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The effect of neem leaves (*Azadirachta indica* A. Juss) application as a natural disinfectant on decreasing number of bacteria and fungi in poultry incubator

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Abstract

Contamination of microorganism in poultry incubator is one of the causes of low egg hatchability. To increase hatchability, maintaining sanitation of poultry incubator is a must. Neem leaves have antibacterial compounds that can be used as a natural disinfectant in poultry incubator. The aim of this research is to determine the effect of neem leaves as a natural disinfectant towards the decreasing number of bacteria and fungi in poultry incubator. Completely randomized design is used as the experimental method with three treatments of neem leaves solution, namely P1 (50%), P2 (75%), and P3 (100%) and six replications. Variables observed of this study were the decreasing number of bacteria and fungi, inhibitory zone on bacterial growth, and identification of fungi in poultry incubator. The result showed that solution of neem leaves with various concentrations had similar effects on the decreasing number of bacteria and fungi in poultry incubator. Based on analysis of variance, all treatments had no significant effect on inhibitory test of coccus bacteria. The higher concentration of neem leaves, the larger the inhibitory zone formed. The treatment of various neem leaves decreased number of bacteria in poultry incubator by 56.16% (P1), 57.94% (P2), and 62.20% (P3), while fungi by 57.63% (P1), 58.11% (P2), and 57.65% (P3). The statistical analysis showed that all treatments had no significant effect on the decreasing number of bacteria and fungi in poultry incubator. Based on fungal identification, neem leaves affected five types of fungi namely; *Aspergillus ochraceus, Penicillium sp., Cladosporium sp., Mucor sp.,* and *Geothricum sp.*

Keywords: Neem leaves, natural disinfectant, bacteria, fungi, poultry incubator

Introduction

Sanitation in hatching machines is a treatment to clean the hatching machine which aims to inhibit growth or reduce microorganisms (bacteria and fungi). Microorganism contamination, such as *Aspergillus* that produce aflatoxins, in hatching machine may reduce the hatchability of eggs (Widiastuti et al. 2003; Eckman & Morgan, 1978). Sanitation on hatching machines is carried out with chemicals such as $KMnO_4$ and formaldehyde. Nowadays, it is known that these chemicals have many negative effects, thus many researchers have been conducted to obtain natural ingredients that can be used as sanitary materials for hatching machines. Neem plants is one of natural ingredients that can be used as sanitary materials.

Neem plants have compounds that can be utilized by humans. Neem leaves contain alkaloid compounds, saponins, tannins, glycosides, tripernoids, flavonoids, reducing sugars, pentoses, carbohydrates (Biu et al. 2009). Saponins are polar-shaped surfactants thereby they break down the fat layer on cell membranes which ultimately causes disruption of cell membrane permeability, so that the process of diffusion of materials or substances needed by bacteria and fungi can be disrupted, eventually cells swell and break (Sugianitri, 2011; Ngajow et al. 2011). Alkaloids will bond strongly with ergosterol to form a hole that causes cell membrane leakage which results in permanent damage to cells and cell death (Mycek et al, 2001). Flavonoids and tannins are phenolic compounds which are able to react with cell walls by entering the cell nucleus, and making all proteins in the fungus denatured so that the fungal cells become damaged and die (Mauseth, 2013).

Several studies have shown that the chloroform neem extract can inhibit the activity of *Fusarium sp.* on dragon fruit plants (Wahdjuni et al. 2016). In addition, methanol extract and ethanol extract of neem leaves with concentrations of 25, 50, 75 and 100% can inhibit the growth of *Aspergillus flavus*, *Alternaria solani*, and *Cladosporium* (Shrivastava and Swarnkar, 2014). Moreover, Bacterial growth such as *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus cereus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumonia*, *Neisseria gonohorreae*, *Proteus mirabilis*, *Vibrio alginolyticus*, *Pseudomonas*, and *Mycobacterium*, are reduced by the extract of neem leaves (Apristiani et al. 2005; Pritima, 2008; Ayini et al. 2014; Sarmiento et al. 2011). Based on the description above, it is expected that the use of neem leaves as a natural disinfectant can reduce the number of bacteria and fungi in hatching machines.

Materials and methods

Making neem leaves solution began with collecting fresh neem leaves as much as 500 grams, then the neem leaves were washed thoroughly and drained. The neem leaves were put into a blender and added 1000 ml of distilled water and mashed. Once smooth, the neem leaves were squeezed and filtered using gauze. The 70% alcohol as much as 10 ml was added. The solution of neem leaves that had been obtained was made into 3 types of concentrations, namely, 50, 75 and 100%.

Sampling was carried out on traditional duck egg hatching machines with an open cup method. Sampling was done by inserting a petri dish containing PDA (Potato Dextrose Agar) and NA (Nutrient Agar) media in an open state, into the hatching machine for 30 minutes, after that the cup was closed and incubated for 24 hours. The colonies formed are then counted.

The experimental design used was a completely randomized design (CRD) with 3 treatments and 6 replications. Testing the treatment effect was tested using paired t test, variance analysis, then followed by tukey test, and orthogonal polynomial test. The variables observed in this study were the number of bacteria and fungi in the hatching machine before sprayed with the solution of neem leaves and after sprayed with a solution of neem leaves.

Results and discussion

The following table shows the percentage decrease in the number of bacteria and fungi in hatching machines. Paired t-test was carried out on each treatment to determine whether or not there was a difference between before and after treatment to decrease the number of bacteria and fungi (**Table 1**).

Table 1 Significance of paired t test on decreasing number of bacteria and fungi.

Treatments	Bacteria		Fungi	
	Percentage of decreasing number	Significance of paired t test	Percentage of decreasing number	Significance of paired t test
P1	56.16	Not significant	57.63	Significant
P2	57.94	Significant	58.11	Not significant
P3	62.20	Significant	57.65	Significant

Note: P1 = 50% concentration of neem leaves; P2 = 75% concentration of neem leaves; P3 = 100% concentration of neem leaves.

The test results on decreasing number of bacteria showed that P1 was not significantly different ($P \geq 0.05$), whereas in P2 and P3 showed a significant difference ($P < 0.05$) between before and after treatment. This means that the 50% neem leaves solution had no significant difference between before and after the treatment in decreasing the number of bacteria. This was presumably because the concentration of 50% was still too low so that antibacterial substances in the solution of neem leaves were not strong enough to reduce the number of bacteria. According to Fardiaz (1992), the ability of an antimicrobial agent was influenced by several factors, one of which was the concentration of antimicrobial substances. The higher the concentration, the higher the number of bacteria was. The test results of the decrease number of fungi showed that P1 and P3 showed significantly different ($P < 0.05$), while P2 showed not significantly different ($P \geq 0.05$) which means there were no differences in the number of fungi before and after treatment. The analysis of variance and orthogonal polynomial test showed that the concentration of neem leaf solution 50, 75 and 100% were not significantly different ($P \geq 0.05$) which means that the three treatments had similar effect in reducing the number of bacteria and fungi with no specific pattern.

Table 2 Average number of inhibitory zone on coccus and bacil bacteria.

Treatments	Coccus bacteria (mm)	Bacil bacteria (mm)
P1	3.12	2.65
P2	4.29	2.75
P3	2.65	2.83

Note: P1 = 50% concentration of neem leaves; P2 = 75% concentration of neem leaves; P3 = 100% concentration of neem leaves

Inhibitory test on bacterial growth was tested to portray the effectiveness of neem leaves solution as disinfectant. Table below showed the result of inhibitory test on coccus and bacil bacteria (**Table 2**). All treatments showed similarity in the ability to inhibit bacteria growth. Based on analysis of variance followed with polynomial test, different concentration of neem leaves had significant effect ($P < 0.05$) on inhibitory test on coccus bacterial growth.

Table 3 showed the identification result of fungi before and after treatments. There were five fungi found in poultry incubator that decreasing after treatments.

Table 3 The identification result of fungi.

Fungi	Before treatment	After treatment	Percentage of decreasing
Aspergillus ochraceus	47	24	44.37
Penicillium sp.	736	148	63.33
Cladosporium sp.	93	46	45.29
Monilia sitophila	0	2	0
Mucor sp.	5	2	50.75
Geothricum sp.	21	15	32.30

The study by Tripathi and Bhatnagar (2012) showed that neem leaves contained protein, polyphenols, carbohydrates, calcium, magnesium, sodium, potassium, phosphorus, and nitrogen. Organic materials such as carbohydrates and nitrogen were nutrients needed by bacteria and fungi as nutrients. Fungi could synthesize proteins by taking carbon sources from carbohydrates (eg glucose, sucrose or maltose), nitrogen sources from organic or inorganic materials, and minerals from their substrates (Fardiaz, 1992).

The effectiveness of neem leaves solution as a natural disinfectant could also be influenced by organic matter found in hatching machines. According to Ducel et al. (2002) organic materials such as serum, blood, pus, feces, or lubricants can interfere with antimicrobial activity. This occurred in two ways, namely to intervene in the chemical reaction between disinfectants and organic materials so that the complex is less germicidal or even non-germicidal. Another way was that organic material would be a barrier to disinfectants.

The number and location of disinfected microorganisms were also one of the factors that influence disinfecting activities. According to Ducel et al. (2002) as long as other conditions remained constant, the greater the number of microbes, the longer it took for disinfectants to eradicate them. This situation reinforced the reason for the need for a cleaning process before disinfection was carried out. According to the Indonesian Ministry of Health (1996), porous surfaces were difficult to disinfect, especially if microorganisms were trapped inside these pores together with organic materials.

Overall, neem leaves could reduce the number of bacteria and fungi on hatching machines. This was presumably because neem leaves contained active compounds that could inhibit microbial growth. The study of Biu et al. (2009) showed that neem leaves contained active compounds, namely alkaloids, saponins, tannins, glycosides, tripernoid, flavonoids, reducing sugars, pentoses, and carbohydrates. Flavonoids and tannins were phenol groups which reacted with cell walls by entering into the cell nucleus, and made all the proteins in the fungus denatured so that the fungal cells became damaged and die (Mauseth, 2013). Saponins were polar-shaped surfactants so that they break down the fat layer on cell membranes which ultimately caused disruption of cell membrane permeability. This results in the process of diffusion of materials or substances needed by fungi could be disrupted, eventually the cells swelled and ruptured (Sugianitri, 2011).

Conclusions

Based on the results about the effect of neem leaves (Azadirachta indica A. Juss) as a natural disinfectant on hatching machines, it can be concluded that the solution of neem leaves at 50, 75 and 100% concentration showed similar effectiveness in reducing number of bacteria and fungi.

References

Apristiani, D. & Astuti, P. (2005). Isolasi komponen aktif antibakteri ekstrak kloroform. Daun mimba (*Azadirachta indica* A. Juss.) dengan bioautografi. *Biofarmasi 3*(2), 43-46.

Ayini, U., Harnina, S., & Dewi, T.C. (2014). Efek antibakteri ekstrak daun mimba terhadap bakteri *vibrio algynoliticus* secara *in vitro*. *Biosaintifika. Journal of Biology and Biology Education 6*(1), 67-75.

Biu, A.A., Yusuf, S.D., & Rabo, J.S. (2009). Phytochemical screening of Azadiractha indica (Neem) (Meliceae) in Maiduguri, Nigeria. *Biosince Research Communications 21*(6), 281-283.

Ducel, G., Febry, J., &Nicolle, L. (2002). Prevention of nosocomial infection. United States: World Health Organization.

Eckman, M.K., & Morgan-Jones, G. (1978). Fungus species isolated from commercial hatcheries in alabama. *Avian Diseases 23*(1), 204-208.

Fardiaz, S. (1992). *Mikrobiologi pangan 1*. Jakarta: Gramedia Pustaka Utama.

Mauseth, J.D. (2013). *Botany: An introduction to plant biology*. 5th eds. Burlington: Jones and Bartlett Learning.

Mycek, M.J., Harvey, R.A., Champe, P.C., & Fisher, B.D. (2001). *Lippincottt's illustrated reviews: Farmacology*. Jakarta: Widya Medika.

Ngajow, M., Abidjulu, J., & Kamu, V.S. (2011). Pengaruh antibakteri ekstrak kulit batang matoa (*Pometia pinnata*) terhadap bakteri *Staphylococcus aureus* secara *in vitro*. *Jurnal MIPA Unsrat Online 2*(2), 128-132.

Pritima, R.A., & Pandian, R.S. (2008). Antibacterial potency of crude extracts of *Azadirachta indica* A. juss (leaf) against microbes causing reproductive tract infections among women. *Current Biotica 2*(2), 193-205.

Sarmiento, W.C., Maramba, C.C., & Gonzales, M.L.M. (2011). An *in vitro* study on the antibacterial effect of neem (*Azadirachta indica*) leaf extract on methicillin-sensitive and Methicillin-resistant *Staphylococcus aureus*. *PIDSP Journal 12*(1), 40-45.

Shrivastava, D.K., & Swarnkar, K. (2014). Anitifungal activity of leaf extract of neem (*Azadirachta indica* Linn.). *Internationa Journal of Current Microbiology and Applied Sciences 3*(5), 305-308.

Sugianitri, N.K. (2011). *Ekstrak Biji Buah Pinang (Areca catechu L.) dapat Menghambat Pertumbuhan Koloni Candida albicans secara in vitro pada Resin Akrilik Heat Cured*. Thesis, Universitas Udayana, Bali.

Tripathi, A.K., & Bhatnagar, M.K. (2012). Physico-chemical study of foliar chemicals of neem (*Azadirachta indica* A. juss) in different seasons, around Amarkantak thermal power plant, Chachai (Madhya Paradesh) India. *International Journal of Science and Research 3*(11), 119-123.

Wahdjuni, S., Puspawati, N.M., & Arista, N.P.R.E. (2016). Isolasi dan identifikasi senyawa aktif antijamur dari daun mimba (*Azadirachta indica* A. juss) sebagai pengendali jamur *Fusarium* sp. pada tanaman buah naga (*Hylocereus* sp.). *Jurnal Kimia 10*(2), 197-203.

Widiastuti, R., Darminto, S., Bahri, S., & Firmansyah, R. (2003). Inokulasi aflatoksin B1 pada telur berembrio dan residunya pada ayam menetas. Pros. Seminar nasional teknologi peternakan dan veteriner. *Bogor 2003*, 462-466.