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The impact of pH and soil nutrients on the total alkaloid content of *Cryptolepis sanguinolenta* (Lindl.) Schtr.

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ABSTRACT

Cryptolepis sanguinolenta (family Periploaceae) raw materials were sampled from some locations in Ghana namely; Pepease, Mamfe and Abonse, and the effect of soil pH and soil nutrients on the quantities of alkaloid in the various plant organs have been studied. The results outcome show that significantly influenced on the alkaloid contents in the plant organs of *C. sanguinolenta* sourced from the different locations. Fluctuations in soil pH and soil nutrients: phosphorus, nitrogen, organic carbon and organic matter varied concurrently with the alkaloidal content levels in *C. sanguinolenta*.

Keywords: *Cryptolepis sanguinolenta*, soil pH, soil nutrients.

INTRODUCTION

Alkaloid levels may vary among plant parts and tissues (Ball *et al*. 1997; Belesky and Hill 1997; Justus *et al*. 1997 and Leuchtmann *et. al*. 2000). As stated by Arechavaleta *et al*. (1989); Huizing *et al*. (1991); Agee and Hill (1994); Malinowski *et al*. (1998), alkaloid production vary with available resources such as soil moisture, nutrients and temperature. It has been observed that alkaloid levels may be influenced by environmental factors. The types and levels of alkaloids appear to depend mostly on endophyte species, strain, or genotype (Siegel *et al*., 1990; Bush *et al*., 1993; Christensen *et al*., 1993).The chemical composition of the analyzed essential oils of *Pilocarpus microphyllus* Stapf. showed qualitative and quantitative variation as influenced by local environmental conditions of soil and seasonal period of collections (Francisca, *et al*., 2003).Elhaak and Migahid (1999) have investigated the total alkaloids in *Euphorbia paralias* L. leaves and stem; and observed that the variations in alkaloids content of leaves, stem and whole shoot were correlated with soil moisture and salinity stresses. According to them great amounts of alkaloids accumulated in the plant leaves and stem under soil moisture stress than under soil salinity stress. Plant leaves were also reported to contain large amounts of alkaloids than the stem under soil moisture stress but the reverse was true under salinity stress.

Nielsen (1965) observed that in West Africa soil pH is not an important factor when it comes to plant growth as compared to those in the temperate regions. Soil pH influences the growth of agronomic plants, which depends on specific pH regimes. Soil pH was noted to be controlled by many factors such as leaching, erosion, etc (Nielsen, 1965)). It has been observed by Lawson (1966) that plant growth depends on the presence of a sufficient quantity of mineral nutrients based on a suitable texture and adequate air as well as water. In an earlier study by Nye (1954), it was concluded that mineral nutrients such as nitrogen, phosphorus, potassium, calcium, sulphur, magnesium, iron, copper, boron, manganese, zinc, etc are always required in varying quantities for plant growth.

Griffin (2002) found that organic matter releases many plant nutrients as it is broken down in the soil, including nitrogen (N), phosphorus (P) and sulfur (S) needed for plant growth. Mineral balance seems to influence alkaloid production, for example, cannabinoid production. Krejci (1970) found increases related to unspecified "poor soil conditions". Haney and Kutscheid (1973) have shown the influence of soil K, P, Ca and N concentrations on Illinois *Cannabis*. These minerals have also been shown to affect the production of cannabidiol (CBD), *delta*-8-THC and cannabinol (CBN), although the latter two compounds are now thought to be spontaneous degradation products of *delta*-9-tetrahydrocannabinol (THC). Kaneshima *et al.* (1973) have demonstrated the importance of optimal Fe levels for the synthesis of THC in Cannabis plants. Latta and Eaton (1975) reported Mg and Fe to be important for *delta*-9-tetrahydrocannabinol (THC) production, suggesting that these minerals may serve as enzyme co-factors. Coffman and Gentner (1975) also corroborated the importance of soil type and mineral content, and observed a significant negative correlation between plant height at harvest and THC levels. Interestingly, Marshman *et al.* (1976) reported greater amounts of THC in Jamaican plants growing in "organically" enriched soils.

Herbalists or Traditional Medical *Practitioners* in Ghana mostly harvest *C. sanguinolenta* roots from the different locations listed above in their quest of preparing potent anti-malaria herbal product. The efficacious nature of their products seem to be at variance in their applications in treating out-patients, is attributed to the varying content of the alkaloidal content (antimalarial ingredient) in the plant organs (Ameyaw *et. al.* 2007).

Therefore, the main aim of this research is to determine the relationship between some topsoil characteristics and the total alkaloid content of the plant organs of the plant species, *C. sanguinolenta*.

MATERIAL AND METHODS

Plant materials used in the study were collected from the environs of Pepease, Mamfe and Abonse in the Eastern Region of Ghana. The three

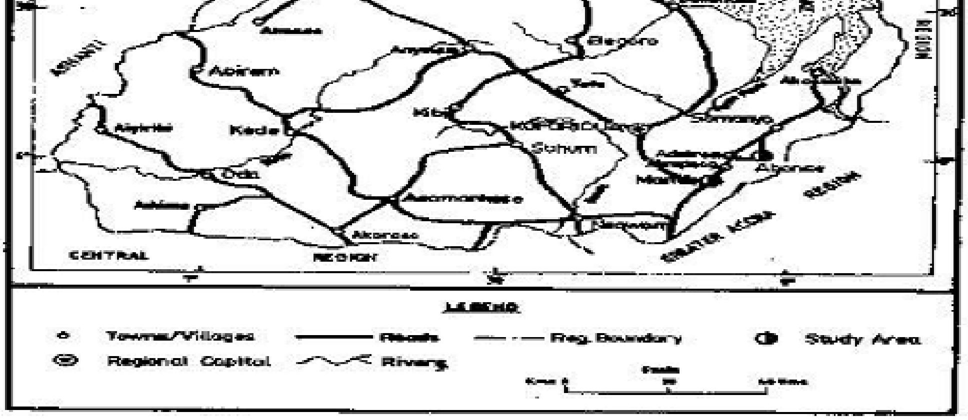

Fig. 2: Map of Eastern Region showing sample collection sites

settlements are located in the Kwahu South and Akuapem North Districts of the Region Fig. 2. The two districts are hilly with the latter district (Akuapem North District) ecologically classified as *Antiaris-Chlorophora* association. The plant species, *C. sanguinolenta* used for this research, were identified among the woody species of the locations.

Soil analysis

At each location (viz: Pepease, Mamfe and Abonse), soil samples were taken around plants within 5cm radius, which have been tagged for study. The soil samples were taken in March, June, September and December 2003. On each occasion, the samples were analysed for pH, available phosphorus, total nitrogen, organic carbon, organic matter and particle size.

Assessment of soil pH

In determining pH, the method developed by IITA (1985) was followed.

Determination of available phosphorus

Available phosphorus was determined using the method developed by IITA (1985).

Determination of total nitrogen

Total nitrogen was determined by Kjeldahl oxidation method (Anderson and Ingram 1989).

Determination of organic carbon

The Walkley-Black method adopted by IITA (1985) was used in determining organic carbon.

Determination of organic matter

The same procedure above for the determination of organic carbon was repeated for the determination of organic matter content. The result obtained was multiplied by the factor 1.724 to give the organic matter content.

Quantitative determination of alkaloid

Quantitative determination of alkaloid of the root, stem and leaf materials of the plant obtained from the three locations were carried out on a monthly basis for 12 months (March 2002 to April 2003). Five replicates were prepared for each plant organ per a location and the mean value computed. The plant materials were air-dried and ground to fine powder using the Manesty disintegrator. Fifty grammes of the powders obtained in each case were soxhlet extracted with hexane for 12 hours to defat the powdered plant materials. The defatted powder in each case was taken and the alkaloid extracted with 500ml of ethanol. The extracts were filtered and concentrated under reduce pressure using a rotary evaporator. The residue was mixed with 200ml of 10% aqueous acetic acid and allowed to stand overnight. The mixture was filtered with Whatman No. 1 filter paper. The filtrate was basified to pH 10 with ammonium solution. The basic mixture was extracted with two equal volumes of 200ml of chloroform. The chloroform extract was dried with anhydrous sodium sulphate and the solvent removed under reduced pressure. The weight of the evaporating dish was deducted from the weight of the evaporating dish plus the alkaloid residue to give the weight of the alkaloid contents. The percentage of alkaloid was calculated using the following formula:

$$\text{Total Alkaloid (\%)} = \frac{W}{Y} \times 100$$

Where

W = weight of alkaloid content extracted

Y = weight of powdered plant material.

RESULTS

The impact of pH and soil nutrients on the total alkaloid content of *Cryptolepis sanguinolenta* (Lindl.) Schtr.

Effect of soil pH and nutrients on the alkaloid content

The data presented in Figs. 3a-c and 7a-c, focus on the effects of pH, available phosphorus, total nitrogen, organic carbon and organic matter on the alkaloid content of *C. sanguinolenta* collected from the different locations.

Effect of soil pH on the alkaloid content

Figs 3a-c shows the effect of the pH of the soils on the alkaloid content of different organs of *C. sanguinolenta* from the different locations. The changes in the pH values of the soils greatly influenced the total alkaloid content of the different organs of *C. sanguinolenta* as presented in Figs. 3a- c.

The gradual increase in the pH of the soils in March and June affected the levels of alkaloid in the root and leaf of *C. sanguinolenta* Fig. 3a. Furthermore, the change in the soil pH between June and September resulted in the stabilization of the level of the alkaloid content of the roots of the plant and a slight drop in the alkaloid content of the stems and leaves of the plant Fig. 3a. Again, a drop in the soil pH between September and December did not cause much change in the level of alkaloids in the root although there was a slight rise of the alkaloid level in the stem whereas the alkaloid content of the leaf showed a further decrease Fig. 3a.

A clear change in the soil pH between March and June resulted in an increase in the alkaloid content of the leaf and a fall in the alkaloid content of the root with a slight drop in that of the stem. Further changes in the soil pH between June and September resulted in a slight increase in alkaloid content of the root and a slight fall in that of both the stem and leaf Fig 3b. A rise in soil pH between September and December resulted in an

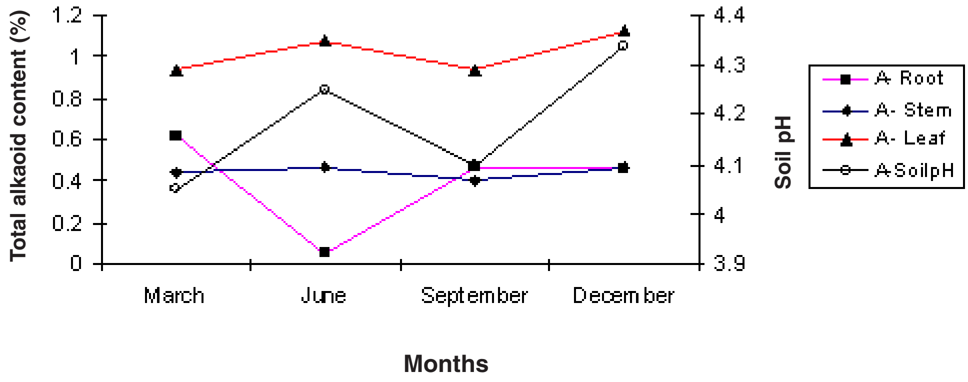

Fig. 3a: The effect of soil pH on the alkaloid content of the plant materials obtained from Pepease.

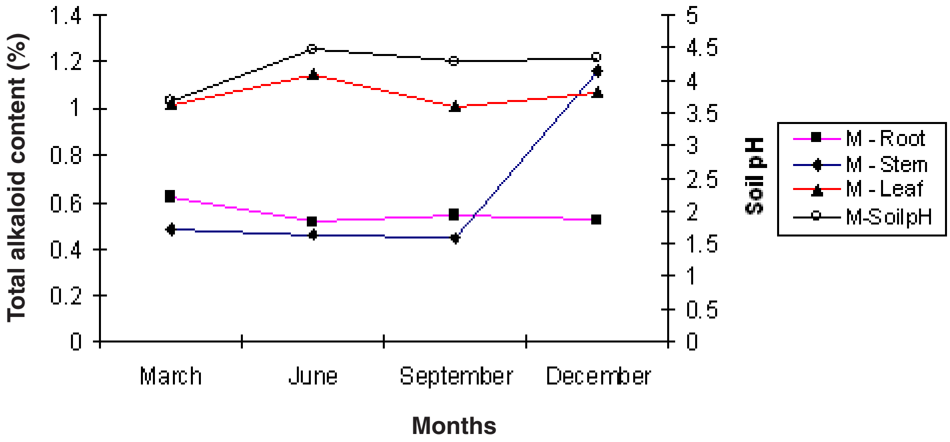

Fig. 3b: The effect of soil pH on the alkaloid content of the plant materials obtained from Mamfe.

increase in alkaloid content of the stem and leaf alkaloid with a slight drop in that of the root Fig. 3b.

Increase in soil pH within the year at Abonse affected the levels of alkaloid in *C. sanguinolenta* Fig. 3c. Thus, the significant increase in the pH values between March and June favoured increases in the alkaloid content of both the leaf and stem whereas there was a decrease in the level of alkaloids in the root of *C. sanguinolenta* as shown in Fig. 3c. Decrease in the soil pH between June and September resulted in an increase in alkaloid content of the root, whereas the concentration of alkaloids in both the stem and leaf decreased Fig. 3c. The significant change in soil pH between September and December also led to an increase in the alkaloid content of both the leaf and stem with a slight reduction in that of the root as reflected in Fig. 3c.

Effect of available soil phosphorus on the alkaloid content

The variations in available soil phosphorus were erratic for all locations namely: Pepease, Mamfe and Abonse. There was an increase in the amount of available phosphorus during the fourth quarter over and above that of the first quarter of the year. The presentations in Figs 4a-c show the effect of the availability of phosphorus in the soils on the alkaloid content of the different organs of *C. sanguinolenta*. An increase in the available soil phosphorus between March and June led to a decrease in alkaloid content in the root while that of

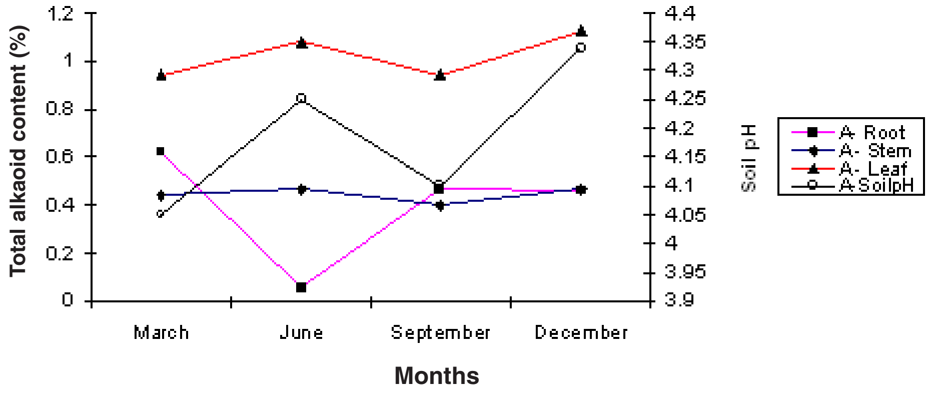

Fig. 3c: The effect of soil pH on the alkaloid content of the plant materials obtained from Abonse.

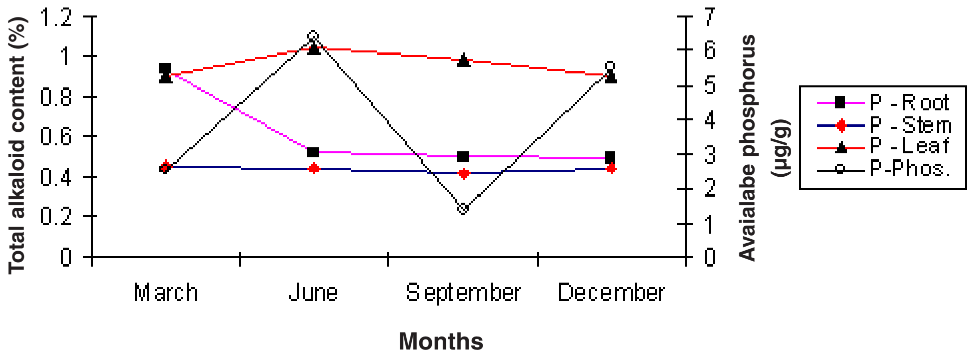

Fig. 4a: The effect of available soil phosphorus on the alkaloid content of the plant materials obtained from Pepease.

leaf increased Fig. 4a. Also, changes in the available soil phosphorus level between June and September resulted in no change in the alkaloid content of the root and a drop in that of the leaf with that in the stem also decreasing slightly Fig. 4a. Further changes in the available soil phosphorus during the last quarter of the year resulted in a slight change the alkaloid content of the root while that in the stem increased with the level in the leaf also decreasing Fig. 4a.

Increase in the available soil phosphorus between March and June resulted in a decrease in the alkaloid content of the root while that of the leaf increased Fig. 4b. A decrease in the available soil phosphorus between June and September resulted in an increase in the root alkaloid content with a drop in that of the leaf and slight reduction in the alkaloid content of the stem of *C. sanguinolenta* Fig. 4b. Again, the change in values of the available phosphorus of the soils during the last quarter of the year also led to a slight decline in the alkaloid content of the root while the alkaloid concentration in both the leaf and stem increased Fig. 4b.

An increase in the available soil phosphorus between March and June led to a sharp decrease in the alkaloid content of the root with increases in alkaloid content in both the stem and leaf Fig. 4c. A decrease in the available soil phosphorus between June and September resulted in an appreciable increase in the alkaloid content of the root and decrease in concentration alkaloids in both the stem and leaf Fig. 4c. Again, a further change of the available soil phosphorus during the last quarter of the year also led to a slight reduction

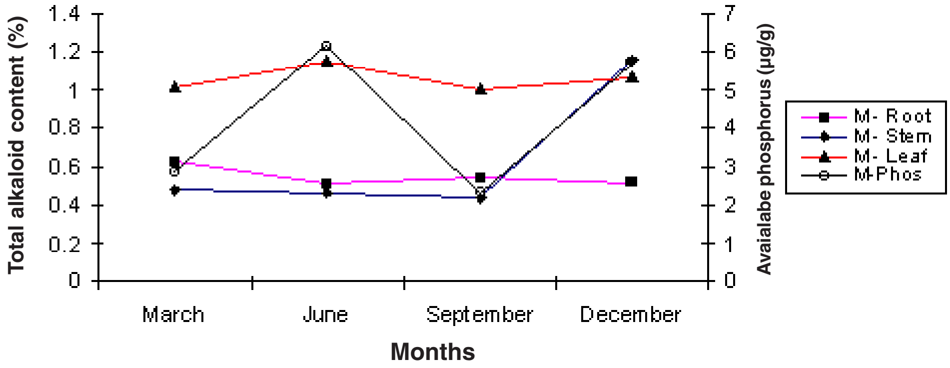

Fig. 4b: The effect of available soil phosphorus on the alkaloid content of the plant materials obtained from Mamfe.

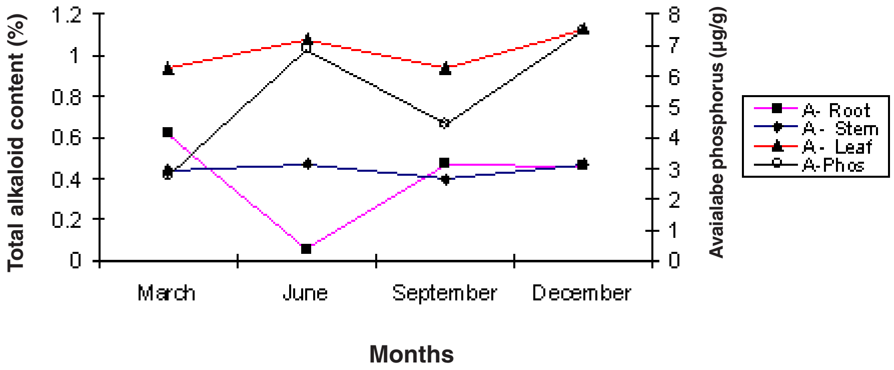

Fig. 4c: The effect of available soil phosphorus on the alkaloid content of the plant materials obtained from Abonse.

in the root alkaloid content with an increase in both the stem and leaf Fig. 4c.

Effect of soil nitrogen on the alkaloid content

The results presented in Figs 5a-c, show the effects of the soil nitrogen on the alkaloid content of *C. sanguinolenta*. Increase in the nitrogen values between March and June affected the alkaloid levels in the root, stem and leaf of *C. sanguinolenta* Fig. 5a. There was a sharp decrease in the total alkaloid content in the root of *C. sanguinolenta* between March and June when the soil nitrogen level increased from 0.1 to 0.2%. This also resulted in only a slight decrease in the alkaloid content of the stem while there was an appreciable increase in alkaloid content in the stem. The slight adjustment of the soil nitrogen between June and September also resulted in a slight fall in the alkaloid content root and stem as well as a drop in that of the leaf Fig. 5a. During the S eptember and December, intervals the alkaloid content of the leaf decreased further although that in the stem increased slightly whereas there was no change in the levels of the alkaloid in the root following the decline in the soil nitrogen content Fig. 5a.

With the exception of the last two quarters, there were no significant differences among the monthly-quantified total nitrogen in the soils obtained from Mamfe within the year (Appendix 2b(v)). However, the slight differences in the soil nitrogen content affected the levels of alkaloid in the root and stem of the plant species during the first two months. This also led to a fall in the root alkaloid content and

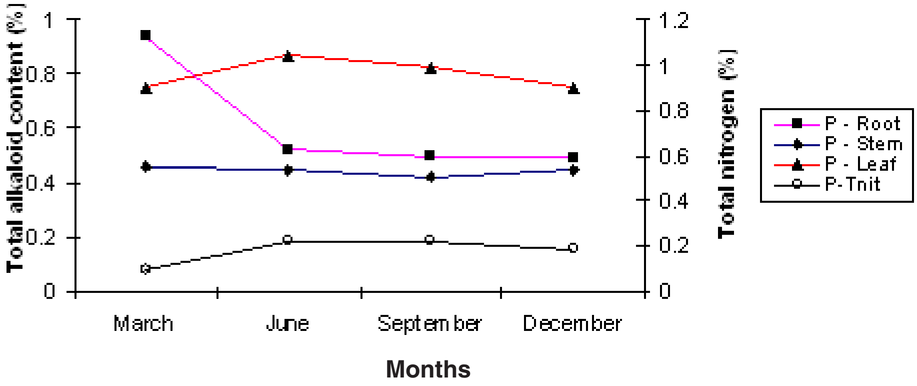

Fig. 5a: The effect of soil nitrogen on the alkaloid content of the plant materials obtained from Pepease

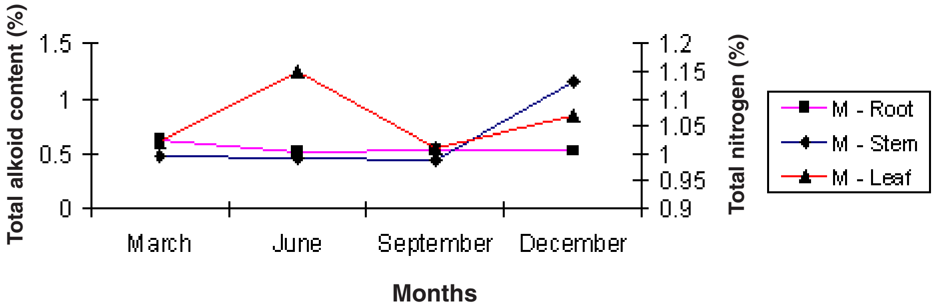

Fig. 5b: The effect of soil nitrogen on the alkaloid content of the plant materials obtained from Mamfe.

a rise in the leaf alkaloid content Fig. 5b. Between June and September there was a drop in the leaf alkaloid content, gradual increase in root alkaloid content and a slight fall in the stem alkaloid content Fig. 5b. Changes in the values of the soil nitrogen during the last two quarters also affected the levels of alkaloid in the plant organs, causing a dramatic increase in the alkaloid content of stem while that in the leaves also increase although there was a decrease in the alkaloid content of the roots Fig. 5b.

The change in soil nitrogen content at Abonse during the first quarter of the year also had dramatic effects on the alkaloid in *C. sanguinolenta* Fig. 5c. The slight change in the soil nitrogen between June and September resulted in reducing the alkaloid content of the stem and leaf although that in the root increased Fig. 5c. The changes in the values of the total nitrogen in the soils during the last quarter of the year also resulted in a slight change in the alkaloid content of the root although that in both the stem and leaf showed an increase in Fig. 5c.

Effect of soil organic carbon on the alkaloid content

The results presented in Figs 6a-c show the effect of the soil organic carbon on the alkaloid content of *C. sanguinolenta*. The organic carbon increased from March to December in the soils of Pepease and Abonse but decreased in the soils of Mamfe. The increase in the organic carbon of the soils in March and June affected the alkaloid content of the root and leaf of the plant species. While the alkaloid content of the leaf went up, that of the root decreased Fig. 6a. A decrease in the organic carbon values between June and September resulted in a reduction of both the alkaloid levels of the stem and

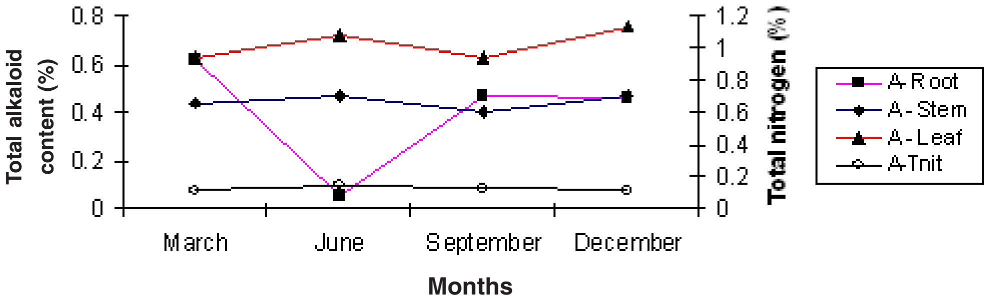

Fig. 5c: The effect of soil nitrogen on the alkaloid content of the plant materials obtained from Abonse.

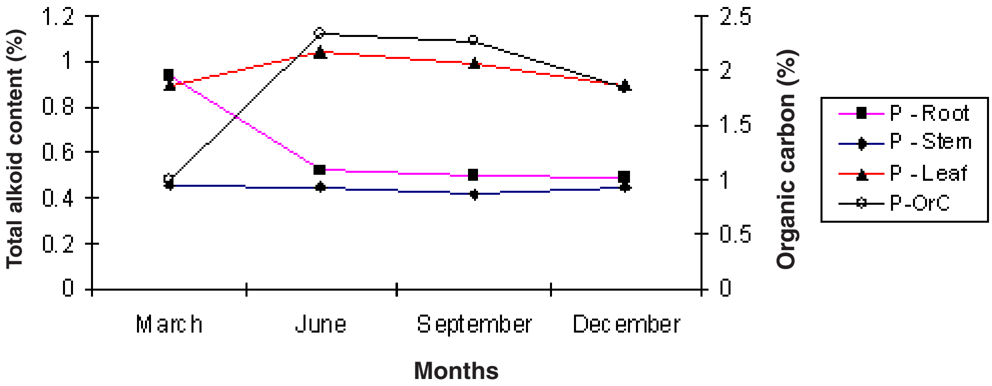

Fig. 6a: The effect of soil organic carbon on the alkaloid content of the plant materials obtained from Pepease.

leaf. Decrease in the values of organic carbon between September and December led to a gradual rise of the alkaloid level of the stem although there was a further drop in the leaf alkaloid content with only a slight drop in the alkaloid level in the root Fig. 6a.

There was a considerable reduction in the organic carbon content of the soils of Mamfe between March and June, which affected the alkaloid contents in the root and leaf of *C. sanguinolenta*. In general, leaf alkaloid content went up as the root alkaloid content declined Fig. 6b. Decrease in the organic carbon values of the soils between June and September resulted in decrease in the alkaloid levels of the stem and leaf with a slight rise of the root alkaloid Fig. 6b. A significant increase in organic carbon content of the soils between September and December led to a gradual rise in the alkaloid content of the stem and leaf of the plant species with a slight fall in the root alkaloid content during the same period Fig. 6b.

The significant increase and fall in the organic carbon values of the soils at Abonse between March and September affected the alkaloid levels in *C. sanguinolenta*. Thus, the significant increase in the organic carbon values between March and June favoured an increase in the alkaloid content of the leaf and stem with a corresponding

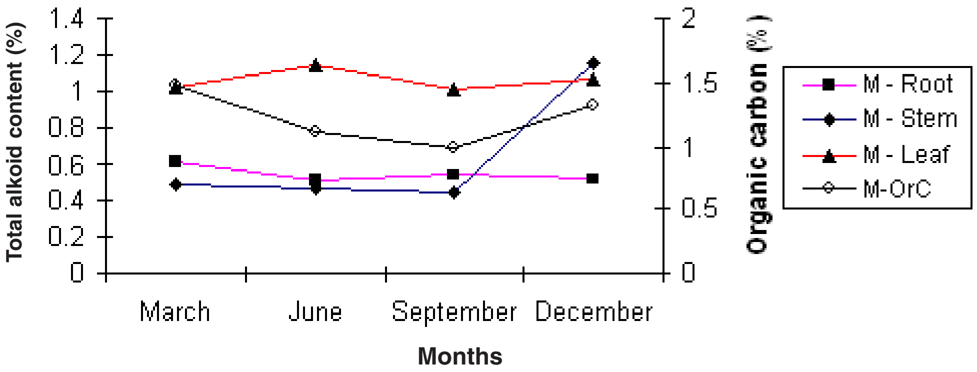

Fig. 6b: The effect of soil organic carbon on the alkaloid content of the plant materials obtained from Mamfe.

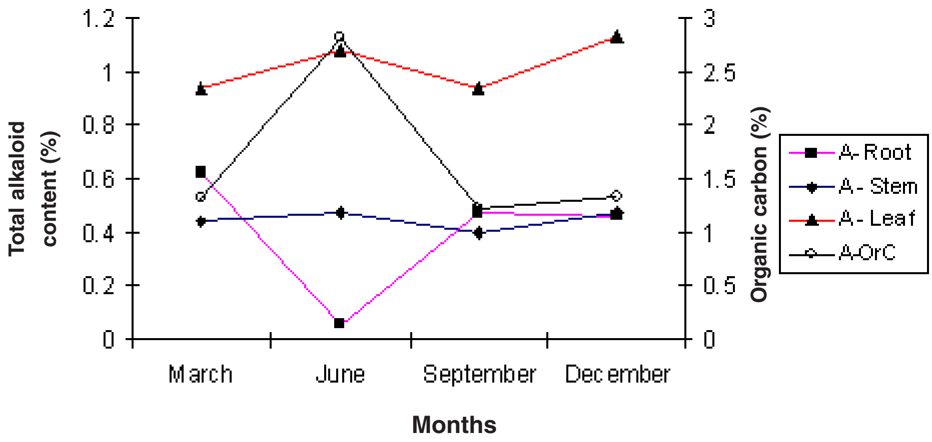

Fig. 6c: The effect of soil organic carbon on the alkaloid content of the plant materials obtained from Abonse.

fall in the alkaloid level of the root of *C. sanguinolenta* at Abonse Fig. 6c. The significant fall in the organic carbon values between June and September resulted in an increase in root alkaloid content with corresponding decrease in both the stem and leaf alkaloid contents of the plant species Fig. 6c. A significant increase in the values of organic carbon between September and December led to a rise in the leaf and stem alkaloid contents with a slight drop in the root alkaloid content Fig. 6c.

Effect of soil organic matter on the alkaloid content

The results presented in Figs 7a-c, show the effects of the soil organic matter on the alkaloid content of *C. sanguinolenta* at Pepease. The significant increase in soil organic matter content between March and June favoured an increase in the leaf alkaloid content with no change in that of the stem Fig. 7a. At the same time, there was a corresponding fall in alkaloid level of the root as shown in Fig. 7a. The significant fall in the organic matter values between June and September led to a decrease in the stem and leaf alkaloid content, with no change in the root alkaloid content Fig. 7a. A further fall in the values of organic matter content of the soils at Pepease between September and December resulted in a rise in the stem alkaloid content and a continued drop in the leaf alkaloid level Fig. 7a.

A significant fall in the organic matter content in the soils at Pepease between March and June led to an increase in the alkaloid content of the leaf with a fall in alkaloid content of the root resulted in stabilizing alkaloid content of the stem

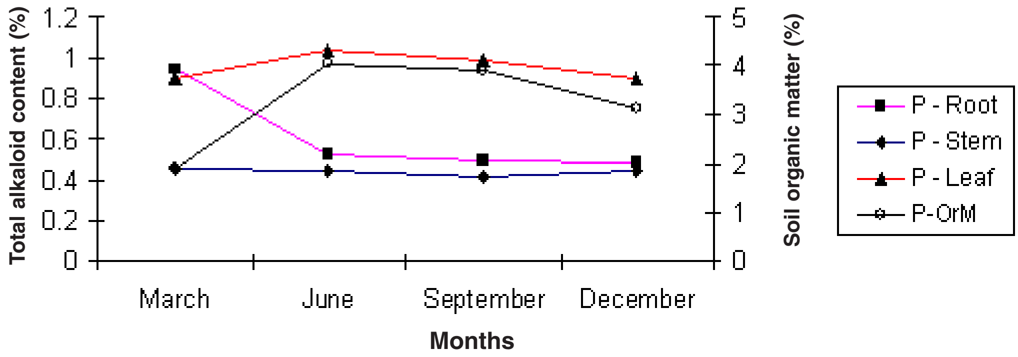

Fig. 7a: The effect of soil organic matter on the alkaloid content of the plant materials obtained from Pepease.

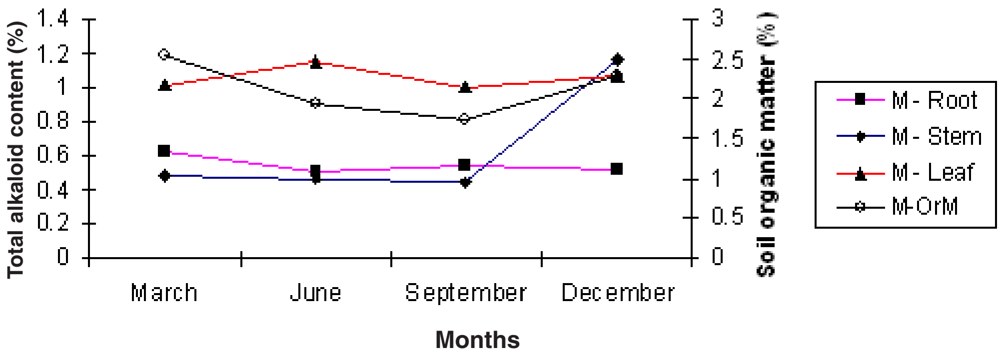

Fig. 7b: The effect of soil organic matter on the alkaloid content of the plant materials obtained from Mamfe.

Fig. 7b. A further fall in the significant values of organic matter content between June and September resulted in a slight increase in alkaloid content of the root with also a slight fall in that of alkaloid content of the leaf and a stabilization in that of the stem Fig. 7b. A significant decrease in the organic matter content between September and December resulted in an increase of the alkaloid content of both the stem and leaf with a slight decrease in alkaloid content of the root Fig. 7b.

The significant increase and fall in the organic matter levels in the soils between March and September affected the synthesis of alkaloid in the plant species. Thus, the significant increase in the amount of organic matter values between March and June favoured increase in the leaf and stem alkaloid contents with a corresponding fall in the synthesis of alkaloid in the root of the plant species as shown in Fig. 7c. The significant fall in the organic matter values between June and September led to an increase in root alkaloid content and decreased in that (i.e. alkaloid content) of both the stem and leaf Fig. 7c. Further decrease in the soils organic matter content between September and December led to an increase in the leaf and stem alkaloid content, with a slight fall of the root alkaloid content as reflected in Fig. 7c.

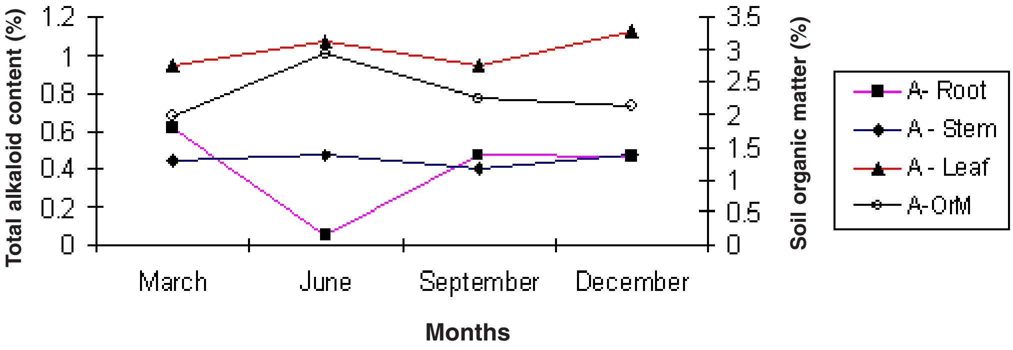

Fig. 7c: The effect of soil organic matter on the alkaloid content of the plant materials obtained from Abonse.

DISCUSSION

The discussion is focused on the topsoil characteristics: pH, available phosphorus, total nitrogen, organic carbon and organic matter affected the alkaloid content of *C. sanguinolenta* as shown in Figs. 3a, b, c and 7a, b, c respectively.

The pH values of the soils obtained from the different locations had influential effects on the alkaloid content of the plant species in all the locations Figs. 3a-c. The pH of a soil affects the activities of microorganisms, which then affect the level of nitrogen, phosphorus and sulphur in the soil. Soil pH regimes contribute to make available the needed soil nutrients in the soils to be absorbed by plant species. The influential aspects of soil pH in making soil micronutrients or mineral elements available through the agency of decomposers, in soils for plants have been explained by Richards (1996) and Brady (1990). According to them, acid soils cause phosphorus to form insoluble compounds with aluminum and iron. Liming soils with low pH dissolve these insoluble compounds and allow phosphorus to be more available for plant uptake.

The available phosphorus in the soils affect the synthesis of alkaloid in the plant species sampled from the locations (Figs. 4a-c). The phosphorus in the soil is absorbed by the plant species to avoid deficiencies or diseases. It contributes to seed germination, photosynthesis, protein formation and almost all aspects of growth and metabolism, which may include the normal synthesis of alkaloid. Haney and Kutscheid (1973) and Krejci (1970) had cited mineral balance as influential agent in the synthesis of alkaloid. They linked the influential abilities of soil potassium (K), phosphorus (P), calcium (Ca) and nitrogen (N) concentrations to the synthesis of cannabidiol (CBD), *delta*-8-THC, cannabinol (CBN) and cannabinoid as well as all the alkaloids in *Cannabis*.

The total nitrogen in the soils like the available phosphorus affect the synthesis of alkaloid in the plant species obtained from the locations Figs 5a-c. As a macronutrient in the soil, nitrogen forms a major component of proteins, hormones, chlorophyll, vitamins and enzymes essential for plant life, as well as, the basic building blocks in the synthesis of alkaloid. Its level in both the soil and plant can greatly influence the alkaloid content in plants, when the total nitrogen concentrations are significantly different in the different localities. Moreover, the report of Haney and Kutscheid (1973) and Krejci (1970) on mineral balance as influential agent in the synthesis of alkaloid is very conclusive in linking the influential abilities of soil potassium (K), phosphorus (P), calcium (Ca) and nitrogen (N) concentrations, to the synthesis of cannabidiol (CBD), *delta*-8-THC, cannabinol (CBN) and cannabinoid, which are alkaloids in *Cannabis*.

The organic carbon of the soils in varying concentrations affected the synthesis of alkaloid in the plant species harvested from the various locations Figs. 6a-c. It may be that the availability of organic carbon, which is usually deduced from organic matter in the soils, could be a contributory factor in determining the presence of certain micronutrients like phosphorus, nitrogen, etc to be absorbed by the plant species in order to avoid deficiencies or diseases that could hinder the synthesis of alkaloid in the plant species. Organic carbon derived from organic matter in the soils as the latter is known to release many plant nutrients as it is broken down in the soil, including nitrogen (N), phosphorus (P) and sulfur (S) as reported by Griffin (2002).

Organic matter concentrations in the soils also greatly affected the synthesis of alkaloid in the plant species harvested from the locations Figs. 7a-c. The effect of organic matter on the synthesis of alkaloid may be linked to the chemical and physical effects of the organic matter of the soil. Chemically, organic matter helps in the release of many plant nutrients, including nitrogen (N), phosphorus (P) and sulfur (S) as it is broken down in the soil. Plants in their metabolism or synthesis of chemical constituents including alkaloid require these micronutrients. The physical effects provided by the organic matter of the soil include the loosening of the soil, which increases the amount of pore space. This has several important effects such as making the soils less compact, improving the soil structure, which implies that the sand, silt and clay particles in the soil stick together, forming aggregates or crumbs (Griffin, 2002).

Therefore, the significantly different concentrations of the organic matter in the soils of the different study locations Table 2 would most probably have been the factor regulating the synthetic levels of alkaloid in the plant species Figs 7a-c.

CONCLUSION

The study centered on the impact of soil pH and soil nutrients on the level of alkaloid content in the plant organs of the plant species. It can be concluded that apart from environmental factors, soil factors (such as soil pH and soil nutrients) are also some of the attributes that control or regulate the levels of alkaloid contents in *C. sanguinolenta*. Therefore, herbalists or collectors of plant parts for medicinal preparations need to study prevailing soil factors that can support plant species to yield efficacious end-products or chemical extracts.

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