Ecological studies on the colocynth, *Citrullus colocynthis* (L.) (Curcurbitaceae) from Shada, Saudi Arabia and its insect repellent properties.

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Abstract: This study attempts to provide an insight into the relationship between *Citrullus colocynthis* and the surrounding soil. Moreover, it aims to investigate the relationship between the various physicochemical properties of soil with each other. The flavonoid compounds in *C. colocynthis* were isolated, identified and tested for possible insect repelling properties. Soil samples were collected and subjected to analysis, while the insect repellent properties of *C. colocynthis* extracts were investigated by testing its components on the red flour beetle, *Tribolium castaneum*. A significant correlation was found between organic matter content and pH, soil moisture and soil textural components. A positive correlation was found between pH value and soil moisture, coarse and fine sand. Conversely a negative correlation was found between pH with silt and clay. Soil moisture increased proportionately with silt and clay content but decreased proportionately with coarse and fine sand content. Quercetin 3-rhamnoside and quercetin were isolated from *C. colocynthis* leaves and had a moderate repellant effect against *T. castaneum* and effectively lowered the population of the beetles by 82.5%. In conclusion, there was a strong relationship between the physicochemical properties of soil. Furthermore, the flavonoid constituents of *C. colocynthis* had potential insecticidal properties.

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1. Introduction

Citrullus colocynthis (L.) Schrad. is a plant of medicinal importance (Meena and Patni, 2008; Aldamegh et al., 2013) and is known to grow extensively and fast in the sandy soils of Arabian Peninsula (Aldamegh et al., 2013). According to El-Ghazali et al., (2010), studies on the pharmaceutical properties of the flora of Saudi Arabia have been neglected for a long time because of the harsh conditions that the Arabian climate presents. *C. colocynthis* is an uncultivated plant that grows all year round. It is a procumbent herb with small yellow flowers, and its fruit very bitter. It grows rapidly in sandy soils and is believed to have anti-tumoral properties. It is also used as an antipyometra in animals (Chaudhary and Al-Jowaid, 1999).

Kim et al., (1995) states that as plant and soil are interdependent, plant diversity affects soil quality in a particular area. Healthy soil depends on the organic contents and it is an important part of soil physical, chemical, and biological fertility matter (Singwane and Malinga, 2012). The study of soil organic and inorganic matter therefore is vital in determining the distribution of the plants (Karim, 2009). Soil properties are persistently subjected to changes brought and the rate at which these occur is largely influenced by the vegetation type and tightness (Wasonga et al., 2003).

The structure of the soil is the main determinant in the functioning of the soil, its capacity to bear plants and animals, alter the quality of the environment especially that of carbon (C) retention and water quality. It is often expressed as the degree of stability of aggregates being a major factor which controls physical, chemical, and biological processes leading the soil dynamics (Bronick and Lal, 2005). In addition, several physical, chemical and biological properties of soil are affected by organic matter. Soil pH, carbonates, nitrates, porosity, and bulky density are among some of the properties affected by organic matter (Karim et al., 2009).

Constituents of phytomedicine can be isolated from plants, which are also suggested to have potent biochemical compounds (Meena and Patni, 2008). The bark, leaves, flowers, roots, fruits, and seeds, for example, can be sources of plant-based natural constituents (Gordon and David, 2001). The combinations of secondary products available in plants grant plant materials their beneficial medicinal effects. However, medicinal properties are displayed by particular plant species or groups and are consistent with this concept as the combination of secondary products in a specific plant is taxonomically distinct (Wink, 1999). These compounds include alkaloids, flavonoids, steroids, phenolics, terpenes, volatile oils etc. Man has been exploiting these natural plant products for use in medicines, cosmetics, dyes, flavors and foods (Meena and Patni, 2008**)**. Arid and semi-arid plants are good sources for the production of various types of secondary metabolites which make them resistant to various environmental stress e.g. scarcity of water, salinity, pathogens (Meena and Patni, 2008**)**. Arid climates are known to activate the production of secondary phytochemical compounds of plants in high concentrations. This presumably made these plants more capable of fighting its natural enemies such as insects, herbivores and diseases (Aldamegh et al., 2013) and also resistant to various environmental stress e.g. scarcity of water, salinity, pathogens (Meena and Patni, 2008**)**.

Therefore, the objectives of this study are to investigate the relationship between the various physicochemical properties of soil (namely, pH, soil structure, moisture, and organic matter) with each other. The extracted and powdered materials of *C. colocynthis* leaves were evaluated for potential insecticidal properties. We also aim to isolate and identify of the different types of flavonoid compounds in *C. colocynthis* and test for possible insecticidal effects.

2. Materials and methods

Soil sample analysis Approximately 3 kg of the soil where *C. colocynthis* were cultivated was collected and assessed for its physicochemical properties. Soil pH was measured (with a soil:water ratio of 1:2) using a pH meter (Denver Instrument Basic pH mv Meter Ultrabasic) (Conklin, 2005), while percent moisture and soil organic matter was measured using oven (SOM) (Wilde et al., 1972). Soil structure was determined by using the sieve method described by Al-Yamani et al., (2006).

Plant collection Leaves of *C. colocynthis* were collected from Shadah, Saudi Arabia. The leaves were harvested manually and stored in plastic zipper bags prior to transportation to the laboratory. The leaves (approximately 15 kg) were air-dried in the laboratory for about two weeks and then ground into fine powder by using a blender (Panasonic MX-337RA).

Plant extraction The ground leaves of *C. colocynthis* leaves (about 30g) were placed into conical flasks and soaked with 240 mL of methanol and n-hexane, respectively for three days. The mixture was filtered through a funnel using filter paper Whatman no. 1 and then separated from the solvent by using a rotary evaporator set at 38°C to obtain an extract. The crude extract was then diluted to the desired concentration prior to the experimental setup and stored in a fridge at −4ºC until use.

Isolation and identification of flavonoids Three hundred grams of the powdered leaves were extracted with methanol and concentrated. Filter papers were used to filter the aqueous methanolic solution. The process was repeated several times and done using ether and a separating funnel and column chromatography was performed according to the method described by Harbone and Mabry (1982). Subsequently, paper chromatography (PC) (filter paper: Whatman no. 1 and 3; developing solvent systems) was performed and the finally thin layer chromatography (TLC) (dimension: 20 x 20 cm; adsorbent: silica gel plates, aglycones: developing solvent system; BPF: benzene, pyriden, formic acid; spray agents: ammonia) was conducted according to the methods described by Medic-Saric et al., (1999). Khogali et al., (2006) method was used to identify flavonoids from leave extracts.

Standard flavonoids Standard flavonoids, (Quercetin, Quercetin 3-rhamnoside) were purchased from Sigma Chemicals Co., St. Louis, MO, USA.

Test insects Tribolium castaneum were collected from subcultures reared on wheat flour in round plastic containers with lids in a closed cabinet at constant temperature of 28.0±0.5°C and 65.0±2.0% relative humidity. One to two weeks old *T. castaneum* beetles were used in all the experiments.

Bioassay with C. colocynthis leaves extract The filter paper impregnation method which was modified accordingly to Liu and Ho (1999), was carried out to investigate the repellency activities of *C. colocynthis* leaves extracts against *T. castaneum*. Filter papers (Whatman No. 1) of 8.5 cm in diameter were cut into half, with one half treated with 0.3 ml of either n-Hexane or methanol extracts of 50% w/w concentration of *C. colocynthis* and the other half of the filter paper (untreated side) was treated with either n-Hexane or methanol, respectively. The pairs of the treated and control filter papers were placed in a plastic Petri dish (9 cm diameter). Next, twenty adult beetles were introduced into the center of the Petri dishes. At every hour until the 6th hour, the numbers of beetles observed on each filter paper were counted and recorded. The experiment was replicated five times. The control consists of placing the beetles on filter papers treated with either n-Hexane or methanol.

Bioassay with C. colocynthis powdered leaves About 0.15g of powdered leaves was mixed into 5g of food substrate. Treated and untreated food substrates (5g each) were placed on opposite sides of a Petri dish. Ten adult *T. castaneum* were introduced into the center of the Petri dish. After 1h, the number of insects found on either side of the Petri dish (treated or untreated substrates) were counted and recorded. This was repeated at an interval of 1h up to the 6th h. The experiment was replicated five times and percentage repellency (PR) values were calculated using the equation below:

$PR = [(N_C - N_T)/ (N_C + N_T)] \times 100$,

where N_C = number of insects in the control area and N_T = number of insects in the treated area.

Bioassay with standard flavonoids Different concentrations flavonoids (0.1, 1 and 5 mg/ml) in 250 ml aliquots in methanol (solvent) of each standard flavonoids was sprayed onto the treated half of a filter paper while the untreated half received 250 ml methanol. Control received 250 ml methanol on each half of filter papers. After drying under a fume hood for 5 min, each filter paper was placed in the bottom of a glass Petri dish which measured 5.5 cm in diameter. Ten adult *T. castaneum* were introduced into the center of each dish. Beetles distributions (either in the treated or untreated area) was observed at 24, 48 and 72 hours after treatment. The experiment was replicated five times and percentage repellency (PR) values were calculated.

Statistical analysis The correlations between all physicochemical characteristics were carried out with Pearson correlation and regression analysis using the curve fitting procedure in Curve Expert Version 1.4 (D. Hyams, Hixson, Tennessee, USA).

The differences in percentage repellency of *T. castaneum* towards hexane and methanol extracts of *C. colocynthis* were analysed using Paired T-test at α=0.05. The same statistical test (Paired T-test at α=0.05) was performed to determine the differences in adult emergence in the treated (*C. colocynthis* powdered form) and untreated (control). The percentage repellency effect of different types of flavonoids at different concentration against *T. castanuem* was subjected to a one-way (ANOVA) analysis of variance and means were separated by using Tukey HSD.

Statistix® Version 7.0 (Analytical Software, Tallahassee, Florida, USA) was used for data analysis in this study.

3. Results and Discussion

Organic matters and soil physicochemical characteristics (Figs. 1, 2 and 3) shows a significant correlation between organic matter (OM) and soil physicochemical characteristics ($P<0.05$). We found a strong positive correlation between OM with pH (Fig. 1), soil moisture (Fig. 2), coarse and fine sand (Figs. 3 a and b).

The amount of OM in the soil surrounding the area where *C. colocynthis* were sampled ranged between 7.99-7.56% of the total soil sample. Soil OM is important in soil biogeochemical processes (Bot and Benites, 2005). Thus, there must be a balance between soil OM protection and soil biological functioning in a productive and healthy soil (Wander, 2004). While our results showed that the pH of soil was slightly alkaline, other authors (Singwane and Malinga, 2012) showed that soils had the tendency of becoming acidic as a result of decay of OM, ammonium and sulfur fertilizers. Findings reveal that in pine and eucalyptus plantations the soil becomes less acidic when the organic matter content decreases. This explains our results, where the OM content in the soil is relatively low, thus the slightly alkaline level of the soil pH.

Our results showed that an increase in OM brought about an increase in soil moisture. However, in literature there is a lack of clarity as to what extent and under what conditions OM and other aggregating agents will improve water storage (Fig. 2). Jamison (1953), studied soils in the Southeastern United States and demonstrated that except for sandy soils, OM content increases were not accompanied by an increase in the capacity of a soil to store available moisture. According to Blanco-Canqui and Lal (2004), soils with high clay content may cause higher SOM protection than sandy soils, resulting in higher total contents of OM. In this study, OM content was higher as the clay content increases.

This study shows that the OM in the soil was found to be positively correlated with coarse and fine sand content (Figs. 3 a and b). The relationship between soil texture and OM decomposition has been widely investigated and results show that the rate of decomposition and net mineralization depend on the accessibility of organic substrates to soil organisms (Oades, 1984; Christensen, 1987; Amato and Ladd, 1992; Hassink, 1996). Although several studies have investigated organic matter decomposition, few have described the relative importance of direct and indirect mechanisms of soil texture control on organic matter stabilization (Srensen, 1981; van Veen et al., 1985). Good soil is characterized by high levels of SOM, which serves several important functions. According to Pimentel et al., (1995), OM eases the formation of soil aggregates, increases soil porosity, and consequently improves soil structure and water infiltration. Moreover, SOM augments the formation of soil aggregates and soil structure (Singwane and Malinga, 2012).

*pH and soil physicochemical characteristics*A significant and positive relationship ($P<0.05$) was found between clay and soil pH. Similarly, there was a strong and positive correlation between soil pH and soil moisture, coarse and fine sand (Figs. 4 and 5 (a and b)).

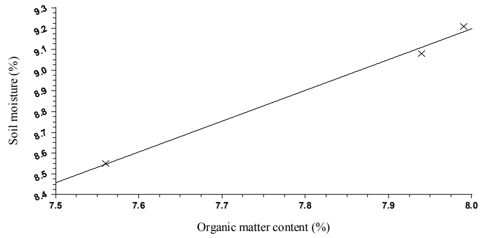

Figure 1: Relationship between soil organic matter content (%) and soil pH level (r^2= 0.6744; y= 0.5335x + 3.356; Linear regression).

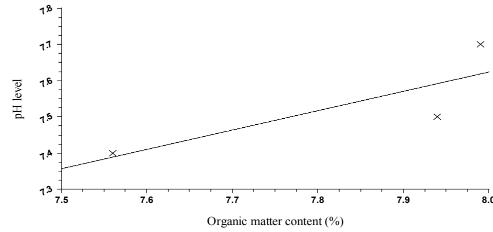

Figure 2: Relationship between soil organic matter content (%) and soil moisture content (%) (r^2= 0.9968; y= 1.4818x - 2.6567; Linear regression)

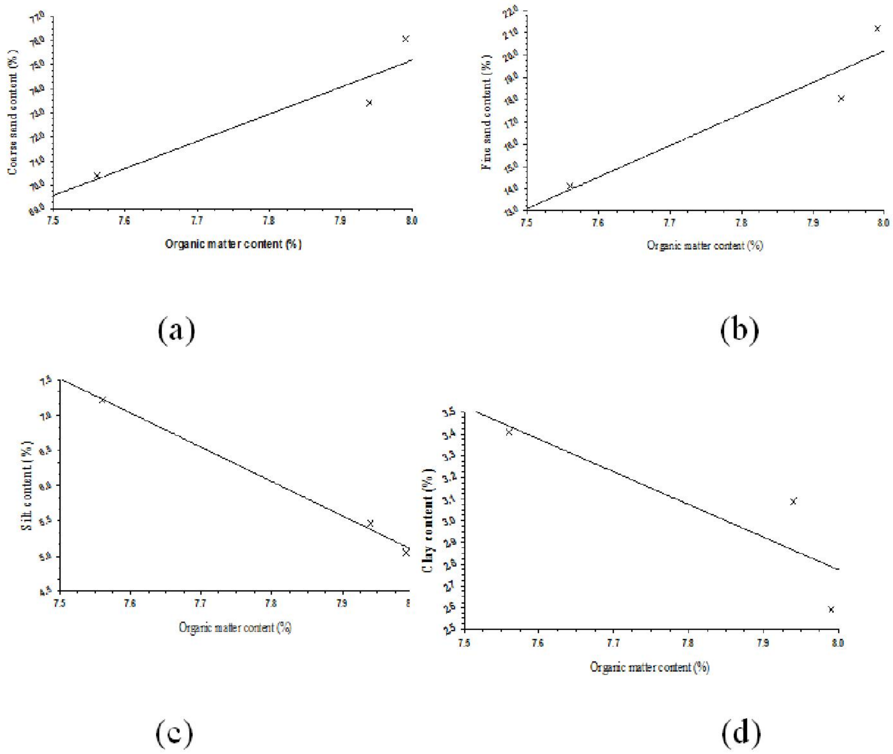

Figure 3: Relationship between soil organic matter content (%) and (a) coarse sand content (%) (r^2= 0.9935; y= 11.2640x - 14.9172; Linear regression), (b) fine sand content (%) (r^2= 0.9935; y= 14.1320x - 92.8769; Linear regression), (c) silt content (%) (r^2= 0.9941; y= -4.8796x + 44.1218; Linear regression) and (d) clay content (%) (r^2= 0.7329; y= -1.5045x + 14.8104; Linear regression).

Conversely, a negative correlation was found between soil pH and silt and clay content ($P<0.05$) (Figs. 5 (c and d)). The pH of the soil samples ranged from about 7.4 to 7.7. Wischmeier and Mannering (1969) reported that an increased pH increases erodibility in a high-silt soil if the structure is very fine or fine granular due to the effect on surface crusting. On the other hand, if the structure is medium or coarse granular, subangular, or angular, erodibility decreases with an increase in pH.

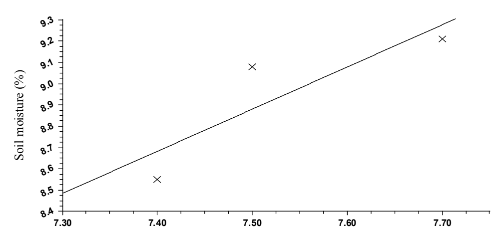

Figure 4: Relationship between soil pH level (%) and soil moisture content (%) (r^2= 0.7473; y= 1.9786x - 5.9586; Linear regression).

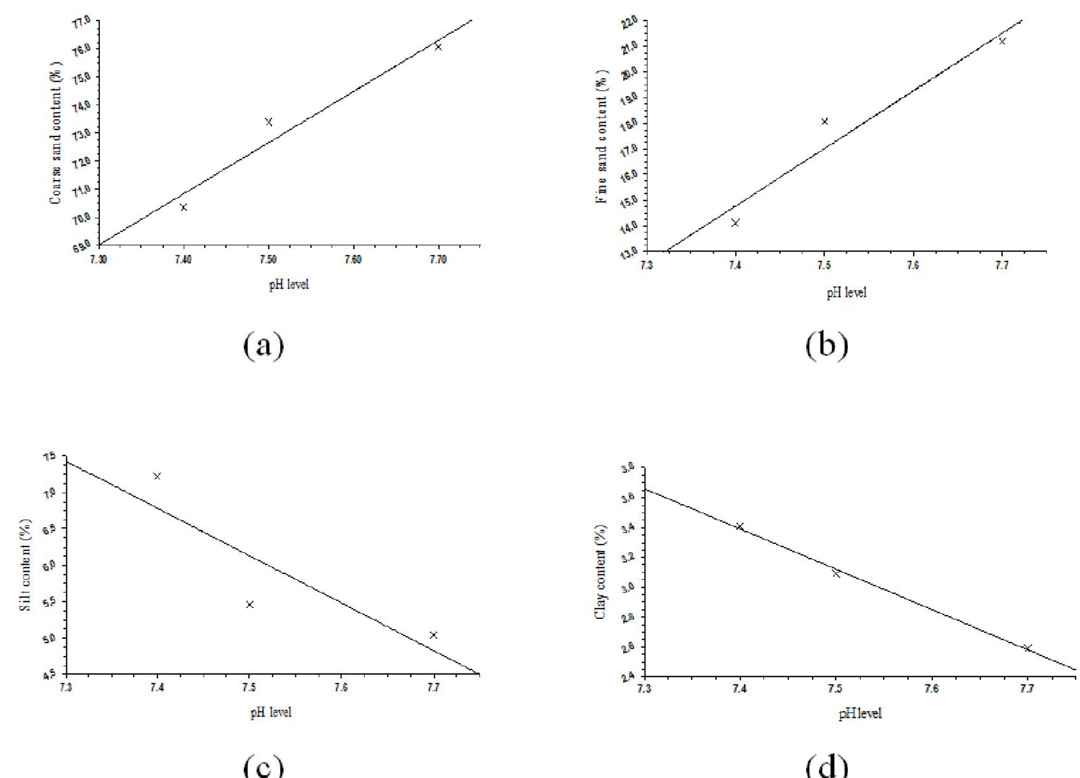

Figure 5: Relationship between soil pH level (%) and (a) coarse sand content (%) (r^2= 0.9509; y= 18.2357x - 64.0957; Linear regression), (b) fine sand content (%) (r^2= 0.9353; y= 22.4000x - 150.9700; Linear regression), (c) silt content (%) (r^2= 0.7442; y= -6.5000x + 55.8800; Linear regression) and (d) clay content (%) (r^2= 0.9959; y= -2.7000x + 23.3700; Linear regression).

Soil pH also affects microbial activity, ion solubility, and clay dispersibility besides influencing plant growth (Haynes and Naidu, 1998). As pH increases particle repulsion, the negative surface charge on clay particles also increases. As a result, it is crucial to control soil pH in soils with dispersive clays (Chorom *et al.*, 1994) owing to the fact that clay particles usually flocculate when pH levels are high (Haynes and Naidu, 1998). Soils with a high pH and carbonate concentration favor the formation of large aggregates (Boix- Fayos *et al.*, 2001). In common practice, lime is added to soil to increase pH thereby causing an increase in microbial activity and crop yields, and consequently higher SOM and increased aggregation (Haynes and Naidu, 1998).

Soil moisture and soil physicochemical characteristics As shown in (Fig. 6), there was a significant correlation between soil moisture and soil physicochemical characteristics (coarse sand, fine sand, silt and clay) (P<0.05). The highest and lowest soil moisture was recorded at approximately 9.21% and 8.55%, respectively. Hamblin (1985) reported that an improved soil structure was in general associated with a positive impact on soil moisture retention properties. Soil structure and texture are purported to affect soil water flow, availability and storage (Pachepsky and Rawls, 2003). Bypass flow in soil is increased by aggregation and interconnected pores, resulting in increased infiltration and reduced runoff. Furthermore, the movement of water into deeper layers of soil as well as leaching is increased (Franzluebbers, 2002; Nissen and Wander, 2003).

The results of our study showed that soil moisture was high when clay and silt were high. Findings from a previous study that analyzed the available moisture storage data for 271 profile horizon samples from soils of northwestern, central, eastern, and southwestern Missouri demonstrated that available moisture storage capacity (A.W.C.) decreased with clay and increased with silt content in soils that were primarily composed of silt. Further, they reported that coarse silt (0.05 to 0.02 mm) was associated with an increase in A.W.C., which was greater than that observed for fine silt (0.02 to 0.002 mm) (Jamison and Kroth, 1958).

We found a strong and negative correlation between the percent of coarse sand and fine sand and soil moisture (Figs. 6 a and b). In general, as soil structure becomes fine, available water storage capacity is thought to increase. The present study found a significant positive correlation between soil moisture and silt as well as clay (P<0.05). It is hypothesized that silty soils should retain more available moisture compared to clay and sandy soils (Jamison and Kroth, 1958). Jamison and Kroth (1958) also stated that there is an inversed linear relationship between available water storage and sand content.

Table 1. Percentage repellency of *T. castaneum* towards methanolic and hexanic extract at 50% (w/v) of *C. colocynthis*. a Means followed by different letter(s) within the same column were significantly different at α =0.05 (Paired t-test).

Treatment	Percentage repellency (%)
Methanolic extract	39.2 ± 2.7a
Hexanic extract	24.0 ± 5.8b

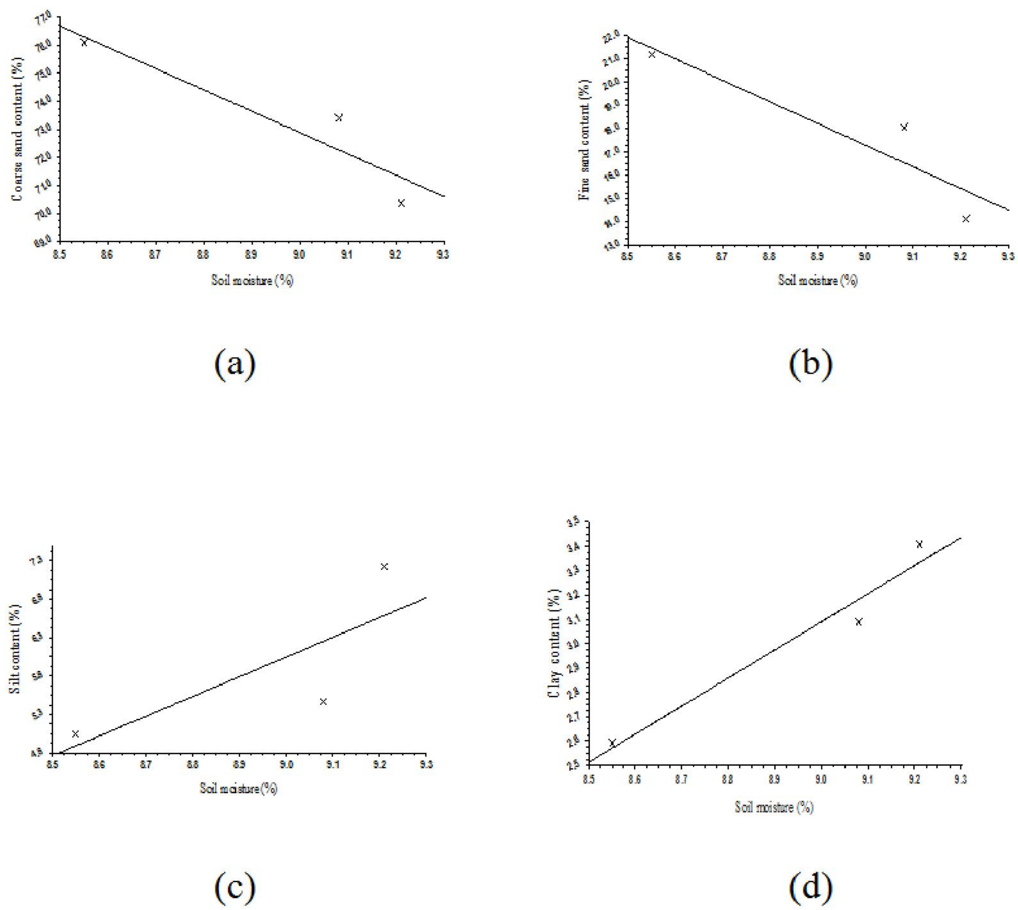

Figure 6 Relationship between soil moisture level (%) and (a) coarse sand content (%) (r^2= 0.8692; y= -7.6178x + 141.4340; Linear regression), (b) fine sand content (%) (r^2= 0.8458; y= -9.3065x + 101.0390; Linear regression), (c) silt content (%) (r^2= 0.7796; y= 2.5665x - 17.0486; Linear regression) and (d) clay content (%) (r^2= 0.9563; y= 1.1560x - 7.3122; Linear regression).

Bioassay with C. colocynthis Table 1 shows the percentage repellency of *T. castaneum* when exposed to either methanolic or hexanic extracts of *C. colocynthis* at 50% (w/v). Repellency activities were found in both types of extracts against *T. castaneum*. The repellency percentages were significantly different (P<0.05), with methanolic extracts recording higher percentage repellency (39.2%) than compared to the hexanic extracts (24.0%). The concentration of the extracts at 50% w/w concentration may not be sufficient to present a high repellency effect against *T. castaneum.* A study by Nadeem et al., (2012) reported that the toxicity of *C. colocynthis* ethanol extracts on *T. castaneum* were found to be dose and exposure time dependent. Therefore, it is probable that a higher concentration can yield a better repellency percentage for our study. It is also probable that the solvents (methanol and ethanol) used in this study to extract the materials from *C. colocynthis* may have yielded less repellent properties. Nadeem *et al.*, (2012) stated that their extract from *C. colocynthis* by using ethanol was efficient in killing *T. castaneum* at a low concentration of 10%. In contrast, our study showed that *T. castaneum* is more susceptible to both the methanolic and hexanic extracts of *C. colocynthis*.

Numerous researches have been done on *C. colocynthis* against a number of pests such as cockroaches, adult honey bee, housefly, cotton leaf worm, bed bug and mosquito (El-Naggar *et al.*, 1989). A study by Seenivasan *et al.*, (2004) showed that the petroleum ether and ethyl acetate seed extracts showed anti-oviposition, F1 adult emergence, and ovicidal and repellent activity against the pulse beetle *Callosobruchus maculatus*.

On average, more than 290 adult beetles were recorded in the control, while only about 51 adult beetles emerged from mediums that had been treated with *C. colocynthis* in the powdered form at 3% (w/w) (Table 2). Peasant farmer and researchers have Claimed the successful use of material of plant origin in insect pest control including spices and powders of plant parts (Pugazhvendan *et al.*, 2009). It clearly shows that the number of emerging adult beetles from the treatment was significantly lower than compared to that in the control (P<0.05). Overall, *C. colocynthis* is effective in decreasing the number of *T. castaneum* as the number of beetles emerging from the control was almost 6-fold of that in the treatment. According to Rehman *et al.*, (2009), *C. colocynthis* was found to inhibit the overall ovipositional rate of the peach fruit fly, *Bactrocera zonata*. This shows that the powdered form of *C. colocynthis* have anti-oviposition properties against *T. castaneum*.

Table 2: Mean (± SE) number of emerging adults of *T. castaneum* at day 60 in the control and treated medium (3% w/w powdered *C. colocynthis*).

	Mean emerging number (±S.E.)	N
C. colocynthis	51.4 ± 13.5a	257
Control	294.8 ± 8.5b	1474

a Means followed by different letter(s) within the same column were significantly different at α =0.05 (Paired t-test).

In this study, quercetin and quercetin rhamnosid were isolated and identified. The phytochemical analysis conducted by Aldamegh *et al.*, (2013) revealed the presence of some bioactive principles for *C. colocynthis.* Meena and Patni, (2008**)** found that quercetin was isolated *in vivo* from the leaf, stem, fruit and root of *C. colocynthis.* The high temperatures of arid areas may favor the production of secondary phytochemical compounds in high concentrations, which are suggested to have a higher potential of fighting insects, herbivores and diseases (Aldamegh *et al.*, 2013).

T. castaneum were exposed to standard flavonoids (quercetin and quercetin rhamnosid) and showed varied results in repellency activities depending on the exposure time and concentration (Table 3). When adult *T. castaneum* were exposed to filter papers treated with quercetin at 0.001% (w/v) and quercetin rahmnosid at 0.01% (w/v), repellency percentage was consistent. The efficacy of the standard flavonoids significantly decreased with prolonged exposure time. However, the repellency percentage for quercetin at 0.01% (w/v) and quercetin rhamnosid at 0.001% was inconsistent at the designated time interval.

Table 3. Mean percentage repellency (%) of *T. castaneum* against two different concentrations of two flavonoids standards at 24, 48 and 72 hr.

Standards	Concentration (%)	Percentage repellency (mean %±S.E.)		
		hr (s)		
		24	48	72
Quercetin	0.001	32.0±2.0a	20.0±1.4b	-8.0±1.4c
	0.01	72.0±0.6a	4.0±1.9c	40.0±1.3b
Quercetin rhamnosid	0.001	12.0±2.3a	-8.0±1.9c	28.0±1.0b
	0.01	32.0±2.1a	4.0±2.2b	-32.0±1.6c

a Means followed by different letter(s) within the same column were significantly different at (p< 0.05; Tukey HSD)

4. Conclusion

In the soil sample, we found a positive correlation between OM content and soil pH level, soil moisture, coarse sand and fine sand content. Conversely, there was a negative correlation between silt and clay content with OM content. Soil moisture was positively correlated to coarse sand and fine sand content but negatively correlated to soil moisture, silt and clay content. Coarse sand and fine sand content decreased as the soil moisture increased but as silt and clay content increased, it increased the soil moisture content. The methanol and n-hexane extracts of *C. colocynthis* showed moderate effectiveness at repelling *T. castaneum*. The population growth of the beetles was clearly affected when they were reared in medium containing *C.*

colocynthis in powdered form, where a lower number of beetles emerged. Inconsistent results were obtained when *T. castaneum* were exposed to standard flavonoids (quercetin 0.01% and quercetin-rhamnosid 0.001%). Nevertheless, as the exposure times increases, a decrease in PR was observed when tested with quercetin 0.001% and quercetin-rhamnosid 0.01%.

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