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Research Article

Morphological and Cultural Characterization of Quarantine Concerned *Phomopsis* spp. Associated with Oilseed Crops

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Abstract

Total twelve isolates representing five species of *Phomopsis* were collected from various oilseed crops representing both exotic as well as domestic collections of India and were examined for morpho-cultural variability. Isolates of *Phomopsis* spp. showed significant difference in colony type, colour, radial growth, shape and size of both α and β spore, pycnidial arrangement, production, colour and days required for intiation. PCA revealed that among different mopho-cultural charcters, few characters such as colony margin, days required for full growth, pycnidial colour as well as days required for pycnidial initiation exhibited strong correlation for explaining variability. Three groups A, B and C were created through neighbour joining cluster analysis based on morpho-cultural characters of *Phomopsis* spp. Group A included both the domestic and exotic isolates of *P. phaseoli*, whereas group C was most diverse group including isolates of four *Phomopsis* spp. collected from different crops and agro ecological regions. This study revealed that morphological variation arises due to adaptation to various ecological zones that may lead to introduction of new race or genotype of *Phomopsis* spp. to the India. This variation among *Phomopsis* spp. can be used in resistance breeding programme to various crops.

Key words: Conidia, crops, cultural, *Phomopsis* spp., pycnidia

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Indian economic is considerably contributed by oil seed by achieving second place next to cereals regarding as agricultural commodity. Oil-seeds contribute nearly 16.75 per cent of the total arable land and produce nearly 11 per cent of the total value of the agricultural products in the country during 2015-2016 (Agricultural Statistics at a Glace 2017). Among the various seed borne diseases of oilseed crops, disease cause by *Phomopsis* is an economical important disease. *Phomopsis* can also infect wide range of crop species with nearly 900 different species (Rossman and Palm-Hernandez 2008). Apart from plant pathogen, *Phomopsis* can also survive in various modes such as saprobes, endophytes and even able to cause diseases in human or animals (Garcia Reyne et al 2011). Oilseed crops suffer from different diseases caused by *Phomopsis* with enumerated symptoms *i.e.*, cankers, diebacks, root rots, fruit rots, leaf spots, blights, decay and wilts (Santos and Phillips 2009). Among various symptom, seed decay cause by *Phomopsis* is an important for plant quarantine. Severely infected seed become shriveled, fractured prolonged, and chalky, but transitory infection in seed due to *Phomopsis* is also very common (Hartman et al 1999). Seed lose their vigour and viability after infection and lead to produce inferior plant in field (Raeisi et al 2011).

Morpho-cultural characters of plant pathogenic fungi were major criteria for taxonomical study; therefore various classical compilations such as monographs were used in morphological taxonomy

(Hyde et al 2010). Similarly, *Phomopsis* spp. were also characterized based on morpho-cultural characters and association with host (Chi et al 2007). *Phomopsis* characterized based on morphological characters such as pycnidia posses ostiole, cylindrical elongated phialides that contain mainly two types of spores, alpha and beta (Rehner and Uecker 1994). The alpha conidia are characterized with fusiform in shape, hyaline, aseptate, biguttulate or unguttulate or multiguttulated. Whereas, beta conidia are characterized with filiform in shape, straight or slightly curved, hyaline, aseptate and unguttulate (Sutton 1980). Interestingly, some species produce third type of conidia named as gamma conidia (Cristescu 2007) are fusiform in shape, hyaline and multiguttulated (Rodeva et al 2009). Conidiophore of *Phomoposis* is hyaline, short, septate, filiform (Crisescu 2003). Various *Phomopsis* species were grouped into two major group W types and G type based on cultural characters on potato dextrose agar (PDA) media such as colony colour, white type of colony for W type and grey or brown type of colony for G type (Kanematsu et al 2000). Further, virulence behavoiur also distinguish them, W type is more virulent for apple, peach and pear in Japan compare to G type (Kanematsu et al 2007). The *Phomopsis* is very important plant pathogenic fungi in imperative necessitate of taxonomic re-evaluation (Rehner and Uecker 1994) for creation of contrast to host association identification formerly used (Zhang et al 1997). Various species or species complex can infected oilseed crop. Zhang et al (1997) identified four species of *Phomopsis/Diaporthe* complex *i.e.*, *D. phaseolorum*, *D. phaseolorum* var. *sojae*, *D. phaseolorum* var. *meridionalis* and *D. phaseolorum* var. *caulivora*. Similarly, four species also identified in association with sunflower as *D. helianthi*, *P. longicolla*, *D. stewartii* and *Diaporthe* spp. (Mathew et al 2012). Few reports on *Phomopsis* complex was obtained on cultural and morphological variability from different countries. Hence, the present investigation on identification of morphological and cultural variability among *Phomopsis* isolates were collected both from indigenously as well as exotic sources.

Materials and Methods

Source of biological materials. Pure cultures of twelve isolates were represented by five *Phomopsis* species were obtained by using modified isolation technique (Akhtar et al 2013) from seeds of various oilseeds crops soybean (*Glycine max*), jatropha (*Jatropha curcas*), safflower (*Carthamus tinctorius*), black locust (*Robinia pseudoacacia*) and with two reference crops, brinjal (*Solanum melongena*), and chilli (*Capsicum annuum*) (Table 1). All the isolates were sub-cultured on PDA

Table 1. Details of *Phomopsis* spp. isolated from seed of different crops

Isolate	*Phomopsis* species	Host	Source/ Place of collection
P1	*P. longicolla*	*Capsicum annuum* (Capsicum)	Seed, ICAR-IIHR, Bangalore
P2	*P. helianthi*	*Carthamus tinctorius* (Sunflower)	Seed, ICAR-NBPGR regional station, Hyderabad
P3	*P. helianthi*	*Carthamus tinctorius* (Sunflower)	Seed, ICAR-NBPGR regional station, Hyderabad
P4	*P. vexans*	*Solanum melongena* (Brinjal)	Fruit, ICAR-IARI, New Delhi
P5	*P. phaseoli*	*Glycine max* (Soybean)	Seed, ICAR-NBPGR regional station, Bhowali
P6	*P. phaseoli*	*Glycine max* (Soybean)	Seed, ICAR-NBPGR regional station, Bhowali
P7	*P. phaseoli*	*Glycine max* (Soybean)	Seed, ICAR-IISR, Indore
P8	*P. phaseoli*	*Glycine max* (Soybean)	Seed, ICAR-IISR, Indore
P9	*P. phaseoli*	*Jatropha curcas* (Jatropha)	Seed, Germany
P10	*P. phaseoli*	*Jatropha curcas* (Jatropha)	Seed, Germany
P11	*P. phaseoli*	*Jatropha curcas* (Jatropha)	Seed, Germany
P12	*P. eres*	*Robinia pseudoacacia* (Black locust)	Seed, Hungary

medium in sterilized Petri plates at 27±1 C and further these purified cultures were stored at 4±1 C for future studies.

Cultural characters. From seven days old culture 4 mm of mycelium disc was incubated to a Petri plate containing 20 mL of PDA and Petri plates incubated at 27±1 C in BOD with three replications for further observations of cultural characters as method described by Rajput and Harlapur (2016). Radial growth was measured after 3 days of incubation as method described by Rajput et al 2017. Based on visual observations colony colour was grouped as W type or G type Kanematsu et al (2007). Days required for complete growth in Petri plates were also recorded along with margin of culture growth wavy to circular. Isolates were also classified based on observed growth parameters as excellent required 4 days for full growth in Petri plates, good required 5-6 days, medium required 6-7 days and slow required more than 7 days.

Morpho-cultural Characters

Conidial characters. The pathogens were identified as per the characteristics described in IMI descriptions of fungi by Mathur and Kongsdal (2003). Both alpha and beta conidia were obtain by sequenzing of pycnidial, collected from 20 days old culture in sterilize distilled water. Conidia slides were prepared under compound microscope at different levels of magnification *i.e.*, 4.0 X to 40.0 X and size and shape of both alpha and beta conidia were measured from NIS element software.

Pycnidial characters. Various pycnidial characters such as pycnidial production (above, underneath or submerged) pycnidial arrangement (randomly and circular), pycnidial of colour (black to brown), days required for pycnidial initiation. were observed. The pycnidial size was measured through NIS element software under sterio microscope.

Data analysis. SAS statistical software ver 9.2 was performed on the data obtain through morpho-cultural characters. Liner mixed model was performed in SAS statistical software ver 9.2 for analysis of isolates interaction, where isolates called as different characters and interaction used as random effects. An inclusive picture of possible mutual interaction among the morpho-cultural characters was obtain through Principle Component Analysis (PCA), whereas through DARwin software ver 6.0 through neighbour joining cluster analysis of different isolates was performed (Singh et al 2018).

Results and Discussion

Cultural variability in *Phomopsis* spp. All five species of *Phomopsis* showed significant variability for cultural characters such as colony colour, radial growth after 3 days of incubation, days required for full growth, margin of colony and type of colony (Table 2). Six isolates consisting of four *Phomopsis* spp. showed light brown colour, including *Phomopsis phaseoli* (P7, P8 and P10) collected from soybean and jatropa, *P. longicolla* (P1) and *P. helianthi* (P2 and P3) isolated from sunflower. Interestingly, yellowish or pinkish pigmentation was also observed in *P. phaseoli* (P7 and P9), where as other isolates showed light brown to dark brown colour of pigmentation except *P. longicolla* (P1) and *P. phaseoli* (P6 and P11). This result concluded that among the *Phomopsis* spp. huge variation was observed exhibited in relation to W and G type of colony. Similar variation was also observed within the isolates of *P. phaseoli,* where most of the isolates were classified as W type, except one isolates each from of jatropa and soybean of *P. phaseoli* (P5 and P10) were be classified as G type. Most of the isolates of *Phomopsis* spp. produced circular margin of colony except exotic isolates of *P. phaseoli* (P10 and P11), *P. eres* (P12) and indigenous isolate of *P. longicolla* (P1) who could produce irregular margin. Isolates of *P. longicolla* (P1), *P. vexans* (P4), *P. eres* (P12) and *P. helianthi* (P2 and P3) were classified as W type. Among the isolates, *P. phaseoli* (P7 and P8) isolated from soybean showed fastest growth, 8.35 cm of radial growth within 3 days of incubation, whereas *P. vexans* (P4) is slowest among them with 2.74 cm of radial growth within 3 days of inoculation. Most of the isolates of *P. phaseoli* exhibited excellent to good growth except *P. phaseoli* (P5) isolated from soybean cultivated in hilly region of India. Isolates of *P. phaseoli* (P7 and P8) also covered entire 90 mm Petri plate within 4 days, where as *P. vexans* (P4) required 15 days. Based on these results, isolates of five *Phomopsis* spp. were grouped into 4

Table 2. Cultural character of different isolates of *Phomopsis* spp.

Isolate	Colony colour		Types of colony	Shape of margin colony	Radial growth after 3 days (cm)	Days required for full growth	Growth
	Upper	Lower					
P1	Light brown	Light creamy white	W	Irregular	6.82	5.0	Good
P2	Light brown	Dark brown	G	Circular	5.85	5.5	Medium
P3	Light brown	Dark brown	G	Circular	5.85	5.5	Medium
P4	Greyish	Greyish	G	Circular	2.74	15.0	Slow
P5	Creamy colour	Dark brown	G	Circular	3.74	7.0	Slow
P6	Creamy white colour	Creamy white colour	W	Circular	6.92	5.0	Good
P7	Light brown	Yellowish to creamy white colour	W	Circular	8.35	4.0	Excellent
P8	Light brown	Yellowish to creamy white colour	W	Circular	8.35	4.0	Excellent
P9	Creamy white colour	Pinkish to creamy white colour	W	Circular	6.87	5.0	Good
P10	Light brown	Black centre with brown margin	G	Irregular	6.32	5.5	Good
P11	Creamy white colour	Creamy white colour	W	Irregular	6.64	5.5	Good
P12	Greyish centre to grey brown colour	Black centre with brown margin	G	Irregular	5.55	6.0	Medium
C.D. (P <0.05)					0.42		

Table 3. Conidial character of different isolates of *Phomopsis* spp.

Isolate	Average spore α size after 15 days (µm)	Range of α spore size (µm)	Shape of α conidia	Average spore β size after 15 days (µm)	Range of β spore size (µm)	Shape of β conidia
P1	4.48	4.18-4.76	Sub cylindrical	0.0	0.0	–
P2	4.56	4.28-4.74	Sub cylindrical	18.68	12.56-23.75	Mostly curved
P3	4.39	4.54-4.66	Sub cylindrical	19.69	16.03-24.41	Mostly curved
P4	3.84	3.11-4.57	Sub cylindrical	12.19	8.62-15.36	Mostly curved few filliform
P5	6.18	4.12-7.30	Sub cylindrical	18.04	11.18-24.45	Mostly Filliform to few curved
P6	4.81	4.40-5.25	Sub cylindrical	17.21	15.53-19.53	Mostly curved to few filliform
P7	5.63	5.27-5.91	Sub cylindrical	16.86	13.59-18.91	Mostly curved
P8	5.57	5.17-5.79	Sub cylindrical	16.65	13.88-20.77	Mostly curved
P9	6.14	5.13-6.73	Sub cylindrical	15.89	14.53-18.89	Mostly curved to few filliform
P10	6.19	5.53-6.93	Sub cylindrical	17.68	16.17-20.33	Mostly curved to few filliform
P11	6.26	5.53-6.94	Sub cylindrical	18.46	12.27-21.17	Mostly curved
P12	0.0	0.0	–	16.91	14.75-18.91	Mostly Filliform to few curved
C.D. (P <0.05)	0.89			1.32		

group (excellent growth, good growth, medium growth and slow growth). All isolates of *P. phaseoli* exhibited excellent to good growth except one isolate. *P. eres* (P12) and *P. helianthi* (P2 and P3) exhibited medium growth at the same time as *P. vexans* (P4) exhibited slow growth.

Morphological Variability in *Phomopsis* spp.

Conidial variability in *Phomopsis* spp. All five species of *Phomopsis* showed significant variability for size of both α and β conidia (Table 3). All isolates produced α conidia, except isolate of *P. eres* (P12). Largest α conidia was produced by *P.*

phaseoli (P11) as 6.26 µm with a range 5.53-6.94 µm, whereas smallest α conidia was produced by *P. vexans* (P4) as 3.84 µm with a range 3.11-4.57 µm. Exotic isolates of *P. phaseoli* (P9, P10 and P11) isolated from jatropa were produce larger α conidia with a range 5.13-6.94 µm as compared to indigenous isolates of *P. phaseoli* (P6, P7 and P8) with a range 4.40-5.91µm, interestingly one indigenous isolate of *P. phaseoli* (P5) also produce larger α conidia. *P. helianthi* (P2 and P3) produce medium size of α conidia with a range 4.28-4.74 µm. Shape of α conidia was not showed any variability among the five species of *Phomopsis;* produce sub cylindrical type of α conidia. All isolates produce α conidia, except isolate of *P. longicolla* (P1). Largest β conidia was observed in *P. helianthi* (P3) as 19.69 µm with a range 16.03-24.41 µm, where as smallest β conidia was observed in *P. vexans* (P4) as 12.19 µm with a range 8.62-15.36 µm. Both indigenous and as well as exotic isolates of *P. phaseoli* did not showed significance difference. Two type of β conidia were produce by five species of *Phomopsis,* either filliform or curved.

Pycnidial variability in *Phomopsis* spp. For morphological variability analysis 12 isolates of *Phomopsis* consisting of five species were grown on PDA and observed significant variability for morphological characters such as pycnidia production in Petriplate, colour of pycnidia, days required for pycnidial initiation and size of pycnidia (Table 4). Three kinds of pycnidia production were observed in five species of *Phomopsis i.e.*, submerged in mycelium, above the mycelium and beneath the mycelium. All exotic isolates of *P. phaseoli* (P9-P11) produce pycnidia in beneath the mycelium, whereas indigenous isolates of *P. phaseoli* (P5, P6, P7 and P8) produced all three type of pycnidia. *P. vexans* (P4) produced pycnidia beneath the mycelium, where as *P. eres* (P12) produced pycnidia both above and beneath the mycelium. *P. longicolla* (P1) and *P. helianthi* (P2 and P3) produces pycnidial in submerged condition. Two kinds of pycnidia arrangement were observed in five species of *Phomopsis i.e.*, circular and randomly. All the isolates of *P. phaseoli* (P5, P7, P8, P9, P10 and P11), *P. helianthi* (P2 and P3) and *P. eres* (P12) showed random arrangement of pycnidia except isolate one of *P. phaseoli* (P6) whereas *P. longicolla* (P1) produced pycnidia in circular arrangement. Brown to black colour pycnidia was produced isolates of *Phomopsis* spp.

Table 4. Pycnidial character of different isolates of *Phomopsis* spp.

Isolate	Pycnidia production in Petriplate	Arrangement of pycnidia	Colour of pycnidia	Days required for pycnidial initiation	Size of pycnidia (µm)	Range of pycnidial size (µm)
P1	Submerged in mycelium	Circular	Brown	10	1087.87	712.76-1205.93
P2	Both submerged and beneath the mycelium	Randomly	Black	10	1132.94	949.18-1597.99
P3	Both submerged and beneath the mycelium	Randomly	Black	10	526.57	306.97-773.18
P4	Beneath the mycelium	Circular at outer layer	Brown	18	128.33	93.46-196.89
P5	Both above and beneath the mycelium	Randomly	Brown	12	887.01	459.02-1511.14
P6	Submerged in mycelium and beneath the mycelium	Circular	Brown	12	644.16	445.88-800.05
P7	Beneath the mycelium	Randomly	Brown	10	445.86	335.08-644.98
P8	Both above and beneath the mycelium	Randomly	Brown	10	667.10	203.4-1044.66
P9	Beneath in mycelium	Randomly	Brown	12	758.45	485.37-1051.92
P10	Beneath in mycelium	Randomly	Brown	11	752.48	465.27-1151.95
P11	Beneath the mycelium	Randomly	Brown	12	982.19	875.57-1019.19
P12	Both above and beneath the mycelium	Randomly	Brown	11	1381.34	857.64-1525.25
	CD ($P < 0.05$)				25.65	

Most of isolates of *Phomopsis* spp. produced brown colour pycnidia except *P. helianthi* (P2 and P3) produced black colour pycnidia. Except *P. vexans* (P4), all isolates of *Phomopsis* spp. initiated pycnidial production within 10 to12 days. The size of pycnidia showed significant variation among the different *Phomopsis* spp. Largest pycnidia was observed in *P. eres* (P12) as 1381.34 µm with a range of 949.18-1597.99 µm, followed by *P. longicolla* (P1) (1087.87 µm) and *P. phaseoli* (P10) (982.19 µm). Smallest pycnidia was observed in *P. vexans* (P4) (128.33 µm) with a range of 93.46-196.89 µm. All the exotic isolates of *P. phaseoli* (P9, P10 and P11) showed similar pycnidia character except size of pycnidia. In contrast all indigenous isolates of *P. phaseoli* (P9, P10 and P11) showed high degree of variation in pycnidial characters.

PCA of morpho-cultural characters. PCA was performed to known the few critical components which can summarize the independent variable that includes the total variance. Inclusive pictures of synergistic morpho-cultural characters were obtained through PCA, whereas various principle components and their individual fraction of variation were explained by eigenvectors (Table 5). The huge variation was observed through PCA indicated that the impact of morpho-cultural characters of *Phomopsis* spp. Biplots obtain through PCA with first couple of principle components were resulting in phenotypic variations among the *Phomopsis* spp. for morphological and cultural characters and the variance measurements among the different variable selected for morpho-cultural variability. The graph obtained through PCA clearly distinguished the characters that were disperse along the axis of two principle components

Table 5. Eigenvectors values for different morpho-cultural parameters of *Phomopsis* spp. corresponding to PC

Variable	Principle components		
	1	2	3
Colony colour upper (CCU)	0.21	-0.37	-0.10
Colony colour lower (CCL)	-0.22	-0.39	-0.30
Types of colony (TC)	0.36	0.10	0.21
Shape of margin colony (SMC)	0.05	0.31	0.07
Radial growth (RG)	-0.41	-0.12	0.13
Days required for full growth (DRFG)	0.33	0.17	-0.31
Growth (G)	0.41	0.11	-0.04
Average spore α size (ASS)	-0.19	0.29	0.05
Shape of α conidia (ASS_0001)	-0.17	0.45	-0.07
Average spore β size (BSS)	0.13	0.00	0.43
Shape of β conidia (BSS_0001)	0.34	-0.21	0.06
Pycnidia production (PP)	0.18	-0.23	0.17
Arrangement of pycinidia (AP)	0.09	-0.05	0.47
Colour of pycnidia (CP)	0.03	0.27	0.35
Days required for pycnidial initiation (DRPI)	0.29	0.12	-0.37
Pycnidial size (PS)	-0.03	-0.36	0.18
Eigenvalue	4.92	3.33	2.82
Proportion	30.71	20.87	17.59
Cumulative Proportion	30.71	51.58	69.16

and highlight the extent of phenotypic variation represented by cluster. The morpho-cultural characters that performed excellent and positively correlated in selected variable are in upper right quadrant of graph. Among the 12 isolates representing five *Phomopsis* spp, *P. helianthi* (P2 and P3) and *P. phaseoli* isolated from soybean and jatropha (P6, P7, P8, P9, P10 and P11) were positively clustered with first couple of principal components (Fig 2). PCA revealed that few cultural characters such as type of colony, shape of margin colony, days required for full growth and growth, whereas couples of morphological characters such as colour of pycnidia and days required for pycnidial initiation were exhibited strong correlation for explaining variability among different isolates of *Phomopsis* spp. (Fig 1). PCA allows avoiding the various characters which exhibit comparable attributes. The eigenvalues were calculated by correlation matrix. By using screen plots of eigenvalue various components were summarized and biplot was created by using of first couple of principle components (Table 4). The first principle components revealed 30.71 per cent of total variation with major contribution of growth, types of colony, shape of β conidia and days required for full growth, whereas in second principle components major contributors are shape of α conidia and shape of margin colony which revealed variability of 51.58 per cent. PCA of first

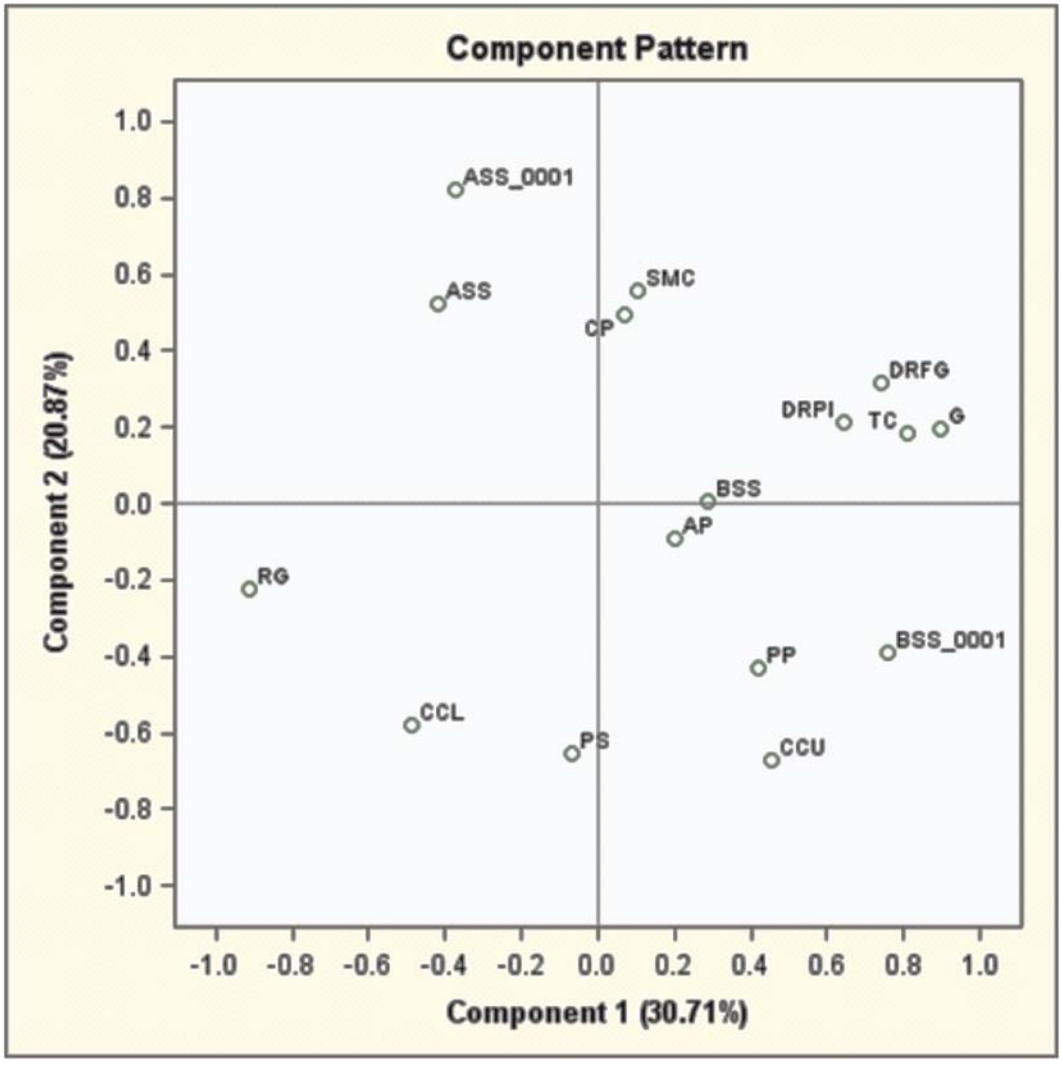

Figure 1. Biplot scores of first two principal components for morpho-cultural characters (n=16). Details of morpho-cultural characters are given in table 4

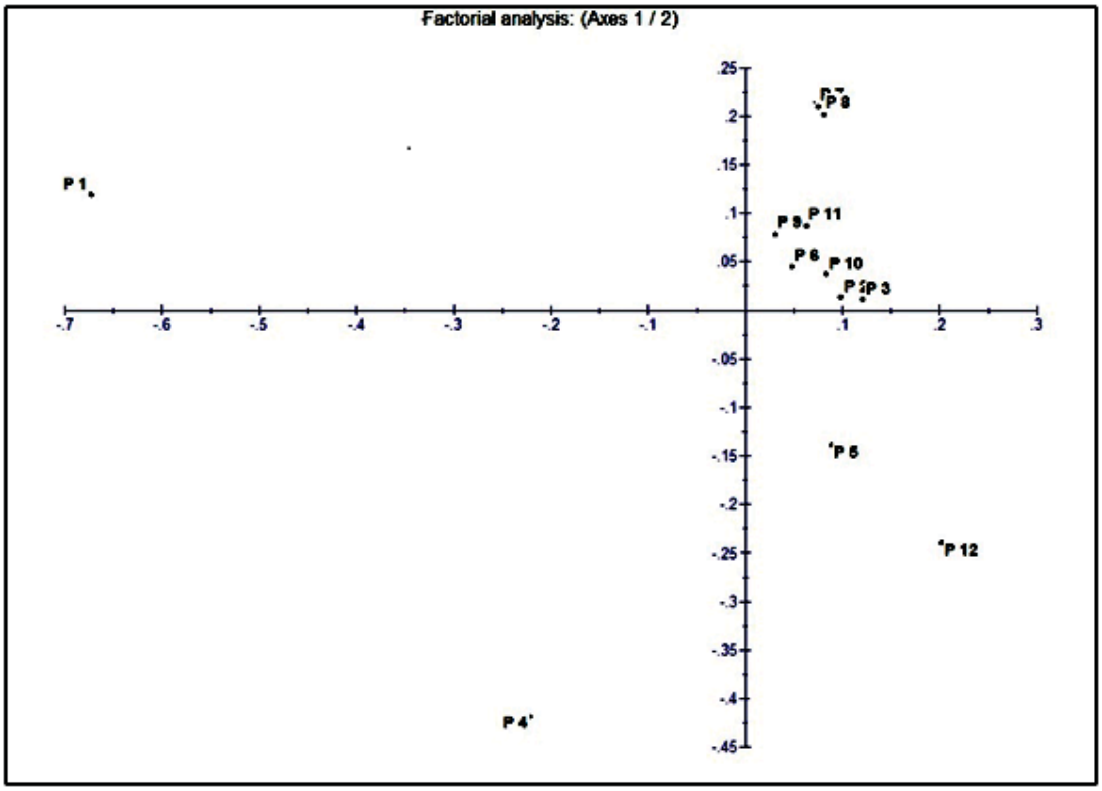

Figure 2. Biplot scores of first two principal components for different *Phomopsis* spp. (n=12). Details of isolates are given in table 1

three principle components also revealed 69.16 per cent of total variation. Arrangement of pycnidia revealed 47.24 per cent of variability and average β spore size revealed 43.07 per cent of variability, were major contributors for third principle components.

Cluster analysis for morpho-cultural characters. Based on 7 cultural characters, 4 conidial characters and 5 pycnidial characters (n=16) were used for cluster analysis of 12 isolates representing five *Phomopsis* spp. (Fig 3). Three groups of 12 isolates of *Phomopsis* spp. were created through cluster analysis with neighbour joining method. Group A included 5 isolates, group B included 2 isolates and group C included 4 isolates. Group A included both exotic and domestic isolates of *P. phaseoli* (P7, P8, P9, P10 and P11) isolated from soybean Jatropha. Group B included only domestic isolates of *P. helianthi* (P2 and P3) isolated from sunflower. Group C is most diverse group, included both exotic and domestic isolates collected from various geographical region and various crops. Group C included 4 different *Phomopsis* spp *i.e., P. phaseoli* (P5), *P. longicolla* (P1), *P. vexans* (P4) and *P. eres* (P12). Among the all isolates *P. phaseoli* (P6) isolated from soybean was unique from remaining isolates. Both PCA and cluster analysis corresponded good to each other, also revealed that *P. phaseoli* (P5), *P. longicolla* (P1), *P. vexans* (P4) and *P. eres* (P12) were distinct from rest of species, which indicate high variability and dissimilarity with other isolates of *Phomopsis* spp.

Various ascomycetes fungus can infect oilseed crops, among them *Phomopsis* is also economical and pathological important fungus with diverse host range and wider adaptability to environment (Udayanga et al 2011). Nearly thousand plant host was reported to cause economical damage by various *Phomopsis* spp. (Rossman and Palm-Hernandez 2008). Among them some *Phomopsis* spp. are of quarantine importance to India and can cause epidemic in India due to lack of resistance sources and some constraint in farmer's practices. Also, huge exchange of plant materials, latent

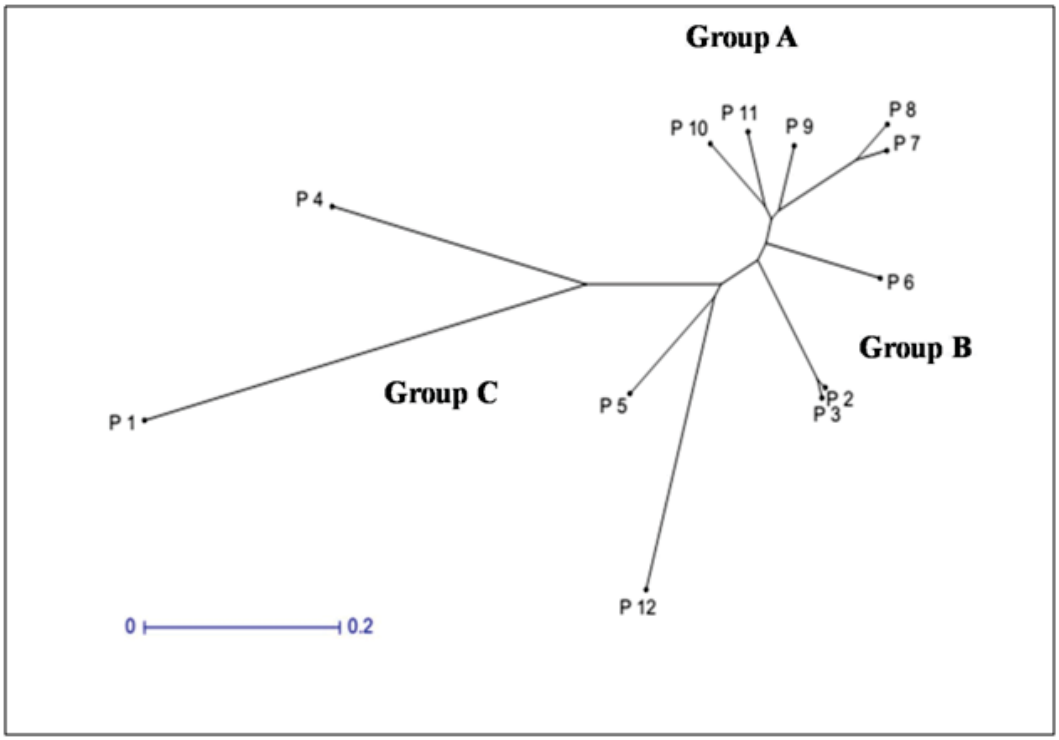

Figure 3. Phylogenetic tree constructed through neighbour joining cluster analysis, for 12 isolates of *Phomopsis* spp. based on morphological, conidial and pycnidial variability. Isolates number represented on terminal. Branch robustness was tested using 1000 bootstrap. Details of isolates are given in table 1.

infection and togetherness in non domesticated host plant in India, always keep India at risk of introduction of new race of phytopathogenic fungus.

In present study, 12 isolates of *Phomopsis* consisting of five species were characterized based on morphological and cultural characters. Our isolates showed significant different in cultural characters such as colony colour from both upper and lower side, types of growth, shape of margin of colony, radial growth after 3 days, days required for full growth, growth pattern, average α spore size and shape and average β spore size and shape. Highly significant difference was also observed in mophological characters such as pattern of pycnidia production, arrangement of pycnidia, colour of pycnidia, days required for pycnidial initiation, and size of pycnidia. Based on colony colour *Phomopsis* spp. classified in two major groups *i.e.*, W or G type. Two isolates of *P. phaseoli* classified as G type, whereas rest of W type this is due to difference in virulence or adaption to host (Kanematsu et al 2000). Similarly different isolates of *Phomopsis* spp. isolated from peach, pears and apples of Japan, were also divided into W and G type based on colony characters and virulence (Kanematsu et al 2007). Gray, brown, yellow and pink pigmentation was observed in *Phomopsis* spp., whereas, margin of the colony also varied from irregular to circular. These variations arise due to influence of light and production of melanin (Brayford 1990, Rajagopal et al 2011). Taxonomical characters such as average size of α and β conidia showed significant variation among the *Phomopsis* spp. and interestingly, little variation was observed within species of *Phomopsis*. The pycnidia were variable in location of production, but most of *Phomopsis* spp. producing pycnidia beneath the mycelium. Among the *Phomopsis* spp., pycnidial production was mostly randomly arranged and brown in colour, whereas require 10 days for initiation. Average size of pycnidia showed

significant variation among the *Phomopsis* spp, with a range of 128.33 µm to 1381.34 µm. Different isolates of *P. phaseoli* were also showed high level of variation in size of pycnidia, with a range of 203.4 µm to 1525.25 µm. Similarly, both conidial and pycnidial characters was matching with previously reported *Phomopsis* spp. (Uecker 1988). PCA and gouping analysis revealed that despite having some similar morphological characters, *P. phaseoli* isolated from both hilly and plain areas of soybean showed high variation. Interestingly, *P. phaseoli* (5) isolated form soybean and *P. eres* (P12) isolated from Jatropa clustered together showed that mophological characters of fungi are influence by ecological ambience or geographic regions. This could happen due to various weed host or soil type which can act as inoculums building agents for fungi (Biswas et al 2008). Both domestic and exotic isolates of *P. phaseoli* isolated from soybean and jatropha grouped together, this revealed that both domestic and exotic isolates have common source of origin. Interestingly, they differ with ecological and geographical condition that revealed the strong inherent potential for survival in diverse agro climatic zones which leads to increase extensive coverage of disease incident all across different susceptible cultivar of oilseed crops of throughout of India. Our investigation yielded three group of *Phomopsis* spp., based on morpho-cultural characters. This revealed the pathogenic variation among different isolates collected from same or different geographical regions. This variation among *Phomopsis* isolates can be used in resistance breeding programme to various crops. This provides the information on natural variation among *Phomopsis* spp., and warrants the need of comprehensive study of *Phomopsis* spp. covering all agro-ecological zones of oil seeds crops of India, especially soybean.

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Compliance with ethical standards

Conflict of interest. The authors declare that they have no conflict of interest.

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