

Doi: [HTTPS://DOI.ORG/10.23910/2/2022.0454a](https://doi.org/10.23910/2/2022.0454a)

## Apple Scab (*Venturia inaequalis* Wint) Management Using a Novel Fungicide Combination in the North-Western Himalayas of India

Shalini Verma<sup>1\*</sup>, H. R. Gautam<sup>1</sup> and Kishore Khosla<sup>2</sup><sup>1</sup>Dept. of Plant Pathology, Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan, H.P. (173 230), India<sup>2</sup>College of Horticulture and Centre of Excellence for Horticultural Research and Extension, Thunag, Mandi, H.P. (175 048), India

### Corresponding Author

Shalini Verma

e-mail: shalinimpp@yaspuniversity.ac.in

### Article History

Article ID: IJEP0454a

Received on 01<sup>st</sup> January, 2022Received in revised form on 30<sup>th</sup> January, 2022Accepted in final form on 08<sup>th</sup> February, 2022

### Abstract

Apple (*Malus domestica*) is commercially most important horticultural crop grown in the north-western Himalayan region of India. The apple scab caused by fungi *Venturia inaequalis* (Cooke) Wint., is a devastating disease of apple aided by cool, moist climate during early spring. The present study on evaluation of effective fungicides against apple scab was undertaken in Himachal Pradesh during the years 2016 and 2017 under natural epiphytotic conditions. The application of carbendazim 25%+flusilazole 12.5% SE (0.08%) significantly decreased the apple scab disease in the present study. This resulted in maximum reduction of per cent conidia and conidial germination of *V. inaequalis* at the concentrations tested. It was superior and effective in comparison to other fungicides. Since the new combination of carbendazim 25% + flusilazole 12.5% SE exhibits systemic activity and both the fungicides have different modes of action, therefore, such a new combination can delay or prevent the build-up of resistance in the pathogen and can be effectively utilized as a promising fungicide for the control of apple scab disease.

**Keywords:** Apple, carbendazim, flusilazole, fungicides, scab, *Venturia inaequalis*

### 1. Introduction

Apple (*Malus domestica*) is the most important horticultural crop grown in the north-western Himalayan region of India. Apple is rich in phytonutrients, antioxidants, Vitamin-C,  $\beta$ -carotene and is consumed fresh or occasionally as a cooked product. In India, apple is primarily cultivated in Jammu and Kashmir, Himachal Pradesh and Uttarakhand states. It is also cultivated to a small extent in Arunachal Pradesh, Nagaland and Sikkim states of India. In India, apple occupies an area of about 307 t ha with a total production of 2.37 million metric tonnes and productivity of 7.72 mt ha<sup>-1</sup> (Anonymous, 2019a). Jammu and Kashmir is the leading state in area and production of apple with highest productivity followed by Himachal Pradesh, Uttarakhand and Arunachal Pradesh. In Himachal Pradesh, area under apple cultivation is 112.6 thousand hectares with a production of 368.6 thousand metric tonnes and productivity of 3.27 mt ha<sup>-1</sup> (Anonymous, 2019b).

Apple, like any other crop, is prone to a variety of diseases caused by fungi, bacteria, viruses, mycoplasmas etc. but fungal diseases are a major problem for commercial apple production in the temperate and humid regions. The fungal pathogens cause leaf spots, leaf blights, blossom blights, fruit spots, fruit rots, root rots, canker and post-harvest decays in

apple. The common foliar disease, apple scab caused by fungi *Venturia inaequalis* (Cooke) Wint., is a devastating disease of apple in the temperate regions of the world with cool, moist climate during early spring. It attacks fruits and leaves, which results in severe reduction in fruit quality and yield (Sandskar, 2003). The apple scab pathogen completes its life cycle in two stages, the imperfect (*Spilosea pomi*) stage on living plant parts and perfect or perithecial (*Venturia inaequalis*) stage on dead fallen leaves. Primary (ascospore) infections are usually limited to one or two distinct spots per leaf, whereas secondary (conidial) infections are often much more numerous. Secondary infections occasionally are so numerous that the entire surface of the leaf appears covered with scab.

The disease is endemic in areas having high humidity and rainfall during the spring and early summer months and pathogen perpetuates under shady and moist conditions (Thakur et al., 2004a, 2004b; 2005a, 2005b; 2008). The insufficient control of apple scab can cause direct infection of fruits and pedicels causing yield and economic loss of up to 70% of the production value (Gupta, 1992). The severe leaf damage can lead to a weakened tree with reduced flower bud formation (Thakur and Sharma, 1999). It can cause 100% yield loss if no control measures are applied (Belete and Boyraz, 2017). Since, growers, suppliers and vendors of apple crop



generally have a zero tolerance towards apple scab; any infection of the disease reduces the quality and marketable fruit yield (Percival and Boyle, 2005).

Among various methods, fungicidal management is the most effective method adopted by apple orchardists to protect their crops from fungal pathogens. The main strategy used for apple scab control in orchards is the frequent application of different recommended fungicides throughout the season. The long-term, extensive use of conventional fungicides has led to the selection pressure on scab pathogen and evolution of resistant strains against the presently recommended fungicides. This stable, heritable adjustment in pathogen populations to a fungicide limits the efficacy and shelf life of the fungicide. It can cause the abandonment of an entire class of fungicides with replacement programmes usually leading to time delays, decreased efficacy and higher control costs. However, the excessive use of fungicides may cause heavy residues on the fruits.

Resistance of micro-organisms to agricultural fungicides was not encountered until about 1970, when resistance development was reported in *V. inaequalis* to dodine from USA (Szkolnik and Gilpatrick, 1969). Since then, several studies have assessed fungicide resistance in *V. inaequalis* to kresoxim-methyl (Olaya and Koller, 1999a, b), myclobutanil (Koller et al., 1991; 1997), thiophanate-methyl (Wicks, 1974; Katan et al., 1983) and demethylation inhibitor fungicides (Braun, 1994).

Due to specific biochemical mode of action of systemic fungicides, single mutation in the target pathogen affects resistance. Consequently, resistance to these fungicides has developed more readily as compared to resistance to older, broad-spectrum protectant fungicides. It causes great apprehension in the farmer's mind regarding the reduced efficacy of these compounds against apple scab due to such a phenomenon when they fail to get desired control of disease and witness unexpected crop losses from their orchards. Benzimidazole fungicides such as carbendazim have been withdrawn from many markets because the pathogens have become resistant to these fungicides. The resistant strains of fungi possess altered  $\beta$ -subunits with a decreased affinity to benzimidazole (Davidse, 1986). However, no curative fungicides with varying modes of action have been introduced for apple scab control since the late 1990s (Chapman et al., 2011). The strategies to delay the development of resistance to the different classes of fungicides under field conditions rely on restricting the number of applications per season of fungicides in each class and mixing or alternating fungicides of different classes (Bowen et al., 2011). The newly developed fungicides having preventive and curative mode of action capable of restricting *V. inaequalis* development for long term can be used. Therefore, the present investigation was carried out to evaluate the bio-efficacy of some existing fungicides and their new combinations against apple scab caused by *V. inaequalis*.

## 2. Materials and Methods

The present study for evaluation of effective fungicides against apple scab was undertaken at the Regional Horticultural Research and Training Station, Seobag- Kullu, Himachal Pradesh, India during the years 2016 and 2017 under natural epiphytotic conditions. The field experiment was laid out in randomized block design on 120 apple plants of variety Starking Delicious of 16-18 years age in an unsprayed orchard having consistently high apple scab incidence in Barshaini area in Manikarn valley of Kullu district. Eight treatments viz. carbendazim 25% + flusilazole 12.5% SE (0.04, 0.06 and 0.08%), carbendazim 50% WP (0.025%), flusilazole 40% EC (0.01%), hexaconazole 5% EC (0.05%) and difenoconazole 25% EC (0.015%) including control (water spray) were undertaken. Each concentration of the fungicides was replicated thrice (five trees/replication). The spray was done four times at 15 days interval along with surfactant (Sandovit™) at 15 ml 10 L<sup>-1</sup>. Untreated check was maintained for comparison. The spray was initiated in the first week of May every year with the appearance of disease symptoms on leaves. For recording data of scab disease on leaves and fruits, sampling was done on 5 branches on each tree, chosen randomly round the trees to assess the percentage of scab on the trees as a whole. A visual key was adopted for the assessment of scab on apple leaves (Croxall et al., 1952a) and fruits (Croxall et al., 1952b) (Table 1 and 2).

Mean percentage of scabbed area per fruit was obtained by multiplying the number of fruits with the mean scab percentage of the group and then adding the product and dividing the figure by the total number of fruits examined.

The per cent disease index (PDI) was calculated according to the equation given by McKinney (1923) as follows:

$$PDI = \frac{\text{Class rating} \times \text{Number of leaves/fruits in a particular class}}{(\text{Total number of leaves/fruits observed}) \times \text{Highest class rating}} \times 100$$

The per cent disease control (PDC) was calculated by adopting the below mentioned method and data were subjected to analysis of variance to calculate the critical significant differences amongst the treatments.

$$PDC = \frac{\text{Disease index in control treatment} - \text{Disease index in treatment}}{\text{Disease index in control (unsprayed) treatment}} \times 100$$

### 2.1. Evaluation of post symptom activity

The post symptom activity is the action of fungicides applied to the plant after the appearance of disease symptoms which arrests or inhibits further progress of the disease. For evaluating such activity, leaves with sporulating lesions from five terminals of each tree in a block of three trees per treatment were selected. The conidia were removed from the sporulating lesions by the pressure of water (4 kg cm<sup>-2</sup>) applied



Table 1: Visual key for the assessment of scab on apple leaves (Croxall et al., 1952a)

Rating (%)	Type and extent of infection
0.01	Occasional units showing scab on 1 or 2 leaves
0.05	Scab spots on one or two leaves in each unit
0.25	Every unit shows up to 5 small spots or their equivalent on about ¼ of the leaves on each unit
0.50	Every unit shows up to 5 small spots or their equivalent on about ½ of the leaves on each unit
1.00	Majority of the leaves infected, a few with about 25% of their area covered with spots
5.00	Almost every leaf infected with the scab areas covering approximately 25% of leaf surface on about ¼ of the leaves
10.00	Every leaf infected with the scab areas covering approximately 25% of leaf surface on about ½ of the leaves
25.00	Every leaf infected with the scab areas covering approximately 50% of leaf surface on about ½ of the leaves
50.00	All leaves infected with scab areas covering almost the entire surface on about 50% of the leaves

Table 2: Visual key for the assessment of scab on apple fruits (Croxall et al., 1952b)

Rating (%)	Type and Extent of infection
0.01	Occasional units showing one or two fruits
0.10	Small scab spots on one or two fruits in each unit
0.50	Small scab spots on about one quarter of the fruit
1.00	Up to 5 small scab spots on about ½ of the fruit in each unit, no cracking
5.00	Scab spots on about ¾ of the fruit in each unit, occasional fruits may show cracking
10.00	Every fruit in each show scabbed area with few cracks
25.00	Every fruit in each unit with large scab spots with up to 1/3 of fruits cracked, fruits more variable in size and on the whole slightly smaller than fruits from the healthy ones
50.00	Every fruit in each unit with large scab spots with up to 1/3 of the fruits severely cracked and of non-marketable grade. Fruits appreciably smaller than that from healthy trees.

by a paint gun sprayer. Afterwards, such leaves were sprayed with a freshly prepared suspension of the test fungicides at the desired rate up to runoff. Polythene bags were tied loose over the terminals to prevent subsequent washout by natural rains. The control trees were left unsprayed for comparison. One leaf per shoot was removed after 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> day of first spray and 9<sup>th</sup>, 11<sup>th</sup> and 14<sup>th</sup> day after giving second spray on the 7<sup>th</sup> day. Five leaf lesion discs of 5 mm diameter were removed with the help of a cork borer and immersed in 5 ml of distilled water. After shaking for one minute, the content was decanted through a muslin cloth. The number of conidia per lesion surface of 5 mm was determined with the help of a haemocytometer and the average of 5 readings was recorded. The per cent reduction in number of conidia was worked out by the formula given below:

$$\text{Per cent conidia number reduction} = \frac{\text{Number of conidia in control} - \text{Number of conidia in treatment}}{\text{Number of conidia in control}} \times 100$$

The viability of conidia was observed by placing 0.1 ml of spore suspension in each of 3 cavity slides maintained in a Petri dish moist chamber and incubated at 20°C for 24 h. The per cent inhibition of conidial germination was calculated by the formula given below:

$$\text{Per cent spore germination inhibition} = \frac{\text{Spore germination in control} - \text{Spore germination in treatment}}{\text{Spore germination in control}} \times 100$$

The data pertaining to experiments were subjected to analysis of variance to calculate the critical differences amongst the treatments as per Gomez and Gomez (1984).

### 3. Results and Discussion

#### 3.1. Bio-efficacy of fungicides against apple scab (*Venturia inaequalis*)

During 2016 and 2017 crop season (Table 3), all the treatments differed significantly with regard to per cent disease index and per cent disease control on leaves and fruits in comparison to untreated control. The minimum disease index (PDI) (0.00%) on leaves occurred for both seasons when the apple crop was sprayed with *carbendazim 25%+flusilazole 12.5% SE* at a concentration of 0.08%. During 2016, it was followed by *carbendazim 25%+flusilazole 12.5% SE* at 0.06% (0.04 PDI), flusilazole 40% EC at 0.01% (0.22 PDI) and difenoconazole 25% EC at 0.015% (0.39 PDI), latter two statistically at par with each other. However, during 2017, *carbendazim 25%+flusilazole 12.5% SE* at a concentration of 0.08% (0.00 PDI) was followed by difenoconazole 25% EC at 0.015% (0.62 PDI) and *carbendazim 25%+flusilazole 12.5% SE* at 0.06% (0.80 PDI) which were statistically at par with each other. The cent per cent mean per cent disease control (PDC) on leaves was

Table 3: Bio-efficacy of fungicides against apple scab (*V. inaequalis*) under field conditions

Treatment	Dose (10 l <sup>-1</sup> of water)	Concentration (%)	Percent Disease Index (20 days after 4 <sup>th</sup> spray)				Percent Disease Control (20 days after 4 <sup>th</sup> spray)						
			2016		2017		2016		2017		Mean	Mean	
			Leaves	Fruits	Leaves	Fruits	Leaves	Fruits	Leaves	Fruits	Leaves	Fruits	Leaves
Carbendazim 25% + flusilazole 12.5% SE	0.015	0.04	1.75 (7.59) <sup>e</sup>	4.71 (12.51) <sup>e</sup>	0.86 (5.31) <sup>c</sup>	1.55 (7.13) <sup>c</sup>	89.76	87.46	88.61	92.75	93.36	93.06	
Carbendazim 25% + flusilazole 12.5% SE	0.022	0.06	0.04 (1.18) <sup>b</sup>	0.80 (5.11) <sup>b</sup>	0.53 (4.15) <sup>b</sup>	0.35 (3.31) <sup>b</sup>	99.76	97.87	98.82	95.53	98.50	97.02	
Carbendazim 25% + flusilazole 12.5% SE	0.03	0.08	0.00 (0.00) <sup>a</sup>	0.00 (0.00) <sup>a</sup>	0.00 (0.00) <sup>a</sup>	0.00 (0.00) <sup>a</sup>	100.00	100.00	100.00	100.00	100.00	100.00	
Carbendazim 50% WP	0.0125	0.025	6.02 (14.18) <sup>f</sup>	10.91 (19.25) <sup>f</sup>	3.99 (11.51) <sup>d</sup>	7.78 (16.16) <sup>d</sup>	64.77	70.97	67.87	66.38	66.71	66.55	
Flusilazole 40% EC	0.004	0.01	0.22 (2.69) <sup>c</sup>	3.67 (11.02) <sup>d</sup>	0.00 (0.00) <sup>a</sup>	0.00 (0.00) <sup>a</sup>	98.71	90.23	94.47	100.00	100.00	100.00	
Hexaconazole 5% EC	0.0025	0.05	0.99 (5.71) <sup>d</sup>	1.19 (6.24) <sup>c</sup>	0.35 (3.36) <sup>b</sup>	0.35 (3.32) <sup>b</sup>	94.20	96.83	95.52	97.05	97.05	97.05	
Difenoconazole 25% EC	0.004	0.015	0.39 (3.57) <sup>c</sup>	0.62 (4.51) <sup>b</sup>	0.00 (0.00) <sup>a</sup>	0.00 (0.00) <sup>a</sup>	97.71	98.35	98.03	100.00	100.00	100.00	
Untreated Control	-	-	17.09 (24.38) <sup>g</sup>	37.58 (37.58) <sup>g</sup>	11.87 (20.11) <sup>e</sup>	23.37 (28.87) <sup>e</sup>							
CD (p=0.05)			(1.01)	(1.42)	(1.11)	(1.39)							

Figures in parentheses are angular transformed values; Figures with different alphabets differ significantly

recorded in carbendazim 25% + flusilazole 12.5% SE at 0.08%. It was followed by carbendazim 25% + flusilazole 12.5% SE at 0.06% (98.82 PDC), difenoconazole at 0.015% (98.03 PDC), hexaconazole 5% EC at 0.05% (95.52 PDC) and flusilazole 40% EC at 0.01% (94.47PDC) on leaves. The minimum mean per cent disease control (67.87) on leaves was recorded in carbendazim 50% WP at 0.025% concentration.

All the fungicide applications on apple trees significantly reduced per cent disease on fruits in comparison to untreated control during 2016 and 2017. The minimum (0.00%) per cent disease index (PDI) on fruits occurred for both seasons when the apple crop was sprayed with carbendazim 25%+flusilazole 12.5% SE at a concentration of 0.08%, flusilazole 40% EC at 0.01% and difenoconazole 25% EC at 0.015%. These were followed by carbendazim 25%+flusilazole 12.5% SE at 0.06% (0.53 PDI) and hexaconazole 5% EC

(0.35PDI) which were statistically at par with each other, during 2016. Similarly, during 2017, these were followed by carbendazim 25%+flusilazole 12.5% SE at 0.06% (0.35 PDI) and hexaconazole 25% EC at 0.015% (0.35 PDI) which were statistically at par with each other. The 100% mean per cent disease control (PDC) on fruits was recorded in carbendazim 25%+flusilazole 12.5% SE at 0.08%, flusilazole 40% EC at 0.01% and difenoconazole at 0.015%. It was followed by carbendazim 25%+flusilazole 12.5% SE at 0.06% (97.02 PDC) and hexaconazole 5% EC at 0.05% (97.05 PDC). The minimum mean per cent disease control (66.55) on fruits was recorded in carbendazim 50% WP at 0.025%.

The pre-mixture combination of carbendazim 25%+flusilazole 12.5% SE was evaluated under field conditions along with the already recommended standard fungicides. The site of action of carbendazim (benzimidazoles) are microtubules



which form an essential part of the fungal cytoskeleton and are active in spindle formation and the segregation of chromosomes in cell division. It alternates helices of  $\beta$ - and  $\alpha$ -tubulins affecting tubulin integrity and consequent degradation of fungal cytoskeleton. It specifically binds to the 3-subunit of tubulin after entering the nucleus and inhibits the dimerization of the  $\alpha$  and 3-subunits to a functional tubulin unit. This disrupts mitosis during cell division at metaphase by distorting the mitotic spindle causing failure of separation of daughter nuclei and consequent cell death (Davidse, 1986). Molecular biology techniques have confirmed  $\beta$ -tubulin as the target site (Fujimura et al., 1990) of carbendazim. The other compound in the combination viz., flusilazole (DMI) acts through the inhibition of the sterol biosynthesis in fungal membrane by removal of the C14-methyl group from 24-methylenedihydrolanosterol or eburicol. This leads to

subsequent accumulation of precursor sterols and reduction in ergosterol biosynthesis. Ergosterol plays a unique role in the maintenance of fungal membrane function, and reduction in its availability results in membrane disruption and electrolyte leakage (Oliver and Hewitt, 2014).

### 3.2. Evaluation of post symptom activity

The perusal of data (Table 4) indicates that the mean maximum reduction of per cent conidial number of *V. inaequalis* was caused by difenoconazole (68.0%) at 0.015% followed by hexaconazole (56.3%) at 0.05%, and carbendazim 25%+flusilazole 12.5% SE (43.9%) at 0.08% while the mean minimum reduction of per cent conidial number was shown by carbendazim (18.5%) at 0.025%. Similarly, the perusal of data (Table 5) indicates that the mean maximum reduction of per cent conidial germination of *V. inaequalis* was shown by

Table 4: In vitro per cent reduction in conidial number of *V. inaequalis* by fungicides

Fungicides	Concentration (%)	Percent reduction in conidial number (days after treatment)							
		1	3	5	7	9	11	14	Mean
Carbendazim 25% + flusilazole 12.5% SE	0.04	16.6 (24.1)	20.8 (27.1)	24.3 (29.5)	26.1 (30.7)	40.7 (39.6)	37.1 (37.5)	32.9 (34.9)	28.3 (31.9) <sup>f</sup>
Carbendazim 25% + flusilazole 12.5% SE	0.06	18.8 (25.7)	24.9 (29.9)	27.8 (31.8)	28.7 (32.4)	35.9 (36.8)	41.3 (39.9)	35.2 (36.4)	30.3 (33.3) <sup>e</sup>
Carbendazim 25% + flusilazole 12.5% SE	0.08	27.4 (31.5)	33.0 (35.1)	39.8 (39.1)	42.3 (40.5)	54.0 (47.3)	57.0 (49.0)	53.8 (47.1)	43.9 (41.3) <sup>c</sup>
Carbendazim 50% WP	0.025	9.1 (17.5)	11.1 (19.4)	16.3 (23.8)	20.0 (26.6)	24.9 (29.9)	25.9 (30.6)	22.2 (28.0)	18.5 (25.1) <sup>g</sup>
Flusilazole 40%EC	0.01	20.4 (26.8)	25.1 (30.0)	28.8 (32.4)	29.5 (32.8)	43.3 (41.1)	48.8 (44.2)	48.3 (43.9)	34.8 (35.9) <sup>d</sup>
Hexaconazole 5% EC	0.05	43.1 (41.0)	55.2 (47.9)	64.9 (53.7)	49.8 (44.9)	61.2 (51.4)	68.2 (55.6)	51.8 (46.0)	56.3 (48.6) <sup>b</sup>
Difenoconazole 25% EC	0.015	50.1 (45.0)	58.8 (50.1)	73.6 (59.0)	63.5 (52.8)	84.7 (66.9)	81.3 (64.3)	64.1 (53.1)	68.0 (55.9) <sup>a</sup>
Mean		46.9 (30.2)	56.0 (34.2)	49.2 (38.5)	44.4 (37.2)	59.9 (44.7)	54.9 (45.9)	44.0 (41.4)	
CD ( $p=0.05$ )									1.5

Figures in parentheses are angular transformed values; Figures with different alphabets differ significantly

difenoconazole at 0.015% (83.5%) followed by carbendazim 25%+flusilazole 12.5% SE at 0.08% (71.8%) and hexaconazole at 0.05% (68.2%) while the mean minimum reduction of per cent conidial germination was shown by carbendazim at 0.025% (4.8%).

In the present investigation, triazole fungicides have shown maximum reduction of per cent conidial number and conidial germination of *V. inaequalis*, as they are sterol demethylation inhibitors which prevent the development of the fungus by inhibiting cell membrane ergosterol biosynthesis closely followed by the new combination carbendazim 25%+flusilazole 12.5% SE as it acts through two different modes

of action. These results demonstrate an inhibitory effect of these fungicides early in the development of fungi and are in agreement with previous studies demonstrating that triazole fungicides have the same primary mode of action namely the impairment of membrane production through the inhibition of ergosterol production (Akers et al., 1990). The inhibition of ergosterol production and consequent reduction in fungal growth apparently also deprived *V. inaequalis* of the ability to induce papilla formation in the host. These morphological changes in test fungi demonstrated delayed spore formation and germinations at the end of the experiment.





Table 5: *In vitro* per cent reduction in conidial germination of *V. inaequalis* by fungicides

Fungicides	Concentration (%)	Percent reduction in conidial number (days after treatment)								
		1	3	5	7	9	11	14	Mean	
Carbendazim 25%+flusilazole 12.5% SE	0.04	31.8 (34.3)	47.3 (43.4)	36.9 (37.4)	50.7 (35.6)	51.9 (46.1)	50.1 (45.1)	34.0 (35.6)	43.3 (39.7) <sup>e</sup>	
Carbendazim 25%+flusilazole 12.5% SE	0.06	42.8 (40.8)	54.7 (47.7)	48.0 (43.8)	43.5 (41.2)	59.5 (50.5)	55.7 (48.2)	45.8 (42.5)	50.0 (45.0) <sup>d</sup>	
Carbendazim 25%+flusilazole 12.5% SE	0.08	65.2 (53.8)	74.4 (59.6)	71.4 (57.6)	66.3 (54.4)	81.9 (64.8)	77.8 (61.8)	66.1 (54.4)	71.8 (58.1) <sup>b</sup>	
Carbendazim 50% WP	0.025	3.8 (11.28)	4.8 (12.7)	5.7 (13.8)	3.9 (11.3)	6.3 (14.5)	5.1 (13.1)	3.9 (11.4)	4.8 (12.6) <sup>g</sup>	
Flusilazole 40%EC	0.01	26.8 (31.2)	41.1 (39.8)	36.1 (36.8)	33.3 (35.2)	43.3 (41.1)	38.9 (38.6)	33.3 (35.2)	36.1 (36.9) <sup>f</sup>	
Hexaconazole 5% EC	0.05	70.1 (56.8)	76.5 (61.0)	63.1 (52.6)	53.2 (46.8)	81.2 (64.3)	72.3 (58.3)	61.2 (51.5)	68.2 (55.8) <sup>c</sup>	
Difenoconazole 25% EC	0.015	88.3 (70.05)	93.2 (74.9)	83.2 (65.8)	76.8 (61.2)	95.3 (77.5)	84.1 (66.5)	63.8 (53.0)	83.5 (66.9) <sup>a</sup>	
Mean		46.9 (42.6)	56.0 (48.4)	49.2 (44.0)	44.4 (40.9)	59.9 (51.3)	54.9 (47.4)	44.0 (40.6)		
CD ( $p=0.05$ )									1.6	

Figures in parentheses are angular transformed values; Figures with different alphabets differ significantly

The new combination of carbendazim 25%+flusilazole 12.5% SE exhibits systemic activity, and both the fungicides in this combination have different modes of action. Therefore, such a new combination can delay or prevent the build-up of resistance in the pathogen and can be effectively utilized as a promising preventive fungicide for the control of apple scab disease. The present results show that the field application of carbendazim 25%+flusilazole 12.5% SE has significantly decreased the apple scab disease and at the same time it also showed maximum reduction of per cent conidial number and conidial germination of *V. inaequalis* at the tested dose. It was found effective and superior to other evaluated fungicides.

#### 4. Conclusion

The use of pre-mixture compounds or fungicides that act through different modes of action will most likely gain importance in future apple scab management strategies. This will certainly reduce the time, labour and control cost of the growers in the event of occurrence of disease during the crop growth period. The present study concludes that four sprays of carbendazim 25%+flusilazole 12.5% SE at 0.08% at 15 days' interval beginning first week of May is an effective treatment against the control of apple scab in Himachal Pradesh. This combination was highly effective and gave maximum per cent disease control of apple scab in orchards.

#### 5. References

Akers, A., Koehle, H.H., Gold, R.E., 1990. Uptake, transport and mode of action of BAS 480 F, a new triazole fungicide.

- Proceedings of the Brighton Crop Protection Conference Farnham, UK: British Crop Protection Council, 837–45.
- Anonymous, 2019a. Area and production of horticulture crops for 2018–19 (National Horticulture Board). <http://nhb.gov.in/Statistics.aspx?enc=WkegdyuHokljEtehJJoqOKWLU79sOQCy+W4MfOk01GFOVQSEvtp9tNHHoiv3p49g> [10.36 AM, 17 December 2021].
- Anonymous, 2019b. Horticulture at a glance 2018-19 (State Department of Horticulture). <http://www.hpgriset.net.gov.in/hpgriset/Horticulture/Default.aspx?SiteID=5&pAGEid=1219> [10.36 AM, 17 December 2021].
- Belete, T., Boyraz, N., 2017. Critical review on apple scab (*Venturia inaequalis*) biology, epidemiology, economic importance, management and defense mechanisms to the causal agent. *Journal of Plant Physiology and Pathology* 5, 1–11.
- Bowen, J., Mesarich, C., Bus, V., Beresford, R., Plummer, K., 2011. *Venturia inaequalis*: the causal agent of apple scab. *Molecular Plant Pathology* 12, 105–122.
- Braun, P.G., 1994. Development and decline of a population of *Venturia inaequalis* and disease buildup. *Plant Disease* 84, 1319–1326.
- Chapman, K.S., Sundin, G.W., Beckerman, J.L., 2011. Identification of resistance to multiple fungicides in field populations of *Venturia inaequalis*. *Plant Disease* 95, 921–926.
- Croxall, H.E., Gwynne, D.C., Jenkins, J.E.E., 1952a. The rapid assessment of apple scab on leaves. *Plant Pathology* 1, 39–41.



- Croxall, H.E., Gwynne, D.C., Jenkins, J.E.E., 1952b. The rapid assessment of apple scab on fruit. *Plant Pathology* 1, 89–92.
- Davidse, L.C., 1986. Benzimidazole fungicides: mechanism of action and biological impact. *Annual Reviews of Phytopathology* 24, 43–65.
- Fujimura, T., Esteban, R., Esteban, L.M., Wickner, R.B., 1990. Portable encapsidation signal of the L-A double stranded RNA virus of *S. cerevisiae*. *Cell* 62, 819–828.
- Gomez, K.A., Gomez, A.A., 1984. Statistical procedures for agricultural research. Second Edition, John Wiley and Sons Inc, New York, USA. 680pp.
- Gupta, G.K., 1992. Apple scab (*Venturia inaequalis*) in India. In: David, B.V. (Ed), Pest management and pesticides- indian scenario, Namrutha Publication, Madras, pp. 67–76.
- Katan, T., Shabi, E., Gilpatrick, J.D., 1983. Genetics of resistance to benomyl in *Venturia inaequalis* isolates from Israel and New York. *Phytopathology* 73, 600–603.
- Koller, W., Parker, D.M., Reynolds, K.L., 1991. Baseline sensitivities of *Venturia inaequalis* to sterol demethylation inhibitors. *Plant Disease* 75, 726–728.
- Koller, W., Wilcox, W.F., Barnard, J., Jones, A.L., Braun, P.G., 1997. Detection and quantification of resistance of *Venturia inaequalis* populations to sterol demethylation inhibitors. *Phytopathology* 87, 184–190.
- McKinney, H.H., 1923. Influence of soil temperature and moisture on the influence of wheat seedlings by *Helminthosporium sativa*. *Journal of Agricultural Research* 26, 196–217.
- Olaya, G., Koller, W., 1999a. Baseline sensitivities of *Venturia inaequalis* populations to the strobilurin fungicide kresoxim-methyl. *Plant Disease* 83, 274–278.
- Olaya, G., Koller, W., 1999b. Diversity of kresoxim-methyl sensitivities in baseline populations of *Venturia inaequalis*. *Pesticide Science* 55, 1083–1088.
- Oliver, R., Hewitt, H.G., 2014. Fungicide performance. In: *Fungicides in Crop Protection*; CABI, UK. 75–91pp.
- Percival, G.C., Boyle, S., 2005. Evaluation of microcapsule trunk injections for the control of apple scab and powdery mildew. *Annals of Applied Biology* 147, 119–127.
- Sandskar, B., 2003. Apple scab (*Venturia inaequalis*) and pests in organic orchards. PhD Thesis, Swedish University of Agricultural Sciences, Alnarp, 39pp.
- Szkolnik, M., Gilpatrick, J.D., 1969. Apparent resistance of *Venturia inaequalis* to dodine in New York apple orchards. *Plant Disease Reporter* 53, 861–864.
- Thakur, V.S., Sharma, R.D., 1999. Apple scab and its management. In: Verma, L.R., Sharma, R.C. (Eds), *Diseases of horticultural crops- fruits*. Indus Publishing Company, New Delhi, pp 54–75.
- Thakur, V.S., Sharma, N., Verma, S., 2005a. Effect of meteorological factors on the development of lesion density in apple scab epidemic in Himachal Pradesh. *The Horticultural Journal* 18, 4–9.
- Thakur, V.S., Sharma, N., Verma, S., 2005b. Present scenario of apple scab in various apple orchards of Himachal Pradesh. *The Horticultural Journal* 18, 134–136.
- Thakur, V.S., Verma, S., Sharma, N., 2004a. Role of meteorological factors in the development of *Venturia inaequalis* pseudothecial protoplasmic content in Himachal Pradesh. *Advances in Plant Science* 21, 269–274.
- Thakur, V.S., Verma, S., Sharma, N., 2004b. Epidemic analysis of saprophytic stages of *Venturia inaequalis* to forecast primary inoculum and infection conditions in Himachal Pradesh. *Plant Disease Research* 20, 131–137.
- Thakur, V.S., Verma, S., Sharma, N., 2008. Role of pseudothecial contents in pseudothecial of *Venturia inaequalis* to monitor apple scab epidemic in Himachal Pradesh. *Advances in Plant Science* 21, 265–270.
- Wicks, T., 1974. Tolerance of the apple scab fungus to benzimidazole fungicides. *Plant Disease Reporter* 60, 818–819.

