Al-Qadisiyah Journal of Pure Science
ISSN(Printed): 1997-2490 ISSN(Online): 2411-3514
DOI: 10.29350/jops
Al-Qadisiyah Journal of Pure Science

Evaluation Of The Effectiveness of Alcoholic Extract of Tobacco Leaves in Controlling The Biting Lice Parasitizing Local Chickens in The Laboratory in Iraq

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Received: 06/05/2026; Accepted: 20/05/2026; Published Online: 20/05/2026.

Abstract

The results of this experimental research have proved that the alcoholic extract of tobacco leaves and the commercial insecticide Wormix Drop showed significant effectiveness in the fight against lice infesting local chickens in a controlled condition. The experiment design was such that it included well-defined negative control and treatment groups to accurately measure the actual effects of the agents under test and reduce confounding variables to a minimum. Moreover, rigorous acclimatization procedures and comprehensive clinical and microscopic analyses were used, which guaranteed the reliability and validity of the results. Such results indicate that the toxic-free extract of tobacco leaves could be an effective alternative or complementary method to using traditional commercial insecticides in the control of external parasites in chickens through a plant-based solution. However, further studies are recommended to evaluate long-term safety and large-scale field applicability.
Group 1 (G1) was treated with 40 mg/kg of an alcoholic extract of banana leaves.

Group 2 (G2) was treated with 80 mg/kg of an alcoholic extract of banana leaves.

Group 3 (G3) was treated with the conventional herbicide Wormix Drop

Group 4 (G4) was left as a positive (infected) group without any treatmen.

Group 5 (G5) was left as a negative (uninfected) group.

Keywords : Nicotiana tabacum; Alcoholic extract; Biting lice; Poultry ectoparasites; Botanical insecticide; In vitro evaluation.

1.Introduction

Lice infestations are a common health problem in birds, causing significant discomfort as the external parasites feed on the bird's blood or skin, leading to a range of adverse clinical and physiological effects. According to(Labrique *et al.*, 2010) and(Permin & Ranvig, 2001), lice feed on feathers, skin, and blood, resulting in feather loss, general weakness, and reduced productivity.While a limited number of lice infestations pose little threat to birds, increased infestation density often leads to the spread of parasites from the natural host to neighboring hosts. This finally leads to increased mortality rates of the affected species with the change in the host (Morand, 2000). Moreover, the size of birds is also a major determinant of the success and survival of parasites because bigger birds offer a variety of habitats, which allow the lice to avoid the immune system of the host. On the other hand, the smaller birds with the lice have the problem of locating a new host, and the rate of mortality is high during the process of translocation (Rozsa, 1997).

3. Materials and Methods

3.1 Collection of Birds

A total of 140 domestic chickens (Gallus gallusdomesticus) infested with lice were collected from local markets in the city of Al-Diwaniyah during the period from the beginning of August 2025 until September of the same year.

3.2 Examination of Birds and Sample Preservation

The birds were examined visually and with the aid of a magnifying lens to isolate lice from feathers in different body regions. Lice were manually collected using forceps and a fine brush (Clayton & Drown, 2001)The isolated samples were then preserved in 70% ethyl alcohol and glycerin following the method described by (**Al-Hab**, **1979**)for subsequent identification.

3.4Preparation of Isolated Samples

The isolated samples were washed with physiological normal saline to remove any residual host contents. Subsequently, the lice were transferred from 70% ethyl alcohol to a cold potassium hydroxide (KOH) solution at a concentration of 10% and left for 24 hours for clarification. The samples were then rinsed with distilled water and immersed in xylene for 1–2 minutes. Afterward, the specimens were mounted on clean glass slides using D.P.X mounting medium, covered with a coverslip, and left to dry prior to microscopic examination.

Experimental study3.5

Fifteen adult local chickens were housed individually under controlled conditions and acclimatized for one week. Three birds were confirmed free of external and internal parasites by clinical examination and microscopic fecal analysis and served as the negative control. The remaining birds were naturally infested with lice, as confirmed by visual inspection of feathers and skin, particularly in the thighs, vent, under-wing, and breast regions. Birds were allocated into five groups (n = 3): two groups treated with an alcoholic extract of Nicotiana tabacum leaves (40 or 80 mg/kg, spray application), one group treated with Wormix Drop, a positive

(infested, untreated) control, and a negative (non-infested) control.Regarding the conventional (chemical) insecticide, it was applied in accordance with the method prescribed by veterinarians and as specified on the product label by the manufacturer.

3.6Application of the Parasitic Insecticide Wormix Drop

Wormix Drop, which contained ivermectin (0.5%), was taken as a standard chemical insecticide to be compared with the alcoholic extract of Nicotiana tabacum leaves. The treatment was given as recommended by the veterinary and manufacturers, with slight modifications to the body weight of adult chickens. Birds were held carefully by means of restraints, and feathers were pulled back at the back of the neck to show the skin. Two drops of the formulation were placed on the skin with the original dropper: one at the skull base and the other one in the line of the mid-back. The first day was the only day of administration of the dose. The birds were kept in clean cages where they were treated and monitored clinically during the experimental period to determine the intensity of infestation by lice and to determine any ill effects.

3.7Infestation Assessment Method

The pretreatment assessment was done to measure the severity of the lice infestation. Assessment was then done again after 1, 3, 7, and 14 days of treatment to see the slow reduction in the number of lice.

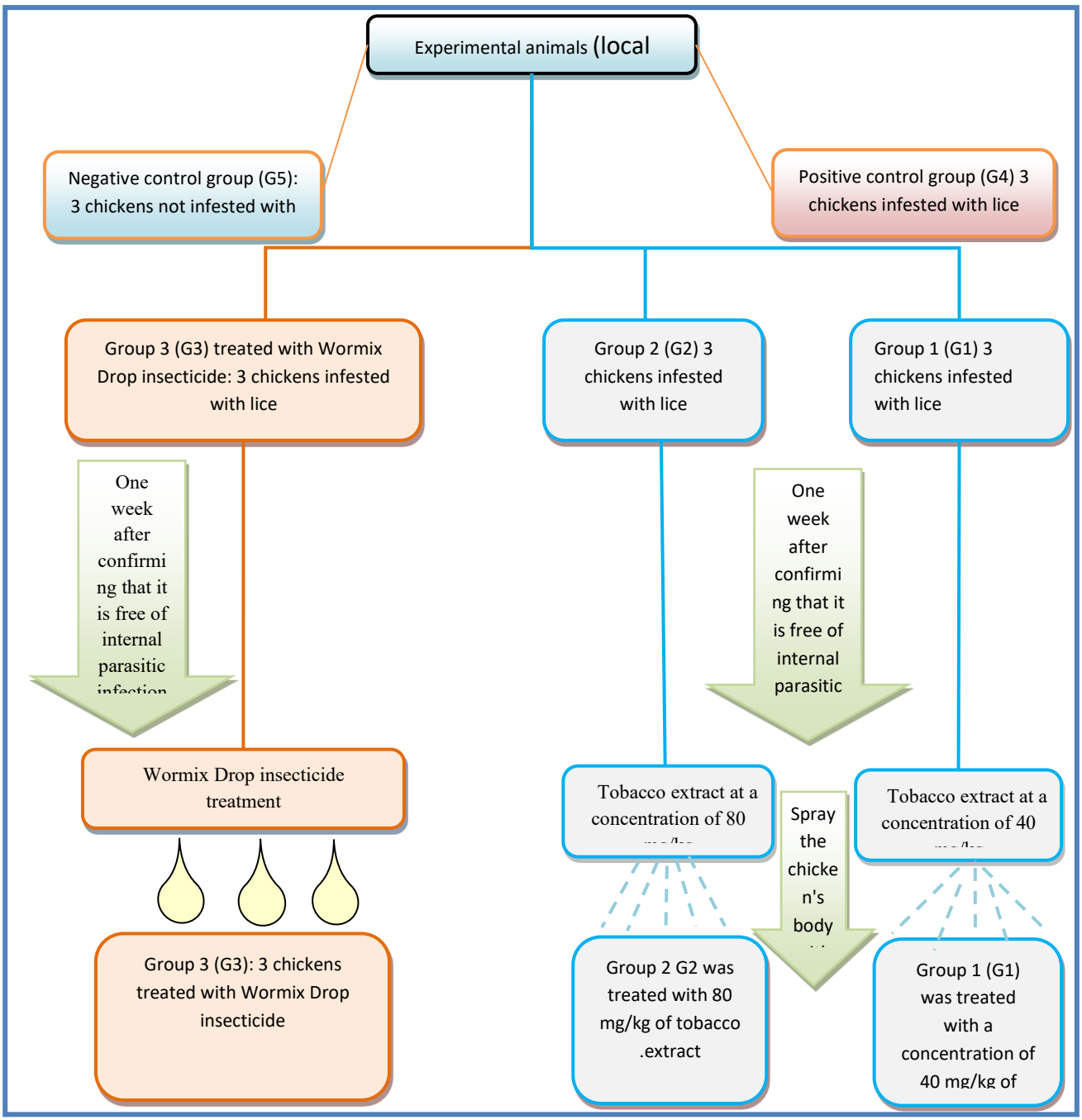
It was counted in the following standard steps:

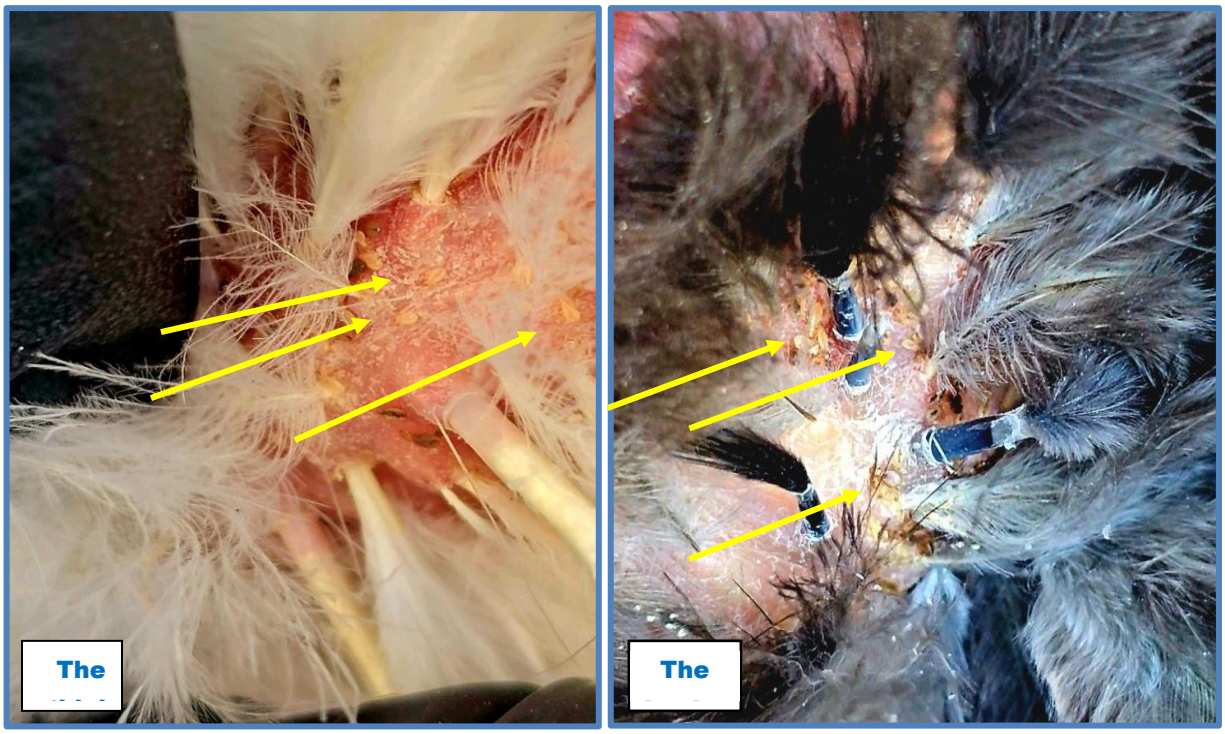
1. Manually restrain the bird and examine common infestation areas: (underwings, back, around the cloaca, and feather base).
2. Divide the body into five fixed examination zones for standardized measurement.
3. Count adult lice and nymphs visible to the naked eye using a hand lens.
4. Record the results for each bird on a separate form, noting the date and type of treatment.
5. Calculate the average infestation for each group and then compare the reduction between measurements before and after treatment.

The treatment effectiveness assessment standard was adopted according to the following equation:

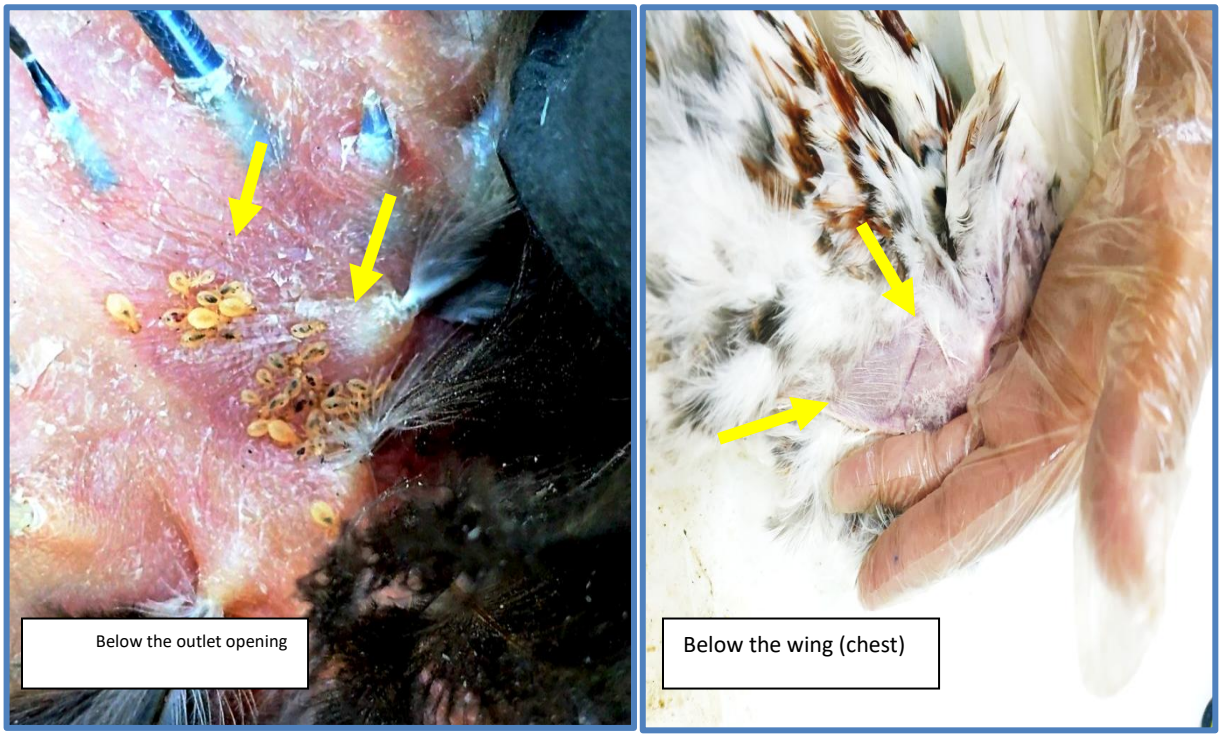
$$\%\mathbf{100} \times \frac{\text{Average iniury before treatment} - \text{Average iniury after treatment}}{\text{Average iniury before treatment}} = \text{Effectivness}\%$$

FigureExperimentalGroupsDesign)

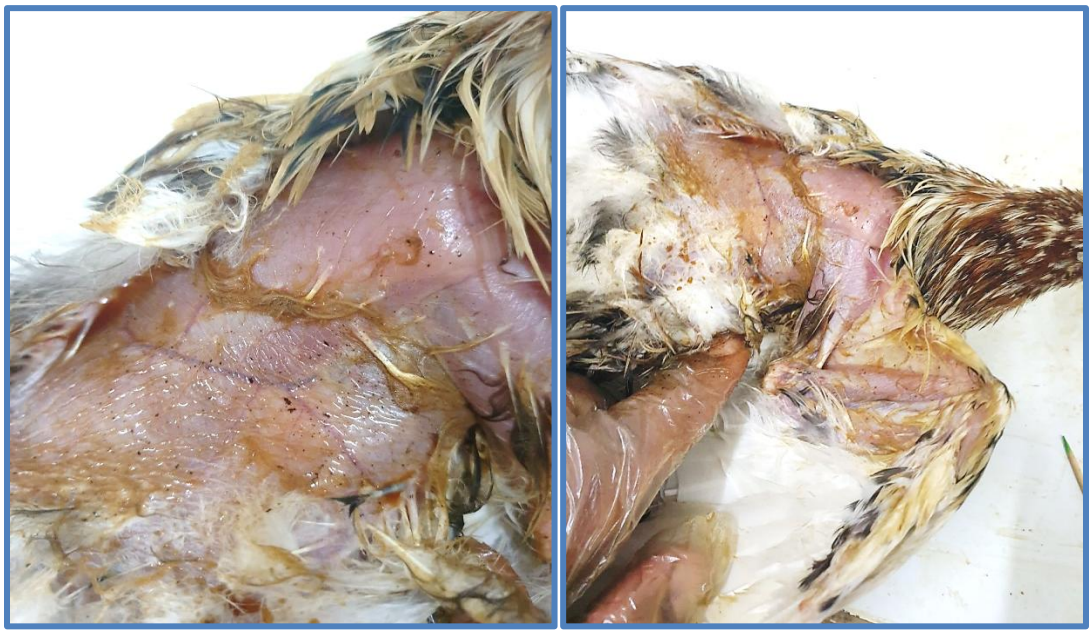




.Natural lice infestation and spread of lice on the groin and back(1-3)picture



Picture3.2 :(Natural lice infestation and spread of lice above the wing exit opening.



Picture3-3The experiment was carried out using the alcoholic extract of tobacco leaves at 80 and 40 mg/kg by spraying it as a mist onto the chicken's body.

4.The result:

4-1Relative therapeutic efficacy (%) of 40% alcoholic tobacco leaf extract against chicken lice during different exposure times

Percentage of treatment efficacy.	Live lice count after treatment	Treatment period	group; Live lice count before treatment	Type of material
10	9	10m	11	Extract tobacco 40% (G1)
70	3	1h	11	
100	0	6h	11	
100	0	24h	11	

The results indicate that the effectiveness of the 40% tobacco extract was clearlyrelated to the exposure time, as the 10-minute treatment showed a limited decrease in the number of lice from (11) to (9) individuals, with a treatment efficiency rate of (10%).

4.2Relative therapeutic efficacy (%) of80% alcoholic tobacco leaf extract against chicken lice during different exposure times

Type of material	Live lice count befor treatment	Treatment period	Live lice count after treatment	Percentage rate of therapeutic efficacy
Extract tobacco 80% (G2)	12	10m	5	50
	12	1h	1	90
	12	6h	0	100
	12	24h	0	100
The lice count in the control group was positive	10			

The results show that using tobacco extract at a concentration of 80% reduced the number of live lice from an average of (12) before treatment to (0) after 6 hours and 24 hours, achieving a therapeutic efficiency of 100%.

4.3Relative therapeutic efficacy (%) of the chemical insecticide WORMIX DROP (Ivermectin 5 mg/ml) against chicken lice during different exposure times

Type of material	Live lice count befor treatment	Treatment period	Live lice count after treatment	Percentage rate of therapeutic efficacy
Wormix Drop (G3)	10	10m	10	0
	10	1h	9	10
	10	6h	8	20
	10	24h	6	40
	10	48h	4	60
The lice count in the control group was positive.	10			

(Campbell, 2018)pointed out the effectiveness of ivermectin and abamectin against external parasites. Ivermectin belongs to the group of "macrocyclic lactones" and is the active ingredient in Wormix Drop. This substance works byParalyzing the neuromuscular system: The substance binds to glutamate-gated chloride channels found in the nerve and muscle cells of the parasites.

4.4Comparison of relative therapeutic efficacy (%) between alcoholic extract of tobacco leaves at different concentrations and the chemical pesticide (WORMIX DROP) against chicken lice over different time periods

Type of material	Time after treatment	Extract tobacco 40%	Extract tobacco 80%	Wormix Drop
Extract tobacco 40% **Extract tobacco 80%** **Wormix Drop** **(G1,G2,G3)**	10m	10	50	0
	1h	70	90	10
	6h	100	100	20
	24h	100	100	40
	48h	100	100	60

The results indicate that:

The highest concentration of the extract (80%) achieved faster and greater effectiveness in killing or inhibiting parasites compared to the lowest concentration (40%).

The effectiveness of the therapeutic agents increased with increasing exposure time from 10 minutes to 48 hours, indicating that longer exposure enhances the antiparasitic effect.

The best results were shown by Wormix Drops, which achieved 100 % efficacy during prolonged intervals (24 and 48 hours), indicating that the active compounds in this product might be stronger or have a greater capacity to bind to parasite receptors.

5 .Discussion:

5.1 Relative therapeutic efficacy (%) of 40% alcoholic tobacco leaf extract against chicken lice during different exposure times

The findings indicated that the therapeutic efficacy of the 40 percent alcoholic extract of tobacco leaves has a strong dependency on the exposure time. A small decrease in the number of lice was witnessed in short contact periods, and this was attributed to inadequate absorption of active compounds. The extension of exposure time to one hour produced an efficacy of 70, which means the development of toxic activity related to the build-up of bioactive substances,

especially nicotine and other alkaloids, which interfere with the cholinergic nervous system of insects, causing them to become paralysed and eventually die. There was a 100 percent mortality after 6 and 24 hours of exposure, which supported the high level of effectiveness of the extract in the presence of long contact conditions. These results are in line with the earlier studies that have indicated the cumulative effect of botanical insecticides and the antiparasitic effect of tobacco extract against ectoparasites with less residual toxicity and environmental hazards. In turn, the current findings affirm the possibility of a tobacco leaf extract with 40 percent content to be used as a feasible and, to a great extent, safe alternative to be used as a part of an integrated control initiative in poultry lice.

5.2Relative therapeutic Efficacy (%) of80% Alcoholic Tobacco leaf Extract against Chicken lice during different exposure times

The extract realized 50% efficacy with only 10 minutes of contact, which demonstrates the strong knockdown of nicotine and other active components in tobacco on the nervous system of the parasites. When the length of contact was increased to one hour, the efficacy reached 90 percent, with complete eradication realized after six hours, which suggests the current scientific trend of biopesticide searching for biopesticides as alternatives to chemical pesticides to reduce their toxicity and resistance to drugs. The potency of the extract is attributed to the fact that nicotine, an alkaloid that is an effective neurotoxin to ectoparasites, is present in large quantities in it. (Gahukar, 2018)Nicotine acts by binding to the nicotinic acetylcholine receptors (nAChRs) of the nervous system of the lice, overstimulating the nervous system, causing convulsions and eventual death.(Quadros et al., 2023).

5.3Relative therapeutic efficacy (%) of the chemical insecticide WORMIX DROP (Ivermectin 5 mg/ml) against chicken lice during different exposure times

The experimental result presented in the table is a laboratory/field experiment that determines the effect of the active ingredient (ivermectin) at different exposure times (10 minutes, 1 hour, 6 hours, 24 hours). It is possible to observe a linear relationship between time and efficacy: the efficacy of the treatment reaches 0 % with exposure of a very short duration (10 minutes) and slowly rises to 40 percent after 24 hours.

Survival rate: The population of lice diminished to 10 (pre-treatment) and 6 (24 hours), which means that there was not an instantaneous reaction but a gradual one.

5.4Comparison of relative therapeutic efficacy (%) between alcoholic extract of tobacco leaves at different concentrations and the chemical pesticide (WORMIX DROP) against chicken lice over different time periods

The table shows the effectiveness of two concentrations of tobacco extract (Nicotiana tabacum) (40 and 80 %) and Wormix eye drops in relation to parasites or worms (the placebos in the case) at various time points (between 10 minutes and 48 hours). The results indicate that:

In comparison with the low concentration (40%), the high concentration (80%) of the extract was found to be faster and more effective in killing or inhibiting movement.

The therapeutic effect of the therapeutic agents was found to rise with the length of exposure between 10 minutes and 48 hours, indicating that more time is beneficial for the effect of antiparasitism.

Wormix eye drops were the most effective treatment, with 100 percent efficacy during a longer duration (24 and 48 hours), which indicated that the active constituents in this product were more effective or had a higher capacity of binding to parasite receptors.

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