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Symptomatological and Morphological Characterization of Downy Mildew (*Plasmopara viticola*) of Grapes in Jammu and Kashmir, India

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A B S T R A C T

Downy mildew of grapes (*Plasmopara viticola*) is wide spread and most destructive disease of grapes (*Vitis vinifera* L.) worldwide. The disease has attained the status of a major disease of grape under Kashmir conditions. The characteristic symptoms observed were small pale yellow translucent spots approximately 0.3mm in size on adaxial leaf surface, which later on coalesced and formed irregular nectrotic patches surrounded by brown halo. On the corresponding abaxial surface, white cottony growth appeared which later turned dirty grey to dark brown ultimately resulted in premature defoliation. Infected shoot tips exhibited shepherds crook symptoms, dried up and droop and young infected berries turned brown, got covered with white cottony growth and withered later on. Whereas, late infected berries remained stunted, developed a purplish grey colour, shriveled and dropped. The tubular coenocytic hyphae produced tree like sporangiospores with four to six branches at right angles and hyaline, ellipsoid and papillated sporangia. The oospores were brownish, circular and germinates by single germ tube.

Introduction

Grape (*Vitis vinifera* L.) is an economically important fruit crop from temperate regions which has been adapted to tropical and subtropical regions of the world occupying an area of 18 million acres with an annual production of about 75 million metric tons and productivity of 27.9 million tons/ hectare globally (Anonymous, 2016). In Jammu and Kashmir, grapes are grown in Kupwara, Baramulla, Bandipora, Ganderbal, Budgam and Srinagar districts covering an area of 226 hectares with production of 966 metric tons (Anonymous, 2019). It is a deciduous crop, rich in sugars, acid, vitamins, minerals and tannins (Radha and Mathew, 2007). Among various diseases, downy mildew caused by *Plasmopara viticola* (Berl. &Curt) Berlese & de Toni is an economically important, wide spread and destructive disease of *Vitis* species in viticulture areas (Williams *et al.*, 2007; Delmotte *et al.*, 2006).

The disease affects all the green parts of the vine namely leaves, shoots, tendrils, inflorescence and bunches (Kenelly *et al.,* 2007) and is serious in climate with abundant rainfall, high humidity and longer periods of wetness on leaves and fruits (Choi *et al.,* 2017). The economic significance of the damage caused by downy mildew is apparent, if the bunches are affected early (Valarmathi and Ladhalakshmi, 2020).When control is poor and/or weather conditions are favourable and no protection against the disease is provided, it causes severe losses due to total or partial destruction of grape bunches, besides secondary influence of foliage loss (Walker and Haesbrock, 2007).The occurrence of downy mildew has been reported recently from the valley (Shahzad *et al.,* 2006) and has assumed an alarming threat to successful cultivation of grapes with an overall incidence and intensity of 43.22 and 22.95 percent, respectively (Sabiha and Shahzad, 2010). So, considering the importance and potentiality of being an epidemic pathogen, the present investigation were attempted to explore the symptomatological and morphological characterization of downy mildew on grapes under field conditions.

Materials and Methods

The symptomatological studies of the disease were studied on grape cultivar "Thompson Seedless" randomly selected in the vineyard of SKUAST-Kashmir, Shalimar. The vines were kept unsprayed throughout the growing season to study the symptoms of downy mildew under natural epiphytotic conditions. First observation was taken as soon as the disease appeared.

The symptom development with respect to size, shape, colour of the lesions on leaves, shoots, tendrils as well as on fruits was recorded continuously at weekly intervals. Size of the lesions was recorded in terms of average lesion size in mm. The morphological characteristics of the causal organism were studied on host (*in vivo*). Semi-permanent slides were prepared from the infected host tissue, stained with cotton blue in lactophenol and observed under microscope (Olympus, Tokyo, Japan) for studying various morphological characters of the pathogen. The important characters studied were

Hyphae - Septation, width and colour

Sporangiophore - Size, shape and colour

Sporangia - Size, shape and colour

Oospore - Size, shape and colour

Results and Discussion

Symptomatological Characterization

During the periodic observations of marked plants (Fig 1 A-I), the initial disease symptoms were noticed in the third week of June on upper leaf surface as small pale yellow translucent (oily) spots measuring 0.5 mm in size. (Fig.1A). Periodic changes in size, shape and colour of the leaves were observed and the results summarized are as in Table 1; Fig.4.

The shape of the lesions was indefinite in first two weeks after which it turned irregular to angular due to coalescing of spots (Fig 1B, D). The colour of the lesion changed from pale yellow to yellow surrounded by chocolate coloured halo and limited by veins in first week of July (Fig. 1C). On the corresponding under surface of the leaf, a delicate dense white cottony growth appeared in the third week of July (Fig. 1E). White cottony growth turned to dirty grey by second week of August (Fig. 1G).

After sporulation, centers dried out and became reddish brown (Fig. 1 F) followed by necrosis of the affected portion (Fig. I H). On the upper leaf surface, lesions enlarged and coalesced covering the entire leaf. In the first week of September, affected leaves turned dark brown and brittle (Fig. 1I) and finally severely affected leaves dropped prematurely. These findings are more or less supported by the observations made by Ashley

(2007) who have reported enlargement of spots with change in colour and shape with the passage of time.

Symptoms of downy mildew on shoots and tendrils of grapes appeared in the first week of July as water soaked spots followed by shiny depressions. The spots turned oily brown on which white cottony growth of the fungus appeared (Fig. 2 A). Young shoots and tendrils after infection became distorted, thickened and curled (Shepherd's crook) (Fig. 2 B) and in the first week of September, infected shoots and tendrils turned brown and died (Fig. 2 C-E). The characteristics symptoms of the disease as observed under natural conditions of inoculum were identical and agreed with those observed by Smith (2020) and Karthick *et al.,* (2019)

Two stages of fruit infection were observed during the growing season. First when the berries were about the size of small peas, young berries at this stage turned light brown, soften and covered with white cottony growth of the fungus. In the second week of July, infected berries turned brownish grey and withered (Brown rot) (Fig. 3A). Secondly, berries infected in late summer (first week of July) did not soften or became covered with white cottony growth of the fungus. Instead turned brownish purple, growth remained stunted and infected berries developed a purplish hue. In the second week of August, infected berries turned dark purplish brown, shrivelled and dropped (Grey rot) (Fig. 3B). Ellis in 2005 also observed similar symptoms on downy mildew infected fruits. Similar reports have also been reported by several other workers (Valarmathi and Ladhalakshmi, 2020; Fisher *et al.,* 2007)

Morphological Characterization

The morphological characters of the pathogen were studied only on the host as *Plasmopara viticola* is an obligate parasite. The various morphological characters of the pathogen (Table 2) observed in host are as under:

Hyphae were tubular, coenocytic, intercellular and hyaline measuring 7-10µm in width (Fig. 4 A). Sporangia were borne on tree like sporangiophores measuring 7-11 x 16.5-585 µm with an average of 8.04 x 308.27 µm derived directly from the mycelium. Each sporangiophores produces four to six branches, at right angles to the main stem at definite intervals and each side branch produce two or sometimes three secondary branches (Fig. 5 C). Branching at right angles is characteristic of *P. viticola* (Kim *et al.*, 2019). Sporangia were hyaline, thin walled formed by swelling of tip of sterigmata and measured 11.30 - 26.39 x 7.54 - 18.85 µm with an average of 22.0 - 13.03 µm. Sporangia were ovoid in shape with a small papilla at the tip giving it a lemon shaped outline (Fig. 5 B). Each sporangium gives rise to biflagellate zoospores. Germination of sporangia by zoospores has been supported by the findings of Choi *et al.,* (2017) and William *et al.,* (2007). In their investigation, a total of 108,000 sporangia were observed, of this total number, only seven sporangia exhibited a germ tube i.e. less than 0.0065 per cent of *P. viticola* sporangia germinated directly. The zoospores escaped from the side of the sporangium opposite its point of attachment, either through an opening in a papilla (Fig. 5 D) or by directly perforating the sporangial wall (Fig 5 E). Oospores were brownish, circular, enveloped by two membranes and surrounded by thickened persistent rough and wrinkled oogonial wall and measured 18-250µm in diameter (Fig. 4 F,G). The oospore germinated by single germ tube terminating in a pyriform sporangium (Fig. 5 H,I). Ronzon and Clerjeau (1988) also reported germination of oospores that terminates into sporangium (macrosporocyst).

The fungus produced characteristics white cottony growth on the corresponding underside of the leaf. Several lesions coalesced under severe infection with the formation of large irregular necrotic patches that turned brittle and defoliate prematurely. On Shoots symptoms appear as water soaked areas which then turned oily brown and become covered with white cottony growth of the fungus. Infected shoot tips thickened; curled (Shepherd's crook) turned brown and die. On fruits two stages of infection occurs- Brown Rot and Grey Rot.

Table.1 Development of symptoms of downy mildew [*Plasmopara viticola* (Burk.&Curt.) Berl.& de Toni] on grapes (cv. Thompson Seedless)

Month/Week	Leaf (Lesion)			Shoot/ Tendril lesion	Berry Lesion
	Size (mm)	Shape and Colour	Sporulation		
June III	0.5	Pale yellow translucent spots on adaxial surface	_	_	_
IV	2.0	Yellow to greenish yellow spots with indefinite borders	_	_	Young berries turn light brown and soften.
July I	6.0	Yellow irregular translucent spots with chocolate coloured halo	_	Water soaked areas on young shoots	Infected berries covered with white cottony growth
II	12.5	Coalescing of spots, besides fading of halo	_	-do-	Brownish grey lesions leading to withering of berries (Brown Rot)
III	35.5	Irregular to angular patches with dense white aerial cottony growth on abaxial leaf surface	+	Water soaked areas with shiny depressions	
IV	47.0	-do-	+	Water soaked areas turning oily brown	Mature berries turned brownish purple
August I	55.0	Centre of lesions becomes reddish brown	+	-do-	Stunted growth of affected berries
II	60.0	Irregular patches becoming necrotic on upper leaf surface	+	White cottony growth appears on shoots and tendrils.	Infected berries develop a purple hue
III	_	Large irregular patches due to coalescing of spots covering most of the leaf area	+	Infected shoot tips and tendrils thicken and curl (Shepherd's crook)	Turn dark purplish brown, shrivel and fall off
IV	_	White fungal growth on the under surface turning dirty gray	+	-do-	_
September I	_	Necrotic tissue dark brown and brittle	+	Infected shoots and tendrils turn brown and died	_
II	_	Severely infected leaves drop prematurely	+	-do-	_
III	_	-do-	+	-do-	_
IV	_	-do-	+	-do-	_

Table.2 Morphological characters of *Plasmopara viticola* (Burk. &Curt.) Berl.& de Toni causing downy mildew of grapes

Structure	Shape and Characteristics	Colour	Size
Hyphae	Tubular, coenocytic, irregular in diameter, hyphal wall thin; contents hyaline and granular. Old hyphae entirely empty of contents while young hyphae densely filled with granular protoplasm.	Hyaline	7-10µm wide
Sporangiophores	Branched with four to six monopodial branching arising at right angle to main axis at definite intervals	Hyaline	7-11 x 16.5-585 µm (Av.8.04 x 308.27)
Sporangia	Lemon shaped; thin walled; papillate formed at the tip of sterigmata.	Hyaline	11.30 – 26.39 x 7.54 – 18.85µm (Av. 22.30 x 13.03)
Oospores	Circular; enveloped by two membranes, surrounded by rough thick wrinkled wall; germination by single germ tube giving rise to pyriform sporangium	Brownish	18-150µm

Fig.1 Symptomatological development of Downy mildew of grapes on leaves

A. Initial small pale yellow spot

B. Enlarging Spots

C. Chocolate coloured halo

D.Coalescing of spots

E. White cottony growth on lower leaf surface

F. Reddish brown centre of lesions

G. White fungal growth turning dirty gray

H. Irregular necrotic patches

I. Necrotic leaf turning dark brown and brittle

Fig.2 Symptomatological development of Downy mildew of grapes on Tendrils

A. Infected shoot turning brown

B. Infected shoot tip thicken

C. Infected shoots turn brown and die and curl (Shepherd' s crook)

D. White cottony growth on tendril

E. Browning and death of Infected tendrils

Fig.3 Downy mildew symptoms of grapes on fruit

A. Brown Rot on berries

B. Grey Rot on berries

Fig.4 Periodical lesion progression of downy mildew on grape leaves (cv Thompson Seedless)

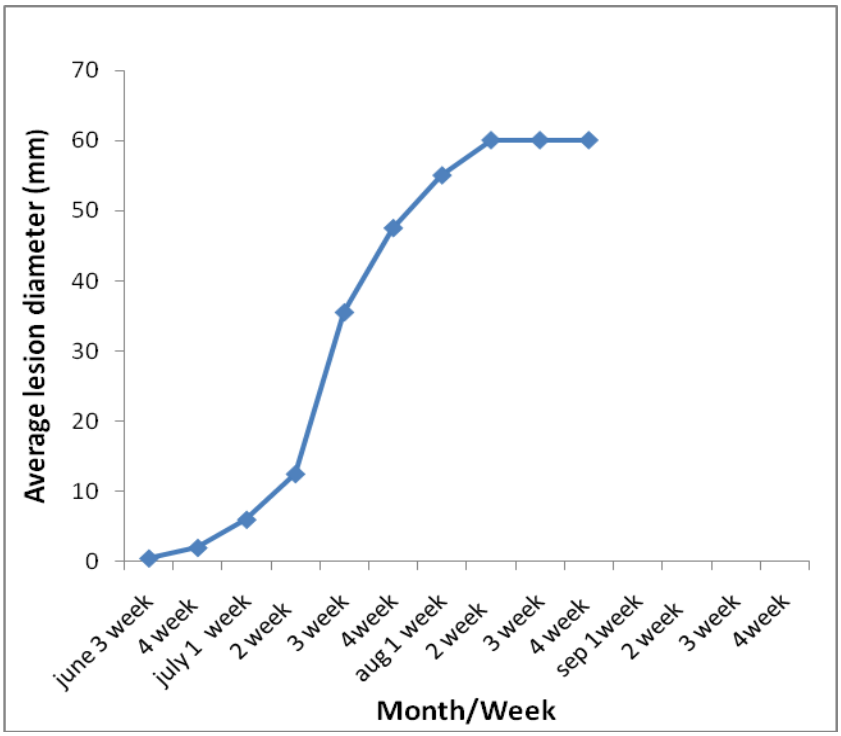
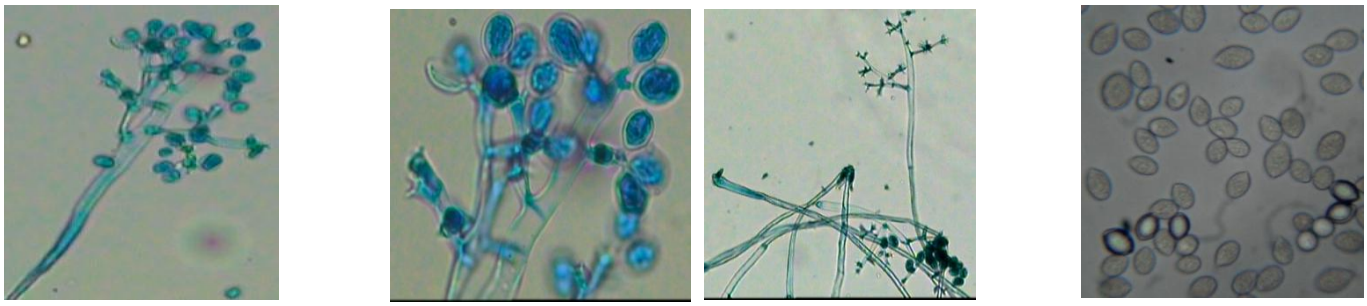
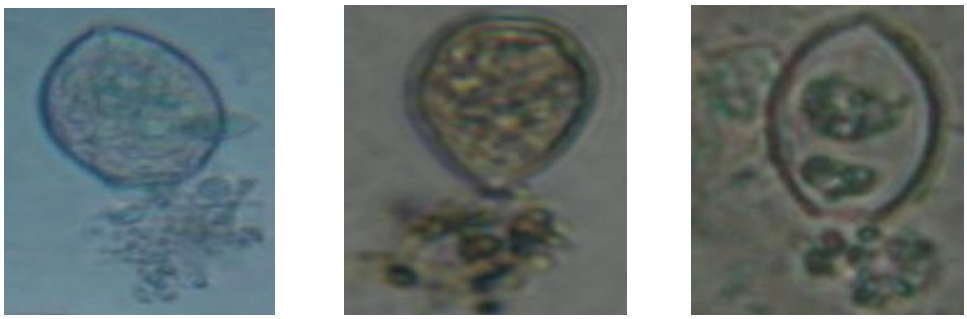


Fig.5 Morphological characters of *Plasmoparaviticola (*40X)

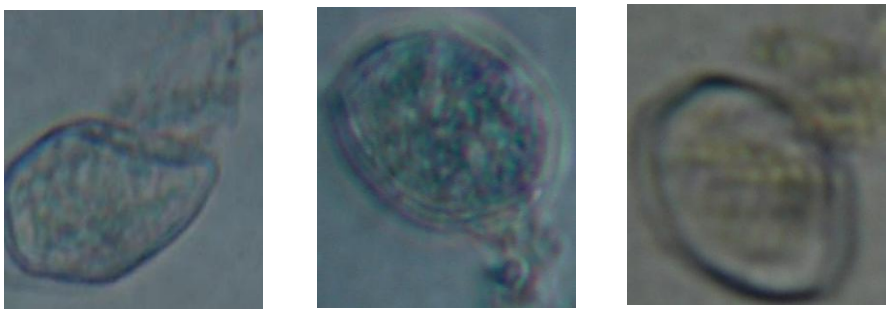
A. Sporangia on sporangiophores B. Tubular coenocytic hyaline mycelium C. Sporangia of *P. viticola*



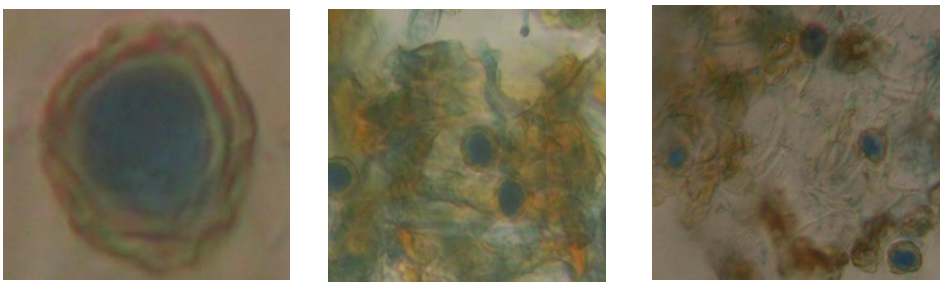
D. Release of zoospores from Sporangium through an opening in the papilla



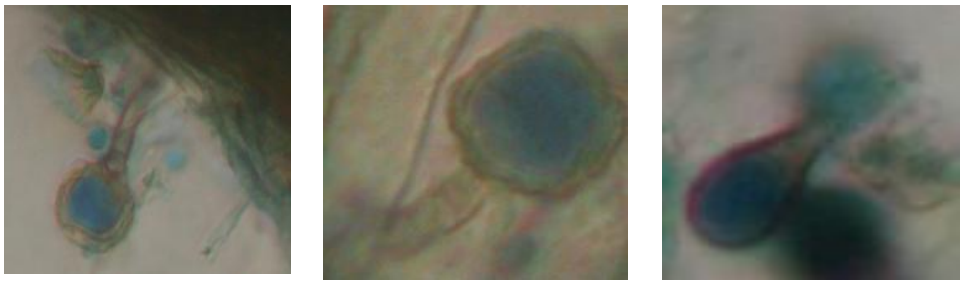
E .Release of zoospores from Sporangium by directly perforating the wall



F. Oospore of *Plasmopara viticola* G Oospores from infected host tissue



H. Germinating oospores I. Germinating oospore giving rise to sporangium



The fungus produced tubular, coenocytic hyaline hyphae measuring 7-10µm in diameter. Sporangiophores (7-11 x 16.5-585 µm average 8.04 x 308.27 µm) were tree like with four to six branches at right angles to the main stem at definite interval, and were derived directly from the mycelium.

Sporangia were hyaline, ovoid, papillated and measured 11.30-26.39 x 7.54-18.85 µm in size with an average of 22.0 x 13.03 µm. Each sporangium produced biflagellate zoospores which escape either through an opening in a papilla or by directly perforating the wall. Oospores of the fungus were brownish, circular (18-150 µm) in size enveloped by two membranes and surrounded by the thick rough and wrinkled wall. The oospore germinated by single germ tube which terminated in a pyriform sporangium. These findings could help in better understanding of the disease and its management in the field.

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Competing Interest

The author declare that there is no competing interest in the publication of this manuscript

Ethical statement

This research did not involve any human and/or animal participants

Conflict of interest

No conflict of interests.

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