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SAFETY EVALUATION OF IVERMECTIN, CYPERMETHRIN, NEEM SEED OIL AND CITRUS OIL APPLICATION ON DOGS INFESTED WITH SARCOPTES SCABIEI VAR. CANIS: SERUM BIOCHEMICAL FINDINGS.

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ABSTRACT

The serum changes accompanying the use of Citrus oil, Neem seed oil, Ivermectin, and Cypermetrin in ameliorating the effects of Sarcopticosis in dogs is scanty in literature. Hence, this study weighs the safety of these therapeutic interventions on dogs. Thirty dogs were enrolled for the study out of which twenty five were infested with sarcoptes mites and the other five were sarcoptes free. Botanical preparations were made and oils were extracted for use. Infested dogs were grouped in fives and were treated as recommended for six weeks. The main effects of the anti-parasitic agents, study duration and their interactions on the plasma biochemistry was evaluated. Total Protein was influenced significantly ($P < 0.05$) by the study duration while Albumin amount was significantly influenced ($P < 0.05$) by the anti-parasitic agent. The anti-parasitic agent and their interactions significantly influenced ($P < 0.05$) the Globulin concentration in dogs. The ALT and ALP were however influenced significantly ($P < 0.05$) by the anti-parasitic agent, study duration and their interactions. The findings of similar miticidal activity from the use of either a synthetic or botanical Acaricide goes to strengthen the need for study especially in the light of therapeutic failures and environmental toxicity.

Keywords: Serum, Botanicals, Synthetic, Acaricides, Sarcoptes, Dogs.

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INTRODUCTION

Canine parasitism still stands as one of the most challenging encounters in maintaining canine welfare and productivity. Its neglect as a tropical disease, its deleterious effects on the hosts, as well as the emerging resistance of these parasite to therapeutic agents all contribute to its burden on pets and companion animal owners. One of the frequently come across parasites is Sarcoptes scabiei var. canis which has been considered both an endoparasite and ectoparasite (Carvalho et al., 2016).

The major effect of this parasite is on the immune system of the hosts where it modulates host's immunity and slackens immune response of host to its presence (Arlian & Morgan, 2017). Its effect on the epidermis is also worth mentioning as it continually tunnels through to successfully develop and complete its life cycle. This developmental journey subsequently births several degenerative and abnormal changes in the stratum spinosum and stratum corneum make up (Arlian & Morgan, 2017). Additionally, several alterations have been documented in the biochemical response of various hosts to the establishment of the parasite. Sarcoptes infested rabbits have been identified with increased ALP, AST, and ALT values (Metwally et al., 2018) while Hafeez et al., (2007) reported an increase in total serum proteins in sarcoptes infested sheep. On the other hand, Aboelhadid et al., (2016) reported a decrease in the total proteins and serum albumin as well as an increase in AST and ALT amounts in sarcoptes infested camels.

The variations in the biochemical responses seen in sarcopticosis in different animals depicts the potential damaging effect of the parasite on certain organs of the system. Hence, an immediate institution of a holistic treatment plan against sarcopticosis can never be over emphasized. However, certain limitations exist to this all-inclusive treatment plan as most of the conventional drugs used against sarcoptes mites are increasingly being resisted by the mites (Currie et al., 2004). In the wake of the emerging mite resistance to the commonly used miticides, some botanical therapies are being evaluated for its miticidal properties. Even though these botanicals possess glycosides, alkaloids, flavonoids, coumarin compounds, vitamins, and tannins (Altaf et al., 2019), their strength in restoring and correcting sarcoptes induced biochemical changes in dogs is still to be determined.

The sequential alterations in serum biochemical parameters of sarcoptes infested dogs has been previously reported (Nwufoh et al., 2019) with remarkable findings of heightened ALP amounts, and a decrease in total protein, albumin, globulin and creatinine amounts. Study thus sought to compare the sequential effects of systemic, topical and botanical therapies on the biochemical parameters of dogs with sarcopticosis as well as investigate the interactions between therapies and organ functions.

MATERIALS AND METHODS

Study design and group allocations

Thirty (30) healthy and mite free dogs were purchased for study. After environmental adjustments and parasite screening, twenty five (25) dogs were infested as earlier described by Nwufoh et al., (2019). The remaining five (5) dogs were assigned as negative control dogs while the twenty five (25) infested dogs were distributed into five groups of fives. The five (5) mite free dogs were

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assigned Group A control dogs. Groups B had five (5) infested dogs earmarked for treatment with Citrus oil. Group C also had five (5) infested dogs set aside for treatment with Ivermectin Super (Ivermectin + Clorsulon-Merial-UEMOA/V/00012/2013/02/28) while Group D dogs (5) were assigned for Neem seed oil treatment. Group E (5 dogs) and Group F (5 dogs) were consigned for treatment with Petroleum jelly and Cypermethrin pour-on (CYPER care-Ancare, 2.5% pour-on solution).

Ointment - Extract preparation of Azadirachta indica and Citrus sinensis

Fresh ripened seeds of Azadirachta indica were harvested from a neem tree at the Federal College of Animal Health, Moor Plantation, Ibadan. Seeds were authenticated by a botanist at the Department of Botany (UIH-23171), University of Ibadan. Neem seeds were dried under room temperature after which aqueous extracts of seeds was prepared. Dried seeds were ground before 500g was boiled in a litre of water for half an hour. Ensuing mixture was filtered and new solvent was added. Procedure was carried out thrice before extract was obtained after solvent evaporation. Extract was then prepared as a 20% ointment with a Petroleum jelly vehicle (w/w) (Shahid et al., 2007).

Extraction of Citrus oil followed the procedure of Neelima et al., (2017). Fresh peels of Citrus sinensis were obtained from a Citrus tree (UIH-23170). Peels were obtained using laboratory knife before being subjected to hydro-distillation method. Distillation procedure involved exposing citrus peels to boiling water for the oil to be released into the water. Essential oils were then further collected by distillation. Finally, the steam and oil vapors were condensed and collected into a Florentine flask. Oil was further prepared as a 20% ointment with a Petroleum jelly vehicle (w/w).

Treatment application

Healthy animals which served as control animals were cleaned with distilled water and saline solution (0.9%) NaCl 1ml/30kg intramuscularly. Hairs around lesion areas of infested animals were shaved before application of treatment. After shaving, crusts were taken out with tepid cleaning with cotton wool and allowed to dry. Neem seed oil, Citrus oil and Petroleum jelly were then generously applied on lesion areas on alternate days till end of experiment.

Ivomec-D (Ivermectin + Clorsulon- Merial-UEMOA/V/00012/2013/02/28) was administered subcutaneously at 1ml per 50kg of body weight. Cypermethrin (CYPER care-Ancare, 2.5% pour-on solution) was administered directly to all lesion areas on the skin of the animals.

Drugs were administered on the first (1st) and fourteenth (14th) day at the neck lateral to the midline. Response to therapy was closely supervised till end of study.

Sample collection

Blood samples for serum biochemical analysis was obtained from selected animals in all groups A, B, C, D, E, F. Blood samples were collected under physical restraint and via the cephalic vein weekly. Blood samples were collected into plain vacutainers and blood was allowed to clot. Serum was subsequently harvested into Eppendorf tubes weekly.

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Serum biochemical investigation

Assessment of total protein (TP) amount was determined with the direct biuret method (Lubran, 1978). Albumin amount was also determined with the bromocresol green method as described by Doumas et al., (1971). With the various serum biochemical kits, Blood urea nitrogen (BUN), Creatinine, Alanine AminoTransferase (ALT), Aspartate AminoTransferase (AST), Alkaline Phosphatase (ALP), and Glucose amounts were evaluated (Randox chemicals, Netherlands).

Statistical analysis

Data obtained from serological analysis were expressed as mean ± Standard Error (SE) and subjected to one-way Analysis of Variance (ANOVA). Significant level of difference among treatment mean was established at 95% confidence limit. Tukey multiple-ranged test was used as post-Hoc test. All statistical analysis was accomplished using IBM SPSS 22® statistical package.

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Serum Biochemical Analysis

Table 1: Main effects of anti-parasitic agents, study duration and their interactions on the plasma biochemistry of Sarcoptes infested dogs

Variable		TP	Alb	Glo	AST	ALT	ALP	BUN	Cre	Glu
Individual treatment mean[a]										
Antiparasitic agent	Study duration (weeks)									
Control	1	7.47	3.20	4.77^{ab}	14.00	96.33^{abc}	126.00^{abc}	29.33	2.07	105.67^{de}
	2	6.53	2.80	4.43^{ab}	16.67	99.67^{abc}	121.67^{abcd}	27.33	1.67	110.00^{bcde}
	3	6.33	3.07	4.10^{abc}	15.67	104.33^{ab}	125.33^{abc}	33.33	1.93	109.67^{bcde}
	4	7.13	3.23	4.23^{abc}	17.00	111.67^{ab}	128.00^{a}	38.33	2.07	114.67^{abcde}
	5	7.63	3.60	4.73^{ab}	15.67	116.00^{a}	130.67^{a}	44.00	2.40	119.67^{abc}
	6	7.00	2.80	4.47^{ab}	15.33	116.33^{a}	125.33^{abc}	33.33	2.00	115.33^{abcde}
Citrus	1	7.93	3.23	3.23^{c}	14.00	104.33^{ab}	118.33^{abcde}	26.00	1.57	109.33^{bcde}
	2	7.63	2.73	4.53^{ab}	14.67	98.00^{abc}	113.00^{abcdef}	26.33	1.43	111.00^{bcde}
	3	7.67	3.27	4.20^{abc}	14.67	107.67^{ab}	116.33^{abcde}	29.33	2.07	113.00^{abcde}
	4	7.97	3.27	4.37^{ab}	14.67	110.00^{ab}	121.00^{abcd}	32.33	2.23	116.33^{abcde}
	5	7.67	3.53	4.67^{ab}	15.00	116.67^{a}	126.67^{ab}	38.33	2.50	118.67^{abcd}
	6	7.53	3.03	4.43^{ab}	11.33	114.33^{a}	121.00^{abcd}	34.33	1.87	117.67^{abcd}

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Table 2: Main effects of anti-parasitic agents, study duration and their interactions on the plasma biochemistry of Sarcoptes infested dogs (continued)

Variable		TP	Alb	Glo	AST	ALT	ALP	BUN	Cre	Glu
Individual mean[a]	treatment									
Antiparasitic agent	Study duration (weeks)									
Ivermectin	1	7.27	4.23	4.23^{abc}	13.33	106.00^{ab}	104.00^{cdef}	37.67	0.70	117.67^{abcd}
	2	7.50	3.47	4.03^{abc}	11.00	73.00^{d}	91.67^{f}	30.00	0.77	90.00^{f}
	3	7.27	2.70	4.17^{abc}	13.33	107.67^{ab}	102.67^{def}	31.00	0.60	104.00^{e}
	4	7.47	2.87	3.67^{abc}	14.33	111.67^{ab}	105.00^{bcdef}	29.00	0.57	108.67^{bcde}
	5	7.70	3.30	3.90^{abc}	16.33	105.67^{ab}	121.33^{abcd}	42.33	0.77	121.33^{ab}
	6	7.87	3.50	3.73^{abc}	15.67	108.67^{ab}	119.00^{abcde}	39.33	0.90	120.00^{abc}
Neem	1	7.47	3.20	4.47^{ab}	13.00	111.33^{ab}	125.33^{abc}	25.33	1.90	111.33^{bcde}
	2	7.43	2.70	4.57^{ab}	14.00	114.00^{a}	126.67^{ab}	27.00	1.57	115.67^{abcde}
	3	7.27	2.63	3.70^{abc}	14.33	116.00^{a}	125.67^{abc}	25.67	1.60	115.00^{abcde}
	4	7.53	2.73	4.23^{abc}	15.00	118.33^{a}	127.67^{a}	31.00	1.87	117.33^{abcd}
	5	7.37	2.93	4.57^{ab}	15.00	112.00^{ab}	130.00^{a}	38.33	2.00	118.33^{abcd}
	6	7.23	3.00	4.27^{abc}	14.67	115.00^{a}	123.67^{abcd}	35.00	2.03	120.67^{abc}

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Table 3: Main effects of anti-parasitic agents, study duration and their interactions on the plasma biochemistry of Sarcoptes infested dogs (continued)

Variable		TP	Alb	Glo	AST	ALT	ALP	BUN	Cre	Glu
Individual mean[a]	treatment									
Antiparasitic agent	Study duration (weeks)									
Vaseline	1	7.63	3.17	4.50^{ab}	15.33	111.00^{ab}	108.67^{abcdef}	36.67	0.77	118.00^{abcd}
	2	6.53	3.57	4.13^{abc}	14.33	90.00^{bcd}	96.67^{ef}	28.00	0.73	110.00^{bcde}
	3	6.97	3.33	4.30^{abc}	14.00	78.00^{cd}	112.00^{abcdef}	29.00	0.60	108.33^{bcde}
	4	7.47	3.00	3.83^{abc}	14.33	109.00^{ab}	114.67^{abcde}	28.67	0.60	111.00^{bcde}
	5	6.83	3.37	4.03^{abc}	15.33	108.67^{ab}	124.33^{abcd}	41.33	0.90	124.67^{a}
	6	7.37	3.27	4.07^{abc}	15.67	110.00^{ab}	120.00^{abcd}	42.33	0.97	121.00^{ab}
Permetrin	1	8.20	3.03	4.50^{ab}	14.67	107.66^{ab}	125.67^{abc}	22.67	1.57	107.67^{cde}
	2	7.33	3.07	4.80^{a}	15.33	112.33^{ab}	126.00^{abc}	26.67	1.47	110.67^{bcde}
	3	7.47	2.40	4.33^{abc}	15.33	109.00^{ab}	126.00^{abc}	27.33	1.70	114.33^{abcde}
	4	8.33	3.07	4.30^{abc}	13.67	110.67^{ab}	125.00^{abcd}	30.33	1.67	116.33^{abcde}
	5	7.40	2.90	4.33^{abc}	16.00	114.33^{a}	127.33^{ab}	33.00	1.80	117.00^{abcde}
	6	7.23	2.73	4.50^{ab}	13.33	116.00^{a}	112.00^{abcdef}	38.33	1.87	120.00^{abc}
Pooled SE		0.06	0.05	0.04	0.19	1.10	1.07	0.66	0.06	0.66

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Table 4: Main effects of anti-parasitic agents, study duration and their interactions on the plasma biochemistry of Sarcoptes infested dogs

Variable										
Means of main effects[b]										
Control		7.65	3.12[lm]	4.46	15.72	107.39	126.17	34.28[lm]	2.02[l]	112.50
Citrus		7.66	3.18[lm]	4.24	14.06	108.50	119.39	31.11[lm]	1.94[lm]	114.33
Ivermectin		7.16	3.34[l]	3.96	14.00	102.11	107.28	34.89[l]	0.72[n]	110.28
Neem		7.16	2.87[m]	4.30	14.33	114.44	126.50	30.39[lm]	1.83[lm]	116.39
Vaseline		7.43	3.28[lm]	4.14	14.83	101.11	112.72	34.33[lm]	0.76[n]	115.50
Permetrin		7.37	2.87[m]	4.46	14.72	111.67	123.67	29.72[m]	1.68[m]	114.33
	1	7.73[x]	3.34	4.28	14.06	106.11	118.00	29.61[y]	1.43[xy]	111.61[yz]
	2	7.51[xy]	3.06	4.42	14.33	97.83	112.61	27.56[y]	1.27[y]	107.89[z]
	3	7.01[y]	2.90	4.13	14.56	103.78	118.00	29.28[y]	1.42[xy]	110.72[yz]
	4	7.13[xy]	3.03	4.11	14.83	111.89	120.22	31.61[y]	1.50[xy]	114.06[y]
	5	7.66[x]	3.27	4.37	15.56	112.22	126.72	39.56[x]	1.73[x]	119.94[x]
	6	7.38[xy]	3.06	4.24	14.33	113.39	120.17	37.11[x]	1.61[x]	119.11[x]
ANOVA: P-*value*										
Antiparasitic agent		0.05	0.01	0.00	0.06	0.00	0.00	0.00	0.00	0.00
Study duration		0.00	0.05	0.05	0.19	0.00	0.00	0.00	0.00	0.00
Antiparasitic agent x Study duration		0.79	0.21	0.00	0.20	0.00	0.01	0.09	0.63	0.00

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Means of different superscripts along the same column are significantly different ($p < 0.05$)TP- Total Protein, Alb- Albumin, Glo- Globulin, AST- Aspartate AminoTransferase, ALT- Alanine AminoTransferase, ALP- Alkaline Phosphatase, BUN- Blood Urea Nitrogen, CRT- Creatinine, and Glucose.

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The Total Protein (TP) was not significantly influenced ($p > 0.05$) by the anti-parasitic agent as well as the interaction between the anti-parasitic agents and study duration (Table 4). However, Total Protein was influenced significantly ($P < 0.05$) by the study duration. While there was no significant variation ($P > 0.05$) in Total Protein of dogs on the 1st and 5th week after treatment, the highest significant level ($p < 0.05$) was recorded at these weeks. The lowest amount of Total Protein was recorded at the 3rd week of treatment.

The Albumin was not significantly influenced ($P > 0.05$) by the study duration as well as the interaction between the study duration and anti-parasitic agents used (Table 4). However, Albumin amount was significantly influenced ($P < 0.05$) by the anti-parasitic agent. There was no significant variation ($p > 0.05$) in the Albumin concentration among dogs treated with Citrus, Neem, Vaseline, Permetrin and the Control group. The Albumin concentration in these groups was however lower significantly ($P < 0.05$) than the dogs treated with Ivermectin.

The study duration did not significantly influence ($P > 0.05$) the Globulin concentration in sarcoptic dogs. That nevertheless, the anti-parasitic agent as well as their interaction significantly influenced ($P < 0.05$) the Globulin concentration in the dogs (Table 4). The Globulin concentration among dogs treated with Neem, Permetrin and the Control group did not differ significantly ($p > 0.05$) (Table 1, and 2) but were significantly higher than those treated with Ivermectin. The highest significant ($P < 0.05$) Globulin concentration was recorded in Permetrin treated dog at the 2nd week of treatment while the lowest recorded Globulin amount was in citrus treated dog at the 1st week of treatment.

The AST was not significantly influenced ($P > 0.05$) by the anti-parasitic agent, study duration and their interaction (Tables 1, 2, 3, and 4).

The ALT and ALP were however influenced significantly ($P < 0.05$) by the anti-parasitic agent, study duration as well as their interactions (Table 4). Dogs treated with Neem seed oil had ALT concentration significantly higher ($P < 0.05$) than other treatment agents and the control (Tables 1, 2, and 3). The lowest significant ($P < 0.05$) ALT concentration was recorded in dog treated with Vaseline. The ALT in dog was also highest significantly ($P < 0.05$) at the 6th week of treatment and lowest at the 2nd week of treatment (Tables 1, 2, and 3). The ALP on the other hand was highest at the 5th and 6th week of various treatments but was not significantly different ($P > 0.05$) in dog treated with Neem and the Control group. ALP levels were lowest in Ivermectin treated dogs at the 2nd week of treatment (Tables 1, 2, and 3).

The Blood Urea Nitrogen (BUN) and the Creatinine were significantly influenced ($P < 0.05$) by the anti-parasitic agent and the study duration but were not influenced significantly ($P > 0.05$) by their interaction (Table 4). The highest significant ($P < 0.05$) BUN was recorded in dogs treated with Ivermectin while dogs treated with Permetrin had the lowest significant ($P < 0.05$) BUN (Table 4). The BUN and Creatinine did not differ significantly ($P > 0.05$) between the 5th and 6th week of treatment and were significantly higher ($P < 0.05$) than the values recorded at the 1st, 2nd and 3rd week after treatment (Table 4).

The Glucose concentration in the plasma of the dog was influenced ($P < 0.05$) by the anti-

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parasitic agent, the study duration and their interaction (Table 4).

There was no significant difference ($P > 0.05$) in the Glucose levels amongst dogs treated with Citrus, Neem, Vaseline and Permetrin (Table 4) even though Glucose amounts were significantly higher ($P < 0.05$) in these groups when compared with dogs treated with Ivermectin (Tables 1, 2, and 3). The dogs at the 5th and 6th week of treatment had glucose concentration that did not vary significantly ($P > 0.05$) and were significantly higher ($P < 0.05$) than glucose amounts recorded at the 1st, 2nd, and 3rd weeks of treatment (Table 4). That nevertheless, the highest and lowest significant ($P < 0.05$) glucose concentrations were recorded in Vaseline treated dog at the 5th week of treatment and Ivermectin at the 2nd week of treatment respectively (Tables 1, 2, and 3).

DISCUSSION

The safety evaluation of Ivermectin, Cypermethrin, Neem Seed Oil and Citrus Oil application on dogs infested with Sarcoptes scabiei var. canis was assessed using serological findings as the pathogenesis of mite infestation in dogs earlier studied revealed marked reduction in Total Protein, Albumin, Globulin and Creatinine amounts. Findings also worthy of note include elevations in Alanine aminotransferase and Alkaline phosphatase as study week progressed (Nwufoh et al., 2019).

The loss of plasma proteins resulting from the exudative dermatitis and incessant fluid meal of the mites (Dadlich and Khanna, 2008) improved as treatment of infested dogs progressed. Cypermetrin treated dogs fared better in Total protein output as several studies have linked cypermethrin to the paralysis and death of mites (Irfan et al., 2003). Study outcomes of continuous improvement of Total protein as treatment lasted further strengthens the need for a more than one application and administration of treatment for total clearance of mites.

Comparatively, Ivermectin use significantly improved the decreased level of albumin in infested dogs. Ivermectin has also been found to possess good insecticidal properties as it interferes with the sodium channel current of the mite thereby birthing delayed repolarization and mite fatality (Irfan et al., 2003). The mite fatality arising from the use of Ivermectin most probably improved the amount of albumin. On the other hand, the use of cypermethrin improved globulin amounts as cypermetrin and ivermectin have been documented to possess similar modes of action (Usha, 2001).

Neem seed oil treated dogs were observed with high amounts of Alanine aminotransferase which is a marker of liver health. While infestation lasted (Nwufoh et al., 2019), ALT amounts were influenced by the establishment of mites and while treatment lasted, ALT amounts were observed highest at the 6th week of treatment. It should however be noted that the lowest amount of ALT was observed at the 2nd week of therapy. Consequent upon this finding, a short duration of therapy would soothe liver functions as longer durations would position the liver for further metabolic and excretory activities. Additionally, the lowest levels of ALT observed in Vaseline treated dogs brings to fore the need of a more gentle approach in addressing the earlier influenced liver function.

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Furthermore, ALP amounts improved from its state while mite infested with the use of Ivermectin (a potent miticidal agent) as it eliminated mites and improved liver function in less than 2 weeks. ALP amounts with the use of Ivermectin were also highest at the 5th and 6th week thus suggesting that most therapies should only be instituted on short term basis. Kidney functions (BUN) were also better enhanced with the use of Neem seed oil and Cypermethrin while Creatinine amounts were lowered with Ivermectin and Vaseline use. Since it was earlier noted that mite establishment did not interfere with the appetite of dogs as seen with the Glucose assay, the various therapeutic approach only improved Glucose amounts as dogs fed better with reduced mite burdens.

The findings of similar miticidal activity from the use of either a synthetic or botanical Acaricide goes to strengthen the need for study especially in the light of therapeutic failures and environmental toxicity. A progressive use of these bio-pesticides would on the long run reduce the harmful effects of the synthetic Acaricide as the use of these Acaricide has been proven to also take a toll on non-target species of organisms in the environment (Rosell et al., 2008).

The use of bio-pesticides alongside synthetic pesticides stands practical and useful as the only cost associated with its use is in its application. This is contrary to the cost of synthetic Acaricide, its availability at all times and its impact on the environment, pet and pet handler.

Conclusively, the readily available and affordable Neem seed oil positions itself as a choice miticide in the face of its swift improvement of parasite induced serum parameters while Cypermethrin and Ivermectin stands ideal as it also improved earlier influenced serum parameters.

It is therefore recommended that more neem trees be planted for the constant availability of neem while the use of synthetic products be regulated to guard against environmental, pet and pet handler toxicities arising from exposure to synthetic products and their target of non-specie organisms.

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