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Research Article

PERPETUATION OF *Diplodia pinea* CAUSING NEEDLE BLIGHT OF BLUE PINE (*Pinus wallichiana*) IN KASHMIR VALLEY

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Abstract: The needle blights are prevalent in almost all the pine growing regions of the world. *Diplodia pinea* was frequently isolated pathogen from blightened blue pine needles in Kashmir valley. The perpetuation studies of the pathogen was carried out on intact as well as on fallen diseased pine needles kept at three depths beneath humus. The studies on the production and viability of *D. pinea* on intact blightened needles as well as on fallen needles, kept at three soil depths (i.e. 0, 5 and 10 cm), revealed the presence of asexual state throughout the storage period. During the whole course of study, teleomorphic state was not observed.

Keywords: Blue pine, *Diplodia pinea*, Kashmir, Needle blight, Perpetuation

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Introduction

Blue pine (*Pinus wallichiana*), is an evergreen arbor of monotypic genus. It belongs to the genus Pinus and family *Coniferae* widely grown in Jammu and Kashmir State. Blue pine is prone to a number of diseases which pose serious threat to its regeneration. The blights especially needle blights are prevalent in almost all the pine growing regions of the world [1]. Different pathogens associated with pine needle blight produce peculiar characteristic symptoms depending upon the nature of pathogen, pine specie, prevailing environmental conditions and tree age. Pathogens have their own mode of survival and overwintering. Most of the needle blight pathogens overwinter on dead needles, bark, wood and cones, both on tree and ground, in the form of pycnidia (fruiting bodies) [2]. The infection cycle begins again in the following spring producing conidia in wet weather [3, 4].

Material and methods

The studies on perpetuation of *Diplodia pinea* was carried out on intact as well as on fallen diseased pine needles kept at three depths beneath humus during the years 2010-2012 at Forest Nursery, Faculty of Forestry, Shalimar, Srinagar (J&K). The previous year's infected needles from intact twigs of Blue pine were recovered from field at fortnightly interval from February during 2011 and 2012. Periodic observations regarding the production of conidia and their viability were taken from February onwards. However, the fallen infected needles of Blue pine were collected in last week of November (2010 and 2011). The needles (approx. 100 needles) were put separately in nylon mesh bags and placed in Forest Nursery beneath humus at 3 depths *viz*., 0, 5 and 10 cm. The needle whorls as per treatment and depth were recovered from the field at fortnightly interval. Periodic observations were taken regarding the production of conidia and their viability from February onwards. Twenty infected needles (4 needle whorls) from intact twigs collected from field and twenty needles from each wire-mesh bag kept at 0, 5 and 10 cm litter depths were randomly selected for the development of conidiomata and ascomata (if any) per needle fascicle. The needles were examined under stereoscopic microscope for the production and type of fruiting body.

To assess spore formation, the needles were crushed in 100 ml sterilized distilled water and strained through a double layered cheese cloth. The filtrate (50 ml) was centrifuged at 6000 rpm for 15 minutes. After centrifugation, the supernatant was discarded and sterilized distilled water was added to make 5 ml of the pellet [5]. The number of conidia/ascospores was counted with the help of a haemocytometer. To evaluate the spore viability, one drop of 50 µl processed sample was placed on a glass slide and incubated in a moist chamber at 25±1°C. The number of spores germinated was recorded after 24 hours incubation under binocular microscope (Leica-Model DME Model 13595XXX). The percent viability of spores was estimated on the basis of formula as:

$$Spore\ viability\ (\%) = \frac{Number\ of\ spores\ germinated}{Total\ number\ of\ spores\ viewed} \times 100$$

Results

The studies on the production and viability of *D. pinea* on intact blightened needles as well as on fallen needles, kept at three soildepths (*i.e*. 0, 5 and 10 cm), revealed the presence of asexual state throughout the storage period. The conidial production and viability on intact twigs of Blue pine revealed insignificant variation during both the years. The data during the year 2011 the number of conidia showed a progressive increase from 3.20 × 10^5 to 6.40 × 10^5 conidia during 1st fortnight of February 2011 to 1st fortnight of March 2011 and thereafter a gradual decrease was observed [Table-1]. The initial conidial viability on intact twigs in year 2011 was 15.33 percent which reached a maximum of with 50.66 percent in the 1st fortnight of March 2011 and thereafter it showed a gradual decrease till 2nd fortnight of June 2011 when only 0.32 × 10^5 conidia with 1 percent viability were observed. A similar trend was also observed on intact needles in year 2012 with a maximum of 6.72 × 10^5 conidia having 53.33 percent viability in 1st fortnight of March 2012 which gradually decreased till 2nd fortnight of June 2012 when only 0.96 × 10^5 conidia with 1.33 percent viability were noticed [Table-2]. A similar trend was observed on the needle kept beneath soil at three depths *i.e*., 0, 5 and 10 cm with the variation that conidial count and viability lasted for lesser

periods. The number of conidia on 1st fortnight of February-2011 at 0, 5 and 10 cm was minimum (2.56×10^5, 1.28×10^5 and 0.64×10^5, respectively) which attained maximum values in the 1st fortnight of March- 2011 (4.16×10^5, $2. \times 10^5$ and 1.60×10^5 conidia, respectively). Thereafter a gradual decrease in conidial count was noticed [Table-2]. Peak conidial viability at 0, 5 and 10 cm depth observed on 1st fortnight of March-2011 was 42.33, 22.66 and 6.00 percent, respectively. Thereafter a conidial viability decrease at all the soil depths with lowest value of 1.33 percent viability was observed on 1st fortnight of May on the needles kept at 0 cm depth. Whereas only 1 and 0.33 percent conidial viability was observed on the needles kept at 5 and 10 cm soil depth on 2nd fortnight and 1st fortnight of April, respectively, in the year 2011, beyond which no conidia were viable at either of the depths. The conidial production and their viability showed almost a similar trend at all the three soil depths in year 2012 [Table-4]. The conidial number on 1st fortnight of February-2012 showed a minimum number of conidia (2.88×10^5, 1.60×10^5 and 0.80×10^5) which reached to a maximum of 4.80×10^5, 2.88×10^5 and 1.92×10^5 conidia at 0, 5 and 10 cm depth respectively on 1st fortnight of March-2012. Thereafter there was a gradual decrease in conidial count. A peak in viability (45.66, 26.33 and 7.66%)was recorded on 1st fortnight of March-2012 at 0, 5 and 10 cm depth. Thereafter a gradual decrease in conidial viability was observed at all the soil depths with lowest of 1.66 percent viability on 1st fortnight of May on the needles kept at 0 cm depth. Whereas, only 1.33 and 0.66 percent conidial viability was observed on needles kept at 5 and 10 cm soil depth on 2nd fortnight and 1st fortnight of April, respectively, in the year 2012, beyond which no conidia were viable at either of the depths. During the whole course of study, teleomorphic state was not observed during both the years.

Discussion

Perpetuation studies were carried out on infected needles on intact twigs as well as on needles kept at 3 depths *i.e.*, 0, 5 and 10 cm during the years 2011 and 2012. Periodic observation revealed that fungus perpetuated in the form of pycnidia. The conidial production and viability in both the years on intact blightened needles was comparatively more than on the fallen needles as well as decreased significantly with an increase in forest litter depths. Similar observation has been reported by [6, 7] who observed that the cones of red and Jack pine collected from the ground yielded fewer conidia of *D. pinea* than cones collected from the canopy. [3] also observed more conidial entrapment from *Pinus resinosa* seedlings, from trees, or from the ground than from the fallen material *i.e.*, litter. The conidial dispersal of *D. pinea* from *Pinus resinosa* was maximum during 16 weeks after springs when rainfall was considerably less but temperature was more ideal for the formation of conidia [8-10].

Table-1 Perpetuation of *Diplodia pinea* and its viability on intact blightened needles of Blue pine during the years 2011

Period of observation		Intact blightened needles	
Month	Fortnight	Conidia ($1x10^5$/ml)**	Viability (%)*
February 2011	1st	3.20 (1.78)	15.33 (23.00)
	2nd	4.80 (2.19)	31.66 (34.19)
March 2011	1st	6.40 (2.52)	50.66 (45.36)
	2nd	5.85 (2.41)	48.33 (44.91)
April 2011	1st	5.12 (2.26)	40.66 (39.57)
	2nd	4.16 (2.03)	20.00 (26.51)
May 2011	1st	3.52 (1.87)	12.00 (20.22)
	2nd	2.56 (1.60)	8.33 (16.74)
June 2011	1st	1.28 (1.13)	4.66 (12.44)
	2nd	0.32 (0.56)	1.00 (5.72)
July 2011	1st	0.00 (0.00)	0.00 (0.00)
CD (P=0.05)		0.10	5.38

** The values in parentheses are angular transformed values*
*** The values in parentheses are square root values*

Two year study revealed that the fungus overwintered as pycnidia on host needles which appear to be the one of the reason for higher conidia production in later year. The finding is in agreement with [11] who reported that *D. pinea* overwinters as mycelium or pycnidia in conifer shoots, bark, cones or litter and conidia disseminate during wet weather in spring through fall. Peak spore production and infection usually coincides with host bud break and shoot and needle elongation. The pathogen can over-winter as pycnidia in or on the previous year's diseased shoots, or as an endophyte of mature pinecones or shoots [2-4].

Table-2 Perpetuation of *Diplodia pinea* and its viability on intact blightened needles of Blue pine during the years 2012

Period of observation		Intact blightened needles	
Month	Fortnight	Conidia ($1x10^5$/ml)**	Viability (%)*
February 2011	1st	3.52 (1.87)	18.00 (25.05)
	2nd	5.12 (2.26)	35.00 (36.22)
March 2011	1st	6.72 (2.59)	53.33 (46.91)
	2nd	6.08 (2.46)	50.66 (45.36)
April 2011	1st	5.44 (2.33)	43.00 (40.93)
	2nd	4.80 (2.19)	25.00 (29.94)
May 2011	1st	3.88 (1.96)	18.66 (25.54)
	2nd	2.88 (1.69)	10.66 (19.01)
June 2011	1st	1.92 (1.38)	5.33 (13.32)
	2nd	0.96 (0.97)	1.33 (6.60)
July 2011	1st	0.00 (0.00)	0.00 (0.00)
CD (P=0.05)		0.12	6.25

** The values in parentheses are angular transformed values*
*** The values in parentheses are square root values*

Conclusion

The perpetuation of *D. pinea* was studied on intact blightened needles as well as on the needles kept at 3 forest litter depths *i.e.*, 0, 5 and 10 cm during the years 2011 and 2012. The periodic observation revealed that fungus perpetuated in the form of pycnidia both on intact blightened needles as well as on needles kept at various soil depths. The conidial production and viability on intact twigs was comparatively more than on the fallen needles on ground as well as decreased significantly with increase in burial depths in forest litter. During the whole course of study, teleomorphic state was not observed during both the years. In the present study, it may be concluded that the fungus *Diplodia pinea* perpetuates in the form of pycnidia on intact blightened needles as well as on forest litter depths which serves as a source of primary inoculum for the next season. The inoculum load of *Diplodia pinea* is reduced by increasing the burial depths of the blightened needles in the forest litter.

Application of research: The research reveals that the field sanitation by the burial of fallen infected needles beneath the soil helps in reducing the inoculum load of the pathogen which causes infection in the next spring.

Research Category: Plant Pathology

Abbreviations:
CD: Critical difference, RPM: Rotations per minute, Approx: Approximately

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Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Table-3 Perpetuation of *Diplodia pinea* and its viability at different forest litter depths during the year 2011

Month	Fortnight	Conidia (1x10^5/ml) **			Viability (%)*		
		0 cm	5 cm	10 cm	0 cm	5 cm	10 cm
February 2011	1st	2.56 (1.60)	1.28 (1.13)	0.64 (0.80)	9.33 (17.74)	5.66 (13.73)	1.66 (7.38)
	2nd	3.20 (1.78)	1.60 (1.26)	1.28 (1.13)	25.66 (30.38)	16.66 (24.04)	3.33 (10.49)
March 2011	1st	4.16 (2.03)	2.56 (1.60)	1.60 (1.26)	42.33 (40.55)	22.66 (28.37)	6.00 (14.14)
	2nd	3.52 (1.87)	2.24(1.49)	0.96 (0.97)	29.66 (32.94)	12.33 (20.51)	2.00 (8.11)
April 2011	1st	2.88 (1.69)	0.96 (0.97)	0.32 (0.56)	15.33 (23.00)	3.00 (9.95)	0.33 (3.28)
	2nd	1.28 (1.13)	0.32(0.56)	-	7.66 (16.03)	1.00 (5.72)	-
May 2011	1st	0.64 (0.80)	-	-	1.33 (6.60)	-	-
	2nd	-	-	-	-	-	-
June 2011	1st	-	-	-	-	-	-
	2nd	-	-	-	-	-	-
CD (P=0.05)		0.11	0.09	0.06	2.23	1.46	0.63

**The values in parentheses are angular transformed values, **The values in parentheses are square root values, -The material was degraded*

Table-4 Perpetuation of *Diplodia pinea* and its viability at different forest litter depths during the year 2012

Month	Fortnight	Conidia (1x10^5/ml) **			Viability (%)*		
		0 cm	5 cm	10 cm	0 cm	5 cm	10 cm
February 2012	1st	2.88 (1.69)	1.60 (1.26)	0.80 (0.89)	11.00 (19.33)	6.66 (14.92)	2.00 (8.11)
	2nd	3.52 (1.87)	1.92 (1.39)	1.60 (1.26)	26.33 (30.82)	18.00 (25.05)	4.33 (11.98)
March 2012	1st	4.80 (2.19)	2.88 (1.69)	1.92 (1.39)	45.66 (42.48)	26.33 (30.82)	7.66 (16.03)
	2nd	3.84 (1.94)	2.56 (1.60)	1.28(1.13)	31.33 (33.98)	15.66 (23.26)	2.33 (8.76)
April 2012	1st	3.20 (1.78)	1.28 (1.13)	0.48 (0.69)	18.66 (25.54)	4.33 (11.98)	0.66 (4.64)
	2nd	1.60 (1.26)	0.64 (0.80)	-	8.33 (16.74)	1.33 (6.60)	-
May 2012	1st	0.96 (0.97)	-	-	1.66 (7.38)	-	-
	2nd	-	-	-	-	-	-
June 2012	1st	-	-	-	-	-	-
	2nd	-	-	-	-	-	-
CD (P=0.05)		0.11	0.09	0.07	2.36	1.68	0.72

**The values in parentheses are angular transformed values, **The values in parentheses are square root values, -The material was degraded*

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