Review Article

# Current Insights on Stemphylium Blight of Lentil with its Management Strategies

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**Abstract** | Stemphylium Blight (SB) triggered by *Stemphylium botryosum* (Wallr.) (teleomorph- *Pleospora herbarum*), first identified in Bangladesh in 1986, is a significant challenge to all the major lentil-growing nations. It induces partial to complete foliar damage and results in about 3/5$^{th}$ to full failure of the lentil yield. The molecular study for the recognition and delineation of species is inevitable as high complexity is seen due to environmental concerns and contrasting morphological characteristics among species. The frequency and intensity of this disease depends on environmental and climatic factors that are mainly favored by high humidity, temperature greater than 22$^{0}$C, and cloudiness. The pathogen mainly persists in crop debris and occasionally in seed during the offseason. Mapping QTLs by utilizing wild varieties and landraces may be used to develop disease-resistant varieties which it is the most efficient, cost-effective, and environmentally sustainable technique. Integrated disease management approaches along with Omics and weather prediction tools may be crucial in avoiding severe SB outbreaks. A comprehensive review article discusses the morphological as well as molecular dimension of pathogens, their effects, pathogenicity, integrated disease management approaches, and future views.

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## Introduction

Lentil (*Lens culinaris* Medik.), one of the classic founder crops is an annual, herbaceous self-pollinating grain legume grown in winter-season belonging to subfamily Papilionoideae of family Fabaceae/Leguminosae (Schaefer *et al.*, 2012). Western Asia is the place where cultivated lentil (2n=14) is assumed to have been first originated and domesticated. Later, it spread quickly to Africa, southern and central Europe, the Mediterranean basin, and Indian subcontinents (Matny, 2015). Seven taxa systematized into four species were described in the most recent classification, namely *L. culinaris* ssp. *culinaris*, *L. culinaris* ssp. *tomentosus*, *L. culinaris* ssp. *orientalis*, *L. culinaris* ssp. *odemensis*, *L. ervoides*, *L. lamottei*, and *L. nigricans* (Wong *et al.*, 2015). They are rich in proteins, minerals, prebiotics, carbohydrates, cholesterol lowering soluble fibers, and are naturally low in phytic acid so often stated as poor's man meat for the lower economic class. Aside from nutritional properties, lentil is important for the dryland cropping system in rotation to cereals because of their N-fixing capacity. Also, its crop residues can be used as feed for animals (Gupta *et al.*, 2013; Subedi *et al.*, 2014). The total cultivated area of lentil is estimated at around 6.10 million hectares with annual production and productivity of 6.33 million tons and 1.04 mt/ha, respectively in the world. Canada, India, USA, Turkey, Australia, Kazakhstan, Nepal, Russian Federation,

Bangladesh, and China are the top 10 lentil producing countries (FAOSTAT, 2020).

Among a widespread range of fungal pathogens infecting the lentil plants, stemphylium blight caused by *Stemphylium botryosum* Wallr. was previously a minor disease but it is increasing tremendously as a serious threat in recent years (Mwakutuya and Banniza, 2010). Over 33 species of *Stemphylium* are known among which *S. botryosum*, *S. solani*, *and S. vesicarium* are very prevalent to cause important crops and fruit trees diseases (Wang *et al*., 2010). *Stemphylium botryosum* also infects many more hosts such as *S. botryosum* f. sp. *lactucae* for lettuce, *S. botryosum* f. sp. *lycopersici* for tomato, and *S. botryosum* f. sp. *spinacia* for spinach but not any such specialty for *S. botryosum* is recognized in pulses so far (Kant *et al*., 2017). In the experiment performed by Vaghefi *et al*. (2020), *S. beticola* and *S. eturmiunum* were recorded in lentil for the first time while *S. astragali* for the first time in Australia. Other species like *S. vesicarium* and *S. simmonsii* were also recorded in lentil. Stemphylium blight of lentil is a defoliating disease which was first recognized in Bangladesh and later reported in many lentil producing countries that causes heavy losses up to 80-92.35% but disease development can be influenced by many environmental factors (Huq and Khan, 2008).

Stemphylium blight can be distinguished from other diseases by morphological and molecular characterization, and the severity of infection along with the symptoms appeared. Blights like Ascochyta blight and Alternaria blight can also be seen in the lentil simultaneously. Sometimes, they can be misdiagnosed with Stemphylium blight as all of them are problematic from flowering to maturity, mostly infecting above ground parts, symptoms look similar, and often the mixed infection is encountered (Dokken-Bouchard, 2011). It can be perplexed with other diseases of lentil such as Ascochyta blight (No dark pycnidia is found in Stemphylium), Sclerotinia rot (No visible white fluffy mold or black sclerotia in Stemphylium blight) or Botrytis blight (No grey fuzzy mold under a magnifying glass in Stemphylium blight) (Isaacs, 2014). So far, the understanding of this disease is missing and very few studies have been carried out on the identification of different species of *Stemphylium*, epidemiology of the pathogens, factors influencing disease growth, host-pathogen interaction, mechanisms of resistance, and population racial composition. Very few researches are published concerning various aspects of Stemphylium blight of lentil (Das *et al*., 2019). Therefore, this article reviews the existing understanding of its economic importance, geographical distribution, etiology, epidemiology, variability, symptomology, disease management strategy alongside the future outlook on the possible activities related to Stemphylium blight of lentil.

*Geographical distribution*

*Stemphylium botryosum* have recorded to be distributed over the world in both pathogenic and saprophytic forms (Brahamanage *et al*., 2018). The disease Stemphylium blight on lentil was first documented during 1981 and was further verified in 1986 from Bangladesh by Bakr and Zahid (1986). From 1990 to current date, Stemphylium blight has observed as a major disease of lentil (Alam *et al*., 2017a). It has been considered as one of the disastrous diseases of lentil production in South Asian countries mostly in Bangladesh, Eastern Nepal, and North-Eastern India (Alam *et al*., 2017a; Chen *et al*., 2009; Subedi *et al*., 2014). It is rising as a serious threat in West and South Asia, North America, and severely spread as a minor disease in Saskatchewan, Canada (Yadav *et al*., 2017). Previous works conducted in Bangladesh and India measured 62% of losses and sometimes complete crop failure was resulted due to extensive defoliation during the early stage of the pod setting (Razzak *et al*., 2018; Alam *et al*., 2017a). Since then, it has got prominence due to its increased occurrence and severity in many other countries like Hungary, Canada, Australia, Egypt, Syria, USA, Iran (Chen *et al*., 2009). The diverse host range including both leguminous and non-leguminous plants indicated the adaptability and existence of the pathogen to diverse environmental conditions (Yadav *et al*., 2017). During 1993, Stemphylium blight was first reported in Nepal. Recently it has been recorded from Banke, Bardiya, Rupandehi, Chitwan, Makwanpur, Bara, Parsa, and Rauthat districts (Subedi *et al*., 2014).

*Economic importance*

The disease causes the loss of green part of leaf and reduction in photosynthetic capacity at flowering and pod filling stage affecting the quantity and quality of grain as well as seed production (Hay *et al*., 2019). Crop loss of 80-92.35% or even complete crop failure in the epidemic year had been recorded from Bangladesh. Similarly, 82.55% disease incidence and 93.4% crop

failure were noted in India (Mandal *et al.*, 2019). Nowadays, the disease is a key challenge not only in Bangladesh but also in northeastern India, Nepal, and other regions of the world causing yield losses up to 100 percent (Islam *et al.*, 2020). After a dry yet very humid growing season in 2007, Stemphylium blight was fairly extreme and widespread. Afterward, it is commonly considered to be rising in Saskatchewan, Canada (Dokken-Bouchard, 2010). The prevalence of Stemphylium blight in most of the varieties was found maximum during the flowering stage followed by pod setting stage and maturity stage but it may differ with varieties. It results in huge economic loss due to the production of low quantity and quality of grain and seed (Razzak *et al.*, 2018).

*Morphological identification of Stemphylium and related genera*

Before the progress of the molecular methods in fungal taxonomy, species were identified mainly upon morphological characterization including colony growth characters; shape, size and color of conidia and conidiophore; number, arrangement, and constriction of conidial septa; length-width ratio; surface ornamentation, etc. (Poursafar *et al.*, 2016; Pryor and Gilbertson, 2000). *Stemphylium* and its related genera *Alternaria*, *Ulocladium*, *Macrosporium* produces phaeodictyosporic conidia (Camara *et al.*, 2002). Simmons (1969) classified many of typical *Alternaria* and *Stemphylium* into *Ulocladium*.

Economically, one of the important genus associated with lentil blight is *Stemphylium botryosum* Wallr (Anamorph) whereas *Pleospora herbarum* is the sexual stage (teleomorph). Moreover, other genus like *Alternaria tenuissiama* and *Ascochyta fabae* f. sp. *lentis* also cause lentil blight (Koike *et al.*, 2001; Tripathi, 2015) but the damage symptoms may slightly differ with genera to genera. Morphological characteristics of different blight causing genus are shown in Table 2. Among them, *Alternaria* and *Stemphylium* are morphologically related species (Pryor and Gilbertson, 2000). *Stemphylium* can be distinguished from others dematious hyphomycete by the presence of swollen apical conidiogenous cells and proliferating conidiophores (Woudenberg *et al.*, 2017).

There are more than 200 species of *Stemphylium* identified that causes different disease in more than 43 genera (Brahamanage *et al.*, 2018). Chiefly, the species are saprophytic, endophytic, or epiphytic in nature (Poursafar *et al.*, 2016). Some species such as *S. botryosum* and *S. globuliferum* or *S. eturmiunum* and *S. vesicarium* seem morphologically similar and misidentified using morphology alone (Olsen *et al.*, 2018). Morphologically distinctive species such as *S. alfalfa*, *S. herbarum*, *S. vesicarium* and other appears in the identical phylogenetic clade (Camara *et al.*, 2002). Minor alterations in the conidial size and shape, ornamentations of the tip of the conidiophore, and patterns of septum development characterized the species *S. botryosum* and *S. globuliferum* (Simmons, 1969). Morphological characterization of different species of *Stemphylium* is shown in Table 1 as well as in Figures 1, 2 and 3.

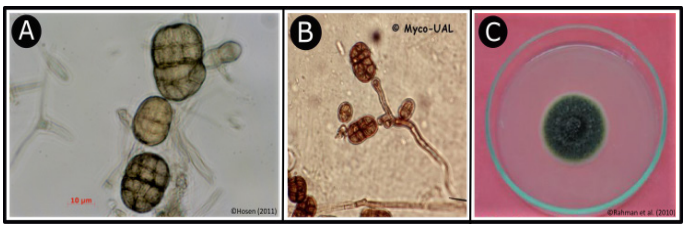


**Figure 1:** *Conidia and conidiophore of S. botryosum (A and B). Colony morphology of S. botryosum (C).*

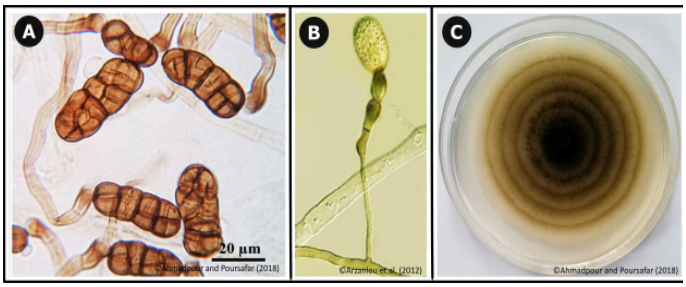


**Figure 2:** *Conidia and conidiophore of S. vesicarium (A and B). Colony morphology of S. vesicarium (C).*


**Figure 3:** *Conidia and conidiophore of S. solani (A and B). Colony morphology of S. solani (C).*

*Culture media for sporulation of pathogens*

Several kinds of culture media can be used for sporulation. Some of them are (1) Potato dextrose agar (39 g Difco PDA); (2) Malt extract agar (20 g malt extract, 1 g protease peptone, 20 g sucrose and 15 g agar); (3) V8 juice agar (V8: 200 mL of Campbell's V8 juice, 4 g $CaCO_3$ and 15 g agar); (4) V8 + PDA (150 mL V8 juice agar, 10 g PDA, 10 g agar); (5) Nutrient agar (20 g agar, 4 g dextrose, and 2 g $KNO_2$); (6) Water agar (20 g agar) and (7) Coon's medium (1.2 g $MgSO_4$, 2.7 g

**Table 1:** *Morphological characterization of different species of Stemphylium.*

| Characters | *Stemphylium botryosum* | *Stemphylium vesicarium* | *Stemphylium solani* |
|---|---|---|---|
| Colony color | Grey Brown or brownish-black | Grey to brownish grey | Subhyaline initially, Grayish green, gradually becoming dark brown |
| Colony shape | Regular without sector; velvety to cottony | Forms circular concentric rings, entire and flat; sparse aerial mycelium | Hairy or velvety, rounded, irregular margin |
| Mycelium character | Aerial, brown to green gray | Branched, septate, smooth, immersed, and superficial, sub hyaline to pale brown | Hyaline, septate |
| Conidiophore | Brown, unbranched, percurrent proliferation, swollen dark and roughened apical cell | Pale to mild brown; Simple or Occasionally one branch; Straight to variously curved; septate, smooth; Roughened, swollen, dark brown apical sporiferous cell with up to 3 proliferations | Single or slight clustered; Tan to light brown; Vesicular tip; 3-10 septa; Apical cell slightly to distinctly swollen; single spore at the apex |
| Conidiophore Size | 19 to 28 × 14 to 19 µm | (30 to 67) × (4 to 5) µm | 28.5 to 197.5 × 3.8 to 6.3 µm |
| Conidia color | Translucent olive brown | Dark brown at maturity | Subhyaline to light brown color |
| Conidia shape | Variously subspherical, oblong or broadly ovoid; Minute echinulate wall, basal scar | Oblong to oval; ellipsoidal immature conidia | Muriform; oblong to ovoid; round basal end; blunt pointed tip |
| Conidial constriction and septation | Constricted at median transverse septum ;1-3 transverse, 1-3or 4 longitudinal septa | Constricted at 1 or commonly 3 septa, 1-5 or 6 transverse and 1-2 or 3 longitudinal septa | Median constriction; 1-8 transverse and 0-5 longitudinal septa |
| Conidia size | 24 to 26 × 33 to 35 µm | 20 to 30 × 12 to 15 µm | 31.3 to 55.0 × 13.8 to 21.5 µm |
| References | Rahman *et al.*, 2010; Simmons, 1969; Koike *et al.*, 2001 | Arzanlou *et al.*, 2012; Simmons, 1969 | Xie *et al.*, 2016; Chai *et al.*, 2014; Kim *et al.*, 2004; Vakalounakis and Markakis, 2013 |

**Table 2:** *Morphological characterization of different blight causing agent in lentil.*

| Characters | *Stemphylium botryosum* | *Alternaria tenuissima* | *Ascochyta fabae* f.sp. *lentis* |
|---|---|---|---|
| Colony characteristic | Grey Brown or brownish-black; Regular without sector; Velvety to cottony | White turn into gray to grayish black; Woolly colonies | Dark aerial mycelium; Gregarious, immersed, and globose pycnidia |
| Conidiophore | Brown; Unbranched; Percurrent proliferation; Swollen dark and roughened apical cell | Pale to pale brown; straight, erect; Sub cylindrical, Septate, Geniculate | |
| Conidia color | Translucent olive brown | Pale | Hyaline |
| Conidia shape | Singe; Variously subspherical, oblong or broadly ovoid; Minute echinulate wall, basal scar | Solitary in short-chain, Straight or curved, beaked up to half-length | Round, one cell slightly larger |
| Conidial constriction and septation | Constriction at median transverse septum, 1-3 transverse, 1-3 or 4 longitudinal septa | 4-7 transverse and many oblique septa | Medially constricted, 0-3 septa |
| References | Rahman *et al.*, 2010; Simmons, 1969; Koike *et al.*, 2001 | Prasad *et al.*, 2017 | Tripathi, 2015; Cromey *et al.*, 1987 |

potassium dihydrogen phosphate and 20 g agar). Any of the media is made to 1L of final volume by adding deionized water. They are autoclaved at 121°C and 15 psi for 30min. For a 20 h day/day photoperiod, the cultivation plates were incubated on cool white fluorescent light (44 µ mol / m2 / s) at 22 °C (Kant *et al.*, 2017). Caudillo-Raiz *et al.* (2017) cultured the pathogen on potato dextrose agar plus V8-PDA medium (10 g potato dextrose agar, 10 g granulated agar, 3 g CaCO3, 150 mL V8 vegetable juice) to a final volume of 1L water and incubated for 7 days at 25°C under continuous light. V-6 juice agar media

was autoclaved at 120°C under 15 psi pressure for 30 minutes to prepare the culture of *Stemphylium botryosum*. To prepare V-6 juice agar, 200 g of six vegetables (tomato, carrot, potato, lettuce leaves, cabbage leaves, and Indian spinach) were blended in blender machine with each of equal amount. Extract was rendered by boiling in water and sieved with a fine cotton cloth. In the extract, dextrose and agar of each 20 g was dissolved. The volume of 1000 ml was prepared by adding distilled water in the extract on a conical flask. Rose Bengal 0.3 g was used to limit the unwanted growth of another fungus (Alam *et al*., 2017b).

*Molecular study of the pathogen*

Morphological characteristics were studied for species identification and delineation. However, the overlapping of morphological traits between the species and their dependence on environmental factors such as temperature and substratum type has created complication in taxonomic study. That is why, the molecular methods are embraced in fungal taxonomy (Poursafar *et al*., 2016). Very limited studies have been done on *Stemphylium* at the molecular level. Despite being the common and universal barcoding region for phylogenetic fungal studies, ITS have only a 73 percent probability to precisely classify a species (Crous *et al*., 2014). ITS is often accompanied by glyceraldehyde-3-phosphate dehydrogenase (*gpd*) region for the study of phylogenetic relations amongst *Stemphylium* species. Phylogenetic analyses of the joint ITS and *gpd* gene validated *S. phaseolina* and *S. variabilis* as two typical phylogenetic species (Wang *et al*., 2010). ITS and sequence data of *gpd* have concluded the phylogenetic relationships among 44 isolates. Among representative 16 species of *Stemphylium*, *S. vesicarium* can distinguish itself from *S. botryosum* by *gpd* gene sequences that are more variable than ITS. However, did not separate *S. vesicarium* from *S. herbarum* and *S. alfalfa* (Camara *et al*., 2002). ITS region, *gpd* gene, and cytochrome b were used to differentiate *Stemphylium vesicarium* from *Stemphylium botryosum*. They were analyzed easily by 3 kb intron present in cytochrome b region in *S. botryosum* but not in *S. vesicarium* (Graf *et al*., 2016). Morphological characteristics based on *gpd* gene revealed *Stemphylium vesicarium* as the causative agent of Purple spot of asparagus, rather than the frequently reported *S. botryosum* (Cunnington and Irvine, 2005). Partial actin (actA), beta-tubulin (tub2), calmodulin (cmdA), translation elongation factor 1-alpha (tef1), glyceraldehyde-3-phosphate dehydrogenase (*gpd*) and DNA-directed RNA polymerase second largest subunit (rpb2) were commonly used protein-coding genes, namely used for the phylogenetic study of the genus *Stemphylium* (Woudenberg *et al*., 2017).

Among two *Stemphylium* spp., *S. botryosum* was found to develop the disease on Canadian lentil. This result was based on the molecular phylogenetic analysis centered on the ITS and *gpd* gene region. It is analogous with other hosts such as faba bean, soybean, bean, pea, alfalfa, coriander, caraway, and fenugreek but the second one still needs extensive taxonomic study (Caudillo-Ruiz *et al*., 2017). Chaisrisook *et al*. (1995) genetically analyzed 5 different isolates of *Stemphylium* from alfalfa using Random Amplified Polymorphic DNA (RAPD) markers. Mehta (2001) conducted the molecular analysis to determine genetic variation using the RAPD markers and ITS of 33 monosporic *S. Solani* isolates (Cotton 28 and Tomato 5). A new pathogen causing flower and leaf blight of *Platycodon grandifloras*, *Stemphylium platycodontis* sp. nov. was determined by phylogenetic analysis and morphology based on ITS, *gpd*, translation elongation factor 1 alpha (EF-1α) and a combined dataset in Korea (Deng *et al*., 2013). Three new taxa, *S. luffae*, *S. lycii*, and *S. cucumis* along with other several species including *S. botryosum* were described by morphological and molecular phylogenetic analyses using ITS and gpd genes (Pei *et al*., 2011). *S. lycopersici* has been formerly reported to cause gray leaf spots of tomato in Taiwan but after the molecular characterization by sequencing the ITS and *gpd* region, it is proposed that *S. lycopersici* caused Stemphylium leaf spot (Huang and Tsai, 2017). Phylogenetic study of *Stemphylium* strains from various hosts was done using loci i.e. ITS, *gpd* gene, EF-1 alpha genes, and the intergenic spacer between *vma*A and *vps*A (Inderbitzin *et al*., 2009).

*Secondary metabolites in host-pathogen interaction*

Secondary metabolites such as phytotoxins generate the effects of the disease in the host but it could also be a vital resource for the fast and precise diagnostic methods. It might also help to identify the safest and fastest remedial treatment (Evidente *et al*., 2019). The toxin named stemphol was produced by *Stemphylium botryosum* of oilseed rape when cultured on rice but about 95% of the toxin was produced on Czapek Dox (CD) and Potato-dextrose (PD) broth (Solfrizzo *et al*., 1994). Barash *et al*. (1983) revealed that stemphyloxin I, a phytotoxin was produced

from certain isolates of *Stemphylium botryosum*. Five metabolites such as stemphylin, stemphyloxin II, stemphyperylenol, stemphol, and stemphol related compound have been detected by chromatographic and spectroscopic data from 33 isolates of *Stemphylium* spp. (Andersen *et al*., 1995). Cytotoxic chemical compounds like dehydrocurvularin, altersolanol, tetrahydroaltersolanol, stemphyperylenol, and microsporin were exhibited from *Stemphylium botryosum* of *Chenopodium album* among which tetrahydroaltersolanol showed effective protein kinase inhibition (Aly *et al*., 2010). *Stemphylium vesicarium*, causing brown spots on European pear produced 2 host-specific toxins (SV toxins I and II) (Singh *et al*., 1999). Similarly, the results from Tanahashi *et al*. (2017) indicated that *Stemphylium* sp. in Japan produced the same SV-toxins as *S. vesicarium* in Europe. Some metabolites such as alterporriol A, alterporriol B, alterporriol D, alterporriol E alterporriol G, alterporriol H, altersolanol A, altersolanol J, altersolanol K, altersolanol L, stemphypyrone, 6 O methylalaternin and macrosporin were also analyzed from *Stemphylium globuliferum* of *Mentha Pulegium* (Debbab *et al*., 2009). *Stemphylium lycopersici* produced non-host selective toxin, macrosporin which is significant to cause leaf necrosis (Trigos *et al*., 2011). Four new tetrahydroanthraquinone derivatives namely, dihydroaltersolanol B, dihydroaltersolanol C, and the atropisomers acetylalterporriol D and acetylalterporriol E were found from the endophytic *Stemphylium globuliferum* of *Juncus acutus* (Liu *et al*., 2015). Stemphypyrone was produced by all strains, whereas only two of the metabolites, orobol and solanapyrone A, were distinct for *S. trifolii* and *S. lancipes* respectively. Stemphyperylenone A was specific to *S. beticola* and *S. simmonsii*. The outcomes from this research also revealed that metabolites alone can differentiate most *Stemphylium* species except for *S. botryosum* and *S. eturmiunum* (Olsen *et al*., 2018).

*Symptomatology*

*Stemphylium* infection in lentil starts mostly during the flowering stage rather than the seedling stage. Initial indications of blight start on the leaflets consist of tiny, pin-headed white or light brown to tan colored spots. Primarily symptom is prominent in the upper canopy only, but under severe infestations entire foliage and stems progressively turn dull yellow (Mwakutuya and Banniza, 2010). Light and dark angular spots scattered on the entire leaf surface. The spot enlarges and coalesce rapidly under ideal conditions thus blighting entire leaves and shoot within 2-3 day. Farmers field possibly will reach brownish color and looks fire burnt within 7 to 10 days (Alam *et al*., 2017a; Taylor *et al*., 2007). Whole plants may defoliate by rapid abscission of infected leaves. Stem bend down and showed special symptoms like a fishing hook. Stem becomes dry and slowly appears ashy white, but the pod remains green (Alam *et al*., 2017a). Extensive growth of white mycelium can be seen on the infected stem (Kant *et al*., 2017). Under severe infection, it reduces plant biomass, seed yield, seed size, result in seed staining and lowers the rate of germination (Chen *et al*., 2009).

The disease symptoms can be mystified with the blights caused by *Alternaria tenussiana* and *Ascochyta fabae* f. sp. *lentis*. *Alternaria tenussiana* shows brownish leaf spot which coalesce to form larger patches and consequently cause blight of entire leaves. It also produces tan to dark brown, circular to crescent spot on pods which later turns into pod blight (Prasad *et al*., 2017). Ascochyta blight is a common blight of Lentil caused by *Ascochyta fabae* f. sp. *lentis*. It produces small tan to dark circular lesions on leaflets, petioles, and stems with indefinite brownish margin initially. The center of the spot is light colored and speckled with pycnidia arranged in concentric rings at maturity. Infected leaflets drop and the apical portion of branches wilt (Cromey *et al*., 1987). Spots formed by ascochyta blight are clearer on pods. Lesions on the stem are brown and elongated which may girdle the stem and finally collapse the entire plant (Tripathi, 2015). These diseases are hardly confirmed by visual symptoms only hence morphological or molecular identification of pathogens may help to identify disease precisely. The symptoms of SB of lentil is shown in Figure 4.

*Disease cycle and epidemiology*

There is very limited knowledge available on the disease cycle and epidemiology of lentil blight caused by *Stemphylium botryosun* (Wallr.) (Caudillo-Ruiz, 2016). Epidemiological study of blight disease caused by *Stemphylium* spp. on other plants can provide some information in the disease cycle. *Stemphylium botryosum* is a necrotrophic fungus and has been reported to over summer successfully on plant debris as mycelium in offseason (Huq and Khan, 2008). Nasir and Bretag (1997) reported the association *of Stemphylium botryosum* with lentil seed. Kaiser and Hannan (1986) isolated *Botrytis cinera, Cladosporium,*

*Alternaria* spp., and *Stemphylium* spp. in very small quantity and *Ascochyta lentis* in a larger amount from lentil seed. Next to the overwintering period, sexual fruiting structures of *Pleospora* (Pseudothecia) are formed on previously infected debris of the plants (Johnson, 1990; Basallote-Ureba *et al.*, 1999). The ascospores and conidia produced on the infected crop debris and infected seed are transferred by various means like air, water and responsible for primary infection on healthy lentil plant (Taylor *et al.*, 2007; Kant *et al.*, 2017; Mwakutuya, 2006) but the consequence of seed-borne inoculum on disease development is not studied (Taylor *et al.*, 2007).

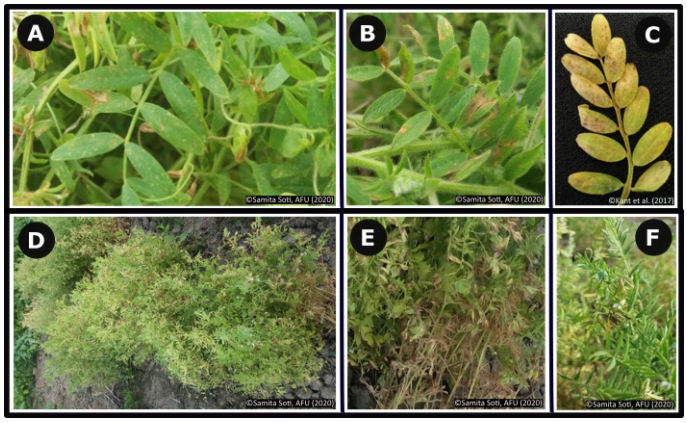


**Figure 4:** *Appearance of tiny Pin head size spots scattered over the leaf surface (A), Spreading chlorotic lesions (B), Whole leaf become chlorotic and dry (C), Infected plants showing completely brightening lower leaves (D,E) and Infected twigs with fishing hook like appearance (F).*

The prevalence of disease during the crop season is affected by various meteorological factors such as atmospheric temperature, relative humidity, rainfall, number of cloudy cover, etc. (Huq and Khan, 2008; Sinha and Singh, 1993). Infection level increases with increasing temperature and wetness duration. The pathogen can be tremendously devastating under high temperature (> 25°C) and persistent wetness periods (> 8 h) in Canada (Mwakutuya, 2006). An average mean temperature of 18± 2°c and morning relative humidity 85% to 90% is appeared to be most favorable to disease development in Indian (Sinha and Singh, 1993). Regions having high humidity, night temperature around 8°C, day temperature above 22°C, and cloudy days promote disease development (Sarker *et al.*, 2004).

The airborne conidia are a secondary source of inoculum. *Stemphylium botryosum* develops airborne spores, which can disperse and damage healthy lentil plants on windy days over large areas (Mandal *et al.*, 2019). The conidia of *Stemphylium vesicarium* strongly adhere to the onion leaf surface and germinate within 6 hours if the condition is favorable. The thin layer of moisture on the leaf surface is required for conidia germination (Aveling and Snyman, 1993). Germination occurs simultaneously on different cells of the same conidium. In alfalfa leaf, germ tubes produce by *Stemphylium botryosum* grow straight and along the juncture of the epidermal cells (Borges *et al.*, 1976). The penetration of leaf surface occurs either directly between epidermal cells or through stomata. Stomatal penetration is more successful that occurs only after the formation of appressoria (Aveling and Snyman, 1993). Extreme disease outbreaks with the possibility to inflict enormous crop losses can be triggered in those areas having favorable meteorological conditions combined with the lack of resistant varieties.

*Disease management*

**Host-plant resistance:** Climate change impacts are predicted to influence the ideal infection state, host specificity, plant infection process, microbial communities in the soil, and pathosystem that eventually alter the magnitude of disease expression (Elad and Pertot, 2014). Among others, host-plant resistance is mostly recognized, cost-effective, and environmentally friendly approach to disease management (Rubiales and Fondevilla, 2010). Wild species and landraces play a key role in the development of disease resistant and the commercial variety of lentil. From the investigation of Podder *et al.* (2013), *L. culinaris* was reported to have high susceptibility and high resistance was found in *L. lamottei* and *L. ervoides* among the seven species of *Lens*. Thicker cuticular and epidermal cell walls, fewer stomata, and a greater amount of epidermal hairs serve as a mechanical shield for pathogens to penetrate by hyphae (Das *et al.*, 2019). The pathogen reaches the host by the means of stomata and form bulging mycelium below the stomata which can be influenced by relative pathogen virulence and environment (Cowling and Gilchrist, 1982). Host-plant resistance plays a vital role in the development of climate-resilient and durable cultivars to cope with the biotic and abiotic stress for sustainable lentil production.

Very few studies have been done in the genetics of SB resistance. A cross between ILL- 6002 (resistant) and ILL-5888 (susceptible) developed 206 F7–derived recombinant inbred lines (RILs). QTL QLG480–81,

two SRAP markers (ME5XR10 and ME4XR16c), and one RAPD marker (UBC34) located on linkage group 4 were significantly associated with the QLG480–81 in both crop years. However, ME4XR16c marker was found to be more tightly linked so used in marker-assisted selection for Stemphylium blight resistance (Saha *et al*., 2010). Resistance against *S. Botryosum* tended to be quantitatively acquired from both field and indoor sampling in the Barimasur-4 × CDC Milestone. The finding of this analysis has indicated that Precoz (one of the parents of Barimasur-4) was resistant to SB (Kumar, 2007). Three QTLs (qSB-2.1, qSB-2.2, and qSB-3) were revealed for SB resistance from the cross between two *L. ervoides* accessions, L01-827A and IG 72815 (Bhadauria *et al*., 2017). The 48, 96, and 144 h post-inoculation (hpi) time was suitable for disease development in *L. ervoides* recombinant inbred lines (RILs) LR-66-637 (resistant to SB) and LR-66-577 (susceptible to SB). Also, calcium transporting ATPase and glutamate receptor 3.2 were found to be potential disease resistance genes (Cao *et al*., 2019). Some resistance genotypes of lentil are presented in Table 3.

*Cultural/ Agronomic measures*

Few written literatures are available about the cultural methods to control the SB of lentil. Non-host crop rotation, destruction of old crop residues, field sanitation, modification of the seeding time, and physical or chemical treatment of the seed can be the best strategies to reduce disease inoculum (Taylor *et al*., 2007; Das *et al*., 2019). From the study of Alam *et al*. (2017b), the highest occurrence of disease (72.50%) was reported from plants grown from October 25 sowing which was statistically equivalent to Nov 1 (63.5%), Nov 15 (62.17%) and Nov 22 (62.17%).

**Table 3:** *Resistance genotypes of lentil against Stemphylium blight.*

| Genotypes/ Varieties and Location | Disease reaction | References |
|---|---|---|
| ILL6408, ILL0133, ILL0379, ILL0426, ILL0427 and ILL0215 (Australia) | Resistant | Kant *et al*., 2017 |
| BLX 07004-12 and BARI masur-7 (Bangladesh) | Resistant | Rahman *et al*., 2015 |
| LL 1370, VL 151, LL 1375, RLG 195, L 4727, L 4769, LL 1397, DL 14-2, VL 526, VL 126, RKL 14-20, IPL 334, L 4710, PL 210 and PRECOZ (West Bengal, India) | Moderately resistant | Mondal *et al*., 2017 |
| BARI Masur 4, BARI Masur 5, BARI Masur 6, BARI Masur 7 and BARI Masur 8 (Bangladesh) | Resistant | Kumar *et al*., 2017 |
| Moitree, HUL 57and IPL 316 (India) | Resistant | |
| Sheetal, Sagun and Khajura Maasuro 3 (Nepal) | Resistant | |
| FLIP 2014-045, ILL 7164, Sagun, RL-4, Khajura Masuro-1, Simal, ILL 10856 and Khajura Maasuro-2 | Moderate resistant | Pokhrel *et al*., 2018 |
| ILL 1704, ILL 6458, ILL 2373 and X93383 | Moderate resistant | Pokhrel *et al*., 2018 |
| ILL 1704, Bari Masoor-4, ILL 6465, RL 62, Bari Masoor-5, Bari Masoor-6, ILL 7978 and Simal | Resistant | Pokhrel *et al*., 2018 |
| Bari Masoor-4, X 94543, RL-79, ILL 6465 and RL 62 (Rampur, Nepal) | Resistant | Pokhrel *et al*., 2018 |
| X 93383, Bari Masoor-4, ILL 1672, ILL 5787, LN 0111, RL 6, RL 9, ILL 7538, ILL 10134 and ILL 10045 (Parwanipur, Nepal) | Moderate resistant | |
| Aarial and RL 79 (Rampur, Nepal) | Resistant | Pokhrel *et al*., 2018 |
| ILL 1704, X 93383, ILL 3490, Simal, ILL 6465, ILL 7978, ILL 9996, RL 27, BARI Masoor 6, NR 99S-95-2-4, IL-1, ILL 6818, ILL 7990, ILL 7723, LN 6137, ILL 72700-1, ILL 7715, LN 0111, ILL 6024, CUMARA, Shishir, X 94-S-38, ILL 2565, NR 2001-71-3, LN 0128, RL 55, RL 94, RL 79, RL 75, NR 2001-71-7, RL 70, RL 58, RL 9, ILL 7538, RL 39, RL 47, RL 95, RL 60, RL 78, ILL 2437, RL 69 and FLIP 2009-59L (Parwanipur, Nepal) | Resistant | |
| ILL 1704, ILL 8603, Shishir, ILL 2712, RL 6, RL 78, RL 75 and RL 60 (Nepalgunj, Nepal) | Resistant | |
| BD-3806 | Resistant | BARI Annual Report, 2015-16 |
| BD-5959 | Resistant | BARI Annual Report, 2017-18 |
| BD-3921, BD-3930, BD-3931 and BARI Masur-7 (Bangladesh) | Highly resistant | Razzak *et al*. 2018 |

However, at Nov 29 (42.17 %) and Dec 6 (30.83 %) sowings, the occurrence of the disease was significantly decreased. The best sowing periods are 2nd to 4th week of November and 2nd to 4th week of October, respectively, in terai and mid-hill of Nepal (Gharti *et al.*, 2014). Wider spacing of 30 cm was found to lower the incidence of disease (Darai *et al.*, 2017).

*Botanicals and biological control measures*

Experimental results of two years have disclosed that *Acorus calamus* and *Xanthozylum armatum* has been efficient botanicals for managing Stemphylium blight of lentil (Subedi *et al.*, 2015b). Similarly, Percent Disease Control (PDC) was greater in *A. calamaus* (46.60%) and *X. armatum* (46.26 percent) versus unsprayed plot (Subedi *et al.*, 2015a).

*Trichoderma viridae* was successful in controlling disease and increasing production (Subedi *et al.*, 2015a). *Trichoderma harzianum* and Provax (chemical fungicide) were found to control the disease however *T. harzianum*, which is soil-safe and environmentally sustainable was suggested to be used for SB management in lentils (Kashem *et al.*, 2013).

*Chemical control measures*

Huq and Khan (2007) performed the in-vivo experiment to conclude Rovral 50WP @ 0.2 % followed by Dithane M-45 @ 0.2 % and Tilt 250EC @ 0.05 % as the useful fungicide to control SB of lentil. Rovral 80 WP from the iprodione group was found best in controlling the disease and also gave the best performance in respect to plant height, number of branch per plant, number of pod per plant, number of seed per pod, thousand seed weight and grain yield (Islam *et al.*, 2019). Shahiduzzaman *et al.* (2015) also revealed that 4 sprays of Rovral WP (0.2%) and Secure 600WG (Fenamidione + Mancozed) (0.2%) at the interval of 7 days controlled the disease. In contrast, recommended fungicide efficiency (Rovral 50 WP) was not as high in both laboratory and field conditions as compared to other fungicides. Tilt 250 EC (0.05%), Bavistin DF (0.1%), and Companion (0.2%) performed better to control radial mycelial growth in the experiment by Uddin *et al.* (2010). Das *et al.* (2017) recommended the foliar spray with Captan 70% + Hexaconazole 5% WP @ 1 g / liter of water twice at 10 days interval. It has lowered the disease incidence by 63.89% and improved lentil yield by 32.86% with the highest net marginal cost-benefit ratio (1:9.8). Darai *et al.* (2017) recommended Dithane M-45 after flowering to manage SB in lentil. The lower Percent Index of Diseases (PDI) was noted in Krilaxyl (36.00), and Mancozeb (37.35), respectively (Subedi *et al.*, 2015a). Consequent 3 sprays of Dithane M-45 @ 2.5g/l of water at 10 days interval, seed rate of 30 kg/ha, fertilizer doze of (20:40:20 NPK kg/ha+ 1 kg/ha basal doze) and improved variety (Lentil black) showed lower disease index (34.95%), higher crop yield (1142.5 kg/ha) with higher BC ratio of 2.42 (Subedi and Neupane, 2018).

*Disease forecasting model*

Stempedia is the weather-based model that plays a vital role in forecasting, evaluating, and mitigating the risk of SB disease of lentil. This model has two most significant parameters-sunshine hours and maximum temperature. It can be used to articulate the principle of application of fungicides and sowing time to obtain maximum returns (Salam *et al.*, 2016). Paudel *et al.* (2020) reported the significant disease pressure in wet conditions for 76% of the surveyed farmers and predicted that more than 60% of conditions of all year favor the disease from stempedia. The findings of the simulation have indicated that these threats could be resolved by earlier plantation. FAST model was developed for forecasting early blight in tomatoes, but it was also evaluated for the scheduling of fungal spray for *Stemphylium vesicarium* on pear (Montesinos and Vilardell, 1992). Tom-cast is also a disease forecasting system, but it was applied for *Stemphylium vesicarium* (Purple spot of asparagus) (Meyer *et al.*, 2000). BSPcast was designed for the prediction of *Stemphylium vesicarium* (Brown spot in pear) which was based on wetness period on leaves and temperature during that period. It also provides the best timing for fungicide application (Llorente *et al.*, 2000).

## Conclusions and Recommendations

In major lentil-producing regions, SB is growing as a devastating disease resulting in over 60 percent of the yield loss in epidemic conditions. Developing sustainable, high-yielding, and disease-resistant varieties is a prerequisite for satisfying global demand for lentils. There is a lot of information lacking on pathogen genetics, pathogenicity, molecular principles, and methods for advanced disease control. It is necessary to exploit the sources of resistance to disease. Wild relatives and landraces. *L. lamottei* and *L. ervoides* are found to be potential resources but very petite is

understood about the molecular basis governing SB resistance in them. Far less research is carried out on the mapping of genes and QTLs, assessment of germplasm, characterizations, analysis of variation, genomic fingerprinting, and marker-assisted breeding. The challenge may be the necrotrophic existence of pathogens, genetic variation, and environmental interaction, but systematic and multidisciplinary strategies offer a stronger plan for effective management of SB. To solve the complex defense mechanisms of *Stemphylium botryosum* in lentil, thorough research on the conventional, molecular as well as integrated omics tools (Figure 5) should be performed. Prediction and forecasting models are also the most effective method for SB management techniques but stempedia is the only model built unique to this pathosystem to date and applies only to a few aspects such as sowing time and scheduling of fungicides. It is therefore necessary to develop a weather-based prediction model that covers several aspects.

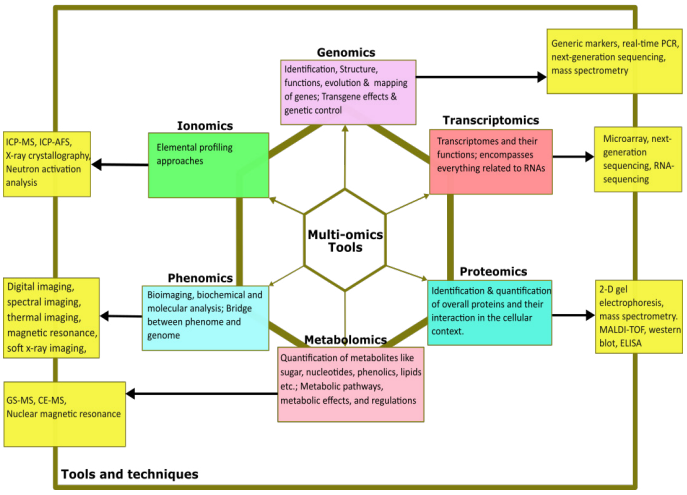


**Figure 5:** *A summary of 'Omics' strategies for the development of SB resistant cultivars in lentil.*

## Novelty Statement

This study brings novelty by cumulating all the information related to Stemphylium blight from previous to recent works along with other related blight pathogens and diseases of lentil.

## Author's Contribution

**Shishir Sharma:** Design of the manuscript; molding the article and major contribution in writing the introduction, molecular studies, management of the disease and conclusion.

**Laxmi Prasad Joshi:** Concept of the study; major contribution in writing the morphological characterization of the pathogens, disease cycle and epidemiology and symptomatology; proof readings.

*Conflict of interest*

The authors have declared no conflict of interest.

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