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Growth and Development of Plant Pathogenic Fungi in Define Media

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ABSTRACT

The fungi showed variation in their growth and development when grown on various nutrient media. This study examines the growth and developments of seven different fungi in define media. The fungi growth was obtained by daily measurement of fresh and dry weights with changes in pH. Two fungi Fusarium sp. and Antrodia sitchensis having slow growth rate in compare to rest of the five and preferred neutral to slightly alkaline condition for their growth. Rest of the five, Aspergillus niger, Curvularia intermedia and three of Macrophomina phaseolina isolates (a, b, c) were found to grow better in slightly acidic condition. Initial pH of the medium changed considerably during the growth of the fungi. The role of pH change in the growth and development of the fungi is discussed.

Keywords: Define media; fungi; growth and development; pH change.

INTRODUCTION

Fungi form a large and heterogeneous eukaryotic group of living organisms characterized by their lack of photosynthetic pigment and their chitinous cell wall [1]. Fungal kingdom contains more than 1.5 million species, but only around 100,000 have so far been described, with yeast, mold, and mushroom being the most familiar [2,3]. Although the majority of fungal species are saprophytes, a number of them are parasitics, in order to complete their biological cycle, animals or plants, with around 15,000 of them causing disease in plants, the majority belonging to the Ascomycetes and Basidiomycetes [4, 5].

Fungal physiology refers to the nutrition, metabolism, growth, reproduction and death of fungal cells. It also generally relates to interaction of fungi with their biotic and abiotic environment, including cellular responses to stress. The physiology of fungal cells impacts significantly on the environment, industry and human health. Fungal metabolism is also responsible for detoxification of organic pollutants and for bioremediation of heavy metals in the environment [6]. The production of many economically important industrial commodities relies on exploitation of fungal metabolism and these include such diverse products as whole foods, food additives, fermented beverages, pharmaceuticals [7], pigments [8], Alkaloids [9], biofuels [10], industrial antibiotics, enzymes [11], vitamins, organic and fatty acids [12, 13] and sterols [14].

The fungus showed variation in growth rate when grown on various nutrient media. In the present study an attempt was made to evaluate seven fungi isolated from different infected host for their growth and development in define media. *Aspergillus niger, Fusarium sp., Antrodia sitchensis, Curvularia intermedia* and three representative isolates

of *Macrophomina phaseolina* (a-Castor, b-Mango, c-Rose) isolated from different infected host were used in this study.

MATERIALS AND METHODS

Isolation of Pathogens

Infected plant materials were taken and put under running tap water for 1-2 h in jar. Then it was washed with 0.1% $HgCl_2$ for 10 min for surface sterilization. The sample was washed with sterilized distilled water 3 to 4 times for removal of $HgCl_2$; with the help of pre-sterilized scalpel and forceps infected part of plant material was cut. The cutting part was inoculated on Potato Dextrose Agar (PDA) plate in aseptic condition and incubated at 28±2 ºC for 24-48 h for growth of pathogens. After their adequate growth, isolation for pure culture was carried out. Pure culture of pathogen(s) was incubated at 28±2 ºC for seven days. Then with the help of (1cm diameter) borer fungal disc were transfer on other PDA plate for radial growth to obtain the same growth pattern. Further this pure culture was preserved for experimentation.

Culture preservation and maintenance

All the cultures were preserved on Potato Dextrose Agar (PDA) slant at 4º C as well as in Distilled water at room temperature. When needed they were grown on PDA plate at 28±2 ºC for seven days and the growing hyphae were used for experimentation.

Preparation of Inoculums

First the fungi were revived from old culture on PDA plates incubated for seven days at 28 ± 2°C. Then with the cup borer (1cm diameter) disc was cut from the peripheral region of the plate and transfer to another plate for radial growth. Seven days old culture was further used for experimentation a disc (1cm diameter) of fungal hyphae was used as inoculum.

Media and culture condition

A define media Murashige and Skoog (MS) (1962) [15] with 2% sucrose supplement was prepared and 50 ml of it filled in each of the 250 ml conical flask. The media was autoclaved for 15 min and allowed to cool to room temperature. The initial pH of the medium was 5.6 ± 0.01. The inoculum was added to each flask and maintained in a stationary condition at 28 ± 2°C for their growth and development. For growth analysis fresh weight dry weight and pH were measured with ±SD, at every 24 h intervals. For each experimental data, three replicates were taken.

RESULTS AND DISCUSSION

Predictive microbiology studies the behavior of micro-organisms under different physico-chemical conditions such as temperature, water activity, pH. It can help the identification of critical points of production and distribution process and optimization of production and distribution chains [16]. Predictive modeling has been extensively used mainly to predict bacterial growth as a function of environmental factors such as temperature, pH and activity of water [17-19]. However, model development of fungal growth has not received the same level of attention as that of bacterial growth [20, 21]. A few studies concerning fungal growth have dealt with the predictive modeling approach [22-25].

In this experiment, seven different fungal isolates were studied for growth in the terms of fresh and dry weights. The growth patterns of these fungi are divided into (i) hyphae initiation phase or lag phase (ii) rapid growth of mycelia or log phase and (iii) declined phase (Fig. 1-7). From seven different fungi studied in this experiment, accumulation of fresh weight in *Aspergillus niger* showed lag phase up to 24 h, the log phase from 24-72 h and declined thereafter. Accumulation in dry weight showed increasing treand up to 72 h and stabilized in later phase (Fig. 1a). Maximum fresh weight (2.32 ± 0.30 g) and dry weight (27 ± 0.02 mg) accumulation was recorded at 72 h. From the initial 5.6 pH, it falls to 2.3 on the second day (48 h), gradually it was decreased and observed 1.6 at seventh day (168 h) (Fig. 1b). *A. niger* is known for its strong acidification of culture media [26-28]. In this study, it is also recorded that pH falls drastically from 48 h onwards in case of *A. niger*. Besides drastic changes in pH, the accumulation of secondary metabolites was also considered being responsible for the inhibition of fungal growth [29, 30]. *A. niger* is known to contaminate dough, processed food, canned food and bakery products [31]. This fungus is one of the most resistant fungi and can grow even in low temperature and at pH 2.5 [32].

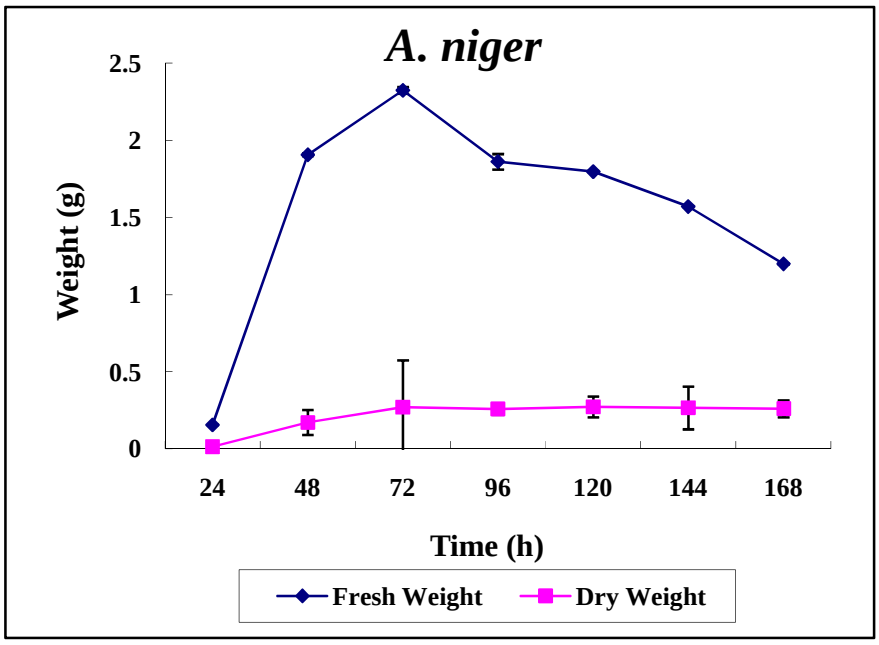

Fig. 1a Changes in fresh weight and dry weight of *A. niger* with age maturation.

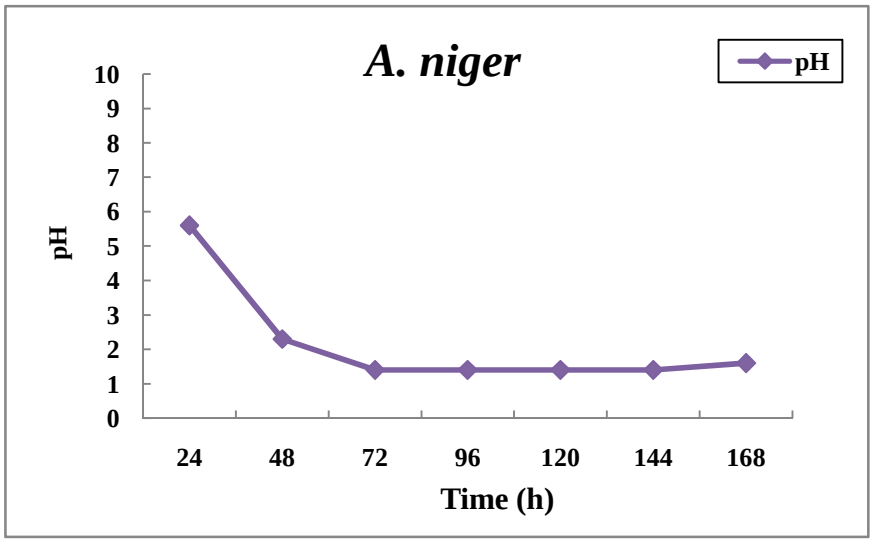

Fig. 1b Changes in pH in *A. niger* during growth period.

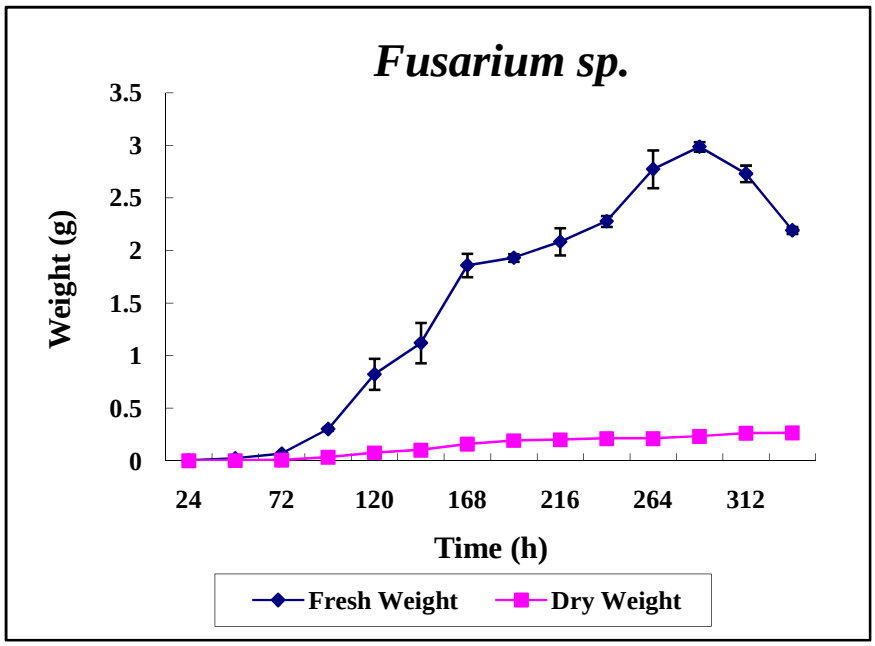

Fig. 2a Changes in fresh weight and dry weight of *Fusarium sp.* with age maturation.

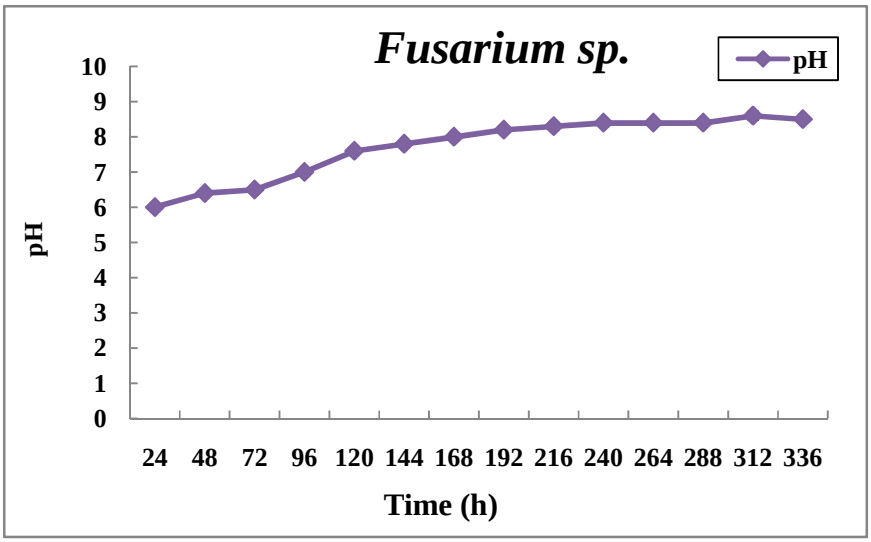

Fig. 2b Changes in pH in *Fusarium sp.* during growth period.

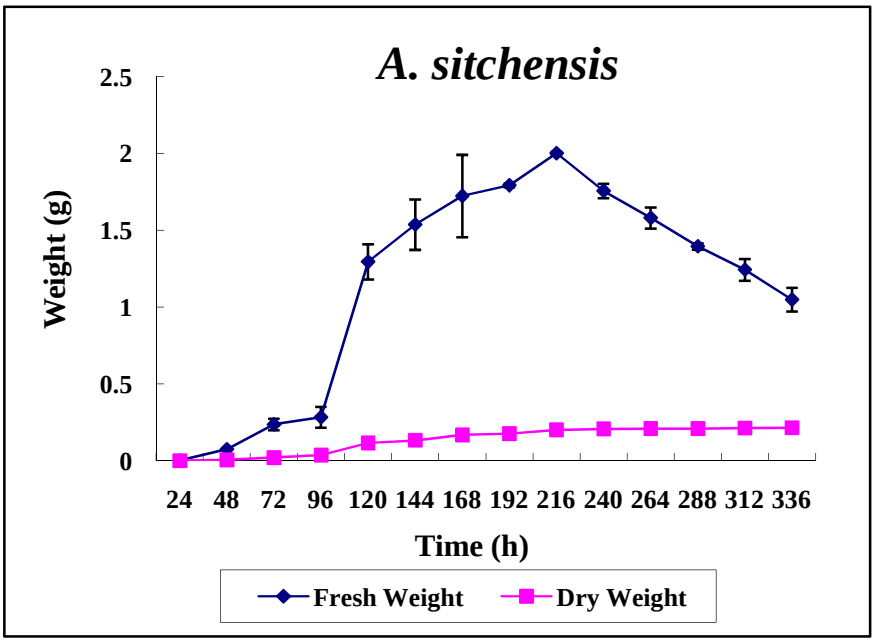

Fig. 3a Changes in fresh weight and dry weight of *A. sitchensis* with age maturation.

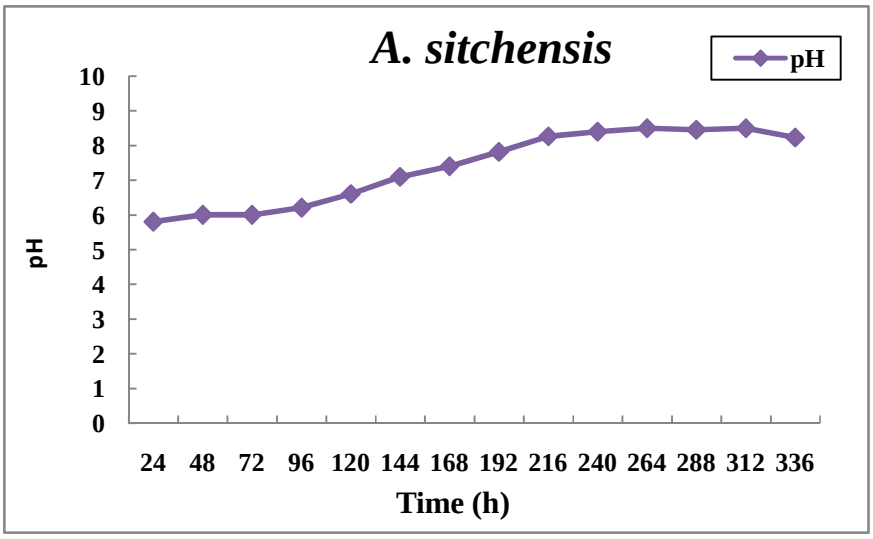

Fig. 3b Changes in pH in *A. sitchensis* during growth period.

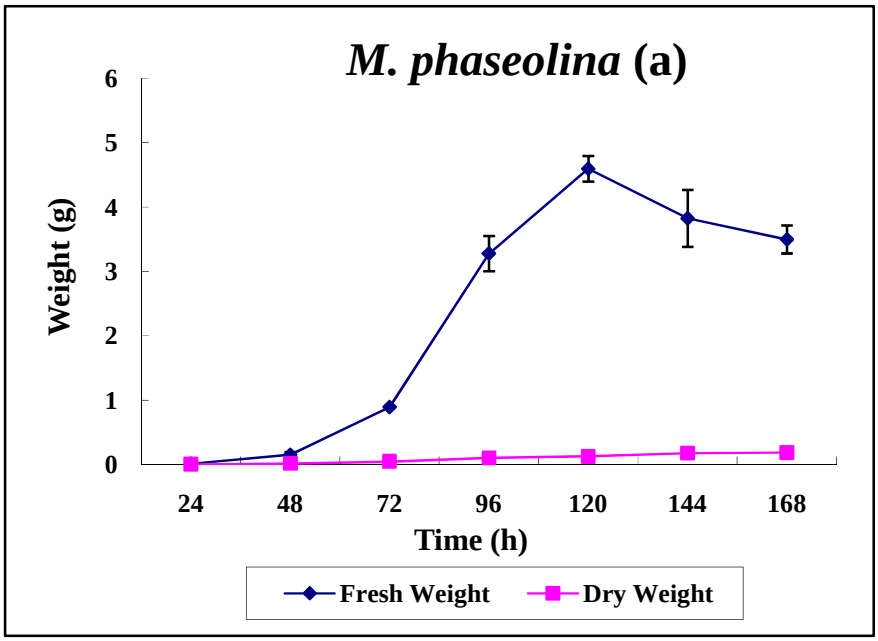

Fig. 4a Changes in fresh weight and dry weight of *M. phaseolina* (a) with age maturation.

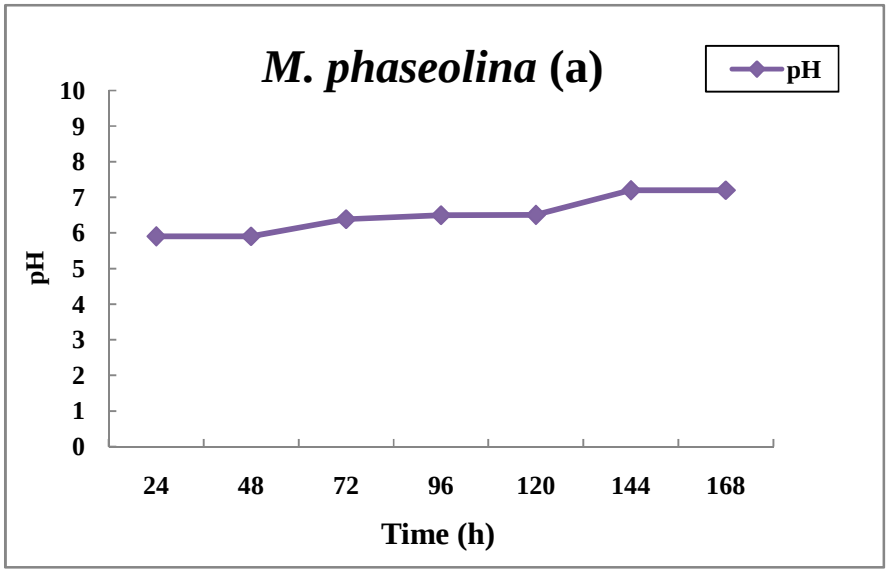

Fig. 4b Changes in pH in *M. phaseolina* (a) during growth period.

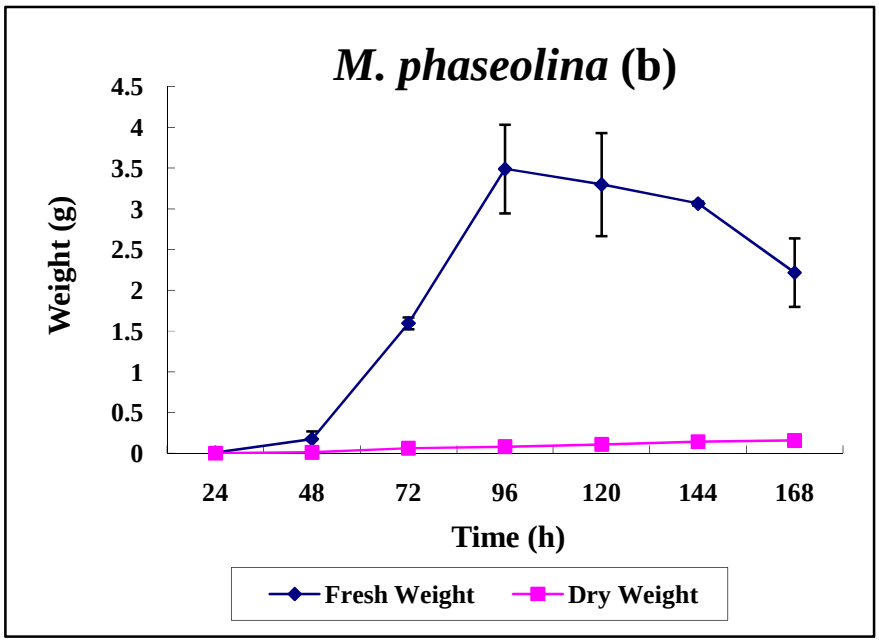

Fig. 5a Changes in fresh weight and dry weight of *M. phaseolina* (b) with age maturation.

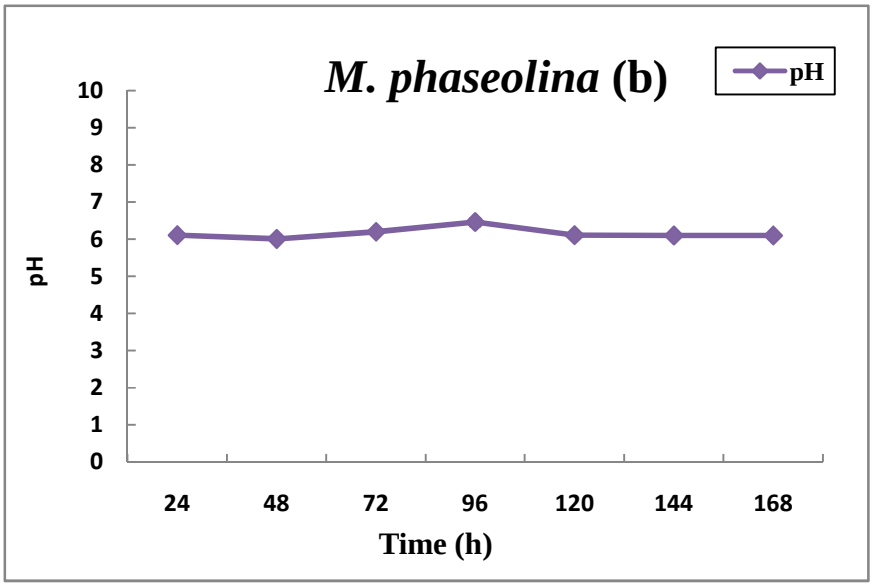

Fig. 5b Changes in pH in *M. phaseolina* (b) during growth period.

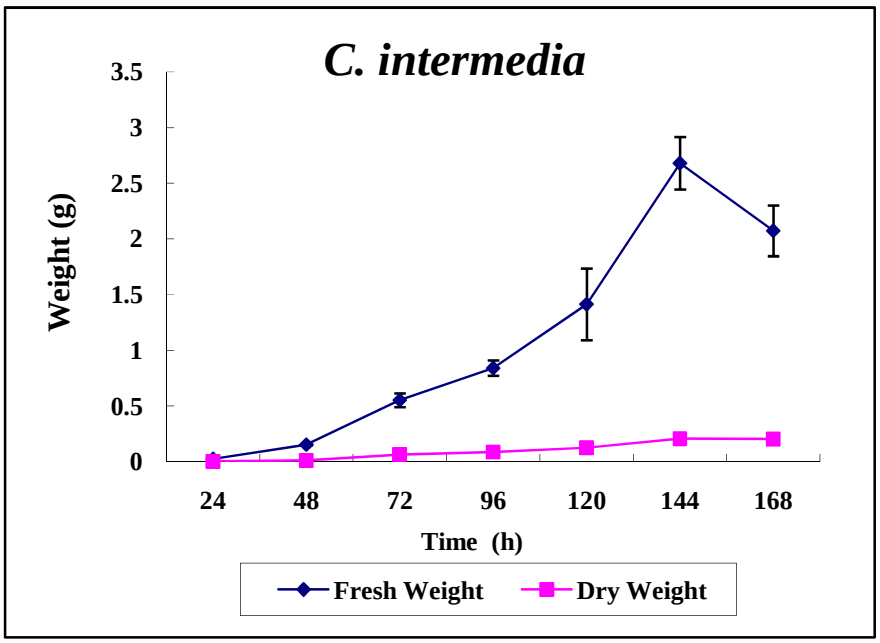

Fig. 6a Changes in fresh weight and dry weight of *C.intermedia* with age maturation.

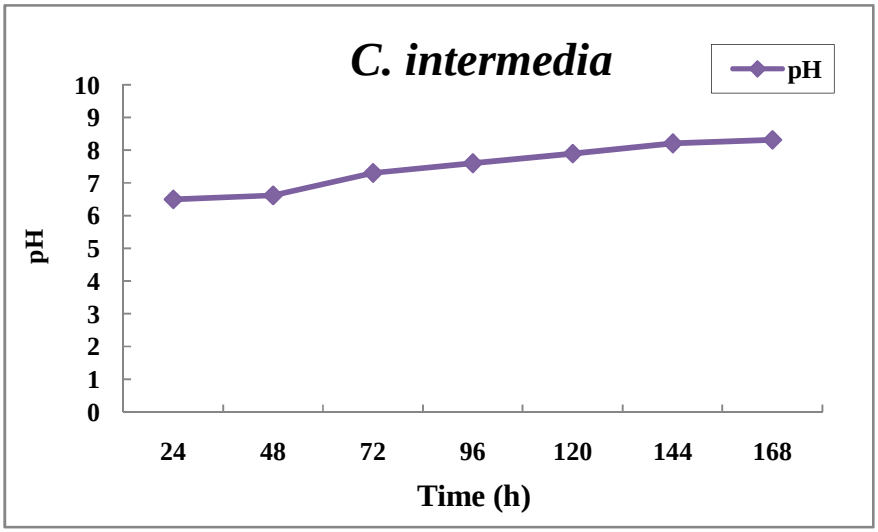

Fig. 6b Changes in pH in *C. intermedia* during growth period.

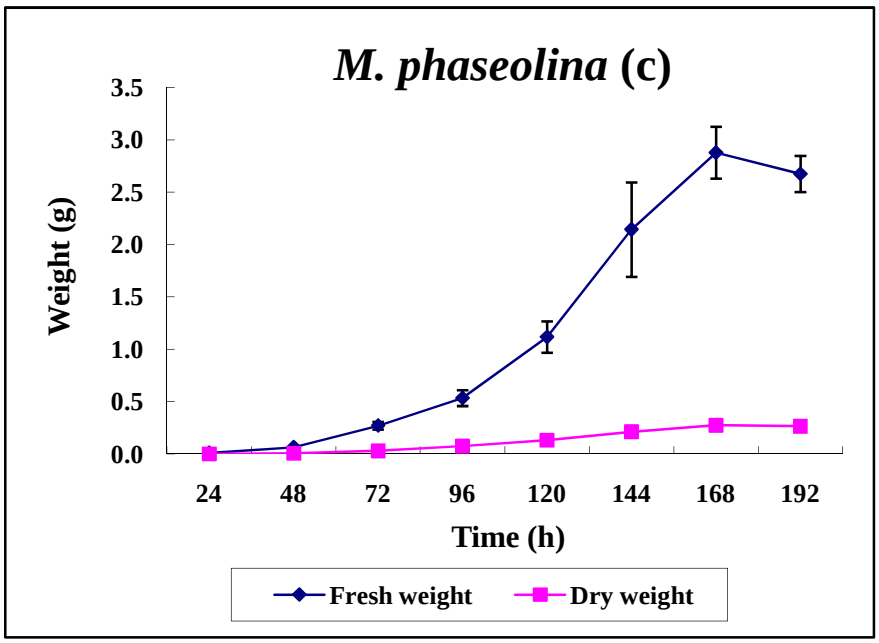

Fig. 7a Changes in fresh weight and dry weight of *M. phaseolina* (c) with age maturation.

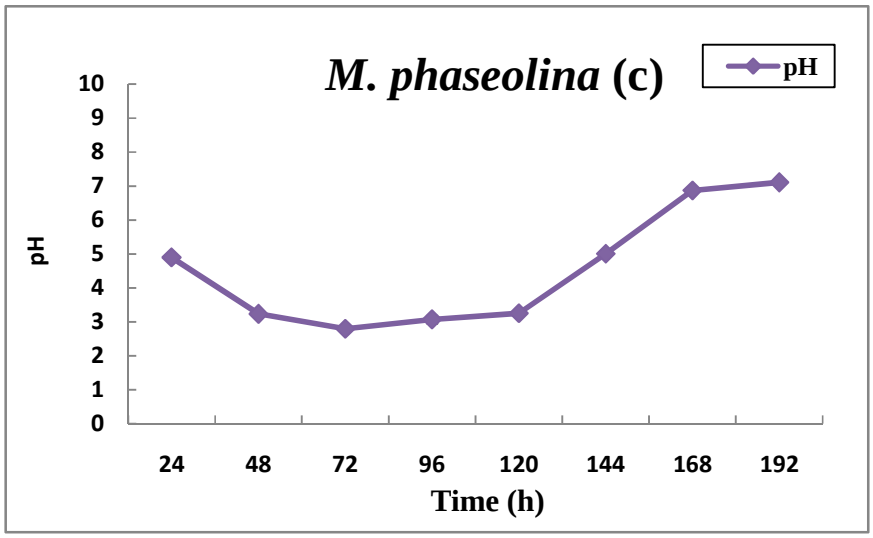

Fig. 7b Changes in pH in *M. phaseolina* (c) during growth period.

On the other hand, growth rate of *Fusarium sp*. and *Antrodia sitchensis* was found slower amongst the all fungi studied (Fig. 2a, 3a). Both the fungi showed lag phase up to 96 h, the log phase was observed from 96 h onwards up to 288 h in *Fusarium sp.* and in case of *A. sitchensis* it was noted from 120-216 h and started declining thereafter. Maximum fresh weight in the log phase was measured as 2.98 ± 0.04 g and 2.00 ± 0.01 g in *Fusarium sp.* and *A. sitchensis*, respectively. The pH of the medium in both the isolates was gradually increased ranging from 5.8-8.5 (Fig. 2b, 3b). Dry weight was also gradually increased in *Fusarium sp.* (2-266 mg) and *A. sitchensis* (1-214 mg). Overall duration of growth and development was higher i.e. double (14 days) as compared to other fungi studied.

Variations in the different isolates of *Fusarium sp.*, was recorded *in vitro* against different cultural and physiological parameters [33, 34]. Agarwal and Sarbhoy [35] reported acidic pH favoring growth of all *Fusarium sp., F. oxysporum and F. solani* and grew best at pH 4.5 and 6.0 while *F. graminearum* and *F. equiseti* at pH 3.5 and 6.5, respectively. Farkya et al. [36] observed maximum growth and sporulation of *F. solani* at 5.5 pH. Supported by Gupta and co-worker [37], pH significantly differentiates the mycelia growth and maximum growth was recorded at pH 5.5, but this was in contrast to our finding, the maximum growth was reported in alkaline pH (8.3±0.01). Their growth was slow in initial stage but as pH become neutral they grow well from 135 h onwards (Fig. 2a).

In this experiment *Macrophomina phaseolina* were isolated from three different host plants. *Macrophomina phaseolina* (a) (isolated from Castor) showed lag phase till the 48 h followed by log phase up to 120 h then declined gradually (Fig. 4a). Maximum fresh weight was recorded 4.5 ± 0.1 g at 120 h. However, a gradual increased in dry weight (1-181 mg) was recorded though out the growth period. The changes in pH ranged from 5.9-7.2 (Fig. 4b). Somewhat similar pattern of growth was also observed in *Macrophomina phaseolina* (b) isolated from the other host (Mango), lag phase was parallel to *M. phaseolina* (a) and in comparison to that log phase was up to 96 h (Fig. 5a). Maximum fresh weight was recorded 3.49 ± 0.5 g followed by decline and the dry weight was continuously increased (1-155 mg) till maturation. The pH of media increased up to 96 h from 6.1-6.4 after that it was steady at 6.1(Fig. 5b). On the other hand, *Macrophomina phaseolina* (c) (isolated from rose) showed lag phase same as the above isolates up till 48 h subsequently followed by log phase up to 168 h then declined (Fig. 7a). The maximum fresh weight was measured 2.87 ± 0.2 g. The pH falls to 2.8 at 72 h then slowly turns to neutral at 192 h (7.1±0.01). The changes in dry weight measured from 1-274 mg. In this the three isolates of same species (*Macrophomina phaseolina*) showed slight variation in their growth. In case of *M. phaseolina* (a, c) the pH turns to neutral at the end of growth phases. But in all the isolate of *M. phaseolina* it was observed that it grew best in pH range 6.4-6.8 (Fig. 4b, 5b, 7b).

In support to this finding, Uppal et al. [38] reported variation in growth of *M. phaseolina* that can tolerate wide range of pH, while the optimum range lies between pH 3.4 and 6.4. The growth of *M. phaseolina* (causal agent of root rot of mulberry) maximum at pH 7.0 but showed marked difference with pH change [39].

Curvularia intermedia showed lag phase of two days (48 h) followed by log phase, gradually increased in fresh weight up to 144 h (Fig. 6a) and declined thereafter. Maximum fresh weight was recorded 2.67 ± 0.2 g and dry weight ranged from 2-207 mg. The pH was increased from 6.5-8.3 at end of growth (Fig. 6b). In this pH range the optimal pH obtained herein study agrees with Sonia et al. [40] who reported *C. pallescens* grow well at pH 6.0.

Growth may be profoundly affected by a number of physical factors like temperature, pH, light, aeration, pressure etc. The pH range (between minimum and maximum values) is greater in fungi than it is in bacteria. Most microorganisms grow best around neutrality (pH-7) and on the other hand fungi, generally prefer slightly acidic conditions for their growth [41], but some species favor neutral to slightly alkaline conditions [42]. Present study revealed that in this define media among the seven fungi, five (*A. niger, C. intermedia* and *M. phaseolina* (a,b,c)) were grew well in slightly acidic condition and their growth was completed all most in 189 h. In case of remaining two, *Fusarium sp*. and *A. sitchensis*, the growth period was double in compared to the rest of the fungi; they grow well in neutral to slightly alkaline condition. On the basis of this study it can be concluded that media and pH have a great impact on growth of fungi.

The metabolic versatility of fungi is exploited by the fermentation industry, to make antibiotics and other high value substances of interest to medicine, agriculture and the chemical industry, to produce enzymes and to carry out specific steps in chemical processes. This study may help to the fermentation industries in production of various metabolites by understanding growth and development of fungi, in define media.

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