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Observation on Pediculosis in Buffalo-Cows with Emphasis on its Impact on Ovarian Activity and Control by Herbal Remedies

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Abstract: Lice infestation has major impacts on the productivity and welfare of livestock. The current study was carried out to throw light on the effect of lice infestation on general health condition and ovarian activity in buffalo-cows with emphasis on its control by an alternative safe way. A total number of 1583 buffalo-cows reared at Lower Egypt was included in this study. Animals were clinically and gynecologically examined and the incidence and intensity of lice infestation were recorded and samples from lice were collected to be investigated at the laboratory. Blood samples were collected for carrying out some relevant analyses. Lice were subjected to different concentrations of *Artemisia herba alba and Lupinus termis* aqueous extracts and toxicity was recorded. After exposure, the supernatant of the lice homogenates was used for determination of some biochemical constituents as well as protein fractionation by Sodium dodecyle sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Some field treatment trials were carried out using herbal extracts in comparison with some commonly used commercial insecticides (Ivomec super, Fasciontel and Dectomax). Results revealed that 27.73% of examined buffaloes were infested with different kinds of ectoparasites, among which, 47.92% were infested by lice (*Haematopinus tuberculatus*) and have inferior body condition scores (BCS), anemia, lymphopenia and eosinophelia with low serum progesterone level, disturbed oxidative status and marked decrease in concentration of iron(Fe), selenium(Se) and zinc(Zn) in their blood as compared to control values. Out of infested animals, 50.25% suffered from ovarian inactivity with low serum progesterone levels. *In vitro* studies revealed that, aqueous extract of seeds powder of *Lupinus* (61.0 – 100.0%) was more effective than aqueous extracts *of Artemisia (*75.7 - 95.6%*)* as indicated by mortality rate of lice. After exposure to herbal extract, biochemical constituents of lice homogenate revealed obvious changes in glucose, protein, triglycerides, catalase, alkaline phosphatase (AlP) and creatinine values as compared to control. Also, SDS-PAGE exhibited differences in bands number and molecular weights in treated lice proteins. Herbal extracts have comparable efficacies in controlling lice infestation (69.23 – 72.0%) as compared with the used pesticides (80 – 83%). It could be concluded that lice infestation have negative effect on general health status and ovarian activity in infested buffaloes. Herbal remedies give good results in controlling of lice infestation and can substitute chemical insecticide.

Key words: Buffalo-cows, Ovarian activity, Lice infestation control, *Artemisia herba alba, Lupinus termis*, *Haematopinus tuberculatus*

INTRODUCTION

Buffaloes are the main source for good quality meat and milk in Egypt, despite this species is mostly reared under harsh socioeconomic conditions and has low productive and reproductive potentials [1].

Ectoparasitism is one of the most important factors that hinder the productive efficiency of farm animals, as it causes hypersensitivity, marked blood loss, secondary infestation, excoriation and ultimately death in few cases. Also, ectoparasites cause indirect harm, particularly when present at high intensities, causing disturbance, increasing levels of behavior such as rubbing and reduced time spent for grazing or ruminating and in some cases, self-wounding [2]. Importantly, some ectoparasites act as vectors for protozoa, bacteria, viruses and helminthes [3-5]. So, ectoparasites even in relatively little number induced negative effects on the welfare and

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productivity of livestock. Economic losses due to ectoparasitism are mainly due to loss in milk and beef production with an equal amount of 10 million dollar in USA [6] as well as due to inferior ovarian activity [1].

Phthiraptera (lice) are specialized insects adapted to parasitize many warm-blooded vertebrates with generally high host specificity. Louse infestation of cattle appears to be common and widespread, whereas, visual examination of calves in Scotland revealed a prevalence of lice infestation in 80% of farms [7]. In south England, clinical inspection of 24 farms showed that 75% of the herds were infested. The chewing louse *B. bovis* was present in 94% of the Norwegian cattle herds, while the sucking louse *L. vituli* was present in 42% of herds [8].

Currently, ectoparasites control strategies are based on chemical insecticides. Nonetheless, in beef and dairy cattle due to problems related to insecticide resistance, drug residues and high costs of insecticides, there has been a growing interest in research concerning the possible use of botanic insecticides as alternatives to synthetic insecticides [9]. Botanical pesticides in general possess low mammalian toxicity and thus constitute least or no health hazards and environmental pollution [10].

Artemisia species have been used worldwide as a rich source of plant derived pesticides [11] as well as tonic, stomachic, stimulant beverage and as antiseptic oils or tinctures for the relief of rheumatic pains [12]. Mixture of the dry leaves of *A. judaica*, *A. oncosperma* and *A.herba alba* are very common anthelmintic drugs in most of North African and Middle East countries.

The genus *Lupinus* (Genistae) is widely distributed, whereas, approximately 300 species are found in the Mediterranean countries, Africa and North and South America [13]. It is used as an insecticide against lice in Iran [14] and *Drosophila melanogaster*, *Ceratitis capitala* and *Spodoptera littoralis* in Egypt [15].

The current investigation was carried out to throw light on lice infestation in Egyptian buffaloes. Emphasis was given to monitor the impact of infestation on general health condition and ovarian inactivity and to carry out some *in vit*ro investigations to find out some safe native herbal remedies for lice control rather than the imported expensive chemical insecticides.

MATERIALS AND METHODS

The present work was carried out during the period of time extended from September 2004 to June 2008 as a part of the National Research Centre Project No. 7120106.

Animals: A total number of 1583 buffalo-cows, kept in small holder farmers at villages of Lower Egypt was included in this study. These animals were fed on Barseem, few amounts of concentrate, crop residues and rice straw. A full case history and owner complaint for each animal was recorded. General health condition was examined and the BCS is recorded as outlined by Ribeiro *et al*. [16]. The prevalence of lice infestation on these animals and its intensity were recorded in a chosen body surface area (5 x5 cm) at the neck region. Lice were collected from animals using special vials according to Kettel [17] to be identified, examined and subjected to *in vitro* study using herbal extract in the laboratory.

Gynecological examination was carried out by inspecting the external genitalia and palpating the internal genital organs through rectum for three successive weeks at least. Buffalo-cows did not show estrous signs at least 6 months after calving during the breeding season (September –March) and have small non functioning ovaries were considered to be suffering from ovarian inactivity. The condition was confirmed later on by monitoring the serum progesterone level. Blood samples were collected with and without EDTA from all animals to study the effect of infestation on hemogram, progesterone level, values of some oxidant/antioxidant markers as well as concentrations of some trace elements.

Plants extract: *Artemisia herba alba* and *Lupinus termis* were obtained from Medicinal and Aromatic Plants Division, Horticultural Research Institute, Agricultural Research Centre, Ministry of Agriculture, Egypt.

Flowers of *Artemisia* (250 gram) were extracted by the aqueous method as outlined by Chiasson *et al*. [18], either by: 1- Boiling in 2L liters of water (250g/ 2 L) for two hours, or, 2-Soaking in 2 liter of water over night. The extracts obtained by the two methods were filtered and stored at -4 °C until used.

The seeds of native *Lupines* were extracted by 2 methods too, either by: 1- Soaking in water (500g/ 2 L) for at least 12 hrs, then boiling until full cooking and left at least another 12 hrs and collection of soaking solution, or 2- Boiling of seeds (500g/ 2 L) and powder of seeds(250g/ 2 L) individually in water, soaking in water for several days to get endogenous alkaloid fractions,[19]. The extractions were filtered and stored at -4 ° C until used.

The aqueous extractions of both herbs were tested against buffalo lice *in vitro*.

Toxicity Test: Toxicity test was carried out on the adult stage (females) of lice. The following concentrations of both soaked and boiled herbal extracts were used:

- *Artemisia*: 12.5, 6.25 and 4.2 g/ 100ml distilled water
- *Lupines*: 25, 12.5 and 6.25g/100ml for seeds and 12.5, 6.25 and 4.2 g/ 100ml distilled water for powder.

Distilled water was used as a control treatment. Treatment was replicated 5 times and each replicate included five lice. The exposure was applied by dipping lice for 30 seconds in each concentration or in distilled water in case of control treatments, transmitted to filter paper. Lice were separated in plastic cups cone (five lice/cup), incubated at the prevailing field temperature, the mortality rate was observed after half to six hours. Calculated mortality percentages were based on occurrence of complete paralysis.

Analysis

Blood: Complete blood picture including erythrogram and leukogram were carried out and anemia indices were calculated [20]. Serum progesterone level was assayed by ELIZA microwell technique using kits from DIMA (Germany). The kit had sensitivity of 2.0 pg/ml with inter- and intra- run precision coefficient of variations of 2.9 and 4085, respectively [21]. Oxidant/ antioxidant markers including: malondialdehyde, MDA [22]; nitric oxide, NO [23]; catalase, CAT [24]; superoxide dismutase, SOD [25]; ascorbic acid, ASCA[26]; reduced glutathione, R-GSH [27]and total antioxidant capacity, TAC [28] were calorimetrically assayed using chemical kits from Bio Diagnostic, Egypt

Trace elements including zinc (Zn), iron (Fe) and copper (Cu) concentrations in diluted serum samples and selenium (Se) in whole blood samples were determined using atomic absorption spectrophotometer (Perkin Elmer, 2380) as outlined by Varley *et al*.[29].

Lice Homogenate: Lice exposed to each concentration of both cold *Artemisia* (6.25 and 4.2g/100ml) or *Lupinus* seeds (12.5 and 6.25 g/100ml) and control (water) treatment were individually taken in 0.01M phosphate buffer saline, pH 7.2 (PBS). Lice were homogenized in an equal amount of PBS then sonicated for 5 minutes under 150 watt interrupted pulse output at 50% power cycle using a sonifier cell disrupter. The sonicated lice were subjected to high speed centrifuge (10000 rpm) for one hr at - 40°C, the resulting supernatant was collected. The supernatant were used for the determination of glucose [30], triglycerides [31], creatinine [32] and alkaline phosphatase [33] using chemical kits supplied by bioMérieux-France and MDSS GmbH Schiffgraben-Germany. Catalase activity was estimated by the method of Aebi [24], using specific kits obtained from Bio-diagnostic, Egypt.

Characterization of Protein Content of Engorged Lice by SDS-PAGE: The protein content of louse samples were determined by the Lowery method [34]. 10% SDS Slab-polyacrylamide gel electrophoresis (SDS-PAGE) and running buffer consisting of 0.5M tris, 1,92M glycine and 10% SDS (pH, 8.3) were used as described by Hames [35].

Field Treatment Trials: A total of 189 buffalo cows was selected to carry out some field treatment trials. Animals were selected to have an average body size fits for breeding and divided into 6 groups as follows: The first group included 10 animals kept without any treatment as a control group. The second group included 26 buffalo-cows, sprayed by cold water extract (12.5g/ 100ml) of *Artemisia.* The third group included 50 buffalo-cows, sprayed by water extract of *Lupinus seeds powder* (12.5 g/100 ml water). Animals in both groups were sprayed day after day for two weeks. The fourth group included 29 buffalo-cows; each was subcutaneously injected with Ivomic Super (Ivermectin-clorsulon,; Meck Sharp and Dohme, the Netherlands) in a dose of 1 ml/ 50 kg live body weight. The fifth group included 49 buffalo-cows, each was subcutaneously injected with Fasciontel 5% (Closantel; Tornel Laboratories,Mexico) in dose of 1 ml/15 Kg. The sixth group included 25 buffalo-cows, each was intramuscularly injected with Dectomex (Doramectin; Pfizer, Egypt) in a dose of 1 ml/50 kg live body weight. The treated buffaloes were observed regularly and checked weekly thenafter for absence of the lice infestation.

Statistical Analysis: Data were computed and statistically analyzed [36].

RESULTS

Prevalence of Lice Infestation: Out of 1583 examined buffalo-cows through this study, 409 animals were infested with different kinds of ectoparasites (27.73%). 197 among these 409 animals (47.92%). were infested with different degrees by lice (*Haematopinus tuberculatus*)

Table 1: Effect of pediculosis on the blood picture in buffalo-cows (Mean±SE)

Heamogram	Parameter	Healthy animals	Animals infectd with lice
Erythrogram	RBCS (10^6/ul)	5.57±0.11	4.11±0.17***
	Hb (g/dl)	14.90±0.34	10.63±0.18 ***
	PCV (%)	36.62±0.18	30.06±0.21***
	MCV (Fl)	36-60±1.93	54.49±1.90***
	MCHC (%)	35.91±0.15	34.03±0.47 **
Leukogram	WBCS(10^3/ul)	6.07±0.36	6.54±0.79
	L (%)	60.16±1.68	51.17±0.51**
	N(%)	39.55±0.18	41.26±0.64*
	E(%)	1.25±0.56	3.74±0.27 ***
	B(%)	0.24±0.14	1.51±0.13
	M(%)	1.25±0.06	2.92±o.26 *

*P< 0.05 **P<0.01 ***P<0.001.

RBCS= Red blood cells, Hb = heamoglobin, PCV= packed cell volume, MCV= Mean corpuscular volume, MCHC = Mean corpuscular heamoglobin concentration, WBCS= white blood cells, L=lymphocytes, N= neutrophiles, E= eosinophiles, B=basophiles, M= monocytes.

Table 2: Effect of pediculosis on oxidant /antioxidant markers in the blood of buffalo-cows (Mean±SE)

Parameter		Healthy animals	Animals infested with lice
Oxidants	MDA(mmol/ml)	1.98±0.90	1.51±0.34
	NO(mmol/L)	20.31±0.38	25.55±1.58 **
Antioxidants	CAT (U/ml)	2.28±0.04	1.067±0.03 ***
	ASCA(ug/dl)	132.17±5.12	97.07±3.64 ***
	SOD (U/ml)	338.16±7.11	326.11±13.18
	GSH-R(mmol/L)	6.38±0.11	3.28±0.83 **
	TAC (mmol/L)	1.43±0.08	0.67±0.06 ***

**P<0.01, ***P<0.001.

MDA= Malodialdehyde, NO= Nitric oxide, CAT= Catalase, ASCA= Ascorbic acid, SOD= superoxide dismutase, GSH-R Glutathion reduced, TAC= total antioxidant

Table 3: Effect of pediculosis on some trace elements concentration in blood of buffalo-cows (Mean±SE)

Elements (µg/dl)	Healthy animals	infected animals
Zn	139.11±2.17	129.77±2.67 **
Cu	78.65±4.13	71.84±3.17
FE	168.40±4.17	143.17±2.12 ***
Se	144.85±0.43	132.58±2.18 ***

**P< 0.01, ***P< 0.001.

Zn=zinc, Cu= copper, Fe= iron and Se= selenium

The intensity of infestation in the chosen surface area at the neck region (5X5 cm) was heavy (>3 insects) in 18%,moderate (2-3 insects) in 32% and light (1 insect) in 50% of the infested animals.

Effect of Infestation on General Health Condition:

- Monitoring of BCS, revealed that lice infested animals have inferior (P<0.05) scores as compared with non infested animals (2.04 ± 0.22Vs. 2.88± 0.28).
- Erythrogram of animals having pediculosis revealed macrocytic hypochromic anemia as compared with healthy non infested animals (Table 1).
- Leukogram of infested animals showed lymphopenia (P< 0.01), eosinophelia (P<0.001) as well as neutrophelia and monocytosis (P<0.05) as shown in Table 1.

Oxidant /Antioxidant Markers: Table 2 shows that animals having lice infestation have higher values of the oxidant NO (P<0.01) and lower values of the antioxidants CAT, ASCA and TAC (P<0.001) as well as GSH-R (P<0.01) in comparison to non infested animals.

Trace Elements: Table 3 shows the marked decrease in concentration of serum iron and selenium (P<0.001) and zinc (P<0.01) in animals infested with lice as compared to healthy non infested buffalo-cows

Prevalence of Ovarian Inactivity:

- Among the total examined animals, 505 suffered from cessation of ovarian activity during the breeding season of buffaloes. Out of these animals, 99 buffalo cows (19.46%) were infested by lice. In the same time, these animals represent 50.25% of the total lice infested animals (197).
- These animals suffering from ovarian inactivity revealed no behavioral signs of estrus after calving since more than 6 months. Moreover, rectal palpation, revealed small size flabby genital organs with absence of physiological structures on the surface of both ovaries.
- In normal cyclic buffalo-cows, serum progesterone level was lower in animals having pediculosis (1.63± 0.27ng/ml) than that of non infested animals (2.89±0.17ng/ml) during the mid luteal phase of the estrous cycle(P< 0.01). In animals suffering from bilateral inactive ovaries, the level was non detectable (< 2pg/ml) in both infested and non infested groups.

Bioassay Test: Results for the four aqueous extracts of *Artemisia* and *Lupinus* are summarized in Figure 1. High mortality rates of lice of were observed in 12.5g/ 100ml

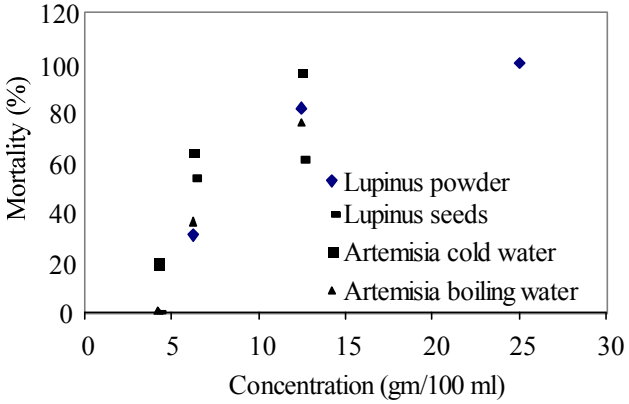

Fig. 1: Mortality rate of *H. tuberculatus* exposed to water extract of *Artemisia* and *Lupinus*

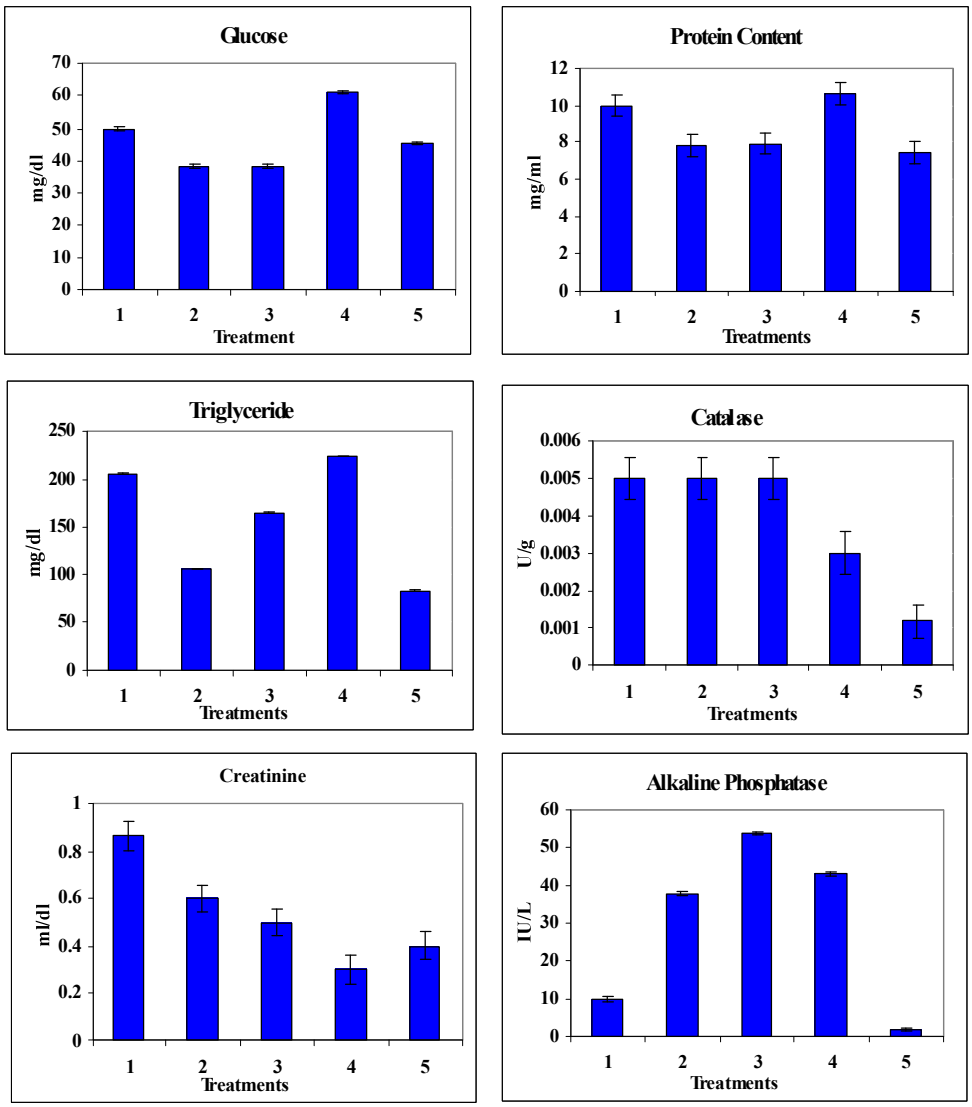

Fig. 2: Some biochemical changes in homogenate of *H. tuberculatus* exposed to *Artemisia* and *Lupinus*
(1) *Artemisia* (6.25g/100ml) (2) *Artemisia* (4.2g/100ml)
(3) *Lupinus* (12.5g/100ml) (4) *Lupinus* (6.25g/100ml) (5) control.

Table 4: Electrophoretic profile of total protein of *H. tuberculatus* exposed to *Artemisia* and *Lupinus* (seeds) extracts

	Artemisia		*Lupinus*	
Control	6.25g /100ml	4.2g /100ml	6.25g /100ml	4.2g /100m
220	223.95	221.96	221.96	221.96
196	190	197.74	194.25	197.74
124.54	165.75	174.6	170.76	174.6
93.7	123.44	150.1	147.46	150.1
66.25	88.84	124.54	119.13	124.54
47.68	44.4	95.38	87.27	95.38
38.18	33.41	42.47	44.01	42.47
30.84	23.62	34.93	33.41	34.93
19.42	17	17.61	24.04	17.61
	16.85		17	

concentration, either if it is cold or hot water extract of *Artemisia* (95.6and 75.7%, respectively). In case of *Lupinus,* high mortality rates were observed for powder (12.5g/100 ml) and seeds (25g/100ml) groups of aqueous extract (100 and 61.2%, respectively). Moreover, the aqueous extract of *Lupinus* powder was the most effective agent among all used extracts of *Artemisia* and *Lupinus*. On the other hand, the mortality rate was zero after exposure to 6.25 g/100ml boiled *Artemisia extract* and 4.2 g/100ml *Lupinus* seeds.

Biochemical Changes in *H. Tuberculatus* Exposed to Water Extract of *Artemisia* and *Lupinus*: The mean values of some selected biochemical parameters in homogenates of *H. tuberculatus* following exposure to herbal remedies are shown in Fig. 2. Glucose value increased ($P<0.05$) after exposure to *Artemisia* (6.25g/100ml) and *Lupinus* (12.5g/100ml). However, this value decreased after exposure to the low concentration of both herbs(4.2 and 6.25g/100ml, respectively) as compared to the control value. Protein content increased ($P<0.01$) after exposure to *Artemisia* and *Lupinus* (6.25g/100ml) *as* compared to control. Values of triglycerides, catalase and alkaline phosphatase increased ($P<0.01$) after all exposures as compared to control. Creatinine values decreased ($P<0.05$) after exposure to *Lupinus* (6.25g/100ml) as compared to control.

Effect of Exposure to *Artemisia* and *Lupinus* on the Electrophoretic Pattern of Lice Protein: Electrophoretic profiles for the total protein of female *H. tuberculatus* exposed to *Artemisia* and *Lupinus* are presented in Table 4. The homogenates of exposed and non-exposed lice reflected appearance and disappearance of protein bands after 30 seconds of dipping. Nine molecular entities were detected in the control with molecular weight ranging between 19.42 - 220 kDa. However, after exposure to 6.25and 4.2% *Artemisia,* the lice SDS dissociated proteins were separated into 9 and 10 protein bands with MW ranging between 16.85 – 223.95 and 17.61 -221.96 kDa, respectively. Also, new protein bands were stimulated after treatment with each concentration of *Lupinus*. There were two common protein bands shared between concentration 6.25% of *Lupinus* and control with MW of 93.7 kDa and between concentration 4.2g of *Artemisia* and 6.25g of *Lupinus* with WM of 221.96 kDa.

Table 5: Use of some herbal extract and pesticides to eradicate pediculosis in buffalo-cows

		Response	
Herb/Drug	No. of Animals	No	%
Untreated control	10	00	00.00
Artemisia	26	18	69.23
Lupinus seeds powder extract	50	36	72.00
Ivomec super	29	24	82.76
Fasciontel	49	41	83.77
Dectomax	25	20	80.00

Field Treatment Trials: Table 5 shows that the aqueous extract of *Artemisia* and *Lupinus* seeds powder had comparable efficacies (69.23 and 72.0%, respectively) in controlling lice infestation while, the efficacy varied from 80 -83% for the used pesticides. The treated animals showed neither adult insects nor its different developmental stages.

DISCUSSION

Poor reproductive performance and parasitism are two serious problems which causing high economic losses in farm animals, especially in the developing countries. A rapid scan of the literature revealed few accounts of epizootics due to arthropods, although it was evident that continuing attacks by arthropods and diseases they transmit cause losses of billions of dollars annually. Too often these losses appear to be overlooked perhaps through ignorance, lack of reporting, or too few carried out trials for insects' control.

The current investigation was carried out to declare the effect of pediculosis in buffaloes under the prevailing Egyptian condition with emphasis on the impact on health condition and the ovarian activity. Also, special effort was given to the control of lice using natural safe remedies.

H. tuberculatus is a principally an ectoparasite of buffaloes as well as of close associated cattle. It causes irritation, rubbing and scratching resulting in patchy hair loss, sores and untidy appearance, blood-loss due to sucking and in serious cases, anaemia, abortion and death of the animal [37].

The current study revealed inferior BCS in animals infested with lice. Previous studies recoded that nutritional status of the host may influence the degree of lousiness, with undernourished animals presenting heavier louse burdens [38]. Also, infested animals do not gain weight at normal rates and may remain stunted and an additional daily weight gain of 250 g has been recorded for treated calves [39]. Moreover, estimated losses in the United States (including control costs) have been cited as between US126.3 -130$ million Dollars [40], together with an estimated loss of 30.9 kg per head in weight for 12 percent of slaughtered cattle [41].

In this study, the prevalence of ectoparasites in the examined buffalo- cows was 27.73% out of which lice infestation was 47.92%, despite, lice infestation recorded such high prevalence of 75 and 94% in English and Norwegian cattle herds, respectively [8]. However, the current prevalence in buffaloes was somewhat not too high, since buffaloes are mainly reared in small holder farms, have thick skin and continuously looked by owners.

In this study, 50.25% out of animals suffering from ovarian inactivity were infested with lice as confirmed by lower level of progesterone. In this respect, it was reported that cessation of ovarian function following parasitic infection is a usual sequence to the decline in general health, fall of appetite, deficiency of micronutrients necessary for sound reproduction and eventually loss of weight and anemia [1,42].

This study showed that animals having pediculosis suffered from macrocytic hypochromic anemia confirmed by the low level of serum iron and copper,which are essential for erythropoiesis. Similar results were recorded by El-khdrawy *et al*.[1]. On the other hand, lymphopenia in the infested animals herein, may indicate that hypoimmune animals are more subjected to parasitic infection than well fed and healthy animals [43]. The eosinophilia is common in parasitic infections as a result of the parasite metabolic secretions. Eosinophils cause pruritus through histamine secretion that consequently cause chemotaxsis of other inflammatory cells such as neutrophiles and monocytes [20].

Results revealed that animals having lice infestation were under oxidative stress as they expressed high values of NO and low values of CAT, ASCA, GSH-R and TAC. In this respect, it was recorded that animals infested with ectoparasites show disturbance of oxidant/antioxidant balance [44- 47]. Neutrophiles and macrophages produce reactive oxidants such as hydrogen peroxide, hypochlorite and oxygen radicals which have potent cytotoxic effects on parasites as well as other pathogenic organisms [48]. Also, it was found that free radicals induce or contribute to adverse effects on the skin through erythema, edema, wrinkling, inflammation, autoimmune reactions, hypersensitivity and keratinization abnormalities [49]. Moreover, Lipid peroxidation can be harmful for skin due to alternations in the membrane structure and permeability [50].

In this study, serum Zn, Cu, Fe and Se concentrations were low in infested animals. These traces are necessary for the function of different enzymes essential for different metabolic processes. There is a positive correlation between deficiency of these micronutrient and poor body condition and increased susceptibility to different diseases and cessation of ovarian functions [51,52].

The efficacies of conventional medicaments against both endo- and ecto-parasitic diseases have been reported with variable success [53]. However, the toxic effects of these chemicals on animal and human [54], the development of resistance to it by target parasites [55] as well as the high cost of drugs [56] pave way for herbal remedies as reasonable alternative. Herbal therapies are natural products, environmental friendly and cheap. The need for alternative, non chemical, control strategies in animal production systems has increased in the last decade due to development of antiparasitic resistant strains of parasite [57]. Proposed alternative approaches against parasitic infections include vaccination [58], nematophagous fungi [59], exploitation of genetic resistance of animals, dietary supplementation of growing animals and the use of bioactive forages [60].

Numerous plants indigenous to Africa in general have been found with amazing medicinal properties. Some are well-evaluated specific active principles against the target parasites, while others are not. It is therefore highly essential that medicinal plants whose properties have not been fully characterized should form a top agenda of top management in developing nations whose citizens are sometimes unable to afford expensive medicine. This policy if pursued will not only preserve the scarce foreign exchange, but also promote the spirit of plant conservation. This can be accelerated by including ethno-veterinary medicine as part of curriculum in veterinary school.

The current results revealed, both the *Artemisia* aqueous extracts possess a fairly good lice controlling property. Moreover, the cold extract (Soaking) is more effective than the hot extract (Boiling). This could be due to boiling has a negative effect on the active component of the herb. Although, the aqueous extract of *Artemisia* was used herein, the obtained results were in line with the finding of Abd-Elshafy *et al*.[61] who found that diethyl ether, ethyl acetate and ethanol extracts of *Artemisia* manifested the highest toxicity against larvae of *Hylomma dromedarii*. Also, Hassanein *et al*. [62] recorded that hexane, chloroform, ethyl acetate and ethanol extracts of *Artemisia* were toxic to the fourth instar larvae of *Spodoptera littoralis* (Boisduval, 1833) (Insecta: Lepidoptera: Nuctuidae). Moreover, Soliman *et al*. [63] found that successive extracts of *Artemisia* with petroleum ether, chloroform, ethyl acetate and ethanol were toxic for the two-spotted spider mite *Tetranychus urticae* (Koch) (Acari: Actinedida: Tetranychidae). In this respect, Mohamed *et al*. [64], detected tannins, serrols, terpenes, flavonoids, alkaloids, saponin, resin, phenols and glycosides in *A. monosperma*.While, Antonious and Hegazy [65] showed that *A. herba alba* has antifeedant effect on the fifth instar larvae of *S. littoralis* and Saber [66] found that *A. monosperma*, have repellency, mortality and oviposition deterrent effects against female of *T. urtica*.

The water extracts of *Lupinus* (powder and seeds) are completely different. The powder extract has a stronger effect in buffalo lice *H. tuberculatus* than the seeds extract. This could be due to the contained alkaloids is completely and effectively extracted by using the powder form. In the same time, it was known that *Lupinus* alkaloids have pronounced effect on the nervous system of insects [67].

The present study revealed marked increases in the alkaline phosphatase, triglycerides and catalase values in all homogenates of *H. tuberculatus* exposed to *Artemisia* and *lupins*. This indicated that lice become under stress after exposure to these herbs. AlP is intracellularly bonded enzyme and the increase in its circulating activity in the body of lice is indicative of cellular destruction [68]. In addition, the increase in creatinine value in all homogenate of herbs exposed lice, except in concentration of 6.25g of *lupins*., indicated the existence of a problem in the excretory system of the insect.

The eletrophoretic pattern of lice protein showed clear differences in bands numbers and molecular weights following exposure to the used extracts of *Artemisia* and *lupins*. These results agree with those reported by Habeeb *et al*. [69].

In the current study, the efficacy of *Artmesia* and *lupinus* extracts were evaluated under the field conditions in comparison with some commonly used anti parasitic drugs used for eradication of buffalo lice. The recovered animals have neither the adult insects nor its different developmental stages. Use of the plant extract gave good insecticidal effect and the recovery rate of the treated animals was 69.23 - 72.0%. On the other side, the used drugs were effective in 80 – 83 % of the cases. Moreover, *Artemesia* species has a remarkable antioxidant activity and is a radical scavenger, which indicates its effectiveness against diseases caused by over production of radicals [12].

It could be concluded that lice infestation have negative effect on general health status and ovarian activity in infested buffaloes. Herbal remedies give good results in controlling of lice infestation and can substitute chemical insecticide as it is cheaper, safer, cause no mammalian toxicity and echo friendly.

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