Research Article

Evaluation of Biological Inoculants against Leaf Blight of Asalio Caused by Alternaria alternata

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Abstract

Asalio an important medicinal plant with significant pharmacological properties has been observed to be generally affected by many fungal pathogens in India. Phosphate-solublizing Fungi (PSF) functions in soil phosphorus cycle by increasing the bioavailability of soil phosphorus for plants and root associated fungi have been known to benefit plants hence considered as plant growth promoting fungi (PGPF). Present investigation Five isolates of *Aspergillus awamori* namely Zinger-Z, Parthenium-P, Red gram-G, Rice leaf sheath-R, Field bean-B isolated from different four rhizosphere and one phylloplane were tested In-vitro and In-vivo conditions. The highest Mycelial growth suppression recorded by (*Aspergillus awamori-Z)* with 44.22 %. The *A.awamori-P* was found highly effective in promoting the physiological parameters viz relative water content, chlorophyll content index, membrane stability index (MSI) and reducing leaf blight incidence.

Keywords: PGPF, Aspergillus awamori isolates, disease suppression in-vitro and in-vivo conditions

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Introduction

Lepidium sativum L. is commonly known as Asalio and Chandrasur. It is belongs to the family brassicaceae, in english it is known as "Garden Cress''. Its posses Seeds, leaves and roots are economically and medicinally important and it is mainly grown for seeds all over India. Asalio is under commercial cultivation in Madhya Pradesh having more than 25000 ha. Due to increase in demand the area under Chandrasur production is being increased year after year. The whole plant is used for secondary syphilis and tenesmus [15] anti-diarrheal and anti-spasmodic[16] hypoglycemic [7] laxative [23] anti-bacterial [4] antioxidant, contraceptive effects[24] and in inflammatory bowel disease [21]. Among them *A. alternata* causes severe leaf spot in the northern Indian plains. Alternaria leaf spot disease symptoms in *L. sativum* are characterized by the appearance of brown necrotic spots on the leaf margin. The necrosis spreads towards the midrib and as a result the leaf curls up and dries, affecting the herb yield. Host Selective Toxins (HST) function as essential determinants of pathogenicity or virulence; Alternaria producing the most important and well known HSTs [12].

The HST group comprises a limited number of phytotoxins that meet the following criteria: (i) the toxin and its producer have similar host specificity; (ii) the virulence of the pathogenic strains is positively correlated to their capacity to produce the toxin and (iii) the toxin is able to produce, in susceptible plants, symptoms characteristic of the disease caused by the pathogen [12]. HSTs are toxic to plant species or cultivars susceptible to the pathogens producing these toxins and there is a correlation between sensitivity to the toxin and susceptibility of the plant to the pathogen [14]. Therefore, organic solution for such menace is indispensible for retaining the therapeutic value of the crop along with higher yield. Phosphorus-solubilizing microorganisms (PSMs) are abundantly available in the rhizosphere of plants [29] where they compete with other organisms for nutrient and space and in-turn provide nutrient for growth and protection from deleterious micro-organism. Many studies have shown an increase in growth and P-uptake by plants through the inoculation of PSMs (one of the component of PGPF) in pot experiments [28] and as well as in field conditions[26].The antagonistic fungi, *Aspergillus* are a common genus in most agricultural fields of India and are ubiquitous fungus with no specific moisture and pH requirements. Therefore, the present investigation was undertaken to overcome the menace of this pathogen, the Plant Growth Promoting Fungi (PGPF) with special reference to *Aspergillus awamori isolates* have been used to manage the disease.

Material and Methods

Collection of diseased specimens and purification of the pathogen

Diseased Asalio plants exhibiting typical symptoms of *Alternaria alternata* infection were collected from the experimental field of AICRP on Medicinal Aromatic Plants and Betelvine of Jawaharlal Nehru Krishi Vishwa Vidyalaya (22°49'- 220 80'N; 78°21'- 80°58'E), Jabalpur in the Central India.

Treatment details of mycoflora used under in-vitro and in-vivo studies

Different PGPF were isolated from the four rhizosphere and one phylloplane of different crops, the five isolates of *Aspergillus awamori* have been screened against *Alternaria alternata* under *in-vitro* and *in- vivo* conditions.

Aspergillus awamori isolates

A. awamori- Z, (*A. awamori*- Zinger isolate), *A. awamori* –P(*A. awamori*- Parthenium isolate), *A. awamori*-G(*A. awamori*-Red gram isolate), *A. awamori* –R(*A. awamori*-Rice leaf sheath isolate) and *A. awamori* –B(*A. awamori*-Field bean isolate).These isolates were used for field experiment was conducted with six treatments and three replications in randomized block design with plot size of two square meters. These beneficial fungi (@ 2ml/m^2) were combined with $ZnSo_4$ (@ 200ppm) were applied in soil. The treatments were designated as T1-(*A.awamori*-R), T2-(*A.awamori*-P)**,** T3-(*A. awamori*-B), T4-(*A. awamori*-Z), T5-(*A.awamori*-G) and T6-(Control).

Evaluation of antagonistic potential of beneficial fungi through dual culture technique

The antagonistic potentials of bioagents such as *Trichoderma* species and *Aspergillus awamori* isolates were evaluated against test pathogen (*Alternaria alternata)*through dual culture technique. A five mm disc of different fungal isolates was cut out from the seven days old culture with the help of a sterile cork borer and placed close to one end of the Petri-plate containing 20 ml solidified PDA medium. At the opposite end, a similar disc from the culture of the pathogen*(Alternaria alternata*)was placed simultaneously. Each treatment was replicated three times and the experiment was repeated twice. The Petri-plates were incubated at 25±2ºC in a BOD incubator and observations were recorded at regular intervals. The Suppression of the growth of the pathogen by the antagonistic fungi was measured after 48 hrs, 72 hrs, 96 hrs and 144 hrs of incubation till both occupies the entire space of Petri-plate. The antagonism was measured on the basis of (mm) suppression and inhibition of the pathogen by the bio agent by the following formula-

Per cent inhibition of growth of the pathogens was calculated by using the following formula.

$$\text{Inhibition} = \frac{\text{Radial growth in control(C)} - \text{Radial growth in the treatment (T)}}{\text{Radial growth in control(C)}}$$

$$\text{Per cent inhibition} = \text{Inhibition} \times 100$$

Relative water content (RWC)

Measurements of RWC were performed on leaves collected from Asalio plants. Leaves were always collected from the mid section of either branches or seedlings, in order to minimize age effects. The leaves were first removed from the stem with tweezers. A sharp razor blade was used to cut the leaf base and leaves were then immediately weighed (fresh mass, FM). The FM obtained from each sample was minimum 1 gram. In order to obtain the turgid mass (TM), leaves were floated in distilled water inside a closed Petri dish. At the end of the imbibition period, leaf samples were placed in a pre-heated oven at 80 ºC for 48 h, in order to obtain the dry mass (DM). Values of FM, TM, and DM were used to calculate RWC, using the following equation [1].

$$\text{RWC (\%)} = [(\text{FM} - \text{DM})/ (\text{TM} - \text{DM})] \times 100$$

A leaf sample was made up of ten to fifteen leaves, collected from the same branch or seedling. For data analysis, each leaf sample was treated as an experimental unit. The experimental units were organized following a Random block design.

Chlorophyll content index

Chlorophyll Content Index was estimated through the portable chlorophyll meter. Fully expanded leaf sample from three places of each plant of different treatments has been selected for estimation of chlorophyll content index. The mean of triplicate readings taken using SPAD-502 (SPAD-502, Minolta, Japan) around the mid-point near the midrib of each sample were recorded for different treatment of Asalio leaf and averaged[19].

Membrane stability index (MSI)

The membrane stability index (MSI) was determined according to the method [5].Leaf discs (0.2 g) of control and treated plants were thoroughly washed in running tap water and double distilled water and were placed in 20 ml of doubled distilled water at 40 ^{0}C for 30 minutes, after that electrical conductivity (EC) was recorded by conductivity bridge (C1). Subsequently, the same samples were placed in boiling water bath (100^{0}C) for 10 minutes and the electrical conductivity was recorded (C2). The membrane stability index was calculated by using the formula:

$$MSI = [1- C1/ C2] \times 100$$

Results

Mycelial growth suppression of Alternaria alternata by Aspergillus awamori isolates

The effect of five isolates of *A. awamori* has been assessed against *Alternaria alternata* mycelial growth at different intervals. The mycelia growth suppressing ability of all the isolates were significantly higher against the test pathogen. The highest (23.99 mm) suppression was recorded with T_1, while least (29.19 mm) suppression was recorded with T_5. The mycelial growth suppressing ability of T_2 and T_3 were higher but alike to each other and were next best to T_1. The significant reduction T_4 (28.43mm) in mycelia growth of *Alternaria alternata* T_4.There was significant increase in mycelia growth of the pathogen from 48 hrs to 120 hrs. But higher growth suppression was recorded thereafter at 144 hrs. Hence, the mycelia growth suppression was significant with each bioagent at different time intervals in comparison to control (**Table 1**). [2] Observed high antagonistic activity of all the isolates of *A. niger* against the test fungi due to their fast mycelia growth and competition for nutrients, hyper parasitic behaviour and mechanical obstruction. P-solubilizing filamentous fungi (like *A. awamori*) are also well-known producers of lytic enzymes and cell-wall-degrading enzymes, such as β-1,3-glucanases, cellulases, proteases, and chitinases are known to be involved in the activity of some microorganisms against phytopathogenic fungi [3, 6, 9].

Table 1 Suppression of mycelial growth *of Alternaria alternata* by *Aspergillus awamori* isolates

***Aspergillus awamori* isolates**	**Growth in (mm)**						
	48 hours	**72 hours**	**96 hours**	**120 hours**	**144 hours**	**Mean-A**	**Percent inhibition**
T_1(*A.awamori*-Z)	26.07 (19.33)	26.80 (20.33)	30.43 (25.66)	20.23 (12.00)	16.41 (8.00)	23.99	44.22
T_2 (*A.awamori*-P)	24.59 (17.33)	29.10 (23.66)	28.40 (22.66)	32.26 (28.50)	25.46 (18.50)	27.96	34.99
T_3(*A.awamori*-G)	24.08 (16.66)	27.74 (21.66)	27.96 (22.00)	31.93 (28.00)	23.57 (16.00)	27.06	37.08
T_4(*A.awamori*-R)	27.03 (20.66)	27.96 (22.00)	29.10 (23.66)	32.23 (28.50)	25.83 (19.00)	28.43	33.89
T_5(*A.awamori*-B)	25.71 (18.83)	28.31 (22.50)	30.00 (25.33)	35.25 (33.33)	26.68 (20.16)	29.19	32.13
T_6Control	30.97 (26.50)	35.46 (33.66)	42.80 (46.16)	50.09 (58.83)	55.75 (68.33)	43.01	
mean	26.41	29.23	31.45	33.66	28.95		
CV	3.38						
FungusCD($P \leq 0.05$)	0.74						
Hours CD($P \leq 0.05$)	0.67						
Fungus x Hours	1.65						

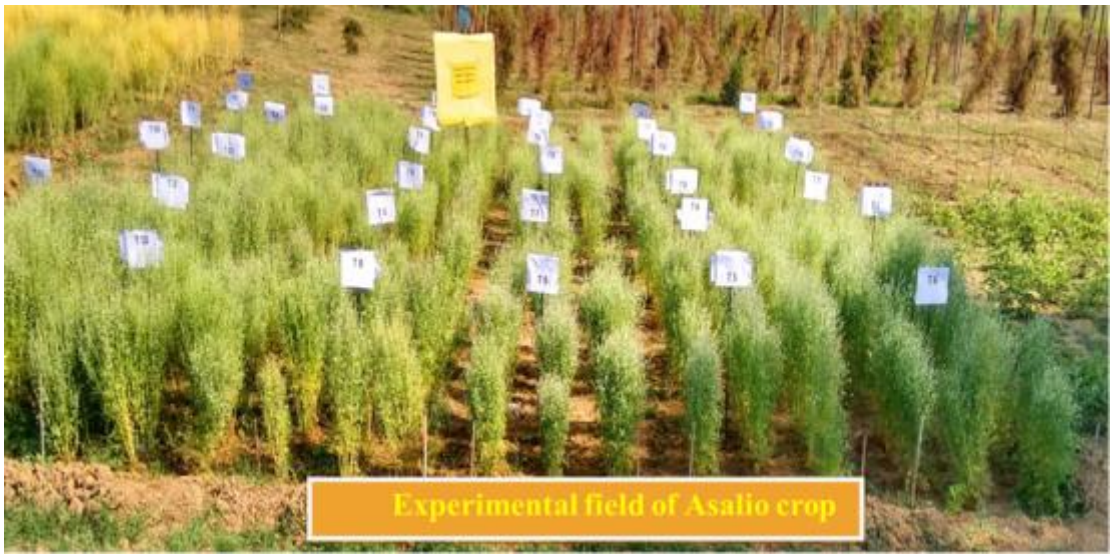
The values in the parenthesis are original value

Impact of biological treatments on physiological and disease incidence of Asalio crop

The inoculation of fungal bioagents along with FYM and $znso_4$ were shown significant on physiological parameters like relative water content (RWC), chlorophyll content, membrane stability index (MSI) and disease index under *in-vivo* conditions (**Table 2**). Although the highest (73%) RWC was recorded in T_2 treatment but was statistically at-par with T_3 (*A.awamori*-B), T_4 (*A.awamori*-z). Similarly, the highest chlorophyll content (49.2%). The highest MSI T_1 (AW-R) 67.66%, T_2(AW-P) 68.33 and lowest T_6(Control) 89.18%, T3(*A. awamori*-B) 89%. The treatments effect of T1 and T2 was almost similar to those of control in reducing the disease incidence (Table 2). Phosphate-solubilizing microorganisms have potential for the biocontrol of plant pathogens [18] as they change insoluble phosphatic compounds into soluble forms [22] thus increasing the growth and yield of crop plants [8, 25]. Several studies have demonstrated that PGPF induce systemic protection against phytopathogens [10, 11]. Phytohormones, salicylic acid (SA), jasmonic acid (JA), and ET play important roles in the induced defense responses [13]. Furthermore, ABA, BS, GAs, and auxins have also been reported to play important roles in plant defense response, but their involvement has been poorly studied [20]. Applications of *A. awamori* cause decline in the pathogen populations and result in enhanced yield was reported [27].

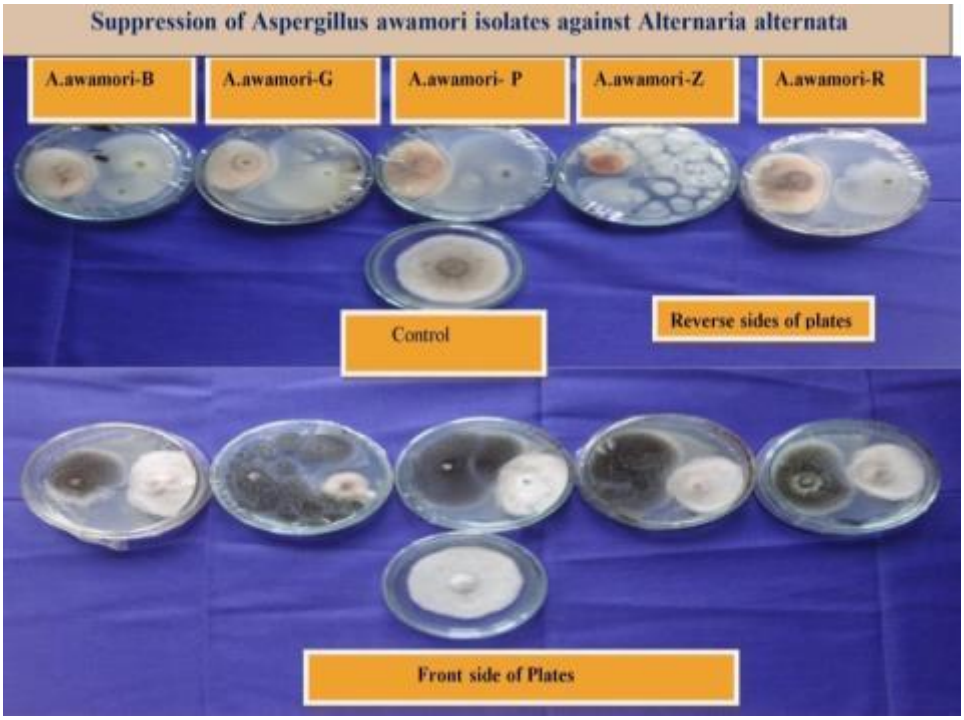
Table 2 Effect of biological treatments on physiological and disease incidence of Asalio crop

Treatments of fungal bio agents	Relative water content (%)	Chlorophyll Content (%)	Membrane stability index (%)	Percent disease index (%)
T_1(*A.awamori*-R)	64	40.65	67.66	37
T_2 (*A.awamori*-P)	73	49.2	68.33	37.31
T_3(*A.awamori*-B)	59.16	46.34	89	52.22
T_4(*A.awamori* -Z)	59.08	41.52	81.32	42.98
T_5(*A.awamori*-G)	67.91	44.9	85.85	43.31
T_6Control	51.33	25.5	89.18	54.04
CV	6.47	5.85	4.16	7.35
Fungus CD($P \leq 0.05$)	7.35	4.4	6.72	5.95



Different level of Aternaria alternata infection on Asalio leaves

Infected plant



Conclusion

The five isolates of *A. awamori* were significant on *Alternaria alternata* in-vitro and in-vivo conditions but A. awamori-P most effective on physiological parameters in field conditions.

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