ORIGINAL ARTICLE

EFFICACY OF GARLIC (*Allium sativum* L.), OREGANO (*Origanum vulgare* L.) AND TURMERIC (*Curcuma longa* L.) AGAINST LARVAL DEVELOPMENT OF *Ascaridia galli* EGGS ISOLATED FROM PHILIPPINE NATIVE CHICKEN

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ABSTRACT

The efficacy of three plant extracts - garlic, oregano and turmeric - in inhibiting larval development of *Ascaridia galli* eggs was evaluated *in vitro.* About 2000-2500 *A. galli* eggs were incubated per treatment and replicated five times. Ethanolic extracts of plant materials were diluted in phosphate buffered saline to form a dose concentration of 50 mg/ml. After 21 days of incubation under room temperature, results revealed that the three plants were as effective as albendazole in inhibiting the development of *A. galli* eggs on the 10th day of incubation. Garlic and turmeric were found to be effective but were no longer as efficient as albendazole on day 14 and day 21. Among the three plants, garlic was the most effective in inhibiting the development of the eggs since it did not go beyond the morula stage until day 21. On the other hand, the number of eggs that formed into larval stage was lower in turmeric than in oregano. This study indicated that garlic, oregano and turmeric can be used to prevent *A. galli* eggs development into L_3 stage and hence, may prevent roundworm infection in chicken. However, supplementation must be programmed in order to retain their efficacy.

Keywords: *Ascaridia galli*, chicken, garlic, larval development, oregano, turmeric

INTRODUCTION

Ascaridia galli is considered as the most common and most damaging parasitic roundworm of domesticated fowls (Permin *et al*., 1999). Females of this parasite lay thick heavy-shelled eggs in the intestine that pass in the feces. The eggs undergo embryonation in the environment until they become infective (Morgan and Hawkins, 1953). Embryonated eggs are very hardy and may live for two years under optimum conditions (Islam *et al*., 2008). Chemical dewormers have been used to decrease the effects of these parasites. However, the drugs are expensive and often unavailable to farmers in rural areas (Kaingu

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et al., 2013). Furthermore, serious disadvantages such as resistance, food residues and environmental pollution have become evident over the past years (Islam *et al*., 2008). These have lead consumers all over the world to clamor for meat products produced organically. The Philippines is no exemption. There is now a growing consumer awareness for safe and healthy food products. The creation of the Philippine National Standard (PNS) for organic agriculture based on the Organic Act of 2010 is a strong proof of this increasing demand.

Evaluation of local plant materials as substitute for synthetic dewormers such as albendazole could contribute to the science-based practices in organic chicken production. As such, the extracts of these local plant materials contain substances that simulates the action of albendazole in terms of destroying and eliminating common roundworms, specifically *Ascaridia galli*. Furthermore, plant materials manifesting these characteristics are abundant in nature and are, thus, cheap.

In vitro screening of plant materials as anthelmintic against *A. galli* has been done in several countries. However, most prevailing studies using these medicinal plants aimed only at controlling the population of adult *A. galli* worms. The threats posed by its eggs are oftentimes neglected. Eggs of *A. galli* are generally hardier and have a higher survival rate in the environment, thus, having a higher infection potential. The present study was designed to evaluate and compare the *in vitro* efficacy of garlic, oregano and turmeric on the inhibition of larval development of *A. galli* eggs from native chickens and compare their effectiveness over albendazole, a standard chemical dewormer.

MATERIALS AND METHODS

The study was conducted from February to March 2014 at the Animal Health Laboratory, Animal and Dairy Sciences Cluster, College of Agriculture, University of the Philippines Los Baños, College, Laguna. Phosphate buffered saline (PBS) was used as the medium for this experiment. It was utilized to maintain the eggs in a viable condition while they are manipulated outside of their regular growth environment. A 1x stock solution of PBS was prepared through the mixture of following reagents: 32 g sodium chloride (NaCl); 0.80 g potassium chloride (KCl); 5.76 g disodium hydrogen phosphate Na_2HPO_4; 0.96 g potassium phosphate KH_2PO_4; 0.53 g calcium chloride $CaCl_2$; and 0.40 g magnesium chloride ($MgCl_2$). Four thousand ml of PBS was made by dissolving and mixing the reagents mentioned earlier in distilled water. The final solution was sterilized by autoclaving for 20 min at 15 psi.

Garlic (*Allium sativum*), oregano (*Origanum vulgare*) and turmeric (*Curcuma longa)* were selected on the basis of their ethnomedical uses and availability. Garlic and turmeric were purchased from a local market in Laguna. Oregano leaves, on the other hand were obtained from a backyard farm. Approximately 2 kg were obtained from each plant material. Only the cloves of garlic, the rhizome part of turmeric and the leaves of oregano were specifically used in this study.

The plant materials were washed several times and sliced thinly. Thereafter, they were oven dried for 3 days at 70°C and then ground to powdered form. About 40 g of each powdered material were soaked individually in 200 ml of 95% ethanol for 5 days with intermittent shaking and then filtered using Whatman No. 1 filter paper. The filtrate for each plant material was concentrated in a rotavapor machine (Rotavapor R-210, BUCHI Labortechnik AG, Meierseggstrasse 40, Postfach CH-9230 Flawil 1, Switzerland) at 80°C. The concentrated thick masses were then weighed and diluted to 50 mg/ml using PBS. The albendazole (albendazole 11.25% oral suspension with 112.5 mg of albendazole per ml, Chemvet Products, Inc., Caloocan City, Metro Manila) was diluted in PBS to form a dose concentration of 50 mg/ml.

Specimens of adult *Ascaridia galli* worms were collected in PBS pH 7.4, from the intestines of Philippine native chickens. The contents of the intestines were carefully squeezed out to obtain live parasites. Thirty (30) mature female parasites were gathered and were washed several times with distilled water to remove all the fecal debris. Eggs were recovered using the method of Kaingu *et al*., (2013) by scraping the uterine portion of the parasites using scalpel while being submerged in PBS. The macerated portions of their body were removed and the remaining eggs were concentrated in 40 ml of PBS. A few drops of 2% formalin were added to the suspension to prevent mold formation in the samples. The product was called egg-PBS suspension.

A total volume of 5 ml concentrated extract inoculated with approximately 2000-2500 eggs was placed in each petri dish. The study utilized five treatments: Treatment 1 - PBS (negative control), Treatment 2 - garlic, Treatment 3 - oregano, Treatment 4 - turmeric and Treatment 5 - albendazole (positive control). The concentration of 50 mg/ml active ingredient was strictly used for all treatments, which were replicated 5 times following a completely randomized design.

All the treatments were placed in petri dishes that were autoclaved for proper sterilization. The upper meniscus of the total volume of suspension in all petri dishes was marked by permanent ink. The petri dishes were placed in one table and kept at room temperature for 21 days. Moist cottons were placed in 4 corners of the table so as to have additional moisture and prevent desiccation. A portion of the petri dishes was kept open to allow aeration for the development of eggs. The temperature and relative humidity ranges for the entire trial were taken from a temperature and relative humidity monitoring device. Throughout the trial, the samples were shaken twice a day to mix evenly the contents of the suspension. The upper meniscus of fluid was maintained by adding PBS if necessary.

The samples were monitored on the 10th, 14th, and 21st day for the development of the larva within the egg. Using a micropipettor, an aliquot of 50 µl suspension were taken from each replicate, placed in glass slide with cover slip and examined under the microscope. Eosin red was used as dye for the treatments with eggs that were hardly seen due to impurities of the suspension. For the basis of classifying the developing eggs, a chart on the stages of development of *A. galli* eggs, adapted from the drawings of Ackert (1931) was used.

In order to simplify the identification and classification of the different stages of the *A. galli* eggs during the development course, the whole process was divided into 2 groups: 1) undeveloped; and 2) developed. Both mature infertile eggs and fertile eggs that had not gone through division process were counted as undeveloped. Eggs in the early and late morula, tadpole, vermiform and larval stages were considered as developed. The number

of eggs counted for each unit was then multiplied with 100 to represent the total number of eggs in the entire 5 ml of suspension. To easily denote the degree of effectiveness among treatments, the specific stage that the eggs have reached were noted.

Laboratory evaluation of anthelmintic activity of garlic, oregano and turmeric was done using *Ascaridia galli* viable eggs recovered. The percentage of larval development was calculated using the formula:

$$\%\ developed\ eggs = \left[\frac{number\ of\ eggs\ counted\ as\ developed}{total\ number\ of\ eggs\ counted}\right] x\ 100$$

The data gathered were first transformed using square root function. The transformed data were analyzed for outliers, normality and homogeneity of variance. Thereafter, data were subjected to ANOVA and Least Significant Difference (LSD) tests at 5% level of significance.

RESULTS AND DISCUSSION

The mean percentage of developed *A. galli* eggs and the degree of their development during the 10th, 14th and 21st day of incubation are shown in Tables 1 and 2. *In vitro* assessment of the activity of garlic, oregano, and turmeric on inhibiting *A. galli* eggs development revealed that their effectiveness was time dependent.

Percentage of developed eggs and degree of development at day 10.

Garlic, oregano, turmeric and albendazole showed significant (P<0.05) overall anthelmintic activity as compared with PBS treated eggs. All treaments obtained 0% development, except PBS treatment, which had 43% developed eggs. At this period of time, the three plant extracts were found to be as effective as albendazole in inhibiting the development of *A. galli*.

Table 1. Mean percentage of *A. galli* eggs counted during the 10th, 14th and 21st day of incubation in different media.

Mean % of developed eggs	Treatment					
	PBS	Garlic	Oregano	Turmeric	Albendazole	CV (%)
Day 10	43.20^{a}	0^{b}	0^{b}	0^{b}	0^{b}	38.75
Day 14	46.81^{a}	30.47^{b}	39.00^{ab}	30.67^{b}	0^{c}	17.71
Day 21	51.04^{a}	25.39^{c}	39.56^{b}	19.31^{c}	0^{d}	13.94

Means with the same superscript in the same row are not significantly different.

In terms of development, the eggs in PBS treatment have already developed into the vermiform and larval stages (43%), although the proportion of the undeveloped (57%) eggs was still higher. On the other hand, treatment of eggs in the three plant extracts and albendazole all remained at their undeveloped state. The early development of eggs in PBS can be explained by the triggering effect of the temperature and relative humidity. According to Ramadan and Znada (1992), at 10 days larva could already be developed considering an optimum environmental condition. Tarbiat (2012) observed in his study that *A. galli* eggs subjected at a temperature of 30°C have developed rapidly and turned into vermiform and larval stages at 7 days of incubation. Meanwhile, under laboratory condition, the relative humidity might have a pronounced effect on the viability of the *A. galli* eggs. Tarbiat (2012) stated that *A. galli* eggs require a highly saturated atmosphere before they could finish the development process. In this experiment, the temperature ranged from 25.4 to 31.6°C while ensuring a more saturated environment (%RH ranged from 42 to 79) through the provision of moist cottons on the corners of the table. Given that optimum conditions were provided for the development of the eggs, it could be herein stated that garlic, oregano, and turmeric did inhibit the development of the eggs.

Percentage of developed eggs and degree of development at day 14.

There was an apparent difference on the percentage of developed eggs at this period. Some of the eggs treated in the three plant extracts have already developed. PBS treated eggs had the highest percentage of development with 46%. However, 100% of the eggs in albendazole treatment still remained at their undeveloped stage. Although treatments with garlic (30%) and turmeric (30%) had developed eggs, they were still found to be significantly (P<0.05) different from PBS treatment, which means that garlic and turmeric extract are still effective in inhibiting *A. galli* egg development but were no longer as efficient as albendazole. At this point, oregano, yielding 40% developed eggs was no longer considered effective, unlike garlic and turmeric. This was due to the fact that it does not differ significantly from PBS treatment group.

The remaining vermiform eggs in PBS treatment have transformed into the early larval (20%) stage and some into coiled and fully mature (26%) infective larvae. Albendazole treated eggs were still undeveloped at this point, yielding 0% development. The least degree of developing eggs was explicitly observed in treatment with garlic. At this treatment, development was observed to be much slower compared with the eggs treated in oregano and turmeric. The eggs in garlic treatment were at the early morula stage or simply at their cell dividing stage only. Majority of the eggs in the entire population were at the 2-cell stage at the proportion of 18% but the degree of development was bounded up to the 5-cell (2%) stage. On the other hand, eggs in oregano and turmeric treatments have extended to the late tadpole and early vermiform stages. Considering the proportion of tadpole and vermiform embryos that have formed, the percentage was significantly (P<0.05) higher in oregano (40%) than in turmeric (31%) treatment. The occurrence of development on the three plant dewormers might be hastened by the decreasing effect of the extract prior to day 14.

Table 2. Comparison of the degree of development of *A. galli* eggs observed during the 10th, 14th and 21st day of incubation.

Day of observation	Treatment				
	PBS	Garlic	Oregano	Turmeric	Albendazole
Day 0[1]	100 % UE	100 % UE	100 % UE	100 % UE	100 % UE
Day 10	57% UE	100 % UE	100 % UE	100 % UE	100 % UE
	43% VS				
Day 14	54% UE	58% UE	60% UE	69% UE	100 % UE
	20% VS	18% 2-cell stage	40% TS	31% TS	
	26% LS	4% 3-cell stage			
		6% 4-cell stage			
		2% EMS			
Day 21	49% UE	75% UE	61% UE	83% UE	100 % UE
	51% LS	11% 2-cell stage	14% VS	12% VS	
		14% LMS	25% LS	7% LS	

[1]Column for day 0 was made to show that all treatments started with undeveloped eggs.
UE: unfertilized egg; VS: vermiform stage; LS: larvated stage; EMS: early morula stage; LMS: late morula stage; TS: tadpole stage.

Percentage of developed eggs and degree of development at day 21.

The results for the last day of observation still showed a high percentage of development in PBS treatment with 51% developed eggs. Treatment of eggs with albendazole was consistent throughout the trial since no development occurred until the last day of trial. It was the most effective among the treatments in terms of inhibiting the development of the *A. galli* eggs. Garlic and turmeric treatments were still significantly different with both PBS and oregano. Even if oregano was found to be significantly different with PBS, it was still not as effective as garlic and turmeric.

All the developed eggs in PBS have transformed into coiled and fully mature infective larva. The degree of development in garlic treated eggs was hastened up to the late morula (6%) stage which can be distinguished by the presence of blastomeres. But predominantly, developed eggs of this treatment were at the 2 cell (11%) stage only. For oregano and turmeric treatments, the eggs have transformed into fully formed vermiform embryo and some into coiled and fully mature infective larva, but then again, the percentage was significantly higher in oregano than turmeric treatment with 40% and 19% developed eggs, respectively. The eggs in albendazole remained undeveloped at a rate of 100% until the last day of the trial.

Based on several *in vitro* studies, the active principles in medicinal plants responsible for the anthelmintic and/or nematocidal activity are non-polar compounds, which are soluble in less polar solvents like ethanol (Ahmad *et al*., 2013; Abdelqader *et al.,* 2012). Furthermore, these plants produce compounds called phytoncides which may be the source of their active components (Li *et al.*, 2009).

Comparing the three medicinal plants that were used in this experiment, garlic extracted from its cloves showed the highest efficacy in inhibiting development of *A. galli* eggs. The effectiveness of garlic could be attributed to its active ingredient, allicin, and also other sulfur containing compounds that have anthelmintic potential (Haciseferogullari *et al.*, 2005). The slow degree of development found in this treatment could be attributed to the fast conversion of alliin to allicin by its enzyme, allinase. On the other hand, its short term efficiency could be derived from the fact that the reactive allicin molecules have a very short half-life (Block, 1985).

Next to garlic was turmeric and its anthelmintic activity could be attributed to the cucurminoid components of the rhizome. Claeson *et al.,* (1993) further stated that its antiparasitic activity is associated with β-dicarbonylic system with conjugated double bonds. Also, the presence of cyclocurcumin, a component of curcumin could be the nematocidal constituent of the extract (Kiuchi *et al.*, 1993).

The least effective among the plant materials was the oregano extract. This plant was only effective for 10 days of incubation. The active components carvacrol and thymol are believed to be the responsible for its anthelmintic property (Windholz, 1976). Oregano extract were more commonly used and proven effective in i*n vitro* studies against bacterial and fungal infections. From this, it could be said that oregano has a greater bactericidal and antifungal effect than being anthelmintic or nematocidal.

Albendazole, on the other hand, showed an overall effectiveness in inhibiting the development of *A. galli* eggs. Once given, albendazole is rapidly converted to its sulphoxide metabolite which is primarily responsible for the systemic anthelmintic activity. Also, it is known to make the cell have an impaired uptake of glucose, leading to depletion of glycogen reserve and reduces stored ATP. Lastly, it binds to nematode β-tubulin, inhibiting polymerization, thus preventing the formation of microtubules and so stopping cell division. The exact mechanism of action underlying the effects of the three medicinal plants remains unknown as they may vary according to the preparation. No studies have been developed yet about their mode of action on the cell but there are claims that medicinal plants, when used as anthelmintic, damage the cell through condensation of cytoplasmic material, making the membranes disintegrate, thus loosing structural integrity.

CONCLUSIONS

Garlic, oregano and turmeric represent promising alternatives to chemical anthelmintic drugs. However, subsequent supplementation must be done in order to retain their efficacy. Among the three plant extracts, garlic was the most effective since it inhibited the development of the eggs to morula stage until the 21st day of incubation. This study recommends the use of varying dose concentrations of the extract to accurately determine the effective dose at which *A. galli* egg development would be inhibited. It is also recommended to perform phytochemical test to detect for the presence and/or absence of compounds in the extracts. An i*n vivo* study of this topic is highly encouraged to be performed to test the efficacy of these plants against adult worms.

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REFERENCES

Abdelqader A, Qaralla B, Al-Ramamneh D and Das G. 2012. Anthelmintic Effects of Citrus Peels Ethanolic Extracts against *Ascaridia galli*. *Vet Parasitol* 188(2): 78-84.

Ackert JE. 1931. The morphology and life history of the fowl nematode *Ascaridia lineata* (Schneider). *J Parasitol* 23(1): 360-379.

Ahmad J, Tanveer S and Zargar B. 2013. *In vitro* anthelmintic activity of *Mentha longifolia* leaves against *Ascaridia galli*. *Glob Veterinaria* 11(1): 112-117.

Block E. 1985. The chemistry of garlic and onion. *Sci Am* 252(3): 114-9.

Claeson P, Panthong A, Tuchinda P, Reutrakol V, Kanjanapothi D, and Taylor WC. 1993. Three non-phenolic diarylheptanoids with anti-inflammatory activity from *Curcuma xanthorriza*. *Planta Med* 59(5): 451-454.

Haciseferogullari HM, Ozcan FD and Calisir S. 2005. Some nutritional and technological properties of garlic (*Allium sativum*). *J Food Eng* 68(1): 463-469.

Islam KR, Farjana T, Begum and Mondal MMH. 2008. *In Vitro* efficacy of some indigenous plants on the inhibition of development of eggs of *Ascaridia galli*. *Bangl J Vet Med* 6(2): 159-167.

Kaingu F, Kibor A, Waihenya R, Shivairo R and Mungai L. 2013. Efficacy of *Aloe secundiflora* crude extracts on *Ascaridia galli in vitro*. *Sust Agr Res* 2(2): 49-53.

Kiuchi F, Goto Y, Sugimoto N, Akao N, Kondo K and Tsuda Y. 1993. Nematocidal activity of turmeric: Synergistic action of curcuminoids. *Chem Pharm Bull* 41(9): 1640-3.

Li Q, Kobayashi M, Wakayama Y, Inagaki H, Katsumata M, Hirata Y, Hirata K, Shimizu T, Kawada T, Park BJ, Ohira T, Kagawa T and Miyazaki Y. 2009. Effect of phytoncide from trees on human natural killer cell function. *Int J Im Pharmacol* 22(4): 951.

Morgan BB and Hawkins PA. 1953. *Veterinary Helminthology*. Minneapolis, United States: Burgess Publishing Company.

Pemin A, Bisgaard M, Frandsen F, Pearman M, Kold J and Nansen P. 1999. Prevalence of gastrointestinal helminths in different poultry production systems. *British Poult Sci* 40: 439-443.

Ramadan HH and Znada ANY. 1991. Morphology and life sistory of *Ascaridia galli* in the domestic fowl that are raised in Jeddah. *J. K. A. U. Sci.* 4: 87-99.

Tarbiat B. 2012. Environmental tolerance of the free-living stages of the poultry roundworm (*Ascaridia galli*). Accessed 1 February 2014. from http://stud.epsilon.slu.se/4764.