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EVALUATION OF SUITABILITY OF PH ON MYCELIAL GROWTH OF *CALOCYBE INDICA* STRAINS

P.K. Dhakad[1,*], Ram Chandra[1], S.K. Patel[1], Sumit Kumar[1], V.K. Sonakar[1] and M.K. Yadav[2]

[1]Mushroom Lab, Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005

[2]Department of Plant Pathology, Janta College, Bakewar, Etawah-206124

Abstract

India has produced more than 291.95 million tons of food grain during 2019-20 and achieved its food security but struggle to achieve nutritional security. So mushrooms production may be a good alternative as a tool to alleviate poverty and bring diversification to agricultural production. Mushrooms are low in calories but rich in protein, carbohydrate, vitamins, minerals, fibers and the mushrooms were nutritionally placed between vegetables and meat. Different strains of milky mushroom having diversified level of pH range for maximize their mycelial growth and production. In present experiment we evaluated five strains of *Calocybe indica* like CIP-18, CIP-19, CIP-20, CI(wild-1) and CI(wild-2) for seven different pH levels (pH 5, 5.5, 6. 6.5, 7, 7.5 and 8). Results shown that the maximum mycelial growth observed for strain CIP-20 at 5 pH (8.6±0.4 cm) than at 7 pH (8.43±0.35) while minimum growth observed at 6 pH (4.53±0.11 cm). The strain CIP-18 and CIP-19 also showed maximum mycelial growth at 5 pH that was 8.27±0.49 cm and 7.37±0.20 cm, respectively. But strain CIP-18 and CIP-19 shown minimum growth at 6 pH that was 4.87±0.28 cm and 4.37±0.20 cm, respectively. The wild strain of *Calocybe indica* CI(wild-1) and CI(wild-2) shown maximum growth at 7.5 pH that was 5.6±0.36 cm and 5.6±0.3 cm, respectively while minimum growth at 6 pH that was 4.23±0.25 cm and 4.17±0.15 cm.

Introduction

India is an agriculture based country and Indian agriculture will continue to be a main strength of Indian economy. India has produced more than 291.95 million tons of food grain during 2019-20 and achieved its food security but struggle to achieve nutritional security. Mushrooms as a crop of economic importance and a tool to alleviate poverty and bring diversification to agricultural production. Mushrooms were considered as healthy, nutritious and luxury food among the rich community because of their unique taste and flavour but now it has also grown as a common man's food. Mushrooms are low in Cholesterol but rich in protein, essential amino acids, carbohydrate, minerals, vitamins, fibers and mushrooms were nutritionally placed between vegetables and meat, so mushrooms are aptly called as slimming foods or vegetable meat.

Milky mushroom (*Calocybe indica*) is a one of the important cultivated mushroom which was introduced and commercialized to the edible mushrooms world from India. Milky Mushroom also known as "DudhChhatta/Dudhiya Mushroom" because it has milky white appearance and large sized sporophores or also known as "white summer mushroom" because of its tropical nature. *Calocybe indica* is more attractive having excellent shelf-life. It was grown on several agricultural wastes and on wide range of temperatures and pH. Different strains of *Calocybe indica* show diversity in adaptation of their suitable wide range of the temperature and pH levels for maximum radial growth of mycelium on PDA medium. Thus, it is essential to evaluate most favourable pH and temperature range for efficient mycelial growth of milky mushroom strains. Singh *et al*. (2015) observe the range of temperature and pH for proper mycelial growth of different strains of *Calocybe indica* on PDA media. Experiment revealed that the *Calocybe indica* strains (CI-6, CI-8, CI-9 CI-10 and APK-2) showed maximum mycelial growth at pH 8.0 followed by 7.5 and 8.5. Experiment also showed maximum mycelial growth at 30^{0}C and minimum at 21^{0}C. In present study, seven different pH ranges were evaluated on PDA medium to determine their effects on mycelial growth of five strains of milky mushroom, *viz*. CIP-18, CIP-19, CIP-20, CI(wild-1) and CI(wild-2).

Material and Methods

Collection of Mushroom Culture

Pure culture of three strains CIP-18, CIP-19 and CIP-20 of

*Corresponding author Email: pkdhakad1989@gmail.com

Calocybe indica were introduced from Dr. Rajendra Prasad Central Agricultural University, Samastipur, Bihar. These cultures were sub-cultured and maintained on PDA medium in a B.O.D. incubator at 30±2ºC temperature for further investigation. A survey was also conducted to nearby forest regions of Varanasi and Mirzapur district of Eastern Uttar Pradesh to collect edible wild milky mushroom Species. After collection of wild Milky Mushroom samples the pure culture of these samples was prepared on PDA medium in "Mushroom Spawn Laboratory" Department of Mycology and Plant Pathology, I.A.S., B.H.U. Varanasi, for the further investigations sub-culture was also maintained.

Maintaining the pH in medium

The PDA media was prepared and pH was maintaining with the help of base (NaOH) and acids (HCl). Firstly make the medium without agar and adjusted the pH (5, 5.5, 6, 6.5, 7, 7.5 & 8) with the help of pH meter. After adjusting the pH mixed the agar-agar and then sterilized the medium in autoclave at 121ºC (15lb/sq. inch pressure) for 30 minutes. After autoclaved melt medium pour in sterilized Petri-plates in laminar flow and took for the solidification of media. After solidifying of medium in laminar flow then inoculate culture in Petri-plates and kept in incubator where temperature was 30ºC and recorded the radial growth of mycelium at 3rd, 7th and 10th day.

Results and Discussion

The effect of seven different pH (5, 5.5, 6, 6.5, 7, 7.5 & 8) on mycelia growth of *Calocybe indica* strains were shown in Table-1. As result shown in Table-1 mycelial growth recorded at 3rd day for strain CIP-18 shown maximum mycelial growth at 5 pH (2.73 cm) followed by 7 pH (2.70 cm), 7.5 pH (2.67 cm), 8 pH (2.53 cm), while minimum from 6 pH (1.90 cm). Strain CIP-19 shown maximum growth at 7.5 pH (2.77cm) than at 8 pH (2.67 cm) but 6.5 pH shown minimum growth (2.17 cm). The maximum mycelial growth from 3rd day observations recorded for strain CIP-20 at 7 pH (3.33cm) followed by 7.5 pH (3.1cm), 5 pH (3.0 cm) while minimum growth observed at 6 pH (2.03 cm).for strain CI(wild-1) maximum growth from 7.5 pH (2.20 cm) than from 5.5 pH (2.10 cm) and minimum growth observed from 7 pH (1.77 cm). CI(wild -2) strain shown maximum growth from 7.5 pH (2.26 cm) while minimum from 5 pH (1.63 cm).

The mycelial growth for five strains of *Calocybe indica* from seven different pH shown in Table-1. The result shown that on 7th day for strain CIP-18 maximum mycelial growth at 7 pH (5.63±0.28cm) and 5 pH (5.63±0.35cm) but at 6 pH shown minimum growth (3.3±0.26 cm). Strain CIP-19 shown maximum growth at 5 pH (5.53±0.05 cm) than at 7.5 pH (5.3±0.1 cm) while minimum at 6 pH (3.27±0.15 cm). The mycelial growth observed from 7th day for strain CIP- 20 shown maximimum growth than other strain. At 7 pH (6.33±0.15 cm) and 5 pH (6.33±0.72cm) strain CIP-20 shown maximum growth while at 6 pH having minimum growth (3.53±0.15 cm). CI(wild-1) strain shown maximum growth at 7.5 pH (4.07±0.56 cm) and minimum at 6 pH (3.17±0.15cm). Strain CI(wild-2) shown maximum mycelial growth at 7.5 pH (4.13±0.30 cm) than at 7 pH (3.73±0.45 cm) while minimum growth at 6 pH (3.1±0.2 cm).

The result presented in Table-1 for 10th day observation shown that the maximum mycelial growth for strain CIP-18 shown maximum growth at 5 pH (8.27±0.49 cm) than 7 pH (7.93±0.25 cm) and 7.5 pH (7.53±0.05 cm) but minimum growth observed from 6 pH (4.87±0.28 cm). Strain CIP-19 shown maximum growth at 5 pH (7.37±0.20 cm) than 6.5pH (6.97±0.20 cm), pH 7(6.77±0.11 cm) and pH 7.5 (6.7±0.1 cm) while minimum at 6 pH (4.37±0.20 cm). Maximum mycelial growth at 10th day observed for strain CIP-20 at 5 pH (8.6±0.4 cm) followed by 7 pH (8.43±0.35 cm), 6.5 pH (7.9±0.1 cm), 7.5 pH (7.87±0.15 cm), 8 pH (6.33±0.11 cm) and 5.5 pH (6.13±0.20 cm) while minimum growth was at 6 pH (4.53±0.11 cm). For strain CI(wild-1) maximum growth was at 7.5 pH (5.6±0.36 cm) than 5.5 pH (5.5±0.26 cm), 5 pH (5.37±0.20 cm) and minimum growth at 6 pH (4.23±0.25cm). The CI(wild-2) strain of *Calocybe indica* shown maximum growth at 7.5 pH (5.6±0.3 cm) followed by 7 pH (5.07±0.41 cm), 5 pH (4.93±0.25 cm), 5.5 pH (4.73±0.35 cm), 8 pH (4.53±0.30 cm) and 6.5 pH (4.43±0.25 cm) while minimum growth observed at 6 pH (4.17±0.15 cm).

Conclusion

Mushroom production is good alternative to achieve nutritional security. Mushrooms production is an importance tool to alleviate poverty and bring diversification to agricultural production. Different strains of milky mushroom having diversified level of pH range for maximize their mycelial growth and production. In present experiment we evaluated five strains of *Calocybe indica* like CIP-18, CIP-19, CIP-20, CI(wild-1) and CI(wild-2) for seven different pH levels. As result shown in Table-1 we found that the maximum mycelial growth observed for strain CIP-20 at 5 pH (8.6±0.4 cm) than at 7 pH (8.43±0.35) while minimum growth observed at 6 pH (4.53±0.11 cm). The strain CIP-18 and CIP-19 also showed maximum mycelial growth at 5 pH that was 8.27±0.49 cm and 7.37±0.20 cm, respectively. But the strain CIP-18 and CIP-19 shown minimum growth at 6 pH that was 4.87±0.28 cm and 4.37±0.20 cm, respectively. The wild strain of *Calocybe indica* CI(wild-1) and CI(wild-2) shown maximum growth at 7.5 pH that was 5.6±0.36 cm and 5.6±0.3 cm respectively while minimum growth at 6 pH 4.23±0.25 cm and 4.17±0.15 cm. The similar result was reported by Krishnamoorthy *et al.* (2015) with studied on best suitable media, temperature and pH for mycelial growth of *C. indica.* They found that potato dextrose agar or malt extract agar medium, 25-35°C was optimum temperature andoptimal pH range, between 5.5 and 8.5 for mycelial growth and production of *C. indica.*

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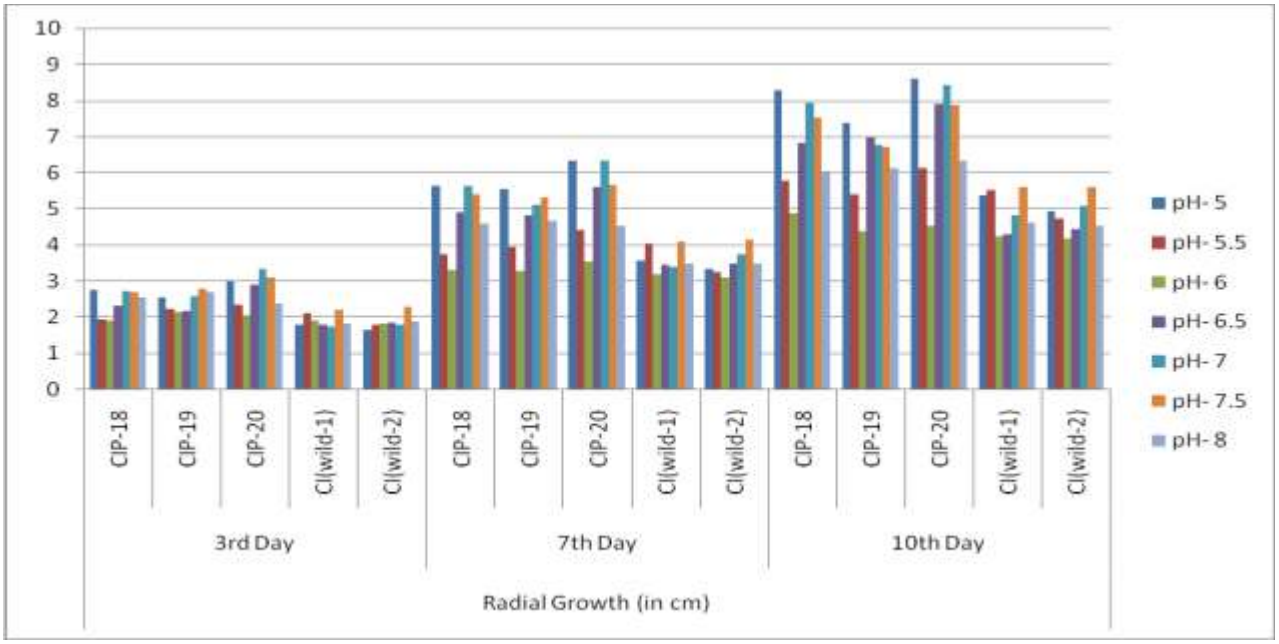

Fig. 1: Effect of different pH on mycelial growth for milky mushroom (*Calocybe indica*) strains

Table 1: Effect of different pH on mycelial growth for milky mushroom (*Calocybe indica*) strains

Strains/ pH	Radial Growth (in cm)														
	3rd Day					7th Day					10th Day				
	CIP-18	CIP-19	CIP-20	CI(wild-1)	CI(wild-2)	CIP-18	CIP-19	CIP-20	CI(wild-1)	CI(wild-2)	CIP-18	CIP-19	CIP-20	CI(wild-1)	CI(wild-2)
pH- 5	2.73±0.15^{a}	2.53±0.23^{ab}	3±0.52^{ab}	1.77±0.23^{c}	1.63±0.20^{b}	5.63±0.35^{a}	5.53±0.05^{a}	6.33±0.72^{a}	3.57±0.15^{ab}	3.33±0.15^{bc}	8.27±0.49^{a}	7.37±0.20^{a}	8.6±0.4^{a}	5.37±0.20^{a}	4.93±0.25^{a}
pH- 5.5	1.93±0.15^{b}	2.23±0.11^{bc}	2.33±0.05^{c}	2.1±0.2^{ab}	1.77±0.21^{b}	3.73±0.32^{d}	3.93±0.05^{e}	4.4±0.1^{c}	4.03±0.23^{a}	3.23±0.32^{c}	5.77±0.37^{d}	5.4±0.2^{d}	6.13±0.20^{c}	5.5±0.26^{a}	4.73±0.35^{a}
pH- 6	1.9±0.1^{b}	2.13±0.05^{c}	2.03±0.05^{c}	1.9±0.1^{abc}	1.8±0.1^{b}	3.3±0.26^{d}	3.27±0.15^{f}	3.53±0.15^{d}	3.17±0.15^{b}	3.1±0.2^{c}	4.87±0.28^{c}	4.37±0.20^{c}	4.53±0.11^{d}	4.23±0.25^{c}	4.17±0.15^{a}
pH- 6.5	2.3±0.1^{ab}	2.17±0.25^{c}	2.9±0.1^{b}	1.77±0.11^{c}	1.83±0.15^{b}	4.9±0.1^{bc}	4.8±0.36^{cd}	5.6±0.17^{b}	3.43±0.20^{b}	3.47±0.20^{bc}	6.83±0.15^{c}	6.97±0.20^{b}	7.9±0.1^{b}	4.3±0.1^{bc}	4.43±0.25^{a}
pH- 7	2.7±0.3^{a}	2.57±0.20^{a}	3.33±0.11^{a}	1.73±0.15^{c}	1.77±0.15^{b}	5.63±0.28^{a}	5.1±0.2^{bc}	6.33±0.15^{a}	3.37±0.25^{b}	3.73±0.45^{ab}	7.93±0.25^{ab}	6.77±0.11^{b}	8.43±0.35^{a}	4.8±0.36^{b}	5.07±0.41^{a}
pH- 7.5	2.67±0.51^{a}	2.77±0.25^{a}	3.1±0.2^{ab}	2.2±0.26^{a}	2.26±015^{a}	5.4±0.5^{ab}	5.3±0.1^{ab}	5.67±0.25^{b}	4.07±0.56^{a}	4.13±0.30^{a}	7.53±0.05^{b}	6.7±0.1^{b}	7.87±0.15^{b}	5.6±0.36^{a}	5.6±0.3^{a}
pH- 8	2.53±0.11^{a}	2.67±0.05^{a}	2.37±0.05^{c}	1.8±0.17^{bc}	1.87±0.15^{b}	4.57±0.25^{c}	4.67±0.20^{d}	4.53±0.15^{c}	3.47±0.30^{b}	3.47±0.20^{bc}	6.03±0.30^{d}	6.13±0.30^{c}	6.33±0.11^{c}	4.6±0.43^{bc}	4.53±0.30^{b}
CD(5%)	0.436	0.327	0.393	0.324	0.289	0.555	0.333	0.553	0.523	0.491	0.535	0.354	0.412	0.528	1.728