

Full Length Research Paper

Efficacy of materials used by resource limited farmers in ethno-veterinary control of fleas in free-range chickens in the Eastern Cape Province, South Africa

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Fleas are commonly controlled using commercial insecticides which are however expensive and inaccessible to resource-limited farmers. This has resulted in farmers resorting to the use of alternative remedies that are cheap and socially acceptable. However, information on the efficacy of these materials on fleas is lacking. The objectives of this study were to determine potential dermal irritation and efficacy of selected materials used in the control fleas in free-range chickens. The materials tested included used engine oil, Jeyes fluid (carbolic acid 13%), and plant material from *Clutia pulchella* and *Calpurnia aurea*. Distilled water and Karbadust (carbaryl 5%) were used as negative and positive controls, respectively. A complete randomized design was used where sixty free-range chickens were allocated to twelve treatment groups with five chickens each. The chickens were artificially infested with fleas, which were counted and recorded daily every morning for seven days. The results show that used engine oil, Jeyes fluid at (76.8%) and *C. pulchella* (100%) caused flea reduction in the order of 100, 99.8 and 85.5% three days post application of test materials. These reduction was similar to those of Karbadust (carbaryl 5%), a commercial insecticide that had a flea reduction of 100%. *C. aurea* (100%) and *C. pulchella* (75%) showed an efficacy of 75.5 and 60%, respectively. The different materials tested exhibited variable efficacy on fleas, where in certain cases it compared well with Karbadust used by farmers. The test materials did not cause any visible signs of irritation on chickens. Despite the efficacy of used engine oil and Jeyes fluid, they are environmental contaminants.

Key words: External parasites, flea control, skin irritation, used engine oil.

INTRODUCTION

Flea infestations cause irritation, restlessness, severe skin infections, reduced productivity and either mild or severe anaemia occasionally resulting in chicken death (Philips, 2005; Mashishi, 2010). Fleas (*Echidnophaga gallinacea*) are a broad based parasite of chickens that can cause ulcerations on soft body parts, like the comb

and wattles (Boughton et al., 2006; Loh and Kabayi, 2011). They also play an important role as intermediate hosts of various parasites, such as *Dipylidium caninum*, and therefore expose free-range chickens to adverse effects of heavy worm burden (Fajimi et al., 2006; Hellmann et al., 2007). This problem with fleas occurs particularly in developing countries where control strategies are deployed irregularly (Kilonzo et al., 2001; Kingori et al., 2010). Similar findings were reported in a study on the occurrence of external parasites. Fleas were found to be more prevalent in chickens where control measures were irregu-

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lar (Moyo, 2009).

The control of fleas has typically been achieved by the use of synthetic contact insecticides, such as carbaryl, permethrin, diazinon and dichlorvos. However, the use of these synthetic chemicals are associated with a number of limitations, including development of resistance in the target organism (Rust, 2005), high cost, residual effects of drugs in meat and undesirable environmental effects (Dalton and Mulcahy, 2001; Rust, 2005). As a result, ethno-veterinary remedies can be used as an alternative to commercial insecticides. Several authors have documented the use of plant-derived products in parasite management with reviews already available both from agriculture (Isman, 2000, 2006) and the veterinary perspective (George et al., 2008). Furthermore, resource-limited farmers have found ethno-veterinary remedies effective in controlling external parasites; however, there is need to validate their efficacy *in vivo* (Dautel, 2004). Therefore, the objectives of the study were to determine the potential dermal irritation effects and the efficacy of selected ethno-veterinary remedies used by resource-limited farmers in the control of fleas in free-range chickens.

MATERIALS AND METHODS

Experiment facility

This study was conducted at the Fort Cox College of Agriculture and Forestry (32°47'11"S; 27°0'08"E) in the Amathole District Municipality, Eastern Cape Province, South Africa. Twelve flea tight Perspex cages 62 cm length, 51 cm width and 47.5 cm height were used. Feed and water troughs were fitted inside the cages. These were placed in a fowl run of 25.4 m², with a temperature range of 27 - 30°C and relative humidity of 70% (Mustapha et al., 2006).

Plant material collection, identification and preparation

Fresh leaves of *Calpurnia aurea*, Voucher No SM01-010/2008 and *Clutia pulchella*, voucher No SM01-011/2008 were collected in May 2009 at Umdeni village in Amatola Basin (32°40'38"S; 26°59'79"E). They were authenticated at the Albany herbarium, Rhodes University, and deposited at the Giffen herbarium of the University of Fort Hare. The materials were prepared according to the methods of farmers recorded in an earlier study (Moyo and Masika, unpublished). Fresh leaves of *C. pulchella* and *C. aurea* were crushed using a meat mincer. The concentrations of the extracts were determined on a weight per volume basis (Yin and Kwok, 2005) to obtain 50 and 75% (w/v). The crushed test materials were squeezed to obtain 100% concentration extracts. The crushed test material was extracted in distilled water overnight at room temperature. Afterward, the materials were strained with a muslin cloth and subsequently filtered using Whatman No. 1 filter paper. The filtrates were placed in capped labeled bottles and kept in a refrigerator at 6°C until use.

Non-plant materials

Jeyes fluid (carbolic acid 13% and sodium hydroxide 1% m/m) (Adcock Ingram, Bryanston South Africa) was diluted using distilled water to make the following concentrations: 19.2, 38.4 and 76.8% (v/v). Used engine oil was not diluted and was drained from the

vehicle. A commercial insecticide, Karbadust (carbaryl 5%; Agro-Serve (Pty) Ltd, Bryanston, South Africa) commonly used by farmers in the study area and distilled water were used as positive and negative control, respectively.

Fleas

Mature fleas, *Ctenocephalides felis* of mixed sex were obtained from Clinvet International, Bloemfontein, South Africa. These fleas were used because they affect both chickens and cats (Roberts and Janovy, 2000) and in addition, it was the only flea species that was cultured having the same age and from the same environment. The fleas were held in small well ventilated plastic vials that were incubated at a temperature of 27 ± 3°C with a relative humidity of 80 ± 10% (Mustapha et al., 2006). Forty eight hours before the experiment, fleas were anaesthetized with CO₂ to facilitate easy distribution into plastic vials. They were randomly allocated to 60 small plastic vials with each vial having approximately twenty fleas used to infest one chicken.

Ethical issues

The experimental protocols were approved by the University of Fort Hare Ethics Committee and were in compliance with international standards.

Animal management

Rats

A total of 48 adult Wistar rats of either sex, weighing between 250 - 300 g were used in the dermal test. The rats were individually caged and fed on commercial rodent pellets (EPOL Feeds Ltd, South Africa). Fresh drinking water was provided *ad libitum*. Rats were kept under standard 12-h day and light cycle, with room temperature of 25°C and with a relative humidity of 70%.

Chickens

Sixty female Venda breed chickens, at an average of 5 months, were bought from one farmer in the Amatola basin. In addition, these chickens were from the same property hence they had the same exposure to fleas prior to the experiment. The chickens were acclimatized for five days in the fowl run before being infested with fleas (Franc and Yao, 2006). Chickens were grouped by treatment and were individually identified. Each individual cage was labeled by treatment. The feed and water was provided *ad-libitum*.

Dermal irritation test

The dermal irritation test was performed according to the procedure of Wolz et al. (2002). The pH of the test materials was determined using the Crison micro pH 2000 meter (Crison Instrument, South Africa). Two parallel patches of 2 × 3 cm were shaved off the dorsum of the rats. Care was taken to avoid bruising the skin. The rats were left for 24 h before the test materials were applied. A brush was used to apply the treatments on to the skin patches; subsequently the sites were covered with a gauze patch and non-irritant tape. The parallel patch served as a negative control to which distilled water was applied, while commercial insecticide Karbadust (carbaryl 5%) was used as a positive control. Three rats were randomly allocated to each of the 16 groups of test materials; each rat was housed in its cage. The test materials were left on the

Table 1. Standards for skin irritation study.

Skin response	Score
Erythema and eschar formation	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet-redness) to slight eschar formation (injuries in depth)	4
Edema formation	
No edema	0
Very slightly edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1.0 mm)	3
Severe edema (raised more than 1.0 mm and extending beyond exposure area)	4
Total possible score for irritation	8

Table 2. pH of test materials.

Material	pH meter reading
Karbadust	7.13
<i>C. pulchella</i> (100%)	5.51
<i>C. pulchella</i> (75%)	5.35
<i>C. pulchella</i> (50%)	5.25
<i>C. aurea</i> (100%)	5.66
<i>C. aurea</i> (75%)	5.46
<i>C. aurea</i> (50%)	5.43
Used engine oil	5.24
Jeyes fluid (19.2%)	8.90
Jeyes fluid (38.4)	8.95-
Jeyes fluid (76.8%)	8.97
Distilled water	6.90

skin patches for 4 h, after which, the residual test substances were swabbed off the area using cotton wool soaked in distilled water. Care was taken not to alter the existing response or the integrity of the epidermis. The animals were examined for signs of erythema, edema and any abnormal signs developing on the skin, which were graded and recorded at intervals of 1, 24, 48 and 72 h after patch removal. Irritation was graded according to a visual scoring scale by the same investigator (Table 1). Initial and terminal weights were recorded to determine changes in rat weight during the experimental period (Kanjanapothi et al., 2004). The pH measurements and dermal irritation test were done to ensure that test materials were safe on the chicken skin (OECD 1992).

In vivo efficacy of test materials

Chickens were individually caged in flea tight Perspex glass cages and a test tube containing 50 fleas was introduced per individual cage to infest the chicken (European medicine Agency, 2007). Chickens were left in the cages for a period of 20 min to allow fleas

to attach before being removed from the cage. This was done two days before the application of the test materials (Franc and Yao, 2006) to allow the parasites to fully attach to the chickens. Twenty-four hours later, the chickens were examined for fleas to determine the infestation level of each chicken before the application of test materials. A complete randomized experimental design was used where 60 free-range chickens were randomly allocated to 12 treatment groups with five birds each. Treatments were allocated to experimental units (cages). Test materials of 3 ml in quantity were topically applied on day zero at infested sites (wattles, combs and eyelids) using a feather (Yazwinski et al., 2005). Attached live fleas were counted every morning for 7 days versus the initial fleas infested to the chickens to determine decrease in flea numbers. The efficacy of test materials was measured relative to the positive control Karbadust (carbaryl 5%). After the experiment, the infested chickens were dusted with Karbadust to remove all remaining fleas.

Statistical analyses

Data obtained were subjected to the General Linear Model Procedures of SAS (2003). The significant differences between least square means were compared using PDIFF test of SAS (2003).

RESULTS

The pH of the materials used ranged from 5.24 to 8.97 (Table 2). Topical application of test materials at different concentrations did not cause any visible irritation on the rats and on chickens. Also, there was no significant difference in the initial and terminal weights of the rats ($P > 0.05$). The materials tested showed various degrees of flea reduction. Twenty-four hours after application of test materials, used engine oil, Jeyes fluid (76.8%) and *C. pulchella* (100%) exhibited significant ($P < 0.05$) mean flea reduction of 85.5, 87.5 and 75.5%, respectively. Some of the test materials reduced flea populations after 24 h. The flea load reduction increased up to day 3 and then remained constant from day 4 to 7 (Table 3). The overall flea load reduction of used engine oil and Jeyes fluid at 76.8% were not significantly different ($P > 0.05$) from that of the positive control Karbadust. The extracts of *C. pulchella* (75%) and *C. aurea* (100%) had an overall mean flea load reduction of 75.5 and 60%, respectively. *C. pulchella* and *C. aurea* at 75 and 100% concentrations produced results, which were not significantly different. On the third day of observation used engine oil, Jeyes fluid (76.8%) and *C. pulchella* (100) had the highest flea load reductions of 100, 85.5 and 99.8%, respectively, and these were not significantly different ($P > 0.05$) from that of the Karbadust.

DISCUSSION

None of the test materials caused any visible signs of irritation at the levels tested on both rats and chickens. The regulatory guidelines for the assessment of dermal irritation require taking pH values of test materials (Worth and Cronin, 2001). The acidic and alkaline properties of compounds are known to play a crucial role in the generation of dermal lesions (Worth and Cronin, 2001). When pH of

Table 3. Daily overall mean flea reduction.

Treatment	Day 1 (N = 50)	Day 2 (N = 50)	Day 3 (N = 50)	Day 4 (N = 50)	Day 5 (N = 50)	Day 6 (N = 50)	Day 7 (N = 50)
Distilled H ₂ O	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
Karbadust	98.4 ± 1.45 ^a	98.8 ± 1.42 ^b	100 ± 0.00 ^c				
Used oil	89.5 ± 1.9 ^a	98.8 ± 1.0 ^b	100 ± 0.00 ^c				
<i>C. pulchella</i> (75%)	60 ± 1.6 ^a	70 ± 1.4 ^b	79.5 ± 1.3 ^c				
<i>C. pulchella</i> (100%)	75.5 ± 1.5 ^a	80 ± 1.3 ^b	85.5 ± 1.2 ^c	90 ± 1.2 ^d			
<i>C. aurea</i> (75%)	39.5 ± 2.5 ^a	45 ± 2.0 ^b	55 ± 1.5 ^c	60 ± 1.4 ^d			
<i>C. aurea</i> (100%)	60 ± 1.25 ^a	70.5 ± 1.1 ^b	80 ± 1.0 ^c				
Jeyes fluid (38.4%)	58.5 ± 1.8 ^a	60 ± 1.5 ^b	65.5 ± 1.2 ^c				
Jeyes fluid (76.8%)	87.5 ± 2.0 ^a	98.5 ± 1.9 ^b	99.8 ± 1.0 ^c				

the test material is less than or equal to 2 or equal to or greater than 11.5, the material may be declared an irritant and no further testing is required (OECD, 1992). Resource-limited farmers have used some of these materials for many years. Overtime and through trial and error, they have managed to establish the correct formulations that are not physically harmful to their animals (Moyo et al., 2009). It should be noted that in this study, test materials were administered in a single application; repeated application for a prolonged period could produce a different effect on the skin.

Some materials used in this study exhibited significant overall mean flea load reduction on chickens. The significant flea reduction exhibited after 3 days post application of test materials indicate that these test materials can be used at 3 day intervals if there is a heavy flea infestation. In this study, the flea load reduction remained constant from day 4 to 7 and this could be due to the fact that the test materials that exhibited flea reduction load of less than 100% were no longer effective. Used engine oil is a mixture of several different chemicals (Wang et al., 2000), including aliphatic hydrocarbons, aromatic hydrocarbons, heavy metal contaminants such as aluminum, tin, lead, nickel and manganese (Achuba and Clarke, 2008). Used engine oil remarkably reduced the number of fleas after 24 h, showing that it can cause flea reduction within a very short period of time. The relatively high efficacy of used engine oil is likely due to its long lasting effects (persistence) on treated sites after treatment (Djouaka et al., 2007). Also, used engine oil contains some toxic compounds like manganese and polycyclic aromatic hydrocarbons used in the formulations of pesticides (Kumar et al., 2008), and these could have contributed to flea load reduction recorded in this study. In addition, the oil film most probably acts by clogging the spiracles causing the parasite to suffocate (Dreyer et al., 1998). As a result, direct contact between the oil and the flea is, therefore, important for effective results.

Jeyes fluid is a household disinfectant that contains tar acids (13% m/m; carboic acid), sodium hydroxide (1%)

and more than 60% water (Igram Adcock, South Africa). In this study, the efficacy of Jeyes fluid varied with concentrations; Jeyes fluid at 76.8% reduced flea load within three days. This could be attributed to its strong smell and phenols (carboic acid) which are toxic and poisonous (Gieger et al., 2000). The use of Jeyes fluid against livestock external parasites has been documented by several researchers (Fourie et al., 2001; Sekokotla, 2005; Rajput et al., 2006; Moyo and Masika, 2008). The detrimental effects of Jeyes fluid to animals and the environment require further investigation and caution needs to be practiced when using this chemical.

Clutia pulchella at 100% concentration had an overall mean flea reduction of 78.6%, which was adequate for reducing flea load on chickens. The findings of the present investigation revealed that *C. pulchella* exhibited flea load reduction. However further studies have to be conducted to determine whether the extract has repellent or insecticidal activities. Currently no other studies reporting the bioactivity of this plant to insect pests are known. *Calpurnia aurea* also exhibited significant efficacy against fleas. This plant has been reported to contain quinolizidine alkaloids such as lupanine and calpurminine (Kubo et al., 1984); lectins, non-protein amino acids and tannins (Fullas, 2001), virgiline and virgiline pyrrolicarboxylic acid ester (Alonso et al., 2000). Lectins, tannins and quinolizidine alkaloids are toxic and poisonous in animal and insects (Sauvlon et al., 2004; Goel et al., 2005; Resta et al., 2008). The efficacy of *C. aurea* on flea load reduction could be attributed to its composition.

These findings confirm farmers' experience on the use of *C. aurea* in controlling fleas. Despite the fact that it showed significant efficacy in this study, information is lacking to support its recommended use against fleas. Previous studies have recorded the use of *C. aurea* in the control of head lice in humans and ticks in cattle (Tadeg et al., 2005); treatment of lumpy skin and scabies of cattle (Amenu, 2007); and its use in the treatment of diarrhea in humans (Teklehaymanot and Giday, 2007). Despite its wide use, however, there is no scientific evidence to sub-

stantiate its efficacy for the anti-parasitic properties.

Conclusions

The materials evaluated in this study did not have any visible irritant effect on rats at the levels tested. Similarly, they did not cause immediate discernible effect on chickens. Some materials like used oil, Jeyes fluid 76.8% and *C. pulchella* at 100% showed significant efficacy at high concentrations against fleas, hence revealing their potential to be used as flea insecticides. The results vindicate the use of the tested materials by resource-limited farmers in the study area. Meanwhile, further work would have to be done to assess the residual effects of these materials, before recommending their widespread use for flea management on chickens.

REFERENCES

- Achuba FI, Peretiemo-Clarke BO (2008). Effect of spent engine oil on soil catalase and dehydrogenase activities. *Int. Agrophy.* 22: 1-4.
- Alonso R, Fernandez-Aranguiz A, Colom K, Herrerad A, Cisterna R (2000). Profile of Bacterial Isolate and Antimicrobial susceptibility. Multicenter study using a one-day cut-off *Rev.Esp. Quimioter.*, 13: 384-393.
- Amenu E (2007). Use and management of medicinal plants by indigenous people of Ejaji area (Chelya Woreda) West Shoa, Ethiopia: An Ethnobotanical approach. MSc Dissertation, Addis Ababa University of School of graduate studies. Addis Ababa, Ethiopia.
- Boughton RK, Atwell JW, Schoech SJ (2006). An introduced parasite, the sticktight flea (*Echidnophaga gallinacean*) and its pathology in the threatened Florida scrub-jay (*Aphelocoma coerulescens*). *J. parasitol.* 92: 941-948.
- Dalton JP, Mulcahy G (2001). Parasite vaccines a reality? *Vet. Parasitol.* 98: 149-169.
- Dautel H (2004). Test systems for tick repellents. *Inter. J. Med. Microbiol.* 293 (37): 182-188.
- Djouaka RF, Bakare AA, Bankole SH, Doannio JMC, Kossou H, Akogbeto MC (2007). Quantification of the efficacy of treatment of *Anopheles gambiae* breeding sites with petroleum products by local communities in areas of insecticide resistance in the Republic of Benin. *Malar. J.* 6: 56 doi: 10.1186/1475-2875-6-56.
- Dreyer K, Fourie LJ, Kok DJ (1998). The efficacy of used engine oil against ticks on cattle. *Onderstepoort J. Vet. Res.* 65: 275-279.
- European Medicines Agency (2007). Guideline for testing and evaluation of the efficacy of antiparasitic substances for the treatment and prevention of tick and flea infestation in dogs and cat. London.
- Fajimi AK, Ayodeji IO, Taiwo AA, Ajayi FT (2006). Evaluation of the lure and kill potential of sand paper leaves (*Ficus exasperate*) Against Fleas of Indigenous Fowls. *World Appl. Sci.J.* 1: 76-79.
- Franc M, Yao KP (2006). Comparison of the activity of selamectin, imidacloprid and fipronil for the treatment of cats infested experimentally with *Ctenocephalides felis felis* and *Ctenocephalides felis strongylus*. *Vet.Parasitol.* 13: 159-164.
- Fullas F (2001). Ethiopian traditional medicine common medicinal Plants in Perspective, 1st edition, USA.
- George D, Guy J, Arkle S, Harrington D, de Luna C, Okello E, Shiel, R, Port G, Sparagano O (2008). Use of plant-derived products to control arthropods of veterinary importance; a review. *Ann.N.Y. Acad. Sci.* 1149: 23-26.
- Goel G, Puniya AK, Aguilar CN, Singh K (2005). Interaction of gut microflora with tannins in feeds. *Naturwissenschaften.* 92: 497-503.
- Hellmann K, Adler K, Parker L, Pfister K, DeLay RL, Rugg D (2007). Evaluation of the efficacy and safety of a novel formulation of metaflumizone in cats naturally infested with fleas in Europe. *Vet. Parasitol.* doi: 10.1016/j.vetpar.2007.08.041.
- Kubo I, Takeshi M, Mutsuo K, Andrew C, Hideo N (1984). Quinolizidine alkaloid from the African Medicinal Plant *Calpurnia aurea*. Molluscicidal activity and structure study by 2D-NMR, *Agric.Chem.* 48: 2839-2841.
- Isman MB (2000). Plant essential oils for pest and disease management. *Crop Protect.* 19: 603-608.
- Isman MB (2006). Botanical insecticides, deterrents and repellents in modern agriculture and an increasingly regulated world. *Annu. Rev. Entomol.* 51: 45-66.
- Kanjanapothi D, Panthong A, Lertprasertsuke N, Taesotikul T, Rujjanawate C, Kaewpint D, Sudthayakorn R, Choochote W, Chaithong U, Jitpakdi A, Pitasawat B (2004). Toxicity of crude rhizome extract of *Kaempferia galangal* L (Proh hom). *J. Ethnopharmacol.* 90: 359-365.
- Kilonzo BS, Ngomuo AJ, Sabuni CA, Mgode GF (2001). Effects of *Azadirachta indica* (Neem) extract on livestock fleas in Morogoro District, Tanzania. *Insect Sci. Applic.* 21 : 89-92.
- Kingori AM, Wachira AM, Tuitoeck JK (2010). Indigenous chicken production in Kenya: A review. *International Journal of Poultry Science.* 9: 309-316, ISSN 1682-8356.
- Kumar KS, Sajwan KS, Richardson JP, Kannan K (2008). Contamination profiles of heavy metals, organochlorine pesticides, polycyclic aromatic hydrocarbons and alkylphenols in sediment and oyster collected from marsh/estuarine Savannah GA, USA, *Marine Pollution Bulletin.* 56 (1):136-149.
- Loh R, Kabayi M. (2011). Stickfast fleas-control and eradication. <http://www.agric.wa.gov.au>.
- Mashishi MSK (2010). External parasites on chickens. <http://www.nda.agric.za>
- Moyo B, Masika PJ (2008). Tick control methods by resource-limited framers and the effects of ticks on cattle in rural areas of the Eastern Cape Province, South Africa. *Trop. Anim. Health Prod.* 41: 517-523.
- Moyo B, Masika P.J, Dube S, Maphosa V (2009). An *in vivo* study of the efficacy and safety of ethno-veterinary remedies used to control cattle ticks by rural farmers in the Eastern Cape Province of South Africa. *Trop. Anim. Health Prod.* 41: 517-523.
- Moyo, S (2009). Alternative practices used by resource-limited farmers to control fleas in free-range chickens in the Eastern Cape Province, South Africa. MSc thesis. Department of Livestock and Pasture. University of Fort Hare, Alice, South Africa.
- Mustapha D, Stephen PF, Daniel S (2006). *Insect repellents: Principles, Methods and Uses.* Bacon Raton: CRC Press. <http://books.google.co.za>. Downloaded 19/09/10.
- OECD (Organisation for Economic Cooperation and Development) (1992). Guideline for testing of chemicals No 404. Acute dermal irritation/corrosion. Organisation for Economic Cooperation and Development: Paris
- Philips JP (2005). Ectoparasites (Poultry). In: The Merck Veterinary Manual. 9th Edn. 50 Anniversary Edition. C.M Kahn, Ed. Merck and CO., INC. Whitehouse, N.J., U.S.A. in Education Partnership with Meril Ltd. A Merck and Aventis Company; 227-272.
- Rajput ZI, Hu S, Chen W, Arijo AG, Xiao C (2006). Importance of ticks and their chemical and immunological control on livestock. *J. Zhejiang Univ. Sci. B,* 7 : 912-921.
- Resta D, Boschini G, D'Aqostina A, Arnoldi A (2008). Evaluation of total quinolizidine alkaloids content in lupin flours, lupin-based ingredients and foods. *Mol. Nutr. Food Res.* 52: 490-495.
- Roberts L, Janovy J (2000). *Foundations of Parasitology*, Sixth edition. U.S.A. McGraw Hill.
- Rust MK (2005). Advances in the control of *Ctenocephalides felis felis* (cat flea) on cats and dogs. *Tre. Parasitol.* 21: 232-236.
- Sauvlon N, Nardon C, Febvay G, Gatehouse AM, Rahbe H (2004). Binding of the insecticidal lectin concanavalin A in pea aphid, *Acyrtosiphon pisum* (Harris) and induced effects of the structure of midgut epithelium cells. *J. Insect Physiol.* 50: 1137-1150.
- Sekokotla MJ (2005). Assessing implementation of veterinary Extension on the control of cattle parasites, in Moretele District, North West province, South Africa. (Unpublished Thesis, University of Pretoria).
- Statistical Analysis Systems Institute (SAS, 2003). SAS user's guide: statistics, version 6. SAS Institute Inc., Cary, NC, USA.
- Tadeg H, Mohammed E, Asres K, Gebre-mariam T (2005). Antimicrobial activities of some selected traditional Ethiopian medicinal

- plants used in the treatment of skin disorders, J. Ethnopharmacol. 100: 168-175.
- Teklehaymanot T, Giday M (2007). Ethnobotanical study of medicinal plants used by people in Zegie Peninsula, Northwestern Ethiopia, J. Ethnobi. Ethnomedic, 3: 12 doi:10.1186/1746/4269.
- Wang J, Jia, CR, Wong CK, Wong PK (2000). Characteristics of polycyclic aromatic hydrocarbon created in lubricating oils. Water Air Soil Pollu. 120: 381-396.
- Wolz E, Schedegger C, Arcelin G (2002). Acute dermal toxicity study on rats. Roche Vitamins Ltd, Report Number 1010389.
- Worth AP, Cronin MTD (2001). The use of pH measurements to predict the potential of chemicals to cause acute dermal and ocular toxicity. Toxicol. 169: 119-131.
- Yazwinski TA, Tucker CA, Robins J, Powell J, Phillips M, Johnson Z, Clark D, Wolfenden R (2005). Effectiveness of various acaricides in the treatment of naturally occurring *Ornithonyssus sylviarum* (Northern fowl mite). Infestations of chickens. Department of Animal Science and Department of poultry Science, University of Arkansas, Fayetteville, Arkansas. J. Appl. Poult. Res.14: 265-268.
- Yin S, Kwok YO (2005). Detection technologies for chemical warfare agents and toxic vapour. Technology and Engineering. CRC Press, USA.