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Comparative Analysis of Some Morpho-cultural Characters of *Venturia inaequalis* (Apple Scab Pathogen) under Kashmir Conditions

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Apple (Malus × domestica Borkh.) 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 *V. inaequalis* and its ability to damage crops poses a serious threat to sustainable apple production. In this study, the morpho-cultural characteristics of *V. inaequalis* were systematically analyzed and compared between the Shopian and Baramullah districts of the Kashmir Valley. Ten isolates from each district were grown on Malt Extract Agar (MEA) and Potato Dextrose Agar (PDA) for comparative analysis. 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 Baramullah district and 34.67 spores/cm² for the district Shopian, respectively. This variation could be attributed to several factors, including genetic diversity within the pathogen populations, differences in altitude, climate, and other environmental factors, 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.

Keywords: Apple; morpho-cultural; greyish-green; malt extract agar; Venturia inaequalis.

1. INTRODUCTION

Apple (*M. domestica*.) 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 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. These variations are expected to have significant implications on pathogen's virulence, fungicide resistance, and overall fitness, emphasizing the necessity for region-specific disease management strategies in apple orchards.

2. METHODS AND MATERIALS

2.1 Sampling for the Isolation of Pathogen

Disease samples of apple were collected from Shopian (33.72º N 78.83º E) and Baramullah (34.14º N 74.26º E) districts in Kashmir (see Table 1) for the isolation of the pathogen (*V. inaequalis*). The 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 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.

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 media and incubated at 20 ± 2 °C. Subsequently, conidia within a 1 cm² area were enumerated under a microscope and subjected to statistical analysis as previously described.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 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 colonies of *Venturia inaequalis* exhibit slow growth on media usually taking several days to develop visible colonies as compared to other fungal pathogens. The colonies shows olivaceous green color initially and black and velvety texture with the advanced growth. In this case 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).

3.1.2 Colony growth

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 highest colony growth was observed in isolate number S-R-1 with the colony diameter of 25.67 on PDA and 30.70 on MEA in isolate number S-K-2 in district Shoipan. In case of district Bramullah the highest colony growth was observed in isolate number B-N-2 with the colony diameter of 30.55 on PDA and 35.77 on MEA in isolate number B-N-1. 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 Spore count: The conidial concentrations of all the isolates on 1 cm² area of media are detailed in Table 2. It is evident from

the table that the highest number of spores were produced by S-Z-1 isolates (37.44), while as lowest spore count was observed in S-P-2 isolate (32.00) in district Shopian. In case of district Baramullah, the highest number of spores were produced by B-Da-2 isolates (30.55), while as lowest spore count was observed in B-Da-1 isolate (25.00). The mean conidial spore density was recorded at 27.84 spores/cm² for the Baramullah district and 34.67 spores/cm² for the Shopian district, respectively.

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

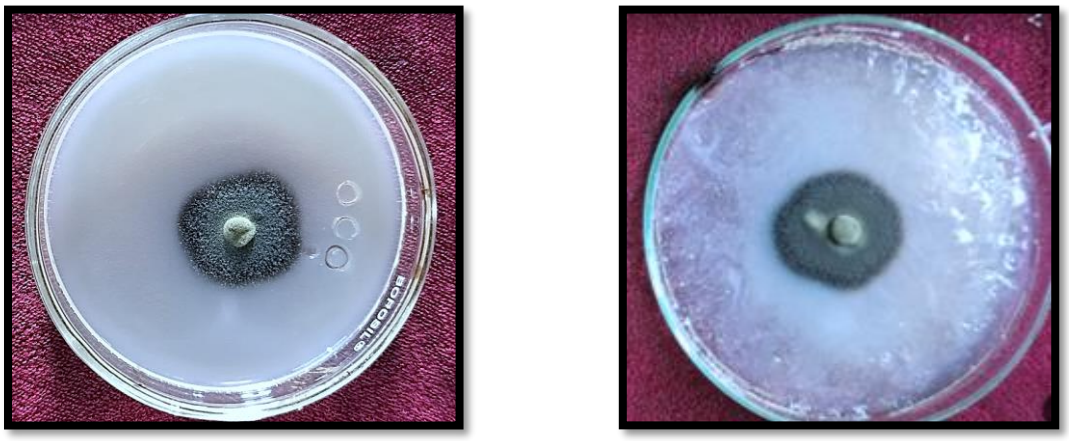

Plate 1. Development of V. inaequalis colonies on PDA for 15 days. Shopian and Baramullah from left to right

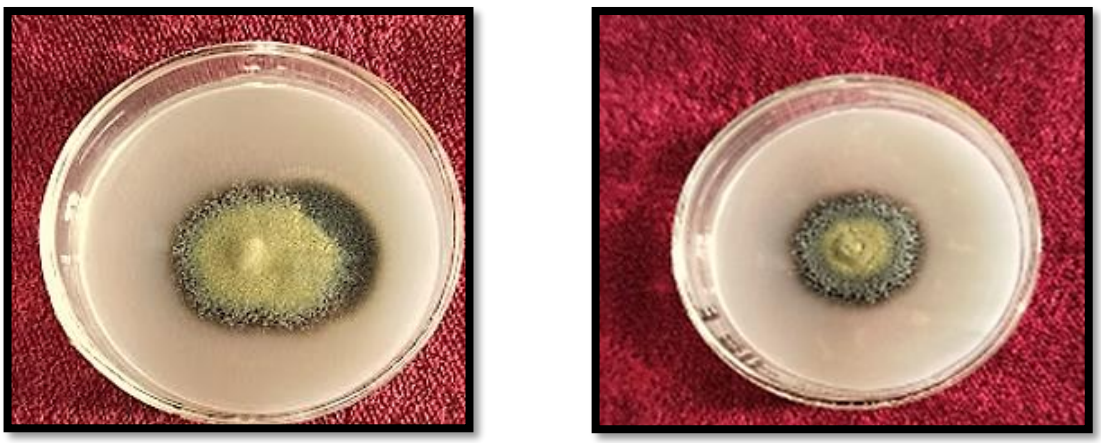

Plate 2. Development of V. inaequalis colonies on MEA for 15 days. Shopian and Baramullah from left to right

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

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

Table 2. Comparison of conidial numbers after 24-48 hours of incubation on 1cm2 area of media in district Shopian and district Baramullah

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

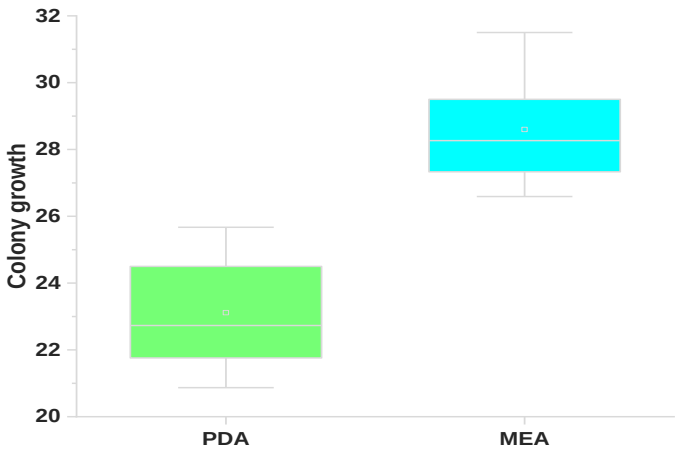

Fig. 1. Comparison of colony sizes (radius measurements in mm) after 10-15 days' incubation on PDA and MEA media in district Shopian

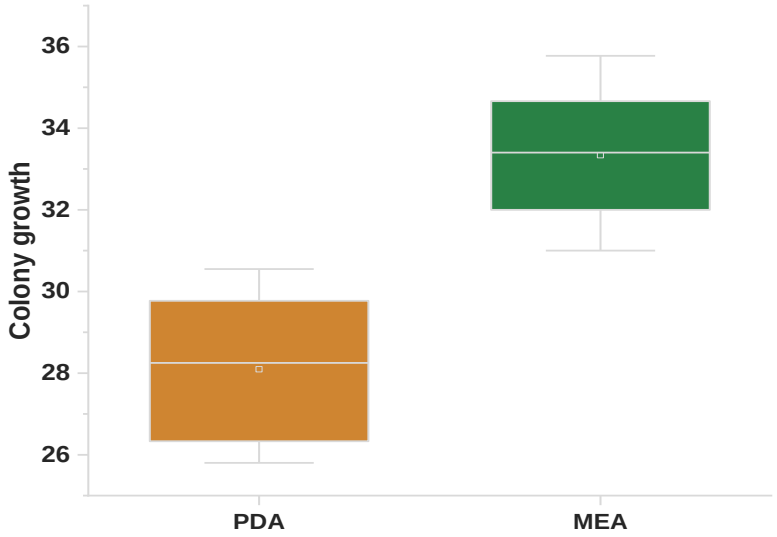

Fig. 2. Comparison of colony sizes (radius measurements in mm) after 10-15 days' incubation on PDA and MEA media in district Baramullah

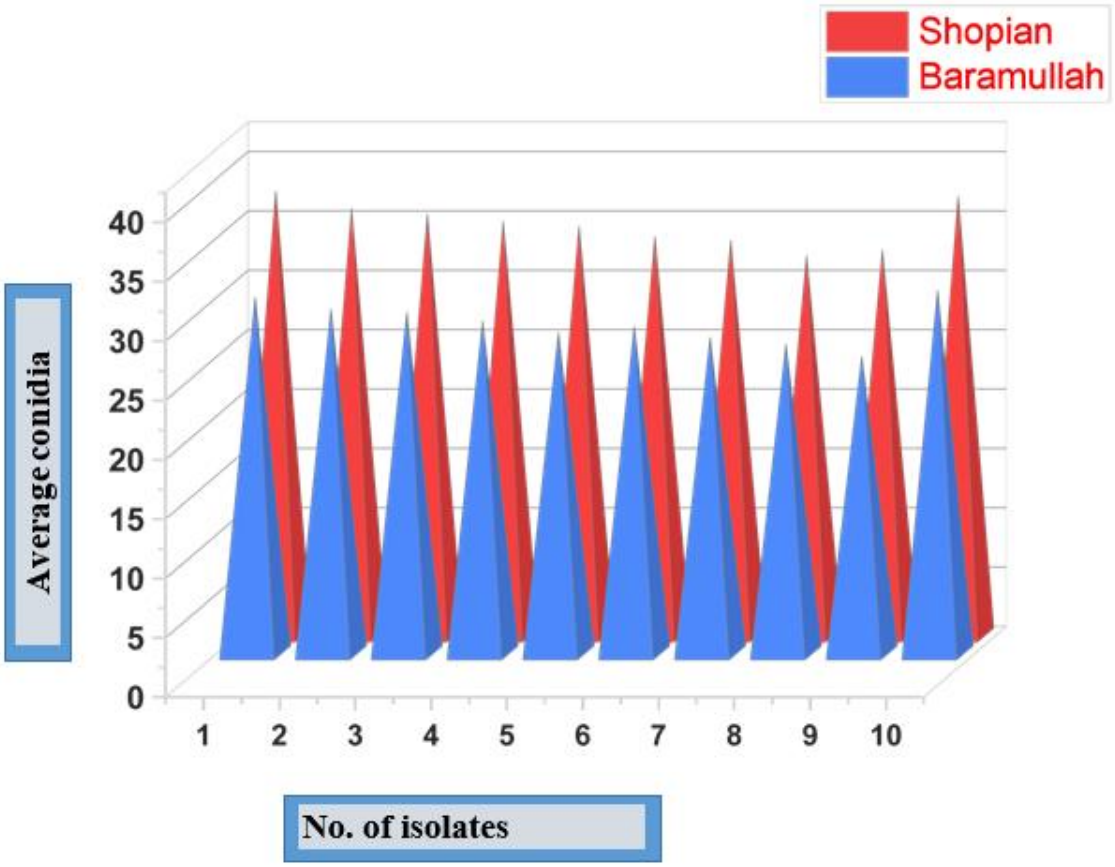

Fig. 3. Comparison of average conidia number in district Shopian and Baramullah

3.2 Discussion

The variation in isolate morphology was observed in all isolates demonstrating statistically significant differences (refer to Table 1). 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. 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. The difference in mean conidial densities per cm² was recorded at 27.84 spores/cm² for the Baramullah district and 34.67 spores/cm² for the Shopian district, respectively. Shopian being the higher-altitude region is characterized by cooler and more stable temperatures that may exhibit reduced growth rates but enhanced sporulation of *Venturia inaequalis* was seen, more likely due to prolonged exposure to conditions favorable for the pathogen's development. The variations are also supposed to be associated with the extensive application of fungicides targeting *V. 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 Roig et al. [13]. *V. inaequalis* is known to rapidly develop resistance to fungicides, a common phenomenon among plant pathogens worldwide [14]. 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.

4. 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Atil A. Apples 101: Nutrition facts and health benefits; 2019.
Available:https://www.
2. Anonymous. Ministry of Agriculture and Farmers Welfare, Department of Agriculture, Cooperation and Farmers welfare, Government of India, Agricultural Statistics at a Glance. 2020;117.
3. Anonymous. Directorate of Economics and Statistics Government of Jammu and Kashmir. Digest of Statistics. 2020a;167-169
4. Gupta SK, Gupta GK. Relative efficacy of scab fungicides in protective spray programme against summer diseases of apple during four years period. Pestology. 1992;16:28-31.
5. Sharma IM, Bhardwaj SS. Canker and foliar diseases of apple. In: Diseases of horticultural crops-fruits: (Eds. Verma LR, Sharma RC). Indus Publishing Company, New Delhi, India. 1999;724.
6. Sharma JN, Verma KD. Integrated management of foliar and fruit diseases of apple in Himachal Pradesh. Journal of Mycology and Plant Pathology. 2005;35: 368-380.
7. Holb IJ, Abonyi F, Buurma J, Heijne B. On-farm and on-station evaluations of three orchard management approaches against apple scab and apple powdery mildew. Crop Protection. 2017;97:109–118.

8. Masoodi KZ, Ahmed N, Mir MA, Bhat B, Shafi A, Mansoor S. Comparative transcriptomics unravels new genes imparting scab resistance in apple (Malus x domestica Borkh.). Functional & Integrative Genomics. 2022;22(6):1315-1330.
9. Jamar L, Lefrancq B, Lateu M. Control of apple scab (*Venturia inaequalis*) with bicarbonate salts under controlled environment. Journal of Plant Diseases and Protection. 2007;114(5):221–227.
10. Gonzalez-Domínguez E, Armengol J, Rossi V. Biology and epidemiology of Venturia species affecting fruit crops. Frontiers in Plant Science. 2017;8: 1496.
11. Sharma IM. Antagonistic effect of fungi associated with apple scab lesions on growth of its pathogen *Venturia inaequalis* (Cke.) Wint. Research Journal of Agricultural Sciences. 2010;1:245-248.
12. Berrie AM, Xu XM. Managing apple scab (*Venturia inaequalis*) and powdery mildew (*Podosphaera leucotricha*) using Adem. International Journal of Pest Management. 2003;49(3):243-249.
13. Roig E, Neumann P, Simon JP. Growth and isoenzyme comparison of fîve isolâtes of Venturia inaequalis. Phytoprotection. 1990;71(2):65-71.
14. Kavak H, Celik A. Comparison of some morphological and physiological characters of apple scab pathogen (*Venturia inaequalis*) in two different agricultural ecology of Turkey. Erwerbs-Obstbau. 2021;63(1).
15. Köller W, Parker DM, Turechek WW, Avila-Adame C, Cronshaw K. A two-phase resistance response of Venturia inaequalis populations to the QoI fungicides kresoxim-methyl and trifloxystrobin. Plant Disease. 2004;88(5):537-544.
16. Bowen JK, Mesarich CH, Bus VG, Beresford RM, Plummer KM, Templeton MD. Venturia inaequalis: the causal agent of apple scab. Molecular Plant Pathology. 2011;12(2):105-122.
17. Beresford RM, Wright PJ, Wood PN, Park NM, Larsen NJ, Fisher BM. Resistance of *Venturia inaequalis* to demethylation inhibitor and dodine fungicides in four New Zealand apple growing regions. New Zealand Plant Protection. 2013;66:274-283.

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