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Nsadzetsen Gilbert Adzemye
1. Laboratory of Animal Production and Health, Institute of Agricultural Research for Development (IRAD), P.O Box 51, Bambui, Cameroon
2. Department of animal production, Faculty of Agronomy and Agricultural Sciences, P.O Box188, Dschang Cameroon

Marc K Kouam
Department of Animal Production, Faculty of Agronomy and Agricultural Sciences, P.O Box188, Dschang Cameroon, Africa

Ambrose Limnyuy Kinso
Laboratory of Animal Production and Health, Institute of Agricultural Research for Development (IRAD), P.O Box 51, Bambui, Cameroon

Munji Victorine Nsongka
Laboratory of Animal Production and Health, Institute of Agricultural Research for Development (IRAD), P.O Box 51, Bambui, Cameroon

Corresponding Author:
Nsadzetsen Gilbert Adzemye
1. Laboratory of Animal Production and Health, Institute of Agricultural Research for Development (IRAD), P.O Box 51, Bambui, Cameroon
2. Department of animal production, Faculty of Agronomy and Agricultural Sciences, P.O Box188, Dschang Cameroon

Evaluation of anthelmintic efficacy of *Cucurbita maxima* (Pumpkin) powder leaves on the gastrointestinal parasites of pigs in mezam division, Cameroon

Nsadzetsen Gilbert Adzemye, Marc K Kouam, Ambrose Limnyuy Kinso and Munji Victorine Nsongka

Abstract
Anthelmintic resistance in pigs has been reported to affect the health and productivity of pigs globally. The objective of this present study was to evaluate the anthelmintic efficacy of commonly used synthetic drugs and one plant (*Cucurbita maxima* (Pumpkin) powder leaves). This study was carried out in Mezam division, North West Region of Cameroon from March to July. We went out for naturally infected pigs and those with epg of ≥ 150 were selected to test the anthelmintic efficacy of three synthetic anthelmintics (ivermectin, levamisole and albendazole) and pumpkin powder leaves. The EPG of each pig was determined using Modified Macmaster technique. A total of 240 naturally infected pigs were used, 160 (99 males and 61 females) were used to test the anthelmintic efficacy of synthetic drugs by Faecal Egg Count Reduction Test (FECRT) while 80 pigs (50males and 30 females) were used for the clinical trial of pumpkin powder leaves. These pigs were placed into five treatment groups and a control each comprising of 40pigs each. Treatments were as follows: Levamisole (8mg/kg/bw/Sc), albendazole (7.5mg/kg/bw/i/m), ivermectin (0.2mg/kg/bw/Sc), *Cucurbita maxima* powder leaves Lower dose (25g/kg/bw/Per os) and *Cucurbita maxima* powder leaves upper dose (50g/kg/bw/per os). Faecal samples were collected on day 0 (before treatment) and days 7th, 14th, 21st, and 28th (post treatment) to determine the Faecal Egg Count Reduction (FECR) per animal. The effects of the synthetic drugs were determined using FECRT according to the guidelines of World Association for the Advancement of Veterinary Parasitology (WAAVP). Composite faecal cultures from both the treatment groups and control were done before and after treatment and L3 lavar isolated using Barman's apparatus while identification was based on morphological features. Ivermectin and levamisole were effective with mean FECR of 96.2% and 94.8% respectively. Albendazole showed resistance with a FECR of 92.4%. The upper dose (50g/kgbw) of powder pumpkin leaves was more effective (72.23%) than the lower dose (25g/kgbw) with FECR of 48.52%. Third stage larvae (L3) of *Hyostrongylus rubidus* (18), *Oesophagostomum spp* (two) and *Strongyloides ransomi* (five) were recovered from pooled faeces before treatment while *Hyostrongylus rubidus* and *strongyloides ransomi* after treatment. *Cucurbita maxima* powder leaves demonstrates striking Anthelmintic properties which can be exploited and further developed to serve as alternative Anthelmintic.

Keywords: Pigs, gastrointestinal parasites, ethnoveterinary, *Cucurbita maxima*, Mezam division Cameroon

1. Introduction
Pig production is very important in most developed and developing countries including Cameroon as it plays a vital role to the nation's economic growth [1], Reported on the importance of pig farming in Cameroon where he projected growth in the pig industry to generate up to 30,000 tons of meat per year with a projected 40,000 tons in 2016 and 45,000 tons by the year 2020. However, gastrointestinal parasitic infections remain a major constraint to livestock production in general and pig production particularly across all agro-ecological zones and production systems in Africa and Cameroon particularly [2]. The effects of these parasites on animals are malnutrition, anaemia, eosinophilia, pneumonia and other disease related losses in production arising from livestock mortality, morbidity, and poor fattening performance as well as reduce milk production in dairy animals which have serious economic

impact on livestock production and food security [3].

Anthelmintic resistance has emerged as a serious problem hindering the successful control and prevention of gastrointestinal nematodes in ruminants and pigs all over the world especially in developing Countries [4]. Therefore effective and steady monitoring of resistance in animals is vital in order to maintain and improve on the efficacy of the current available veterinary anthelmintics and ensure their availability to veterinary practitioners and livestock farmers thereby increasing productivity of pigs. Although several studies have been carried out on anthelmintic resistance in ruminants and pigs in most parts of the world [5, 6] and in Northern Cameroon [7] scanty information still exist on in Cameroon and Mezam division particularly especially on local breeds of pigs. Because of growing resistance to most commonly used synthetic anthelmintics, there is increasing interest in exploiting potentials of plants as source of anthelmintics. Medicinal plants have been reported to contribute as high as 80% therapeutic medications in developing countries [8, 9]. These plants are economical, efficient, easily available, sustainable, environmental friendly and safe to use with almost no side effects [10] which has led to increase in ethnoveterinary practice especially amongst local farmers in many developing countries including Cameroon. The seeds of *Cucurbita maxima* have been proven to have anthelmintic properties in ruminants [11] but not much work has been done on the leaves of this plant especially on gastrointestinal (GIT) parasites of pigs in Mezam Division where the plant is commonly used by local pig farmers as an anthelmintic has prompted our interest in this plant.

2. Materials and Methods

2.1. Drugs

Three synthetic drugs (Albendazole, Levamisole, Ivermectin 1%) each chosen from the basic three classes of broad spectrum anthelmintics (benzimidazoles, macrocyclic lactones and imidazothiazoles) manufactured by a registered pharmaceutical company (LOBS International) were used for the study.

2.2. Plant materials and their preparation

Fresh leaves of *Cucurbita maxima* were used. The leaves were harvested 4weeks after planting and from different villages within Mezam Division, North-West region of Cameroon. After harvesting, the leaves were air dried for one week. The plant samples were tied together and hang to expose the plant to air at ambient temperature not to force dried the plant materials using high temperature, hence heat liable compounds were preserved. After drying, the leaves were reduced to smaller particles using a hammer mill [12].

2.3. Experimental Animals

A total of 240 naturally infected pigs selected from farms within Mezam Division were used for the clinical trial. 160 pigs (99males and 61females) were used to test the anthelmintic efficacy of synthetic drugs while the remaining 90 pigs (50males and 30females) were used for the trial. Animals selected were those that have not been dewormed for at least three months with parasite egg per gram (EPG) of ≥ 150. Pregnant animals, sick animals, and young animals (< 3months) were excluded from the study.

2.4. Treatment Protocol

Faecal egg counts expressed as egg per gram of faces were done on faecal samples collected from pigs across Mezam Division. On this basis, 240 pigs having a minimum of 150epg were selected and placed randomly in five treatment groups (TG) and 1 control group of 40 pigs each. Treatment group one (TG1) was given 0.2mg/kgb. w. of Ivermectin, TG two; 8mg/kg bw of Levamisole, TG three; 10mgkgb.w of Albendazole, TG four; 25g/kg/bw of pumpkin powder leaves, TG five; 50g/kg/bw of pumpkin powder leaves and the control group was not given any treatment. The powder leaves of Pumpkin were incorporated into small quantities of feed and fed directly to the individual pigs very early in the morning before they were given their normal daily rations and water for three consecutive days. This was to ensure that the pigs consume the plant extract when they were still hungry. Ivermectin was administered subcutaneously, Albendazole orally while Levamisole was given intramuscularly.

2.5. Collection, Transportation and Storage of Samples.

At day zero, fresh faeces was collected directly from the rectum by gently inserting a gloved lubricated index finger into the rectum of each animal a day prior to treatment according to the recommendations of [13]. Samples were collected in sample containers, labelled, stored in a flask with ice parks and transported to the Animal Health and Production laboratory of IRAD BUMBUI, for analysis. After treatment, faecal samples were collected at days 7th, 14th, 21st, and 28th [13]. Samples that were not analysed within each day of collection were preserved in 10% formalin.

2.6. Determination of Fecal Egg Count per gram of faeces.

The faecal egg count was determined per gram of feces using the Modified Mac Master technique as previously described [14]. In brief, 2g of faecal material was mixed thoroughly with 28ml of floatation solution (sugar with specific gravity 1.27) in a beaker using a spatula. The fecal suspension was filtered through a strainer into a second beaker and after stirring the filtrate with Pasteur pipette, a sub-sample was drawn with the pipette which was used to fill the compartments of the McMaster counting chambers. The slide was examined using a compound microscope at 10 x 10 magnification.

2.7. Determination of the efficacy of the drugs

The fecal egg count reduction test (FECRT) was used to determine the efficacy of the drugs tested. The FECRT was calculated using the formula:

FECRT% = (T1 – T2/T1) ×100, where, T1 = Pre-treatment EPG, T2 = post-treatment EPG.

EPG = number of eggs in first chamber + number of eggs in the second chamber× 50

2.8. Coproculture and larval identification

Composite faecal samples (1g) from each of the treatment groups and the control was cultured while larval isolation and identification done according to methods previously described by [15] Briefly 1g of fecal material from each animal in the treated groups and control group in each of the study sites was collected and composite fecal cultures formed and allowed for 21 days at room temperature and larva (L3) isolated using Barman, s apparatus and identified using morphological features.

2.9. Statistical analysis

The data collected was entered in to Microsoft excel sheets for analysis. It was analysed using the Statistical Package for Social Science version 23 (SPSS Inc, Chicago, IL, USA). The

chi-square test was used to determine the 'goodness of fit' between observed and expected results at 95% confidence interval. P values of > 0.05 were considered significant. Post hoc ANOVA test was used to compare mean values for faecal egg counts or egg count reductions at 95% C.I.

3. Results

3.1. Changes in mean fecal egg count before and after treatment with synthetic anthelmintics

The mean FEC decreases steadily in all the treatment groups but remains unchanged for the control group.

Table 1: Changes in mean fecal egg counts post treatment

DRUGS	Pretreatment(D0)	D7	D14	D21	D28
Ivermectin(n=30)	746.67±406.61	556.67±261.21	21.67±31.30	283.33±142.83	556.67±178.46
Levamisole (n=30)	1208.33±507.881008.33	1008.33±457.52	52.33±49.01	238.51±238.50	568.33±257.80
Albendazole(n=29)	1010.0±448.25	825.00±327.15	66.67±46.113	391.67±129.38	620.0±164.31
Control	1356.67±784.08	812.33±293.80	1666.67±602.78	1258.33±725.4	1796±502.78

Do= Day0, D7= Day7, D14= Day21, D28= Day28.

3.2. Anthelmintic efficacy of synthetic drugs using Fecal Egg Reduction Test (FERT)

The results show that ivermectin and levamisole were effective with FECR of 96.2% and 94.8% at 95% C.I while albendazole was less effective with FECR of 89% at 95% C.I.

Table 2: Anthelmintic efficacy of synthetic drugs using FECRT (Coles *et al*; 2000)

Treatments (Drugs)	Control	Ivermectin	Levamisole	Albendazole
Number Treated	28	30	29	30
Variance of FEC	295260.92	57359.19	118604.02	70764.37
Mean FEC Pre-Treatment	1156.67±106.64	746.67±74.24	1208.33±92.73	1010.00±81.84
Mean FEC Post-Treatment	1566.67±110.05	21.67±5.72	53.33±8.95	66.67±8.42.
Mean% EPG Reduction	NA	96.21%	94.81%	92.41%
Upper 95% C. I	NA	98.43	96.62	94.95
Lower 95% C. I	NA	93.98	92.99	89.88
Interpretation	NA	NR	NR	R

NA= Not applicable. C. I= Confidence Interval. NR = Non-Resistance, R= Resistance

3.3. Comparative Effects of the synthetic drugs on different days post treatment using FECRT

Compared with the treatment group, there was a significant decline (P<0.05) in EPG between day zero (Pretreatment) and days 7th, 14th, 21st, and 28th (post treatment) for pigs treated with Ivermectin. However, pigs treated with Levamisole and Albendazole showed a significant difference in fecal egg count reduction between Day0 (pre-treatment) and days 14th, 21st, and 28th post treatment.

Table 3: Comparative effects of synthetic drugs on mean parasite egg count over the study period

Agent	Days post treatment	Mean Difference	P Value	95% C. I	
				Lower Bound	Upper Bound
Ivermectin	Day0 Vs. Day 7	190.00±61.84*	.021	19.18	360.82
	Day0 Vs. Day 14	463.33±61.84*	.000	292.51	634.16
	Day0 Vs. Day 21	725.00±61.84*	.000	554.18	895.82
	Day0 Vs. Day 28	190.00±61.84*	.021	19.18	360.82
Levamisole	Day0 Vs. Day 7	200.00±88.92	.168	-45.64	445.64
	Day0 Vs. Day 14	755.00±88.92*	.000	509.36	1000.64
	Day0 Vs. Day 21	1155.00±88.92*	.000	909.36	1400.64
	Day0 Vs. Day 28	640.00±88.92*	.000	394.36	885.64
Albendazole	Day0 Vs. Day 7	185.00±68.68	.060	-4.74	374.74
	Day0 Vs. Day 14	618.33±68.68*	.000	428.59	808.07
	Day0 Vs. Day 21	943.33±68.68*	.000	753.59	1133.07
	Day0 Vs. Day 28	390.00±68.68*	.000	200.26	579.74

* Means within a row with superscripts differ significantly (P< 0.05).

3.4. Anthelmintic Efficacy of *Cucurbita maxima* (Pumpkin) powder leaves on gastrointestinal parasites of pigs.

Table below present results of anthelmintic efficacy of *Cucurbita maxima* powder leaves. The interpretation of the results is based on the WAAVP recommendations. Based on this, the upper dose *of Cucurbita maxima* was effective (72.10%) while the lower dose (25g/kgbw) was less effective (48.50%).

Table 4: Anthelmintic efficacy of *Cucurbita maxima* powder leaves using FECRT (Coles *et al*; 1992)

Curcumbita maxima	Control	Upper dose(25g/kg.bw)	Lower dose(50g/kg.bw)
Number Treated	28	30	29
Mean FEC Pre-Treatment	1156.67±106.64	1065.00±74.79	1206.67±72.66
Mean FEC Post-Treatment	1158.33±114.10	688.33±61.43	568.33±33.43
Mean% EPG Reduction	NA	72.10%	48.50%
Upper 95% C. I	NA	84.31	70.32
Lower 95% C. I	NA	77.58	50.578

NA=Not Applicable, C. I= Confidence Interval

3.5. Comparative effects of *Cucurbita maxima* powder leaves on mean Fecal Egg Count (FEC) before and after treatment

Table present results of the comparative effect *Cucurbita maxima* leaves extract before and after treatment on Fecal Egg Count (FEC). There was a significant difference in Fecal Egg Count between day 0 and days 14 and 21 while there was no significant difference in Fecal Egg Count between days 7 and28 for upper and lower doses.

Table 5: Comparative effects of *Cucurbita maxima* powder leaves on mean Fecal Egg Count before and after treatment.

***Cucurbita. M* Powder leaves**	**Days post treatment**	**Mean Difference**	**P value**	**95% C. I**	
				Lower Bound	**Upper Bound**
Lower Dose(25kg/kg/bw)	Day0 Vs. Day 7	91.67±61.85	.788	-131.68	315.02
	Day0 Vs. Day 14	376.67±61.85*	.000	153.32	600.02
	Day0 Vs. Day 21	646.67±61.85*	.000	423.32	870.02
	Day0 Vs. Day 28	136.67±61.85	.443	-86.68	360.02
Upper Dose(50kg/kg/bw)	Day0 Vs. Day 7	-180.00±65.71	.053	-361.52	1.52
	Day0 Vs. Day 14	458.33±65.71*	.000	276.82	639.85
	Day0 Vs. Day 21	821.67±65.71*	.000	640.15	1003.18
	Day0 Vs. Day 28	180.00±65.71	.053	-1.52	361.52

* Means within a row with superscripts differ significantly ($P<0.05$).

3.6. Third stage larvae isolated from treatment and control groups before and after treatment.

L3 larva identification was based on morphological features. For pigs treated with synthetic drugs, *Hyostrongylus rubidus* and *Oesophagostomum* spp were recovered from pre-treatment and post treatment faecal cultures while pigs treated with pumpkin (*Curcumbita maxima*) powder leaves, *Hyostrongylus rubidus*, *Oesophagostomum spps* and *Strongylus spps* were recovered from pre-treatment and post treatment faecal cultures as shown on table five.

Table 6: Third stage Larvae isolated and identified from pool faecal samples before and after treatment with synthetic drugs

Anthelmintic agent	**Nematode species**	**Number of larvae per pool sample**				
		Day 0	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Control(n=40)	*Hyostrongylus rubidus*	18	10	7	13	15
	Oesophagostomum	2	1	0	0	0
	Strongyloides ransomi	5	0	0	0	0
Ivermectin(n=40)	*Hyostrongylus rubidus*	25	0	0	0	0
	Oesophagostomum spps	0	0	0	0	0
	Strongyloides ransomi	10	0	3	5	4
Levamisole (n=40)	*Hyostrongylus. rubidus*	15	0	0	0	4
	Oesophagostomum spps	0	0	0	0	0
	Strongyloides ransomi	3	0	0	0	0
Albendazole(n=40)	*Hyostrongylus rubidus*	20	0	0	0	0
	Oesophagostomum spps	2	3	5	3	4
	Strongyloides ransomi	6	3	5	4	7

Table 7: Third stage Larvae isolated and identified in pool samples of pigs treated with *Cucurbita maxima* powder leaves

***Cucurbita maxima* powdered leaves**	**Nematode species**	**Number of larvae per pool sample**				
		Day 0	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Control(n=40)	*Hyostrongylus rubidus*	18	10	7	13	15
	Oesophagostomum Strongyloides ransomi	2	1	0	0	0
Lower Dose(n=40)	*Hyostrongylus. rubidus*	17	7	5	0	3
	Oesophagostomum	0	0	0	0	0
	Strongyloides ransomi	6	4	7	8	10
Upper Dose (n=40)	*Hyostrongylus rubidus*	13	9	0	3	2
	Oesophagostomum spp	0	0	0	0	0
	Strongyloides ransomi	5	2	3	4	7

4. Discussion

As the level of resistance of most gastrointestinal parasites of pigs to synthetic anthelmintics increases on daily basis, efforts are being put in place to exploit naturally occurring compounds that are produced by plants and animals in their metabolic pathways. The aim of this study was to test the anthelmintic efficacy of three synthetic broad-spectrum anthelmintics (Ivermectin, Levamisole, and Albendazole) and powder leaves of pumpkin (*Curcumbita maxima*). Results obtained from FECRT indicated a FECR of 96.21%, 94.81% and 92.41% for ivermectin, Levamisole and albendazole respectively at 95% C.I. These findings agree with the report of [16] who reported high efficacies with ivermectin and levamisole. The results however defer from those of [17, 18] who reported anthelmintic resistance to Pyrantel and levamisole by *Oesophagostomum spp* in pigs. [19, 20] also reported resistance to pyrantel, levamisole and benzimedazoles in *Oesophagostomum species*. Based on WAAVP guidelines [21] for interpretation of anthelmintic efficacy, ivermectin and Levamisole were effective in pigs in Mezam division while Albendazole showed resistance to most of the gastrointestinal parasites in pigs. Resistance development to Albendazole may be attributed to extensive use of the drug perhaps below the recommended dose or using

it alone or in combination with other drugs. The effects of the synthetic drugs were also compared on different days post treatment which showed a significant decline in EPG between day 0 (pre-treatment) and days 7th, 14th, 21st and 28th post treatment. This signified that ivermectin and Levamisole can be used as a drug of choice in infections that requires immediate drug action. The results also show that pre-treatment EPG counts of untreated infected group (control) showed no statistical significant change ($p>0.05$) on days 7th, 14th, 21st and 28th post treatment. Percentage efficacies of Lower dose (25g/kgb. w) and Higher Dose (50g/kg/b.w) of *Cucurbita maxima* were 48.51% and 72.23% respectively**.** Based on the recommendations of WAAP guidelines [22] for interpretation of anthelmintic efficacy, the upper dose of the plant was effective against gastrointestinal parasites of pigs in Mezam Division. The trial showed that the plant has anthelmintic activities in pigs. Therefor the use of this plant as an anthelmintic will help to reduce gastrointestinal parasitic infections in pigs as well as over dependent on synthetic drugs most of which have already developed resistance to gastrointestinal parasites of pigs. Some authors suggest that secondary metabolites, such as cucurbitacin B, cucurbitin, cucurmosin, saponins and sterols might play a crucial role in affecting G.I. nematodes. However, no further studies have confirmed these assumptions yet. L3 larva identification was based on morphological features of the L3 larvae. For synthetic drugs group, Nematodes of genera *Hyostrongylus rubidus*, *strongyloides ransomi* and *Oesophagostumum* were recovered from pre-treatment and post treatment pool samples. In the three treatment groups, *Hyostrongylus rubidus* larvae was isolated from the levamisole group post treatment indicating resistance to this drug. *Oesophagostomum* and *strongyloide ransomi* were also isolated from the albendazole group signifying resistance to the drug. These results agree with those of [22] who reported resistance of *strongyloides spps* and *hyso strongyloides* spps to levamisole. In the *pumpkin* (*curcumbita maxima*) group, nematodes of genera *Hyostrongylus* and *Oesophagostomum* were recovered from pre-treatment and post treatment faecal cultures. The presence of L3 larva of *Hyostrongylus rubidus* and *Oesophagostomum* spp in post treatment faecal cultures suggest anthelmintic resistance by these parasites. This is in conformity with the work of [23] who reported the existence of resistance to albendazole by *Oesophagostomum* spp.

5. Conclusion

Based on the objectives of this work and results obtained, the following conclusions were drawn. Amongst the three commonly used anthelmintics by pig farmers in Mezam Division, Ivermectin and Levamisole were the most effective with percentage efficacies of 96.21% and 94.82% respectively. Albendazole has developed resistance to most of the pig parasites in Mezam division. We therefore recommend ivermectin and levamisole as the drug of choice for treatment of helminthosis cases in Mezam Divison. Ivermectin reduces the parasite egg significantly between days 7th and 14th post treatment and therefore recommended for use in chronic cases of helminthosis in pigs. It is also effective against ectoparasites as reported in some works. *Cucurbita maxima* powder leaves upper dose (50g/kgbw) was very effective (72.21% efficacy) against gastrointestinal parasites of pigs. Due to the side effects associated with synthetic anthelmintics, their expensive nature and scarcity we recommend this plant to farmers as an alternative anthelmintic. Though not as effective as the conventional anthelmintics, it has numerous advantages such as availability, little or no side effects, low production cost, safe administration, cheaper and most importantly environmental friendly. Therefore, based on the results of this work, *Cucurbita maxima* powder leaves may be an important alternative treatment for both standard and ecological methods of livestock rearing.

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7. References

1. Dieumou FE, Tandzon DPT. Feed management in Pig production and an attempt for improvement: A case study of Babadjou locality in the Western Region of Cameroon. Journal of Applied Life Sciences International. 2017; 12(3):1-9.
2. FAO. Rome, Italy: Global perspectives on nematode parasites control in ruminant livestock: the need to adopt alternatives to chemotherapy, with emphasis on biological control; [PubMed], 2002, 104
3. Roepstorff A. Ascaris suum in Pigs: Population Biology and Epidemiology. Dr. Diss. The Royal Vet. Agri. University, 2003, 112
4. Buttar BS, Hari HS, Singh NK, Jyoti M Haque, Rath SS *et al*. Emergence of Anthelmintic resistance in an organized sheep farm in Punjab. J. Vet. Parasitol. 2012; 26:69-71.
5. De Souza AP, Ramos CI, Bellato V, Sartor AA, Schelbauer CA. Resistência de helmintosgastrintestinais de bovinos an anti-helmínticos no Planalto Catarinense. Cien. Rural. 2008; 38:1363-1367.
6. Demeler J, Van Zeveren AM, Kleinschmidt N, Vercruysse J, Höglund J, Koopmann R *et al*. Monitoring the efficacy of ivermectin and albendazole against gastrointestinal nematodes of intestinal cattle in Northern Europe.Vet. Parasitol. 2009; 160:109-115
7. Ebene N Jean, Onyali IO, Mingoas JP, Pougue H Bayemi, Mfopit MY *et al*. Zoopharmacology In: Wynn S.G, and Fougere (Eds). Veterinary Herbal medicine. Library of Congress Cataloging-in Publication Data. 695. 2007, 7-15.
8. Joy PP, Thomas J, Mathew S, Skaria BP. Medicinal Plants. Tropical Horticulture, (Eds. Bose, T.K., Kabir, J., Das, P. and Joy, P.P.). Naya Prokash, Calcutta. 2001; 2:449-632
9. Jia W, GAO W, Tang. Antidiabetic herbal drugs officially approved in China. Phytother. Res. 2003; 17:1127-1134.
10. Hossen MJ, Uddin MB, Ahmed SSU, Zhiling Y, Cho JY. Traditional Medicine/Plants for the Treatment of Reproductive Disorders in Asia Nations. Pak. vet. J. 2016; 36:127-133.
11. Strickland VJ, Potts W, Krebs GL. Pumpkin kernel and garlic as alternative treatments for the control of *Haemonchus contortus* in sheep. Animal Prod. Sci. 2009; l49:139-144.
12. Borhan MZ, Ahmad R, Rusop M Mohd, Abdullah S. Impact of Nano powders on Extraction Yield of Centella asiatica. Adv. Mater. Res. 2013; 667:246-250.

13. Coles GC, Jackson F, Pomroy WE, Prichard RK, Von Samson-Himmelstjerna G *et al*. The detection of anthelmintic resistance in nematodes of veterinary importance. Vet Parasitol. 2006; 136:167-185.
14. Barger IA, Miller JE (ed.), Klei TR. The role of epidemiological knowledge and grazing management for helminth control in small ruminants. International Journal for Parasitology. 1999; 29(1):41-47.
15. Zajac AZ, Conboy GA. Veterinary Clinical Parasitology 8th Edition, 2012, 8-11.
16. Ayoade GO. Adejinmi JO, Abiola JO, Lucas F. Efficacy of some anthelmintics used in porcine practice in Ibanda, Nigeria. African Journal of Biomedical Research, 2003; 6(2).
17. Roepstorff A, Bjorn H, Nansen P. Resistance of *Oesophagostomum spps* in pigs to pyrantel citrate. Vet parasitol. 2004; 20:477-481.
18. Bjorn H, Monrad J, Nansen P. Anthelmintic resistance in nematode parasites of sheep in Denmark with special emphasis on levamisole resistance in *Ostertagia, Circumcincta* Acta Vet Scand. 1991; 32:145-154.
19. Sangster NC, Whitlock HV, Russ IG, Gunawan M, Griffin DL, Kelly JD *et al*. *Trichostrongylus Colubriformis* and *Oestertagia Circumcincta* resistant to Levamisole, morantel, tartrate and thiabendazole. Occurrence of field strains. Res Vet. Sci. 1979; 27:106-110.
20. Geary TG, Hosking BC, Skuce PJ, Von Samson-Himmelstjerna G, Maeder S, Holdsworth WP *et al*. WAAVP Guideline on anthelmintic combination products targeting nematode infections of ruminants and horses. Vet Parasitol. 2012; 190: 306-316.
21. Coles GC, Bauer C, Borgsteede FH, Geerts S, Klei TR, Taylor MA *et al*. World Association for the Advancement of Veterinary Parasitology (WAAVP) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 1992; 44:35-44.
22. Wolstenholme AJ, Fairweather I, Prichard R, von Samson-Himmelstjerna G, Sangster NC. Drug resistance in veterinary helminths. Trends Parasitol. 2004; 20:469-476.
23. Maingi N, Bjørn H, Thamsborg S, Bøgh H, Nansen P. Anthelmintic resistance in nematode parasites of sheep in Denmark. Small Rumin. Res. 1997; 23:171-181.