The Pharma Innovation Journal 2023; 12(7): 3585-3588

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(7): 3585-3588

www.thepharmajournal.com
Received: 03-04-2023
Accepted: 04-05-2023

Sanjay Kumar
School of Agriculture, Dev Bhoomi Uttarakhand University, Dehradun, Uttarakhand, India

Manisha Phogat
School of Agriculture, Dev Bhoomi Uttarakhand University, Dehradun, Uttarakhand, India

Navneet Kumar
School of Agriculture, Dev Bhoomi Uttarakhand University, Dehradun, Uttarakhand, India

Brajesh Kumar
School of Agriculture, Dev Bhoomi Uttarakhand University, Dehradun, Uttarakhand, India

Corresponding Author:
Manisha Phogat
School of Agriculture, Dev Bhoomi Uttarakhand University, Dehradun, Uttarakhand, India

Evaluating the effectiveness of bio-control agents in suppressing early blight (*Alternaria solani*) in tomato plants

Sanjay Kumar, Manisha Phogat, Navneet Kumar and Brajesh Kumar

Abstract
Early blight disease, caused by *Alternaria solani*, poses a significant challenge to tomato cultivation, leading to substantial economic losses and reduced yields. Conventional fungicides have been the primary means of control, but their indiscriminate use has resulted in resistant strains and environmental concerns. This research presents an innovative integrated management strategy that harnesses the potential of botanical extracts and biocontrol agents to combat early blight disease sustainably. The study isolated and identified *A. Solani*, evaluated the antifungal efficacy of botanical extracts, and tested the inhibitory effects of biocontrol agents. The results demonstrate promising control capabilities, suggesting that a combined approach can offer a balanced, eco-friendly, and cost-effective solution for safeguarding tomato crops and promoting sustainable agriculture.

Keywords: Alternaria solani, environmental concerns, eco-friendly, safeguarding tomato

Introduction

The tomato (*Lycopersicon esculentum* Mill) stands as an iconic vegetable and an indispensable horticultural crop, celebrated globally for its versatility in adapting to diverse climatic conditions and its remarkable nutritive significance. With a history dating back to the mid-19th century, tomatoes have remained a staple in the human diet due to their remarkable flavour and nutritional value. In 2014-2015, the world witnessed an astounding annual production of 163.4 million tonnes of tomatoes, covering an area of 4.8 million hectares. In India, tomatoes are cultivated across 791.0 thousand hectares, yielding a commendable productivity of 22.0 tonnes per hectare. However, the state of Haryana, occupying a modest area of 30.0 thousand hectares, faces challenges in maintaining comparable production levels as compared to other states like Madhya Pradesh and Andhra Pradesh. One of the most significant adversaries faced by tomato cultivators is the devastating early blight disease, primarily caused by the pathogen *Alternaria solani*. This fungal disease has been responsible for substantial economic losses, reducing yields by up to 78-80%. The disease manifests through characteristic irregular leathery lesions with concentric rings on leaves, posing a severe threat to the plant's health and productivity. The conventional method of controlling early blight disease has long relied on the indiscriminate use of fungicides, leading to fungicide-resistant strains and environmental hazards. Therefore, there is an urgent need for a more sustainable and eco-friendly approach to manage this agricultural challenge effectively. This research endeavours to present a pioneering integrated management strategy that harnesses the potential of botanical extracts and biological control agents in mitigating early blight disease in tomato plants. By tapping into the synergistic effects of these natural formulations, we aim to enhance the plant's internal resistance against abiotic and biotic factors, ultimately achieving a balanced, environmentally conscious, and cost-effective solution for safeguarding tomato crops. In pursuit of this objective, our study focuses on the isolation, identification, and pathogenicity verification of the early blight pathogen, *Alternaria solani*. Furthermore, we evaluate the efficacy of selected botanical extracts in suppressing the infection in tomato plants, paving the way for a sustainable integrated disease management approach. Through this innovative research, we aspire to contribute to the advancement of agriculture and the sustainable production of tomatoes, bolstering food security and fostering ecological harmony.

Materials and Methods

A. Evaluation of different plant extracts against *A. Solani*

The five plants used are listed in Table 1. Datura (*D Stramonium*), Neem (*A Indica*), Eucalyptus (*E Globulus),* and Lantana (*L Camera*) were collected from Dehradun.

The Garlic (*A Sativum*) was obtained from the local vegetable market in Dehradun.

Table 1: List of botanical plants

S. No.	Common Name	Botanical Name	Family	Plant Parts
1.	Datura	*Datura stramonium*	Solanaceae	Leaves
2.	Neem	*Azadirachta indica*	Meliaceae	Leaves
3.	Eucalyptus	*Eucalyptus globulus*	Myrtaceae	Leaves
4.	Lantana	*Lantana camera*	Verbenaceae	Leaves
5.	Garlic	*Allium sativum*	Amaryllidaceae	Cloves

B. Details of the layout plan

1. Number of treatments: 17.
2. Number of replications: 3.
3. Spacing: Row to Row: 40 cm.
4. Spacing: Plant to Plant: 15 cm.
5. Plot size: 1.5×1.5 m^2.
6. Date of sowing: 19 February 2023.
7. Experimental design: CRBD.

C. Preparation of *solani* inoculum for application

The 10-day-old culture of *A. solani* grown on PDA media was flooded with 15 ml of sterile distilled water, and colonies of *A. Solani* were scraped aseptically with the help of a sterile rubber spatula. The resultant suspension was filtered through double layers of sterilised cheesecloth, and the final concentration was adjusted to 4 105 conidia per ml using a hemocytometer. The prepared inoculum was used for foliar spraying.

D. Foliar application

The individual applications of *T. viride* (10 g/lit), Flusilazole (0.04 ml/lit), Kasugamycin (0.04 ml/lit), Garlic (20 ml/lit), Neem (20 ml/lit), and the combined application of Kasugamycin + Neem (0.04 ml/lit + 20 ml/lit), Flusilazole + Garlic (0.04 ml/lit + 20 ml/lit), Kasugamycin + Garlic (0.04 ml/lit + Garlic + Neem (20 ml/lit) were applied as foliar sprays. A battery-operated knapsack sprayer with a hollow cone nozzle was used for foliar spray.

Result

A. Collection of diseased samples and isolation of pathogens

The data pertaining to observations of the plant parts of tomatoes showing the typical early blight symptoms were collected from the farmer's fields, which are located in the eight villages of Dehradun. During the collection of samples, the symptoms were found on all aerial parts of the plant, including the leaves and stem. The lesions on leaves were observed to be angular, oval, or circular in shape. The stem lesions were found as rectangular, dark, and slightly sunken blotches. The spots, when made into slides and observed under the light microscope, were found to be dark muriform conidia of the pathogen. The isolates were purified by a single-spore isolation technique. Moreover, one culture of *A. solani* was obtained from the Indian Type Culture Collection (ITCC), New Delhi, with ITCC No. 3720. The obtained culture was maintained in PDA slants and stored at 4 °C for further investigation.

B. Cultural characteristics of different isolates of solani

The results of the cultural characteristics of eight isolates of *Alternaria solani* on the PDA medium Isolates of *A. solani* differed with respect to their cultural characteristics. The isolates of *A. solani* grown on PDA showed variation in their colony colour, character, margin, and zonation. Different types of colonies colours, like dark black, greenish-brown, greenish-white, brownish, greyish-white, light grey, light black, and black colonies, were observed in PDA culture media. Mostly, the colonies had smooth or rough colony characters with regular and irregular margins. The smooth colony character was observed in five isolates, *viz.*, AsP, AsK, AsN, Asd, and AsS; the rough colony character was observed in three isolates, *viz.*, AsD, AsR, and AsV. The colony margins varied from regular to irregular. Regular margins were observed in six isolates, *viz.*, AsP, AsK, AsN, Asd, AsS, and AsV. Whereas the other two isolates had irregular margins. Among eight isolates, seven were found without zonation and one had zonation.

C. Test of pathogenicity of *solani* isolates

All eight isolates of *A. solani* were tested for pathogenicity by the following Koch's postulate method: These isolates were subjected to pathogenicity tests on susceptible cells under *in vitro* conditions using the detached leaf technique and showed typical early blight symptoms. All isolates of *A. solani* were able to produce characteristics of early blight symptoms, like concentric rings on leaves. The infected leaves showed brown spot-like symptoms that may be angular, oval, or circular in shape.

D. Effects of various plant extracts on conidial germination of *solani*

The antifungal activity of aqueous extracts of five plants, such as Datura (*Datura stramonium*), Garlic (*Allium sativum*), Neem (*Azadirachta indica*), Eucalyptus (*Eucalyptus globulus)*, and Lantana (*Lantana camera)*, was evaluated under *in vitro* conditions against conidial germination of *A. solani through the* microscopic slide technique (Rodrigues *et al*. 2010) [30]. All plant extracts at 5, 10, 15, and 20% concentrations were found to be significantly effective in reducing the conidial germination of *A. solani* as compared with the control. The highest percent of conidial germination (98.28%) was measured in control over all the plant extracts at all concentrations.

E. *In vitro* effects of biocontrol agents on the radial growth of *solani*

The antagonistic activity of four biocontrol agents, such as *Trichoderma viride*, *Trichoderma harzianum*, *Bacillus subtilis,* and *Pseudomonas fluorescens,* was screened under *in vitro* conditions against *A. solani* through a dual culture method on PDA. The two fungal biocontrol agents and the two bacterial biocontrol agents A critical perusal of data pertaining to the effect of four biocontrol agents on radial growth (mm) and percent inhibition of mycelial growth of *A. solani* against control *in vitro* results revealed that all biocontrol agents significantly inhibited the radial growth of the *A. solani* pathogen when compared with the control treatment. Results revealed that the maximum radial growth was recorded in the control treatment (90 mm). Among four biocontrol agents, minimum radial growth was found in *T. Viride* (12.76 mm), followed by *B. Subtilis* (15.56 mm), while maximum radial growth was observed in *P. Fluorescens* (27.06 mm), followed by *T. Harzianum* (18.13 mm). The maximum inhibition percent of radial growth of *A. Solani by the* dual culture technique was observed in the case of *T. Viride* (85.72%), followed by *B. subtilis* (83.11%), after 7 days of inoculation at 252 ºC. The least inhibition percent of radial growth of *A. Solani* was observed in the case of *P. Fluorescens* (69.93%), followed by *T. harzianum* (79.85%).

Table 2: *In vitro* efficacy of various biocontrol agents against *A. solani*

Biocontrol agents	Radial growth (mm)	Inhibition %
Trichoderma viride	12.76±1.23e	85.82
Trichoderma harzianum	18.13±0.80c	79.85
Pseudomonas fluorescens	27.06±1.02b	69.93
Bacillus subtilis	15.20±1.08d	83.11
Control	90.0±0.00a	-
SE (m)	0.541	
CD (5%)	1.727	

Results are expressed as means of three replicates; indicating the standard deviation of the means. Different letters showed significant differences in treatment results according to Duncan’s multiple range test at p 0.05.

Discussion

Among different sectors, the agricultural sector has played a pivotal role in boosting the economic growth and development of any country. Also, the agricultural sector firmly provides food security, generates employment, and boosts national income (Mozumdar, 2012; Pawlak and Kolodziejczak, 2020) [36-37]. However, despite this importance, the agriculture sector continuously faces a wide array of problems, particularly climate change (Cramer *et al*., 2018) [38]. Climate change creates various problems, such as decreased crop production, disrupted food availability, rainfall variation, and changes in extreme weather events, and also affects the factors responsible with respect to disease incidence, such as the progress of plant growth, pathogen resurgence, decrease in beneficial microbe population, and development of plant diseases (Sharma *et al*., 2019a; Maurya *et al*., 2022) [39, 40]. Plant diseases are generally caused by different types of detrimental microbes, such as fungi, bacteria, viruses, and nematodes (Ratnadass *et al*., 2012) [41]. Early blight symptoms are seen at all stages of plant growth. Symptoms appear first on leaves as dark brown to black lesions with concentric rings (Kumar *et al*., 2018) [42]. The conidia survive on the soil surface and on the old, dry lower leaves of the plant and spread under favourable conditions. Potato is severely affected by early blight, causing a significant impact on quality and tuber yield loss of up to 50% annually (Landschoot *et al*., 2017) [43]. Several researchers and scientists have attempted to manage early blight disease. A successful disease-reducing strategy could comprise a single practise but to get long-term solutions to combating disease losses, it requires the combined application of various control strategies (Khoury and Makkouk, 2010; Holtappels *et al*., 2021) [31, 32]. However, the disease can be managed by the use of a considerable number of fungicides and biocontrol agents combined with other management practices (Gholve *et al*., 2014; Wavare *et al*., 2016; Gopi *et al*., 2020) [33-35].

Conclusion

The combined application of biocontrol agents and plant extracts showed significant potential in the management of early blight in tomatoes caused by *A. Solani*. The outcomes obtained from the present study indicated that a carefully planned combined application of biocontrol agents and botanical extracts may even be more productive than a single method. It can be used as a better alternative strategy for disease management of early tomato blight under sustainable agriculture. Therefore, the farmers may be advised to take an integrated approach to achieving higher profitable production and effective management of early blight disease in tomatoes.

References

1. Abo-Elyousr KAM, Sobhy I, Abdel-Hafez I, Abdel-Rahim I. Isolation of Trichoderma and evaluation of their antagonistic potential against Alternaria porri. J Phytpathol. 2014;162:567-574.
2. Alsafadi F, Al-Fadil TA, Trabi BA. Evaluation of some local tomato genotype’s reaction to early blight disease caused by *Alternaria solani*. Arab J Plant Protection. 2012;30(1):139-141.
3. Bessadat N, Benichous S, Kihal M, Henni DE. Aggressiveness and morphological variability of small spore Alteneria species isolated from Algeria. Journal of Experimental Biology and Agricultural Sciences. 2014;2:2.
4. Bhaliya CM, Jadeja KB. Efficacy of different fungicides against Fusarium solani causing coriander root rot. The Bioscan. 2014;9(3):1225-1227.
5. Bhat HA, Rayees KA, Qazi NA, Dar NA, Ganie SA. Status and symptomatology of Alteneria leaf blight (*Alternaria alternata*) of Gerbera (Gerbera Jamisonii) in Kashmir valley. African Journal of Agriculture and Research. 2013;8(9):819-823.
6. Bora P, Bora LC, Deka PC, Borkotoki B, Sharma AK, Dutta HS, *et al*. Efficacy of *Pseudomonas fluorescens* and Trichoderma viride based IDM Powder for management of bacterial wilt disease of ginger, Internat. J. Plant Sci. 2016;11(2):180-186.
7. Bruggen AHCV, Finckh MR. Plant diseases and management approaches in the organic farming system. Annual review of phytopathology. 2016;54:25-54.
8. Chohan S, Perveen R, Mehmood MA, Naz S, Akram N. Morpho-physiological studies, management and screening of tomato germplasm against *Alternaria solani*, the causal agent of tomato early blight, Int. J. Agric. Biol. 2015;17:111118.
9. Desta M, Yesuf M. Efficacy and Economics of Fungicides and their Application Schedule for Early Blight (*Alternaria solani*) Management and Yield of Tomato at South Tigray, Ethiopia. J Plant Pathol Microb. 2015;6:268.
10. EI- Mohamedy RSR, Abdalla AM. Evaluation of antifungal activity of *Moringa oleifera* extracts as natural fungicide against some plant pathogenic fungi *in vitro*. Journal of agricultural technology. 2014;10(4);963-982.
11. Ghassan F, Singh AS, Harbant Madya, Syarhabil M, Ahmad. Extracts some plants on controlling green m old of orange and on post-harvest quality parameters. World Applied Sciences Journal. 2013;22(4):564-570.
12. Gomathy S, Ambikapathy V. Antagonistic activity of fungi against Pythium debaryanum (Hesse) isolated from chilli fields soil. J. Advances in ap. Sci. Res. 2011;2(4):291-297.
13. Gondal AS, Ijaz M, Riazl K, Khan AR. Effect of

different doses of fungicides (Mancozeb) against Alterneria leaf blight of tomato in tunnel. J. Pl. Pathol and Microbiol. 2012;3(3):1-3.
14. Horticultural Statistics at a glance; c2015. Available from: http://nhb.gov.in/area-pro/horst_galance_2016.pdf [2015 Nov 18].
15. Koley S, Mahapatra SS. Evaluation of culture media for growth characteristics of *Alternaria solani*, causing early blight of tomato, Journal of Plant Pathology and Microbiology J Plant Pathol Microbiol; c2015. S:1. DOI: 10.4172/2157-7471.S1-005.
16. Lee HB, Patriarca A, Magan N. Alterneria in food ecophysiology, mycotoxin production and toxicology. Microbiology. 2015;43(2):93-106.
17. Lubaina AS, Murugan K. Evaluation of Biological Efficacy of Trichoderma Species Isolates against Alternaria Leaf Spot Disease of Sesame. Inter. Journal of Applied Biology and Pharmaceutical Technology. 2015;6(1):17-24.
18. Madhavi M, Kavita A, Vijayalaxmi M. Studies of Alternaria porri (Ellis) Ciferri pathogenic to Onion (*Allium cepa* L.), Archives of Applied Sci. Res. 2012;4(1):1- 9.
19. Malusa E, Pinzari F, Canfora L. Efficacy of biofertilizers: Challenges to improve crop production. Microbial inoculants in sustainable agricultural productivity; c2016. Available Online: DOI: 10. 1007/978-81-322-2644-4_2
20. Meitei KM, Bora GC, Singh SJ, Sinha AK. Impact of *Alternaria solani* (Early blight) on cultivated tomato (*Solanum lycopersicum* L.) in North-eastern region of India and identification of early blight disease resistant tomato genotypes. 2015;7(2):672-680.
21. Rai S, Kashyap LP, Kumar S, Srivastava KA, Pramod RV. Identification, characterization and phylogenetic analysis of antifungal Trichoderma from tomato rhizosphere. Springer Plus. 2016;5:1939.
22. Rajput AQ, Shahzad S. Growth and sporulation of *Trichoderma polysporum* on organic substrates by addition of carbon and nitrogen sources. Pak. J. Bot. 2015;47(3):979-986.
23. Sadana D, Didwania N. Bioefficacy of fungicides and plant extracts against *Alternaria solani* causing early blight of tomato. International conference on plant, Marine and environmental conference, Kuala Lumpur, Malaysia; c2015 Jan. p. 1-2.
24. Sahu RK, Pattnaik MM, Kar M. Bioefficacy of some plant extracts on growth parameters and control of diseases in Lycopersicum esculentum, Asian J. Plant Sci. Res. 2012;2(2):129-142.
25. Sahu DK, Khare CP, Patel R. Eco-friendly management of Early blight of tomato using botanical plant extracts, Journal of Industrial pollution control; c2015.
26. Sallam NMA. Control of tomato early blight disease by certain aqueous plant-extracts, Plant Pathol. J. 2011;10:187-191.
27. Selim ME. Effectiveness of Trichoderma biotic applications in regulating the related defence genes affecting tomato early blight disease, Essential oil, Journal of Plant Pathology and Microbiology. 2015;6:311 DOI: 10.4172/2157-7471.1000311.
28. Woo SL, Ruocco M, Vinale F, Nigro M, Marra R. Trichoderma-based products and their widespread use in agriculture. Open Mycol J. 2014;8:71-126.
29. Woudenberg JHC, Groenewald JZ, Binder M, Crous PW. Alternaria redefined Mycology. 2013;75:171-212.
30. Rodrigues JL, Serpeloni JM, Batista BL, Souza SS, Barbosa F. Identification and distribution of mercury species in rat tissues following administration of thimerosal or methyl mercury. Archives of Toxicology. 2010 Nov;84:891-6.
31. Khoury WE, Makkouk K. Integrated plant disease management in developing countries. Journal of Plant Pathology; c2010 Dec 1. p. S35-42.
32. Holtappels D, Fortuna K, Lavigne R, Wagemans J. The future of phage biocontrol in integrated plant protection for sustainable crop production. Current Opinion in Biotechnology. 2021 Apr 1;68:60-71.
33. Gholve VM, Tatikundalwar VR, Suryawanshi AP, Dey U. Effect of fungicides, plant extracts/botanicals and bioagents against damping off in Brinjal. African Journal of Microbiology Research. 2014 Jul 23;8(30):2835-48.
34. Wavare A, Ubale S. Parametric investigation for PP homopolymer (1110MAS) in plastic injection moulding process using Taguchi method. IJERGS. 2016;4(2):323-31.
35. Gopi CV, Vinodh R, Sambasivam S, Obaidat IM, Kim HJ. Recent progress of advanced energy storage materials for flexible and wearable super capacitors: From design and development to applications. Journal of Energy Storage. 2020 Feb 1;27:101035.
36. Mozumdar L. Agricultural productivity and food security in the developing world. Bangladesh Journal of Agricultural Economics. 2012;35(454-2016-36350):53-69.
37. Pawlak K, Kołodziejczak M. The role of agriculture in ensuring food security in developing countries: Considerations in the context of the problem of sustainable food production. Sustainability. 2020 Jul 7;12(13):5488.
38. Cramer W, Guiot J, Fader M, Garrabou J, Gattuso JP, Iglesias A, *et al*. Climate change and interconnected risks to sustainable development in the Mediterranean. Nature Climate Change. 2018 Nov;8(11):972-80.
39. Sharma P, Mehta M, Dhanjal DS, Kaur S, Gupta G, Singh H, *et al*. Emerging trends in the novel drug delivery approaches for the treatment of lung cancer. Chemico-biological interactions. 2019 Aug 25;309:108720.
40. Maurya A. Running lean. O Reilly Media, Inc; 2022 Feb 23.
41. Ratnadass A, Fernandes P, Avelino J, Habib R. Plant species diversity for sustainable management of crop pests and diseases in agro ecosystems: A review. Agronomy for sustainable development. 2012 Jan;32:273-303.
42. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X. Molecular evolutionary genetics analysis across computing platforms. Molecular biology and evolution. 2018 Jun;35(6):1547.
43. S Landschoot, Carrette J, Vandecasteele M, De Baets B, Höfte M, Audenaert K, *et al*. Boscalid-resistance in Alternaria alternate and Alternaria solani populations: An emerging problem in Europe. Crop Protection. 2017 Feb 1;92:49-59.