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Physiological study of *Cladosporium carphophilum* causing scab in almond

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Abstract
In order to determine the best solid medium for growth and sporulation of *C.carpophilum*, the fungus was grown on five different media viz., corn meal agar, Czapek's dox agar, oat meal agar, potato dextrose agar, and Richard's synthetic agar. It indicates that the fungus *C. carpophilum* grows best on potato dextrose agar medium with a maximum colony diameter of 19.50 mm followed by oat meal agar (18.75 mm) and best sporulation on potato dextrose agar (1.15×10^6/ml) The fungus under study was able to grow on wide range of temperature. However, maximum colony diameter of 20.0 mm was observed at 25ºC which was statistically at par with colony diameter of 19.50mm and 18.25mm observed at 20 and 30ºC respectively and best sporulation was produced at 25ºC (1.20×10^6/ml). The fungus under study grew throughout the pH range of 4.5 to 8.5. However, maximum colony diameter of 19.25 mm and best sporulation of 1.18×10^6/ml was recorded at pH 5.5.

Keywords: physiology, cladosporium, carpophilum, scab, almond

Introduction
During the last few years, the almond orchadists of the Kashmir Valley have been facing scab like serious disease problem on almond plant. The disease affects fruits and lead to premature leaf fall resulting in low productivity and poor fruit quality. Later this disease was identified as scab of almond caused by *Cladosporium carphophilum* and reported first time in India in 2017 (Kacho *et al* 2017) [7]. The disease has been reported to be causead by *C. carpophilum* Thum. and occurs in other parts of the world (Pammel, 1892; Anderson,1956) [1]. Since it is a new disease to India, the present study was carried out to know its physiology.

Material and Methods
To ascertain the best medium, optimum temperature and most favourable pH of the medium for the growth and sporulation of *C.carpophilum* following studies were undertaken as per the technique adopted by Gonzales (2013), in Division of Plant Pathology Labortary, SKUAST-K, Shalimar, during 2015-16.

Effect of media
Five different solid media viz., potato dextrose agar, corn meal agar, Czapek's dox agar, oat meal agar, and Richard's synthetic agar were evaluated with respect to their support for growth and sporulation of the test fungus. Each of the media to be evaluated were prepared as per their composition and sterilized in an autoclave at 15 lbs pressure per square inch for 15 minutes. Each test media (30 ml) was poured into sterilized petriplates under aseptic conditions. The culture discs (3 mm dia.) were cut with a sterilized cork borer from a 30 days old actively growing fungus culture and were aseptically transferred to a petriplates containing different test media. The inoculated plates were incubated at 25±1ºC. The experiment was laid out in a completely randomized design (CRD) having three replications for each treatment. The observations for colony diameter and sporulation were recorded after 30 days of incubation respectively. The sporulation was studied by homogenizing thoroughly 1 mm of mycelial mat in 1 ml of distilled water. The suspension, thus obtained was used for counting the number of spores with the help of haemocytometer.
The medium supporting the best growth as well as sporulation of the test fungus was selected as a basal medium for further studies.

Effect of temperature
Effect of temperature on the growth and sporulation of the fungus was studied on the medium, which supported best growth and sporulation (potato dextrose agar medium).

The medium was prepared by mixing the ingredients as mentioned 3.2. The petriplates each containing 20 ml of sterilized basal medium (PDA) were inoculated with a 3 mm culture disc of test fungus and then incubated at five different temperatures viz., 15, 20, 25,30 and 35 (±1ºC). Three replications of each treatment were maintained in complete randomized design (CRD). Methods adopted for recording observations on colony diameter and scale adopted for grading sporualtion were same as in effect of media.

Effect of pH

In order to determine the optimum pH level for better growth and sporulation of the test fungus, the pH of basal medium was adjusted at desired levels viz., 4.5, 5.5, 6.5, 7.5 and 8.5 with the help of pH meter using N/10 hydrochloric acid (HCl) and N/10 sodium hydroxide (NaOH) solutions. Inoculation and observation were made in similar way as in effect of media.

Result and Disscusion

Effect of media

In order to determined the best media for growth and sporulation of *C.carpophilum* the fungus was grown on five different solid media viz., potato dextrose agar, oat meal agar, corn meal agar, Czapek's dox agar and Richard's synthetic agar medium. The fungus *C. carpophilum* grows best on potato dextrose agar medium with a maximum colony diameter of 19.50 mm followed by oat meal agar 18.75 mm (Table. I).The results further indicate that the fungus gave best sporulation on potato dextrose agar (1.15×10^6/ml) followed by corn meal agar (1.10×10^6/ml) and oat meal agar (1.04×10^6/ml) and minimum sporulation was recorded on Richard's synthetic (0.80×10^6/ml) followed by Czapeck's dox agar (0.96×10^6/m as was also reported by Schweizer (1958) [13] for *C.carpophilum* and *C.cerasi* causing scab disease in peach and cherry respectively. Sahan (2003) and Kata (2004) [8], also observed that potato dextrose agar a preferred medium for growth and sporulation of *C.musae* responsible for leaf speckle in banana. Since carbon constitutes about half of dry weight of the fungus and is not only the main structural element but a chief source of energy as well (Cochrane, 1958) [3]. The fungus also sporulated best on corn meal agar and oat meal agar medium. These findings are in agreement with the observations made by Hall (1984) [6], who obtained maximum sporulation of *C.allii-cepae* on corn meal agar. Since corn meal and oat meal agar have less easily degestable carbohydrates, most of the dematiaceous fungi sporulated better on corn meal agar and oat meal agar as observed by Charles (2012) [2].

Effect of temperature

Temperature not only effect the growth and sporulation of fungus but all the biological activities of the organism are influenced and the influence usually varying between and within different genera and species and among different strains and/or geographical isolates of the same species (Cochrane, 1958) [3]. In the present study, the fungus under study grew on a wide temperature range of 15-35±1ºC but maximum growth and sporulation was obtained at 25±1ºC followed by that at 20±1ºC (Table 2). These findings are in agreement with the observation of Lawrence and Zehr (1992) who also obtained maximum radial growth and sporulation of *C.carpophillum* at 25ºC. Similar observation was also noticed by Darell (1995) in *C.caryigenum* causing scab of pecan nut. Domigues *et al.* (2013) [4] while conducting physiological study of *Fusicladium eriobotryae*, causing scab of loquate, the best growth and sporulation was obtained at 20ºC.The fungus grew poorly at 35±1 which is in accordance with the finding of Mohammed *et al* (2015) [10] who reported that the fungus *C.herburum* did not grow at 35ºC.

Effect of pH

The hydrogen ion concentration of the substrate medium is known to control the degree of dissociation in inorganic ions in culture and hence their uptake into the fungal thallus, thus significantly influencing the fungal growth and proliferation. Sometimes complete inhibition of growth and sporulation may occure on media which are too acidic or too alkaline(Graffin1996). The fungus has been found to grow well within a pH range of 4.5-8.5 but the maximum growth and sporulation was achieved at pH 5.5 followed by pH 6.5 and 4.5 (Table3). The optimum pH range for growth and sporulation of the fungus was 4.5-6.5 indicating that the fungus preferred an acidic medium for growth and sporulation. The results are in close association with the finding of Rafal *et al.* (2012) [12] who also found good growth of *Cladosporium* sp. at pH range of 5-6.

Table 1: Effect of different culture media on growth and sporulation of *Cladosporium carpophilum* Thum. after 30 days of incubation at 25±1ºC.

Medium	*Average colony diameter (mm)	**Sporulation ($x10^6$/ml)
Potato dextrose agar	19.50 (4.42)[a]	1.15
Oat meal agar	18.75 (4.33)[a]	1.04
Corn meal agar	15.2 (3.90)[b]	1.10
Czapeks Dox agar	11.25 (3.35)[c]	0.96
Richards synthetic agar	8.75 (2.89)[d]	0.80
SEm±	0.113	–
CD (p=0.05)	0.375	–

Values in parenthesis are square root transformation
Values superscripted by same letter(s) are statistically identical
*Average of 04 replications
**Average of 10 observations

Table 2: Effect of different temperature regimes on growth and sporulation of *Cladosporium carpophilum* Thum. After 30 days of incubation on PDA.

Temperature ±1 (ºC)	*Average colony diameter (mm)	**Sporulation ($x10^6$/ml)
15	8.75 (2.96)[b]	1.01
20	19.50 (4.42)[a]	1.04
25	20.00 (4.47)[a]	1.20
30	18.25 (4.27)[a]	0.88

35	3.75 (1.94)[c]	0.64
SEm±	0.107	–
CD (p=0.05)	0.381	–

Values in parenthesis are square root transformation
Values superscripted by same letter(s) are statistically identical
*Average of 04 replications
**Average of 10 observations

Table 3: Effect of different temperature regimes on growth and sporulation of *Cladosporium carpophilum* Thum. After 30 days of incubation on PDA

Temperature ±1 (ºC)	*Average colony diameter (mm)	**Sporulation (x10^6/ml)
15	8.75 (2.96)[b]	1.01
20	19.50 (4.42)[a]	1.04
25	20.00 (4.47)[a]	1.20
30	18.25 (4.27)[a]	0.88
35	3.75 (1.94)[c]	0.64
SEm±	0.107	–
CD (p=0.05)	0.381	–

Values in parenthesis are square root transformation
Values superscripted by same letter(s) are statistically identical
*Average of 04 replications
**Average of 10 observations

Table 4: Effect of different pH levels on growth and sporulation of *Cladosporium carpophilum* Thum. after 30 days of incubation at 25±1 ºC on PDA

pH levels	*Average colony diameter (mm)	**Sporulation (x10^6/ml)
4.5	14.25 (3.77)[c]	0.86
5.5	19.25 (4.39)[a]	1.18
6.5	15.75 (3.97)[b]	1.12
7.5	11.25 (3.35)[d]	0.70
8.5	7.25 (2.69)[e]	0.30
SEm±	0.038	–
CD (p=0.05)	0.103	–

Values in parenthesis are square root transformation
Values superscripted by same letter(s) are statistically identical
*Average of 04 replications

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