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## Research Article

### Ectoparasitic Effects of Citronella Grass (*Cymbopogon nardus*) and Lemongrass (*Cymbopogon citratus*) Shampoo Against Mites (*Dermanyssus gallinae*) of Native Chickens (*Gallus gallus domesticus*)

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## ABSTRACT

This study was conducted to produce an herbal shampoo using the extracts of citronella grass, lemongrass, and mixed of both grasses. It was conducted to find out which among the produced herbal shampoos was the effective against red mites of native chicken and their time observed to kill mites. In addition, it was conducted to determine the cost analysis of using the different grasses as a shampoo. Sixty laying native chickens naturally infested with mites were used in the study. The chickens were grouped randomly and distributed to five (5) treatments. Herbal shampoos were prepared before bathing the native chickens; the bathing process was begun and lasted for four (4) weeks with seven (7) days interval. Overall the positive control or the commercial shampoo Wash Out Intense® was the most effective among all other treatments with 92.83% efficacy. The notable efficacy of the commercial shampoo was followed by the herbal shampoo produced which was the citronella shampoo then the lemonella shampoo followed by lemongrass shampoo, with 76.14%, 71.14%, and 68.80% efficacy respectively. The treatment that shows the lowest observed time to kill mites was the positive control (Wash Out Intense®) which consists the effective components to kill mites, it was then followed by T3 the citronella shampoo, T5 the lemonella shampoo and lastly T4 the lemongrass shampoo. Lastly the cost analysis of making shampoo using citronella, lemongrass and mixed of both grasses' extracts revealed that were less economical than the commercial shampoo available.

**Keywords:** Citronella grass, herbal shampoo, lemongrass, red mites

## Introduction

The Philippines is an agricultural country wherein poultry production contributed most

to the progression of animal enterprises today.

Traditionally, native chicken production began as a backyard enterprise and raised by the local

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farmers but has turned to more advanced operations and instant output. As modernization occurs in the Philippines, many health advocates preferred the traditional way of raising native chicken and grew organically. Native chicken (*Gallus gallus domesticus*) has a unique characteristic that the local farmers like to raise; their meat and egg are savory that the Filipino and foreign consumers enjoy. Native chickens are free ranged and said to be hardy and well-adapted in the tropical temperature country. They are resistant to common illness and sturdy. Despite these good characteristics, they are susceptible to common ectoparasite infestation because of their roaming activity.

As they roam, native chickens can be mites or "red mites" (*Dermanyssus gallinae*) is increasingly implemented in dermatological complaints in non-avian hosts, like the caretaker and owners of the chicken's backyard. Many commercial miticides are available in the market. Still, most of these miticides are harmful chemicals to the native chickens and the one handlers, owners, or caretakers.

Different herbs plants can be an alternative miticide with a minimal side effect that can help our environment while preventing the native chicken from this kind of external parasite. Citronella grass (*Cymbopogon nardus*) and Lemongrass (*Cymbopogon citratus*) are native to a tropical country like the Philippines, and these grasses can repel mosquitoes and other arthropods in general. Hence, this study aimed to produce a natural based shampoo product, extracted from citronella grass and lemongrass and their combination, which were used against chicken mites

### **Objectives of the study**

This study was conducted to:

- [1] Produce herbal shampoo with Citronella, Lemongrass, and mixture of both grasses' extracts;
- [2] Determine the efficacy of citronella grass, lemongrass, and mixed shampoo against mites of native chicken;
- [3] Determine the time required to kill mites in native chicken using different shampoo preparation; and
- [4] Determine the cost analysis of using citronella grass and lemongrass shampoo.

### **Time and Place of the Study**

The study was conducted on the first semester of the School Year 2020-2021, from September to November 2020 at Antonio's residence, Sinilian 3rd Camiling, Tarlac. Identification of mites was conducted at Parasitology Laboratory at Tarlac Agricultural University, Malacampa, Camiling, Tarlac.

### **Methods**

#### **Experimental Animals**

A total of 60 laying native chickens naturally infected with mites regardless of weight, were used in the study. Each chicken was tagged with a leg band- different colors of yarn for identification according to treatments.

#### **Experimental Layout and Treatment**

Experimental chickens were randomly distributed into five (5) treatments, replicated four (4) times with three (3) native chickens in each replication, following the Complete Randomized Design (CRD). The different treatments were as follows:

Treatment 1 - Negative Control (Tap Water)

Treatment 2 - Positive Control [Commercial Shampoo- Wash Out Intense® (Permethrin+ Piperonyl butoxide+ D- panthenol)]

Treatment 3 - 100 ml Shampoo = 50% Citronella grass extract + 50% liquid castile soap

Treatment 4 - 100 ml Shampoo = 50% Lemongrass extract + 50% liquid castile soap

Treatment 5 - 100 ml Shampoo = 25% Citronella grass extract + 25% Lemongrass extract + 50% liquid castile soap

### **Housing**

The cages used were made of bamboo and green plastic mesh. There were 20 cages; each cage has three (3) native chickens and were labeled according to treatment and replication. The floor space of each cage measures three (3) by three (3) ft, and cages were four (4) feet high with a floor elevation of one (1) foot. Each cage was provided with feeding and watering bowls and bedding.

### **Management of Experimental Animals**

The experimental animals were fed with commercial layer feeds twice a day, every seven (7) in the morning, four (4) in the afternoon, and were provided clean water and refreshed every feeding time. Cages were cleaned regularly.

### **Gathering and Extraction of Citronella grass and Lemongrass**

Citronella grass and lemongrass, 2 kilograms each, were harvested, collected, and weighed using a weighing scale. The collected citronella grass and lemongrass were washed with tap water to remove the debris and dirt and air dried before they were cut into small pieces. The cut citronella grass and lemongrass were separately stored in a plastic container until the extraction. The ¼ kg of cut citronella grass were put into the blender until the grasses were fully grinded. The ground grasses were put into a clean cloth and manually squeezed until fully extracted. This procedure was continued until 50 ml of the extract were attained. The extract was filtered using a strainer and put into a clean bottle, and was stored in the refrigerator until the making of shampoo. The process in the making citronella extract was the same in the process in securing extract from lemongrass.

### **Preparation of Shampoo with Citronella grass and Lemongrass**

The liquid castile soap, bottled extract of citronella grass and lemongrass measuring cup, mixer, and the container were prepared and measured according to the required quantity in the formulation. The 50 ml citronella grass extract was poured out into the mix together with the 50 ml liquid castile soap. The solution was mixed well until the desired consistency is acquired. The solution or shampoo was poured into a clean bottle and was set aside. The procedure was repeated to the lemongrass extract

and the mixed grasses. The set-aside shampoos were poured into a separate clean bottle and were labeled, citronella shampoo, lemongrass shampoo, and lemonella shampoo.

### **Counting of the number of Mites before Bathing the Native Chickens**

The chickens were taken out from their cages, every parts of each chicken from different treatments were checked where the red mites were seen. The counted number of red mites seen from one (1) quadrant of the body was multiplied four (4) times.

### **Application of Different Shampoo in Mite infested Chickens**

The different treatments were applied by bathing the experimental birds. Each formulated shampoo of 10 ml was mixed in a pail with one (1) gallon of tap water and were mixed well until some lather were seen. Each bird was dipped twice in the bathing solution until the feathers were wet. After bathing, the experimental bird was lifted and allowed to stand on a clean white cloth inside the separate cage until the chicken is dry. The number of dead mites that fell on the white cloth after bathing were counted with hand tally counter, and were recorded. The live mites that fell on the white cloth after bathing were observed and the time until they die were also recorded. In addition, the number of mites that fell in the water were filtered using a white cloth and also counted with the hand tally counter.

The number of dead mites left on one quadrant of the experimental bird's body were also calculated and multiplied by four; both numbers were added to get the estimated number of mites in the whole body. The bathing process was repeated after seven (7) days for four (4) weeks.

To determine the percentage of the efficacy of the anti-parasitic property of the prepared bathing solutions, the following formula was used:

$$\% \text{ Efficacy} = \frac{\text{Number of dead mites after treatment} \times 100}{\text{Total number of mites}}$$

### **Identification of the Parasites**

The mites were collected from the different parts of each one (1) native chicken (head,

wings, body, and tail) per cage. The collected mites were put into a small plastic container and were traveled to Parasitology Laboratory

at Tarlac Agricultural University, Malacampa, Camiling, Tarlac for identification and confirmation.

### Data Gathered

The following data were gathered during the study:

1. The percentage of the efficacy of different shampoos after its application in chickens;
2. The effect of shampoos with Citronella grass, Lemongrass, and a mixture of both kinds of grass on chicken mites on day 1, day 8, day 15, and day 22;

3. The time required to kill the red chicken mite using the different treatments, and;
4. The cost of shampoo using Citronella grass and Lemongrass.

### Data Analysis

The data gathered were analyzed using Analysis of Variance (ANOVA), and the difference among treatment means were determined using the Least Significant Difference (LSD). All types of analysis were made by using the STAR (Statistical Tool for Agricultural Research) software.

## Results and Discussion

Table 1. Number of Mites before Administration of Treatments

TREATMENTS	Day 1	Day 8	Day 15	Day 22
T1 (Negative Control)	17.33 <sup>a</sup>	20.08 <sup>a</sup>	22.33 <sup>a</sup>	31.33 <sup>a</sup>
T2 (Positive Control)	17.66 <sup>a</sup>	8.66 <sup>b</sup>	4.66 <sup>c</sup>	1.33 <sup>b</sup>
T3 (Citronella Shampoo)	20.00 <sup>a</sup>	10.66 <sup>b</sup>	6.66 <sup>bc</sup>	1.33 <sup>b</sup>
T4 (Lemongrass Shampoo)	17.33 <sup>a</sup>	12.00 <sup>b</sup>	8.00 <sup>b</sup>	1.33 <sup>b</sup>
T5 (Lemonella Shampoo)	21.33 <sup>a</sup>	20.08 <sup>a</sup>	7.66 <sup>bc</sup>	3.33 <sup>b</sup>

CV = Means with the same letter are not significantly different at 6385 level of significance by Tukey's Honest Significant Difference (HSD) Test

The table showed that on day one (1) there was no significant difference among the number of mites of chickens in five (5) treatments. However, table showed a slight numerical difference with T5 (Lemonella Shampoo) having the highest mean of 21.33 followed by T3 (Citronella Shampoo) with 20.00, the rest were almost the same. On day eight (8) T1 (Negative Control) and T5 (Lemonella Shampoo) had no significant differences and had the highest number of mites having both 20.08, also the table revealed that T2 (Positive Control), T3 (Citronella Shampoo), and T4 (Lemongrass Shampoo) were not significantly different to each other but significantly different to both T1 (Negative Control) and T5 (Lemonella Shampoo) having a means of 8.66, 10.66 and 12.00 respectively. Though T1 (Negative Control) and T5 (Lemonella Shampoo) have the same mean, T1 (Negative Control) on the other hand showed an increased in number of mites after the first bathing, while T5

(Lemonella Shampoo) showed a decrease in number of mites after the first bathing. On day 15 T1 (Negative Control) had the highest mean of 22.33, pointed that chickens in T1 (Negative Control) has the highest number of mites among the other treatments. It also revealed that T3 (Citronella Shampoo), T4 (Lemongrass Shampoo), and T5 (Lemonella Shampoo) were not significantly different to each other but there was a numerically difference to their means having 6.66, 8.00, and 7.66 respectively. However, T2 (Positive Control) and T3 (Citronella Shampoo) were not significantly different to each other nevertheless their means have a numerical difference, having a means of 4.66, and 6.66 respectively.

On day 22 table showed that the chickens in T1 (Negative Control) who was the only one different among other treatments, having the highest number of mites with the mean of 31.33. On the other hand, T2 (Positive Control), T3

(Citronella Shampoo), T4 (Lemongrass Shampoo), and T5 (Lemonella Shampoo) have no significant difference, however T2 (Positive Control) to T4 (Lemongrass Shampoo) have the same means of 1.33 which showed that it was lower than T5 (Lemonella Shampoo) with a mean of 3.33.

In terms of each treatment every week, the number of chicken mites in T1 (Negative Control) were increasing with a means of 17.33, 20.08, 22.33, and 31.33; T2 (Positive Control) showed that the number of chicken mites were decreasing every week having 17.66, 8.66, 4.66 respectively; T3 (Citronella Shampoo) had a mean of 20.00 on day 1, mean of 10.66 on day 8, mean of 6.66 on day 15, and mean of 1.33 on day 22, that shows a high drop of mites very week after bathing with citronella shampoo; Means of 17.33, 12.00, 8.00, and 1.33 in T4 (Lemongrass Shampoo) that also shows a decreasing initial numbers of chicken mites every week; Lastly T5 (Lemonella Shampoo) have a means of 21.33, 20.08, 7.66, and 3.33 on day 1, 8, 15, and 22 respectively.

On the other hand, Treatment 2 the positive control- Wash Out Intense® shampoo showed a high decrease on number of mites for 22 days. According to Toynton *et al.*, (2009), commercial shampoo Wash Out Intense® had a composition called permethrin, it is an insecticide in the pyrethroid chemical family that acts on the nervous system of insects. It interferes with sodium channels to disrupt the function of neurons, and causes muscles to spasm, culminating in paralysis and death of the parasite.

The decrease in number of mites was then followed by the using the citronella shampoo, lemonella shampoo, and lemongrass shampoo respectively. According to Chowański *et al.*, (2016) citronella and lemongrass have a composition of alkaloid that can affect mites at all levels of biological organization, but their action generally disturbs cellular and physiological processes, e.g., by altering redox balance, hormonal regulation, neuronal signalization or reproduction.

Table 2. Difference of treatments in terms of mortality rate of mites

TREATMENTS	Day 1	Day 8	Day 15	Day 22
<b>T1 (Negative Control)</b>	3.00 <sup>c</sup>	3.75 <sup>b</sup>	3.08 <sup>b</sup>	3.00 <sup>a</sup>
<b>T2 (Positive Control)</b>	16.41 <sup>a</sup>	7.50 <sup>ab</sup>	4.66 <sup>ab</sup>	1.33 <sup>a</sup>
<b>T3 (Citronella Shampoo)</b>	14.33 <sup>ab</sup>	7.33 <sup>ab</sup>	3.36 <sup>ab</sup>	1.33 <sup>a</sup>
<b>T4 (Lemongrass Shampoo)</b>	12.08 <sup>b</sup>	8.08 <sup>a</sup>	5.83 <sup>a</sup>	1.16 <sup>a</sup>
<b>T5 (Lemonella Shampoo)</b>	16.25 <sup>a</sup>	7.75 <sup>a</sup>	5.16 <sup>ab</sup>	2.75 <sup>a</sup>

CV= Means with the same letter are not significantly different at 4.6385 level of significance by Tukey's Honest Significant Difference (HSD) Test

On day 1 after bathing, the table showed that T2 had the highest mortality rate of chicken mites having a mean of 16.41 which had no significant difference to T5 and T3 with a mean of 16.25 and 14.33. However, T3 had no significant difference to T4 with a mean of 12.08, which all of the treatments were far-off comparable to a mean of 3.00 of T1.

On day 8, T4 with a mean of 8.08 and T5 with a mean of 7.75 were significantly different to T1 with a mean of 3.75 but not significantly different to T2 and T3 with a means of 7.50 and 7.33.

On day 15, after the bathing process of native chickens, T4 got the highest mortality rate of mites of 5.83 which was not significantly different to T5, T2 and T3 with a means of 5.16, 4.66 and 3.36 respectively, on the other hand T1 with a mean of 3.08 had a significant difference among all other treatments. It showed that all treatments except the negative control were comparable and have an effect killing the mites of native chickens. On the day 22, post-bathed table revealed that all treatments had no significant differences to each other, however based from Table 1. On day 22, T1 had the highest initial

number of mites of 31.33 which showed after bathing that only 3.00 mites were dead, which is very comparable to the initial number of mites of all other treatments. Negative control, the tap water, was not effective to kill chicken mites because it has no parasitic effect that can kill the red mites.

The result was connected and supported based from the discussion in Table 1. Treatment 2 the positive control- Wash Out

Intense® shampoo, showed a high mortality rate of mites for 22 days According to Toynton *et al.*, (2009), commercial shampoo Wash Out Intense® had a composition called permethrin, it is an insecticide in the pyrethroid chemical family that acts on the nervous system of insects. It interferes with sodium channels to disrupt the function of neurons, and causes muscles to spasm, culminating in paralysis and death of the parasite.

**Table 3. Difference of treatments in terms of percent of efficacy**

TREATMENTS	Day 1	Day 8	Day 15	Day 22
<b>T1 (Negative Control)</b>	19.36 <sup>c</sup>	19.12 <sup>b</sup>	13.77 <sup>d</sup>	9.58 <sup>b</sup>
<b>T2 (Positive Control)</b>	91.4 5 <sup>a</sup>	79.87 <sup>a</sup>	100.00 <sup>a</sup>	100.00 <sup>a</sup>
<b>T3 (Citronella Shampoo)</b>	79.53 <sup>ab</sup>	71.73 <sup>a</sup>	53.30 <sup>c</sup>	100.00 <sup>a</sup>
<b>T4 (Lemongrass Shampoo)</b>	69.61 <sup>b</sup>	66.47 <sup>a</sup>	73.41 <sup>b</sup>	65.69 <sup>a</sup>
<b>T5 (Lemonella Shampoo)</b>	76.39 <sup>ab</sup>	61.05 <sup>a</sup>	65.87 <sup>bc</sup>	81.25 <sup>a</sup>

CV= Means with the same letter are not significantly different at 6385 level of significance by Tukey's Honest Significant Difference (HSD) Test

Data on day 1 post-bathed of native chickens, T2 had showed the highest percent of efficacy among all other treatments with 91.45 percent. However, it has no significant difference among T3 and T5 with 79.53 percent and 76.39 percent wherein these two treatments had no significant with between T4 while T1 had the lowest percent of 19.36 and had a significant difference among all other treatments. On day 8, only T1 had a significant difference among all other treatments which means all other treatments are not significantly different to each other, however T2 had the highest percent of efficacy of 79.87, followed by T3, T4, and T5 with 71.73 percent, 66.47 percent, and 61.05 percent, respectively.

On day 15, T2 showed a remarkable efficacy of 100.00 percent which had a significant difference among other treatments, T4 and all treatments are comparable to each other as showed in Table 3 on day 15 in terms of percentage efficacy.

According to Sbhatu *et al.*, (2020) shampoo formulations with higher proportion (40 to 50% v/v) of *A. adigratana* gel were found to have comparable characteristics and qualities with a marketed shampoo. They fall within the range of

acceptable quality parameters of commercial shampoos. In general, the positive control or the commercial shampoo Wash Out Intense® have the composition of (Permethrin+ Piperonyl butoxide+ D-pantenol) was the most effective among all other treatments. Permethrin is an insecticide in the pyrethroid chemical family, it acts on the nervous system of insects. It interferes with sodium channels to disrupt the function of neurons, and causes muscles to spasm, culminating in paralysis and death (Toynton *et al.*, 2009); Insects have enzymes in their bodies that break down some insecticides. Piperonyl Butoxide stops some of these enzymes and allows insecticides more time to work. This means insects are less likely to recover from the combination of PBO and certain insecticides (Cross *et al.*, 2017).

The remarkable efficacy of the commercial shampoo was followed by the herbal shampoo produced which was the citronella shampoo, its important compounds includes alkaloids, volatile and non-volatile terpenoids, flavonoids, carotenoids and tannins (Oladeji *et al.*, 2019). Lemongrass has such compounds of phytosterols, anthocyanin, amino acids, organic acid, phenolic compounds, volatile components, fatty acids,

fumesol, flavonoids, isovalerenic aldehyde, methylheptenone, valeric esters, L-linanool, furfural, isopulegol, p-coumaric acid (Avoseh *et al.*, 2015). In addition, Chowański *et al.*, (2016) showed that alkaloid can affect insects at all levels of biological organization, but their action generally disturbs cellular and physiological processes, e.g., by altering redox balance, hormonal regulation, neuronal signalization or reproduction. Appendix table 7 showed some of the pharmacological evidence of citronella grass (*Cymbopogon nardus*) and lemongrass (*Cymbopogon citratus*).

It showed all citronella, lemongrass and lemonella shampoos are considered as herbal shampoos according to Shivanand *et al.*, (2010) since herbal shampoo is a cosmetic preparation

that uses herbs from plants, and it is meant for washing of hair and scalp just like the regular shampoo. Not just only in the usage of humans but can be applied to domestic animals also it is an alternative to the synthetic shampoo available in the market. In addition, Oladeji *et al.*, (2019) stated that the therapeutic potential of medicinal herbs could be associated to the presence of phytochemicals or secondary metabolites. These compounds are evenly spread in medicinal plants. According to Avoseh *et al.*, (2015),

*Cymbopogon* genus including citronella (*Cymbopogon nardus*) and lemongrass (*Cymbopogon citratus*) in different countries shows high applicability as a common tea, medicinal supplement, insect repellent, insecticide, in flu control, and as anti-inflammatory and analgesic.

Table 4. Difference of treatments in observed time (minutes) to kill the red chicken mite

TREATMENTS	Day 1	Day 8	Day 15	Day 22
<b>T1 (Negative Control)</b>	7.50 <sup>a</sup>	15.50 <sup>a</sup>	15.75 <sup>a</sup>	41.50 <sup>a</sup>
<b>T2 (Positive Control)</b>	5.00 <sup>c</sup>	5.00 <sup>b</sup>	5.00 <sup>c</sup>	5.00 <sup>b</sup>
<b>T3 (Citronella Shampoo)</b>	6.00 <sup>b</sup>	8.00 <sup>b</sup>	8.00 <sup>b</sup>	9.00 <sup>b</sup>
<b>T4 (Lemongrass Shampoo)</b>	6.50 <sup>b</sup>	8.50 <sup>b</sup>	9.25 <sup>b</sup>	10.25 <sup>b</sup>
<b>T5 (Lemonella Shampoo)</b>	6.00 <sup>b</sup>	8.00 <sup>b</sup>	9.00 <sup>b</sup>	10.00 <sup>b</sup>

CV= Means with the same letter are not significantly different at 2.1314 level of significance by LSD.

Data on day 1 post-bathed native chickens, T1 had showed the longest time in terms of minutes among all other treatments with 7.50. T3, T4 and T5 have no significant difference, however T4 shows a slight numerical difference in time. Among other treatments T2 shows a significant difference with 5.00 minutes. On day 8, only T1 had a significant difference among all other treatments with 15.50 which means all other treatments are not significantly different to each other, however T2 and T4 shows a slight difference in time with 5.00 and 8.50 respectively.

On day 15, T1 shows the longest observed time of kill the mites with 15.75 which had a significant difference among other treatments. Followed by T4, T5 and T3 with the means of 9.25, 9.00, and 8.00 respectively, however these 3 treatments have no significant difference to

each other. While T5 shows the shortest time observe to kill the mites with 5.00. On day 22, T2, T3, T4, and T5 were significantly different with the means of 5.00, 8.00, 9.25, and 9.00 respectively, however T1 shows a high significant difference to other treatments with a mean of 41.50. Day 22 shows that T1 with 41.50 has the longest observed time to kill mites and has a significant difference among other treatments. On the other hand, T2, T3, T4 and T5 have no significant difference to each other with a means of 5.00, 9.00, 10.25, and 10.00 respectively.

The table shows that the T1 the negative control shows the consistency having the longest observed time to kill the red mites since it is the only treatment that has no fatal property to the red mites. The treatment that shows the shortest observed time to kill mites was the T2 the positive control which consist the effective

components to kill mites according to the discussions above, it was then followed by T3 the citronella shampoo, T5 the lemonella shampoo and lastly T4 the lemongrass shampoo.

Cost analysis of making shampoo using citronella, lemongrass and mixed of both grasses'

extracts revealed that were less economical than the commercial shampoo available. The cost analysis of using citronella and lemongrass was showed on the table below.

Table 5. Cost analysis of citronella, lemongrass and lemonella shampoos

COST ANALYSIS				
MATERIALS	PRICE	PRICE	PRICE	PRICE
	T3 CITRONELL A SHAMPOO ( <u>Php</u> )	T4 LEMONGRASS SHAMPOO ( <u>Php</u> )	T5 LEMONELLA SHAMPOO ( <u>Php</u> )	COMMERCIAL SHAMPOO (Wash Out Intense®) ( <u>Php</u> )
60 ml of coconut liquid castile soap	54	54	54	
Grasses (Citronella and Lemon grass)	50	50	50	
Electricity	5	5	5	
Labor	100	100	100	
<b>TOTAL EXPENSES</b>	<b>209 (120 ml)</b>	<b>209 (120 ml)</b>	<b>209 (120 ml)</b>	<b>64 (40 ml)</b>
<b>COST OF 10 ML SHAMPOO</b>	<b>18</b>	<b>18</b>	<b>18</b>	<b>16</b>

## Summary

The study was conducted to produce an herbal shampoo using the extracts of citronella grass, lemongrass, and mixed of both grasses. It found out which herbal shampoos which was the most effective compared with commercial shampoo (Wash Out Intense®) against mites of native chicken and their time observed to kill mites. In addition, the cost analysis was determined in using citronella grass and lemongrass as a shampoo against chicken mites.

60 laying native chickens naturally infested with mites from different areas around Tarlac were used in the study, the chickens were grouped randomly and distributed to five (5) treatments. Chickens were managed accordingly with feeding with commercial layer feeds twice a day, every seven (7) in the morning, four

(4) in the afternoon, and were provided clean water and refreshed every feeding time their cages were also cleaned regularly. The mites infesting were collected and traveled to at Parasitology Laboratory at Tarlac Agricultural University, Malacampa, Camiling, Tarlac. Herbal shampoos made out of citronella, lemongrass, and mixed of both grasses extract was prepared before bathing the native chickens. After the identification and confirmation of the mites, the bathing process were made, lasted for four (4) weeks with seven (7) days interval. Before bathing the number of mites of native chickens were counted and recorded; after bathing the dead mites were also counted and recorded.

The efficacy data showed that from day one (1) to day 22, T2 the positive control- Wash Out Intense® shampoo was the most effective

shampoo (from 79.87- 100.00%). T3 (citronella shampoo) with the means of 79.53, 71.73, 53.30, and 100.00 percent from day 1, 8, 15, and 22 respectively. The lemonella shampoo with the means of 76.39, 61.05, 65.87, and 81.25 percent, and lemongrass shampoo with the means of 69.61, 66.47, 73.41, and 65.69 percent on day 1, 8, 15 and 22 respectively. On the other hand, T1 the negative control (tap water) demonstrated the lowest percentage of efficacy on day 1 to 22 with the means of 19.36, 19.12, 13.77, and 9.58 percent. According to Sbhatu *et al.*, (2020) shampoo formulations with higher proportion (40 to 50% v/v) of *A. adigratana* gel were found to have comparable characteristics and qualities with a marketed shampoo. As for the herbal shampoos produced having a greater than 50% efficacy, they were then comparable to the Wash Out Intense®, also they fall within the range of acceptable quality parameters of commercial shampoos.

Wash Out Intense® have the composition of (Permethrin+ Piperonyl butoxide+ D- panthenol) was the most effective among all other treatments with 92.83% efficacy. The notable efficacy of the commercial shampoo was followed by the herbal shampoo produced; a) citronella shampoo, b) lemonella shampoo, c) lemongrass shampoo, with 76.14%, 71.14%, and 68.80% efficacy, respectively. The effectiveness of the different treatments also relied to the observed time to kill the red mites, table 4 shows that the T1 the negative control showed the longest observed time to kill the red mites since it has no fatal property to the red mites. The treatment that showed the shortest observed time to kill mites was the T2 (positive control), T3 (citronella shampoo), T5 (lemonella shampoo) and T4 (lemongrass shampoo).

Cost analysis of making shampoo using citronella, lemongrass and mixed of both grasses' extracts revealed that were less economical than the commercial shampoo available.

## Conclusion

This study produced herbal shampoos from the grasses citronella, lemongrass, and mixture of both grasses' extracts. It was found out that the three (3) shampoos were effective as a main component of the shampoo against red

chicken mites. The citronella shampoo was the most effective comparable to the commercial shampoo (Wash out intense®) used. Thus, shampoos produced using citronella, lemongrass and mixed of both grasses could be used and could be cost effective compared to the commercial shampoo used.

## Recommendation

Based on the result of this study, these are the following recommendations:

1. The use of mixed grasses (lemonella) shampoo were effective as an herbal shampoo against red mites of native chicken.
2. Increasing the concentration or percent of lemongrass extracts are recommended to increase the efficacy of lemongrass shampoo.
3. Further study to different species of birds using the different produced herbal shampoo.
4. Further study to other species of ecto-parasites of birds or poultry using the citronella, lemongrass, and lemonella shampoo.
5. The proper disposal of the mites or other ecto-parasite must be included to other study.
6. Further study of the quality characteristics of the herbal shampoo produced.

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