SIMPLE CULTIVATION OF EFFECTIVE MICROORGANISMS TO REDUCE NUMBER OF FLIES IN LOCAL CHICKEN COOP

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Abstract

Effective microorganisms (EM) were used in many biological applications, such as the making of organic fertiliser and bioremediation. Some chicken farmers make their effective microorganisms due to simple preparation and to reduce the production cost. However, the effectiveness of the EM in an actual chicken coop was hardly found reported. In this research, the effectiveness of a commercial EM (EM-1) was compared with self-prepared EM made from the fermentation of pineapple (EM-Pineapple) and milk (EM-Milk). This experiment was run at a chicken coop with a capacity of 100 chickens. The number of flies in the coop was collected and counted before, and after the EM was sprayed in the chicken coop. After-treatment, EM-1 reduced flies' number about 62.27%. EM-Milk decrease flies' number to 55.00% while EM-Pineapple reduces the flies to 50.34%. Although all the EM showed a reduction of flies' number after the treatment, EM-1 is the most effective compared to other EMs. However, statistical analysis shows that the effectiveness among the treatments is similar. This finding suggested that simple cultivation of EM can prevent foul odour and economically reduces the number of flies in the small-scale chicken coop.

Keyword: Chicken Coop, Effective Microorganisms, Flies, Milk, Pineapple

Introduction

Poultry Industry and Management

Based on Afizah (2017) in the year 2014, the production of poultry in Malaysia has shown a significant demand for chicken meat, eggs, and other poultry products. This industry is the primary supply of protein source to the country besides beef and fisheries. In an economic perspective, the poultry industry gives a considerable amount of income to the gross national product.

Due to the benefits of the poultry sector, the breeding of poultry, mainly the chicken needs a significant amount of land, proper breeding management and adequate supply of chicken feed and water. Chicken farming usually located away from the residential area due to the waste management system. However, for village folks, the farmers have limited ground area to build a distance coop from their houses. Besides land shortage, many of the coops are build next to the farmer houses to provide convenient and flexible time for the farmers to care and feed the chicken while running their routine chores. The villagers have this small chicken farming at their houses to provide food for the family and to generate some money from nearby consumers.

Although the chicken farm may valuable to the farmers, as mentioned earlier, the chicken farms or coops need proper waste management to cater to the environmental and health issue. Chicken farming invites many unwelcoming pests, such as flies (Hamdan et. Al., 2012). The infestation of pest and foul smell of chicken waste raise a massive problem to the farmers. Due to the economic deficit, an affordable and effective resolution should be adopted to solve the issue. Hence, this study proposes a cheaper, environmentally friendly, and biologically acknowledged answer to the farmers using local microorganisms' culture from cheap and accessible resources.

Effective Microorganisms

Due to waste management, farmers can use friendly microorganism or EM in their chicken coop. This solution includes a few types of bacteria such as lactic acid bacteria, yeast actinomyces and photosynthetic bacteria (Sangakkara, 2014). Thus, this solution can be used without polluting the environment with pesticide or synthetic substance that negatively impact the soil in long-term practical. The other benefit of using the EM solution, since it contains natural mutualistic bacteria, thus, prevent the chances of poultry poisoning.

EM are well-known microbial that have shown many benefits in several industries (Satyanarayana et al., 2012). EM products are already on the market, but the price is quite high. For the small-scale farmers, buying a commercial EM product will incur their cost. Thus, some of the farmers produce their EM by applying knowledge from the internets (Kay, 2012; Lyfe, 2017). Based on Tanaka et al., (2006), the research produced lactic acid from rice. The study proved that farmers could make EM solution using inexpensive resources. Hence, by preparing their EM, farmers with the low budget would able to apply EM on their coop.

Materials and Methods

Preparation of EM Culture

Approximately 150 g of 5% broken rice was used to obtain rice water. The rice was soaked and washed with 1.5 L of distilled water for three times. The rice water was left undisturbed for three to four days to allow precipitation. Three layers of rice water were formed, and the middle layer was carefully harvested. The EM-Milk was prepared by mixing rice water with fresh milk by a ratio of 3:1. The mixture was fermented in a container for five days, and a greenish solution was formed under a white layer. The greenish layer was harvested and next added with 200 ml of molasses. The mixture was homogeneously mixed and kept in a low light intensity cabinet. The mix was further fermented for four days until some gasses developed in the bottle.

The EM-Pineapple was prepared by adding 200 g of small diced semi-ripe pineapple flesh with 200 ml of rice water and top up to 1 litre with distilled water. The mixture was then added with 200 ml of molasses and thoroughly mixed. Next, the mixture was kept in the same cabinet with EM-Milk until some gasses were developed in the bottle.

The preparation of EM-1 EMRO was according to the manufacturer suggestion, with was 1:1:4, respectively to EM-1 stock solution to molasses to distilled water). The mixture was stored until some gasses were developed in the container.

Chicken Coop Treatments

For the treatment, a coop at Kg Bolok Hilir, Lanchang, Pahang was used as the project site, and the coordinate of this site was 3.5304099 N, 102.1548516 E (degree & decimal minutes). The coop size was about 16.72 m^2. Due to the large surface area, the EM solution was spurted to all surface of the coop floor and wall using WP16 16 L Agriculture Knapsack Backpack Pressure Sprayer. Before the treatment, the EM solution was diluted with distilled water by the ratio of 1:20. As for standardisation, the total amount of each EM solution that was used in this treatment was 8.4 L.

Flies and Environment Data Collection

The flies were obtained by trapping them on Adhesive Sticky Trap Paper, STS brand. Two gridded flytraps were put in the coop before and after three days of treatment for each data collection. The total number of flies was counted and recorded accordingly.

The relevant environment data such as temperature, relative humidity (RH), aeration/air velocity, light intensity and weather were also recorded daily using an Envirometer. Some weather information on raining and wind flows were obtained from an online report from the Malaysia Meteorological Department, Ministry of Science, Technology, and Innovation.

Microbial Isolation and Screening

The EM solution was brought to the laboratory for isolation and enumeration. For the isolation process, the EM solution was diluted to a serial of dilution up to the dilution of 10^{10} using a sterilised saline solution. The diluted EM was placed on two different types of selective agar plates, which were Mac Conkey agar and MRS agar to screen on the microorganisms' diversity. The plated agar was incubated for two days in 35ºC incubator. The microorganisms growth from the agar plates were observed by staining methods, and the images were recorded via Dino-eye Capture 2.0.

Results and Discussion

Effect of EM Treatments on Flies Number

The flies were captured successfully using the paper flytrap, as shown in **Table 1**. The total fly number before treatment was 729 flies. After the respective treatments, the number was decreased. EM-1 reduces 62.27% flies, followed by EM-Milk 55.00% and EM-Pineapple 50.34%. Based on Table 1, EM-Milk result was toward EM-1, so it was selected as the best self-prepared EM.

Table 1 Number of flies before and after treatments

	Before Treatment	**After Treatment**		
	Control	EM-1	EM-Pineapple	EM-Milk
Fly Number	729	275	362	328

This solution can be assumed as efficient toward reducing fly and odour. The treatment located at a chicken coop, it has a foul odour because of the decaying chicken waste. Based on Santi et al., (2015) flies were attract toward gas that produces by the decomposition process of protein, for example, decaying of the animal body or animal waste. The reduction of flies can be used as the measurement, as the foul odour was decreased using EM treatment.

IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY) was used to determine the hypothesis whether the treatments, including the control, show any significant difference in reducing the flies' number. A Kruskal-Wallis test showed that there was a significant difference in mean ($H = 44.619$, $p < 0.00$). A post hoc test was conducted to test the pairwise comparisons. The results found that the control was found a significant difference to all the treatments. However, comparison among the treatments showed no difference, which means the treatments are equally effective in reducing the number of flies captured. **Table 2** shows the pair-wise comparison with Dunn-Bonferroni test together with Bonferroni correction as the post hoc test.

Table 2 Pair-wise comparison of control and EM treatments

Sample 1	**Sample 2**	**Test Statistic**	**Standard Error**	**Standard Test Statistic**	**Sig.**	**Adjusted Sig.**
EM-1	EM-Milk	-8.900	7.339	-1.213	0.225	1.000[b]
EM-1	EM-Pineapple	-10.575	7.339	-1.441	0.150	0.897[b]
EM-1	Control	45.425	7.339	6.190	0.000	0.000[a]
EM-Milk	EM-Pineapple	-1.675	7.339	-0.228	0.819	1.000[b]
EM-Milk	Control	36.525	7.339	4.977	0.000	0.000[a]
EM-Pineapple	Control	34.850	7.339	4.749	0.000	0.000[a]

Sig. – significance

Environment Data Collection

The experiment was conducted in a chicken coop, located in a natural environment of Lanchang, Pahang. Due to the exposure of the treatments, results were prone to be affected by the environmental factors. Thus, factors such as temperature, humidity, light intensity, and air were observed and recorded. The climate news from the Meteorological Department of Malaysia and newspapers were also obtained to support the atmosphere profile. Anon (2018) reported that cold weather might occur while Sukaimi (2018) reported that the country might be strike with hot weather. Thus, the environment data were collected to monitor drastic climate change during the proceeded experiment.

Based on **Table 3**, before the treatment, the temperature was in the range of 28.5°C-30.0°C. The temperature in control treatment session in range 30°C-31°C. While for pineapple treatment, it ranges from 30.7°C-32.5°C and milk treatment, it ranges from 30.1°C-31.0°C. Light intensity at the treatment area was around 1400-1444 Lux. The humidity was in the range of 30%-37% RH, and the air velocity was constant at 0 m/s. All data were obtained at the same time with the flies' collection data for three consecutive days for each treatment.

Table 3 Range of temperature, light intensity, humidity, and air velocity throughout the experiment

	Value Range
Temperature	28.5°C- 32.5°C
Light Intensity	1400-1444 Lux
Humidity	30%-37% RH
Air Velocity	0 m/s

The temperature was measured to observe suitability of temperature for bacteria growth. Based on Hudecova (2011), *Lactobacillus* species such as *L. rhamnosus* grow their colony at 25°C-35°C. Also, humidity measurement was essential to avoid the growth of mould in the chicken coop. In the experiment, mould was not visible, and this in agreement with Johansson (2012), which stated mould would not grow below than 75% RH.

Microbial Screening

As the EM-Milk was found better than reducing the number of flies compared to EM-Pineapple, the EM-Milk solution was subsequently brought to the microbial isolation. From a series of dilution, 1 ml of the diluted solution was grown on two types of plate, MacConkey, and MRS agar. No growth was found appeared at the MacConkey plate, while 15 colonies were exhibited at the MRS plate at dilution of 10^5. Thus, using a standard formula for CFU/ml, the result showed the undiluted EM-Milk was calculated as 1,500,000 CFU/ml. The colonies were isolated and proceeded with Gram staining. The observation of the cells found they were rod shapes and blue colour. Thus, the colonies were assumed to be Gram-positive, *Lactobacillus* species. Using a Dino Eye, a photo was captured at 1000X total magnification with some measurements on the length and width of the cells. The length and the width of the cell as shown in **Figure 1** were found 1 - 2µm which is similar to the size of *Lactobacillus* (Valík, Medveďová, & Liptáková, 2008)

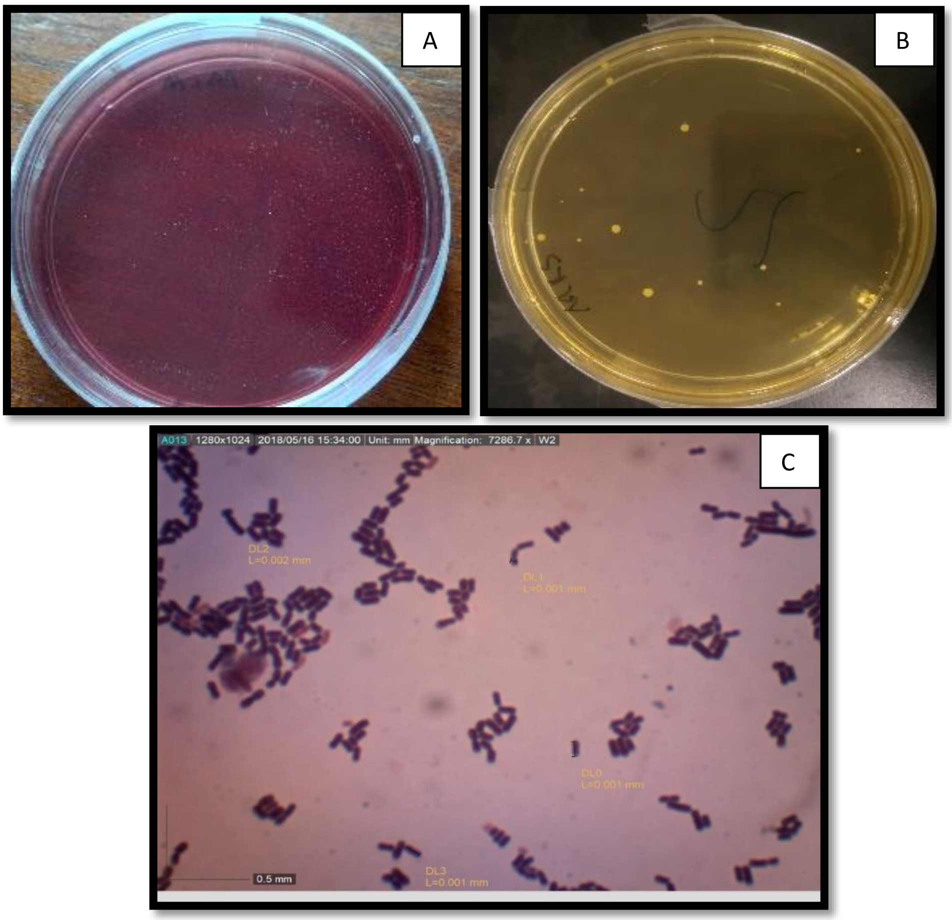

Figure 1 MacConkey agar (A), MRS Agar (B) and bacterial image at 1000X via Dino Eye (C)

Based on Hasman et al., (2015), MacConkey can screen and allowed the Gram-negative bacteria to grow. The composition in this agar such as crystal violet and bile salt inhibits the growth of Gram-positive. Whereas, MRS agar was used to grow *Lactobacilli* bacteria (Bujalance et al., 2006). The composition in this agar such as sodium acetate inhibits the growth of competing bacteria other than *Lactobacilli*. Due to the compositions, it helps in selectively isolate, screening, and pure culturing bacteria from the EM solution.
For the morphological and the size of the bacteria, the colonies were observed using a microscope. Gram staining, regardless of the staining procedure (Siguenza et al., 2019), provides the bacteria with specific colour followed by their species category, Gram-positive and Gram-negative. Gram-negative and Gram-positive bacteria appear pink and blue colour, respectively. Besides, the stain makes better visibility of the cells' structure for their morphology identification.

Conclusion

Comparison of three types of EM shows a similar effect on the reduction of flies' number with no significance value under a 95% confidence interval. EM-Milk was chosen as the best self-prepared EM because it reduced a higher number of flies than the EM-Pineapple. However, EM-1 outcompete EM-Milk to 7.27%. The isolation and screening of culture from EM-Milk showed a suggestion of the bacterial type of *Lactobacillus* sp. at $1.5\,X\,10^6\,CFU/ml$. Simple cultivation of EM from milk and pineapple have proven to be effective in reducing the number of flies in the local small-scale chicken coop. Future recommendation for the study would be on the optimisation of the medium composition for the microbial cultivation. The optimum medium composition will reduce the cost but at the same time able to achieve maximum yield.

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Conflict of interests

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