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Research Article

Effects of Deltamethrin on Mortality, Feeding Behaviour and Oviposition in the UK *Culicoides* Species and at UK Environmental Temperature

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ABSTRACT

Biting midges (Culicoides nubeculosis), is a vector of Bluetongue virus which causes Bluetongue disease in man and domesticated ruminants. World Health Organization (WHO) laboratory assays were used to evaluate effects of temperature on susceptibility of *Culicoides nubeculosis* to deltamethrin. In 'treatment temperature experiment', the effect of temperature was observed for 24 hours following an hour exposure of *C. nubeculosis* to varying concentrations of deltamethrin while in '24 hours post treatment temperature experiment', effect of temperature was observed for 24 hours of 1 hour exposure of *C. nubeculosis* to test insecticide. Subsequent experiments with *C. nubeculosis* that survived after 24 hours post-exposure to sub-lethal doses of deltamethrin as well as control group were used to evaluate blood-feeding and egg production rates. Results indicated significant effects on mortality of biting midges in post treatment temperature experiment but insignificant in treatment temperature experiments. The difference in blood feeding rates between deltamethrin-exposed biting midges and the control group was statistically insignificant (P>.05) but eggs laid by control group were significantly more in number than those lay by exposed (P<.05). These results will be useful in vector control of bluetongue viral infections in humans and ruminants.

Key words: Biting midges, Vector, Bluetongue virus, Deltamethrin, Vector management

INTRODUCTION

Bluetongue is a non-contagious viral disease of ruminants caused by a virus which belongs to the genus *Orbivirus* within the family Reoviridae. Bluetongue virus replicates in all ruminants, but severe disease is mostly restricted to sheep. The disease which manifests as fever, inflammation and ulceration of mucosal membrane of nasal cavity and mouth causes severe morbidity and mortality in affected livestock (Papadopoulos *et al*., 2009). Although sheep are most severely affected, cattle are the main reservoir of the virus; and infected cattle are very important in the epidemiology bluetongue since they show no symptom and can remain infected for up to 100 days. In 1944 after extensive searching, a dipterous fly in the genus *Culicoides* and family Ceratopogonidae was implicated as biological vector of bluetongue (Toit, 1944). Incriminatory experiments were done by injecting homogenized field-collected midges into sheep and horses. Within 7 days, animals had developed disease symptoms consistent with Bluetongue virus (BTV) and African horse sickness virus (AHSV). Both male and female *Culicoides* feed on plant juices but only females feed on blood which they require for egg maturation (Birley and Boorman, 1982). In the wild, female biting midges acquire BTV when they ingest a blood meal from a viraemic vertebrate host. Extrinsic incubation period which is the interval between virus ingestion and subsequent ability of biting midges to transmit virus depends on temperature and takes about 10 days at 25ºC (Mullens *et al*., 2004). Infected females usually remain so for life (Mellor, 1990; Mellor *et al*., 2000). Susceptibility to infection and the subsequent replication and dissemination of viruses in *Culicoides* is determined by range of hereditary and environmental factors (Mellor *et al*., 2000). In the majority of species adult activity is nocturnal and activity is greatest when evening and night-time conditions are warm, humid and calm (Onyido *et al*., 2010). According to Mellor *et al*. (2000), about 1300 to 1400 species are within the genus *Culicoides*, however only 30 of them have been incriminated as vectors of BTV. The Biting midges are usually exophagic and exophilic; nonetheless some species such as *C. obsoletus* and *C. dewulfi* have shown endophagic behaviour. Apart from Antarctica and New Zealand, *Culicoides* is found in every other part of the world, but they are most frequently

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present in warm, damp and muddy areas which are rich in organic matter as well as abundant in animal hosts (Onyido *et al*., 2010; Mellor *et al*., 2000). In Enugu, south-eastern Nigeria the biting midges are most active on hot and still days particularly throughout October and the first week of November (Onyido *et al*., 2010). In Africa BTV is transmitted by *Culicoides imicola* which is the most widely spread midge species on the globe (Purse *et al*., 2005). In Europe BTV is transmitted by *Culicoides obsoletus* (Purse *et al*., 2005) while in Australia it is transmitted mostly by *C. fulvus* (Standfast *et al*., 1985). However transmission is not limited to the mentioned species only. Bluetongue virus transmission was restricted to areas where competent vector species occur - broadly the tropical and subtropical parts of the world, between latitudes 35ºS and 40ºN. Within the zones transmission is seasonal, and often occurs between late summer and late autumn when adult vectors are abundant (Mellor *et al*., 2000) as the climate condition is conducive for the vector. In countries where the disease occurs there has been enormous impact on sheep production. Tabachnick, (1996) reported that United States lost about 25 million US dollars in trade losses alone in 1996. An outbreak in the Netherlands accounted for a total loss of €196.4 – €207.4 million in 2006 and 2007 respectively (Velthulis *et al*., 2010) due to mortality, reduced production during protracted convalescence including poor wool growth and reduced reproductive performance.

Control measures for BTV include restriction of animal movement, vector control and vaccination. However, vaccination remains a subsidiary tool at present as the only available vaccine is for sero-type 8 only and does not confer cross-protection against the other 23 strains. Vector control measures can involve manipulating the environment to remove larval breeding sites, housing of livestock in screened building or controlling adult midges by treating either resting sites such as animal housing or host animals with insecticides (Carpenter *et al*., 2008). Control of adult biting midges through the use of insecticide is the most widely practiced vector control measure. During the outbreak of BTV-8 in Europe deltamethrin was the main insecticide used to control *Culicoides*. Deltamethrin pour-on (Mehlhorn *et al*., 2008) was applied along the back of the livestock. However, the effect of deltamethrin on UK *Culicoides* species and at UK environmental conditions has not been assessed. It has been observed that temperature can have a large effect on insecticide susceptibility (WHO, 1998) and also affect the efficacy of the compound. Previous works on susceptibility of biting midges to different insecticides were done on tropical and sub-tropical species, and assays were carried out at room temperature (Mehlhorn *et al*., 2008; Schmahl *et al*., 2008; Schmahl *et al*., 2009a; Schmahl *et al*., 2009b; Papadopoulos *et al*., 2010; Venial *et al*., 2011). The major aim of this study was to determine the effects of deltamethrin on mortality, feeding behaviour and oviposition in the UK *Culicoides* species and at UK environmental temperatures.

MATERIALS AND METHODS

Biting midges: *Culicoides nubeculosis* was used in the present study because it is often used as a model organism in laboratory insecticide testing (Venial *et al*., 2011) prior to fieldwork. Despite being classed as a "non-vector species" (Mellor *et al*., 2000), it is a good alternative where large numbers of midges are required as it is only one of two species that reproduces successfully under laboratory conditions. *Culicoides nubeculosis* were provided by the Pirbright Institute (Formerly the Institute of Animal Health, Pirbright UK). To reduce morbidity and mortality, female midges were sent as late stage pupae to the London School of Hygiene and Tropical Medicine (LSHTM). At LSHTM they were stored at 24ºC - 25ºC in the incubator with cotton wool soaked in 10% glucose solution. Biting midges emerged as adult within two days. Studies have shown that age, sex and blood feeding status influence susceptibility of insects to insecticides (Chareobviriyaphap *et al*., 2006), therefore only 1-3 days old non-blood fed midges were used in the experiment.

WHO tube Bioassay: Susceptibility of laboratory-reared *C. nubeculosis* to deltamethrin at different concentrations was assessed using modified WHO bioassay test kit. Also effect of temperature during exposure of *C. nubeculosis* to deltamethrin and 24 hours after exposure to deltamethrin was evaluated. WHO standardized bioassay test kit consists of two tubes; the exposure tube which is marked with red dot and the holding tube which marked with green dot. The two tubes were connected using a double ended connection and a plastic slide was inserted into the double ended connection in order to keep the midges in either exposure tube or holding tube when desired. Ends of the tubes were covered with fine-mesh net (instead of the provided wire mesh screen) which prevented the midges from escaping during the experiment. Also 10% sugar solution soaked in cotton wool was kept on the fine-mesh net during the experiment. Metal clip which is also part of WHO bioassay kit was used to keep filter papers within the tubes in place.

Deltamethrin: Pure deltamethrin (in powder formulation) procured from Sigma Aldrich Company was the insecticide used in the experiment. To produce the stock solution for the experiment 0.02g of deltamethrin was mixed with 0.02l of acetone-silicon solvent to obtain 1% stock solution (20mg/20ml). Acetone-silicon mix was created by adding 33ml of silicon to 67ml of acetone. Silicon was added to prevent crystallization of deltamethrin and aid the spread of the compound across the filter paper. The desired deltamethrin concentrations (0.05%, 0.025%, 0.005%, 0.0025%, 0.0005% and 0.00025%) were obtained through serial dilution. To create 0.05%, 1ml of 1% stock solution was mixed with 19ml of acetone-silicon mix. 0.02percent (0.025%) was created by mixing 10ml of 0.05% and 10ml of acetone-silicon mix. 0.005percent (0.005%) and 0.0025% were created by adding 4ml of 0.025% to 16ml of acetone-silicon mix, and by adding 10ml of 0.005% to 10ml acetone-silicon mix respectively. While 0.0005% was created by adding 4ml of 0.0025% to 16ml of acetone-silicon mix, 0.00025% was created by adding 10ml of 0.0005% to 10ml silicon-acetone mix. Under drying chamber each sample concentration (2ml) was pipette evenly on different Whatman No.1 filter papers (12 x

15cm). Filter papers were suspended over cardboards with pins to aid drying and avoid loss of insecticide. Control filter papers were impregnated with 2ml of acetone-silicon mix only; both insecticides impregnated filter papers and acetone-silicon impregnated filter papers were left to dry overnight under drying chamber.

Susceptibility test procedure: In the experiment, 4 replicates of susceptibility testing were carried out for each concentration. Seven sheets of non-treated Whatman No.1 filter papers (12 x 15cm) rolled into a cylinder shape were placed in holding tubes (one per tube with green dot) and secured into position with a metal clip. The tubes were attached to slide units. Approximately 25 active female biting midges were aspirated from holding pots into each holding tube through the filling hole in the slide. After transferring the midges the slide unit was closed and holding tubes set in upright position for 5 minutes to allow the midges to acclimatize. Filter papers impregnated with different concentrations of deltamethrin were rolled into a cylindrical shape and placed in exposure tube (one per each tube). Metal clip was inserted into each exposure tube. Acetone-silicon mix impregnated filter papers were placed in the exposure tubes as control. Metal clips were also used to secure the filter papers in place within the tubes. The exposure tubes were attached to other end of the slide unit (Plate 1).

Plate 1: Intrinsic susceptibility test of *Culicoides nubeculosis* using WHO susceptibility test

For each repeat (6 different concentrations and 1 control) twenty-five (25) *C. nubeculosis* were transferred from the pot into the holding tubes. After 5 minutes midges were transferred into the exposure tube by sliding open the dividing slide and gently tapping and blowing the midges into the exposure tube. Immediately all the midges were transferred into the exposure tube the slide was closed. Biting midges were kept in the exposure tubes which were set in vertical position with the mesh-screened end uppermost for a period of one hour (which is standard WHO exposure time); when the exposure time elapsed, biting midges were transferred back into the holding tube by tapping gently on the tube and blowing. After transferring the exposed midges into the holding tube exposure tubes detached from the slide unit. Cotton wool soaked in 10% sugar solution was placed on the mesh-screened end of the holding tubes. Holding tubes were kept in the incubator set at 25˚C. After 1 hour numbers of midges which were seen on their sides or on their backs (knock down) were recorded. Mortality was determined after 24 hours by lack of either movement, the insect lying on its side or inability to respond to a slight tapping on holding tube.

Temperature experiment: Two forms of experiment were done to evaluate the effect of temperature on susceptibility of *C. nubeculosis* to deltamethrin. In the first experiment biting midges were exposed to deltamethrin using WHO bioassay tubes following the same procedure described above. During the experiment exposure tubes (with biting midges) were kept at the desired temperature for 1 hour (the standard WHO exposure period), this experiment is referred to as "treatment temperature experiment" In the other temperature experiment which is referred as "post treatment temperature experiment" biting midges exposed to deltamethrin impregnated filter paper as described in the susceptibility test was kept at desired temperature (after 1 hour exposure to deltamethrin) for 24 hours. During the course of the experiment temperature was monitored using Brannan thermometer. Temperature 20˚C was obtained in the laboratory room. During the experiment temperature of the room was recorded at 1 hour interval to avoid bias. 15 degrees Celsius was obtained when the temperature control knob of the fridge was set at 5 but 10˚C was obtained when the temperature control knob of the fridge was set at 1. Since 15˚C and 10˚C was obtained in fridge temperature was not monitored at 1 hour interval.

Blood feeding experiment: To determine if exposure to deltamethrin will affect blood feeding and subsequently egg production of *C. nubeculosis*, investigation was conducted using biting midges that survived after 24 hours post exposure to each sub lethal concentrations (0.005%, 0.0025%, 0.0005% and 0.00025%) and control. Hemotek membrane system was used to blood feed the midges. Hemotek membrane system comprises of hemotek system, metal membrane unit/meal reservoir, paraffin and rubber band; used to attach the paraffin to meal reservoir. Ten biting midges that were still active after exposure to the above mentioned concentrations were aspirated from the WHO bioassay tubes after 24 hours of exposure to deltamethrin. Pottered midges were placed into different laboratory pots. The pots were covered with fine-mesh net with a small hole. The meal reservoir was filled with horse blood (de-fibrinated horse blood, TSC biosciences) using a pipette and paraffin stretched over it as a membrane; the paraffin was secured in place using a rubber band. The prepared meal reservoir was screwed to the heat transfer plate. The heat transfer plate was plugged to the power unit and blood (contained in the feeder) was allowed to heat up for five minutes before use. The feeder was placed on the fine-mesh covered pot and biting midges allowed to engorge for 10 minutes (Plate 2).

Blood feeding females were then placed in the freezer for two minutes to immobilize them. When two minutes elapsed biting midges were removed from the freezer and placed into transparent a10cm diameter by 1cm deep Petri dish. Petri dish was covered with a transparent lid to keep midges in place in case the effect of cold temperature wears off and they become active. Observation was done at 20× magnification under a dissecting microscope to determine the proportion that fed. Biting midges are counted as fed if they have distended abdomen that is conspicuously red. Number of biting midges that took a

Plate 2: Blood feeding experiment using hemotek membrane system

blood meal after ten minutes was recorded. After counting was completed biting midges were transferred back into pots. Engorgement rate was calculated as number of blood fed biting midges divided by total number of biting midges in the pots, multiply by 100. However, partial blood feeding was not taken into account as all blood feed midges was considered fully engorged. As biting midges can only lay eggs in humid substrate, water saturated cotton wool was placed at the bottom of pots and damp filter paper placed on it. The saturated cotton wool maintained high humidity and filter paper served as oviposition site. Also filter paper prevented the midges from becoming trapped in the wet cotton wool fibre. Pots were covered with fine-mesh net. Approximately 10 biting midges were held in each pot for a period of eight days. Cotton wool soaked with 10% glucose solution was placed on the fine-mesh net covering each pot to provide nourishment for the period of experiment. Pots were kept at 25ºC in an incubator for eight days and sugar solution was provided daily. On eight day filter papers were removed from pots and allowed to dry. Number of eggs on the filter papers was counted. Eggs were identified as clusters of black/brown objects on the filter paper. Filter paper was placed in Petri dish under hand lens and number of eggs counted and recorded for each replicate.

Data analyses: Statistics package used to analyze data obtained in the experiment was SPSS. Probit analysis using SPSS was used to obtain the LC_{50} and LC_{90} of *C. nubeculosis* to deltamethrin. Analysis of variance conducted in SPSS was used to check for significant difference in both the blood feeding experiment and egg production experiment. After data analysis result was considered significant if the P value obtained is < .05. Two-way analysis of variance (univariate) was conducted in SPSS to evaluate the effect of temperature and deltamethrin on 24hour mortality. As recommended by the WHO, replicates with mortality rates of the control >20% were excluded in the analyses. For replicates with mortality rates of the control > 5% and < 20%, the mortality were corrected using Abbott's formula (corrected mortality = 100 (observed mortality – control mortality) / (100 – control mortality).

RESULTS AND DISCUSSION

There was positive correlation between concentration of deltamethrin and 24 hour mortality, as 24 hour mortality increased with increase in deltamethrin concentration. Mortality observed at 24 hour post-exposure to deltamethrin was highest at 0.05% while the least mortality was observed at 0.00025%. Less than 50% mortality was observed for concentrations 0.005% and less. Mortality rate observed for biting midges in the control group was 12% which was 4% higher than the mortality rate observed among the biting midges exposed to 0.00025% (Figure 1).

Lethal Concentration 50 (LC_{50}) was the dose required to kill half the members of a tested population after specified test duration and LC_{90} is the dose required to kill half the members of tested population after specified test duration ($LC_{50=}$ 0.005%, LC_{90} = 0.31%). When compared to 24 hours mortality, 1 hour knockdown was higher across the concentrations and control as well (Figure 2).

As 1 hour knockdown did not correspond with 24 hour mortality biting midges knocked down would not necessarily be dead within 24 hours post mortality. For example up to 50% of biting midges that were knocked down at 0.005% became active after 24 hours. In the investigation it was observed that *C. nubeculosis* was susceptible to deltamethrin even at concentrations lower than approved field dose. This observation is consistent with the findings by (Venial *et al*., 2011). Knockdown and mortality increased with increase in deltamethrin concentration which indicated that at certain dose such as 0.0005% and 0.00025% *C. nubeculosis* were unaffected by deltamethrin. When compared to biting midges exposed to silicon-acetone impregnated filter paper (control), biting midges that were exposed to deltamethrin impregnated filter paper were more knocked down (after 1 hour) or killed (after 24 hours) showing that effect was due to deltamethrin, for example up to 90% of biting midges exposed to 0.05% and 0.025% were killed compared to only 12% mortality observed in control group. High mortality as suggested by (Venial *et al*., 2011) could be due to lack of prior exposure of the laboratory reared *C. nubeculosis* to insecticide. Some colonies of laboratory bred insects are more susceptible to insecticide than field collected insects because they were collected from the field before widespread insecticide use, and therefore it is important to verify these results with wild caught flies. The findings could then justify use of data obtained from experiment conducted using laboratory reared organisms in making assumptions about field organisms. The LC_{90} (0.031%) obtained in this study exceeds 0.001% of Venial *et al*. (2011) and 0.02% obtained by Brand (unpublished) despite the three studies using the same laboratory strain of *C. nubeculosis* provided by Pirbright Institute (formerly IAH). The reason for this variation could be difference in the number of biting midges used in the experiment as this study used 25 biting midges for each replicate, while (Venial *et al*., 2011) and Brand (unpublished) used 15 biting midges and 20 biting midges respectively for each replicate. Again, the present study did four replicates but each of the above mentioned studies did three replicates. As demonstrated by Brand (unpublished) there is association between exposure period and 24 hour mortality, as 24 hour mortality increases with increase in exposure period. One hour exposure might not be appropriate for testing intrinsic susceptibility of biting midges because the time frame was designed for indoor residual spraying against mosquitoes, therefore there is possibility that biting midges may not be in contact with host that long. In that

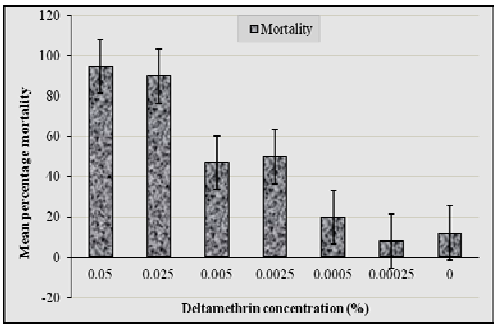

Fig. 1: Mean (±SE) percent mortality of *Culicoides nebeculosis* on 24 hour post-exposure to differing concentrations (%) of deltamethrin.

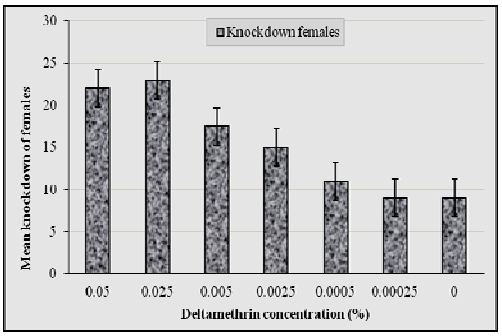

Fig. 2: Mean (±SE) knockdown of female *Culicoides nebeculosis* on 1 hour exposure to differing concentrations (%) of deltamethrin.

case higher concentration maybe required to obtain LC_{90} in short exposure period. But first, the length of time biting midges are in contact with the host needs to be confirmed so that actual dose response of biting midges to different insecticides can be obtained.

Deltamethrin is used in both temperate and tropical regions to control vector but if activity of the insecticide is affected by temperature there is chance that concentration that produced 90% mortality in one region may either cause higher mortality or lower death in another region. In this study effect of temperature on efficacy of deltamethrin on *C. nubeculosis* was evaluated in the treatment temperature experiment and was found that temperature did not significantly affect mortality of midges during 1 hour exposure. However study by (Kaushalya and Edelson, 2004) to evaluate the effect of temperature on efficacy of insecticides to differential grasshopper showed that while the activity of *Beauveia bassiana* is temperature dependent (mainly because is a biological control agent and study has shown that they have reduced activity at low temperature ≤10ºC and high temperature >25ºC) other insecticides and insect growth regulators used in the study showed little or no difference. Variation in the outcome could also be due to difference in experiment procedure. In the present study WHO exposure tube was left at the desired temperature for 1 hour during the exposure and when exposure time elapsed biting midges was transferred into holding tube and placed in incubator (set at 25˚C) after which knockdown was counted (after 1 hour) and mortality (after 24 hours). But the above mentioned study kept insecticide impregnated-leaves at desired temperature for 24 hours before exposing insect to it for 1 hour. There is tendency that if impregnated-filter paper is left at desired temperature for 24 hours or more there maybe difference in mortality and knockdown. Hence any variation recorded will be as a result of temperature effect. For the experiment where biting midges were kept at the desired temperature for 24 hours after 1 hour exposure to deltamethrin, it was found that temperature significantly affected mortality between 25°C and 10°C but there is no difference between 25°C and other temperature range (20°C and 15°C). Possible explanation for this could be that biting midges that were knocked down by insecticide could not recover because of the low temperature. Reports have shown that at low temperature midges are unable to survive which may explain while high mortality occurred at 10°C and also while there is no bluetongue virus transmission during winter.

On post treatment temperature experiment (temperature at which the insect was kept for 24 hours after 1 hour exposure to deltamethrin), there was variation in LC_{50} and LC_{90} obtained when deltamethrin-exposed *C. nubeculosis* were kept at different temperature for 24 hours (recovery time). Based on the LC_{50} and LC_{90} obtained for each of the temperature experiment, biting midges exposed to deltamethrin at 10˚C and 20˚C were more susceptible (LC_{90} =0.02% for the two temperature experiment) than biting midges exposed to deltamethrin at 25˚C and 15˚C with LC_{90}=0.31 and LC_{90}= 0.14 respectively (Table 1).

Statistical analysis showed that there was significant difference ($F=1.981$, $df=18$, $p<0.05$) between 24 hour mortality at different temperatures in the Post treatment temperature experiment while 24 hour mortality was significantly higher at 10°C temperature when compared to 25°C (which is the control temperature) other temperature range was not statistically significant (Figure 3).

When temperature was maintained during 1 hour exposure of *Culicoides nubeculosis* to deltamethrin (Figure 4), it was found that there was no significant difference between 24 hour mortality at different temperatures ($df= 18$, $f= 0.838$, $p>0.05$).

Blood feeding experiment conducted with *C. nubeculosis* exposed to sub lethal doses of deltamethrin and *C. nubeculosis* in the control group showed that blood feeding rate was highest in the control group (with 76% engorgement rate) followed by biting midges exposed to deltamethrin concentration 0.0005% with 75%. While 73% and 51% are the engorgement rate for biting midges exposed to deltamethrin concentrations 0.00025% and 0.0025% respectively, 40% (which is the least blood feeding rate) is the engorgement rate for biting midges exposed to deltamethrin concentration 0.005% (Figure 5).

Statistical analysis showed that there was no significant difference in the rate of blood feeding between biting midges exposed to 0.005% deltamethrin and control group ($P=0.22$, $F=2.23$, $df=7$). Biting midges in the control group produced highest number of eggs (1316) while biting midges that were exposed to deltamethrin concentration 0.005% produced the least number of eggs (87). Biting midges that were exposed to deltamethrin concentrations 0.0025%, 0.0005%, 0.00025% produced 216, 491, 787 number of eggs respectively (Figure 6).

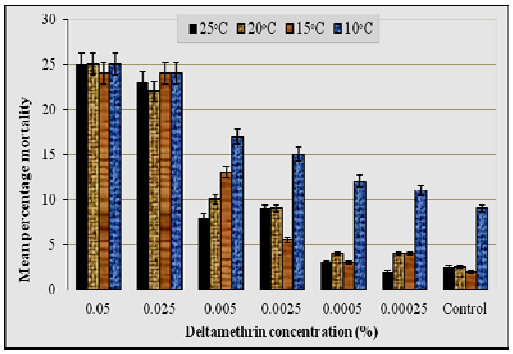

Fig. 3: Mean percentage 24-hour mortality of *Culicoides nebeculosis* exposed to differing concentrations (%) of deltamethrin at different post-treatment temperatures kept for 24 hours.

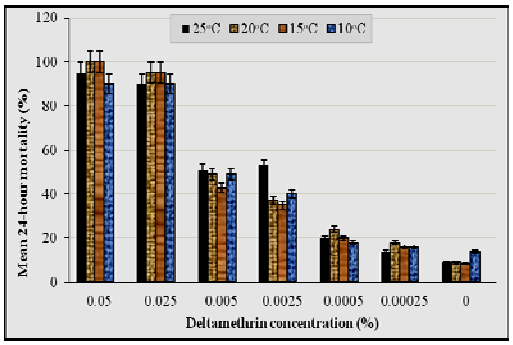

Fig. 4: Mean percentage mortality of *Culicoides nebeculosis* at 24 hour post-exposure to differing concentrations (%) of deltamethrin-impregnated filter papers at different treatment temperatures.

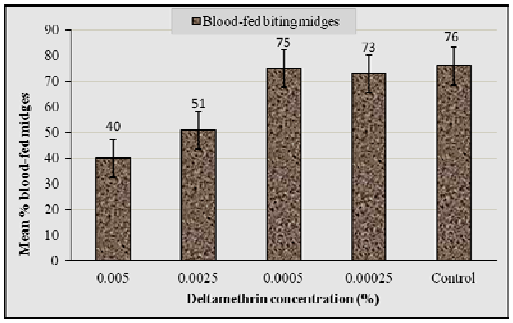

Fig. 5: Mean (± proportional SE) percent number of of blood-fed biting midges in deltamethrin-exposed and control groups.

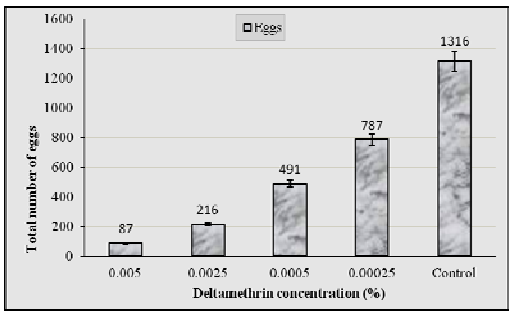

Fig. 6: Total number of eggs produced by biting midges exposed to deltamethrin and control group.

Table 1: Mortality of *Culicoides nubeculosis* after 24 hours of exposure to different concentrations of Deltamethrin at different temperatures

Temperature	Replicates	LC_{50}	95% CI	LC_{90}	95% CI
25˚C	4	0.003	0.002; 0.004	0.31	0.18; 0.62
20˚C	4	0.002	0.001; 0.004	0.20	0.11; 0.56
15˚C	4	0.002	0.001; 0.003	0.14	0.08; 0.32
10˚C	4	0.00	0.00; 0.001	0.20	0.006; 0.595

In Figure 6, the number of eggs increased with decrease in deltamethrin concentration. Statistical analysis using one way analysis of variance (ANOVA) showed that there was significant difference in the number of eggs produced by deltamethrin exposed and unexposed female biting midges ($F=6.007$, $df = 19$, $P<0.05$) which indicated that deltamethrin may have effected egg production. Also, analysis between each exposure group and control group showed that there was statistically significant difference in the number of eggs produced by biting midges exposed to 0.005% concentration and control group ($F=19.662$, $df=7$, $P<0.05$). Again, the number of eggs produced by biting midges exposed to 0.0025% was statistically significant when compared to the number of eggs produced by biting midges in the control group ($F=16.3$, $df = 7$, $P>0.05$). However biting midges exposed to 0.0005% and 0.00025% did not produce number of eggs that was statistically different in comparison with the number of eggs produced by the biting midges in the control group. Outcome was stated as statistically significant if the *p*-value obtained in the analysis was less than 0.05.

The result of blood-feeding experiment suggested that prior exposure to deltamethrin will affect blood feeding rate of *C. nubeculosis*. Therefore onward transmission of Blue Tongue Virus during an outbreak could be prevented if biting midges were unable to take blood meal after contact with insecticide treated livestock. Insecticide can interfere with blood feeding by disrupting the ability of insects to locate food due to reduced olfaction. It was observed that even though blood feeding rate in control group was higher than the blood feeding rate obtained in concentration 0.005% (the maximum deltamethrin concentration) by 36%, statistical analysis showed that the difference was insignificant. Overall observation showed that blood feeding rate did not reduce with increase in deltamethrin concentration neither was there any significant difference in the rate of blood feeding between exposed and unexposed biting midges. This observation is consistent with the report by Mullens *et al*. (2000) that 57% of biting midges exposed to untreated hair as well as 47% of biting midges in the exposure group took blood meals and also reported that insecticide did inhibit blood feeding. In the case of Mullens *et al*. (2000), variation in the result was attributed to the concentration of insecticide used during the experiment. Some experimental approaches used to evaluate the blood feeding rate of biting midges which include laboratory bioassays exposed insects to treated hair, feeding laboratory insects *in vitro* on blood through treated hair, and feeding insects directly on treated animals in small cup-type containers. These procedures differ from the method used in the current study in the sense that this study evaluated blood feeding rate after 24 hours post exposure to insecticide. In study

by Rumpf *et al.* (1998), hair-blood-feeding bioassays were used to evaluate the engorgement rate of *C. sonorensis* (BTV vector in USA) through 5% permethrin. Although this goes forth to show that there is chance that previously knocked-down midges that became active still have the ability to feed and potentially able to transmit BTV if they are infected.

On egg-laying, it will be valuable if exposure to insecticide reduces fecundity as vectors that succeeded in taking blood meal (from insecticide treated animal) and are still able to produce egg will produce low number of eggs thus resulting to reduction in vector population. The result for this study showed that there was no significant difference in the number of eggs produced by biting midges exposed 0.0005%, 0.00025% and number of eggs produced by unexposed biting midges. However, biting midges that were exposed to 0.005% and 0.0025% produce smaller number of eggs; 87 and 216 respectively, which was significantly lower statistically when compared to the number of eggs (1316) produced by biting midges in the control group. The result might be that biting midges exposed to concentrations higher than 0.0005% may have reduced longevity thus did not live long enough to lay eggs. Again it could be hypothesized that fecundity reduced with increase in deltamethrin concentration but further study will be needed to confirm this. In contrast to the observation in this investigation, Mullens *et al*. (2000) reported that exposure to sub lethal dose of insecticide did not affect egg production as difference in the number of eggs produced by biting midges that feed through 5% permethrin- treated hair and those that fed through untreated hair was not statistically significant. Possible reason for the difference could be the concentrations of insecticide used; in this study low concentration was used (0.005%, 0.0025%, 0.0005% and 0.00025%) but the above mentioned study used 5% permethrin. As this is the first study to look at effect of deltamethrin on fecundity of biting midges the result could serve as a baseline for future study. Also, study by Rumpf *et al*. (1998) to determine the effect of different classes of insecticides and insect growth regulators on several life parameters of *Micromus tasmaniae*, including fecundity, found that even though sex ratio, longevity and fecundity were affected by Insect growth regulators, none of these parameters were affected by insecticides- including pyrethroid based cypermethrin. Proportion of females in the pots was greater than males, with the possibility that some of the females did not mate. Future study can correct this by having equal number of both sexes.

Conclusion

This study has demonstrated under UK environmental conditions the effect of deltamethrin on mortality, feeding behaviour and oviposition in the UK Biting midges *Culicoides* species which vectors Bluetongue virus responsible for Bluetongue disease in man and domesticated ruminants. There was significant effects on mortality of biting midges in post treatment temperature experiment but insignificant in treatment temperature experiments. Though there was insignificant effect between the blood feeding rates of deltamethrin-exposed and deltamethrin-unexposed biting midges, the eggs laid by the unexposed were significantly more in number than those laid by the deltamethrin-exposed female biting midges. These results will be useful in vector control of bluetongue viral infections in humans and ruminants. Finally, some of the limitations to be considered with regards to this study were that the sample size for the blood feeding experiment maybe small. So future study may consider more replicates for each concentration in order to get substantial data for robust analysis. Again humidity was not taken into account during the temperature experiment and it might have played a role in mortality of biting midges.

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