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ORIGINAL ARTICLE

Feeding preferences of the field cricket *Scapsipedus icipe* (Orthoptera: Gryllidae) for different species of *Commelina*

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Key words. Crickets, edible insects, Commelina, Commelinaceae, food security, Sub-Sahara Africa

Abstract. The field cricket, Scapsipedus icipe Hugel and Tanga (Orthoptera: Gryllidae) is edible and could be used to reduce malnutrition in Sub-Saharan Africa. As the demand for cricket products is increasing, there is a need to find cost-effective ways of rearing this cricket using locally available and affordable sources of food. This study evaluated the feeding preferences of the field cricket S. icipe for 11 species of dayflowers (Commelina spp.; Commelinaceae), using no-choice and multiple-choice experiments in controlled environments. Leaf feeding rates and the nutrient content and phytochemicals of the plants were determined. The ANOVA results indicate significantly higher feeding rates when offered Commelina petersii and C. forskaolii, than Commelina sp. and C. purpurea in no-choice experiments. Multiple-choice experiments ranked C. petersii as the most preferred species followed by C. forskaolii and the two reference species, C. benghalensis var. benghalensis (non-hybrid variant) and C. benghalensis (hybrid variant). The Spearman correlation and PCA revealed positive significant associations between leaf feeding and Ca and NDF content of leaves and a negative significant association between Ca and NDF. A high Ca/low NDF content was recorded for C. petersii and a low Ca/high NDF content for C. purpurea. The beta regression analysis and a biplot identified six phytochemical constituents influencing leaf feeding: phenols, alkaloids, tannins, glycosides, saponins and anthraguinones. Phenols stimulate feeding by S. icipe on C. petersii and C. forskaolii, whereas the tannins and alkaloids in Commelina sp. and C. purpurea, acted as deterrents. Nutrient content and phytochemicals are two important factors determining the suitability of species of Commelina for the field cricket S. icipe. Based on the results of this study, the leaves of C. petersii are highly recommended as a source of food for the mass rearing of this field cricket (S. icipe) and boosting entomophagy in Sub-Saharan Africa.

INTRODUCTION

Entomophagy is growing in popularity globally as a novel source of food and feed with a great potential for contributing to food security (Van Huis et al., 2013). The main reason for consuming insects is to supplement the source of animal and plants-based proteins, which is expected to increase due to the growth in world population that is projected to reach 9 billion by 2050 (van Huis & Oonincx, 2017). As the population increases, natural resources (e.g., land, water) used for protein production are being degraded and become insufficient for supporting the increasing population. In order to fulfil the nutritional needs of this growing population, the possibilities of using edible insects as a potential source of protein for present and future generations has been explored (Van Huis et al., 2013; Tao & Li, 2018). Edible insects have advantages over alternative sources of protein, such as beef, in their high nutritional content, high feed conversion efficiencies and high fecundity. In addition, it is more environmentally friendly and less expensive to rear insects for food.

The field cricket, Scapsipedus icipe Hugel and Tanga (Orthoptera: Gryllidae) (Tanga et al., 2018) is edible and could be used to reduce malnutrition at local and global levels. It is a native of Kenya and well adapted to the tropical climate of Africa including Madagascar (Tanga et al., 2018; Magara et al., 2021). This insect is highly nutritional in terms of protein, fat, fibre, mineral and vitamins (Murugu et al., 2021). As food for humans, this cricket can be fried and eaten as a snack or processed into flour and still remain highly nutritional. It can also be offered as feed for other insects (Mwale et al., 2022), or as food for livestock, such as pigs (Miech et al., 2017). Like other Gryllidae, S. icipe can feed on a variety of foods including agricultural by products, vegetable materials, commercial food, forage and even weeds (Tyree et al., 1976; Ayieko et al., 2016; Miech et al., 2016; Orinda et al., 2017; Oloo et al., 2020;

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Murugu et al., 2021; Vaga et al., 2021). While agricultural by products and vegetable feeds are expensive for farmers in Sub-Saharan Africa, local resources, such as, agricultural waste, forage and weeds are natural and affordable diets for crickets. This could be used to address the problem of food security by exploiting a source of high qualityprotein (Hanboonsong et al., 2013; Miglietta et al., 2015). Provision of local plant resources as feed for crickets will also reduce the competition with green-crop production for human consumption (e.g., kales, cabbage, vine of sweet potato). Currently, there is little information on what local plants S. *icipe* feeds on, except that reported by Magara et al. (2019), which reports it can feed on fish offal, wheat bran and crops produced for human consumption such as soybean, pumpkin leaves and carrots. Using local plants (e.g., from weed farms) as low-cost diets for this field cricket will enhance and assure sustainability of its mass production for improving the nutritional well-being of Kenyan as well as people generally in Sub-Saharan Africa. Insects that feed on plants are referred to as "herbivorous" or "phytophagous" insects.

During the last few decades, several studies have assessed the use of local plants as food for various species of crickets in captivity (e.g., Tyree et al., 1976; Miech et al., 2016; Choo et al., 2017; Kinyuru & Kipkoech, 2018; Vaga et al., 2021). For instance, the house cricket, Acheta domesticus L. (Orthoptera: Gryllidae) in Europe will feed and successfully develop on flowering Lamium album L. supplemented with Trifolium pratense L. (Vaga et al., 2021). Similarly, the Cambodian field cricket, Teleogryllus testaceus (Walker, 1869) (Orthoptera: Gryllidae) can be successfully reared on the farm weed Cleome rutidosperma DC. (Miech et al., 2016). For Sub-Saharan Africa, Kinyuru & Kipkoech (2018) report that A. domesticus can be reared on several weeds and recommends Commelina benghalensis L., with a high protein content, because it is its most preferred species. Thus, plant-food selection by crickets varies greatly depending on species of cricket, environment and species of plant. Finch & Collier (2008) categorize plant selection into "host plant finding or location" and "host plant acceptance as food". Many studies indicate that odour, visual cues like colour and phytochemicals are used by insects for locating host plants (e.g. Prokopy et al., 1983a, b; Stanton, 1983; Kostal & Finch, 1996). In contrast, other studies indicate that phytochemical and nutrient content are more important in food discrimination and selection (e.g. Bernays, 1995; Chapman & De Boer, 1995; Simpson et al., 1995; Ying et al., 2003; Matthews & Matthews, 2010). Nevertheless, Ying et al. (2003) report that some insects can even differentiate between plants based only on their phytochemical and nutrient content. Generally, phytochemicals act as repellents or attractants for herbivorous insects, whereas the nutrient contents of plants are important for their development and survival (Bernays & Chapman, 1994). However, the presence or relative concentrations of such bio-active compounds and nutrients varies across taxonomic groups of plants with some herbivorous insects having a higher preference for particular plant families or even genera (Ward et al., 2003; Chapman, 2009; Capinera, 2014). This study focused on preferential feeding of crickets on species in the plant genus Commelina commonly known as "Dayflowers" . Plants in this genus are herbaceous annuals or perennials belonging to the Commelinaceae and distributed from tropical to warm-temperate parts of the world (Wilson, 1981; Faden, 1998). These species propagate both sexually (seeds) and asexually (vegetative), with vegetative propagation highly plastic and adaptable for rapid production and uniform plant growth (Budd et al., 1979; Ecker & Barzilay, 1993; Webster & Grey, 2008; Yang & Kim, 2016). To optimize the utilization of local plants, such as, weeds that are affordable, accessible, available and nutritious for crickets, it is necessary to better understand their preferences. The objective of the present study was to determine the preference of a cricket for different species of Commelina. The relationship between nutrient content and phytochemicals in species of Commelina and the preference of the crickets was also investigated.

MATERIAL AND METHODS

Scapsipedus icipe

Colonies of *Scapsipedus icipe* were obtained from Jaramogi Oginga Odinga University of Sciences and Technology (JOOUST) insect farm at Bondo, Kenya. The crickets were reared in different screenhouses at temperatures ranging between 28°C to 36°C, relative humidity between 59–77% (RH) and a photoperiod of 12 h (Fig. 1). They were fed on a commercial diet (chicken mash) obtained from Unga Farm Care (E.A.) Ltd FUNGO[®] Grower Mash, Nairobi, Kenya.



Fig. 1. S. icipe (A) adult, (B) one month and one week old nymph and (C) rearing facility.

Three batches of eggs of S. icipe were incubated in three 100litre plastic buckets (950 eggs/buckets). The eggs were placed on humid cotton wool in the buckets and covered with 1 mm mesh net to prevent predators eating the crickets and their escaping. To provide a refuge, egg trays (29 cm \times 29.5 cm) were placed vertically in the buckets. Drinking water was provided ad libitum in the form of moist cotton wool in a 16 cm saucer, which was changed every two days. Other conditions, such as, cleaning, disinfection and control of predators was monitored every day following the procedure in the cricket rearing handbook of Orinda et al. (2021). Crickets were supplied with commercial diet for a period of 30 days, starting from day 14 post-hatching (PH). One month old nymphs of the same body size were used in the preference experiments (Orinda et al., 2021). Mortality of crickets was recorded daily in each treatment and dead insects replaced immediately by live ones from backup buckets, which each contained 15 crickets reared in the same food-plant/treatment as those selected for the preference experiments. Prior to providing the crickets with leaves of Commelina they were deprived of food for 16 h to increase their hunger.

Source of plant material

A total of 11 species of Commelina were obtained from different agroecological zones in Western Kenya (Runyambo et al., 2022). Correct species names were verified at the East African Herbarium (EAH) of the National Museums of Kenya. Each of these species (Table 1, Fig. 2) was grown in a plot of $1 \text{ m} \times 1$ m, replicated three times and watered every day for a month before use in the feeding experiments. No pesticides or fertilizers were used. Fresh leaves were cut from the first to third nodes in the apex of each Commelina plant for feeding to the crickets. In addition, fresh leaves of each of the 11 species were harvested for analysis of their nutrient content and phytochemicals. Prior to feeding, leaves of each species were rinsed with clean water and then left to dry for 10 min. During the experiments, the crickets were provided with fresh leaves every day. It is important to note that only leaves of Commelina plants were supplied as food in this experiment because they were considered to contain the nutrients essential for the development of herbivorous insects (Dethier, 1954).

Experimental design

To evaluate the feeding of crickets on the leaves of different species of *Commelina*, two experiments were carried out. Nochoice and multiple-choice experiments were conducted over a period of ten days from 22th April to 1st May, 2021. In the nochoice experiment 11 species of *Commelina* were the treatments, whereas in multiple-choice experiment four species of *Com*- *melina* were the treatments. A randomized complete block design with three replicates was used in each experiment.

Leaf feeding

No-choice experiment

The purpose of this experiment was to evaluate the rate of feeding of the cricket on the leaves of the most and least preferred species of *Commelina*. Preferences of crickets were assessed relative to two reference species, COMBE1 and COMBE2. In this experiment, crickets were fed 1.5 grams, which was based on a preliminary experiment.

Multiple-choice experiment

This experiment aimed to rank the most preferred species identified in no-choice experiments. Hence, it was done in a similar manner as the no-choice experiment, but there was no reference species. The four most preferred species of *Commelina* were compared with one another. A preliminary experiment indicated that 0.8 grams should be fed to the crickets in this experiment.

Calculation of leaf area

Calculation of total leaf area (cm²), consumed leaf area (cm²) and percentage of leaf consumed (%) were measured using Leaf-Byte: mobile application (version 1.3.0; Getman-Pickering et al., 2020) on Apple iPad mini-3 tablet. The leaves were measured flat before and after feeding using a transparent glass protector, model iPad mini tablet. In order to obtain accurate pictures taken at an angle, a white background scale with 4 black dots arranged in a square (10 cm spacing) was used.

Nutrient contents analysis

Fresh leaves of each species of *Commelina* were harvested, oven dried at 65°C for 24 h, airdried for 24 h and then converted to powder using a blender (Sinbo SHB 3090 Turbo Blender). The powder for each species was passed through a 45 mm aperture sieve. Approximately 25 grams of fine powder of each species of *Commelina* were placed in polyethene bags and kept airtight prior to nutrient analysis at the nutritional laboratory, Faculty of Veterinary Medicine in the Department of Animal Production, University of Nairobi. Nutrient analysis was done using proximate components, Van Soest system for fibre fractions and minerals.

Proximate components and Van Soest system for fibre fractions

Proximate analysis was done using 5 grams of the powder of each plant (Kirk & Sawyer, 1980). The analysis included determination of moisture content (Mc) conversion to dry matter (DM), ash (ASH) content, crude protein (CP), ether extract (EE), crude fibre (CF) and nitrogen free extract (NFE) according to the stand-

Table 1. Name of plant species included in this study, their code, life history (A – annual, P – perennial, A/P – short-lived perennial) and origin.

Plants species	Code	Life history	Origin of species (county and location)
Commelina africana L. var. africana	COMAF	Р	Siaya county (near JOOUST)
Commelina benghalensis L. var. benghalensis (non-hybrid variant)	COMBE1	A/P	Siaya county (Waguso)
Commelina benghalensis L. (hybrid variant)	COMBE2	A/P	Kisumu county (Korando)
Commelina diffusa Burm. f.	COMDI	A/P	Siaya county (Waguso)
Commelina erecta L. var. livingistonii	COMEL	Р	Siaya county (Waguso)
Commelina forskaolii Vahl	COMFO	А	Siaya county (Waguso)
Commelina kotschyi Hassk.	COMKO	A/P	Kisumu county (Kashule coloa)
Commelina latifolia A. Rich. var. latifolia	COMLF	Р	Siaya county (Warianda)
Commelina petersii Hassk.	COMPE	Р	Siaya county (Abawa)
Commelina purpurea C.B. Clarke	COMPU	Р	Homabay county (Kendu bay)
Commelina sp.	COMSP	Р	Homabay county (Kisui)

Source: Runyambo et al. (2022).



COMDI

COMEL

COMAF

COMPU





COMSP

COMKO

COMPE

Fig. 2. The species of Commelina used in the preference tests.

ard methods of the Association of Official Analytical Chemists (AOAC, 1998). Moisture content was determined by heating the sample in an oven at 105°C for 12 h, cooled in desiccants at 60°C and weighed. Ash content, which indicates the mineral content, was determined by incinerating samples at 550°C in a muffle furnace, which were then cooled and weighed. Crude protein was determined using the Kjeldahl method and the values multiplied by 6.25. Ether extract of the samples were also obtained. Crude fibre content was obtained by successive digestion of defatted

samples using 1.25% sulphuric acid and 1.25% sodium hydroxide solutions (Kirk & Sawyer, 1980). Nitrogen free extract (NFE) was obtained by subtracting the percentage of the above determinations from 100%.

Van Soest analysis was used to determine values of neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), levels of cellulose and hemicellulose. NDF, ADF and ADL were determined using the Van Soest & Robertson (1985) method.

Table 2. Phytochemicals present in leaf e	tracts of species of Commelina	(number of samples $n = 2$).
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Species codes	Phenols	Glycosides	Steroids	Alkaloids	Flavonoids	Tannins	Terpenoids	Saponins	Anthraquinones
COMPU	_	+	_	+	+	+	+	+	_
COMLA	_	_	+	_	+	+	+	_	+
COMDI	+	+	+	+	+	+	+	+	-
COMFO	+	+	+	+	+	+	+	_	+
COMPE	+	+	+	+	+	+	+	_	_
COMEL	+	+	+	+	+	+	+	+	+
COMKO	+	_	_	_	+	+	+	+	+
COMBE 1	_	+	+	+	+	_	+	+	+
COMBE 2	_	+	+	+	+	_	+	+	+
COMSP	+	+	+	_	+	+	+	+	_
COMAF	-	-	+	-	+	+	+	+	_

Note: (+) - Present; (-) - Absent.

Mineral composition

One gram of leaf powder was placed in a muffle furnace at 550° C for 1 h and the ash was dissolved in hot 10% of hydrochloric acid and nitric acid (ratio 3 : 1) and diluted with 100 ml of distilled water. Content of the various minerals (iron – Fe, zinc – Zn, calcium – Ca, magnesium – Mg, sodium – Na, potassium – K, manganese – Mn and copper – Cu) were determined using atomic absorption spectrometry (AAS) (Shimadzu, AA-6300, Tokyo, Japan) according to Association of Official Analytical Chemists methods (AOAC, 1998).

Extraction and analysis of phytochemicals

Leaves of 11 species of *Commelina* were harvested and then left to dry for 7 days to induce the production of bio-active compounds. The dried leaves were powdered using a blender (Sinbo SHB 3090 Turbo Blender) and passed through a 45 mm sieve. The powders were kept in air-tight polythene bags in cool-dry place until required for the laboratory analysis at the nutritional laboratory, faculty of veterinary medicine, department of animal production, University of Nairobi. Confirmatory qualitative tests for nine phytochemicals (phenols, alkaloids, glycosides, tannins, steroids, flavonoids, terpenoids, saponins and anthraquinones) were done using standard methods Yadav & Agarwala (2011), Evans (2009), Sofowora (1993) and Harborne (1973).

Statistical analysis

Data were checked for normality and homoscedacity using Shapiro-Wilk and Bartlett tests. A logarithmic [log(x+1)] transformation was applied to data where necessary. Non-parametric tests were used for non-normally distributed and non-homoscedastic data after transformation. Differences in the rate at which the crickets fed on the leaves, comparisons of leaf nutrient concentrations and mortality of crickets were tested using ANOVA and Kruskal-Wallis tests at different critical values of alpha (α = 0.0001, $\alpha = 0.001$, $\alpha = 0.01$ and $\alpha = 0.05$). Spearman correlation was used to determine associations between the percentage of the leaves consumed and leaf nutrient content at a critical value of α = 0.05. Principal component analysis (PCA) using standardized variables (factor loading < .28) revealed patterns of relationship between species and nutrient contents. Beta regression model was used to determine the phytochemicals that influence leaf feeding at a critical value of $\alpha = 0.05$. This type of regression is an extension of the generalized linear model and most suitable for situations in which the dependent variable, proportion of the leaves eaten, is positive and recorded at intervals, 0 to 1 and the endpoints 0 and 1 can be excluded (Ferrari & Cribari-Neto, 2004; Cribari-Neto & Zeileis, 2010). Seven independent variables: phytochemicals either present or absent, and dependent ones: proportions of the leaves eaten were computed. Flavonoids and terpenoids were not included in the analysis as both were only recorded once. Probit functional link for conditional means and log functional link for conditional scales using OIM (Observation Information Matrix) of standard error type was applied. The marginal effect assessed the relative importance of each independent variable in explaining the variation in the dependent variable. A biplot was used to display the relationship between significant phytochemicals from the regression model and species.

The beta regression is parametric and assumes beta distribution with the dependent variable follows a density distribution

$$g(\mu_t) = \sum_{i=1}^{\kappa} x_{ti} \beta_i = \eta_t$$

where $\beta = (\beta_1, ..., \beta_k)^T$ is a vector of unknown regression parameters $(\beta \in \mathbb{R}^k)$ and assumed to $x_{i1,...,n}, x_{ik}$ are observations on *k* covariates (k < n), which are assumed fixed and known. Finally, *g*(.)

Table 3. Variation in the consumption of different species of *Commelina* by *S. icipe* in no choice experiments compared with that recorded for the two reference species (COMBE 1 and COMBE 2, and individually).

COMBE 1 as reference		COMBE 2 as reference						
Dunnett's multiple comparisons test	Mean	Dunnett's multiple comparisons test	Mean					
COMER	30.53ns	COMER	30.53ns					
COMAF	33.81ns	COMAF	33.81ns					
COMPE	38.76***	COMPE	38.76**					
COMPU	19.30****	COMPU	19.30****					
COMDI	34.11ns	COMDI	34.11ns					
COMBE 2	32.35ns	COMFO	37.52*					
COMFO	37.52***	COMKO	31.62ns					
COMKO	31.62ns	COMSP	26.93*					
COMSP	26.93ns	COMLA	29.74ns					
COMLA	29.74ns	COMBE 1	29.98ns					
COMBE1	29.98	COMBE 2	32.35					

Note: Comparisons of means using Dunnett's test at different significant levels: ****P < 0.0001; ***P < 0.001; **P < 0.01; *P < 0.05, ns – not significant.

is strictly monotonic and twice differentiable link function that maps (0,1). The probit function $g(\mu)$ was used $g(\mu) = \varphi^{-1}(\mu)$, where $\varphi(.)$ is the cumulative distribution function of standard random variable.

ANOVA and Kruskal-Wallis tests were done using Prism Software (version 8.0.2, GraphPad Inc., San Diego, CA), Spearman correlations, principal component analysis, beta regression model and biplot using STATA 14.2 software (Stata Corp LLC, TX, USA).

RESULTS

Leaf feeding

The response of *Scapsipedus icipe* to the leaves of the different species of *Commelina* in no-choice and multiple-choice experiments differed.

No-choice

Of the 11 species of *Commelina* tested, a highly significant (F = 8.316, df = 10, P = 0.0002; F = 8.316, df = 10, P = 0.0082) higher rate of feeding was recorded for COMPE when the crickets were provided with only one species of *Commelina*, compared to the rates recorded for COMBE1 and COMBE2 (Table 3). Similarly, a significantly higher (F = 8.316, df = 10, P = 0.0010; F = 8.316, df = 10, P = 0.0404) feeding rate was recorded for COMFO than COMBE1 and COMBE2. Unlike the suitable species, highly significant (F = 8.316, df = 10, P < 0.0001; F = 8.316, df = 10, P = 0.0284) low rates of feeding were recorded for COMPU and COMSP, compared with that recorded for the two-reference species. Other species of *Commelina* did not differ significantly (P > 0.05) when compared with the two-reference species.

Multiple-choice

When crickets were given a choice of the four most preferred species of *Commelina* in the no-choice experiment (COMPE, COMFO, COMBE1 and COMBE2), the feeding rate recorded for COMPE was highly significantly the

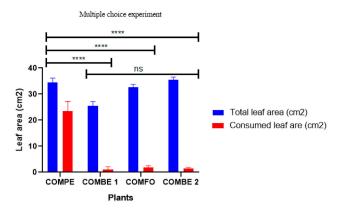


Fig. 3. Area of leaf of four species of *Commelina* consumed by the cricket relative to the total leaf area provided. Turkey's Honest Significant Difference test (THD) was used to compare the means with the p-values. ****P < 0.0001, ns – not significant.

highest (F = 37.87, df = 3, P < 0.0001) (Fig. 3). Based on this result, COMPE was ranked as the most suitable species followed by COMFO, then COMBE1 and COMBE2.

Insect mortality

Mortality of the crickets in the no-choice experiment was very low (9.69%) (Kruskal-Wallis test, Kruskal-Wallis statistic = 32.33, P = 0.450) and no deaths were recorded in the multiple-choice experiments.

Nutrient content of species of Commelina

The nutrient contents of the species differed significantly (P < 0.05), except for the minerals magnesium, copper and zinc (Table S1, S2). Five principal components (PC1, PC2, PC3, PC4 and PC5) are greater than one in the scree plot [eigenvalue with (Rho) > 1; Fig. S1], and together explained 88.83% of the variability. These five components accounted for 35.46%, 18.25%, 14.75%, 11.78% and 8.59%, respectively. Five major nutrient groups were

Table 4. Principal component analysis (PCA) of the nutrient content of *Commelina* plants with standard deviation (SD), percentage of variation explained and cumulative percentage related to each principal component eigenvector.

Nutrient content			Principa	al Component Eige	envalues	
Variable		PC1	PC2	PC3	PC4	PC5
DM	Dry matter	0.2412	0.0472	0.3387	0.2204	-0.336
ASH	Ash	0.1869	0.0277	-0.076	0.6385	-0.1065
EE	Ether extract	0.0257	0.4529	0.2877	0.0948	0.381
CP	Crude protein	-0.2675	0.2879	-0.2684	0.0011	-0.0347
NFE	Nitrogen free extract	-0.1688	-0.2703	0.3419	-0.4464	0.1368
NDF	Neutral detergent fibre	0.375	0.1976	-0.1717	-0.1724	0.0626
ADF	Acid detergent fibre	0.2953	-0.0163	-0.3282	0.0056	0.3803
ADL	Acid detergent lignin	0.2654	0.239	0.2397	-0.0907	-0.4632
Hemicellulose	_	0.2752	0.2485	0.071	-0.2715	-0.2091
Cellulose	_	0.278	0.2415	-0.325	-0.2374	0.1527
Na	Sodium	0.1347	-0.3429	0.2174	0.1525	0.2797
К	Potassium	0.1388	-0.4283	-0.3158	0.1293	-0.2075
Са	Calcium	-0.2735	0.2916	0.2019	0.297	0.2156
Mn	Manganese	0.3158	-0.1689	0.3105	-0.122	0.1255
Fe	Iron	0.3781	-0.0696	0.1357	0.1561	0.3114
SD		0.22	0.26	0.26	0.27	0.26
% of Variance		35.46	18.25	14.75	11.75	8.59
Cumulative %		35.46	53.7	64.45	80.24	88.83

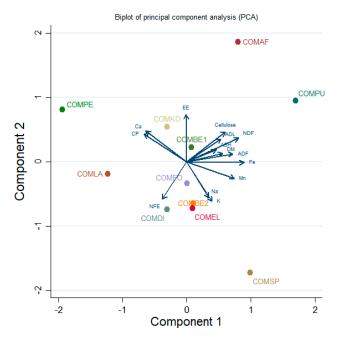


Fig. 4. Biplot of the first two principal components (component 1, explained variance 35.58%; component 2, explained variance 18.56% and total explained variance 54.14%), representing the distribution of the variation in the nutrient contents of different species of *Commelina*: dry matter (DM), neutral detergent fibre (NDF), crude protein (CP), ash (ASH), cellulose, acid detergent fibre (ADF), ether extract (EE), nitrogen free-extract (NFE), acid detergent lignin (ADL), manganese (Mn), calcium (Ca), potassium (K), sodium (Na) and iron (Fe) according to the factor loadings (eigenvector value > 0.28) of the components. Scientific names of species are listed in Table 1.

identified: (NDF, Mn and Fe) in PC1, (K, Na, EE and CP) in PC2, (DM, NFE and cellulose) in PC3, (ash and Ca) in PC4 and (ADF and ADL) in PC5 (Table 4). The distribution of the species in the biplots of the PCA indicate that COMPE and COMLA contain more CP and COMPE more Ca. However, these two species were low in Fe, Mn and cellulose, ADL and NDF. COMKO had an intermediate level of CP, Ca and EE. COMBE1 had an intermediate level of EE, cellulose and ADL. COMEL, COMBE2 and COMFO had intermediate levels of EE, Na and K. As for COMPU, it had high levels of cellulose, ADF, ADL, NDF and DM. COMDI had high values for NFE. COMSP and COMAF were far from one another and the other species in terms of the level of nutrients (Fig. 4).

A positive correlation (Spearman's *rho* = 0.6364, P < 0.05) between the percentages of the leaves consumed and their calcium content was significant. In addition, there was a significant positive correlation (Spearman's *rho* = 0.6273, P < 0.05) between the percentages of the leaves consumed and their neutral detergent fibre content. There was, however, a significant negative correlation (Spearman's *rho* = -0.6091, P < 0.05) between the calcium and neutral detergent fibre content and significant positive correlation between the calcium and protein content of the leaves (Spearman's *rho* = 0.6455, P < 0.05) (Table S3).

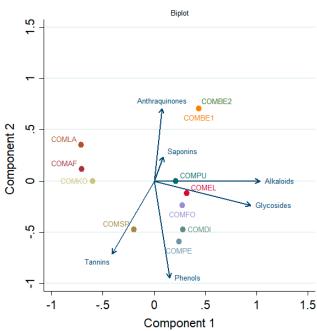


Fig. 5. Biplot of the relationships between phytochemicals, results of beta regression analysis and species of *Commelina* (component 1, explained variance 32.90%; component 2, explained variance 28.57% and total explained variance 61.47%). Scientific names of species are listed in Table 1.

Phytochemicals

Flavonoids and terpenoids were present in leaves of all the species of *Commelina* tested. There were no phenols, glycosides, alkaloids or anthraquinones in COMAF and COMLA, and no tannins or phenols in COMBE1 and COMBE2. Saponins and anthraquinones were not recorded in COMPE, whereas COMFO only lacked saponins. The other plants didn't differ much in either the presence or absence of different phytochemicals (Table 2).

The likelihood ratio test statistic of the beta regression analysis was statistically significant (P < 0.05). This indicates that this model fits the results well and that phenols have a highly significant positive (P < 0.001) influence on leaf feeding and steroids not significant positive (P > 0.05) effect. Glycosides, alkaloids and tannins had highly significant negative (P < 0.001) effects on leaf feeding, whereas saponins and anthraquinones had only significant negative (P < 0.05) effects. Marginal effects indicate an increase or decrease in the magnitude of the dependent variable with a-unit increase in each of the independent variables. Hence, an increase in phenols is associated with an increase in the percentage of the leaves consumed, whereas increases in alkaloids, glycosides, tannins, saponins and anthraquinones were associated with decreases in the percentage of the leaves consumed. For example, a 1% change in the independent variable is associated with an increase in the percentage of the leaves consumed of 0.10% when there is an increase of 1% in phenol content, whereas 1% increase in alkaloids decreased leaf feeding by 0.13% (Table 5). The biplot shows the association between phytochemicals and species of *Commelina*. The arrows indicate the extent of

Table 5. F	Results of the Beta	regression used	to determine	the phytoch	nemicals inf	fluencing lea	af feeding.
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	•			•	0		
Variables	Coeff.	Std. Err.	z	P-value	95% Conf. Interval		
Leaf feeding					Lower	Upper	
Phenols	0.5070	0.1160	4.33	0.001	0.277	0.7362	
Glycosides	-0.7541	0.1994	-3.77	0.001	-1.146	-0.3622	
Steroids	0.2041	0.1202	1.70	0.090	-0.0315	0.0439	
Alkaloids	-0.6231	0.1579	-3.95	0.001	-1.0278	-0.2908	
Tannins	-0.6593	0.1880	-3.51	0.001	-0.4085	-0.005	
Saponins	-0.2067	0.1029	-2.01	0.045	-0.4085	-0.005	
Anthraquinones	-0.2571	0.1032	-2.49	0.013	-0.4594	-0.0549	
Cons	-0.1154	0.3008	-0.38	0.701	-0.7051	0.4742	
Scale							
Cons	4.8249	0.4251	11.35	0.001	3.9916	5.6582	
Average Marginal effect	s: Model OIM						
Variables	dy/ex	Std. Err.	z	P-value	95% Cor	if. Interval	
Phenols	0.0913	0.0220	4.14	0.001	0.0481	0.1345	
Glycosides	-0.1778	0.0450	-3.94	0.001	-0.2662	-0.0894	
Alkaloids	-0.1335	0.0346	-3.85	0.001	-0.0655	-0.2015	
Tannins	-0.1663	0.0459	-3.62	0.001	-0.2564	-0.0021	
Saponins	-0.0459	0.0223	-2.05	0.040	-0.0897	-0.0021	
Anthraquinones	-0.0461	0.0178	-2.58	0.010	-0.0811	-0.0110	

Note: Regression diagnostics: number of observations, 11; likelihood ratio χ^2 (p value), 18.99 (0.0082); log likelihood, 20.36.

the variation in the phytochemical content. The longer the arrow and the direction, the greater the variation. Based on the significant phytochemicals identified by the beta regression analysis, phenol in COMPE and COMDI was strongly associated and moderately associated in COMFO. Moreover, the tannins, glycosides and alkaloids, respectively, in COMSP, COMEL and COMPU were strongly associated. The species COMBE1 and COMBE2 were associated by both saponins and anthraquinones. As for COMLA, COMAF and COMKO, they were not associated with any of the significant phytochemicals (Fig. 5).

DISCUSSION

This study evaluates the preferences of *S. icipe* for feeding on different species of *Commelina* in a screenhouse. It also reports the link between the nutrient content and chemical constituents of leaves with this cricket's preference for feeding on the leaves of particular species of *Commelina*.

Based on rates feeding COMPE is the most suitable plant for rearing *S. icipe* followed by COMFO, and COMPU was the least suitable. The other species that were less consumed than the two-reference species, COMBE1 and COMBE2, however, are not inedible. The rate of feeding recorded for these species indicated that when suitable plants are scarce and the crickets are hungry, they are likely feed on many of them. In addition, the low mortality of crickets fed on leaves of species of *Commelina* in this study, indicates they are a good quality food. Nevertheless, food selection by crickets is complex and involves visual, olfactory, habitat, intraspecific, celestial (sun and sky), magnetic of the field and leaf nutrient cues (Tyree et al., 1976; Horch et al., 2017; Ugolini, 2021; Vaga et al., 2021; Kuo & Fisher, 2022).

The effect on the growth and survival of crickets of feeding on weeds is well studied (e.g., Tyree et al., 1976; Miech et al., 2016; Choo et al., 2017; Kinyuru & Kipkoech, 2018; Ng'ang'a et al., 2020; Vaga et al., 2020, 2021; Kuo & Fisher, 2022), whereas the relationship between feeding and nutrient content is less investigated. Nevertheless, there is some information on the components of some plants provided as food or incorporated in mixed diets for crickets (Miech et al. (2016) and Vaga et al. (2020, 2021), which indicates nutrient content is important. The results presented reveal a significant positive association between the percentage of the leaves eaten and Ca and NDF, and significant negative association between Ca and NDF, which indicates the key roles of these two nutrients for S. icipe. Moreover, the PCA confirmed these results as it revealed an inverse relationship between the concentrations of NDF and Ca in COMPE and COMPU. Indeed, the most consumed species (COMPE) contained a high concentration of Ca and low NDF, whereas the least consumed (COMPU) contained a low concentration of Ca and high NDF. These results are in accordance with the results of Vaga et al. (2021) in which the house cricket A. domesticus preferred L. album that has a low NDF. In addition, Acheta prefers fresh-cut T. pratense with a low NDF to late-cut T. pratense with high NDF (Vaga et al., 2020). In contrast, Miech et al. (2016) report that the Cambodian field cricket T. testaceus is tolerant of the high fibre contents of its most preferred species, Cleome rutidosperma. Furthermore, the results on the role of fibre are inconsistent, with some studies reporting a high fibre content associated with high feeding and high performance (Tyree et al., 1976; Veenenbos & Oonincx, 2017) and others high fibre contents and low feeding and low performance (Nakagaki & Defoliart, 1991; Orinda et al., 2017). Hence, the effect of fibre content on the rate of feeding in crickets is not clearly understood, and more studies are needed. In this study, the low level of feeding on COMPU, could be attributed to S. icipe having to spend more time chewing its more fibrous leaves (Faden, 2012). The current study also showed that the nutritional profile of species of Commelina is rich in CP, DM, NFE and minerals (Ca and Mn). Magara et al. (2019) and Murugu et al. (2021) report that these nutrients are important for the growth and development of the field cricket S. icipe. The crude protein contents of COMPE and COMBE1 were higher than that reported for C. rutidosperma (22.2%) fed to Teleogryllus crickets (Miech et al., 2016) and L. album (22%) + T. pratense (19.9%) in mixed diets for Acheta crickets (Vaga et al., 2021). According to Bawa et al. (2020), the protein content of cricket diets is crucial for their growth and development despite low concentrations of nutrient such as EE and minerals (Fe, Zn, Mg, and Cu). The house cricket, A. domesticus can be successfully reared on a Commelina diet (known as COMBE1) (Kinyuru & Kipkoech, 2018). In the preference tests, crickets preferred COMPE, which has higher calcium content than the other species. With respect to the Ca content of crickets, Murugu et al. (2021) compare Ca content of S. *icipe* to that of plants (e.g., sorghum, maize, wheat, kidney bean) and animal (e.g., beef, goat, chicken, eggs) sources and conclude that Ca content of S. *icipe* is, with the exception of kidney beans and eggs, higher. Hence, consumption of S. *icipe* reared on diets such as COMPE rich in Ca could increase the availability of calcium, especially for children, and reduce the effects of calcium deficiency in low-income countries in Sub-Saharan Africa. It should be noted that the Commelina plants used in the present study were harvested from various agroecological zones in Western Kenya and cultivated at the JOOUST crop farm. Thus, it cannot be excluded that their nutritional content will differ if grown at other geographic locations with different soil profiles.

Commelina plants are widely used in medicine as they are a source of bioactive compounds. Leaf extracts of species of Commelina contain alkaloids, flavonoids, steroids, terpenoids, volatile oils, saponins and tannins of which flavonoids are the most frequent and abundant (e.g., Ghosh et al., 2019; Kansagara & Pandya, 2019; Ezeabara et al., 2020; Bussmann et al., 2021; Islam et al., 2021). In the current study, flavonoids and terpenoids were detected in all species, indicating that crickets fed Commelina could be a good source for humans of some important antioxidants and antibacterial substances (Grabmann, 2005; Panche et al., 2016). In addition, the chemical constituents of the leaves of Commelina had a crucial role in the feeding of S. icipe. The relationship between phytochemicals and feeding preferences of crickets are poorly investigated compared to those of Orthoptera, such as, Acrididae. This is possibly because most Acrididae are more devastating pests than Gryllidae. For Orthoptera, there are several examples of the chemical constituents of leaves being important in determining their feeding preferences. Bernarys & Chapman (1994) and Sanjayan & Ananthakrishnan (1987) report examples of chemical constituents of plants acting as stimulants or deterrents for feeding in Orthoptera. The results of the current study indicate that phenols are likely to increase leaf feeding and alkaloids, glycosides, tannins, anthraquinones and saponins decrease leaf feeding, that is,

phenols acted as stimulants and alkaloids and tannins as deterrents. These results are consistent with those reported for Acrididae (e.g., Harley & Thorsteinson, 1967; Mole & Joern, 1994; Dini & Owen-Smith, 1995; Wallace, 2013). For example, phenols stimulate feeding in the grasshopper, Melanoplus bivitattus (Harley & Thorsteinson, 1967; Wallace, 2013) and alkaloids and tannins deter feeding in the locust, Locustana pardalina and two grasshoppers, Ageneotettix deorum and Phoetaloites nebrascensis (Mole & Joern, 1994; Dini & Owen-Smith, 1995). While substances that stimulate feeding can be specific, at high concentrations they can act as deterrents (Chapman, 2009). In the present study only the phytochemicals that influenced the feeding of crickets are reported. There is a need for more quantitative data on the chemicals in *Commelina* plants as these plants were not completely rejected by S. icipe despite containing some deleterious chemicals. The low mortality of the crickets indicates they are well adapted to deal with deleterious chemicals. Herbivorous insects in general are well adapted to deal with phytochemicals in their diet, e.g., by rapid excretion, detoxification and avoiding ingesting toxins (Brattsten, 1988; Schoonhoven et al., 2005).

CONCLUSION

This study provides an insight into the importance of nutrients and phytochemicals in determining the suitability of species of Commelina as food for the field cricket S. icipe. The results indicate that this cricket has a strong preference for particular species of Commelina, with C. petersii and C. forskaolii the most suitable followed by the two reference species, C. benghalensi var. benghalensis (nonhybrid variant) and C. benghalensis (hybrid variant), and the least preferred Commelina sp. and C. purpurea. There were positive associations between leaf feeding, Ca and NDF, and negative associations between Ca and NDF. C. petersii has a high Ca/low NDF content, whereas C. purpurea has a low Ca/high NDF content. Six phytochemicals (phenols, alkaloids, tannins, glycosides, saponins and anthraquinones) influenced the leaf feeding of S. icipe, with the phenols in C. petersii and C. forskaolii acting as stimulants, and the tannins and alkaloids in Commelina sp. and C. purpurea acting as deterrents. The low mortality of the cricket recorded in this study indicate this insect thrives on Commelina-based diets, which are a good source of CP for their growth and development. For the mass rearing of this cricket the leaves of C. petersii are highly recommended.

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COMPETING INTERESTS. The authors declare that they have no competing interests.

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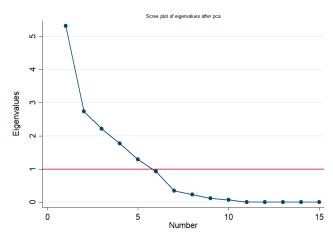


Fig. S1. Scree plot eigenvalues of the first five principal components.

Table S1. Results of ANOVA's of 10 nutrients including Van Soest's fibre analysis. Data reported as Means (\pm SE) and coefficient of variation (CV) and analysed after a logarithmic transformation [log(x+1)]. Number of samples (n = 2).

Species	%DM	%ASH	%EE	%CP	%NFE	%NDF	%ADF	%ADL	% Hemicellulos	e % Cellulose
COMPU	90.53±0.03ª	24.14±0.005	3.25±0.01	21.67±0.005ª	25.6±2.03 ^f	73.36±0.03	28.95±0.01	3.25±0.03 ^{cd}	44.41±0.00	29.64±0.02
COMLA	89.24±0.07	14.83±0.06	2.07±0.06ª	27.53±0.025	34.89±0.15 ^{ad}	58.18±0.03	18.54±0.01	2.43±0.02ª	39.64±0.02 ^b	18.83±0.01
COMDI	90.08±0.01 ^b	12.41±0.01	2.59±0.005 ^{cd}	20.81±0.005	45.55±0.13 ^{cd}	59.22±0.02	17.85±0.04	2.73±0.02 ^b	41.375±0.01	18.65±0.04
COMFO	91.25±0.03 ^d	19.60±0.02	1.70±0.03	21.91±0.05 ^b	31.23±0.02 ^{ab}	58.545±0.03	19.31±0.04	3.31±0.01 ^d	39.235±0.07	19.065±0.02
COMPE	90.08±0.03 ^b	16.76±0.02	3.85±0.03 ^f	25.10±0.04	37.74±0.06 ^{be}	48.715±0.07	15.37±0.10ª	1.84±0.02	33.34±0.03ª	14.465±0.02
COMEL	90.42±0.03 ^{ac}	13.10±0.03	2.09±002ª	22.44±0.02	39.90±0.01a ^{cd}	57.53±0.03	24.30±0.01	2.03±0.01	33.23±0.02ª	22.725±0.03
COMKO	$90.30 \pm 0.05^{\text{bc}}$	15.91±0.01	2.90±0.02 ^e	23.93±0.02	33.50±0.04 ^f	62.56±0.02	16.35±0.04	3.14±0.02°	47.205±0.02	23.46±0.01
COMBE1	$90.47{\pm}0.02^{\text{ac}}$	32.89±0.02	2.43±0.02 ^{bc}	23.20±0.01	23.06±0.02 ^{be}	57.13±0.03	19.81±0.01	2.37±0.01ª	36.32±0.05	17.83±0.03
COMBE2	91.20±0.05 ^d	20.80±0.01	2.76±0.01 ^{de}	21.77±0.01 ^{ab}	40.07±0.09 ^{ab}	54.87±0.03	15.19±0.02ª	3.86±0.01	39.68±0.01 ^b	15.45±0.02
COMSP	90.59±0.02ª	22.55±0.01	2.37±0.02 ^b	20.54±0.02	38.00±0.01°	61.36±0.03	20.86±0.02 ^b	2.66±0.02 ^b	42.5±0.01	16.54±0.01
COMAF	91.28±0.03 ^d	21.30±0.04	3.82±0.06 ^f	23.70±0.03	29.36±0.07	68.525 ± 0.005	20.91±0.03 ^b	4.37±0.08	47.61±0.03	23.21±0.04
% CV	0.61	2.15	16.56	1.52	13.66	0.86	2.91	12.34	1.04	2.11
P-value	<0.05	<0.05	< 0.05	< 0.05	< 0.05	< 0.05	<0.05	<0.05	<0.05	<0.05

Note: Means for a species sharing the same superscript letter are not significantly different at *P* < 0.05; Crude fibre was not included in the analysis due to the consideration of the Van Soest's fibre systems.

Table S2. Kruskal-Wallis test of the mean % content of eight minerals recorded in different species of *Commelina*. Data are Means (±SE) and coefficient of variation (CV). Number of samples (n = 2)

Species	Na% Mean	K% Mean	Ca% Mean	Mg% Mean	Zn% Mean	Mn% Mean	Fe% Mean	Cu% Mean
COMPU	0.0130±0.002	0.1700±0.000	0.4700±0.010		0.0036±2.000e-004		0.2450±0.005	0.0019±3.000e-004
COMLA	0.0039±1.000e-004	0.1700±0.010	1.4600±0.020	0.4300±0.000	0.0034±3.000e-004	3.2300±0.010	0.0048±2.000e-00	40.0014±3.000e-004
COMDI	0.0073±1.000e-005	0.1200±0.010	0.4800±0.000	0.3800±0.020	0.0030±0.001	5.5770±0.000	0.1100±0.000	0.0018±0.000
COMFO	0.0036±0.000	0.1700±0.000	1.4300±0.000	0.4900±0.020	0.0030±0.001	4.1280±0.002	0.0560±0.047	0.0033±0.001
COMPE	0.0100±0.000	0.0970±0.001	6.2000±0.100	0.4300±0.020	0.0050±0.001	2.9800±0.000	0.0430±0.001	0.0021±3.000e-004
COMEL	0.0013±1.000e-004	0.1700±0.000	1.5100±0.020	0.4400±0.010	0.0040±0.001	6.3040±0.004	0.1400±0.030	0.0028±2.000e-004
СОМКО	0.0009±1.000e-006	0.1400 ± 0.000	1.1500±0.020	0.5000±0.100	0.0039±1.000e-004	4.1650±0.000	0.0810±0.003	0.0026±1.000e-004
COMBE 1	0.0050±0.001	0.1700±0.000	1.9500±0.020	0.3600±0.020	0.0043±2.000e-004	4.2030±0.001	0.1500±0.000	0.0037±2.000e-004
COMBE 2	0.0130±0.002	0.1700 ± 0.000	1.7100±0.030	0.4400±0.000	0.0040±0.001	6.3040±0.002	0.1400±0.001	0.0028±3.000e-004
COMSP	0.0522±3.000e-004	0.1850 ± 0.005	0.3200±0.010	0.3200±0.000	0.3800±0.000	7.0150±0.001	0.2460±0.000	0.0018±1.000e-004
COMAF	0.0018±0.000	0.1000 ± 0.000	2.8220±0.001	0.4900±0.010	0.0045±0.001	6.7220±0.002	0.1960±0.001	0.0023±3.000e-004
%CV	87.47	25.38	19.96	67.69	239.53	0.76	177.13	158.91
Kruskal Wallis statatistic	20.80	19.21	20.64	16.91	11.79	20.81	18.23	20.07
P-value	0.0225*	0.0377*	0.0237*	0.0765ns	0.2994ns	0.0225*	0.0286*	0.0512ns

Note: Significant difference assessed at p-values. * – P < 0.05; ns – not significant, P > 0.05.

Table S3. Results of the Spearman's correlation assessing the relationships between different nutrient contents and percentage of the leaves consumed.

	DM	ASH	EE	CP	NFE	NDF	ADF	ADL	Cellulose	Na	к	Са	Mn	Fe	% leaves consumed
DM	1.0000														
ASH	0.6105*	1.0000													
EE	0.0319	0.2273	1.0000												
CP	-0.4009	-0.2273	0.1545	1.0000											
NFE	-0.3007	-0.6636*	-0.1364	-0.3091	1.0000										
NDF	0.2916	0.1818	0.1545	-0.2909	-0.3455	1.0000									
ADF	0.3371	0.3545	-0.1545	-0.2727	-0.4455	0.5455	1.0000								
ADL	0.7153*	0.2636	0.1545	-0.2909	-0.1636	0.5364	0.0273	1.0000							
Cellulose	0.1549	-0.0273	0.0273	0.0636	-0.4636	0.7727*	0.5818	0.3818	1.0000						
Na	0.0868	0.4419	0.164	-0.5695	0.2096	-0.123	-0.082	-0.018	-0.5604	1.0000					
К	0.2484	0.3519	-0.6791*	-0.4659	-0.0198	0.0248	0.3569	-0.02	-0.0397	0.3378	1.0000				
Ca	0.0364	0.0364	0.3273	0.6455*	-0.1455	-0.6091*	-0.2636	-0.164	-0.3273	-0.2642	-0.5106	1.0000			
Mn	0.5571	0.2415	0.0364	-0.6378*	0.2597	0.3462	0.4237	0.3645	0.0638	0.2078	0.3031	-0.2323	1.0000		
Fe	0.589	0.6697*	0.2005	-0.6378*	-0.205	0.4647	0.6333*	0.2825	0.1731	0.3813	0.3825	-0.287	0.8174*	1.0000	
% leaves consumed	0.2141	0.2818	0.2636	0.0727	-0.1455	0.6273*	-0.1818	-0.155	-0.3818	0.123	-0.2131	0.6364*	-0.2278	-0.0547	1.0000

Note: Significant difference assessed at p-value. * – P < 0.05; significant variables are in bold.