# Comparative analysis of some Morpho-cultural characters of *Venturia inaequalis* (Apple Scab Pathogen) under Kashmir conditions


## Abstract

Apple (*Malus domestica*) represents one of the most widely cultivated and economically important temperate fruit crops in the world. However, its cultivation faces significant challenges due to various fungal pathogens, particularly apple scab inflicted by *Venturia inaequalis*, which has a profound impact on the yield and quality of apple, resulting in substantial economic loss. The pervasive nature of ~~*Venturia*~~ *V. inaequalis* and its ability to damage crops poses a serious threat to sustainable apple production. In this study, the morpho-cultural characteristics of ~~*Venturia*~~ *V. inaequalis* were systematically analyzed and compared between the Shopian and Baramullah districts of the Kashmir Valley. The colonies were characterized by a compact hue ranging from greyish-green to blackish and exhibited a densely velvety texture. Mycelial growth was predominantly aerial, with some isolates displaying partial irregularity in colony margins, while others exhibited greater uniformity. The mean colony radii for potato dextrose agar and malt extract agar media was measured as 23.01 mm and 28.50 mm for the Shopian district, and 28.09 mm and 33.33 mm for the Baramullah district, respectively. The mean conidial spore density was recorded at 27.87 spores/cm² for the Shopian district and 34.67 spores/cm² for the Baramullah district, respectively. This variation could be attributed to several factors, including genetic diversity within the pathogen populations, the selection pressure exerted by fungicide applications, the emergence of novel pathogen races, and phenotypic plasticity driven by the differing agricultural practices and microclimatic conditions in the respective regions.


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Keywords: Apple, Morpho-cultural, Greyish-green, Malt extract agar, *Venturia inaequalis*


## 1. INTRODUCTION

Apple (*Malus* × *domestica* Borkh.) the premier table fruit of the temperate world, also known as the king of the temperate fruits, is a cross-pollinated fruit crop, that belongs to the genus Malus of the family Roseaceae and order Rosales. It is a widely grown fruit crop in temperate regions of the globe and is the most favored fruit of people owing to its affordability and nutrient profile of 85.56 g water, 52.0 kcal energy, 0.26 g protein and 13.81 g carbohydrate (1) India is the fifth largest producer of apple with production and productivity of 2.78 million tonnes and 9.00 tonnes ha-$^{1}$ respectively, from an area of 0.309 million ha (2). In India, commercial apple cultivation is predominantly concentrated in the regions of Jammu and Kashmir, Himachal Pradesh, and Uttarakhand, collectively contributing to 99 percent of the nation's total apple production. Amongst them, Jammu and Kashmir are the major contributors concerning area and production with 164854 hectares and 2026472 metric tonnes annually, respectively (3). Respectively. Apple is affected by a large number of diseases and pests, which

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are mostly present in all apple producing regions of the world viz: apple scab (*Venturia inaequalis*), Alternaria leaf blotch (*Alternaria mali*), powdery mildew (*Podosphaera leucotricha*), marssonina leaf blotch (*Marssonina coronaria*), root rot (*Rosellinia necatrix*), collar rot (*Phytophthora cactorum*) and cankers caused by various fungi (4, 5, 6 ). Among them, fungal diseases especially apple scab inflicted by *Venturia inaequalis* Cook (Winter) is the most catastrophic which significantly affect the production and quality of fruits, leading to great economic loss (7). The disease results in 30-40 per cent fruit loss annually and even complete loss at some places (8). The infection begins in spring when the ascospores are released from the crop debris and cause primary infection in young emerging apple leaves. Conidia are then produced from the lesions caused by primary inoculum infections and continue the cycle of secondary infection in leaves and fruits. The pathogen impacts leaves, flowers, buds, shoots, and fruits, resulting in economic losses of up to 70 per cent by diminishing yield and fruit quality (9, 10). In Kashmir Valley, the release of the ascospores can begin as early as late March and may continue over several weeks (11). The symptoms appear as transparent pin head size spots that turn to brown or black velvet with the advancement of the disease. (12). Also, the variations in colony pigmentation, adhesion, aerial development, and colony margins have been frequently observed in V. inaequalis thus indicating the emergence of new races of *Venturia inaequalis* (13). The objective of this study was to identify the variations by evaluating morpho-cultural characters of *V. inaequalis* while considering the differences in ecology and chemical management of apple scab.

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## 2. METHODS AND MATERIALS

### 2.1. Sampling for the Isolation of Pathogen

Disease samples of apple (*Malus domestica* M.) were collected from various districts in Kashmir (see Table 1) for the isolation of the pathogen *Venturia inaequalis* (Cooke) Wint. These samples were securely packaged with labels detailing the isolate name, variety, age, location, and time of collection, and subsequently transported to the laboratory for further analysis. An infected sample, including a section of adjacent healthy tissue, was aseptically excised into 5 mm discs and placed in Eppendorf tubes containing 1.5 ml of sterile distilled water. After 2 minutes of vigorous agitation, conidia were released into the water and uniformly distributed onto the surface of pre-prepared water agar, which was then incubated at 20 ± 2 °C for 24 hours. The Petri dishes were examined microscopically, and germinated conidia were identified and marked. The marked regions were excised and transferred to new Potato

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Dextrose Agar (PDA) and Malt Extract Agar (MEA) media, which were then incubated at 20 ± 2 °C for 10-15 days to obtain pure cultures. The following morphological characteristics were assessed as follows: colony color, colony diameter, and conidial abundance

List 1 : Number of isolates and codes of *V. inaequalis* isolates obtained from apple-grown areas of Shopian and Baramullah

| S.No. | Survey | Isolate code | S. No. | Survey | Isolate code |
|---|---|---|---|---|---|
| 1 | Shopian/Zainapora | S-Z-1 | 1 | Baramullah /Nowpora | B-N-1 |
| 2 | Shopian/ Zainapora | S-Z-2 | 2 | Baramullah/ Nowpora | B-N-2 |
| 3 | Shopian/Ramnagari | S-R-1 | 3 | Baramullah Sangrama | B-S-1 |
| 4 | Shopian/ Ramnagari | S-R-2 | 4 | Baramullah Sangrama | B-S-2 |
| 5 | Shopian/ Kachdora | S-K-1 | 5 | Baramullah/Nathipora | B-Na-1 |
| 6 | Shopian/ Kachdora | S-K-2 | 6 | Baramullah Nathipora | B- Na-1 |
| 7 | Shopian/Pinjura | S-P-1 | 7 | Baramullah/ Delina | B-D-1 |
| 8 | Shopian/ Pinjura | S-P-1 | 8 | Baramullah/ Delina | B-D-2 |
| 9 | Shopian/ Reshnagri | S-Re-1 | 9 | Baramullah/Dangerpora | B-Da-1 |
| 10 | Shopian/ Reshnagri | S-Re-2 | 10 | Baramullah/Dangerpora | B-Da-2 |

2.2 Identification and comparison of colony characteristics, colony growth rate, and conidial Spore count

Ten isolates from each district were evaluated for colony color, mycelial morphology, margin delineation and colony growth. The assessment involved transferring 5 mm agar plugs from their respective stock cultures to malt extract agar and potato dextrose agar for comparative analysis. Following incubation for 10-15 days at 20 ± 2 °C, the above characteristics were assessed both macroscopically and microscopically. Colony diameters for each isolate was recorded in millimeters, and a t-test was utilized to compare the three replicates of isolates from each district. To evaluate potential variations in conidial spore counts, a drop from the conidial suspension was then distributed evenly on the surface of pre-prepared water agar and incubated at 20 ± 2 °C for 24 hours. Subsequently, conidia within a 1 $cm^2$ area were enumerated under a microscope and subjected to statistical analysis as previously described.

## 3. RESULTS AND DISCUSSION

### 3.1 Colony Morphology

Based on macroscopic and microscopic evaluations, the colonies derived from *Venturia inaequalis* isolates exhibited predominant colour on both Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA). The majority of the colonies were characterized by a compact hue ranging from greyish-green to blackish and exhibited a densely velvety texture. Mycelial growth was predominantly aerial, with some isolates displaying partial irregularity in colony margins, while others exhibited greater uniformity. (see Figs. 1 and 2). Colony growth rates following a 10-15day incubation period on Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) are summarized in Table 1, with colony diameters recorded in millimeters. The mean colony radii for PDA and MEA were as follows: 23.01 mm and 28.50 mm for the Shopian district, and 28.09 mm and 33.33 mm for the Baramullah district, respectively. Conidial concentrations of the isolates from various sources on 1 $cm^2$ of water agar after 24 hours of incubation are detailed in Table 2. The mean conidial spore density was recorded at 27.87 spores/$cm^2$ for the Shopian district and 34.67 spores/$cm^2$ for the Baramullah district, respectively. The variation in isolate morphology was observed in all isolates demonstrating statistically significant differences (refer to Table 1). These results are consistent with the findings of (14) who almost observed similar values that are at par with my results. These variations are supposed to be associated with the extensive application of fungicides targeting Venturia inaequalis in apple orchards across different regions, as well as the nutrient availability from different growth and are most likely attributed to different races, as suggested by different researchers (13) *Venturia inaequalis* is known to rapidly develop resistance to fungicides, a common phenomenon among plant pathogens worldwide. This suggests a high likelihood of morphological and physiological variations among different isolates (15, 16,17) which supports our results. The optimal growth conditions for *Venturia inaequalis* on different media demonstrated that the most rapid development occurred on MEA compared to PDA, with this trend being relatively uniform across all isolates. This highlights the physiological response of *V. inaequalis* to varying nutrient media. The observed variations in colony morphology and growth rates may have significant implications for the pathogen's virulence, fungicide resistance, and overall fitness, emphasizing the necessity for region-specific disease management strategies in apple orchards.

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Discuss further the results morphological observation per site, per isolates if possible
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Discuss the MEA and PDA in comparison with their composition and their effect on fungal growth

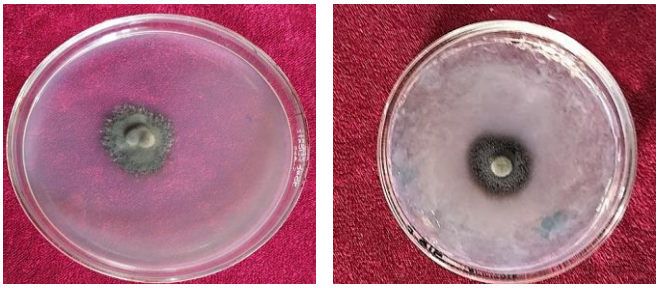

Plate 1: Development of V. inaequalis colonies on PDA for 15 days. Shopian and Baramullah from left to right

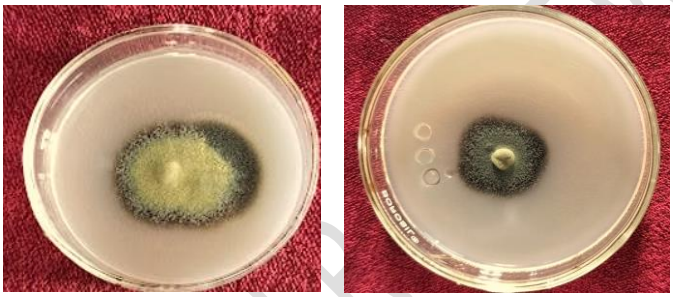

Plate 2: Development of *V. inaequalis* colonies on MEA for 15 days. Shopian and Baramullah from left to right

Table 1: Comparison of colony sizes (radius measurements in mm) after 10-15 days' incubation in PDA and MEA media

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| Isolates | Growth rate of colonies in different mediums (mm) | | Isolates | Growth rate of colonies in different mediums (mm) | |
|---|---|---|---|---|---|
| | PDA | MEA | | PDA | MEA |
| S-Z-1 | 24.50 | 30.70 | B-N-1 | 30.00 | 35.77 |
| S-Z-2 | 25.29 | 28.03 | B-N-2 | 30.55 | 34.00 |
| S-R-1 | 25.67 | 28.50 | B-S-1 | 29.00 | 34.66 |
| S-R-2 | 23.68 | 27.33 | B-S-2 | 29.77 | 33.80 |
| S-K-1 | 22.63 | 26.59 | B-Na-1 | 28.60 | 35.44 |
| S-K-2 | 21.76 | 31.50 | B- Na-1 | 27.90 | 33.00 |
| S-P-1 | 22.83 | 29.50 | B-D-1 | 27.00 | 32.50 |
| S-P-2 | 20.87 | 29.33 | B-D-2 | 26.00 | 32.00 |
| S-Re-1 | 21.43 | 26.81 | B-Da-1 | 26.33 | 31.00 |
| S-Re-2 | 22.47 | 27.67 | B-Da-2 | 25.80 | 31.20 |
| Average | 23.01 | 28.50 | Average | 28.09 | 33.33 |
| STDEV | 1.63 | 1.64 | STDEV | 1.74 | 1.67 |
| SE± | 0.52 | 0.52 | SE± | 0.55 | 0.53 |
| t test | Significant | Significant | t test | Significant | Significant |

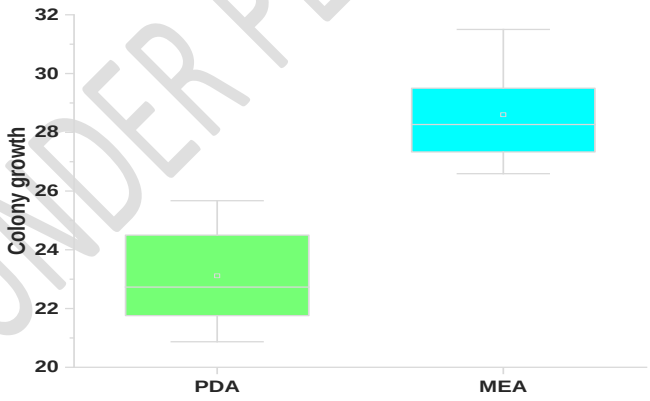


Fig 1: Comparison of colony sizes (radius measurements in mm) after 10-15 days' incubation on PDA and MEA media in district Shopian

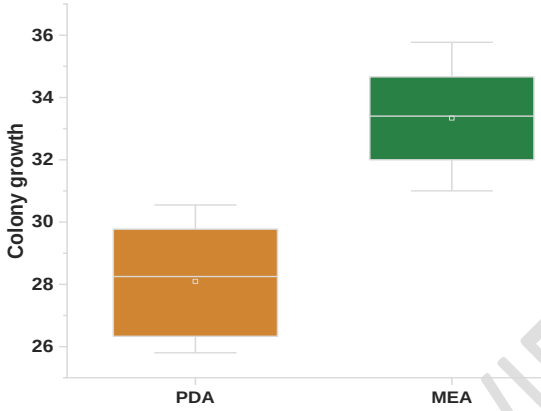


Fig 2: Comparison of colony sizes (radius measurements in mm) after 10-15 days' incubation on PDA and MEA media in district Baramullah

Table 2: Comparison of conidial numbers after 24-48 hours of incubation in 1 cm2 area of water agar in district Shopian and district Baramullah

| **Isolates** | **Average Spore No.** | **Isolates** | **Average Spore No.** |
|---|---|---|---|
| S-Z-1 | 30.00 | B-N-1 | 37.44 |
| S-Z-2 | 29.00 | B-N-2 | 36.00 |
| S-R-1 | 28.70 | B-S-1 | 35.50 |
| S-R-2 | 28.00 | B-S-2 | 34.90 |
| S-K-1 | 27.00 | B-Na-1 | 34.50 |
| S-K-2 | 27.55 | B- Na-1 | 33.60 |
| S-P-1 | 26.60 | B-D-1 | 33.30 |
| S-P-2 | 26.00 | B-D-2 | 32.00 |
| S-Re-1 | 25.00 | B-Da-1 | 32.50 |
| S-Re-2 | 30.55 | B-Da-2 | 37.00 |
| Average | 27.84 | Average | 34.67 |
| STDEV | 1.57 | STDEV | 1.74 |
| SE | 0.52 | SE | 0.58 |
| t test | Significant | t test | Significant |

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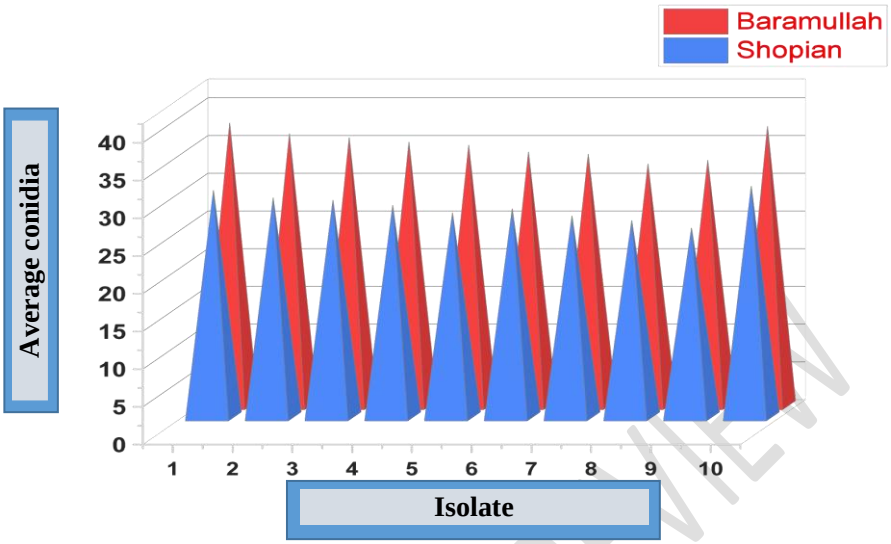


Fig 3: Comparison of average conidia number in district Shopian and district Baramulla

CONCLUSION

The best growth conditions of the *V. inaequalis* were evaluated in the artificial medium, it was observed that the fastest development was realized on MEA followed by PDA. This situation is the same for both, this somehow reveals the physiological response of *V. inaequalis* to these nutrition mediums. The variations in colony morphology, growth rates, and conidial production have significant implications on pathogen's virulence, fungicide resistance, and overall fitness, emphasizing the necessity for region-specific disease management strategies in apple orchards.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

**References**


1. Atil, A**.** 2019. Apples 101: Nutrition facts and health benefits. https://www.

2. Anonymous, 2020. Ministry of Agriculture and Farmers Welfare, Department of Agriculture, Cooperation and Farmers welfare, Government of India, *Agricultural Statistics at a Glance*. pp. 117

3. Anonymous, 2020a. Directorate of Economics and Statistics Government of Jammu and Kashmir. *Digest of Statistics* pp. 167-169

4. Gupta, S. K. and Gupta, G. K. 1992. Relative efficacy of scab fungicides in protective spray programme against summer diseases of apple during four years period. *Pestology* **16**: 28-31

5. Sharma, I. M. and Bhardwaj, S. S. 1999. Canker and foliar diseases of apple. ***In****: Diseases of horticultural crops-fruits*: (Eds. L. R. Verma and R. C. Sharma). Indus Publishing Company, New Delhi, India, pp. 724.

6. Sharma, J. N. and Verma K. D. 2005. Integrated management of foliar and fruit diseases of apple in Himachal Pradesh. *Journal of Mycology and Plant Pathology* **35:** 368-380.

7. Holb, I. J., Abonyi, F., Buurma, J. and Heijne, B. 2017. On-farm and on-station evaluations of three orchard management approaches against apple scab and apple powdery mildew. *Crop Protection* **97**: 109–118

8. Masoodi, K.Z., Ahmed, N., Mir, M.A., Bhat, B., Shafi, A., Mansoor, S., Rasool, R.S., Yaseen, M., Dar, Z.A., Mir, J.I. and Andrabi, S.M., 2022. Comparative transcriptomics unravels new genes imparting scab resistance in apple (Malus x domestica Borkh.). *Functional & Integrative Genomics*, ***22***(6): 1315-1330.

9. Jamar, L., Lefrancq, B. and Lateu, M. 2007. Control of apple scab (*Venturia inaequalis*) with bicarbonate salts under controlled environment*. Journal of Plant Diseases and Protection* **114**(5): 221–227

10. Gonzalez-Domínguez, E., Armengol, J. and Rossi, V. 2017. Biology and epidemiology of *Venturia* species affecting fruit crops. *Frontiers in Plant Science* **8**: 1496.

11. Sharma, I. M. 2010 Antagonistic effect of fungi associated with apple scab lesions on

growth of its pathogen *Venturia inaequalis* (Cke.) Wint. *Research Journal of Agricultural Sciences* **1**: 245-248.

12. Berrie, A. M. and Xu, X. M. 2003. Managing apple scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha)* using Adem. *International Journal of Pest Management* **49**(3): 243-249

13. Roig, E., Neumann, P. and Simon, J.P., 1990. Growth and isoenzyme comparison of fîve isolâtes of *Venturia inaequalis*. *Phytoprotection*, **71**(2): 65-71.

14 Kavak, H. and Celik, A., 2021. Comparison of Some Morphological and Physiological Characters of Apple Scab Pathogen (Venturia inaequalis) in Two Different Agricultural Ecology of Turkey. *Erwerbs-Obstbau*, *63*(1).

15. Köller, W., Parker, D.M., Turechek, W.W., Avila-Adame, C. and Cronshaw, K., 2004. A two-phase resistance response of *Venturia inaequalis* populations to the QoI fungicides kresoxim-methyl and trifloxystrobin. *Plant Disease*, ***88***(5): 537-544.

16. Bowen, J.K., Mesarich, C.H., Bus, V.G., Beresford, R.M., Plummer, K.M. and Templeton, M.D., 2011. *Venturia inaequalis*: the causal agent of apple scab. *Molecular plant pathology*, **12**(2); pp.105-122.

17. Beresford, R.M., Wright, P.J., Wood, P.N., Park, N.M., Larsen, N.J. and Fisher, B.M., 2013. Resistance of *Venturia inaequalis* to demethylation inhibitor and dodine fungicides in four New Zealand apple growing regions. New Zealand *Plant Protection*, **66**: 274-283.