Synergistic interaction of Artemisia cina n-hexanic extract and Tagetes lucida ethyl acetate extract on Haemonchus contortus

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Research Article

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Abstract

The present study aimed to analyze the possible synergistic activity among the active extracts from *Artemisia cina* and *Tagetes lucida* combinations on the *Haemonchus contortus* nematode parasite of sheep. The work was carried out in vitro on eggs and infective larvae 3 of *H. contortus*. Also, the characterization of the extracts was made with HPLC. The results were analyzed with SAS 9.1 apply the anova and Tukey test, and the lethal concentration (LC) LC_{50} and LC_{90} were determined with regression analysis employed Proc Probit of SAS 9.1. Additionally, the FT was calculated with LC_{50} and LC_{90} to determine the synergistic effect. The results demonstrated high efficacy of the two plants studied on both nematode stages egg and larvae L3, as well as of their combinations in where the best inhibition hatch egg was obtained with 50/50% combination of each plant and the best larvae mortality were obtained with 25% *A. cina* and 75% *T. lucida* at 10 mg/ml, additionally this combination showed synergistic effect. In conclusion the two plants are an option as anthelmintic non pharmacological due to high bioactive effect and due to their synergistical response.

INTRODUCTION

Gastroenteric helminths are the cause of major parasitical diseases in grazing ruminants. These disorders cause damage to animal health and welfare in addition to reducing pro-ductive performance such as milk production, weight gain, carcass quality and fertility, among others (Charlier et al., 2014). Haemonchosis is a parasitic disease with high importance in small ru-minants. The pathophysiology of *Haemonchus contortus* produces hard damage due to its blood-feeding activity and as consequence anaemia, weight loss, and in presence of hy-peracute infection the sudden death (Besier et al., 2016). Due to the rapid development and increase an-thelmintic resistance (AR) in nematodes populations worldwide and, in the order to di-minish the damage caused by these parasites in production system the search for phar-macological and non-pharmacological tools, are necessary (Torres-Acosta et al., 2012; Yuan et al., 2019; Bull et al., 2022). Within these strategies of management are included the use of selective deworming with aid of indicators like the body condition score (Calvete et al., 2020) and FAMACHA colour chart; this strategy allows a proportion of the population not exposed to the chemical anthelmintic contributing to retard the AR growth (Arece-García et al., 2016). Other practices are focused in the populations resistant like the use of secondary metabolites from biological organisms, for example nematophagous fungi (Ocampo-Gutiérrez et al., 2021; Valdez-Uriostegui et al., 2021) or ruminal bacteria (Nazish et al., 2021).

On the other hand, the plants with secondary metabolites have displayed important anthelmintic activity on gastrointestinal nematodes including *H. contortus* (Davuluri et al., 2020; Minho et al., 2020). The Asteraceae family has a wide number of species and are being described for their benefits in the health both human and animal production. These plants contain several secondary compounds like terpenoids, flavonoids and other molecules that have been reported with anticancer, antimalarial and anthelmintic activities (Rolnik and Olas et al., 2021; García-Hernández et al., 2022). Artemisinin is a sesquiter-pene lactone obtained from Artemisia annua that demonstrated efficacy against malaria re-sistant to chloroquine and since 1950 until present day; there are discovery more proper-ties of artemisinin (Sülsen et al., 2021, García-Hernández et al., 2022).

Artemisia cina is commonly known as Levant wormseed, is an herbaceous plant that has been traditionally used as an anthelmintic agent in many parts of the world, especially in the Mediterranean and Middle Eastern regions. Several studies have shown the efficacy of *A. cina* in controlling various parasitic infections. For instance, a study found that the aqueous and ethanolic extracts of *A. cina* had a significant effect on reducing the number of eggs of gastrointestinal nematodes in sheep (Nematollahi et al., 2021). Similarly, another study reported that the ethanolic extract of A. cina was highly effective in controlling gastrointesti-nal nematodes in goats (Eguale et al., 2011).

Moreover, A. cina has also been found to be effective against other parasitic infections such as malaria, leishmaniasis, and schistosomiasis (Bero et al., 2009; Al-Salahy et al., 2013; Gonçalves et al., 2020). The active compounds pre-sent in A. cina, such as santonin, artemisinin, and their derivatives, have been identified as the main contributors to its anthelmintic activity (Bero et al., 2009). A study with an A. cina aqueous extract, which was administered to sheep naturally infected with Moniezia spp, recorded a 100% efficacy (faecal egg count reduction) on at 9th day post-administration (Bashtar et al., 2011). Additionally, an *A. cina* n-hexane extract was assessed against *H. contortus* infective larvae and caused a mortality of 80% at 2 mg/ml. In this same research, the authors administered the *A. cina* n-hexane at 2mg/kg to gerbils artificially infected with *H. contortus* and, they found a 100% efficacy (Higuera-Piedrahita et al., 2021).

Tagetes lucida is other genus (Asteraceae) extensively studied as anthelmintic since is rich in some phenolic acids and other metabolites like coumarins and terpenes (Porras-Dávila et al., 2021). This plant is commonly known as Mexican “Pericón”, is a flowering plant that has been tradi-tionally used for various medicinal purposes, including its cytotoxic activity that can re-sult in anthelmintic potential (Mejía-Barajas et al., 2012).

Studies have reported the efficacy of *T. lucida* in controlling parasitic infections. For example, a study founded that the ethanolic extract of *T. lucida* had a significant effect on reducing the number of eggs of gastrointestinal nematodes in sheep (Rojas-Velázquez et al., 2015). Similarly, an-other study (Rivera-Meza et al., 2016) reported that the methanolic extract of *T. lucida* had a high anthelmintic activity against the gastrointestinal nematode *H. contortus* in sheep.

Moreover, *T. lucida* has also been found to be effective against other parasitic infections such as malaria and leishmaniasis (Luna-Herrera et al., 2005; Bero et al., 2009). The active compounds present in *T. lucida*, such as tagetone, piperitone, and their derivatives, have been identified as the main contributors to its anthelmintic activity (Rojas-Velázquez et al., 2015).

A number of investigations, making combinations of chemical anthelmintics (albendazol and levamisole) with extracts from plants (terpenes) have improved the nema-tocidal effect on gastrointestinal nematodes (GIN) and this suggests a possible synergistic effect (Silva et al., 2021). The combination of natural products with chemical anthelmintics could con-tribute to the control of GIN with anthelmintic resistance reports It is well-known that natural compounds from plants have a wide range of biological activities, including antiparasitic activity. However, most studies focus on isolated compounds or extracts from single plants, which may not be sufficient to achieve optimal therapeutic effects. Therefore, studying the synergistic effects of different plants could lead to the development of more effective and safer antiparasitic treatments (Katiki et al., 2017; Lanusse et al., 2018).

Several studies have demonstrated the potential benefits of studying plant synergies for antiparasitic activity. For example, a study by (García-Rubio et al., 2017) investigated the antileishmanial ac-tivity of four plant extracts and their combinations. The study found that the combination of two extracts showed a synergistic effect and exhibited greater antileishmanial activity than each extract alone.

Similarly, a study investigated the antischistosomal activity of extracts from three different plants, both alone and in combination. The study found that the combination of all three extracts exhibited a synergistic effect, with a greater reduction in worm burden than each extract alone (Ndjonka et al., 2019).

Moreover, a recent review by (Gbènou et al., 2021) summarized the potential of plant synergies for antiparasitic activity. The review highlighted that plant synergies can improve efficacy, reduce toxicity, and prevent the development of resistance in parasites. The aim of the pre-sent study was to evaluate the interaction of plants Artemisia cina (n-hexane extract) and *Tagetes lucida* (Ethyl acetate extract) on *in vitro* assays effect against *Haemonchus contortus* for identify synergistic activity among these extracts.

MATERIALS AND METHODS

Plant material

At the Hunab laboratory, a purchase was made of 10 kg of the fresh pre-flowering leaves and stems of *A. cina* O. Berg ex Poljakov (Asteraceae). To authenticate the plant, a voucher specimen was examined by Dr. Alejandro Torres-Montúfar and deposited at the herbarium of Facultad de Estudios Superiores Cuautitlán (FES-C) UNAM, México under voucher no 11967. The plant was cultivated under specific conditions, including 80% humidity, 24°C temperature, and pH 6.3 soil. *Tagetes lucida* aerial parts were harvested in El Cerrillo Piedras Blancas locality in the Toluca Municipality, Mexico State (19.42984 N, -99.669345 W). One specimen of this plant was deposited in the Herbarium of the Centre for Research in Biodiversity and Conservation, Universidad Autónoma del Estado de Morelos (UAEM-Mexico), under voucher code number 33784.

Artemisia cina extract obtaining

Five kilograms of dried *A. cina* leaves and stems were ground and placed in crystal containers with a capacity of 1000 mL. To extract the compounds, n-hexane was used and the mixture was kept at room temperature (23–25°C) for 48 h. The extract was filtered using Whatman No. 4 paper and the solvent was removed by low-pressure distillation through a rotary evaporator (Heidolph Laborota 4000, Heidolph Instruments, Schwabach, Germany). Finally, the extract was lyophilized and stored at 4°C for phytochemical and biological assays.

Tagetes lucida extract obtaining

Five kilograms of dried *T. lucida* was macerated in ethyl acetate for 48 hours. The ex-tract was filtered using Whatman No. 4 paper and the solvent was removed by low-pressure distillation through a rotary evaporator (Heidolph Laborota 4000, Heidolph Instruments, Schwabach, Germany).

Egg hatching inhibition (EHI) test

The EHI test was performed with *Haemonchus contortus* eggs obtained from a lamb previously infected with the para-site (5000 infective larvae singular dose, FESC strain, Mexico). The eggs were separated from faeces (30–50 g), with clean water through sieves of 140, 74 and 37 μm, and were cleaned by density gradient with 40% saccharose. The assays were performed in 96-well microtitration plates. Each treatment was tested three times considering four repetitions by replicate (n = 12). The combinations (treatments, *A. cina* extract: *T. lucida* extract) were assigned as follow: 1) Combination 100:00, 2) Combination 0:100, 3) Combination 50:50 and 4) Combination 75:25 and 5) Combination 25:75. Each combination was tested at 1–5 mg/ml. Additionally, distilled water and Tween 20 (at 1%) were used as negative controls and Ivermectin (5 mg ml/mL) was used as positive control.

Fifty microliters of an aqueous suspension containing 100 ± 20 *H. contortus* eggs were deposited in each well (experimental unit). Then, 50 μl aliquots of the treatments or controls were deposited, giving a total volume of 100 μl. The plates were incubated at room temperature (25–28°C) for 48 h. After this time, the hatching process was stopped using 10 μl of Lugol´s solution. The total eggs and larvae (first and second stage) of each well were counted, and the egg hatching inhibition percentage (EHI %) for each treatment was de-termined using the following formula:

(1)

$$EHI\% = \left(\frac{numberofeggs}{numberofeggs + numberoflarvae}\right) x100$$

Larval mortality assay

The larvicidal activity of the combination extracts was performed using *H. contortus* infective larvae (L3), which were obtained from a donor animal. The L3 were obtained from faecal cultures by modified technique of Corticelli and Lai (1963). The assays were performed using 96-well microtritation plates. The treatments were designed as follow: 1) Combination 100:00, 2) Combination 0:100, 3) Combination 50:50 and 4) Combination 75:25 and 5) Combination 25:75. Each combination was tested at 1–10 mg/ml. Additionally, distilled water and Tween 20 (at 1%) were used as negative controls and Ivermectin (5 mg/ml) was used as positive control. The plates were incubated at room temperature (18–25°C) for 24 h. After this period, the total larvae alive or dead of each well were counted in the optic microscopy Hinotek Lab model L1200. Then, the mortality percentages were estimated based on the following formula:

(2)

$$MortalityLarvae\% = \left(\frac{numberdeadlarvae}{numbersofdeadlarvae + numberofalivelarvae}\right)x100$$

Statistical analysis

The data of IEH and mortality percentages were analyzed through ANOVA based on a completely randomized design by the general linear model in SAS program. The dependent variables were the combinations and controls. Tukey´s test was performed to identify significance differences among treatments at 0.05 significance level. The treatments with a dependent effect on the concentration were subjected to regression analysis to estimate the lethal concentrations (LC) 50 and 90 using a Probit analysis (SAS, 2014).

2.7 Synergic interaction analysis

In the order to characterize the interaction between the combinations of the extracts *A. cina* and *T. lucida* an isobolographic analysis was used following the methodology de-scribed by (Tallarida et al., 1997). According to this method, the lethal concentrations 50 and 90 of each ex-tract (assay individual) and their combinations were considered to determine the synergic effect. Likewise, the interaction magnitude of the combinations, the total fraction of LC of the *A. cina* extract and *T. lucida* extract and their combinations were determined according to the following formula:

(3)

$$TF = \left(\frac{LCA.cinaextractinthecombination}{LCA.cinaextractalone}\right) + \left(\frac{LCT.lucidaextractinthecombination}{LCT.lucidaextractalone}\right)$$

Where:

TF = total fraction LC = lethal concentration

Values close to 1 indicate a possible additive effect and < 1 indicate a super-additivity or synergism effect.

RESULTS

Compounds with antiparasitic activity of *Artemisia cina* and *Tagetes lucida*

n-hexane extract of *Artemisia cina* previously reported the presence of lignans with potent antiparasitic activity, like isoguaiacine and norisoguaiacine, the compounds were report-ed by Higuera-Piedrahita et al. (2023) and they were elucidated by magnetic resonance.

On the other hand, our study group previously determined, using the HPLC technique, the major compounds of *Tagetes lucida*, the most abundant being phenolic compounds such as caffeic acid, ferulic acid, coumaric acid and chlorogenic acid (Díaz-Medina et al., 2021).

Ovicidal activity

The ovicidal activity of the both extracts in individual form and their combinations are shown in the Table 1. The individual evaluation of *T. lucida* ethyl acetate extract showed the best egg hatch inhibition close to 90% at 2.5 mg/ml. While the *A. cina* n-hexane ex-tract exhibited the lower IEH with the three-concentration used. The combination 50/50 present IEH almost 100% with all the concentration used (1, 2.5 and 5 mg/ml).

Table 1

Haemonchus contortus egg hatch inhibition percentages (EIH%) after 48 h exposure to *Artemisia cina* and *Tagetes lucida* extracts and their combinations.

	Means of eggs and larvae (L1 or L2) recovered		.
Treatment	Larvae ± s.d	Eggs ± s.d	EHI% ± s.d
Artemisia cina (mg/mL)			
1	81.1 ± 8.8	23.1 ± 12.6	21.17 ± 8.2 [d]
2.5	65.8 ± 13.9	9.3 ± 4.2	7.8 ± 8.3 [e]
5	66.0 ± 31.8	10.4 ± 13.5	15.2 ± 15.2 [de]
Tagetes lucida (mg/mL)			
1	37.0 ± 10.2	89.0 ± 6.8	71.0[b] ± 5.6[b]
2.5	9.2 ± 7.5	72.7 ± 12.2	89.0 ± 8.3 [a]
5	4.0 ± 3.5	109.1 ± 12.7	96.5 ± 2.9 [a]
50% *A. cina*/ 50% *T. lucida*			
1	5.0 ± 3.1	109.9 ± 22.9	95.6 ± 2.7 [a]
2.5	4.4 ± 3.7	64.3 ± 7.8	93.7 ± 5 [a]
5	2.2 ± 2.8	70.4 ± 14.3	97.3 ± 3.2 [a]
75% *A cina*/ 25% *T. lucida*			
1	93.8 ± 19.6	13.0 ± 6.7	12.3 ± 6 [de]
2.5	4.4 ± 3.7	41.9 ± 13.2	55.5 ± 14.6 [c]
5	6.0 ± 3.1	13.0 ± 6.7	90.9 ± 5.1 [a]
25% *A cina*/ 75% *T. lucida*			
1	28.0 ± 11.7	72.2 ± 18.6	71.4 ± 13 [b]
2.5	6.2 ± 4.4	73.0 ± 11.5	92.4 ± 4.7 [a]
5	4.8 ± 4.2	98.7 ± 19.5	95.7 ± 3.4 [a]
Ivermectin (5 mg/mL)	0.2 ± 0.7	80.4 ± 14.4	99.7 ± 0.8 [a]
Distilled water	85.1 ± 14.6	3.9 ± 1.9	4.2 ± 1.6 [e]

* Means with same letter are similar statistically $P \leq 0.05$, s.d.= standard deviation

Means of eggs and larvae (L1 or L2) recovered	.
Variation coefficient	11.68
R^2	0.96
* Means with same letter are similar statistically P ≤ 0.05, s.d.= standard deviation	

Mortality larval

The higher larvae mortality was obtained used the uncombined extracts with the concentration of 10 mg/ml (Table 2). This same response was achieved with the combination 25/75% of *A. cina* and *T. lucida* respectively using 10 mg/ml. The 1 mg/ml concentration showed lower mortality in all treatments except for *T. lucida*. These reveal a dose-response relationship of the extracts on the *H. contortus* larvae. The mortality found in the present study of ivermectin against *H. contortus* was 100% due to the strain tested is susceptible.

Table 2
Haemonchus contortus infective larvae mortality after 24 h exposure to *Artemisia cina* and *Tagetes lucida* extracts and their combinations.

Treatment	**Means died and lives larvae recovered**		**% Larvae mortality ± s.d.**
	Lives ± sd	**Died ± sd**	
Artemisia cina (mg/ml)			
1	72.8 ± 9.5	11.8 ± 3.5	13.6 ± 3^{hi}
2.5	67 ± 6.2	26.2 ± 7.2	29.2 ± 8.1^{ghi}
5	66.6 ± 6.5	35.8 ± 7.8	37.3 ± 2.5^{efgh}
10	10.3 ± 2.3	78.6 ± 12.9	88.2 ± 3.7^{ab}
Tagetes lucida (mg/ml)			
1	69.3 ± 17.6	30.7 ± 7	31.6 ± 5 ^{fgh}
2.5	45.3 ± 23.6	52.1 ± 15.7	54.4 ± 8.8 ^{def}
5	25.8 ± 9.0	68.4 ± 15	70.4 ± 8.8^{bcd}
10	9.3 ± 6.02	56.3 ± 20	85.4 ± 4.7^{ab}
50% *A. cina/* 50%*T. lucida* (mg/ml)			
1	72.7 ± 13	17.3 ± 3.3	20.1 ± 4.7^{hi}
2.5	53.2 ± 6.7	61.9 ± 26.6	50.0 ± 10.9^{defg}
5	29.4 ± 10.1	50.2 ± 14.3	59.8 ± 11.4^{cde}
10	12.1 ± 7.6	51 ± 23.5	81.2 ± 6.7^{abc}
75% *A. cina*/25%*T. lucida* (mg/ml)			
1	76 ± 8.9	19.44 ± 3.4	20.0 ± 1.1^{hi}
2.5	76.5 ± 9.6	32.8 ± 13.4	29.2 ± 6.7^{ghi}
5	29.7 ± 10.4	45 ± 27.3	55.4 ± 15.8^{def}
10	25 ± 7	71.7 ± 24.1	72.8 ± 11^{bcd}
25% A. cina/ 75% T. lucida (mg/ml)			

*Means with same literal are statistical similar. $p \leq 0.05$.

Treatment	Means died and lives larvae recovered		% Larvae mortality ± s.d.
	Lives ± sd	Died ± sd	
1	61.5 ± 23.4	23.3 ± 11.5	28.5 ± 8.4 ^{ghi}
2.5	50.8 ± 11.8	50.4 ± 4.7	50.2 ± 8.9^{defg}
5	18.7 ± 18.3	58.7 ± 32.8	72.8 ± 13.4^{bcd}
10	10.6 ± 2.1	71.3 ± 6.7	87.1 ± 1.2^{ab}
Distilled water	87 ± 8.6	5 ± 1	5.8 ± 1.6^{i}
Ivermectin	0 ± 0	116 ± 11.8	100 ± 0 ^{a}
Variation coefficient	18.3		
R^2	0.91		
*Means with same literal are statistical similar. $p \leq 0.05$.			

Lethal Concentration 50 y Lethal Concentration 90

The LC_{50} and LC_{90} calculated for the *A. cina* and *T. lucida* extracts are shown in Table 3. The *T. lucida* extract showed the LC_{50} with minor concentration and the *A. cina* extract alone presented the major LC_{50}. The LC_{90} by contrast was obtained for *A. cina* extract.

Table 3
Lethal concentrations (LC) 50 and 90 of *A. cina* and *T. lucida* extracts on larvae 3 of *H. contortus.*

Treatment	LC50	IC limit 95% (lower-upper)	Conc. atribuided to *A. cina / T.lucida*	LC90	IC limit 95% (lower-upper)	Conc. atribuided to *A. cina / T. lucida*
T. lucida	**1.52**	1.2–1.9		**17.78**	12.9–27.9	
A. cina	**6.7**	6.2–7.1		**10.6**	9.9–11.7	
50 *A. cina* / 50 *T. lucida*	**3.3**	2.9–3.7	1.65/1.65	**18.4**	14.6–24.9	9.2/9.2
75 *A. cina*/ 25 *T lucida*	**4.36**	3.8-5.0	3.7/1.09	**26.4**	19.9–37.9	19.7/6.6
25 *A. cina* /75 *T lucida*	**2.4**	2.1–2.7	0.6/1.8	**14.07**	11.3–18.5	3.5/10.5
IC = Confidence interval						

The proportion of LC_{50} and LC_{90} attributed for each extract according to the proportion added in the combination is presented in the next table, with this information the isobologram were drawn (Fig. 1).

Synergistic activity

The synergistic activity is showed in Fig. 1. According to estimates for LC_{50}, the extracts are more lethal when are combined, the isobologram reflect that the concentration needed for cause 50% of mortality in *H. contortus* larvae is lower in all combination than in extracts alone, the lines of the three combinations pass under the line of LC_{50} of the extracts alone, suggest synergistic activity.

The LC_{90} isobologram is in Fig. 2. In this graphic show the lines for the LC_{90} of extracts from *A. cina* and *T. lucida* alone and their combinations, the line of the combination 50% *A. cina* and 50% *T. lucida* too the 25% *A. cina* and 75% *T. lucida* passed under the line of LC_{90} of extracts alone. The other combination (75/25) not show the same tendence.

The TF (Total Fraction) for each combination is presented in Table 4. This table show the effect in all combination and both LC_{50} and LC_{90} are sub-additive (antagonism). Moreover for 25:75 *A. cina*: *T. lucida* combination extracts CL_{90} the effect demonstrated was synergistic.

Table 4
Total Fraction (FT) extracts from *Artemisia cina* and *Tagetes lucida* combinations according to the proportions used, for CL_{50} and CL_{90}.

Combination	Proportion	Concentration lethal		Total fraction	Effect
		A. cina	*T. lucida*		
A. cina : T. lucida$_{CL50}$	50:50	1.65	1.65	1.3	Sub-additive (antagonic)
	75:25	3.27	1.09	1.2	Sub-additive (antagonic)
	25:75	0.6	1.8	1.3	Sub-additive (antagonic)
A. cina : *T. lucida*$_{CL90}$	50:50	9.2	9.2	1.3	Sub-additive (antagonic)
	75:25	19.2	6.6	2.2	Sub-additive (antagonic)
	25:75	3.5	10.5	0.9	Synergistic

Discussion

Synergistic effects of plant extracts have gained considerable attention as potential alternative treatments for parasitic infections in animals. Plants are known to contain a variety of bioactive compounds, and their combination may enhance their efficacy as antiparasitic agents. Several studies have reported the benefits of using plant extracts in combination to treat parasitic infections in animals. For instance, a study by (Kar et al., 2017) demonstrated that the combination of *Piper longum* and *Tinospora cordifolia* extracts showed a synergistic effect against gastrointestinal nematodes in sheep. Similarly, a study by Fikru et al. (2016)

found that the combination of extracts from various plants, including *Achyranthes aspera*, *Vernonia amygdalina*, and *Lantana camara*, had a synergistic effect against gastrointestinal nematodes in goats.

The synergistic effects of plant extracts may be due to their ability to target different stages of the parasite's life cycle or different physiological mechanisms. In addition, the combination of compounds may lead to increased bioavailability and better absorption, resulting in higher efficacy against parasitic infections (Oliveira et al. 2019). Moreover, the use of plant extracts in combination may help to reduce the development of resistance to the treatment, as it is less likely for parasites to develop resistance to multiple compounds simultaneously (Kamaraj et al., 2019).

Synergistic effects on metabolites from plants of the genus Artemisia and secondary metabolites from other plants have been previously evaluated and identified. The use of essential oil of *Artemisia herba alba* and *Lavandula angustifolia* has an antibacterial effect against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* at 20 µl/mL not combined and 0.5 and 0.1 µl/mL respectively when evaluated in combination, showing synergistic activity. (Moussi et al., 2020). In addition, a trial conducted on antibiotic-resistant *Staphylococcus aureus* strains with a combination of several flavonoids from *A. rupestris* and norfloxa-cin showed a TF of less than 0.5 (synergistic effect) (Lan et al., 2021). Furthermore, in a study two of the major methoxylated compounds from the dichloromethane fraction of *Tagetes lucida* plant showed a possible synergistic effect between coumarins and flavonoids against fungi (Céspedes et al., 2006). These previews can help to explain the results displayed in the present work, in that some combination among the extracts from the two studied plants appear synergistic activity.

Artemisia cina has been previously reported to have activity against *H. contortus*. *A. cina* 30 CH (centesimal Hahnemannians) administered to sheep naturally infected with gas-trointestinal helminths reduced egg counts by 69% per gram, and also inhibited 100% of egg hatching in vitro (Higuera-Piedrahita et al., 2020). On the other hand, the n-hexane extract has been the most active against H. contortus larvae (Higuera-Piedrahita et al., 2021). Based on these reports, the use of this type of extract was selected in the present study. Recently, two lignan compounds were identified as respon-sible for the activity against H. contortus, 3'-Demethoxy-6-O-demethylisoguaiacine and norisoguaiacine, in this extract (Higuera Piedrahita et al., 2023). However, the anthelmintic effect found in that study for the hexane extract was greater than that found in the present study, as it exhibited 80% larval mortality at 4 mg/ml (Higuera-Piedrahita et al., 2023), while in our study a similar effect was found using 10 mg/ml.

On the other hand, metabolites present in the aerial part of the plant *T. lucida* have been reported, mainly coumarins, flavonoids, and terpenes (Porras-Dávila et al., 2022). These compounds have shown anti-inflammatory effects (Porras-Dávila et al., 2022) as well as antimicrobial effects from 450 mg/ml using petroleum ether and chloroform extracts on bacteria such as *Escherichia coli*, *Enterobacter alcalifaciens*, *Staphylococcus aureus*, among others (Capunzo et al., 2003). In other studies, its effect against fungi such as *Aspergillus niger*, *Fusarium sporotrichum* among others has also been demonstrated, obtaining the highest activity in the Methanol/Dichloromethane fraction, and identifying coumarins as the compounds with the greatest effect (Céspedes et al., 2006). On the other hand, *T. lucida* essential oil was applied against the nematode *Nacobbus aberrans* in tomato plants, achieving an 85% inhibition of gall formation by this nematode when applied at 10 mg/ml of essential oil (Zarate-Escobedo et al., 2018). These previous studies could help explain the anthelmintic effect found using this plant.

CONCLUSIONS

Artemisia cina and *Tagetes lucida* extracts have the capacity to act synergistically against the *Haemonchus contortus* nematode, due to their high anthelmintic activity and similar active compounds, for which it is necessary to continue their study.

Declarations

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COMPETING INTERES

The authors have non financial interests to disclose.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection and first draft of the manuscript were performed by Itzel Santiago Figueroa, Rosa Isabel Higuera Piedrahita and Agustín Olmedo Juárez. And all authors commented on previous versions of the manuscript, help to discussed the results and contributed, to final versión of manuscript. All authors read and approved the final manuscript.

DATA AVAILABLE

Data will be made available on reasonable request.

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Figures

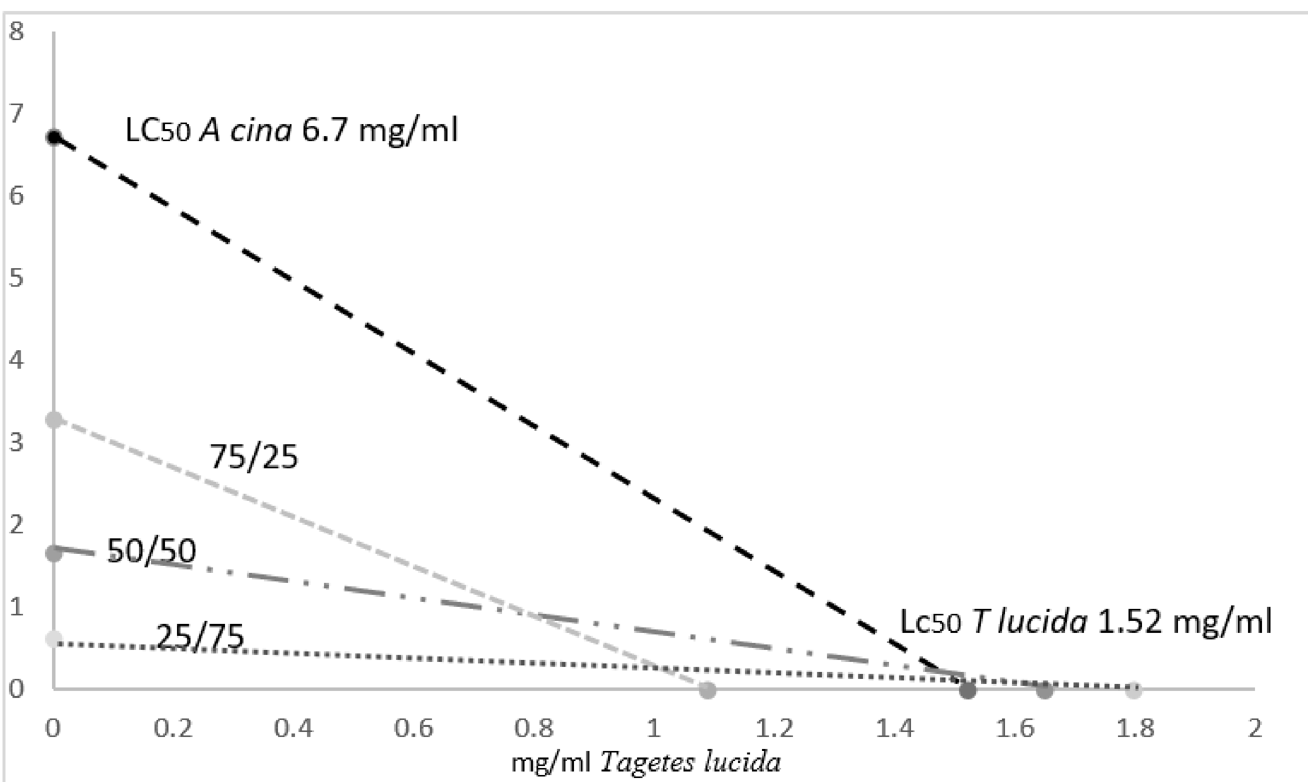

Figure 1

Isobologram of lethal concentration 50 ofArtemisia cina, Tagetes lucida extracts and some combinations on H. contortus L3.

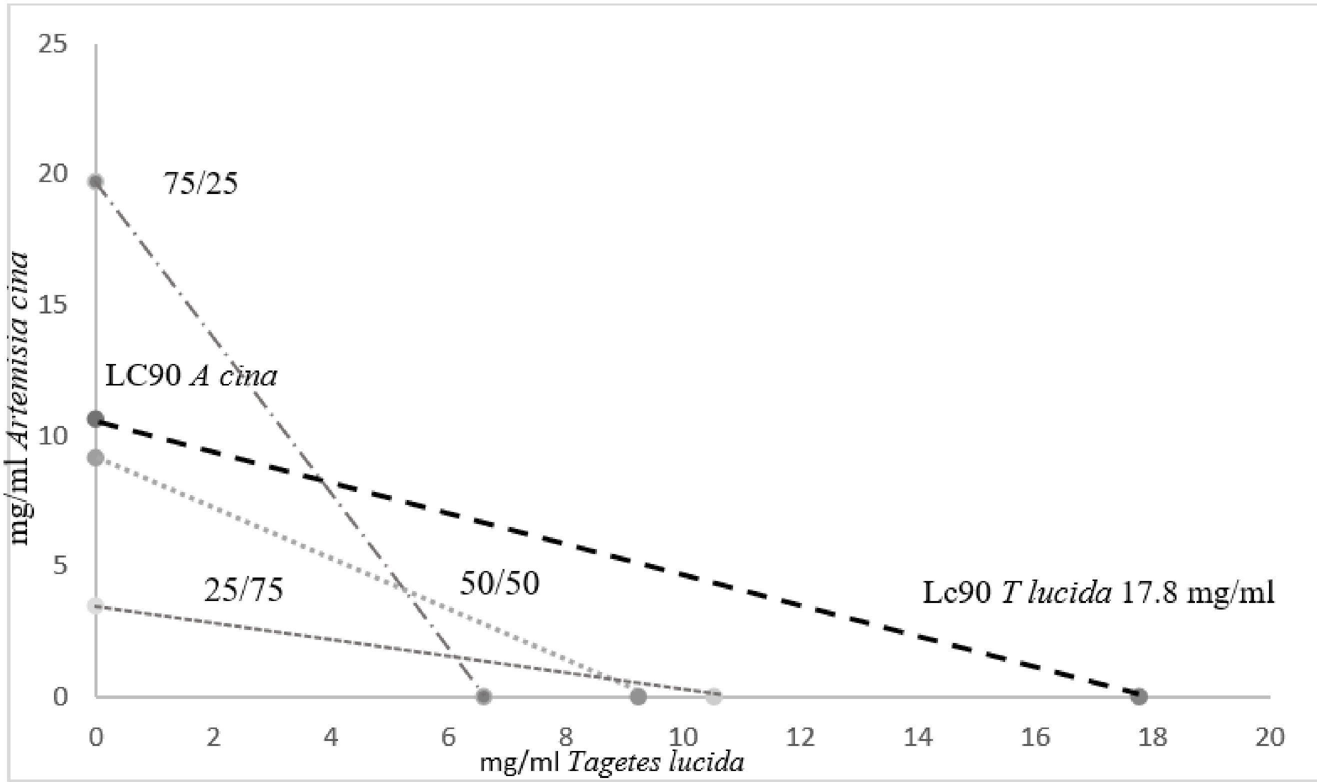

Figure 2

Isobologram of lethal concentration 90 of A. cina and T. lucida extracts and some of their combinations on H. contortus larvaes 3.