Ife Journal of Agriculture, 2016, Volume 28, Number 1

PHYSICOCHEMICAL BASIS FOR RELATIVE SUSCEPTIBILITY OF TWO JUTE MALLOW MORPHOTYPES TO *Acraea eponina* CRAMER

RAIFU, I.T. and SOYELU, O.J.*
Department of Crop Production and Protection, Obafemi Awolowo University, Ile-Ife 220005, Nigeria
**Corresponding author: jlekan2001@yahoo.co.uk; +234 7063250322*

ABSTRACT

The leaves of Corchorus olitorius L. are rich in antioxidants, fatty acids, minerals, vitamins and mucilaginous polysaccharides and it was reported in a preliminary study that larval Acraea eponina (Cramer) showed preference for C. olitorius morphotype with serrated leaves (NC1) compared to the morphotype with smooth-edged leaves (NC2). The current study was, therefore, carried out to determine physicochemical properties of C. olitorius that mediate preference of A. eponina for NC1. The proximate and mineral analyses of leaves harvested from the two morphotypes were carried out using standard methods of food analysis. Viscosity of mucilage extracted from the leaves of the two morphotypes was also measured using a viscometer. The results of proximate composition showed that NC1 contained higher amount of crude protein (37.47%) which is essential for tissue formation and rapid growth of the immature larvae. The C. olitorius with serrated leaves also had higher levels of ash (12.59%) and moisture (95.66%) compared to NC2. A similar trend was observed in the mineral content with NC1 containing higher levels of manganese (5.15 mg/100g; needed for formation of insect cuticle), iron (15.71 mg/100g), copper (0.79 mg/100g), magnesium (38.98 mg/100g) and chromium (0.62 mg/100g). However, mucilage from the leaves of NC2 was found to be more viscous, a factor that may reduce the desirability of A. eponina for the morphotype. The results showed that NC1 has a superior nutritional quality necessary for growth and development of the larval A. eponina and this is responsible for the preference for this morphotype by foraging larvae.

Key words: Acraea eponina, Corchorus olitorius, morphotypes, nutritional composition, minerals, viscosity

INTRODUCTION

An immense variety of leafy vegetables is eaten in different parts of the world today and they play an important role in human nutrition by being outstanding sources of protein, energy, vitamins, minerals and hormone precursors (Kmiecik *et al*., 2004; Antia *et al*., 2006). Nigeria is endowed with many indigenous leafy vegetables which spread across the estimated cultivable land area of 71.2 million hectares (Opabode and Adebooye, 2005). In 2013, Nigeria produced 11.9 million tonnes of vegetable which represented 15.4% of the total production in Africa (FAOSTAT, 2015).

The jute mallow, *Corchorus olitorius* L. (Malvaceae), is one of the popular leafy vegetables eaten in Nigeria. It is generally known as *ewédú* among the Yoruba people of the southwest, *ahuara* in the eastern Igboland and *malafiya* or *rama* among the Hausas in the northern part (Akoroda, 1988; Musa *et al*., 2010; Islam, 2013). When harvested young, the leaves are flavourful and tender; older leaves tend to be more woody and fibrous, making them less ideal for consumption. The vegetable belongs to a genus of about 40-100 species of flowering plants and its leaves, which are simple, may have smooth, lobed or slightly serrated edges (Olawuyi *et al*., 2014). Tropical Africa has been suggested as the center of origin for *C. olitorius* because of the huge genetic diversity observed in the region, with a secondary center of diversity in the Indo-Burmese region (Fondio and Grubben, 2004).

Leaves of *C. olitorius* contain over 17 active nutrient compounds including protein, α-tocopherol, ascorbic acid, riboflavin, folic acid, flavonoids, glutathione, phenols, fatty acids, minerals, mucilaginous polysaccharides, β-carotene, iron and calcium (AVRDC, 2009; Islam, 2013). In addition to serving nutritional functions the nutrient compounds protect man against activities of free radicals thereby reducing incidence of heart disease, cancer, diabetes, and hypertension (Islam, 2013; Yokoyama *et al*., 2014). Despite the numerous benefits derived from *C. olitorius*, its production is hampered by a number of insect pest species.

The small orange acraea, *Acraea eponina* (Cramer) (Lepidoptera: Nymphalidae), is a major pest of the jute mallow and it is capable of causing a yield loss of 25-100% (Jiggins *et al*., 2003). The larval instars of the pest defoliate leaves of the jute mallow thereby reducing the quality and market value. In severe cases, the leaves are rendered unfit for human consumption (Akoroda, 1985; Schippers, 2000). Opabode and Adebooye (2005) reported that detailed studies on the preference of insect pests for different morphotypes of the jute mallow have not been carried out in Nigeria. A preliminary study (Akintuyi, 2015) showed that the jute mallow morphotype with serrated leaves attracted more *A. eponina* larvae than the morphotype with smooth-edged leaves. The current study which serves as a follow-up to Akintuyi's findings was, therefore, designed to determine the relationship between physicochemical properties of jute mallow leaves and the observed pattern of *A. eponina* infestation with a view to explaining the feeding preference.

Materials and methods

Field establishment

Seeds of *C. olitorius* morphotypes with serrated (NC1) and smooth-edged (NC2) leaves obtained from the National Horticultural Research Institute (NIHORT), Ibadan were scarified with warm water before being planted in the field at the Obafemi Awolowo University Teaching and Research Farm (7° 28' N, 4° 33' E and 224 m above the sea level). The morphotypes were established on separate 5 m × 5 m plots with a distance of 30 cm between rows and 2 m between plots. The NC1, known as *oníyaya* in Yoruba, has shiny green leaves with sharply serrated blades while NC2 (*amúgbádú*) has lanceolate leaves with smooth edges. Leaves of the two morphotypes were harvested and processed separately for laboratory analyses at 5 weeks after planting.

Proximate analysis

Leaf moisture content

Freshly harvested leaves of the two jute mallow morphotypes were placed in separate paper bags and weighed using a sensitive balance. These were then oven-dried to a constant weight at 70°C for 24 h. After drying, each paper bag with its contents was weighed again and percent moisture content of each morphotype was determined using the formula:

$$\% \text{ Moisture} = \frac{\text{Weight of sample before drying (g)} - \text{Weight of sample after drying (g)}}{\text{Weight of sample before drying (g)}} \times 100\%$$

Nutrient and mineral analyses

Freshly harvested leaves of the two jute mallow morphotypes were placed in separate paper bags and oven-dried at 40°C for 24 h. The dried leaves were ground into fine powder using a laboratory pestle and mortar and stored at 4°C inside well labeled air-tight bags. Proximate composition of the powdery leaf samples was determined as described by Ndlovu and Afolayan (2008). Ash determination involved the incineration of each sample in a muffle furnace (Naber Industrieofenbau, Bremen, Germany) at 600°C for 3 h. The materials were then removed from the furnace and allowed to cool in a desiccator. The final weight of each sample was taken using the Mettler-Toledo analytical balance and percent ash content was determined using the equation:

$$\% \text{ Ash} = \frac{\text{Weight of crucible} + \text{Ash (g)} - \text{Weight of empty crucible (g)}}{\text{Initial weight of sample taken (g)}} \times 100\%$$

Crude fat determination was achieved by exhaustively extracting the samples with diethyl ether and using the formula:

$$\% \text{ Fat} = \frac{\text{Weight of flask} + \text{Oil (g)} - \text{Weight of empty flask (g)}}{\text{Initial weight of sample taken (g)}} \times 100\%$$

Crude fiber was estimated from the loss in weight of the crucible and its contents on ignition after ashing, following the sequential extraction of the samples with 1.25% sulphuric acid and 1.25% sodium hydroxide. Crude protein was determined using the microkjeldal nitrogen method which involved the digestion of 0.5 g of sample with sulphuric acid and a catalyst followed by calorimetric determination of nitrogen. The value of nitrogen was multiplied by 6.25 to obtain percent crude protein. The total carbohydrate content was obtained by subtracting the values of total ash, crude fiber, fat and protein from the total dry matter (Antia *et al*., 2006).

The calcium, chromium, copper, iron, magnesium, manganese and nickel contents were measured using an Atomic Absorption Spectrophotometer (AAS) after acid digestion of the samples (Antia *et al*., 2006).

Extraction of mucilage

Mucilage was extracted from freshly harvested leaves following the method described by Ahiakpa *et al*. (2014). The leaves were washed thoroughly to remove debris, weighed and chopped into small pieces. A 1.5 volume of distilled water was added to the leaves and the mixture was blended using a domestic mixer. After the blending, 2 volume of distilled water was added and the mixture was filtered using a muslin cloth to extract mucilage. The filtrate was centrifuged at 4000 rpm for 30 min and 3 volume of ethanol was added to the resulting clear greenish solution to precipitate the mucilage.

Viscosity of extracted mucilage

The mucilage extracted from each jute mallow morphotype was poured into a small plastic container and spindle 2 of a NDJ-85 Viscometer was inserted into it. The viscometer was set at 60 rpm and as the spindle revolved in the mucilage, the resistance it encountered within the measuring bowl was electronically conveyed to the measuring head and this generated results which were plotted into the software interface. Viscosity was measured in mPa^{-s} (milli-Pascal per second).

Statistical analysis

The proximate composition, mineral and viscosity data obtained were subjected to one-way analysis of variance (ANOVA) at 0.05 level of probability using SAS v. 9.1 (SAS, 2002). All samples were analyzed in triplicates and mean values were separated using Tukey's HSD except the viscosity values which were separated using the Fischer's LSD.

Results

The proximate composition and thickness of mucilage varied significantly between the two jute mallow morphotypes. *Corchorus olitorius* with serrated leaves (NC1) had a higher ($p < 0.05$) moisture content of 95.7% compared to 84.2% of that with smooth-edged leaves (NC2). The NC1 also contained higher levels of ash ($p < 0.05$) and crude protein ($p < 0.01$). However, NC2 was richer than NC1 in fat ($p < 0.05$) and carbohydrate ($p < 0.01$) contents (Table 1). The mineral composition of the two morphotypes presented in Table 2 showed that NC1 had significantly higher levels of copper (0.79 mg/100g), chromium (0.62 mg/100g), iron (15.71 mg/100g), magnesium (38.98 mg/100g) and manganese (5.15 mg/100g) while calcium (57.46 mg/100g) and nickel (0.80 mg/100g) were found to be more abundant in NC2. In addition, the viscosity of mucilage extracted from the leaves of smooth-edged morphotype was found to be more than 7-fold of that extracted from serrated *C. olitorius* (Table 3).

TABLE 1: THE NUTRITIONAL COMPOSITION OF TWO MORPHOTYPES OF THE JUTE MALLOW, *Corchorus olitorius*

Constituent (%)	Morphotype with serrated leaves (NC1)	Morphotype with smooth-edged leaves (NC2)
Moisture Content	95.66[a]	84.16[b]
Ether Extract	6.63[b]	7.74[a]
Ash Content	12.59[a]	12.04[b]
Crude Fiber	4.97[a]	5.10[a]
Crude Protein	37.47[a]	27.28[b]
Dry Matter	91.50[a]	90.86[a]
Carbohydrate	29.84[b]	38.70[a]

Mean values with similar alphabets in the same row are not significantly different at 0.05 level of probability (analysis of variance followed by Tukey's test).

TABLE 2: THE MINERAL COMPOSITION OF TWO MORPHOTYPES OF THE JUTE MALLOW, *Corchorus olitorius*

Corchorus olitorius	Copper	Manganese	Magnesium	Iron	Calcium	Nickel	Chromium
Morphotype with serrated leaves (NC1)	0.79[a]	5.15[a]	38.98[a]	15.71[a]	56.78[b]	0.00[b]	0.62[a]
Morphotype with smooth-edged leaves (NC2)	0.68[b]	3.45[b]	38.35[b]	12.79[b]	57.46[a]	0.80[a]	0.00[b]

Mean values with similar alphabets in the same column are not significantly different at 0.05 level of probability (analysis of variance followed by Tukey's test). The minerals were quantified in mg/100g.

TABLE 3: THE VISCOSITY OF MUCILAGE EXTRACTED FROM TWO MORPHOTYPES OF THE JUTE MALLOW, *Corchorus olitorius*

Corchorus olitorius	Viscosity (mPa^{-s})
Morphotype with serrated leaves (NC1)	17.53
Morphotype with smooth-edged leaves (NC2)	129.13
$LSD_{0.05}$	3.69

Discussion

The nutritional quality of the jute mallow leaves is superior to those of the fruits and stems (Ndlovu and Afolayan, 2008) and the leaves naturally serve as sources of food for the defoliating larvae of *A. eponina*. The two morphotypes examined in the current study differed greatly in their physicochemical properties and, to a large extent, this variation was responsible for the feeding preference of *A. eponina*. For optimum growth and development, insects require the macronutrients (protein, carbohydrate, lipid, water) and the micronutrients (vitamins, minerals, trace elements) (Barbehenn *et al*., 1999) and these were found to be generally more abundant in the serrated jute mallow morphotype.

Protein is the most limiting macronutrient required by insects for growth and reproduction, and protein quality may play a role in host plant specialization (Felton, 1996). Proteins also play central roles in all metabolic processes and in the structure and function of muscles and other tissues. In addition, protein and amino acids are the primary sources of the 10 essential amino acids which are required by *Acraea* spp. for egg production and larval development (Ogbalu, 2001). Lepidopterous larvae typically contain 80-90% water and they, therefore, require a high moisture intake to maintain their body (Chown and Nicolson, 2004). Nearly half of the dietary water intake is incorporated into tissues during rapid growth (Reynolds *et al*., 1985; Martin and Van't Hof, 1988) and larvae can be limited by lack of water (Scriber, 1977). Indeed, Scriber (1979) suggested that low leaf water may be more limiting than low protein content. The jute mallow morphotype with serrated leaves is a richer source of moisture for larvae of *A. eponina* and this may be one of the reasons for heavier pest infestation observed on its leaves. A suitable source of water is very crucial to larval development because they do not compensate for a decline in leaf moisture levels by increasing their feeding rates (Slansky, 1993). The growth performance of leaf-chewing larvae is strongly correlated with leaf water content (Slansky and Scriber, 1985; Woods and Harrison, 2001) and there is a consistent tendency for larval survival and growth rate to be reduced on water-stressed foliage. It is apparent that plant water deficits can have complex effects on the behaviour and ecology of herbivorous insects.

Minerals cannot be biosynthesized; so if an insect requires a mineral, that mineral must be present in the diet in adequate amount and appropriate form (Cohen, 2004). Caterpillars are known to require appreciable amounts of potassium, phosphate and magnesium (Mattson and Scriber, 1987). Magnesium functions in the glycolysis pathway involved in conversion of carbohydrates to yield energy and in numerous enzyme actions in other pathways (Lehninger *et al*., 1993). In addition, magnesium stimulates insect feeding responses while calcium is involved in muscular excitation and regulation of muscle responses to stimuli. An array of trace elements known to function as cofactors for specific enzyme reactions is also required. Of primary importance are iron, zinc, manganese, and copper, which have been found to be essential for several insects (Lehninger *et al*., 1993). Many essential metabolic activities such as production of 20-dehydroxyecdysone (an ecdysis hormone), formation of cuticle and conversion of stored chemical energy to useful adenosine triphosphate (ATP) energy are dependent on iron (Cohen, 2004). The cuticle of the mandibular cusps in lepidopterous larvae is often hardened by the presence of zinc or manganese (Chapman, 1998). Manganese is a cofactor in several enzyme actions, especially with metalloenzymes such as arginase and ribonucleotide reductase while copper is a cofactor in several enzyme processes, including those involving cytochrome oxidase (Lehninger *et al*., 1993). The jute mallow with serrated leaves was found to be richer in these important minerals and they most likely influenced the feeding preference of larval *A. eponina*.

Viscosity, a measure of resistance of a liquid to shear forces and flow, was found to be higher in the mucilage extracted from jute mallow with smooth-edged leaves. Borrell (2006) reported that the rate of feed intake in insects is dependent on viscosity of the fluid rather than its sweetness and the rate of intake decreases with increasing viscosity (Krenn, 2010; Kim *et al*., 2013). Insects find it easier to feed on less viscous food sources and this is one of the reasons larval *A. eponina* preferred the jute mallow with serrated leaves.

In conclusion, the jute mallow morphotype with serrated leaves had physical and chemical properties that were more beneficial to the growth and development of *A. eponina*. The intrinsic ability of adult *A. eponina* to select the morphotype with superior nutrient composition is very crucial to the process of host selection. Vegetable farmers are, therefore, advised to be more proactive when cultivating jute mallow morphotype with serrated leaves in order to avoid heavy infestation by *A. eponina*.

Acknowledgement

The authors appreciate assistance rendered by Dr. R.E. Okonji of the Department of Biochemistry and H.A. Adeniran of the Department of Food Science and Technology, Obafemi Awolowo University.

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