Original Research Article

Selection of the best performing media for thegrowth and development of *Cordyceps militaris* mycelia

ABSTRACT:

The genus *Cordyceps,* also known as Keerajadi or Yarsa-gumbu in the vernacular language, has been used for years as a medicinal fungus for augmenting endurance, longevity and vitality. For thousands of years, it has been recognised as an essential ingredient under "Sowa-Rigpa System of Medicine" as well as the Chinese Traditional Medicine and has immense broad spectrum biological value and activity. *Cordyceps* mushroom have higher market price which is due to scarcity in availability and higher global market demand. In the present investigation different media i.e., Agar, Optimised Nutrient Broth (ONB), Chitosan + Malt Extract Agar (MEA), Chitosan + Agar + Optimised Nutrient Broth, Kenknights and Munnaiers (K&M), Malt Extract Agar (MEA), Malt Extract Agar + Optimised Nutrient Broth, Oat Meal Agar (OMA), Rice Extract Agar (REA), Rice Extract Agar + Optimised Nutrient Broth, Rose Bengal Agar (RBA) were used to identify the growth of *Cordyceps militaris* mycelial growth. Here in this experimentation, we find the best media having fastest mycelial growth. In comparison to Malt Extract Agar mycelial growth of *Cordyceps militaris* was increased on Kenknightsand Munnaiers media by 23.25% on 14th day and 28.03% on the 21st day.

Keywords: *Cordycepsmilitaris*, Mycelial growth rate, Radial growth, Mycelial density, pH value

1. INTRODUCTION:

Cordyceps existence is known since 2000 BC also known as Keerajadi, Yarsagumba/Years Gumpa, Dong-Chong Xia-Cao (winter-worm summer-grass), Keera Ghas, Keera Jhar, Chyou Kira, Himalayan Viagra, Himalayan Gold, Tocheikasa in Japan and Sanjeevani Booti by the Indian local people(Tuli et. al. 2014). The earliest reference of the medicinal usage of Yarsagumbahave been documented in Sowa-Rigpa System of Medicineby Nyamnyi Dorje a Tibetan doctor and lama who lived 1439-1475. The use of *Cordyceps* as a medicinal herb can be traced back to 15th century, where it was included in traditional Chinese Materia Medica (a compendium of medicinal substances used in traditional Chinese medicine). *Cordyceps* was first introduced to western society in 17th century and was originally described by Carl Linnaeus in 1753 as *Clavaria militaris* L., 1753.Elias Magnus Frieschanged the name to *Cordyceps militaris* (L.) Fr. (1818). *Cordyceps* name have been derived from twoGreek words'κορδύληkordýlē' meaning 'club' and'κεφαλή'cephali'head'. *Cordycepsmilitaris* spores are produced internally inside a sac-like structure called ascus. *Cordyceps* belongs to phylum Ascomycota classified in the order *Hypocreales*. This entomopathogenic (insect parasitizing) fungus is primarily found on the head of the soil-dwelling larva of*Hepialusarmoricanus*Oberthur(Lepidoptera) moths and is although infrequently observed growing

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on other moth species (Kai yue,*et al.* 2013). *Cordyceps* grows parasitically on insects and small arthropods (lepidopteron larvae, pupae of insects, ants, spiders, etc) infects and kills them by using their bodies as substrate for growth and reproduction. The infection of *Cordyceps* starts when the conidia of *Cordyceps*which secrete certain hydrolytic enzymes like lipase, proteases, chitinase which lead to dissolution of the cutin (a heteropolysaccharide made with the polymerization of *N*-acetyl glucosamine through 1–4 β-linkage) and hence providing it nutrition (Tuli *et. al.* 2014), (Wang *et al.* 2005).*Cordyceps* is used for revitalization of various systems of body and is considered as some of the oldest herbs used traditionally.

Since ancient times, people in China, Tibet, Nepal and India have taken *Cordyceps* spp. to help their bodies adjust to the harsh conditions of the mountains, including low ambient temperature, high atmospheric pressure and lack of oxygen in the air. It has tonic effects considered as adaptogen, which helps in simulation of immune system and have the ability to reduce fatigue. For now, *Cordyceps* can be extensively used in modern system of medicine and is of great interest in medicinal application. It is a medicinal mushroom which has abundant source of natural product with several biological uses. It is increasingly popular as a dietary supplement, particularly for athletes and fitness enthusiasts. Since, it enhances physical performance and increases energy. *Cordyceps* contain several compounds that have the potential to improve oxygen uptake and endurance, as well as have anti-inflammatory and antioxidant effects.

Fruiting bodies of *Cordyceps* contain fat soluble vitamins (vitamin A and vitamin E) and water-soluble vitamins (vitamin B2, vitamin B3 and vitamin C).*Cordyceps* have been reported in reliving from symptoms of chronic bronchitis, asthma, influenza aviral infection, inflammation, kidney disorders and other respiratory disease/infections (Zhu *et al.* 1998), (Ohta, *et al.* 2007). Numerous natural dietary extracts from *Cordyceps* mushrooms have been demonstrated to be effective in the induction of cell cycle arrest and apoptosis on cancer cells in vitro (Jin *et al.*, 2008). Water extract of *Cordyceps militaris* (CM) have been reported to inhibit tumor cell proliferation via arresting the cell cycle at the G2/M phase and induce apoptosis through upregulation of p53, p21 and cyclin B1, as well as the activation of caspase-8, caspase-9 and caspase-3. (Yang *et al.*, 2012) It can be used to improve blood lipid(fat) levels and treating arrhythmia (irregular heart beat). It has tonic effects considered as adaptogen, help in simulation of immune system and have the ability to reduce fatigue. For now, *Cordyceps* can be extensively used in modern system of medicine and is of great interest in medicinal application. It is a medicinal mushroom which have abundant source of natural product with several biological usages.

1.1. Natural Habitat:

Cordyceps militaris is found in tropical regions and humid temperate climates broadly distributed throughout Europe, North America, East and Southeast Asia.*Cordyceps militaris* have similar medicinal properties as*Cordyceps sinensis* which majorly grows at high altitude Himalayan Alpine Meadows. *Cordyceps sinensis* is mostly found in China's, Sichuan, Qinghai, Yunnan, Gansu and Tibet Autonomous Region provinces.*Cordyceps* spp. has been found in Asia, North America and Europe,

mostly in countries such as China, Nepal, Bhutan, Thailand, Vietnam, Korea and Japan (*Tuli et. al. 2014*). *Cordyceps sinensis* growsparticularly in some specific habitat and often inhabits the soil of a grassland at an elevation of 3500 to 5000 m. It is naturally distributed in the high-altitude grassland of Nepal, Bhutan, India and Tibetan plateau of China. This species is also found in some areas of the Uttarakhand (Garhwal and Kumaon regions), Sikkim, Arunachal Pradesh Himalayas viz. Chamoli District, Pithoragarh District,Suthol, Kanol, Munsiyari-Darchula region, Niti-Mana Valley, Chiplakot, Ultapara, Brahmkot, Najari, Nagnidhura,Laspa region, Lachen, Lachung and Gnathang (Bharat*et al.* 2020), (Tuli *et. al.* 2014),(Caplins, *et al.* 2017), (Negi *et. al.* 2012).The collection and gathering of this fungus by many of the Bhotiya communities has exploded and have emerged as a lucrative yet high-risk livelihood strategy(Caplins, *et al.* 2017).Local dweller and tourists come to these Himalayan alpine meadows in the harvesting season and stay there for months in tented colonies, which overall leads to soil erosion damaging the top layer of soil and pollution due to burning of packaged cooking food wraps and campfire are common. Further, soil erosion is common which can overall lead to channel formation in the meadow in longer duration.

Cordyceps militaris commonly called as orange caterpillar fungus can be cultured on both liquid and solid media. *Cordyceps militaris* is considered as a promising substitute of *Cordyceps sinensis* because of similar medicinal properties, chemical capacities and also have several higher levels of certain bioactive components. (Chou, *et al.* 2024)In the recent years its artificial cultivation isin demand, where production cost and cultivation period can be optimised. Also, it has been reported by several papers that*Cordyceps militaris* have shorter cultivation time and lower production costsin the artificial cultivation.(Chou, *et al.* 2024) Ascomycota-classified *Cordyceps* spp. mushrooms have a long history of use in Asian ethnomedicine due to their adaptogenic and tonic properties, as well as their capacity to lessen weariness and character to boost the immune system in humans. Due to parasitic activity of moth's larva in the arid alpine environment condition and over-exploitation of*Ophiocordyceps sinensis* now called as *Cordyceps sinensis*have led to risktoward unnatural extinction.*Cordyceps sinensis*is designated as vulnerable species as per IUCN Red list of threatened species in 2019 and China biodiversity red list 2018 (Wang *et al* 2021). Vulnerable species means a species at high risk of human-caused (un-natural) extinction without further human interventions(Mace, *et al.* 2008). Since the outcome ofCOVID consumption of naturally available *Cordyceps sinensis*has increasedand is expected to grow from $1.2 billion in 2023 to $1.23 billion in 2024 at a compound annual growth rate (CAGR) of 9.8%. (*Cordyceps sinensis* Global Market Report 2024) The continuous increase in global demand posesa serious ill-effect on the climate and biodiversity (habitat destruction) of the area in the hills. So, there is an urgent need for its artificial cultivation.

2. MATERIAL AND METHODS:

2.1. Experimental Design:

The experiments were conducted in the Plant stress and molecular cell biology laboratory, Department of Molecular Cell Biology and Biotechnology, College of Basic Sciences and Humanities, Govind Ballabh Pant University of Agriculture and Technology in India. The experiments were arranged in a completely randomized Design with four replicates per treatment.

2.2. Culture collection and maintenance:

Pure Culture of *Cordyceps militaris*(DMR 1163) was obtained from ICAR-Directorate of Mushroom Research (DMR), Solan, Himanchal Pradesh which wascultured and maintained on Malt Extract Agar medium.

2.3. Preparation of culture media:

List 1 :Componentsof culture media

Contents of media (in gm)	Agar	Agar + ONB	Chitosan + Agar + ONB	MEA	MEA + ONB	Chitosan + MEA	REA	REA + ONB	OMA	RBA	K&M
Agar	20	20	20	20	20	20	20	20	12.5	15	15
Chitosan	-	-	10	-	-	10	-	-	-		
Dextrose	-	30	30	20	50	20	-	30	-	10	1
Magnesium Sulphate	-	0.75	0.75		0.75		-	0.75	-	0.5	0.1
Malt Extract	-	-	-	20	20	20	-	-	-	-	-
Mono Potassium Phosphate	-	-	-	-	-	-	-	-	-	1	-
Oat Meal	-	-	-	-	-	-	-	-	60	-	-
PapaicDigest of Soyabean Meal	-	-	-	-	-	-	-	-	-	5	-
Peptone	-	7.5	7.5	1	8.5	1	-	7.5	-	-	-
Potassium Chloride	-	-	-	-	-	-	-	-	-	-	0.1
potassium Dihydrogen Phosphate	-	1.5	1.5	-	1.5	-	-	1.5	-	-	0.1
Rose Bengal	-	-	-	-	-	-	-	-	-	0.05	-
Sodium Nitrate	-	-	-	-	-	-	-	-	-	-	0.1
Tri Ammonium Citrate	-	1.5	1.5	-	1.5	-	-	1.5	-	-	-
Vitamin B1	-	0.075	0.075	-	0.075	-	-	0.075	-	-	-
Vitamin B12	-	0.015	0.015	-	0.015	-	-	0.015	-	-	-
White Rice Extract	-	-	-	-	-	-	20	20	-	-	-
Yeast Extract	-	4.5	4.5	-	4.5	-	-	4.5	-	-	-
D. D. Water (in L)	1	1	1	1	1	1	1	1	1	1	1

All the Eleven media were prepared as per above mentioned composition.Where, Agar, Agar+ONB(Optimised Nutrient Broth), Chitosan + Agar + ONB, MEA (Malt Extract Agar),MEA (Malt Extract Agar) + ONB, Chitosan + MEA,REA (Rice Extract Agar), REA + ONB, OMA(Oat Meal Agar), RBA(Rose Bengal Agar), K&M (Kenknights and Munnaiers), gm (grams), L (litre)

2.4. Sterilization of the media:

Sterilization of the media was done at 121˚C &15 psi.

2.5. Inoculation and Incubation:

The Slants obtained by the DMR were used to prepare mother culture petri-plate of internal size 85 mm to the volume mark of 25 ml. Further these mother culture petri-plate was used to inoculate eleven media in four replicates. Here, for the inoculation cork-borer of 5 mm size was used to punch agar plug with mycelial mat. Which was pasted invertedly on the media by the help of the inoculation needle. These petri-plates were sealed by parafilm tape and were placed in the incubator maintained at 18˚C for further reading of growth.

2.6. Measurement of Radial Growth, Mycelial Growth/Day and Mycelial density:

Mycelial growth was determined with Digital vernier calipers, the diameter of the radial growth of mycelium was marked and noted on 7^{th},14^{th} and 21^{st} dayat the drawn quadrant axis of the replicates.For, theAverage mycelial growth rate is defined as the mycelial growth obtained on 7^{th}, 14^{th} and 21^{st} day divided by number of days**(Fig. 1)**. Mycelial density is the mycelia present over the surface of the agar (Mahadevan and Shanmugasundaram). For the mycelialdensity/fluffinessthe following rating was used:

+ = Low; ++ = Moderate;+++ = Abundant;+++ = Abundant;++++ =Very Abundant

2.7. pHAdjustment/Standardization:

From the above output, we have decided the best performing medium and on the basis of the mycelial growth. Further, the selected media was run over different pH (pH 4, pH 5, pH 6, pH 7, pH 8 & pH 9) on agar and liquid media which supported the radial growth, mycelial growth. The pH was taken on 7^{th}, 14^{th} and 21^{st} day after inoculation (DAI). In the liquid media the result was taken on the basis of the freshweight, dry weight and the moisture content. The acidityand basicity of the media was maintained by 3N NaOH and 3N HCl.

2.8. Statistical analysis:

One Factor Analysis of Variance (ANOVA) was conducted for the experiments withmean significance difference ($P<0.05$) among the treatments by the use of OPSTAT (Sheoran et. al. 1998). Each value is expressed as mean and SE (n=4).

3. RESULT AND DISCUSSIONS:

Mushrooms are found naturally in the environment and are restricted to certain regions depending on their requirement of specific temperature, humidity, food, daylight etc. For fulfillingincreasing demand of mushrooms for consumption they are grown in several artificial environmental conditions. There are several factors like shelf life, crop time, spawn quality, mycelial run rate, etc. which can be looked for

improving the quality of mushroom grown by farmers. In order to culture any mushroom in in-vitro laboratories it is required to search the best components which can increase the performance and quality of the spawn. Here, we have focused on the performance of the mycelium over different media. Selecting the best suited medium which can promote a better growth and can overallreduce the time of crop/flushes. In the present study*Cordyceps militaris* was cultured successfully in the laboratory environment. In the present investigation different mediai.e., Agar, Agar+Optimised Nutrient Broth(ONB), Chitosan + Malt Extract Agar(MEA), Chitosan + Agar + ONB, Kenknights and Munnaiers (K&M), Malt Extract Agar(MEA), Malt Extract Agar(MEA) + Optimised Nutrient Broth(ONB),Oat Meal Agar(OMA), Rice Extract Agar(REA),Rice Extract Agar + Optimised Nutrient Broth,Rose Bengal Agar (RBA)were used to identify their effect on *Cordyceps militaris* mycelial growth. Themycelium radial growth was recorded at the 7th, 14th and 21st day. The readings were pooled data of two trials to get precise results**(Table 1)**. The results showed mycelial radial growth/radial diameter of *Cordyceps militaris*on 21st day was ranked highest on Kenknight and Munnaiers media with 8.22 cm ± 0.02, the second highest radial growth was obtained in rice extract agar with 7.74 cm±0.13 and for the third highest radial growth for Agar+ Nutrient Broth at 6.96 cm ± 0.21**(Graph 1)**.For the mycelial growth per day was calculated with dividing the growth with number of days. The highest growth rate/day on 21st dayforKenknight and Munnaiersmedium at 0.39 cm±0.00 cm, second highest was of Rice Extract Agar at 0.37 cm±0.01 and third highest was of Agar and Nutrient Broth at 0.33 cm±0.1**(Graph 2)**. It was also observed that the growth rate for the Kenknight and Munnaiers was highest on the 14th day 0.43cm ±0.01in comparison to 7th 0.40cm±0.01 and 21st day 0.39cm±0.0.The results derived from the experimentation on submerged liquid media in static condition provided highest fresh weight and dry weight in Rice extract medium (Fw=4.04gm±0.11 and Dw=0.472gm±0.009) the second highest in the malt extract media (Fw=1.24±0.07 and Dw=0.16±0.008) while the least fresh weight and dry weight was obtained in Kenknight and Munnaires media (Fw=0.78±0.02 and Dw=0.065±0.001)**(Table 2; Graph 3A)**. Moisture content percentage was highest in KenknightMunnaiers (91.7%), second highest in Rice Extract Media (88.34%) and third highest in Malt extract media (88.84%)**(Graph 3B)**. In comparison to the solid media the static liquid media did not gave the similar result we can justify this as the media was submerged having less movement.

For the mycelial density Agar was the lowest (1/+), Kenknight and Munnaier was at moderate (2/++). Malt extract Agar, Oat meal agar, Rice extract Agar, Rice extract Agar + Optimized Nutrient Broth and Rose Bengal agar were abundant (3/+++) in mycelial density. Chitosan MEA, Chitosan + Agar +ONB and MEA+ONB were very abundant (4/++++) in mycelial density.

The pH of a medium plays a vital role in the growth of the biomass and it also play an important role in production of different metabolites. Metabolic activity of the cells varies with the change in pH value of the media. It should also be taken into consideration that the supportive pH value varies from media to media for the specific strain of mushroom (Kaur *et al.* 2023). Also, it should be noted that there is no existences of systematic relation between growth of the biomass and bioactive metabolite production (Dudekula et. al 2020, Elisashvili 2012).The best performing medium (i.e., K&M, REA,

MEA) on the basis of radial growth was chosen for the pH value analysis (pH 4, pH 5, pH 6, pH 7, pH 8, pH 9)**(Table 3; Graph 4 and 5)**. Here the control Kenknight and Munnaiers media was taken as control with pH of 6.87 as given by the manufacturer. The highest growth was noted at pH 5, pH 6 and pH 7. This provides an indication that the media is supportive to mycelium in the pH range 5-7.

The limited availability and high global market demand of this medicinal mushroom has led to its extremely high market price. Overall, exploitation by the local hunters for gathering of this medicinal mushroom causes a direct negative impact on the Himalayan biodiversity. To overcome all this to some extent we suggest much more should be done/ inclination is required towards the growth and development of *Cordyceps militaris* where it can be cultivated artificially to compete with the global market demand of *Cordycepssinensis.*To meet the market demand against *Cordyceps sinensis,* the artificially grown *Cordyceps militaris* can be cultivated which have similar compounds as *Cordyceps sinensis*. This may eventually reduce pressure on biodiversity. Further, over-exploitation of *Cordyceps sinensis* by the local gatherers causes soil erosion which initially starts with sheet erosion turning into rill erosion because of heavy rain-fall and gets converted into gully erosion. Further, widespread of this causes soil depletion by surface water flow finally leading to the formation of channels in the meadows.

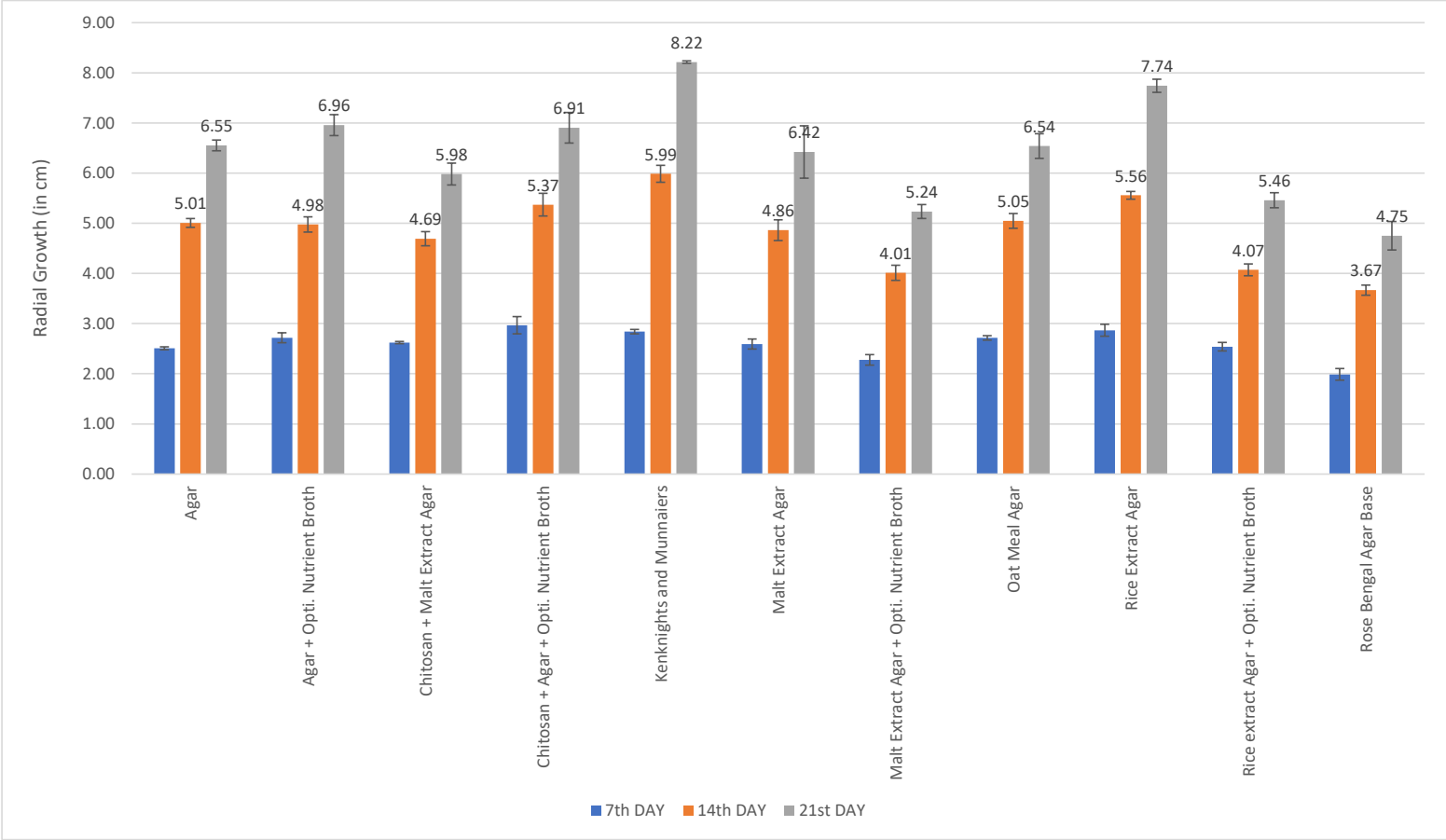
4. Conclusion

This paper has shown in-comparison to Malt Extract Agar, mycelial growth of *Cordyceps militaris* was increased on Kenknights and Munnaiers media by 23.25% on the fourteenth day and 28.03% on the twenty-first day. The comparison of average radial growth rate and mycelial growth rate per day of *Cordyceps militaris* mycelium shows Kenknights and Munnaiers media is faster in comparison to Malt Extract Agar. Overall, achieving the fastest mycelium run rate in comparison to current cultures used in cultivation of *Cordyceps militaris* may improve production outputs.

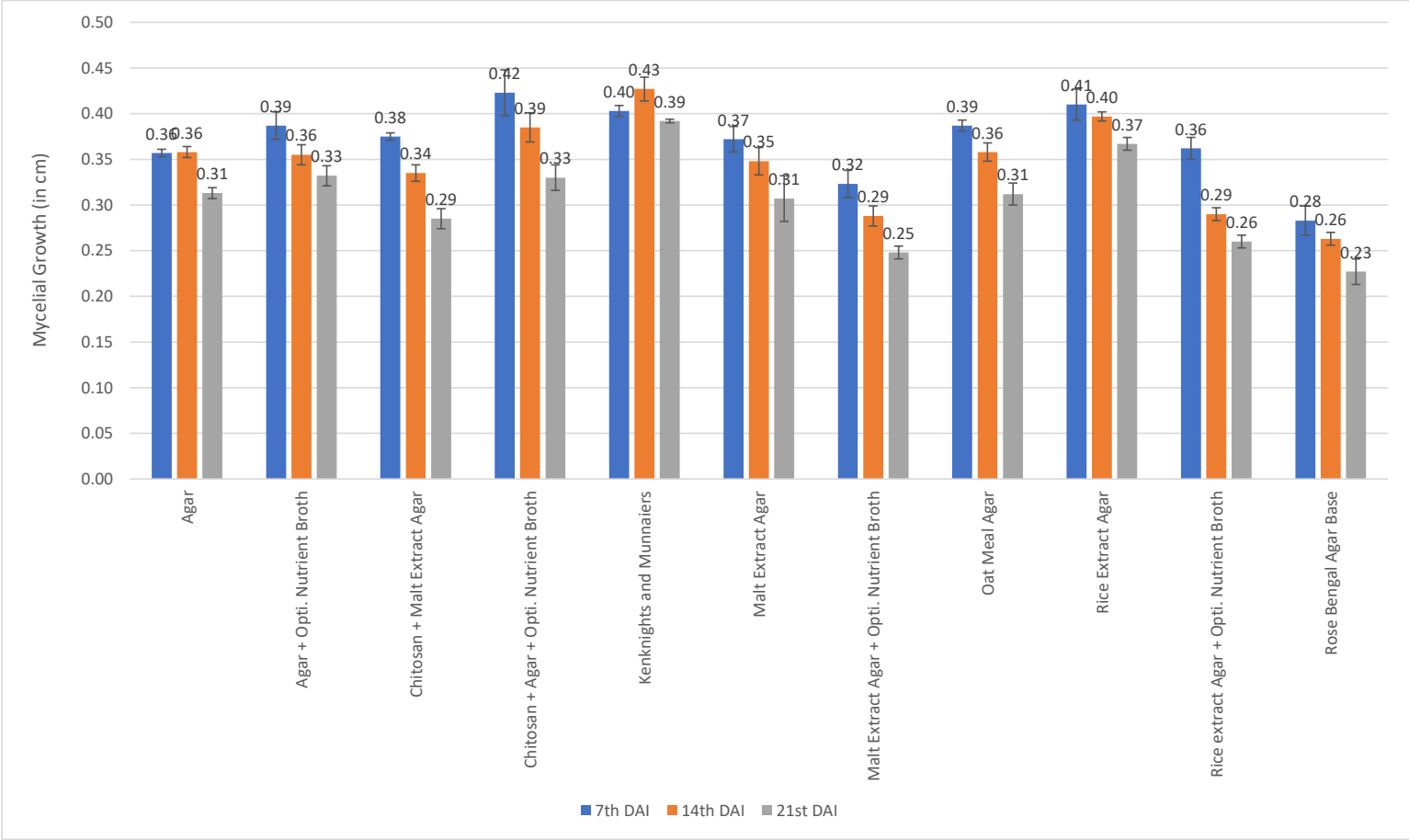
Treatment	Radial growth of Mycelium on						Average Mycelial Growth Rate per Day for					
	7th DAY		14th DAY		21st DAY		7th DAI		14th DAI		21st DAI	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Agar	2.51	0.03	5.01	0.09	6.55	0.11	0.36	0.00	0.36	0.01	0.31	0.01
Agar + Optimised Nutrient Broth (ONB)	2.72	0.10	4.98	0.15	6.96	0.21	0.39	0.02	0.36	0.01	0.33	0.01
Chitosan + Agar + ONB	2.97	0.17	5.37	0.23	6.91	0.31	0.42	0.03	0.39	0.02	0.33	0.01
Malt Extract Agar	2.59	0.10	4.86	0.21	6.42	0.52	0.37	0.01	0.35	0.02	0.31	0.03
Malt Extract Agar + ONB	2.28	0.11	4.01	0.15	5.24	0.14	0.32	0.02	0.29	0.01	0.25	0.01
Chitosan + Malt Extract Agar	2.62	0.02	4.69	0.14	5.98	0.22	0.38	0.00	0.34	0.01	0.29	0.01
Rice Extract Agar	2.87	0.12	5.56	0.08	7.74	0.13	0.41	0.02	0.40	0.01	0.37	0.01
Rice extract Agar + ONB	2.54	0.09	4.07	0.12	5.46	0.15	0.36	0.01	0.29	0.01	0.26	0.01
Oat Meal Agar	2.72	0.04	5.05	0.15	6.54	0.25	0.39	0.01	0.36	0.01	0.31	0.01
Rose Bengal Agar Base	1.99	0.12	3.67	0.10	4.75	0.28	0.28	0.02	0.26	0.01	0.23	0.01
Kenknights and Munnaiers	2.84	0.04	5.99	0.17	8.22	0.02	0.40	0.01	0.43	0.01	0.39	0.00
C.D.	0.27		0.43		0.70		0.04		0.03		0.03	
SE(m)	0.1		0.15		0.25		0.01		0.01		0.01	
SE(d)	0.14		0.21		0.35		0.02		0.02		0.02	
C.V.	8.99		7.59		9.38		9.11		7.48		9.44	

Each value expressed as mean and SE where n=4.Days After Inoculation (DAI)

Table 1:Pooled Average Radial Growth and average mycelial growth rate per day of *Cordyceps militaris* on different synthetic media at 7th, 14th and 21st day for two trials.



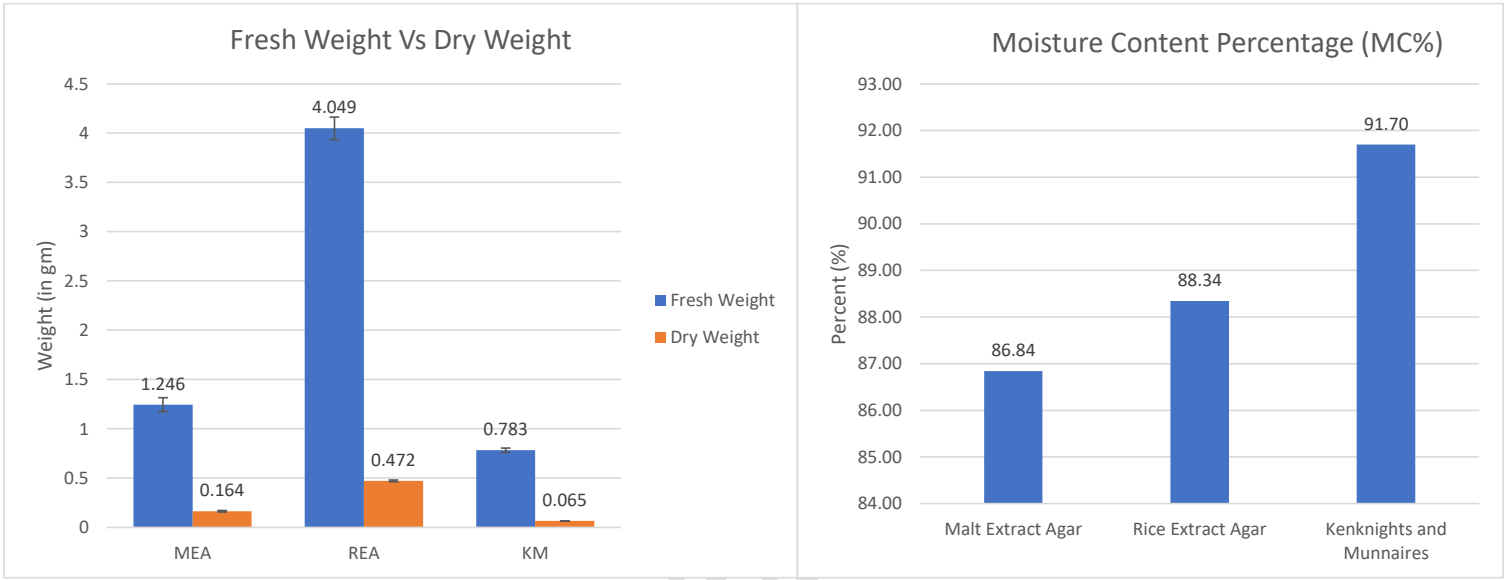
Graph 1: Average radial growth rate of mycelium of *Cordyceps militaris* on different synthetic media at 7th, 14th and 21st day.



Graph 2: Average mycelial growth rate per day on different synthetic media upto 7th,14th and 21st days after inoculation (DAI).

Media	Mean Fresh Weight	S. E	Mean Dry Weight	S. E	MoistureConcentration (MC%)
Malt Extract Agar	1.246	0.07	0.164	0.008	86.84
Rice Extract Agar	4.049	0.11	0.472	0.009	88.34
Kenknights and Munnaires	0.783	0.02	0.065	0.001	91.70

Table 2: Fresh Weight, Dry weight and Moisture Content (in %) of mycelium of *Cordyceps militaris*harvested from different liquid media. (MEA, REA and K&M)

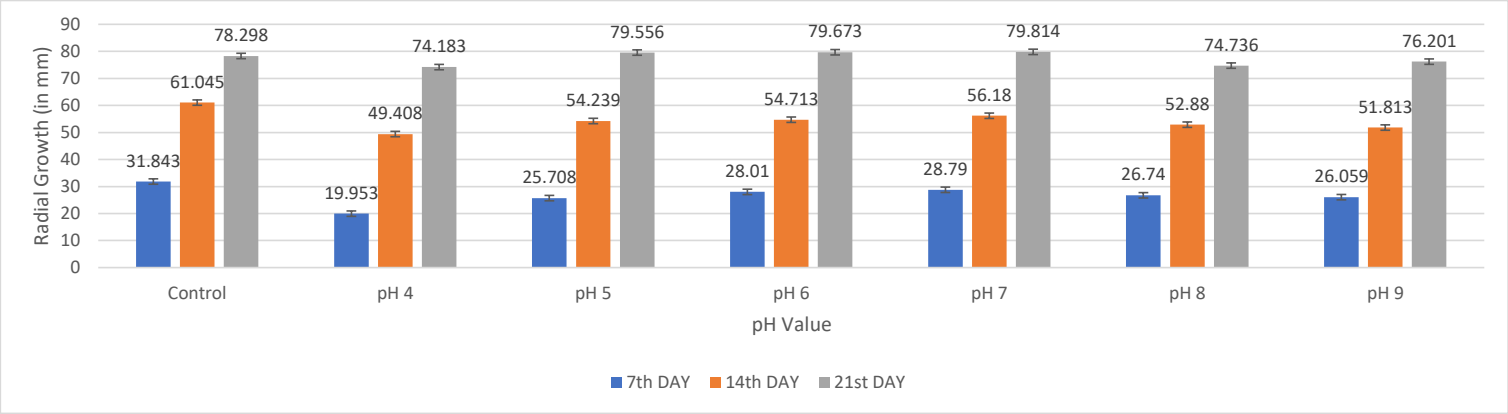


(A) **(B)**

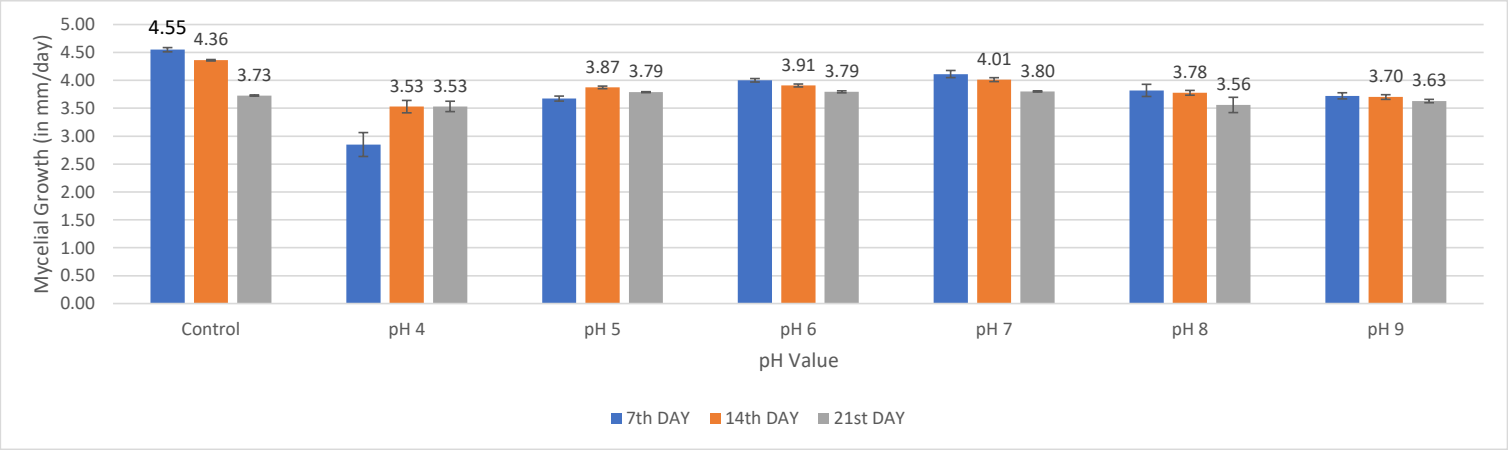
Graph 3: (A) Fresh Weight and Dry weight; (B) Moisture Content (in %) of mycelium of *Cordyceps militaris*harvested from different liquid media. (MEA, REA and K&M)

Treatment	Radial growth of Mycelium on						Average Mycelial Growth Rate per Day for					
	7th DAY		14th DAY		21st DAY		7th DAI		14th DAI		21st DAI	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Control (pH6.87)	31.84	0.27	61.05	0.19	78.30	0.23	4.55	0.04	4.36	0.01	3.73	0.01
pH 4	19.95	1.50	49.41	1.56	74.18	1.96	2.85	0.21	3.53	0.11	3.53	0.09
pH 5	25.71	0.31	54.24	0.33	79.56	0.18	3.67	0.05	3.87	0.02	3.79	0.01
pH 6	28.01	0.22	54.71	0.37	79.67	0.38	4.00	0.03	3.91	0.03	3.79	0.02
pH 7	28.79	0.45	56.18	0.49	79.81	0.23	4.11	0.06	4.01	0.04	3.80	0.01
pH 8	26.74	0.77	52.88	0.60	74.74	2.87	3.82	0.11	3.78	0.04	3.56	0.14
pH 9	26.06	0.39	51.81	0.58	76.20	0.64	3.72	0.06	3.70	0.04	3.63	0.03
C.D.	2.23		2.33		4.36		0.30		0.15		0.19	
SE(m)	0.74		0.78		1.46		0.10		0.05		0.06	
SE(d)	1.05		1.10		2.06		0.14		0.07		0.09	
C.V.	5.75		2.92		3.76		5.21		2.66		3.48	

Table 3:Average Radial Growth and average mycelial growth rate per day of *Cordyceps militaris* obtained over different pH value on K&M media at 7th, 14th and 21st day.



Graph 4: Radial growth of Mycelium on K&M over different pH.

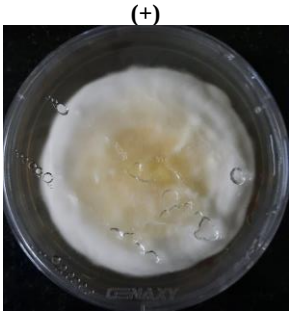


Graph 5:Mycelial growth per day on K&M over different pH.

Agar
(+)

Agar + ONB
(++++)

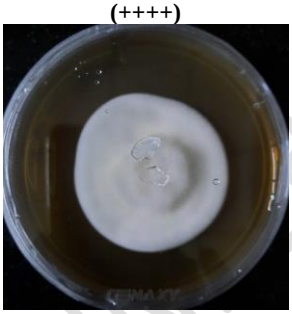
Chitosan + MEA
(++++)



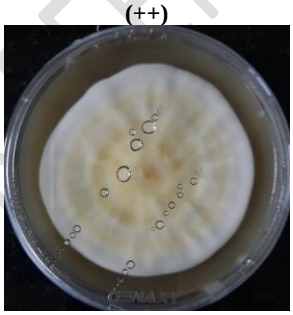
Chitosan +Agar+ ONB
(++++)

Kenknights and Munnaiers
(++)

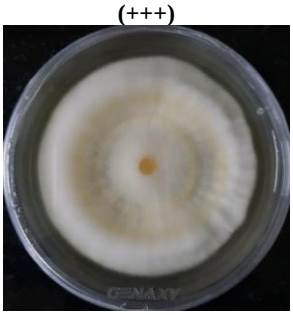
Malt Extract Agar
(+++)



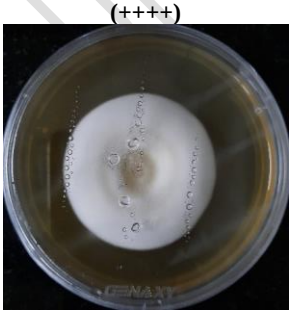
MEA + ONB
(++++)



Oat Meal Agar
(+++)



Rice Extract Agar
(+++)



REA + ONB
(+++)

Rose Bengal Agar
(+++)

Fig. 1: Mycelial density
+= Low
++= Moderate
+++= Abundant
++++=Very Abundant

STATEMENTS OF DECLARATION:

Consent
Not Applicable

Ethical approval

Not Applicable

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