Cattle flies are external, blood-sucking parasites. Their control can be done by using repellents from plant extracts. The objectives of the research were, to determine the number of flies that infest cattle and to evaluate how long the aqueous extract of leaves and seeds of *M. oleifera* took to make effect. Through the study, fly counts on the wither or hump region were taken between 8:00-9.00 am and 5:00-6:00 pm. To obtain the aqueous extract, 2.5 kg of leaf and seed powder were used, diluted in 20 L of distilled water. The solution was left to stand for 24 h in a dark room. With the liquid obtained, four clinically healthy bovines, two females of the Gray Brahman and Suizbu breeds and two bulls of the Guzerat and Red Brahman breeds, were sprayed until the aqueous extract runoff. Once the treatment was applied, the fly count was performed on the surface of the animals. During the three days of the study, a total of 1,256 flies were counted in the four animals, with an average of 608 flies on days 1 and 3 of the experiment, while on day 2 it decreased to 52 flies, with an average of 10 flies in the morning, and 42 in the afternoon. The spraying with the extract of leaves and seeds prevented the flies from landing on the animals; however, a long-lasting repellent effect was not observed.

**ABSTRACT**

Cattle flies are external, blood-sucking parasites. Their control can be done by using repellents from plant extracts. The objectives of the research were, to determine the number of flies that infest cattle and to evaluate how long the aqueous extract of leaves and seeds of *M. oleifera* took to make effect. Through the study, fly counts on the wither or hump region were taken between 8:00-9:00 am and 5:00-6:00 pm. To obtain the aqueous extract, 2.5 kg of leaf and seed powder were used, diluted in 20 L of distilled water. The solution was left to stand for 24 h in a dark room. With the liquid obtained, four clinically healthy bovines, two females of the Gray Brahman and Suizbu breeds and two bulls of the Guzerat and Red Brahman breeds, were sprayed until the aqueous extract runoff. Once the treatment was applied, the fly count was performed on the surface of the animals. During the three days of the study, a total of 1,256 flies were counted in the four animals, with an average of 608 flies on days 1 and 3 of the experiment, while on day 2 it decreased to 52 flies, with an average of 10 flies in the morning, and 42 in the afternoon. The spraying with the extract of leaves and seeds prevented the flies from landing on the animals; however, a long-lasting repellent effect was not observed.

**CONTRIBUTION/ORIGINALITY:** This study shows the potential of aqueous *M. oleifera* leaves and seeds extract as an alternative to the chemical management for the control of cattle flies in southwestern Mexico.

**1. INTRODUCTION**

Vector control is the primary means of combating these medically important ectoparasites. However, the problems associated with the continued use of synthetic insecticides and the increased concern for the protection of the environment necessitate a reduction in the use of synthetic products and the search for insecticides that are ecologically sound and safe for the environment [1]. In this way, plant products can be used as natural insect repellents and biopesticides to repel or kill arthropods that feed on blood [2].

The Moringa tree (*Moringa oleifera* Lam.) is native to the Indian subcontinent, grows well in the humid tropics, as well as hot, dry lands with poor soil fertility, and is adapted to drought [3]. It is a very useful tree since almost
all its parts can be used for industrial, food, and medicinal purposes; even its leaves, flowers, and fresh pods are used as feed for livestock [3]. On this last point, it is important to highlight that according to pharmacognostic and toxicological studies in animals [4, 5] and there were no toxic signs or death of the individuals during the experimentation.

In recent times, more attention has been paid to studies of natural pesticides in pest and vector control [1]. Repellents play an important role in protection against bites, an effective repellent must reduce the contact of the vector with the potential host to interrupt the transmission of diseases; in addition, it must not be toxic or irritating and must have a long-lasting effect [6]. Some plant products have been used as repellents against gastrointestinal nematodes [7] mosquitoes [8] ticks [9] and flies [10]. Therefore, this research focuses on the repellent effect of the aqueous extract of leaves and seeds of M. oleifera against flies on cattle.

2. MATERIALS AND METHODS

2.1. Study Location

The present study was carried out at the El Lucero ranch, located in the town of Colonia Miguel Alemán, municipality of Cuajinicuilapa, Guerrero, México. Located at coordinates 16°33'00'' north latitude and -98°31'54'' west longitude at 17 meters above sea level. The climate is classified as Aw and is called dry sub-humid tropics. Rainfall has monsoon-like characteristics due to the invasion of hot and humid air masses from the sea, with a rainfall range of 1100 to 1300 mm [11].

2.2. Collection and Preparation of the Material

Leaves and seeds of M. oleifera were used. The samples were collected manually directly from the tree, which is in the municipality of Cuajinicuilapa, Guerrero. The samples were transported in a plastic container to the Nutrition Laboratory of the Faculty of Veterinary Medicine and Zootechnics No. 2 of the Autonomous University of Guerrero. The seeds were removed from the pods and the leaves from the branches, washed with distilled water, and allowed to dry. Subsequently, each sample was weighed with a precision scale (Rhino Bapre-3). The seeds were stored in a sack; while the leaves were weighed in quantities of 1 Kg and placed in brown paper bags of 15.5 x 38.5 cm until used.

Subsequently, the seeds were dried by exposure to sunlight for two days, from 10:00 a.m. to 4:00 p.m. After that time, they were placed in a plastic basket, with a capacity of 10 kg. Subsequently, they were ground with a manual mill (Rel-Rey-2095). Once the sample was ground, it was stored in brown paper bags of 15.5 x 38.5 cm with a capacity of 1 kg. The leaves were dried in an oven (Felisa-FE-293A) at a temperature of 32 ºC for 48 h, being manipulated twice a day, in the morning and the afternoon. After that time, the leaves were ground in a manual mill.

2.3. Animals and Fly Count

Animals management was done following the regulations welfare criteria [12, 13]. Briefly, four clinically healthy bovines were used; two females of the gray Brahman and Suizbu breeds (±375 kg) and two stallions of the Guzerat and red Brahman breeds (±600 kg). One week before the study, the interaction between flies and cattle was observed (8:00 a.m. to 6:00 p.m.), to determine the time of greatest presence of flies. Once the time of greatest dipteran activity is established (8:00 a.m. to 9:00 a.m. and 5:00 p.m. to 6:00 p.m.); 78 h before and 24 h after spraying the extract, photographs were taken with a digital camera (Sony, DSC-W800/BC 20.1 MP) of the withers or hump region. To facilitate the counting of the flies, photography and a grid template were used [14]; in this way, the total number of specimens perched on each animal was counted, before and after spraying Figure 1.
2.4. Extraction and Spraying Procedure

The aqueous extract technique was chosen, as it is a benign solvent [1]. For this, 2.5 kg of leaf and seed powder were weighed, placed in containers with a capacity of 20 L, and distilled water was added until they were full. It was left to rest in a dark room for 24 h, and once the rest time had elapsed, the liquid was filtered through a manual sprinkler. Subsequently, the animals were sprinkled with the total solution until runoff. Once the treatment was applied, the fly count was performed on the surface of the animal. The moment of the evaluation was during the hours of the greatest presence of flies in the environment.

2.5. Data Analysis

The results of the number of flies regarding the days before, during, and after the spraying of the extract in the animals are shown as descriptive statistics.

3. RESULTS

During the three days of the study, a total of 1,256 flies were counted, with an average of 608 flies on days 1 and 3 of the experiment, while on day 2 it decreased to 52 flies. On the day before spraying, the total number of flies was 807, while on the day after the total was 397. In the case of the gray Brahman, during the previous day up to 225 flies were counted, which decreased to only 4 flies and subsequently increased to 16. For its part, in the red Brahman, 125 flies were counted during the previous day, which decreased to only 3 flies and increased to 59 on the following day. Meanwhile, in the Guzerat the previous day 350 flies were observed, which decreased to 45 flies, and on the following day increased to 270. Finally, in the Suizbu 107 flies were counted during the previous day, while
the day 0 the presence of dipterans was not observed, which increased to 16 the following day. shows the behavior of the flies during the intervention.

**Figure 2.** Flies behavior during the repellent effect.

4. DISCUSSION

Regarding the effect of *M. oleifera* on the fly count, it is known that most plants contain chemical compounds that are used to prevent attacks by insects. These chemicals are grouped into nitrogenous compounds (primary alkaloids), terpenoids, phenols, proteinase inhibitors, and growth regulators, which gives the plant an important repellent characteristic. Although, as mentioned, the primary function of these compounds is a defense against insects, many of the aforementioned elements are also effective against other biting dipterans, including flies [15]. In this way, Bennett, et al. [16] found in their study on the chemical composition of *M. oleifera*, that this plant is rich in several substances such as glucosinolates, isothiocyanates, flavonoids, anthocyanins, proanthocyanidins, and cinnamates and it is proven that the aforementioned products exert a repellent effect [1, 2, 17, 18]. However, in the present study, it is not possible to establish what type of substance is the one that favors the repellency effect, therefore, it is suggested to carry out further studies to determine which compounds are found in the extract.

It is important to highlight that, according to toxicological studies, there have been no toxic signs or death of individuals during oral ingestion of *M. oleifera* in animal studies [4, 5] nor in humans [19]. Repellents are substances that act locally or at a distance, deterring an arthropod from flying, landing, or biting human or animal skin [20] reported that, the extract of the leaves of *M. oleifera* is a good repellent agent for the control of *Aedes aegypti*, however, efficacy depends on dose rates and exposure interval. In their studies, Ferreira, et al. [42]; Prabhu, et al. [6], and Ohia, et al. [8] demonstrated that the seed extract of *M. oleifera* has a repellent action against *Aedes aegypti, Anopheles gambariae*, and *Anopheles stephensi*, respectively. The phytochemicals derived from the extracts of *M. oleifera* are effective agents for the control of vector mosquitoes and therefore, they can be used for integrated pest management programs, such as fly control. There are studies in which the repellent effect of *M. oleifera* on flies has been evaluated; in this case, *Musca domestica*. For example, Emeribe, et al. [21] revealed that a moringa leaf extract is an excellent option as a repellent in fresh mango fruits (*Mangifera indica*). In the case of the study carried out by Nisar, et al. [22] the results showed that the botanical extracts tested have significant potential to alter biological
parameters, such as reducing reproductive potential in the house fly. Finally, it can be added that plant extracts that have properties for the control of housefly populations can be used in the different genera and species of flies that affect the livestock. There is evidence that hair color influences fly count. For example, in the Guzerat breed the color can be silver ash or steel ash, even almost black and the quarters, both front and rear, are always darker than the rest of the body [23]. In the racial patterns of the Brahman breed, two lines can be seen, the Red Brahman with uniform red hair in different shades, with the anterior and posterior third generally darker tending to black in males and in females the fur is lighter [24]. The White or Gray Brahman has predominantly white fur, especially in females; although the ash color can be found in the front part of the body, in the neck, and even in the hump ACCGC [25]. Sancho, et al. [26] mention that, unlike white-coated animals, dark-colored animals are more attracted to mosquitoes and flies. In the case of the horn fly, Dayton, et al. [27]; Steelman, et al. [28] mention that the load in cattle is different between breeds, and that this difference is associated with the characteristics of the hair, as well as the amount of sebum in the skin. In addition, Herrera and Arguedas [29] found that there is a high correlation between coat color and tick and fly infestation. Such is the case reported by Oliveira, et al. [30]; Silva, et al. [31] these authors observed higher counts of the dipterans in animals with dark or reddish coats than in animals with white coats. Regarding the genetic factors associated with resistance to ectoparasites, Gasparin, et al. [32] report that some chromosomes are involved in the case of the expression of resistance to ticks by bovines. In addition, Prayaga and Henshall [33] mention that there are heritability factors regarding resistance to ectoparasites. Therefore, the ability of cattle to transmit or inherit resistance to ectoparasite infestation to their offspring is variable [34].

5. CONCLUSION

The present investigation concluded that the spraying with the extract of leaves and seeds prevented the flies from landing on the animals. However, a long-lasting repellent effect was not observed; For this reason, it is necessary to apply the solution and evaluate it for more hours, in addition to implementing or adding elements that allow a greater permanence of the effect on the hair and skin of the animals. It is recommended to use ecological products to avoid the use of chemical products, carry out more studies using an adherent to improve the duration of the product on the hair and skin of animals; in addition, carry out studies in which mixtures with extracts of other plants are used.

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REFERENCES

[1] O. Chinenyenwa and A. Godson, "Bio-insecticidal efficacy of Moringa oleifera on the malaria vector, Anopheles and toxicity evaluation on fish behaviour," International Journal of Mosquito Research, vol. 4, pp. 85-92, 2017.

[2] C. Vongsombath, "Botanical repellents and pesticides traditionally used against haematophagous invertebrates in Lao PDR," Doctoral Dissertation, Acta Universitatis Upsaliensis, 2011.

[3] B. Moyo, P. J. Masika, A. Hugo, and V. Muchenje, "Nutritional characterization of Moringa (Moringa oleifera Lam.) leaves," African Journal of Biotechnology, vol. 10, pp. 12925-12933, 2011. Available at: https://doi.org/10.5897/ajb10.1599.

[4] P. M. Ferreira, A. F. Carvalho, D. F. Farias, N. G. Cariolano, V. M. Melo, M. G. Queiroz, and J. G. Machado-Neto, "Larvicidal activity of the water extract of Moringa oleifera seeds against Aedes aegypti and its toxicity upon laboratory animals," Proceedings of the Brazilian Academy of Sciences, vol. 81, pp. 207-216, 2009.
N. Guaycha-Pérez, C. Jaramillo-Jaramillo, S. Cuenca-Buele, J. Tocto-León, and I. Márquez-Hernández, "Preliminary pharmacognostic and toxicological studies of leaves, stem and root of moringa (moringa oleifera Lam.)," *Science Magazine UNEMI*, vol. 10, pp. 60-68, 2017.

K. Prabhu, K. Murugan, A. Nareshkumar, N. Ramasubramanian, and S. Bragadeeswaran, "Larvicidal and repellent potential of Moringa oleifera against malarial vector, Anopheles stephensi Liston (Insecta: Diptera: Culicidae)," *Asian Pacific Journal of Tropical Biomedicine*, vol. 1, pp. 124-129, 2011.Available at: https://doi.org/10.1016/s2221-1691(11)60009-9.

M. P. Abreu, J. A. García, Y. L. Leyva, Y. Roche, M. Molina, A. Sanavria, and A. H. Da Fonseca, "In vitro effect of aqueous extracts of Moringa oleifera and Gliricidia sepium on the development of exogenous phases of gastrointestinal strongyles of sheep," *Journal of Animal Health*, vol. 36, p. 28, 2014.

C. Ohia, G. Ana, and O. Bolaji, "Larvicidal activity of aqueous extract of Moringa oleifera Seeds on Anopheles gambiae and its effects on Poecilia reticulata," *Agrosearch*, vol. 13, pp. 176-185, 2013.Available at: https://doi.org/10.4314/agrosh.v13i3.18.

J. Rosado-Aguilar, K. Arjona-Cambranes, J. Torres-Acosta, R. Rodríguez-Vivas, M. Bolio-González, A. Ortega-Pacheco, and A. Aguilar-Caballero, "Plant products and secondary metabolites with acaricide activity against ticks," *Veterinary Parasitology*, vol. 238, pp. 66-76, 2017.

A. J. Valencia-Botán, N. Bautista-Martínez, and J. A. López-Buenfil, "Use of aqueous extracts of neem, Azadirachta indica A. Juss, in the oviposition of the Mexican fruit fly Anastrepha ludens Loew (Diptera: Tephritidae) in Valencia orange," *Phyto sanitary*, vol. 8, pp. 57-59, 2004.

D. M. E. García, "Modifications to the Köppen climate classification system: To adapt it to the conditions of the Mexican Republic. Mexico. UNAM, Institute of Geography. Retrieved from https://www.igg.unam.mx/geoigg/biblioteca/archivos/memoria/20190917100949.pdf," 2004.

LFSA, "Federal animal health law. Official Gazette of the federation DOF 02-16-2018. Mexico DF. Retrieved from http://www.diputados.gob.mx/LevesBiblio/pdf/LFSA_190218.pdf," 2018.

Humane Farm Animal Care Welfare Standards, "Beef cattle." Retrieved from: https://2gmsag2k4ou3ll8b41b7v2qp-wpengine.netdna-ssl.com/wp-content/uploads/HFAC-Beef-Cattle-Standards_20.3-1.pdf, 2021.

Y. Zhong, J. Gao, Q. Lei, and Y. Zhou, "A vision-based counting and recognition system for flying insects in intelligent agriculture," *Sensors*, vol. 18, p. 1489, 2018.Available at: https://doi.org/10.3390/s18051489.

M. F. Maia and S. J. Moore, "Plant-based insect repellents: A review of their efficacy, development and testing," *Malaria Journal*, vol. 10, pp. 1-15, 2011.Available at: https://doi.org/10.1186/1475-2875-10-s1-s11.

R. N. Bennett, F. A. Mellon, N. Foidl, J. H. Pratt, M. S. Dupont, L. Perkins, and P. A. Kroon, "Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees Moringa oleifera L.(horseradish tree) and Moringa stenopetala L.," *Journal of Agricultural and Food Chemistry*, vol. 51, pp. 3546-3553, 2003.Available at: https://doi.org/10.1021/jf0211480.

F. O. Alao, T. A. Adebayo, and A. O. Olaniran, "Bioefficacy of Moringa oleifera and Anacardium occidentale against insect pests of watermelon (Citrullus lanatus Thumb) and their effects on watermelon fatty acid profile," *Acta Fytotechnica and Zootechnica*, vol. 21, pp. 84-92, 2018.Available at: https://doi.org/10.15414/afz.2018.21.03.84-92.

J. H. Díaz, "Chemical and plant-based insect repellents: Efficacy, safety, and toxicity," *Wilderness & Environmental Medicine*, vol. 27, pp. 153-163, 2016.Available at: https://doi.org/10.1016/j.wem.2015.11.007.

S. J. Stohs and M. J. Hartman, "Review of the safety and efficacy of Moringa oleifera," *Phytotherapy Research*, vol. 29, pp. 796-804, 2015.Available at: https://doi.org/10.1002/ptr.5325.

I. Mgbemena, T. Ebe, A. Nnadozie, and K. Ekeanyanwu, "Repellent activities of the methanolic leaf extracts of Moringa oleifera and Stachydraphe indica against Aedes aegypti mosquito," *Journal of Pharmacy and Biological Sciences*, vol. 10, pp. 77-81, 2015.
E. O. Emeribe, J. O. Asagwara, and G. O. Edemobi, "The potentials of Moringa oleifera L. leaf extracts as Musca domestica repellent on fresh Mangifera indica L. fruit in Owerri, Imo State," *International Journal of Applied Research and Technology*, vol. 6, pp. 107-115, 2017.

M. S. Nisar, M. A. Ismail, H. Ramzan, M. M. Maqbool, T. Ahmad, H. A. Ghrumh, A. Khalofah, J. Kmet, M. Horvát, and S. Farooq, "The impact of different plant extracts on biological parameters of housefly (Musca domestica (Diptera: Muscidae)): Implications for management," *Saudi Journal of Biological Sciences*, vol. 28, pp. 3880-3885, 2021. Available at: https://doi.org/10.1016/j.sjbs.2021.03.070.

J. C. Martínez González, F. A. Lucero Magaña, S. P. Castillo Rodríguez, and E. Ortega Rivas, "Estimation of some genetic growth parameters in the Guzerat breed in Mexico," *Zootecnia Tropical*, vol. 27, pp. 049-056, 2009.

AMCC, "Mexican association of Zebu Breeders, Racial Patterns. Retrieved from: https://cebumexico.com/patrone-raciales/," 2021.

ACCGC, "Colombian association of Zebu Cattle Breeders. Retrieved from https://www.asocceu.com/index.php/razas/brahmán," 2021.

E. Sancho, M. Caballero, and I. Ruíz-Martínez, "The associated microflora to the larvae of the human bot fly Dermatobia hominis L. Jr. (Diptera: Cuterebridae) and its furuncular lesions in cattle," *Memories of the Oswaldo Cruz Institute*, vol. 91, pp. 293-298, 1996.

S. Dayton, C. A. Hayden Brown jr, E. E. Gbur, and G. Tolley, "Interactive response of the horn fly (Diptera: Muscidae) and selected breeds of beef cattle," *Journal of Economic Entomology*, vol. 84, pp. 1275-1282, 1991. Available at: https://doi.org/10.1093/jee/84.4.1275.

C. Steelman, M. Brown, E. Gbur, and G. Tolley, "The effects of hair density of beef cattle on Haematobia irritans horn fly populations," *Medical and Veterinary Entomology*, vol. 11, pp. 257-264, 1997. Available at: https://doi.org/10.1111/j.1365-2915.1997.tb00404.x.

M. L. Herrera and E. B. Arguedas, "Comparison of two racial groups of cattle in terms of incidence of ticks (acari: Ixodidae) and torsos (diptera: Oestroideae)," *Nutrición Animal Tropical*, vol. 8, pp. 1-9, 2014.

M. Oliveira, M. Alencar, R. Giglioti, M. Beraldo, F. Aníbal, R. Correia, L. Boschini, A. Chagas, T. Bihassi, and H. Oliveira, "Resistance of beef cattle of two genetic groups to ectoparasites and gastrointestinal nematodes in the state of São Paulo, Brazil," *Veterinary Parasitology*, vol. 197, pp. 168-175, 2013. Available at: https://doi.org/10.1016/j.vetpar.2013.06.021.

A. M. Silva, M. M. Alencar, L. C. Almeida, and W. J. Barioni, "Artificial infestation of Boophilus microplus in beef cattle heifers of four genetic groups," *Genetics and Molecular Biology*, vol. 30, pp. 1150-1155, 2007. Available at: https://doi.org/10.1590/s1415-47572007000600020.

G. Gasparin, M. Miyata, L. Coutinho, M. Martínez, R. Teodorico, J. Furlong, M. Machado, M. Silva, T. Sonstegard, and L. Regitano, "Mapping of quantitative trait loci controlling tick (Rhipicephalus [Boophilus] microplus) resistance on bovine chromosomes 5, 7 and 14," *Animal Genetics*, vol. 38, pp. 453-459, 2007. Available at: https://doi.org/10.1111/j.1365-2052.2007.01634.x.

K. Prayaga and J. Henshall, "Adaptability in tropical beef cattle: Genetic parameters of growth, adaptive and temperament traits in a crossbred population," *Australian Journal of Experimental Agriculture*, vol. 45, pp. 971-983, 2005. Available at: https://doi.org/10.1071/ea05045.

C. M. Esquivel-Villalobos, "Breed, hair and skin as a function of animal welfare," *Livestock World*, vol. 1, pp. 73-85, 2012.