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**IN - VITRO MANAGEMENT OF LEAF SPOTS OF OATS (*AVENA SATIVA* L.) CAUSED BY *HELMINTHOSPORIUM AVENAE***

**M Amin Mir<sup>1</sup>, MD Shah<sup>2</sup>, Sheikh Arshid<sup>1</sup>**

<sup>1</sup>Uttaranchal College of Science and Technology, Dehradun, India.

<sup>2</sup>Sheri-e-Kashmir Institute of Agricultural Science, Srinagar Kashmir, India.

**ABSTRACT**

Oats (*Avena sativa*) is the most important rabbi fodder crop and in Kashmir, it is grown as a winter fodder crop in vacated paddy fields and young orchards. Survey of major oat growing areas viz., Ganderbal, Srinagar and Anantnag of Kashmir in spring 2012 and 2013 revealed the leaf blight incidence and severity ranging from 19.0, 12.2 and 35.5% respectively. The severity was measured as per the scale of Mayee and Datar (1986) at panicle initiation to grain maturity stages of growth. The blighted lesions on examination yielded the constant association of *Helminthosporium avenae* which was isolated on potato dextrose agar (PDA) and produced fluffy arial brownish mycelial growth. The anomorph was encountered in all assays. The conidiospores, arising singly 2-4 groups, were straight or flexuous, frequently geniculate, dark and septate, measured 110-126 $\mu$ m  $\times$  8-12 $\mu$ m. The conidia were sub-hyaline or pale yellow, 1-6 septate (average four ) and 30-82 $\mu$ m (average 56  $\mu$ m)  $\times$  08-12  $\mu$ m (average 10.4  $\mu$ m) in dimension cylindrical with round apex bearing inconspicuous scar similar to that described by Ellis (1971). The perfect stage has not been found yet in Kashmir.

**Keywords:** *Helminthosporium avenae*, *Avena sativa*, Kashmir, *In-Vitro*.

**INTRODUCTION**

Oats (*Avena sativa*) is one of the most important fodder and grain crops of Kashmir. It occupies about 0.143 million hectare area with a production of 0.322 million tonnes, the annual contribution from Kashmir being 0.217 million tonnes from area of 0.015 million hectare with an average production of 1.02 metric tonnes per hectare during 2010-2012 which is far below from international average and better from national average. For evolutionary reasons, most of the pathogens that attack oats have a close relationship with the plant. So, in most areas where the environment suits the crop, it also favours the occurrence of the diseases that these pathogens cause. Some pathogens, however, are less adapted to the species or less robust in these environments, and they cause less serious, or sporadic, diseases.

Oats provide one of the richest sources of the dietary soluble fiber beta-glucan, providing 5.0 g (oatmeal) to 7.2 g (oat bran) per 100 g serving.13 both are also valuable sources of total dietary fiber, which ranges

from 9.9-14.9 g per 100 g serving. Oats also contain more lipids (5-9%) than other cereal crops and are rich in unsaturated fats, including the essential fatty acid linoleic acid. Oats contain unique antioxidants, called avenan thramides, as well as the vitamin E-like compounds, tocotrienols and tocopherols [1]. Soluble fiber from oats, when incorporated into a low-glycemic diet, can improve postprandial glycemic and insulinemic responses in both non-insulindependent diabetes mellitus and healthy subjects [1-3]. More than 12 published studies report that oats, consumed as oat bran, oatmeal, or isolated beta-glucans, reduce both fasting and postprandial blood glucose and insulin levels.

The DASH (Dietary Approaches to Stop Hypertension) study demonstrated that a diet high in whole grains, fruit, vegetables and low fat dairy, and restricted in fat, lowers BP in hypertensive individuals [4]. Dietary consumption of oats is consistent with the DASH recommendations, and may confer a benefit due to its fiber content.

Eeckhout EV et al [5] carried out the effect of rate and timing of fungicide applications on incidence and severity of sheath blight grain yield of rice [6] mentioned the compatibility and co-toxicity of selected insecticides and fungicides against borne plant hopper and leaf spot of oat. Crown rust is the most harmful disease that affects oats and it is distributed worldwide, having been observed in all areas where these crops are grown [7]. Crown rust is one of the most important oat diseases in Brazil, Argentina and Uruguay. Grain yields are negatively correlated with crown rust severity [8] and may be reduced by as much as 50 percent in susceptible cultivars [9]. It is caused by *Puccinia coronata* f.sp. *avenae*, a heteroecious macrocyclic rust [10]. One of the particularities of this disease is its capacity to attack several plant species. The uredial and telial phases occur on oats and other grasses, including all species of oats (*Avena* spp.), *Secale cereale*, *Hordeum vulgare*, *Lolium* spp., *Festuca* spp. and *Bromus inermis*, among others; the spermagonial and aecial phases occur on Rhamnus bushes, the alternate host [11].

The causal agent is *Puccinia graminis* Pers. f. sp. *avenae* Eriks. and Henn., which attacks all species of oats, including wild oats [12]. Stem rust is a widespread disease of oats, occurring almost everywhere they are grown [13]. In Canada, it occurs almost every year in some provinces, including in Ontario, Quebec, Manitoba and eastern Saskatchewan, causing severe crop losses [14]. In Australia, it can be devastating, causing crop losses up to 100 percent [15]. In South America, stem rust epidemics are typically at the end of the crop season, from booting stage onwards, when the temperature is warmer. In Argentina, where *A. sativa* is used as fodder, it is particularly serious because their cycle is longer than that in Brazil, where the importance of the disease has diminished because oats are harvested earlier (personal observation of the author).

## MATERIALS AND METHODS

All the chemicals used in this investigation were of analytical reagent (AR) grade and were purchased from Sigma Merck. De-ionized water was used for the complete study. All the glassware and equipment used for handling were stabilized properly prior to use.

### Survey of Oat Growing Areas

Extensive survey of oat growing areas in these districts viz., Srinagar, Anantnag and Ganderbal of Kashmir valley was carried out in mid march (at maximum tillering to booting stages of oat crop) during the year 2012-2013, for ascertaining the status of leaf spot disease (*Helminthosporium avenae*) of oats (*Avenae sativa* L.). Three zones in each district and three villages under each zone were selected. Five farmer's fields were randomly selected in each field were observed for per cent disease incidence and intensity.

### Isolation

Spots of the oat plants from each location, showing typical spot symptoms, were for isolation of the pathogen. The leaf spots of oat bits (3-4mm) containing diseased and healthy spot portions, were cut with sterilized scissors, surface sterilized in 0.01 % mercuric chloride (HgCl<sub>2</sub>) solution for 1 minute, rinsed 2-3 times in sterilized distilled water, blotted dry and placed aseptically on potato dextrose agar (PDA) medium (Appendix-1). Four such bits were placed on PDA in each petri dish and incubate for two to three days at 28±2°C.

### Purification and identification

The hyphal tips emanating from the tissue bits were cut off and transferred to PDA in Petri plates for purification and further growth of the pathogen. For identification, morphology characteristics such as shape, size and colour of sclerotia, branching type, septation and colour of mycelium, presence/absence of moniloid cells, growth rate etc, were observed under compound microscope and compared with standard description given by Parmeter and Whitney (1970).

### Maintenance

Single sclerotial culture of all the isolates were cultured separately on PDA for various including Anastomosis grouping, pathogenicity test, *in vitro* evaluation etc. The master cultures of these isolates were maintained on PDA slants in test tubes at 10±2°C (Bolkan and Biberiro, 1985).

### Pathogenicity test

The pathogenicity of all the fungal isolates was established by following Koch's postulates (starr, 1984).

### Disease Management

#### Management through Botanical and Chemical Fungi toxicants

The botanicals and chemical fungi toxicants evaluated against leaf spots of oats caused by *H.avenae* are given in table 2.

## RESULTS

The present studies on leaf spots (*H.avenae*) of oats were conducted in Division of Plant Pathology and Rice Research and Regional Station of Sher-e Kashmir University of Agriculture Science and Technology of Kashmir with the objectives of ascertaining the disease status, mode of pathogen perpetuation and management strategy for containing the disease.

### Disease status

Survey of the major oat growing areas of three districts viz., Srinagar, Ganderbal and Anantnag of Kashmir was conducted during 2012 and 2013 to assess the status of leaf spots (*H. avenae*) of oat.

The information generated during survey of the three major oat growing Districts of Kashmir during 2012 revealed the prevalence of leaf spot of oat in all the districts (table 1).

The overall scenario of oat leaf spot in the three districts surveyed is represented in fig. 2. Over the year the mean disease incidence (35.97%) and intensity (12.01%) was the highest in district Anantnag followed by those in district Ganderbal (33.74 and 10.81%, respectively) and Srinagar (29.24 and 9.39%, respectively).

Five botanicals and ten chemical fungi toxicants were evaluated *in-vitro* against *Helminthosporium avenae*, the incitant of oat leaf spot.

### **In-vitro evaluation**

#### **Botanicals**

The aqueous leaf extracts of five botanicals at 10, 20, 30, 40 and 50 per cent concentration were evaluated for their effect on mycelial growth.

#### **Effect on mycelial growth**

*Helminthosporium avenae* was allowed to grow *in-vitro* on PDA amended separately with various concentrations of aqueous extracts of different botanicals and observed for their effect on mycelial growth. The data (Table 3) revealed that all the test botanicals caused significant inhibition of the mycelial growth of the test of the fungus. On an average, Artemisia exhibited the maximum mean inhibition of 94.15% followed by Datura with an average growth inhibition 87.44%. Mint followed in efficacy against the pathogen showing an average inhibition of 85.39%, while least mean inhibition was exhibited by cannabis (79.43%) among the test botanicals. There also existed significant differences among different concentrations. In general, the higher concentration of 50% caused maximum mean mycelial inhibition of 97.59%. However, as the concentration was reduced, mean mycelial growth inhibition was also reduced such that a 20% concentration of aqueous extracts caused mean mycelial inhibition of only 83.44% which further decreased to 68.13% at 10% concentration.

There existed a significant interaction between the botanicals and their test concentrations. At 10% concentration, the highest mycelial growth inhibition was exhibited by Artemisia (80.00%) followed by Datura (75.33%), while Cannabis and Mint each exhibited the least mycelial growth inhibition (60.00%). At 20 % concentration as well, Artemisia exhibited highest inhibition (93.33) followed by Datura (82.92%), while Cannabis and Mint, each caused the least (79.16%). Artemisia exhibited highest (97.33%) mycelial inhibition at 30% concentration followed by Datura (88.33%) while Cannabis exhibited the least (81.33%) inhibition. At 40% concentration, Artemisia completely (100.00%) inhibited the mycelial growth followed by Datura and Mint (94.00% each), whereas Cannabis exhibited least inhibition

(83.33%) at the same concentration. Artemisia and Garlic completely (100.00%) inhibited the mycelial growth of *Helminthosporium avenae* at 50% concentration followed by Mint (98.33%), while Cannabis caused only 93.33% mycelial inhibition at the same concentration.

#### **Non-systemic fungi toxicants**

Six non-systemic fungi toxicants were evaluated at 50, 100, 250, 500 and 1000  $\mu\text{g a.i. ml}^{-1}$ . Concentration for their effect *in-vitro* mycelium growth of the test pathogen (*Helminthosporium avenae*)

#### **Effect on mycelial growth**

*Helminthosporium avenae* was grown on PDA amended separately with desired concentration of test fungi toxicants and the mycelial growth inhibition was recorded. The data (Table 4) revealed that all the test fungi toxicants significantly inhibited the mycelial growth at all the test concentrations. On an overall mean basis, Mancozeb, proved the most effective exhibiting mean mycelial growth inhibition of 95.26% followed by Capton and Dodine causing 93.80 and 93.66% mean inhibition, respectively, while Copper oxychloride proved the least effective among test non-systemic fungi toxicants showing only 44.08% mean inhibition. In general, the efficacy varied significantly with change in fungicide concentration. The maximum inhibition of 96.56% was achieved at 1000  $\mu\text{g a.i. ml}^{-1}$  which decreased as the fungi toxicant concentration was lowered recording a minimum of 66.83% at a concentration 50  $\mu\text{g a.i. ml}^{-1}$  concentration.

There also existed a significant interaction between fungi toxicants and their concentration. Capton, Dodine, Mancozeb and Antrocol caused complete (100%) growth inhibition at 500  $\mu\text{g a.i. ml}^{-1}$ , Zineb and Copper oxychloride provide only 95.0 and 84.41 inhibition even at the highest concentration of 1000  $\mu\text{g a.i. ml}^{-1}$ . All the fungitoxicants caused more than 50% growth inhibition at lowest concentration of 50  $\mu\text{g a.i. ml}^{-1}$  except copper oxychloride which registered only 90% growth inhibition at this concentration.

#### **Systemic fungi toxicants**

Four systemic fungi toxicants each at 10, 20, 30 40 and 50  $\mu\text{g a.i. ml}^{-1}$  were evaluated for their effects on mycelial growth of *Helminthosporium avenae*.

#### **Effect on mycelia growth**

*Helminthosporium avenae* was grown on PDA amended separately with desired concentration of the test fungi toxicants and the mycelia inhibition was recorded. The data presented in Table 5, revealed that all the test fungi toxicants significantly inhibited the mycelia growth at all the test concentrations. On an overall mean basis, Carbendazim and Hexaconazole were most effective resulting in complete (100.00%) mycelia growth inhibition followed by Diniconazole showing 98.58%, Difenocazole

inhibition showed the least efficacy with 91.25% mean mycelia growth inhibition. In general, the efficacy varied significantly with change in fungicidal concentration. The highest mycelial growth inhibition of 95.05% was exhibited at a concentration of 50  $\mu\text{g a.i ml}^{-1}$  and the lowest of 82.65% at 10  $\mu\text{g a.i ml}^{-1}$ . There also existed a significant interaction between fungi toxicants and their

concentration. Carbendazim and Hexaconazole provided complete (100%) growth inhibition at lowest test, concentration of 10  $\mu\text{g a.i ml}^{-1}$ , while Difenocozole and Diniconazole provided such a level of inhibition respectively at 30 and 40  $\mu\text{g a.i ml}^{-1}$ . Difenocozole and Diniconazole provided more than 90% growth inhibition at 30  $\mu\text{g a.i ml}^{-1}$ .

**Table 1. Incidence and intensity of oat leaf spots (*H.avenae*) in three districts of Kashmir valley during 2012**

District	Zone	Villages	Disease Incidence (%)	Disease Intensity (%)
Srinagar	Soura	Ahmadnagar	24.5	8.19
		Nouwshera	25.5	8.79
		Buchpora	27.5	8.88
	Hazatbal	Nigeen	37.0	11.13
		Gullabagh	33.0	10.18
		Saderbal	34.5	10.36
	Srinagar	Tailbal	25.0	8.34
		Batpura	26.0	8.55
		Shalimar	25.5	8.5
		<b>Mean</b>		<b>28.72</b>
Ganderbal	Lar	Takibal	28.5	9.06
		Safapora	30.0	9.81
		Wakura	30.0	9.62
	Ganderbal	Behama	32.0	10.0
		Nunner	33.5	10.55
		Tulmulla	35.5	11.36
	Kangan	Kangan	38.0	13.33
		Prang	37.0	12.22
		Wasun	33.0	10.55
		<b>Mean</b>		<b>33.11</b>
Anantnag	Kulgam	Khudwani	37.5	11.82
		Sarandoo	37.0	11.82
		Kulgam	38.5	13.69
	Achabal	Achabal	37.5	11.29
		Khundroo	37.0	12.96
		Imoh	35.5	10.29
	Dooru	Veerinagh	27.5	9.25
		Dooru	27.5	9.25
		Larkipora	36.5	12.03
		<b>Mean</b>		<b>35.0</b>
	<b>Overall mean</b>		<b>32.27</b>	<b>10.42</b>

**Table 2. Incidence and intensity of oat leaf spots (*Helminthosporium avenae*) in three Districts of Kashmir valley during March 2013**

District	Zone	Villages	Disease Incidence (%)	Disease Intensity (%)
Srinagar	Soura	Ahmadnagar	26.0	8.83
		Nouwshera	26.5	8.88
		Buchpora	28.5	9.06
	Hazatbal	Nigeen	38.0	11.12
		Gullabagh	34.0	10.44
		Saderbal	35.5	10.55
	Srinagar	Tailbal	26.5	8.61
		Batpura	27.5	8.66
		Shalimar	26.0	8.55

	Mean		29.77	9.42
<b>Ganderbal</b>	Lar	Takibal	29.5	9.25
		Safapora	33.0	10.55
		Wakura	30.5	9.81
	Ganderbal	Behama	33.0	10.00
		Nunner	35.5	10.29
		Tulmulla	37.0	11.47
	Kangan	Kangan	39.0	13.69
		Prang	38.0	12.69
		Wasun	33.5	10.66
		Mean		34.38
<b>Anantnag</b>	Kulgam	Khudwani	40.5	14.06
		Sarandoo	38.5	13.57
		Kulgam	41.5	14.44
	Achabal	Achabal	38.5	12.03
		Khundroo	40.0	13.88
		Imoh	37.5	11.47
	Dooru	Veerinagh	29.0	10.75
		Dooru	28.0	9.25
		Larkipora	39.0	13.60
		Mean		39.94
	Overall mean		33.69	10.66

**Table 3.** Per cent mycelial growth inhibition of oat leaf spot pathogen (*Helminthosporium avenae*) grow on PDA amended separately with aqueous leaf extracts of different Botanicals *in-vitro*

Botanical name	English name	Local name	Aqueous leaf extracts concentration (%)					
			10	20	30	40	50	Mean
<i>Artemesia indica</i>	Mugort	Tethwan	80.00 (63.44)	93.33 (75.20)	97.33 (82.30)	100.00 (90.00)	100.00 (90.00)	94.16 (79.87)
<i>Cannabis sativa</i>	Cannabis	Bhang	60.00 (50.77)	71.96 (62.83)	81.33 (64.39)	83.33 (65.90)	93.33 (75.03)	79.43 (66.31)
<i>Datura stramonium</i>	Datura	Datur	75.33 (60.21)	82.92 (65.59)	88.33 (70.02)	94.00 (76.22)	96.33 (78.95)	87.44 (70.11)
<i>Mentha arvensis</i>	Mint	Pudna	65.33 (53.92)	82.66 (65.42)	86.33 (68.30)	94.00 (76.22)	98.33 (82.57)	85.39 (69.28)
<i>Allium sativum</i>	Garlic	Ruhan	60.00 (50.77)	79.16 (62.83)	95.00 (67.21)	92.33 (64.43)	100.00 (90.00)	83.32 (67.04)
Mean			68.13 (55.82)	83.44 (66.37)	87.66 (70.44)	92.73 (80.13)	97.59 (83.31)	-
CD (p=0.05)								
Botanical			=	(0.32)				
Concentration			=	(0.32)				
Botanical × concentration			=	0.72				

**Table 4.** Per cent mycelial growth inhibition of oat leaf spot pathogen (*Helminthosporium avenae*) grow on PDA amended separately with different concentrations of non-systemic fungi toxicants *in-vitro*.

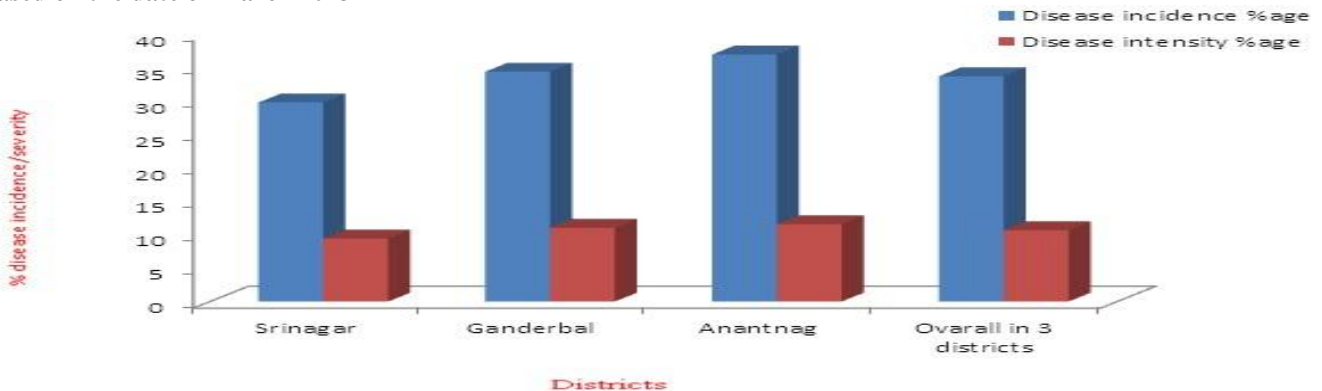
Fungi toxicants	Fungi toxicants concentration (µg a. i. ml-1)					
	50	100	250	500	1000	Mean
Capton	85.00 (67.21)	87.33 (69.17)	96.61 (79.52)	100.00 (90.00)	100.00 (90.00)	93.80 (79.58)
Copper oxychloride	9.33 (17.72)	23.75 (29.16)	30.67 (36.44)	66.25 (54.48)	84.41 (67.83)	440.8 (41.14)
Dodine	58.00	90.00	93.33	100.00	100	93.66

	(67.21)	(71.56)	(75.05)	(90.00)	(90.00)	(78.76)
Mancozeb	83.33 (70.02)	91.66 (72.88)	96.66 (79.52)	100.00 (90.00)	100.00 (90.00)	95.25 (80.48)
Antrocol	70.00 (56.78)	73.33 (58.90)	76.67 (61.11)	100.00 (90.00)	100.00 (90.00)	83.99 (71.36)
Zineb	63.33 (52.73)	66.67 (54.73)	75.00 (59.99)	91.66 (73.22)	95.00 (77.08)	78.33 (63.55)
Mean	66.83 (55.29)	72.06 (58.09)	79.16 (62.84)	92.98 (81.28)	69.56 (84.15)	-
CD (p=0.05)						
	Fungi toxicant	=	(1.4)			
	Concentration	=	(1.05)			
	Fungi toxicant × concentration	=	3.54			

**Table 5. Per cent mycelia growth inhibition of *Helminthosporium avenae* grown on PDA amended separately with different concentrations of various systemic fungi toxicants *in-vitro***

Fungitoxicant	Fungi toxicant concentration $\mu\text{g a.i ml}^{-1}$					mean
	10	20	30	40	50	
Carbendazim	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Difenoconazole	78.33 (62.26)	88.33 (70.02)	94.17 (76.04)	96.67 (79.52)	98.75 (83.58)	91.25 (74.28)
Diniconazole	96.25 (78.84)	97.92 (81.79)	98.75 (83.63)	100.00 (90.00)	100.00 (90.00)	98.58 (84.85)
Hexaconazole	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Mean	94.65 (80.27)	97.92 (57.95)	98.23 (84.91)	99.16 (87.38)	99.68 (88.39)	-
CD (P=0.05) = (0.34)						
Fungi toxicant Concentration = (0.27)						
(Fungi toxicant concentration) = 0.77						

**Figure 1. Status of leaf spot (*Helminthosporium avenae*) of oat at different locations in three district of kashmit valley based on the date of March 2013**



**DISCUSSION**

The leaf spots of oat caused by *Helminthosporium avenae* is a disease with wide geographic distribution and now occurs throughout the temperate and tropical oat producing areas, being most prominent where oat is grown under intense, high fertility production systems although earlier considered a minor

disease. It has assumed the status of a major disease in the oat growing tracts of India and, therefore, is one of the major biological constraints in the profitable oat production.

Establishing the status of the disease and the resultant yield losses due to it, are the pre-requisites for deciding at the adaption of disease management practices,

and in fact from the basis component of decision making in integrated management. The objectives was achieved by undertaking surveys of three important oat growing districts viz., Srinagar, Ganderbal and Anantnag, of Kashmir valley during the present investigation. The disease incidence during 2012 and 2013 cropping seasons in the surveyed fields in the three districts of Kashmir was 32.27 and 33.69% with intensities of 10.42 and 12.57% respectively. The highest disease incidence (35.97%) and intensity (12.01%) over the years was recorded in district Anantnag, while the lowest overall disease incidence (29.42%) and intensity (10.81%) was recorded in district Srinagar. Location-wise, overall highest disease incidence (40.00%) and intensity (13.06%) was recorded at Kulgam in district Anantnag while the lowest disease incidence (25.25%) and intensity (8.53%) was recorded at Ahmadnagar in district Srinagar. The variation in disease incidence and intensity in various villages surveyed could probably be attributed to use of indigenous and relatively resistant/susceptible varieties, monoculturing, application of mainly nitrogenous fertilizers, and occasional or rather neglected spray programmes. The higher levels of disease recorded in 2013 compared to that in 2012 seems to be because of higher temperature and fair amount of precipitation received during 2013 (Appendix-II), that

resulted in congenial condition for pathogen growth and proliferation and thus predisposed that host to the infection.

In other part of the state as well, leaf spot incidence of around 28% and intensity of 8% has been reported in R.S pura Jammu in 1995 and 1996. The variation in leaf spot of oat has also been attributed to different soil types, temperature and humidity and mode of perpetuation of the pathogen. The continued prevalence of the disease throughout the surveyed areas in Kashmir valley necessitates a sound scientific basis be established for devising the measures for effective and eco-friendly management of this ailment with minimum dependence on effective fungicides.

Among the four systemic fungitoxicants evaluated *in-vitro* against *Helminthosporium avenae*, Carbendazim and Hexaconazole were most effective resulting in complete (100.00%) mycelia growth inhibition followed by Diniconazole showing 98.58%, Difenocozole inhibition showed the least efficacy with 91.25% mean mycelia growth inhibition. In general, the efficacy varied significantly with change in fungicidal concentration. The highest mycelial growth inhibition of 95.05% was exhibited at a concentration of 50  $\mu\text{g a.i ml}^{-1}$  and the lowest of 82.65% at 10  $\mu\text{g a.i ml}^{-1}$ .

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