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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Residual Activity of *Metarhizium anisopliae* or Plant Extracts on Laying Hens for *Menacanthus stramineus* Lice Control by Dipping

E.S. Pablo[1], A.L.M. Sandoval[1], M.R. Fernández[2], E. Morales[1], O. Prado[3], G. Tellez[4] and M.T.M. Quintero[5]
[1]Departamento de Producción Agrícola y Animal,
Universidad Autónoma Metropolitana, D.F. 04960, México
[2]Instituto Nacional de Investigación Forestales Agrícolas y Pecuarias CENID
Parasitología de Jiutepec, Morelos, Mexico
[3]Laboratorio de Producción Avícola, FMVZ, Universidad de Colima, Tecomán, Colima, 28100
[4]Department of Poultry Science, University of Arkansas, Fayetteville AR 72701, USA
[5]Departamento de Parasitologia, FMVZ, Universidad Nacional Autónoma de México, D.F. 04510, Mexico

Abstract: In the present study, eighty-four Hy-Line W36 laying hens in two experiments were distributed in 7 treatments with 3 replicates of four hens each. Each treatment, hens received 3 dipping/2 min every 48 h. Residual activity was done by counting lice one month after dipping. Treated hens with no live lice were reinfested with 20 lice and repeated during three months. In experiment one, aqueous suspensions of three plant extracts were tested as dips for control of MS lice: a) Neem (Azadirachta indica) 500 ppm; b) Ruda (Ruta graveolens) 11,700 ppm; or c) Solanacea (Ardisia solanacea) 50,000 ppm; d) Negative Control (water). After the first dipping, a significant difference ($p<0.05$) in the number of dead lice were observed in the hens that received Neem (84.1%) or Solanacea (98.1%), however, after the second and third dipping, all treated groups showed a significant increase in the number of dead lice compared with the control. Average after the 3 dips was: Neem (93.6%); Ruda (85.2%); Solanacea (98.2%); Control (49.1%). One month later, all 3 treated groups had 0 lice compared with 38 lice in the control group. Counts of live lice at two months after first reinfestation were: Neem (0); Ruda (1); Solanacea (43); Control (51). Counts of live lice at three months after second reinfestation were: Neem (0); Ruda (15); Solanacea (NA); Control (60). In experiment two, 3 aqueous suspensions were tested: group 1) Ruda tincture 50,000 ppm; group 2) Coumaphos 1,000 ppm; or group 3) *M. anisopliae* 50,000 ppm. After the first dipping, a significant difference in the number of dead lice were observed in the hens that received Coumaphos (100 %), however, no significant differences were observed between treatments after the second and third dipping Counts of live lice one month later were: group 1 (2 lice); group 2 (0 lice); group 3 (38 lice). Counts of live lice at two months after first reinfestation were: group 1 (13) and group 2 (16). The results of the present study suggest that some alternative bio-control methods for lice in laying hens are effective.

Key words: *Metarhizium anisopliae*, lice, hens, bio-control, plant extracts

INTRODUCTION

Chewing lice (Phthiraptera: *Amblycera*, *Ischnocera*) are important poultry ectoparasites. Living mainly on the skin, amblyceran lice may cause irritation of the skin, restlessness, overall weakening, cessation of feeding, loss of weight, inferior laying capacity and skin lesions that may become sites of secondary infection (Mullen and Durden, 2002; Sychra *et al*., 2008; Wall and Shearer, 2001). The most pathogenic are hematophagous species-*Menacanthus stramineus* and *Menacanthus cornutus*. They may cause anemia, heavy multi-focal skin lesions or even dead of infested birds (Prelezov and Groseva, 2006). With regard to the economic importance of chewing lice on poultry, various aspects of their biology have been studied, such as distribution on the host body, population dynamics, geographical distribution or economic harmfulness (Bradley *et al*., 2009; Fabiyi, 1996; Trivedi *et al*., 1991). Furthermore, these parasites may transmit agents of harmful diseases (bacteria, viruses and Protozoa) to man and/or to his domestic animals (Vreeken-Buijs *et al*., 1998).

Several control methods including chemical control agents have been recorded. More than 35 compounds including organochlorines, organophosphates, pyrethroids, and carbamates have been applied against the poultry ectoparasites (Nordenfors *et al*., 2001; Thind and Ford, 2007). At the same time some alternative control methods such as the biological control using the bacterium *Bacillus thuringiensis* were also applied. The latter method was proven to be also effective against a number of dust mites (McKeen *et al*., 1988; Hassanain *et al*., 1996). Plant extracts can be used for scientific testing, to find out what nutrients or chemicals are in the plant. Several plant extracts have indicated to have efficacy against mite and lice in poultry (Fathy *et al*., 2008; George *et al*., 2008). The objectives of the present

study were to evaluate the effectiveness of alternative bio control methods for lice in laying hens.

MATERIALS AND METHODS

Plant extracts: Plant extracts were reproduced and adapted from Paye, 2000.

Experimental animals: The present study was conducted at the Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias INIFAP CENID Parasitología, Jiutepec, Morelos. Eighty-four Hy-Line W36 laying hens in two experiments. Previous to the study, ectoparasites form these birds were sampled and identified at the Entomology Laboratory of the College of Veterinary Medicine, UNAM using the Hoyer's Mounting Medium (Evans and Browning, 1955). Only *Menacanthus stramineus* was identified and reported to be present in these birds. Each treatment, hens received 3 dipping/2 minutes every 48 h. Residual activity was done by counting lice one month after dipping. Treated hens with no live lice were reinfested with 20 lice and repeated during three months.

Experimental design trial 1: In this trial, forty eight laying hens were distributed in 4 treatments with 3 replicates of 4 hens each. Aqueous suspensions of three plant extracts were tested as dips for control of MS lice: a) Neem (*Azadirachta indica*) 500 ppm; b) Ruda (*Ruta graveolens*) 11,700 ppm; or c) Solanacea (*Ardisia solanacea*) 50,000 ppm; d) Negative Control (water).

Experimental design trial 2: In experiment two, thirty six laying hens were distributed in 3 treatments with 3 replicates of four hens each. Aqueous suspensions tested were: group 1) Ruda tincture 50,000 ppm; group 2) Coumaphos 1,000 ppm; or group 3) *M. anisopliae* 50,000 ppm.

Data analysis: In trial one, a 4 x 3 factorial analysis was performed to evaluate the four extracts tested and dipps. In trial two, a 3 x 3 factorial analysis was performed to evaluate the three extracts tested and dipps. Statistical significance was considered at $p<0.05$ (SAS Institute, 1988).

RESULTS AND DISCUSSION

The two most common ectoparasites found in caged laying operations are the chicken body louse (*Menacanthus stramineus*) and the northern fowl mite (*Ornithonyssus sylviarum*). The presence of both these parasites on the same hen host has rarely been observed. Because the economical importance of these pests in poultry production, improved control measures, including herbal or biological, must be pursued to avoid economic losses and achieve sustainable control (Bradley *et al*., 2009; Fabiyi, 1996; Trivedi *et al*., 1991). Table 1 summarizes the umber of dead lice counted in seven anatomical regions of hens treated with three plan extracts in trial 1. After the first dipping, a significant difference ($p<0.05$) in the number of dead lice were observed in the hens that received Neem (84.1%) or Solanacea (98.1%), however, after the second and third dipping, all treated groups showed a significant increase in the number of dead lice compared with the control. Average after the 3 dips was: Neem (93.6%); Ruda (85.2%); Solanacea (98.2%); Control (49.1%).

One month later, all 3 treated groups had 0 lice compared with 38 lice in the control group. Counts of live lice at two months after first reinfestation were: Neem (0); Ruda (1); Solanacea (43); Control (51). Counts of live lice at three months after second reinfestation were: Neem (0); Ruda (15); Solanacea (NA); Control (60), these data is summarized in Table 3. Table 2 summarizes the number of dead lice counted in seven anatomical regions of hens treated with three different products in trial 2. After the first dipping, a significant difference in the number of dead lice were observed in the hens that received Coumaphos (100%), however, no significant differences were observed between treatments after the second and third dipping. Counts of live lice one month later were: group 1 (2 lice); group 2 (0 lice); group 3 (38 lice). Counts of live lice at two months after first reinfestation were: group 1 (13) and group 2 (16) suggesting that the tincture of Ruda with 50,000 ppm was numerically better than Coumaphos. Several chemical compounds are still used to control ectoparasites in poultry (Meyer-Kühling *et al*., 2007). However, there arise questions onto the safety of the administration of these products. Phoxim residues in eggs were shown by using high performance liquid chromatography diode array analysis after treatment of stocked poultry housing facilities (Hamscher *et al*., 2007). Moreover, propoxur residues in eggs were determined when applying the same analytical method

Table 1: Number of dead lice counted in seven anatomical regions of hens treated three plan extracts in experiment 1

Treatment	Dip 1	Dip 2	Dip 3	Average
Control (Water)	29.7±3.4^{e}	53.7±18.3^{de}	63.9±8.4^{bcd}	49.1±7.52^{b}
Neem (500 ppm)	84.2±5.3^{abcd}	96.7±1.4^{ab}	100±0.0^{a}	93.6±2.6^{a}
Ruda (11,700 ppm)	61.4±10.2^{cde}	95.2±2.6^{abc}	99.3±0.4^{a}	85.3±6.0^{a}
Solanacea (50,000 ppm)	98.9±0.70^{a}	96.3±2.3^{ab}	99.6±0.4^{a}	98.3±0.8^{a}
Average	68.6±7.25^{b}	85.5±6.3^{a}	90.7±4.4^{a}	

Values presented as mean ± SE. Means presented represent the. Different letters indicate significant differences between treatments ($p<0.05$). In each treatment, hens received 3 dipping/2 min every 48 h

Table 2: Comparison of the number of dead lice counted in seven anatomical regions of hens treated with three different products in experiment 2

Treatment	Dip 1	Dip 2	Dip 3	Average
Ruda (50,000 ppm)	67.6±7.1[b]	89.7±3.6[a]	100.0±0.0[a]	85.8±4.7[b]
Control (Coumaphos 1,000 ppm)	100.0±0.0[a]	100.0±0.0[a]	100.0±0.0[a]	100.0±0.0[a]
M. anisopliae (50,000 ppm)	66.5±7.9[b]	95.9±2.3[a]	98.9±0.7[a]	87.1±5.1[b]
Average	78.1±5.6[b]	95.2±1.8[a]	99.6±0.26[a]	

Values presented as mean ± SE. Means presented represent the. Different letters indicate significant differences between treatments ($p<0.05$). In each treatment, hens received 3 dipping/2 min every 48 h

Table 3: Residual activity of the treatments in experiment 1 and 2 one month after dippings

	30 days		60 days		90 days
Treatment	Conteo	Reinfestacion	Conteo	Reinfestacion	Conteo
Control (Water)	38	0	51	0	60
Neem (500 ppm)	0	20	0	20	0
Ruda (11,700 ppm)	0	20	1	20	15
Solanacea (50,000 ppm)	0	20	43		
Ruda (50,000 ppm)	2	20	13		
Control (Coumaphos 1,000 ppm)	0	20	16		
M. anisopliae (50,000 ppm)	38				

Residual activity was done by counting lice one month after dipping. Only treated hens with no presence of live lice were reinfested with 20 lice and repeated during three months

indicating an amount of residues above the allowed limits for eggs in the European Union (Hamscher *et al*., 2007). Therefore a future ban of the use of chemical acaricides is realistic, especially in consideration of the forthcoming of more restrictive animal welfare legislation for the production of more safe meat and eggs in a nonpolluted environment (Chauve, 1998). Moreover, repeated long term chemical control may induce resistance in the mite strains. The a application of the *B. thuringiensis* against the red mites infesting poultry is also not advisable, since the exotoxin produced by the bacteria is toxic also to vertebrates (McKeen *et al*., 1988). Therefore shifting priority to control mites by effective and safe natural products is extremely recommended. The results of the present study suggest that some alternative bio-control methods for lice in laying hens are effective.

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