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Article

# Effect of Five Concentrations of Aqueous Extracts of *Pleurotus ostreatus* P. Kumm and *Tagetes minuta* L. on the Mortality of Two Nematodes in a Laboratory Setting.

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## ABSTRACT

The nematode attacks affect several plant species of Ecuadorian crops. There are fungi and plants with nematicide ability that have agricultural interest. This study assessed the effect of five concentrations of aqueous extracts of *Pleurotus ostreatus* and *Tagetes minuta* on the mortality of *Meloidogyne* spp. and *Panagrellus redivivus* nematodes in a laboratory setting. The aqueous extracts were obtained through hydro distillation where concentrations of 0%, 0.5%, 5%, 25%, 50% and 100% were prepared. A wholly randomized single-factor design was used for the *P. ostreatus* extract and a bifactorial for the *T. minuta* extract (leaves and flowers). The number of dead individuals was evaluated, and the efficacy and $LC_{50}$ were determined. *T. minuta* leaf extract showcased higher nematicide activity against *P. redivivus* with an $LC_{50}$ of 8.03 ppm; when applied to *Meloidogyne* sp., the extract showed nematicide activity with an $LC_{50}$ of 0.01 ppm. For *P. ostreatus* extract, the greatest nematicide activity against *P. redivivus* was an $LC_{50}$ of 1.22 ppm and nematicide activity against *Meloidogyne* sp., was an $LC_{50}$ of 0.01 ppm. The aqueous extract of *T. minuta* flowers showed low nematicide activity and the aqueous extract of *T. minuta* leaf showed the best nematicide activity.

**Keywords:** nematicide; *Tagetes minuta*; *Pleurotus ostreatus*; *Panagrellus redivivus*; *Meloidogyne* sp.

## INTRODUCTION

Nematodes are one of the phytopathogens that cause significant losses in global agricultural production, with estimated losses of up to one hundred billion dollars annually[1]. This group of phytoparasites is characterized by its diversity, complexity, and wide distribution across all productive agroecosystems worldwide[2]. These qualities and peculiarities enable them to induce other diseases[3], potentially causing annual losses between 11 and 14%, or even higher if another pathogen is introduced[4].

Ecuador has not been exempt from nematode attacks; reports from Ecuadorian fields indicate the presence of: *Meloidogyne incognita*, *Meloidogyne javanica*, *Meloidogyne arenaria*, *Meloidogyne hapla*, *Meloidogyne graminicola*, *Rotylenchulus reniformis*, and *Nacobbus aberrans*[5]. These nematodes affect plant species that are part of the daily diet of every Ecuadorian, as well as those destined for export, such as watermelon, melon, cucumber, sugarcane, corn, tomato, onion, pineapple, papaya, passion fruit, rice, summer flowers, etc.

Given this situation, one nematode control alternative has been synthetic chemical nematicides such as carbamates and organophosphates. However, these have shown alarming side effects, high toxicity, residue

persistence, and development of resistance[6]. Therefore, for several years now, there has been a search for more benign control methods for the environment and humans [7].

Various research projects are currently being conducted for the biological control of different phytopathogens[1,4,8]. One of these alternatives is the use of plant extracts[9,10,11], which are characterized by their biological origin, biodegradability, and minimal negative impact on human health and the environment. These extracts have shown the ability to act through their metabolites and exhibit nematicidal[12,13,14], insecticidal, acaricidal, or herbicidal activity. In this research, we worked with aqueous extracts of *Tagetes minuta* and *Pleurotus ostreatus*.

One of the extracts that have shown promising results as nematicide is garlic bulbil extract, which reduced galling index by 73%, egg and juvenile production by 80%, and female population by 94%[15]. A reality close to our area regarding integrating a biological control alternative has been the use of the Tagetes genus, which has pesticide characteristics related to its allelopathic function[13].

*Pleurotus ostreatus* is a fungus native to China but has been distributed worldwide except for the Arctic[11]. This fungus is known as oyster mushroom and is considered a health promoter and environmental restorer[16]. *Tagetes minuta* is an annual aromatic herb that grows in temperate grasslands and mountainous regions of South America[8]; it is known for being arthropod repellent and having herbicidal, nematicidal, insecticidal, fungicidal, antiviral properties, etc. According to research carried out, these particularities are due to its components: ocimenones (Z) and (E), piperitone, piperitenone, limonene, tagetone and caryophyllene[17]. Dihydrotagetone inhibits the hatching of *Meloidogyne incognita* eggs by 72 to 79% in 14 days; in the case of juveniles (Z)-β-ocimene is lethal in 72 hours[18].

Knowing the work with plant extracts in essential oils, which require complex processes to obtain, this research used the aqueous extract, which is the unstable liquid emulsion of saturated vapor and essential oil[10] of *Tagetes minuta* and *Pleurotus ostreatus* to evaluate the nematicide effect of the aqueous extracts of *Pleurotus ostreatus* and *Tagetes minuta* in the laboratory and to determine the best $LC_{50}$ of *Pleurotus ostreatus* and *Tagetes minuta* on *Meloidogyne* spp. and *Panagrellus redivivus* in the laboratory.

*Panagrellus redivivus* is a free-living nematode that lives in humid environments in a state of fermentation and can feed on various cereals. Thanks to its qualities, tiny size, rapid growth, short life cycle, high fecundity, and easy handling, it is one of the most used nematodes in research work[19]. For its part, *Meloidogyne* spp. is an endoparasitic phytonematode that completely penetrates the root to feed, develop and reproduce through eggs[20], it is characterized by causing galls, damaging the root system and causing dwarfism, chlorosis, wilting, defoliation or premature senescencese[2].

## MATERIALS AND METHODS

*T. minuta* was collected in the community San José de Cunduana, Licán Canton, Province of Chimborazo (Longitude 1°37'40 "S, Latitude 78°43'17"W, Altitude 3085 masl). 0.5 kg of leaves and 1 kg of fresh flowers were collected from native crops; they were cut into pieces of 1 cm long, placed in ziploc bags (17.7cm x 19.5cm) separately and transported in a flex-foam thermal cooler (Century-T40-3).

*P. ostreatus* and *P. redivivus* were cultured and provided by the store of the project "Study of the diversity of nematophagous fungi associated with the rhizosphere of tomato (*Solanum lycopersicum* L.) in three locations in the Province of Chimborazo", executed during 2018 and 2019 at the Biological Sciences Laboratory. *P. redivivus* was raised in a nutrient medium of oats in 250 g per 200 ml of sterile distilled water. After 15 days, an aliquot of the *P. redivivus* culture was taken with a flat-tip brush (0 Ø) and placed in a 100 ml glass bottle with sterile distilled water, this process was repeated until the suspension was opaque. Next, a 10 ml sample was placed in petri dishes (90 mm Ø) and 50 nematodes were fished for each of the experimental units with the help of round-tipped brushes (0 Ø - 4/0 Ø) (Figure 1).

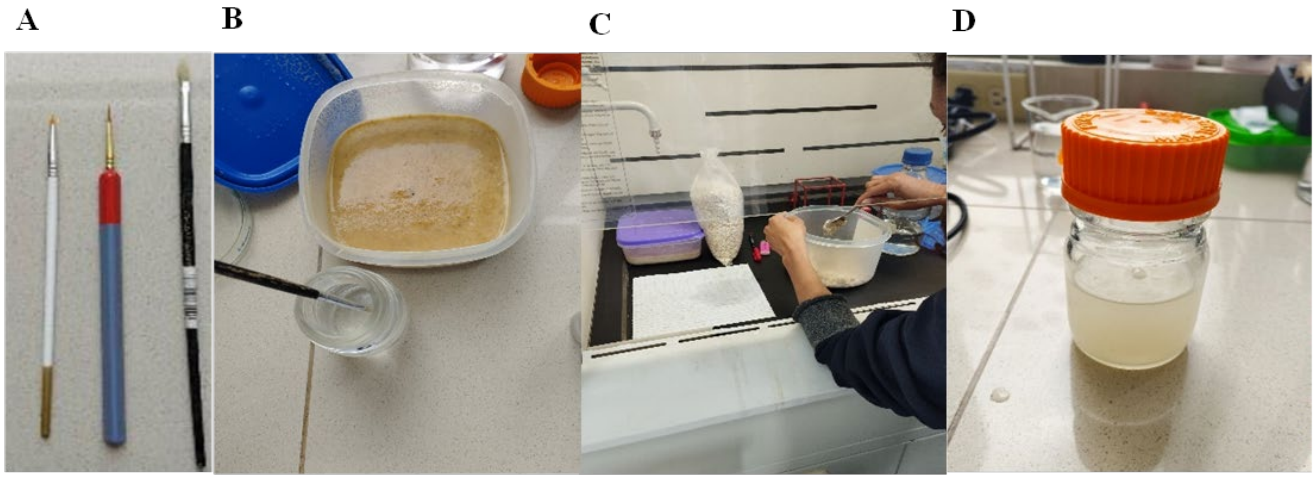


**Figure 1. Nematode fishing brushes (A), Culture of *P. redivivus* (B), Inoculation of *P. redivivus* (C), Suspension of *P. redivivus* (D).**

Both plant materials were processed in the Laboratory of Pharmaceutical Technology of the Faculty of Sciences at ESPOCH, Riobamba Canton, Chimborazo Province (01°38'51 "S, 78°40'59"W, altitude: 2850 masl)., the hydro-distillation method used by Silva[21] was applied: in a two 500 ml mouths, the chopped plant material was placed with hot water up to 3/4 parts of the funnel's capacity, in such a way that all the material was submerged, the heat was applied to start boiling and steam dragging, obtaining; as a result the aqueous extract. Once the aqueous extracts were received, the concentrations were prepared at 0%, 0.5%, 5%, 25%, 50% and 100% with sterile distilled water, each in 50 ml screw-top glass bottles containing. They were stored in a refrigerator (LG- Side by side of 615 l) at 5°C.

*Meloidogyne* sp. was obtained from infected root samples with nodules in greenhouse plantations of red bell pepper (*Capsicum annuum*) around 4 months old, located in the sector Quillan Loma Alto, Izamba Parish, Tungurahua Province (01°13'11"S, 78°33 '38"W, altitude 2672 masl). The root with nodules was placed in ziploc bags (17.7cm x 19.5cm) and transported in a flex-foam thermal cooler (Century-T40-3). The tray method for the extraction of Coyne and Claudios[22] nematodes was used as a principle with the following adaptations: The roots were washed to remove the adhering soil and cut into 1 cm long segments, 10 g of roots were placed on a napkin (23cm x 24cm) folded in 4 parts, later a tie was made, forming a kind of tea bags, which were suspended in a 500 ml glass jar with saline solution at 0.9% during 24 hours (Figure 2D).

After 24 hours, juvenile 2 (J2) was briefly identified using a stereoscope (Motic SMZ-171). The morphological characteristics detailed by Jaraba, Lozano and Suárez[23] and the illustrations by Carmona and Padilla[24] were utilized as a guide. It was also considered that when working with the females of *Meloidogyne* sp. the result was youth 2 (J2). Finally, 10 ml of the 0.9% saline solution was taken. The tea bag with nodules was suspended in glass petri dishes (90 mm Ø) and 20 nematodes were fished for each experimental unit with a traditional fishing instrument (Figure 2A).

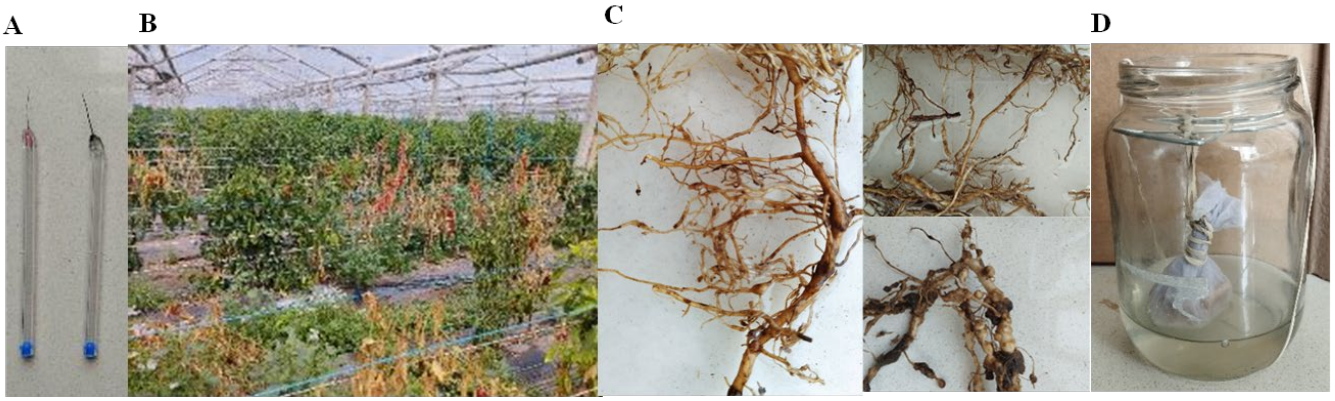


**Figure 2. Nematodes fishers (A), Pepper crop with nodules. (B), Root nodules with *Meloidogyne* (C), Adapted method of Coyne and Claudius (D).**

Bioassays were carried out in the Biological Sciences Laboratory of the Faculty of Natural Resources at ESPOCH. The number of juveniles 2 (J2) of the nematodes *P. redivivus* and *Meloidogyne* sp were obtained. For each of the experimental units, 6 ml of the different concentrations prepared (0%, 0.5%, 5%, 25%, 50% and 100%) of the aqueous extracts of *T. minuta* leaf-flower and *P. ostreatus*, was placed in each of the sections of the plastic tripetri boxes (90 mm Ø) according to the treatment. Each experimental unit counted the number of dead and alive J2 nematodes with the help of the stereoscope. Data were recorded every 4 h during the day, and the following efficacy equation of Abott[25] was applied.

$$Efficacy = \frac{IT-it}{IT}\ x\ 100 \quad (1)$$

Where:
IT= Live nematodes in the control
it= Live nematodes in the treatment

The Lethal Concentration 50 ($LC_{50}$) was estimated through a regression based on the efficacy of the different concentrations of the aqueous extracts of *T. minuta* and *P. ostreatus* on the mortality of the populations of *P. redivivus* and *Meloidogyne* sp. The $LC_{50}$ was determined using the EC50 Estimator library of the R programming language of the RStudio version 2022.07.2 program.

The data analysis was performed for each exposure time, for which two types of experimental designs were used: A bifactorial complete randomized design for the aqueous extracts of *T. minuta* leaf and flowers and a completely randomized single factor for the aqueous extract of *P. ostreatus*, in both cases with five concentrations (0%, 0.5%, 5%, 25%, 50% and 100%) and two nematodes (*Panagrellus redivivus* and *Meloidogyne* sp.).

## RESULTS

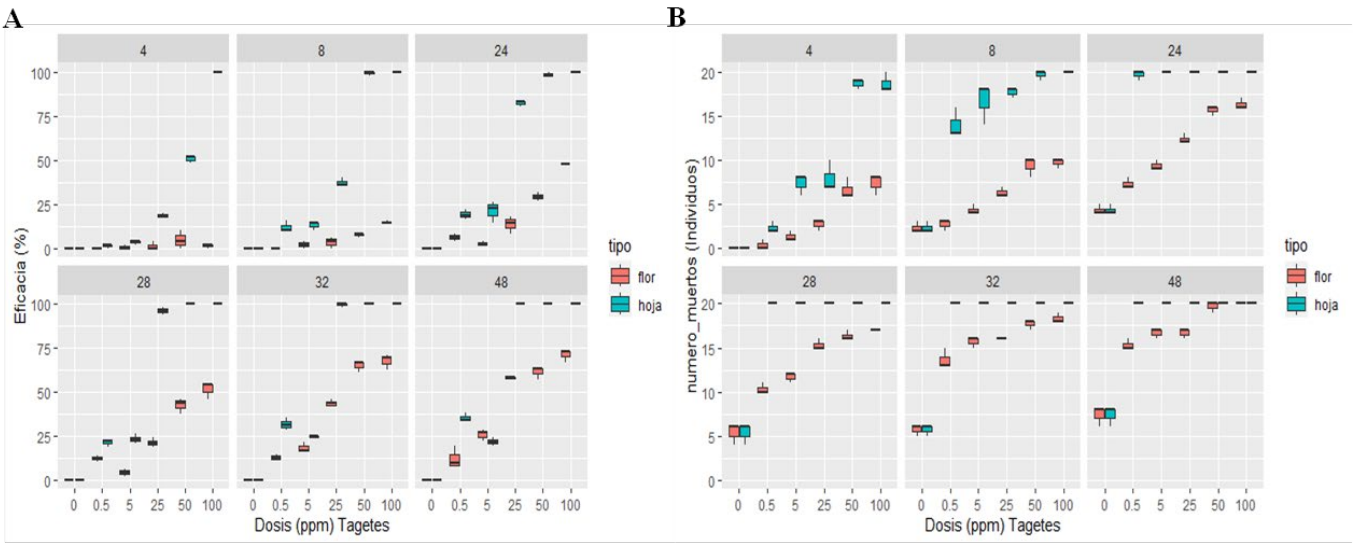


**Figure 3. Aqueous extract of *Tagetes minuta* leaf and flower against *Panagrellus redivivus* (A), Aqueous extract of *Tagetes minuta* leaf and flower against *Meloidogyne* sp. (B).**

Figure 3 (A) shows the efficacy of the aqueous extract of *T. minuta* leaf and flower on the mortality of the nematode *P. redivivus* during 48 h of exposure. The efficacy of the aqueous extract of *T. minuta* leaf was higher than that of flowers; this reached an efficacy in mortality more significant than 50% of the population of *P. redivivus* from the first 4 hours of exposure in concentrations of 50% and 100% whose efficacy was 50.57% and 100% respectively. Finally, after 48h of exposure, it reached an efficacy of 100% in the concentrations of 25%, 50% and 100%. On the other hand, the aqueous extract of *T. minuta* flower in none of the

concentrations managed to reach an efficacy of 100% in the mortality of *P. redivivus*; the maximum efficacy reached was 71% in the 100% concentration.

Figure 3 (B) shows the efficacy of the aqueous extract of *T. minuta* leaf and flower on the mortality of the nematode *Meloidogyne* sp. during 48 h of exposure, showing that the efficacy of the aqueous extract *T. minuta* leaf was superior to that of flowers, this reached an efficacy more significant than 50% in the mortality of the population of *Meloidogyne* sp. from the first 4h of exposure in the 50% and 100% concentrations with the efficacy of 93.33% in both cases and finally reached an efficacy of 100% after 48h of exposure in the concentrations of 0.5%, 5%, 25%, 50% and 100%. The aqueous extract of *T. minuta* flower reached an efficacy of 100% in the highest concentration at 100%.

The results of the mortality caused by *P. redivivus* and *Meloidogyne* sp. by the aqueous extracts of *T. minuta* leaf and flower showed through an analysis of variance at 48 h of exposure that in both cases the factors analyzed concentration (0%, 0.5%, 5%, 25%, 50%, 100%) and type (flower-leaf) were highly significant, therefore with the Tukey Test it was concluded that for the *P. redivivus* nematode the best concentrations were: 100%, 50% and 25%; and for the nematode *Meloidogyne* sp. the best concentrations were 100% and 50%; in both cases the best type of extract coincided with the aqueous extract of *T. minuta* leaf.

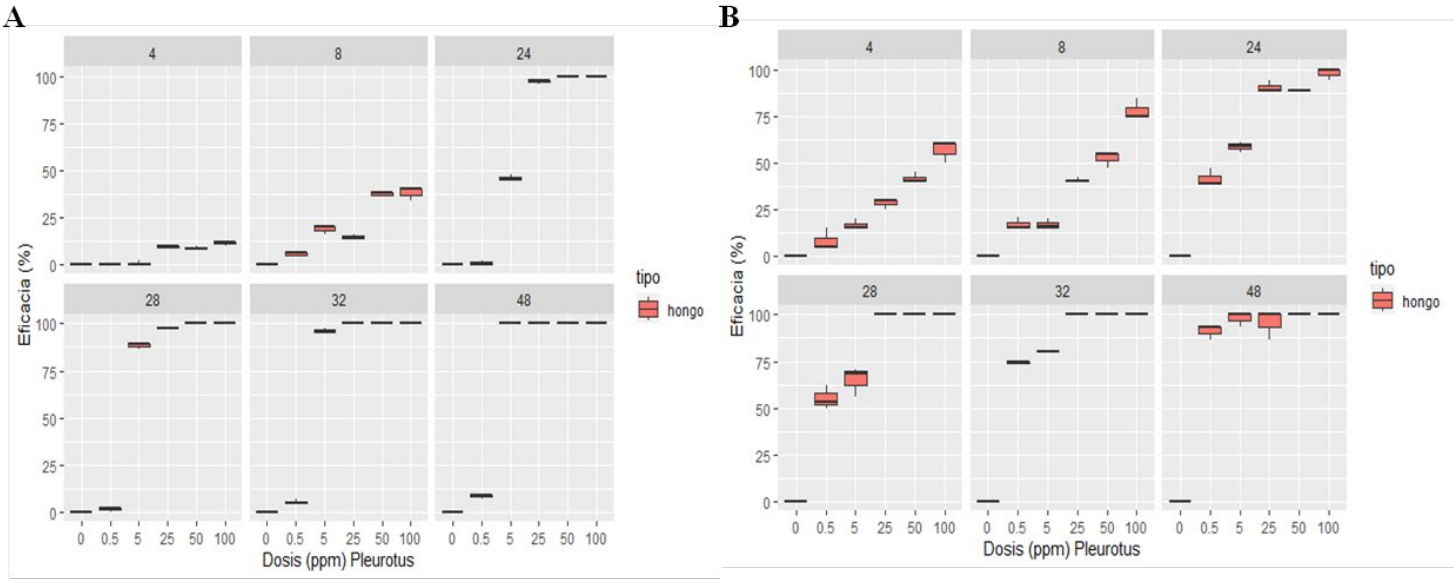


**Figure 4. Aqueous extract of *Pleurotus ostreatus* against *Panagrellus redivivus* (A), Aqueous extract of *Pleurotus ostreatus* against *Meloidogyne* sp. (B).**

Figure 4 (A) shows the efficacy of the aqueous extract of *P. ostreatus* on the mortality of the *P. redivivus* nematode during 48 h of exposure, showing that the effectiveness of said extract was greater than 50% at 24 h of exposure in concentrations at 25%, 50% and 100% with efficacy of 97.09%, 100%, 100% respectively and at 48h the efficacy of the aqueous extract on the mortality of *P. redivivus* was 100% in the concentrations at 5%, 25%, 50% and 100%.

Figure 4 (B) shows the efficacy of the aqueous extract of *P. ostreatus* on the mortality of the *Meloidogyne* sp. nematode during 48 h of exposure; this indicates that the extract presented an efficacy more significant than 50% at 4h of exposure in the 100% concentration with an efficacy value of 56.67% and at 48h the concentrations at 0.5%, 5%, 25%, 50% and 100% achieved an efficacy of 91.11%, 97.78%, 100% and 100% respectively.

The results of the mortality caused to *P. redivivus* and *Meloidogyne* sp. by the aqueous extract of *P. ostreatus* showed through an Analysis of Variance at 48 h of exposure that in both cases the factor analyzed concentration (0%, 0.5%, 5%, 25%, 50%, 100%) was highly Significant therefore with the Tukey test it was concluded that the best concentrations for both cases were: 100%, 50%, 25% and 5%.

| Time | ***T. minuta* leaf** | | ***T. minuta* flower** | | ***P. ostreatus*** | |
|---|---|---|---|---|---|---|
| | *P. redivivus* | *Meloidogyne* sp. | *P. redivivus* | *Meloidogyne* sp. | *P. redivivus* | *Meloidogyne* sp. |
| **4** | 132.15 | 306.74 | 15.66 | 40.10 | 14.21 | 481.06 |
| **8** | 26.58 | 7.42 | 830.58 | 67.39 | 217.24 | 607.09 |
| **24** | 13.04 | 0.01 | 246.32 | 251.81 | 5.39 | 20.47 |
| **28** | 8.03 | - | 35.01 | 115.59 | 2.40 | 11.97 |
| **32** | 13.20 | - | 95.16 | 11.76 | 1.48 | 3.30 |
| **48** | 19.22 | - | 37.38 | 50.59 | 0.89 | 0.07 |

**Table 1. $LC_{50}$ of the aqueous extract of *Tagetes minuta* and *Pleurotus ostreatus* against nematodes.**

According to treatments against *P. redivivus*, Table 1 shows that the lowest aqueous extract of *T. minuta* leaf was 8.03 ppm at 28h of exposure with a minimum limit of - 4.03 ppm and a maximum of 20.10 ppm, while in the case of the aqueous flower extracts the lowest $LC_{50}$ was 15.66 ppm after 4 hours of exposure with a minimum limit of -112.42 ppm and a maximum of 143.74ppm.

The highest $LC_{50}$ in the aqueous extract of the leaf was 132.15 ppm in the first 4 hours of exposure with a minimum limit of -160.14 ppm and a maximum of 424.43 ppm; on the other hand, the aqueous extract flower was 830.58 ppm after 8 hours of exposure with a minimum limit of -5929.97 ppm and a maximum of 7591.14 ppm.

The lowest $LC_{50}$ of *P. ostreatus* aqueous extract was 0.89 ppm at 48h of exposure, with a minimum limit of 0.84 ppm and a maximum of 0.95 ppm. In comparison, the highest $LC_{50}$ was 217.24 ppm after 8 hours of exposure with a minimum limit of -2776.20 ppm and a maximum of 3210.68 ppm. In this case, the $LC_{50}$ decreases as time passes.

According to *Meloidogyne* sp. results, the lowest aqueous extract of *T. minuta* leaf was 0.01 ppm at 24h of exposure with a minimum limit of 0.01 ppm and a maximum of 0.02 ppm. In contrast, in the aqueous flower extracts, the lowest $LC_{50}$ was 11.76 ppm after 32h of exposure, with a minimum limit of -194.39 ppm and a maximum of 217.91 ppm.

The highest $LC_{50}$ in the aqueous extract of the leaf was 306.74 ppm in the first 4h of exposure with a minimum limit of -4633.86 ppm and a maximum of 5247.33 ppm; on the other hand, the aqueous extract of flowers was 251.81 ppm after 24 hours of exposure with a minimum limit of -1972.92 ppm and a maximum of 2476.54 ppm. In the case of the aqueous extract of the leaf, it reaches its $LC_{50}$ in just 24 hours of exposure.

The lowest $LC_{50}$ of *P. ostreatus* aqueous extract was 0.07 ppm at 48h of exposure, with a minimum limit of 0.02 ppm and a maximum of 0.11 ppm. In comparison, the highest $LC_{50}$ was 607.09 ppm after 8 hours of exposure with a minimum limit of -3935.96 ppm and a maximum of 5150.14 ppm; in this case, the $LC_{50}$ decreases as time passes.

## DISCUSSION

The aqueous extract of *T. minita* leaf presented an efficacy of more than 50% in 48 h of exposure in concentrations of 25%, 50% and 100% against *P. redivivus* (Figure 1) and in concentrations of 0.5%, 5%, 25%, 50% and 100% in the case of *Meloidogyne* sp. (Figure 1). This result is similar to that of Murga[26], where the aqueous extract of *T. minuta* was used to control *Meloidogyne incognita* in paprika pepper. They concluded that *T. minuta* leaf could limit root nodulation by eliminating infective J2 juveniles up to 50%. In the same way, Iannacone[27] was observed to it cause a mortality more significant than 50% in eggs and J2 juveniles.

The efficacy to a greater or lesser degree of the aqueous extracts of *Tagetes* is directly associated to the presence of secondary metabolites, according to the evaluations made by Zygadlo[28], the aqueous extract of *T. minuta* leaves present dihydroxyacetone, (E)-tagetone and limonene; instead, the extract of flowers only have

(E)-tagetone, a reason that could explain the low efficacy of the flowers aqueous extract. However, these metabolites are toxic to specific organisms and microorganisms[18]; therefore, toxicity depends on the percentage in which it is present, and this, in turn, relies on the geographical location and abiotic factors such as high and low temperatures, drought, alkalinity, salinity, UV light, etc[29].

Aqueous or oily extracts that have terpenes with phenolic, hydroxyl, or carboxylic groups have been characterized by having greater nematicide activity[14]; the genus *Tagetes* presents these groups and in the case of *T. minuta* it gives the group of terpenes. These same metabolites have a mechanism of action that increases lipid peroxidation rates, causing an induction of oxidative stress[30].

The aqueous extract of *T. minuta* leaf presented the best results with both *P. redivivus* and *Meloidogyne* sp. due to its secondary metabolites. Dihydrotagetone and (E)-tagetone have been the most found in investigations related to the nematicide action of the genus *Tagetes* towards nematodes. When comparing the nematicide action of the extract of *Tagetes zypaquirensis* with a nematicide of chemical origin (carbofuran), the extract showed a similar action, reducing the populations of *Meloidogyne* spp. Therefore, they highlighted that this extract can become an alternative for managing root nodules[13].

Limonene is a metabolite capable of reducing the hatching of *Meloidogyne incognita* eggs by 72 to 79% in 14 days[18]. In this case, the essential oil was used, so there was the presence of (Z)-β-ocimene, which was attributed to the mortality of J2 juveniles in 72 hours; possibly, this metabolite is also found in the aqueous extract used and is the explanation for the high mortality rate of nematodes since it contains a minimum percentage of essential oil[31]. Limonene is one of the metabolites present in the genus *Tagetes* and stands out for causing the mortality of the J2 of *Meloidogyne* sp. linked to the percentage in which it is present[9].

The best $LC_{50}$ of the aqueous extract of *T. minuta* was that of the leaf; for *P. redivivus* it was 8.03 ppm (0.008 mg/ml) at 28h, and for *Meloidogyne* sp., it was 0.01 ppm (0.00001 mg/ml) after 24 hours of exposure (Table 1). The results of the $LC_{50}$ are different from those found by Herrera and Sandoval[32], who determined that the best $LC_{50}$ for *Meloidogyne* sp. of the ethanolic extract of *T. minuta* was 0.0017 mg/ml, possibly due to the type of extract handled, geographic location, and abiotic factors[29] since the data correspond to plant tissue collected in Peru. Zarate[33] determined the $LC_{50}$ of the essential oil extract of *Tagetes lucida*, a species of the *Tagetes* family, which was 0.06 mg/ml, which reduced between 63 and 80% of tomato root galls.

Regarding the exposure time of the $LC_{50}$, this is related to the time required by the metabolism of the nematode to unfold the secondary metabolites or with the speed of action of the metabolites of the oil inside the nematode[34]. In this case, it is related to the secondary metabolites present in the aqueous extract. Despite the lack of research on aqueous extracts, the values found for the $LC_{50}$ of the aqueous extract of *T. minuta* constitute a valuable reference for the management of plant substances.

The aqueous extract of *P. ostreatus* presented an efficacy more significant than 50% *against P. redivivus* (Figure 2) in concentrations of 5%, 25%, 50% and 100%, in the case of *Meloidogyne* sp. (Figure 2) in concentrations of 25%, 50% and 100% in both cases at 48h of exposure. Similar results show an efficacy in the mortality of 65.2% of *Globodera pallida*[12]. In addition, this aqueous extract of *P. ostreatus* was reported as having the ability to reduce the number of galls caused by *Meloidogyne incognita,* and they highlighted that this extract could be a promising measure for the control of this type of phytonematodes[35].

The efficacy of the aqueous extract of *P. ostreatus* on the mortality of both nematodes during 48 h of exposure it presented specific peaks of activity, in the case of *P. redivivus* the aqueous extract had a peak of efficacy at 24 h, while with *Meloidogyne* sp. it occurred in the first 4 h of exposure, in both cases the efficacy increased until reaching 100%, a similar investigation reported that *Pleurotus ostreatus* has a peak of activity during the first 4 h to 24 h of exposure[12].

*P. ostreatus* showed a high mortality rate of the nematodes, which is attributed to its toxic characteristics, which are typical of the genus *Pleurotus* sp. known to present several species with nematophagous activity, this activity is manifested through the system of production of immobilizing toxin reserves that they present in toxocysts that are produced laterally on the hyphae[36]. In the same way, Armas[37] identified these toxins,

finding in the case of *P. ostreatus* trans-2-decenedioic acid that corresponds to the nematotoxin called NRRL 3526[38].

The high mortality rate is due to the nematophagous activity that *P. ostreatus* presents, which begins when the nematotoxin present in the aqueous extract comes into contact with the nematode and immobilizes it, quickly digests it, producing hyphae that grow chemotropically and invade the oral cavity, the anus and the cuticle of the nematode[38,39]. The best $LC_{50}$ of the aqueous extract of *P. ostreatus* for *P. redivivus* was 0.89 ppm and for *Meloidogyne* sp. 0.07 ppm in both cases after 48 hours of exposure (Table 1).

After the review of the research that has been carried out on this subject, all of them have aimed to determine the efficacy of the aqueous extract as a nematicide, but the $CL_{50}$[37,40,41] has not been defined. Despite this, each of the investigations emphasizes the particular mode of action and the efficacy of *P. ostreatus*, which is related to its ability to secrete trans-2-decenedioic acid and some proteases that have not yet been described[42]. The extract has a high nematicide efficacy, so it is advisable to take precautions when this type of extract is taken to the field since the effect of the metabolites can change once they interact with other molecules found in the soil[43].

## CONCLUSIONS

The aqueous extracts of *Pleurotus ostreatus* and *Tagetes minuta* leaf and flower showed a nematicide effect on *Panagrellus redivivus* and *Meloidogyne* sp. The aqueous extract of *Tagetes minuta* leaf was better than that of flowers; an efficacy of 100% of the aqueous extract of *Tagetes minuta* leaf was achieved against *Panagrellus redivivus* in concentrations of 25%, 50% and 100% for *Meloidogyne* sp. in concentrations 0.5%, 5%, 25%, 50% and 100% after 48h of exposure. In the case of the aqueous extract *Pleurotus ostreatus*, an efficacy of 100% was achieved against *Panagrellus redivivus* in the concentrations 5%, 25%, 50% and 100%, for *Meloidogyne* sp. in concentrations 0.5%, 25%, 50% and 100% at 48h of exposure.

The best lethal Concentration 50 ($LC_{50}$) of the aqueous extract of *Tagetes minuta* leaf and flowers was that of leaves; for *Panagrellus redivivus,* the $LC_{50}$ of 8.03 ppm in 28 h of exposure and for *Meloidogyne* sp., the $LC_{50}$ of 0.01 ppm in 24 h of exposure. Regarding the aqueous extract of *Pleurotus ostreatus*, the best $LC_{50}$ against *Panagrellus redivivus* was 1.22 ppm in 48 h of exposure and against *Meloidogyne* sp. 0.01 ppm in 48 h of exposure.

**Author Contributions:** Madison Chango: collection and analysis of data in the laboratory, presentation of results, discussion and writing of the draft article. Gabriela Rosero: laboratory data collection, review, editing and translation of the article. Norma Erazo: direction of the research project and laboratory methodology. Pablo Álvarez: approach to the experimental design and statistical analysis.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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