MORPHOLOGICAL CHARACTERIZATION OF KENYAN NATIVE *MACROLEPIOTA* SPP OF MUSHROOM AND THE EFFECT OF SUPPLEMENTED MILLET AND SORGHUM GRAINS IN SPAWN PRODUCTION

C.Mbaluto[1, 3], S. Runo[1], W. Wanyoike[2], D. Otieno[3] and C. Onyango[3]
[1]Department of Biochemistry and Biotechnology, Kenyatta University, Nairobi, Kenya
[2]Botany Department, Jomo Kenyatta University of Agriculture Science and Technology, Nairobi, Kenya
[3]Food Technology Division, Kenya Industrial Research and Development Institute, Nairobi, Kenya
E-mail: crismbaluto@gmail.com

Abstract
Kenya is rich in mushroom biodiversity that has not been fully exploited. Rural communities consume wild edible species especially during the rainy seasons. Utilization of mushrooms collected from the wild requires adequate description of useful phenetic features and morpho-cultural characteristics which create basis for molecular approaches. The aim of this study was to morphologically characterize *Macrolepiota* spp. and develop grain propagation spawns. Twenty one accessions were collected with assistance of local residents. Fruiting bodies were subjected to macro and micro-morphological characterization. Propagation spawns were developed from pure mycelial culture. The spawning experiment was arranged in completely randomized design with three replicates. Morphological analysis showed close similarity between indigenous and exotic species. Microscopic analysis of spores and external features of mycelia colonies did not reveal significant differences. Spawn production showed that wheat bran supplemented with sorghum and millet grains can be successfully colonized by mycelia to produce high quality spawn. This study has established new wild edible mushroom species in Kenya. Based on conventional morphological taxonomy the Kenyan *Macrolepiota* species from Aberdare National Forest Reserve is described for the first time as a new Basidiomycota. The species is edible. However, domestication protocols can be developed to address food insecurity and supplement families' income. Formation of a checklist to be used as a reference is vital as the fungal diversity keeps on changing.

Key words: Macrolepiota, basidiomycota, spawn, edible species, food insecurity, checklist, Kenya

1.0 Introduction

Mushrooms are macrofungi usually distinguished by forming fruiting bodies commonly visible to naked eye (Tibuhwa *et al.,* 2011; Rajaratnan and Thiagarajan, 2012). Mushroom studies have long been of interest to scientist and communities due to their significant roles (Ram *et al.,* 2010; Tibuhwa et al., 2011). They form integral part of the forest environment and ecosystems such as forest flourishing through saprobic life. Mushrooms have enormous use generally in human life welfare as well as in food industries. Edible mushrooms provide a wide range of mineral and vitamins. The total nutrient content vary significantly among species (Tibuhwa *et al*., 2011). It is amicable that a diverse fungal population contributes to a diverse diet for wild life and human.

Cultivation provide an opportunity to improve local farmers livelihoods and reduce dependence on natural resources. The sustainabilty of mushrooms is thus important to maintain and promote productivity of crop land, range lands and forest and may be critical for maintanance of biodiversity and livelihoods (Tibuhwa *et al*., 2011). Despite the importance of mushrooms in both natural and agro-ecosystems, little is known about their community structure and dynamics especially in the tropics. Mushrooms in the tropic are under studied, relatively under utilised and very little information on the diversity is known (Tibuhwa *et al*., 2011). On the other hand, fungal diversity is usually overlooked during consideration of management of forest ecosystems, yet successful conservation efforts in any ecosystem may require understanding mushrooms communities in terms of ecology and distribution. Classification of macrofungi is constantly anguished by contradictions. This is due to the lack of complete knowledge about all the fungal organisms (Harrington 1990). In Europe Jaworska (2010) recorded 17 *Macrolepiota* species. The genus contains some edible species, which has been an interest to cultivate by researchers knowledge of this genus in Kenya is poor and fragmentary.

According to Ge., *et al*., [2010], several species of *Macrolepiota* were recorded from China (Vellinga and Yang 2003; Shao and Xiang 1981; Zang *et al.,* 1996; Bi *et al.,* 1997; Mao 2000 and Teng 1996), literature on some of these records has very limited information in the descriptions and information.

Based on extensive morphological examination of *Macrolepiota* in MountKenya and the affinities to other species of *Macrolepiota* are presented and discussed. With the ever increasing population and shrinking land, secondary agricultural vocations are going to occupy a prominent place to fill the void of quality food requirements. The demand for quality food and novel products is increasing with the changes in life style and income. Mushroom cultivation fits very well into functional foods free from synthetic chemicals category. Diversification in any farming system imparts sustainability. Mushrooms are one such component that not only impart diversification but also help in addressing the problems of quality food, health and environmental sustainability. However, this study was aimed at characterizing wild edible mushrooms commonly consumed in Aberdare national forest reserve Kenya forest region, thus diversify knowledge and recommend for domestication adaptations.

2.0 Materials and Methods

2.1 Study Area: Aberdare National Forest Reserve in Kenya

The study area was based at Abadare National forest reserve in Kenya, located in central Kenya. (Figure 1).

2.2 Ethynomycological Study

Structured questionnaire were used for ethynomycological survey. Data obtained was for indigenous knowledge; preservation methods and other ethnomycological utilization of wild edible mushrooms in the area were investigated. Information was collected from 27 respondents; detailed results will be presented in a separate study.

2.3 Collection and Preservation of Fruiting Bodies

A five days field trip was made to the forest and covered five reserves of the park (Figure 1) in the month of August 2012. Mushroom collection in the 5 forest reserve yielded 21 accessions. On the study site, collection positions were determined using GPS (Global Positioning System-Garmin *etrex-20* Garmin, USA), on spotting the mushroom fruiting bodies. Photographs were taken using a digital camera (Cyber-Shot DSC-W550 Sony Corporation Japan) and types of vegetation around were recorded as described by KFS. The woody substrates lying on the ground were also identified to the generic level and wherever possible to the species level. Ecological parameters like temperature and relative humidity were also noted. Some of the fresh fruiting bodies were harvested then dehydrated using silica gel and kept in air tight plastic bags until further analysis and molecular studies.

2.4 Macroscopic and Microscopic Characterisation

Morphological characterization of the 21 accessions of Macrolepiota was done in the field as well as in the laboratory on the basis of Basidiocarp colour, size, shape, texture margin, exterior surface of the fruit body, presence of stipe and its attachment to the substrates, cap edge curliness, the fruit body fleshiness when fresh and dry, Hymenial structure, Ring presence or absence, developmental stages forms, as well as nature of growth and spore print [20, 23]. Spores were used for microscopic characterization. Stains used included; Cotton blue, Congo red solution and Melzers reagent. Observations were made at X40, X100 and X400 magnification of a bright field biological microscope. Microscopic features of the Basidiospore observed included; size, shape, stain reaction and symmetry. Pictures were taken using a digital camera mounted on the microscope.

2.5 Comparative studies and identification of the *Macrolepiota* sp

Identification of specimens was done using published works on Basidiomycetes mushrooms such as (Ge, *et al.,* 2010; Vellinga, 2003; Vellinga, *et al.,* 2003). The materials studied were as follows: Kenya: Aberdare National Forest Reserve-Ragati, Zaina, Mathioya, Kabage and Zuti.

2.6 Development of Grain Spawns

Mycelia obtained from tissue culture was used to develop grain spawns. Millet and sorghum grains supplemented with either wheat or kopakula at varying combinations. The grains were boiled in fresh clean water (1:1.5 w/v) for 30 min to soften and eliminate chances of germination before and upon utilization for spawn production. A total of nine different treatments (150 g each) were used as shown in (Table 1). Grains ratio; 1, 1:1, 4:1 and 2:2:1 were used. The weighed grains, wheat bran and koppa kula were then thoroughly mixed by hand. Each grain formulation (150 g) was mixed with Calcium carbonate powder at a ratio of 3g/Kg to regulate their *p*H. To produce grain spawn of 50% moisture level, water was added at a rate of 25 ml per 150 g formulation. Grain combinations

were put in 250 ml heat resistant glass bottles and autoclaved (*All American*: Wisconsin Aluminum Foundry Co., Inc; Manitowoc) for 15 min at 121^{o}C. Bottles were allowed to cool in a sterile lamina flow hood after shaking them to loosen and evenly distribute wet and dry grains. Ten 9 mm agar pieces were carefully transferred to upper surfaces of prepared grains for each grain combination. Inoculated grain bottles were tightly secured using moist cotton wool and covered with sterile aluminium foil and bottle lids. They were incubated in dark at optimal temperature until they were fully colonized. Spawn production experiments were laid out in a Completely Randomized Design (CRD) and replicated three times.

3.0 Results and Discussion

3.1 Ecology

The fruiting bodies exhibited different ecological characteristics (Table 2). Sibounnavong *et al.,* (2008) reports specimens at different stages of development should be collected by digging (not pulling) them up so as not to damage their bases. Those attached on dead logs and woods, efforts should be made to scrape a piece of the wood or bark on which the specimen is attached. In this study accession RMK-04 was growing gregarious on soil. The surrounding vegetations were *Vitex kiniensis* (Meru Oak).

Accession ZMK-02 had some species growing Caespitose and gregarious, in mixed forest (plantation, grassland and indigenous) blocks. The surrounding vegetation comprised of *Copressus lustanica* (*Copressuceae), Vitex kiniensis, Croton macrostachyus, Albizia gummifera, Olea europaea, Polycia kikuyuensis, Cordia africana, Prunus africana, Cassipuree molosonia, Juniperus procera and Croton megalocapus*. Accession RMK-08 was growing singly (solitary) on soil. Surrounding vegetation was *Copressus lustanica.* The woodlands were also dominated by unknown vegetations. This agrees with Kuo (2007) who reported that macrolepiota are saprobic, growing alone or scattered in woods or at the edges of woods, or in pastures, on trails and other disturbed ground areas. They appear in late summer.

3.2 Morphological Characteristics

Phenetic features observed on various fruiting bodies were compared with literature reports (Ge *et al*., 2010; Kuo 2007) who worked on conventional *Macrolepiota* genus identification. In agreement with the results, the cap of mature fruit-body when fresh ranged 100-215 mm in diameter while the young fruit bodies were 25 mm. Young caps were closed parabolic to oval shape. Slightly grown fruiting bodies caps were convex, some appressed apex. While, mature caps were applanate (plano-spread) with a small, rounded umbo dark brown to brown in colour while aging. Cap surfaces were covered with clearly visible brownish tile like arranged scales. The apex surfaces were smooth. Edges of caps were slightly tomentous to entire. The young fruit bodies had in-rolled margins. The fruiting body was thin for RMK-04 and thick for ZMK-02 and RMK-08. Two main basidiocarps colours were observed, whitish (accessions RMK-08 and ZMK-02) and brownish (accessions RMK-04). However, some accessions bore lighter or darker shades of these colours and were grouped together with the predominant colour (Table 3).

The stipes were slim, cylindrical, hollow inside, bulbous at the base except for accession RMK-04 which was un-swollen, 25-270 mm high and 10 mm wide. The base had maximum diameter of up to 40 mm. The stipe bore whitish colour shades (Table 4), with small brownish scales characterised by zigzag pattern occurred on some accessions especially mature accessions. Stipe was centrally attached to the cap, fibrous with simple base attachment to the host substrate. This was in agreement with Kuo (2007) findings.

The remnants of complete velum made on the stipe a double ring, which was relatively stable, and observable only on mature accessions. The ring was positioned towards the stipe apex and was movable along the stipe. Ring was absent on accession RMK-04. The flesh was white when fresh and cream when dry. Ring size ranged between 1-20 mm with rubbery texture. It was membranous for accession ZMK-02 and upturned for accession RMK-08 [Table 5]. Ring was only observable on all accessions with convex to plano basidiocarps.

The hymenophore was composed of the lamellae and lamellulae in intercalcated version except for all young accessions which had their cap closed. The lamellae were crowded, bulging out and free except for accession RMK-04 which had adnexed lamellae. Both lamellae and their margins were white except for accession RMK-04 which

bore brown shades. Accessions RMK-08 had thin gills while RMK-04 and ZMK-02 had broad lamellae, all of which were entire (Table 6).

Spores were white, elliptical and asymmetrical in colour, shape and symmetry respectively. Under magnification X400 the lengths were 11.30±0.72, 11.70±0.58 and 13.15±0.67 for RMK-04, ZMK-02 and RMK-08 respectively. The widths were 7.67±0.50, 8.35±0.66 and 8.80±0.51 for ZMK-02, RMK-04 and RMK-08 respectively. Stain reaction observed was Cynophillic, Congophilous for Cotton Blue and Congo Red. It was Inamyloid for RMK-04.Accession ZMK-08 and RMK-08 Dextrinoid in Melzers reagent.

3.3 Spawn Development

Mycelia established after 2 days after inoculation. Ramification progressed down the formulations at different rates. Pure [100%] sorghum grain gave significantly ($p<0.05$) the fastest rate of colonization of 8-10 days. This partially agreed with (Onyango *et al*., 2011), who reported *Auricularia* spp colonized in 10 day though for a formulation [Table 8]. This was followed by 50% millet and 50% sorghum and 100% millet which lasted 10-13 days. The slowest rate ($p<0.05$) of colonization was witnessed in kopakula supplemented millet which took 24 days. As reported (Onyango *et al.,* 2011) rapid mycelia growth observed may be attributed to a greater food reservoir in large sorghum grains, whereas the smaller millet grains provided a greater number of inoculation points. High rates of colonization are attributed to mycelia getting the most suitable ratio of mixture with a high reservoir of energy and all the nutritional ingredients such as carbon, nitrogen, lipids and minerals (Onyango *et al*., 2011). Vigorous substrate colonization by the mycelium during spawn run is desirable because it reduces mushroom cropping time and may allow mycelium to outgrow competitors in the substrate (Royse 1997 in Onyango *et al.,* 2011). Bran supplementation gave significantly faster growth rate ($p<0.05$) compared to Kopakula. Bran provides a protein rich medium which can increase rate of mycelia growth two-fold (Onyango *et al*., 2011).

4.0 Conclusion

Based on the data represented in this study, it's evident that within Mount Kenya Forest and Aberdare reserves there is rich biodiversity of mushrooms. It can be deduced that within the restricted area there are more edible mushrooms which are not yet explored. This calls for conservation of these ecosystems. This would aid in conservation of the various edible fungal germplasm for more research studies thus address the food insecurity and low family income. Finally it would also reduce human pressure on the natural ecosystems.

Acknowledgment

Authors are grateful to Commonwealth Scientific Industrial Research Organisation (CSIRO) who sponsored the project, Kenya Forest Service [KFS] and Kenya Wildlife Service (KWS) for support in accessing to forest reserves during sampling and collection of mushrooms.

References

Ram, R. C., Pandey, V. N. and Singh, H. B. (2010). Morphological characterization of edible fleshy fungi from different forest regions. *Indian Journal of Scientific Research,* **1** (2), pp. 33-35.

Tibuhwa, D. D., Muchane, M. N., Masiga, C. W., Mugoya, C. and Muchai, M. (2011). An inventory of macrofungi and their diversity in the Serengeti- Masai Mara Ecosystem, Tanzania and Kenya. *Journal of Biological Sciences*, **11**(6), pp. 399-410.

Rajaratnam, S and Thiagarajan, T. (2012). Molecular characterization of wild mushroom. *European Journal of Experimental Biology*, **2** (2), pp. 369-373.

Harrington, F. A. (1990). *Sarcoscypha*in North America (Pezizales, Sarcoscyphaceae). *Mycotaxonomy*, **38**, pp. 417–458.

Tibuhwa, D. D. (2011). Morphology and taxonomy of *Sarcoscyphaolololosokwaniensis*sp. nov.: A new Ascomycota species from Serengeti National Park-Tanzania. *Journal of Yeast and Fungal Research*, **2** (1), pp. 1 – 6.

Singer, R. (1948) ('1946'). New and interesting Species of Basidiomycetes. Papers Michigan Academy of Science, *Arts and Letters*, **32**, pp. 103–150.

Ge, Z. W., Yang, Z. L. and Vellinga, E. C. (2010). The genus *Macrolepiota* (Agaricaceae, Basidiomycota) in China. *Fungal Diversity*, **45**, pp. 81–98.

Vellinga, E. C., de Kok, R. P. J. and Bruns, T. D. (2003). Phylogeny and taxonomy of *Macrolepiota*(Agaricaceae) Phylogeny and taxonomy of *Macrolepiota*(Agaricaceae) *Mycologia,* 95(3), 2003, pp. 442–456.

Vellinga E. C. (2003). *Chlorophyllum* and *Macrolepiota (Agaricaceae)* in Australia. *Australian Systematic Botany,* **16**, pp. 361-370.

Sysouphanthong, P., Hyde, K. D., Chukeatirote E, Vellinga, E. C. (2011). A review of genus *Lepiota* and its distribution in Asia. *Current Research in Environmental & Applied Mycology,* **1** (2), pp. 161– 176.

Singer, R. (1959). Dos generos de hongosnuevospara Argentina. *Bol Society Argentina Botany*, **8**, pp, 9–13.

Vellinga, E.C , Yang, Z. L. (2003). Volvolepiota and Macrolepiota—*Macrolepiotavelosa*, a new species from China. *Mycotaxon,* **85**, pp, 183–186.

Kirk, P. M., Cannon, P. F., Minter, D. W., Stalpers, J. A. (2008). Dictionary of the fungi, 10thedn. CABI, Wallingford.

Jaworska, J. (2010). *Macrolepiotaolivascens*, a new species for Poland. *ActaMycologia* **45**(1), pp. 67-72.

Shao, L. P., Xiang, C. T. (1981) ('1980'). The study on the Macrolepiota spp. in China. *Journal of Northeastern Forestry Institute,* **4**, pp. 35–38.

Zang, M., Li, B., Xi, J. X. (1996). Fungi of Hengduan Mountains. Science, Beijing, in Chinese.

Bi, Z. S., Li, T. H., Zhang, W. M., Song, B. (1997). A preliminary agaric flora of Hainan Province. Guangdong Higher Education, Guangzhou, p 388, in Chinese.

Mao, X. L. (2000). Themacrofungi in China. Henan Science and Technology, Zhengzhou, p 719, in Chinese.

Teng, S. C. (1996). In: Korf RP (ed) Fungi of China, Mycotaxon Ltd, Ithaca, New York, pp. 453–456.

Leonard, P.L. (ed) (2010). A Guide to Collecting and Preserving Fungal Specimens for the Queensland Herbarium. Queensland Herbarium, Department of Environment and Resource Management, Brisbane.

Sibounnavong, P., Cynthia, C. D., Kalaw, S. P., Reyes, R.G. and Soytong, K. (2008). Some species of macrofungi at Puncan, Carranglan, Nueva Ecija in the Philippines. *Journal of Agricultural Technology*, **4** (2), pp. 105-115.

Kuo, M. (2007). *Macrolepiotaprocera:* The parasol mushroom. Available on: *http://www.mushroomexpert.commacrolepiota_procera.html* (Accessed on; 17-06-2013).

Srivastava, B., Dwivedi, A. K. and Pandey, V. N. (2011). Morphological Characterization and Yield Potential of Termitomyces spp. Mushroom in Gorakhpur forest Division*. Bulletin of Environment, Pharmacology & Life Sciences,* **1**(1), pp. 54 – 56.

Royse, D. J. (1997). Specialty Mushrooms and their Cultivation; *Horticulture Review* **19**: pp, 59-97.

Onyango B.O., Palapala V. A., Arama P. F., Wagai S. O. and Gichimu B. M. (201). Morphological characterization of Kenyan native wood ear mushroom [Auricularia auricula (L. ex Hook.) Underw.] and the effect of supplemented millet and sorghum grains in spawn production, *Agriculture and Biology Journal of North America*, **2**(3). pp, 407-414.

Tables

Table 1: Grain and supplement formulations for spawning

S/N	Grain and supplement formulation
1.	1 Sorghum
2.	4 Sorghum + 1 Wheat bran
3.	4 Sorghum + 1 Koppa kula
4.	1 Millet + 1 Sorghum
5.	2 Sorghum + 2 Millet + 1 Wheat bran
6.	2 Sorghum + 2 Millet + 1Koppa kula
7.	1 Millet
8.	4 Millet + 1 Wheat bran
9.	4 Millet + 1 Koppa kula

Table 2: Macrolepiota sp natural habitation characteristics

Accession	Growth	Host substrate	Type of forest	Vegetation
[1]RMK-04	Gregarious	Soil	Indigenous	*Vitex kiniensis*
[2]ZMK-02	Caespitose/ Gregarious	Soil	Mixed	All vegetation
[3]RMK-08	Solitary	Soil	Plantation	*Copressus lustanica*

[1]GPS location: S 0°22.564'; E 37°09.456'; Altitude 6733 ft
[2]GPS location: S 0°25.564'; E 36°49.470'; Altitude 7767 ft
[3]GPS location: S 0°22.013'; E 37°09.496'; Altitude 6837 ft

Table 3: Morphological characteristics of cap for the Macrolepiota sp

Phenetic feature	RMK-04[1]	ZMK-02[2]	RMK-08[3]
Shape	Convex	Convex-plano	Convex-plano
Colour	Brownish	Whitish grey	Whitish grey
Surface features	Squamulose	Squamulose	Squamulose
Apex	Appressed	Umbonate	Umbonate
Margin	Entire	Entire	Entire
Diameter[±]	1 Cm	11.5 Cm	10 Cm
Flesh	Thin	Thick	Thick

[1]GPS location: S 0°22.564'; E 37°09.456'; Altitude 6733 ft
[2]GPS location: S 0°25.564'; E 36°49.470'; Altitude 7767 ft
[3]GPS location: S 0°22.013'; E 37°09.496'; Altitude 6837 ft

Table 4: Morphological characteristics of stipe for the Macrolepiota sp

Phenetic feature	RMK-04[1]	ZMK-02[2]	RMK-08[3]
Attachment to cap	Central	Central	Central
Colour	White	White	White
Length	30 mm	125 mm	150 mm
Longitudinal Shape	Cylindrical	Cylindrical	Cylindrical
Base shape	Un-swollen	Bulbous	Bulbous
Surface features	Smooth	Smooth	Smooth
Consistency	Fibrous	Fibrous	Fibrous
Base attachment	Simple	Simple	Simple
Flesh	Hollow	Hollow	Hollow

[1]GPS location: S 0°22.564'; E 37°09.456'; Altitude 6733 ft
[2]GPS location: S 0°25.564'; E 36°49.470'; Altitude 7767 ft
[3]GPS location: S 0°22.013'; E 37°09.496'; Altitude 6837 ft

Table 5: Morphological characteristics of ring for the Macrolepiota sp

Phenetic feature	RMK-04[1]	ZMK-02[2]	RMK-08[3]
Present/absent	Absent	Present	Present
Shape	-	Membranous	Up turned
Size	-	2 mm	15 mm
Consistency	-	Rubbery	Rubbery
Colour	-	White	White
Position on stipe	-	Top	Top
Attachment to stipe	-	Movable	Movable

[1]GPS location: S 0°22.564'; E 37°09.456'; Altitude 6733 ft
[2]GPS location: S 0°25.564'; E 36°49.470'; Altitude 7767 ft
[3]GPS location: S 0°22.013'; E 37°09.496'; Altitude 6837 ft
[4]- : Features were absent

Table 6: Morphological characteristics of Hymenia for the Macrolepiota spp

Accession No.	RMK-04[1]	ZMK-02[2]	RMK-08[3]
Structure	Gills	Gills	Gills
Attachment to stipe	Adnexed	Free	Free
Gills colour	Brown	Whitish	Cream
Margin texture	Entire	Entire	Entire
Margin colour	Brown	Whitish	Cream
Lamellulae	Intercalcated	Intercalcated	Intercalcated
Thickness	Broad	Broad	Thin
Spacing	Spaced	Crowded	Crowded

[1]GPS location: S 0°22.564'; E 37°09.456'; Altitude 6733 ft
[2]GPS location: S 0°25.564'; E 36°49.470'; Altitude 7767 ft
[3]GPS location: S 0°22.013'; E 37°09.496'; Altitude 6837 ft

Table 7: Spore morphology for Macrolepiota sp as observed at (X400)

Phenetic feature	RMK-04[1]	ZMK-02[2]	RMK-08[3]
Spore characteristics			
Colour (fresh)	White	White	White
Shape	Ellipsoid	Ellipsoid	Ellipsoid

Symmetry	Asymmetric	Asymmetric	Asymmetric
Size at (X400)			
Length (µm)	11.30±0.72	11.70±0.58	13.15±0.67
Width (µm)	8.35±0.66	7.67±0.50	8.80±0.51
Stain reaction			
Cotton blue	Cyanophillic	Cyanophillic	Cyanophillic
Congo red	Congophilous	Congophilous	Congophilous
Melzers reagent	Inamyloid	Inamyloid	Dextrinoid

[1]GPS location: S 0°22.564'; E 37°09.456'; Altitude 6733 ft
[2]GPS location: S 0°25.564'; E 36°49.470'; Altitude 7767 ft
[3]GPS location: S 0°22.013'; E 37°09.496'; Altitude 6837 ft

Table 8: Time taken (days) for mycelial establishment on grain spawn formulation

		Accession		
No.	**Grain formulation**	**RMK04**	**ZMK02**	**RMK08**
1.	100%Sorghum	10.50±0.71^{b}	9.0±0.00^{a}	8.50±0.71^{a}
2.	100%Millet	13.50±0.71^{de}	9.50±0.71^{a}	11.0±0.0^{abc}
3.	50%Millet+50%Sorghum	13.0±1.41^{cd}	10.0±0.00^{a}	10.0±0.0^{ab}
4.	80%Millet+20%KopaKula	0.0±0.0^{a}	24.50±0.71^{d}	25.0±1.41^{g}
5.	80%Millet+20%Wheat bran	15.0±0.0^{e}	14.50±0.71^{b}	14.50±0.71^{cde}
6.	80%Sorghum+20%KopaKula	0.0±0.0^{a}	21.50±0.71^{c}	21.50±2.12f
7.	80%Sorghum+20%Wheatbran	11.50±0.71^{bc}	10.0±1.41^{a}	9.0±1.41^{a}
8.	40%Sorghum+40%Millet+20%KopaKula	0.0±0.0^{a}	24.0±1.41^{d}	23.0±141^{fg}
9.	40%Sorghum+40%Millet+20%Wheatbran	12.50±0.71^{bcd}	9.0±1.41^{a}	13.0±0.0^{bcd}
10.	CV [%]	8.37	5.9	6.21
11.	LSD ≤ 0.05	2.0	2.0	3.0

Results are expressed as Mean±SD in days for three determinations per accession at optimal mycelia establishment conditions.

Figures

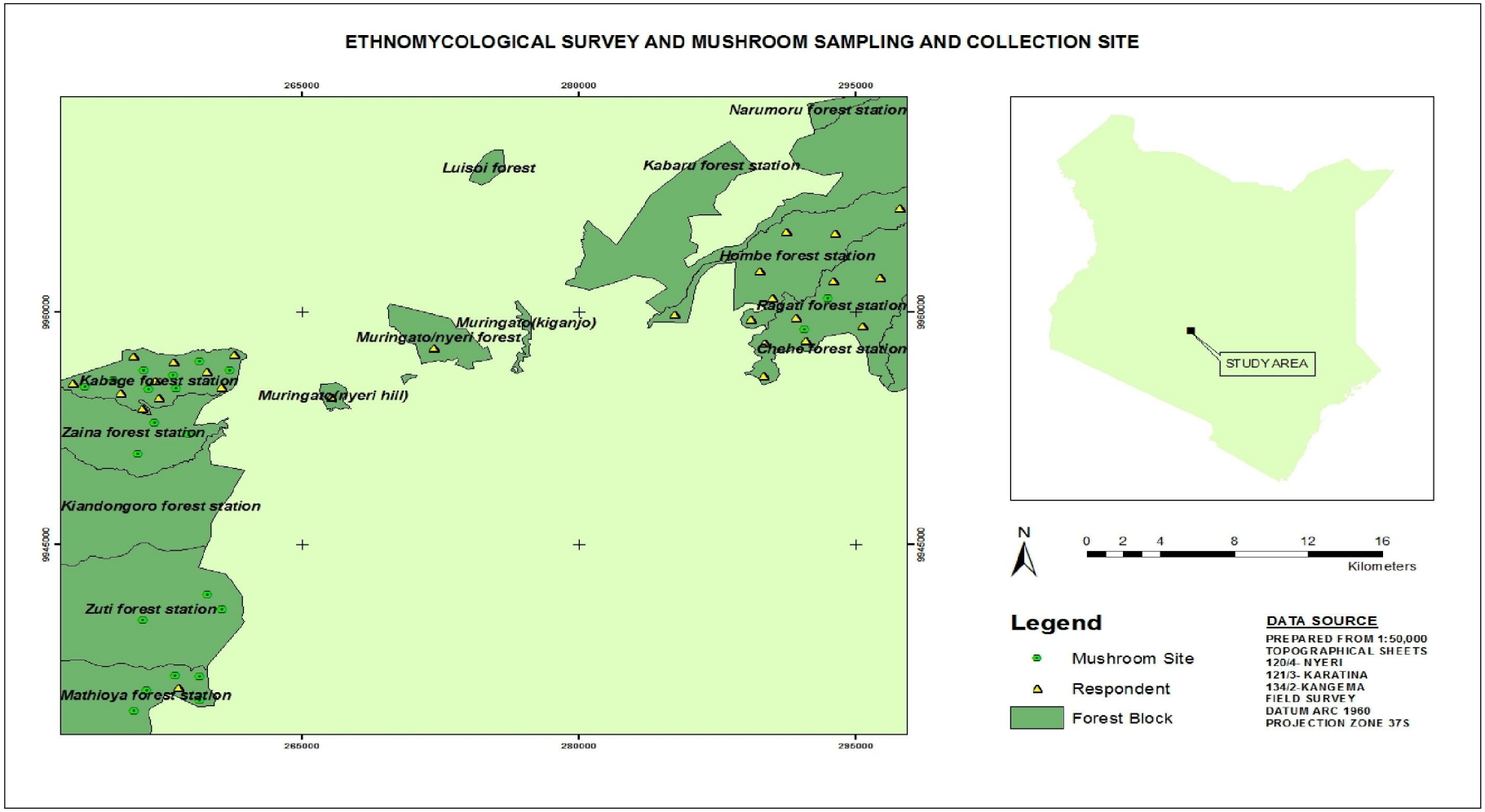

Figure 1: Ethnomycological survey and mushrooms sampling and collection site