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Phoma Blight of Soybean in Kashmir: Etiology, Relative Yield Losses and Critical Stage of Management Intervention

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ABSTRACT

Background: Soybean leaf blight has been viewed as a possible biotic stress responsible for decreasing yield potential of soybean in Kashmir over the years. Therefore, its etiology and recording associated yield loss were deemed necessary corresponding to baseline findings for deciding any chemical or non-chemical intervention in disease management.

Methods: Field and laboratory investigations were undertaken during crop periods of 2019 and 2020. Disease specimens were collected from various locations across valley and the pathogen was isolated on Potato Dextrose Agar medium. To identify the host range of soybean isolate, nine pulse crops were tested under controlled conditions (Tem. 22±3°C and RH>80%). The relative yield loss and critical stage of chemical intervention were assessed by exposing soybean populations to disease at different phenological stages which created five different levels of disease in a field experiment laid in RBD with four replications.

Result: *Phoma sojicola* (syn. *Ascochyta sojicola*) was associated pathogen of this disease of soybean in Kashmir. Typical disease symptoms on leaves comprised of roughly circular and brown lesions (up to 20 mm diameter) with concentric rings having interspersed pycnidia. Most often the diseased tissue fall apart and gives it a shot hole expression. Radial daily mycelial growth of the pathogen was 3.62 mm colony was grey olivaceous in the centre and light olivaceous towards periphery. Conidia were hyaline, oblong to ellipsoidal and 1-2 celled with average dimensions of 7.98 × 2.87 µ. Chlamydospores were brown olivaceous, globose to sub-globose with average dimensions of 16.89 × 9.30 µ. After artificial inoculation, *Phaseolus vulgaris*, *Vigna sinensis, V. radiata*, *V. mungo*, *Cicer arietinum, Vicia faba* and *Cajanus cajan* manifested disease symptoms after variable incubation periods of 8-13 days, while as *Lens esculenta* and *Pisum sativum* resisted the invasion. Early appearance of the Phoma blight (V-1 stage) in soybean can reach a terminal intensity of 67.16 per cent besides causing 51.72 per cent yield loss. Tolerable yield loss (5.63%) was achieved only when the crop was maintained disease free up to R-3 (beginning pod development) stage.

Key words: Ascochyta, Etiology, Glycine, Phoma, Sojicola, Soybean.

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] belongs to family Fabaceae and is an economically significant crop worldwide. It is known as 'Golden bean' or the 'Super legume' for its well-recognized food value (Kulcheski *et al*., 2016). It was also referred as "Gold of 20th century" on account of its easy cultivation, high benefit-cost ratio and less requirement of nitrogen fertilizer (Khutate *et al*., 2005). Soybean, native to the northeast regions of China and the Korean peninsula (Lee, 2015), is grown in fifty countries around the world (Boerma and Specht, 2004). It is grown foremost for its protein content and secondarily for oil, thus can either be classified as food bean or as oil bean (Ivanov *et al*., 2009). Jammu and Kashmir was leading in production of soybean in 1940s and 1950s with annual production of 502 tonnes on 702.80 ha (Shurtleff and Aoyagi, 2012). As this region is facing an acute oilseed deficit and animal feed shortage, a strong need is felt for the exploitation of this multipurpose crop to the maximum of its potential. However, like in many other pulses, the occurrence of biotic stress in soybean leads to the reduction of its yield to varying extents. In India, losses in soybean due to various diseases have been estimated to the extent of 12 per cent of total production and the major diseases reported are rust, wilt, leaf spot, rot and powdery mildew besides bacterial and viral diseases (Jahagirdar, 2019). Ascochyta blight is among serious diseases of cool climate pulses and it may lead to total crop failure in a disease favouring environment. In India, this disease is largely distributed in the Indo-Gangetic Plains and is known to occur widely in North-Western Plain Zone covering Jammu, Punjab, Haryana, Western UP and North-West Rajasthan, causing a yield loss of about 50-90 per cent (Grewal and Pal, 1986). In Kashmir valley, the incidence of leaf blight in soybean resembling Ascochyta blight has emerged as a predominant disease during past few years. Keeping in view the importance of this versatile crop and lack of base line data about this leaf blight, region specific

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studies were undertaken *vis-a-vis* important aspects like etiology of the disease, host range of the pathogen and assessment of associated yield loss.

MATERIALS AND METHODS

This study was conducted in Faculty of Agriculture (SKUAST-K) at Wadura (J and K) during 2019 and 2020 crop seasons. The associated fungus was isolated in axenic culture by standard tissue bit transfer method (Yadav, 2015) and its pathogenicity was confirmed on 4 week old healthy potted soybean plant. Spore suspension, adjusted to approximately 5×10^6 spores ml^{-1} was used for spot inoculation in a 'C' pattern on fully expanded leaves under greenhouse conditions comprising moderate temperature (22±3°C) and high relative humidity (>80%). The plants were regularly observed for development of typical disease symptoms. Moreover, associated symptoms of the disease *viz*., date of first appearance of disease and the subsequent developmental changes, were also recorded on naturally infected soybean plants (cv. Shalimar soybean-1) maintained unsprayed under field conditions. The morphology comprised of micrometry and the observations like shape and colour of conidia, picnidia, chlymadospore and mycelium. Staining and mounting of fungal structures was done by using lactophenol cotton blue solution (Weeks and Padhye, 1982). Micrometry was conducted with a calibrated compound microscope aided by Magvision software.

Most commonly grown pulses in Kashmir *viz*., common bean (*P. vulgaris*), cow pea *(V. sinensis),* green gram (*V. radiata* L.), black gram (*V. mungo* L.), pea (*P. sativum*) and also other less popular pulses like chick pea (*C. arietinum*)*,* lentil (*L. esculentis*), broad bean (*V. faba*) and pigeon pea (*C. cajan*) were tested for their post inoculation response to the soybean isolate (*P. sojicola*) under controlled conditions (Tem. 22±3°C and RH>80%) in a poly house. Healthy seeds of each pulse crop were surface sterilized and direct-seeded into pre-sterilized garden soil in plastic trays. The plants were inoculated 30 days after sowing (DAS) with spore suspension (5×10^6 conidia ml^{-1}) of the pathogen prepared afresh. The plants were regularly observed up to 3 weeks after inoculation for recording observation on incubation period, shape, size and colour of disease spot wherever the symptom developed.

The yield reduction associated with this disease was assessed by comparing grain yields attained in plots variedly affected by the leaf blight and that of plots where the disease was managed at minimum level with frequent chemical interventions. Five levels of disease (D_1-D_5) were created by exposing five different unit populations (P_1-P_5) of the soybean (Cv. Shalimar soybean-1) to *P. sojicola* at different phenological stages representing respective five treatments (T_1-T_5) each with four replications in RBD. Disease was first initiated in P_1 and plants surrounding blocks and the rows serving as buffer zone between plots by spray inoculation with a spore suspension (5×10^6 conidia ml^{-1}) of the pathogen 20 days after sowing. Until a plant population were exposed to pathogenic invasion, it was maintained free of disease by foliar application of carbendazim + mancozeb 75 WP @ 0.25% as given below. This way we could also determine the critical period of disease management during a cropping season besides predicting terminal disease level vis-à-vis starting of this disease at a particular phenological stage.

Treatment	Details	
	Fungicidal intervention (Days after sowing)	Exposure to Phom blight (Days after sowing/ crop stage)
T_1	No fungicidal spray	20/ V1 – 1st node
T_2	20	35/ V4 - 4th node
T_3	20 and 35	50/ R1-beginning bloom
T_4	20, 35 and 50	65/ R3- beginning pod
T_5	20, 35, 50, and 65	80/ Maturity

Observations on disease were recorded at physiological maturity of the crop. Disease intensity was recorded on 15 plants in each plot on the basis of diseased plant area while using 0-9 generalised disease scoring scale (0 = No disease, 1 = less than 10% diseased plant area, 2 = 11-20 %, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70 %, 8 = 71-80% and 9 = more than 80% diseased plant area) and was calculated by using the Mckinney's formula (McKinney, 1923) while as the disease incidence was worked out by using the formula (Number of diseased units)/(Total number of units examined).

RESULTS AND DISCUSSION

While proving the pathogenicity of the fungus, the disease symptoms became visible 7 to 8 days after inoculation in the desired 'C' pattern presented in Fig 1. Symptoms comprised of small brown spots which developed into characteristic roughly circular lesions having pale whitish centre surrounded by dark brown margin. The ring pattern was also associated with these lesions and took it about 20 days to manifest the typical blight symptoms. Morphological characteristics of the pathogen are presented in Fig 2.

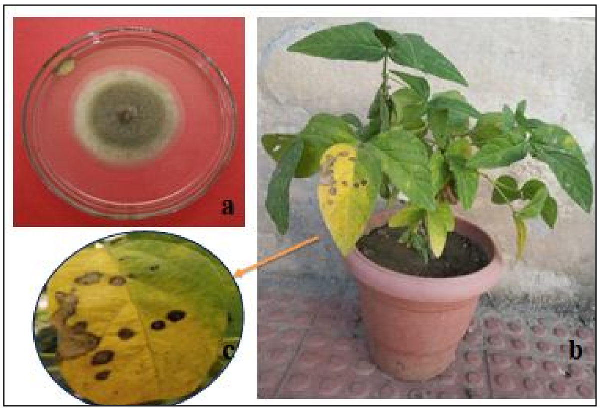


Fig 1: Axenic culture of *P. sojicola* on PDA (a); Symptoms in 'C' pattern after artificial inoculation on soybean leaf (b and c).

Conidia were mainly aseptate, (sometimes one-septate), hyaline, oblong to ellipsoidal slightly constricted at septum, rounded on both ends measuring 6.52 - 12.30 × 2.11-4.67 µm (av. 8.25 × 2.89 µm). Conidia formed in axenic culture on PDA were relatively with average dimensions of 7.98 × 2.87 µm. Conidia were released from pycnidia in the form of long chains called 'cirrhus'. Pycnidia were dark brown with a diameter of 67.01-255.43 µm (av. 146.34 µm). Pycnidia in axenic culture on PDA were larger than those observed on host leaf having average diameter of 213.87 µm. These were glabrous, olivaceous to olivaceous black, ostiolate, globose to sub-globose. Some occurred solitary and a few confluent, scattered irregularly on the colony surface or sometimes clustered in distinct sectors. Chlamydospores observed in culture were spherical having diameter of 20.10 µm and also cylindrical to sub-globose with average dimensions of 9.30 × 16.89 µm. Chlamydospores were olivaceous to olivaceous brown, terminal or intercalary, occurring singly or in chains or in aggregation and started appearing after 10 days of incubation at 25±1°C. Mycelium was septate, sub-hyaline to grey olivaceous with average diameter of 4.08 µm having 4-6 septa per 100 µm hypha length. The morphological characteristics of this isolate were more or less similar to those earlier described by Abramov (1931), Boerema *et al*. (1997) and Kovics *et al*. (1999) for *Phoma sojicola* (syn. *Ascochyta sojicola*).

Cultural properties of the fungus are presented in Fig 3. First visual growth of the pathogenic fungus on PDA was observed after 24 hours of incubation at 25±1°C. The fungus showed a steady growth and covered 72.4 mm diameter of PDA surface within 10 days @ 3.62 mm average daily radial growth colony an appeared greenish to grey olivaceous with fluffy texture and prominent black pycnidia visible throughout. Kovics *et al*. (1999) recorded more or less similar observations similar when *P. sojicola* was cultured on Oat Agar, Malt Agar and Corn Agar media. The little variation in the colony characteristics can be attributed to variable sources and forms of nutrients in these media and also to the possible varying existence of *P. sojicola* isolates in time and space.

Observations on symptom development as recorded on leaves, stem and pods of soybean are presented in Fig 4. The disease initially appeared as small brown spots of 2-3 mm diameter on leaves during 4th week of June in a month old crop. These spots increased in size steadily and reached maximum diameter of about 19.53 mm in next 3 weeks @ 0.88 mm day^{-1} uninterrupted by veins. The spots developed on leaf margins as well as on leaf lamina and those on margins attained characteristic 'v' shape. Necrotic leaf spots were surrounded by yellow halo and also reflected prominent concentric irregular bands of brown shades and finally turned dull grey to tan. Most often the necrotic area was partly or wholly shed apart resulting in short hole expression. Premature defoliation was also recorded in the subsequent disease development. Later on, disease symptom also developed on stem and pods. On stem the lesions were

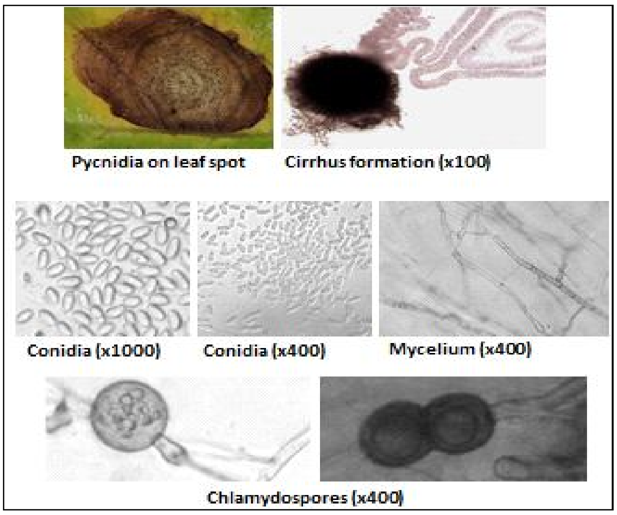
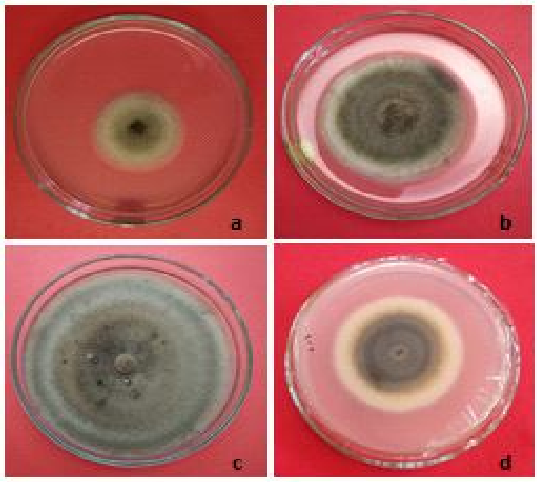
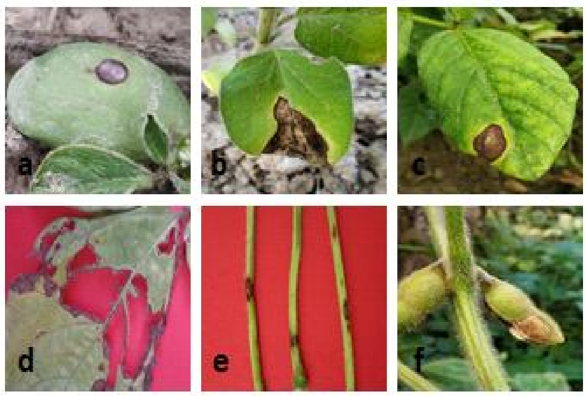


Fig 2: Morphological characteristics of *P. sojicola*, a soybean isolate.



a): 4 days old, b): 7 days old, c): 10 days old and d) underside of 8 days old colonies on PDA.

Fig 3: Colony characteristics of *P. sojicola* causing blight of soybean.

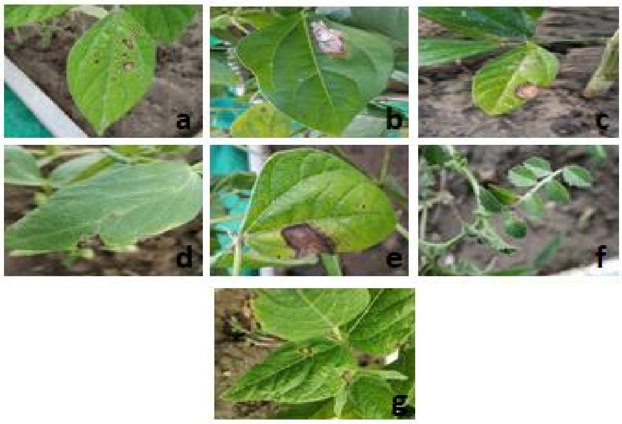


a) Lesion on cotyledon leaf, b) V-shaped lesion, c) Mature lesion on leaf, d) Coalescing of lesions and holes left after diseased area has fallen out, e) Lesions on stem and f) Lesion on soybean pod.

Fig 4: Symptoms on soyben due to *P. sojicola*.

brown, elliptical and measured (4.50-11.50 × 3.20-4.33 mm) with brownish to purplish margin and white greyish centre. Developing pods showed roughly circular to oval brown lesions with light brown centre and narrow dark brown margin attaining maximum size of 8-15 mm. The pycnidial production was abundant and supported by leaf, stem as well as pod lesions. These results were somewhat similar to those described by Kovics *et al*. (1999) for *P. sojicola* on soybean. However, there was no mention of yellow halo in their description. It cannot be denied either as according to Kim and Chen (2019) ascochitine, a secondary metabolite produced by *Ascochyta*, can produce such yellow discolouration in the affected leaf tissue. Varying existence of *P. sojicola* isolates and variation in host genotypes in time and space manifesting variable disease symptoms cannot be denied either. Moreover, infection of pods and the underlying seed hints at its seed borne nature though crop debris, particularly the stem and pods, may support its survival in field the most.

Investigations on host range of *P. sojicola* revealed that this isolate can infect most of the commonly grown pulses viz. common bean, cow pea, green gram, black gram, chick pea, broad bean and pigeon pea in a favourable environment (Table 1 and Fig 5). Manifestation of symptoms by the host cultivars of these pulses was variable with respect to incubation period, rate of lesion development and its appearance. Incubation period was found minimum (8 days) in common bean and maximum (13 days) in pigeon pea cultivars while as the rate of lesion development after appearance of initial symptoms was maximum (0.70 mm day^{-1}) on broad bean. These pulse cultivars falling in the host range of *P. sojicola* may be lacking resistance gene against this pathogen and also *Phoma* spp. produce



a) Green gram, b) Cowpea, c) Pigeon pea, d) Broad bean, e) Blact gram, f) Chickpea and g) Commom bean.

Fig 5: Symptom manifestation by various pulse cultivars after inoculation with *P. sojicola*.

Table 1: Host range of the soybean isolate, *P. sojicola,* among other pulses.

Test plant cultivar	Disease reaction	Incubation period (IP) in days	Maximum lesion size and growth rate (post IP)	Disease symptoms
Common bean (WB-1634)	+ve	8	4-5 mm after 9 days @ 0.39 mm day^{-1}	Circular to roughly circular, light brown lesion with definite dark brown margin; presence of pycnidia conspicuous.
Chick pea (Shalimar chickpea-1)	+ve	10	3 mm after 5 days @ 0.40 mm day^{-1}	Circular spot with light brown centre and dark brown outline with pycnidia visible through naked eye.
Cow pea (Shalimar cowpea-1)	+ve	12	11 × 8 mm after 13 days @ 0.65 mm day^{-1}	Irregular whitish lesion surrounded by dark brown margin; visible pycnidia present throughout.
Green gram (Shalimar moong-1)	+ve	12	5-6 × 5 mm after 7 days @ 0.64 mm day^{-1}	Roughly circular light brown lesion with definite dark brown margin supporting numerous pycnidia throughout.
Black gram (Shalimar blackgram-1)	+ve	12	5 × 3.5 mm after 7 days @ 0.46 mm day^{-1}	Roughly circular, brown lesion with dark brown margin having visible pycnidia.
Broad bean (SKUA-VFA-101)	+ve	11	11 × 5 mm after 10 days @ 0.70 mm day^{-1}	Sub-circular lesion having conspicuous concentric rings and interspersed pycnidia.
Pigeon pea (WP-1)	+ve	13	5 × 2.5 mm after 6 days @ 0.64 mm day^{-1}	Oblong to sub-circular light brown lesion surrounded by dark brown margin. Concentric rings more towards the periphery and the pycnidial distribution visible throughout the lesion.
Lentil (Shalimar masoor-1)	-ve		-	-
Pea (Shalimar pea-1)	-ve		-	-

Table 2: Yield loss in soybean vis-à-vis Phoma leaf blight under field conditions.

Disease initiation stage	Terminal disease incidence (%)	Terminal disease intensity (%)	Seed yield (grams/plot)*	Yield loss over check (%)
T1: V-1	82.81 (66.18)	67.16 (55.11)	181.50	51.72
T2: V-4	65.12 (54.05)	39.55 (38.92)	260.35	30.74
T3: R-1	53.32 (46.90)	28.85 (32.41)	310.25	17.46
T4: R-3	26.52 (30.79)	8.98 (17.26)	354.75	5.63
T5: Maturity (Check)	9.48 (17.62)	2.96 (9.68)	375.90	-
CD($P\leq0.05$)	7.3	4.1	22.5	
SE(d)	3.3	1.8	10.2	

*2 m × 1 m plot size; Values in parenthesis are arc-sine transformed values.

Table 3: Simple correlation coefficients between soybean yield and Phoma leaf blight.

Variables	Disease incidence	Disease intensity	Yield
Disease incidence	1		
Disease intensity	0.97*	1	
Yield	-0.96*	-0.99*	1

*Significant at ($p\leq0.05$).

polyketide-derived secondary metabolites (ascochitine toxin) which is considered responsible for significant toxicity in a range of legumes. Lentil and pea were found non-hosts either due to their insensitive response to ascochitine or their non-host resistant factor. According to Newman and Derbyshire (2020), necrotrophic fungi showing broader host range have highly modified molecular toolkit that comprises of mechanisms to modulate host reactive oxygen species, pH and detoxification of wide range of host-derived antifungal secondary metabolites. This too can be a reason for compatible intereaction of *P. sojicola,* a soybean isolate, and most of the pulses tested in present investigation. The findings of Frenkel *et al*. (2007) that *P. pinodella* was pathogenic to *Pisum sativum, P. fulvum, Cicer judaicum, C. arietinum, C. reticulatum, C. pinnatiûdum* and *C. bijugum* also support the present findings. Similarly, Van der Aa *et al*. (2000) reported that *P. exigua* var. *exigua* has wide host range and can infect more than 200 host genera. Moreover, these results also corroborate the findings of Keirnan *et al*. (2020) who has reported that *P. koolunga* cause disease lesions on a wide range of legumes in controlled environment.

Data on yield loss in soybean vis-à-vis leaf blight reveals that the disease levels were significantly different from each other besides revealing significant impact of disease initiation stage on leaf blight development and subsequent yield loss (Table 2). Maximum disease incidence (82.81%) and intensity (67.16%) were recorded in soybean population where disease initiated at first node stage (V-1). There was proportionately highest yield loss of 51.72 per cent when compared with check (T5) where significantly lower levels of terminal disease incidence and intensity were observed. Yield loss of 30.74 and 17.46 per cent was recorded when disease initiated at V-4 and R-1 stages, respectively. Disease incidence (26.52%) and intensity (8.98%), recorded in soybean population where disease first appeared at R-3 stage, were again significantly higher as compared to check. However, the yield harvested from these two populations was statistically at par. The reduction in yield was due to loss of photosynthetic area and the significant difference in yields of different plant populations in this case was quite possible as the disease is polycyclic in nature and earlier infection was supposed to support more inoculum build up and subsequent disease development. These findings are supported by a comparable report of Xue *et al*. (1997) that inoculation of plants at flowering caused yield loss of 24-34 per cent compared to inoculation at pod filling stage where it caused only 19 per cent of yield loss. The population that was maintained free of disease upto R-3 stage suffered a tolerable loss of 5.63 per cent. Further, the data was subjected to statistical analysis in order to find out the extent of relation between yield and disease parameters (Table 3). The analysis matrix showed highly significant and negative correlation of yield with disease incidence and disease intensity. It is supported by the findings of Chang *et al*. (2007) who reported a negative relationship between yield per plant and Ascochyta blight severity in chickpea. The findings of present study also corroborate the findings of Nene (1981) that Ascochyta blight in beans caused yield loss upto 50-70 per cent and Shahid *et al.* (2008) reported that Ascochyta blight significantly reduced chickpea seed yield, quality and caused yield losses of about 100 per cent in susceptible cultivars. Similarly, the yield losses due to Phoma blight in soybean can be much more under disease favouring weather in Kashmir, hence it should be considered a significant limiting factor in soybean production. Therefore, need to be evaluated for successful establishment of this potential pulse/ oilseed crop in Kashmir.

CONCLUSION

The associated pathogen was identified to be *Phoma sojicola* (Abramov) Kovics, Gruyter and Aa. This soybean isolate has a good number of other pulses to infect besides soybean and the identified susceptible cultivars of those pulses should not be recommended in the areas where soybean succumbs to *P. sojicola*. However, pea and lentil cultivars can be recommended in such areas and incorporated in the cropping sequence with an objective of reducing population build-up of this pathogen. Phoma blight is a major disease of soybean in Kashmir and has a potential to cause 0.894 units decrease in soybean yield with every unit increase in its intensity. Crop stages up to R-3 stage should be considered a critical period for management of this disease and suitable management strategies be recommended as soon as the disease appears during this period.

Conflict of interest

The authors declare that they have not any conflict of interest in publishing this research article.

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