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Full Length Research Paper

Practices of farmers in processing and marketing of crayfish in Akwa-Ibom State, Nigeria

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The study was undertaken to assess farmer's practices in processing and marketing of crayfish in Akwa-Ibom State, Nigeria. Ninety crayfish farmers systematically selected from two zones, four blocks and twelve circles formed sample for the study. An interview schedule was used to collect data while percentage, mean score and multiple regression were used in data analysis. Findings show that the majority (95.6%) of the respondents processed their crayfish by smoking, using fire wood (87.7%). On the average, they produced 12.52 bags (1bag=19 kg) of crayfish monthly and packaged/stored them in big cellophane (73.3%) and raffia (72.2%) bags. Greater proportion (81.1%) of the respondents sold their crayfish after processing to retailers (61.1%) at local markets (91.1%). They earned ₦169,000 (approximately 551 US Dollars) and made expenses worth ₦57,400 (approximately 187 US Dollars) monthly from crayfish business. Hence, their monthly profit was ₦111,600 (approximately 364 US Dollars). Eighty four percent of the respondents indicated dry season and specifically November (55.6%) as season and month of the highest sale of crayfish. Age (t= 2.372; p= 0.021) and quantity (bags) of crayfish processed in a month (t= 3.032; p= 0.003) were determinants of monthly income of the crayfish farmers. Inability to pay for labour during processing due to lack of cash (M=1.79) and having eye problem due to smoke from the open fire and backache due to prolonged bending down during smoking/processing of crayfish (M=1.78) were major challenges of the respondents in processing of crayfish. Unavailability of credit and competition from other crayfish marketers (M=1.62 each) were major challenges of the respondents in processing of crayfish. The study recommends that extension agents, researchers and business administrators should teach and boost the competencies of the farmers on modern ways of processing and marketing of agricultural products through government and non-government sponsored trainings and workshops. This will boost both quality of crayfish and agricultural products processed, marketed, consumed locally and possibly create opportunity for their exportation and more income.

Key words: Farmers, processing, marketing, crayfish, Akwa-Ibom State, Nigeria.

INTRODUCTION

Crayfish is one of the aquatic animals and a dominant decapods in many freshwater and even terrestrial habitats playing important community roles through their mobility, behaviour and omnivory. Their main habitats are cool or warm high quality streams and lakes, warm lower quality wetlands, semi-terrestrial swamps and temporary

wetlands (burrowers), and cave ecosystems (Reynolds et al., 2013). According to the authors, crayfish may tolerate broad temperature, dissolved oxygen and salinity ranges. Being an important crustacean consumed all over the world, they are usually prepared for consumption by smoking, and occasionally preserved by sun-drying. It is also a common delicacy in the diet among the people of the Southern Western Nigeria (Joseph, 2011). Crayfish may also be available at all the seasons, relatively cheap, affordable and suitable to supply adequate nutrients to cater for infants estimated daily nutrient requirements to eradicate protein energy malnutrition (PEM), in the developing countries (Joseph, 2011). It is a clean and very low carbohydrate food (Grace, 2010) and has a super healthy combination of nutrients from its almost pure form of protein to its healthy amount of omega-3 fatty acids which we now know are among the most beneficial fats we can eat (FAO, 2009). Meals containing crayfish play a great role in the development of humans in the world especially in the lives of people in the developing countries where other protein sources are grossly inadequate and comparatively costly (Nkang, 2014a). Experimentally, protein derived from crayfish and fish based diet is as good as that obtained from meat (Nkang, 2014b). Consumption of crayfish together with products of plants origin which are poor in some amino acids such as lysine and thiamine enables not only a complete utilization of plant protein, but also improves the content of the diet (Ele, 2014).

Crayfish key roles and attributes in ecosystems include indicators or surrogates for water quality, bio-indicators for communities or habitats, keystone controllers of trophic webs and ecological engineers. Protected crayfish may also act as umbrella species for the conservation of communities (Reynolds et al., 2013). They play important role in food chain by feeding on living and dead plants and smaller creatures/invertebrates as well as serving as food for fish and mammals.

They are normally harvested and processed from ponds and natural waters. The harvesting of crayfish entails farmers harvesting crayfish using canoe, traps and baits (Center for Environment Human Rights and Development (CEHRD), 2007). Use of baits is the most reliable method for harvesting crayfish (CEHRD, 2007). These baits are used in harvesting crayfish after, the baits are fixed between the nets and the nets are placed in the water which attracts the crayfish. After harvest, crayfish can be consumed fresh but they are perishable in this form and need to be preserved through processing especially when they cannot be consumed or sold immediately.

Fish processing refers to the processes associated with fish and fish products between the time fish are caught or harvested, and the time the final product is delivered to the customer (Swahn, 2009). Although the term refers specifically to fish, in practice, it is extended to cover any aquatic organisms harvested for commercial purposes, whether caught in wild fisheries or harvested from aquaculture or fish farming (Swahn, 2009). Crayfish are processed differently in various countries that use them because of their various needs. For example in countries like USA, China and Australia harvested crayfish are generally packed into open-mesh vegetable sacks for refrigeration, storage and transportation (Vance, 2009). Crayfish may be kept for hours in water for evacuation of food from intestinal tract before storage. This procedure has a dual effect of increasing the attractiveness of the product to the consumer while also increasing quality for storage and transport (Jose, 2002). In African countries like Nigeria, Ghana and South Africa crayfish are normally smoked, sun dried or salted (Adeosun, 2007). Smoking is the removal of most of the water from the flesh. It is also employed by the harvesters to reduce wastage due to decomposition. Crayfish are sometimes sun-dried, especially during the dry season which corresponds to the peak period for this fishery. They are usually spread on top of a mat for drying in the sun, or over an oven in a smoke house (Moses, 2013).

This crustacean has been used as a major source of income because of its high demand in the markets. Its market shifted from local consumption in rural areas to higher volume markets in cities such as Baton Rouge, New Orleans and beyond (Taylor, 2009). In the study area (Akwa-ibom State, Nigeria) crayfish has provided business and economic activities for the fishermen, crayfish dealers as well as consumers of crayfish (Enang, 2014). Crayfish, both industrial and artisanal, are major sources of both direct and indirect employment. This include crayfish capture/production, processing for local and export markets and jobs associated with gear sales/repair and cold storage facilities (Essuman, 2009). These and other value chain activities help to reduce post-harvest losses and boost economic returns from crayfish enterprise.

Unfortunately, crayfish farmers in rural areas still adopt old ways of crayfish processing which are unhygienic and affect the quality of the crayfish through microbial contamination (Nieland, 2004). Also, some farmers lack experience in the modern and proper way/method of processing (Teitze, 2014). Those living in rural areas normally use the same facilities that were used before in processing because of lack of fund to afford a new one. Farmers most times lack the proper storage facilities to

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store their processed crayfish which may lead to spoilage of the product (Njie, 2002), deterioration of crayfish during processing, heavy loss and poor yield (Teitze, 2014).

Aquaculture has been described as the fastest growing animal food-producing sector and to outpace population growth in the world (FAO, 2012). Unfortunately, crayfish are neglected compared to other aquatic animals because it is believed to yield low profit in terms of sales and it is usually called a poor man's business (Flake, 2007). The fishery sector (crayfish inclusive) is still characterized by rising import bills, low output, high post-harvest losses and the marketing methods used by traditional/local farmers that involves spreading of crayfish on the floor using raffia bags is un-hygienic and leads to spoilage of crayfish (Bassey et al., 2013).

Lack of availability of credit for crayfish marketers lowers efficient marketing and does not facilitate proper utilization of crayfish marketing resources and the adoption of crayfish marketing innovations (Oladapo et al., 2007). Poor market structure (instability), poor road network, and poor access to credit/finance are also factors that affect marketing efficiency. Government policies on the fish sector seem to be directed towards increasing production with little emphasis laid on marketing (Ukoha, 2003). Whereas the activities (harvesting, processing and marketing) are interlinked and driven by their forces in such a manner that a harvester/processor is motivated when there is ready market for his/her goods and vice versa.

One of the ways of improving the quality of agricultural products will be to carry out periodic investigation on the existing value chain activities (harvesting, processing, preservation, storage, marketing, etc.) in agriculture. This study will therefore provide relevant information on the activities of the farmers in processing and marketing of crayfish as well as determinants of income from the enterprise and challenges in processing and marketing of crayfish with a view to expose lapses/abnormalities that need to be tackled in order to bring/ improve efficiency in the sector and agriculture at large.

METHODOLOGY

The study area

The study was carried out in Akwa-Ibom State, Nigeria. The state has a population of 3,920,208 and a total land mass of 6,900 km^2 (National population commission (NPC), 2006). It is located in the coastal southern part of the country, lying between latitudes 4°32′N and 5°33′N, and longitudes 7°25′E and 8°25′E. The state is bordered on the east by Cross River State, on the west by Rivers State and Abia State, and on the south by the Atlantic Ocean and the southernmost tip of Cross River State (Ikono, 2016). This area is favourable for live-stock and fish production. Thus, most of the inhabitants are either full time or part time livestock/fish farmers. They produce different livestock like poultry, pigs, snails, rabbits, fish (crayfish, catfish, etc) and other aquatic animals. The state consists of thirty-one (31) local government areas and six agricultural zones namely Oron, Abak, Ikot-ekpene, Etinan, Eket and Uyo agricultural zones (Ikono, 2016).

Population and sampling procedure

All crayfish farmers in the zone constituted the population for the study. A multi-stage sampling technique was employed in selecting the respondents for the study. In stage one, two agricultural zones namely Oron and Eket were purposively selected from the six agricultural zones in the state.

In stage two, two blocks were purposively selected from each of these zones (Mbo and Okobo) from Oron (Ibeno and Onna) from Eket giving a total of 4 blocks.

In stage three, three circles were purposively selected from each of the blocks which are: Brahma clan, Uteffiong and Ibaka fron Mbo block, Ube-okobo clan, Ebighi-edu clan and Atabong clan from Okobo block, Iwokpom, Inuayerikot and Nkpanak from Ibeno block and Awa clan, Oniong clan and Nung ndem from Onna block giving a total of 12 circles.

In stage four, 8 crayfish farmers were purposively selected from each circle giving a total of 96 respondents for the study. The purposive selection done at each stage aimed at capturing areas and people who were more involved in catching, processing and production of crayfish in the state.

Data collection

Data for the study were collected from respondents through the use of structured interview schedule that were administered by the researcher and other research assistants to the respondents. The interview schedule contained relevant questions based on the objectives of the study. In order to elicit information on their processing activities, the respondents were requested to indicate processing practices they engage in crayfish production. For example methods of processing, equipment used in processing, reasons for processing, methods of storage/packaging, etc. They were also requested to indicate marketing activities that they do such as when, where, whom and how they market their crayfish as well as estimated income and expenditure per month from crayfish production. Determinants of income of respondents on crayfish enterprise were captured using multiple regression through examination of variables like: estimated monthly income in naira, marital status, primary occupation, sex among others.

Data on challenges respondents face in processing and marketing of crayfish were collected using a modified Likert-type scale of three points with responses as: "serious (S)= (2), not serious (NS)= (1), not at all (NA)= (0)". The values on the Likert-type scale were added up to get 3, which was divided by 3 to get a mean of 1.0. Any variable with a mean score higher or equal to 1.0 was regarded as a major challenge while variable with mean score less than 1.0 was regarded as minor challenge. Some challenges that were measured under processing include: lack of processing facilities and inadequate knowledge on processing while price fluctuation, poor infrastructure, loss of capital due to debtors were some of the challenges that were measured under marketing.

Data were analysed with percentage, mean score and multiple regression. The regression model is stated in explicit form as follows:

$$Y = \alpha + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_5X_5 + \beta_6X_6 + \beta_7X_7 + \beta_8X_8 + \beta_9X_9 + \beta_{10}X_{10} + U$$

where Y = income of crayfish farmers, α = constant term, $\beta_1 - \beta_{10}$= regression coefficients, $X_1 - X_{10}$= (independent variables), X_1 = age (years), X_2 =years spent in formal education (years), X_3 = sex (male = 1, female = 0) (Dummy), X_4= marital status (Married=1, not

married=0), X_5= primary occupation (farming=1, not farming=0), X_6= years of experience in marketing crayfish (years), X_7= household size (number), X_8= access to agricultural related information (yes=1, no= 0), X_9= extension agents visit in 2015 on agricultural matters, X_{10} = quantity (bags) of processed crayfish produced in a month (kg), and U = error term.

RESULTS AND DISCUSSION

Respondents' activities in processing of crayfish

Methods of processing crayfish

Entries in Table 1 show that the majority (95.6%) of the respondents processed crayfish by smoking; 35.6% processed by washing in salt water; while 20.0% processed by sun drying. These farmers may have relied mainly on smoking method probably because it gives crayfish a desirable taste and increases the consumer's desire or demand for it. According to Agwumba (2009), smoking of crayfish is the alternative method used when sun-drying is impossible because of the frequent rains during the rainy season.

Sources of heat energy for processing

Data in Table 1 also reveal that the majority (87.7%) of the respondents used fire wood while 38.9% used solar energy as sources of heat energy for processing of crayfish. Since majority of the respondents used fire wood as source of heat energy for processing, this practice may not be sustainable and ideal especially in this era of climate change and its negative impact that necessitates afforestation and deemphasizes indiscriminate felling of trees. Using modern and improved methods that saves time, reducing drudgery and use of energy efficient source like machines that can be operated with electricity or solar energy, may be better options. However, the finding agrees with Agwumba (2009) who stated that crayfish landings are smoked immediately when brought to shore; the process of smoking fish occurs by using fire wood in a smoke house.

Reasons for processing

Result in Table 1 also shows that 37.8% of the respondents processed crayfish in order to store them for a long time, 25.6% processed crayfish to increase market value and income while 24.4% processed crayfish to improve taste. Processing of crayfish in order to avoid deterioration and spoilage becomes inevitable when the product cannot be sold fresh or immediately after harvest probably due to lack of buyers and transportation facilities to move the product to bigger/urban markets where they can be sold. Through processing, handling of crayfish is easier, the shelf life is increased and crayfish is put into a form that is acceptable to the buyers/consumers.

Methods of storage/packaging of crayfish

Table 1 shows that majority (73.3%) of the respondents packaged/stored their crayfish in big cellophane bags, 72.2% stored in raffia bags, while 6.7% of the respondents stored in basins. This implies that some of the crayfish farmers still use old methods of storing/packaging of crayfish which may be detrimental to the product and human health. The use of plastic and laminated packaging bags to store crayfish is the most reliable method of packaging. This is because they are designed to prevent dehydration and oxygen penetration which invariably controls deterioration.

Quantity (bags) of crayfish produced in a month:

Result in Table 1 reveals that greater proportion (34.4%) of the respondents produced 11 to 15 bags, 27.8% produced between 6 and 10 bags, 24.4% produced 1 to 5 bags, 4.4% produced 16 to 20 bags, while 8.9% produced more than 20 bags of crayfish in a month. The mean number of bags of crayfish they produced in a month was 12.52 bags (1 bag = 19 kg).

Activities in marketing of crayfish

When crayfish is marketed

Data in Table 2 also reveal that the majority (81.1%) of the respondents sold their crayfish after processing, while 58.9% sold theirs' immediately after harvest. Most of the respondents may have sold crayfish after processing because processed crayfish appear to be durable, generally accepted by people and yield more returns.

Type of buyers/customers

Entries in Table 2 reveal that 61.1% of people that purchase the crayfish were retailers, 48.9% were wholesalers, 38.9% were consumers, while 4.4% were exporters. Retailers are in closer link with the consumers and sometimes with the farmers/producers. In a normal scenario, they play very crucial role in marketing of agricultural commodities by bringing agricultural products to the door step of immediate users, assessing and transferring their reactions about the product to the farmers for adjustment and taking feedback from farmers to consumers. Optimum/maximum utilization of this network/synergy will lead to efficiency in production, processing and marketing of agricultural products. In the case of crayfish, when this role is abused, purchasing of crayfish by retailers leads to uncoordinated market structure which is characterized by instability, exploitation

Table 1. Distribution of respondent based on their activities on processing of crayfish (Field Survey, 2016).

Variable	Percentage
***Method of processing crayfish**	
Smoking	95.6
Sun drying	20.0
Salting	4.4
Washing	35.6
Cleaning	5.6
Sorting	2.2
***Source of heat energy for processing**	
Sunshine	38.9
Oven	1.1
Firewood	87.7
Ebanda	17.8
***Reasons for processing crayfish**	
Improve taste	24.4
Prolong storage	37.8
Increase income	25.6
Increase market value	25.6
***Methods of storage/packaging of crayfish**	
Plastic containers	1.1
Polythene/cellophane bags	73.3
Sachets	1.1
Bags	72.2
basins	6.7
Quantity (bags) of crayfish produced in a month (a bag=19 kg)	**12.52**
1-5	24.4
6-10	27.8
11-15	34.4
16-20	4.4
>20	8.9

of crayfish farmers and low economic return for the farmers unlike selling directly to the consumers which ensures profit and a stable market price (Igwe, 2009).

Marketing venue

Entries in Table 2 reveal that the majority (91.1%) of the respondents sold crayfish at local markets, while 46.7% of the respondents sold at urban markets. This finding tends to suggest that farmers in the area have access to local markets than urban markets. This may be due to ignorance, lack/poor access road and lack of money for transportation of products to urban marketing sites. Selling at local markets may lead to further exploitation of crayfish farmers and may not afford them opportunity to interact with co-producers and buyers from different regions for exchange of information, technologies and innovations, especially those related to their enterprise.

Monthly income from crayfish enterprise

Data in Table 2 also indicate that 55.7% of the respondents earned below ₦ 100,000; 18.2% earned between ₦101,001and ₦200,001; 12.5% earned ₦200,002 to ₦300,002; 9.1% earned between ₦300,003 and 400,003; while 4.5% earned between ₦400,004 and ₦500,004. The mean monthly income of the respondents was ₦169,000 (approximately 551 USD). Although, this amount may not be up to the globally approved minimum wage per month, it may be said to be relatively high

Table 2. Distribution of respondents based on their activities in crayfish production (harvesting, processing and marketing) n=96 (Field Survey, 2016).

Variable	Percentage	Mean
***when crayfish is marketed**		
Immediately after harvest	58.9	
Immediately after processing	81.1	
After storage	1.1	
When cash is needed	2.2	
***Marketing venue**		
Local markets	91.1	
At home	1.1	
Place of work	3.3	
Urban market	46.7	
***Type of buyers/customers**		
Consumers	38.9	
Exporters	4.4	
Retailers	61.1	
Wholesalers	48.9	
Monthly income on crayfish enterprise		
<100,000	55.7	
100,001-200,001	18.2	
200,002-300,002	12.5	169,000
300,003-400,003	9.1	
400,004-500,004	4.5	
Monthly expenditure on crayfish enterprise		
<100,000	83.8	
100,001-200,000	13.8	57,400
200,001-300,000	1.2	
300,001-400,000	1.2	
Major expenses in marketing crayfish		
Rented shop	28.9	
Transportation	80.0	
Feeding	12.2	
Nylon bags	6.7	

*Multiple responses.

compared to what is obtainable among rural farmers especially in developing countries. For example average monthly income of melon farmers in Enugu Ezike Agricultural Zone Enugu Sate, Nigeria was ₦7,455.4 (approximately 45 USD) (Iwuchukwu et al., 2016).

Monthly expenditure on crayfish production

Data in Table 2 also reveal that majority (83.8%) of the respondents incurred below ₦100,000 expenses in crayfish production while respondents that spent ₦100,000 to ₦200,000 on crayfish production accounted for 13.8%. The mean monthly expenses on crayfish production was ₦57,400 (approximately 187 USD) implying that the respondents made profit of ₦111,600 (approximately 364 USD) from crayfish enterprise.

Expenses on marketing of crayfish

Data in Table 2 show that the majority (80.0%) of the respondents stated that their major expenses in marketing of crayfish was incurred in transportation. 28.9% of the respondent indicated rented shop while feeding was the major expense made by 12.2% of the

respondents in marketing of crayfish. This is supported by Ajiboye and Afolayan (2009) who stated that transportation is the major factor in marketing of fish and it is one of the expensive means in marketing crayfish. The author further stated that availability of transport facilities is a critical investment factor that stimulates the marketing growth of crayfish through increased accessibility, its efficiency and effectiveness.

Other marketing activities of the respondents

Strategies for marketing crayfish

Entries in Table 3 show that majority (70.0%) of the respondents indicated packaging while 62.2% indicated displaying in the markets as their strategies of marketing crayfish. Thus, respondents relied mainly on packaging and displaying of crayfish in the market as their marketing strategies. Tobor (2005) opined that packaging contributes in marketing crayfish by making the product appealing to the consumer and extending its shelf life. Regrettably, the crayfish farmers made little or no effort towards advertising crayfish which would have boosted the positive effect of packaging as it will create awareness on the availability of the product and attract more customers and hence more income.

Seasons of highest and lowest sales

Table 3 also shows that the majority (84.0%) of the respondents indicated dry season, while 16.0% of the respondents indicated wet season as the season for highest sale of crayfish. Fish/crayfish are not easy to harvest during wet season probably due to increase in the volume of natural water where crayfish are caught/harvested. Consequently, there may be reduction in quantity of crayfish harvested and marketed during wet season. On the other hand, crayfish farmers are likely to sell their crayfish faster with more profit during wet season because demand of the commodity may outstrip supply during this season. However, Idiong (2009) asserted that fish farmers attain the highest sales during wet season because they (fish) are hard to harvest in wet seasons and therefore scarce in the markets.

Months of highest and lowest sales

Entries in Table 3 show that greater proportion (55.6%) of the respondents indicated that they obtain their highest sales in November, while 27.8% of the respondents indicated December as the month for highest sales. November and December are among the “Ember months” which are regarded as months for preparation and celebration of Christmas respectively by Christians which dominated the study area. Also, these months mark end of the year and people all over the world shop during this time in preparation of the New Year celebration. Specifically in Nigeria, products like fish, crayfish and meat are sold easily and at a higher price during these months.

Data in Table 3 further show that greater percentage (41.1%) of the respondents indicated that August is the month of lowest sale, 21.1% indicated July, 18.9% indicated June, while 14.4% of the respondents indicated September as the month of lowest sales. These months mark period of rainy season in Nigeria where harvesting and even drying/processing of crayfish are difficult due to increase in size of water and shortage of heat energy either from sunshine or fire wood to dry or process the crayfish. So activities of crayfish catching/harvesting, processing and marketing are likely to be low during this time.

Mode of selling of crayfish

Entries in Table 3 further show that greater percentage (68.9%) of the respondents sold their crayfish by bargaining with purchasers, 47.8% sold crayfish on a fixed price, while 6.7% sold on an auction price. Most times, selling of agricultural products in developing countries like Nigeria does not involve fixing of price because prices of these products vary, depending on season and availability of the product in the market. Therefore, farmers bargain with buyers in order to market their goods. Bargaining in fish marketing is a type of negotiation in which the buyer and seller of a good or service debate the price and exact nature of a fish product. If the bargaining produces agreement on terms, the transaction takes place (Enang, 2014).

Determinants of revenue from crayfish enterprise

The regression result in Table 4 shows that there was a significant relationship ($F= 3.397$; $p<0.05$) between the socio-economic characteristics of the crayfish farmers and their monthly income from crayfish enterprise. Specifically, age ($t= 2.372$; $p= 0.021$) and quantity (bags) of crayfish processed in a month ($t= 3.032$; $p= 0.003$) were positively significant and influenced the monthly income of the respondents from crayfish enterprise. This means that change in age of farmers and quantity (bags) of crayfish processed/produced in a month will change monthly income of farmers from crayfish enterprise.

Age of the respondents positively influence their monthly income from crayfish enterprise agrees with findings of Anyawale and Oluwasola (2008) that state that age of farmers, all things being equal has a positive impact on crayfish enterprise size, earnings, ability to

Table 3. Distribution of respondents based on other marketing activities (n=96) (Field Survey, 2016).

Variable	Percentages	Mean
***Strategies for marketing crayfish**		
Advertising	3.3	
Packaging	70.0	
Displaying in the market	62.2	
Marketing in stalls	4.4	
Season of highest sales		
Dry	84.0	
Rainy	16.0	
***Month of highest sales**		
January	23.3	
February	8.9	
March	5.6	
April	2.2	
May	1.1	
June	4.4	
July	1.1	
August	14.4	
September	1.1	
October	3.3	
November	55.6	
December	27.8	
Month of lowest sales		
January	1.1	
April	1.1	
May	2.2	
June	18.9	
July	21.1	
August	41.1	
September	14.4	
October	5.6	
November	8.9	
December	4.4	
***Mode of selling crayfish**		
Bargaining	68.9	
Fixed	47.8	
Auction	6.7	

*Multiple responses.

take risks and adoption of modern innovation which they perceive to be capable of yielding higher income. The positive significant relationship between quantity (bags) of crayfish processed in a month and the monthly income from crayfish enterprise is expected because income of the respondents depend mostly on quantity of crayfish processed such that when more crayfish are processed, more bags of crayfish will be expected and hence more income. In corroboration, Nkang (2014a) stated that the number of bags of fish/crayfish sold in a month determines if the fish farmer is making a profit or loss for that month.

Number of years spent in acquiring formal education, sex, marital status, primary occupation, years of experience in marketing crayfish, household size, access to agricultural related information and number of times extension agents visited in 2015 on agricultural matters did not influence income from crayfish enterprise. This

Table 4. Factors influencing monthly income/revenue of crayfish farmers (Field Survey, 2016).

Variable	Coefficient	T-statistics	Probability
Constant	-	633	0.529
Age	0.328	2.372	0.021
Number of years spent in acquiring formal education	-0.152	-1.287	0.202
Sex	0.146	1.267	0.209
Marital status	0.123	0.949	0.346
primary occupation	-0.006	-0.062	0.951
Years of experience in marketing of crayfish	-0.138	-1.076	0.286
Household size	0.093	0.770	0.444
Access to agricultural related information	-0.042	-0.408	0.684
Number of times extension agents visited in 2015 on agricultural matters	-0.003	-0.026	0.980
Quantity (bags) of crayfish processed/produced in a month	0.324	3.032	0.003

R^2=0.333, $\overline{R}^2$=0.235, F-value=3.397, (P<0.05 so it's significant). *Significant.

Table 5. Mean scores of perceived challenges in processing of crayfish (Field Survey, 2016).

Challenges in processing of crayfish	Mean	SD
Lack of processing facilities	1.18*	0.86
Inadequate knowledge on processing of crayfish	0.52	0.71
Climate change effect on t processing of crayfish	1.43*	0.80
Poor road for transportation of product from harvesting to processing site	1.42*	0.71
Inadequate equipment for processing of crayfish	1.07*	0.74
Microbial contamination caused by use of old processing facilities	0.90	0.69
Drudgery associated with the task	1.49*	0.87
Inhaling of carbon monoxide during processing	1.65*	0.60
Undesirable odour that comes with crayfish during processing	1.43*	0.70
Reduction of the size of harvested crayfish after processing	0.65	0.68
Loss of money and property due to incessant fire incidents caused by smoking in open fire	1.76*	0.49
Inability to pay for labour during processing due to lack of cash	1.79*	0.52
Need to use costly mangrove in smoking crayfish to give the desired taste to it	0.72	0.93
Having eye problems and backache due to smoke from the open fire and prolonged bending down during smoking/processing of crayfish	1.78*	0.45
Lack of efficient modern processing facilities provided privately or by government	1.53*	0.82

*Major challenges.

implies that these variables did not add to the ability to predict the monthly income earned from crayfish enterprise in the study area.

Challenges respondents encounter in processing of crayfish

Entries in Table 5 reveal that major challenges of the respondents in processing of crayfish were inability to pay for labour during processing due to lack of cash (M=1.79), having eye problems (due to smoke from the open fire) and backache (due to prolonged bending down) during smoking/processing of crayfish (M=1.78), loss of money and property due to incessant fire incidents caused by smoking in open fire (M=1.76), inhaling of carbon monoxide during processing (M=1.65), lack of efficient modern processing facilities (M=1.53), drudgery associated with the task (M=1.49), undesirable odour that comes with crayfish during processing (M=1.43), climate change negative effect on the processing of crayfish (M=1.43), poor road for transportation of the product from harvesting to processing site (M=1.42), lack of processing facilities (M=1.18) and inadequate equipment for

Table 6. Mean scores of perceived Challenges in marketing of crayfish (Field Survey, 2016).

Challenges in marketing of crayfish	Mean	SD
Inadequate storage facilities of crayfish not sold after marketing	1.29*	0.83
Quality deterioration when not sold immediately and consequent reduction of price and income	1.15*	0.77
Poor infrastructure used for crayfish marketing	1.12*	0.88
Unfavourable government policies on crayfish marketing	1.44*	0.83
Lack of availability of credit for crayfish marketers	1.62*	0.78
High perishability of the product	1.21*	0.79
Huge competition from other crayfish marketers	1.62*	0.79
High bargaining and lack of purchasing power	1.49*	0.86
High price fluctuation	1.54*	0.84
Unsuitable position of market	0.92	0.93
Competition with other more valued aquaculture like fish	0.80	0.83
Undesirable odour of crayfish	1.33*	0.86
Dirtiness of business	1.44*	0.83
Attraction of flies and other undesirable creatures	1.61*	0.79
Lack of storage facilities	1.46*	0.87
Limited market outlets and poor marketing information	0.74	0.87
Loss of capital due to debtors	1.49*	0.86

processing of crayfish (M=1.07). The findings suggest that the respondents are still using old way of processing which involves use of fire wood to smoke or dry the crayfish for preservation. This method may be said to have aggravated other problems. For example, in using old method of processing crayfish, the farmer has to purchase firewood and employ people to help in processing crayfish because the task is stressful with other health implications/problems associated to it. When the farmer does not sell part of the harvested crayfish in fresh, he/she may lack cash to settle the wages of the labourers because the farmer is yet to market the crayfish after processing. Secondly, employment and payment of these labourers increase cost of production which can be handled/overcome by reducing the income of the farmers or increasing the price of the commodity in the market which may cause inflation. Also, when the fire used in smoking the crayfish is not properly monitored or managed it can lead to fire disaster where the crayfish and other valuable properties including cash can be lost. It is important to note also that the old method of processing crayfish using fire wood encourages deforestation and desertification that aggravate climate change and its negative impact on the universe and specifically on processing of crayfish as indicated by these respondents.

In support of the finding, Njie (2002) opined that rural farmers most times lack proper processing facilities which may be caused by lack of funds, while Silvia (2015) asserted that an open environment is an efficient way of smoking/drying fish to reduce fire outbreaks and reduce inhaling of carbon monoxide which is harmful to the processor.

Some factors that the respondents perceived as minor challenges to processing of crayfish include: inadequate knowledge on how to process crayfish (M=0.52), microbial contamination caused by the use of old processing facilities (M=0.90) and reduction of the size of harvested crayfish after processing (M=0.65).

Challenges respondents encounter in marketing of crayfish

Entries in Table 6 reveal that the major challenges of respondents in marketing of crayfish include: unavailability of credit for crayfish marketers (M=1.62), competition from other crayfish marketers (M=1.62), attraction of flies and other undesirable creatures by crayfish during marketing (M=1.61), high price fluctuation (M=1.54), high bargaining and lack of purchasing power (M=1.49), loss of capital due to debtors (M=1.49), lack of storage facilities (M=1.46), dirtiness of the business (M=1.44), unfavourable government policies on crayfish marketing (M=1.44), undesirable odour of crayfish (M=1.33), inadequate storage facilities for crayfish not sold after marketing (M=1.29), perishability of the product (M=1.21), quality deterioration when not sold immediately and consequent reduction in price and income (M=1.15), and poor infrastructure used for crayfish marketing (M=1.12). Marketing of crayfish, fish and other fish products in a developing country like Nigeria is not easy especially during rainy season even when they have been processed into dry form. This is because of poor/lack of adequate storage facilities to maintain or improve the quality of the product when they

cannot be sold easily or immediately due to glut in the market or other reasons. Sometimes when the facility is there, other factors like unstable power supply, lack of personnel and mismanagement of the facility may constrain the use of the facility. Consequently, when farmers rely on local method of marketing by exposing the goods and keeping the ones they could not sell in the bag for the next market, the quality of crayfish deteriorates especially when storage is prolonged. At this point crayfish may produce undesirable odour that will scare people and attract other bad creatures. The farmer may find it difficult to market the good and where possible at reduced price probably lower than the cost price. Worst still, when the buyer cannot pay cash at the point of purchasing the good or decides to pay part of the money or in bits or unable to pay at all, the farmer loses capital and income and may lose interest in the business especially when he lacks credit that will help to resuscitate the business. In line with the findings, Gittenger (2004) stated that despite the profitability of crayfish marketing, it has been on the decline due to the problems of lack of storage facilities, quality deterioration which results to price reduction and unavailability of credit for crayfish marketers, etc. Bassey et al. (2013) also noted that the fishery sector (crayfish inclusive) is still characterized by rising import bills, low output, high post-harvest losses and marketing methods used by traditional farmers that is the spreading of crayfish on raffia bags and on the floor is un-hygienic and leads to crayfish spoiling since it is highly perishable.

Some minor challenges in marketing of crayfish as enumerated by the respondents include: unsuitable position of market (M=0.92), competition with other more valued aquaculture like fish (tilapia) (M=0.80) as well as limited market outlets and poor marketing information (M=0.74).

CONCLUSION

Based on the findings of the study, the following conclusions were drawn: Majority of the respondents processed crayfish using old/ traditional method of smoking with logs of firewood. They stored processed crayfish in cellophane bag and sold them to retailers in the local markets. On monthly basis, the respondents realized ₦169,000 (about 551 US Dollars) and made expenses worth ₦57,400 (about 187 US Dollars) in crayfish business hence they made profit of ₦111,600 (364 US Dollars) from the enterprise. Age of farmers and quantity of crayfish processed/produced were the determinants of income from crayfish enterprise. The major challenges respondents encountered in processing were inability to pay for labour employed during processing and health issues (eye problems and backache). Unavailability of credit and competition from other crayfish marketers were constraints in marketing of crayfish.

RECOMMENDATIONS

Extension agents should teach and encourage crayfish farmers to discard old method of processing crayfish using firewood and embrace new and improved processing technologies so as to reduce drudgery and consequent health challenges associated with old processing method, improve quality of processed crayfish and maintain stable ecosystem that will discourage desertification and climate change. Where crayfish farmers cannot afford new processing technologies, government and non- government agencies should subsidize the cost or supply them as incentives to these farmers.

Government and other stakeholders in fishing, ought to develop market information and marketing infrastructure to enhance more market accessibility of crayfish products. They should also sponsor trainings and workshops geared towards building capacities of farmers especially crayfish farmers on marketing. These trainings and workshop should be anchored by business administrators and extension agents. The emphasis will be on marketing strategies like packaging, advertising, sourcing of information on marketing, ideal market for selling of agricultural products etc. Knowledge and skills gained from these expositions will boost the competencies of the farmers on marketing of agricultural products for higher quality and income.

Favourable financial policy that will help farmers especially crayfish farmers to access credit in form of loan and over draft at low interest rate should be made by policy makers, while Central Bank of Nigeria will ensure compliance by all the banks especially agriculture banks. In this way, these farmers can have money to settle expenses in crayfish business, purchase good processing equipment, store their products when there is poor market or glut in the market and transport their products to urban market for quicker sale and more income.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Effect of modified spacing arrangements, fertilizer use and legume intercrop on prevalence of cassava brown streak disease in Western Kenya

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Cassava is an important food security crop in Western and Coastal counties of Kenya. As a food security crop, it is continuously cultivated with minimal inputs. Its production is constrained by factors like declining soil fertility, poor agronomic practices, pests and diseases. Cassava brown streak disease (CBSD) is a viral infection attacking the cassava crop causing yield losses of up to 100%. The current study was intended to determine the effect of planting technologies on the prevalence of CBSD in two agro-ecological zones of Western Kenya: lower midland (LM1) and upper midland (UM1). Various spacing arrangements, four fertilizer regimes, legume intercrop and improved cassava cultivars were tested in a randomised complete block design (RCBD) with each site as a replicate. Data was collected on pathogen population and disease incidences and severity, and cassava and legumes yields. Results showed no effect of modified spacing and legume intercrop on CBSD incidence. However, incidences varied by cassava cultivar (9 to 59%) and fertilizer application (3 to 41%). Low CBSD incidences (3 to 16%) were observed over time in management strategies involving fertilizer NPK 17:17:17 suggesting that vigour enhancement may have contributed to low CBSD incidences. Low incidence of CBSD on improved cultivars indicates that CBSD can be mitigated through crop improvement technologies such as breeding for resistance to diseases. Intercropping cassava with beans and modification of spacing did not demonstrate an effect on CBSD incidence. However, 2 m× 0.5 m spacing arrangement can compensate for rising land pressure in Western Kenya and areas facing similar problem.

Key words: Cassava, cropping arrangements, intercrop, yields, cassava brown streak disease (CBSD), Western Kenya.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is widely cultivated in Asia, Africa and Southern and Central America. Sub Saharan Africa (SSA) is the world's largest cassava producer and its production is estimated at 57% (160 million tonnes) of total cassava produced worldwide. Major cassava producers in Africa include Nigeria, Democratic Republic of Congo, Ghana, Angola and Mozambique, (FAOSTAT, 2015). In East Africa, Tanzania, Rwanda and Uganda are the major producers of cassava. Kenya's annual cassava production is relatively low at 0.8 million tonnes (FAOSTAT, 2015; MoA, 2015) and supports the livelihood of 2.5 million

people in eastern, coastal and western regions of the country (MoA, 2012). Low yield in Kenya is due to declining soil fertility (MoL, 2009; Anneke et al., 2010; Mutoko et al., 2014), lack of high yielding cultivars (Mwango'mbe et al., 2013), poor agronomic practices, pests and diseases (Braima et al., 2000; Munga et al., 2012). Cassava brown streak disease (CBSD) is an important disease prevalent in Eastern, Central and Southern Africa and is caused by two species of *Ipomovirus*; cassava brown streak virus and Ugandan cassava brown streak virus (Mbanzibwa et al., 2011; Winter et al., 2010). The disease is spread by planting infected cuttings (Storey, 1936; Mohammed et al., 2012) and whiteflies (*Bemisia tabaci*) within the field (Storey, 1939). Various management strategies including evaluation of germplasm in different agro-ecological zones and breeding for resistance have been attempted with limited success. Since CBSD was first reported in Northern Tanzania (Storey, 1936), the disease has been endemic to the East African coast (Storey, 1936; Hillocks et al., 2001). However, with less restrictive quarantine measures (FAO, 2013), the disease has now spread to other East African countries (Legg and Boumeester, 2010) including Uganda, Kenya and Burundi and even in areas beyond the coastal strip where the disease is endemic (Alicai et al., 2007). In Western Kenya, Mware et al. (2009) reported disease incidences of between 64 and 100% with a severity score of 2 to 3 on a scale of 1 to 5 in Bondo and Teso areas of Siaya and Busia Counties, respectively. Osogo et al. (2014) reported disease incidences of 30% in Busia County with severity score of 2 to 4.

An effective method of controlling diseases in cassava plants is the use of resistant cultivars. Improved Cassava Mosaic Disease (CMD) resistant cultivars are available and widely cultivated in Western Kenya. Unfortunately, the CMD resistant cultivars have not been consistent in their tolerance to CBSD (Obiero et al., 2010). Efforts to mitigate CBSD by developing tolerant cultivars are ongoing (Ogwok et al., 2012; Vanderschuren et al., 2012; Rwegasira and Chrissie, 2012; Abaca et al., 2013; Woyengo et al., 2013), meanwhile farmers continue to plant susceptible cultivars.

In Western Kenya cassava is grown during the first two months after the onset of the long rain (March to June) and short rain seasons (August to November). The crop is usually propagated by stem cuttings and stays in the field for one year hence receiving two peak periods of rain during its growth cycle. Propagation by stem cuttings enhances disease build up in the subsequent cropping cycle (Yadav et al., 2011). Crop protection practices such as intercropping (Boudreau, 2013), efficient use of nutrients, and planting disease-resistant cultivars (Anneke, 2010; Fairhurst, 2012) can contribute to disease mitigation (Giller et al., 2011), improved crop productivity (FAO, 2013) as well as help attain food security (Agegnehu et al., 2008; Dietrich et al., 2012).

The main objective of this study was to determine the effect of modified spacing arrangements, fertilizer use and legume intercrop on prevalence of cassava brown streak disease in Western Kenya.

MATERIALS AND METHODS

Experimental sites

This on-farm study was conducted in Busia (Mundika N00° 06' 44.154", E034° 27' 16.794"), Kakamega (Elwakana N00°16' 46.44", E34° 33' 15.24"), Vihiga (Demesi N00°6' 20.04", E34° 44' 24.64") and Bungoma (Lutacho N00° 40' 26", E034° 48' 58") counties of Western Kenya. Altitude ranged between 1330 and 1611 masl. The selected sites are characterised by bi-modal rainfall distribution which allows for crops to be grown twice a year; during the long rains (March to June) and the short rains (August-November).

Experimental design

A randomized complete block design (RCBD) was adopted with each site being a replicate. The gross plot size of experimental plots was 4 m × 7 m and the net plot size was 3 m × 3 m. Plots were separated by 1-m wide pathways for accessibility.

Experimental treatments consisted of three cultivars of cassava: MM96/4271 (NASE 14) and Migyera (TMS 30572) which are resistant to CMD and Merry Kaluore as a local susceptible cultivar. Improved cultivars MM96/4271 and Migyera were intercropped with a grain legume (bean, *Phaseolus vulgaris* L.) commonly grown (cultivar KK8) in the experiment sites and three fertilizer applications: NPK 17:17:17, NPK 17:17:17+K, and NPK 17:17:17+N+K were used (Table 1).

MM96/4271 and Merry Kaluore were planted in mono crop (plots 1 and 2) using 1 m × 1 m spacing to compare the effect of using improved (CMD resistant) and local cassava cultivar under non-fertilized condition.

Spacing arrangement was modified to 2 m × 0.5 m where cultivar MM96/4271 was planted without intercrop (plot 3) and with intercrop (plots 5, 6 and 8). The most recent improved cassava cultivar Migyera was planted in plot 7 using NPK 17:17:17+K to compare the effect of planting improved cassava cultivar under near optimal mineral fertilization.

One kilogram (equivalent to 200 kg/ha) of fertilizer NPK (17:17:17) was spread evenly in three plots (5, 6, 7 and 8). Plots 6, 7 and 8 also received 1 kg each of Murate of potash (KCl). In addition, one plot (plot 8) received 1 kg of CAN (Table 1). All the inputs were incorporated into the soil by shallow digging with care not to cross to neighbouring plots.

The trial was established in September, 2014 at the onset of rain

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Table 1. Treatments used in planting technologies trial.

Plot number	Management strategy			
	Cultivar	Spacing (m^2)	Fertilizer	Intercrop
1	Merrykaluore	1 × 1	No	No
2	MM96/4271	1 × 1	No	No
3	MM96/4271	2 × 0.5	No	No
4	MM96/4271	2 × 0.5	No	KK8
5	MM96/4271	2 × 0.5	NPK 17:17:17	KK8
6	MM96/4271	2 × 0.5	NPK 17:17:17+K	KK8
7	Migyera	2 × 0.5	NPK 17:17:17+K	KK8
8	MM96/4271	2 × 0.5	NPK 17:17:17+N+K	KK8

Figure 1. Picture showing cassava-bean intercrop arrangement.

in the short rain season. Fresh cuttings of about 20 cm were planted by burying most of the cassava cutting at an angle. Three rows of beans were planted in between cassava rows at spacing of 50 cm between rows × 10 cm between plants (Figure 1).

The rows of beans were reduced to two during the long rain season (April) of 2015 and were planted 75 cm inwards from the cassava rows to cater for the cassava canopy.

Field selection and land preparation for the on-farm trials and maintenance of the trial were done by the farmers. Planting was done by farmers, agriculture extension officers and researchers.

Scoring pests and diseases

Data on pests, diseases and other relevant agronomic parameters such as emergence and plant height was collected on cassava. Damage by important cassava pest like Cassava Green Mites (CGM) and by diseases such as Cassava Anthracnose Disease (CAD), Cassava Mosaic Disease (CMD), Cassava Brown Streak Disease (CBSD) and Cassava Bacterial Blight (CBB) (IITA, 1990; Legg et al., 2006) was calculated as a proportion:

Disease incidence = Number of infected plants / Total Number observed × 100

Percentage of green mite infested plants was calculated as follows:

Percentage of infested plants = Number of plants with CGM damage / Total Number observed × 100

Severity for both pests and diseases was scored on a scale of 1 to 5 where 1 represents no symptom and 5 represents severe infection/infestation (Hahn et al., 1980; Muimba, 1982; Muyolo 1984; Legg and Bouwmeester, 2010) at 3, 6 and 9 months after planting.

Total count of whiteflies (*B. tabaci*) per plot was recorded between 0600 and 0800 h when the insects are fairly immobile (Ariyo et al., 2005).

Yield data

At harvest, all roots in the net harvestable area were harvested and counted. The harvested roots were sorted into two groups; marketable roots and non-marketable roots. Marketable roots are those roots that can be sold while non-marketable roots, cannot be sold and include small roots, roots damaged by pests or harvesting implements and rotten roots. Fresh weight for both marketable and non marketable roots was recorded and used to calculate fresh cassava yield in tons per hectare.

Yields for beans were recorded at harvest maturity but before shattering. Seed weight was taken after seeds had been threshed, winnowed and dried to moisture content of 14% as recommended

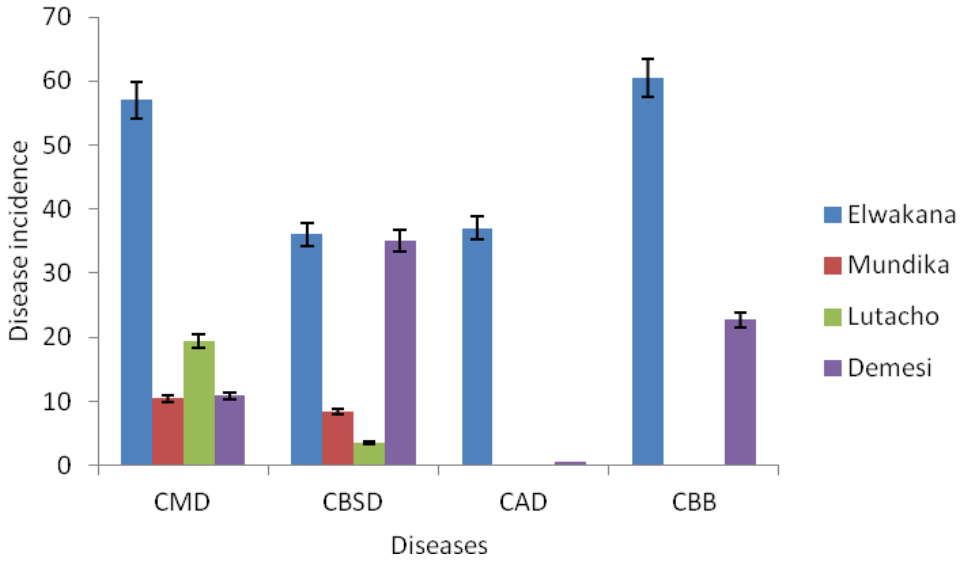

Figure 2. A bar graph showing other diseases observed on cassava across sites.

by Ogutu et al. (2012).

Statistical analyses

Generalised linear models (GLM) procedure of the SAS package version 9.1 was used to compare means by testing for effect of site, modified spacing, fertilizer use and cultivar on CBSD prevalence, cassava fresh yields and legume yields. Least significant difference was used to separate significant difference at p=0.05.

RESULTS

Pests observed on planting technologies trial

Mean whitefly populations per plot for the cropping period ranged between 1 and 14 across sites (Lutacho < Demesi < Mundika < Elwakana) but were similar for the three cassava cultivars and fertilizer levels. Whitefly populations on individual plants ranged between 0 and 62 as follows: Lutacho (0-6), Mundika (0-27), Demesi (7-30), and Elwakana (10-62).

Whiteflies are important pests since they transmit CBSD; however CGM was also found to be prevalent at Mundika (16.7%), Demesi (20.8%) and Elwakana (66.6%) sites. Improved cultivars Migyera and MM96/4271 had more CGM infested plants (45.3 and 27%, respectively) compared to the local cultivar Merry Kaluore which had 1%. The highest number (50%) of CGM infested plants was recorded in plots where NPK 17:17:17 + KCl + CAN fertilizers were used. The 2 m × 0.5 m cropping arrangement recorded more plants with CGM infestation per given time of evaluation than 1 m × 1 m cropping arrangement.

Diseases observed in planting technologies trial

Disease incidence varied across sites ($p<0.05$**, Mean≤60%) with CMD, CBSD occurring in all sites. Disease incidences were the highest at Elwakana site (CBB 60%, CMD 57%, CAD 37%, CBSD 36%) followed by Demesi (CBSD 35.1%, CBB 22.7% and CMD 10.9%). Mundika and Lutacho sites had low (<19%) disease incidences (Figure 2).

CBSD symptoms observed on planting technologies trials

Foliar symptoms of CBSD were observed across sites. Mundika and Lutacho sites had the lowest foliar incidences of CBSD (<11%), while Demesi and Elwakana had the highest incidences (36 to 50%). All the four sites had CBSD severity score of 2.

The only root symptom observed on all cultivars across sites was root necrosis. Cultivar Merry Kaluore had the highest number (mean) of damaged roots (13) followed by improved cultivars MM96/4271 (6) and Migyera (2). The severity score for root necrosis for Merry Kaluore was 3 across sites, while MM96/4271 and Migyera had root severity score of 2. Demesi site was not evaluated for root necrosis, because the farmer harvested early.

Effect on different planting technologies on CBSD incidence

Although statistically, there was no significant difference

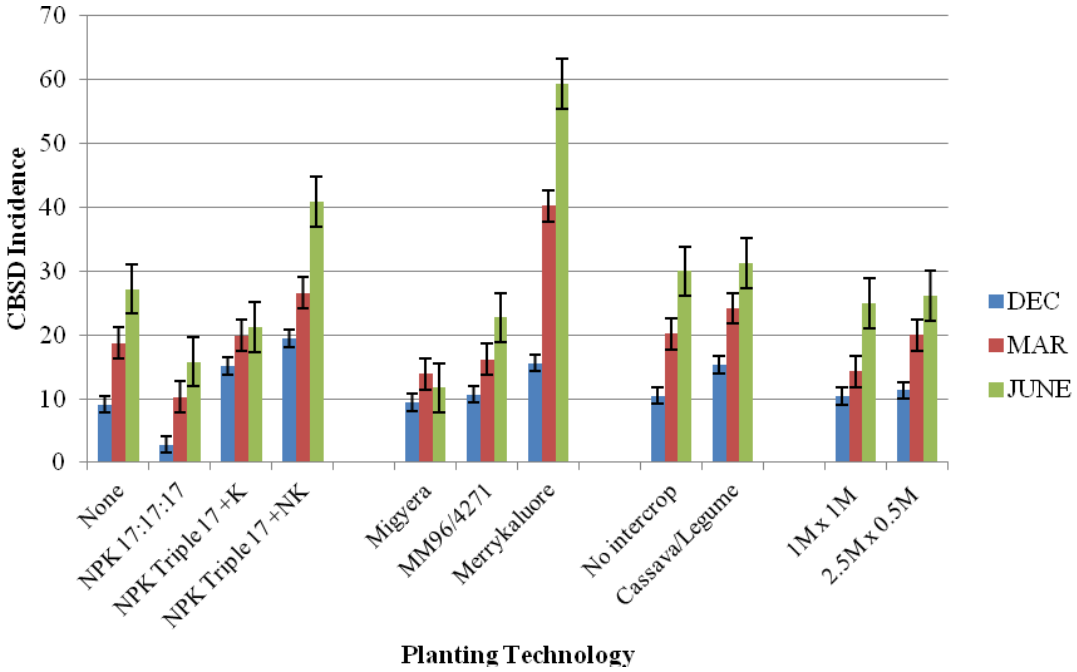

Figure 3. CBSD incidence in relation to planting technology.

(p>0.05) in incidence of CBSD in planting technologies involving modified spacing and legume intercrop, it varied for planting technologies involving improved and local cassava cultivar and also fertilizer application (Figure 3).

CBSD incidence in relation to cassava cultivar

Disease incidence varied by cultivar from 9 to 59%. At 9 months after planting (MAP), the local cultivar (Merry Kaluore) had high incidence of CBSD (59%) while the two improved cultivars; MM96/4271 and Migyera had low CBSD incidences of 23 and 12%, respectively (Figure 3). The severity score was 2.

CBSD incidence in relation to fertilizer application

Low CBSD incidences ranging between 3 and 16% were observed over time for management strategies involving fertilizer NPK 17:17:17. During the cropping period, the incidence of CBSD did not vary (15 to 21%) for fertilizer application NPK 17:17:17+ K, while for NPK 17:17:17 + N + K, disease incidence ranged between 20 and 41% (Figure 3).

CBSD incidence in relation to spacing arrangement

Disease incidence did not vary by spacing. For spacing arrangement of 1 m × 1 m, CBSD incidence ranged from 10 to 25%, while for 2 m × 0.5 m spacing arrangement it ranged from 11 to 26% across sites (Figure 3).

CBSD incidence in relation to intercropping

Disease incidence on leaves did not vary for cassava-bean intercrop (15 to 31%) or for cassava monocrop (11 to 30%) (Figure 3). Root symptoms of CBSD observed on cassava planted in cassava-bean intercrop at Lutacho and Elwakana sites had severity score of 2 and 3, respectively.

Effect of planting technologies on cassava yields

Cassava yields for panting technolgies tested ranked in ascending order were: Mundika (2.4 to 9.4 tons/ha)>Lutacho (6.1 to 16.9 tons/ha)>Elwakana (15 to 29 tons/ha). For management strategy where fertilizer NPK 17:17:17 and KCl were used, Elwakana site had higher (22.5 tons/ha) cassava yields than Mundika (5.3 tons/ha) and Lutacho (6.1 tons/ha) sites. Mundika had the lowest (4.7 tons/ha) cassava yields for cassava intercropped with beans. Cassava planted in 2 m × 0.5 m spacing yielded higher (mean 13.2 ton/ha) than in 1 m × 1 m (mean 9.3 tons/ha) spacing. At Elwakana, planting technology involving improved cultivar MM96/4271 planted using fertilizer NPK 17:17:17, CAN and KCl had the highest yields of 29 tons/ha (Table 2). Grain yields for beans intercropped with cassava cultivar Migyera were

Table 2. CBSD incidences and yield of cassava planted using different planting technologies across sites.

Site	Fertilizer regime			Intercrop		Spacing	
	NPK 17:17:17	NPK 17:17:17 + KCl	NPK 17:17:17 + CAN + KCl	No intercrop	Intercrop	1 m × 1 m	2.0 m × 0.5 m
Mundika	8.6 (10.3)	5.3 (16.6)	9.4 (26.5)	2.4 (26.4)	4.7 (26.9)	8.8 (15.6)	2.4 (15.3)
Elwakana	17.5 (16.3)	22.5 (21.2)	29 (40.9)	20 (30.1)	17 (31.2)	15 (25.2)	20 (26.9)
Lutacho	10.9 (2.8)	6.1 (15.4)	11.3 (19.7)	16.9 (10.9)	15 (15.3)	11.8 (9.5)	16.9 (10.9)

Figures in brackets are CBSD incidences.

higher (1.2 t/ha) than for those intercropped with MM96/4271 (0.8 tons /ha).

DISCUSSION

The application of chemical fertilizers has been reported to have an effect in controlling pests and diseases to a reasonable extent (Ezulike and Ugwu, 2005; Amtmann et al., 2008; Satti, 2012). Plant vigour enhancement due to fertilizer use has been explained as the cause behind ability of plants to withstand pests and diseases (Neuenschwander et al., 1990; Satti, 2012). The effect of NPK fertilizer application on pests and disease incidences and severity in the trials varied depending on site and cultivar. High incidence of CGM at Demesi and Elwakana sites could be attributed to the spread of the pest from neighbouring cassava field (Yaninek, 1989) since green mites are specific to *Manihot* species (Kogan et al., 1999). Improved cassava cultivars had higher percentage of CGM infested plants than local cultivar Merry Kaluore suggesting that the mites have preferences for some cultivars.

Use of NPK 17:17:17 and NPK 17:17:17 + KCl fertilizers resulted in decrease in incidence of green mites. Similar findings of decrease in green mite pressure due to NPK fertilizer application were reported by Anneke et al. (2010).

More plants were infested with CGM in spacing arrangement of 2 m × 0.5 m than 1 m × 1 m spacing. Plants in 0.5 m plant to plant space were very closely knit to each other. This could have contributed to ease of the movement of pests from plant to plant.

One of the major constraints to production of cassava, in sub-Sahara Africa is cassava brown streak disease (Winter et al., 2010; Bigirimana et al., 2011). The disease is transmitted by whiteflies (*B. tabaci*) and use of infected cuttings. Whitefly is the most important vector for cassava viruses and therefore whitefly populations affect CBSD incidence (Thresh and Otim-Nape, 1994; Maruthi et al., 2004). Whitefly population per plant ranged between 0 and 62 and varied by site (Lutacho < Demesi < Mundika < Elwakana). Mundika and Lutacho sites had low CBSD incidence (<11%). Low incidence of CBSD could be associated with low virus pressure because whiteflies must be viruliferous to transmit the virus (Polston and Capobianco, 2013). High CBSD incidences (36 to 50%) at Demesi and Elwakana sites are associated with high virus pressure (Mware et al., 2009; Legg et al., 2011).

Although plants were being evaluated for CBSD, other diseases infecting cassava were also assessed. High incidence of CMD at Elwakana site was associated with influence of whiteflies migrating from infested neighbouring cassava fields, which concurs with the findings of Uzokwe et al. (2016). Disease incidence varied across sites ($p<0.001$, Mean≤60%). High disease incidences at Elwakana and Demesi sites could be due to high whitefly populations while low incidences (≤19%) at Mundika and Lutacho are associated with low whitefly populations.

Local cultivar Merry Kaluore had the highest number of CBSD damaged roots per plot than improved cultivars (MM96/4271 and Migyera). Disease expression (necrosis) in root begins as small yellow/brown corky patches which increase in size and number as the plant grows. In sensitive cultivars, the continued increase in corky patches eventually covers the entire root (Hillocks, 2004). The study findings concluded that Merry Kaluore is among the cultivars that are susceptible to CBSD infection and risks total yield loss due to root necrosis.

Low CBSD incidence was observed in plots where fertilizer NPK 17:17:17 was used. Although the use of fertilizer does not control cassava diseases, its use is more rational when disease-resistant (improved) cultivars are used as they

are more responsive to fertilizer application than local cultivars (Anneke et al., 2010; Fairhurst, 2012). Plant vigour enhancement due to fertilizer may have contributed to low disease incidence. Disease incidence did not vary by spacing or where cassava was intercropped with beans. This implies that CBSD cannot be managed by varying the spacing of cassava crops.

Yields for beans intercropped with cassava cultivar Migyera were higher than those intercropped with MM96/4271. Migyera is characterized by an open growth habit which allows for light to penetrate to the ground, while MM96/4271 has compact growth pattern which intercepts light.

Cassava planted using 2 m × 0.5 m spacing arrangement yielded higher (13.2 t/ha) than cassava planted using 1 m × 1 m spacing arrangement (9.3 t/ha). According to FAOSTAT (2015) report in Kenya, average cassava yield for cassava planted in 1 m × 1 m monocrop is 13.5 t ha^{-1}. The insignificant change in cassava yields as well as the increased bean yields is an indication that cassava can be intercropped with beans using 2 m × 0.5 m cropping arrangement without negatively affecting the cassava yields. Similar findings by Pypers et al. (2011) indicated that cropping arrangements of 2 m × 0.5 m increased grain legume yields without negatively affecting cassava yields.

Cassava cultivar MM96/4271 planted using fertilizer NPK 17:17:17 + CAN + KCl had the highest yield of 29 ton/ha. This suggests that the cultivar could be more responsive to fertilizer applied than Migyera and Merry Kaluore (Fairhurst, 2012).

Conclusion

Planting technologies such as fertilizer use, modification of spacing and intercropping cassava with legumes have no effect on prevalence of CBSD. Low incidence of CBSD on improved cultivars indicates that CBSD can be mitigated through crop improvement technologies such as breeding for resistance to diseases.

Contrary to earlier recommendations that cassava should be established at onset of rain (Toro and Atlee, 1980; Ekanayake et al., 1997), the study found that this may be applicable to cassava pure stand cropping and not in cassava-legume intercrop arrangements.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Use of house cricket to address food security in Kenya: "Nutrient and chitin composition of farmed crickets as influenced by age"

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House cricket is currently introduced for scaled-up production in farming systems in Kenya and other parts of the world, as an alternative source of animal proteins. The aim of this study was to assess the nutritional composition in farmed cricket as influenced by age in order to ascertain the optimal harvesting time for possible utilization of crickets in improving child nutrition in Kenya. Sampling was carried out between weeks 4 and 13. The moisture content was analysed by drying method, chitin by sodium hydroxide digestion, protein content by estimation of total nitrogen, crude fat by soxhlet extraction method, ash by muffle furnace incineration, available carbohydrates by subtraction, and energy by calculation method. The crude protein mean ranged from 36.00 to 60.00 g/100 g, chitin 2.20 to 12.40 g/100 g, total lipids 12.00 to 25.00 g/100 g, over the 13 weeks period. Minerals concentration was optimum at week 9, with magnesium 1.30 to 11.30 mg/100 g, calcium 1.40 to 19.70 mg/100 g, and zinc 0.20 to 16.60 mg/100 g. Findings from this study indicate that farmed cricket would be best harvested between weeks 9 and 11, when the protein and mineral content is optimum. Nutrients available in farmed crickets show that farmed crickets can be used in child food ingredients to improve child nutrition.

Key words: Farmed crickets, proximate, protein, fatty acid, omega 3, omega 6, minerals, child nutrition.

INTRODUCTION

Food security in Kenya is still a challenge and there is need for agriculture diversity to help Kenyan population get sufficient food (Mburu et al., 2016; Kimiti et al., 2016). Food and nutrition security affects most countries in the world and is directly linked to other problems such as human health and poverty (Pritchard, 2016).

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Millions of children in the world are currently affected by acute malnutrition (Bliss et al., 2016; Bartz et al., 2014; Black et al., 2013), with half of the deaths in children below 5 years being caused by under nutrition or nutrition related complications (Black et al., 2013). High prevalence of malnutrition, characterised by stunting early in life has been demonstrated in the Kenyan population by studies done recently, and lack of proteins is rated high in Kenyan children (Kimani-Murage et al., 2015; M'Kaibi et al., 2015). There has also been increased cases in morbidity and mortality due to poverty and food insecurity (Cohen et al., 2015).

While the major source of protein for human consumption is plant and animal proteins (meat, pork, fish, milk and eggs) which is rated higher in quality, and therefore preferred by many people (Ertl et al., 2016; Martens et al., 2013; Cook and Monsen, 1976). These animal proteins are however expensive and not easily available (Van Huis, 2013). The high cost of animal protein is partly contributed by increasing demand for animal protein directly attributed to sharp population rise (Konyole, 2014). This has prompted search for alternative sources which include insects.

Insect consumption has been practiced among Kenyan communities since ancestral times, and more so, when there was food insufficiency (Evans et al., 2015). In the recent years, there is great interest in insect consumption all over the world, since they have been found to contain high nutrient content (van Huis, 2015; Halloran et al., 2015). The only difference in the past and present in relation to edible insects, is that in the past, insects as food was considered a hobby where people in few communities would go to the forest to collect them when the season allowed for non-commercial home consumption compared to the recent times where they are increasingly sold in the markets and on the streets (Durst and Hanboonsong, 2015).

In Kenya, use of insect for food is an ancient tradition in many communities, and currently a more sort after delicacy in the western region of Kenya, (Ayieko et al., 2012; Kinyuru et al., 2009; Christensen et al., 2006). People are used to collecting insects in the wild, this was largely carried out by women and children, and insect farming is thus a new idea among the Kenyan population (Rumpold and Schlüter, 2013; Kinyuru et al., 2012; Bukkens, 1997).

Insects have a varied biochemical composition of both macronutrients and micronutrients (Finke, 2016). Crickets in particular, offer a highly economical protein source (Caparros Megido et al., 2016), and therefore, sustainable solution to existing and looming issues of malnutrition. Better nutrition can be achieved by production and distribution of high quality protein (Kelemu et al., 2015). To help meet growing demands of quality nutrition as the world population grows, insects are good for children especially undernourished children, due to high protein and fatty acid content (Acosta and Fanzo, 2012), and plenty of minerals such as iron, selenium, copper, magnesium and zinc. Cricket farming is a feasible venture and has demonstrated success in other countries for example in Thailand (Hanboonsong et al., 2013).

Cricket rearing requires simple locally available raw materials, which includes egg trays, which is importance if the local community will adopt rearing of crickets in an easy affordable way. Currently, cricket farming is still under small-scale production in most parts of the world (Halloran et al., 2016), but people are slowly adopting to cricket rearing largely for cash at the local markets and this is why there is increased interest in cricket rearing.

Cricket rearing has been shown to be economical in water and feed consumption, since they consume little water and food. Cricket rearing is also time efficient and an environmentally safe way of alternative protein generation as they produce less greenhouse gases (Caparros Megido et al., 2016). Crickets have a high feed conversion rate, therefore providing high quality nutrients, which in turn are cheaper, efficient and environmentally sound protein source when compared with traditional livestock protein sources, which are expensive to maintain (Payne et al., 2015). Crickets could therefore be used to curb food insecurity and malnutrition in developing countries. For this to happen, they should be incorporated in the day to day diet. However, to improve widespread utilization, they have to be reared in large-scale and in a sustainable manner in order to supply a wider population (Van Huis et al., 2015).

The aim of this study was to assess the nutritional and chitin composition of farmed cricket as influenced by age in order to ascertain the optimal harvesting time for maximum nutrient content in an effort to incorporate them in the diets to improve child nutrition. Crickets' rearing was preferred for this study since crickets are easier and cheaper for mass produce.

METHODOLOGY

Cricket production

This study was carried out at Jomo Kenyatta University of Agriculture and Technology (JKUAT). The rearing was carried out in simple fabricated cages that mimicked the crickets natural environment (Hanboonsong, 2013; Loranger and Bertram, 2016). The nymphs were fed on 21 g/100 g protein feed, commercially available as a starter diet. After two weeks, the diet was alternated with commonly available vegetables and weeds such as pumpkins leaves, cassava leaves, morning glory leaves, black jack and sukuma wiki depending on the availability of the green leaves since crickets are known to be herbivores (Lundy and Parrella, 2015). At four weeks, the 21 g/100 g protein commercial feed was replaced with 14 g/100 g protein commercial feed and supplemented with green leaves for their life span.

For this study, one pen was used to breed crickets under the same structure to minimize variations in environmental conditions

and feeding. All crickets were fed on the same diet and maintained under similar conditions with average temperature of 25°C throughout the study period.

Sample collection and preparation

Sampling was done every week from week four to week 13. Three independent samples were collected weekly on days 1, 3 and 5 of the week. One kilogram of harvested cricket yielded three samples which formed samples representing the week.

After collection, the crickets were frozen at -20°C overnight, then washed in tap water and rinsed in distilled water. The crickets were then oven dried at 50°C for 72 h. When the crickets were dried with moisture content 4.00 to 6.00g/100g, the crickets were crushed to very small fine particles (250 mm), to get the cricket powder. When 1 kg of the harvested crickets was oven dried, approximately 400 g of dry weight was obtained. The cricket powder was stored at -20°C and during analysis only the required amount was taken from the freezer. All analysis was carried out in triplicate.

Proximate analysis

Standard methods for proximate analysis were adopted in this study. To determine crude proteins, total nitrogen obtained by micro-Kjeldahl method was used; percentage nitrogen was multiplied by 6.25 N factors to obtain crude protein content in crickets (AOAC, method 990.03). Crude fat was obtained by Soxhlet extraction method (AOAC, method 920.39). AOAC, method 940.28 was used to determine fatty acid profile, and calculation of omega 3 and 6 fatty acid in the crude fat.

Ash determination was done by 550°C muffle furnace incineration (AOAC, method 920.48). To determine the available minerals in the sample, AOAC method 984.27 was used. Mineral analysis was determined by dry ashing and measured by flame atomic absorption spectrophotometry (Shimadzu 6300 AAS AA/AE Spectrophotometer) (AOAC, 2005). Quantification of minerals was done using commercial standards (Sigma-Aldrich Chemie, Steinheim, Germany). In-house controls were also used by using a vacuum packed controlled sample stored at -20°C.

Available carbohydrate content was calculated from the proximate values by subtraction method. Energy levels from the proximate analysis, was obtained by Energy content = (Crude protein × 4) + Crude fat × 9) + (Available carbohydrate × 4) (AOAC, 2005).

Chitin extraction

To extract chitin from crickets, 150 g of ground cricket powder was placed in 1000 ml beaker containing 4% boiling sodium hydroxide and the mixture was boiled for 2 h for the powder to undergo deproteinization to remove sugar and protein components (Toan, 2009; Lertsutthiwong et al., 2002). The content was allowed to cool for 30 min, followed by centrifuging at 10000 revolutions per minute, for 10 min to separate the solid part containing chitin from the liquid part containing sodium hydroxide, sugar and proteins components (Galed et al., 2005).

To dissolve minerals from the centrifuged residue, 600 ml of 1% hydrochloric acid was added and the residue placed in a rotor shaker (Sanyo electric company limited, Japan) rotating at 40 rpm for 24 h at room temperature (Trung et al., 2006). The content was then subjected to centrifugation and the liquid part disposed while the remaining residue was treated with 500 ml of 2% boiling sodium hydroxide for 1 h to decompose albumin (Puvvada et al., 2012). The content underwent centrifugation and the liquid part that contained albumin was discarded. The brown solid residue was washed three times with boiled distilled water to remove the remaining polysaccharides and sodium hydroxide, and then dried at 70°C for 60 h after which the extracted chitin was kept in zip lock bags at -20°C until use.

Amino acid analysis

Approximately, 2.00 g of the cricket flour sample was used for amino acid analysis. The sample was defatted using methanol before hydrolysing using 6 M hydrochloric acid. Amino acid analysis was done using ion exchange chromatography, the hydrolysed sample was injected into the amino acid analyser (Technicon Instrumentation Corporation, Dublin, Ireland.), for separation and characterization of amino acids, using the Technicon sequential multi sample amino acid analyser (Ertingshausen et al., 1969).

RESULTS

Figure 1, shows the nutrient and energy content of cricket based on harvesting age. Cricket which were four weeks old were chosen as the starting point for cricket harvesting since crickets which were less than four weeks old were too small to be handled, and this would mean harvesting so many crickets to obtain the required mass. Protein content was on a staggering rise from week 4 to 11 and dropped at weeks 12 and 13. The protein content in cricket increased as the cricket aged, though the increase is not linear and maximum protein content was seen between week 9 (58.30 g/100 g), week 10 (59.74 g/100 g) and week 11 (60.40 g/100 g). The rise in total lipids is slow when compared with cricket protein content rise (Figure 1), the lowest lipid content was seen at week 13 (12.00 g/100 g), while the highest was at weeks 9 and 10 at 25 g/100 g. Cricket chitin content was on steady rise as the crickets aged and at week 13 chitin content was at maximum 12.33 g/100 g, of the total cricket content. Ash content was almost constant across all ages with approximate composition of between 4.00 g/100 g and 5.00 g/100 g content; there was no defined rise in ash content. Initially, available carbohydrate was high at 39.23 g/100 g at four weeks, then drops drastically to 14.60 g/100 g at week 6, before increasing again at week 7 (Figure 1). Energy levels were high between 1827.03 kJ/100 g at week 4 and rose to 1807.49 kJ/100 g at week six. Optimum energy content is seen at week nine, 1774.02 kJ/100 g.

Table 1 shows mineral content in farmed crickets; mineral content starts at a higher level and slightly drops as the crickets aged and finally rose at weeks 11, 12 and 13. There is no defined rise or drop in mineral contents: magnesium optimum levels of 12.00 mg/100 g was seen at week 12, calcium 11.33 mg/100 g at week 13, copper 10.67 mg/100 g at week 12, iron at 0.04 mg/100 g at week 13, and zinc 19.33 mg/100 g at week 11.

Figure 2 outlines the major fatty acid fractions of cricket

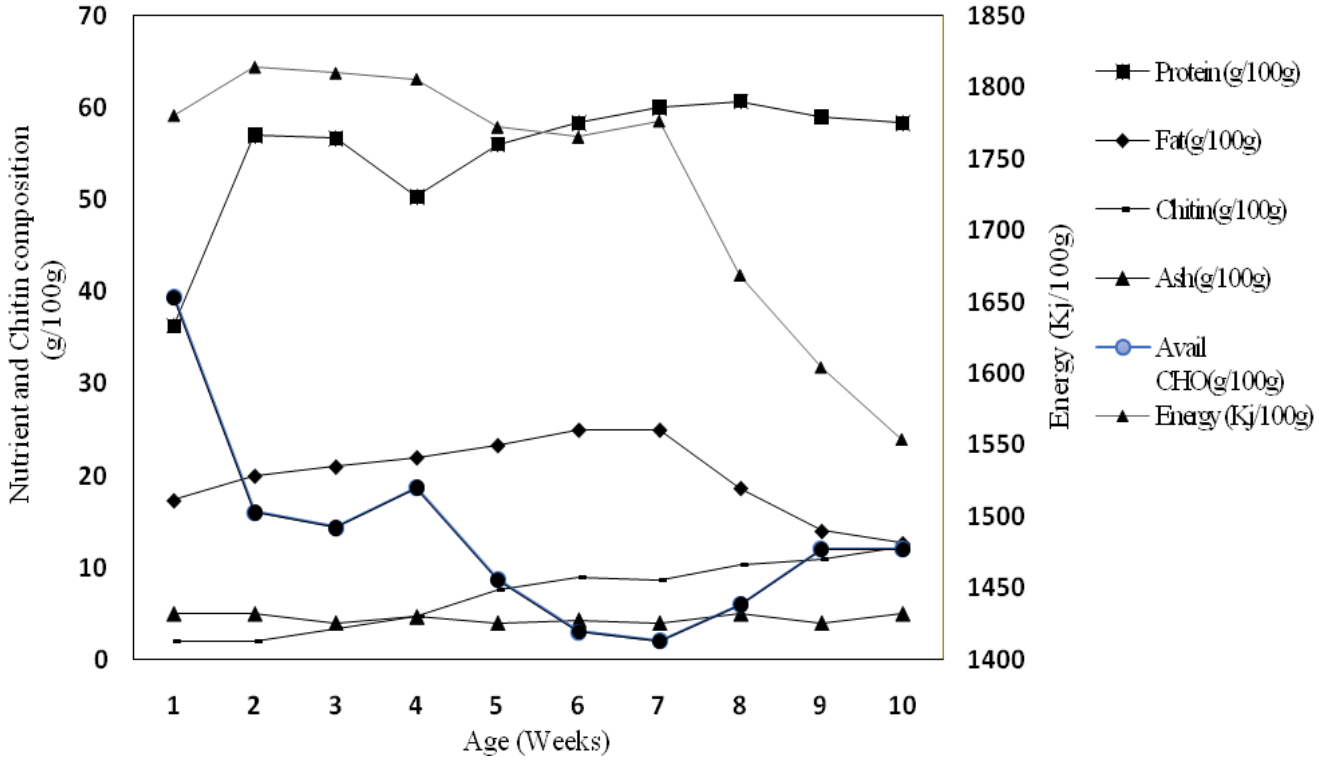

Figure 1. Proximate,chitin and energy content of crickets harvested at different ages.

Table 1. Mineral composition (mg/100 g) of cricket harvested at different ages.

Age in weeks	Magnesium	Calcium	Copper	Iron	Zinc
4	11.33	5.67	8.67	0.03	15.00
5	9.00	2.67	3.67	0.01	12.33
6	10.33	1.67	9.33	0.02	17.00
7	9.33	1.67	6.00	0.01	15.00
8	9.00	1.33	4.67	0.03	14.67
9	9.67	11.00	5.67	0.03	15.33
10	9.33	1.67	4.33	0.01	14.33
11	9.33	2.00	5.67	0.02	19.33
12	12.00	8.00	10.67	0.03	14.67
13	9.67	11.33	5.33	0.04	15.67

oil, which clearly show that cricket fat is rich in good fats which includes omega 6 and 3. Crickets had polyunsaturated fatty acids (PUFAs) ranging from 2.00 to 5.00 g/100 g, the PUFAs increased from week 4 to reach a maximum of 5.00 g/100 g at week 7 and drops again to 3.00 g/100 g at week 13 (Figure 2). Saturated fatty acid (SATU's) increased as the cricket aged from 48.29 g/100 g at week 4 to 63.88 g/100 g at week 6 and then drops to 46.12 g/100 g at week 13. Mono-unsaturated fatty acid (MUFAs) increased from week 4 to 12 and slightly reduces at week 13.

Table 2 shows the fatty acid composition of cricket oil at different ages. When cricket fatty acid profile is further grouped into omega 3 and 6 and the ratio between omega 6 and 3 calculated, the ratio ranged from 0.33 to 2.40.

Table 3 shows the amino acid content of cricket protein in mg/g protein. Out of the total amino acids, 13 amino

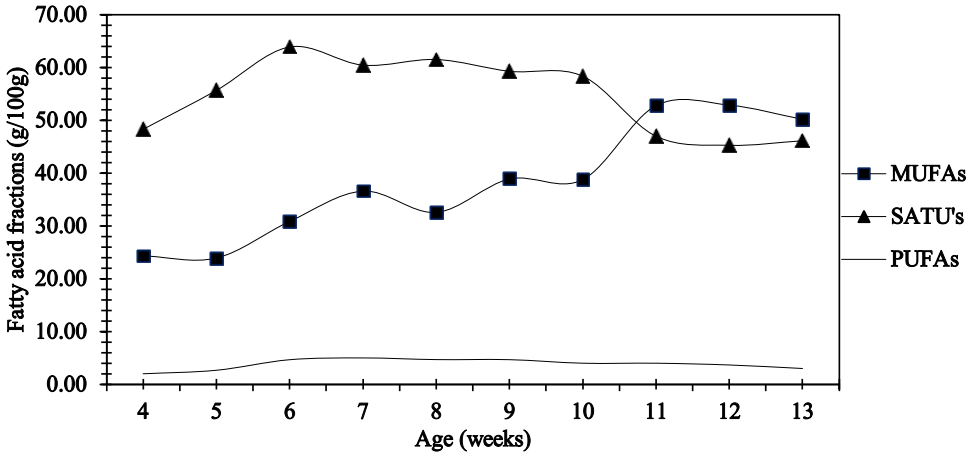

Figure 2. Fatty acid fractions in cricket oil with age in weeks. SATU's, Saturated fats; MUFAs, monounsaturated fats; PUFAs, polyunsaturated fats.

Table 2. Fatty acid composition of cricket oil at different ages.

Age (weeks)	Fatty acid g/100 g							
	Palmitic acid	Oleic acid	Linoleic acid	Linolenic acid	Arachidic acid	EPA	DHA	Omega 6/Omega 3 ratio
4	18.05	23.95	2.08	1.00	1.00	1.00	1.00	1.04
5	18.74	23.00	6.40	1.00	1.00	1.00	1.67	2.40
6	19.96	29.58	5.97	1.00	1.00	1.33	3.33	1.28
7	32.19	35.39	1.63	1.00	1.00	2.00	3.00	0.33
8	28.15	31.26	5.47	2.00	1.67	2.00	2.68	1.17
9	24.97	37.45	8.30	2.00	1.33	1.67	3.00	1.78
10	25.04	37.06	5.00	3.00	2.00	1.00	3.00	1.25
11	27.78	51.17	3.00	3.00	1.33	1.00	3.00	0.75
12	28.00	51.52	3.00	3.00	1.33	1.00	2.67	0.82
13	26.69	48.89	3.33	3.00	2.00	1.00	2.00	1.11

Table 3. Amino acid contained in cricket protein in mg/g protein.

Compound	Amount	Recommended mg/g protein (3-10) years
Histidine	ND	16.00
Arginine	67.23	-
Lysine	ND	48.00
Glutamine	ND	-
Serine	ND	-
Glutamic acid	ND	-
Proline	15.83	-
Valine	12.47	40.00
Methionine	11.32	22.00
Tyrosine	11.32	22.00
Isoleucine	ND	31.00
Leucine	17.27	61.00
Phenylalanine	13.68	22.00

(Ricardo et al., 2015).

acids were analysed and 7 out of the 13 had detectable values.

DISCUSSION

This study has demonstrated that house cricket has high protein content up to about 60.67 g/100 g of the total nutrient content, based on daily value in 8368.00 kJ diet for children above four years and adults (Krauss et al., 2000; Mann and Truswell, 2012); 100 g of cricket serving per day would provide the required 50 g protein. Since the crickets were fed on locally available diets, it is clear that the high protein content can be achieved by local farmers who would like to adopt the simple diet and this makes cricket rearing for protein supplementation a venture worth exploitation in Kenya and other part with similar setups as Kenya. Most children suffer from malnutrition due to insufficient or lack of proteins and therefore crickets provide an opportunity to help these children.

The results correlated well with other studies that have shown high protein content in insects (Kinyuru et al., 2015; Collavo et al., 2005). Since protein content declined in weeks 12 and 13, it would be of importance to consider weeks 9, 10 and 11 have the optimal time for cricket harvesting if the target is to get the highest protein content. In a study carried out by De Foliart et al. (1982), in the earlier decades, crickets were found to contain high protein content of 54.00 g/100 g, this study has demonstrated that cricket protein content can be higher than 54 g/100 g.

Crude fat content of between 12.67 and 25.00 g/100 g in house cricket, is within the range observed by Narzani and Sarmah (2015) where crude fat content in insect seemed to vary from 1.00 g/100 g to 40.00 g/100 g (Narzari and Sarmah, 2015). If children would be given 100 g serving of crickets per day, the crude fat content would provide about 38% of the total daily requirements (65 g/100 g). Other studies have shown low fat content in crickets, when compared with fat content in termites (Payne et al., 2015). Low fat content in cricket would be of importance for industrial exploitation of cricket, since high fat content hinders procesability and shelf life of food products, hence reduce its mass production and market potential (Kinyuru, 2014). Crickets therefore demonstrate high market potential and extended shelf life when compared with termites in relation to the overall fat content and their inclusion in food to supplement protein and fat especially in complementary food.

Insects are known to contain chitin as the major fibre, and therefore, in the current study, chitin was quantified from crickets. Chitin content in insects has been found to range from 4.00 g/100 g to 21.00 g/100 g (Finke, 2015). In the current study, the chitin content ranged between 2.00 g/100 g and 12.00 g/100 g which is the lowest at the tender age and increases as cricket age. This would be the highest contributor of hardened wings as the crickets' age increase. Cricket harvested at weeks 12 and 13 with the highest chitin content were hard to process since the cuticle was rigid and therefore the powder obtained was rough 510 mm as compared to 250 mm in weeks 4 to 11. Rough cricket flour would greatly affect the end products made from cricket powder and therefore it would be better to harvest crickets before too much hardening of the cuticle between weeks 9, 10, and 11. Chitin also did not contribute to energy content and the content rises, while the energy content declined, and this is why at weeks 12 and 13 the energy content was low.

Ash content was below 10.00 g/100 g in the current study, recent studies on insects have indicated that the ash content in insect is less than 10.00 g/100 g (Rumpold and Schlüter, 2015). In the past, studies have demonstrated that crickets having varied in nutrient content (Belluco et al., 2013), with content of available carbohydrates ranging from 1.00 g/100 g to 47.00 g/100 g (Narzari and Sarmah, 2015). In the current study, carbohydrate content was within this range, and as the age increased, the carbohydrate levels steadily decrease over time especially when protein levels and chitin content increased. At week 7, there is a drop in most nutrients which seem to be the lowest at this age; it would be of importance for future scientist to find out if the drop has any correlation with start of egg laying in cricket.

Previous studies have shown insects to be energy dense with up to 2418.35 kJ/100 g of energy (Finke, 2013). The energy content fluctuates, which is attributed majorly to the fluctuations in other proximate contents. The energy levels in cricket started off at a high level and when fat content drops the energy content also drop. As much as the energy contents drops, the drop is not much since the overall energy content ranges from 370.01 kJ/100 g and 1807.49 kJ/100 g. This study shows crickets are energy dense food source, and can easily be an option to curb malnutrition in energy deficient children in developing world where malnutrition rates are still high (Black et al., 2013), as long as the crickets are harvested at the right age to ensure a balance of proximate nutrients, minerals and energy.

Cricket mineral composition shows that crickets are not just energy dense food, they are also good for curbing high mineral deficiency in Kenyan children, since past studies have indicated high mineral deficiency in children (Duong et al., 2015). The data obtained from this study compared well with other studies that have analysed mineral content of crickets (Rumpold and Schlüter, 2015; Payne et al., 2016; Taufek et al., 2016).

Crickets oil contains lauric acid 1.5% which is found in breast milk and coconut oil and is converted to monolaurins which protects children from disease (Dayrit, 2015). Crickets contain myristic acid 3% which is also contained in cow milk (Mazhitova et al., 2015). Poly

unsaturated fatty acid (PUFAs) content in crickets is an advantage especially in human nutrition since PUFAs in diet has been linked to anti-obesity and increased activity in children (Średnicka-Tober et al., 2016). Cricket meal would provide higher percentage of saturated fatty acid at 46 g/100 g when compared with 33 g/100 g in beef and 34 g/100 g in chicken (Ton et al., 2015), while PUFAs was at 5 g/100 g in crickets which is the same as PUFAs percentage in beef (Ton et al., 2015; Średnicka-Tober et al., 2016). Supplementing children diet with cricket would give the children the needed lipids which would ensure more health and increased activity in children.

Oil containing omega 3 and 6 fats has been rated positive in child's diet. The oil found in cricket has omega 3 and 6 fatty acid which clearly indicates that feeding children with cricket would boost their good fat content. Past studies have indicated that fatty acid is more desirable when the ration of omega 6:3 is lower or almost equal to one (Yang et al., 2006); human beings are believed to have evolved on omega 6:3 balanced diet of 1:1 (Oddy et al., 2004) and increase ratio of omega 6:3 promotes obesity in children (Simopoulos, 2016). Growing children need sufficient amounts of omega 3 and 6 (Brenna et al., 2015). Therefore, cricket meal is important if adopted to feed children to improve child nutrition.

There is need for higher protein content in children to be able to meet the required amino acid patterns. Malnourished children require slightly higher protein intake, to enable them get required amino acids (Ricardo et al., 2015). Since cricket contain high protein content, its use in improving child nutrition is likely to boost the amino acid requirements in moderately malnourished children. Farming of crickets can be achieved in drought stricken areas, because cricket requires small amount of space and water. Since drought is a major constrain in Kenyan agriculture (Huho and Mugalavai, 2010), exploitation of crickets can address food security.

CONCLUSIONS AND RECOMMENDATIONS

The optimum age for farmed cricket harvesting would be between week 9, 10, and 11, when the protein, total lipids and mineral content is optimum. At this age chitin content is slightly lower hence better procesability and finer flour which would greatly enhance the end product made from cricket. Cricket omega 6 to 3 ratio makes cricket oil good for child nutrition. This study recommends the rearing of crickets and the use of cricket in child nutritional interventions for its high protein content, which is often the major cause of child under nutrition.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Records of sub family Scelioninae (Hymenoptera: Platygastridae) from oriental region with description of one new species

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A new species from genus *Cardalnamannus* and re-description of species from genus *Encyrtoscelio* Dodd (Hymenoptera: Platygastridae) is being described, which were collected by sweet net collection from the state of Uttarakhand, India. The species *Cardalnaumannus ramamurthyum* sp. nov. is described as new species and re-description of species *Encyrtoscelio apterus* (Szelényi) for the first time from Uttarakhand, India.

Key words: Encyrtoscelio, New species, Uttarakhand India.

INTRODUCTION

The genus, *Cardalnaumannus* was enacted by Mineo et al. (2011) with species, *Gryon amleticus* and classified in the new tribe Dyscritobaenini of the Family Platygastridae. The genus is reported only from Australia with only one species, *Gryon amleticus*. The host and biology is not known (Mineo et al., 2011). The other genus, *Encyrtoscelio* was enacted by Dodd with type species *Encyrtoscelio mirissimus* Dodd. Recently, the genus was keyed by Lê (2000), Rajmohana (2006) and Kononova and Kozlov (2008). The present study described *Cardalnaumannus ramamurthyum* sp. nov. with re-description of *Encyrtoscelio apterus* (Szelényi) from Oriental region for the first time.

MATERIALS AND METHODS

Specimens were collected during the course of the survey programme, during the months of October 2011 and March 2012 in and around Pantnagar (Uttarakhand) area. Morphological terminology follows Masner (1979; 1980), Johnson and Masner (1985) and István et al. (2007). Antenna, wings and legs were mounted in Canada balsam after overnight immersion in 10% KOH and exposure to 70, 80 and 99% ethyl alcohol and clove oil. Photographs of wings were taken with the help of Leica Live Image Analyzer set up developed by Olympus. Scanning electron microscopy (SEM) was done on Jeol JSM6610LV/A/LA (Japan optical electrical limited) after 24 nm thick palladium coating in a JFC1600 Sputter Coater (Japan optical electrical limited) at 6 x 10^{-2} mbar; and images were taken at 23-24 Pa, between 150 and 370x.

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All images were processed in Adobe Photoshop 7.0. The types, *C. ramamurthyum* and *E. apterus* are temporarily retained in the Entomological Museum, G. B. Pant University of Agriculture & Technology, Pantnagar and will be deposited in the National Pusa Collection, Division of Entomology, Indian Agricultural Research Institute, New Delhi (NPC) shortly.

RESULTS

Description of species

***Cardalnaumannus ramamurthyum* sp.nov.**

Holotype: One female specimen was examined; body length: 0.83 mm; forewing: length 0.68 mm; width 0.42 mm; hind wing: length 0.10 mm; width: 0.028 mm; body: yellowish to deep brown/black; eyes: brown; radicle: light yellow; antennae: yellow except clava dark brown; mandibles: dark brown; mesosoma and metasoma: black; fore wings: infuscate; hind wings: hyaline with veins dark brown; coxae: dark brown, femur: brownish, last tarsomere: dark brown. Head: transverse with imbricate/areolate sculpture length slightly wider than long in frontal aspect (Plate 1: 1 and 2); frontovertex width: 3.0x; the total head length (34.0:11.1); preoccipital carinae distinct and praeoccipital area pass sharply over the occiput; ocelli are arranged in acute triangle; lateral ocelli 4 to 5 mm away from inner eye orbits, they either border the vertex or are some millimiters distant from it; scrobe is present or indicated by a smooth area, usually not margined by keel; POL 1.69x longer as OOL; OOL : POL : LOL= 6.8:11.5:7.4. Compound eyes are medium in size and densely pubescent; antennal toruli is situated well below the lower eye margin; occipital carina is complete; genal carinaeis absent; frons without a depression; frons width > eye height > malar space (21.9:15.6:11.4).

Antenna (Plate 1: 3): 12 segmented, with 5 segmented clava; scape is 5.3x as long as wide; antennal segments in relative proportions (length: width) from scape: 17.0:3.2, 4.5:2.6, 2.3:2.5, 2.8:1.6, 2.2:2.9, 1.7:3.6, 1.6:4.2, 2.9:4.8, 1.9:5.3, 2.9:5.4, 2.5:4.1, 4.1:3.3. Mesosoma (Plate 1: 4): is 1.32x longer than wide (28.5:21.3); skaphion is absent; epomial carina is absent; mesoscutum and scutellum with rich sculpture may be areolate rugose, without notauli; metanotum bulges medially to give rise to the dorsellum, with pubescence; prepectus not prominent. Legs are smooth; fore tibial spur is long, curved and bifurcated. Fore wings (Plate 1: 6) are lanceolate, with complete venation; distal sections of both subcostal and marginal veins strongly downcurved before reaching the linkage point; 2.76x as long as wide (11.6:4.2); 1.1x longer than hind wing length; marginal fringe long; SMV 8x longer than MV; proportions of (length) SMV:MV:PMV:STG; 4.8:0.6:1.3: 1.1. Hind wings: 3.75x as long as wide (10.5:2.8) with blunt apex; SMV is complete. Metasoma: 1.63x longer than its greatest width (40.3:24.0); elongate; T1 with striations on one side; T2 striate but not reaching posterior half; specillum is prominent.

Male: Not known.

Holotype: Female dissected and mounted on slide. India, Uttarakhand, Pantnagar, sweepnet collection forests areas, 23-x-11, Hym. platy. Nr. KA15, coll. Kalmesh.

Paratype*:* Nil

Etymology: Named after Dr. V. V. Ramamurthy for his outstanding contribution to insect taxonomy in India.

***Encyrtoscelio apterus* (Szelényi)**

Pachyscelidris aptera (Szelényi, 1941: 163) by monotypy and Original description: Synonymized by Encyrtoscelio apterus (Masner, 1957: 306).

Female: Body length is about 0.69 mm; body: black; eyes: brown; radicle: yellow; antennae: dark brown, except scape and pedicel light brown; mandibles: dark brown; mesosoma and metasoma: black; wings: absent; coxae: black, femur and tibia: brownish; last tarsamere: dark brown.

Head (Plate 2: 1 and 2) length: 1.12x wider than long in frontal aspect (30.3:34.2), reticulate: sculptured; frontovertex length is 2x the total head width (16.4:32.1); ocelli: arranged in acute triangle; compound eyes are large and lightly pubescent; antennal toruli: situated well below the lower eye margin; occipital carina complete; 3-4 distinct genal carinae present; frons width > eye height > malar space (25.3:13.3:11.8); mandibles: very long, 1.13x long as malar space (13.4), wide, blunt at apex and sharpened on inner side and forming a 'U' shape by both mandibles. Antenna (Plate 2: 3): 12 segmented; scape is 5.3x as long as wide, 8x as long as radicle; antennal segments in relative proportions (length: width) from scape: 16.1:3.4, 5.0:3.3, 5.1:3.5, 4.1:3.8, 4.0:3.7, 3.6:3.4, 3.6:3.9, 4.4:4.0, 4.5:4.2, 4.4:4.3, 4.1:4.3, 7.6:3.6. Mesosoma (Plate 2: 4): 2.1x wider than long (33.2:27.6); skaphion is absent; mesoscutum and scutellum with reticulate sculpture, without notauli; metanotum posteriorly unarmed. Legs are smooth, fore tibial spur is long, curved and bifurcated. Fore wings and hind wings: absent (individuals are apterous). Legs are smooth, tibial spur is short (Plate 2: 5 and 6). Metasoma (Plate 2: 1 and 5): 1.63x wider than its greatest length (31.6:19.3); metasoma plump, with same sculpture as that of mesosoma.

Male: Not known

Holotype: Female dissected and mounted on slide. India, Uttarakhand, Kiccha, sweepnet collection forests areas, 03-iii-12, Hym. platy. Nr. KA33, coll. Kalmesh.

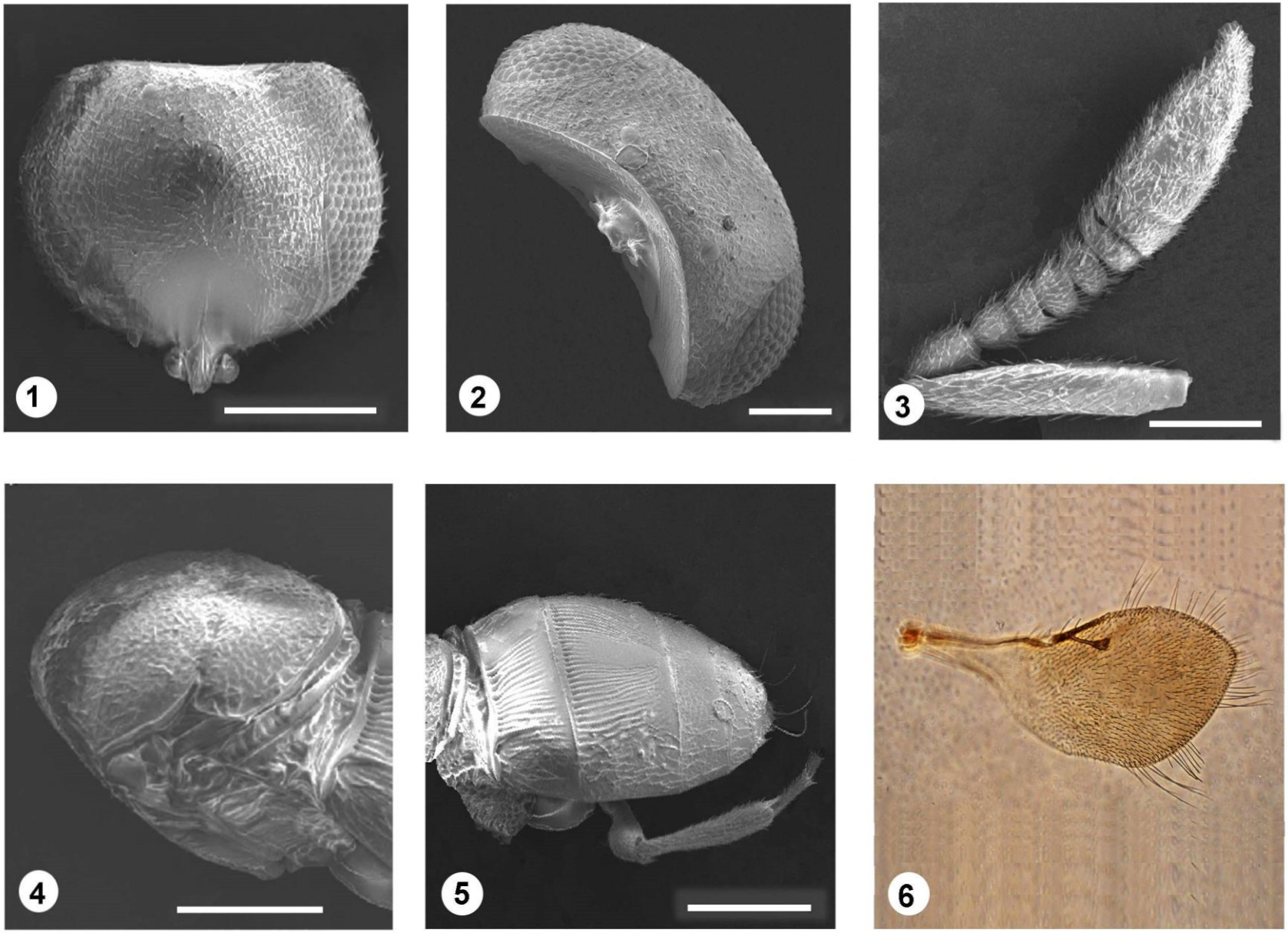

Plate 1. *Cardalnaumannus ramamurthyum* sp. nov. 1: Head in frontal view; 2: Head in dorsal view; 3: Antenna; 4: Mesosoma; 5: Metasoma; 6: Fore wing.

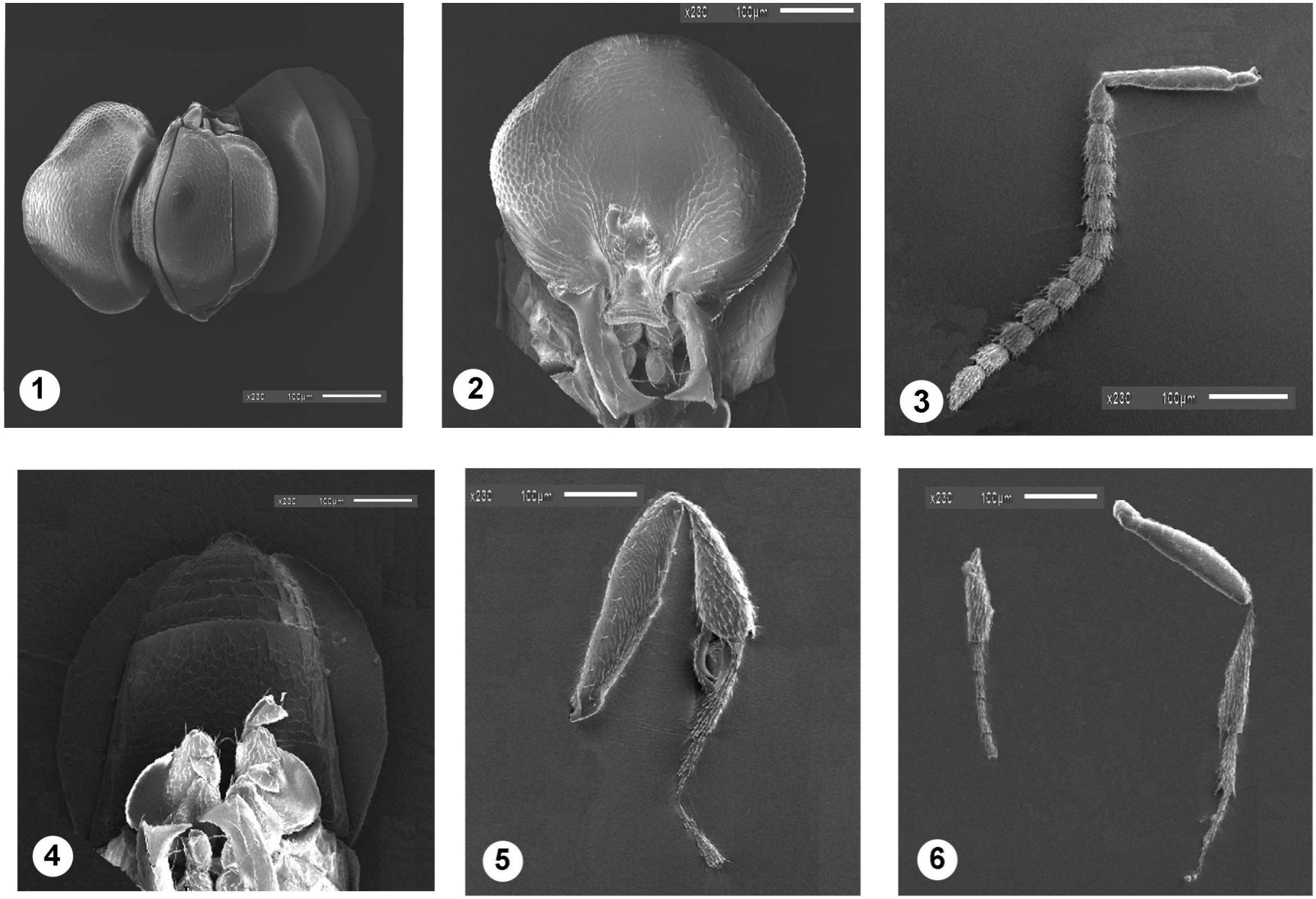

Plate 2. *Encyrtoscelio apterus* (Szelenyi). 1. Body rofile; 2. Head in frontal view; 3. Antenna; 4. Metasoma in dorsal view; 5. Fore leg; 6. Mid and hind leg.

Paratype: 1 female dry mounted.

DISCUSSION

The *Cardalnaumannus* may be distinguished from other genera in the *Dyscritobaeini* except for *Okapa* because of the absence of the fanlike striation on the cheeks; the lateral ocelli are closer to the median ocellus than to the eyes and there is specillum (Mineo et al., 2011). The only genus with some degree of similarity with *Encyrtoscelio* is the frons distinctly projecting forward between eyes which is *Breviscelio* Sundholm but frontal ledges are deeply notched or sinuate medially (Rajmohana et al., 2011). Both genus described have been barely studied in India, this study shows existence of the diversity in the Oriental region.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Abbreviations

POL, Posterior ocellar line; **LOL,** lateral ocellar line; **OOL,** ocular ocellar line; **F1, F2,... F10,** antennal flagellomeres 1, 2,… 10; **T1, T2, ... T5,** metasomal tergites 1, 2,… 5; **S1, S2,** metasomal sternites 1 and 2; **SMV,** sub marginal vein; **MV,** marginal vein; **PMV,** postmarginal vein; **STG,** stigmal vein.

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Full Length Research Paper

Genetic diversity among muskmelon (*Cucumis melo* L.) germplasm in Bangladesh as revealed by microsatellite markers

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Information about genetic variation has become more critical and deciding factor for any breeding and improvement effort,is difficult inefficient and inaccurate when based on morphological traits only. Therefore, this investigation was conducted using nine microsatellite DNA markers to evaluate genetic variation among 96 muskmelon germplasm in Bangladesh. All nine microsatellite markers were polymorphic. Lower values detected in observed than expected heterozygosity for all loci, indicating homozygous condition in the sample population. A total number of 28 alleles were generated by the nine primers across 96 germplasm. The locus TJ10 showed the highest number of alleles (5), whereas CMGA104, CMCT44, CMAG59, CMTA134a and J27 were generated lowest (3) number of alleles.Allele size ranged between 98 bp (CMCT44) to 198 (CMCTT144). The Polymorphism Information Content (PIC) valuesranged from 0.117 to 0.770for the locus CMCTN86and TJ10, repectively. Values of genetic differentiation (Fst) and gene flow (Nm) ranged from 0.535 to 1.000 with an average of 0.776 and from 0.000 to 0.218 with a mean value of 0.072, respectively. Broad genetic base was found among the muskmelon germplasm used in this study. The average pair-wise Nei's genetic distance value was 0.605. The highest genetic dissimilarity coefficient (GD=2.300) was between the germplasm AHM-241 and IAH-102, whereas germplasm IAH-251 and IAH-259 comprised lowest genetic diversity (GD =0.000). In the UPGMA dendrogram, among 96 germplasm of muskmelon 84 grouped in cluster "I" and other 12 in cluster "II".

Keywords: Muskmelon, Microsatellite (SSR) marker, Polymorphism, Genetic diversity, Germplasm

INTRODUCTION

The muskmelon (*Cucumis melo* L., 2n=24) is one of the most nutritive and commercially important cucurbit in the world. Generally, it is considered as a crop of tropical and subtropical regions, extensive cultivation observed in temperate climates. Desert and savannah regions of Africa, Arabia, southwestern Asia and Australia are the

origin of wild populations of muskmelons. Based on the muskmelon largest consumers across the world, the United States is one of them. According to Boriss et al. (2014), Americans consume 27 pounds per year of melons in an average; in which 8.7 pounds is cantaloupe. Muskmelon is normally eaten as a fresh fruit, salad, or dessert with ice cream or custard. In Bangladesh, people consume both unripe and ripe fruits. Unripe fruits are consumed as salad and processed food as soup, stew, curry, stir-fry or pickle. Mature ripe fruits are eaten fresh as a desert fruit and sometimes slightly processed as canned, syrup, jam or dehydrated slices (Malek et al., 2012). Moreover, it is a source of polyphenol antioxidants, chemicals which can regulate the formation of nitric oxide, a vital chemical for prevention of heart attacks.

In Bangladesh, it is considered as a minor but the most common fruit crop of Cucurbitaceae family. It is cultivated all over the country in Bangladesh. According to the latest statistics provided by BBS (2016), it was indicated that the area and production of muskmelon in Bangladesh are 4047 ha and 53000 tons, respectively and contributes 1.13% to total fruit production in Bangladesh.

Despite the variation in habit, size, shape, colour, maturity time and yield observed in Bangladeshi muskmelon germplasm, very little work has been done on genetical improvement of this crop. Muskmelon being predominantly andromonoecious, is a cross pollinated crop and provide ample scope for utilization of the hybrid vigor. Assessment of genetic variability and selection of suitable genotypes is the foremost criterion for genetical improvement of crop species. Plant Genetic Resources Centre (PGRC) of Bangladesh Agricultural Research Institute (BARI) has conserved different types of muskmelon germplasm collected from different parts of Bangladesh. Islam et al. (2017) reported that a total of 131 germplasm of muskmelon were collected and conserved within 2015 to 2016 in PGRC, BARI. Among these collected germplasm, diverse germplasm could be used as a source of genetic material for improving the yield, earliness, uniformity, quality and resistance to biotic and abiotic stresses in muskmelon. Conventionally, morphological markers called descriptors were used for varietal identification and genetic diversity analysis in plants which is time-consuming and expensive, requiring large areas of land and skilled personnel, and are often subjective due to environmental influences (Singh et al., 2004). However, the level of polymorphism for morphological characteristics in elite germplasm is sometimes too limited and inadequate to allow for variety/genotype discrimination (Geleta et al., 2004). In that situation, DNA marker is a one stop solution to combat these problems.

Several researchers have used successfully a variety of DNA markers to characterize the genetic diversity of melons such as isozymes (Staub et al., 1997; Akashi et al., 2002), restriction fragment length polymorphism (RFLPs) (Zheng et al., 1999), random amplification of polymorphic DNAs (RAPDs) (Garcia et al., 1998; Stepansky et al., 1999; Mliki et al., 2001; Lo´pez-Sese et al., 2003; Staub et al., 2004; Sensoy et al., 2007; Tanaka et al., 2007, Nhi et al., 2010; Soltani et al., 2010), amplified fragment length polymorphism (AFLPs) (Garcia-Mas et al., 2000, Yashiro et al., 2005), inter-simple sequence repeat (ISSR) and simple-sequence repeat (SSR) (Katzir et al., 1996; Staub et al., 2000; Danin-Poleg et al., 2001; Lo´pez-Sese´ et al., 2002; Monforte et al., 2003; Nakata et al., 2005; Tzitzikas et al., 2009; Raghami et al., 2014; Trimech et al., 2015) using diverse germplasm from different locations worldwide. Of all classes of DNA based marker, SSR markers represented by the repeats of 1-6 nucleotide-long DNA motifs arranged in tandem, have been considered one of the most powerful Mendelian markers (Jarne and Lagoda, 1996) because of their high reproducibility, co-dominance inheritance, multi-allelic character, and extensive genome coverage (Powell et al., 1996). The polymorphism of SSRs, primarily resulting from the variation of repeat numbers, can be easily detected by a simple PCR technique. In this context, the aim of the present study is to determine the genetic diversity of the muskmelon germplasm in Bangladesh and to find out the phylogenetic relationships among the 96 germplasm using Simple Sequence Repeat (SSR) markers.

MATERIALS AND METHODS

Plant materials

Ninety six germplasm collected from different sources were used as plant materials (Table 1). However, variation among these germplasm based on fruit skin colour, size and shape are given in Figure 1.

Plant sample and extraction of genomic DNA

Diversity at molecular level was studied at the Molecular Biology Laboratory, Plant Genetic Resources Centre of Bangladesh Agricultural Research Institute, Gazipur using SSR markers. Young, fresh, disease and insect free leaves were used for DNA extraction. The genomic DNA was isolated from a bulk of 3-week old seedling leaf tissues taken from 5 plants from each germplasm using SDS and phenol: chloroform: IAA followed by alcohol precipitation described by Saghai-Maroof et al. (1984) with some modifications. Apart from usage of liquid nitrogen, the leaf sample was cut into

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Table 1. List of germplasm investigated in this experiment with their collection area and biological status.

S/N	Coll. no.	Location of collecting site (Upazilla and District)	Sample status	Sample source	S/N	Coll. no.	Location of collecting site (Upazilla and District)	Sample status	Sample source
01	AMA-04	Kaliakoir, Gazipur	TC	FS	49	IAH-23	Mirpur, Dhaka	AC	MC
02	AMA-28	Mirzapur, Tangail	TC	FS	50	IAH-25	Mirpur, Dhaka	AC	MC
03	AMA-87	Trisal, Mymensingh	TC	FS	51	IAH-26	Keranigonj, Dhaka	TC	FS
04	AMA-145	Bhaluka, Mymensingh	TC	FS	52	IAH-27	Keranigonj, Dhaka	TC	FS
05	AMA-205	Gaforegone, Mymensingh	TC	FS	53	IAH-28	Nobabgonj, Dhaka	TC	FS
06	AMA-234	Gaforegone, Mymensingh	TC	FS	54	IAH-29	Nobabgonj, Dhaka	TC	FS
07	AMA-255	Muktagacha, Mymensingh	TC	FS	55	IAH-30	Nobabgonj, Dhaka	TC	FS
08	AMA-405	Sreebordi, Sherpur	TC	FS	56	IAH-31	Nobabgonj, Dhaka	TC	FS
09	AMA-411	Sreebordi, Sherpur	TC	FS	57	IAH-100	Sadar, Gazipur	AC	RS
10	AHM-186	Kaliakoir, Gazipur	TC	FS	58	IAH-101	Sadar, Gazipur	AC	RS
11	AHM-202	Kaliakoir, Gazipur	TC	FS	59	IAH-102	Sadar, Gazipur	AC	RS
12	AHM-203	Kaliakoir, Gazipur	TC	FS	60	IAH-103	Sadar, Gazipur	AC	RS
13	AHM-222	Dhamrai, Dhaka	TC	FS	61	IAH-123	Sreepur, Gazipur	TC	FS
14	AHM-232	Dhamrai, Dhaka	TC	FS	62	IAH-175	Sadar, Gazipur	AC	RS
15	AHM-234	Dhamrai, Dhaka	TC	FS	63	IAH-179	Sadar, Gazipur	AC	RS
16	AHM-235	Dhamrai, Dhaka	TC	FS	64	IAH-183	Sadar, Gazipur	AC	RS
17	AHM-236	Dhamrai, Dhaka	TC	FS	65	IAH-184	Sadar, Gazipur	AC	RS
18	AHM-237	Dhamrai, Dhaka	TC	FS	66	IAH-185	Sadar, Gazipur	AC	RS
19	AHM-238	Dhamrai, Dhaka	TC	FS	67	IAH-186	Sadar, Gazipur	AC	RS
20	AHM-239	Dhamrai, Dhaka	TC	FS	68	IAH-189	Sadar, Gazipur	AC	RS
21	AHM-240	Dhamrai, Dhaka	TC	FS	69	IAH-192	Sadar, Gazipur	AC	RS
22	AHM-241	Dhamrai, Dhaka	TC	FS	70	IAH-195	Sadar, Gazipur	AC	RS
23	AHM-247	Dhamrai, Dhaka	TC	FS	71	IAH-196	Sadar, Gazipur	AC	RS
24	AHM-260	Sadar, Manikganj	TC	FS	72	IAH-202	Sadar, Gazipur	AC	RS
25	MAH-26	Sonargoan, Narayangonj	TC	FS	73	IAH-203	Sadar, Gazipur	AC	RS
26	MAH-47	Rupgonj, Narayangonj	TC	FS	74	IAH-208	Mirpur, Dhaka	AC	MC
27	MAH-55	Rupgonj, Narayangonj	TC	FS	75	IAH-209	Mirpur, Dhaka	AC	MC
28	MAH-58	Rupgonj, Narayangonj	TC	FS	76	IAH-210	Mirpur, Dhaka	AC	MC
29	MAH-66	Rupgonj, Narayangonj	TC	FS	77	IAH-213	Mirpur, Dhaka	AC	MC
30	IAH-01	Mirpur, Dhaka	AC	MC	78	IAH-214	Mirpur, Dhaka	AC	MC
31	IAH-02	Mirpur, Dhaka	AC	MC	79	IAH-215	Mirpur, Dhaka	AC	MC
32	IAH-03	Mirpur, Dhaka	AC	MC	80	IAH-216	Mirpur, Dhaka	AC	MC
33	IAH-04	Mirpur, Dhaka	AC	MC	81	IAH-218	Mirpur, Dhaka	AC	MC
34	IAH-05	Mirpur, Dhaka	AC	MC	82	IAH-219	Mirpur, Dhaka	AC	MC
35	IAH-08	Mirpur, Dhaka	AC	MC	83	IAH-223	Mirpur, Dhaka	AC	MC

Table 1. Contd

36	IAH-09	Mirpur, Dhaka	AC	MC	84	IAH-229	Mirpur, Dhaka	AC	MC
37	IAH-10	Mirpur, Dhaka	AC	MC	85	IAH-231	Mirpur, Dhaka	AC	MC
38	IAH-11	Mirpur, Dhaka	AC	MC	86	IAH-232	Mirpur, Dhaka	AC	MC
39	IAH-12	Mirpur, Dhaka	AC	MC	87	IAH-233	Mirpur, Dhaka	AC	MC
40	IAH-13	Mirpur, Dhaka	AC	MC	88	IAH-237	Mirpur, Dhaka	AC	MC
41	IAH-15	Mirpur, Dhaka	AC	MC	89	IAH-238	Mirpur, Dhaka	AC	MC
42	IAH-16	Mirpur, Dhaka	AC	MC	90	IAH-241	Mirpur, Dhaka	AC	MC
43	IAH-17	Mirpur, Dhaka	AC	MC	91	IAH-247	Mirpur, Dhaka	AC	MC
44	IAH-18	Mirpur, Dhaka	AC	MC	92	IAH-248	Badamtali, Dhaka	AC	MC
45	IAH-19	Mirpur, Dhaka	AC	MC	93	IAH-249	Badamtali, Dhaka	AC	MC
46	IAH-20	Mirpur, Dhaka	AC	MC	94	IAH-251	Badamtali, Dhaka	AC	MC
47	IAH-21	Mirpur, Dhaka	AC	MC	95	IAH-259	Badamtali Dhaka	AC	MC
48	IAH-22	Mirpur, Dhaka	AC	MC	96	IAH-260	Badamtali, Dhaka	AC	MC

Coll. no.: Collector's number; TC: Traditional cultivar; AC: Advanced cultivar; FS: Farm store; MC: Market; RS: Retail shop.

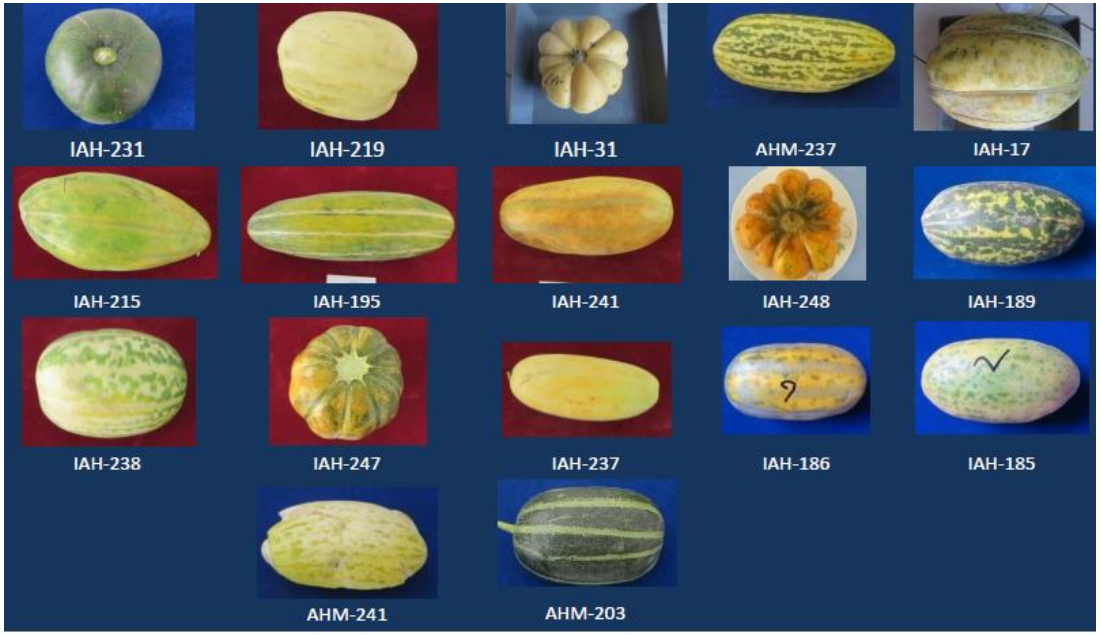

Figure 1. Variability in fruit type among collected muskmelon (*Cucumis melo* L.) germplasm in Bangladesh.

Table 2. List of primers uses in this study.

S/N	Locus	Forward primer	Reverse primer	Ann. T. (°C)	Expected size (bp)	Reference
1	CMAG59	ttgggtggcaatgaggaa	atatgatcttccatttcca	48	124	Katzir et al. (1996)
2	CMGA104	ttactgggttttgccgattt	aattccgtattcaactctcc	48	125	Danin-Poleg et al. (2001)
3	CMCTT144	caaaaggtttcgattggtggg	aaatggtgggggttgaatagg	51	192	Danin-Poleg et al. (2001)
4	CMTA170a	ttaaatcccaaagacatggcg	agacgaaggacggttagcttt	51	125	Danin-Poleg et al. (2001)
5	CMTA134a	acgtgcttcagtaaacatg	ccgacattgaaaaccaacttc	51	159	Danin-Poleg et al. (2001)
6	CMCT44	tcaactgtccatttctcgctg	ccgtaaagacgaaaacccttc	51	104	Danin-Poleg et al. (2001)
7	TJ10	tacgaggaaaacgcaaaatca	tgaacgtggacgacattttt	53	155	Henane et al. (2015)
8	TJ27	aagcggaac-aagctcatctc	caaaagcatc-aattgcttgaa	55	170	Henane et al. (2015)
9	CMCTN86	tgtgacagttatcaaggatgc	aagggaatgcatgtggac	53	175	Henane et al. (2015)

Ann. T. (°C), Annealing temperature (°C).

small pieces and digested with homogenization buffer containing Tris-50 mM, EDTA-25 mM, NaCl-300 mM, 1% SDS and deionized water was used. It was incubated at 65ºC for 30 min, extracted with phenol: chloroform: isoamyl alcohol (25:24:1), precipitated with ice-cold and extra pure isopropyl alcohol and purified with absolute ethanol (Plus sodium acetate, 3M) and 70% ethanol chronologically. DNA sample of each muskmelon germplasm was dissolved in 50 µl of TE buffer in 1.5-ml Eppendorf tube. When the DNA pellet was totally dissolved in TE buffer, 4 µl RNaseA (10 mg/ml) with isolated DNA was added and incubated at 37°C for 30 min (Tilahun et al., 2013). Finally, DNA sample was stored at -20°C.

Quantification and optimization of DNA concentration

The presence of genomic DNA was confirmed on 1% agarose gel qualitatively. The gels were visualized under UV light and photographed using photo documentation system (UV Transilluminator, Uvitec, UK). All of the DNA samples were found to be in good quality in this study. The amount of genomic DNA was quantified using UV spectrophotometer (Thermo Fisher Scientific, USA) at 260 nm. The original DNA concentrations of each muskmelon germplasm were measured following the formula described by Sumon et al. (2014).

Selection of microsatellite/SSR primers

Nine SSR primer pairs described previously (Katzir et al., 1996; ; Danin-Poleg et al., 2001; Henane et al., 2015) were used in the present study for microsatellite analysis (Table 2). All 9 primers pairs showed better responsiveness with clear and expected amplified product sizes.

PCR standardization and amplification

Amplification reactions were performed in 10-µL volumes containing 5X Green GoTaq Reaction Buffer (Promega, USA), 15 mM $MgCl_2$, 1.25 U Taq DNA polymerase (Thermo Fisher Scientific, USA), 0.4 mM each of the dNTPs (NEB, USA), 10 µM forward and reverse primers and 50 ng template DNA. The mixtures were prepared at 0°C and transferred to the thermal cycler. Amplification reactions of SSR loci were carried out in a Mastercycler nexus Gradient thermal cycler (Eppendorf, Germany), using a program consisting of an initial denaturation step of 3 min at 94°C followed by 35 cycles of 45 s at 94°C, 1 min at 48 to 55°C and 1 min at 72°C; the program ended with a 8 min elongation step at 72°C. PCR products were stored at 4°C prior to analysis.

Gel electrophoresis and visualization of PCR products

PCR-products were electrophoresed on a 5% denaturing polyacrylamide gel containing 19:1 acrylamide: Bis-acrylamide, 10X TBE buffer, 10% APS and UltraPure TEMED. Electrophoresis was done using the Triple Wide Mini-Vertical Electrophoresis System, MGV-202-33 (CBS Scientific, USA). The gel was run at 80 to 90 V for a specified period of time depending on the size of amplified DNA fragment (usually 1 h for 100 bp). Upon loading of PCR products, 20°C temperature was maintained by a cooling system (Julabo, Germany). When electrophoresis was completed, the gel was stained with ethidium bromide (10 mg/ml) for 30 min. Finally, the stained gel was soaked in deionized water for 5 min. The individual bands were visualized under UV light and scored for analysis (Sumon et al., 2014).

Preparation of microsatellite data matrix for analysis

SSR markers were attained due to codominant nature microsatellite markers; it could be applied to distinguish both homozygous and heterozygous genotypes in individual plants. Individual alleles (bands) at the microsatellite loci were scored and single data matrix constructed for all loci as described by Molla et al. (2016). The constructed data matrix was used to calculate statistics of genetic variation and cluster analyses were carried out based on the genetic distance (Nei, 1972); unweighted pair group method with arithmetic mean (UPGMA) dendrogram constructed using computer program POPGENE (Version 1.31) (Yeh et al., 1999) and the relationships among the germplasm was determined. The PIC value was calculated using PIC = 1- Σf^2_{ij}; where fij is the frequency of the i^{th} allele for the j^{th} SSR locus (Anderson et al., 1993). PIC values express the discriminating ability of the particular. These values varied based on the number of alleles per locus and the relative frequencies of those alleles in the population. Allelic lengths were estimated using the software DNA FRAG version 3.03 (Nash, 1991).

RESULTS AND DISCUSSION

According to DNA amplification patterns, all nine

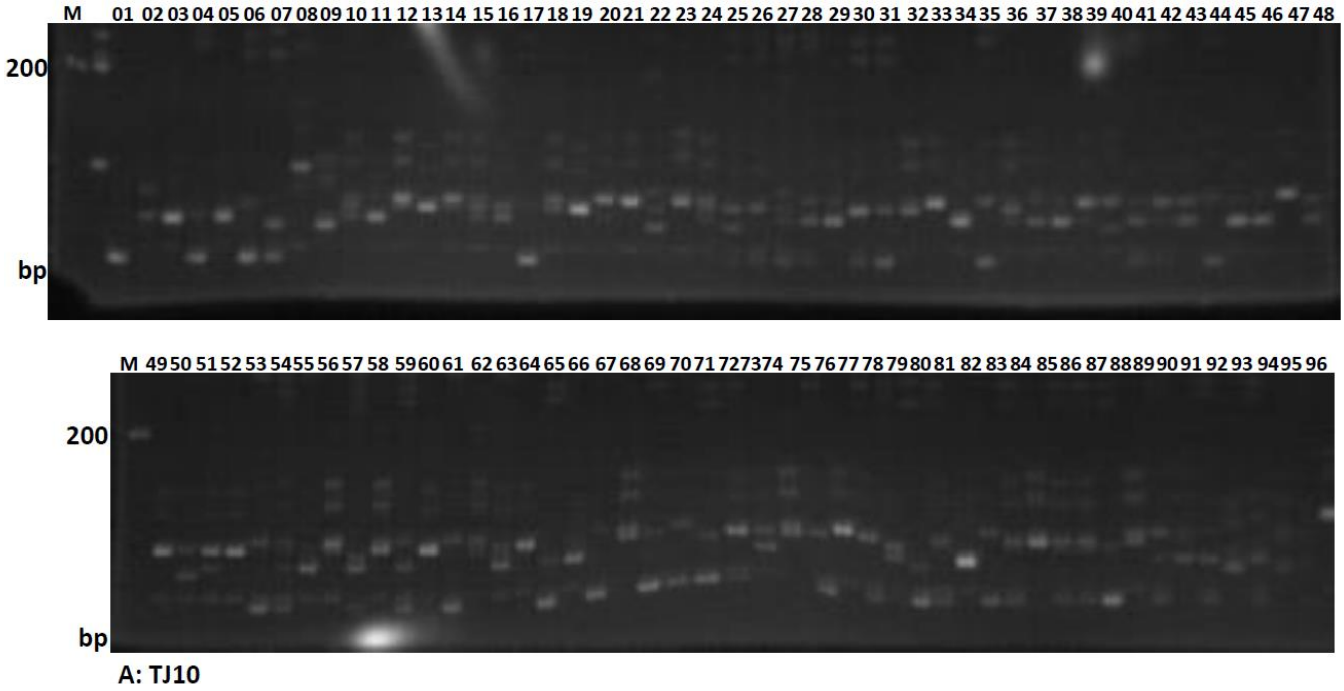

Figure 2. Microsatellite profiles of 96 muskmelon genotypes at locus TJ10; M: molecular wt. marker (100 bp DNA ladder, L: Lane); L01: AMA-04; L02: AMA-28; L03: AMA-87; L04: AMA-145; L05: AMA-205; L06: AMA-234; L07: AMA-255; L08: AMA-405; L09: AMA-411; L10: AHM-186; L11: AHM-202; L12: AHM-203; L13: AHM-222; L14: AHM-232; L15: AHM-234; L16: AHM-235; L17: AHM-236; L18: AHM-237; L19: AHM-238; L20: AHM-239; L21: AHM-240; L22: AHM-241; L23: AHM-247; L24: AHM-260; L25: MAH-26; L26: MAH-47; L27: MAH-55; L28: MAH-58; L29: MAH-66; L30: IAH-01; L31: IAH-02; L32: IAH-03; L33: IAH-04; L34: IAH-05; L35: IAH-08; L36: IAH-09; L37: IAH-10; L38: IAH-11; L39: IAH-12; L40: IAH-13; L41: IAH-15; L42: IAH-16; L43: IAH-17; L44: IAH-18; L45: IAH-19; L46: IAH-20; L47: IAH-21; L48: IAH-23; L49: IAH-25; L50: IAH-25; L51: IAH-26; L52: IAH-27; L53: IAH-28; L54: L55: IAH-30; L56: IAH-31; L57: IAH-100; L58: IAH-101; L59: IAH-102; L60: IAH-103; L61: IAH-123; L62: IAH-175; L63: IAH-179; L64: IAH-183; L65: IAH-184; L66: IAH-185; L67: IAH-186; L68: IAH-189; L69: IAH-192; L70: IAH-195; L71: IAH-196; L72: IAH-202; L73: IAH-203; L74 IAH-208; L75: IAH-209; L76: IAH-210; L77: IAH-213; L78: IAH-214; L79: IAH-214; L80: IAH-216; L81: IAH-218; L82: IAH-219; L83: IAH-223; L84: IAH-229; L85: IAH-231; L86: IAH-232; L87: IAH-233; L88: IAH-237; L89: IAH-238; L90: IAH-241; L91: IAH-247; L92: IAH-248; L93: IAH-249; L94: IAH-251; L95: IAH-259; L96: IAH-260

microsatellite markers used in this analysis were found to be polymorphic. One typical SSR profile is shown in Figure 2. Analysis of the variability parameters for the 9 SSRs in the 96 muskmelon germplasm are shown in Table 3. A total of 28 alleles were identified among all muskmelon germplasm with the 9 SSRs loci scanned herein, with an average of 3.11 allele per locus, varying from two for ‘CMCTT144’ ‘CMTA170a’ and ‘CMCTN86’ to 5 for ‘TJ10’ loci (Table 3) which is lower than that reported by ; Danin-Poleg et al. (2001), and higher than the result obtained by Henane et al. (2015). Variation of allele sizes ranged from 98 to 198 bp. Highest number of observed alleles (5) were found at the locus TJ10 among the 96 muskmelon germplasm ranging in size from 141 to 160 bp followed by 4 alleles (132 to 157 bp and 98 to 113 bp) and 3 alleles (131 to 141 bp, 230 to 302 bp, 150 to 162 bp and 171 to 187 bp) at the loci CMGA104, CMCT44, CMAG59, CMTA134a, and J27, respectively. However, lowest number of alleles (2) size ranging from 184 to 198, 122 to 136 and 170 to 187 bp was detected for the loci ‘CMCTT144’ ‘CMTA170a’ and ‘CMCTN86’, respectively (Table 3). All the nine SSR loci were found to be polymorphic, proving their effectiveness for genetic analysis of muskmelon germplasm. Five alleles expected length of 124 bp for CMAG59 and 6 alleles expected length of 125 and 192 bp for CMGA104 and CMGA144, respectively in muskmelon cultivars were reported (Katzir et al., 1996; Danin-Poleg et al., 2001). Parallel observed allelic lengths were estimated compare to previous study although some variation raised might be due to mutation of di-nucleotide repeat units.

Heterozygosity can be considered as an indicator for the measurement of genetic variability. It expresses level of variation that exists in the population and how that variation is allocated across the alleles of an analyzed locus. Lower values of heterozygosity indicate small genetic variability. In general, small numbers of heterozygous individuals were observed for all SSRs, with an average of 0.227, ranging between 0.000 and 0.583 (Table 3). Expected heterozygosity (He, average 0.493) values for each SSR locus, considering all studied germplasm, were always higher than the observed

Table 3. Variability of SSR markers used for muskmelon germplasm genetic analysis.

Locus	No. of allele	Allele sizes (bp)	Major allele frequency	Observed heterozygosity	Expected Heterozygosity**	PIC
CMAG59	3	131, 137, 141	0.660	0.000	0.504	0.501
CMGA104	4	132, 138, 147, 157	0.323	0.000	0.725	0.721
CMCTT144	2	184, 198	0.863	0.269	0.234	0.233
CMTA170a	2	122, 136	0.875	0.000	0.220	0.219
CMTA134a	3	150, 155, 162	0.469	0.583	0.642	0.639
CMCT44	4	98, 103, 106, 113	0.531	0.417	0.587	0.584
TJ10	5	141, 147, 150, 154, 160	0.318	0.344	0.775	0.770
TJ27	3	171, 178, 187	0.484	0.432	0.634	0.631
CMCTN86	2	170, 187	0.938	0.000	0.118	0.117
Mean	**3.11**	**-**	**0.607**	**0.227**	**0.493**	**0.490**

**Nei's (1973) expected heterozygosity. PIC, Polymorphism information content.

heterozygosity (Ho), representing homozygous individuals in population samples. The observed heterozygosity is the proportion of heterozygous individuals in population samples; expected heterozygosity is the probability of an individual being heterozygous in any locus. In this study, the highest observed heterozygosity values (Ho=0.432) were attained with locus TJ27 (Table 3). Mean observed heterozygosity per locus in the studied germplasm tested herein was higher than those in Lo´pez-Sese´ et al. (2002) and Tzitzikas et al. (2009), probably due to the greater diverse germplasm used in the present study. Higher levels of heterozygosity would be expected because of primarily studied germplasm that had been developed by local farmers and, therefore cross-pollination might occur with other accessions. In this study, lower values of heterozygosity were observed, probably due to lack of intercrossing between them or with other accessions, a high rate of self-pollination. Another possibility is that the accessions originated from small populations or high levels of inbreeding (Raghami et al., 2014).

The PIC values represent the variation of allele and provide an estimation of discriminating ability of the marker. It is reflected by the number of alleles at a locus and also relative frequencies of these alleles. The genetic diversity of the studied germplasm might have an effect in variation of PIC values and high ratio of traditional cultivars used in this investigation might be a reason for raising the PIC values.

It is important to indicate that the selection by breeders have increased the frequency of the alleles or allelic combination with favorable effects at the expense of the others, eventually eliminating many of them (Cao et al., 1998). The estimated PIC values of nine SSR markers analyzed with 96 muskmelon germplasm were higher than zero. It is indicated that polymorphic and informative markers tested in this investigation was capable to describe genotypic variation of those germplasm. PIC values for nine SSRs ranged from 0.117 to 0.770 (Table 2), with an average of 0.490. Four of these SSRs were very informative (PIC > 0.6), with the highest PIC value recorded for TJ10 (0.770) and followed by CMGA104, CMTA134a and TJ27. Validations of informative markers depend on the level of polymorphism of this specific marker which is extremely useful for genetic studies (Sundaram et al., 2007).

Observed and effective number of alleles was also different in the present investigation. The mean number of observed allele and effective alleles for SSR loci with 96 muskmelon genotypes were 3.111 and 2.393, respectively (Table 4). These results were supported by previous study. For instance, 3.5 alleles using 30 SSR primers on 13 genotypes were reported by Danin-Poleg et al. (2001), while Lo´pez-Sese´ et al. (2002) found 2.4 alleles on 15 Spanish melons, and Tzitzikas et al. (2009) 2.47 alleles on 14 Greek and Cypriot melons. Monforte et al. (2003) detected 6.3 alleles on 27 wild and cultivated melons, which was divergent to the mean number of allele for reference genotypes in this study (3.111). This high value was due to various subspecies of melons which they examined.

Genetic differentiation (Fst) values ranged from 0.535 to 1.000 with a mean value of 0.776 and gene flow (Nm) values were found in the ranges 0.000 to 0.218 with an average of 0.072 (Table 4). In this study, comparatively higher values of genetic differentiation and lower values of gene flow were observed among the nine SSR markers which are indicative of diversity among the genotypes as most of the studied genotypes were of land races and local cultivars. The mean Shannon's information index (I) for all loci for 96 muskmelon germplasm were 0.869, and ranged from 0.046 to 1.000 (Table 4).

From these results, SSR markers can be used effectively to estimate genetic distances among genotypes. The mean genetic distance was 0.674 between Iranian cultivated melon (Raghami et al., 2014)

Table 4. Summary of genetic variation statistics for all loci.

Locus	Observed number of alleles (na)	Effective number of alleles (ne)	Shannon's Information Index (I)	Genetic differentiation (Fst)	Gene flow (Nm)*
CMAG59	3	2.005	0.860	1.000	0.000
CMGA104	4	3.583	1.321	1.000	0.000
CMCTT144	2	1.303	0.395	0.535	0.218
CMTA170a	2	1.280	0.377	1.000	0.000
CMTA134a	3	2.768	1.059	0.543	0.210
CMCT44	4	2.403	0.993	0.643	0.139
TJ10	5	4.356	1.536	0.777	0.072
TJ27	3	2.707	1.046	0.665	0.126
CMCTN86	2	1.133	0.234	1.000	0.000
Mean	**3.111**	**2.393**	**0.869**	**0.776**	**0.072**

*Nm = Gene flow estimated from Fst = 0.25(1 - Fst)/Fst.

and 0.285 between Spanish melon (Lopez-Sesé et al.,2002), while in the present study, it varied from 0.000 to 2.300 with an average of 0.605 (Figure 3). Different genetic background represents higher genetic distance values between germplasm pairs. However, least/nil values might be found in case of reverse genetic background. This variability in genetic distance values can play vital role for the enhancement of resources Bangladeshi muskmelon and sustainable use for genetical improvement. Genetic distance UPGMA dendrogram is built on SSR markers data referring to dissimilarity coefficient among the germplasm. On the basis of cluster study, the total genotypes were distributed into two main clusters; I and II. These two main clusters were further sub-divided into 17 sub-groups. Among these 17 sub-groups, 15 fell in main cluster I and the remaining two sub-groups were part of main cluster II (Figure 3). Upon subsequent separation, pair-wise estimates of dissimilarity ranged from 0.000 to 2.300 and the average similarity among all 96 germplasm was 0.605. Germplasm AHM-241 and IAH-102 had the highest genetic dissimilarity coefficient (GD=2.300) which was grouped in sub-cluster G6 and G17, respectively. Subgroup G6 gathered 15 genotypes in which genotypes IAH-251 and IAH-259 comprise sharp similarity (GD=0.000) that makes one think “synonymy phenomenon” may be the same germplasm, but having undergone two different names depending on the collection area. Similarly, diverse germplasm were grouped in different cluster in the dendrogram, because of variation in morphological traits and/or geographical distribution. For instance, germplasm AHM-241 and IAH-102 showed distinct variation in their fruit morphology (Figure 1).

Conclusion

Taken as a whole, the present study clearly show that studied germplasm with this broad genetic diversity could play an important role in the preservation and enhancement of muskmelon genetic diversity. Inter-mating genotypes from the major distinct gene pools could provide new genetic recombination to exploit in various development programme. In spite of the variability observed in this study, the 28 alleles of the 9 SSR loci were not adequate to discriminate all the 96 germplasm of muskmelon. For instance, AHM-222 vs. AHM-234 and IAH-175 vs. IAH-229 germplasm pair were genetically closely related for the loci analyzed. It is emphasized that these germplasm showed variation in some morphological traits like fruit skin, colour intensity, skin hardness of fruit, fruit shape and flesh colour (Islam et al., 2017). In situations where a set of pre-established markers were not able to differentiate accessions from a given species, Jakse et al. (2005) suggested that additional markers have to be used to reveal polymorphisms. Hence, it would be better to use higher number of primers for the creation and construction of an appropriate genetic relationship, germplasm identification and analysis of genetic variation. Moreover, it is necessary to use both morpho-physiological traits and molecular traits together to distinguish important germplasm for further genetical improvement of this crop species.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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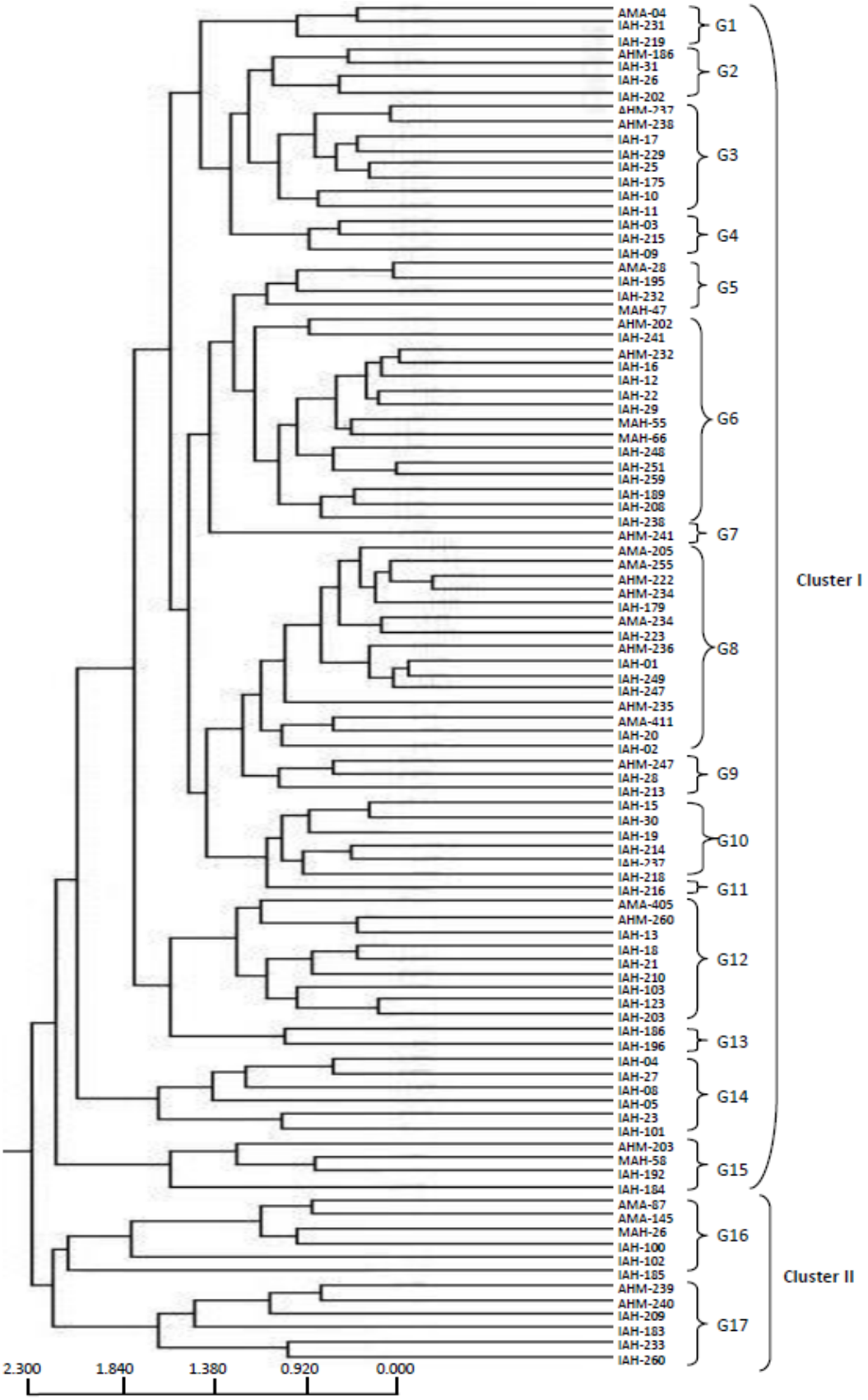

Figure 3. UPGMA cluster analysis based on Nei's (1972) genetic distance, showing diversity and relationship among 96 muskmelon germplasm .

and Melon in Bangladesh".

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