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RESEARCH ARTICLE

GROWTH MEDIUM OPTIMIZATION AND GENETIC ADAPTATION OF A WILD EDIBLE AGARICUS MUSHROOM SPECIES IN UGANDA

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Abstract

In this study, experiments were carried out to optimize the growth medium of a selected wild edible Agaricus mushroom. The objectives of this study were to select the most popular edible Agaricus sp. sold in open food markets, and to determine the quantitative composition of the substrate formulation for optimal yield. The most popular species was found to be Agaricus bisporus and fresh samples were used to produce spawn for the experiments to determine the substrate quantitative composition, randomized complete design (RCD) was used. Molasses, chicken manure, and maize bran were varied in ten different formulations. Each formulation was replicated four times. Ammonium nitrate, Rice straw and gypsum remained constant in different formulations. It was also verified that yield of the mushrooms varied within the different formulations. The best formulation was formulation 5, this formulation produced a mean mushroom weight of 3.190±1.54 and the least produced a mean mushroom weight of 1.5±0.41.

Introduction:-

Mushrooms are large macro fungi. Studies estimate there are about 1.5 million fungal species, with around 14,000 producing mushrooms. Of these, roughly 7,000 are edible, and over 3,000 are considered prime edibles. Only 200 have been experimentally grown, 100 economically cultivated, 60 commercially, and about 10 on an industrial scale. Additionally, 2,000 species are believed to have medicinal benefits. By 1999, global production of cultivated edible mushrooms exceeded 7 million tons (Chang and Miles, 2004). While mushrooms have potential health benefits, including disease prevention like hypertension and cancer (Namuwaya, 2023), less than 1% is poisonous, so identifying these species is crucial. Edible mushrooms are nutritious, low in calories and fats, and rich in proteins, vitamins, and minerals (Istitute, 2022).

Cultivated varieties include Shiitake, Oyster, paddy straw and Button mushrooms. Production growth is highest in Asia, followed by Europe, Oceania, and Africa. Mushroom growth depends on environmental conditions and substrate composition, with water content being a crucial factor (Dream, 2023).

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In Uganda, Oyster mushrooms are commonly grown on substrates like cotton waste and other agricultural wastes like rice straw, whereas Agaricus bisporus (Button mushrooms) are not cultivated here because they are still wild and due to different substrate requirements and cultivation conditions using the exotic strains (Nshemereirwe, 2004). Oyster cultivation challenges include finding suitable substrates and producing mushroom spawn. Supplementing substrates with nutrients can enhance yields, and using agricultural waste for compost can aid in environmental sustainability and poverty reduction. Spent mushroom substrates can also be repurposed as animal feed and soil conditioners (Pope, 2000).

Materials and Methods:-

Field sampling for the most popular Agaricus species.

A questionnaire was used to gather data on the most popular edible Agaricus mushroom species from Nakasero and Kafu open markets in Uganda. Mushroom dealers were either given the questionnaire or interviewed orally, if they sold Agaricus species. The markets were visited twice, with the sampling conducted over two days each visit. A total of ten dealers from Kafu and eight from Nakasero were interviewed.

Dealers were first observed to determine if they sold Agaricus species based on their taxonomic features. Only those who sold Agaricus mushrooms plus other mushrooms were interviewed, and their responses were recorded. The ability of respondents to provide detailed information was assessed based on their knowledge and economic context. Samples of Agaricus species sold at the markets were collected for identity confirmation and further laboratory analysis.

Sample Identification

Fresh Agaricus mushroom sample with morphological characteristics of a typical Agaricus bisporus mushroom was selected among the many which were brought from the sellers that were found in Kafu and Nakasero open markets on the days of sampling. They were transported to UIRI lab sorted and cleaned. At the Button Stage, the pileus (cap), margin, and shape, pileus diameter, color, texture, any scales on the pileus, as well as bruising effects on the cap edge, habitant, season, and taste after cooking were observed and evaluated. At the Mature Stage, the pileus, margin, and shape changes, including lamellae (gills) for color and attachment and the Stipe, on appearance, bruising color changes, and veil color assessed and observed too. By placing the mushroom cap, gills down, on white paper covered with a glass, a spore print was made. The spore pattern and color were analyzed after overnight. From the selected Agaricus mushroom sample, aseptically in the bio safety cabinet, tiny tissues were cut from the fruit body and inoculated on prepared sterile Potato Dextrose Agar media (PDA) plates. After 3-5 days of incubation, the culture was purified through a series of sub cultures to form pure mother culture. The mother culture was triplicated. One well and fully grown petri dish with mycelia was sent to a lab in Mexico for detailed microscopic analysis using a Reichert-Auspria microscope at 400x and at 1000x magnification. This involved examining the color and appearance of the basidia and spores after staining. (Singh et al., 2011).

Spawn preparation,

Spawn was prepared using the second fully grown petri dish of Agaricus mycelia; Sorghum grains were washed and soaked for 12 hours then boiled for 25 minutes. Excess water was drained and the grains dried. 200 grams of Sorghum grain were filled in the spawn bottles and Autoclavable bags and autoclaved at 121 °C for 30 min. After cooling down in the bio safety cabinet, three pieces of active mycelia from Potato Dextrose Agar media were aseptically cut 2–3 cm in diameter and inoculated into the spawn media bottles and bags. The spawn media bottles were incubated at 25 ± 1 °C. 14-21 days after inoculation, and once the white mycelia fully covered Sorghum grains, they were then considered to be viable mother spawn. In a sterile environment of the bio safety cabinet, the mother spawn was multiplied through inoculation of the mother spawn on similar sterilized sorghum grains bags and spawn bottles and were incubated (Singh et al., 2011).

Preparation of substrate formulations

The substrate was composed of six components (Rice straw, Maize bran, Molasses, Gypsum, Ammonium Nitrate and Chicken Manure) in 10 formulations, in a randomized complete design (RCD).

Tab 1:- Ten substrate formulations used in the study.

Study nutrient sources materials	STUDY FORMULATIONS									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Rice straw (g)	3400	3400	3400	3400	3400	3400	3400	3400	3400	3400
Molasses (g)	180	190	200	210	230	240	280	320	80	130
Ammonium nitrate (g)	30	30	30	30	30	30	30	30	30	30
Gypsum (g)	48	48	48	48	48	48	48	48	48	48
Maize bran(g)	110	120	130	140	160	170	200	280	150	60
Chicken manure (g)	980	990	1000	1010	1030	1040	1200	1300	1020	920

Ten different recipes tested, each different by amounts of molasses, chicken manure, and maize bran. Ammonium nitrate, rice straw, and gypsum are kept constant at 30g, 3400g, and 48g in all recipes respectively. These ingredients were chosen due to their accessibility and effectiveness as nitrogen sources, affecting mushroom yield.

The substrate underwent a series of turns to provide aeration in the original heap made. The first turn was on the fourth day, the second on the seventh day, and the third on the tenth day an interval of three days. Gypsum was added on the eighth turn. The piles were monitored for ammonia smell, and after confirming its absence, the substrates were packed in plastic bag gardens and steam pasteurized at 70°C for eight hours respectively, then cooled gradually to 50°C over three hours, and finally to 26°C over three days in a clean room (Singh et al., 2011).

Mushroom spawning, casing and pinning

Compost plastic bag gardens were opened in the spawning room where spawn was mixed uniformly with approximately 5kg of compost in each garden. Spawning was conducted at 25°C. After two-three weeks, once the compost was fully colonized, a mixture of cooled sterilized casing soil (dark swamp soil) and calcium carbonate of 3-4 cm layer was applied on top of the compost (Mushrooms s. P., 2023). The compost was maintained at 70% moisture through regular moisture spraying. The growing environment was kept at 25°C with relative humidity (RH) maintained at 70-85%, though natural climate fluctuations affected the conditions. The growing room temperature was monitored and kept between 22-25°C by also spraying water on the walls and floor of the growing room (Singh et al., 2011).

Harvesting

Button mushrooms were harvested before they opened, adhering to both commercial practices and international standards (Agriculture, 2023). Daily data was recorded, consistent with previous research. To detach the mushrooms from the mycelia, they were gently rotated between the fingers. Casing soil was carefully removed, and each mushroom's fresh weight and height were recorded.

Spent mushroom substrate (SMS)

After the third flush, the spent mushroom substrate (SMS) was removed from the growing bags, steamed to eliminate pathogens and pests, and then used as manure in a garden for tomatoes and maize to enhance soil structure (Agronomy, 2020).

Data analysis

Descriptive statistics summarized the questionnaire responses from mushroom dealers. The mean height and weight data of mushrooms were analyzed using ANOVA, with the means compared using the Tukey's HSD test. Non-linear regression analysis, conducted with the XL-STAT version 2011, assessed the relationship between substrate composition and mushroom performance. Principal component analysis (PCA), performed with PAST 2011 software, examined how different substrate compositions in the formulations were associated and visualized the relative compositions of molasses, maize bran, and chicken manure across the eight formulations.

Results:-

In the survey, a total of 18 respondents were interviewed from Kafu (10) and Nakasero (8) markets. These were selling a wide variety of mushrooms together with Agaricus species such as 'Obutuzi', and 'Ebitosha', (Runyankole), 'Obubala', 'Obusukusuku' and'Obukokwe' (Luganda). The results indicated a higher distribution of the mushroom dealers in Kafu as compared to Nakasero market. Agaricus species sold in the markets were identified through checking and observation. Hence majority of the dealers (77.8%) had the popular Agaricus (Kinorya), while 22.2% had other Agaricus species they were sure were edible but did not know their local names.

Most mushrooms (66.7%) were collected during the rainy season, while the least were collected during the dry season (Fig 1).

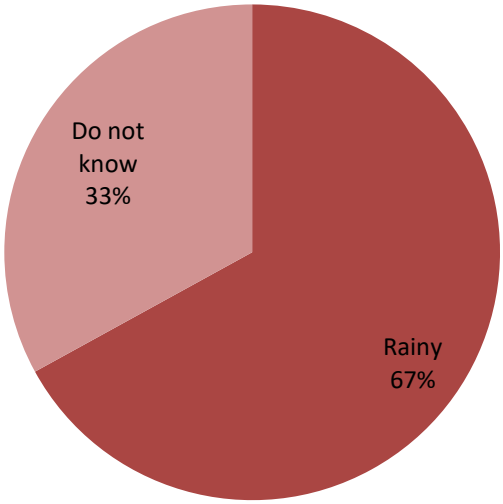

Fig 1:- Periods of collecting mushrooms in the study markets.

Sample Identification

The cap was 4 cm in diameter, convex with the incurved margin, becoming a plane in age. The surface was light brown with innate scales. When it was bruised, it became brown. It did not change color when it was put in KOH and had a mild mushroom odour and taste. Gills were free and pinkish and in the mature stage were dark brownish. The stripe was 5 cm tall, 1.5 cm thick, smooth below the ring, white and bruised brownish. Attached to the stripe was a smooth white superior ring and it was bulbous at base bruising slowly brown (Fig 2). The mushrooms had white, firm flesh with brownish bruises. They were found scattered in grassland near Lake Bunyonyi in Kabale district, following heavy rainfall. The area, used by cattle for grazing, had abundant dung manure compost and was undeveloped with dumped waste for many years (Fig 2). A brown spore print was printed on the white paper as above, and after a microscopic examination, the spores were 4.6-7 x 4.5-5.5µm, brown, oval shaped and smooth on the margin (Fig 2). The microscopic examination also showed a clear basidium having two brown, oval shaped and smooth margined spores (Fig 2). The mushroom was identified as Agaricus bisporus

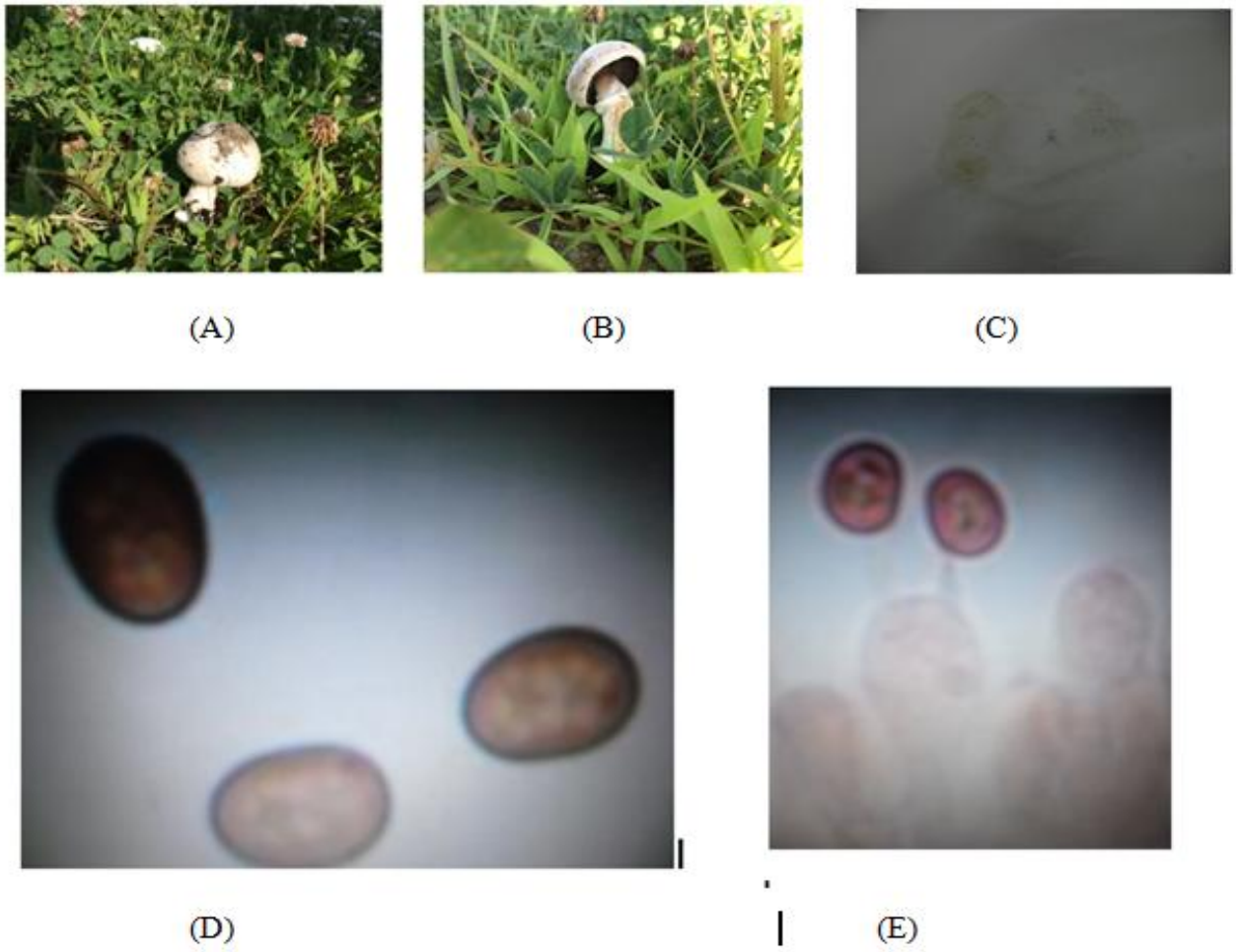

Fig 2:- (A) The button stage of the wild Agaricus,(B) Wild Agaricus maturity stage , (C) A spore print for the wild Agaricus, (D) Brown spores after microscopic view of the spores from the study sample, (Magnification X1000), (E) The microscopic examination of the basidium having two spores from the study sample, (Magnification X1000)

The quantitative composition of the substrate formulations for Agaricus mushroom on artificial medium.

During composting, temperatures began rising after 5 days. By the eighth turning, the compost reached a dark brown color with a sweet smell and no ammonia odor. The most common contaminant was green mold, Trichoderma spp., which appeared in 2 out of 10 formulations, leading to green patches on the substrate. These contaminated substrates were removed and disinfected. Two and half weeks after spawn running, the substrate was fully colonized. One week after casing, the compost, mycelia started growing upwards (Fig 3). After a total of 15 days, the compost and casing were fully colonized, and fruiting began (Fig 3). The first flush of fruit bodies was the best in terms of weight and height, with subsequent flushes showing a decline in these metrics. After the third flush, one fruit body was left to observe veil formation and dark gills (Fig 3). Formulation 5 yielded the highest quantity, it consisted of 3400g Rice straw, 230g maize bran, 30g ammonium nitrate, 48g gypsum, 160g molasses, and 1032g chicken manure.

Fig 3:- (A) An opened bottle showing white mushroom spawn used in the study, (B) packets containing spawn used in the study, (C) Agaricus mycelia colonizing the compost, (D) Colonizing mycelia after putting casing soil, (E) Agaricus mycelia producing pins in the substrate (F) Agaricus bisporus growing at the button stage, (G) Cultivated Agaricus mushroom in its mature stage

Effect of substrate composition on the number of fruiting bodies

Eight fruited with the mushroom Agaricus in the three flushes of harvest. In comparison, the cultivated mushrooms in the study looked whiter and slightly bigger than the wild mushroom studied mushroom sample. The number of fruit bodies formed significantly and varied in different formulations ($F_{7,86.571} = 10.185$, $P \leq 0.0001$). The highest mean number of fruiting bodies was observed in Formulation 5 and 8 and the lowest mean number of fruiting bodies was in Formulation 1 (Figure 4).

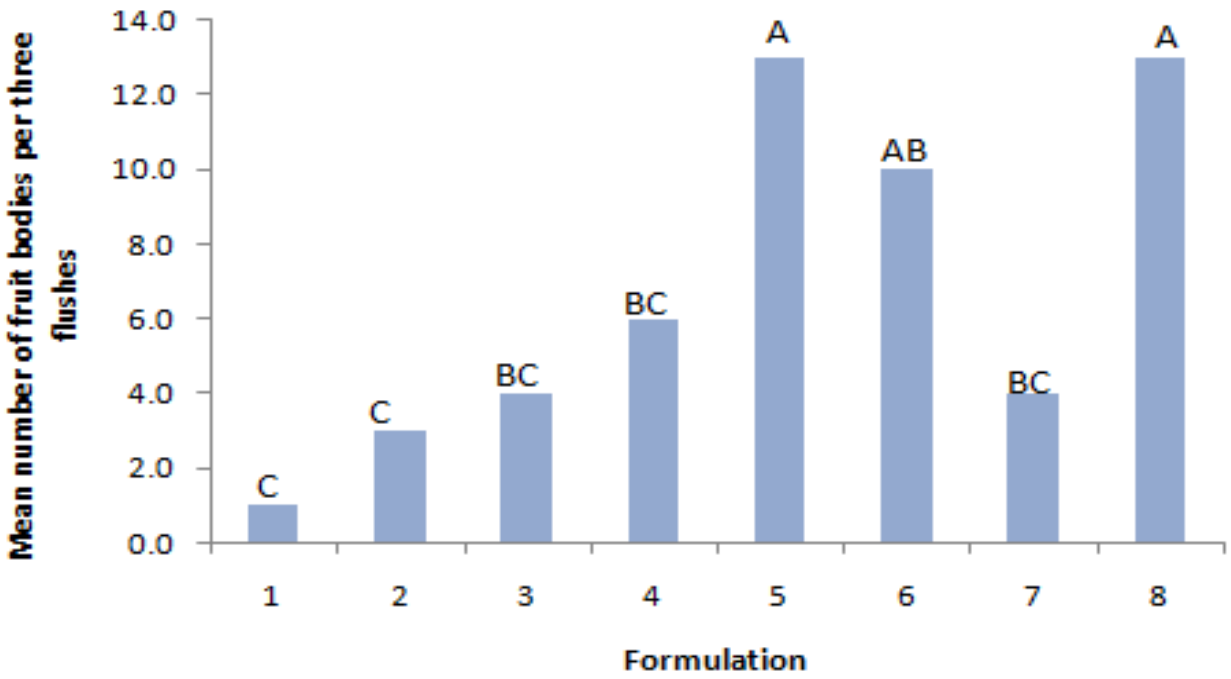

Fig 4:- The ffect of substrate composition on the number of fruiting bodies.

Mean fungal height for eight formulations respectively varied significantly ($F_{7,208} = 5.144$, $p = 0.0001$) (Fig. 5 and 6). The highest mean height was recorded in formulation 6 (3.190±1.54 cm) and the least was in formulation 1 (1.4±0.216 cm). The largest variance in height performance is recorded in formulations 5, 6 and 8, while the least is in formulation 1 as indicated by the size of the error bars (Fig 5).

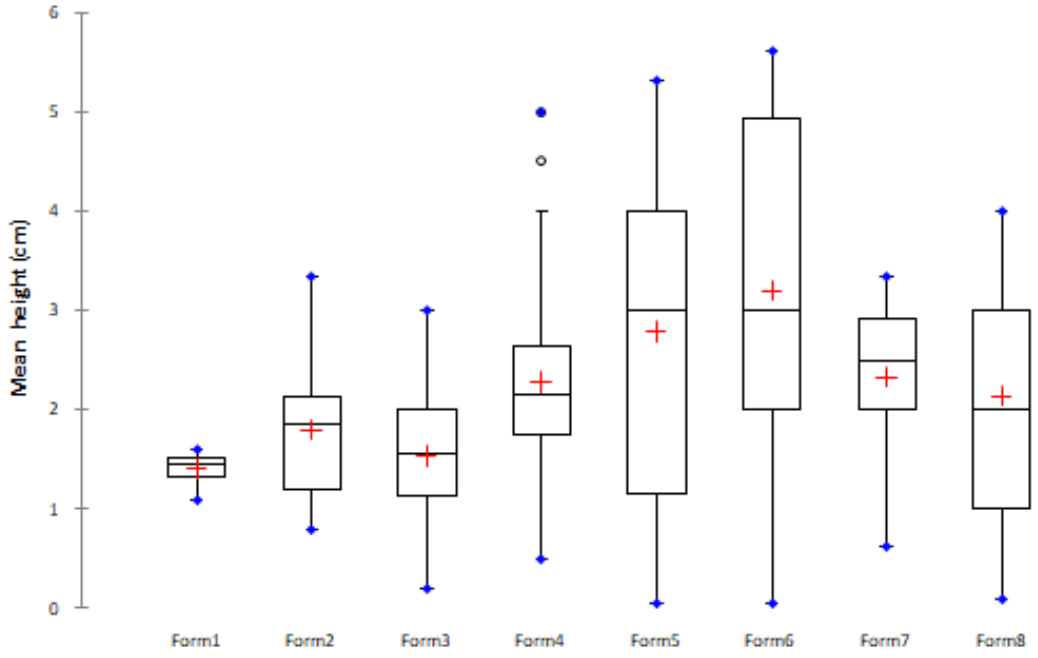

Fig 5:- Variability in mean height (cm) of mushroom performance in eight substrate formulations (Form = Formulation).

The comparison of mean heights showed that formulations 8, 2, 3, and 1 were not significantly different from each (Table 2), but they were significantly different from formulations 7 and 4 (Table 3). The later formulations also were not significantly different from each other. Formulation 6 was significantly better than all the other formulations (Fig 4).

Tab 2:- Analysis for variability in height for eight formulations experimented Model parameters, Variable Height (cm).

Source	Value	Standard error	t	Pr > |t|
Intercept	2.134	0.183	11.64	< 0.0001
Formulation 1	-0.734	0.661	-1.11	0.268
Formulation 2	-0.337	0.367	-0.92	0.359
Formulation 3	-0.601	0.367	-1.64	0.103
Formulation 4	0.141	0.317	0.446	0.656
Formulation 5	0.648	0.254	2.548	0.012
Formulation 6	1.057	0.272	3.887	0
Formulation 7	0.189	0.367	0.515	0.607
Formulation 8	0	0		

Tab 3:- Analysis for variability in weight for the eight formulations experimented Model parameters, Variable Weight (g).

Source	Value	Standard error	t	Pr > |t|
Intercept	1.743	0.166	10.518	< 0.0001
Formulation 1	-0.243	0.597	-0.407	0.685
Formulation 2	0.186	0.331	0.560	0.576
Formulation 3	-0.066	0.331	-0.198	0.843
Formulation 4	0.435	0.287	1.515	0.131
Formulation 5	1.110	0.230	4.832	< 0.0001
Formulation 6	0.643	0.246	2.618	0.009
Formulation 7	0.223	0.331	0.673	0.501
Formulation 8	0.000	0.000		

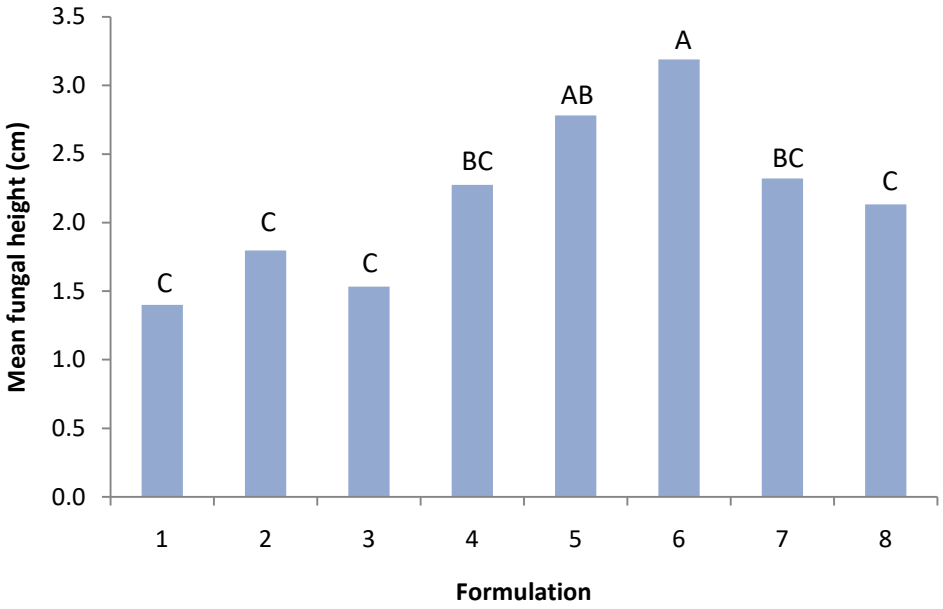

Fig 6:- Graph showing affects of different substrate formulations on the fungal height (cm).

Relative composition of molasses, maize bran and chicken manure among the eight formulations

The Principal Component Analysis (PCA) showed that formulation 6, 5 and 4 respectively had higher association with molasses and maize bran, while formulation 7 was more associated with chicken manure (Fig. 7). The rest of the formulations had significantly low proportions of the variable nutrients (maize bran, molasses and chicken manure).

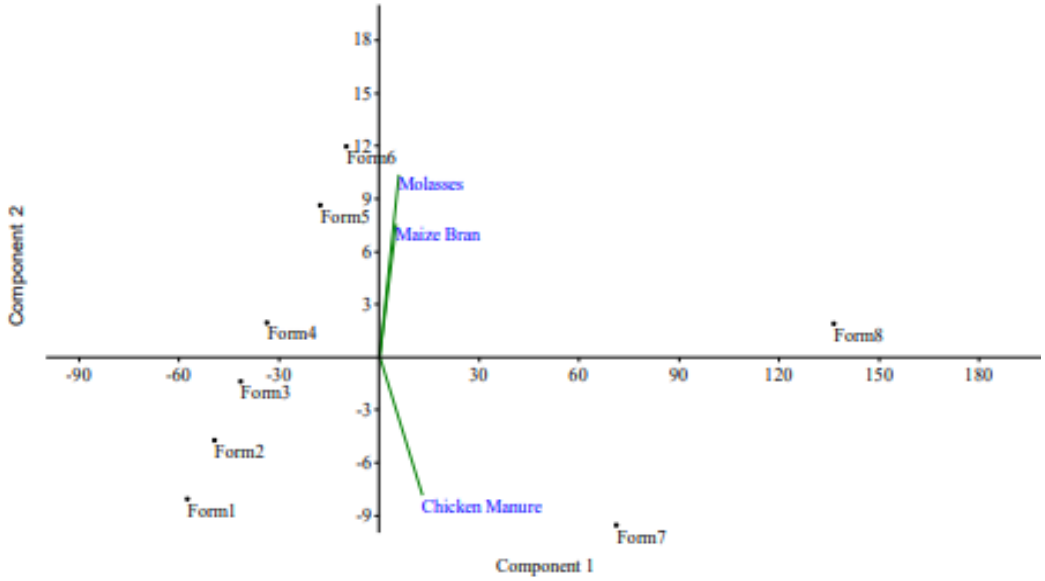

Fig 7:- PCA graph showing the relative composition of molasses, maize bran and chicken manure among the eight formulations'.

Affect of substrate composition on the fungal weight

Fungal mean weight from the eight formulations respectively varied significantly ($F_{7,208} = 4.553$, $p = 0.0001$) (Fig. 4.10 & 4.11). The highest mean weight was recorded in Formulation 5 (2.853±0.99 g) and the least was in

formulation 1 (1.5±0.41 g). The largest variance in yield performance was recorded in Formulations 6, 3, 2 and 8, while the least was in Formulation 1(Fig. 8)

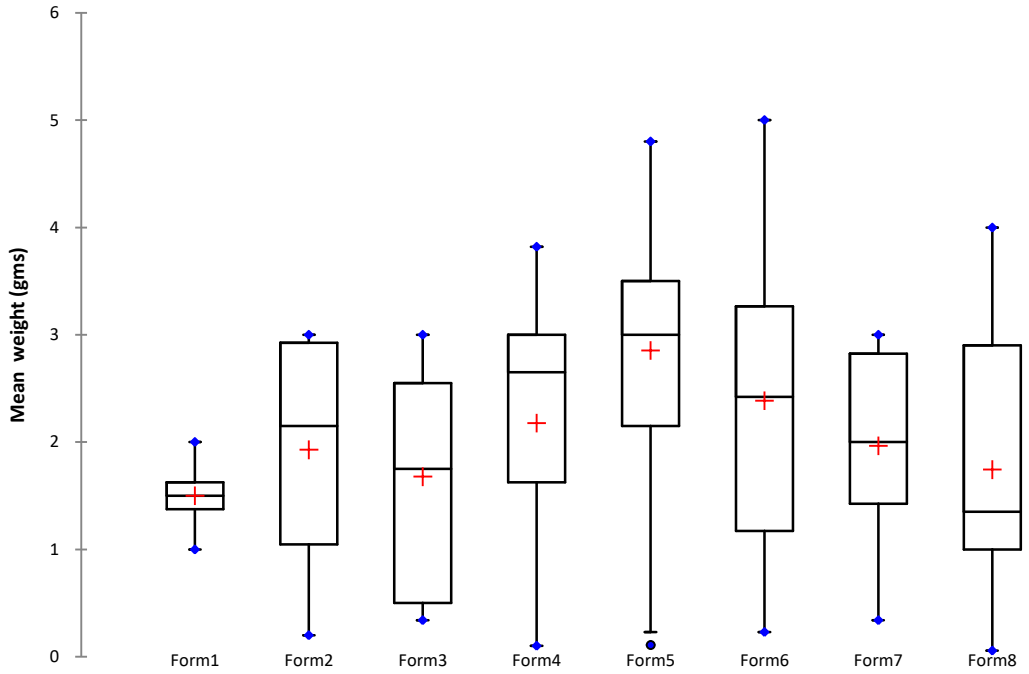

Fig 8:- Variability in mean weight (g) of mushroom performance of the eight substrate formulations (Form = Formulation).

The comparison of mean weights showed that formulations 6, 4, 7 and 2 were not significantly different from each other (Table 3) but they were significantly different from formulations 8, 3, and 1. Formulations 8, 3 and 1 were not significantly different from each other, while formulation 5 was significantly better than all the other formulations. (Fig. 8)

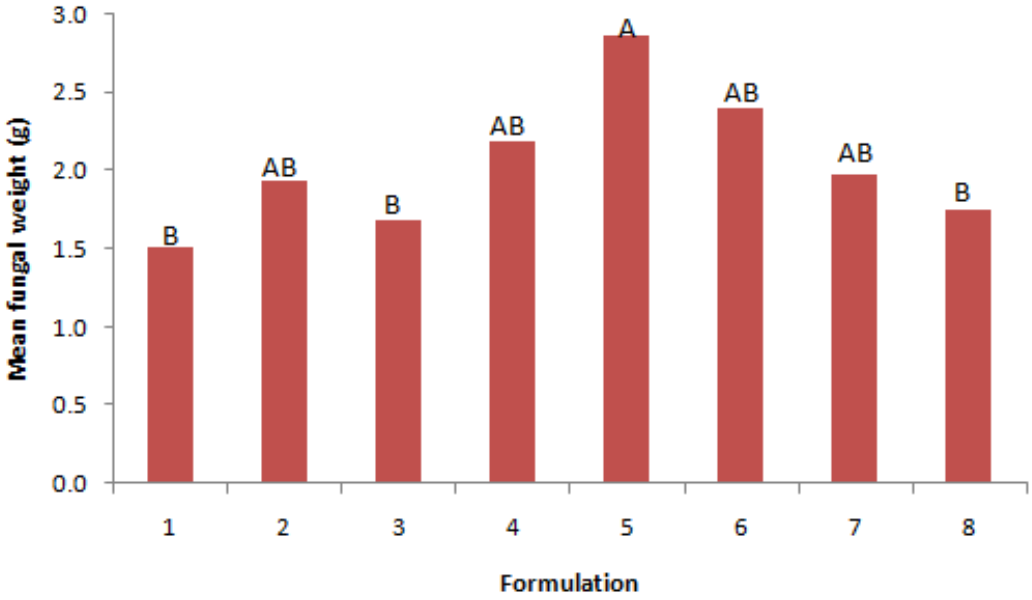

Fig 8:- Effect of different substrate formulations on the fungal weight (g)

Optimal substrate formulations for Agaricus mushroom production

The relationship between the variables; substrates, height and weight of the Agaricus mushrooms is shown in non-linear models in graphs (Fig 9 and Fig 10). All the models for the relation were significant ($P \leq 0.01$). Maize bran showed the biggest contribution to the height and weight of the mushrooms (R^2 =0.75 and 0.64). Molasses ranked second both for height (R^2 =0.68) and weight (R^2 =0.51) explaining variability respectively (Fig 9 and Fig 10). Chicken manure ranked last both in height and weight contribution. Substrates explained 94% (R^2 =0.94) variability in mushroom mean height and 77.4 %(R^2 =0.77) variability in fungal mean weight.

This resulted into mushroom fruit bodies' growth proportional to the availability of nutrients in the different formulations.

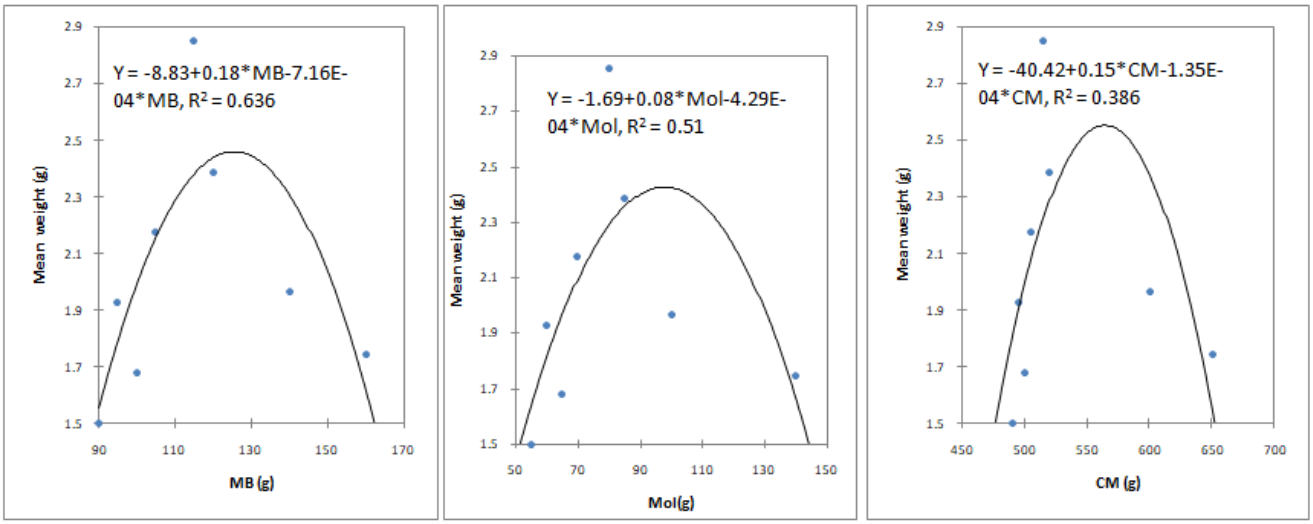

Fig 4.9:- Model distribution of yield performance for the different substrate formulations in relation to mean weight (g). CM=Chicken manure, MB=Maize bran and Mol=Molasses.

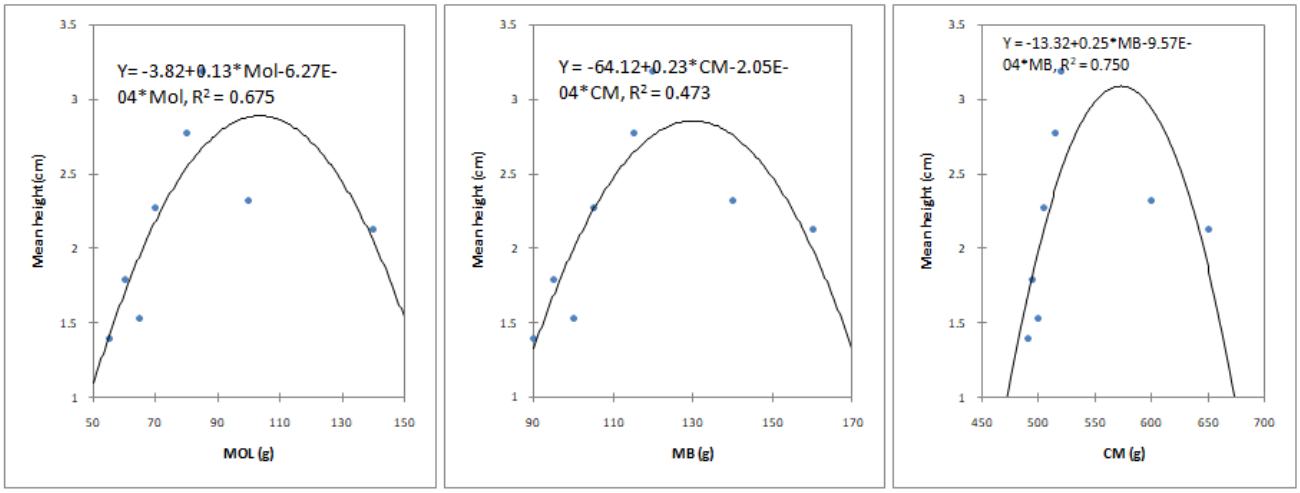

Fig 10:- Model distribution of yield performance for the different substrate formulations in relation to mean height (cm) CM=Chicken manure, MB=Maize bran and Mol=Molasses.

Discussion:-

Agaricus bisporous mushrooms which make up 31% of global mushroom production (Singh et al., 2011), are not cultivated in Uganda. However, local markets feature various wild mushrooms, with Agaricus (kinorya) most popular in Kafu compared to Nakasero likely due to its higher customer traffic. Most mushroom collection occurred during the rainy season, with dealers uncertain about collection times. The study identified the mushrooms as

Agaricus bisporus. Cultivation in artificial growth media showed low fruit body yields, influenced by factors like substrate moisture, nutrient type, and temperature fluctuations. Optimal temperatures for fruit body development ranged from 10-25°C, with significant yield variations observed due to environmental conditions and contamination (Agriculture, 2023). Substrate supplementation with molasses, maize bran, and chicken manure enhances mushroom yield by increasing substrate temperature and nitrogen content (El-Mashad et al., 2003). Over use of supplements can lead to overheating and contamination. Variability in fungal height and yield was attributed to low humidity, temperature fluctuations, and the type of organic supplements used (Mushroom, 2023). In conclusion, the study emphasizes the need for better cultivation technologies and substrate quantity choices, as well as improved control of environmental conditions to enhance better Agaricus bisporus mushroom yields.

Conclusion and Recommendations:-

This study focuses on Agaricus bisporus, locally known as Kinolya in Uganda, which is the most popular edible wild mushroom sold in Kafu and Nakasere markets. It is seasonal, typically appearing during the rainy season, and is a leading economical variety on global markets. The research developed an optimal substrate formulation for cultivating Agaricus bisporus in Uganda, which includes Rice straw, maize bran, ammonium nitrate, gypsum, molasses, and chicken manure. Formulation 5, with specific quantities of these ingredients, yielded the best results but still produced relatively small mushrooms, possibly due to other factors affecting growth. The study highlights the need to promote and invest in mushroom cultivation to boost economic growth, job creation, and dietary improvements in Uganda. It suggests further refinement of substrate formulations and studies on the mushroom's ecology and interaction with casing layers.

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