Available Online at EScience Press
Plant Protection
ISSN: 2617-1287 (Online), 2617-1279 (Print)
http://esciencepress.net/journals/PP

EVALUATING THE BIOCONTROL EFFICACY OF SELECTED BOTANICAL EXTRACTS AGAINST BACTERIAL SPOT OF TOMATO

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ARTICLE INFO

Article history
Received: 19th June, 2023
Revised: 4th August, 2023
Accepted: 8th August, 2023

Keywords
Azadirachta indica
Berberis vulgaris
Bacterial spot
Cannabis sativa
Plant Extracts
Tomato

ABSTRACT

Tomato crops are vulnerable to various bacterial diseases, including bacterial spot, which is caused by *Xanthomonas perforans*. This bacterial pathogen can have a severe impact on tomato plants, leading to lesions and spots on leaves, stems, and fruits. Consequently, it diminishes both yield and quality. The objective of the current study is to assess the efficacy of extracts derived from *Cannabis sativa*, *Berberis vulgaris*, and *Azadirachta indica* in combating bacterial spot disease in tomato plants. These plant extracts were examined for their potential as natural biocontrol agents, aimed at curbing the bacterial spot pathogen and reducing disease incidence. The antibacterial activity of selected aqueous plant extracts was tested using three concentrations (5%, 10%, and 15%) against *X. perforans*. The inhibition zone technique yielded significant results. Among the tested plant extracts, the highest inhibition zones were observed using *C. sativa* extract at concentrations of 15% and 10%, resulting in zones of 15 mm and 13 mm, respectively. Similarly, in a greenhouse experiment, the 15% concentration of *C. sativa* exhibited the most effective treatment. Among the three tested plant extracts, *C. sativa* demonstrated the highest efficacy in terms of Area under the Disease Progress Curve (AUDPC) severity, with values of 59.4%, 55.6%, and 51.3% observed at concentrations of 5%, 10%, and 15%, respectively. The findings of the study suggest that the plant extracts possess significant antibacterial activity, rendering them potentially valuable as effective components in integrated management programs for the sustainable control of tomato bacterial spot disease.

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INTRODUCTION

Worldwide tomato (*Solanum lycopersicum*) production stands at 182,256,458 metric tons, cultivated over an area of approximately 4,762,457 hectares (FAO, 2021). Tomato is a valuable and nutritious addition to a well-balanced diet, providing approximately 23 kilocalories per 100 g of dry weight. It is abundant in essential minerals, vitamins such as vitamin C, potassium, folic acid, and carotenoids (Perveen et al., 2015). Tomato crop is confronted with numerous bacterial diseases

(Hyder et al., 2018a; Hyder et al., 2018b), and one of them is bacterial spot, which is caused by *Xanthomonas* species (Mehmood et al., 2023; Stall et al., 2009). To manage bacterial diseases the primary method currently employed is the use of copper bactericides. These are applied either alone or in combination with other pesticides and antibiotics, notably streptomycin. However, it is worth noting that alternative approaches are sought to develop sustainable and environmentally friendly control measures for these bacterial diseases (Inam-ul-Haq et al., 2016; Mehmood et al., 2023).

Efficient control of plant diseases through appropriate disease management methods is of utmost importance for crop growers, environmentalists, farmers, implementers, and policy makers. Plant diseases can significantly reduce crop yields, causing considerable damage to plants and leading to substantial losses. Hence, implementing effective disease control measures is crucial to mitigate these adverse impacts. The strategies for managing plant diseases primarily rely on sanitary practices, timely pesticide applications, and a significant dependence on agrochemicals, particularly pesticides (Klaus-Joerger et al., 2001; Kumar et al., 2007) while alternative control measures for plant diseases are becoming increasingly important in modern agriculture to reduce the reliance on chemical pesticides and promote sustainable farming practices including biological control using plant growth promoting rhizobacteria (Bibi et al., 2017; Shahzaman et al., 2017; Shahzaman et al., 2016; Shahzaman et al., 2015), host resistance (Bibi et al., 2022), commercial bio-products (Shakoor et al., 2015) and botanical extracts (Mbega et al., 2012).

Various plant species, including *Eucalyptus globulus*, *Datura stramonium*, *Ocimum* spp., *Salix* spp., *Rosmarinus officinalis*, *Cydonia oblonga*, and *Foeniculum vulgare* and many others have demonstrated successful utilization in controlling a wide range of plant diseases through the application of their leaf extracts or organic soil amendments (Kayani et al., 2012; Mukhtar et al., 2013; Saeed et al., 2021; Jabbar et al., 2022). Both chemical compounds and plant extracts have shown the ability to induce resistance in plants against various causal pathogens, encompassing bacteria, fungi, viruses, and nematodes (Bashir et al., 2020; Fallanaj et al., 2015; Mehmood et al., 2022).

Furthermore, it has been discovered that plant extracts can trigger a defensive response in infected plants. The mechanism of action of these plant extracts against bacterial pathogens may involve enhancing natural host defense mechanisms, such as elevating the activity of antioxidant enzymes like peroxidases and polyphenol oxidase, as well as promoting the accumulation of phenolic compounds (Hassan et al., 2007; Jabeen et al., 2022; Srivastava et al., 2011).

The primary objective of this study was to evaluate *in vitro* effectiveness of extracts from three plant species against *Xanthomonas perforans*, as well as to investigate their potential in suppressing bacterial spot disease in tomato plants under both laboratory and greenhouse conditions.

MATERIALS AND METHODS

Pathogenic Isolates

Pathogenic bacterial isolate of *Xanthomonas perforans* was collected from the culture collection of already identified and preserved cultures of Department of Plant Pathology, University of Poonch Rawalakot. Furthermore, the pathogenicity was confirmed according to the standard procedures adopted by Abo-Elyousr and El-Hendawy (2008).

Preparation of plant extract

To prepare the plant extract stock, 30 g each of *Cannabis sativa*, *Berberis vulgaris*, and *Azadirachta indica* leaves were taken that were cut into small pieces and boiled with 100 ml of sterile distilled water at 100°C for 5-10 minutes. After boiling, the mixture was filtered through Whatman filter paper to obtain the crude extract. The filtrate was then stored at 4°C as a stock solution for further experimentation (Manik et al., 2020).

In vitro evaluation of plant extracts against pathogen

The plant extracts were tested against *Xanthomonas perforans* using the inhibition zone technique. Three different concentrations (5%, 10%, and 15%) were prepared by mixing 5 ml, 10 ml, and 15 ml of the plant extract with 100 ml of sterilized double-distilled water. Nutrient agar media plates were used to streak *X. perforans*. Afterward, sterilized blotter paper was soaked in the prepared concentrations of plant extracts and placed on the inoculated plates. For the control treatment, blotter paper dipped in distilled water was used. The plates were then incubated at 28°C for 24 hours. After the incubation period, the growth inhibition zones were observed and measured (Abo-Elyousr and Asran, 2009; Elbeshehy et al., 2015).

Greenhouse evaluation of selected plant extracts against pathogen

Susceptible tomato seedlings were transplanted into

pots. After one week of transplantation, the tomato seedlings were inoculated with a virulent *X. perforans* isolate. Then, one week after pathogen inoculation, 100 ml of different concentrations (5%, 10%, and 15%) of selected plant extracts were done. Each treatment was repeated five times, following a completely randomized design. The pots containing the seedlings were placed in a greenhouse for 8 weeks and plants were carefully examined to assess disease parameters. Sterile distilled water was used as a negative control. No mineral fertilizers or pesticides were utilized during the study (Vicente and Roberts, 2003). By utilizing the severity and number of leaflets data, the area under the disease progress curve (AUDPC) was computed. This calculation involved integrating and evaluating the obtained data using the following equation (Mello et al., 1997);

$$\text{AUDPC} = \sum \frac{(y1 + y2)}{2} \times (t2 - t1)$$

Where, y1 and y2 refer to two successive assessments of disease intensity performed at times t1 and t2, respectively.

Data analysis

The data obtained from the pot study conducted in the greenhouse was analyzed using analysis of variance (ANOVA) with a completely randomized design. Since the greenhouse provided a uniform and consistent meteorological condition without any variations, a CRD was employed for the pot study.

RESULTS AND DISCUSSION

Pathogen and their virulence

From the preserved cultures collection, a total of 5 bacterial isolates were successfully revived by streaking onto fresh nutrient agar plates. The plates were then incubated at a temperature of 25±2℃, and the expected translucent growth characteristic of *Xanthomonas Perforans* was observed. Subsequently, the bacterial isolates were subjected to gram staining, revealing that they were gram-negative. Additionally, the positive loop test further confirmed their identity as *X. Perforans*. The findings of this study align closely with the previous research conducted by Aiello et al. (2013); Mehmood et al. (2023) and Abrahamian et al. (2021). They also identified *X. perforans* as a gram-negative, aerobic, rod-shaped bacterium with a single flagellum. This devastating pathogen is responsible for causing bacterial spot disease in tomatoes and thrives in environments with relatively high humidity and elevated temperatures.

Following the initial screening, all the five isolates underwent further virulence studies on fresh tomato fruits. Inoculation of fresh bacterial cultures was done followed by incubation period of 3-4 days; it was observed that all the isolates exhibited virulence, as evident from the appearance of clear symptoms of bacterial spot on the tomato fruits (Table 1). The results were consistent with the previous findings that the bacterial spot of tomato affects all aerial parts of the tomato plant, including the fruits. The symptoms involve the formation of irregular, circular water-soaked lesions, which can reach up to 3-4 mm in diameter (Jones and Miller, 2014).

***In vitro* evaluation of selected plant extracts**

Three concentrations (5%, 10% and 15%) of three plant extracts were used for inhibition zone technique showed significant results but the maximum inhibition zone was observed 15 mm and 13mm using the concentration of 15% and 10% respectively of *Cannabis sativa* plant extract comparing the control treatment (Figure 1). The findings presented in this study are consistent with the results reported by Abo-Elyousr and Asran (2009) that the garlic (*Allium sativum*) a plant extract exhibited a potent antibacterial activity against bacterial wilt *in vitro*, ranking it as the most effective, followed by *Datura* spp. and *Nerium oleander* extracts in decreasing order of efficacy.

Table 1: Virulence study of bacterial isolates on fresh tomato fruits.

S. No.	Isolates	Response
1	XP-2	+
2	XP-3	+
3	XP-8	+
4	XP-9	+
5	XP-11	+

Similarly, the results were supported by the previous similar findings of Bashir et al. (2020) that the food

poisoning technique was employed to assess fungal growth inhibition using plant extracts. Among the tested plant extracts, *M. azedarach* exhibited the highest efficiency, with a remarkable 73.18% inhibition at a concentration of 20%. It was followed by *C. sativa*, *D. stramonium*, and *A. millefolium*, which displayed inhibition rates of 55.79%, 37.12%, and 31.9%, respectively, in comparison to the control (88.2%).

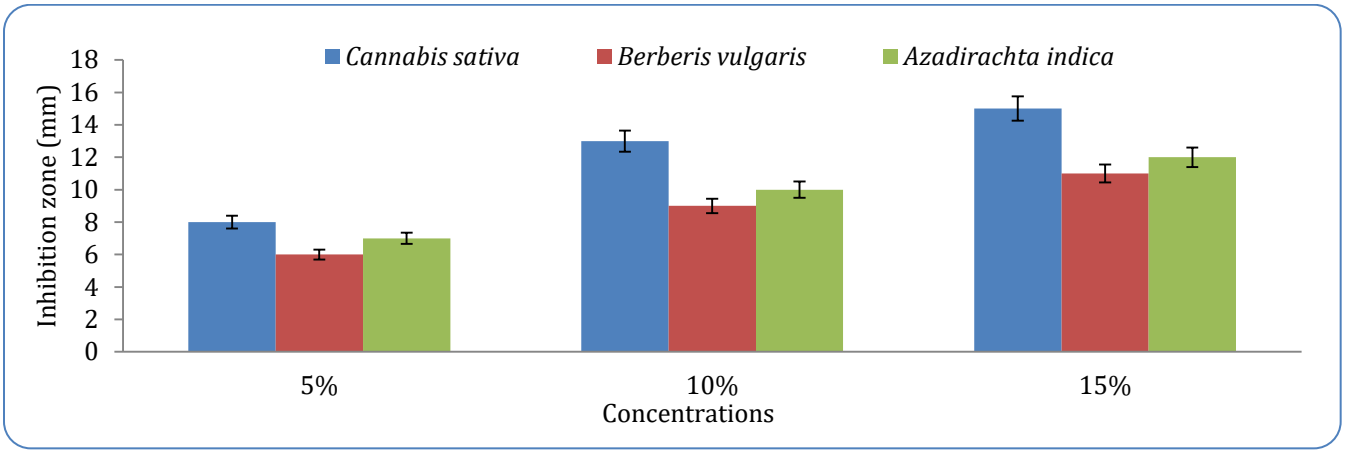

Figure 1: *In vitro* evaluation of different concentrations of selected plant extracts on the growth of *X. Perforans*.

Greenhouse evaluation of selected plant extracts

In the pot experiment, the concentrations of plant extracts from *C. sativa* and *A. indica* showed significant reductions in bacterial spot severity on tomato plants. The most effective treatment was observed with *C. sativa* at a 15% concentration, closely followed by *C. sativa* at a 10% concentration. On the other hand, *B. vulgaris* demonstrated the least disease suppression impact on bacterial spot of tomato, resulting reduction in disease severity when compared to the control treatment. Among the three tested plant extracts, *C. sativa* proved to be the most effective, showing maximum efficacy of AUDPC for severity that was 59.4%, 55.6%, and 51.3% at concentrations of 5%, 10%, and 15%, respectively (Figure 2). In the *in vivo* pot experiment, the control, which involved the use of the distilled water, exhibited no efficacy against bacterial spot of tomato with 96-97% AUDPC.

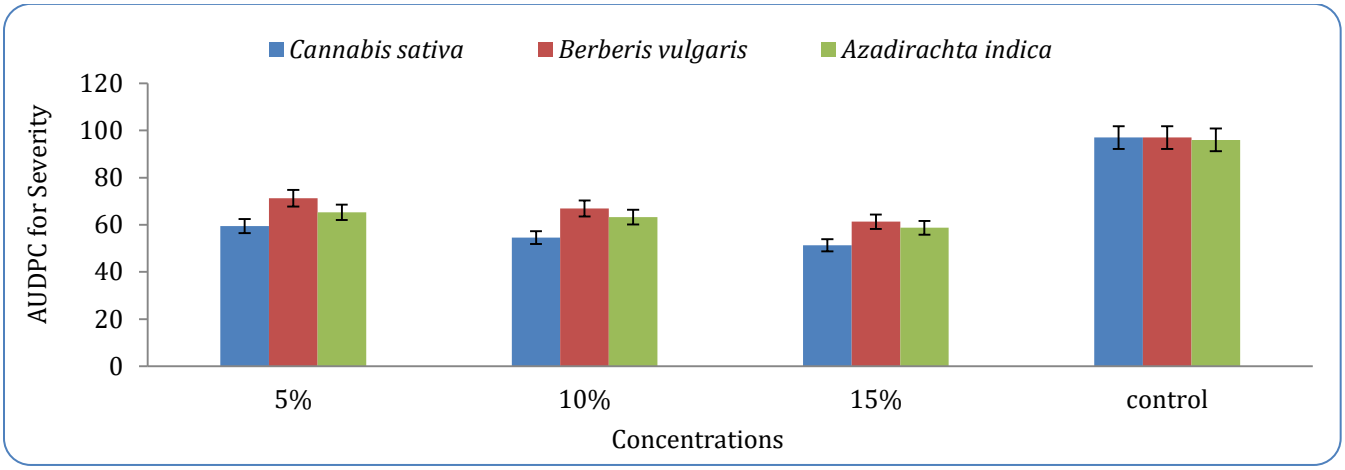

Figure 2: Effect of different concentrations of plant extracts on % disease incidence of *X. Perforans* on tomato.

The need to reduce dependency on commercial fungicides has led researchers to explore alternative and effective approaches. Plant extracts have emerged as a promising substitute for fungicides due to their demonstrated antifungal properties and eco-friendly nature. Various plants have been found to produce

secondary metabolites such as phenolics, terpenes, tannins, flavonoids, essential oils, alkaloids, polypeptides, and lecithin, which exhibit antifungal actions. These compounds play a significant role in the physiology of plants and have been shown to contribute to the reduction of early blight in previous studies (Kagale et al., 2004).

The results of the current study was parallel to the previous findings that evaluated the control of seed-borne infection of bacterial leaf spot (BLS) in tomatoes caused by *X. perforans*, tomato seeds were subjected to treatment with aqueous extracts from 84 different plant materials. In the *in vitro* assays, it was found that 20.2% of the tested plant extracts completely inhibited the growth of *X. perforans* when seed washings from treated seeds were plated on Nutrient Agar. In the *in planta* experiments, a notable 17.8% of the tested plant extracts led to a 100% reduction in BLS incidence in tomato seedlings (Mbega et al., 2012). Similarly, during the greenhouse experiment, *M. azedarach* exhibited the highest reduction in early blight disease severity at a concentration of 20%, achieving a remarkable 67.93% reduction. It was followed by *C. sativa*, *D. stramonium*, and *A. millefolium*, with reductions of 53.63%, 43.28%, and 31.78%, respectively. In comparison, the disease severity in the control group was 87.3% (Bashir et al., 2020).

CONCLUSION

In conclusion, the study demonstrated the potential of *Cannabis sativa* extracts, particularly at a concentration of 15%, as a valuable natural biocontrol agent against *Xanthomonas perforans*, offering a promising avenue for integrated management of tomato bacterial spot disease.

AUTHORS' CONTRIBUTIONS

BM, MJ and MAB designed and conducted experiments. HQ, KT, MRK and MTY helped in collecting and conducting data from the experiments. SS, TZ and RMB edited the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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