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Research Article

Measuring the Rate of Fungal Surface Area Spread in Homogenous Commercial and Heterogenous Non-Commercial Media

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Abstract

The effect of various nutritional factors on fungal growth in cultures made from over-the-counter perishable items (Nutritional Heterogeneity) was explored in the current set of research, and results were compared with lab-grade/commercial media properly calibrated for its growth. Agaricus bisporus (white button mushroom), an edible basidiomycete (filamentous fungi), was chosen for this purpose because it's easily available along with many other favorable properties. A trade-off between the generation of exoenzymes (for an extracellular breakdown of organic material in nitrogen-lacking settings) or sporulation and its mycelium expansion determines the growth efficiency (amount of biomass generated per unit substrate) of fungal cultures. Fungal Growth and a reduced carbon-to-nitrogen ratio are positively correlated [1]. Growth discrepancies (measured by the rate of increase of surface area) in different noncommercial media can also be explained by the presence of various carbohydrate sources, nitrogen sources, and other compounds. We tested the fungal Growth in both organic (glycine) and inorganic source of nitrogen.

Keywords: *Agaricus bisporus*; Fungal Growth Media; Non-Commercial; Carbon To Nitrogen Ratio; Organic/Inorganic Source Of Nitrogen; Surface Area

Abbreviations

CN: Carbon to Nitrogen Ratio; CDA: Czapek-Dox Agar; SDA: Sabouraud Dextrose Agar; YMA: Yeast Malt Agar

Introduction

Our understanding of the everchanging taxonomically and morphologically complex members of the kingdom of Fungi is at a subpar level compared to the depths reached in the animal, plant kingdom, and single cellular organisms. Fungal structures in nature are fascinating because their existence, morphology, and contribution to nutrient cycling depend heavily on factors that don't necessarily affect bacteria the same way. A lower C: N ratio has been shown to stimulate fungal growth, while the addition of nitrogen has been negatively correlated to bacterial growth. Fungal communities also thrive in lower pH values where most soil bacteria cannot survive [2,3]. Variations in morphology and chemical composition induced in the same species due to several biotic and abiotic stresses emphasizes their resilience to tolerate deviations in factors like (pH, humidity, temperature, light, carbon-nitrogen source, and presence of bacterial colonies in the immediate vicinity), presenting a unique feature. This area can be explored in terms of the production of secondary metabolites (SMs), [4] which is a prominent area of research in mycology. It's of economic interest since substances like enzymes, antibiotics, vitamins, amino acids, and steroids are extensively used in countless fields. Cholesterol-lowering statins, immunosuppressive, anti-rejection transplant drug cyclosporin, and antibiotic penicillins are some bioactive compounds obtained from fungal species [5,6]. These secondary metabolites, previously thought to be waste products [6], have broad-spectrum functions in the ecological setting such as protection, pathogenicity, communication signals, and inhibition/promotion of quorum sensing (QS). Bacterial-fungal biofilm contamination is common in clinical venues, *Candida* spp. cause the majority of infections related to medical implant devices [7]. Since filamentous fungi co-exist with bacteria and interfere with QS to protect themselves, it suggests that QS could be a new drug target to control bacterial diseases (Quorum Quenching). Secondary Metabolites are low to high-weight molecular compounds that are reaction end products and not essential for Growth and survival in artificial media. SMs are produced in response to a stimulus that often evokes a response in signal transduction pathways, epigenetic regulation, or global/specific transcription regulators, which ultimately activate silent SM gene clusters [4]. These genes involved in the biosynthetic pathway of secondary metabolites cluster together in the genome as biosynthetic gene clusters (BCGs). Bioinformatic analysis of this has revealed a tremendous potential for new antibiotics since new antibiotics have not kept pace with the development of antibiotic-resistant infections [5,8]. Moreover, actinomycetes have been the main source of antibiotics, and basidiomycetes or filamentous fungi have not been studied to the same extent, even though they are

prolific producers of SMs [5]. Various strategies have been devised to produce SMs in the lab, mainly by mimicking the natural environment, then changing abiotic conditions, or confronting the specimen with other microorganisms (Co-cultivation). A Morphological engineering technique called inorganic Microparticle Enhanced Cultivation (MPEC) has been used on basidiomycetes to upregulate specific enzyme production. It takes into account how hyphae agglomerate in the presence of Al_2O_3 microparticles [9]. Fungal morphologies affect the titers of produced metabolites to a great extent. It has also been observed that fungi can readily tolerate morphogenetic imprecisions/imperfections like asymmetrical pileus, lamellar dysplasia, and other abnormal development [10]. In some cases, compounds present in young hyphae may not be present in older structures. Apical dominance has been correlated to a high Ca gradient at hyphal tips [11]. Therefore, it is of taxonomic and industrial importance to record the growth pattern of one species on several media [12,13], including both commercially available lab grade and media of non-synthetic origin. This will aid in identifying common recognizable patterns in vegetative growth, cell wall structure, sporulation, and colony morphology influenced due to the presence or absence of substances regardless of their metabolic utility. Additionally, attempts have been made to reconcile morphological versions with their effect on the growth trajectory of the fungus. Mycelial Growth has been recorded in Carbon (C) limited environments (again 1 of the 6 factors) and proposed as a model simplified with quasi-static approximations. Fungal invasion-dependent key parameters were identified: C use efficiency and substrate decomposition rate [14]. It was found, that C use efficiency is lower along with decomposition rate at hyphal tips if the substrate is C-rich. [14]. Similarly, these problems have been approached with first-order and Michaelis-Menten kinetics. Interestingly, models have been designed to incorporate multifarious sensing, thigmotropism (contact sensing at the tips), and tip-to-tip anastomosis controlled via a supply of nutrients [15]. Thus it's a challenge to link increasing amounts of genetic and sub-cellular data with morphology and growth. In this study, we investigated and measured the vegetative growth of white button mushrooms in commercial and noncommercial media. We revisited the importance of fungi and performed a straightforward experiment suggesting that they can be easily multiplied on less expensive noncommercial media, which can also be tested for which source is readily metabolized (Carbon-Nitrogen source). We also focused on how the nature of a nitrogen (N) source or C-N source affects the surface area spread. We also looked into other factors such as pH and the C:N ratio to infer better with the constant temperature.

Materials and Methods

Petri dishes (8cm diameter) were cleaned with soap water, autoclaved at 121°C at 15 psi for 20-30 mins depending on whether the autoclave was preheated or not, and clean dry with toilet paper (supposedly cheaper than avg. tissue paper) after sprayed with 70% ethanol to maintain maximum sterility. White button mushrooms and perishable vegetables for noncommercial media were ordered from the nearest convenience store; three commercial media: (i) Czapek Dox Agar (CDA) [Sucrose 30g; $NaNO_3$ 2g; K_2HPO_4 1g; $MgSO_4$ + H_2O 0.5g; KCl 0.5g; $FeSO_4$ + $7H_2O$ 0.01g; Agar 15 g; Total 49.01g in Distilled H_2O 1 L] (ii) Sabouraud Dextrose Agar (SDA) [Dextrose (Glucose) 40g; Mycological, peptone 10g; Agar 15g; Total 65g in Distilled water H20 1 L] (iii) Yeast Malt Agar (YMA) [Peptic digest of animal tissue 5g; Yeast extract 3g; Malt extract 3g; Dextrose 10g; Agar 20g; Total 20.5g in 490ml] were procured from HiMedia Laboratories Pvt. Ltd.. All the procedures were performed in the Bioengineering Lab provided by VIT, Bhopal (23°4'19"" N Latitude; 76°49'46" E Longitude).

Media preparation-incubation-measuring growth

For noncommercial media preparation, a standard amount of 50g of all perishable items was measured on a weighing balance i.e. Carrot (*Daucus carota*), Lemon (*Citrus × Limon*), Wheat flour (*Triticum*), Rice (*Oryza sativa*), Coffee grounds (*Coffea arabica*), Tomato (*Solanum Lycopersicum*), Potato (*Solanum tuberosum*), Cucumber (*Cucumis sativus*) then taken in 100ml of water. Items were added in 100ml of water in chopped form, or as it is, and heated on a hot plate for 30 mins at near-boiling temperature, later, the constituents were stirred or crushed using a spatula to release and extract the nutrients better. The liquid component was filtered using a metal mesh or sieving cloth. Using the unitary method, dextrose and plain Agar were added following the standard 20g/l. Additional dextrose was added since naturally chosen vegetables have low carbohydrate content compared to commercial media. Certain noncommercial media made out of potato, tomato, cucumber, and corn were chosen to add NH_4Cl or glycine for the experiment since these media show promised mycelial Growth. The Standard followed for the addition of NH_4Cl and glycine is 2g/l and 6g/l, respectively**.** Finally, the prepared solutions were plugged with cotton and wrapped in paper before autoclaving at 121°C at 15 psi for 30 mins. Pouring was performed in the Laminar flow following all sterilization protocols. Inoculation procedures were followed initially using tissue from the pileus from the basidiocarp (fungal fruiting body) of the white button mushroom. In subsequent plating, we either used mushroom pieces or inoculated mycelium pieces from previously grown media. Mushrooms were stored at -20 [2] degrees celsius to ensure viability. All culture media was incubated for at least three days at 31±1 Growth was also observed under a phase-contrast microscope while vegetative primordia were growing in the culture. Non Commercial media batches containing 3 dishes each were also prepared: (i) Control (Dextrose + Agar) (ii) (Dextrose + Agar + NH_4Cl) (iii) (Dextrose + Agar + Glycine). Rates of the expanse of surface area spread were calculated using an open-source image manipulation software GIMP (GNU Image Manipulation Program) (Raster-based image editing software). Images of different magnification needed to be scaled accordingly, a basic image of the petri dish without the cap was chosen as a scale (reference) image wherein other images would be imposed to match the number of pixels to the reference scale image; this allowed us to measure the surface area spread in terms of pixel value which was then converted to cm^2 values. We resorted to using GIMP since

there are very few less sophisticated parameters to measure fungal Growth [1]. Measuring the diameter of the colony was cumbersome for asymmetrical colonies, and CFU (colony forming units) as a measure is inapplicable.

The procedure used to calculate Cn ratio

For noncommercial media, it was necessary to know the broth base nutrient content. CN ratio being an important metric for this experiment, it was doubly impertinent to have at least a rough idea of the various nutritional compounds present. This was provided by the extensive database of the US Department of Agriculture's Food Data Central - https://fdc.nal.usda.gov/. This is a robust database of specific and quantitative nutritional information on fruits and vegetables.

From here, the amount of carbon and nitrogen present in a 100 g sample of the hydrated sample was calculated analytically. Hydrated samples were picked as opposed to dry samples as our experiment involved broth bases dehydration. Specific inadequacies were identified, such as listing total dietary fiber quantity instead of specific varieties. These were addressed by representing dietary fibers with cellulose, and the CN ratio was calculated.

Statistical analysis

We calculated the change in the surface area of the primordia since its a better measure to determine if the constituents in the culture are promotive or inhibitive to its Growth. Raw data were collected from the images that gave the surface area in cm2 for 4 days. The procedure followed to get the surface area from the pixel value is as follows

- The number of pixels in the scale image consisting only of the outline of the plate without the lid was obtained using the image manipulation software.
- No. of pixels in scale image = 408254 px
- The petri dish diameter without the lid (8cm, radius r=4cms) was used to find the area:
- πr^2=50.28571 cm^2
- Naturally, in the image, this area will be occupied by the total number of pixels i.e 408254, using this we can find the area under 1 pixel
- N*A=50.28571
- Where N= number of pixels, A = Area of 1 pixel
- Area(A) comes out to be: 0.000123 cm^2
- Now, using the program, different images of the other resolutions were resolved at a pixel value of the order of the reference scale image; a path was drawn manually only around the area covered by the fungal colony, and the number of pixels in that colony was determined.
- The pixel value is then multiplied by each pixel's Area (A) to obtain the cm^2 spread.

Results

Different surface areas were averaged out in a culture containing more than 1 colony, and the growth was treated as one for CDA-1. The first day's change in surface area was calculated (ΔS= Surface Area on Day 2-Surface Area on Day 1). It was also observed that large size of the specimen inoculated (whether from the fruiting body or previously grown media), the initial change in surface area is high, as seen in Corn Control (Corn Broth + Agar + Dextrose) figure (1a), Cucumber NH_4Cl (Cucumber Broth + Agar + Dextrose + NH4Cl) figure (1b), Tomato Glycine (Tomato Broth + Agar + Glycine) figure (1c) and Potato Control (Potato Broth + Agar + Dextrose) figure (1d). Also, change in surface area at a low rate initially or in later stages can be accounted for morphology switching so that fungus is more efficient in colonizing new substrate sources. Nevertheless, we observed that all media supported growth, and change can be visually seen from Day 1 to Day 4 except for ground coffee beans media which registered no growth. Changes in surface area in all noncommercial media with different treatments show a common pattern, glycine enriched media stimulate the consistent highest growth then NH_4Cl enriched followed by the control. Figure (1e) represents a growth comparison between 2 batches of CDA and two noncommercial media with added NH_4Cl, CDA (wholly synthetic inorganic in nature with inorganic salts) compared with noncommercial media containing NH_4Cl). Figure (1g) represents a growth comparison between commercial and noncommercial glycine, potato, and cucumber show progressive growth aided by glycine addition which is both a carbon and nitrogen source. Growth observed in potato glycine media is at par with SDA commercial media. The growth profile and the changes in growth rate are characteristic of a particular noncommercial media; changes in growth in control of different media don't show much deviation or abruptly higher growth except for potato, which shows changes in the growth rate of higher-order, perhaps due to presence of additional diversity in carbohydrate sources like starch in significant quantities and a lower C: N ratio supporting the fungal growth (Table2) Other than potato, controls of all noncommercial media performed the same, primarily due to equal concentration of dextrose in it figure (1h) figure 2. The addition of glycine or NH4Cl does change the C: N ratio and influences fungal Growth. Glycine-enriched media shows a consistent increase in growth. Growth response in the culture is in the following order Glycine Media > NH_4Cl Media > Control Media. Still, this result is subject to additional complexities as we further read more literature on the effect of the organic and inorganic nitrogen sources on forest fungal communities and other sources (under discussion)**.** Different batches of commercial media show similar growth trajectories suggesting our system's accuracy. SDA-2 was intentionally inoculated with mycelium strands previously grown in a media. SDA-2, CDA-1, and CDA-2 were directly inoculated with tissue from the fruiting body figure 3. As seen in table 1, commercial media tends to outperform noncommercial media due to the substrate-rich environment and lower C: N ratio (Table 2). Noncommercial media is very relevant, as inferred from our experiment because its naturally low concentrations make the growth profile more sensitive to the addition of testable sources of nutrition. Commercial media support the fast cultivation of the specimen. However, noncommercial media can be customized for that by adding more nutrients. Table 3 represents the pH of dif-

ferent media; as per our observation, fungi tolerate a wide range of pH (pH 4 to 6.5), and it's not as much of a limiting factor when compared with nutrition. It must be noted that the C:N ratio will be the same if both the concentration of C and N sources is proportionately decreased or increased. However, that would manifest in the growth profile as fungus would not support a faster growth rate in culture containing significantly lower concentrations of both carbon and nitrogen sources even though the ratio is constant.

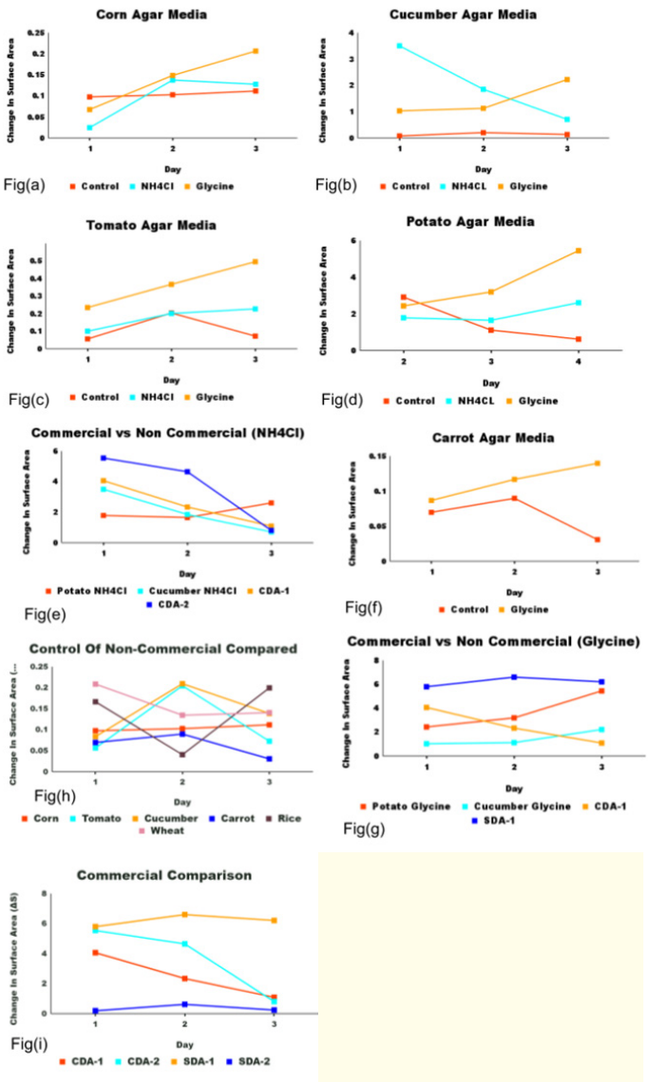

Figure 1: The line graph plots depict the change in the surface area of the fungi growing in the respective culture for 3 consecutive days. On the X axis, we have the days, and the Y axis represents the change in surface area in cm2 unit:

(a) Line graph plot growth rate comparison in corn agar, (b) in cucumber agar, (c) tomato agar, (d) potato agar, (e) Line graph plot growth rate comparison between commercial and noncommercial media for NH4Cl enriched, (f) in carrot agar culture media for control and glycine enriched, (g) Line graph plot growth rate comparison between commercial and noncommercial media for glycine enriched, (h) Line graph plot growth rate comparison between all the controls of noncommercial media, (i) Line graph plot growth rate comparison in different commercial media

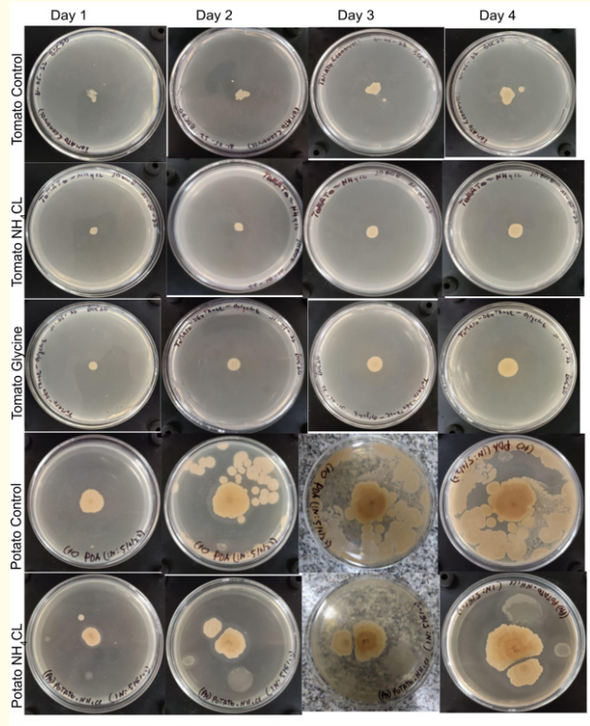

Figure 2a: Image observed under phase contrast microscope showing the fungal growth and morphology with various media.

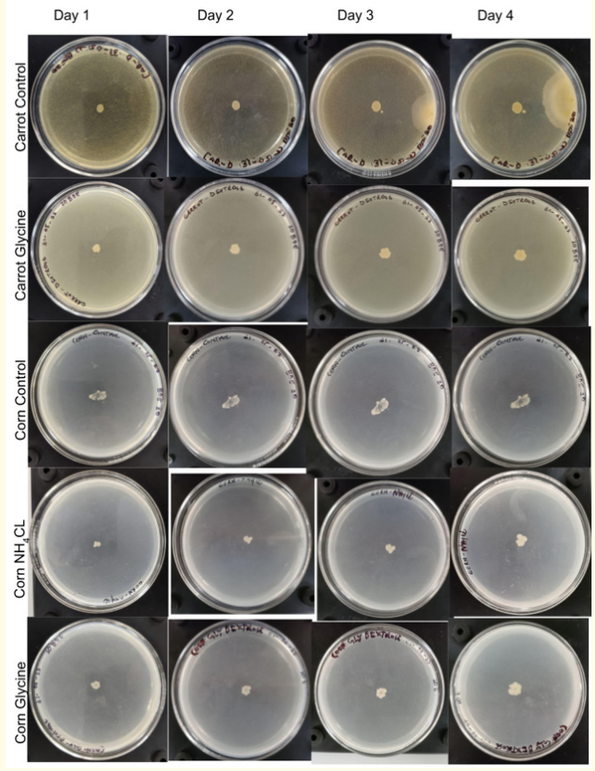

Figure 2b: Image observed under phase contrast microscope showing the fungal growth and morphology with various media.

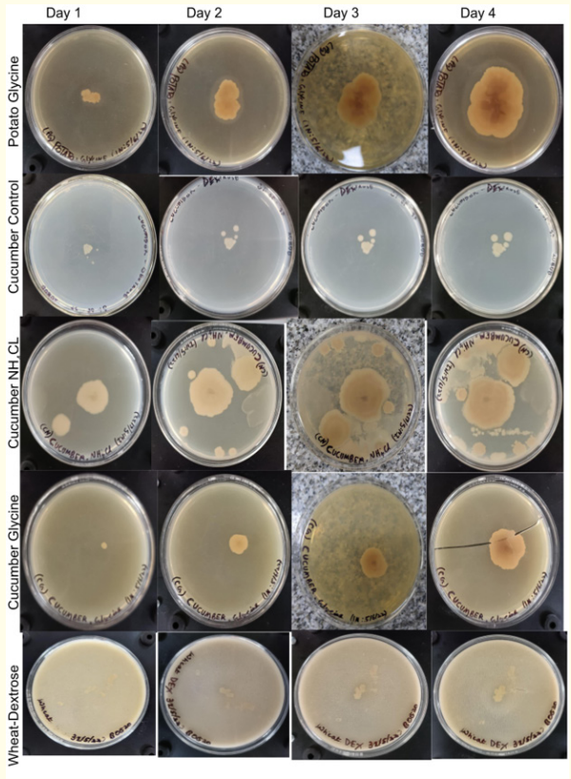

Figure 2c: Image observed under phase contrast microscope showing the fungal growth and morphology with various media.

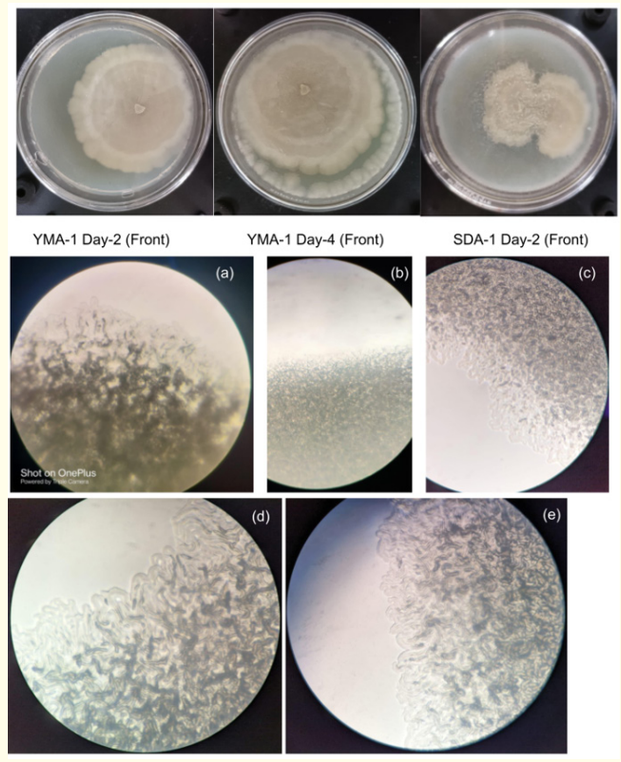

Figure 3: Observed under phase contrast microscope at varying magnification (a) Potato Control (b) Corn control (c) CDA (d) YMA (e) SDA.

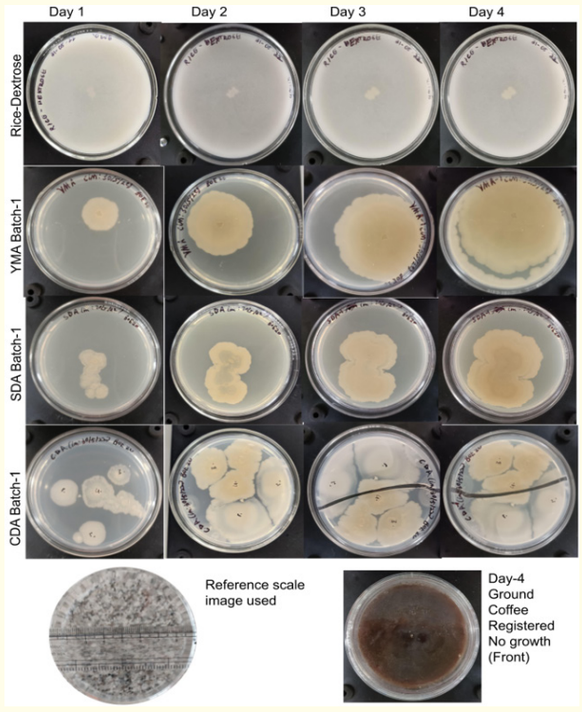

Figure 2d: Image observed under phase contrast microscope showing the fungal growth and morphology with various media.

Media	Surface Area (cm^2)			
Day	**1**	**2**	**3**	**4**
CDA-1	2.466	6.538	8.885	9.983
SDA-1	5.100	10.19	17.524	23.774
YMA-1	4.528	14.505	24.342	47.661
CDA-2	0.718	6.271	10.93	11.741
SDA-2	0.807	1.008	1.635	1.885
YMA-2	0.523	0.854	1.323	2.128
TOM DEX	0.362	0.419	0.639	0.712
TOM NH4CL	0.193	0.294	0.496	0.724
TOM GLY	0.298	0.534	0.902	1.399
CUC DEX	0.258	0.342	0.552	0.691
CORN DEX	0.444	0.542	0.645	0.757
CORN NH4CL	0.151	0.176	0.314	0.442
CORN GLY	0.203	0.271	0.420	0.627
WHEAT DEX	0.287	0.496	0.631	0.772
CAR DEX-1	0.191	0.261	0.351	0.382
CAR DEX-2	0.215	0.302	0.419	0.559
RICE DEX	0.298	0.465	0.506	0.706
PDA-2	1.929	4.850	5.968	6.594
POT NH4CL	1.074	2.867	4.523	7.139
POT GLY	0.748	3.189	6.393	11.853

SDA-3	1.522	15.749	18.772	21.039
YMA-3	3.605	13.382	21.179	26.746
CUC NH4CL-2	3.585	7.096	8.953	9.666
CUC GLY-2	0.112	1.148	2.218	4.509

Table 1: Represents individual surface area values in cm^2 for all media.

Media	C: N Ratio (Approx)
Tomato	21:1
Potato	30:1
Corn	137:1
Cucumber	9:1
SDA	35:1
CDA	38:1

Table 2: Represents Calculated C:N ratios (addition of dextrose, NH4CL, Glycine not included).

Media	pH
Carrot	4.90
Lemon	2.10
Wheat	4.70
Rice	5.20
Coffee	4.00
Tomato	3.40
Potato	4.70
Cucumber	4.10
CDA	6.34
YMA	5.35
SDA	4.78

Table 3: The pH values measured using a pH meter.

Discussion

Our results align with the hypothesis that adding nitrogenous substances that support growth does stimulate growth response. These results also align with previous studies [1,16] (one of which used NH_4NO_3 as the sole nitrogen source). Culturing basidiomycetes is an exceptionally complicated task, as these strains demonstrate a wide range of morphologies dependent on the age of mycelium, cultivation media composition, pH shifting, and other stresses [9]. It is easier to characterize the growth of another fungus in different mycota from their coloration, easily identifiable sporulation, etc [13]. It has been found that the C: N ratio has more influence on fungal growth than the carbon concentration itself as seen in figure (h) (comparing the performance of controls in carrot and corn,carrot has lower C:N ratio and corn reportedly has higher C:N ratio suggesting higher concentration of carbon table (2), since non commercial media have intrinsically lower concentration of substrates, growth rates of both the cultures are similar. The carbon concentration in the two did not make a significant difference), a C:N ratio of 160:1 produces the poorest fungal growth but high spore yields even though the optimal C:N ratio is strain-dependent. 10:1 to 40:1 remains the appropriate C:N ratio for the highest fungal biomass production than 80:1 to 160:1 [16]. Sporulation requirements remain unclear; some literature suggests stressful environments initiate sporulation, and sporulation requirements are more stringent [13] compared to regular growth. We did not observe sporulation in the basidiomycete within the span of 4 days (Images a, b, c, d, e) When N is a limiting factor, fungi expend more energy to reallocate N by autolysis and subsequent hydrolysis [17] of cell components to produce exoenzymes necessary to degrade surrounding C- rich substrate, which may reduce the C-use efficiency and increase the N-use efficiency. N-use efficiency (NUE) describes the partitioning of organic N between growth and the release of inorganic N to the environment, which is N mineralization [18]. It has been found that microbes retain most immobilized organic N (high NUE) when N-limited, resulting in lower N mineralization. According to the theory of ecological stoichiometry: low substrate C:N ratio (N sufficient conditions), strictly homeostatic organisms have low NUE but high CUE (C-use efficiency), in contrast, at high substrate C:N ratio (N-deficient), they lower their CUE while increasing NUE [18]. Similar activity has been observed in fungi, for example at high sucrose concentrations, a single hyphal tip can access more C than in the case of low concentration and thus the C use efficiency is lower [14]. However, the speculation on N immobilization i.e higher organic N is immobilized in N deficient conditions (high NUE) contradicts a work on fungal species that implies that a higher amount of N is immobilized into fungal biomass as a reason behind the stimulated growth in lower C:N ratio [1], thereby possibly reducing N losses. Most studies on soil N cycling have focused on N mineralization, rather than on the breakdown of organic N between incorporation into microbial biomass and release as ammonium. Understanding agro-ecosystems and SOM (Soil organic matter) dynamics become crucial from an industrial perspective to get massive yields. The main decomposition pathways in the soil can be either fungal or bacterial [19]. Knowledge of which pathway is predominant in a soil sample could explain a lot about its mechanism. Other studies claim that organic matter with a high C: N ratio stimulates fungal growth and thus increases soil's F:B (Fungal to bacteria) ratio. Inorganic nitrogen fertilization has been reported to reduce the F:B ratio [19]. Another study explains that cessation of N-fertilizer use can cause a shift from bacterial to fungal-dominated systems [1, in-text citation]. Microbial activity is the highest when the substrate's C: N ratio matches the microbe's demands. Accordingly, because of the higher C:N ratio of fungi compared to bacteria, grazing by fungivores results in a lower N mineralization rate than grazing by bacterivores [19]. Another study found a negative correlation between N addition and bacterial growth, while N stimulated fungal growth [3]. A possible explanation for the N inhibition of bacterial growth was given as a negative effect on pH by adding NH_4NO_3. Fungi can stand low pH (Table 3: pH of the culture used in this experment) better than bacteria, and the F: B biomass ratio decreases with increasing pH. The effect of organic nitrogen addition is not well understood. Organic nitrogen

could present itself in various labile forms like amino acid to complex forms like insoluble aromatic compounds. A study recorded a consistently enhanced growth pattern for a labile organic nitrogen source (Glycine, same as our experiment figure (a-d and f), but this pattern was not observed for complex organic nitrogen forms [20]. Thus, the decomposition of complex organic nitrogen becomes taxa-dependent, affecting the growth response in different saprotrophic fungal communities. In regards to using amino acid as an organic source of nitrogen, there has been a case where *Penicillium janthinellum* was able to grow on glycine as a sole source of carbon and nitrogen [21] which is metabolized by the glycerate pathway. The results suggested that its Growth was influenced by the concentration of glycine, a concentration of 1-5g/l produced (approx.) the same mycelial yield of 7.0% 30h after inoculation of the fungus; higher concentrations resulted in a progressive decline in mycelial yield to 4.5% using 10g/l of glycine. The decline has been attributed to the ammonia end product producing more than the buffering capacity of the medium, possibly interfering with toxicity or pH. Moreover, it has been suggested that zoosporic fungi do not have the metabolic machinery to catabolize single amino acids rapidly as sole sources of both carbon and nitrogen [22] since they registered poor growth (not tested for glycine). Amid studies with mixed results, it has been shown that if some fungi rely on organic sources of nitrogen, then they are likely to be more readily isolated from soil under perennial vegetation [22]. Our results for a batch of a particular noncommercial media, for instance, cucumber, are subject to several factors figure (b). The addition of NH4Cl might lower the pH, or the increasing presence of chloride ions might inhibit further consistent growth. Some studies have observed poor or moderate growth for CDA for a strain related to chloride ion presence. Also, mycelia growth on different nitrogen sources was found to be highest in sodium glutamate-containing medium and lowest in ammonium chloride-containing medium. In the same study, LCA (Lignocellulose Agar) was most suitable for heavy sporulation, whereas PDA produced the most visible colony morphology [13]. A work also claims that the inclusion of sucrose instead of dextrose in potato sucrose agar for *Fusarium* cultures means that the carbohydrate is not so readily available to the fungus, and sporulation is thereby stimulated [12]. , As per these studies, excess sporulation could be that the presence of substrates like sucrose, cellulose, and lignin requires energy-intensive decomposition, which entails high complexity of chemical reaction cascades that may be responsible for sporulation. Additionally, the presence of simple sugars like glucose/dextrose causes glucose repression or the "glucose effect" in fungi, which manifests itself by turning off a large number of genes [23]; this is thought to be an energy-saving response as it primarily affects enzymes used to metabolize other carbon sources which are dispensable in the presence of glucose. Understanding this process is largely based on genetic data; it is unknown how it may impact the phenotype or the quality of the fungal community. Surprisingly, ground coffee media registered no growth or increase in the surface, which is interesting because a Belgium-based eco-startup has cultivated other basidiomycetes like oyster mushrooms and Permafungi that uses coffee grounds and mixes it with spores of the fungi along with straw and cultivates it in growth bags. Our experiment becomes a source of many hypotheses, and the results add to the literature on this topic.

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Conflict of Interest

The authors have no conflicts of interest to disclose.

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