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Prevalence and Pathogenicity of the Isolates of *Fusarium oxysporum* f.sp. *lentis* in Different lentil Grown Areas of Uttarakhand and Uttar Pradesh

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ABSTRACT

Background: Different lentil-growing regions in India have reported that the lentil wilt poses a serious threat to crop cultivation because it can result in 100 percent crop losses. The fungus *Fusarium oxysporum* f.sp. *lentis* is the pathogen responsible for the disease. This infection is carried through the seed and soil.

Methods: To understand the current status of the wilt pathogen in the total of twenty-two lentil-growing areas in Uttarakhand and Uttar Pradesh, the present study was conducted over the course of the two consecutive years 2017-18 and 2018-19, to track the incidence and prevalence of the disease. Observations were made on the numbers of diseased plants and number of infected fields to record incidence and prevalence of the lentil wilt, repectively. Moreover, twenty two isolates were collected to examine the different spores, pathogenicity levels and degree of correlation between both of them.

Result: The data showed that the pathogen was present in every area at 100 per cent prevalence, with a disease incidence within 6.22-26.25 per cent. Data derived from 100 per cent availability of the pathogen inoculum in all lentil-growing regions over the course of two years predicts the emergence of the wilt disease threat to the crop. The observations indicate that the pathogen's inoculum level had increased as the incidence of the disease increased during the subsequent crop season. It was also observed that spore size somewhat affects the pathogenicity of the isolates.Therefore the present study was conducted to observe the impact of wilt pathogen on different lentil grown areas and to alarm the farmers about this emerging threat.

Key words: Disease Incidence, *Fusarium oxysporum* f.sp. *lentis,* Pathogenicity**,** Prevalence, Survey.

INTRODUCTION

In some parts of the world, lentil (*Lens culinaris* Medik), a member of the leguminosae family, is cultivated for its nutrient-rich seeds and eaten as a pulse. It is the second-most nutrient-dense pulse crop after chickpeas and contains between 24 and 26 per cent protein, between 57 and 60 per cent carbohydrate, between 3.2 and 1.3 per cent fibre and is also high in minerals like calcium, phosphorus and iron, which each have a concentration of 300 mg per 100 g. (69 mg per 100 g). One of the many diseases that damage lentils is wilt, which is caused by the fungus *Fusarium oxysporum* f.sp. *lentis*, a vascular pathogen. Wilt is one of the most serious diseases that afflict lentils. Huge losses in the lentil crop were attributed by Hamdi and Hassanein (1996) to the *Fusarium oysporum* f.sp. *lentis* wilt disease in India. In numerous Indian states, the disease is seriously threatening lentil production and resulting in enormous losses in crop yield. Some Indian states, including Assam, Punjab, Haryana, Uttar Pradesh, Bihar, West Bengal and Rajasthan, were badly affected by this disease (Chaudhary *et al.* 2010). Chaudhary and Amarjit's (2002) analysis of the Sangod Tehsil's soil in Kota, Rajasthan found the sever disease incidence in lentil grown areas. Almost every nation that grows lentils experiences extensive Fusarium wilt disease. Ahmad (2010) stated that crop variety, growth stage and prevailing environmental conditions all affect the amount of yield losses caused by wilt. According to Sharma *et al.* (2016), no location in Madhya Pradesh that was surveyed had no incidence of lentil wilt. The review of this disease prompted the current study to investigate and evaluate the prevalence and incidence of wilt disease in the main lentil-growing regions of Uttarakhand and Uttar Pradesh which will assist us in reducing disease occurrence and to alert the farmers about the impending threat to lentils.

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MATERIALS AND METHODS

Survey of areas and collection of data

The Twenty two lentil grown areas of Uttarakhand and Uttar Pradesh were surveyed altogether during the year2017-18 and 2018-19 (Table 2). At each place five fields were surveyed randomly and the each farmer's field to be surveyed was marked with a square of 1 m × 1 m, diagonally across the field at five spots. The plants showing yellowing, drying and wilting as the peculiar symptoms of disease in each marked spot were counted along with the total no. of plants. The infected plants (Fig 1) were uprooted and collected separately in envelopes containing blotter sheet and marked clearly showing the details of location. The collected samples were then brought to the Pulse Pathology Laboratory in the Dept. of Plant Pathology, GBPUAT, Pantnagar. The isolates of the pathogen was isolated on PDA medium and further purified using "Single Spore Isolation" method (Zhang *et al.* 2013). Formula for disease prevalence and incidence is given below.

Disease prevalence (%) =

$$\frac{\text{No. of fileds showing lentil wilt}}{\text{Total number of fields visited}} \times 100$$

Disease inicidence (%) =

$$\frac{\text{No. of wilted plants}}{\text{Total number of plants observed}} \times 100$$

Pathogenicity of the Isolates

Inoculum was prepared in the erlenmayer flasks of 250 ml capacity filled with the 100 g of sand maize mixture (10 gmaize and 90 g of sand) as described by Vasudeva and Srinivasan (1952). The prepared inoculum was thoroughly mixed with sterilized soil @ 20 g/kg soil. Susceptible lentil variety L-9-12 was used for the testing. The Koch's Postulate was also performed for the same. The disease incidence was recorded by using the diseases incidence scale (Table 1) given by Rakhonde *et al.* (2017) with some modification.

RESULTS AND DISCUSSION

Survey of lentil grown areas for wilt disease assessment

From surveyed twenty two lentils grown areas none was found free from the wilt incidence, thus 100 per cent prevalence of the disease was observed. Disease was found frequent and severe in all surveyed lentil growing areas, however, the incidence (Table 2) of the disease was varied from 4.5 per cent to 25 per cent in different localities during the year 2017-18 and 8-27.5 per cent during the year 2018-19. The variation may be due to the several factors like rainfall, relative humidity, temperature, diverse cultivars used, sowing dates and even this may also attributed to the existence of pathogenic variability. The higher disease incidence in some localities may be either due to favourable environmental conditions or susceptibility of the cultivars. The factors altogether must build up the inoculum and subsequently resulted in increased disease severity in some localities as shown in Table 2 where incidence of the disease found increased in the next crop season. The maximum wilt disease incidence (Table 2) was recorded in Shantipuri (26.25%) followed by Halduchaur (23.96%), Pantnagar (22.18%), Bazpur (21.25%), Ramnagar (19.75%) and Kichcha (18.26%). The minimum wilt disease incidence was recorded in Bareilly (6.25%), Dhampur (7.73%) and Chakrata (9.23%). Survey and prevalence studies of lentil wilt were done earlier by several scientists in India and one of them was Chaudhary *et al.* (2010) who surveyed 116 districts of nine lentil growing areas in India and revealed the 0.7 to 9.3 per cent disease incidence at reproductive stage of the plant. Similarly, studies done by Sharma *et al.* (2016) revealed the status of lentil wilt at poding and flowering stage of the crop in three districts of Northern Madhya Pradesh and observed the prevalence of lentil wilt in all the areas surveyed and disease incidence recorded was ranged from 6.21 per cent to 19.37 per cent, however, disease incidence recorded in the present work was found more than the disease incidence recorded in the previous studies; the reasons behind that may be well establishment of the pathogen in the soil due to continuous cultivation of the lentil in the same field year after year or the varieties used by the farmers are becoming susceptible for the pathogen due to the evolution of the pathogen.

Pathogenicity of the isolates

The twenty-two isolates of pathogen were isolated on the PDA medium and further multiplied on the sand maize meal, thereafter, artificially inoculated in the autoclaved soil (20 g/kg soil) separately for their evaluation. The first symptom of diseaseappeared at 30 days after sowing of lentil susceptible variety. The symptoms like yellowing of the plant from the basal older leaves were first appeared in the pot having the inoculum of isolate from Rudrapur

Table 1: Categorization of the isolates of *Fusarium oxysporum* f.sp. *lentis* on the basis of disease incidence under glass house conditions.

Category	Percentwilt	Isolates
Non-pathogenic isolate (NPI)	0%	Nil
Weakly pathogenic isolate (WPI)	1-20%	Nil
Moderately pathogenic isolate (MPI)	21-50%	Nil
Highly pathogenic isolate (HPI)	51-70%	FOL4, FOL7, FOL8, FOL15
Extremely pathogenic isolate (EPI)	>70%	FOL1, FOL2, FOL3,FOL14, FOL6, FOL9, FOL10, FOL11, FOL12, FOL13, FOL14, FOL16, FOL17, FOL18, FOL19,FOL20, FOL21, FOL22

Table 2: Prevalence, incidence and pathogenicity of wilt disease in lentil grown areas of Uttarakhand and Uttar Pradesh during 2017-18 and 2018-19.

	Area surveyed	Plant stage for survey	Disease prevalence	Disease incidence (%) (2017-18)	Disease incidence (%) (2018-19)	Mean disease incidence (%)	Increase in the disease incidence	Pathogenicity (%)
FOL1	Baheri	Seedling	100	15.55	17.55	16.55^{g}	2^{l}	$91.24\ (9.60)^{e}$
FOL2	Bazpur	Flowering	100	20.0	22.5	21.25^{d}	2.5^{k}	$82.22\ (9.10)^{g}$
FOL3	Bareilly	Flowering	100	4.5	8.00	6.25^{s}	3.5^{i}	$88.00\ (9.36)^{f}$
FOL4	Bhowali	Seedling	100	9.25	13.25	11.25^{o}	4^{h}	$57.78\ (7.66)^{k}$
FOL5	Chakrata	Flowering	100	6.45	12.00	9.23^{q}	5.55^{e}	$98.88\ (9.98)^{a}$
FOL6	Dana	Seedling	100	13.09	15.00	14.05^{kl}	1.91^{l}	$75.56\ (8.72)^{h}$
FOL7	Dhampur	Flowering	100	5.45	10.00	7.73^{r}	4.55^{f}	$55.56\ (7.43)^{l}$
FOL8	Gadarpur	Pre flowering	100	10.05	14.3	12.18^{n}	4.25^{gh}	$62.22\ (7.91)^{j}$
FOL9	Gaulapar	Seedling	100	9.45	16.79	13.12^{m}	7.34^{b}	$82.22\ (9.12)^{g}$
FOL10	Halduchaur	Pre Flowering	100	23.25	24.67	23.96^{b}	1.42^{m}	$95.55\ (9.34)^{j}$
FOL11	Kanpur	Seedling	100	14.75	17.25	16.00^{hi}	2.5^{k}	$73.33\ (8.62)^{i}$
FOL12	Kathgariya	Flowering	100	13.0	16.00	14.50^{j}	3^{j}	$94.45\ (9.23)^{cd}$
FOL13	Kichchha	Flowering	100	17.26	19.25	18.26^{f}	1.99^{l}	$93.33\ (9.35)^{d}$
FOL14	Kotwali	Seedling	100	6.79	13.78	10.29^{p}	6.99^{c}	$83.78\ (9.21)^{g}$
FOL15	Naugaon	Seedling	100	5.55	14.25	9.90^{pq}	8.7^{a}	$51.11\ (7.20)^{m}$
FOL16	Pantnagar	Seedling	100	21.35	23.00	22.18^{c}	1.65^{m}	$71.78\ (8.62)^{i}$
FOL17	Pithoragarh	Flowering	100	6.85	14.00	10.43^{p}	7.15^{bc}	$94.55\ (9.60)^{bcd}$
FOL18	Purola	Pre flowering	100	13.25	17.66	15.46^{i}	4.41^{fg}	$96.25\ (9.85)^{b}$
FOL19	Ramnagar	Seedling	100	19.00	20.5	19.75^{e}	1.5^{m}	$87.78\ (9.36)^{f}$
FOL20	Rudrapur	Flowering	100	7.00	13.05	10.03^{p}	6.05^{d}	$93.36\ (9.71)^{d}$
FOL21	Shanti puri	Flowering	100	25.0	27.5	26.25^{a}	2.5^{k}	$75.56\ (8.54)^{h}$
FOL22	Tarkulha	Flowering	100	9.75	17.00	13.38^{lm}	7.25^{bc}	$95.56\ (9.71)^{bc}$
	Grand mean		100	12.57	16.69	14.63	4.12	1.94
	LSD (5%)	-	-	-	-	0.06	0.05	0.14

*Values written in brackets are transformed value of the per cent values.

*Values followed by different letters are significantly ($p<0.05$) different from each other.

Table 3: Growth rate and spore size of different isolates of *Fusarium oxysporum* f.sp. *lentis*.

Area surveyed	Growth rate	Av. size of microconidia (µm)		Av. size of macroconidia (µm)		Av. size of chlamydospores (µm)		Factor B Av. size of spores (µm)	
		Length	Width	Length	Width	Length	Width	Length	Width
Baheri	Fast	3.97^{p}	0.9^{BCD}	8.50^{h}	1.25^{vwx}	7.09^{j}	2.78^{hi}	6.52^{E}	1.64^{gh}
Bazpur	Fast	2.78^{vwxy}	1.4^{stu}	11.06^{d}	1.64^{q}	3.46^{qr}	5.74^{b}	5.76^{G}	2.92^{b}
Bareilly	Fast	3.38^{r}	1.22^{wxy}	19.33^{B}	2.04^{op}	2.21^{BC}	1.46^{rstu}	8.30^{C}	1.57^{hi}
Bhowali	Fast	2.55^{xyzA}	1.02^{ABC}	7.63^{i}	1.08^{yzA}	2.98^{tuv}	1.67^{q}	4.38^{M}	1.25^{k}
Chakrata	Fast	3.35^{r}	1.05^{A}	8.35^{h}	1.32^{uvw}	3.88^{p}	2.47^{l}	5.19^{IJ}	1.61^{gh}
Dana	Fast	3.23^{rstu}	1.4^{stu}	6.27^{l}	1.12^{xyzA}	2.17^{BC}	3.2^{e}	3.89^{O}	1.90^{e}
Dhampur	Medium	2.67^{wxyz}	0.73^{FGH}	6.75^{k}	1.06^{A}	3.20^{rstu}	3.04^{f}	4.20^{N}	1.61^{hi}
Gadarpur	Fast	3.21^{rstu}	0.86^{DEF}	5.84^{m}	0.8^{DEFG}	3.34^{r}	2.54^{jk}	4.13^{N}	1.40^{j}
Gaulapar	Fast	2.62^{wxyz}	1.07^{zA}	10.21^{e}	1.37^{tuv}	9.32^{f}	2.16^{mn}	7.38^{D}	1.53^{i}
Halduchaur	Fast	4.76^{o}	1.38^{tuv}	19.08^{b}	2.63^{jk}	2.45^{zAB}	7.69^{a}	8.76^{B}	3.9^{a}
Kanpur	Fast	2.99^{stuv}	0.64^{H}	5.83^{m}	0.75^{EFGH}	4.94^{n}	1.96^{p}	4.58^{L}	1.11^{l}
Kathgariya	Medium	2.78^{vwxy}	0.78^{EFGH}	10.20^{e}	2.05^{op}	2.97^{uv}	4.44^{c}	5.31^{I}	2.45^{c}
Kichchha	Fast	2.98^{tuv}	1.15^{xyzA}	9.19^{fg}	1.59^{qr}	6.61^{k}	2.46^{l}	6.26^{F}	1.73^{f}
Kotwali	Medium	2.27^{ABC}	1.25^{vwx}	6.00^{lm}	1.66^{q}	3.28^{rs}	4.39^{c}	3.85^{O}	2.93^{b}
Naugaon	Fast	3.27^{rst}	0.88^{CDE}	5.53^{n}	1.12^{xyzA}	1.45^{D}	2.81^{gh}	3.41^{P}	1.71^{fg}
Pantnagar	Slow	1.47^{D}	1.05^{A}	7.64^{i}	1.35^{tuv}	2.31^{AB}	0.89^{CDE}	3.80^{O}	1.41^{j}
Pithoragarh	Fast	1.69^{D}	0.47^{I}	12.27^{c}	1.05^{A}	2.67^{wxyz}	1.67^{q}	5.54^{H}	1.60^{hi}
Purola	Fast	5.43^{n}	1.21^{wxyz}	20.37^{A}	1.67^{q}	2.84^{vwx}	2.21^{m}	9.45^{A}	2.08^{d}
Ramnagar	Fast	2.86^{vw}	1.38^{rst}	8.99^{g}	0.78^{EFGH}	2.84^{vwx}	1.96^{p}	4.89^{K}	2.09^{d}
Rudrapur	Medium	3.95^{p}	0.84^{DEF}	7.46^{i}	0.73^{FGH}	3.69^{pq}	1.54^{qrs}	5.03^{JK}	2.02^{d}
Shanti puri	Medium	3.31^{r}	1.04^{AB}	7.60^{i}	1.12^{xyzA}	2.73^{vwxyz}	3.36^{d}	4.54^{LM}	2.37^{c}
Tarkulha	Medium	2.54^{yzA}	0.68^{GH}	10.12^{e}	1.39^{stu}	2.00^{C}	2.53^{jk}	4.88^{K}	1.73^{f}
Factor A	-	3.09^{c}	1.02^{c}	9.73^{a}	1.93^{b}	3.56^{b}	2.86^{a}	5.46	1.94
LSD (Spore length)		Factor A=0.06	Factor B=0.16	Factor A×B=0.29					
LSD (Spore width)		Factor A=0.03	Factor B=0.08	Factor A×B=0.14					

*Values followed by different letters are significantly ($p<0.05$) different from each other.

followed by the isolates from Bhowali, Bazpur, Pithoragarh, Pantnagar,Kanpur, Dhampur, Kathgariya,Purola, Dana, Shantipuri, Kotwali, Tarkulha, Ramnagar, Bareilly, Gaulapar, Naugaon, Gadarpur, Kichcha, Chakrata, Halduchaur and Baheri. The pathogen isolates from Ramnagar showed maximum disease incidence *i.e.* 98.88 percent followed by Halduchaur isolate (96.25%), Naugaon isolate (95.56%), Purola (95.55%) and Bhowali isolate (94.45%) (Table 2). Minimum disease incidence was recorded for the isolate of Bareilly (51.11%) followed by Gadarpur isolate (55.55%).

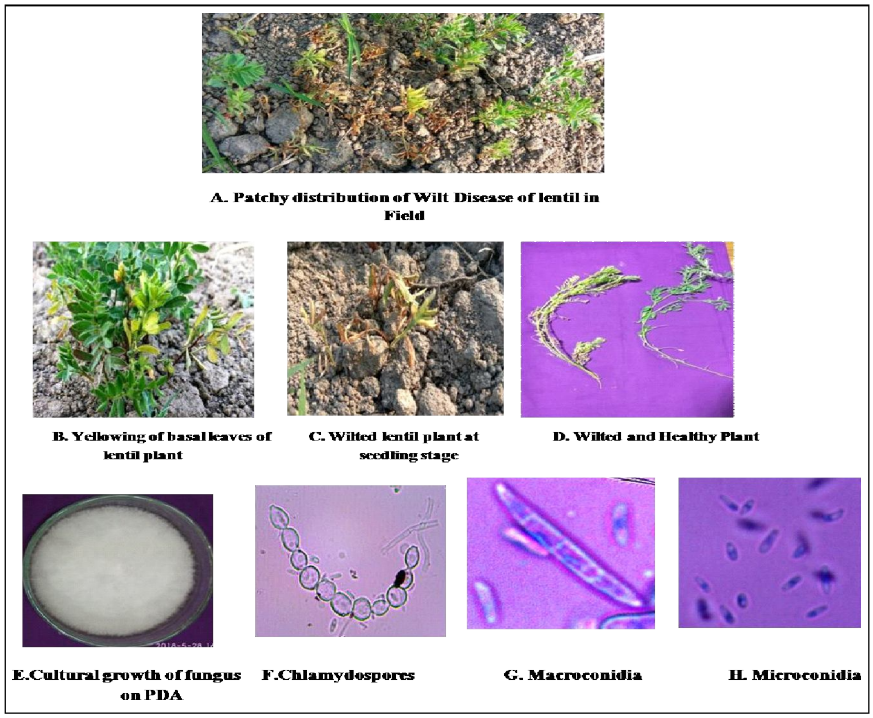

Fig 1: Wilt disease symptoms on lentil (A, B, C, D), isolated fungal culture on PDA medium (E) identification of fungus *Fusarium oxysporum* f.sp.*lentis* on the basis of spores (F, G, H).

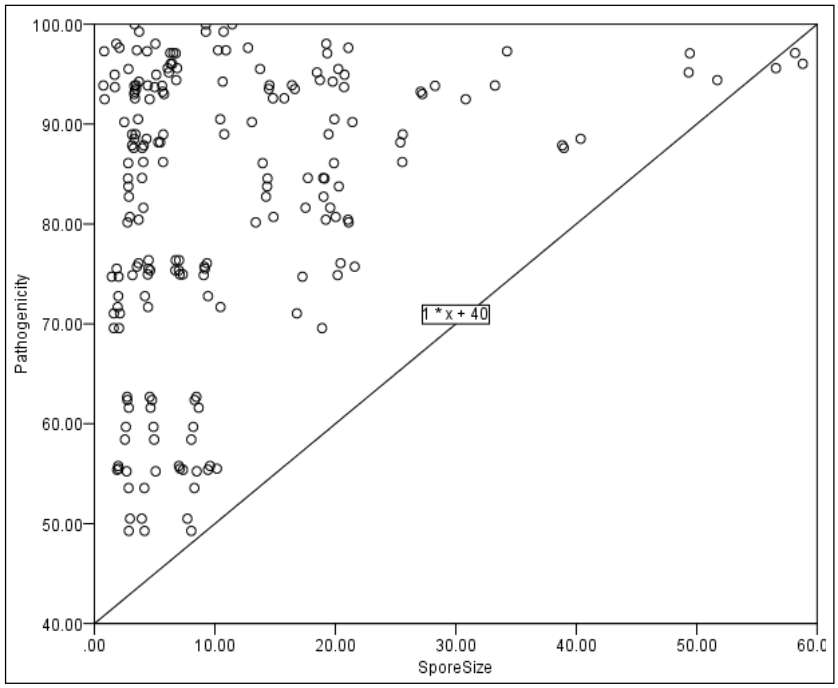

Fig 2: Moderate degree of Correlation between spore size and pathogenicity of*Fusarium oxysporum* f.sp. *lentis* isolate(Pearson's Correlation coefficient= 0.30).

The symptoms of the disease observed on the infected plants were similar to the symptoms observed by Das *et al.* (2022). The categorization (Table 1) of the pathogen isolates was done based on their disease incidence recorded. Conidial and mycelial characters of the isolates were similar to the characters described byTiwari *et al.* (2018). It was also observed that the appearance of the disease was more; for the isolates having fast growth pattern. They were able to infect the plants early as a comparison to others and the size of conidia was also found to have some impact on the appearance of the disease (Table 2,3; Fig 2).The correlation studies between the spore size and pathogenicity revealed the moderate degree of correlation between the spore size and pathogenicity with value of 0.30 *i.e*. Pearson's correlation coefficient (Fig 2).The results obtained were in accordance with Rakhonde *et al*. (2017) with some modification they identified the five categories of the *Fusarium oxysporum* f.sp.*ciceri* isolates based on their pathogenic behavior. Sharma and Agnihotri (1972) categorized the isolates into 'A', 'B' and 'C' groups, based on their virulence in lentil crop. Naimuddin and Chaudhary (2009) reported the pathogenic variability of the pathogen *Fusarium oxysporum* f.sp. *lentis* isolates and range of percent mortality of plants between 18.33 to 80 percent and revealed a wide range of variability in pathogenic character of isolates of *Fusarium oxysporum* f.sp. *lentis.* The present study also exhibited the wide range of variability in the pathogenic characters of the isolates of *Fusarium oxysporum* f.sp. *lentis.*

CONCLUSION

The wilt disease of lentil is one of the major diseases of lentil. It is caused by soil and seed borne fungus *Fusarium oxysporum* f.sp. *lentis.* As, the pathogen survives on the seeds of the crop as well as in the soil, it causes severe losses of the crop yield. Once the pathogen establishes in the soil it is capable of propagating itself in soil and difficult to eradicate easily, even the infected seeds are capable of introducing the disease in uninfected areas. Present study revealed the status of this disease in lentil growing areas of Uttarakhand and Uttar Pradesh. The study showed hundred percent prevalence of the disease as well as varying degree of disease incidence, thereby, showcased that the pathogen has been established in all lentil growing areas observed with cultivation of lentil. The observations recorded showed that in the coming years there may be chances of development of this disease as a big menace for the crop cultivation. The data also revealed that at the present situation we can manage the disease with minimal management strategies with low input cost. Therefore, the study concluded that the wilt disease of lentil can be emerged as big threat for lentil cultivation, so it is necessary to be prepared with all suitable management strategies of the disease.

Conflict of interest: None.

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