IJRAR.ORG E-ISSN: 2348-1269, P-ISSN: 2349-5138

INTERNATIONAL JOURNAL OF RESEARCH AND ANALYTICAL REVIEWS (IJRAR) | IJRAR.ORG
An International Open Access, Peer-reviewed, Refereed Journal

A Review on Biological control of Alternaria blight disease of Soyabean: A Promising and Ecofriendly approaches to Chemical Fungicide

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Abstract:

Soybean cultivation has gained global recognition due to its versatility as an edible oil seed, protein source, and vegetable. The high content of free unsaturated fatty acids in soybean oil has sparked interest in various industries, including pharmaceuticals. However, challenges in production and productivity have emerged due to environmental and biological factors, with diseases such as *Alternaria* blight posing a significant threat. Chemical fungicides have been traditionally used to manage this disease, but concerns over environmental impact and resistance have led to a search for sustainable alternatives. Biological control methods, utilizing beneficial microorganisms like *Pseudomonas spp*. and *Bacillus spp*., show promise in effectively managing Alternaria blight in soybeans.

Keywords: Soybean, Alternaria blight, *Alternaria alternata*, Chemical fungicides, biocontrol control, *Pseudomonas spp.*, and *Bacillus spp.*

Introduction:

Soybean (*Glycine max* L.) is cultivated worldwide for its seed, which is generally used for oil production and protein concentrate for livestock feeding. It was also described as Dolichossoja. The origin of soybeans is still a point of debate, but it was reported to be native to eastern Asia (Dupare *et al.*, 2008). USA, China, and Brazil are more producers of soybean than India, despite having the highest land cultivated in the world (Dupare *et al.*, 2014). In India, the soybean was introduced by China, and nowadays it is cultivated in Maharashtra, Rajasthan, Karnataka, Andhra Pradesh, Gujarat, and Chhattisgarh. Among all the states, Maharashtra and Madhya Pradesh contribute 88 % of total production in the country, and average productivity is 946 kg/ha and 1125 kg/ha, respectively (Pathak, 2017).

Among all crops in India, the soybean crop accounts for 14% of the gross cropped area in the country (Agarwal *et al.*, 2013). With the increase in demand for edible oil from soybeans, the country is trying to increase the productivity and production of the crop. In the form of edible oil, the soybean comprises one third and in protein, they make up two-thirds of the world (Bellaloui *et al.*, 2011). The soybean is not only used as an edible oil and protein source; it is also consumed as a vegetable and is known as "Maodou" in China and "Edamame" in Japan. The vegetable form of soybean is rich in phytochemicals and can be used as a functional food (Bashir, 2023). The malnutrition problems in the world can be minimized by using Maodou as it provides RDA of proteins, essential fatty acids, linoleic acid and linolenic acid, folates, vitamin A and vitamin E, minerals like magnesium, potassium, calcium, iron, zinc, manganese, and coenzymes for the biosynthesis of enzymes like carotinoids, folate, copherol, and isoflavanoids (Agyenim-Boateng et al., 2023).

Constraints in Soybean Production:

Due to the vast applicability of the soybean, soybean crop cultivation and production need to be enhanced, but farmers face a lot of challenges in cultivating the soybean. In India, the cultivation of crops is done in the kharif season, although other varieties are also developed for the rabi season; hence, farmers are mainly dependent upon the rainfall. The non-availability of quality trait seeds of improved varieties, the management of insect pests, and complex types of diseases are among the major problems facing farmers (Dupare *et al.*, 2010). The irregular behavior of abiotic stresses such as monsoons, heavy geographical variability in rainfall, and biotic stresses like infections by fungal, bacterial, viral pathogens, and nematodes (Agarwal *et al.*, 2013) affects soybean cultivation and production.

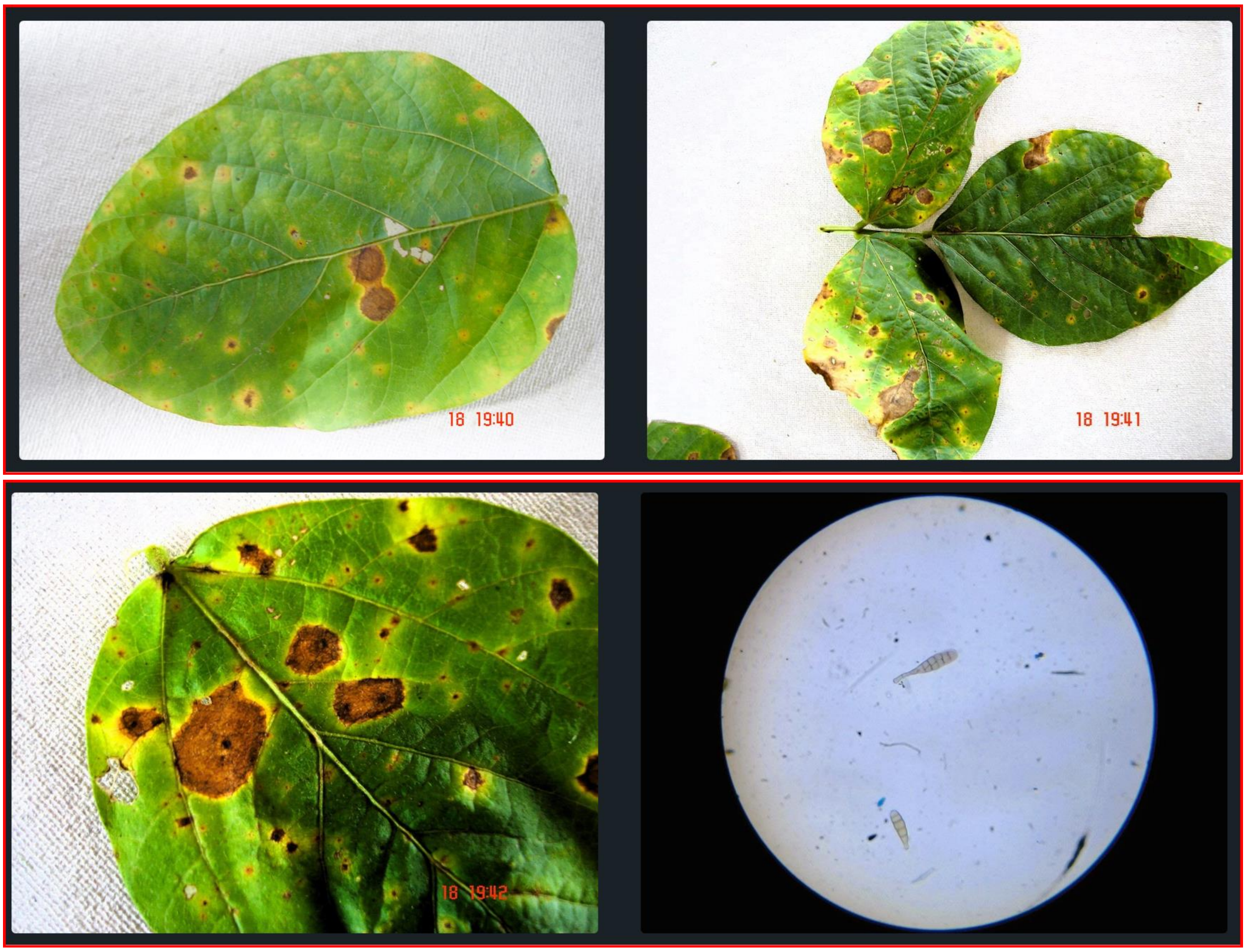
Bacterial diseases like bacterial blight (*Pseudomonas syringae pv. glyc*ine), bacterial pustules (*Xanthomonas campestris pv. glycines*), Bacterial tan spot (*Corynebacterium flaccumfaciens* pv. *flaccumfaciens*), Viral diseases like Alfa alfa mosaic (Alfalfa mosaic virus), Bean yellow mosaic (Bean yellow mosaic virus), soybean crinkle leaf (soybean crinkle leaf virus), fungal diseases like Downey mildew (*Peronospora manshurica*), Fusarium root rot (*Fusarium sp.*), Powdery mildew (*Microspore diffusa*), brown spot (*Septoria glycines*), anthracnose (*Colletotrichum truncatum*), and *Alternaria* leaf spot (*Alternaria alternata*) are among the most prevalent in the world. (Singh *et al.*, 2020; Fagodiya *et al.*, 2022). Majorly the aerial parts of soybeans generally get infected by *Alternaria* which causes serious damage to crop production and the quality of the seeds (Fagodiya & Trivedi, 2021).

Among the 130 diseases observed at various stages of soybean crop growth globally, 35 diseases are economically important under different agro-conditions in India, out of which about 13 are transmitted through seed (Gupta, 2004). However, huge crop losses are mainly incurred by fungal diseases, which impair quality and yield. Alternaria leaf spot of soybean is common in Illinois during the late growing season (Chamberlain, 2011). In India, Shrivastva and Gupta (2001) reported leaf spot disease of soybean caused by *A. alternata*.

Alternaria Blight of Soybean:

Alternaria is ubiquitous fungal genus found worldwide, with more than 300 species described based on phylogenetic and morphological bases. The *Alternaria* genus belongs to the phylum Ascomycota (Mamgain *et al.*, 2013). It is saprophytic, pathogenic and endophytic in nature (Wang *et al.*, 2022). It affects majorly plant families like Cucurbitaceae, Brassicaceae, Solanaceae, and vegetables like tomato, cabbage, cauliflower, broccoli, Napa cabbage, bok choy, collard (Mamgain *et al.*, 2013; Rakhmonov *et al.*, 2023).

Alternaria blight infected soybean plants displayed necrotic, circular to oval and dark brown spots on the upper surfaces of the lower leaves (Kamthane and Rakh, 2013; *Ustun et al.*, 2019). On foliage, the disease symptoms develop brown necrotic spots with concentric rings and yellow halo, large necrotic lesions that eventually coalesce and consume the entire leaf in advanced stages (Photo Plate 1). Infected leaves eventually dry out and drop prematurely. The disease-infected seeds are small, shriveled and characterized by a dark, irregularly spreading sunken area (Bhosale *et al.*, 2014). One of the significant aspects of the biology of an organism is the morphological and physiological characteristics of an individual within a species, which are not fixed. The variability is a conventional phenomenon of the fungus *A. alternata* with a considerable range of variation in conidium morphology observed as changes in spore size and shape, septation, colour, ornamentation, mycelial growth, sporulation, and pathogenicity.



Management Practices for Alternaria Blight of Soybean

Alternaria blight, also known as *Alternaria* leaf spot, is a fungal disease that can affect soybeans. It is caused by several species of the fungus *Alternaria*, most commonly *Alternaria alternata*. Here are some management practices that would be used to control *Alternaria* blight ((Singh, 2023).

Cultural Practices:

1. Plant resistant varieties: Choose soybean varieties that are resistant to *Alternaria* blight. This is the most effective way to manage the disease.
2. Crop rotation: Rotate soybeans with other crops, such as corn or wheat, to help reduce the inoculum level of the fungus in the soil.
3. Tillage: Deep tillage can help to bury crop residues, which can harbor the fungus. However, excessive tillage can also damage the health of the soil.
4. Avoid overhead irrigation: Overhead irrigation can splash spores of the fungus onto leaves, promoting disease development. Use drip irrigation instead, if possible.
5. Destroy infected plant debris: After harvest, destroy infected plant debris by composting or burning. This will help to reduce the inoculum level of the fungus in the soil.

Chemical Control:

Fungicides can be applied to soybeans to control Alternaria blight. However, they should only be used as a last resort, as they can be expensive and have negative impacts on the environment and human health. In field conditions, the management of alternaria disease was most effectively done by using a combination of Azoxystrobin (8.3 %) + Mancozeb (66.7 %) wg at 0.36 % + Neem oil at 0.5 % (Fagodiya *et al.*, 2022).

The synthetic fungicide Hexaconazole showed 100 % growth inhibition of *Alternaria alternata* at 30 ppm followed by 70% inhibition at 20 ppm and 60% inhibition at 10 ppm (Saqib *et al.*, 2020). Fagodiya *et al.*, (2021) suggested that early sowing of seeds was predominantly affected by disease and hence farmers should practice delayed sowing of soybean crops after rainfall i.e. 18th to 19thJuly. The use of high quality, disease free seeds treated with strobilurin fungicide can also reduce disease severity (Borah and Deb, 2022).

Also, seed treatment is a very important operation in soybeans considering the number of fungal, bacterial, and viral diseases that causes a considerable reduction in plant population and thereby yield. Hence, farmers are advised to treat soybean plant seed at the time of sowing using premixed fungicides like Azoxystrobin 2.5% + Thiophanate Methyl 11.25% + Thiamethoxam 25% FS (1 ml/kg seed) or Penflufen + Trifloxystrobine 38 FS (1 ml/kg seed) or Fluxapyroxad 333 g/l FS (1 ml/kg seed) or Carboxin 37.5 + Thiram 37.5 (3g/kg seed) or Carbendazim 25%+ Mencozeb 50% WS (3g/kg seed) (Singh, 2023).

Biological control of *Alternaria* Blight of Soybean:

Biological control offers a promising approach, utilizing naturally occurring organisms or their products to suppress disease. Biological control is regarded as a reliable and safe alternative to chemicals. This review explores the current state of knowledge on biological control strategies for Alternaria blight in soybeans and other crops.

In 1977, Fravel and Spurr isolated sixteen bacterial isolates from tobacco leaf surfaces and screened *in vitro* for effects on spore germination and germ tube development of three Alternaria alternata isolates. Most bacterial isolates hinder the number of germ tubes, and the length and branching of the longest germ tube. Among sixteen bacterial isolates, the five most inhibitory to the growth of *A. alternata* were selected for foliar biocontrol applications. A *Pseudomonas maltophilia* isolate was found to be ineffective in altering lesion severity. Two bacterial isolates reduced lesion severity significantly. One isolate, *Bacillus cereus subsp. mycoides,* effectively controlled tobacco brown-spot lesion development in a controlled environment. Similarly, microscopic observations of the leaf surface showed that conidial germination was 10 % in the presence of *Bacillus cereus subsp. mycoides* whereas 98% germinated in the control and produced brown spot symptoms on tobacco leaf.

Similar results were reported by Ali *et al.,* (2016) who showed that the culture filtrates of *B. subtilis* and *B. amyloliquefaciens* exert strong antagonistic activity against *Alternaria* species due to the presence of the antimicrobial cyclic LP families fengycin-A and fengycin-B.

Praveen *et al.,* (2020) carried out in vitro antifungal activity of six fungal and 10 bacterial bio-agents against *Alternaria alternata* inciting little millet leaf blight disease. Among the fungal bio-agents, *Trichoderma harzianum* (ThB5) and among the bacterial bio-agents, *Bacillus velezensis* (P42) showed mycelial growth inhibition of 75.18 and 84.75%, respectively. In research conducted by Raut *et al.,* (2021) screened *Bacillus amyloliquefaciens* subsp. *amyloliquefaciens strain* RLS19 against *A. macrospora* by the dual culture technique. *Bacillus sp*. significantly killed the radial mycelial growth of *A. macrospora,* that is, 98.08%. On finding the mechanism, it was revealed that *B. amyloliquefaciens subsp. amyloliquefaciens* strain RLS19 produced diffusible non-volatile secondary metabolites, siderophore, and chitinase enzyme, which made contributions to inhibiting the mycelial growth of *A. macrospora* during dual culture screening. In addition, the gas chromatography–mass spectrometry evaluation of the crude extract discovered the presence of three bioactive compounds, specifically acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-; pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-, and 2,5-Piperazinedione, 3,6-bis(2-methylpropyl)- with outstanding antimicrobial, antibacterial and antifungal activity, which contributed to the inhibition of *A. macrospora*.

Also, Ahmad *et al.,* (2023) isolated *Bacillus subtilis* strain Y17B from soil and observed significant antifungal activity against *Alternaria alternata,* the causative agent of fruit rot cherry. To reveal the antimicrobial activity of this strain, a PCR-based study detected the presence of antifungal lipopeptide (LP) biosynthetic genes in genomic DNA. UPLC Q TOF mass spectrometry analysis detected the LPs as surfactin (m/z 994.64, 1022.68, and 1026.62), iturin (m/z 1043.56), and fengycin (m/z 1491.85) in the extracted LP crude of *B. subtilis* Y17B. An *in vitro* antagonistic study demonstrated the efficiency of LPs in inhibiting *A. alternata* growth. Microscopy (SEM and TEM) studies showed the alteration of the morphology of *A. alternata* in the interaction with LPs. *In vivo* test results revealed the efficiency of LPs in reducing the growth of the *A. alternata* pathogen. The overall results highlight the biocontrol potential of LPs produced by *B. subtilis* Y17B as an effective biological control agent against *A. alternata* fruit rot of cherries. *in vivo* and *in vitro* investigation of Soliman *et al.,* (2023), the fungicidal potential of *Bacillus amyloliquefaciens*

RaSh1 (MZ945930) against pathogenic *A. alternata*. In vitro, the results revealed that RaSh1 exhibited strong antagonistic activity against A. alternata. In addition to this, we inoculated pepper (*Capsicum annuum* L.) plants with *B. amyloliquefaciens* RaSh1 and infected them with *A. alternata*. As a result of *A. alternata* infection, which generated the highest leaf spot disease incidence (DI), the plant's growth indices and physio-biochemical characteristics significantly decreased, according to our findings. Our results also showed the abnormal and deformed cell structure using light and electron microscopy of *A. alternata*-infected leaves compared with other treatments. However, DI was greatly reduced with *B. amyloliquefaciens* RaSh1 application (40%) compared to pepper plants infected with *A. alternata* (80%), and this led to the largest increases in all identified physio-biochemical parameters, including the activity of the defense-related enzymes.

Karonji *et al.*, (2024) tested the biocontrol potential of bacterial agents from soil planted with Rosecoco bean plants infected with *A. alternata* and evaluated the bacterial biocontrol activity against *A. alternata* under greenhouse conditions. B. subtilis and B. velezensis bacterial biocontrol agents significantly suppressed disease severity by 20% and 21.2% on the 45th day, respectively.

Mechanism of Biocontrol Action:

Biological control is regarded as a reliable and safe alternative to chemicals (Li *et al.*, 2016). Some bacterial species have been reported as promising biological control agents due to their strong antagonistic activity. They possess multiple modes of action and produce a wide variety of biologically active compounds with antifungal potential against different preharvest and postharvest diseases (Gond *et al.*, 2015; Mnif *et al.*, 2016).

Antibiosis: Antagonistic microbes produce enzymes and metabolites that damage or kill *A. alternata*. Among these antagonistic bacteria, *Bacillus* is well known for its production of broad-spectrum antimicrobial compounds, plant growth promotion, enhancement of plant biomass, and induction of systemic resistance in plants against plant pathogenic fungi (Ongena and Jacques, 2008; Alina *et al.*, 2015; Fatima *et al.*, 2023). *Bacillus* species have recently emerged as an interesting source of biocontrol agents for postharvest fungal disease management. These species showed different antifungal mechanisms, including the production of antifungal volatile organic compounds (VOCs) and lipopeptides (LPs), the induction of disease resistance, and nutrient competition (Wang *et al.*, 2022b). *B. amyloliquefaciens*, *B. subtilis*, *B. licheniformis*, and *B. cereus* have been reported to produce LPs with a high antifungal activity (Lawrance *et al.*, 2014; Zhao *et al.*, 2017; Basit *et al.*, 2018; De Souza *et al.*, 2018). Three families of LPs, iturin, surfactin, and fengycin, have been reported to be inhibitory against pathogenic fungi and oomycetes (Stein, 2005; Ongena and Jacques, 2008).

Competition: Beneficial microbes compete with *A. alternata* for space and nutrients, limiting its ability to establish itself.

Induced Systemic Resistance (ISR): PGPR triggers physiolo Dupare gical changes in the plant that enhance its defense responses against various pathogens, including *A. alternata*.

Advantages of Biological Control:

Environmentally friendly: Reduces reliance on chemical fungicides, minimizing environmental pollution and risks to human health.

Sustainable: Encourages natural ecosystem functions and promotes long-term soil health.

Reduced risk of fungicide resistance: Diversifies control strategies, mitigating the development of resistance in A. alternata.

Conclusion:

Biological control holds immense potential for managing *Alternaria* blight in soybeans in an environmentally sustainable manner. Continued research and development efforts are needed to address current challenges and improve the field efficacy, scalability, and regulatory framework for these promising natural solutions.

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