Impact of Various Media, Temperature, pH and Light Intensity on the Cultural Conditions of Hericiumerinaceus

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Abstract: Hericiumerinaceuscan strengthen the spleen, nourish the stomach, calm the mind, and combat cancer. The studies were carried out in vitro to investigate the possibilities of various medium, temperature, pH and Light intensity. Present study carried out on two strains of H. erinaceous (DMRX-779 and DMRX-780) for evaluation of their cultural characteristics on different media, temperature, pH and light intensity. Among the selected media PDA and WEA showed better mycelia growth. For analysis of temperature (25ºC) were recorded best followed by 20ºC temperature for both the strains and variable pH (5, 6, 8 and 7) was recorded. Light serves as a significant growth factor that has been assessed for the growth of selected strains for the first time. Yellow light promoted fruiting bodies initiation while blue light favors rapid colonization in petriplates. Both strains could be further improved for commercial and economic purposes based on these considerations.

Keyword: Growth, Hericiumerinaceus, Strains, Mycelia, Media, Temperature, pH and Light intensity.

1. Introduction

Growing mushrooms is a sustainable way to handle solid waste. It is clear that growing mushrooms contributes to the biological deterioration of natural resources and that they are a healthy, high-protein diet. Mushrooms are a nutritious dietary source as they are rich in proteins, vitamins, fibers and minerals and low in carbohydrates, fat and cholesterol **(Lau et al.,2012; Phillips et al., 2011; Thawthonget al., 2014; Ulziijargal and Mau 2011)**. Additionally, recent advances in our understanding of mushroom cultivation have helped to advance the technologies used in their production **(Puri, 2011)**.The lion's mane mushroom, or Hericiumerinaceus, has attracted a lot of interest

because of its culinary and therapeutic uses **(Zhang et al., 2019)**.

Traditional Chinese medicine (TCM) has frequently prescribed Hericium erinaceus , also known as "Houtou" or "Shishigashira" in China and "Yamabushitake" in Japan, due t o its demonstrated health benefits.The species can be found in North America, Asia, and Europe in the northern hemisphere **(Thongbaiet al., 2015)**.Since ancient times. Traditional Chinese medicine and cuisine have made use of the edible and therapeutic Hericium erinaceus fungus **(Liu et al., 2021)**. Among the chemical components of H.erinaceus are terpenoids, phenolics, steroids, pyranones, fatty acids and alkaloids. **(Atilaet al., 2021)**reproted that about 80 small molecular compounds have been found and extracted from H.erinaceus.

Hericium erinaceus (Bull.) Pers. 1797 is a member of the class Agaricomycetes, ord erRussulales, and familyHericiaceae **(Kirk et al., 2008)**.To investigate the effect of media, temperature, pH and light intensity on the cultural conditions of Hericiumerinaceus, a controlled experiment was conducted. Media, temperature, pH and light intensity composition are crucial factors influencing the cultural condition of H. erinaceous.

2. Materials and Method

2.1. Multiplication and Maintenance of pure culture of Hericiumerinaceus

For the current study, two strains namely DMRX-779 and DMRX-780had been used which were taken from the ICAR-Directorate of Mushroom Research, Solan, Himachal Pradesh and in vitro experiments were done at Laboratory of Department of Plant Pathology, School of Agricultural Sciences,Shri Guru Ram Rai University, Pathribagh, Dehradun during 2022-2024. Both the strains were further maintained on Potato Dextrose Agar Medium at 25ºC.

2.2. Effect of Different Culture Media on mycelial growth of Hericiumerinaceus

To evaluate the effect of different medium on growth of the mushroom (fungus), six different semi-synthetic and synthetic media were used.Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), Oat Meal Agar (OMA), Wheat Extract Agar (WEA), Sorghum Meal Agar (SMA) and Czapeck's Dox Aagr (CDA) were prepared according to the standard literatures and the constituents are available easily in the locality. Final pH of the medium was adjusted to neutral 7 and the medium was autoclaved at 15 psi pressure for 20 minutes. Media were poured into pre-sterilized petridishes for solidifying and after that 5mm discs of 10 days old pure culture of H.erinaceus were cut with the help of cork borer and inoculated into each solid media and incubated at 25ºC±2 for 15days. Three replications were maintained.

2.3. Effect of Different Temperature on mycelial growth of Hericiumerinaceus

5mm discs of 7 days old test mushroom was inoculated in the sterilized petridishes containing 20 ml of PDA medium. The medium was incubated at different temperature viz, 10ºC, 15ºC, 20ºC, 25ºC, 30ºC and 35ºC for 15 days. Three replications were maintained. For the study different temperature effect on mycelial growth of mushroom, this experiment was conducted in two stages adjusting three incubators at 10ºC, 15ºC and 20ºC. After taking observation in 15 days second set of experiment was conducted by adjusting the incubators to 25ºC, 30ºC and 35ºC. After period of experimentation both the data were combined to get the result.

2.4. Effect of Different pH on mycelial growth of Hericiumerinaceus

For the study of different pH level effect on radial mycelial growth,PD broth of pH (5,6,7,8,9 and 10) were prepared by adding required amount of HCL and NaoH. After maintaining the different pH agar- agar was added to the broth. The medium was autoclaved at 121.6ºC/15psi for 20 minutes. After sterilization medium was poured into the three sterilized petriplates and put into the laminar air flow for 20 minutes. After 30 minutes 5 mm discs of 10 days old pure culture of H.erinaceus were cut with the help of cork borer and inoculated into each solid media and incubated at 25ºC±2 for 15days. Three replications were maintained.

2.5. Effect of Different Light intensity on mycelial growth of Hericiumerinaceus

To evaluate the effect of different light intensityon mycelial growth of the mushroom (fungus) six different colour light has been taken for the study. Colour light are as follow:[blue(450-495nm), green (495-570nm), yellow(570-590nm), red(620-750nm) , artificial day light (ADL) and dark condition.A 5mm disc of both the strains was inoculated in the centre of Petri plates with PDA medium. The plates with the three replicateseach incubated at 25ºC for the parameters.Different light intensity effect and observations were recorded at three days intervals starting from 3rd days of inoculation and observed up to 15th day.

3. Results and Discussion

3.1. Effect of strains on mycelial growth of H. erinaceus

The results were concluded as per the Table 1.thatstrain DMRX- 780 found better than the DMRX-779. As data has been taken on different days of inoculation are 3rd, 6th, 9th and 15th and found strain DMRX-780 showed better growth (6.344cm), whereas DMRX-779 growth was (5.883cm) on the final day comparatively.Strain DMRX-780 has significantly better than DMRX-779.

3.1.1. Effect of media on mycelia growth of H. erinaceous

Among six media PDA and WEAshowed maximum growth (8.417cm) followed by OMA(7.217cm), minimum growth was recorded in SMA (6.167cm) which was at par to MEA (6.85) whereas, no growth was found in CDA on 15thdays of inoculation.PDA medium showed significant result over than OMA, SMA and CDA.

3.1.2. Interaction effect of strains and media on mycelial growth of H. erinaceus

The result presented in Table 2. revealed that the studies of effect of different culture media on mycelial growth of both the strains were tested for radial growth and measured in three days of interval. The result showed that on the 15th day of inoculation strain DMRX-779 is highly significant to WEA media over to all five treatments whereas, strain DMRX-780 is highly significant to PDA media over to MEA, SMA and CDA media. The mycelial growth on PDA mediawas obtained fast and full growth (8.5cm). Strain DMRX-780 showed maximum growth on PDA (8.5cm) and WEA (8.333 cm) followed by OMA (8.33cm) while minimum growth observed on MEA (6.3cm) while, the mycelial growth for the strain DMRX-779 show maximum growth on WEA (8.5cm) followed by PDA (8.3cm) and minimum growth observed in SMA (5.7cm). No growth observed in CDA medium for both the strains.Similar result in accordance to Julian et al., (2018), found that PDA was the most effective medium for promoting H. erinaceus maximum mycelial development (56.95mm), as was noted on the tenth day after inoculation at a temperature of 28–30°C.Similarly, Mihai and associates (2016), reported that Potato Dextrose Agar (PDA) or two percent malt extract agar were used to cultivate Hericiumerinaceus mycelial cultures in petri dishes at 25 °C. Mycelium growth reaches its peak after a week.

3.2. Effect of strains on mycelial growth of H. erinaceus

It has been observed in the Table 3. that both the strains of H. erinaceusfavours25°C temperatures for the mycelial growth. No or less variation found in this parameter of the study.

3.2.1. Effect of temperature on Mycelial growth of H. erinaceus

The results presented in Table 3. concluded that among the six temperatures the most suitable temperature for the mycelial growth of fungus is 25°C while 10°C and 35°C showed unfavourable temperature or no growth.

3.2.2. Interaction effect of strains and temperature on mycelial growth of H. erinaceus

Diametrically temperature effect on mycelial growth presented in (Table 4). It has been observed that among the six different temperatures tested on PDA medium, mycelia growing at 25°C showed significantly the maximum and full growth(8.5cm) by both the

strain, while at 10°C,15°C and 35°C there was no growth, minimum growth at 20°C (2.5 cm) followed by 30°C (4.06cm)showed by strain DMRX-779 and DMRX-780 showed minimum growth (2.5 cm) at 15°C.Due to low and high temperature less or negligible growth were observed in both the strains, which were accordance to the findings of Singh et al. (2022). They also investigated that how different temperature ranges affected H. erinaceus mycelial growth on PDA medium and concluded that after 16 days of incubation, the maximum growth (90.00 mm) was recorded at 25°C, followed by 20°C (73.00 mm), 30°C (66.33 mm), and 35°C (44.67 mm).

3.3. Effect of strains on mycelial growth of H. erinaceus

It has been showed in the Table 5. that on the 15th day of inoculation strain DMRX-779 had maximum growth (7.072 cm) than strain DMRX-780 (7.106 cm) comparatively.

3.3.1. Effect of pH on Mycelial growth of H. erinaceus

Six tested pH has been taken for the present study except 9 and 10 pH all other pH showed fast and full growth on 15th day of inoculation and favour growth and development of H. erinaceus.

3.3.2. Interaction effect of strains and pH on mycelial growth of H. erinaceus

The results presented in Table 6. revealed that the studies of effect of different pH on mycelial growth of strains (DMRX-779and 780) of H. erinaceuspH were tested for the radial growth and measured three days interval. The result showed increased or significant growth on acidicpH i.e. 5,6 pH for both the strains while, minimum growth observed in alkalinepH i.e. 9 and 10 on 12th days of inoculation. 8pH also showed significant growth might be effect of agar-agar. Full growth of pertiplates has been seen in 15th day of inoculation of both the strains. Similar to the findings of **Shruti et al. (2022)**, revealed that pH 5, 5.5 and 6 showed maximum growth by recording 0.83 cm, 0.82 cm and 0.82 cm/day respectively followed by pH 6.5 (0.7cm/day), pH 7(0.65cm/day), pH 7.5(0.52cm/day) suitable pH for Pleurotuseryngii.

4.4. Effect of strains on mycelial growth of H. erinaceus

No variation was found between both the strains (DMRX-779 and DMRX-780) in light intensity parameter of the study.

4.4.1. Effect of different light colour on Mycelial growth of H. erinaceus

Among six different light colour,blue, green colour light and dark show significant effect over yellow, red and ADL.

4.4.2 Interaction effect of strains and pH on mycelial growth of H. erinaceus

The results presented in Table 8. revealed that the study of effect of different colour light on mycelial growth of strains (DMRX-779and 780) of H. erinaceus. Different colour light was tested for the radial growth and measured three days interval. In case of strain DMRX-780 dark (no light) was taken as control and showed significant effect with yellow, red and ADL while maximum growth was found with green (8.5cm), blue and dark light.
Strain DMRX-779 showed significant effect with yellow and green colourwhile maximum growth was found in Blue and Red colour which is at par to green and dark (no light). Whereas, minimum growth was found in ADL.Similar to the findings of **Chutimanukulet al, (2025)** according to their study, the growth and possible bioactivity of H. erinaceusmycelium grown on a red sorghum substrate may be influenced by the light spectrum, especially blue light. Among the all-other treatments, blue light encouraged increased mycelial biomass, fastest mycelial expansion,density and colonization reaching the maximum diameter of 5cm by day 15, with a sharp increased observed between 6 and 12 days. **Wang et al,(2020)** concluded an experiment that red and green light also increased bioactivity, though to a much smaller extent.

4. Conclusion

Nutshell both the strains were found suitable for particular growth factors. Strain DMRX-780 found better than DMRX-779. Particularly PDA medium was best at temperature 25 °C followed by 5pH, 6pH, 7pH and 8pH was best suitable for mycelial growth . Light factor promoted fast colonization and fruitingbody initiation in pertiplates only which might be further enhance for commercially and economically.

Table 1: Mycelial growth (cm) of Hericiumerinaceus on different media and days at 25^{0}C temperature

Treatment	Mycelial Growth (cm) Days After Inoculation				
	3rd	6th	9th	12th	15th
STRAINS					
DMRX-779	0.156	1.783	2.778	4.783	6.011
DMRX-780	0.128	1.828	2.956	5.211	6.344
SEm±	0.018	0.050	0.064	0.086	0.080
CD at 5%	NS	NS	NS	0.252	0.235
MEDIA					
PDA	0.367	2.8	4.4	6.733	8.417
MEA	0.167	2.333	3.55	6.083	6.85
OMA	0	1.517	2.667	4.933	7.217
WEA	0.317	2.85	4.05	6.483	8.417
SMA	0	1.333	2.533	5.75	6.167
CDA	0	0	4.4	6.733	0
SEm±	0.030	0.087	0.110	0.149	0.139
CD at 5%	0.089	0.257	0.324	0.436	0.407

Table 2: Interaction effect of strains and media on mycelial growth of H. erinaceuson PDA medium

Strains/Media	Mycelial Growth (cm) Days After Inoculation									
	3rd		6th		9th		12th		15th	
	DMRX-779	DMRX-780	DMRX-779	DMRX-780	DMRX-779	DMRX-780	DMRX-779	DMRX-780	DMRX-779	DMRX-780
PDA	0.267	0.467	2.7	2.9	3.833	4.967	6.367	7.1	8.333	8.5
MEA	0.2	0.133	2.5	2.167	3.8	3.3	6.433	5.733	7.4	6.3
OMA	0	0	1.3	1.733	2.3	3.033	3.433	6.433	6.1	8.333
WEA	0.467	0.167	3	2.7	4.4	3.7	6.833	6.133	8.5	8.333
SMA	0	0	1.2	1.467	2.333	2.733	5.633	5.867	5.733	6.6
CDA	0	0	0	0	0	0	0	0	0	0
CD at 5% a*b SEm±	0.126 0.043		0.363 0.124		0.458 0.156		0.617 0.210		0.576 0.196	

*Average of three replications

Table 3: Mycelial growth (cm) of Hericiumerinaceus on different temperature and days on PDA medium

Treatment	Mycelial Growth (cm) Days After Inoculation				
	3rd	6th	9th	12th	15th
STRAINS					
DMRX-779	0.117	0.628	1.167	2.022	2.489
DMRX-780	0.206	0.994	1.872	3.100	3.867
SEm±	0.030	0.045	0.040	0.070	0.035

CD at 5%	0.089	0.132	0.117	0.204	0.103
Temperature					
10 °C	0	0	0	0	0
15 °C	0.1	0.45	0.833	1.033	1.25
20 °C	0.067	0.733	1.8	4.017	4.817
25 °C	0.433	2.317	4	6.767	8.433
30 °C	0.367	1.367	2.483	3.55	4.567
35 °C	0	0	0	0	0
SEm±	0.053	0.078	0.069	0.121	0.060
CD at 5%	0.155	0.228	0.203	0.354	0.178

Table 4: Interaction effect of strains and temperature on mycelial growth of H. erinaceusdays on PDA medium

Strains/ Temperature	**Mycelial Growth (cm) Days After Inoculation**									
	3rd		**6th**		**9th**		**12th**		**15th**	
	DMRX-779	**DMRX-780**	**DMRX-779**	**DMRX-780**	**DMRX-779**	**DMRX-780**	**DMRX-779**	**DMRX-780**	**DMRX-779**	**DMRX-780**
10 °C	0	0	0	0	0	0	0	0	0	0
15 °C	0	0.2	0	0.9	0	1.667	0	2.067	0	2.5
20 °C	0	0.133	0.667	0.8	1.633	1.967	2.433	5.6	2.5	7.133
25 °C	0.4	0.467	1.733	2.9	3.033	4.967	6.433	7.1	8.367	8.5
30 °C	0.3	0.433	1.367	1.367	2.333	2.633	3.267	3.833	4.067	5.067
35 °C	0	0	0	0	0	0	0	0	0	0

CD at 5% **a*b** **SEm±**	NS 0.075	0.322 0.110	0.287 0.098	0.501 0.171	0.251 0.086

*Average of three replications

Table 5: Mycelial growth (cm) of Hericiumerinaceus on different pH and days on PDA medium at 25°C

Treatment	**Mycelial Growth (cm) Days After Inoculation**				
	3rd	**6th**	**9th**	**12th**	**15th**
STRAINS					
DMRX-779	0.406	2.156	4.811	6.483	7.106
DMRX-780	0.339	2.044	4.717	6.378	7.072
SEm±	0.016	0.046	0.030	0.027	0.019
CD at 5%	0.048	NS	0.089	0.078	NS
pH					
5pH	0.6	2.633	5.633	7.6	8.5
6pH	0.55	2.517	5.533	7.533	8.5
7pH	0.383	2.383	5.383	7.383	8.5
8pH	0.533	2.583	5.600	7.55	8.5
9pH	0.05	1.083	3.233	4.333	4.333
10pH	0.117	1.400	3.200	4.183	4.2
SEm±	0.028	0.079	0.053	0.046	0.033
CD at 5%	0.082	0.232	0.155	0.135	0.098

Table 6: Interaction effect of strains and pH on mycelial growth of H. erinaceuson PDA medium

Strains/pH	Mycelial Growth (cm) Days After Inoculation									
	3rd		6th		9th		12th		15th	
	DMRX-779	DMRX-780	DMRX-779	DMRX-780	DMRX-779	DMRX-780	DMRX-779	DMRX-780	DMRX-779	DMRX-780
5pH	0.667	0.533	2.700	2.567	5.700	5.567	7.667	7.533	8.5	8.5
6pH	0.600	0.500	2.567	2.467	5.600	5.467	7.6	7.467	8.5	8.5
7pH	0.367	0.400	2.400	2.367	5.433	5.333	7.433	7.333	8.5	8.5
8pH	0.567	0.500	2.633	2.533	5.633	5.567	7.567	7.533	8.5	8.5
9Ph	0.100	0.000	1.167	1.000	3.267	3.200	4.4	4.267	4.4	4.267
10pH	0.133	0.100	1.467	1.333	3.233	3.167	4.233	4.133	4.233	4.167
CD at 5% a*b	NS		NS		NS		NS		NS	
SEm±	0.040		0.112		0.075		0.065		0.047	

*Average of three replications

Table 7: Mycelial growth (cm) of Hericiumerinaceus on different Light intensity and days on PDA medium

Treatment	Mycelial Growth (cm) Days After Inoculation				
	3rd	6th	9th	12th	15th
STRAINS					
DMRX-779	0.311	1.722	3.483	5.911	8.089
DMRX-780	0.283	1.778	3.739	5.917	7.928
SEm±	0.036	0.073	0.097	0.128	0.060
CD at 5%	NS	NS	NS	NS	NS
Light					

Intensity					
Blue	0.633	2.3	4.383	6.083	8.45
Green	0.3	1.9	4.067	6.383	8.383
Yellow	0.083	1.617	3.483	5.217	7.65
Red	0.3	1.333	2.6	5.633	7.767
ADL	0	0.75	2.75	5.7	7.417
Dark	0.467	2.6	4.383	6.467	8.383
SEm±	0.062	0.127	0.168	0.221	0.104
CD at 5%	0.18	0.372	0.492	0.649	0.306

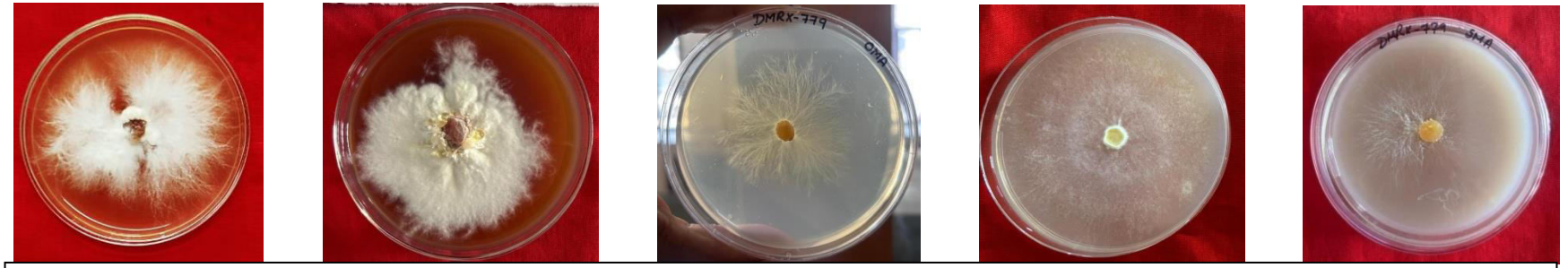
Table 8: Interaction effect of strains and light intensity on mycelial growth of H. erinaceus on PDA medium

Strains/ light intensity	Mycelial Growth (cm) Days After Inoculation									
	3rd		6th		9th		12th		15th	
	DMRX-779	DMRX-780	DMRX-779	DMRX-780	DMRX-779	DMRX-780	DMRX-779	DMRX-780	DMRX-779	DMRX-780
Blue	0.7	0.567	2.7	1.9	4.333	4.433	6.267	5.9	8.5	8.4
Green	0	0.6	1.0	2.8	3.133	5.0	6.033	6.733	8.267	8.5
Yellow	0.167	0	1.367	1.867	3.4	3.567	5.5	4.933	7.8	7.5
Red	0.467	0.133	1.8	0.867	2.4	2.8	5.533	5.733	8.5	7.033
ADL	0	0	0.733	0.767	2.733	2.767	5.6	5.8	7.1	7.733
Dark	0.533	0.4	2.733	2.467	4.9	3.867	6.533	6.4	8.367	8.4

CD at 5%					
a*b	0.257	0.526	0.696	NS	0.433
SEm±	0.088	0.179	0.237	0.312	0.148

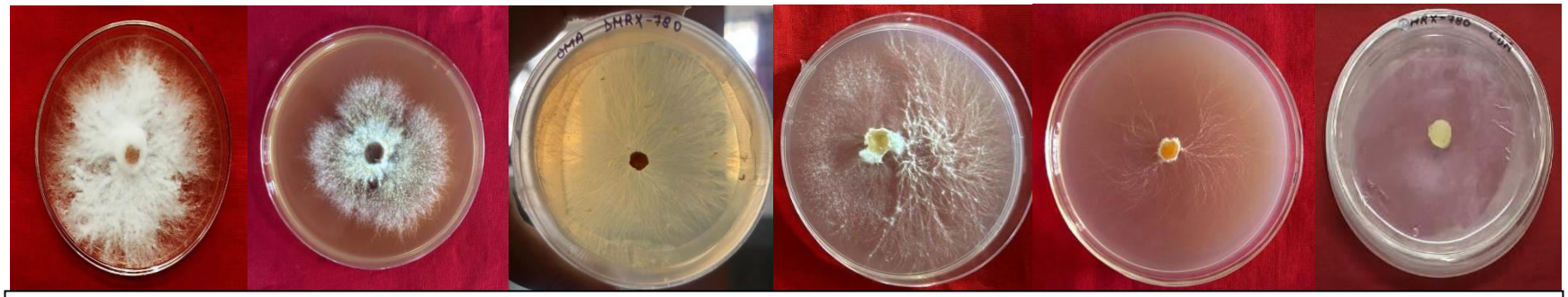
*Average of three replications

Fig.1. :Growth of Hericiumerinaceuson Different Medium after15 Days of Inoculation.



PDA MEA OMA WEA SMA CDA

STRAIN- DMRX-779



PDA MEA OMA WEA SMA CDA

STRAIN- DMRX-780

Fig.2. :Growth of Hericiumerinaceuson Different Temperature at 15 Days After Inoculation.

10ºC	15ºC	20ºC	25ºC	30ºC	35ºC

STRAIN- DMRX-779

10ºC	15ºC	20ºC	25ºC	30ºC	35ºC

STRAIN- DMRX-780

Fig.3.:Growth of Hericiumerinaceuson different pHat 15 Days After Inoculation.

5pH	6pH	7pH	8pH	9pH	10pH

STRAIN- DMRX-779

5pH	6pH	7pH	8pH	9pH	10pH

STRAIN- DMRX-780

Fig.4.:Growth of Hericiumerinaceuson different Light Intensity at 15 Days After Inoculation.

BLUE GREEN YELLOW RED ADL DARK

STRAIN- DMRX-779

BLUE GREEN YELLOW RED ADL DARK

STRAIN- DMRX-780

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