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Table of Contents: Volume 11 Number 4 July 2017

ARTICLES

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Full Length Research Paper

Sorption of Cypermethrin from Alfisol and Inceptisol using acidified simulated rainwater in a soil column set-up

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Cypermethrin is a synthetic, pyrethroid insecticide and used for agricultural practices. The leaching of this pesticide through soil is of great concern because of the possibility of contaminating ground and surface water. The mobility of pesticide in soil being the main environmental reservoir varies among soil orders and the concept is still under investigation. Hence, this study was aimed at investigating the sorption capability of Alfisol and Inceptisol at different soil depth when leached with simulated rainwater of different pH values. The leachates were determined for cypermethrin concentration using a new UV/Visible spectrometry method. The CEC, organic matter content of Alfisol were 4.05±0.03 cmol/kg and 1.74±0.02%, while the corresponding characteristics of Inceptisol were 4.45±0.05 cmol/kg and 1.03±0.01%, respectively. pH of Alfisol and Inceptisol soil orders were 5.92 and 6.25, respectively. Simulated rainwater (pH 4) of 50, 100 and 150 mL leached out 37.0±0.1, 43.1±0.1 and 59.4±0.2% of cypermethrin, respectively from Alfisol at soil depth of 10 cm. At pH 6.8, the corresponding volumes of simulated rainwater leached out 31.8±0.1, 35.4±0.1 and 37.4±0.1% of cypermethrin, respectively. For each soil order, these proportions of sorbed cypermethrin decreased progressively as soil depth increased from 10-30 cm. The proportions of cypermethrin sorbed by Inceptisol were more than the corresponding proportions sorbed by Afisol. Afisol has high clay and organic contents, which might be responsible for its surpassing potential to sorb cypermethrin compared to Inceptisol.

Key words: Pyrethroid insecticide, leaching, Alfisol-Inceptisol soil orders, simulated rainwater, UV/Visible spectrometry.

INTRODUCTION

Increase in food production is a major target of all countries as the world population increased yearly (Saravi and Shokrzadeh, 2011). The demand for food by the entire world population cannot be met without improving crop production. Technological advances involving the utilization of agrochemicals have contributed considerably to increasing yields and regular crop production (Aubertot et al., 2007). The process of

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increasing crop production currently demands for the application of herbicides, insecticides, fungicides, fertilizers and soil amendments in higher quantities than in the past (Adeyinka and Mustapha, 2005). The demand for the use of pesticides is not restricted to developing countries. Crop productivity has increased dramatically in most developed countries such as United Kingdom and USA where wheat and corn yield increased (Akhtar et al., 2009). French farming has also developed production system based on the application of pesticides (Aubertot et al., 2007). The use of organochlorine pesticide has since been banned for agricultural practices in all these developed countries, but is still in use in developing countries like Nepal (Sharma et al., 2013), Egypt (Ibitayo, 2006) and Nigeria (Aiyesanmi and Idowu, 2012; Ize-Iyamu et al., 2007). As much as pesticides play a vital role in agricultural setting, adverse consequences to non target organism as well as human often occur (Vijverberg and vanden Bercken 1990).

The applied pesticide retained by soil is often released through a number of mechanisms into ground water and surface water. Weakly sorbed pesticides are lost from soil through storm run-off and percolation mechanisms (Ismail et al., 2013). The transport and fate of pesticides in soil are greatly affected by many processes such as volatilization, leaching, sorption, chemical and biological degradation (Hayward et al., 2009; Nolan et al., 2008). Of all these processes, sorption of pesticides readily occur as either rainwater or irrigation water percolates the soil column to affect the environment. Among the available pesticides, cypermethrin is one of the most stable pyrethroids whose soption potential in soil is still generating research interest. It was reported that pyrethroid is unlikely to undergo significant migration because of its strong tendency to adsorb to soils and organic matter. However, cypermethrin has been found in groundwater in France (Legrand et al., 1991) and surface water and sediment in the United Kingdom (House et al., 1991). The migration of cypermethrin to the environmental media seems to be generating public interest and concern due to its increasing use and potential effects on food crop and aquatic system. This concern necessitated further investigation to its sorption capacity in different types of soil. The sorption of pesticides is always influenced by some soil properties such as soil type, soil texture, organic matter content, soil pH and temperature. Several studies on cypermethrin have been reported, which include toxicity, occupational exposure and environmental impact studies (Abu-Zreig et al., 2000; Chauhan et al., 2012; Ismail and Kalithasan, 2004; Ismail et al., 2013; Kaufman et al., 1981; Persson et al., 2008; Rani et al., 2014). There is still dearth of data on the sorption of cypermethrin with respect to soil type and nature of the solution that leaches the pesticide. Therefore, the objective of this study was to investigate the extent to which simulated rainwater solutions of different pH values can leach cypermethrin through different soil orders such as Alfisol and Inceptisol packed at different depths in a column. In a way, the understanding of how to define such soil orders better based on the adsorption capacity to immobilize pyrethroid insecticide will be established.

MATERIALS AND METHODS

Chemicals and standard solution preparation

Commercial grade of cypermethrin (10% Emulsifiable Concentrate) were obtained from Jubaili-Agrotec, Beirut Lebanon. Appropriate dilutions of the 10% emulsifiable concentrate of cypermethrin were done with ethanol and used for the experiments. The analytical grade of cypermethin (100 µg/mL) was obtained from Sigma Aldrich, Germany. For the calibration curve, working standard solution of 1.0 – 6.0 µg/mL were prepared by appropriate dilution of the standard stock solution with ethanol. Alfisol and Inceptisol which are cypermethrin-free soil classes were collected from Forestry Research Institute of Nigeria.

Soil sampling, preparation and characterization

The soil samples were collected in an aluminium foil and air-dried. The air-dried samples were manually ground to a fine powder mortal and passed through a 2 mm mesh. The physicochemical properties of the soil as shown in Table 1 were determined using the standard methods of analysis. The pH of soil samples was determined in a soil to water (1:1) suspension using a glass electrode. Organic matter contents of the soil samples were determined using the Walkley-Black method (Nelson and Sommers, 1982). The soil texture analysis was carried out using hydrometer method. Cation exchange capacity (CEC) of the soils were analysed by the sodium acetate method. The physicochemical analyses of the soil order were replicated five times.

Procedure for soil column set up

Three glass columns of 2.5 cm internal diameter each were used in the leaching experiments. The air-dried sieved soils were uniformly packed in each column to depths of 10, 20 and 30 cm, respectively. Another set of glass column was set up for control experiment Soil columns were tapped uniformly to ensure compact packing of the soil in the columns to the required depth. Each of the packed columns was clamped and supported by a retort stand.

Each column was packed with Alfisol and soaked with de-ionized water above soil level. The de-ionized water was then allowed to drain completely. Each column was spike with 5 mL of 10 mg/L cypermethrin. After 1 h, the three columns were eluted with varying volume of rainwater of pH 4.0 and 6.8 separately. The rainwater was collected without passing through roof material over some days and the pH was adjusted to 4.0 and 6.8 using 0.1 M HNO_3 to simulate the common pH range of rainwater acidity data. Clean rainwater has a pH value of 5.0-5.5 but can become much more acidic when combine with oxides of sulphur and nitrogen to give pH value of 4.0. The understanding of the acidification phenomenon of rainwater as available in the literature necessitated the choice of the pH values (Esmen and Fergus, 1976). Cypermethrin was leached down the Afisol with 50, 150 and 150 mL of simulated rainwater. The leachates were collected in a glass flask and cypermethrin concentrations were determined by UV/Visible Spectrometry (Spectro UV/Vis spectrophotometric version 3.5 Labomed Inc.). This procedure was replicated five times for Alfisol and repeated

Table 1. Physicochemical properties of Alfisols and Incetptisol.

Parameter	Alfisol	Inceptisol
pH	5.92 ± 0.02	6.25 ± 0.03
Cation exchange capacity (cmol/kg)	4.05 ± 0.03	4.45 ± 0.05
Organic matter (%)	1.74 ± 0.02	1.03 ± 0.01
Total organic carbon (%)	1.00 ± 0.01	0.60 ± 0.01
Sand (%)	19.2 ± 0.2	88.0 ± 0.3
Silt (%)	61.6 ± 0.4	4.8 ± 0.1
Clay (%)	19.2 ± 0.2	7.2 ± 0.1
Classification of soil	Sandy-loam	Sandy

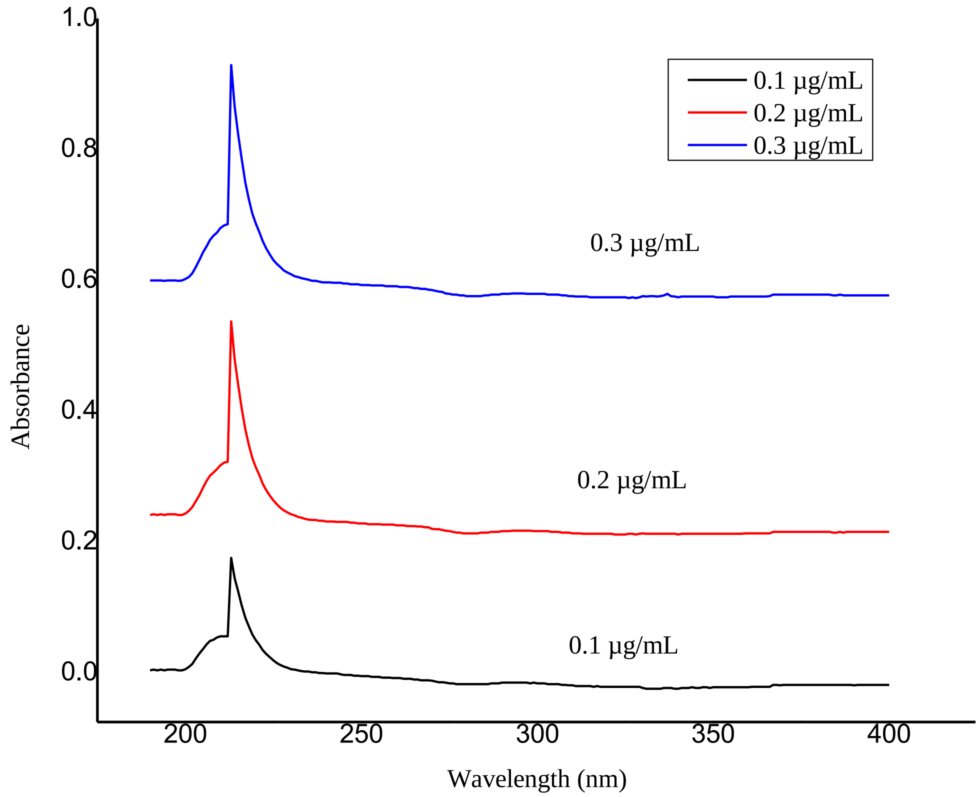

Figure 1. UV/Visible scanned spectrum of cypermethrin standards of different concentrations.

for Inceptisol. The columns for the control experiment at various soil depths were run with rainwater without spiking with cypermethrin.

Instrumental analysis

A sensitive spectrophotometric method was employed to determine the concentration of cypermethrin (Janghel et al., 2007). The wavelength for the UV/Visible spectrometric determination of cypermethrin was determined by scanning the absorbance of 0.1, 0.2 and 0.3 µg/mL of cypermethrin standard solutions. The solutions were prepared by adding 1 mL of 20% NaOH to little volume of each standard solution in a 25 mL volumetric. The solutions were kept for 10 min at room temperature for complete hydrolysis of cypermethrin. 1 mL of 0.1% potassium iodide in acidic medium was added followed by 1 mL leuco crystal violet indicator. The librated iodine selectively oxidizes the leuco base dye to liberate iodine. The solutions were kept for 15 min for full colour development and made up to the mark with ethanol. The absorbance values of the solutions were scanned through the wavelength range of 190 to 800 nm (Figure 1). The reagent blank had negligible absorbance at this wavelength. This procedure was followed for standard working solutions prepared for the calibration curve. Calibration curve was prepared using working standard solutions of 1.0 – 6.0 µg/mL prepared by appropriate dilution of cypermethrin standard stock solution of 100 µg/mL with ethanol. Ethanol was used because of the low solubility of cypermethrin in water. The absorbance values of the solutions were measured at

Table 2. Proportion (%) of cypermethrin leached by simulated rainwater of pH 4.0 from Alfisol and Inceptisol at various soil depths.

Soil depth (cm)	Alfisol			Inceptisol		
	50 mL	100 mL	150 mL	50 mL	100 mL	150 mL
10	37.0 ± 0.1[a]	43.1 ± 0.1[b]	59.4 ± 0.2[c]	39.5 ± 0.2[b]	46.6 ± 0.1[b]	66.6 ± 0.1[b]
20	35.4 ± 0.2[a]	36.5 ± 0.1[a]	40.1 ± 0.1[b]	37.0 ± 0.2[a]	43.5 ± 0.1[b]	63.2 ± 0.2[a]
30	32.1 ± 0.2[a]	35.7 ± 0.1[a]	37.7 ± 0.1[a]	35.2 ± 0.1[a]	38.1 ± 0.1[a]	62.6 ± 0.1[a]

Mean values along the column with different supercript (a,b,c) are significantly different.

213 nm against a reagent blank. The corresponding concentrations of cypermethrin were determined from the calibration curve whose R^2 value was 0.9847.

Procedure for cypermethrin analysis in leachates

1 mL of 20% sodium hydroxide was added to a 25 mL volumetric flask containing 1 mL of the leachate. After 10 min, 1 mL of 0.1% potassium iodide was added in acidic medium followed by 1 mL leuco crystal violet. The solution was shaken and allowed to stand for 15 min for full colour development. A light crystal violet solution obtained was made up to the mark with ethanol and the absorbance was measured at 213 nm against a reagent blank. The cypermethrin concentrations in the leachates were deduced from the calibration curve.

RESULTS AND DISCUSSION

Physicochemical characteristics of soil

Sorption behavior of pesticide is influenced by soil properties and pesticide characteristics, which are equally essential for the assessment of environmental fate of pesticide Both Alfisol and Inceptisol were acidic in nature, with pH of 5.92±0.06 and 6.25±0.03, respectively. Based on the soil texture composition, Alfisol was classified as sandy loam while Inceptisol as sandy. This implies that cypermethrin can permeate Inceptisol more than Alfisol due to its larger pores, thus allowing cypermethrin to move through rapidly. The higher permeability of Inceptisol over Alfisol can make it more sensitive than Alfisol that has low leaching potential. The clay content of Alfisol was observed to be almost thrice the content found in Inceptisol. Surface inorganic and organic functional groups in soil that play significant role in sorption process are associated with clay. Such functional groups include phenolic, carbonyl, and aluminol which are chemically reactive molecular units that have potential of binding unto pesticides such as cypermethrin. The fact that Inceptisol permits more pesticide movement rapidly is corroborated by its low organic matter content of 1.03±0.01% compared with Alfisol that has organic matter content of 1.74±0.02%. Organic matter improves soil water holding capacity and its high content for Alfisol compared to contents found in Inceptisol may be responsible for Afisol surpassing ability over Inceptisol to sorb cypermethrin. This suggests that Inceptisol was more sensitive to ground water contamination.

Impacts of simulated rainwater on the mobility of cypermethrin

The amount of rainfall is an essential factor in determining how far a pesticide can migrate down the soil. Simulated rainwater leached out appreciable proportion of cypermethrin from Alfisol and Inceptisol packed at various depths. The proportions of cypermethrin leached out with rainwater of pH 4 from Alfisol of depth 10 cm were 37.0±0.1, 43.1±0.1 and 59.4±0.2%, respectively. This proportion decreased as soil depth increased from 10 to 30 cm (Table 2). The corresponding proportions at soil depth of 30 cm were 32.1±0.2, 35.7±0.1 and 37.7±0.1%, respectively. For Inceptisol, rainwater of pH 4 leached out 39.5±0.2, 46.6±0.1 and 66.6±0.1% of cypermethrin for soil depth of 10 cm. These proportions of cypermethrin leached out for soil depth of 10 cm were significantly higher than (P < 0.05) the corresponding proportions for soil depth of 30 cm.

With the increasing volume of rainwater, the proportion of cypermethrin leached out increased for all the soil depths. This implies that more of cypermethrin applied on a farmland can be leached down soil depth by heavy downpour on the farmland compare to when the rain is not heavy. This seems to be different from the report that cypermethrin would remain mostly in top (0-10 cm) layer of Malaysian soils and that only small concentration (0.5 mg/kg) could be detected at 50 cm soil depth (Chai et al., 2012). Figures 2 and 3 show the concentrations of cypermethrin sorbed by the soil orders with soil depth. There was a progressive increase in the concentration of cypermethrin sorbed by both Alfisol and Inceptisol as soil depth increased.

Effects of pH on the mobility of cypermethrin in soil

Cypermethrin of 32.1 – 59.4% and 35.2 – 66.6% were leached from soil depth of 10 -30 cm containing Alfisol and Inceptisol respectively when eluted with the simulated rainfall of pH 4.0 (Table 2). In comparison, less proportions of cypermethrin was leached in Alfisol(26.2 - 37.4%) and Inceptisol (28.7 - 40.1%) when eluted with

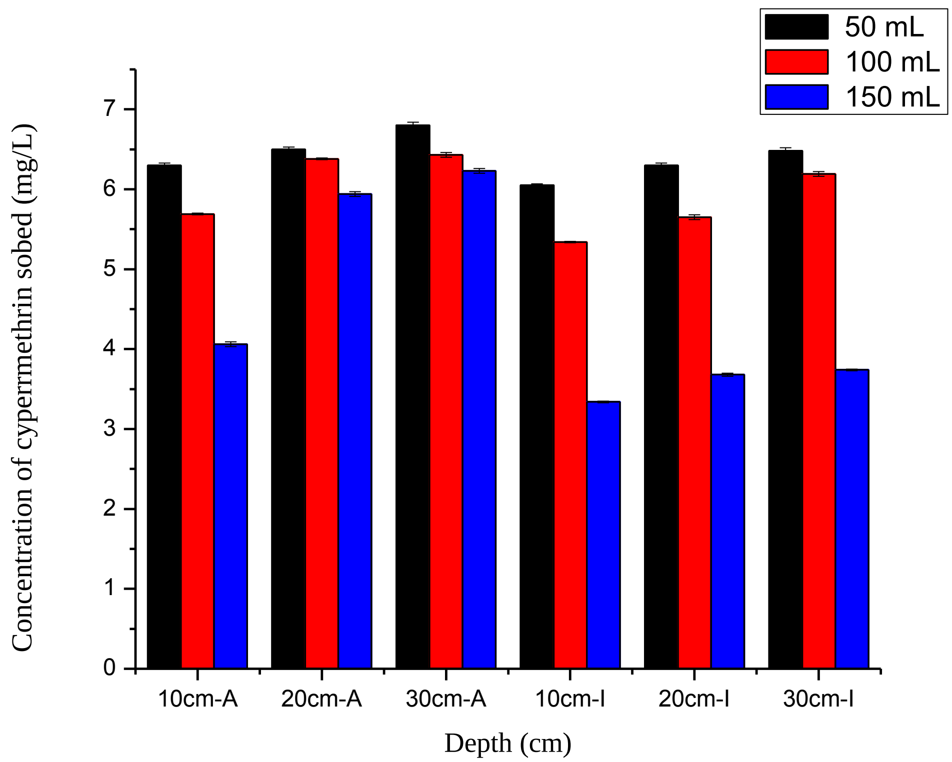

Figure 2: Variation of concentrations of cypermethrin retained by Alfisol (A) and Inceptisol (I) for rainwater of pH4

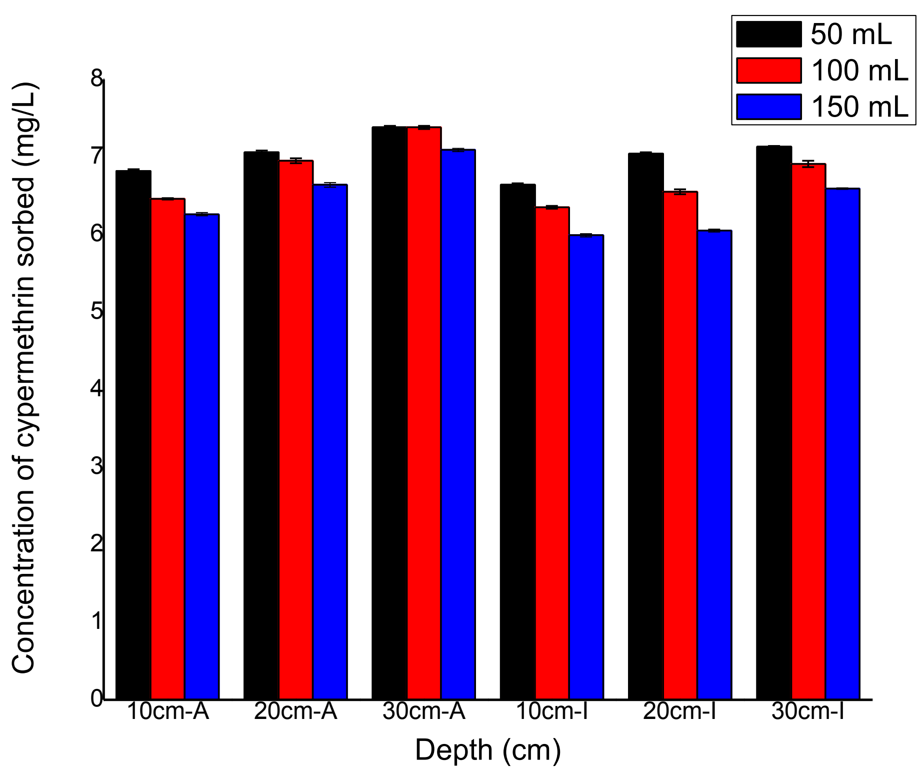

Figure 3. Variation of concentrations of cypermethrin retained by Alfisol (A) and Inceptisol (I) for rainwater of pH 6.8.

Table 3. Proportion (%) of cypermethrin leached by simulated rainwater of pH 6.8 from Alfisol and Inceptisol at various soil depths.

Soil depth (cm)	Alfisol			Inceptisol		
	50 mL	100 mL	150 mL	50 mL	100 mL	150 mL
10	31.8 ± 0.1[b]	35.4 ± 0.1[c]	37.4 ± 0.1[b]	33.6 ± 0.1[b]	36.5 ± 0.1[b]	40.1 ± 0.2[b]
20	29.4 ± 0.2[a]	30.5 ± 0.1[b]	33.6 ± 0.1[b]	29.6 ± 0.2[a]	34.5 ± 0.2[b]	39.5 ± 0.1[b]
30	26.2 ± 0.1[a]	26.9 ± 0.2[a]	29.1 ± 0.1[a]	28.7 ± 0.1[a]	30.9 ± 0.1[a]	34.1 ± 0.1[a]

Mean values along the row with different supercript (a,b,c) are significantly different.

simulated rainfall of pH 6.8 (Table 3). The proportion of cypermethrin leached from both soil orders at pH of 4.0 were much higher than the corresponding proportions leached at higher pH of 6.8. It can be deduced that more of cypermethrin is expected to be leached at lower pH than at neutral pH of the rainwater. This conforms to the observation on degradation of cypermethrin, malathion and dichlorovos in water and in tea leaves (Lin et al., 2012). In the study, more proportion of cypermethrin was leached from tea leaves when distilled water of pH 3.5 was used than when pH 6.8 was used for elution. Hydrolysis of cypermethrin by photolysis to either ester or nitrile acid metabolites occurs at higher pH particularly in alkaline condition. The photoproducts exert more toxic effect on beneficial and target insects (Al-Mughrabi et al., 1992).

Conclusion

Inceptisol has high leaching potential compared with Alfisol. Retention of cypermethrin in Alfisol cores was comparatively more than the retention found in Inceptisol. The surpassing ability of Inceptisol over Alfisol for leaching process of cypermethrin is attributable to its low clay and soil organic matter contents. There is likelihood that cypermethrin moves feely in Inceptisol because of its sandy nature to contaminate nearby groundwater and surface water. Sorption of pesticide by Alfisol and Inceptisol are pH dependent. More of the insecticide leached in both soils at lower pH than at neutral pH of rainwater. The texture of both Alfisol and Inceptisol played a crucial role in the mobility of cypermethrin through the soil.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Comparative evaluation of the antipsoriatic activity of *Acalypha wilkesiana, Culcasia scandens* with *Kigelia africana* using the mouse tail model

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The mouse tail model was used to measure and compare antipsoriatic activity of *Acalypha wilkesiana* and *Culcasia scandens* with that of earlier reported *Kigelia africana* stem methanol extract by the same authors, with the objective of finding out which of these plant extracts can be a better drug option for the treatment of psoriasis. The results obtained showed that topically administered extracts (50-200 mg/ml) induced a significant and dose-dependent increase in %orthokeratosis in the epidermis of the mice tails. % orthokeratosis values were 35.5-43.4 (*A. wikesiana*), 29.7-47.4 (*K. africana*), 31.9-36.5 (*C. scandens*) for the methanol ointments; 29.3-36.2 (*A. wikesiana*), 32.3-58.2 (*K. africana*), 29.40-56.2 (*C. scandens*) for the hexane extracts. In general, the methanol extracts produced higher % othokeratosis. No deterioration in the general condition of the mice in any group was observed. However, erythema was observed on the tails of the mice on which the *K. africana* stem methanol extract ointment (200 mg/ml) was applied. No tail erythema was observed in any other group. Application of the ointments resulted in the softening of the tails. In general, the irritation potentials of the ointments were relatively low when compared to that induced by dithranol a drug commonly used in the treatment of psoriasis. Only the *A. wilkesiana* methanol extract ointment (200 mg/ml) showed greater than 40% drug activity. Thus, *A. wilkesiana* appears to be the better plant for use in possible drug development for the management and cure of psoriasis because *A. wilkesiana* ointment showed more prospects of being an antipsoriatic topical agent when compared to *C. scandens or K. africana,* as the drug activity of the methanol extract of this plant was greater than 40% and quite similar to that of *K. africana* without the corresponding irritation potential or erythema.

Key words: Psoriasis, mouse-tail model, *Acalypha wilkesiana*, *Culcasia scandens*, *Kigelia africana*, dithranol drug activity, irritation potential.

INTRODUCTION

Psoriasis is a chronic auto-immune skin disorder that affects 2% of the world population. The chronic, recurrent, psychological and complex nature of the disorder means that more efficient therapies are being sought (Singhal and Kansara, 2012; Dwarampudi et al., 2012; Suresh et al., 2013), in spite of the large number of treatment options available (Wong and Koo, 2012; Papp et al., 2012). In this study, we have scientifically and objectively assessed the effects of local plants used in the management and treatment of psoriasis in Nigeria and some other countries in Africa using the mouse tail model.

Treatment of psoriasis often begins with topical treatments which involves the use of medications in creams or ointments that are applied to the skin and scalp. The challenges with the common topical agents include side effects like thinning of the skin, changes in skin colour, bruising and dilated blood vessels as those observed with the use of corticosteroids and the irritation caused by vitamin D analogues.

The importance of herbal remedies to the African Society includes the opportunity for cheaper treatment options, the presence of herbalist in the communities, and the use of herbs that are familiar and have always been used in those societies (Sambo, 2010; Kofi-Tsekpo, 2004; Elujoba et al., 2005; Mills et al., 2005; Oyeneye and Orubuloye, 1985).

The common herbs used in several parts of Nigeria for the treatment of Psoriasis and related diseases include *Acalypha wilkesiana, Kigelia africana syn. Kigelia pinnata and Culcasia scandens* (Fawehinmi et al., 2013; Haruna et al., 2013).

A. wilkesiana also known as Red Acalypha is locally and commonly used for the treatment of psoriasis especially in infants. A 50% aqueous ethanol extract of the plant revealed the presence of gallic acid, corilagin and geranin as compounds responsible for its antimicrobial activity (Alade and Irobi, 1993; Adesina et al., 2000).

K. africana also known as sausage tree or Worsboom is grown generally in the tropics. It has a wide variety of medicinal uses which includes the treatment of psoriasis (Olatunji and Atolani, 2009). Its varied medicinal properties may be due to the presence of numerous secondary metabolites. These compounds include irinoids, flavonoids, naphthoquinones and other volatile constituents (Sangita et al., 2009).

C. scandens is a climber that grows in the African tropical forests. It is used to treat psoriasis because of its strong anti-inflammatory and analgesic properties. The methanolic extract has been found to exhibit strong anti-inflammatory properties. Phytochemical analysis of the extract revealed the presence of reducing sugars, carbohydrates, alkaloids, glycosides, saponins, tannins, flavonoids and an unsaturated lactone ring of steroids (Okoli and Akah, 2000)

Cell proliferation is an important part of the pathogenesis of psoriasis. The mouse tail model is considered an adequate model for the study of the progress of this pathogenesis (Sebok et al., 2000; Singhal and Kansara, 2012; Schaper et al., 2013). In this model, the regular foci of parakeratosis, occurring in the adult mouse tail is used to study the ability of different topically delivered agents to produce orthokeratosis in these parakeratotic areas.

The antipsoriatic effect of *A. wilkesiana* and *C. scandens* as topical agents were evaluated using the modified albino mouse tail model (Bosman et al., 1992; Ledon et al., 2007) which had been used earlier to evaluate the effect of *K. africana* previously carried out in our lab (Oyedeji and Bankole-Ojo, 2012). The activity of *K. africana* reported was considered for comparative study and the data on the *K. africana* stem was used because of the high drug activity of this extract.

MATERIALS AND METHODS

Plant samples

Acalypha wilkesiana leaves and *C. scandens* leaves and stem were collected at the botanical garden of the University of Ibadan, Ibadan, Nigeria. All plant samples were subsequently identified by the Assistant Chief Plant Technologist and the officer-in-charge of the botanical garden. The plant samples were air dried and ground using an electric grinder. The *C. scandens* leaves and stem were ground together to form a composite sample.

Chemicals

Analytical grade methanol and hexane were used as solvents in the extraction process. Blue seal Vaseline was used as the vehicle for the plant extracts and as control.

Laboratory animals

The forty (40) male albino mice used for the study were purchased at Covenant farms, Iwo Road, Ibadan, Nigeria. The animals were then transferred to cages in the Experimental Animal Unit of the Faculty of Veterinary Medicine, University of Ibadan where they were kept and reared throughout the period of the study. They were sacrificed when they were 12 weeks and had an average weight of 10.53 g and an average normal length of 7 cm. The experiments were carried out in accordance with the ethical guidelines for investigations in laboratory animals [EE directive of 1886(86/609/EEC)].

Extraction process

Methanol and hexane extracts were obtained from the ground samples using a soxhlet extractor. The extracts were subsequently concentrated using a rotatory evaporator.

The modified mouse tail test

Ointments containing the plant extract and vehicle (Blue Seal Vaseline) were prepared to contain varying concentrations (200, 100 and 50 mg/ml) of the plant extract in the vehicle. 0.1 ml of the

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Table 1. Percent orthokeratosis for tails treated with methanol and hexane ointments.

Plant extract	Methanol ointment concentration (mg/ml)	% Orthokeratosis	Hexane ointment concentration (mg/ml)	% Orthokeratosis
	50	35.5 ± 3.1	50	29.3 ± 2.7
Acalypha wikesiana	100	37.1 ± 2.3	100	35.5 ± 4.0
	200	43.4 ± 4.4	200	36.2 ± 2.1
	50	29.7 ± 1.6	50	32.3 ± 3.6
Kigelia africana	100	35.5 ± 1.7	100	50.1 ± 2.4
	200	47.4 ± 2.0	200	58.2 ± 1.62
	50	31.9 ± 2.1	50	29.40 ± 3.2
Caulcasia scandens	100	36.5 ± 2.4	100	56.2 ± 1.6
	200	35.7 ± 2.1	200	29.40 ± 3.2

Each measurement is a mean of triplicate values.

ointment was rubbed on the proximal parts of the tails of the mice. To ensure a good treatment contact time of 2 to 3 h, plastic cylinders were slipped over the tails of the animals to cover the treated portions and keep treatment in place. Tails treated with vehicle and tails left untreated were used as Controls 1 and 2, respectively. The animals were treated once daily in the morning hours for 2 weeks. Three animals were used per dosage group. At the end of the treatment, the animals were killed by cervical dislocation, the tails were cut-off and longitudinal sections of tails were prepared and stained with haematoxylin for histological examination (Ledon et al., 2007)

Histological examination

The presence of a granular layer or isolated granular layer cells induced in the previously parakeratotic skin areas were examined on 10 sequential scales of the albino mouse tail. Measurements were carried out at the border of the scales with a semi-automatic image evaluation unit.

Drug activity and percent orthokeratosis

The length of the granular layer (A) and the length of the scale (B) were measured to quantitatively evaluate the drug activity and percent orthokeratosis in those parts of the adult mouse tail, which normally have a parakeratotic differentiation. For each animal, 10 sequential scales were measured and the results given in % orthokeratosis per scale. Three animals were taken for one drug concentration or control group. Thus, 30 individual orthokeratosis values were obtained per test group. Per animal and per group mean and standard error of the mean were calculated.

$$\%\ Orthokeratosis = (A/B)\ x\ 100$$

$$\%\text{Drug activity} = \frac{Mean\ Ok\ of\ treated\ group - Mean\ OK\ of\ Control\ Group\ x\ 100}{100 - Mean\ OK\ of\ the\ control\ group}$$

OK = Orthokeratosis

Epidermal thickness

The distance between the dermo-epidermal borderline and the beginning of the horny layer was measured to obtain epidermal thickness. Five measurements per animal were made in every 10 scales and the mean of the different animals was calculated. The change in epidermal thickness was then calculated. The percentage change in the epidermal thickness is often regarded to be representative of the extent to which a substance causes irritation (Ledon et al., 2007).

$$\%\Delta\ Epidermal\ thickness = \frac{ET\ of\ treated\ group - ET\ of\ control\ group\ x100}{100 - ET\ of\ control\ group}$$

ET=Epidermal thickness

RESULTS AND DISCUSSION

The profiles of the percent orthokeratosis as shown on Table 1 indicate that the topically administered extracts induced a significant and dose-dependent increase in orthokeratosis in the epidermis of the mice tails. No deterioration in the general condition of the mice in any group was observed. However, erythema was observed on the tails of the mice on which the *K. africana* stem methanol extract ointment (200 mg/ml) was applied. No tail erythema was observed in any other group. Application of the ointments resulted in the softening of the tails. The induction of a granular layer by the topically administered plant extracts was measured in previously para-keratotic scale regions in the mice tails. In the tail skin samples of the control groups, lack of granular layer in the epidermal stratum was observed as expected. Epidermal thickness is regarded as a parameter indicating skin irritation. Thus, the larger the increase in epidermal thickness induced, the more likely the ointment is going to cause irritation on the human skin. In general, the irritation potentials of the ointments are relatively low when compared to dithranol (Agrawal et al., 2013) which is commonly used in treatment of psoriasis (Figures 1 and 2).

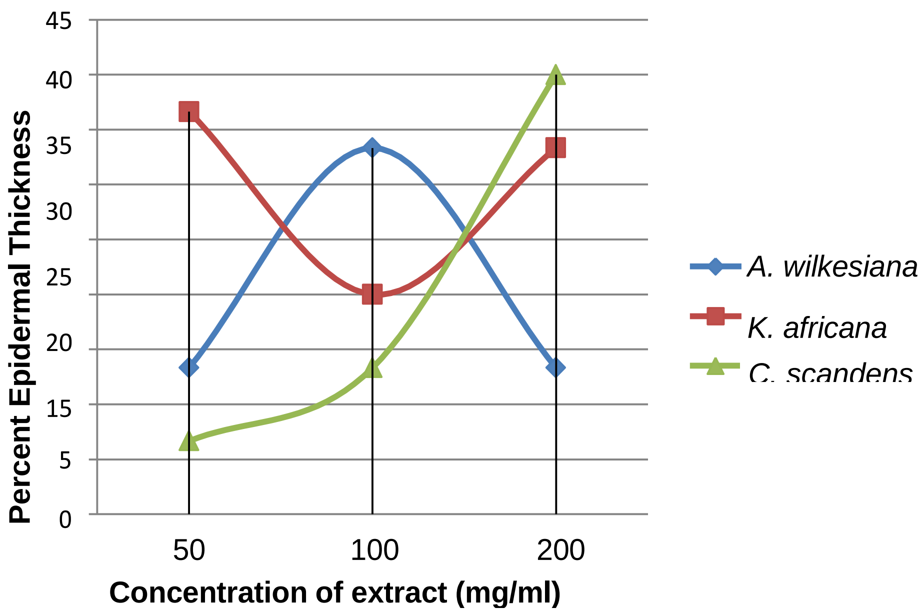

Figure 1. Change in percent epidermal thickness with concentration of methanol extract.

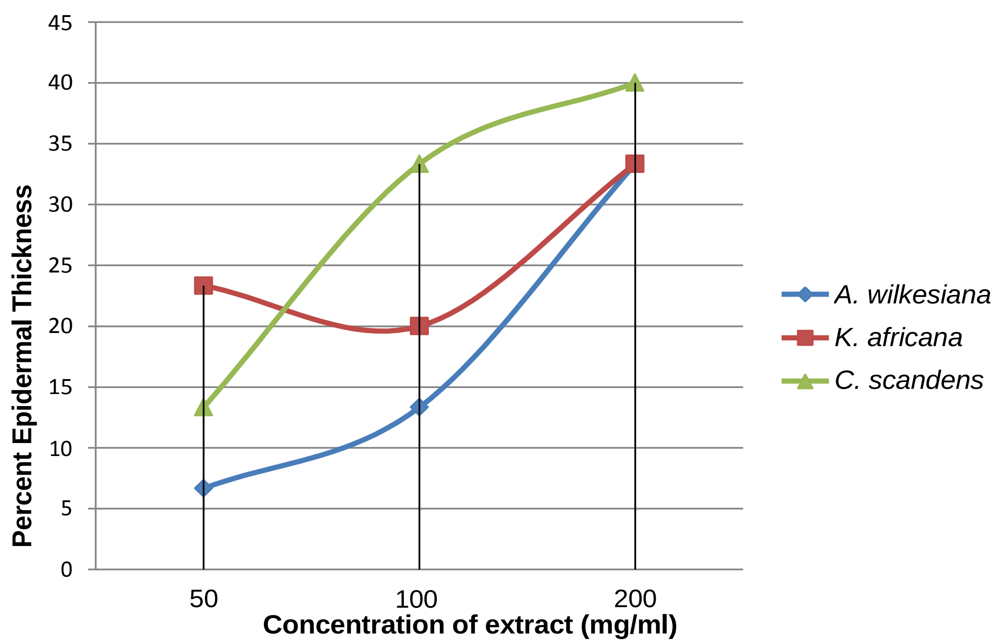

Figure 2. Change in percent epidermal thickness with concentration of hexane extract.

Acalypha wilkesiana ointments

A. wilkesiana ointments exhibited dose-dependent drug activity (Figures 1 and 2). The methanol ointments generally exhibited higher drug activity than the hexane extracts. Any particular progression was not observed for the irritation potential of the *A. wilkesiana* methanol extract ointment. For the hexane extract ointment, the irritation potential increased gradually with increasing extract concentration. No erythema was observed on the treated tail skin or elsewhere.

Culcasia scandens ointments

C. scandens had the least relative drug activity for both the methanol and hexane extract ointments. A steady rise in the irritation potential of both the methanol and hexane ointments was observed (Figures 1 and 2). The *C.a scandens* methanol ointments had a lower irritation potential compared to the hexane ointments.

Comparison of the drug activity of *Acalypha wilkesiana*, *Caulcasia scandens* and *Kigelia africana*

Only the *A. wilkesiana* methanol extract ointment (200 mg/ml) showed greater than 40% drug activity which could be compared to the activity of *K. africana* ointments, thus, suggesting that it can be used in antipsoriatic drug development. The irritation potential of the *A. wilkesiana* hexane ointments was close to that of the *K. africana* stem hexane ointments, suggesting that it would be better to use the methanol extract that had a lower irritation potential with comparable drug activity The *C. scandens* ointments all had lower than 40% drug activity.

Conclusion

Using the modified mouse tail model, the *A. wilkesiana* showed more prospects of being an anti-psoriatic topical agent when compared to *C. scandens* and *K. africana* as its drug activity was quite similar to that of *K. africana*, while its methanol extract had a lower irritation potential and did not cause erythema. However, more pre-clinical tests need to be carried out on the three plants using other models to ascertain their anti-psoriatic activity and how they respond to other aspects of psoriasis, considering the complex nature of the disease.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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