**In vitro study on physiological growth and effect of different artificial inoculation methods on incubation period of *Alternaria porri* (Ellis) Cifferi causing purple blotch of onion**

**ABSTRACT**

*Alternaria porri* causing purple blotch of onion is major constrains in onion cultivation and causes a yield loss ranging from 2.5 to 87.8 per cent. The aim of this study was to find the effect of different inoculation methods on incubation period and to know the best solid media, temperature and pH for the mycelial growth of *Alternaria porri*. The maximum incubation period was observed for symptoms development in Soil inoculation (6.02 days) followed by Wound inoculation (4.93 days) and Syringe inoculation (4.47 days). The minimum incubation period was observed in Foliar spray inoculation (2.38 days). Among the solid media maximum radial growth of the fungus was recorded in PDA (89.97 mm) followed by Oat meal agar (89.67 mm) while minimum radial growth was observed in Corn meal agar media (53.82 mm). The result of pH indicated that pH 5 have maximum mycelial growth (87.0 mm) followed by pH 6 (84.0 mm) and the minimum growth was observed in pH 9 (61.0 mm). The results of temperature revealed that maximum growth (88.95 mm) of the pathogen was observed at 30℃ followed by 25℃ (85.97 mm). A sudden fall in mycelial growth was observed at 35℃ (78.95 mm) and 20℃ (61.22 mm).

Keywords: *Alternaria porri*, artificial inoculation methos, media, pH, temperature

**INTRODUCTION**

Onion (*Allium cepa*), is a herbaceous biennial plant that belongs to genus Allium, family Alliaceae. The chromosome number of onion is 16 (2n). It is commonly known as “Queen of the kitchen,”due to its highly valued ﬂavor, aroma, and unique taste, and the medicinal properties of its ﬂavour compounds (Sunil Prateek et. al 2017). Onion is believed to be originated in South-western Asia, being the centre of domestication and variability from where it was spread first across the world and has been cultivated for over 4700 years as annuals for bulb production (Etana et al. 2019). China and India are the primary onion growing countries, followed by the USA, Egypt, Iran, Turkey, Pakistan, Brazil, the Russian Federation, and the Republic of Korea (FAO, 2012). Onion is one of the major crops in India famous as a potential source of earning in the country which is grown in about 1285 hectare area with an approximate annual production of 23262 tonnes. In 2018-2019 onion production is recorded to be around 23.62 million tonnes (MT) as against 23.26 MT in 2017-18 (The Economic Times Agriculture, 2019). According to NHB (2017-2018) report, the cultivable area of onion in Uttarakhand is approximately 294.83 thousand ha with a total production of 1712.90 MT. Onion, is an export oriented crop in which, out of total export, 50% of the earning comes from Rabi onion followed by 30% and 20% from late-kharif and kharif onions respectively (Bal et al. 2017).

Allium contains more than 780 species (Burnie et al.,1999) with large diversities in morphological characters. The plant is either biannual or perennial (depending on the cultivar), and smells when crushed (WHO, 1999). The plant has shallow adventitious ﬁbrous roots, bulb, and tubular leaves. The vegetative growth of the crop is supported by lower temperature and short photoperiod whereas bulb development requires high temperature with longer photoperiod (Sarnobat et al. 2020). Green onions are also called scallions which are eaten for their immature bulb and green foliage (Singh et al. 2018). The onion bulb ranges in shape from ﬂat to globular to oblong, and the onions are usually of three colors: red, white, and yellow (Fritsch, 2005). The fruits are capsule and contain black seeds. The whole bulb of onions are a good source of (+)-S-alk(en)yl-L-cysteine sulfoxide and γ-glutamyl peptide, which together account for over 70% of the total sulfur in onions (Lawson, 1996). The predominant ﬂavor precursor is isoalliin, which accounts for more than 80% of the total amount of alk(en)yl cysteine sulfoxides. (Jones et al., 2004). Onions are a very common and rich source of dietary ﬂavonoids, and contain three diverse and highly valuable phytochemicals in perfect proportion: ﬂavonoids, fructans, and organosulfur compounds. These compounds are believed to provide beneﬁcial effects for human health. Ascorbic acid is the most abundant vitamin found in the onion bulb, with a concentration of 1 mg/g dry weight (Breu, 1996). Onion contains steroidal saponins (Carotenuto et al., 1999), which prevent absorption of cholesterol in the intestine. Evidence from several investigations suggests that the biological and medical functions of alliums are mainly due to their high organosulfur compounds content (Augusti and Mathew, 1974). In addition to these, certain steroidal saponins and sapogenins, such as β-chlorogenin, have been shown to have a role in biological and pharmaco-logical activities, such as having antifungal, antibacterial, antitumor, anti-inﬂammatory, antithrombotic, and hypo-cholesterolemic properties (Lanzotti, 2006). Onion contains thiosulphate, a compound that is effective in killing common bacteria viz., *Pseudomonas aeruginosa* (Kumar et al. 2010).

Onion crop is attacked by bacteria, fungi, nematodes, viruses, phytoplasma, phanerogamic plant parasite and many other miscellaneous diseases and disorder. Among the various foliar diseases of onion, purple blotch caused by *Alternaria porri* is major constrains in onion cultivation. The disease yield loss ranging from 2.5 to 87.8 per cent during Kharif season (Shrivastava et al., 1994). Kharif onion has got poor productivity due to constant drizzling and cloudy weather which leads to occurence of disease (Kale and Ajjappalavara, 2014). The pathogen is polyphagus infecting crop like onion, garlic, shallot and other Allium crops (John et al. 2018). The pathogenicity of *Alternaria spp*. is due to production of host specific or nonspecific toxins that may induce disease. These toxins are mainly secondary metabolites that destroy susceptible cultivars by leaf necrosis (Dar et al. 2020). Purple blotch is the most important disease in Northern India, which causes considerable loss in seed crop as well as bulb crops (Mishra and Gupta, 2012). Nolla (1927) proposed the name “Purple blotch” and he named the causal organism as *Alternaria alli* which was later renamed as *Alternaria porri*. The pathogen belongs to phylum Ascomycotina, Class Dothidiomycetes and Order Pleosporales (Kirk et al.2008). Temperature, relative humidity and host-nutrition play an important role in infection (Khare and Nema, 1982). High relative humidity (80 to 90%) and optimum temperature (24+2°C) are needed for further development of purple blotch disease symptoms causing considerable yield losses up to 20- 60 percent and extent of loss depends on time of infection and stage of crop growth. Survey on the disease in the field showed the extent of purple blotch disease affecting the crop and quality of the bulbs is widespread particularly in rainy season / high moisture conditions (Ravichandran et al. 2017). (John et al. 2018) reported losses of about 50 to 100 per cent with relative occurrence of *Alternaria porri*.

Symptomatic expression of purple blotch disease first appears on leaves with 2–3 mm in diameter of whitish water soaked lesions, these lesions enlarge, coalesce, zonate and lesions turn brown to purplish colour under favourable conditions. Seldom, lesion surface covered by black fruiting bodies under humid conditions (Verma and Sharma 1999). The older plant tissues more susceptible than younger plants leaf blades to the fungus infestation. As on disease advances the lesions enlarged to form concentric rings, girdling of leaf and stem cause down fall of plant shoot (Muimba-Kankolongo 2018). The infected leaf showed a significant decrease in the quantity of the crude protein, fat, fibre and ash as compare to healthy leaf (Shehu and Aliero, 2010). The fungus prolongs their existence on seed, leaves, stalks and soil surface and survives in crop debris as dormant mycelium and can remain viable for 12 months (Lawande et al.). It spread by the punctures made by the thrips, opening of stomata pores and epidermal layers. (Muimba-Kankolongo 2018)

**MATERIAL AND METHODS**

The present investigation were carried out to isolate the test pathogen, to evaluate different methods of inoculation on symptom development, to check the effect of different media, temperature and pH on growth of test pathogen The present investigation was conducted in the Laboratory of Department of Plant Pathology, College of Horticulture (VCSGUUHF), Bharsar, Pauri Garhwal, Uttarakhand.The details of Experimental material used and methothodology adopted are described below.

**Isolation of pathogen**

Infected leaves of onion with purple blotch were collected from Organic block, Bharsar and used for isolation of the pathogen. The leaves and floral stalks of onion showing typical symptoms of disease purple blotch were collected and packed into clean polyethylene bags. The sample are then transferred to laboratory to isolate and identify the pathogen. The sample collected were washed with tap water and air dried and infected lesions with healthy part were cut into small pieces of about 2 cm each with the help of sterilized scissors and then surface sterilized by immersing in 0.1% sodium hypochloride solution for 30 seconds. These pieces were thoroughly washed in at least three changes of sterilized distilled water to remove the residue of sodium hypochloride and then aseptically the leaf pieces are transferred to Petri plates containing sterile potato dextrose agar medium. After that, incubation is done at 25±2℃ temperature and observed daily for mycelia growth of the fungus. After one week profuse growth of the fungus was observed. The fungus was purified through frequent sub-culturing and pure culture was maintained on slants and stored in refrigerator at 4℃ for further studies. Colonies which developed from the pieces were identified by microscopic observation by taking mycelial character as means for identifying the pathogen.

**Evaluation of different artificial inoculation methods on symptom development**

In vitro experiments were conducted in which artificial inoculation methods were used to evaluate the incubation period and type of symptoms developed by a fungal species. The experiment was conducted in Department of Plant Pathology, laboratory according to Koch’s postulates. Healthy host plants were selected and thoroughly cleaned with sterilized distilled water. The conidia of the test pathogens were taken from freshly prepared ten days old culture and then it was suspended in sterilized water to obtain 10 conidia per ml. Different inoculation methods were followed namely soil inoculation, foliar inoculation, syringe inoculation and wound inoculation on potted plants grown in sterilized soil. Un-inoculated healthy onion plants were kept as control. Observations of the plants were made regularly for the appearance and development of symptoms. Within 7-10 days of inoculation leaves showed typical *Alternaria* symptoms. After that re-isolation was done from diseased tissues of artificially infected plants using PDA plate technique. Then the isolate obtained was compared with the original culture for confirmation of same pathogenic isolates which were inoculated.

**Growth characters on different solid media**

The variation in the cultural characters of *Alternaria porri* studied on different solid media for the growth of fungus. The growth characters of the fungus was studied on the following solid media namely Potato dextrose agar (PDA), Oat meal agar (OMA), Malt extract agar, Corn meal agar and Richard’s agar. 20 ml of each medium were poured into 90 mm diameter Petri plates after sterilization at 121℃ on 15 pounds pressure for 15 minutes. After that, inoculation of plates with 5 mm disc of fungal growth were done under laminar air flow. Then such plates are incubated at 25±2℃. Each treatment were replicated four times. The fungal colony was measured after 2, 4, 6, 8 and10 days of inoculation.

 **Growth on different pH**

Potato Dextrose Agar was used as basal medium. Conical flask of 100 ml capacity were taken and pouring of 20 ml PDA were done in each flask. The pH of the media was maintained to different level *viz*., 5.0, 6.0, 7.0, 8.0 and 9.0 by adding 1 M solution of HCL and NaOH. At each pH level four replications were maintained. The plates were incubated at 25±2℃ and fungal colony was measured after 7 days of incubation.

**Growth on different temperatures**

Potato dextrose agar was used as basal medium. Petri plates containing sterilized basal medium were inoculated with 4 mm mycelial disc of the pathogen and then incubated at various temperatures *viz*., 15℃, 20℃, 25℃, 30℃ and 35℃ in different incubators. In each case, four replications were maintained. The fungal colony were measured after 7 days of incubation.

**STATISTICAL ANALYSIS**

The data obtained for different parameters under laboratory investigation was analyzed by using standard statistical procedure in the completely randomized design. The mean value of data were subjected to analysis of variance as described by Gomez and Gomez (1984) by using MS excel and OPSTATE. The data obtained for different season during the field investigation were analysed by using standard statistical procedure in the completely randomized block design (RCBD). The statistical analysis is carried out for each observed character under the study using MS-Excel and OPSTATE.

**RESULT AND DISCUSSION**

 **Isolation of pathogen**

The leaves and floral stalks of onion showing typical symptoms of disease purple blotch were collected and isolated with moist chamber method and PDA plate method. The pure culture was obtained by single spore isolation technique and the fungus was purified through frequent sub-culturing and pure culture was maintained on slants and stored in refrigerator at 4℃ for further studies.

 **Identification of pathogen**

The test pathogen was identified on the basis of morphological characters like colony, mycelium, conidiophore and conidia.

**Colony:** Colony was fast growing, usually ashey grey, fluffy, circular in the beginning and later turning into dark greenish olive with abundant sporulation.

 **Conidiophores:** Conidiophores arise singly or in groups and are straight or flexuous, sometimes geniculate, septate, and pale to brown. They are upto 120 µm long with one or several well defined conidial scars.

**Conidia:** Conidia usually occur singly and are straight or curved and taper to a beak that is commonly about the same length or slightly larger than the body of the conidium. The size of the conidia ranges from 100-300 µm with the broadest part 15-20 µm thick.

**Effect of different inoculation methods on symptom development**

All the methods of inoculation shown in Table 1 showed significant difference as compared with control. The incubation period ranged from 0.00 to 6.02 days. The maximum incubation period was observed for symptoms development in T2 (Soil inoculation) (6.02 days) followed by T5 (Wound inoculation) (4.93 days), T4 (Syringe inoculation) (4.47 days). The minimum incubation period was observed in T3 (Foliar spray inoculation) (2.38 days).

The initial symptoms of the disease were observed on leaves as small water soaked lesions that quickly develop white centres. Later, these lesions enlarge, coalesce, become zonate and brown to purple that extend upward and downward. The margin was reddish to purple in colour and surrounded by yellow zone. The uninoculated plant leaves does not showed any symptoms of the disease. Above mentioned symptoms were also reported by Verma and Sharma (1999).

**Growth study of pathogen on different culture media**

Effect of different media on mycelial growth of the pathogen is tabulated in table 2. Among the solid media maximum radial growth of the fungus was recorded in PDA (89.67 mm) while minimum radial growth was observed in Corn meal agar media (53.82 mm). Our finding that PDA showed maximum growth are in aggrement with earliar findings of Chethana et al. (2018); Pradnyarani and Kulkarni (2015); Raju and Mehta 1982 and Tahira et al (2019). Priya et al. (2018) reported that Czapeck’s agar supported maximum radial growth followed by PDA.

**Growth study on different pH**

In this experiment, the pathogen *Alternaria porri* was tested against for its tolerance at acidic and basic pH ranging between 5.0-9.0 and the result is shown in table 3. The result revealed that pH 5 have maximum mycelial growth (87.0 mm) followed by pH 6 (84.0 mm) and the minimum growth was observed in pH 9 (61.0 mm). Growth of *A. porri* was found maximum at pH 5 and minimum at pH 9 which is in conformity with the report of Madavi et al. (2012). Growth of *A. porri* was maximum at pH 5 was also proved by Yadav et al. (2017); Saeed et al (1995); Jash et al (2003); Agale et al (2014); Vijaylakshmi et al (2012); Ramjegathesh and Ebenezar (2012) from their findings.

**Growth study on different temperature**

Table 4 shows the effect of different temperature range on mycelial growth of the pathogen. Maximum growth (88.95 mm) of the pathogen was observed at 30℃ followed by 25℃ (85.97 mm). A sudden fall in mycelial growth was observed at 35℃ (78.95 mm) and 20℃ (61.22 mm). However, minimum mycelial growth (4.10 mm) was noticed at 15℃. The present findings are consistent with those of Pradnyarani and Kulkarni (2015) who reported the maximum growth of *Alternaria porri* at 30℃ and miminum at 15℃. The result obtained that 15℃ showed least growth of the pathogen are in agreement with the earlier studies of Somappa et al. (2013). Tahira et al. (2019) observed that 28℃ is optimum for colony growth and sporulation.

|  |  |  |  |
| --- | --- | --- | --- |
| T.No | Treatments | Incubation period (days) ± S.E.(m) | Type of symptoms |
| T1 | Control | 0.00±0.00 | No symptoms |
| T2 | Soil inoculation | 6.02\*±0.22 | Brown lesions |
| T3 | Foliar spray inoculation | 2.38\*±0.15 | Brown lesions |
| T4 | Syringe inoculation | 4.47\*±0.32 | Brown lesions |
| T5 | Wound inoculation | 4.93\*±0.41 | Brown lesions |
|  | SE(d) | 0.37 |  |
|  | C.D.(0.05) | 0.84 |  |

Table 1 Effect of different inoculation methods on symptom development of *A. porri*

⁎Significance at 5% level of significance as compared with control.

Table 2 Effect of different media on mycelial growth (mm) of the pathogen

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| T. No | Media | 2 days± S.E.(m) | 4 days± S.E.(m) | 6 days± S.E.(m) | 8 days± S.E.(m) | 10days± S.E.(m) |
| T1 | Potato dextrose agar | 31.05\*±0.13 | 50.97\*±0.07 | 72.92\*±0.08 | 84.22\*±0.12 | 89.97\*±0.02 |
| T2 | Oat meal agar | 28.10\*±0.09 | 44.65\*±0.06 | 64.95\*±0.11 | 80.10\*±0.09 | 89.67\*±0.04 |
| T3 | Malt extract agar | 19.97\*±0.04 | 32.95\*±0.06 | 46.70\*±0.08 | 63.15\*±0.08 | 68.72\*±0.08 |
| T4 | Corn meal agar | 20.02\*±0.07 | 23.72\*±0.08 | 37.77\*±0.11 | 46.60\*±0.15 | 53.82\*±0.06 |
| T5 | Richard’s media | 26.02\*±0.07 | 41.30\*±0.09 | 64.55\*±0.13 | 79.30\*±0.14 | 89.35\*±0.06 |
|  | SE(d) | 0.12 | 0.10 | 0.15 | 0.17 | 0.08 |
|  | C.D.(0.05) | 0.27 | 0.23 | 0.32 | 0.38 | 0.18 |

\*All the treatments are significant as compared with each other

Table 3 Effect of different pH on mycelial growth (mm) of the pathogen

|  |  |  |
| --- | --- | --- |
| T. No. | pH | Growth (mm)± S.E.(m) |
| T1 | 5 | 87.00\*±0.09 |
| T2 | 6 | 84.05\*±0.06 |
| T3 | 7 | 79.30\*±0.24 |
| T4 | 8 | 74.25\*±0.13 |
| T5 | 9 | 61.05\*±0.11 |
| S.E.(d) |  | 0.20 |
| C.D.(0.05) |  | 0.43 |

\*All the treatments are significant as compared with each other

Table 4. Effect of different range of temperature on mycelial growth (mm) of the pathogen

|  |  |  |
| --- | --- | --- |
| T. No | Temperature (℃) | Growth (mm) ± S.E.(m) |
| T1 | 15 | 4.10\*±0.10 |
| T2 | 20 | 61.22\*±0.12 |
| T3 | 25 | 85.97\*±0.08 |
| T4 | 30 | 88.95\*±0.11 |
| T5 | 35 | 78.95\*±0.10 |
| S.E.(d) |  | 0.15 |
| C.D.(0.05) |  | 0.33 |

 \*All the treatments are significant as compared with each other

 CONCLUSION

 Onion is one of the most important bulbous vegetable crop of global importance. The limiting factor affecting the yield of onion is purple blotch. In view of destructive nature of pathogen the study on effect of different inoculation methods, different media, pH and temperature on the growth of *Alternaria porri* were carried out. Among different inoculation methods the maximum incubation period for symptom development was observed in soil inoculation and the minimum incubation period was observed in foliar spray inoculation. In vitro potato dextrose agar showed maximum growth of the pathogen while minimum growth was observed in corn meal agar. Among different pH levels tested the maximum growth was showed by pH 5 while minimum was observed in pH 9. Out of five different temperature , the maximum growth was recorded at 30℃ while 15℃ the minimum growth of the pathogen. The present investigation will be helpful for different workers studing the pathogen *Alternaria porri*.

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