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Management of *Alternaria* Leaf Blight (*Alternaria alternata*) of *Aloe vera* by talc-based formulation of a potential antagonist

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Aloe vera (L.) Burm. f. is one of the important medicinal plants in the Asphodelaceae family and is native to North and East Africa. Several districts of West Bengal, India, have been affected by a leaf blight disease caused by the fungus *Alternaria alternata*. *Pseudomonas aeruginosa* strain WS-1 an antagonist isolated from the healthy rhizosphere of *Aloe vera*, demonstrated both *in vitro* and *in vivo* biocontrol efficacy against the pathogen. It has been observed that foliar application of a talc-based formulation of the antagonist to the field of *A. vera* reduced disease severity by 80% compared to untreated control.

Keywords: *Alternaria alternata*, *Aloe vera,* biocontrol, leaf blight, *Pseudomonas aeruginosa* WS-1.

INTRODUCTION

Aloe vera (L.) Burm.f. *(Aloe barbadensis)* belongs to the family Asphodelaceae. Of the different medicinal plants, *Aloe vera* has been used for therapeutic purposes since ancient times to treat different human ailments and disorders, along with its health, beauty and skin care properties (Kumar *et al.* 2019, Babu and Noor, 2020). *Aloe vera* is a perennial, succulent, xerophytic shrub, has been studied thoroughly and reported with various bioactive compounds like amino acids, anthraquinones, enzymes, sugars, polyphenols, minerals and vitamins (A, B, C and E) (Quispe *et al.* 2018, Khajeeyana *et al.* 2019, Martínez - Sanchez *et al.* 2020). Numerous research has looked at *Aloe vera* as an alternative medicine due to its abundance of bioactive phytochemicals with considerable therapeutic potentials such as antioxidant, antidiabetic, neuroprotective, cytotoxic, anti-inflammatory properties and many more (Farid *et al.* 2021; Budiastutik *et al.* 2022; Kaur *et al.* 2022; Tong *et al.* 2022). Many herbal drugs and drinks have been formulated from *A. vera* plants for the maintenance of good health.

The treatment of sores and wounds, skin cancer, skin disease, cold and cough, constipation, pile, fungal infection, etc. using aloe vera gel has been reported to be quite helpful (Djeraba and Quere, 2000; Olusegun, 2000; Lanka, 2018). Topical application of aloe vera may also be effective for genital herpes and psoriasis (Pandey *et al.* 2016). Evidence supports the use of *Aloe vera* for the healing of first to second degree burns (Maenthaisong *et al.* 2007; Sánchez *et al.* 2020). A number of glycoproteins present in aloe vera gel have been reported to have anti-tumor and antiulcer effects and to increase proliferation of normal human dermal cells (Yagi *et al.* 2003; Pandey *et al.* 2016; Lanka 2018).

Medicinal and aromatic plants are attacked by number of phytopathogens viz. fungi, bacteria, viruses and nematodes, leading to significant quantitative and qualitative loss (Katan, 2017). It has been reported that most of the diseases of medicinal plants are fungal in origin (Paul and Singh, 2002). Survey results confer that the

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Alternaria blight diseases are very common in medicinal plants cultivated in various districts of West Bengal, India. The disease protection measures of medicinal plants are still restricted to the application of various chemical fungicides, which do not fit strictly with the basic theory of usefulness of herbal drugs, and the residual effects of different chemicals eventually contaminate the purity of such plant drugs. This is also of serious concern from environmental point of view (Sharma *et al.* 2004; Stuart *et al.* 2018). Therefore, disease management of medicinal plants in the field by biological control agents are gaining importance.

Pseudomonads are considered important rhizosphere organisms, wherein considerable research is underway globally to exploit their potential. Fluorescent pseudomonads help to protect crops from pathogens, in the maintenance of soil health, and are metabolically and functionally more diverse (Choudhury *et al.* 2009). A wide range of fluorescent pseudomonads have been reported for having *in vitro* and *in vivo* biocontrol potentiality against a wide range of phytopathogens (Maiti *et al.* 2012; Sen *et al.* 2009, 2012; Anupama *et al.* 2015). Here, attempts have been made to evaluate *in vitro* and *in vivo* antagonistic activity of a bacterium, *Pseudomonas aeruginosa* strain WS-1 against leaf blight disease of *A. vera.*

MATERIALS AND METHODS

Organisms

The pathogenic organism was isolated from the diseased leaves of *A. vera* as a pure culture on potato dextrose agar medium (PDA) and identified as *Alternaria alternata* further confirmed by the Agharkar Research Institute, Pune, India (Maiti *et al.* 2007). The culture was maintained in the same medium and stored at 4°C for further study. The biocontrol agent *Pseudomonas aeruginosa* strain WS-1 was obtained from our laboratory culture stock which was previously isolated from the rhizosphere of healthy *A. vera* plant from the experimental plant garden of Department of Botany, St. Joseph's College, India by serial dilution technique. It was identified as per Bergy's manual and further confirmed by microbial type culture collection (MTCC), Chandigarh, India. The antagonist was subcultured and maintained on tryptic soy agar (TSA) medium for subsequent use.

Dual culture bioassay

The antagonist *P. aeruginosa* WS-1 from 24 h old culture (10^7 cells /ml) was streaked in the peptone (10 g/L) glucose (20 g/L) agar (20 g/L) (PGA) plate as circular / O and semicircular / U pattern. Then mycelial disc (5 mm diameter) of 3 days old culture of *A. alternata* was subsequently inoculated at the center of O or U-shaped region on the PGA plates (Randhawa *et al.* 2002). Inoculation only with the pathogen served as control. The plates in triplicate were incubated at 30°C for 5 days and diameter of colony growth was measured at every 24 h intervals. Light microscopic studies were also performed to detect physical and / or morphological changes of mycelia.

Talc based formulation and survival of the antagonist

Talc based formulation of the antagonist was prepared using the method developed by Vidyasekaran and Muthamilan (1995). *P. aeruginosa* WS-1 was found to be tolerant against streptomycin at 50 µg / ml. The isolate was grown in Kings B medium supplemented with streptomycin (50 µg / ml) for 48 h on a rotary shaker (150 rpm) at 30°C. The bacterial suspension (8×10^9 colony forming unit (cfu) / ml) was mixed with sterile talc (400 ml / kg) containing carboxymethylcellulose (10g / kg) and air dried (approximately to 35% w/w, moisture content). The formulation was stored at 4°C for up to 180 days. Survival of the bacterial population in the formulation was assayed at 30-day intervals using King's medium B supplemented with streptomycin (50 µg / ml) by dilution plating.

Field studies

The Experimental plant garden of Department of Botany, St. Joseph's College, India was used consecutive two years i.e., 2020 and 2021 for the field experiments when the environmental

conditions were conducive (March to August) for the rapid spread of *A. alternata* on *A. vera.* The trial was conducted as a randomized complete block design with three replicate plots (3 × 4 m^2) and twenty-five plants per plot. Well-rotted farmyard manure was mixed well into the soil before planting the saplings. Two months-old disease-free saplings were transplanted to the random blocks during mid March allowing *Alternaria* leaf blight to develop naturally (Silva *et al.* 2004). The talc-based formulation of *P. aeruginosa* WS-1 was prepared by dissolving it in water (4 g / L) allowing it to settle for 1 h, and filtering the solution through muslin cloth. The filtrate was applied as a foliar spray using a low volume sprayer at the beginning of transplant and repeating every 15 days for six months, i.e., up to the mid of September 2020 and 2021. Plots sprayed with the talc-based carrier without the biocontrol agent served as the control. Thirty plants from each plot were rated for disease severity at 15-day intervals starting at the time of transplant till next six months using a 0-5 rating scale (Kishore *et al.* 2005), where 0 = healthy leaves; 1 = small brownish spots appeared on upper surface; 2 = spots found on both upper and lower surfaces; 3 = larger spots with concentric rings; 4 =spots enlarged to touch margin of the leaves lead to browning and defoliation; and 5 = plants severely affected, more than 50% of leaf defoliated.

Statistical analysis

The disease severity data were statistical analysed by using analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) to find out significance level at 1% (p<0.01).

RESULTS AND DISCUSSION

Interaction of P. aeruginosa WS-1 against A. alternata in dual culture

Significant growth inhibition of *A. alternata* by *P. aeruginosa* WS-1 was observed in dual culture. Growth of mycelia was restricted near bacterial growth and continued away from it. The growth inhibition of *A. alternata* remained proportionate with an increased incubation period of upto 5 days. Quantitatively *P. aeruginosa* WS-1 inhibited the growth of *A. alternata* by 76.75% and 67.34% in circular and semicircular streaks after 120 h of incubation respectively (Fig 1). Closer microscopic examination of the mycelia at the interaction zone with *P. aeruginosa* showed signs of shriveling, growth deformities, swelling, fragmentation, short branching and lysis.

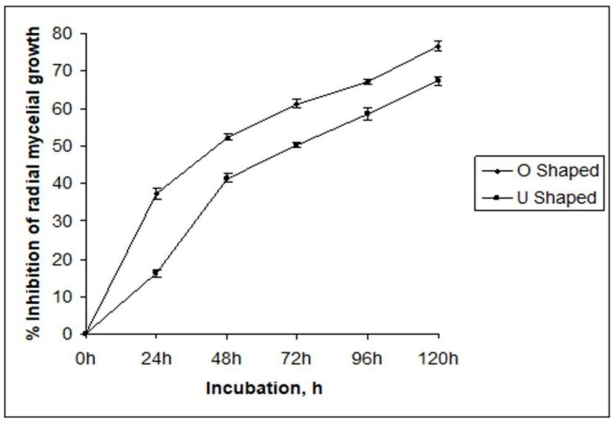

Fig. 1. Inhibition of *A. alternata* by *P. aeruginosa* WS-1 under dual plate culture using circular (O) and semicircular (U) method. Each point represents the mean ± SE (standard error) of three separate experiments, each in triplicate.

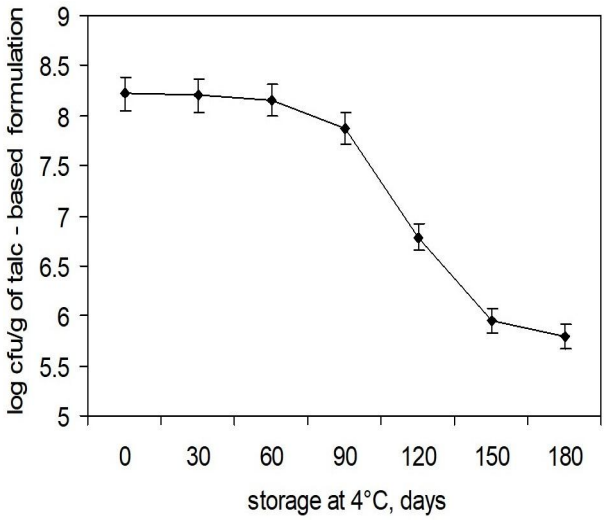

Fig. 2. Survival of *P. aeruginosa* WS-1 in talc-based formulations stored at 4°C. Each point represents the mean ± SE (standard error) of three separate experiments, each in triplicate

Survival of P. aeruginosa WS-1 in talc-based formulation

The survival of *P. aeruginosa* WS-1 in the talc-based formulation at 4°C was monitored for 180 days, (Fig. 2). With time, the initial population of *P.*

Table 1: Efficacy of talc-based formulation of *P. aeruginosa* WS-1 for the control of leaf spot disease of *A. vera* caused by *A. alternata* in 2020 and 2021. Talc-based formulation was applied as foliar sprays on date of transplantation and at an interval of 15 days till 180 days.

Days after transplantation	Disease index 2020		Disease index 2021	
	Control	Treated	Control	Treated
0	0	0	0	0
15	0.267±0.008	0.12±0.006*	0.203±0.009	0.162±0.009
30	0.777±0.009	0.305±0.008*	0.704±0.014	0.308±0.007*
45	1.755±0.007	0.347±0.012*	1.46±0.016	0.321±0.011*
60	2.276±0.012	0.475±0.011*	1.977±0.024	0.452±0.019*
75	2.825±0.015	0.575±0.014*	2.01±0.041	0.58±0.006*
90	3.215±0.011	0.665±0.023*	2.76±0.029	0.691±0.014*
105	3.6±0.013	0.725±0.022*	3.04±0.021	0.7±0.027*
120	3.8±0.014	0.765±0.013*	3.62±0.024	0.768±0.025*
135	4.12±0.022	0.82±0.019*	4.1±0.022	0.801±0.034*
150	4.2±0.031	0.85±0.015*	4.18±0.034	0.824±0.043*
165	4.34±0.032	0.91±0.017*	4.23±0.019	0.927±0.024*
180	4.7±0.029	0.93±0.018*	4.42±0.027	0.91±0.016*

Data with an asterisk in each row differ significantly with control according to DMRT test (P<0.001).

aeruginosa WS-1 (8.3 log cfu / g) in the talc-based formulation decreased gradually. During storage no significant decrease in the viable population was observed. Subsequently, there was a gradual decline in the population. A total of approximately 30% decrease in the colony forming unit was estimated on 180th day after storage at 4°C.

Field studies

Talc formulation of *P. aeruginosa* WS-1 was evaluated in the field on *A. vera* for two successive seasons. After the 2nd and 3rd spray application of WS-1, new symptoms of *Alternaria* leaf blight were inhibited in the treated plots. The mean disease index in control field reached to 4.7 and 4.42 in 2020 and 2021, respectively, at the time of harvest, where more or less all plants were severely affected and more than 50% leaves were defoliated (Table 1). On the contrary, the disease index in WS-1 treated fields at harvest only reached 0.93 and 0.91 in 2021 and 2022 respectively; indicating disease severity reduction of 80 %.

Overall observations during this work suggested that *P. aeruginosa* WS-1 had the potentiality to control *A. alternata* both *in vitro* and *in vivo*. It has been reported that the same antagonist had the ability to control leaf blight disease of *Withania somnifera*, caused by *Alternaria dianthicola* (Maiti *et al.*, 2012). Furthermore, *P. aeruginosa* WS-1 showed antifungal activity mainly by the release of secondary metabolites like siderophore, HCN and lytic enzymes (Maiti *et al.* 2012). Similar type of observations was made earlier by Kapsalis *et al.* (2008) and Ramyasmruthi *et al.*(2012) who demonstrated that the exposure of selected phytopathogenic fungi to lytic enzymes such as chitinase and protease could result in the degradation of the fungal cell wall. According to

several studies, HCN, siderophore, chitinase and protease produced by fluorescent pseudomonads are known to inhibit the growth of some fungal pathogens (Bhatia *et al.* 2003; Kapsalis *et al.* 2008; Ramyasmruthi *et al.* 2012).

Fluorescent pseudomonads have been reported to stay alive in certain formulations (Mugilan *et al.* 2011). The populations of fluorescent pseudomonads did not decrease in talc mixture with 20% xanthane gum after storage for four months at 4°C (Novinscak and Filion, 2020). In the present study, we showed that the strain WS-1 could live well in talc-based formulation stored at 4°C for 6 months. Moreover, the success of any biocontrol formulation not only depends on the ability of the biocontrol agent to survive in the formulation, but also its capacity to survive on the host plant to which it is applied. Our talc-based formulation of the antagonist applied every 15 days during the season, in two consecutive seasons successfully reduced symptoms of *Alternaria* leaf blight on *A. vera* by 80%. This ultimately might help the farmers to limit the use of hazardous fungicides and simultaneously saving their crops. Finally, a consistent biocontrol for aerial plant diseases based on an integrated biological control approach should be developed as a result of the combined efforts of academic, federal, and private sector experts.

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