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DISEASE STATUS AND MICROBIAL CONTAMINATION OF VEGETABLES IN BUEA MUNICIPALITY, SOUTHWEST REGION OF CAMEROON

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ABSTRACT
Vegetable consumption in Buea and its environs has increased in recent times. Constraints towards its production must be taken care of. This study investigates the fungi contamination of vegetables and to identify the taxonomic classes. Survey of 40 vegetable fields was carried out and samples from five preferred vegetables (bitterleaf, waterleaf, huckleberry and amaranthus) were cultured for fungi using Sabouraud Dextrose Agar medium. The results revealed that vegetables farming is a female-dominated venture (64.8% females), of age between 31 to 40years and educated. Vegetable production is challenged by fungal diseases with varied symptoms including; necrosis, leaf spots, leaf curl, and mosaic. All the isolates of fungi culture were identified to be aseptate fungi of the class Zygomycetes. Conclusively, vegetable production is facing threats due to diseases especially that of fungi in the class Zygomycetes. Therefore, it is important for the government to provide the farmers with agrochemicals to curb vegetable diseases management in view to lower diseases and microbial infections.

KEYWORDS: Vegetables, Diseases, Fungi, Contamination, Buea Municipality.

INTRODUCTION
Vegetables play a significant role in human nutrition, especially as sources of vitamins (C, A, B1, B6, B9, E), minerals, dietary fibre and phytochemicals (Yahia et al., 2019). Vegetables provide a healthy and balanced diet especially in developing countries (Septembre-Malaterre et al., 2018). It contributes to household food security, income generation, poverty alleviation, and strengthening the economic base of cities (Asongwe et al., 2014; Chihambakwe et al., 2018; Ridwan et al., 2022) and help in weight loss due to their low-fat content and are powerful antioxidants that have been shown to protect the eyes against light induced damage (Dias, 2012). In addition to nutritional and health benefits, vegetables bring an aesthetic value to the table (Koike et al. 2007).

The amount of vegetable losses due to diseases varied from place to place because of the existence of different pathogens (Imathiu, 2021). Among the constraints limiting vegetable production in Buea, diseases are very prominent, given the optimum condition for their development and have been implicated to be one of the causes of low vegetable productivity (Arrey et al., 2016). Low production in yields encountered in vegetable production could be attributed to pest and diseases. The crop is susceptible to bacteria, fungi, viruses and nematodes that cause significant losses to vegetable producers throughout the world (Kromann et al., 2014). The magnitude of yield loss depends upon the intensity of the disease. Losses are more severe in developing than developed nations of the world (Enyiukwu et al., 2014). It is estimated that around 85% of the diseases of vegetables are caused by fungi or fungal-like pathogens. Therefore, fungi constitute the largest disease-causing plant pathogens damaging vegetables crops in term of growth and yield. However, very little survey on fungi disease contamination of vegetables has been done previously in Buea Municipality. The incidence and distribution of pathogens on vegetables in this Municipality has to be regarded as important, resulting to a drop in the quantity produced which is less than demanded. In view of the above facts, the present study was undertaken to evaluate the disease situation and the microbial contamination of vegetables grown in Buea Municipality. There is need to identify the diseases of vegetables and fungi pathogen class and develop control measures. The objective of the study reported here was therefore to identify diseases, isolate, characterize and identify the fungi classes associated with vegetables in Buea.

MATERIALS AND METHODS

Study Site

The study was carried out in Buea municipality, Fako Division, South West Region, Cameroon. Buea is located in the humid forest with monomodal rain fall zone in the Southwest Region of Cameroon. It spanned from Longitude $8^0$13 to $11^0$14 E and Latitude $3^0$48 and $6^0$11 N. The area has a humid tropical climate. The mean annual rainfall and temperature is about 2085mm and 28^oC respectively. The Relative humidity is 86% and sunshine is 900 to 1200 hrs per annum (Bechem and Mbella, 2019). The soil type is mainly volcanic and relatively fertile and therefore intensively cultivated.

Field Surveys

A survey was carried out in vegetable growing sites in this study area. Interviews coupled with direct observations were conducted with the owners of selected farms. Forty farms proportionally distributed along the axes of Buea were considered as representative sample of vegetable farms in Buea based on the size of the farm. A farm was considered if it had a farm size of at least $5m^2$ with vegetable beds on it. Semi - structured questionnaires were administered to farm owners to gather information on demography, farm size, vegetable type, agronomic practices etc.

Disease Assessment

In each farm, a 2m x 2m subplots were randomly identified. The total number of vegetable plants and the number of infected plants per subplots and types of symptoms were recorded. Photographs of symptom types were taken and disease symptoms observed were described. The mean infection in each sub-plot and the incidence of symptom types per vegetables types were analysed using simple mathematic means.

The disease incidence per vegetable type was calculated using the formula stated by Getachew et al, (2014).

$$I = \frac{Number\ of\ plant\ with\ infection}{Number\ of\ plants\ observed} X100$$

Where I= incidence.

Phenotypic data on host reaction were recorded in terms of disease symptom expression following a five-point scoring scale. Using the symptom severity scores, percentage severity of vegetable type was estimated according to González-Pérez *et al*. (2011) as:

$$Severity\ (S)\ (\%) = \frac{\sum AB}{5\sum B} \times 100$$

A- Disease class 1, 2, 3,4 and 5

B- Number of plants evaluated.

The symptom type for each score is shown on Table 1

Table 1: Scoring system for disease on vegetables in Buea Municipality.

Severity Class	Description
0	Healthy.
1	Disease symptom not extremely distinct and little yellowed or necrotic spots.
2	Mild symptoms on one or more leaves, with little necrosis or mosaic.
3	More symptomatic leaves per plant. Severe symptoms, widespread on plant.
4	Severe symptoms and more noticeable stunting.
5	Very severe stunting, obvious and significant amount of necrosis, chlorosis and wilting

Isolation of Fungi pathogens from diseased vegetable leaves

Sample collection of vegetables

The vegetables used in this experiment were selected based on farmers' preference in the surveyed fields (huckleberry, waterleaf, bitterleaf and amaranthus). The fresh leaf samples of both symptomatic and asymptomatic leaves of the different vegetables were randomly harvested from the fields using a sharp knife. Harvesting was done when the vegetables were void of surface moisture and the pathogen is still active. Samples were carefully packed in Ziploc bags and immediately transported to the Life Sciences Laboratory of the University of Buea, for fungi isolation.

Culture media preparation

Saboraud Dextrose Agar (SDA) was use for the isolation of fungi and was prepared following the manufacturer's instruction (Liofilchem). The medium was allowed to cool and 131.25µL of Gentamycin was added. The mixture was stirred gently by swirling the flask, before pouring 15ml into each 9mm sterile petri dishes. The medium was allowed to solidify before inoculation was done.

Sample preparation

Isolation of fungi was based on the method described by Zainab and Shinkafi (2016). The vegetable leaf samples were washed with running tap water to remove dust and debris and were surface sterilized with 70% ethanol for 1 min. The leaves were cut into small pieces of 1x1 cm using a sterile scalpel under aseptic conditions. They were then immersed in 10% sodium hypochlorite solution for 3 mins. This was followed by rinsing with 70% alcohol for 1 min. The samples were finally rinsed in several changes of sterile distilled water and blotted dry on sterile tissue paper. Each plate was inoculated with 4 pieces of the surface sterilized leaves. Inoculation was done in triplicates, giving a total of 12 plates. The petri dishes were sealed with parafilm wax and wrapped in aluminium foil. The inoculated plates were incubated at room temperature (25℃) for 7 days to enhance fungal growth and sporulation.

Subculture

Distinct fungi colonies from primary cultures were sub-cultured on fresh SDA plates at room temperature to obtain pure cultures. Inoculated plates were sealed with parafilm wax and wrapped in aluminium foil and incubated at room temperature (25 °C) in the dark for 0ne week.

Cultural Characterization

For cultural characterisation, the method of Bechem and Afanga, (2017) was used. From each fungus isolated in pure culture, 5 mm mycelia discs was transferred to the centre of sterile petri dishes containing fresh SDA. Inoculated plates were incubated for 7 days. Colony diameter was measured using a graduated 30 cm ruler and the mean diameter was obtained using the formula:

$$\text{Colony diameter} = \frac{\textit{sum of colony diameter in triplicate}}{\textit{Total number of replicates}}$$

Morphological Characterization

The cultural appearances (colony colour, texture, margin, form, elevation and aerial hyphae) were noted on SDA. As a reference point for the description of the forward and reverse tints of the colonies on culture media, the Munsell colour chart was used. A pictorial atlas for identification of fungi by Watanabe (2002) were equally used in the description of colonies.

Identification of Fungi isolated

For the identification of the isolated fungi, a drop of 10% potassium hydroxide stain was placed on a clean slide.

A mounted needle was used to remove a small portion of the mycelium from the fungal cultures and placed on the stained slide and covered gently cover slip. The slide was then examined under the microscope. Morphological characteristics of the fungi (presence/absence of septum in hyphae and spores' occurrence) were observed and noted.

RESULTS

Farm survey

All the forty questionnaires administered were completed, giving a percentage of 100% success rate. Based on success rate, 64.9% were females and 35.1% were males. The result also revealed that majority of the informants were between the ages of 31 and 40 years and the least were at the age > 60 years, figure 1.

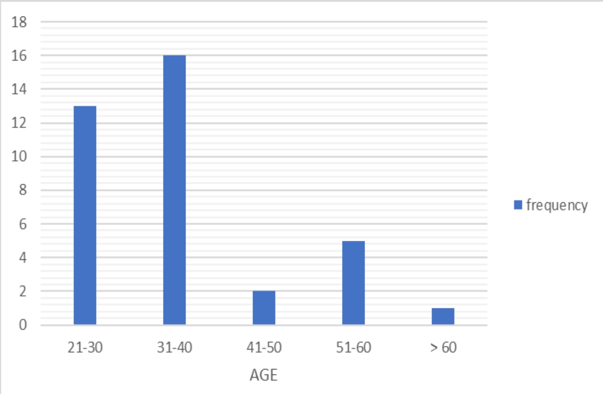


Fig. 1: Age Distribution of Respondents.

From the survey, it was observed that with respect to educational standard, all the farmers had attained formal education. 56.8% of them had attained primary education, 29.7% had secondary education and 13.5% had attained tertiary education.

The vegetables had preference by the farmers depending on the demand by the consumers. Ten different types of vegetables were grown in the study area. Huckleberry was found in thirty farms either in mono-cropping or mixed-cropping fields Figure 2.

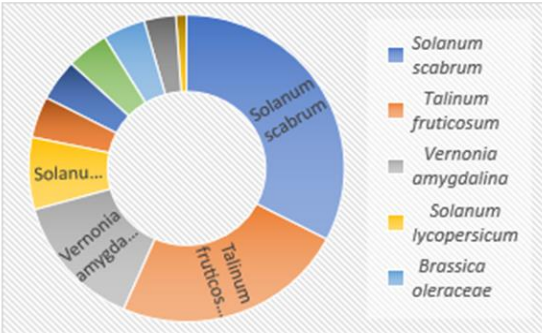


Figure 2. Frequency of Farmland on Which Different Vegetables are Grown.

The farm sizes ranged from 25m^2 to 900m^2. None of the farms were up to a hectare. Most of the field (75%) had farm sizes of between 100 and 500m^2. Few fields had sizes greater than 850cm^2 (7.5%).

Out of the 40 farms that were surveyed, 3 had mono-cropping practice, 8 mono- mixed cropping and 29 had mixed cropping system with other plant species other than vegetables. A greater proportion of the mixed-cropping fields were owned by females. Tomato, cabbage and waterleaf were the vegetables observed as lone crops on the mono-cropped fields.

Fungal disease symptoms

Varied disease symptoms were observed during the study on the different vegetable types. The leaf symptoms ranged from rot, necrosis to chlorosis on the different vegetable types. Some of the symptoms are shown on figure 2.

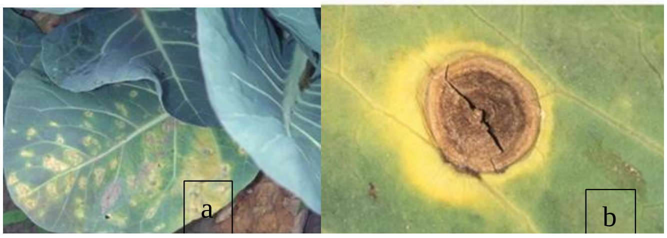


Fig. 2.1: Fungal Symptoms of Cabbage.

Fig. 2.2: Fungal Disease Symptoms of Cucumber.

Fig. 2.3: Fungal Disease Symptoms of Okra.

Fig. 2.4: Fungal Disease Symptoms of Bitterleaf.

Fig. 2.5: Fungal Disease Symptoms of Huckleberry.

Fig. 2.6: Fungal Disease Symptoms of Waterleaf.

Fig. 2.7: Fungi Disease of Amaranthus.

Disease incidence was higher in mono-cropped fields than the intercropped fields. The incidences of disease symptom varied among the different vegetables. The incidence ranged from 11.1 to 36.36%. Diseases incidence observed during the survey indicated that there were less infected okra plants than other vegetables. Cucumber had the highest incidence Table 3.

Table 3: Disease incidence on different vegetables grown in Buea municipality.

SN	Vegetable	Mean number of plants considered	Mean number of infected plants	Disease incidence (%)
1	Huckleberry	51	10	19.61
2	Waterleaf	53	13	24.52
3	Cabbage	12	2	16.66
4	Bitterleaf	43	10	23.25
5	Green	9	2	22.22
6	Okongobong	6	1	16.66
7	Cucumber	11	4	36.36
8	Okra	9	1	11.11
9	Pepper	10	3	30
10	Tomato	18	3	16.66

Fungal Disease Severity on Different Vegetables

Infected vegetables showed various disease severities. The disease severity increases from 0.2% in okra to 2.6% in waterleaf (Fig 3).

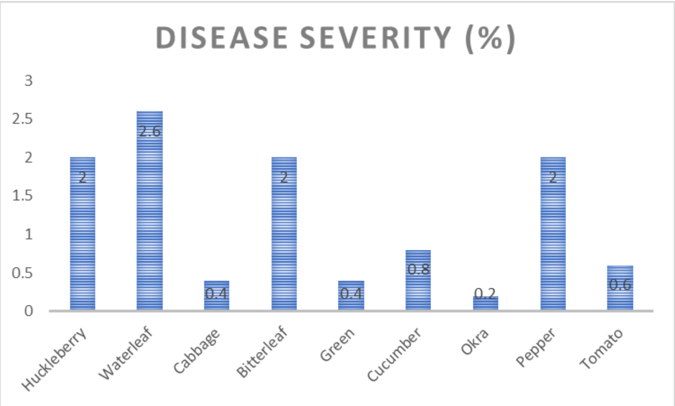


Figure 3: Mean Fungal Disease Severity for Different Vegetables.

Morphological Characterization of Fungi Isolates

The details of the cultural characteristics are shown on table 5 below. The research findings indicates that Bitterleaf (BL) and Amaranthus (AM) showed growth preference on SDA (66.7%). While waterleaf (WL) and huckleberry (HB) replicates showed growth preference of 100% on SDA. Maximum colony diameter of 85mmwas observed with isolates BL, HB and the lowest 25mm was observed in the isolate WL.

Macroscopic Observation of Fungi Isolates

The cultural appearance (colony colour, margin, elevation, form, growth pattern, zonation, surface texture and aerial hyphae) were observed (Table 4) and Figure 5.

Table 4: Cultural Characteristics of Fungi Isolates on SDA Culture Media.

Sample Code	Vegetable	Colony diameter	Surface texture	margin	elevation	Zonation	Colony shape	Colony tints	
								Surface	reverse
WL1	Waterleaf	75m m	Smooth	Irregular	Flat	Irregular and spreading	Irregular	Grey	yellow
WL2	Waterleaf	27m m	Mucoid	Irregular	Flat	Irregular and spreading	Filamentous	Grey	Yellow
WL3	Waterleaf	25m m	Glistering	Entire	Raised	One centralized ring	Circular	Grey	Yellow
HB1	Huckleberry	30m m	Mucoid	Filamentous	Raised	One centralized ring	Filamentous	Grey	Buff
HB2	Huckleberry	85m m	Powdery	Entire	Flat	Distinct zones of black and brown edges	Circular	Black-brownish	Buff
HB3	Huckleberry	85m m	Powdery	Entire	Flat	Distinct zones grey with white edges	Circular	Grey, black and white	Buff
AM1	Amaranthus	30m m	Smooth	Irregular	Raised	One centralized ring	Irregular	Grey	Yellow
AM3	Amaranthus	60m m	Mucoid	Entire	Raised	One centralized ring	Curled	Pink and blue	Red
BL2	Bitterleaf	85m m	Buttery	Entire	Flat	One centralized ring	Curled	Green,yellow, pink, white and grey	Yellow, red and black (Hues)
BL3	Bitterleaf	80m m	Mucoid	irregular	Raised	Irregular and spreading	Filamentous	Grey	Yellow

The surface view of the vegetables varied in the replicates as shown on figure 5.

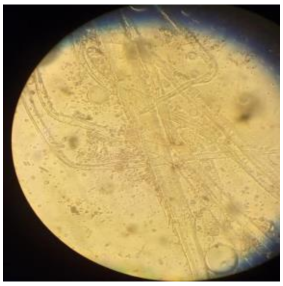
Waterleaf subculture (left: front view; right: back view)

Microscopic view of WL

Bitterleaf subculture (left: back view; right: front view)

Microscopic view of BL

Huckleberry subculture (left: front view; right: back view)



Microscopic view of HB

Amaranthus (left: front view; right: back view)

Microscopic view of AM

Figure 5: Morphological View of the Vegetable Isolates.

Identification of fungi isolates

Identification of isolates was based on micro-morphological characters. The isolates were all identified to taxonomic classes, based on these characters. All the fungi isolate had aseptate hyphae, an indication that they probably belong to Phylum Zygomycetes. All isolates produced spores except BL, that the spores were not visible.

DISCUSSION

Vegetables cultivation is assuming an increasingly important commercial role, especially for the low-income inhabitants. Both men and women are involved in the cultivation and selling of vegetables. All the respondents had attained formal education with the majority of them having attained primary and secondary education. The selected vegetable species are in agreement with Arrey et al., 2023 as preferred by the community of the study area. Knowledge on the management of a disease is very important in the incidence and control of that particular disease, thus the low educational level of most of the farmers could be a disadvantage in adopting improved agronomic practices on disease management of their vegetables. Diseases have always been a major cause of reduced quality and quantity of plants worldwide. Incidence and severity are the tools for measuring the diseases. Report from this survey has shown that there is a high incidence of fungal diseases in vegetables in the study area. Generally, all the vegetable types and the farms surveyed had diseases. Due to lack of farm space, different cropping patterns were observed in the fields surveyed. This may have facilitated the survival and propagation of these pathogens since fungi are able to survive in alternate hosts. The highest mean incidence was observed on cucumber and the lowest was on okra. Though some farms were well managed, diseases still occurred. The incidence of diseases should be a cause for concern, since these diseases are known to be of economic importance in developing countries (Arrey et al., 2023). The environmental conditions generally have an appreciable effect on the incidence of diseases which is generally higher in cold and moist areas. The incidence of the infection is hardly surprising, since host abundance; couple with increasing farming activities in the field in this location may lead to a greater spread of mechanical transmitted infections. Okolle et al. (2014) reported on the good management practices which can be put in place for the control of fungi infection in this study area, even though it has been difficult to the farmers due to lack of fundings. Fungi diseases of this study were caused by aseptate fungi as opposed to study by Saha and Tayung (2022), who observed septate fungi isolated pathogens belonging to the fungal classes Oomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes on diseased vegetables in India. Equally, Udoh et al., (2015) isolated a number of fungi species from some edible fruits and vegetables that were responsible for post-harvest spoilage of some vegetables. Fungi diseases has been observed in other crop in this study area. The zygomycetes have a host range of habitat, mostly on the soil. It can be evident that this class of fungi must be habiting the soil in this study site. This is an indication that the pathogen maybe existing in the soil of this area and must be considered when cultivation for any crop is to be carried out in a large scale. Zygomycetes are fungi species that commonly affect vegetables since they are found in the soil and are characterised by rapid germination.

CONCLUSION

Fungal foliar diseases have become a global threat to vegetable production. They are responsible for yield and economic losses of commercial vegetable crops. From our findings, it is likely that due to lack of land and seed source, farmers constantly farm on the same field and use seeds from their fields which might have been infected with fungal spores, resulting to fungal disease situation of the vegetables. Vegetable contamination is likely due to non-septate hyphae fungus (Zygomycetes) which must be taken into account by future vegetable growers. Therefore, it is necessary for rapid identification of disease-causing fungal pathogens in vegetable crops for proper management and control to be put in place for large scale production of vegetables in Buea Municipality.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

ETB conceived, designed and supervised the research, DBA, MEBT and CA carried out the work, collected data and samples from the field, analysed the samples in lab-based experiments. DBA and MEBT contributed in drafting the manuscript. All authors proof read the manuscript.

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REFERENCES

1. Yahia E. M, Gracia-solis. P and Celis M. E. M. Contribution of fruits and vegetables to Human Nutrition and Health postharvest physiology and biochemistry of fruits and vegetables, 2019. Elsevier, Amsterdam, Netherland.
2. Septembre-Malaterre. A, Remize. F, and Poucheret. P, "Fruits and vegetables as a source of nutritional compounds and phytochemicals: change in bioactive compounds during lactic fermentation". Food Research International, 2018; 104: 86-99.
3. Asongwe GA, Yerima BPK, Tening A.S. Vegetable production and the livelihood of farmers in Bamenda Municipality, Cameroon. Intl. J. Current Microbiology and Applied Science, 2014; 3(12): 682-700.
4. Chihambakwe M, Mafongoya P, Jiri O. Urban and Peri-Urban Agriculture as a Pathway to Food Security: A Review Mapping the Use of Food Sovereignty. Challenges, 2018; 10(1): 1-12.
5. Ridwan Mukaila, Abraham Falola, Sheu-Usman Oladipo Akanbi, Angela Ebere Obetta, Lynda Ogechi Egwue, Tochukwu Linda Onah. Effects of vegetable production on income and livelihood of rural households in Nigeria. Mustafa Kemal University Journal of Agricultural Sciences, 2022; 27(2): 213-223.
6. Dias João Silva. Nutritional Quality and Health Benefits of Vegetables: A Review Food and Nutrition Sciences, 2012; 3: 1354-1374.
7. Koike, S.T., Gladders, P., Paulus, A.O. 2007. Vegetable diseases: a colour handbook. Gulf Professional Publishing, 2007.
8. Imathiu S. Neglected and underutilized cultivated crops with respect to indigenous African leafy vegetables for food and nutrition security. Journal of Food Security, 2021; 9(3): 115-125.
9. Arrey, D. B., Mih, M. A. and Essomo, S. E. Sugarcane Germplasm Collection in Western Cameroon. American Journal of Life Sciences, 2016; 4(6): 139-145.
10. Kromann, P., Miethbauer, T., Ortiz, O. and Forbes, G. A. Review of potato biotic constraints and experiences with integrated pest management interventions. In Integrated pest management, 2014; 245-268). Springer, Dordrecht.
11. Enyiukwu, D.N., Awurum, A.N., Nwaneri, J.A. Efficacy of plant-derived pesticides in the control of myco-induced postharvest rots of tubers and agricultural products: A review. Net J. Agricult. Sci., 2014; 2: 30-46.
12. Bechem E. E. T. and Mbella C. E. J. A Survey of Symptoms of Fungal Disease in Sugarcane (Saccharum officinarum L.) in Buea, in the South West Region of Cameroon. Annual Research & Review in Biology, 2019; 31(3): 1-12.
13. Getachew Gashaw, Tesfaye Alemu and Kassahun Tesfaye. 2014. Disease incidence, severity and yield loss of finger millet varieties and invitro mycelial growth inhibition of P. grisea using biological antagonists and fungicides. Journal of Applied Biosciences, 2014; 73: 5883– 5901.
14. González-Pérez, J.L., Espino-Gudiño, M.C., Torres-Pacheco, I., Guevara-González R.G., Herrera-Ruiz, G. and Rodríguez-Hernández, V. Quantification of virus syndrome in chilli peppers. African Journal of Biotechnology, 2011; 10(27): 5236-5250.
15. Zainab, M. B.1 and Shinkafi, S. A. 2016. Isolation and Identification of Fungi Responsible for Leaf Spots Disease of Mango (Mangifera indica Linneaus) in Sokoto State, Nigeria. Bayero Journal of Pure and Applied Sciences, 2016; 9(2): 166 – 173.
16. Bechem E. T. and Afanga Y. A. Morphological and molecular identification of fungi associated with corm rot and blight symptoms on plantain (Musa paradisiaca) in macro-propagators. Int. J. Biol. Chem. Sci., 2017; 11(6): 2793-2808.
17. Watanabe Tsuneo. Soil and Seed Fungi; Pictorial Atlas of Soil and Seed Fungi. Morphologies of Cultured Fungi and Key to Species. By. 2nd edn. CRC Press, Boca Raton. 2002; ISBN 0 8493 1118 7.
18. Arrey D. B., Oben T. T., Essomo, E. S., Afanga Y. A. and Bechem, E. E. T. Leafy vegetables production, marketing and constraints in Buea Subdivision of the Southwest Region of Cameroon. International Journal of Agriculture and Food Science, 2023; 5(1): 34-40.
19. Okolle, N., Ijiang, P. T. and Ngome, F. A. 2014. Evaluation of Farmer's Knowledge on Pests and Diseases of Vegetables and Their Management Practices In three different Agroecological Zones in Cameroon. Justin (AgroVital Services & Consulting – AgroViSc Cameroon) Requested by and submitted to Victor Afari-Sefa (AVRDC – The World Vegetable Center), 2014.
20. Saha S and Tayung K. Survey and identification of some fungi diseases of vegetable crops of Kamrup (M) district of Assam, India. Journal of Micropathological Research, 2022; 60(4): 557-564.
21. Udoh, I. P., Eleazar C. I., Ogeneh B. O. and Ohanu M. E. Studies on fungi responsible for the spoilage/ deterioration of some edible fruits and vegetables. Advanced Microbiology, 2015; 5: 285-290.