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# Jasmonic Acid Mediated Management of Necrotrophic Pathogen Alternaria Leaf Spot in Soybean

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## ABSTRACT

The Field trial was conducted during *Kharif* season for two consecutive years (July–October 2017 and 2018) at three different locations namely; Amravati, Yavatmal and Katol under Dr. Panjabrao Deshmukh Krishi Vidyapeeth., Akola, Maharashtra, India. The nine treatments were designed in randomized block design replicated thrice to determine the potential use of an elicitor for Alternaria leaf spot management compared with standard fungicide. The treatments include two foliar sprays of either abiotic elicitors alone namely Salicylic acid (SA) @ 100 ppm, Benzoic acid @ 100 ppm, Humic acid @ 1%, Jasmonic acid @ 100 ppm, Potassium nitrate ($KNO_3$) @ 1% or biotic elicitors *Trichoderma viride* @ 1%, *Pseudomonas fluorescens* @ 1% alone or recommended fungicide Propiconazole 0.1% alone at crop flower initiation stage and at 60 days after sowing. Field trials results shows the Propiconazole 0.1% significantly superior in management of disease upto 44.72 % followed by Jasmonic acid @ 100 ppm in controlling the disease upto 40.01 % and was found at par with propiconazole 0.1% in respect to percent disease index, percent disease control and seed yield. Highest percent disease index and least percent disease control and yield recorded in control. Elicitor Jasmonic acids induce protective, therapeutic treatments reduced the severity of disease advocated suppressing necrotrophs pathogen *Alternaria alternata*, the eco–friendly alternatives to fungicides.

KEYWORDS: *Alternaria*, jasmonic acid, disease manegment, soybean, leaf spot

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**Data Availability Statement:** Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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## 1. INTRODUCTION

Soybean (*Glycine max* L. Merrill) is the world's most important miracle seed legume often called the golden bean contains highest protein (40–42%). Currently United States tops the global soybean production contributes 35% of total produce. Global food and nutritional security goal are today's major priority. The biotic, abiotic and climate change are the major threat for soybean crop production. Leaf spot incited by *Alternaria alternata* reported most prevalent destructive necrotrophic pathogen in soybean. The most destructive *Alternaria alternata* caused significant infection at later growth stages of soybean crop causes premature defoliation of leaf resulted in heavy yield loss (Borah and Deb 2022). *Alternaria alternata* appears during first week of September when crop is in vegetative growth stage as brown necrotic spots with concentric rings on foliage. These spots coalesce and form large necrotic areas. The infected leaves eventually dry out and drop prematurely (Fagodiya et al., 2022). Considering the economic importance of soybean crop, destructive nature of the pathogen, the management of this pathogen is difficult due to unavailability of commercial resistant varieties against the disease. The control of biotic stresses like fungal, bacterial, or viral infections is largely based on the use of harmful fungicides, bactericides, and insecticides that toxic to plant invaders, causative agents. However, the harmful effect of this pesticide's end product on the environment and human health leads to strong needs for the search of new, safe means of disease management. Plant has natural phenomenon of induced resistance to protect them from disease. Similar action was performed by synthetic compounds in plants called elicitors. Elicitors are the compounds, that activates the defence mechanism in plants and are separate from each other based on their source, nature and molecular structure (Thakur and Singh, 2013, Prsi´c and Ongena, 2020). The chemical elicitors are the compounds that do not kill pathogens but trigger plant defense mechanisms. The chemical elicitors are salicylic acid, benzoic acid, humic acid, Jasmonic acid that produce phenolic compounds and release various defense–related enzymes in plants and induce resistance against biotic stress (Ali et al., 2021). The upsurge in concentrations of phenolics in plant tissues are in response to infections by pathogens (Bhattacharya et al., 2010). Salicylic and benzoic acids have plays important role in inducing resistance against several biotic stress (Abdel-Monaiem, 2010). Benzoic acid having potential antimicrobial activities (Li et al., 2020). Humic acid is applied effectively in enhancing natural resistance against plant pathogens (Afifi, 2017). Jasmonic acid (JA) the products of linolenic acid, which are produced through the induction of octadecanoid pathway induce the biosynthesis enzymes induce resistance to biotic stress (Jankiewicz, 2003, Schaller et al., 2005, Lumba and Culter, 2010). Potassium nitrate plays an important role as the entry and development of pathogens in to potassium–deficient plants as a result of physical and metabolic changes is counteracted by an increased defence (Amtmann et al., 2008, Sugimoto et al. 2009). Bio agents are one of the alternative strategies in plant system for disease management (Raymaekers et al., 2020). Species such as *Trichoderma, Pseudomonas, Bacillus* species, and other microbes of the rhizosphere shows antagonist activity of many plant pathogens. Genus *Pseudomonas* is found to be capable of inducing systemic resistance in Arabidopsis. (Meena et al., 2016). In this paper, we report the results of extensive field trial conducted over three different locations for two consecutive years to determine the potential use of an elicitor for Alternaria leaf spot management compared with standard fungicide check and be recommended as a viable alternative to chemical pesticides.

## 2. MATERIALS AND METHODS

The field experiment was conducted during Kharif season for two consecutive years (July–October 2017 and 2018) at three different locations namely Regional Research Centre, Amravati (20.9300° N, 77.7500° E), Agricultural Research Station, Yavatmal (20.3899° N, 78.1307° E) and Regional Fruit Research Station, Katol (21.2733° N, 78.5860° E) with nine treatments in three replications at randomized block design under the university Dr. Panjabrao Deshmukh Krishi Vidyapeeth., Akola, Maharashtra, India.

Treatments details:-

1. Salicylic acid (SA) @ 100 ppm at flowering and at 60 DAS
2. Benzoic acid @ 100 ppm at flowering and at 60 DAS
3. Humic acid @ 1% at flowering and at 60 DAS
4. Jasmonic acid @ 100 ppm at flowering and at 60 DAS
5. Potassium nitrate ($KNO_3$) @ 1% at flowering and at 60 DAS
6. *Trichoderma viride* @ 1% talc at flowering and at 60 DAS
7. *Pseudomonas fluorescens* @ 1% talc at flowering and at 60 DAS
8. Propiconazole 0.1% at flowering and at 60 DAS
9. Control (Water spray)

Soybean cultivar variety JS–335 was sown at 45×05 $cm^2$ spacing in plot size 3.6×6 $m^2$ (8 lines of 6 metre length). Two spray as per above treatments applied at flowering (45 DAS) and at 60 DAS. Observations on foliage alternaria leaf spot disease were recorded 10 days after each spraying i.e., 55 DAS and 70 DAS. The crop was raised as per recommended package of practices and protective irrigation was given as and when required.

*2.1. Calculation of percent disease index (PDI)*

The above rating scales or grades are utilized for the calculation of PDI using the following formula of Wheeler, 1969 and yield of soybean (kg $ha^{-1}$) recorded and analysis was done using standard statistical methods.

Percent Diseases Index=(Sum of numerical rating/Total number of plants×Maximum grade)×100 ...1

Percent disease control (PDC) was worked out by applying the formula

Percent disease control=(PDI in control plot−PDI in treatment plot/ PDI in control plot)×100 ...2

Percent disease index was calculated by using uniform method given by (Anonymous, 2014). Disease scoring was done using 0–9 scale (Joshi et al., 2007). At harvest of the crops, an observation on seed yield was recorded in all the treatments and yield data was presented (kg $ha^{-1}$) basis.

## 3. RESULTS AND DISCUSSION

*3.1. Percent disease index and disease control after first spray*

The *Alternaria alternata* infected leaves symptoms shows brown necrotic spots yellow halo with concentric rings on foliage. The pooled data at three different locations for two years after first spray shows that, the significant percent disease index in control is upto 22%. The treatment Propiconazole 0.1% showed significantly minimum percent disease index and highest percent disease control over rest the treatments at all the three locations in Katol 7.18, Amravati 8.44 and Yavatmal 11.85 and highest percent disease control i.e., 49.53 followed by Jasmonic acid @ 100 ppm, percent disease index at Katol 8.15, Amravati 9.59 and Yavatmal 13.26 and par with Propiconazole 0.1%. Jasmonic acid @ 100 ppm recorded 43.08 % disease control over water control. Similar result was observed in field evaluation of fungicides for the management of Alternaria leaf blight of sunflower (Kgatle et al., 2020). In a plant's immune system, the plant hormone jasmonic acid (JA) and its derivatives (JAs) have been documented as key regulators that play vital roles in plant defence responses to pathogens (Pieterse et al., 2012) and acts as protective agrochemicals, used for spraying and protects cultivated soybean crop. These elicitors are capable of inducing the expression of pathogenesis–related genes in plants through induced systemic resistance or acquired systemic resistance channels. Systemic Jasmonic acid modulation in mycorrhizal tomato plants and its role in induced resistance against *Alternaria alternata* (Nair et al., 2015). *Pseudomonas fluorescens* @ 1% talc was at third position. *Pseudomonas* is found to be proficient of inducing systemic resistance in Arabidopsis (Beneduzi et al., 2012). Salicylic acid (SA) @ 100 ppm was at fourth position. Salicylic acid (SA) is a key plant hormone required for establishing resistance to many pathogens (Ding and Ding 2020). Potassium nitrate ($KNO_3$) @ 1% was at fifth position. Foliar spray of potassium nitrate will help in suppression of Alternaria leaf blight of cotton (*Gossypium hirsutum*) in northern Australia (Bhuiyan et al., 2007). Biotic elicitor *Trichoderma viride* @ 1% talc was at sixth position. Spraying of *Trichoderma viride* was used for management of pod blight of soybean (Wasule et al., 2022). Benzoic acid @ 100 ppm was at seventh position. Benzoic acid is an organic aromatic monocarboxylic acid having potential antibacterial and antifungal activities (Li et al., 2020). Humic acid @ 1% was at eight positions. Humic acid is a suspension, based on potassium–humates, used successfully in plant production as a plant growth stimulant and for enhancing natural resistance against plant diseases and pests (Afifi, 2017). Maximum per cent disease index of *Alternaria alternata* at 55 DAS recorded in water spray control at Yavatmal 22.33 (Table 1). The biotic and abiotic agents applied have been

Table 1: Effect of spray at flowering on % disease index and disease control

| Sl. No. | Treatment | % Disease index recorded at 55 DAS Pooled of two years | | | Average mean 55DAS | % Disease control |
|---|---|---|---|---|---|---|
| | | Amravati | Yavatmal | Katol | | |
| $T_1$ | Salicylic acid (SA) @ 100 ppm | 11.09 (3.33) | 15.58 (3.95) | 9.80 (3.13) | 12.16 | 33.00 |
| $T_2$ | Benzoic acid @ 100 ppm | 12.52 (3.54) | 16.99 (4.12) | 10.49 (3.24) | 13.33 | 26.56 |
| $T_3$ | Humic acid @ 1% | 12.96 (3.60) | 17.36 (4.17) | 10.20 (3.19) | 13.51 | 25.56 |
| $T_4$ | Jasmonic acid @ 100 ppm | 9.59 (3.10) | 13.26 (3.64) | 8.15 (2.85) | 10.33 | 43.08 |
| $T_5$ | Potassium nitrate ($KNO_3$) @ 1% | 14.53 (3.81) | 18.09 (4.25) | 11.01 (3.32) | 14.54 | 19.89 |
| $T_6$ | Trichoderma viride @ 1% talc | 11.50 (3.39) | 16.11 (4.01) | 10.40 (3.22) | 12.67 | 30.19 |
| $T_7$ | Pseudomonas fluorescens @ 1% talc | 10.85 (3.29) | 15.78 (3.97) | 9.76 (3.12) | 12.13 | 33.17 |
| $T_8$ | Propiconazole 0.1% | 8.44 (2.91) | 11.85 (3.44) | 7.18 (2.68) | 9.16 | 49.53 |
| $T_9$ | Control (Water spray) | 17.41 (4.17) | 22.33 (4.73) | 14.70 (3.83) | 18.15 | |

*Figures in parentheses are arc sine transformed value shown to protect plants by inducing ISR against pathogens (Reglinski and Walters, 2009).

*3.2. Percent disease index and disease control after Second spray*

At later growth stages these *Alternaria alternata* spots coalesce and form large necrotic areas. The infected leaves eventually dry and drop prematurely. The significant damage in control is upto 24%. The pooled data after second spray for treatment Propiconazole 0.1% was more effective that recorded the percent disease control up to 44.72 and was significantly superior over rest of the other treatment. Propiconazole (0.1%) achieved minimum disease index in Katol 10.09, Amravati 10.39 and Yavatmal 13.96 followed by Jasmonic acid @ 100 ppm, was at par with Propiconazole 0.1% with percent disease index in Katol 10.68, Amravati 11.64 and Yavatmal 15.07 and recoded 40.01% disease control. De Vos et al., 2006 reported that Jasmonic acid (JA) acts against necrotrophic pathogen by triggering induced systemic resistance and induces defence causing genes expression in *Alternaria thaliana* leaves challenged with fungal pathogens. Jasmonic acid protects oats against infection caused by *Erysiphe graminis* f. sp. *hordei*, and similarly protects the tomato and potato plants against *Phytophthora infestan* (Schweizer, 1993). It enhances protection against the fungi *Alternaria brassicicola* and *Botrytis cinerea* the necrotrophic pathogens (Rowe et al., 2010). Jasmonic acid has the ability to induce the production of enzymes polyphenoloxidase and peroxidase in date palm seedlings that provide resistance against *Fusarium oxysporum* f. sp. *albedinis* (Jaiti, 2009). Percent disease index were considerably lower in abiotic and biotic elicitor–sprayed plant as compared to control (Thakur et al., 2016). Maximum percent disease index was observed in water spray control confirms the protective role of this abiotic and biotic elicitor against Alternaria leaf blight disease. Elicitor–based strategy was used for efficiently management of fungal diseases under field conditions in the soybean crop (Chalfoun et al., 2018). Treatment *Pseudomonas fluorescens* @ 1% talc was at third position in respect of per cent disease index and percent disease control. Maximum per cent disease index was observed in water spray control i.e. in Katol 17.22, Amravati 20.77 and Yavatmal 24.32 (Table 2) confirms the protective role of abiotic and biotic elicitor used against Alternaria leaf blight disease. The efficacy of induced resistance under field conditions is variable in field applications (Reglinski and Walters, 2009).

Table 2: Effect of spray at 60 DAS on % disease index and disease control

| Sl. No. | Treatment | % Disease index recorded at 70 DAS pooled of two years | | | Average mean 70 DAS | % disease control |
|---|---|---|---|---|---|---|
| | | Amravati | Yavatmal | Katol | | |
| $T_1$ | Salicylic acid (SA) @ 100 ppm | 13.01 (3.61) | 17.59 (4.19) | 13.32 (3.65) | 14.64 | 29.51 |
| $T_2$ | Benzoic acid @ 100 ppm | 14.64 (3.83) | 18.97 (4.36) | 13.80 (3.71) | 15.80 | 23.93 |
| $T_3$ | Humic acid @ 1% | 14.95 (3.87) | 19.01 (4.36) | 12.57 (3.55) | 15.51 | 25.32 |
| $T_4$ | Jasmonic acid @ 100 ppm | 11.64 (3.41) | 15.07 (3.88) | 10.68 (3.27) | 12.46 | 40.01 |
| $T_5$ | Potassium nitrate ($KNO_3$) @ 1% | 16.57 (4.07) | 20.07 (4.48) | 13.77 (3.71) | 16.80 | 19.11 |
| $T_6$ | *Trichoderma viride* @ 1% talc | 13.52 (3.68) | 18.17 (4.26) | 12.99 (3.60) | 14.89 | 28.31 |
| $T_7$ | *Pseudomonas fluorescens* @ 1% talc | 12.93 (3.60) | 17.68 (4.20) | 12.11 (3.48) | 14.24 | 31.43 |
| $T_8$ | Propiconazole 0.1% | 10.39 (3.22) | 13.96 (3.74) | 10.09 (3.18) | 11.48 | 44.72 |
| $T_9$ | Control (Water spray) | 20.77 (4.56) | 24.32 (4.93) | 17.22 (4.15) | 20.77 | |

*Figures in parentheses are arc sine transformed value

*3.3. Seed yield*

The pooled data results of two foliar sprays of either abiotic, biotic elicitors or chemical fungicide on seed yield in soybean was shows that the treatment Propiconazole 0.1% foliar application found superior in respect of maximum yield 1279.7 kg $ha^{-1}$ over the rest of the other treatment. Two foliar sprays of Jasmonic acid @ 100 ppm resulted in 1186 kg $ha^{-1}$ yield and was at par with Propiconazole 0.1% (Table 3). Water-treated control plants produced significantly lower yield i.e. in Katol 923, Amravati 750 and Yavatmal 898 kg $ha^{-1}$ (Table 3) as compared to fungicide and elicitor foliar spray plants. Abiotic and biotic elicitor's foliar application was able to reduce per cent disease index of alternaria leaf spot compared to the untreated control which resulted in incresed yield in all treatment as compare to untreted control concluded the elicitor was able to induce the defense response in soybean plant. The role of Jasmonic acid (JA) in induction and improvement of the basal resistance against several fungal pathogens and acts as a potential activator of induced resistance against Karnal bunt in developing spikes

Table 3: Effect of spray at flowering and at 60 DAS on seed yield

| Sl. No. | Treatment | Seed yield (kg $ha^{-1}$) | | | Mean |
|---|---|---|---|---|---|
| | | Amravati | Yavatmal | Katol | |
| $T_1$ | Salicylic acid (SA) @ 100 ppm | 904 | 1089 | 1265 | 1086.0 |
| $T_2$ | Benzoic acid @ 100 ppm | 824 | 1052 | 1151 | 1009.0 |
| $T_3$ | Humic acid @ 1% | 812 | 1040 | 1142 | 998.0 |
| $T_4$ | Jasmonic acid @ 100 ppm | 1080 | 1160 | 1318 | 1186.0 |
| $T_5$ | Potassium nitrate ($KNO_3$) @ 1% | 858 | 1080 | 1253 | 1063.7 |
| $T_6$ | *Trichoderma viride* @ 1% talc | 846 | 1068 | 1167 | 1027.0 |
| $T_7$ | *Pseudomonas fluorescens* @ 1% talc | 926 | 1114 | 1290 | 1110.0 |
| $T_8$ | Propiconazole 0.1% | 1213 | 1296 | 1330 | 1279.7 |
| $T_9$ | Control (Water spray) | 750 | 898 | 923 | 857.0 |

*Figures in parentheses are arc sine transformed value

of wheat (Mandal et al., 2006). Soybean cultures treated with Jasmonic acid achieved the activation of enzymes encoding genes such as phenylalanine ammonium lyase, chalcone synthase and stilbene synthase, these proteins are involved in plant defense against pathogens (Belhadj et al., 2008). Jasmonate-induced protein was capable of reprogramming protein translation for increasing abiotic and biotic stress tolerance (Rustgi et al., 2014). Jasmonates are recognized to play important role in biotic stress responses against nectrotrophic pathogens and wounding (Santino et al., 2013).

## 4. CONCLUSION

Management of *Alternaria alternata,* by jasmonate mediated signalling pathway defence response prove to be most effective strategical approach. The two foliar sprays of Jasmonic acid @ 100 ppm at flowering and at 60 DAS resulted in suppression of leaf spot *Alternaria alternata* with respect to percent disease index, percent disease control and higher seed yield. The Application Jasmonic acid @ 100 ppm prove to be safer, non–toxic, environmentally friendly, and minimize the use of chemical fungicide for disease management.

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