*Int.J.Curr.Microbiol.App.Sci* (2018) *7*(8): 575-586

International Journal of Current Microbiology and Applied Sciences
*ISSN: 2319-7706* **Volume 7 Number 08 (2018)**
Journal homepage: http://www.ijcmas.com

**Original Research Article** https://doi.org/10.20546/ijcmas.2018.708.063

# Pre Leaf Fall Spray of Chemical, Cow Urine and Fungal Antagonists on Spring Ascospore Production of the Apple Scab Pathogen, *Venturia inaequalis*

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**Keywords**

*Venturia inaequalis* (Cooke.) G. Wint, antagonists, Cow urine, Uria apple

**Article Info**

***Accepted:*** 06 July 2018
***Available Online:*** 10 August 2018

**A B S T R A C T**

A scabbed leaf collected on 30 April was the most effective date that results 25 percent partial decomposition. The partial decomposition rate was observed low in the month of May with collected leaves on 30 Dec. (0.37 %), 30 Jan. (3.51 %), 28 Feb. (9.76%), and 30 March (18.01 %) at Jochira, Gangotri Fruit valley of District Uttarkashi, Uttarakhand. The interaction between collection date and category was found highly significant. Eighteen micro-organisms were isolated and identified to parasitize saprophytically on apple leaves but only four isolates namely, *Athelia bombacina*, *Trichoderma harzianum*, *Chaetomium globosum*, and *Myrothecium roridum* were further evaluated for their effect on reducing primary inoculums of *V. inaequalis*. A single pre-leaf fall spray of 5 % urea was significantly decomposed the over wintered leaves, and were on par or superior in effectiveness as compared to the 100 % cow urine, antagonists and carbendazim. Similarly, the application of urea at 3 and 5 percent was significantly proved better over others for reducing the pseudothecial formation (97.31, 78.72 %) and the discharge of ascospores (98.04, 95.66 %) from over wintered leaves. Among the four antagonists, *A. bombacina* was highly effective in reducing the development of pseudothecia (69.57%) and ascospore productivity (84.31%).The application of Sterol-biosynthesis inhibiting fungicides at a lower dose gave maximum (83 to 89 %) inhibition of ascospores discharge as compared to other fungicides was also recorded. This study indicated that urea/ antagonist (*Athelia bombacina*) spray could be safely applied during leaf fall stag of apple tree and orchard floor for the management of primary inoculum of *V. inaequalis*

## Introduction

Scab, caused by the fungal pathogen *Venturia inaequalis* (Cooke.) G. Wint, is considered to be the single most important disease of apple *(Malus domestica Borkh.)* is several production areas of the world. The pathogen survives during the winter in the diseased leaves that fall on the floor of orchard. As the temperature rises above 10°C in late February to March, the fungus enter the sexual stage and produces black structure, pseudothecial initial within the leaf tissues. Following a distinct rest, the pseudothecium continues to mature resulting in the development of asci and ascospores. Ascospores are the primary sources of the inoculum in most of the apple growing countries including. India (Singh, 2005; Gupta, 1975, 1979; Thakur and Sharma, 1999; Singh, 2006). Most research on scab has been focused on the control of primary infection and has resulted in spray schedules that essentially are based upon weather condition that influence ascospore maturation (Gadoury and MacHardy, 1986; Singh and Kumar, 1999) and infection (MacHardy and Gadoury, 1986), regardless of the inoculum potential. The relation between the reductions of the primary inoculum and scab severity has been clearly demonstrated by several authors (MacHardy, 1996; MacHardy *et al.,* 2001; Palmiter, 1946; Gadoury and MacHardy, 1986; Singh *et al.*, 1995; Singh and Pal, 1996). The effect of various chemicals, including beneficial micro-organism form soil and Urea (Carisse *et al.,* 2000; Gupta, 1987a & b, 1989; Thakur and Gupta, 1991; Singh, 2006; Thakur and Sharma, 1999) were investigated. Use of microorganisms as biocontrol agents against *V. inaequalis* is also likely to be least expensive and safer then the chemicals. Biological control avoids development of pathogen strains resistant to fungicides and bactericides, which has became a major problem throughout the world (Dekker and Geogopolous, 1982). Over 117 species of fungi have been isolated from orchards of several apple-producing regions of Uttarakhand and some of these fungal isolates were effective for the inhibition of pseudothecia and ascospores production using in vitro leaf disc assays (Singh, 2006). Carisse *et al.,* (2000) studied the potential of the five fungal isolates to reduce the inoculum production of the *V. inaequalis* under orchard conditions and to compare them with known antagonists, *Athelia bombacina* and Urea. Work so far done in India has remained confined to the use of chemicals and fungal antagonists in the pre leaf fall spray so as to break the life cycle of apple scab pathogen and obtained effectively in the control of primary scab (Gupta, 1989; Thakur and Sharma, 1999; Verma and Gupta, 1992; Singh and Kumar, 1999; Singh, 2006). The objective of this study also undertaken with the same aim to evaluate the efficacy of different group of new chemicals and of an organic substance against the ascigerous stage of *V. inaequalis*. Another objective was to evaluate the potential of the four fungal isolates to reduce airborne ascospore production under orchard conditions and to compare them with urea and EBI fungicides.

## Materials and Methods

### Leaf litter decomposition

Over wintering senescent apple leaves, which were severely infected with scab, were collected on last week of November 2004 and 2005 from unsprayed orchards located at Jochira, Gangotri Fruit valley of District Uttarkashi, Uttarakhand. 10 gm of senescent fallen apple leaves were kept in mushlene cloth bag (35.5 × 27.5 cm, 1 mm mesh size), which represented 5 treatments consisting of 3 replications. These samples were allowed to overwinter on the orchard floor. At an interval of 30 days, 3 mesh bags were brought to the laboratory for recording leaf litter decomposition and isolation of fungi by serial dilution method.

The leaf litter decomposition rate was assessed at five intervals on 30 December, 30 January, 28 February, 30 March and 30 April. Each leaf was catagorised (1- 4 scale) according to decomposition of leaf contents (%) as 1, intact; 2, partially decomposed; 3, complete decomposition of lamina with left-out midribs and 4, complete decomposition. Non - treated sample were maintained for composition as checks.

Antagonists were collected from Plant Pathology section, College of Forestry & Hill

Agriculture, Hill Campus, Ranichauri, Tehri. These antagonists were isolated and identified by Singh (2006). The same antagonists were grown on potato dextrose agar medium at 25ºC for 14 days in sterilized Petri plates. The concentrations of antagonists of selected isolate were prepared in 0.01M phosphate buffer with the help of haemocytometer.

## Application of urea, cow urine and spore suspension of antagonists

Scab infected leaves collected from Jochira, were treated with freshly Urea, fungal isolates, Cow urine and broad-spectrum fungicides, Carbendazim. Total nine treatments including control were given to the overwintering leaves of Gangorti fruit belt. Each treatment consisted of three replications and in each replication 10 gm of over wintering leaves were taken. Hundred ml of Urea solution, spore suspension of each antagonists, Cow urine and fungicides were applied in the university laboratory (Jhalla) by spraying both surface of leaves (adaxial and abaxial) spread over the sterilized paper of each treatment. The each treatment were applied with the help of a hand operated atomizer operating at discontinues 0.93 dynes per square centimeter pressure until they were uniformly wet. In the control 100 ml of sterilized distilled water was sprayed.

The treated leaves were allowed for half an hour to dry at room temperature. Each replication was then sealed in square nylon mesh bag and was left after treatment on the same day of its collection to overwinter on the surface of the orchard floor in a randomized Complete Block Design. The blocks represented different locations on the ground in the apple orchard. These nylon mesh bags containing leaves were allowed to over winterd on the orchard floor till the pink bud to petal fall stage of apple (last week of April to May, 2006 and 2007) in the subsequent spring as suggested by Westwood (1978). Overwintered treated and untreated leaves in nylon mesh bag were brought to the laboratory for extent of decomposition, pseudothecial maturity and ascospore productivity.

EBI fungicides were evaluated in pre-leaf fall sprays at Jochira of Gangotri fruit belt. Each test chemicals were sprayed on apple trees a few days prior to general leaf fall in 3rd week of November and 200 leaves per treatment packed in nylon mesh bags were allowed to overwinter on orchard floor. These leaves were brought to the laboratory during next year in the last week of April and May, and were examined for ascospore discharged and productivity.

## Results and Discussion

The effect of collection date on decomposition category irrespective of location also revealed that 11.33 per cent leaves were partially decomposed falling in category 2, whereas 88.68 percent leaves remained intact. With the progress of over wintering as observed in the leaves collected on 30 December, only 0.37, 3.51, 9.76, 18.01, and 25.00 per cent leaves were partially decomposed on 30 January, 28 February, 30 March and 30 April in both the year. The complete decomposition of leaves with left out midribs and complete decomposition with left out petioles falling in the categories 3 and 4 were not observed at all the locations (Table 1). The interaction between collection date and category which was found significant ($CD_{0.05}$ = 5.13) and further confirms the results earlier reported by Burchill (1968) and Ross and Burchill (1968).

## Effect of urea, antagonists, Cow urine and fungicides

All the treatment were found to be effective in apple leaf litter decomposition significantly and reducing the ascigerous stage of *V.*

*inaequalis* (Table 2). The overall mean values indicated that Urea (5%) sprayed leaves was most effective for rate of decomposition as per categories (1-4), was 3.06 per cent leaves were intact, 8.38 percent partially decomposed, 14.52 per cent left with mid-ribs and 75.94 percent completely decomposed as compared to 74.64 per cent intact and 25.95 partially decomposed leaves in untreated samples (Fig. 1).

The decomposition rate for carbendazim and cow urine samples indicated 24.38 and 20.24 percent leaves were completely decomposed with left out petiole (Category 4) followed by 50 per cent leaves falling in category 3 (complete decomposition of lamina with left out midribs) and category 2 (partially decomposed), respectively. The interaction between treatment and category further revealed that antagonists (*A. bombacina*, *T. harzianum*, *C. globouom*, and *M. roridum)* treated leaves showed to be similar decomposition in categories 4, 3, 2, and 1, respectively. The pooled mean values of *Myrothecium roridum* indicated that 17.99, 47.00, 24.90, and 10.39 percent leaves followed by *Athelica bombacina* 10.62, 29.79, 51.22, and 8.94 percent leaves were observed in categories 1, 2, 3 and 4, respectively (Fig 1).

The present findings thus reveal significant effect between the treatments and their decomposition. Maximum decomposition of overwinterd apple leaves was observed in Urea (5%) followed by Urea (3%) and cow urine. Similar results have been seen in urea for initiating leaf decomposition (Burchill, 1968; Singh and Kumar, 1999) and enhancing leaf microflora for competitive degradation and decomposition (Ross and Burchill, 1968; Singh, 2006). Gadoury and MacHardy (1982) obtained similar results who suggested that the cold temperature climates characteristics of apple growing area, do not favour rapid leaf decomposition and hence pose a major obstacle to a beneficial effect to urea treatment (Carisse *et al.*, 2000). The minimum leaf decomposition was observed in *Trichoderma harzianum* where 5.88 percent leaves where completely decomposed followed by the 32.49, 43.12, and 18.92 percent leaves falling in category 3, 2, and 1, respectively. Ruinen (1961) and Preece and Dickinson (1976) also reported that phylloplane fungi, which contribute in the colonization of leaves, also contribute in the decomposition of leaves after senescence.

The mean values of *Athelia bombacina* and *Chaetomium globosum* showed 8.92 percent leaves were completely decomposed with left out petiole followed by 46.79 left with midrib portion, 33.94 percent partial and 11.38 percent intact leaves as compared to 74.64 percent intact and 25.95 percent partially decomposed in checks. Thakur and Sharma (1999) also observed C. *globosum* effective in decomposition of apple leaves during overwintering at both low and high altitudes. Young and Andrews (1990) observed the decomposition of overwintered apple leaves while recovering *A. bombacina* from inoculated leaf pieces. The extent of decomposition was upto 96 percent.

There was almost complete decomposition of the leaves having been treated with 5% urea, while no such apparently effect on leaves treated with cow urine and carbendazim was observed through these were equally effective in suppressing ascospore discharged Gupta, (1989) obtained almost complete decomposition of the overwintered leaves being treated with 5 per cent Urea and Cow urine. Therefore, it is reasonable to expect a relatively faster rate of litter decomposition in the urea treated leaves followed by cow urine and carbendazim. The carbendazim was considered as standard protect ant (Table 2, Fig. 1).

**Fig.1** Effect of Urea, cow urine and antagonist on apple leaf decomposition in orchard

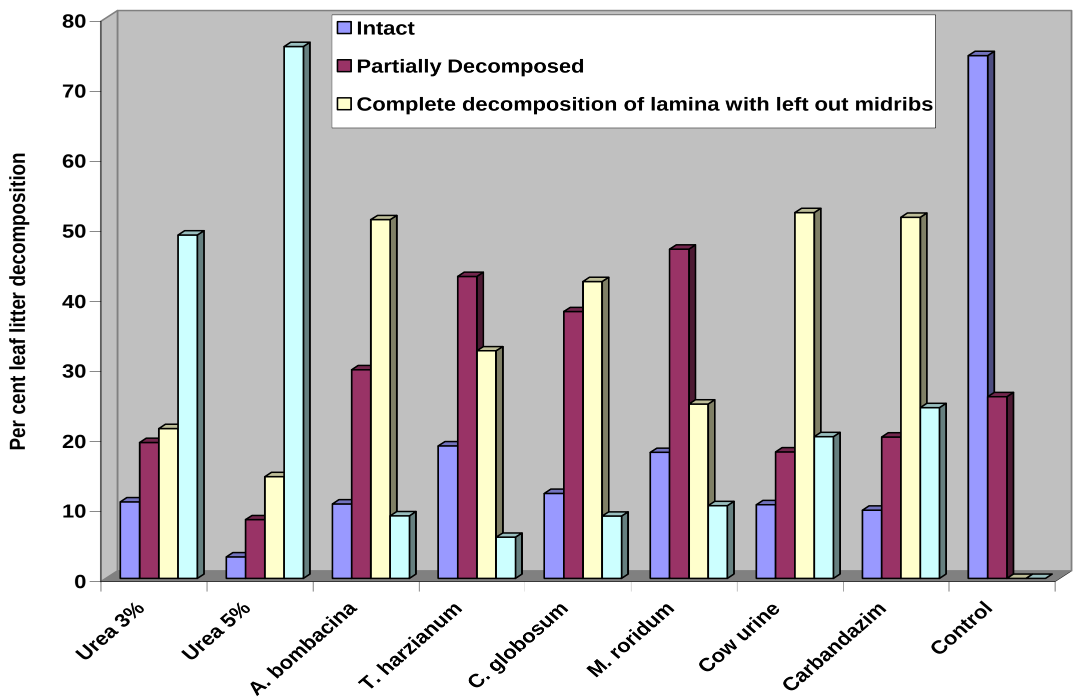


**Fig.2** Effect of a single pre-leaf fall spray on pseudothecia and ascospore productivity of *Venturia inaequalis*

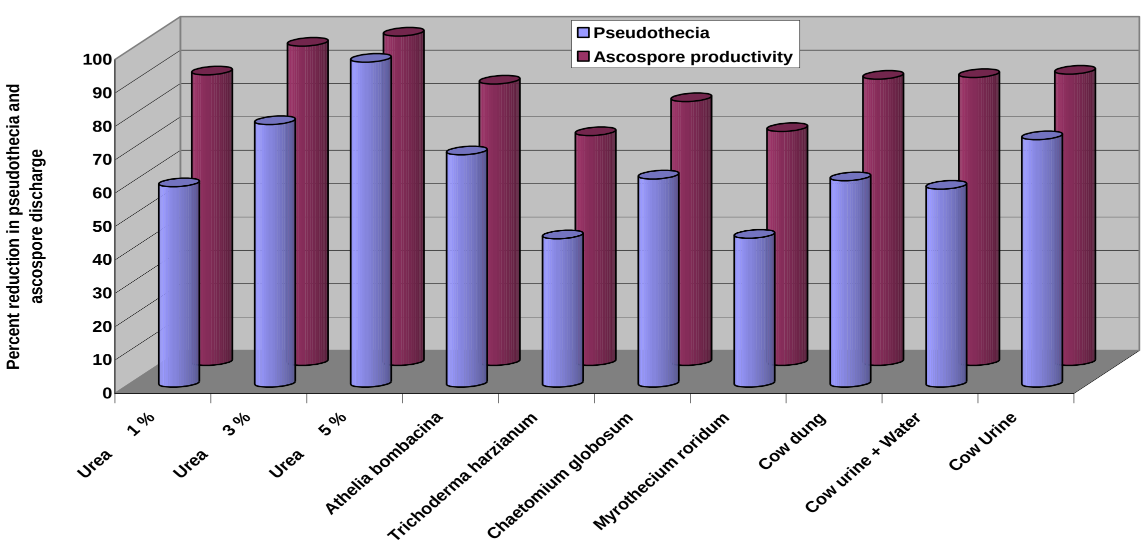

**Table.1** Effect of different time interval on decomposition of over wintering apple leaves

| Collection date | Category (% decompositions) | | | | | | | | Mean | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| December 30, | 99.27 | 00.73 | 0.00 | 0.00 | 100 | 00.00 | 0.00 | 0.00 | 99.63 | 00.37 | 0.00 | 0.00 |
| January, 30 | 97.34 | 02.66 | 0.00 | 0.00 | 95.62 | 04.38 | 0.00 | 0.00 | 99.48 | 03.51 | 0.00 | 0.00 |
| February, 28 | 91.14 | 08.85 | 0.00 | 0.00 | 89.35 | 10.67 | 0.00 | 0.00 | 90.25 | 09.76 | 0.00 | 0.00 |
| March, 30 | 78.74 | 21.30 | 0.00 | 0.00 | 85.29 | 14.71 | 0.00 | 0.00 | 82.01 | 18.01 | 0.00 | 0.00 |
| **April, 30** | 72.82 | 27.26 | 0.00 | 0.00 | 77.25 | 22.75 | 0.00 | 0.00 | 75.04 | 25.00 | 0.00 | 0.00 |
| Mean | 87.86 | 12.16 | 0.00 | 0.00 | 89.50 | 10.50 | 0.00 | 0.00 | 88.68 | 11.33 | 0.00 | 0.00 |

Replicated data transformed in Angular transformation

Significant at 5%

| | | | |
|---|---|---|---|
| Collection date category | 5.14** | 3.25** | 7.27** |
| Category | 4.45** | 2.81** | 6.29** |
| Collection date× category | 3.62** | 2.29** | 5.13** |

Category: 1, Intact; 2 partially decomposed; 3 complete decomposition of lamina with left out midribs; 4 complete decomposition with left out petiole.

**Table.2** Effect of urea, cow urine and antagonists on apple leaf litter decomposition in orchard

| Treatment | Cons. (%) Spore/ ml. | Category (%) decomposition | | | | | | | | Mean | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2005-2006 | | | | 2006-2007 | | | | | | | |
| | | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| **Urea** | 3% | $12.31^{bc}$ | $20.65^{bc}$ | $15.67^{b}$ | $53.60^{d}$ | $09.54^{bc}$ | $18.17^{b}$ | $27.12^{c}$ | $44.48^{e}$ | 10.92 | 19.41 | 21.39 | **49.04** |
| **Urea** | 5% | $3.54^{a}$ | $09.35^{a}$ | $16.55_{b}$ | $71.89^{e}$ | $02.58^{a}$ | $07.42^{a}$ | $12.49^{b}$ | $79.99^{f}$ | 03.06 | 08.38 | 14.52 | **75.94** |
| ***Athelia bombaccina*** | $7.5\times10^{2}$ | $10.06^{ab}$ | $32.68^{c}$ | $50.43^{f}$ | $07.72^{b}$ | $11.19^{bc}$ | $26.91^{bc}$ | $52.02^{e}$ | $10.17^{b}$ | 10.62 | 29.79 | 51.22 | **08.94** |
| ***Trichoderma*** | $7.5\times10^{2}$ | $16.25^{bc}$ | $43.73^{bc}$ | $34.20^{d}$ | $06.01^{b}$ | $21.60^{de}$ | $42.52^{e}$ | $30.79^{c}$ | $05.75^{ab}$ | 18.92 | 43.12 | 32.49 | **05.88** |
| ***Chaetomium globossom*** | $7.5\times10^{2}$ | $10.67^{b}$ | $42.75^{c}$ | $41.59^{e}$ | $06.63^{b}$ | $13.62^{cd}$ | $33.43^{d}$ | $43.16^{d}$ | $11.15^{b}$ | 12.14 | 38.09 | 42.37 | **08.89** |
| ***Myrothecium roridum*** | $7.5\times10^{2}$ | $18.19^{c}$ | $53.52^{d}$ | $25.23^{c}$ | $02.42^{a}$ | $17.79^{de}$ | $40.49^{e}$ | $24.57^{c}$ | $18.36^{c}$ | 17.99 | 47.00 | 24.90 | **10.39** |
| **Cow Urine** | 100% | $13.60^{bc}$ | $18.72^{b}$ | $62.13^{g}$ | $05.87^{b}$ | $07.45^{ab}$ | $17.39^{b}$ | $42.32^{d}$ | $34.62^{d}$ | 10.52 | 18.05 | 52.22 | **20.24** |
| **Carbendazim** | ˉ0.10% | $10.78^{b}$ | $21.88^{bc}$ | $53.45^{f}$ | $13.98^{c}$ | $08.68^{bc}$ | $18.47^{b}$ | $39.68^{d}$ | $34.79^{d}$ | 09.73 | 20.17 | 51.06 | **24.38** |
| **Control** | - | $70.90^{d}$ | $29.30^{c}$ | $00.00^{a}$ | $00.00^{a}$ | $78.39^{f}$ | $22.60^{bc}$ | $0.00^{a}$ | $00.00^{a}$ | 74.64 | 25.95 | 00.00 | **00.00** |
| **Cd at 5%** | - | **06.99**** | **0625**** | **7.10**** | **4.95**** | **4.98**** | **5.56**** | **07.73**** | **07.03**** | | | | |

Replicated data transformed in Angular transformation.

**Table.3** Effect of pre leaf fall spray of urea, cow urine and antagonists on pseudothecial development and ascospore productivity

| **Treatment** | **Conc. (%)** | **Pseudothecia inhibition (%)** | | | | | | **Ascospore productivity inhibition (%)** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **2004** | **ROC** | **2005** | **ROC** | **2006** | **ROC** | **2004** | **ROC** | **2005** | **ROC** | **2006** | **ROC** |
| Urea | 1% | 23.00$^{e}$ | 60.34 | 25.66$^{cde}$ | 60.52 | 25.00$^{de}$ | 59.01 | 812.00$^{cd}$ | 87.50 | 910.00$^{cd}$ | 87.00 | 919.00$^{b}$ | 86.68 |
| Urea | 3% | 11.33$^{b}$ | 80.46 | 16.00$^{bcd}$ | 75.38 | 12.00$^{b}$ | 80.32 | 241.00a | 96.29 | 329.33$^{b}$ | 95.29 | 317.33$^{a}$ | 95.40 |
| Urea | 5% | 02.00$^{a}$ | 96.55 | 03.00$^{a}$ | 95.38 | 0.00$^{a}$ | 100.00 | 052.66$^{a}$ | 99.18 | 078.33$^{a}$ | 98.88 | 135.00$^{a}$ | 98.04 |
| *Athelia bombaccina* | $7.5\times10^{6}$ | 15.33$^{bcd}$ | 73.56 | 18.33$^{bcd}$ | 72.30 | 22.33$^{de}$ | 63.39 | 1025.00$^{c}$ | 84.23 | 1071.00$^{cd}$ | 84.07 | 1104.00$^{b}$ | 84.00 |
| *Trichoderma harzianum* | $7.5\times10^{6}$ | 33.66$^{f}$ | 41.96 | 34.66$^{f}$ | 46.67 | 34.00$^{g}$ | 44.26 | 2093.00$^{e}$ | 67.08 | 2120.00$^{f}$ | 69.71 | 2136.00$^{d}$ | 69.04 |
| *Chaetomium globossom* | $7.5\times10^{6}$ | 20.00$^{cde}$ | 65.51 | 23.33$^{bcde}$ | 64.10 | 26.00$^{de}$ | 57.37 | 1400.00$^{d}$ | 78.46 | 1410.00$^{e}$ | 79.85 | 1466.00$^{c}$ | 78.75 |
| *Myrothecium roridum* | $7.5\times10^{6}$ | 31.66f | 45.41 | 31.66$^{f}$ | 51.29 | 38.66$^{h}$ | 36.62 | 1866.00$^{e}$ | 71.29 | 2043.00$^{f}$ | 70.81 | 2190.00$^{d}$ | 68.26 |
| **Cow-dung** | 50+50 | 20.00$^{cde}$ | 65.51 | 21.00$^{bcde}$ | 67.79 | 29.33$^{ef}$ | 51.91 | 910.00$^{c}$ | 86.00 | 923.00$^{cd}$ | 86.01 | 1072.00$^{b}$ | 84.46 |
| Cow Urine+ Water | 50+50 | 21.00$^{de}$ | 63.79 | 25.66$^{cde}$ | 60.52 | 28.33$^{ef}$ | 53.55 | 900.00$^{c}$ | 86.15 | 917.00$^{cd}$ | 86.90 | 986.66$^{b}$ | 85.70 |
| Cow Urine | 100 | 14.66$^{bc}$ | 74.72 | 15.33$^{bcd}$ | 76.41 | 17.66$^{c}$ | 71.04 | 823.00$^{c}$ | 87.33 | 853.33 $^{cd}$ | 87.80 | 919.33$^{b}$ | 86.67 |
| Control | - | 58.00$^{g}$ | | 65.00$^{g}$ | | 61.00 $^{i}$ | | 6500 $^{f}$ | | 7000 $^{g}$ | | 6900$^{e}$ | |

**Table.4** Mean no of pseudothecia and ascospore productivity in 2004, 2005 and 2006

| **Treatment** | **Conc. (%)** | **Mean no of** | | | |
|---|---|---|---|---|---|
| | | **Pseudothecial formation** | | **Ascospore productivity** | |
| | | **Mean** | **Roc** | **Mean** | **Roc** |
| Urea | 1% | 24.55 | 59.96 | 880.33 | 87.06 |
| Urea | 3% | 13.11 | 78.62 | 295.88 | 95.64 |
| Urea | 5% | 01.66 | 97.29 | 88.66 | 98.69 |
| *Athelia bombaccina* | $7.5\times10^{6}$ | 18.66 | 69.57 | 1066.66 | 84.31 |
| *Trichoderma harzianum* | $7.5\times10^{6}$ | 34.10 | 44.39 | 2116.33 | 68.87 |
| *Chaetomium globossom* | $7.5\times10^{6}$ | 23.11 | 62.31 | 1425.33 | 79.04 |
| *Myrothecium roridum* | $7.5\times10^{6}$ | 33.99 | 44.57 | 2033.00 | 70.10 |
| Cow dung | 50:50 | 23.44 | 61.78 | 968.33 | 85.75 |
| Cow urine + Water | 50:50 | 24.99 | 59.25 | 934.55 | 86.26 |
| Cow Urine | 100 | 15.88 | 74.10 | 865.22 | 87.28 |
| Control | - | 61.33 | | 6800.00 | |

## **Table.5** Effect of pre leaf fall sprays of chemical on pseudothecial production and ascospore productivity of *Venturia inaequalis*

| Chemical | Conc. (%) | Pseudothecia / disc | | | | | | Ascospore productivity / 100 leaves | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2004 | Roc | 2005 | Roc | 2006 | Roc | 2004 | Roc | 2005 | Roc | 2006 | Roc |
| Carbendazim | 0.10 | 9.00$^{a}$ | 84.48 | 10.66$^{a}$ | 83.60 | 09.60$^{a}$ | 85.00 | 759.33$^{a}$ | 87.34 | 0766.00$^{ab}$ | 88.56 | 0712.33$^{ab}$ | 89.96 |
| Myclobutanil | 0.10 | 24.66$^{edef}$ | 57.48 | 25.33$^{bcd}$ | 61.03 | 24.33$^{bcde}$ | 61.98 | 1400.00$^{abcd}$ | 76.66 | 1490.00$^{d}$ | 77.76 | 1361.00$^{e}$ | 80.83 |
| Mancozeb | 0.30 | 28.66$^{defg}$ | 50.58 | 34.66$^{defg}$ | 46.67 | 27.66$^{cde}$ | 56.00 | 2134.00$^{f}$ | 64.43 | 2158.00$^{e}$ | 67.79 | 2163.00$^{g}$ | 69.53 |
| Bitertanol | 0.10 | 27.33$^{defg}$ | 22.87 | 30.66$^{dg}$ | 52.83 | 26.00$^{bcde}$ | 59.37 | 1062.00$^{abcd}$ | 82.30 | 1105.00$^{bc}$ | 83.50 | 1100.00$^{bcd}$ | 84.50 |
| Carbendazim+ Mancozeb | 0.1+0.3 | 16.33$^{b}$ | 71.84 | 19.33$^{bc}$ | 70.26 | 19.33$^{bc}$ | 69.79 | 1408.33$^{bcd}$ | 76.52 | 1637.66$^{d}$ | 75.55 | 1461.66$^{ef}$ | 79.41 |
| Thiophenate methyl | 0.10 | 29.00$^{efg}$ | 50.00 | 34.00$^{e}$ | 47.69 | 24.00$^{bcd}$ | 62.50 | 1467.66$^{e}$ | 75.53 | 1491.66$^{d}$ | 77.73 | 1470.00$^{ef}$ | 79.29 |
| Penconazole | 0.05 | 23.33$^{cde}$ | 59.77 | 27.00$^{cdef}$ | 58.46 | 21.00$^{bc}$ | 67.18 | 844.66$^{a}$ | 85.92 | 0885.00$^{ab}$ | 86.79 | 0878.33$^{bcd}$ | 87.62 |
| Defenaconazole | 0.015 | 22.00$^{bcd}$ | 62.06 | 24.00$^{bcd}$ | 63.07 | 21.66$^{bc}$ | 66.15 | 820.66$^{a}$ | 86.32 | 851.00$^{ab}$ | 87.29 | 0805.66$^{bc}$ | 88.65 |
| Flusilazole | 0.01 | 15.37$^{ab}$ | 73.50 | 16.33$^{ab}$ | 74.87 | 17.33$^{ab}$ | 72.92 | 689.33$^{a}$ | 88.51 | 722.00$^{a}$ | 89.22 | 0706.00$^{a}$ | 90.05 |
| Fenarimole | 0.10 | 19.66$^{bc}$ | 66.10 | 21.66$^{bc}$ | 66.67 | 20.00$^{bc}$ | 68.75 | 1021.00$^{abc}$ | 82.98 | 1086.00$^{b}$ | 83.79 | 0950.66$^{bcd}$ | 86.61 |
| Chlorothalonil | 0.30 | 24.66$^{cdef}$ | 57.48 | 26.66$^{cde}$ | 58.98 | 25.33$^{bcd}$ | 60.42 | 1415.00$^{bcd}$ | 76.41 | 1442.33$^{cd}$ | 78.47 | 1182.33$^{abc}$ | 83.34 |
| Hexaconazole | 0.03 | 29.33$^{efg}$ | 50.00 | 33.66$^{e}$ | 48.21 | 26.33$^{bcde}$ | 58.85 | 1015.00$^{ab}$ | 83.08 | 1070.00$^{ab}$ | 84.02 | 1085.00$^{bcd}$ | 84.71 |
| Copper oxy chloride | 0.4 | 30.66$^{fg}$ | 17.13 | 31.33$^{de}$ | 51.80 | 32.33$^{de}$ | 49.48 | 1776.00$^{ef}$ | 70.40 | 1785.00$^{cd}$ | 73.35 | 1688.33$^{ef}$ | 76.22 |
| Copper hydroxide | 0.4 | 33.33$^{g}$ | 42.53 | 35.00$^{h}$ | 46.15 | 33.66$^{e}$ | 47.40 | 2400.00$^{h}$ | 60.4 | 2420.00$^{e}$ | 63.88 | 2024.33$^{g}$ | 71.48 |
| **Control** | **-** | **58.00$^{h}$** | | **65.00$^{i}$** | | **64.00$^{f}$** | | **6000.00$^{i}$** | | **6700.00$^{f}$** | | **7100.00$^{h}$** | |

## **Table.6** Average data for the different chemical sprays on the pseudothecial production and ascospore productivity of *Venturia inaequalis*

| Chemical | Conc. (%) | Mean | | | |
|---|---|---|---|---|---|
| | | Pseudothecia | | Ascospore productivity | |
| | | Mean | ROC | Mean | ROC |
| Carbendazim | 0.10 | 9.75 | 84.35 | 745.88 | 88.69 |
| Myclobutanil | 0.10 | 24.77 | 60.25 | 1417.00 | 78.53 |
| Mancozeb | 0.30 | 30.32 | 51.35 | 2151.00 | 67.40 |
| Bitertanol | 0.10 | 27.99 | 55.09 | 1089.00 | 80.05 |
| Carbendazim+ Mancozeb | 0.1+0.3 | 12.88 | 79.33 | 1502.55 | 77.24 |
| Thiophenate methyl | 0.10 | 29.00 | 53.47 | 1476.44 | 77.62 |
| Penconazole | 0.05 | 23.77 | 61.86 | 879.33 | 86.67 |
| Defenaconazole | 0.015 | 22.55 | 63.82 | 825.77 | 87.48 |
| Flusilazole | 0.01 | 16.34 | 73.78 | 705.77 | 89.30 |
| Fenarimole | 0.10 | 20.44 | 67.20 | 1019.22 | 84.55 |
| Chlorothalonil | 0.30 | 25.55 | 59.00 | 1346.55 | 79.59 |
| Hexaconazole | 0.03 | 29.66 | 52.41 | 1056.77 | 83.98 |
| Copper oxychloride | 0.4 | 31.44 | 49.55 | 1749.77 | 73.48 |
| Copper hydroxide | 0.4 | 33.99 | 45.46 | 2281.44 | 65.43 |
| Control | - | 62.33 | | 6600.00 | - |

## Effect of urea, cow urine and antagonists against the perfect stage of *V. inaequali*

Ten treatment namely, urea (1, 3, 5 %), *Athelia bombacina*, *Trichoderma harzianum*, *Chaetomium globosum*, *Myrothecium roridum* ($7.5\times10^6$ spore / ml.), Cow dung (50: 50), Cow urine (100 %) and Cow urine + water (50: 50) were tested for suppressing the ascigerous stage of *V. inaequalis* at Jochira, Harsil. It is clear from data, that all the test treatments were significantly effective ($cd_{0.05}$ 6.65 (2004) 5.39 (2005) 4.02 (2006)) in reducing the pseudothecial formation and ascospore productivity (Table 3).

Urea at 5 per cent concentration gave 97.29 per cent to nearly complete suppression of pseudothecial formation and 98.69 percent reduction in ascospore productivity, whereas urea 3 per cent gave 78.62 and 95.69 percent reduction in pseudothecial formation and ascospore productivity during the year 2004-2006 (Table 4).

Therefore, the application of urea at 3 or 5 per cent concentration was useful for reducing the pseudothecial formation and the discharge of ascospores from overwintered leaves. The data as incorporated in figure 2 revealed that 100 per cent cow urine provided a 74.10 per cent suppression of pseudothecial formation and 87.28 per cent ascospores productivity as was also obtained with 50 per cent cow urine, cow dung, 7.5 x $10^6$ spore /ml of *Athelia bombacina*, *Chaetomium globosum*, *Myrothesium roridum* and *Trichoderma harzianum*. Among the four antagonists, *A. bombacina* was highly effective in reducing the pseudothecial formation (69.57%) and ascospore productivity (84.31%) where minimum ascospore productivity was observed followed by 68.87, 70.10, and 79.04 per cent *T. harzianum*, *M. roridum*, *C. globosum* and in control, respectively. *T. harzianum* was least effective (Fig. 2).

The complete inhibition of pseudothecial maturity and ascospore discharge with 3 and 5 per cent urea as reported here was confirmatory to the earlier finding (Gupta, 1977, Burchill *et al.*, 1965, Verma and Gupta, 1992, Thakur and Sharma, 1999, Singh, 2005, 2006), whereby he had obtained 86 to 93 percent inhibition by pre leaf fall spray under Kashmir valley, Himanchal Predesh and Uttarakhand conditions. Similarly, variable results were reported by several workers from different place of world (Burchill, *et al.*, 1965; Gupta 1979; Vojvodic, 1970; Singh and Kumar, 1999). These studies reveal that the urea sprayed leaves had turned dark brown, and most of them were in decomposed and disintegrated state. Gupta (1989) obtained complete inhibition of ascospores in Himachal Pradesh due to Cow urine pre leaf fall spray. This report also confirms the present finding cow urine can both directly and indirectly effect pseudothecial development and ascospore productivity of *V. inaequalis.*

It could be seen from Table 5 that all the test chemicals were significantly effective in reducing the ascospore discharge. Among sterol-biosynthesis inhibiting fungicides, Flusilazole, Defenconazole, Penconazole, Carbendazim Bitertanol, Fenarimole, and Hexaconazole were gave maximum (83 to 89 %) inhibition of ascospores discharge in three consecutive years. The effectively of SBI chemicals of 0.01 percent flusilazole providing more number of pseudothecia but was able to inhibits maximum ascospores discharge effectively in comparison to systemic fungicides, carbendazim. In three year of testing, Flusilazole, Defenconazole, Penconazole, Carbendazim Bitertanol, Fenarimole, and Hexaconazole were found equally effective (Table 6). However, the application of SBI Chemicals at different concentration was useful for reducing the discharge of ascospores from overwintered leaves. Pseudothecial formation and ascospores productivity was lower with 0.4 per cent of copper hydroxide and 0.3 percent of Mancozeb. Variable results on the pseudothecial formation and suppression of ascospores with SBI chemicals spray in autumn have been reported by several workers from different countries (Gupta, 1979; 1987a, 1987b; Verma and Gupta, 1992).

## References

Burchill, R. T. 1968. Field and laboratory studies of the effect of Urea on ascospore production of *Venturia inaequalis (*Cke.) wint. *Ann. Appl. Biol.* 62: 297-307.

Burchill, R. T. and Williamsons, J. C. 1971. Comparison of some new fungicides for the control of scab and powdery mildew of apple. *Plant Pathol.* 20: 173-176.

Burchill, R. T., Hutton, K. E. Crosse, J. E. and Garrett, C. M. E. 1965. Inhibition of the perfect stage of *Venturia inaequalis* (Cooke.) Wint. by urea. *Nature* 205: 520-521.

Carisse, O., Philion, V., Rolland, D. and Bernier, J. 2000. Effect of fall application of fungal antagonists on spring ascospore production of apple scab pathogen, *Venturia inaequalis*. *Phytopathology* 90: 31-37.

Crosse, J. E., Garrett, C. M. E. and Burchill, R. T. 1968. Changes in the microbial population of apple leave association with the inhibition of the perfect stage of *Venturia inaequalis* after urea treatment. *Ann. Appl. Biol.* 61: 203-216.

Dekker, J. and Geogopolous, S. G. (eds) 1982. Fungicide resistance in crop protection. PUDOC, Wageningen, 273 pp.

Gadoury, D. M and MacHardy, W. E. 1982 b. A model to estimate the maturity of ascospore of *Venturia inaequalis*. *Phytopathology* 72: 901-904.

Gadoury, D. M. and MacHardy, W. E. 1982a. Effect of temperature on the development of pseudothecia of *Venturia inaequalis*. *Plant Dis.* 66: 468.

Gadoury, D. M. and MacHardy, W.E. 1986. Forecasting ascospore dose of *Venturia inaequalis* in commercial apple orchards. *Phytopathology* 76: 112-118.

Gupta, G. K. 1987b. Investigation on the effect of urea and fungicides in suppressing the ascigerous stage of apple scab pathogen. *International J. Tropical Plant Dis.* 5: 93-97.

Gupta, G. K. 1975. Epidemiology, forecasting and control of apple scab *(Venturia inaequalis* (Cke.) Wint.). *Pesticide* 9: 31-34.

Gupta, G. K. 1977. Occurrence of black rot canker (*Botryosphaeria quercuum* (Schw.) Sacc. of apple trees in India. *Prog. Hort.* 9: 29-30.

Gupta, G. K. 1979. Role of on season, post-harvest and pre leaf fall sprays in the control of apple scab (*Venturia inaequalis*). *Indian J. Mycol. Pl. Pathol* 9: 141-149.

Gupta, G. K. 1987a. Apple scab and its management. *Indian Horticulture* 32: 48-52.

Gupta, G. K. 1989. Pre leaf fall sprays of chemicals and cow urine in suppression of ascospore. *Pesticide* 23: 23-24.

MacHardy, W. E, Gadoury, D. M. and Gessler, C. 2001. Parasitic and Biological fitness of *Venturia inaequalis*: relationship to disease management strategy. *Plant Dis.* 85: 1036-1051.

MacHardy, W.E. 1996. Apple scab: Biology, epidemiology and management. APS Press. The American Phytopathological Society St. Paul, Minnesota, 545 pp.

MacHardy, W.E. and Gadoury, D. M. 1986. Pattern of ascospore discharge by

*Venturia inaequalis*. *Phytopathology* 76: 985-990.

Palmiter, D. H. 1946. Ground treatment as on aid in apple scab control. N. Y Agriculture experimental Station. *Bulletin* 714. 27 pp.

Preece T. F. and Dickinson, C. H. 1976. Microbiology of Ariel Plant Surfaces. Academic Press, New York, 669pp.

Preece, T. F. and Dickinson, C. H. (eds.). 1976. Microbiology of Aerial plant surfaces. *Academic Press*, New York.

Ross, R. G. and Burchill, R. T. 1968. Experiment using sterilized apple leaf discs to study the mode of action of urea in suppressing perithecia of *Venturia inaequalis* (Cke.) Wint. *Ann. Appl. Biol.* 62: 279-296.

Ruinen, J. 1961. The *phyllosphee I:* an ecologically neglected mildew plant Soil 15: 81-109.

Singh K P., Kumar, J. and Singh, H. B. 2001. Curative and protective action of ergosterol-biosynthesis inhibiting fungicides in relation to infection periods against apple scab in Uttaranchal Himalayas. *Indian J. Plant pathol.* 19: 34-38.

Singh K. P. 2006. Investigation on development of bio-control measures for the management of saprophytic stage of apple scab pathogen *Venturia inaequalis* in Uttaranchal Himalayas. *ICAR, Report* New Delhi, 53pp.

Singh, A. 2006. Epidemiology and management of apple powdery mildew in the Uttaranchal Himalayas. Ph. D. *Thesis*, HNBGU Srinagar, Uttaranchal 221pp.

Singh, K. P. 2005. Integrated management of apple scab, through development of scab warning system in Uttaranchal. NATP-CGP report, ICAR, New Delhi.46 pp.

Singh, K. P. and Kumar, J. 1999. Efficacy of different fungicidal spray schedules in combating apple scab severity in Uttar Prates Himalayas. *Indian Phytopathology* 52: 142-147.

Singh, K. P. and Kumar, J. 1997. Maturation and discharge of ascospores of *Venturia inaequalis* in central Himalayas of India (*Abstr*.) International conference of Integrated Plant Disease Management for Sustainable Agriculture. *Indian Phytopathological Society*.p 386.

Singh, K. P. and Kumar, J. 1998. Forecasting maturity and discharge of ascospore of *Venturia inaequalis*, an ecological approach for management of apple scab in Uttar Pradesh Himalayas. (*Abstr.*).

Singh, K. P. and Kumar, J. 1999. Efficacy of different fungicidal spray schedules in combating apple scab severity in Uttar Pradesh Himalayas. *Indian Phytopathol.* 52: 142-147.

Singh, K. P. and Kumar, J. 1999. Studies on ascospore maturity of *Venturia inaequalis*, the Apple scab pathogen, in Central Himalayas of India. *Indian J. Mycol. Pl. Pathol.*29: 408-415.

Singh, K. P. and Kumar, J. 2005. Integrated pest management of apple scab GBPUA&T *Tech. Bulletin* p 34.

Singh, K. P. and Kumar, J. 2006. Integrated management of apple scab, through development of scab warning system in Uttaranchal. NATP-CGP Reprt. ICAR, New Delhi, 46.pp.

Singh, K. P. and Kumar, J. 2007. *Prediction of ascospore maturation of Venturia inaequalis in central Himalayas*. Proc. *Third Asian conference on plant pathology*. Yogakarta, Indonesia. 45-46 pp.

Singh, K. P. and Pal R. 1996. Studies on maturity pattern of ascospore of *Venturia inaequalis* in U.P hills (*Abstr*.). *Indian Phytopathological Society*.

Singh, K.P., Kumar, J. and Pal, R. 1995. Integration of chemicals for the

management of apple scab and sanjose scale, the two important constraints to apple production in U.P. hills of India. (*Abst*r.) *National Symposium, Indian Phytopathological Society*.
Thakur, V. S. and Gupta, P.K. 1991. Apple phylloplane microbes as antagonists to *Venturia inaequalis*. *Indian Phytopathol.* 44: 34 (*Abstr*.).
Thakur, V. S. and Sharma, R. D. 1999. Effect of urea on microbial degradation of apple leaf litter and its relationship to the inhibition of pseudothecial development of *Venturia inaequalis*. *Indian J. Agril. Sce*. 69: 147-151.
Verma, K. D. and Gupta, G. K. 1992. Effect of pre leaf fall sprays of urea and fungicides in suppressing the ascigerous stage of apple scab pathogen in Himachal Pradesh. *Plant Dis. Res.* 7: 68 - 70.
Vojvodic, D. 1970. Ispitivanje uticaja kasnog jesenjeg prskanjana form*ranje peritecija Venturia inaequalis (Cke.)* Wint. *Zast. Bilja* 21: 151-155.
Westwood, M. N. 1978. *Temperate-zone pomology*. Freeman, W. H. and Co. San Fransisco. C.A. pp. 330-331.
Young, C. C. and Andrews, J. H. 1990. Recovery of *Athelia bombacina* from apple leaf litter. *Phytopathology* 80: 530-535.
Young, C. S. and Andrews, J. H. 1990. Inhibition of pseudothecial development of *Venturia inaequalis by* the basidiomycete *Athelia bombacina* in apple leaf litter. *Phytopathilogy* 80: 536-542.

**How to cite this article:**

Prasad, R.K., K.P. Singh and Gupta, R.K. 2018. Pre Leaf Fall Spray of Chemical, Cow Urine and Fungal Antagonists on Spring Ascospore Production of the Apple Scab Pathogen, *Venturia inaequalis*. *Int.J.Curr.Microbiol.App.Sci.* 7(08): 575-586.
doi: https://doi.org/10.20546/ijcmas.2018.708.063