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# Drying fish preference assessment and efficacy of semiochemicals as repellents to blow fly *Chrysomya megacephala* (F.) (Diptera: Calliphoridae) during sun drying of fish

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**ABSTRACT:** *Chrysomya megacephala* (F.) is a major pest of fish after harvest and during the processing stage. Existing management strategies are inadequate in curbing the fly menace especially in processing sites with poor hygenic conditions. The present study attempted to evaluate the preference of the *C.megacephala* in drying fish stages and also evaluated the efficacy of synthetic compounds of some fish based semiochemicals which are identified as repellents. The results of the study indicate that salt cured fish after one- day drying is the most preferred choice for the flies. All synthetic repellents attempted to control the flies, were found to be effective as repellents and had given about 50% suppression of the population compared to control. Urethane (Ethyl carbamate) had shown the maximum repellency (67%) followed by Hexanal (52.6%) and Diphenyl ether (52%). Dimethyl benzothiophene and N, N-dimethyl acetamide also exhibited 43.3 % to 48.2% repellency in alleviating the flies. Results of the preference study provide information to processors about right time to take adequate precaution while sun drying of fish. The study also revealed the possibility of utilising tested synthetic analogues in population suppression of blowflies with an effective dose optimisation before application. 

**KEY WORDS:** *Chrysomya megacephala*, pest of dry fish, stage preference, repellency

## INTRODUCTION

Associated with agriculture and allied production, fisheries play the role of critical contributor to food supply in terms of economic and social security with nutritionally significant animal protein. Fish is a highly perishable commodity and undergoes step by step spoilage and loss after harvest. According to Gethu *et al.* (2016) physical losses that occur due to under-utilization after landing, losses in nutritional value due to decomposition, quality losses that occur due to spoilage, market force loss due to inadequacy

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between demand and supply, losses due to traditional processing methods and losses due to insect infestation which contribute to physical and quality loss are the various forms of post- harvest losses that occur. Under the influence of hot and humid conditions of tropical countries, insect infestation and other biological agents contribute to large scale physical losses and quality deterioration of processed fish (Khan and Khan, 2001). The pest species complex which incur loss to the sector is dominated by blowflies (Diptera: Calliphoridae), flesh flies (Diptera: Sarcophagidae) and hide beetles (Coleoptera: Dermestidae and Cleridae) (Johnson and Esser, 2000). Each insect group attacks the fish at various stages in the processing and storage of which blowflies are attracted to the fish after harvest, during the salting and sun-drying stages.

After harvest, 1-2 day old fish is highly preferred for oviposition and breeding blow fly *C. megacephala*, the major pest of fish and other blowflies (Nowsad, 2010).The existing management strategies have not yielded any effective result to overcome the menace. The present study aims to evaluate the preference of *C. megacephala* towards the drying stages of fish and also attempts to evaluate the effectiveness of some identified fish based semiochemicals as repellents against blowflies during sun drying of fish. These semiochemicals were identified from post mortem and salt cured and sundried stages of Indian mackerel by headspace gas chromatography mass spectrometry (HS-GCMS) studies and the related repellency behavior of blow fly *C. megacephala* are determined by electroantennographic and olfactometer bioassay studies.

## MATERIALS AND METHODS

### Preparation of dry fish samples and preference study:

Indian Mackerel (*Rastrelligar kanagurta*) was procured from local fish markets of Cochin. The fish samples of different drying stages used for study were prepared by dry salting method. Dry salting (i.e., a layer of fish followed by layer of salt) was done with 25% (w/w) salt for 24 hrs. The samples were then sun dried and designated as salt cured fish without drying (SCF) salt cured and 1 day dried fish (SCDF 1D), salt cured and 2 day dried fish (SCDF 2D), salt cured and 3 day dried fish (SCDF 3D) salt cured and 4 day dried fish (SCDF 4D) based on duration of drying and used for further study.

Oviposition preference study was conducted in fly rearing cages. The preference study was conducted as a multiple choice test. About 30 g of the salt cured and different fish samples were placed in Borosil petriplates (9.5cm Ø) and these were randomly placed in the cages each time to avoid any positional effect. Twenty 8-10 day old gravid females and 10 males of *C. megacephala* were released in each cages and allowed to freely oviposit on their choice of different dried fish samples for 6 hrs. The plates were removed after and weight of egg mass deposited on each sample in petriplate was weighed using a digital electronics balance (Sartorius BP 211D). The experiment was conducted in triplicates and repeated three times.

**The test insect:** A lab reared population of the blow fly *C. megacephala* were used in the present study. They were established in the animal house of Indian Council of Agricultural Research - Central Institute of Fisheries Technology, Cochin (ICAR-CIFT). Initially a few adults were collected from residential complex of CIFT located at Thevara, Cochin (9.9426° N, 76.2986° E) on a fish offal trap. The adults were brought to the animal house of the institute and maintained at 29 ± 2 °C, 60 ± 5 % R.H and photoperiod of 12:12 (L: D) hr., the condition of animal house. Further multiplication was done on the fish (Indian mackerel) as a rearing medium. The adult flies were reared in insect rearing cages (45 × 45 × 45cm) with wood sheet base, glass top, three sides covered with cloth of which two sides were having circular whole fitted with sleeves to facilitate serving of the food and cleaning. The front door of the cage was made of acrylic sheet. Adults were provided with fish, sucrose crystals, and water soaked absorbent cotton in petriplates. Feeding was provided *ad libitum*. Flies were reared for few successive generations, authenticated the species identification by an insect taxonomist and used for the study.

**Test chemicals:** The synthetic compounds used for testing were procured from chemical companies Sigma Aldrich, Merck and Avra Synthesis.

**Evaluation of repellency effect of synthetic analogues of fish based semiochemicals:**

The repellency effect of the fish based semiochemicals were evaluated in small fish drying yard following the methodology adopted by Aak *et al.* (2010) and Zhu *et al.* (2017) with modifications. A fish drying yard of size (2m x 2m) was prepared for the study. Polythene sheet was spread on the floor in which gutted fish for drying spread over exposed to sunlight (Plate 1). The semiochemical compound (4 ml) was sorbed on non absorbant cotton fibres in a porous eppendorf tube which served as a dispenser of the volatile, was kept hanging just above the fish (Plate 1) using a thread which was tied on a thread suspended along the drying area. To sample the flies visiting the drying yard, yellow sticky insect traps (Pest Control of India) of (size 50 cm x 25 cm) 2 nos, adhered on a piece of carton were kept hanging at borders of the drying area. The traps were placed for 6 hrs. in the drying yard till sunlight faded. Afterwards, the traps were removed and the number of flies caught on the traps were recorded (Plate 2 & 3). A control plot sampling was done by placing the traps alone over the field. The experiment was duplicated for each compound and repeated twice. The number of flies caught in the trial was counted and determined the mean value. The efficacy of the tested chemicals were determined by calculating the reduction in the number of flies caught in each trial compared to control. The repellency percentage was calculated according to the formula of Mohammed *et al.* (2016).

$$\text{Repellency (\%)} = (Nc\text{-}Nt)/Nc * 100$$

where Nc = No. of flies caught in control and Nt = No. of flies caught in each treatment.

Statistical Analysis: The data analysis was performed in IBM SPSS Statistic version 20. One-way ANOVA at 5% level of significance was performed to compare the means of population of flies trapped in treated and control plots. Tukey's multiple comparison tests were used for post-hoc analysis. The results are expressed as mean ± standard deviation.

## RESULTS AND DISCUSSION

**Assessment of drying stage fish preference of *C. megacephala:***

Multiple choice preference study of *C. megacephala* conducted with salt cured fish and with successive stages of drying reveals significant differences in their preference. Among the choices, the fish dried one day after a day's salt curing (SCDF 1D) obtained the maximum eggs which was

**Plate 1.** Drying plot with repellent & trap (Experimental)

**Plate 2.** Yellow sticky trap with trapped flies (Control)

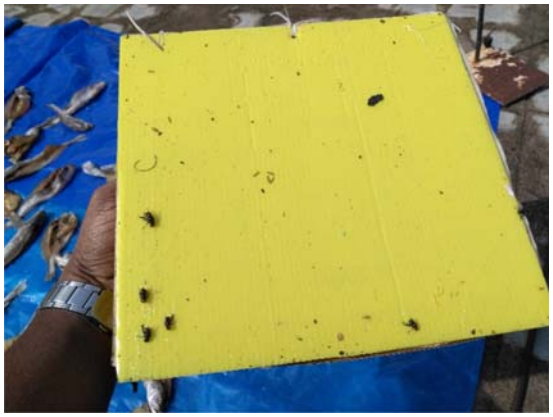

**Plate 3.** Yellow sticky trap with trapped flies (Experimental)

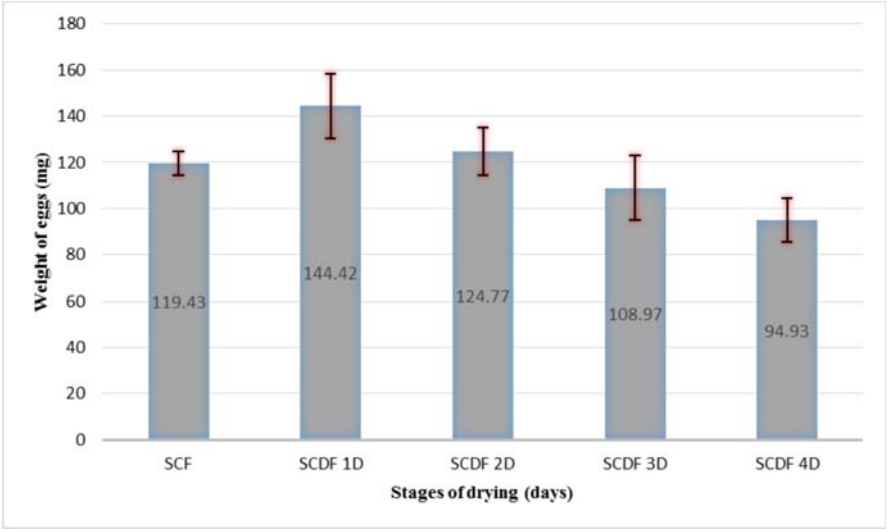


**Fig. 1** Oviposition preference of *C. megacephala* on fish drying stage

SCF (Salt cured fish), SCDF 1D (Slat cured and 1day dried fish), SCDF 2D (Slat cured and 2day dried fish), SCDF 3D (Slat cured and 3day dried fish), SCDF 4D (Slat cured and 4day dried fish)

significantly higher compared to others ($p<0.05$). The mean weight of eggs laid on this was 144.42 ± 13.98 mg. The weight of eggs laid on 2 day dried salt cured fish (SCDF 2D) was 124.77 ± 10.53 which also was statistically significant, but was homogenous with SCF ($p>0.05$) where the mean wt. of eggs laid was 119.43 ± 5.13 mg. SCDF 3D and SCDF 4D had obtained the least weight of eggs (108.97 ±14.09, 94.93 ± 9.57 respectively) which was not statistically significant ($p>0.05$) (Fig.1).

The obtained result is in accordance with some of the previous studies conducted by Walker and Wood (1986) and Sachithanandan *et al.* (1986). They reported that during the first two days of sun drying, fish is highly preferred by blowflies and opined that this attraction might be due to the high and preferred moisture content compared to more dried samples. Nowsad (2010) reported a similar effect that fish is more susceptible to blowfly attack during the early stages of drying when moisture content is high. Esser (1988, 1990) observed that in multiple choices, *C. megacephala* preferred to lay eggs on fish with low salt concentration. These facts can be attributed to the obtained result of present study as 1-2 day dried samples will have less salt concentration and more moisture content compared to 3day dried and 4day died samples due to

reduction in water content. Moreover, blowflies possess salt sensitive receptors (Gillary, 1966; Proctor, 1972) which might have enabled them to detect SCDF 1D and SCDF 2D which are with low salt concentrations and high moisture content among the given choices. Esser (1992) and Johnson (1997) reported that salting may result in the production of volatiles which makes fish more attractive to blowflies. Clucas and Ward (1996) also opined that blowflies are attracted to fish visually and by odours or volatile compounds released from the fish. Apart from as a preservative by reducing water activity ($a_w$) and arrest of microbial growth (Horner, 1997), Harris and Tall (1994) reported that salt enhances the sensory properties such as aroma and flavour in fish foods which also might be the influential factor in the present result.

**Efficacy of synthetic analogues of fish based semiochemicals as repellent against blowflies:**

Among the five synthetic repellent compounds tested, all had shown effect in reducing the fly population visiting the fish drying yard compared to control which was higher and significant. The most significant reduction in fly number was given by urethane with 67% repellency and a mean no of 18.25 ± 1.0606 flies trapped in. Followed by that, Hexanal and Diphenyl ether, were significantly low from control where the mean no. of flies trapped were 26.5 ± 2.1213 and 26.75 ± 3.8890 respectively with 52.6 and 52.2% repellency and was homogenous. N, N-Dimethyl acetamide and Dimethyl benzothiophene also given significant reduction in fly numbers with 48.2 and 43.3% repellency and a reduction in fly nos. as 29 ± 2.1213 and 31.75 ± 3.1819 compared to control where the mean no. of flies trapped were 56 ± 4.2426, and significantly higher (Table 1).

The initial studies on the repellent effect using common substances on necrophagous insects including blowflies was done by Marchenko (1988) where he reported that paint or gas dropped on clothing induce delayed carrion colonization. The repellency effect of various indigenous oils was tested by Subramanian and Mohanan (1980) against blowflies including *C. megacephala* which revealed that lemongrass oil and camphor in ground nut oil and eucalyptus oil are good repellents. Likewise neem product azadiractin application in drying fish provided more than 80% repellency to *C. megacephala* from fish (Xia *et al.,* 2010). Charabidze *et al.* (2009) reported petroleum spirit, perfume and mosquito repellent citronella has repellency effect on cadaver insects and delay their visiting time while in a closed environment blowfly *Calliphora vicina* has repellency to petroleum spirit, mosquito repellent citronella, hydrochloric acid and paradichlorobenzene. While in the present study purely synthetic compounds of fish based semiochemicals, Hexanal, Diphenyl ether, Urethane (Ethyl carbamate), Dimethyl benzothiophene and N, N-dimethyl acetamide are used as repellents. Angioy *et al.* (1987) previously opined that Hexanal has repellent activity to blowfly *Protophormia*

Table 1. Efficacy of fish based semiochemicals as repellent against blowflies

| Semiochemical tested | No. of flies trapped Mean ± SD | Repellency (%) |
|---|---|---|
| Hexanal | $26.5 \pm 2.1213^{b}$ | 52.6 |
| Urethane (Ethyl carbamate) | $18.25 \pm 1.0606^{c}$ | 67.0 |
| Diphenyl ether | $26.75 \pm 3.8890^{b}$ | 52.2 |
| N, N- Dimethyl acetamide | $29 \pm 2.1213^{b}$ | 48.2 |
| Dimethyl benzothiophene | $31.75 \pm 3.1819^{b}$ | 43.3 |
| Control | $56 \pm 4.2426^{a}$ | 0 |

Different superscripts in column indicates significant difference between treatment means ($p<0.05$)

*terraenovae* and the present study is in accordance with that and Hexanal has given significant repellency effect (52.6%) indicated by the reduction in catch up to 50% compared to control. The repellency effect of Hexanal was identified by Douglas *et al.* (2005) and they reported that a chemical odourant emitted during breeding season of crested auklets *Aethia cristatella*, in which Hexanal is a dominant constituent as it repels mosquitos and other ectoparasites (Douglas *et al.,* 2001, 2005). According to Dethier (1954), Garson and Winnike (1968) there occurs a linear relationship between concentration of aliphatic aldehydes and attraction repulsion behaviour of blow flies. These studies with the result revealed in the present study support the potential of Hexanal to be used as a repellent against blowflies for their management.

Diphenyl ether also provided significant reduction in the number of flies caught in the present experiment indicating the repellency effect of the compound which was 52%. The 50% reduction of the blow flies trapped in the field trap indicate the repellency potential of the compound towards eliminating the flies. The chemical is a reported repellent against insects (Debboun *et al.,* 2006) and Rutledge (1988) had a similar repellency result report with diphenyl ether against mosquitos and horseflies for 3 and 6 hours respectively. Diphenyl ether compounds proved to have knock down effect and mosquitocidal activity to many species (Hueter *et al.,* 2016). Shamsi *et al.* (1990) also evaluated the repellency effect phenyl compounds against blowflies *C. rufifacies* and *Lucilia cuprina* using a modified cone trap method and reported that biphenyl and phenyl phenol provided 70 - 90% repellency effect.

The most significant reduction in fly catch was given by urethane (Ethyl carbamate) among all the synthetic of semiochemicals experimented as repellents. The compound had repellency effect up to 67% and had reduced the fly numbers to three fold in comparison to control field which demonstrates the efficacy and proposes the potential of the compound as a candidate for blow fly management. The repellency effect of ethyl carbamate was reported earlier by Fergusen and Alexander (1953) and substituted carbamate Icaridin (Picaridin) is also a well- known repellent against various insects. Dimethyl benzothiophene and N, N-Dimethyl acetamide are the two other compounds resulted repellency effect with reduction in the no. of flies in sampling compared to control. The repellency or toxicity of the chemical has not been revealed anywhere while it is reported that the derivative benzothiophene is vapour toxic to mosquito *Aedes aegypti* (Koehler and Patterson, 2007) and the presence of benzothiophene with in naphthalene balls which is a well-known insect repellent modifies its toxicity (Pajaro-Castro, 2017). These studies support the results of the present study about the efficacy in repelling blow flies with Dimethyl benzothiophene. Similarly, there are no reports about the repellency effect of N, N- dimethyl acetamide (Acetamide) to any insects. However, it is structurally similar to N, N-diethyl-m-toluamide (DEET) (Snyder *et al.,* 1986) which is an effective repellent to many insects including some blow flies (Zhang *et al.,* 2011). DEET is the active ingredient in many commercial insect repellent products against mosquitoes, flies, ticks, fleas and other biting insects (Rivera-Cancel *et al.,* 2007). So the effective repellency exhibited by the acetamide to blowflies can be attributed due to this structural similarity, which will ensure similar property and it has given about 50% reduction of flies compared to control.

In conclusion, salt cured fish after one day sun drying is the most susceptible stage for blow fly attack. This assessment result would facilitate the traditional fish processors to intervene with remedial measures and take adequate precautions to protect the drying fish. Indiscriminate use of chemical pesticides and use of banned chemicals are reported to be in use for controlling blow flies, where the present study is a preliminary attempt that stands out as novel with the use of synthetics of fish semiochemicals. The data obtained unveils the effectivity of used compounds as repellents that can be utilized further effectively for the blow fly management. All the tested compounds were found effective in reducing the population to 50% or more. However, further dose optimization study at field levels using effective and advance dispensing

system with optimal rate of dispensing are pre requisite for correct evaluation of the effectivity. This would prompt the development of a semiochemical based management strategy to overcome the fly menace in fish drying yards and related areas with poor sanitary conditions.

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