



Journal of Biological Sciences

ISSN 1727-3048

science
alert

ANSI*net*
an open access publisher
<http://ansinet.com>

Effect of Administration of the Essential Oil from *Tagetes minuta* L. Leaves in Wistar Rats

¹O.O. Odeyemi, ¹M.T. Yakubu, ²P.J. Masika and ¹A.J. Afolayan

¹Department of Botany, University of Fort Hare, Alice 5700, South Africa

²Agricultural and Rural Development Research Institute, University of Fort Hare,
Alice 5700, South Africa

Abstract: The effects of the essential oil from *Tagetes minuta* leaves at 125, 250, 375 and 500 $\mu\text{L kg}^{-1}$ b.wt. on some biochemical parameters of Wistar rats were studied. There was no significant difference in packed cell volume, mean corpuscular hemoglobin concentration, monocytes, lymphocytes, eosinophils, basophils, serum alanine transaminase, liver and kidney body weight ratios. However, the 125 $\mu\text{L kg}^{-1}$ b.wt. resulted in significant decrease in red blood cell and hemoglobin, whereas the same dose produced increase in mean corpuscular volume, mean corpuscular hemoglobin, neutrophils and large unstained cell. Sodium, inorganic phosphorus, conjugated bilirubin, albumin, globulin and total protein at all the doses were not affected while potassium, chloride, urea, creatinine and total bilirubin concentration were increased at certain doses. Whereas the activity of serum gamma glutamyl transferase was significantly increased at all the doses, those of serum aspartate transaminase were increased only at 125 and 500 $\mu\text{L kg}^{-1}$ b.wt. The kidney-body weight ratio was increased only at 375 and 500 $\mu\text{L kg}^{-1}$ b.wt. The result showed that the oil of *T. minuta* had a mild effect on the parameters investigated at certain doses. This dose and parameter specific effects may influence the use of the essential oil from *T. minuta* as an insecticide against *Sitophilus zeamais* in maize grains meant for human and animal consumption.

Key words: *Tagetes minuta*, essential oil, biochemical parameters, insecticide, *Sitophilus zeamais*

INTRODUCTION

In sub-Saharan Africa, maize (*Zea mays* L.) is one of the most nutritional crops; however, proper storage of grains continues to be a challenge for subsistence farmers (Govender *et al.*, 2008). Insects are one of the major causes of qualitative and quantitative losses in agricultural stored products. Poor storage techniques and some environmental factors encourage the growth of insect population in farm stores. Farmers and grain traders in sub-Saharan Africa are then forced to sell stored produce prematurely because of deterioration due mostly to insect damage. Producers have expressed the need for a relatively cheap and safe method of insect control (Stathers *et al.*, 2002).

The heavy reliance on the use of conventional insecticides has led to problems of insect resurgence, resistance, negative impact on non-target organisms, health and environmental hazards. These have raised concern among the public for the need to search for a safe and environmentally friendly pest control options (Duke *et al.*, 2003).

There is now a growing interest in the exploration of the natural vegetation for possible alternatives (Jovetic, 1994). Botanicals are important in insect management. They are known to provide effective control against insects that have become resistant to other insecticides (Weinzierl, 2000). Several plants have been found to possess insecticidal activities against a wide range of agricultural pests. *Tagetes minuta* (L.) (Mexican marigold) is a member of the family Asteraceae, which are well known for their insecticidal activities (Broussalis *et al.*, 1999; Tomova *et al.*, 2005). Previous study from our laboratory revealed that the oil from *Tagetes* plant had a strong contact effect against the stored maize weevil. However, there is dearth of toxicological information on the essential oil. Therefore, the present study was conducted to evaluate the toxicological effect of the essential oil from *T. minuta* leaves in rats.

MATERIALS AND METHODS

Plant material and authentication: Samples of the plant, collected from Nkonkobe municipality, Eastern Cape, South Africa in March, 2008, were authenticated by

Professor DS Grierson of the Department of Botany, University of Fort Hare. A voucher specimen (Kem 02/2008) was deposited at Giffen herbarium of the University. This study was conducted between April and May, 2008.

Experimental animals: Twenty Wistar rats of both sexes weighing between 200 and 230 g were obtained from the Animal House of the Agricultural and Rural Development Research Institute (ARDRI), University of Fort Hare, South Africa. The rats were housed in polypropylene cages placed in well-ventilated house conditions. They were maintained on EPOL brand pelleted feed (EPOL feeds, South Africa Ltd.) and tap water *ad libitum*. The study was carried out following approval from ethical committee on animal use and care of University of Fort Hare, Alice.

Assay kits and chemical reagents: The assay kits for creatinine, urea, calcium, phosphorus, albumin, bilirubin, alkaline phosphatase, gamma glutamyl transferase, alanine and aspartate transaminases were obtained from Roche Diagnostic GmbH, Mannheim, Germany.

Preparation of essential oil: The procedure described by Asekun *et al.* (2007) was used. Briefly, fresh leaves from *T. minuta* were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. The oil was stored in air-tight container at room temperature before administration.

Animal grouping and administration of essential oil: The rats were randomly grouped into five consisting of 4 rats each. Group A (control) received orally, 1 $\mu\text{L kg}^{-1}$ b.wt. of distilled water for 14 days while Groups B, C, D and E received 125, 250, 375 and 500 $\mu\text{L kg}^{-1}$ b.wt. of the essential oil, respectively, for same number of days. The oil and the distilled water were administered daily at 0900-1000 h.

Preparation of serum: The method described by Yakubu *et al.* (2005) was used. Briefly, under ether anaesthesia, the neck area of the rats was quickly shaved to expose the jugular veins. The veins after being slightly displaced (to avoid contamination with interstitial fluid) were sharply cut with a sterile scapel blade and an aliquot of the blood was collected into BD vacutainer sample bottles for the haematological analysis. The remainder was allowed to clot for 10 min at room temperature and then centrifuged at 1282 xg for 5 min using Hermle Bench Top Centrifuge (Model Hermle, Z300, Hamburg, Germany). The sera were stored frozen overnight before being used for the assay. The heart, kidney and liver were thereafter removed and weighed to determine their organ body weight ratio.

Determination of biochemical parameters: Adopting the method of Tietz *et al.* (1994), the levels of creatinine, uric acid, calcium, chloride, sodium and potassium ions, phosphorus and urea were determined. Total cholesterol, LDL-C, HDL-C, triglyceride, albumin, bilirubin, protein, alkaline phosphatase, gamma glutamyl transferase, alanine and aspartate transaminase were determined in the serum using assay kits from Roche Diagnostics, GmbH, Germany on Roche modular (model P800) Mannheim, Germany. The Advia 2120 (Bayer, Germany) was used for the haematological parameters.

Statistical analysis: Data obtained were subjected to one way Analysis of Variance (ANOVA) and means were separated by the Duncan Multiple Range Test. Percentage data were transformed to arcsine before analysis. Significant levels were tested at 5%.

RESULTS

The result on the haematological parameters, function indices of the kidney and liver as well as the serum enzymes showed that the essential oil has mild effect at some specific doses. Whereas the administration of *T. minuta* essential oil did not produce any significant difference in packed cell volume, mean corpuscular hemoglobin concentration, monocytes, lymphocytes, eosinophils and basophils at all the doses investigated, there were alterations in the remaining haematological parameters at specific doses. While the 125 $\mu\text{L kg}^{-1}$ b.wt. resulted in significant decrease in red blood cell and hemoglobin, the same dose also produced increase in the concentration of mean corpuscular volume, mean corpuscular hemoglobin, neutrophils and large unstained cell. On the other hand, the 250 $\mu\text{L kg}^{-1}$ b.wt. significantly decreased the red blood cell and haemoglobin whereas the platelet and large unstained cells were increased. While the 375 $\mu\text{L kg}^{-1}$ b.wt. only increased the white blood cell count, the highest dose (500 $\mu\text{L kg}^{-1}$ b.wt.) produced increase in large unstained cells (Table 1).

Although, the essential oil did not produce any significant effect on sodium, inorganic phosphorus conjugated bilirubin, albumin, globulin and total protein content of the animals at all the doses investigated, there were significant increases at certain doses in the concentrations of potassium, chloride, urea, creatinine and total bilirubin (Table 2).

While there was no significant difference in the liver and heart body weight ratios at all the doses investigated, the 500 $\mu\text{L kg}^{-1}$ b.wt. produced increase in kidney-body weight ratio (Fig. 1).

Table 1: Effect of the essential oil of *Tagetes minuta* L. on haematological parameters of rats (N = 4)

Haematological parameters	Doses ($\mu\text{L kg}^{-1}$ b.wt.)				
	Control	125	250	375	500
WBC ($\times 10^9 \text{ L}^{-1}$)	5.24 \pm 1.05 ^a	4.25 \pm 0.27 ^a	5.24 \pm 0.47 ^a	7.82 \pm 1.18 ^b	5.24 \pm 0.42 ^a
RBC ($\times 10^{12} \text{ L}^{-1}$)	9.14 \pm 0.15 ^a	8.32 \pm 0.29 ^c	8.40 \pm 0.16 ^{bc}	8.98 \pm 0.25 ^{bc}	9.04 \pm 0.20 ^{ab}
Hb (g dL ⁻¹)	16.13 \pm 0.25 ^a	15.35 \pm 0.48 ^{ab}	15.20 \pm 0.04 ^b	16.03 \pm 0.31 ^{ab}	15.25 \pm 0.10 ^{ab}
PCV (l L ⁻¹)	0.54 \pm 0.01 ^a	0.51 \pm 0.02 ^a	0.51 \pm 0.01 ^a	0.53 \pm 0.01 ^a	0.52 \pm 0.01 ^a
MCV (fl)	58.43 \pm 0.49 ^{bc}	61.40 \pm 0.62 ^a	60.23 \pm 0.85 ^{ab}	59.20 \pm 0.93 ^{bc}	57.23 \pm 0.78 ^c
MCH (pg)	17.68 \pm 0.11 ^b	18.48 \pm 0.17 ^a	18.08 \pm 0.32 ^{ab}	17.90 \pm 0.20 ^{ab}	16.90 \pm 0.28 ^c
MCHC (g dL ⁻¹)	30.20 \pm 0.25 ^a	30.05 \pm 0.24 ^a	30.00 \pm 0.40 ^a	30.18 \pm 0.30 ^a	29.55 \pm 0.13 ^a
Platelet ($\times 10^9 \text{ L}^{-1}$)	955.25 \pm 32.23 ^b	876.50 \pm 22.82 ^b	1052.75 \pm 7.15 ^a	879.25 \pm 32.84 ^b	937.50 \pm 32.53 ^b
Neutrophils (%)	12.31 \pm 1.38 ^{bc}	16.39 \pm 0.66 ^a	14.60 \pm 1.45 ^{ab}	10.23 \pm 0.73 ^c	14.28 \pm 0.43 ^{ab}
Monocytes (%)	28.97 \pm 2.33 ^a	37.27 \pm 2.57 ^a	32.61 \pm 2.82 ^a	29.77 \pm 5.60 ^a	36.33 \pm 3.23 ^a
Lymphocytes (%)	54.17 \pm 3.17 ^a	42.18 \pm 2.09 ^a	48.74 \pm 4.62 ^a	53.75 \pm 6.12 ^a	42.88 \pm 4.17 ^a
LUC (%)	14.02 \pm 0.59 ^b	17.73 \pm 0.81 ^a	17.36 \pm 0.12 ^a	16.09 \pm 0.72 ^{ab}	18.46 \pm 1.77 ^a
Eosinophils (%)	6.25 \pm 0.67 ^{ab}	6.27 \pm 0.30 ^{ab}	5.70 \pm 0.85 ^b	4.86 \pm 0.31 ^b	7.15 \pm 0.53 ^a
Basophils (%)	3.41 \pm 0.49 ^a	2.71 \pm 0.14 ^a	2.66 \pm 0.31 ^a	2.71 \pm 0.14 ^a	3.00 \pm 0.49 ^a

Means with the same superscripts as control across the rows are not significantly different ($p > 0.05$). WBC: White Blood Cell, RBC: Red Blood Cell, PCV: Packed Cell Volume, Hb: Hemoglobin, MCV: Mean Corpuscular Volume, MCHC: Mean Corpuscular Hemoglobin Concentration, and LUC: Large Unstained Cell

Table 2: Effect of the essential oil of *Tagetes minuta* L. on the liver and kidney function indices of rats (N = 4)

Parameters	Doses ($\mu\text{L kg}^{-1}$ b.wt.)				
	Control	125	250	375	500
Sodium (mmol L ⁻¹)	138.25 \pm 0.65 ^a	140.00 \pm 0.71 ^a	138.00 \pm 0.71 ^a	138.20 \pm 1.81 ^a	139.500 \pm 1.71 ^a
Potassium (mmol L ⁻¹)	5.23 \pm 0.18 ^b	5.73 \pm 0.11 ^a	5.23 \pm 0.13 ^b	5.58 \pm 0.09 ^{ab}	5.450 \pm 0.14 ^{ab}
Chloride (mmol L ⁻¹)	107.50 \pm 1.19 ^b	109.50 \pm 0.87 ^{ab}	110.50 \pm 0.50 ^a	109.25 \pm 0.85 ^{ab}	110.750 \pm 0.85 ^{ab}
Inorganic phosphorus (mmol L ⁻¹)	3.15 \pm 0.06 ^a	3.18 \pm 0.17 ^a	3.03 \pm 0.16 ^a	3.13 \pm 0.05 ^a	3.150 \pm 0.06 ^a
Urea (mmol L ⁻¹)	5.88 \pm 0.05 ^{bc}	5.80 \pm 0.21 ^c	6.60 \pm 0.25 ^a	5.90 \pm 0.28 ^{bc}	6.500 \pm 0.11 ^{ab}
Creatinine (mmol L ⁻¹)	53.75 \pm 1.03 ^b	51.00 \pm 1.35 ^b	51.50 \pm 1.50 ^b	51.50 \pm 1.85 ^b	60.250 \pm 2.72 ^c
Total bilirubin ($\mu\text{mol L}^{-1}$)	4.00 \pm 0.41 ^{ab}	6.00 \pm 1.47 ^a	4.50 \pm 0.50 ^{ab}	2.75 \pm 0.48 ^b	5.250 \pm 1.31 ^{ab}
Conjugated bilirubin ($\mu\text{mol L}^{-1}$)	0.95 \pm 0.03 ^a	1.75 \pm 0.25 ^a	1.25 \pm 0.25 ^a	0.98 \pm 0.03 ^a	1.480 \pm 0.50 ^a
Albumin (mmol L ⁻¹)	18.00 \pm 0.41 ^a	18.50 \pm 0.50 ^a	19.25 \pm 0.48 ^a	18.50 \pm 0.29 ^a	18.750 \pm 0.48 ^a
Globulin (mmol L ⁻¹)	41.25 \pm 0.48 ^a	39.25 \pm 0.85 ^{ab}	37.50 \pm 0.50 ^b	40.50 \pm 0.6 ^a	39.250 \pm 0.63 ^{ab}
Total protein (g L ⁻¹)	59.25 \pm 0.25 ^a	57.75 \pm 1.31 ^a	56.75 \pm 0.85 ^a	59.00 \pm 0.71 ^a	58.000 \pm 1.08 ^a

Means with the same superscripts as control across the rows are not significantly different ($p > 0.05$)

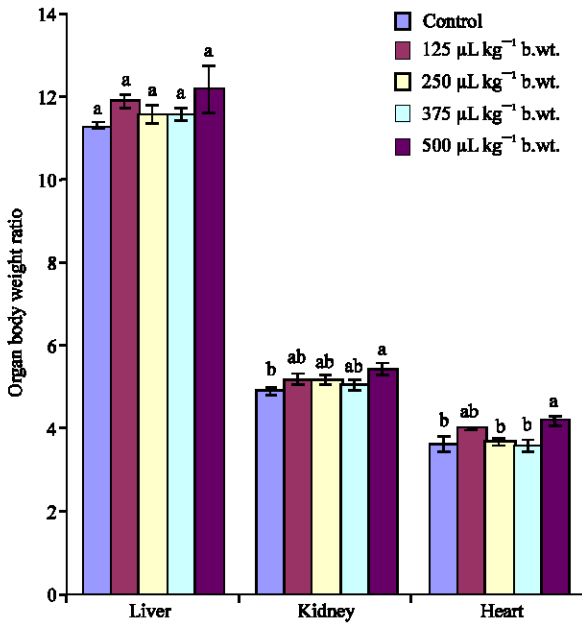


Fig. 1: Effect of the essential oil of *Tagetes minuta* L. on some organ body weight ratio of rats. Bars with the same letter(s) as control for same organ are not significantly different ($p > 0.05$)

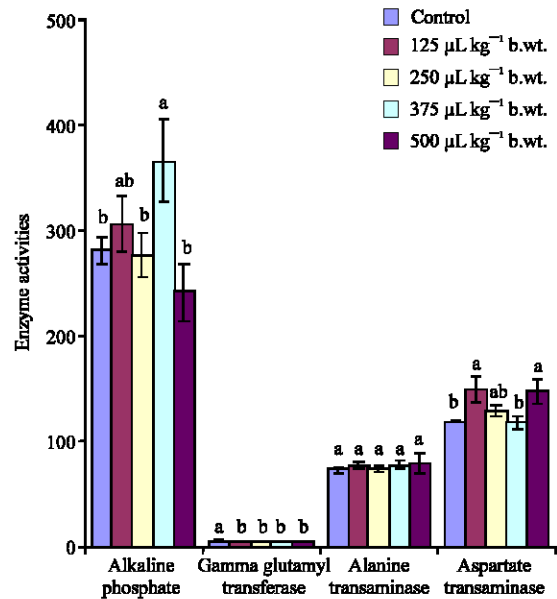


Fig. 2: Effect of the essential oil from *Tagetes minuta* L. on some enzyme activities of rat serum. Bars with the same letter(s) as control for each enzyme activity are not significantly different ($p > 0.05$)

Administration of the essential oil did not produce any significant effect on the serum alanine transaminase activity at all the doses, but increased the activity of γ -glutamyl transferase. The essential oil also resulted in increase in aspartate transaminase activity only at 125 and 500 $\mu\text{L kg}^{-1}$ b.wt. and alkaline phosphatase activity at 375 $\mu\text{L kg}^{-1}$ b.wt. only (Fig. 2).

DISCUSSION

Assessment of haematological parameters can be used to determine the extent of deleterious effect of a foreign compound including plant extracts on the blood. It can also be used to explain blood relating functions of a plant extract or its products (Yakubu *et al.*, 2007). The non-significant effect on packed cell volume, mean corpuscular haemoglobin concentration, monocytes, lymphocytes, eosinophils and basophils is an indication that the percentage of blood volume taken up by the red blood cell was not altered (Ganong, 2001). It may also be that the concentration of haemoglobin in the cell was not altered or that the oil did not interfere with the effector cells of the immune system. The alterations in the red blood cells, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, neutrophils and large unstained cells at 125 and 250 $\mu\text{L kg}^{-1}$ b.wt. may suggest destruction of matured red blood cells and this may affect the oxygen carrying capacity of the blood at this dose (McLellan *et al.*, 2003). Mean corpuscular volume and mean corpuscular haemoglobin have particular importance in the diagnosis of anaemia in most animals (Coles, 1986). The increase in these parameters at this dose may imply that the oil is capable of inducing anaemia. However, since the higher doses did not bring about alteration in these parameters, it may be logical to infer that the animals were only trying to adapt to the effect of the oil at the lower doses.

The serum proteins and electrolytes evaluated in this study are useful parameters to indicate impairment in the functional capacity of the liver and kidney. Bilirubin, an index of liver damage is the major breakdown product of red blood cells. The increase in total bilirubin might affect the ability of the liver to transform bilirubin to the bile pigment-bilirubin glucuronide (Naganna, 1989). Such elevated level of bilirubin is an indication of impairment in the liver functional capacity (Moudgil and Narang, 1989). The fact that there was no alteration in the levels of albumin, globulin and total protein indicate mild, selective and localized toxicity of the essential oil on the liver.

Potassium is a major component of cardiac function. The increase in serum potassium ion observed with 125 μL dose suggests a possible adverse effect on the sodium pump that maintains the constancy of the extracellular concentration of potassium. Similarly, the

increase in chloride ions at 250 and 500 μL doses suggests tubular dysfunction (Chawla, 1999). Urea is the major nitrogen-containing metabolic product of protein catabolism. The increase in serum urea content at 125 and 250 μL doses may be attributed to impairment in the urea cycle (Yakubu *et al.*, 2003). Such increase in serum urea concentration indicates renal dysfunction. Creatinine is a metabolic by-product of muscle metabolism. The increase in serum creatinine content at the highest dose of the essential oil suggests glomerular and tubular dysfunction (Chawla, 1999). Therefore, the dose specific effect produced by the essential oil on the indices of kidney damage investigated in this study suggests selective toxicity.

Organ body weight ratio is a marker of cell constriction and inflammation (Moore and Dalley, 1999). The non-significant effect on the liver and heart-body weight ratios suggests that the essential oil did not cause inflammation or constriction of the hepatocyte and cardiac cells. However, the increase in the kidney-body weight ratio at 500 $\mu\text{L kg}^{-1}$ b.wt. only suggests inflammation of the nephron. This further show the mild and selective toxicity of the oil.

There are many enzymes found in the serum that did not originate from the serum. During tissue damage, some of these enzymes find their way into the serum, probably by leakage (Wills, 1985). Serum enzyme measurements are therefore a valuable tool in clinical diagnosis, providing information on the effect and nature of pathological damage to any tissue. Therefore, the increase in serum γ -glutamyl transferase activity at all the doses, aspartate transaminase activity at 125 and 500 $\mu\text{L kg}^{-1}$ b.wt. and alkaline phosphatase activity at 375 $\mu\text{L kg}^{-1}$ b.wt. may indicate tissue damage leading to leakage of tissue enzymes to the serum. Such leakage from the tissue may adversely affect the normal functioning of these enzymes such as adequate transportation of required ion or molecules across the cell membrane (Akanji *et al.*, 1993), transamination reaction involving the transaminases, glutathione metabolism and resorption of amino acids by γ -glutamyl transferase (Kaplan and Pesce, 1996).

The result of the present study has shown that the oil of *Tagetes minuta* is of mild toxicity. The alterations produced at certain doses on some of the indices of liver and kidney function, haematological parameters and kidney-body weight ratio suggest that the effect of the essential oil from *Tagetes minuta* leaves is dose and tissue index selective.

ACKNOWLEDGMENT

The authors are grateful to the National Research Foundation, South Africa, for financial support.

REFERENCES

- Akanji, M.A., O.A. Olagoke and O.B. Oloyede, 1993. Effect of chronic consumption of metabisulphite on the integrity of the kidney cellular system. *Toxicology*, 81: 173-179.
- Asekun, O.T., D.S. Grierson and A.J. Afolayan, 2007. Effects of drying methods on the quality and quantity of the essential oil of *Mentha longifolia* L. subsp. *Capensis*. *Food Chem.*, 107: 995-998.
- Broussalis, A.M., G.E. Ferraro, V.S. Martino, R. Pinzon, J.D. Coussio and J.C. Alvarez, 1999. Argentine plants as potential source of insecticidal compounds. *J. Ethnopharmacol.*, 67: 219-223.
- Chawla, R., 1999. Kidney Function Tests. In: *Practical Clinical Biochemistry. Methods and Interpretation*. 2nd Edn., JAYPEE Brothers Medical Publishers (P) Ltd., New Delhi, India, ISBN: 8180611086, pp: 90-92.
- Coles, E.H., 1986. *Veterinary Clinical Pathology*. In: *Health Science Division, Saunders, W.B. (Ed.) Elsevier Science, Philadelphia, USA., ISBN: 0721618286, pp: 10-42.*
- Duke, S.O., S.R. Baerson, F.E. Dayan, A.M. Rimando and B.E. Scheffler *et al.*, 2003. United States department of agriculture-agricultural research service research on natural products for pest management. *Pest Manage. Sci.*, 59: 708-717.
- Ganong, W.F., 2001. *Review of Medical Physiology*. 20th Edn., Lange Medical Books/McGraw-Hill Medical Publishing Division, London, ISBN: 0-8385-8282-6, pp: 414-417.
- Govender, V., T.A.S. Aveling and Q. Kritzinger, 2008. The effect of traditional storage methods on germination and vigour of maize (*Zea mays* L.) from northern KwaZulu-Natal and southern Mozambique. *S. Afr. J. Bot.*, 74: 190-196.
- Jovetic, S., 1994. Natural pyrethrins and biotechnological alternatives. *Biotechnol. Dev. Monit.*, 21: 12-13.
- Kaplan, L.A. and A.J. Pesce, 1996. *Clinical Chemistry: Theory, Analysis and Correlation*. Vol. 8, 3rd Edn., A Harcourt Health Science Company, Mosby, London, ISBN: 0-8151-5243-4 pp: 140-152.
- McLellan, S.A., D.B.L. McClelland and T.S. Walsh, 2003. Anaemia and red blood cell transfusion in the critically ill patient. *Blood Rev.*, 17: 195-208.
- Moore, K.L. and A.F. Dalley, 1999. Structure of the Penis. In: *Clinically Oriented Anatomy*, 4th Edn. Williams L. and A. Williams (Eds.). Woller Klummer Corporation, Philadelphia, ISBN:0683061410 pp: 287-299.
- Moudgil, K.D. and B.S. Narang, 1989. The Liver and the Biliary System. In: *Textbook of Biochemistry and Human Biology*, 2nd Edn., Talwar, G.P., L.M. Srivastava and K.D. Moudgil (Eds.). Prentice-Hall of India Limited, New Delhi, India, ISBN: 8120319656, pp: 271-273.
- Naganna, B., 1989. Plasma Proteins. In: *Textbook of Biochemistry and Human Biology*. 2nd Edn. Talwar, G.P., L.M. Srivastava and K.D. Moudgil (Eds.). Prentice-Hall of India Private Ltd., New-Delhi, ISBN: 8120319656, pp: 59-61.
- Stathers, T.E., B.M. Mvumi and P. Golop, 2002. Field assessment of the efficacy and persistence of diatomaceous earths in protecting stored grain on small-scale farms in Zimbabwe. *Crop Prot.*, 21: 1033-1048.
- Tietz, N.W., E.L. Prude and O. Sirgard-Anderson, 1994. *Tietz Textbook of Clinical Chemistry*. 2nd Edn., W.B. Saunders Company, London, ISBN: 0721656102, pp: 1354-1374.
- Tomova, B.S., J.S. Waterhouse and J. Doberski, 2005. The effect of fractionated *Tagetes* oil volatiles on aphid reproduction. *Entomol. Exp. Applied*, 115: 153-159.
- Weinzierl, R.A., 2000. Botanical Insecticides, Soaps and Oils. In: *Biological and Biotechnological Control of Insect Pests*, Rechcigl, J.E. and N.A. Rechcigl (Eds.). Lewis Publisher, New York, ISBN: 1566704790, pp: 1-12.
- Wills, D.E., 1985. *Biochemical Basis of Medicine*. 3rd Edn., John Wright and Sons Ltd., Bristol, England, ISBN: 9780750620130, pp: 267-268.
- Yakubu, M.T., L.S. Bilbis, M. Lawal and M.A. Akanji, 2003. Evaluation of selected parameters of rat liver and kidney function following repeated administration of yohimbine. *Biokemistri*, 15: 50-56.
- Yakubu, M.T., M.A. Akanji and A.T. Oladiji, 2005. Aphrodisiac potentials of the aqueous extract of *Fadogia agrestis* (Schweinf. Ex Hiern) stem in male albino rats. *Asian J. Androl.*, 7: 399-404.
- Yakubu, M.T., M.A. Akanji and A.T. Oladiji, 2007. Haematological evaluation in male albino rats following chronic administration of aqueous extract of *Fadogia agrestis* stem. *Pharmacog. Mag.*, 3: 34-38.