the mentioned groups for six weeks and neem oil was sprayed on the litter twice a week for six weeks.

EXPERIMENTAL DESIGN: One hundred and ninety two layers were divided randomly into eight treatment groups. Each treatment had two replicates containing twelve layers per replicate. The experiment was as follows:

EVALUATED PARAMETERS: The experimental parameters evaluated were - fly larval count and pupicidal activity.

FLY LARVAL COUNT: The viability of the fly larvae was evaluated in the chicken faecal material of each group. The faecal material below the cages in each group was randomly subdivided into six equal areas. Samples of 100 gm of faeces from each subdivided area were taken on zero day before treatment (0 DBT), 1st, 3rd, 5th, 14th, 28th and 42nd day during treatment (end of 2nd, 4th and 6th week) and the fly larva was counted.

PUPICIDAL ACTIVITY: The pupicidal activity was evaluated in vitro by spraying neem oil on the larvae collected from control group. Fresh third instar larvae (thirty numbers) were collected from control group and were introduced into separate transparent plastic boxes and provided with layer feed. The top area of the plastic box was covered with a fine muslin cloth for ventilation. Neem oil emulsion (5%) was sprayed on 3rd day and the pupa was counted on 5th day in the treatment group. Control group was treated with soap solution (vehicle) alone similar to treatment group. This experiment was replicated six times and treatment group was compared with control.

STATISTICAL ANALYSIS: Data were subjected to statistical analysis under one way anova method.

RESULTS: The present study was attempted to evaluate the larvicidal and pupicidal effects of neem products (neem seed and neem oil) in comparison with cyromazine in poultry. A pilot study was conducted to ascertain the optimal larvicidal effect of neem oil and it was found that 5% was able to produce good larvicidal effect. Larval count was carried out on 0th, 1st, 3rd, 5th, 14th, 28th and 42nd day. Pupicidal effect was evaluated by in vitro method on 5th day. Fly density measurement was done on 0th, 21st and 42nd day.

FLY LARVAL COUNT: The house fly larvae of all groups were counted on 0th, 1st, 3rd, 5th, 14th, 28th and 42nd day and presented in Table-1. There was no significant difference between the treatment groups at the end of zero, first, third and fifth day.

On fourteenth day, compared to control (T1) larvicidal activity was significantly higher in T8, T7, T3, T5, T2 and T6 wherein cyromazine and neem seed were incorporated in the feed. The larvicidal activity of T3, T5, T2 and T6 were comparable with T4. However, there was no significant difference between T4 and T1.
On twenty eighth day, the larvicidal activity of T2, T3, T4, T5, T6, T7 and T8 were significantly higher than T1. However, there was significant difference in larvicidal activity between T4 (neem oil spray alone) and T1. On forty second day, Larvicidal activity of T2, T3, T4, T5, T6, T7 and T8 were significantly higher than T1. However larvicidal activity of other treatment groups was significantly higher than T4.

PUPICIDAL COUNT: The house fly pupae were counted by in vitro method on 5th day of treatment and presented in Table-2. There exists significant difference between the treatment group and control.

RESULTS AND DISCUSSION

In the present study, fly larval count for larvicidal activity was compared among all treatment groups on 0th, 1st, 3rd, 5th, 14th, 28th and 42nd day. The larvicidal activity on 0th, 1st, 3rd and 5th day did not reveal any significant difference between treatment groups when compared to control (T1).

On 14th day of treatment, the larvicidal activity was significant in T2, T3, T5, T6, T7 and T8 of which T8 (combination of neem seed, neem oil spray and cyromazine) showed the highest larvicidal activity. All the feed incorporated treatments showed significant larvicidal activity than that of T1 and T4. Larvicidal activity of neem oil spray (T4) did not differ significantly from that of other feed incorporated treatments. However, spraying of neem oil alone did not have significant effect on larvicidal activity when compared to that of the feed incorporated treatments on 14th day of treatment. From this observation, it was found that larvicidal activity was more effective when cyromazine was added to the feed. Both diflubenzuron and cyromazine were equally effective in controlling fly proliferation in bird faeces when experimenting with Musca domestica. Further, the author stated that these were more effective when added to feed than when powdered over the faeces. However, the use of cyromazine was more effective when applied over the faeces than when added to poultry feed.

On 28th and 42nd days of treatment, the larvicidal activity in all the treatment groups was higher when compared to control (T1). The larvicidal activity was significant in T8, T7, T3, T5, T6, T2 and T4. Oral administration of cyromazine showed larvicidal activity. 74-81% reduction in the population of houseflies occurred over a 5 weeks field trial, using 2% cyromazine against second and third larval instars which confirmed the larvicidal effects of cyromazine.

The larvicidal effect of cyromazine incorporated treatments may be due to possible mechanisms which disrupt the growth and development of insects and arthropods. The authors also inferred that IGR mainly affected the development of immature stages, and disrupted metamorphosis and reproduction. The above larvicidal effects of cyromazine incorporated feed were also observed in chickens that addition of IGR (cyromazine) to the feed of laying chickens under different housing conditions resulted in quick decrease in the number of larvae in the excrements of the birds, while no adverse effect was detected upon their natural predator's population.

Administration of neem seeds through feed caused highest larvicidal activity and it might be due active compounds of neem which had insect growth regulation and repellency against insects. This was in accordance with the studies made in neem seeds which contain approximately 99 biologically active compounds of which azadirachtin, nimbin, nimbidin and nimbolides are major molecules. Many of these products besides acting as insect growth regulators and fly repellent do have antifeedant, ovicidal and fecundity suppression effects. It kills insects by many different methods and hence possesses good insecticidal effect. Administration of neem products through feed and spraying neem oil on litter material caused highest larvicidal activity. Spraying of neem oil on litter (T4) possessed good larvicidal activity during 28th and 42nd day of treatment.

Similar effects were made in neem oil against louse while spraying neem and pungam oil which possessed good lousicidal activity at the dose rate of 5 ml per bird. It had also been concluded that the ectoparasiticialcid efficacy of neem and pungam oil was comparable to that of potent synthetic pyrethroid, cypermethrin. The pupicidal activity was observed in vitro with third instar larva against neem oil spray on 5th day. Neem oil treated group showed higher pupicidal activity compared to control group. Based on the result, administration of neem seed kernel and cyromazine with or without neem oil spray had higher insecticidal effect than treatment with cyromazine alone on fly control. The integrated pest management was necessary to control the flies who concluded that the best treatment for house flies resistant to cyromazine was biological + cultural + chemical with localized applications.
of topical cyromazine\textsuperscript{18}.

**CONCLUSION**

There was no significant changes in larvicidal activity on 0\textsuperscript{th}, 1st, 3\textsuperscript{rd} and 5\textsuperscript{th} days. All the feed incorporated treatments (T2, T3, T5, T6, T7 and T8) possessed good larvicidal activity on 14\textsuperscript{th}, 28\textsuperscript{th} and 42\textsuperscript{nd} day. Mere spraying of neem oil did not show any larvicidal activity on 14\textsuperscript{th} day of treatment but on 28\textsuperscript{th} and 42\textsuperscript{nd} day of treatment it possessed good larvicidal effect. Pupicidal activity was significant in neem oil spray on 5\textsuperscript{th} day by in vitro method when compared to control group.

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