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Research Article
Field Trial using Combined Treatment of Garlic and Organic Spray Formula for Flie's Control and Animal Defensive Behavior Alleviation in Cattle Farm

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Abstract

Background and Objective: Resistance of flies to conventional insecticides used led to renewed interest in organic pesticides as alternative management tools for flie's control. This study aimed to determine the flie's activity in cattle environment and evaluate the repellent efficacy of combined treatment of garlic based formula, pour-on and organic spray on the suppression of fly population on animals and in their environment respectively. **Materials and Methods:** A cross-sectional study was carried out on cattle farm during the period from February-May, 2015. The daily average of microclimatic factors was measured. Monitoring of flie's count pre and post-treatment was done using (visual observation and photographic tool) for animals and (sticky cards) in their environment. The effectiveness of tested formulae was determined by calculating the percentage reduction in flie's attack rate and animal's defensive behavior. **Results:** The highest population of flie's activity on both dairy cows and their environment was recorded in April and May months (260.0±5.28, 253.0±4.30, 457.0±7.14 and 485.0±7.32) respectively. Calves barn, stall corner and animal stall appeared as predilection sites for flie's activity (503.33±7.4, 473.0±5.3 and 383.66±4.81, respectively). The percentage reduction in average flie's count was significantly different at $p<0.05$ on both calves, beef cattle and their environment (31.1, 42.6, 43.2 and 47.9%, respectively). Animal's defensive behavior decreased post-treatment especially for tail flicks (26.3, 23.5, 11.1 and 11.6%, respectively) and skin twitching (81.6, 72.5, 90.8 and 65.1%). **Conclusion:** A combined treatment of garlic based formula pour-on animals with organic spray on their environment was effective at time interval once/week in knocking down flie's population and consequently reduced its impact on the animal health and alleviated animal's defensive behavior.

Key words: Environment, flies control, garlic as repellent, defensive behavior, livestock animals

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Flies are a serious pest in animal production. They cause financial loss by affecting the health and comfort of animals and reduce feed intake impacting weight gains, milk production, milk fat content[1,2] and its presence gives a bad impression to visitors and it is also a notorious vector that can transmit highly contagious diseases to both animals and humans[3], by many deadly antibiotic-resistant zoonotic pathogens[4,5]. No doubt that, the availability of vast quantities of manure, animal units provide a favorable environment for the breeding site and settling of different types of fly such as, the common housefly (*Musca domestica*), stable flies (*Stomoxis calcitrans*) and other flies that bite or cause annoyance to cattle and thus can impair growth rates or milk production resulting in reduced feed conversion efficiency which may be due to increased energy demands caused by fly attack[6].

Flie's monitoring is sophisticated as counting fly specks on paper placed throughout a barn, observing animal housing areas and the environment for the presence of adult flies. Cow's environment should be monitored including calf housing, stall corner, accumulated wet bedding in pens, lagoons, feed storage areas, water trough and animal's carcasses before and/every 2 weeks during fly season begins[7].

Behavior is generally the animal's "First line of defense" in response to environmental change. Observations of animal behavior can provide us with a great information about animal's requirements[8]. Understanding how pest flies influence the behavior of cattle can help in clarifying the importance of this issue in cow welfare and comfort and potentially to animal productivity. Cattle defensive behavior can range from tail flicking, leg stamping, head throwing and skin twitching to evasive displacement and bunching behavior in an effort to lessen parasite attack intensity[9,10].

The global problem of fly resistance to conventional insecticides has resulted in renewed interest in organic pesticides as alternative management tools for flie's control[11]. The key to fly control is management whereas the manure is handled in a way to minimize fly breeding and fly populations should be managed for prevention[12]. Control strategies of the pest which depend on insecticide for fly control are decreasing due to increased insecticide resistance and environmental constraints[13]. While, organic pesticides can be home made, they are less expensive, easy to use and harmfulness[14]; it can be used in the application of integrated pest management program (IPM) for flie's control[15]. Garlic as organic pesticides has insecticidal properties and is a key ingredient in many organic pest control sprays[16]. To improve the efficacy of the organic pesticides, it can be added to water, paraffin and liquid soap. Whereas, liquid soap acts as a surfactant without which the spray will drain from the surface, paraffin acts as a solvent[17]. In scientific studies, garlic has successfully destroyed mosquito larvae and certain species of ticks and has repelled mosquitoes, flies and fleas[18], however, their application in cattle farms was not scientifically evaluated. The objective of this study was aimed to determine flies activity during study period followed by field trial to evaluate the repellent efficacy of a combined treatment of garlic based formula, pour-on and organic spray on animals and in their environment respectively for flie's control and animal's defensive behavior alleviation.

MATERIALS AND METHODS

Study location and period: This study was conducted on a cattle farm in Beni-Suef province (coordinates: 29°04'N 31°05'E), Egypt during the period from February-May, 2015. The study area is characterized by two distinctive seasons: Summer (May-October) and winter (November-February). The climate is dry with low rainfalls during winter and sometimes study area is exposed to some hot blowing dust-laden wind during the period between March and June, which known as the khamasine depressions[19,20].

Study animals: A total of 200 livestock animals [Friesian cows (n = 121), beef cattle (n = 55) and calves (n = 24)] were housed separately in 14 partially sheltered yards on an earthy floor at stocking rate 9.0 m^2 cow^{-1} and 3.0 m^2 $calf^{-1}$. The straw bedding beneath animals was removed once/three months. The yards were provided with water troughs and fixed manager of concrete cement. There is no control program for flies adopted except spraying of animals at the beginning of summer season with deltamethrin at a concentration of (0.1%) on animals and (1%) for their environment once a time. Flies have a role in the occurrence of some health problems in the farm where several cases of myiasis and mastitis were reported during the study.

Study design: A cross-sectional study was conducted on a cattle farm to determine flies activity during a study period from February-May, 2015 followed by field trial to evaluate the repellent efficacy of combined treatment of garlic based formula, pour-on and organic spray on the suppress of a fly population on both animals and in their environment respectively. Daily averages of microclimatic factors (ambient temperature (°C), relative humidity (%) and

air speed (knots h^{-1}) were recorded throughout the study period. Monitoring of flie's count pre and post treatment was done using two means (visual observations and photographic tool) whereas 6 similar sites were selected on the animals body (the neck, shoulder, backline, abdomen, limbs and tail), which are particularly attractive to flies. Meanwhile, in the environment, flies were collected by using sticky cards. The effectiveness of tested formulae was determined by calculating the percentage reduction in flies attack rate in addition to fly evasive (defensive) behavior.

Measuring of microclimatic factors: The daily average (±SE) of ambient temperature (°C) and relative humidity (RH%) in the study area were measured diurnal and nocturnal by using clock thermo-hygrometer [Model 302, measuring range -20 to 50°C] and (20-90%) meanwhile digital anemometer (VanE probe microprocessor digital meter) n 233569, accuracy +2.0%+1.0 d, resolution 0% was used for measuring of air speed.

Monitoring of flie's count: The average numbers of flies on both animals and in their environment were monitored. In the environment, the flies were collected by using sticky cards, where 28 cards were distributed at equal distances throughout the farm as the method described by Jacobs *et al.*[21]. A thick sticky transparent substance was used as adhesive glue which was obtained from Insects Research Institute, Dokki, Egypt and it consisted mainly of starch and water. The sticky cards were transferred directly to a laboratory and the numbers of flies on each card were counted. Meanwhile, the average numbers of flies on livestock animals were monitored three times per week by two means (visual observations and photographic tool):

- **Visual observation:** The average numbers of flies were determined at 6 similar sites on the animals body (the neck, shoulder, backline, abdomen, limbs and tail) during the daylight (9.0 am-4.0 pm) by visual observation as a method described by Lysyk[22] and Gerry *et al.*[23]
- **Photographic tool:** Flies were counted by using a digital camera as method described by Fraga *et al.*[24] where the average flie's number was assessed on the same different body parts of animals mentioned in visual observation by taking 3-5 photographs/site in each time

Evaluation of the repellent efficacy of tested formulae

Animals used and management: Thirty days field trial was conducted in the investigated farm during May, 2015. A total of 48 healthy cows of different breeding purposes (beef cattle and calves) were selected and assigned to two equal groups (n = 24). Each group was kept separately in a partially sheltered yard with a concrete floor provided with drinking water buckets and feeding manager. The average numbers of flies were counted 15 days before trial as control next after application of garlic based formula, pour-on animals and organic spray formula in their environment in the next 15 days followed by counting the flies at specific intervals in the environment and calculated per animals.

Tested formulae: Two natural formulae were tested. Garlic based formula, pour on treated animal and organic spray formula were applied in surrounding environment.

Garlic based formula: About 500 g of chopped garlic clove was soaked overnight with 200 mL paraffin oil and strained. Two liters of vinegar were added to the mixture then stored in a tightly closed glass bottle. About 100 mL liquid soap was added to the mixture immediately before using the method described by Stephen[25]. The mixture diluted with 72 L water for using in pour-on of animals.

Organic spray formula: It was prepared according to Stephen[25] as one part of vinegar was added to 4 parts of water then 100 mL of liquid soap was added to the mixture immediately before spraying.

Method of application: The repellent efficacy of two natural formulae was assessed by calculating the percentage reduction in the flies attack rate and animal's defensive behavior. The basic step in flie's control is improving hygienic measures in cattle farm. All manure was collected from animal stalls, paddocks and yards every day. Routinely wet cleaning or pressure wash of wall, floor and corner using warm water before using organic spray formula. Six similar sites on the animals body were selected (i.e., the neck, shoulder, backline, abdomen, limbs and tail), which are particularly attractive to flies and targeted for the application of garlic based formula. The animals were washed with cleaned water then garlic was applied on the back line and different sites of animal's body, pour-on (2 L $animal^{-1}$) twice at the beginning of a study and next at 7th day of the study. Three sticky cards/group were held in a defined place as previously described. A visual assessment of the initial reduction of fly population was carried out during the 1st hours after application by counting flies on animal's body and their environment for 2 weeks post-treatment, whereas observations were made during

5-7 h day^{-1} just post treatment. To assess the persistent efficiency of tested formulae, monitoring of flie's count was conducted three times per day throughout the study period. The flies attack rate to animals was counted at intervals of 30 min over 4 h period. Flies in their environment and at both sides of the animal were counted from a distance of 1 m away from the animals, a proper distance for not disturbing flies that came to attack the animals. Defensive behavior was also used as a tool in assessing the repellent efficiency. Whereas, it was focally recorded by direct observation from outside the yard by using a digital camera without animal disturbance[26] prior and post-trial for about an hour for each animal twice per day, one morning and one afternoon for two successive days. Four replicates were subjected to observation, each replicate contains three animals. The defensive behavior patterns [Tail flicking, skin twitching, leg stamping (Strikes, kicking), ear flicks and head throwing] were recorded according to Dougherty *et al.*[27] and Ralley *et al.*[9] (Supplementary Table S1):

$$\text{Repellency Index (RI)} = \frac{(\text{Nc-Nt})}{\text{Nc}} \times 100$$

where, Nc is the number of flies infesting cattle's (in the pre-treatment day) and Nt is a number of flies in the treatment.

Statistical analysis of data: The data were recorded on Microsoft Excel spreadsheet then prepared for analysis. The mean values of flie's attack/animals in relation to their environment and evaluation of the efficacy of repellent formulae for flie's control were calculated by use of descriptive statistics, the non-parametric test (Chi-square test and Friedman test). Meanwhile, the behavioral data were analyzed by (ANOVA) and using Tukey's test. Heteroscedastic data were compared by a non-parametric statistical method Wilcoxon (two related samples) using statistical package for social sciences SPSS (v.20.0).

Table S1: Defensive behavioral patterns measured in livestock animals

Behavioral patterns	Description
Tail flicking	The movement of the tail to the animal's side or back
Skin twitching	An involuntary reaction to irritation on the animal's skin
Leg stamping (Strikes, kicking)	The movement of the leg in an effort to dislodge biting flies and is a movement not related with locomotion
Ear flicks	The movement of ears in an attempt to dislodge the flies
Head throwing	The movement of the head towards the body directed towards either the sides or front legs

RESULTS

Role of climatologic factors in enhancement of flie's activity: The daily averages of microclimatic factors (temperature (°C), relative humidity (%) and airspeed (knots h^{-1}) were significantly different among months of study period where the highest averages of ambient temperature (°C) were recorded during May (26.8±0.62°C) and April month (20.7±1.4°C), while February and March months had the lowest average temperatures (13.7±0.73 and 16.8±0.51°C, respectively). Moreover, there was a significant fluctuation in the average of relative humidity (%) as months of February and April recorded the highest values (63.9±1.86 and 63.8±2.13%, respectively) while the lowest averages were observed in March and May (59.9±1.41 and 49.1±1.38%, respectively). Regarding the average values of airspeed (knot h^{-1}) measured during study months, April recorded the highest airspeed rate (5.4±2.0) followed by March and February (4.6±1.6 and 4.4±0.10, respectively) (Fig. 1).

Flie's count in cow's environment, the rate and distribution of flie's attack to animals: The mean values (±SE) of flie's count in cow's environment/24 h during study period (Table 1) revealed gradual increase in the flie's activity in cow's environment in February (343.66±6.87) and March (325.0±5.73) followed by a significant increase ($p<0.01$) during months of April and May (457.0±7.14 and 485.0±7.32, respectively). Moreover, the total average of fly's activity in cow's environment was (400.42±6.2) during study period.

Such results in Fig. 2 shows the total averages of flies count in different housing compartments. It was observed that higher daily average of flie's number in cow's surroundings at $\chi^2 = 12.0$, ($p \leq 0.01$). Moreover, calves barn followed by stall corner and animal stall appeared as predilection sites for flies that attract the highest flie's number (503.33±7.4, 473.0±5.3 and 383.66±4.81, respectively) followed by water trough and gutter (286.33±3.1 and 175.0±2.53, respectively).

Table 1: Mean values (±SE) of flie's count in cow's environment/24 h during study period (120 days)

Month	Flie's count (Mean±SE)/24 h
February	343.66±6.87^{ab}
March	325.00±5.73^{ab}
April	457.00±7.14^{b}
May	485.00±7.32^{a}
Total	400.42±6.20
p-value	<0.01

^{a,b}Within the same row, proportions with different superscript letters differ significantly at $p<0.01$

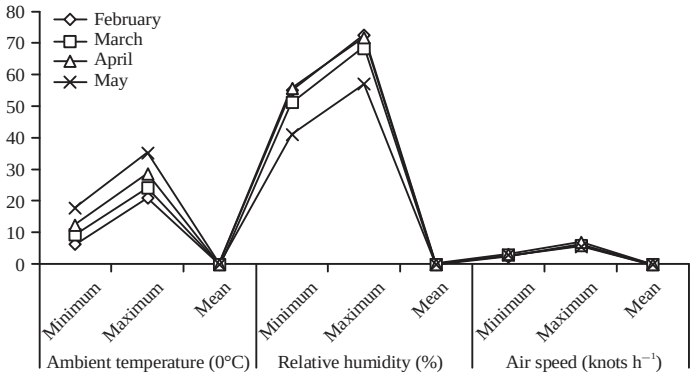

Fig. 1: Mean values of microclimatic factors in examined farm throughout study period

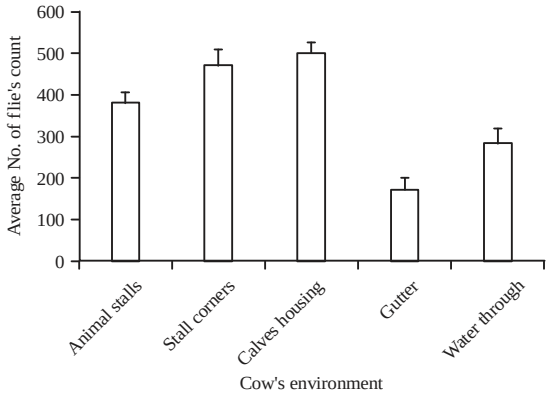

Fig. 2: Total average of flie's count (±SD) in the animal's environment throughout study period

Table 2: Mean values (±SE) of flie's count on animals/24 h during study period

Cows (Months)	Dairy	Calves	Beef
February	119.67±3.23	124.33±6.25	113.33±6.48
March	171.00±5.50[a]	147.00±3.71[b]	144.67±6.56[b]
April	260.00±5.28[a]	248.00±6.32[b]	158.67±4.73[ab]
May	253.00±4.30[a]	265.33±7.65[a]	221.38±7.38[b]
Total	215.92±10.73[a]	196.16±7.84[b]	160.50±9.56[ab]
p-value	<0.01		

[a,b]Within the same row, proportions with different superscript letters differ significantly at p<0.001

The rate and distribution of flie's attack to animal's body were detected by using both visual observation and photography in Table 2. It can be recorded that the highest rates of flie's attack occurred in dairy cattle (215.92±10.73) followed by calves and beef cattle (196.16±7.84 and 160.50±9.56, respectively) at p≤0.01. The same table clarified the distribution of flies in relation to months of study which indicated that the highest flies attack rate for resident calves and dairy cattle occurred during May month (265.33±7.65 and 253.00±4.30, respectively). However, the lowest rate of flies attack to animals (calves, dairy cattle and beef cattle) recorded during February month was (124.33±6.25, 119.67±3.23 and 113.33±6.48, respectively).

Table 3: Mean values (±SE) of flie's attack/animals in relation to flie's number in their environment prior field trial (15 days)

Flie's count (Mean±SE)	Animals		Environment	
days prior trial	Calves	Beef cattle	Calves	Beef cattle
1st	275.00±2.56[a]	220.0±5.20[b]	203.67±6.11	183.00±2.080
3rd	241.67±4.89	232.67±4.23	171.36±4.06[b]	207.67±6.83[a]
5th	251.23±5.31[a]	200.57±3.61[b]	289.67±5.85[a]	200.67±4.27[b]
7th	312.00±3.78	239.00±4.43	326.33±6.24[a]	262.33±6.25[b]
9th	304.00±4.78[a]	264.00±3.67[b]	381.67±6.47[a]	340.67±7.60[b]
11th	311.33±3.51[a]	235.34±4.35[b]	362.34±5.71[a]	321.67±4.87[b]
13th	303.67±5.92	341.00±5.33	438.01±6.81[a]	326.67±8.95[b]
15th	286.63±9.25[b]	328.33±8.71[a]	401.22±7.53	372.46±10.28
Total	265.33±7.65	221.38±7.38	307.71±9.08	276.52±9.760
p-value	<0.05		<0.05	

[a,b]Within the same row, proportions with different superscript letters [t]differ significantly at p<0.05

Flie's attack rate/animals in relation to flie's number in their environment pre and post-field trial: Referring to the mean values (±SE) of flie's attack/animals in relation to flie's count in their environment pre-field trial (Table 3) showed that the total mean values of flie's attack rate to calves body were higher than beef cattle (265.33±7.65 and 221.38±7.38 respectively). Moreover, the total mean values of flie's count in calve's environment were higher (307.71±9.08) compared to beef cattle (276.52±9.76).

On the other hand, post-field trial, the mean values (±SE) of flie's attack/animals in relation to flie's count in their environment (Table 4) proved that there was a significant decrease at (p<0.01) in the mean values of flie's count on calve's body (182.71±8.34) compared to prior trial (265.33±7.65). Referring to comparing the mean values of flie's count in their surrounding environment pre and

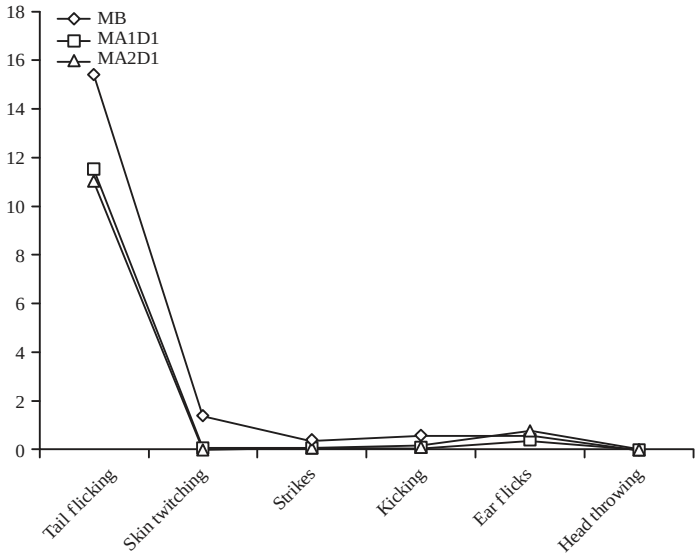

Fig. 3: Mean values of animal's defensive behavior during morning hours throughout study period

Table 4: Mean values (±SE) of flie's attack/animals in relation to flie's number in their environment post field trial (15 days)

	Animals				Environment			
	Calves		Beef cattle		Calves		Beef cattle	
Flie's count days in trial	Post-trial	Repellency (%)	Post-trial	Repellency (%)	Post-trial	Repellency (%)	Post-trial	Repellency (%)
1st	180.00±3.80	34.5[a]	131.67±3.11	40.1[b]	104.33±3.38	48.8[a]	102.67±4.31	43.9[b]
3rd	171.00±5.94	29.2[b]	140.34±4.82	39.7[a]	110.67±7.96	35.4[b]	111.33±5.06	46.4[a]
5th	157.34±4.09	37.4	128.00±2.42	36.2	163.67±4.09	43.5	101.23±6.49	49.5
7th	207.67±3.41	33.4[b]	140.36±3.53	41.4[b]	195.33±3.75	40.0	137.33±7.85	47.7
9th	216.33±2.90	28.8[b]	154.35±4.81	41.5[a]	193.23±4.59	32.9	184.34±5.31	45.8
11th	222.00±4.42	28.7[b]	136.67±5.14	41.9[a]	245.67±3.75	32.1[b]	176.67±6.27	45.1[a]
13th	201.65±6.39	33.6[b]	173.67±4.37	47.2[a]	269.33±3.46	38.6[b]	170.00±9.01	47.8[a]
15th	191.42±10.12	33.2[b]	181.31±9.46	44.8[a]	253.24±6.51	36.9[b]	201.32±8.91	45.9[a]
Total	182.71±8.34	31.1[b]	127.00±5.26	42.6[a]	174.61±5.06	43.2[b]	143.95±7.87	47.9[a]
p-value	<0.05							

[a,b]Within the same row, proportions with different superscript letters differ significantly at p<0.05

post-field trial revealed a significant decrease in flie's number at the end of study period at p<0.05 (174.61±5.06) as compared to prior trial (307.71±9.08). The percentage reduction in flie's number on animal's body and their surrounding environment were (31.1 and 43.2%, respectively). On the other hand, the mean values (±SE) of flie's count on beef cattle body and in their surrounding environment showed a significant decrease in flie's count at (p<0.05) on both animals body and in their surrounding environment were (127.0±5.26 and 143.95±7.87, respectively) as compared to before trial (221.38±7.38 and 276.52±9.76, respectively). Moreover, the percentage reduction in flie's number on animal's body and their surrounding environment were (42.6 and 47.9%, respectively).

Animal's defensive behavior pre and post-field trial: The animal's defensive behavior pre and post-treatments at morning was revealed a non-significant decrease in tail flicking, skin twitches, strikes and kicking post-treatments at morning hours, only ear flicks were decreased post first application but they increased after the second application, also head throwing decreased only post first application (Fig. 3).

The defensive behavior at afternoon hours showed a non-significant decrease in tail flicking, skin twitching and kicking post first application. Also, there was a non-significant increase in total tail flicking and ear flicks post second application (Fig. 4).

The animal's defensive behavior frequency per minute pre and post-trial (Table 5) showed a reduced total tail flicks post

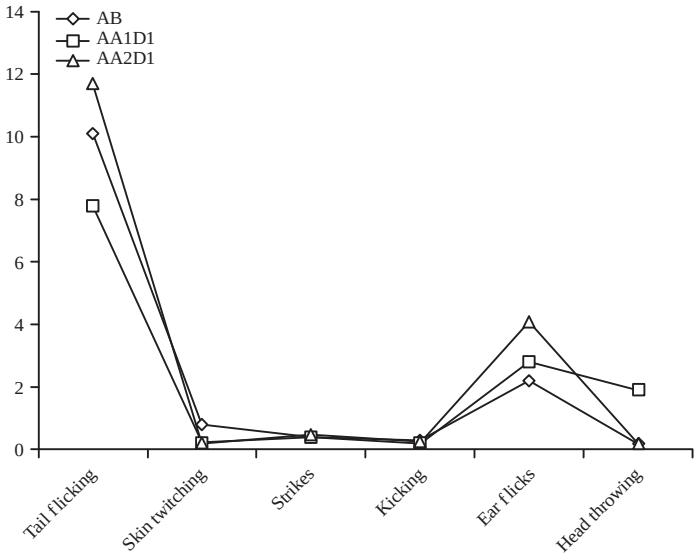

Fig. 4: Mean values of animal's defensive behavior during afternoon hours throughout study period

Table 5: Mean values (±SE) of animal's defensive behavior prior and post field trial

Defensive behavior	Before	Day 1 (1st week)	Day 2 (1st week)	Day 1 (2nd week)	Day 2 (2nd week)
Tail flicking	12.8±2.1	9.4±1.5	9.8±1.8	11.3±2.1	11.3±1.9
Skin twitching	1.1±0.5	0.2±0.1	0.3±0.1	0.1±0.0	0.4±0.2
Strikes	0.4±0.1	0.3±0.1	0.3±0.1	0.3±0.2	0.4±0.3
Kicking	0.5±0.1	0.2±0.0	0.4±0.1	0.2±0.0	0.3±0.1
Ear flicks	1.4±0.4	1.8±1.0	2.4±1.1	2.5±1.0	3.9±1.7
Head throwing	0.1±0.0	1.1±1.0	0.1±0.0	0.1±0.1	0.2±0.0

treatment by garlic based formula by 26.3% at the 1st day of treatment, 23.5% at the 2nd day of treatment, 11.1% after the second application and 11.6% at 2nd day post-second application. Also, skin twitching fly evasive element was reduced by 81.6, 72.5, 90.8 and 65.1% at the observation days respectively. Strikes and kicking also reduced by (28.9, 31.6, 23.7 and 5.3%) and (63.0, 21.7, 60.9 and 26.1%) for strikes and kicking at the observation days, respectively. There was a non-significant increase in ear flicks.

DISCUSSION

Fly prevention and control on animal production units are necessary to prevent the transmission of pathogens that could affect animal and human health and the maintenance of good hygiene. The role of climatologic factors in enhancement of flies activity in Fig. 1 showed the highest averages of flie's number in cow's environment were recorded during April and May months that had averages of ambient temperature, relative humidity and air speed which provided optimal conditions for flies survival multiplication and spreading through air movement such finding coincides with that obtained by Cruz-Vazquez *et al.*[28] who found that relative humidity had an important influence on stable fly's population (first peak) and temperature had a definitive effect on decreasing phase of its population. Moreover, Kaufmann *et al.*[1] reported that in warm humid conditions (>29°C) the life cycle of pest flies may takes 9-10 days compared with 21-28 days when it is cooler (21°C). An adult female can lay 100 or more eggs every 4 days for up to 3 weeks and generally up to 500 eggs in a life time. Eggs hatch within hours if conditions are right (70% moisture, high temperature). WHO[29] reported that fly densities are highest at mean temperatures of 20-25°C; they decrease at temperatures above and below this range. On the contrary, Jin and Jaal[30] noticed that flie's abundance was inversely proportional to relative humidity and total rainfall.

The highest daily mean values of flies count in animals environment was revealed in May followed by April month to reach its minimal value during February. It was noticed that the flaring up of flie's number and its activity were significantly increase in summer months and influenced by the type and hygienic status of the animal house, such finding came in agreement with that obtained by Ngeon-Klan *et al.*[31]

who found that flie's population reached their peak activities in summer months. Such results could be attributed to the hygienic practices in housing system and high stocking rate, faulty drainage system, improper ventilation beside the lack of awareness about risks of flies or their control in this farm. These results were in agreement with Day[32] who found that sanitation should be the first line of defense against house flies and other filth-breeding fly species. Under optimum conditions, house flies can complete their life cycle (egg to adult) in as few as 9 days. By adhering to a strict manure management program throughout the period of greatest fly activity (i.e., the spring and summer months) it is possible to disrupt the life cycles of these pests. Mohammed *et al.*[33] observed that the highest rate of flie's attack on dairy cattle and calves was reflecting the hygienic conditions of their environment.

From the results obtained in Fig. 2 it has been found that manure heap, stall corners, drainage water, calf barn and feed store represent potential sources for flie's breeding and dispersal in livestock farms followed by spilled feed, animals stall and water trough . The current results are parallel to that achieved by Adams[12] who noticed that the predilection sites for house flie's breeding were a calving area, drainage, storage area and generally any place where food was spilled or manure accumulation was allowed to become moist.

The highest rate of flie's attack in examined farm may reflect the hygienic condition in this farm because animal's environment may act as a sustainable reservoir for flie's survival and spread in cattle farm. This result was in agreement with Mohamed[34] who showed that the beef cattle followed by dairy cows were at higher risk of flie's attack particularly those kept under poor housing condition (208.7±44 and 181±41.6, respectively).

The fly population should be managed for prevention and control, prevention includes sanitation, habitat destruction and good house design. Sanitation is at least (75%) of a fly control program. No insecticide can be effective against flies as long as breeding sites exist[12]. Study results in Table 4 revealed that improvement of animal's environment with regular application of organic vinegar spray and using garlic based formula makes cows unattractive to flies and mosquitoes so that it will avoid areas of the body that have been treated with this formulas and achieving percentage reduction in flie's count (reached to 47.9%) in surrounding environment and (42.6%) on body of beef cattle. Moreover, repellents don't kill flies and mosquitoes but the best repellents will provide protection from bites for a long period of time (>5 h) with a single application. Whereas other authors Prowse *et al.*[35] studied exposure of the two target dipteran pests, *Delia radicum* (L.) and *Musca domestica* L., to different concentrations of garlic juice revealed variability in insecticidal effect across life stages. The LC_{50} values recorded for *Musca domestica* were: eggs (7 day exposure) 1.6%; larvae (24 h exposure) 10.1%; larvae (24 h exposure) 4.5% and adults (24 h exposure) 2.2%. Findings in this study were in agreement with Edwin *et al.*[36] who pointed to help limit fly problems. It is imperative to improve environmental sanitation and hygiene which will then eliminate the conditions which are favorable to fly breeding. Solid concrete floors with drains should be constructed; dung should be cleaned out. Moreover, flies soil the inside and outside houses with their feces. They can also have a negative psychological impact because their presence is considered a sign of unhygienic conditions[29].

Fly-repelling responses such as tail flicking, foot stamping, skin twitching, ear flicks and head throwing are attempts to dislodge the flies and the frequency of these activities increases as fly densities increase. Tail flicking is effective at removing fly located on the animal's sides, back and rear legs[9]. Multiple studies have found tail flicking to be the most frequent biting fly avoidance response in comparison to other common behaviors such as ear flicking, skin twitching and leg stamping[9,10,37,38]. The results showed decreased tail flicking and skin twitching frequency in treated cattle than prior treatment, such result was supported by the flies count reduction on animal's body that presented in Table 4. Moreover, Woolley[38] recorded that total tail flicks, partial tail flicks and full tail flicks were reduced by (44.8, 38.7 and 51.4%, respectively) in cows treated with the fly repellent and skin twitches were the second most abundant fly avoidance behavior observed. Treated cows had a (54.0%) reduction in skin twitches, relative to untreated cows. Head throws, leg stamps and side licks were all reduced on treated cows by (78.9, 45.1 and 33.3%) respectively, in comparison to untreated cows.

Ear flicks and head throwing findings might be at least partly due to having a number of "Out of sight" scans per animal especially during the cattle eating from the manager so they have been recorded. Also, the head had no such attention in pouring the garlic juice as the other body parts, so we cannot depend on their data in determining the spray efficacy. From the data presented in Table 1, it has been noted that the flie's population was high during 24 h of the study period and throughout the evaluation of the fly evasive behavior during morning and afternoon hours during the observation days in Fig. 3, 4, it has been found that a significant decrease in main fly evasive responses during morning and afternoon hours meanwhile, there was non-significant increase in the total tail flicking afternoon in

the second application which may be attributed to the increase in fly population in the surrounding environment at these days in comparison with the previous days of application which was supported by the data presented in Table 3.

CONCLUSION

A combined treatment of garlic based formula pour-on animals with organic spray on their environment are effective at time interval once/week in knocking down flie's population and consequently reduced its impact on the animal health and alleviated animal's defensive behavior. Tested formulae safety need to be fully evaluated, but there were no abnormal health observations or skin irritations related to treatment observed in the treated animals.

SIGNIFICANCE STATEMENTS

- Monitoring of fly population on livestock animals and in their environment
- Field trial to evaluate the repellent efficacy of garlic based formula, pour-on and organic spray formula on the suppression of fly population on animals and in their environment
- Defensive behavior was used as a tool in assessing the repellent efficiency
- The effectiveness of tested formulae was determined by calculating the percentage reduction in fly attack rate in addition to measuring animal's defensive behavior

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