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Study on the Consumption Index and Growth Rate of *Acrotylus humbertianus* Saussure on Different Diet under Scanning Electron Microscope

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Abstract

Acrotylus humbertianus Saussure is a major agriculture pest in Sindh. This species consumes a wide variety of food plants from different families. In order to test its preference on different diets a conformity chemical analysis has been done under Scanning Electron Microscope (SEM) for the first time. The maximum consumption index (CI) of all stages *A. humbertianus* on cabbage was recorded 0.042-0.78 mg/day followed by 0.2-0.42mg/d on sugarcane and 0.019-0.43mg/d on maize, while least CI was calculated i-e 0.016-0.39 mg/day on mix diet. Beside this, growth rate (GR) of *A. humbertianus* on these food plants indicates that (GR) was highest on sugarcane i-e 0.016-0.25mg/d followed by 0.02 -0.62mg/d and 0.013-0.35mg/d on cabbage and maize respectively, while it was 0.011-0.32 mg/d on mixed diet. The first and second instars of *A. humbertianus* differed significantly in their consumption, indices on all four host plants and the highest consumption index was recorded on adult female fed on cabbage (0.78), while the growth rate (GR) of *A. humbertianus* on four plants i-e sugarcane, cabbage, maize and mixed diet was observed highest on cabbage i-e 0.040mg/ g day followed by maize, sugarcane and mixed diet i-e 0.031 mg/g day, 0.029 mg/g day and 0.020 mg/g day respectively. From nymphal stages highest growth rate was recorded in second and third instars i-e 0.043 mg/g day, fed on cabbage.

Keywords: Agriculture pest, Food plants, Preference, Growth rate, Consumption index

Introduction

Grasshoppers are the polyphagous insects and cause considerable damage to varieties of crops in Pakistan. The genus *Acrotylus* having multidimensional importance in agriculture related areas. It reduces the average productivity of plants and damages the host plants seriously. *Acrotylus* badly affect farmer earning capacity by eating their crops such as cereals, grains, vegetables, oil plants, sugarcane, rice, maize and different grasses. It also reduces nutritional value of plants by eating their leaves and soft parts. Furthermore, they migrate from one crop to nearby grasslands and can cause massive losses to crops. Among the numbers of pest species *A. humbertianus* reported as a major pest of vegetables, sugarcane, rice, maize and grasses etc. Ahmed ^[1] found that *A. humbertianus* damage crops like sorghum, maize and seedling of cotton and also feed on leaves of common weeds like Dila, Baru and Cabbage leaves during winter. Research so far, conducted on *A. humbertianus* has mainly concentrated on its taxonomic status ^[2, 3, 4, 5, 6, 7, 8]. Furthermore, Riffat and Wagan ^[9] carried the work on the effects of various plants on nymphal development and egg production in *Hieroglyphus perpolitata* they stated that life-activities of insects are significantly affected by feeding on different host plants. Similarly, Bernays and Chapman ^[10] reported that survivability of insect also depends upon the type of food. Various studies in areas such as biology, ecology, behavior and biomass ^[11, 12] have been done on different species of grasshoppers. Although, various authors carried work on the different aspects of *A. humbertianus* such as Williams ^[13], Kaufman ^[14], Gangwere ^[15], Cherynkovsky ^[16], Pandian and Devi ^[17], Cates and Orians ^[18], Baily and Mukherji ^[19], Wagan ^[20], Suresh ^[21], Boucher and Varady-Szabo ^[22] but none of these focused on the feeding activities preference of *A. humbertianus* on different diet. Besides, it's all havoc, it is very beneficial in man products because of its high reproductive potential and gregarious status; this insect can be utilized as non-conventional sources of animal's protein-supplement for fishes. For utilization of this natural resource, biomass

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production through mass culture is important. To obtain a high amount of biomass, studies on the growth rate, as well as their survivability rate, are very essential because rapid growth and less mortality will directly influence their mass culture and biomass production. On the other hand growth is directly related to the digestion/assimilation of food. The type and nature of the food also plays vital role in growth of individual. However, there are no detailed studies on the effect of diet on growth and survivability of *A. humbertianus* has been carried out before this. This research focused on growth rate and survivability of this species fed on preferred food plants with a view to obtain basic information for biomass production of *A. humbertianus* from this region present investigation has been designed for first time.

Material and methods

Sampling, sorting and rearing of various stages of *Acrotylus humbertianus*

A large stock of *Acrotylus* ranging from different stages nymph to adult, collected periodically from vast agricultural area of Matiari district (Latitudes 25° N and Longitude 68° E) during the year 2015 in different month of the year (from May to November) samples were captured earlier hours of the day before 12 O' clock and some were also collected in evening time between 5 O' clock to 7 O'clock. Random sampling was done from different fields of Cotton, Sugarcane, Grass, Maize, Alfalfa, and Rose plant. *Acrotylus* were collected with insect hand net (8.89 cm in diameter and 50.8 cm in length) and some by hand picking. They were immediately transferred to in plastic jars and offered different varieties of plants for feeding as they survive for longer time. The insect jar was also made conducive to keep specimens comfort. The collected specimens were sorted out into different developmental stages. They were transferred into different plastic jars where they were given different food plants. They were kept and reared isolate in 4 liters plastic jar at normal room temperature where temperature range was between 28± 2 °C to 39±2 °C and relative humidity was 28±2% to 55 ± 2% for rearing of insects method describe by Riffat and Wagan [23] was adopted. The identified specimens kept in plastic jar with food plants that were moist by water spray and hard stick was also kept in jar as well in the cage in order to imitate natural environment as *Acrotylus* feel it as natural home and comfort easy. All jars and cages were kept in hygienic conditions for this plastic jars were sterilized weekly kept in sun light for ample time (about 6 hours); whilst bottom paper sheet of plastic jar was changed daily, and all die insects were removed.

Observation under Scanning Electron Microscopy (EDS)

Analyzing of various elements occurrences in tested food plants under Scanning Electron Microscopy/ EDS has been done at the Centre for Pure and Applied Geology, University of Sindh, Jamshoro. For this experiment 09 samples of different plants i-e *Zea mays* (Maize), *Saccharum officinarum* (Sugarcane), *Sorghum bicolor* (Jowar), *Cynodon dactylon* (Grass), *Brassica oleracea* (Cabbage), *Citrus limon* (Lemon), *Solanum melanogena* (Brinjal), *Carica papaya* (Papaya), *Rosa indica* (Rose) were randomly collected from fields of Matiari district Sindh, Sindhi Language Authority Hyderabad and from the animal fodder shops Qasimabad, Hyderabad fresh leaves of all these plants brought to laboratory after keeping it for 12-14 hours photoperiod under the sun for evaporation of water when these leaves become fully dry up after that they have been analyzed in Scanning Electron Microscope (SEM) Laboratory for noting the higher and low chemical

composition of different types of elements present in these plants in order to know that why the insect gave more preference to this plant and why they rejected them.

Experimental procedure

The procedure was initialized with the smooth cutting of core chips and their mounting sample stub of Scanning Electron Microscopy (SEM) using a conductive double sided carbon solution tape. The sample stub then was mounted in the sample chamber of (JEOL JSM-6490 LV Model) Scanning Electron Microscope which is also equipped with an extra and important accessory component of Bruker EDS (Energy Dispersive X-Ray Spectrometer). Scanning Electron Microscope take about 15-20 minutes to develop vacuum when development of vacuum shown on screen of computer then start to select the portion of sample for magnification and for EDS one by one. All plants (09) sample were placed on conductive double sided carbon solution tape which were placed on sample stub. Samples were marks with different numbers then start to see the magnified images and elemental composition of the plant leaves.

The suitable operational parameters of SEM were put to obtain the fine focusing at high and desired values of magnifications of the samples. In the end the analysis was followed by the elemental determination of the samples, both qualitative and quantitative. For that EDS was activated. After reaching an ideal magnification of X80, the samples were chemically analyzed. The elemental composition was determined in the form of different peaks heights for qualitative study and at the same time the results of sample analysis were achieved in tabular and graphic forms for the quantitative analysis as well (Fig: I a-i) .

Formula for growth rate and consumption index

Growth Rate (GR) = (Weight gained by insect) / duration of feeding Period X (means weight of insect during feeding period).

Consumption index (CI) = (For calculation of CI the daily food consumption of the insect as well faecal matter production) were measured.

Statistical analysis

Data obtained from experimental groups was subjected to one way analysis of variance (ANOVA) (SPSS 16.0 Soft-ware) with repeated measures and significant means were determined using Least Significant Difference Range Test (LSD) this test was used to compare the means of various treatments.

Results

Consumption index (CI) and growth rate (GR) of *Acrotylus humbertianus* on top preferable plants

Table (I) showed that *A. humbertianus* was faster developed on four plants i-e sugarcane, cabbage, maize, mixed diet that may be due to quality or quantity of consumed diet or both. Hence consumption of various hosts by the nymph of *A. humbertianus* was estimated. The maximum consumption index (CI) of all stages *A. humbertianus* on cabbage was recorded 0.042 - 0.78 mg/day followed by 0.2-0.42mg/d on sugarcane and 0.019-0.43mg/d on maize, while least CI was calculated i-e 0.016-0.39 mg/day on mix diet. Beside this, growth rate (GR) of *A. humbertianus* on these food plants indicates that (GR) was highest on sugarcane i-e 0.016-0.25mg/d followed by 0.02 -0.62mg/d and 0.013-0.35mg/d on cabbage and maize respectively, while it was 0.011-0.32 mg/d on mixed diet. When subjected to one way ANOVA, a

significant difference in the consumption index on all stages of *A. humbertianus* on four host plants was obtained (Table. I).

The first and second instars of *A. humbertianus* differed significantly in their consumption indices on all four host plants and the highest consumption index was recorded on adult female fed on cabbage (0.78), while the growth rate (GR) of *A. humbertianus* on four plants i-e sugarcane, cabbage, maize, mixed diet was observed highest on cabbage i-e 0.040mg/ g day followed by maize, sugarcane and mixed diet i-e 0.031 mg/g day, 0.029 mg/g day and 0.020 mg/g day respectively. From nymphal stages highest growth rate was recorded in second and third instars i-e 0.043 mg/g day, fed on cabbage. A gradual increase in growth rate from fourth to sixth instar was recorded during the post-embryonic development. The highest growth rate noted during adult phase in the female could be attributed to the pre-ovipositional period, during which the insect develops for efficient reproduction in the following stage (Table I).

Observation through scanning electron microscope (SEM)

Showing that spectrum acquisition of sugarcane leaves observation in Fig. (I a) it indicates that normal weightage percentage of Oxygen was highest i-e 62.30 followed by 32.69 of Carbon opposing to this least percentage were obtain for Silicon, Potassium, Aluminum and Chlorine i-e 3.13,1.64,0.19,and 0.19 respectively. Spectrum Acquisition of Lemon was normal weightage percentage of Oxygen was highest 75.70 followed by 22.79 of Carbon opposing to this least percentage i-e 0.83, 0.62, 0.04 and 0.02 were for Potassium Calcium, Aluminum and Silicon respectively (Fig. I b). As for as chemical composition of rose is concerned normal weightage percentage of Oxygen was highest i-e 45.10 followed by 30.46 of Carbon and least percentage i-e 17.60, 1.67, 1.35, 0.52 and 0.46 was calculated for Fluorine, Potassium, Magnesium, Silicon and Aluminum respectively (Fig.I c). However, observation regarding papaya showed that spectrum acquisition percentage weightage of Oxygen was i-e 64.92 followed by 28.36, of carbon and least percentage i-e 3.33, 1.30 0.96 0.31 0.23 0.16 and 0.05 of Potassium, Calcium, Magnesium, Sulfur, Chlorine, Silicon, Phosphorus and Aluminum respectively (Fig. I d).

Beside this, normal weightage percentage of Oxygen was highest i-e 64.18 followed by 21.82 of Carbon opposing to this least percentage i-e 2.76 2.62 2.42 2.01 1.23 1.06 0.93 0.48 and 0.48 for Indicum, Potassium, Silicon, Sodium, Chlorine, Aluminum, Phosphorus and Sulphur respectively was noted on the maize plant leaves. (Fig.I e). The normal weightage percentage of Oxygen was highest i-e 65.98 followed by 22.77 of Carbon opposing to this least percentage i-e 3.92 2.48 1.67, 1.15 0.97 0.39 and 0.23 for Silicon, Potassium, Indicum, Sodium, Chlorine, Aluminum and Sulphur respectively was noted on grasses (Fig. I f).Beside this, for Cabbage leaves normal weightage percentage of Oxygen was noted highest i-e 61.77 followed by 30.75 of Carbon opposing to this, least percentage i-e 3.29 1.50 0.80 0.71 0.30 and 0.18 for Potassium, Indicum, Sulphur, Calcium, Phosphorus and Chlorine respectively (Fig. I g). As for as jowar observation were concerned its normal weightage percentage of Oxygen on jowar was highest i-e 63.29 followed by 24.07 of Carbon and least percentage i-e 15.51 1.93 1.53 1.48 1.21 1.14 1.05 0.57 and 0.36 was noted for Silicon, Aluminum, Indicum, Sodium, Magnesium, Calcium, Chlorine, Iron and Sulphur respectively (Fig.I h). Scanning Electron Microscopy data regarding brinjal chemical analysis showed that normal weightage percentage of Oxygen was highest i-e 61.75 followed by 25.65 for Carbon

on contrary to this it was 3.67, 1.68, 1.64, 1.60, 0.93, 0.85, 0.82, 0.71 and 0.69% for Potassium, Aluminum, Calcium, Indicum, Silicon, Chlorine, Magnesium, and Molybdenum respectively (Fig. I i).

Overall observation shows that sugarcane proved to be most preferable by *A. humbertianus*. As for as it element value is concerned it having the greater carbon concentration i-e 32.69%, Oxygen value is 62.30% and least Silicon value with 3.13% as for as cabbage diet is concerned it having normal Carbon value 30.75% with Oxygen ratio of 61.77% and Potassium ratio was 3.29%. However, in case of sugarcane Potassium ratio was 1.64% and cabbage having low Silicon value 0.26 (it might be ignorable). 3rd top preferable diet was maize it comprise on 21.82% Carbon, 64.18% Oxygen and has significant high value of Silicon i-e 2.42% was noted, when comparison carried with sugarcane and cabbage. Similarly, Potassium value i-e 2.62% was recorded in maize and it was greater in cabbage with 3.29% and there badly rejected food plants were lemon, rose and papaya the observation showed that lemon having 22.79% carbon and high oxygen percentage is i-e 75.70 and Potassium with 0.83% and Silicon was present with 0.02%. Beside this, normal Carbon range in papaya was 28.36%, Oxygen ratio 64.91%, Potassium with 3.33% and least ratio of Silicon i-e 0.23% was obtain in papaya through SEM ((Energy Dispersive X-Ray Spectrometer).

Discussion

This species consumes a wide variety of food plants from different families. However, survivability of *A. humbertianus* fed on these plants did not differ remarkably, although their survivability was higher on *S. officinarum* followed by *B. oleracea* and *Z. mays* at any stage of lifetime *Z. mays*, *S. officinarum*, *S. bicolor*, *C. dactylon*, *B. oleracea* and *S. melanogena* considering their life history trials. Observation under scanning electron microscope (SEM) indicates that sugarcane proved to be most preferable by *A. humbertianus*. Bernays and Chapman^[24] reported grasshopper feeding taxa as oligo-trophagous because they just feed on Poaceae and different species of this family. But for same concept Otte and Joern^[25] used polyphagus term. Oligophagus means feeding on limited range of plants within the species of one family and other plants reject completely. According to Otte and Joern^[25] oligophagus are those insects that feed on limited plants of different families. Polyphagus means feeding on wide range of plant species of different families.

Uvarov^[26] has reported that the range of plants tested in laboratory experiment may not coincide with tested plants in the field this observation was concerned with open field and study was analyzed from the foregut content or fecal material that egested out from the body. Ingestion and selection of food depend upon many factors i-e quality and acceptability of food plants depend on physio-chemical factors and feeding behavior of the insects which is regulated by neuro-physiological processes and odour of the plant and also he observed that mortality was highest during the 1st instars and gradually decrease towards the last nymphal instars. This because of less defensive mechanism compared to later instars. Similarly, present research shows that *A. humbertianus* has less sustainability so recorded more mortality rate in 1st instars in compare to last instars that having more defensive mechanism. During field survey it was also noted that insects continually left the various food plants and after that re-oriented for feeding. Grasshoppers were highly mobile and capable of a great deal of different activities the net effect of the activity was that to move from one plant to another plants. If the less

preferable the available food plants, the less feeding was noticed. The lower the density of the available plants, the greater the distance covered in each move. During the present investigation, it was found *A. humberianus* accepts variety of food plants and there is a great variation in the variety of food plant it accepts as prefer food. Beside this, field observation showed that grasshopper walked straight towards the food plants and examined it by touching its leaves with antennae and forelegs. After carefully examination of the plant, the insect took a small bite of the plant feeding remained continued, if the test plant got accepted. In case of non-acceptance, the grasshopper left the branch without further eating. Different host plant were offered to *A. humberianus* some were accepted and some were rejected by grasshopper present study suggests that it must be due to variation in the nutritional quality of host plants. Present study might be helpful to identify nature of damage to particular part of plant and its favorite host plant.

Different food plants also affect the breeding activity of insect it might be due to the reason that breeding areas of insects are mostly found in the overgrazed pastures of in the sparsely distributed vegetation and because of overgrazing by goatsheep and cattle because they provide suitable places for oviposition and must have succulent and palatable green

vegetation for the young nymphs, at their emergence in monsoon during month of mid-July to August Parihar [27] observed that absence of succulent food of mixture resulted in marked reduction in breeding activity and at the time of death. The present study based on the feeding behavior of *A. humberianus* in field as well as in laboratory the importance of feeding behavior in every field of entomology cannot be over emphasized because it constitutes, a key concept for understanding the main feeding habits of insect in relation to them surrounding vegetation. Beside this, it also tracks out the path of their evolutions and mandibles adaptation and afford reliable information for the proper comprehension of insect feeding with relation to its other life parameters. Current research may be fruitful to fill gap in scientific literature about feeding behavior of *Acrotylus* species and its quality of damage to host plant this attempt has been carried out first time hopefully, it will boost fresh biological literature regarding area of proposed research.

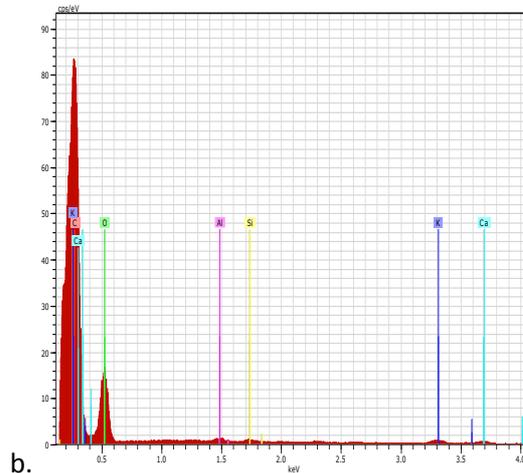
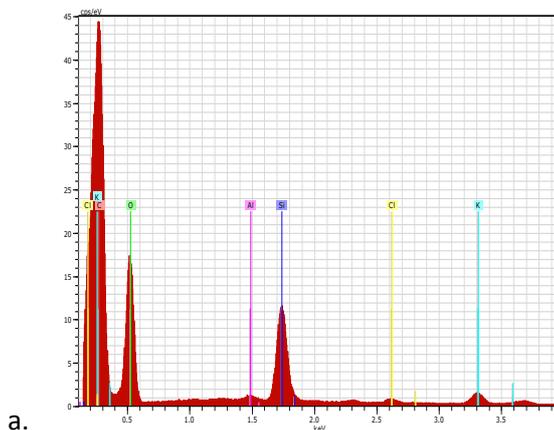
Acknowledgement

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Table 1: The consumption index (CI) and growth rate (GR) of *Acrotylus humberianus* on four top preferable plants

| Instars | FUI | Sugarcane (Mean ±SD) mg/day | Cabbage (Mean ±SD) mg/day | Maize (Mean ±SD) mg/day | Mix diet (Mean ±SD) mg/day |
|--------------|------|---|---|---|--|
| I | CIGR | 0.40±0.012 ^c 0.019±0.0021 ^f | 0.51±0.011 ^d 0.020±0.0012 ^f | 0.29±0.013 ^b 0.013±0.0008 ^c | 0.26±0.012 ^a 0.011±0.0014 ^e |
| II | CIGR | 0.42±0.037 ^c 0.016±0.0019 ^e | 0.58±0.032 ^d 0.043±0.0025 ^f | 0.031±0.015 ^b 0.024±0.0019 ^g | 0.17±0.012 ^a 0.019±0.0014 ^f |
| III | CIGR | 0.25±0.028 ^a 0.21±0.0022 ^c | 0.042±0.0021 ^c 0.049±0.0027 ^f | 0.40±0.014 ^c 0.046±0.0041 ^f | 0.30±0.014 ^b 0.018±0.0038 ^e |
| IV | CIGR | 0.20±0.23 ^{ab} 0.25±0.0033 ^e | 0.25±0.020 ^c 0.031±0.0039 ^g | 0.23±0.030 ^k 0.021±0.0023 ^f | 0.17±0.014 ^a 0.012±0.0023 ^e |
| V | CIGR | 0.22±0.026 ^c 0.033±0.0041 ^g | 0.29±0.010 ^d 0.037±0.0025 ^g | 0.019±0.0017 ^b 0.029±0.0022 ^f | 0.016±0.013 ^a 0.022±0.0029 ^f |
| VI | CIGR | 0.26±0.019 ^b 0.035±0.0025 ^f | 0.31±0.0022 ^c 0.38 ±0.0019 ^f | 0.27±0.022 ^b 0.35±0.0025 ^c | 0.018±0.019 ^a 0.26±0.0031 ^e |
| Adult Male | CIGR | 0.04±0.024 ^b 0.031±0.0025 ^f | 0.62±0.031 ^c 0.045±0.0040 ^l | 0.35±0.027 ^a 0.036±0.0034 ^g | 0.37±0.023 ^{ab} 0.022±0.0028 ^e |
| Adult Female | CIGR | 0.42±0.017 ^{ab} 0.052±0.0039 | 0.78±0.015 ^c 0.62±0.002 ^c | 0.43±0.022 ^b 0.047±0.0042 ^f | 0.39±0.037 ^a 0.32±0.0029 ^e |

Note: FUI- Food utilization Indices; CI-Consumption Index; and GR- Growth Rate Values represent mean ± S.D (n=6). Lower case letters in rows represent results which are statistically not separable at P< 0.05 level.



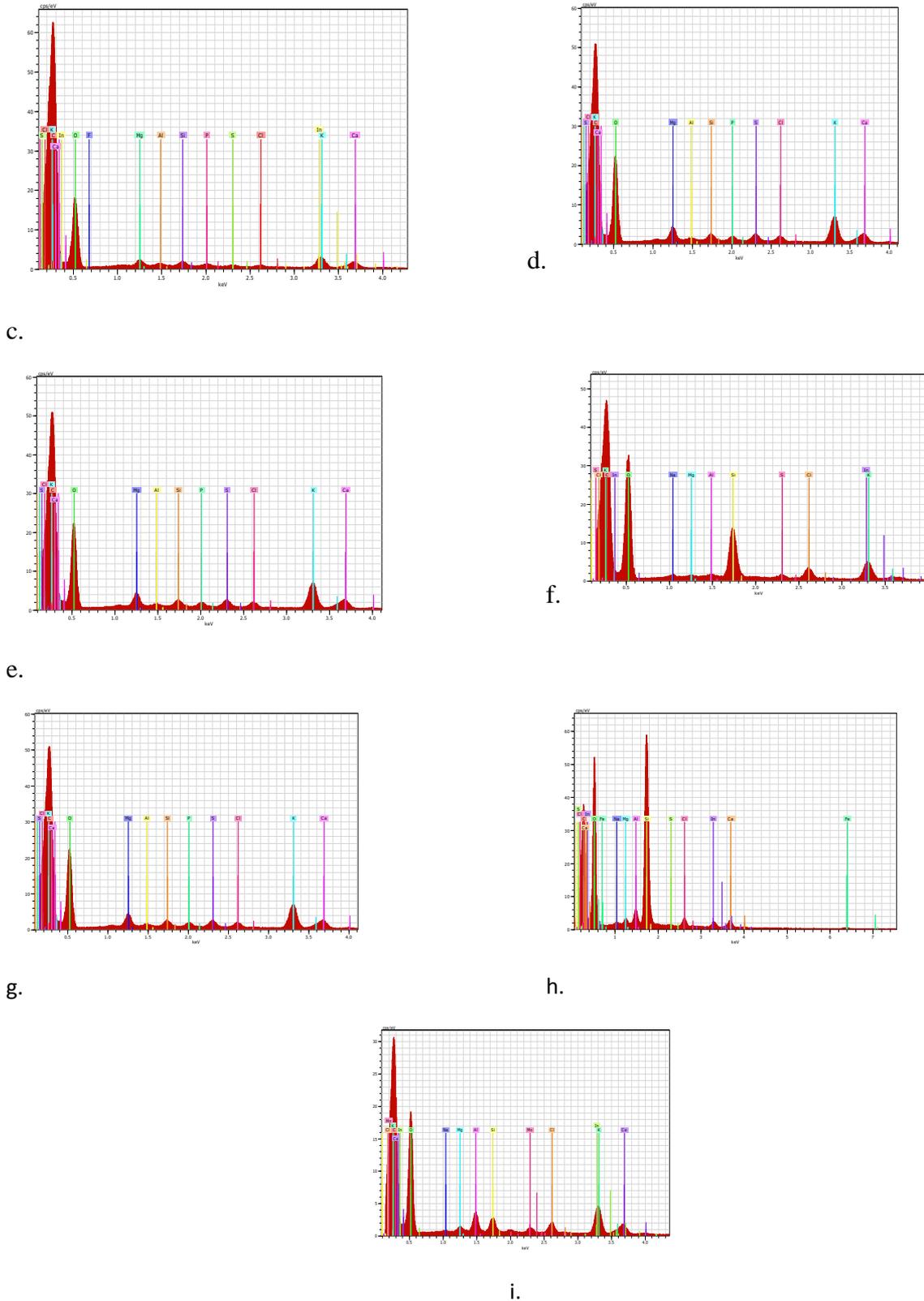


Fig 1: The elemental determination in various food plants under scanning electron microscope (a) Sugarcane, (b) lemon leaves, (c) Rose leaves, (d) Papaya leaves, (e) Maize leaves, (f) grass leaves, (g) Cabbage leaves, (h) Jowar leaves, (i) Brinjal.

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