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Research Article

Effect of Floor Cleaner Combination of Alcohol Ethoxylate-Sodium Lauryl Ether Sulfate and Combination of Carbol-Pine Oil on *Ascaris lumbricoides* Eggs

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Abstract

Disinfectants are chemical agents used in disinfection in liquid form or solution form and are well-known as microbicidal, fungicidal, and virucidal but still unknown as parasitocidal, especially the effect to *A. lumbricoides*. In Indonesia, the prevalence of ascariasis is about 30,4% and still high. Ascariasis is caused by *A. lumbricoides* helminth that human swallows in egg form. *Ascaris lumbricoides* egg's characteristics are hydrophobic and sticky, making it easy to stick on the floor, household, and skin. This research aims to find the difference between the combination of the effects of Alcohol Ethoxylate-Sodium Lauryl Ether Sulfate and the combination of Carbol-Pine Oil to *A. lumbricoides* eggshell and larva development as prevention and to break the life cycle chain of *A. lumbricoides*. The research results showed no effects from Alcohol Ethoxylate-Sodium Lauryl Ether Sulfate combination and Carbol-Pine Oil combination to *A. lumbricoides* eggshell and embryo development.

Keywords: Disinfectants, ascariasis, concentration, eggshell, larva development.

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INTRODUCTION

Worm disease is still a public health problem in Indonesia because it is classified as a disease that has received less attention (neglected disease). Worms are caused by intestinal nematodes, which in their life cycle require soil to develop into an infective form (soil-transmitted helminths - STH). These groups include *Ascaris lumbricoides* which causes ascariasis; *Trichuris trichiura* causes trichuriasis; *Strongyloides stercoralis*, which causes strongyloidiasis and hookworms; *Ancylostoma duodenale* and *Necator americanus*, which causes ankylostomiasis and necatoriasis.¹ STH prevalence in Indonesia is relatively high, around 58.5% and caused mainly by *A. lumbricoides*, 30.4%. In some areas, in Sumatra the prevalence of ascariasis is around 78%, Kalimantan 79%, Sulawesi 88%, West Nusa Tenggara 92%, and West Java 90%.² The high prevalence of ascariasis is partly due to the ability of female *A. lumbricoides* worms to produce as many as 200 000 eggs/day, which are excreted in faeces.³

In the soil, the eggs will develop into infective eggs that contain larvae. When humans ingest the infective eggs, the eggs will hatch into larvae in the small intestine. The larvae will penetrate the intestinal mucosa, then be carried by the portal blood flow and follow the blood flow to the lungs and up to the pharynx. The larvae in the pharynx will stimulate the cough reflex so that the larvae are swallowed back and enter the small intestine. In the small intestine, the larvae

will develop into adult worms.^{4,5}

A. lumbricoides eggs have three protective layers: the innermost layer, the middle layer, and the outermost layer, which function as a defence against adverse conditions or environments for the eggs. The innermost layer of *A. lumbricoides* eggs is the lipoprotein layer. There is a space called the perivitelline space in this layer, which contains perivitelline fluid and larvae. In this room, there is also a layer of chitin which is the middle layer of the egg and functions as a layer that gives shape to the egg, and the outermost layer is the vitelline layer which contains glycoproteins or is called the albuminoid layer. Eggs of *A. lumbricoides* are also hydrophobic and easy to adhere to, allowing the eggs to adhere to various objects such as floors, household furniture, fruits and vegetables and human skin.^{5,6} The development of *A. lumbricoides* eggs depends on the soil, humidity, temperature, rainfall, wind, exposure to sunlight and oxygen. Eggs develop well at soil temperatures between 25-30°C, high humidity, not exposed to direct sunlight. In addition, the wind can accelerate the drying of eggs and facilitate the spread of eggs through the dust. Furthermore, the type of clay, sandy, loose and mossy, is suitable for egg development because this type of soil is rich in oxygen which is suitable for egg development. Eggs cannot develop or even die at low humidity in soil types that do not contain much oxygen, exposure to direct sunlight and high rainfall.⁷ In addition, the disinfectant can also kill the eggs of

A. lumbricoides. The 10% povidone-iodine content in the disinfectant was proven to be able to kill *A. lumbricoides* eggs after six weeks of incubation.⁸

Alcohol ethoxylate is a non-ionic surfactant that works by denaturing micro-organism proteins (MO). Alcohol plays a role in protein denaturation, while ethoxylate is a moisturizing agent that accelerates the denaturation of MO protein. Sodium lauryl ether sulfate (SLES) is a natural anionic surfactant with bacteriostatic properties against gram-positive bacteria, is microbicidal against human immunodeficiency virus (HIV) type 1 and is functional but is not effective in killing gram-negative bacteria. Carbol or other names of phenol is a disinfectant that has bactericidal, fungicidal, viricidal, and tuberosidal properties.^{9,10} Pine oil resulting from hydrodistillation of several types of pine species has antimicrobial properties against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, and *Candida albicans* fungi.¹¹ Therefore, research is needed to determine the effect of the combination of alcohol ethoxylate-sodium lauryl ether sulfate and carbol-pine oil contained in floor cleaners sold in the market on *A. lumbricoides* eggs.

Based on the background of the research above, the problem answered in this study is "Do PL I and II disinfectants affect *A. lumbricoides* eggs?" The purpose of the study was to determine whether PL I and II disinfectants affected *A. lumbricoides* eggs.

LITERATURE REVIEW

Soil-transmitted helminths (STH) are a group of species that infect humans with soil as a transmission medium and part of their life cycle.¹² There are four species in the STH group that most commonly infect humans, including *A. lumbricoides*, *T. trichiura*, *A. duodenale*, *N. americanus*. *Ascaris lumbricoides* was the most common cause with about 807 to 1121 million cases, followed by *T. trichiura*, around 604 to 795 million cases and *N. americanus* and *A. duodenale* around 576 to 740 million cases.^{2,13} Soil-transmitted helminths are widespread in several countries with tropical and subtropical climates, such as America, sub-Saharan Africa, China and East Asia. More than 1.5 billion people from 24% of the world's human population are reported to have been infected with STH globally, with more than 267 million preschool-aged children and more than 568 million school-aged children infected living in endemic areas. Soil-transmitted helminths can infect humans through vegetables contaminated with infective eggs, the behaviour of children who like to play on the ground, the habit of putting their hands in their mouths, and through drinking water contaminated with infective eggs. STH infection can cause health problems in humans, including anaemia, malnutrition, decreased appetite, dysentery, diarrhoea and loss of essential substances needed by the body such as protein, iron, and vitamin A.¹⁴

Ascariasis is a disease caused by infection with *A. lumbricoides* or roundworms. Humans are the definitive host of *A. lumbricoides*, and infection can occur via the oral-faecal route by consuming food or water that has been contaminated with infective eggs. In addition, poor hygiene behaviour such as eating habits without washing hands first is also a way of transmitting STH.^{15,16}

On a global scale, the prevalence of ascariasis is still relatively high. CDC, United States reported in 2013 that about 807 to 1121 million human beings had been infected with *A. lumbricoides* worms with the highest morbidity found in children. In Asia, such as India, more than 50% of adults are infected with *A. lumbricoides* worms, while in

Malaysia, it is about 25.7% of the population infected. In the Philippines, about 54.5% of children are infected.¹⁷ The prevalence of ascariasis in Indonesia is still high, which varies in various regions. In Sumatra the prevalence is around 78%, Kalimantan 79%, Sulawesi 88%, West Nusa Tenggara 92%, and West Java 90%. It is evidenced by the research and development workshops P2B2 Tanah Bumbu in 2008 and 2009 in 13 districts and cities of South Kalimantan Province. The study found that 10% of schoolchildren suffer from ascariasis. Another study on elementary school children in grades IV, V, and VI conducted in the coastal area of Makassar city in 2013 found that around 34% had ascariasis. Another study conducted on students of SD Negeri 29 Purus Padang, West Sumatra, found that 33% had ascariasis.¹⁸

Risk Factors for Ascariasis-There are several risk factors that can cause infection with *A. lumbricoides*, namely: a) The habit of not washing hands before eating and after defecating; b) Defecation behavior is not in the right place, causing soil and water contamination by *A. lumbricoides* eggs which can be easily swallowed through the habit of putting hands in mouth and through drinking or food such as vegetables that are not processed correctly, or washing vegetables using water contaminated with *A. lumbricoides* infective eggs; c) Poor home sanitation and lack of access to clean water; d) The unavailability of waste water disposal facilities and proper bathing, washing and latrine facilities; e) Climatic factors, in areas with high rainfall and warm environmental temperatures, can help accelerate the development of *A. lumbricoides* eggs; e) The low level of knowledge due to low education, especially parents, will affect the healthy behavior of all family members regarding sanitation and personal hygiene; and f) Immune status, immunocompromised individuals, especially HIV/AIDS, will have cellular immune dysfunction, thereby facilitating infection with *A. Lumbricoides*.¹⁹

The morphology of *A. lumbricoides* is divided into two: egg morphology, which consists of two types of eggs, namely fertile and infertile eggs, which can sometimes be accompanied by an albuminoid layer or without an albuminoid layer (decortication) and the morphology of adult worms. In *A. lumbricoides* eggs, there are three protective layers: the lipoprotein layer composed of 25% protein and 75% ascaroside. Ascaroside is a glycoside bond composed of glucose bonds with alcohol that cannot be penetrated by a substance soluble in water, fat, or even gas. Then, a lipoprotein layer forms a space containing perivitelline fluid and larvae called the perivitelline space. After the vitelline space, there is a thick, protein-rich layer called the chitin layer. This layer gives shape to the eggs of *A. lumbricoides*. After that, the outermost layer protects the eggs of *A. lumbricoides*, namely the vitelline layer. This layer is composed of glycoproteins and acylated, which will later form a uterine layer. This layer will turn into the uterus.²⁰ *A. lumbricoides* eggs consist of fertilized eggs or fertile eggs and unfertilized eggs, and the fertilized egg will continue the worm's life cycle until it reaches the adult stage in the human intestine. Meanwhile, unfertilized eggs cannot continue the parasite life cycle because no embryo can develop into larvae.

Fertilized eggs (fertile): Round or ovoid with a thick protective layer measuring 60-70 m x 40-50 m. Brown or yellow-brown in colour. The egg contains a single cell separated by a thick protective layer. On the egg wall, there is mammized albumin which serves as a protective layer. Unfertilized eggs (infertile): They are longer than fertilized eggs, triangular or kidney-like, with a thin protective layer 85-95 m x 35-45 m. Contains a mass of globules, granules, and the mammary layer's shape is more varied than that of a

fertilized egg. Adult worms: Cylindrical in shape, male worms measuring 15-30 cm x 0.2-0.4 cm and female worms measuring 20-35 cm x 0.3-0.6 cm. The posterior part of the female worm is straight, while the male worm is curved. Has three lips at the anterior end of the female and male worms.²¹

Life Cycle of *Ascaris lumbricoides*: Adult worms live in the small intestine. A female worm can lay about 200 000 eggs per day, then be expelled with faeces. Eggs that come out consist of fertilized and unfertilized eggs. Unfertilized eggs can be ingested by humans and digested by the human digestive system but do not cause clinical manifestations because unfertilized eggs are not infective. Unlike the unfertilized egg, the fertilized egg contains an embryo that will develop and become infective in approximately three weeks. The development of eggs is very dependent on environmental conditions. Soil with high humidity and warm temperatures is an excellent medium for developing eggs into infective forms. When humans swallow the infective eggs, the eggs will hatch, and the larvae will penetrate the intestinal mucosa, then carried by the portal blood flow and follow the bloodstream to the lungs. Then the larvae will settle in the lungs and develop into mature larvae for 10 to 14 days. Mature larvae will penetrate the alveolus wall, enter the alveolus cavity, then ascend the bronchial tract to the bronchi, trachea, and pharynx. When the larvae are in the pharynx, a cough reflex occurs so that the larvae are swallowed and enter the small intestine. Mature larvae will develop into adult worms in the small intestine and can live for one to two years.

Pathology and Clinical Manifestations: Clinical manifestations that arise in patients with ascariasis occur due to adult worms and larvae found in the patient's body, including a) Pulmonary ascariasis, also known as Loeffler's syndrome, is caused by larvae migrating in the lungs. Symptoms include coughing up blood, shortness of breath, fever, eosinophilia and on chest X-ray; b) Intestinal ascariasis, occurs due to the presence of adult worms in the patient's intestines; c) Malabsorption and intestinal obstruction; and c) Hepatobiliary ascariasis, occurs due to adult worms that enter and exit the bile duct from the duodenum actively. Symptoms that arise in this disease are a pain in the right hypochondrium that is continuous or intermittent, jaundice, high fever, upper abdominal pain, vomiting, chills, hypotension and hepatomegaly.²²

Diagnosis of Ascariasis: The diagnosis of ascariasis can be confirmed by direct stool examination. One gram of the patient's fresh faeces was taken and made preparations that were then dripped with distilled water and observed under a microscope. The presence of eggs in the stool on direct examination confirms the diagnosis of ascariasis. In addition to direct stool examination, other techniques can be used to establish the diagnosis of ascariases, such as thick Kato-Katz preparations, simple sedimentation and flotation examination.²³

Treatment of Ascariasis: Several types of drugs can be used to treat ascariasis patients: a) Albendazole and mebendazole, which can be used as individual treatments or as a preventive measure against worms en masse for adults and children (The dose for children and adults is the same, namely a single dose of 400 mg after meals orally for albendazole and a single dose of 500 mg or twice a day 100 mg for three days orally for mebendazole); and b) Ivermectin, is another type of drug that can be given as a treatment for ascariasis sufferers and also as a preventive measure against worms that is only found in the United States. The dose that can be given is 150 to 200 mcg/kg in a

single oral dose.²⁴

Disinfection And Disinfectant: Disinfection is the process of eliminating all pathogenic micro-organisms, except for bacterial endospores. Chemical or physical agents used in disinfection in the form of liquids or solutions are called disinfectants. Based on the concentration, disinfectants are divided into three types, namely: 1). high-level disinfectant that can eliminate all types of micro-organisms (MO) in a short duration except for endospore bacteria, 2). medium-level disinfectants that can kill mycobacteria, vegetative bacteria, some types of viruses, and some fungi but cannot kill bacterial endospores, and 3). low-level disinfectant that can kill vegetative bacteria, some types of viruses, and some types of fungi. Chemical-based disinfectants are most often used in disinfection. The active substances contained in chemical disinfectants can be a combination of several active substances or only consist of one type of active substance.²⁵

Disinfectant Phenol Coefficient: The disinfectant phenol coefficient measures the ability of phenol as an antimicrobial agent in a disinfectant to kill bacteria compared to standard phenol. A disinfectant can be said to be successful in killing bacteria if it has a phenol coefficient > 1, and if a disinfectant has a phenol coefficient = 1 or < 1, then the disinfectant can be said to have failed in killing bacteria and can make bacteria resistant to phenol. Therefore, the higher the phenol coefficient of a disinfectant, the better it will kill bacteria.²⁶

Ethoxylate Alcohols: Ethoxylate alcohols (AE) are non-ionic surfactants that contain hydrophobic alkyl bonds and belong to alkoxyate alcohols separated by an ether group in an ethyl oxide (EO) bond and have the chemical structure R (OCH₂CH₂), with R as the alkyl group. Which is filled by 8 to 18 long carbon (C) bonds. Ethoxylate alcohol is widely used as an active substance in disinfectants, detergents, household and industrial furniture cleaners. As a disinfectant, AE works by denaturing the protein in MO, which is the role of alcohol and EO, a moisturizing agent to accelerate denaturing MO protein.²⁷

Sodium Lauryl Ether Sulfate: Sodium lauryl ether sulfate (SLES) is a naturally occurring anionic surfactant derived from coconut and palm tree seeds and contains a mixture of sodium alkyl sulfate with lauryl alkyl sulfate. Sodium lauryl ether sulfate has the chemical formula C₁₈H₃₇NaO₇S and is used in liquid detergents and surfactants such as liquid bath soap, toothpaste, and shampoo can be used as a cleaning agent for household and industrial furniture. As a surfactant, SLES has bacteriostatic properties against gram-positive bacteria, is microbicidal against human immunodeficiency virus (HIV) type 1 and is functional but not effective in killing gram-negative bacteria.²⁸

Carbol and Pine Oil: Carbol or phenol is a disinfectant containing hydroxyl chemicals that bond to carbon atoms and form aromatic rings. Carbol has the chemical formula C₆H₅OH and is used as an antiseptic agent in surgical instruments in hospitals and as a cleaning agent for floors and toilets. As a disinfectant, Carbol has bactericidal, fungicidal, viricidal, and tubercidal properties. In killing germs, carbolic acid will enter through the cell wall of germs and disrupt the protein metabolism process in germs so that there will be the inactivation of enzymes necessary for germ metabolism. Pine oil results from the hydrodistillation process of several types of pine species such as *Pinus armandii*, *Pinus strobus*, *Pinus sylvestris*, *Pinus brutia*. Pine oil is used as a fragrance in cosmetics and household cleaning tools, as a flavouring agent in food. In addition, in the world of health, pine oil which is the result of hydrodistillation of several types of pine species, also has beneficial functions, such as pine oil from *P. strobus* used as

cough medicine. *P. sylvestris* has antipyretic properties that can reduce fever, and *P. sylvestris* has antimicrobial properties against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, and *Candida albicans* bacteria which are used as additives in disinfectants for cleaning floors or household furniture.²⁹

Effect of Disinfectant on Ascaris Eggs: Based on research conducted by Oh et al. [18] using several types of commercial disinfectants containing active substances such as ethanol 99% and 70%, methanol 99% and 70%, povidone-iodine 10%, cresol 3%, 0.2% and 0.02% sodium hypochlorite and 5% chlorohexidine mixed with saline, and exposed to intact and decorated *A. lumbricoides* eggs. Then the eggs were observed under a microscope for six weeks on each disinfectant with a different concentration. It was reported that ethanol, chlorohexidine, and methanol could not inhibit egg development during six weeks of incubation. Cresol 3%, sodium hypochlorite 0.2% and 0.02% were only able to inhibit the development of embryos from *Ascaris* eggs in the third week of incubation because during the third week of incubation, *A. lumbricoides* larvae were not found in the sample. Only povidone-iodine inhibited embryogenesis in *Ascaris* eggs from the first week of incubation to the sixth week of incubation. It can be concluded that only a few of the disinfectants that have been used as disinfectants to clean furniture or floors in the house were able to kill *A. lumbricoides* eggs, depending on the active substances and concentrations contained in these commercial disinfectants.

RESEARCH METHODOLOGY

This study is an analytical study that compares the content of active substances in floor cleaners. We will compare the combination of disinfectant alcohol ethoxylate-sodium lauryl ether sulfate with carbol-pine oil in floor cleaners, and this research was conducted in the Parasitology laboratory of the UKI Medical Faculty. This research was conducted from September 2017 to October 2017. The sample of the study was *A. lumbricoides* eggs from patients with ascariasis. The tools used in this study were: phenol reagent (as control), distilled water, physiologic salt, sterile preparations and lids, microscope, sterile tube, sterile digital micropipette with an accuracy of 1000 l and 500 l, sterile dropper pipette, refrigerator. Bunsen, Matches, Sterile measuring tube, Measuring tube rack, Sterile beaker, Sterile measuring cup, Centrifuge, Lysol solution, Entelan, Sterile gloves, Mask, Lab coat, Logbook, 100 ml sterile sedimentation tube, Sterile

syringe, and Permanent markers. On stool examination, the research material used was *A. lumbricoides* eggs derived from fresh faeces containing *A. lumbricoides* eggs. The disinfectant used was a combination of alcohol ethoxylate-sodium lauryl ether sulfate in-floor cleaner I (PL I) and carbolpine oil in-floor cleaner II (PL II). The results of observing eggs for one month were collected and processed using a data processing program. First, the normality test was carried out on the data studied using the Kolmogorov-Smirnov test. If the data under study has met the requirements of the normality test, it is continued by conducting the One Way Anova test. However, suppose data does not meet the normality test requirements in the Kolmogorov-Smirnov test. In that case, the data can be processed using the Kruskal Wallis test to distinguish the effectiveness of PL I and PL II against *A. lumbricoides* eggs if the Kruskal Wallis test obtained a calculated p-value of <0.05, followed by the Mann Whitney test to find out whether there was a significant difference in the data. Conditions for normality test of a data if the significance value or p-value > 0.05.

RESULT AND DISCUSSION

This study studied the effectiveness of the disinfectants contained in two commercial floor cleaners against *A. lumbricoides* eggs. PL I (alcohol ethoxylate-sodium lauryl ether sulfate) and PL II (carbol-pine oil) were tested in various concentrations, including the concentration recommended by the manufacturer, higher concentration and lower concentration. The test concentrations are the concentrations recommended by the manufacturer (1.5%), higher (6% and 3%) and lower concentrations (0.75%, 0.40% and 0.2%). Observations made for one month showed that all concentrations of PL I and PL II did not affect the development of *A. lumbricoides* eggs (Tables 1 and 2). Most of the eggs remain intact, and the embryos within them develop into larvae enclosed in an intact egg wall. The intact egg wall allows the embryo to continue to develop into a larva. It seems that despite being incubated with a combination disinfectant alcohol ethoxylate-sodium lauryl ether sulfate (PL I) and carbol-pine oil (PL II) for one month, the eggs still develop into an infective form which, if ingested can still cause new infections. It can be concluded that the two combinations of disinfectants in the cleaning fluid did not affect the eggs, both in terms of wall integrity and larval development (Anova $p > 0.05$). Thus, H_0 in this study was accepted that the disinfectant did not affect *A. lumbricoides* eggs.

Table 1: Effects of various concentrations of PL I disinfectant on *A. lumbricoides* eggs

Week		Concentration					
		6%	3%	1,50%	0,75%	0,40%	0,20%
1	Embryo	7	7	7	6	5	4
	Larvae	0	0	0	0	0	0
	Damaged	1	0	0	0	1	0
2	Embryo	7	7	7	6	5	4
	Larvae	0	0	0	0	0	0
	Damaged	1	0	0	0	1	0
3	Embryo	4	4	2	4	4	3
	Larvae	2	1	2	1	2	1
	Damaged	1	0	0	1	1	0
4	Embryo	3	3	1	2	2	2
	Larvae	2	2	3	3	3	2
	Damaged	1	0	0	1	1	0
5	Embryo	3	3	1	1	0	0
	Larvae	1	2	4	3	5	4
	Damaged	1	0	0	1	1	0

Note: *factory-recommended concentration (1.5%)

Table 2: Effects of various concentrations of PL II disinfectant on *A. lumbricoides* eggs

Week		Concentration					
		6%	3%	1,50%	0,75%	0,40%	0,20%
1	Embryo	5	8	6	7	6	8
	Larvae	0	0	0	0	0	0
	Damaged	1	1	1	0	0	0
2	Embryo	5	8	6	7	6	7
	Larvae	0	0	0	0	0	0
	Damaged	1	1	1	0	0	0
3	Embryo	4	5	6	6	4	5
	Larvae	0	1	0	0	1	2
	Damaged	2	2	1	0	0	0
4	Embryo	3	4	6	4	2	4
	Larvae	0	2	0	2	3	3
	Damaged	3	2	1	0	0	0
5	Embryo	3	2	3	1	2	2
	Larvae	0	3	3	5	3	5
	Damaged	3	2	1	0	0	0

Note: *factory-recommended concentration (1.5%)

Based on table 1, in the first and second weeks of the PL I disinfectant, only one damaged egg was found, namely at a concentration of 6% and 0.40%, where this has not been able to ensure that the active substances contained in the PL I disinfectant can inhibit the development eggs of *A. lumbricoides*. Then in the third week, there was a significant change in egg development, where eggs in an embryonic state in the previous week had turned into larvae in all concentrations of the PL I disinfectant. In the fourth week, *A. lumbricoides* eggs continued to develop and more and more turned into larvae by increasing the number of larvae at concentrations of 3%, 1.5%, 0.75%, 0.40%, and 0.20%. The larvae number continued until the last week of the study, where all embryo eggs found in the early weeks had turned into larvae at concentrations of 0.40% and 0.20%. It also occurs at high concentrations of PL I disinfectants such as at concentrations of 6%, 3% and 1.5%, where the embryonic eggs found in the early weeks of the study, half of which had become larvae.

Based on table 2, in the first and second weeks of PL II disinfectant with a concentration of 6%, 3%, and 1.5%, one damaged egg was very different at low concentrations, where no damaged eggs were found. In the third week, there was an increase in the damaged eggs (one egg) in the PL II disinfectant with a concentration of 6% and 3%. Furthermore, the increase in the number of damaged eggs was only found in PL II disinfectant with a concentration of 6%, but on the contrary, eggs which in the early weeks were still in embryonic form had changed into larvae at concentrations of 3%, 0.75%, 0.40% and 0.20% in the fourth week. Eggs that turned into larvae continued to increase until the last week of the study, such as at a concentration of 1.5%, which in the first week there were no egg changes, but in the last week, there were three changes in eggs that became larvae. At a 1.5% concentration and at a 0.75% and 0.20% concentration, an increase of eggs turned into infective larvae.

There are three variables used in data processing: *A.*

lumbricoides embryos, *A. lumbricoides* larvae, and damaged egg walls. The three variables were tested for normality using the Kolmogorov-Smirnov test to determine whether the data was normal or not. The value of normality requirements in data if the calculated p value > 0.05 (p-value normal). From the results of the Kolmogorov-Smirnov test on PL I data, it was found that the calculated p-value of *A. lumbricoides* embryos was 0.684. It can be concluded that the distribution of data contained in the *A. lumbricoides* embryo variable was normal so that the One Way Anova test could be performed. The calculated larval variable of *A. lumbricoides* p-value is 0.075, which means the data distribution is normal, and then the One Way Anova test can be performed. The damaged egg wall variable has a calculated p-value of 0.001, meaning that the data on the damaged egg wall variable can only be processed using the Kruskal Wallis test because the data distribution is not normal.

One Way Anova test was conducted to assess the difference in the mean effect of various concentrations of PL I on the embryos and larvae of *A. lumbricoides*. From the One Way Anova test results, it was found that the p-value of the *A. lumbricoides* embryo was 0.597, so H_0 was accepted because the p-value of the *A. lumbricoides* embryo count > standard p-value (0.05). So it can be concluded that various concentrations of PL I did not affect *A. lumbricoides* embryos. While the larval variable of *A. lumbricoides*, the calculated p-value is 0.885, then H_0 is accepted because the p-value of the calculated larvae of *A. lumbricoides* > standard p-value (0.05). So it can be concluded that various concentrations of PL I did not affect the larvae of *A. lumbricoides*.

Kruskal Wallis test was conducted to assess the difference in the mean effect of various concentrations of PL I on damaged egg walls. From the results of the Kruskal Wallis test, it was found that the calculated p-value of the damaged egg wall was 0.000, which means that H_0 is rejected or H_1 is accepted. Then the Mann Whitney test was carried out to

assess whether there was a significant difference in the data. From the Mann Whitney test results, at a concentration of 6% and 0.40%, the calculated p-value is 0.003, and 0.75% is 0.05, then H1 is accepted. Of the three concentrations, only one egg experienced egg wall damage at each concentration, so it can be concluded that various concentrations of PL I did not significantly damage the egg wall of *A. lumbricoides* so that the development from embryo to larvae continued.

The Kolmogorov-Smirnov test carried out the data contained in PL II to determine whether the distribution of the data was normal or not. From the results obtained, the embryo variable *A. lumbricoides* has a calculated p-value of 0.466. It means that the data contained in the variable embryo *A. lumbricoides* is classified in a normal distribution because the calculated p-value is > standard p-value (0.05), so the One Way Anova test can be carried out to determine the difference in the average effect of various concentrations of PL II on embryo *A. lumbricoides*. Variable larvae of *A. lumbricoides* and damaged egg walls had p-values of 0.001 and 0.011. Then the p-value of the two variables < p-value standard (0.05), so that the two variables can only be processed using the Kruskal Wallis test.

From the One Way Anova test results on the data contained in the embryo variable *A. lumbricoides*, it was found that the calculated p-value was 0.766. Then H₀ is accepted because of the calculated p-value > standard p-value (0.05). So it can be concluded that there is no effect of various concentrations of PL II on *A. lumbricoides* embryos. The same thing was also found in the larval variables of *A. lumbricoides* and damaged egg walls after the Kruskal Wallis test. It was found that the calculated p-value of the two variables was 0.344 and 0.000. The calculated p-value of the larval variable of *A. lumbricoides* > from the standard p-value, then H₀ for the larval variable of *A. lumbricoides* is accepted. It was concluded that various concentrations of PL II had no effect in inhibiting the development of *A. lumbricoides* larvae. While the variable egg wall is damaged, H1 is accepted, so it must be continued with the Mann Whitney test to assess whether there is a significant difference in the data. From the Mann Whitney test results, it was found that only three concentrations affected damaging the egg wall of *A. lumbricoides*, namely concentrations of 6%, 3%, and 1.5%. The P-value obtained at a concentration of 6%, and 3% is 0.005 and 1.5% is 0.003. So H1 is accepted, so it can be concluded that the effect of various concentrations of PL II in damaging the egg wall is not significant.

In a study conducted by Alfiah et al.³⁰ regarding the effectiveness of various concentrations of alcohol ethoxylate on larvae and pupae of *Aedes aegypti* incubated and observed for one week's development, it was found that ethoxylate alcohol did not affect larvae and pupae of *A. aegypti*. In contrast to the research conducted by Alfiah et al., research conducted by Glover et al.³¹ regarding the effectiveness of ethoxylate alcohol against the cell membrane walls of the bacteria *Proteus mirabilis*, *Staphylococcus aureus* and *Saccharomyces cerevisiae*, it was found that ethoxylate alcohol was able to increase the permeability of the cytoplasmic membrane and cause cell death in the three bacteria. Another study conducted by Beerse et al.³² regarding the effectiveness of sodium lauryl ether sulfate in killing gram-positive bacteria. It was found that sodium lauryl ether sulfate can kill gram-positive bacteria because gram-positive bacteria do not have a protective cell membrane compared to gram-negative bacteria. So, sodium lauryl ether sulfate can easily penetrate the cell membrane of gram-positive bacteria and damage the cell metabolism of gram-positive bacteria.

Research conducted by Yang et al.¹¹ regarding the activity of pine oil as a disinfectant found that pine oil is used as an active substance in providing aroma in a disinfectant and has antimicrobial, insecticidal properties. Yang et al.'s research is also supported by research conducted by Zeynep et al.³³ regarding the effectiveness of pine oil as an antimicrobial and insecticide. In this study, pine oil was proven to be able to kill several types of bacteria such as *Klebsiella pneumoniae*, *Escherichia coli* and *S. aureus* and *Ephestia kuehniella* eggs by changing the permeability of cell membranes of bacteria and *E. kuehniella* so that pine oil can easily penetrate cell membranes and damage the process cell physiology and chemistry. Borneman et al.³⁴ also researched the effect of carbolic acid on the growth of *Ruminococcus albus* and *Ruminococcus flavefaciens* bacteria. It was found that phenol could penetrate cell walls and inhibit metabolic processes resulting in a slowdown in the growth process of these bacteria. These studies prove that ethoxylate alcohol, sodium lauryl ether sulfate, carbolic acid, and pine oil can kill several types of bacteria. The four active substances work by penetrating bacterial cell membranes and damaging cell metabolism, especially in gram-positive bacteria, which have a thinner cell membrane than gram-negative bacteria with two protective layers, namely lipoprotein and lipopolysaccharide layers. It allows the four active substances to easily penetrate the cell membrane of gram-positive bacteria and damage cell metabolic processes.^{4,9,11} As in Tables I and II, it can be seen that there is an increase in the number of larvae at various concentrations of PL I and II every week. It indicates that the combination of the active substance alcohol ethoxylate-sodium lauryl ether sulfate in PL I and carbol-pine oil in PL II did not significantly inhibit the development and damage the egg wall of *A. lumbricoides*. The egg wall layer of *A. lumbricoides* consists of 3 layers: the lipoprotein layer, the chitin layer, and the vitelline layer. The lipoprotein layer is the innermost layer and consists of 25% protein and 75% ascaroside. Ascaroside is a glycoside bond composed of glucose bonds with alcohol that cannot be penetrated by a substance soluble in water, fat, or even gas. The chitin layer is thick and rich in protein, and the vitelline layer is composed of glycoproteins and isolated. The three layers of the wall that are the factors causing the active substances contained in PL I and PL II cannot have a significant effect in damaging the walls and inhibiting the development of *A. lumbricoides* eggs. The active substances contained in the two-floor cleaners cannot penetrate the egg walls and destroy the metabolic process of *A. lumbricoides* which is how the disinfectant works.^{5,6,9,11}

CONCLUSION

This research concludes that PL I disinfectant has no effect on *A. lumbricoides* eggs, and PL II disinfectant affects *A. lumbricoides* eggs. Thus, it is suggested that the number of eggs is more and more homogeneous for further research. Exposure of eggs to disinfectants should be done in minutes or less than 24 hours, and the effect of CO₂ and viscosity may be factors that need to be taken into account in future studies. The use of the same wet preparation for one month to examine the effect of PL I and PL II has limitations. The number of eggs studied for various concentrations is not the same, which may affect the study's final results.

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