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Population Dynamics of *Culicoides* spp. (Diptera: Ceratopogonidae) Midges Influences Dairy Animals' Production

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ABSTRACT

The substantial number of body movements by the host as a response to bites by an insect pest *Culicoides* spp. is thought to exert considerable stress to the animals. Also such preventive body movements by the animals consume certain amount of additional energy to be spent on it, resulting in reduced milk yield in dairy animals. Investigations were undertaken during the year 2017-2018, covering almost all three seasons of the year and correlation and regressions was worked out between body movements undertaken in response to *Culicoides* spp bites, annoyance and worries and its impact on milk yield in cows and she buffaloes. Annoyance and worries observed from the bite of *Culicoides* spp. resulted in increase in body movements and decrease in milk production. Data of the present investigation showed in case of cows, reduction in average body movements from 25.40 to 14.10 resulted in 3.60% increase and from 14.10 to 9.67 reduction in average body movements resulted in 9.12% increase in milk yield. Likewise in case of buffaloes reduction in the body movements from 33.40 to 25.88, 14.68 and 9.89 resulted in 6.77, 10.67 and 15.15 % hike in milk yield, respectively. Thus *Culicoides* spp. population found was positively correlated with body movements indicating rise in pest population results in rise in body movements and negatively correlated with average milk yield exhibiting inversely proportionate relationship.

HIGHLIGHTS

- *Culicoides* flies population was found influenced by environmental factors.
- Annoyance by these flies causes significant reduction in milk productions.
- Control of these flies will reduce the losses of energy spent on reflective body movement.

Keywords: *Culicoides* spp, population, body movements, meteorology, average milk yield

Insect pests of livestock particularly different species of dipteran flies such as *Culicoides* midges or *Simulium* flies or *Phlebotomus* flies or mosquitoes impact the livestock husbandry in three ways. Firstly it can be realised as annoyance and worries to animals and blood loss from direct biting and feeding; secondly as acting vectors for several debilitating and harmful diseases; and thirdly as expenditures additionally incurred on their control (Taylor *et al*., 2012). There are several globally distributed families of Diptera that harass farm animals; these include the Culicidae, Ceratopogonidae, Muscidae, Oestridae, Simuliidae and Tabanidae (Kamut and Jezierski, 2014). High populations of various insect species, especially common in the summer, may adversely affect farm animal health because of the transmission of contagious and parasitic diseases and the induction of insect allergies (Talavera *et al.,* 2018). Blood-sucking insects may compromise animal welfare and cause economic losses

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due to the annoyance and distress suffered by the animals, feeding interruptions and energy requirements for avoidance or defence behaviours initiated by the animals in response to the insects. Consequently, significant decreases in production traits, such as weight gain in beef cattle or milk yield in dairy cattle, can be expected when blood-sucking insects are numerous. A significant decrease in milk production by dairy cattle caused by insects feeding on cattle blood was ascertained by several previous reports (Altunsoy and Kilic, 2012).

Amongst the dipteran pests, *Culicoides* spp midge are the important human and animal pests with great economic significance (Kamut and Jezierski, 2014). *Culicoides* (Diptera: Ceratopogonidae) are commonly known as biting / bloodsucking midges or gnats (1-3 mm) exhibit a huge biodiversity with more than 1300 species described worldwide of which some 96% are hematophagous (Borkent, 2014a, b) and hence these midges are also a source of nuisance through the bites of females. Their presence can, therefore, hinder the economic development of some regions, hampering agricultural and forestry activities, as well as tourism development (Searle *et al.*, 2014).

About 63 different species of *Culicoides* have been reported from different states of India which predominantly include *C. imicola, C. oxystoma*, *C. peregrines*, *C. schultzei,C. actoni, C. anopheles, C. palpifer, C. huffi, C. innoxius, C. arakawaea, C. circumscriptus* as the predominant ones (Narladkar, 2012; Archana *et al*., 2016). These midges are of great concern because they transmit bluetongue (BT), African horse sickness virus (AHSV), epizootic hemorrhagic disease virus (EHDV), equine encephalitis virus (EEV), akabane virus, bovine ephemeral fever virus and Schmallenberg disease (Hoffmann *et al*., 2012; De Regge *et al*., 2012). However, Blue Tongue disease in sheep has special status which restricts free trade of animals accounts for 125 million US Dollar revenue annually. BT is a viral disease causing morbidity and mortality in sheep, cattle and wild ruminants, including deer, sambar and bluebull. BT is endemic in many parts of India (Chanda *et al*., 2019) and its outbreak in Tamilnadu during the monsoon season of 1997- 1998 caused the death of 300,000 sheep and goats and occurrence of this disease in many parts of India over the last few decades has affected millions of sheep and goats and other livestock (Ilango, 2006). Vector potential of these flies in India in terms of transmission of various viral and protozoan diseases of livestock was reported through several studies (Chanda *et al*., 2019) of which bovine ephemeral fever and blue tongue have been considered a major hindrance in ruminant husbandry (Lee, 2019; Rupner *et al*., 2020).

Ecological aspects and the development cycle of *Culicodes,* it is similar to mosquitoes; females lay eggs, which will develop into four larval stages, followed by a nymph stage. Emerging adults will mate, and both sexes feed on nectar, whereas only females take a blood meal on vertebrates for egg maturation. The only difference is about the nature of larval habitat. The larvae of these midges are not aquatic and can be found in humid/mud substrates enriched with organic matter: wet soil ecotone between aquatic and terrestrial habitats (coastal salt mud flats, freshwater vegetated swamps, *etc*.), dung pats or moist decaying vegetative material (Medlock *et al*., 2018). The abundance of *Culicoides* midges was increased manifold at both before and after sunrise and sunset and the preferred sites for animal biting include back region of the animals. However their resting sites were preferably the walls of animal house. Breeding sites of these *Culicoides* species was observed in the vicinity of animal habitation. High concentration of larvae was observed in the wet soil, least contaminated with dung and urine, along the edges of sluggish drains around the cattle sheds. Moderate water content (50 %), low pH, low organic matter content and high electric conductivity milihos /cm^3 (soluble salts) were also reported favourable for breeding of *Culicoides*. At room temperature the pre-oviposition period, incubation period, larval period, pupal stage for *Culicoides peregrinus* and *Culicoides schultzei* were 48 and 56 hrs, 28 and 42 hrs, 10 and 13.66 days, 48 and 48 hrs, respectively. *C. schultzei* required larger period (19.83 days) to complete life cycle as compared to *C. peregrinus* (15.17 days). Duration of life cycle indicates that, several generations of these midges in one fly season are possible. Earlier studies on bionomics of *Culicoides* revealed that meteorological factors such as maximum temperature, more number of bright sunshine hours, low rainfall and high wind velocity deterred; while, minimum temperature, high relative humidity and rainfall favoured build-up of *Culicoides* populations significantly (Purse *et al*., 2015; Grimaud *et al*., 2019).

The annoyance and worries in the animals caused due to some of the dipteran insects were found contributing to the unnecessary / unproductive energy consumption through increased defensive movements of animals to get rid of them (Sumner *et al*., 2017). The responsive movements of tail, ear pinna, neck and skin by the animals to repeated painful bites and sitting of these midges, distract animals from their feeding along with blood loss due to sucking by midges (Grogan and Lysyk, 2015). All these add to the psychological disturbances which ultimately leads to reduced production and reproduction performances (Rozo-Lpez *et al.,* 2021; Narladkar and Shivpuje, 2014a). It was also reported that reduced fly population on dairy cattle and the reduced performance of avoidance behavior due to insecticide repellant use has a good impact on welfare and health condition of dairy animal (Purse *et al.,* 2015). Owing to these, an earlier estimate reported 18.97% (Narladkar and Shivpuje, 2012) milk production losses. On the basis of this estimate, Narladkar (2018) projected the economic losses due to these midges in milk production to the extent of ₹ 95463 crores in India during a year 2012-2013. In the same study, it was further mentioned that, during 2012-2013 the average milk production per animal was 7.02 L/day from crossbred cow, 2.36 L/day from indigenous/ ND cow and 4.80 L/day milk from Indian buffalo and the projected milk loss @18.97% was 1.33 L/day amounting to ₹ 50.54 cow/ day when milk rate was considered as ₹ 38/l, 0.45 L/day loss amounting to ₹ 17.10 per cow/day and -0.91 L/day amounting to ₹ 35.48 per buffalo/day, respectively (Narladkar, 2018).

Therefore present study was planned to establish association between population dynamics of *Culicoides* species, body movements and its net effect on milk yield in cattle and buffaloes with two principle objectives as- (a) to validate those observations mentioned in the preceding paragraphs and considering that the population of *Culicoides* is higher than any hematophagus fly species in the shed and particularly during resting period of animals, and (b) to validate the results of, controlled experiment previously conducted, wherein, it was observed that preventing entry of these midges in animals' houses through netting resulted significantly less (almost 9 times) body movements and thus improved health and production of dairy animals under the shed to the extent of 18.97% (Narladkar and Shivpuje, 2014b)- under natural/ field conditions.

METHODOLOGY

Location of the present study

The agro-ecology of Parbhani town where the study was conducted is sample site which resembles with the rest of Marathwada region of the Maharashtra state, India. This region locates between Latitude 17^o 35' N – 20^o 40' N Longitudes 70^o 40' – 78^o 15' E , MSL – 40.9 meter, comprising of eight districts in the Deccan Plateau Zone, is basically an intense agrarian region having rural setting. Climatologically Marathwada region is categorized as semi-arid on annual basis. It is humid to per-humid during monsoon, sub-humid to semi-arid during winter and arid during summer season. The corresponding distribution pattern of annual rainfall (500-1100 mm) is 75-85, 10-15 and 6-10 per cent, respectively. The wet monsoon (weeks 23-44, 4th June –4 Nov) period alternates with long rain free cold winter (weeks 45-9, 5th Nov. – 4th March). The (12-18^{o}C) minimum temperature and 40-45^{o}C max. Is observed during the summer (weeks 10-22, 5th March – 3rd June), having appreciable temporal and spatial variability, a typical characteristics of semi-arid climate. The thermo-aero dynamics conditions are conducive for existing agro-eco-systems during monsoon and winter, with moderate and severe limitations of soil moisture availabilities during winter and summer seasons. The results of current study can be applicable to other regions with similar agro ecological setup.

Animals and animal shed included in the study

The animal sheds involved the current study were of gable type (50x30 feet) accommodating 20 cattle or buffaloes separately in tail to tail manner. One light trap was used for each shed of cattle or buffalo which was hung on an iron pole in the middle of shed at 25 feet from ground. Collection of *Culicoides* midges were conducted once in a week from livestock sheds during the period of June 2017 –May 2018, covering all the three seasons of a year. Collection was done for five minutes, by employing light trap, during the dawn and dusk times and at night times (20.00 hrs to 21.00 hrs), the time when bites of *Culicoides* spp were reported to be at its peak (Lehiy *et al.,* 2018). Collected midges were immediately transferred in specimen bottles containing 70% alcohol. These were brought to the laboratory of Department of Veterinary

Parasitology of the college and were speciated with help of zoom stereoscope supported by standard morphological keys and were counted.

Collection of *Culicoides* midges by light trap

Collections were undertaken using down draught light trap (220 V) equipped with 8 watt black light tube OVI type (developed by Onderstepoort Veterinary Institute). Light traps were hung in the vicinity of animals for 5 min at the dawn and the dusk and at night times (20.00 hrs to 21.00 hrs). The *Culicoides* midges were attracted to UV light of the light trap and were collected in the 50 ml sized test tubes. Count was recorded once in a week consecutively for 50 times from buffalo shed and 34 times from cattle shed in a calendar year covering all the three seasons occurring in this region. This way 150 readings from buffalo shed and 102 readings from cattle shed were taken. On each day of observation three readings *i.e.* at dawn, dusk time and night time (in between 20.00 hrs and 21.00 hrs) were recorded. In the present study UV blue emitting light of wavelength 450-495 nm was used. The standard procedure for collection of *Culicoides*, use of light trap, type, size, height etc all was adapted from Medlock *et al*. (2018). Compared to lamps which emit narrow wavelengths of light; mosquitoes, *Culicoides* and sand flies have generally been found to be more attracted by short wavelengths, such as ultra violet, blue (450–495 nm) and green (495–570 nm) light (Wilson *et al*., 2021). Even if the techniques to collect larvae (substrate sampling or emergence traps) do exist, they are time-consuming and laborious. Therefore, the sampling of populations is mostly carried out using adult collections (Medlock *et al*., 2018). One light trap was employed in a cattle shed and one in a buffalo shed. Two feet in height including collecting cloth chamber and collecting tube (Photograph attached). Similar type of light trap was used by earlier workers (del-Río *et al*., 2013; Narladkar and Shivpuje, 2014; Archana *et al*., 2016).

Recording of observations on body movements

Simultaneously at the time of collection, movements of various body parts of the animals were recorded for 5 min for one animal by employing a video camera (SONY HDR-CX130) and video was shot from a distance without disturbance to animals. Data of video footage was transferred to a computer and body movement counts were recorded with the help of hand tally counter while observing the video. Movements of body parts such as swinging of tail, shaking of ear pinna, neck rotations and skin wagging were counted. In addition restlessness and disregard to feeding both at resting and at sitting position were recorded. Data generated was further utilized for establishing relationship. Milking cows and buffaloes were also included for recording of body movements due to bites of *Culicoides* spp. The collection of *Culicodes* midges and recording of body movements were performed once in a week consecutively for 50 times from buffalo shed and 34 times from cattle shed.

Meteorological data

Different meteorological parameters such as Temperature ^{o}C (Max, Min), Humidity (AM, PM), Rainfall (mm) and Bright sunshine hrs in a day are collected from Meteorological observatory, Vasantrao Naik Marathwada Agricultural University, Parbhani.

Collection of data on milk yield

Six productive cows of Red Kandhari breed weighed 300-400 kgs and six productive buffaloes of Marathwadi breed of 400 – 500 kgs of weights maintained at the Instructional Livestock Farm of the college were included for collection of data on milk yield. Daily milk yield data were obtained from the farm records for selected cows and buffaloes. In addition; the data on body movements due to bites of midges generated during a research project completed earlier in the department were also considered as reference (Narladkar, 2012).

STATISTICAL ANALYSIS

Data generated and meteorological data were subjected to correlation and regression to obtain the impact of *Culicoides* on milk yield and energy consumption of cows and buffaloes with the help of WASP 2.0 provided online by ICAR – CCARI, Goa (Jangam and Wadekar, 2020).

RESULTS

Prevalent *Culicoides* spp.

Following species of *Culicoides* spp midges were collected from cattle and buffalo sheds, were identified and confirmed. The species were:—

1. *Culicoides peregrinus* Kieffer, 1910
2. *Culicoides schultzei* Enderlein, 1908
3. *Culicoides actoni* Smith, 1929

During the study period total 50 observations and 34 observations were recorded in buffaloes and cows, respectively. The average population of *Culcoides* spp. midges observed was 69.420 ±7.197 on buffaloes and 51.824 ±5.525 in cows. The population of *Culicoides* spp midges has shown significant correlation with total body movements, minimum temperature and relative humidity. It indicates that, when minimum temperature and humidity are at optimum level, *Culicoides* population is on rising trend and it directly results in increasing body movements as a consequence of painful bites, annoyance and worries. Accordingly these observations established the significant correlations between *Culicoides* midge population, body movements and resultant average milk yield (Table 1). It also shows that with the reduction in the body movements, there is quite an increase in milk yield of animals. For cows, reduction in body movements from 21-30 to 11-20 resulted in 3.60% increase and from 11-20 to 00-10 has been resulted in 9.12% increase in milk yield. Likewise in case of buffaloes, reduction in the body movements from 31-40 to 21-30, 11-20 to 9-10 resulted in 6.77, 10.67 and 15.15 % increase in milk yield, respectively.

Total body movements

Total body movements showed significant correlations with rainfall, minimum temperature and relative humidity with positive magnitude. However, total body movements have shown a significantly negative correlation with bright

Table 1: Body movements undertaken by cow or buffalo in response to number of *Culicoides* spp biting and its impact on milk yield

Range of Body Movements	Number of Observations	No of Culicoides observed within five minutes Mean±SE (Min-Max)	Body movements in response to *Culicoides* spp bite					Average milk yield per cow/week or buffalo/week	% hike in milk yield
			Ear movements Mean±SE (Min-Max)	Neck movements Mean±SE (Min-Max)	Tail movements Mean±SE (Min-Max)	Body movements Mean±SE (Min-Max)	Total body movements Mean±SE (Min-Max)		
Cows									
21-30	05	104.80±13.71 (51-124)	5.60±0.81 (4-8)	2.60±0.51 (1-4)	14.40±0.93 (12-17)	2.40±0.40 (1-3)	25.40±1.50 (21-29)	13.92±0.25 (13.1-14.6)	—
11-20	20	50.60±5.11 (30-103)	3.70±0.21 (2-5)	1.35±0.19 (1-3)	8.00±0.36 (5-11)	1.10±0.18 (0-3)	14.10±0.48 (11-19)	14.43±0.15 (13.2-15.5)	3.60
01-10	09	25.11±1.12 (21-31)	2.67±0.33 (1-4)	1.22±0.15 (1-2)	5.00±1.5 (4-7)	0.89±0.20 (0-2)	9.67±0.24 (8-10)	15.19±0.16 (14.2-15.8)	9.12
Buffaloes									
31-40	05	161.60±24.04 (104-217)	8.60±0.75 (7-11)	4.20±0.49 (3-6)	19.00±1.30 (17-24)	3.60±0.40 (3-5)	33.40±1.17 (31-37)	16.70±0.24 (16.0-17.38)	—
21-30	08	115.38±35.13 (51-166)	5.63±0.53 (4-8)	2.75±0.37 (1-4)	15.63±0.98 (12-20)	2.13±0.35 (1-3)	25.88±93 (21-29)	17.83±0.22 (16.5-18.50)	6.77
11-20	28	53.71±5.57 (30-139)	3.86±1.04 (2-6)	1.36±0.12 (1-3)	8.32±0.39 (4-13)	1.14±0.14 (0-3)	14.68±0.47 (11-20)	18.48±0.12 (17.0-19.38)	10.67
01-10	09	26.22±1.48 (21-34)	2.78±0.36 (1-4)	1.22±0.15 (1-2)	5.11±0.39 (4-7)	0.89±0.20 (0-2)	9.89±0.11 (9-10)	19.13±0.16 (18.3-19.75)	15.15

% hike in milk yield is calculated with reference to milk yield recorded during maximum number of body movements; Average milk yield/cow/week: Total milk yield per cow during one week and total six cows or six buffalo's milk yield has been recorded; Total body movements=Ear movements+ Neck movements+ Tail movements+ body movements.

sun-shine hours, evaporation rate and average milk yield. Negative correlation with average milk yield signifies that increased body movements result in unnecessary expenditure of energy and it results in lowering down of milk production (Table 1-3).

Average milk yield

Average milk yield has shown significant negative correlation with total body movements, maximum temperature, minimum temperature and EVP (Table 1-3). In the current study all the factors observed in buffaloes showed correlation with most of the meteorological factors *at par* with cattle; however two marked differences were noted from cattle. First, *Culicoides* population has shown negative significant correlation with average milk production. Second, the total body movements showed negative non-significant correlation with maximum temperature and bright sun shine hours (Table 3).

Impact of meteorological factors on *Culicoides* spp. population

From the regression analysis it was found that, relative

Table 2: Correlation matrix and test of significance between *Culicoides* spp. population, environmental factors, total body movement and average milk yield in the Cows

	CP	TBM	RF	TMX	TMN	RHM	RHE	ER	BSS	WS	AMY
CP	1.000										
TBM	0.840*	1.000									
RF	0.653	0.624*	1.000								
TMX	-0.031	0.059	-0.008	1.000							
TMN	0.491*	0.456*	0.351*	0.650*	1.000						
RHM	0.188	0.089	0.069	-0.846*	-0.368*	1.000					
RHE	0.538*	0.370*	0.377*	-0.696*	0.025	0.731*	1.000				
ER	-0.012	0.124	0.048	0.960*	0.638*	-0.860*	-0.671*	1.000			
BSS	-0.256	-0.340*	-0.233	0.441*	-0.018	-0.462*	-0.570*	0.408*	1.000		
WS	-0.146	0.009	0.053	0.171	0.219	0.014	0.037	0.219	-0.031	1.000	
AMY	-0.325	-0.544*	-0.188	-0.390*	-0.562*	0.188	0.102	-0.453*	0.187	-0.080	1.00

CP: *Culicoides* spp. Population; TBM: Total body Movements; RF: Rain fall (mm); TMX: Maximum temperature (^{o}C); TMN: Minimum temperature (^{0}C); RHM: Relative Humidity morning (%); RHE: Relative humidity evening (%); ER: Evaporation rate (mm); BSS: Bright sun shine (Hrs); WS: Wind Speed (Kmph); AMY: Average milk yield per cow(litres); * significant correlation.

Table 3: Correlation matrix and test of significance between *Culicoides* spp. population, environmental factors, total body movement and average milk yield in the buffaloes

—	CP	TBM	RF	TMX	TMN	RHM	RHE	ER	BSS	WS	AMY
CP	1.000										
TBM	0.867*	1.000									
RF	0.183	0.243	1.000								
TMX	-0.231	-0.225	0.026	1.000							
TMN	0.594*	0.657*	0.237	0.259	1.000						
RHM	0.395*	0.461*	0.372*	-0.771*	0.205	1.000					
RHE	0.630*	0.675*	0.367*	-0.582*	0.576*	0.823*	1.000				
ER	-0.365*	-0.316*	-0.411*	0.824*	0.081	-0.827*	-0.654*	1.000			
BSS	-0.390*	-0.523	-0.226	0.464*	-0.472*	-0.650*	-0.796*	0.528*	1.000		
WS	-0.049	0.147*	0.421*	0.143	0.358*	0.229	0.244	0.022	-0.068	1.000	
AMY	-0.685*	-0.778*	-0.054	-0.021	-0.646*	-0.195	-0.438*	0.021	0.420*	-0.168	1.000

CP: *Culicoides* spp. Population; TBM: Total body Movements; RF: Rain fall (mm); TMX: Maximum temperature (^{o}C); TMN: Minimum temperature (^{0}C); RHM: Relative Humidity morning (%); RHE: Relative humidity evening (%); ER: Evaporation rate (mm); BSS: Bright sun shine (Hrs); WS: Wind Speed (Kmph); AMY: Average milk yield per cow(litres); * significant correlation.

humidity at evening time and rainfall has positive correlation with *Culicoides* population for cattle (Table 4).

Impact of increased body movements on milk production

Regression analysis presented in the Table 5, indicates significant correlation of milk yield with body movements (t = -2.156 for cows and t = -2.334 for buffaloes) and bright sun-shine hours (t = +2.266 for cows and t = +2.154 for buffaloes). The outcome obtained from multiple regression analysis revealed that, milk production is positively affected by rainfall, maximum temperature, morning and relative humidity at evening hours and bright sunshine. Results of the present study also revealed a highly significant positive correlation between *Culicoides* population and body movements which signifies that the increased body movements performed by cows and buffaloes are absolutely in response to *Culicoides* population and against the nuisance created by them (Table 6). Hence, it can be inferred that *Culicoides* population, body movements and average milk production are the strongly interlinked factors with each other. Though the magnitude of correlation between *Culicoides* spp. population and milk production is non-significant in case of cows and significant in case of buffaloes (Table 7), it is with negative sign, indicating milk production is substantially affected by *Culicoides* population. Milk production has significant negative correlation with body movements (Table 8) indicating the impact of body movements on milk production.

DISCUSSION

It was hypothesized that the annoyance, worries and other psychological disturbances caused due to the bites and swarming of dipteran biting flies around animals' body are responsible for undesirable spending of additional energy towards making preventive movements to get rid of these flies which has been reflected in hike in milk yield. During present study in case of cows, reduction in body movements from 21-30 to 11-20 resulted in 3.60%

Table 4: Regression analysis and regression equation between *Culicoides* spp. population and environmental factors in cattle and buffaloes Dependable variable: *Culicoides* spp. population

	Cattle					Buffaloes				
Independent Variables	**Average**	**Reg. coefficients (b)**	**Standard Error(SE(b))**	**T Test**	**T table (0.05)**	**Average**	**Reg. coefficients (b)**	**Standard Error(SE(b))**	**T Test**	**T table (0.05)**
RF	2.667	1.662	0.617	2.717*	2.060	3.212	-0.147	0.676	-0.217	2.020
TMX	33.941	2.502	3.965	0.637	2.060	33.222	-2.447	4.712	-0.511	2.020
TMN	14.900	0.190	2.282	0.085	2.060	17.490	8.223	2.530	3.248*	2.020
RHM	69.417	1.024	0.765	1.347	2.060	74.260	-1.262	1.014	-1.249	2.020
RHE	26.208	1.811	0.825	2.208*	2.060	38.160	0.867	0.831	1.032	2.020
ER	6.032	6.659	7.144	0.934	2.060	5.420	-11.806	5.383	-2.191*	2.020
BSS	9.108	1.295	3.196	0.400	2.060	7.690	7.940	2.481	3.197*	2.020
WS	3.341	-10.736	3.700	-2.892*	2.060	3.694	-10.600	4.094	-2.591*	2.020
Intercept (a) = -175.290 Coefficient of determination (R Square) = 0.728 Multiple Correlation Coefficient (R) =0.852 Standard Error = 19.4						Intercept (a) = 110.348 Coefficient of determination (R Square) = 0.691 Multiple Correlation Coefficient (R) =0.832 Standard Error = 30.510				
Regression Model: CP = -175.290 + (1.662) × RF + (2.502) × TMX + (0.190) × TMN +(1.024) × RHM + (1.811) × RHE + (6.659) × ER + (1.295) × BSS + (10.736) × WS + 19.484						**Regression Model:** CP = 110.348 + (-0.147) × RF + (-2.447) × TMX + (8.223) × TMN + (-1.262) × RHM + (0.867) × RHE + (-11.806) × ER + (7.940) × BSS + (-10.600) × WS + 30.510				

CP: *Culicoides* spp. Population; TBM: Total body Movements; RF: Rain fall (mm); TMX: Maximum temperature (^{o}C); TMN: Minimum temperature (^{0}C); RHM: Relative Humidity morning (%); RHE: Relative humidity evening (%); ER: Evaporation rate (mm); BSS: Bright sun shine (Hrs); WS: Wind Speed (Kmph); AMY: Average milk yield per cow(litres); * significant correlation.

Table 5: Regression analysis and regression equation between *Culicoides* spp. population, environmental factors, total body movement and average milk yield in cattle and buffaloes Dependable variable: Average milk yield

	Cattle					Buffaloes				
Independent Variables	**Average**	**Reg. coefficients (b)**	**Standard Error(SE(b))**	**T Test**	**T table (0.05)**	**Average**	**Reg. coefficients (b)**	**Standard Error(SE(b))**	**T Test**	**T table (0.05)**
CP	69.420	-0.003	0.004	-1.663	2.023	69.420	-0.006	0.003	-1.542	2.023
TBM	17.480	-0.052	0.025	-2.156*	2.023	17.480	-0.065	0.028	-2.334*	2.023
RF	3.212	0.002	0.015	0.183	2.023	3.212	0.005	0.012	0.281	2.023
TMX	33.226	0.041	0.083	0.568	2.023	33.222	0.047	0.082	0.477	2.023
TMN	17.490	-0.032	0.058	-0.651	2.023	17.490	-0.021	0.046	-0.584	2.023
RHM	74.260	0.003	0.012	0.232	2.023	74.260	0.003	0.017	0.243	2.023
RHE	38.160	0.022	0.016	1.480	2.023	38.160	0.023	0.012	1.391	2.023
ER	5.420	-0.086	0.109	-0.834	2.023	5.420	-0.078	0.104	-0.710	2.023
BSS	7.690	0.122	0.054	2.266*	2.023	7.690	0.116	0.051	2.154*	2.023
WS	3.694	-0.130	0.088	-1.697	2.023	3.694	-0.123	0.079	-1.639	2.023

Cattle	Buffaloes
Intercept (a) = 17.634	Intercept (a) = 17.867
Coefficient of determination (R Square) = 0.719	Coefficient of determination (R Square) = 0.727
Multiple Correlation Coefficient (R) =0.849	Multiple Correlation Coefficient (R) =0.856
Standard Error = 0.532	Standard Error = 0.527
Regression Model:	**Regression Model:**
AMY = 17.634 + (-0.003) × CP + (-0.052) × TBM + (0.002) × RF + (0.041) × TMX + (-0.032) × TMN + (0.003) × RHM + (0.022) × RHE + (-0.086) × ER + (0.122) × BSS + (-0.130) × WS + 0.532	AMY = 17.867 + (-0.006) × CP + (-0.065) × TBM + (0.005) × RF + (0.047) × TMX + (-0.021) × TMN + (0.003) × RHM + (0.023) × RHE + (-0.078) × ER+(0.116) × BSS + (-0.123) × WS+0.527

CP: *Culicoides* spp. Population; TBM: Total body Movements; RF: Rain fall (mm); TMX: Maximum temperature (^{o}C); TMN: Minimum temperature (^{o}C); RHM: Relative Humidity morning (%); RHE: Relative humidity evening (%); ER: Evaporation rate (mm); BSS: Bright sun shine (Hrs); WS: Wind Speed (Kmph); AMY: Average milk yield per cow (litres); * significant correlation.

Table 6: Regression analysis and regression equation between *Culicoides* spp. population and total body movement in cattle and buffaloes Dependable variable: Total body movements:

	Cattle					Buffaloes				
Independent Variables	**Average**	**Reg. coefficients (b)**	**Standard Error(SE(b))**	**T Test**	**T table (0.05)**	**Average**	**Reg. coefficients (b)**	**Standard Error(SE(b))**	**T Test**	**T table (0.05)**
CP	51.825	0.139	0.017	8.907*	2.037	69.420	0.129	0.017	11.984*	2.011

Cattle	Buffaloes
Intercept (a) = 7.334	Intercept (a) = 8.526
Coefficient of determination (R Square) = 0.713	Coefficient of determination (R Square) = 0.745
Standard Error = 2.913	Standard Error = 3.832
Regression Model:	**Regression Model:**
TBM = 7.334 + (0.139) × CP + 2.913	TBM = 8.526 + (0.129) × CP + 3.832

CP: *Culicoides* spp. Population; TBM: Total body Movements; * significant correlation.

Table 7: Regression analysis and regression equation between *Culicoides* spp. population and average milk yield in cattle and buffaloes
Dependable variable: Average Milk yield

	Cattle					Buffaloes				
Independent Variables	**Average**	**Reg. coefficients (b)**	**Standard Error(SE(b))**	**T Test**	**T table (0.05)**	**Average**	**Reg. coefficients (b)**	**Standard Error(SE(b))**	**T Test**	**T table (0.05)**
CP	51.825	-0.003	0.008	-1.920	2.037	69.420	-0.019	0.008	-6.512*	2.011
Intercept (a) = 14.937						Intercept (a) = 19.140				
Coefficient of determination (R Square) = 0.104						**Coefficient of determination (R Square) = 0.465**				
Standard Error = 0.708						Standard Error = 0.651				
Regression Model:						**Regression Model:**				
AMY = 14.937 + (-0.003) × CP + 0.708						AMY = 19.140+(-0.019) × CP + 0.651				

CP: *Culicoides* spp. Population; AMY: Average milk yield per cow(litres); * significant correlation.

Table 8: Regression analysis and regression equation between average milk yield and Total body movements in cattle and buffaloes
Dependable variable: Average Milk yield

	Cattle					Buffaloes				
Independent Variables	**Average**	**Reg. coefficients (b)**	**Standard Error(SE(b))**	**T Test**	**T table (0.05)**	**Average**	**Reg. coefficients (b)**	**Standard Error(SE(b))**	**T Test**	**T table (0.05)**
TBM	14.582	-0.079	0.025	-3.650*	2.037	17.480	-0.095	0.015	-8.699*	2.011
Intercept (a) = 15.642						Intercept (a) = 19.919				
Coefficient of determination (R Square) = 0.292						Coefficient of determination (R Square) = 0.616				
Standard Error = 0.627						Standard Error = 0.558				
Regression Model:						**Regression Model:**				
AMY = 15.642 +(-0.079) × TBM + 0.627						AMY = 19.919 +(-0.095) × TBM + 0.558				

AMY: Average milk yield per cow (litres); TBM= Total body movements * significant correlation.

increase and from 11-20 to 00-10 has been resulted in 9.12% increase in milk yield. Similarly, in case of buffaloes reduction in the body movements from 31-40 to 21-30, 11-20 to 9-10 resulted in 6.77, 10.67 and 15.15 % increase in milk yield, respectively. Previous report also supports that reduction in body movements to minimum or almost nil resulted in increased milk yield to the extent of 18.97 per cent (Narladkar and Shivpuje, 2014).

Light traps for *Culicoides* spp.

As far as collection of *Culicoides* is concerned, UV light trap remain the most time efficient and widespread method to assess *Culicoides* presence and abundance at a wide scale. UV light traps collect a fraction of the *Culicoides* populations, mostly host-seeking females. The assessed abundance depends to a large degree on the trap location, including distance to animals, height above ground level, etc. (Lysyk *et al*., 2014). Viennet *et al*. (2012; 2013) showed that UV light trap collections were linearly correlated to attack rates on animals for several *Culicoides* species. This showed that abundance assessed by UV light trap collections may still be useful for risk assessment: abundance data based on UV light trap collections are likely to be broadly representative of biting rates (in identifying order-of-magnitude differences in population size according to spatial or temporal variation) and hence abundance data collected with UV light traps have been used widely to manage animal movements (Medlock *et al*., 2018).

Total body movements

Present study observations indicated that, total body movements showed significant correlations with rainfall, minimum temperature and relative humidity with positive magnitude. It means that increase in rainfall, minimum temperature and relative humidity help to build-up *Culicoides* population thereby increase in the body movements in response to bites of higher number of midges. However, total body movements have shown a significantly negative correlation with bright sun-shine hours, evaporation rate and average milk yield. It may be due to the natural phenomenon that during bright sun shine hrs animal rests under shed in calm and quite state, hence lowered body movements. Negative correlation with average milk yield observed during the present study signifies that increased body movements result in unnecessary expenditure of energy and it results in lowering down of milk production (Table 1-3).

Average milk yield

Data summarized in the Tables (1-3) shows that, average milk yield has significant negative correlation with total body movements, maximum temperature, minimum temperature and EVP, Because these factors lead to rise in Thermal-Humidity Index (THI) and severe thermal stress resulting in lowered milk production which is concurrent with the earlier opinions (Kohli *et al*., 2014; Behera *et al*., 2018).

Impact of meteorological factors on *Culicoides* spp. population

Data from the Table (4) indicated that relative humidity at evening time and rainfall has positive correlation with *Culicoides* population for cattle. However it is well established that rainfall and humidity are favorable for propagation of dipteran insects through providing more breeding sites (Gao *et al*., 2017). Wind speed has significant negative impact on *Culicoides* population. It can be justified that, during high wind speed midges like *Culicoides* cannot settle on body of animal or shed and hence their count goes down and it has reflected in significant negative correlation. In case of buffaloes an unusual significantly positive correlation between *Culicoides* population, minimum temperature and bright sun shine time has been noted (Table 4); which is beyond our understanding and hence could not be justified or explained. Significant negative correlation with evaporation rate can be justified as breeding of *Culicoides* takes place in the mud along the banks of drainage channels and evaporation of moisture from such breeding sites definitely causes reduction in midge population.

Present study was planned to test the hypothesis that, rise in population of livestock pests greatly affects the milk production by several direct and indirect ways. In the present study all observations are described by taking representative pest species as *Culicoides* spp midges. These midges require temperature range of 12 to 32ºC and RH range of 72-86% as most favorable factors (Halder *et al*., 2013). Meteorological data collected of this region matches to this range except in too hot summer months, thus, rest of the period of the year is suitable for growth and prevalence of these midges. Earlier studies conducted by Narladkar and Shivpuje (2014) have reported that, due to bite of *Culicoides* spp there was reduction in rumination hours, sleeping hours and resting hours. Also considerable amount of energy is consumed in producing movements of body parts like tail, ear pinna, neck, skin *etc.* to wipe out these midges from the body, all of which collectively lead to reduced milk yield (Narladkar and Shivpuje, 2014). However, the rise in *Culicoides* spp. population on the body of cattle cannot happen independently. It involves a complex mechanism of insect physiology and phenology, both are totally dependent on the climatic/environmental parameters (Geohagen *et al*., 2014). Environmental parameters play important role in monitoring *Culicoides* population as direct effect (diagonal) and in combination with each other (off-diagonal). The environmental parameters are interlinked with each other and thus, they influence on the insects population (Searle *et al*., 2013). Climate changes have significant impact on natural environment and it gets reflected by affecting their range, phenology, activity, number of generations and survival (Geohagen *et al*., 2014; Nwaerema, 2020). Being an ectothermic, insects are highly dependent on thermal conditions of the surrounding environment. Hence, besides food from host, climate conditions are basic factors for insect population (Jaworski and Hilszczański, 2013). Similarly insects as poikilothermic animals change their activity visibly depending on the temperature of the surrounding environment (Searle *et al*., 2014).

Impact of increased body movements on milk production

Regression analysis summarized in Table 5, indicated significant correlation of milk yield with body movements and bright sun shine hrs. However it has non-significant correlation with rest of the variables. It is herewith validated that total body movements have strong correlation with milk production and is inversely proportionate (Sumner *et al*., 2017; Narladkar and Shivpuje, 2014). The results of current study proposed that increased energy consumption for preventive movements contributes to this reduced milk yield which is also validated statistically.

Results of the multiple regression analysis undertaken during present study revealed that, milk production is positively affected by rainfall, maximum temperature, morning and relative humidity at evening hours and bright sunshine. However, it is a natural phenomenon that rainfall helps in improving the availability of green pastures after a long pause of hot and dry summer months and this contributes to improved health of livestock and resultant increase in milk production.

Narladkar and Shivpuje (2012) studied the effect of fly proof net shed on the milking buffaloes and advocated that, if buffaloes are protected from *Culicoides* bite during night time can reduce their body movements to 22.16 from 196.50 (observed in the buffaloes which were not protected from *Culicoides* bite by using shed net) within 10 minutes. Further they also recorded that due to such reduction in body movements; milk yield was improved by 18.97 per cent. Their observations corresponds with the observations obtained in the present study. Likewise, Brewer *et al.* (2021) reported improved health parameters as results of interventions to keep the sheds free of dipteran flies and also iterated the need to evaluate impact on weight gain and milk yield of these interventions. A 2-3 fold increase in milk yield was reported in a success story published by FAO (2013) on "Netting flies and mosquitoes protects livestock, boosts milk yields in Rome," by using insecticide-impregnated nets. This particular success story incidentally supports the findings of the present investigation. Due to use of nets, it was reported that biting insect population dropped substantially, which resulted in reduced annoyance and worries in the form of curtailment of body movements and saving of energy and ultimately resulted in increasing the quantum of milk yield. Therefore present study also suggests that control of these midges is a mandatory step to obtain optimum milk yield.

Summarily the results of present study may be interpreted considering certain limitations which include that these results are from field animals and no controlled trial was attempted in the current study which might have revealed a little altered observations. Yet the current observations realised the need for such controlled study by including various management groups as well as minimising the effect of other insect pests and physiological variations of animal subjects.

CONCLUSION

Combining together the observations of present study along with earlier reports it is concluded that there is defined and strong correlation between *Culicoides* midge population, body movements and resultant average milk yield in cattle and buffaloes which is dependent on and varies according to environmental parameters. Secondly to achieve the optimum milk yield, it is recommended to use some type of protection to the milking animals, either in the form of control of *Culicoides* midges by following IPM tactics or use of fly proof net shed.

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