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# Induction of defense related proteins in okra by the mycoparasitic fungi *Ampelomyces* against *Erysiphe cichoracearum*

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ABSTRACT

Powdery mildew caused by *Erysiphe cichoracearum* is a destructive disease worldwide. With increasing awareness of possible deleterious effects of fungicides on the ecosystem, biological control now appears to be a promising strategy for managing diseases in a range of crops. The most explored agents for biocontrol of powdery mildew in okra is the mycoparasite *Ampelomyces quisqualis*. In this study, three effective isolates of biocontrol agent *Ampelomyces*; TNAU-AQ101, TNAU-AQ103 and TNAU-AQ109 were evaluated for the induction of systemic resistance in okra against *Erysiphe cichoracearum* in comparison with fungicide Dinocap. The activity of defense enzymes *viz.,* peroxidase, polyphenol oxidase and β-1, 3-glucanase were found to be significantly higher in powdery mildew inoculated okra plants treated with talc formulation of TNAU-AQ101 at 5g $l^{-1}$ two sprays when compared to healthy and inoculated control. Reduction in the incidence of powdery mildew was positively linked to increase of defense enzymes activity in okra treated with talc formulation of TNAU-AQ101 at 5g $l^{-1}$ two sprays. The results reveal that application of *Ampelomyces* have a practical significance on the management of powdery mildew of okra.

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## INTRODUCTION

Okra or Bhendi [*Abelmoschus esculentus* (L.) Moench] is an important vegetable of the world's oldest cultivated crops and being produced in the tropical and sub-tropical low land regions of Asia, Africa, America and warmer parts of Mediterranean regions. Okra is affected by many diseases among which powdery mildew is the most damaging and severe one which continue to pose a serious threat to okra production by inflicting heavy losses (Bharat, 2013). The constraints associated with the use of fungicides and resistant cultivars have led to the search of alternative methods to control powdery mildews which include the use of biocontrol agents.

*Ampelomyces* is the most studied biocontrol agent of powdery mildews (Kiss, 1997). These were among the first mycoparasites to be used as biocontrol agents of powdery mildew fungi. *Ampelomyces* acts against

powdery mildews through mycoparasitism and it destroys the invaded powdery mildew colonies (Kiss, 2008). Penetration of the host cell wall by *A. quisqualis* is likely to involve both enzymatic and mechanical processes (Angeli *et al*., 2012). The biocontrol agents bring about Induced Systemic Resistance (ISR) through fortifying the physical and mechanical strength of cell wall as well as changing physiological and biochemical reaction of host leading to synthesis of defense chemicals against challenge inoculation of pathogens.

Keeping in mind these facts present study was done to assess the ISR mediated resistance in okra plants inoculated with the powdery mildew pathogen and treated with biocontrol agents.

## MATERIAL AND METHODS

**Assay of defense related enzymes:**

*Glass house studies*:

Efficacy of the biocontrol agent *Ampelomyces* spp. against powdery mildew pathogen was studied in okra plants artificially inoculated with powdery mildew under glass house conditions.

*Inoculation of powdery mildew conidia in pot culture experiments*:

Inoculation of the powdery mildew conidia was performed as described by Vimala (2005). Conidial suspension of powdery mildew was prepared by scrapping the conidia from the infected leaves of okra. The conidial suspension concentration was adjusted to 5 x$10^6$ $ml^{-1}$ by using a haemocytometer. The conidial suspensions were sprayed separately on healthy leaves of okra in glass house. The plants were then covered by polythene bag for 24 hours to maintain high humidity for disease development. The development of the disease was observed periodically upto 10 days.

**Sample collection :**

The leaf samples were collected at three days interval starting from zero to nine days after inoculation with powdery mildew pathogen and treated with *Ampelomyces* spp. A separate pathogen inoculated and un-inoculated control were also maintained.

**Enzyme extraction:**

One gram of leaf sample was homogenized with 2 ml of 0.1M sodium phosphate buffer 0.1 M (pH 7.0) at 4 $^0$C. The homogenate was centrifuged for 20 min at 10,000 rpm. Protein extracts prepared from okra tissues were used for the assay of peroxidase, polyphenol oxidase and β-1, 3-glucanase enzymes.

**Colorimetric assay :**

*Assay of peroxidase (PO)*:

Assay of PO activity was carried out as per the procedure described by Hammerschmidt *et al.* (1982). The reaction mixture consisted of 2.5 ml of a mixture containing 0.25 per cent ($vv^{-1}$) guaiacol in 0.01 M sodium phosphate buffer, pH 6.0 and 0.1 M hydrogen peroxide. Enzyme extract (0.1 ml) was added to initiate the reaction, which was followed observation calorimetrically at 470 nm. Crude enzyme preparations were diluted to give changes in absorbance at 470 nm of 0.1 to 0.2 absorbance units $min^{-1}$. The boiled enzyme was used as blank. Activity was expressed as the increase in absorbance at 470 nm $min^{-1}$ $mg^{-1}$ of sample.

*Assay of polyphenol oxidase (PPO)*:

The polyphenol oxidase activity was determined as per the procedure given by Mayer *et al.* (1966). The reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5) and 200 µl of the enzyme extract. To start the reaction, 200 µl of 0.01 M catechol was added and the activity was expressed as change in absorbance $min^{-1}mg^{-1}$ of sample.

*Assay of phenylalanine ammonia-lyase (PAL)*:

PAL activity was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nm. Sample containing 0.4 ml of enzyme extract was incubated with 0.5 ml of 0.1 M borate buffer, pH 8.8 and 0.5 ml of 12 m M L-phenylalanine in the same buffer for 30 min at 30 $^0$C. The amount of trans-cinnamic acid synthesized was calculated using its extinction co-efficient of 9630 $M^{-1}cm^{-1}$ (Dickerson *et al.*, 1984). Enzyme activity was expressed in fresh weight basis as n mol trans-cinnamic acid $min^{-1}$ $mg^{-1}$ of sample.

*Assay of* S*-1, 3-glucanase*:

One gram of okra leaf tissue was extracted in 5 ml of 0.05 M sodium acetate buffer (pH 5.0). The homogenate was centrifuged at 20,000 rpm for 10 min at 4 $^0$C and the supernatant was used as enzyme source. β-1,3-glucanase activity (E.C. 3.2.1.39) was assayed by

the laminarin-dinitrosalicylic acid method (Pan *et al*., 1991). The reaction mixture consisted of 62.5 µl of 4 per cent laminarin (Sigma) and 62.5 µl of enzyme extract. The reaction was carried out at $40^0$ C for 10 min. The reaction was stopped by adding 375 µl of dinitrosalicylic acid and heating for 5 min in boiling water, vortexed and its absorbance was measured at 500 nm. The enzyme activity was expressed as µg glucose released $min^{-1}$ $mg^{-1}$ of sample.

*Assay of chitinase*:

The colorimetric assay of chitinase was carried out as per Boller and Mauch (1988). Reagents used were colloidal chitin, snail gut enzyme, dimethyl amino benzaldehyde (DMAB) and buffer.

**Activity gel electrophoresis:**

*Peroxidase* (*PO*):

To study the expression pattern of different isoforms of peroxidases in different treatments, activity gel electrophoresis was carried out. For native anionic polyacrylamide gel electrophoresis, resolving gel of 8 per cent and stacking gel of 4 per cent were prepared. After electrophoresis, the gels were incubated in the solution containing 0.15 per cent benzidine in 6 per cent $NH_4Cl$ for 30 min in dark. Then drops of 30 per cent $H_2O_2$ were added with constant shaking till the bands appear. After staining, the gel was washed with distilled water and photographed (Sidhu *et al.,* 1984).

*Polyphenol oxidase* (*PPO*):

Enzyme was extracted by homogenizing one gram of leaf tissue in 0.01 M potassium phosphate buffer (pH 7.0). The homogenate was centrifuged at 20,000 rpm for 15 min at 4 $^0$C and the supernatant was used as enzyme source. After native electrophoresis, the gel was equilibrated for 30 min in 0.1 per cent p-phenylene diamine in 0.1 M potassium phosphate buffer (pH 7.0) followed by 10 mM catechol in the same buffer. The addition of catechol was followed by a gentle shaking which resulted in appearance of dark brown discrete bands (Jayaraman *et al.*, 1987).

## RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

**Induction of defense enzyme activity:**

The induced systemic resistance in okra plants inoculated with the powdery mildew pathogen and treated with the biocontrol agent *Ampelomyces* spp. was studied in glass house condition and the results were presented here under.

**Induction of peroxidase (PO) activity:**

The peroxidase activity was estimated from the leaves of okra plants inoculated with the pathogen and treated with talc formulation of *Ampelomyces* spp. The activity of peroxidase increased significantly upto five days in all the treatments and thereafter declined. The maximum peroxidase activity was observed in TNAU-AQ101 at 5g $l^{-1}$ two sprays with 0.783 changes in

**Table 1 : Induction of peroxidase activity in okra plants inoculated with pathogen and treated with biocontrol agents under glass house condition**

| Sr.No. | Treatments | PO activity (changes in absorbance $min^{-1}$ $g^{-1}$ of sample) at 470 nm | | | | |
|---|---|---|---|---|---|---|
| | | Days after inoculation | | | | |
| | | 1 | 3 | 5 | 7 | 9 |
| $T_1$ | TNAU-AQ101 at 5g $l^{-1}$ one spray | $0.525^c$ | $0.653^c$ | $0.751^c$ | $0.625^c$ | $0.530^c$ |
| $T_2$ | TNAU-AQ101 at 5g $l^{-1}$ two sprays | $0.589^a$ | $0.697^a$ | $0.783^a$ | $0.699^a$ | $0.572^a$ |
| $T_3$ | TNAU-AQ103 at 5g $l^{-1}$ one spray | $0.487^d$ | $0.515^d$ | $0.714^d$ | $0.612^d$ | $0.519^d$ |
| $T_4$ | TNAU-AQ103 at 5g $l^{-1}$ two sprays | $0.543^{bc}$ | $0.657^{bc}$ | $0.761^{bc}$ | $0.637^{bc}$ | $0.532^{bc}$ |
| $T_5$ | TNAU-AQ109 at 5g $l^{-1}$ one spray | $0.383^e$ | $0.467^f$ | $0.650^e$ | $0.535^e$ | $0.479^e$ |
| $T_6$ | TNAU-AQ109 at 5g $l^{-1}$ two sprays | $0.547^b$ | $0.677^b$ | $0.767^b$ | $0.645^b$ | $0.545^b$ |
| $T_7$ | Dinocap (0.1%) two sprays | $0.349^f$ | $0.476^e$ | $0.585^f$ | $0.521^f$ | $0.463^{ef}$ |
| $T_8$ | Inoculated control | $0.295^g$ | $0.325^g$ | $0.432^g$ | $0.557^g$ | $0.459^f$ |
| $T_9$ | Uninoculated control (Healthy) | $0.227^h$ | $0.235^h$ | $0.241^h$ | $0 .232^h$ | $0.229^g$ |

*Values are means of four replications

Data followed by the same letter in a column are not significantly different from each other according to Duncan's multiple range test at P=0.05

absorbance min$^{-1}$ g$^{-1}$ of fresh leaf sample. The activity of the enzyme increased upto 5$^{th}$ day and declined thereafter. The next best treatment was TNAU-AQ109 at 5g l$^{-1}$ two sprays with 0.767 changes in absorbance min$^{-1}$ g$^{-1}$ of fresh leaf sample followed by TNAU-AQ103 at 5g l$^{-1}$ two sprays with 0.761 changes in absorbance min$^{-1}$ g$^{-1}$ of fresh leaf sample. Healthy and pathogen inoculated control recorded lesser PO activity (Table 1).

The results obtained in this study were also similar to the earlier reports of many workers. Amer *et al.* (2015) observed that cucumber plants treated with *Ampelomyces* spp. (three times) showed increased peroxidase activity by 109 per cent compared with untreated control, while two times treated plants increased only by 101.5 per cent of untreated plants. Peroxidase activity reached its maximum increase at 6 hrs and continued until the end at 72 hrs with three time treated plants. He concluded that two and on time application of *Ampelomyces* spp. lead to an increase in peroxidase activity to its maximum after 24 and 3 hrs, respectively, when compared to control and declined, thereafter.

### Induction of poly phenol oxidase (PPO) activity:

The PPO activity also increased significantly upto five days in all the treatments and thereafter declined. The maximum PPO activity was observed in TNAU-AQ101 at 5g l$^{-1}$ two sprays with 1.113 changes in absorbance min$^{-1}$ g$^{-1}$ of fresh leaf sample. The activity of the enzyme increased upto 5$^{th}$ day and declined, thereafter. The next best treatment was TNAU-AQ109 at 5g l$^{-1}$ two sprays with 0.836 followed by TNAU-

**Table 2 : Induction of *Polyphenol oxidase* activity in okra plants inoculated with pathogen and treated with biocontrol agents under glass house condition**

| Sr. No. | Treatments | PPO activity (changes in absorbance min$^{-1}$ g$^{-1}$ of sample) at 495 nm | | | | |
|---|---|---|---|---|---|---|
| | | Days after inoculation | | | | |
| | | 1 | 3 | 5 | 7 | 9 |
| $T_1$ | TNAU-AQ101 at 5g l$^{-1}$ one spray | 0.632$^d$ | 0.719$^{cd}$ | 0.733$^{cd}$ | 0.665$^d$ | 0.545$^c$ |
| $T_2$ | TNAU-AQ101 at 5g l$^{-1}$ two sprays | 0.781$^a$ | 0.947$^a$ | 1.113$^a$ | 0.931$^a$ | 0.805$^a$ |
| $T_3$ | TNAU-AQ103 at 5g l$^{-1}$ one spray | 0.557$^e$ | 0.675$^d$ | 0.685$^d$ | 0.525$^e$ | 0.487$^d$ |
| $T_4$ | TNAU-AQ103 at 5g l$^{-1}$ two sprays | 0.673$^c$ | 0.738$^{bc}$ | 0.759$^c$ | 0.712$^c$ | 0.612$^{bc}$ |
| $T_5$ | TNAU-AQ109 at 5g l$^{-1}$ one spray | 0.378$^f$ | 0.472$^f$ | 0.514$^f$ | 0.437$^f$ | 0.409$^e$ |
| $T_6$ | TNAU-AQ109 at 5g l$^{-1}$ two sprays | 0.697$^b$ | 0.779$^b$ | 0.836$^b$ | 0.785$^b$ | 0.639$^b$ |
| $T_7$ | Dinocap (0.1%) two sprays | 0.305$^g$ | 0.483$^e$ | 0.595$^e$ | 0.454$^g$ | 0.311$^f$ |
| $T_8$ | Inoculated control | 0.221$^h$ | 0.253$^g$ | 0.375$^g$ | 0.243$^h$ | 0.217$^g$ |
| $T_9$ | Uninoculated control (Healthy) | 0.115$^i$ | 0.137$^h$ | 0.141$^h$ | 0.135$^i$ | 0.116$^h$ |

*Values are means of four replications
Data followed by the same letter in a column are not significantly different from each other according to Duncan's multiple range test at P=0.05

**Table 3: Induction of penylalanine ammonia lyase activity in okra plants inoculated with pathogen and treated with biocontrol agents under glass house condition**

| Sr. No. | Treatments | PAL activity (µg of cinnamic acid min$^{-1}$ g$^{-1}$of sample) at 290 nm | | | | |
|---|---|---|---|---|---|---|
| | | Days after inoculation | | | | |
| | | 1 | 3 | 5 | 7 | 9 |
| $T_1$ | TNAU-AQ101 at 5g l$^{-1}$ one spray | 0.712$^d$ | 0.753$^d$ | 0.795$^d$ | 0.752$^c$ | 0.647$^{bc}$ |
| $T_2$ | TNAU-AQ101 at 5g l$^{-1}$ two sprays | 0.785$^a$ | 0.891$^a$ | 0.975$^a$ | 0.946$^a$ | 0.860$^a$ |
| $T_3$ | TNAU-AQ103 at 5g l$^{-1}$ one spray | 0.627$^e$ | 0.706$^e$ | 0.725$^e$ | 0.687$^d$ | 0.531$^d$ |
| $T_4$ | TNAU-AQ103 at 5g l$^{-1}$ two sprays | 0.738$^c$ | 0.782$^c$ | 0.831$^c$ | 0.765$^{bc}$ | 0.633$^c$ |
| $T_5$ | TNAU-AQ109 at 5g l$^{-1}$ one spray | 0.531$^f$ | 0.637$^f$ | 0.647$^g$ | 0.595$^e$ | 0.493$^e$ |
| $T_6$ | TNAU-AQ109 at 5g l$^{-1}$ two sprays | 0.757$^b$ | 0.835$^b$ | 0.862$^b$ | 0.782$^b$ | 0.675$^b$ |
| $T_7$ | Dinocap (0.1%) two sprays | 0.434$^g$ | 0.521$^g$ | 0.687$^f$ | 0.521$^f$ | 0.472$^f$ |
| $T_8$ | Inoculated control | 0.379$^h$ | 0.316$^i$ | 0.528$^h$ | 0.492$^g$ | 0.386$^g$ |
| $T_9$ | Uninoculated control (Healthy) | 0.286$^i$ | 0.327$^h$ | 0.341$^i$ | 0.317$^h$ | 0.292$^h$ |

*Values are means of four replications.
Data followed by the same letter in a column are not significantly different from each other according to Duncan's multiple range test at P=0.05

AQ103 at 5g $l^{-1}$ two sprays with 0.759 changes in absorbance $min^{-1}$ $g^{-1}$ of fresh leaf sample. Healthy and pathogen inoculated control recorded lesser PPO activity (Table 2).

Renuka (2003) reported that the PPO activity was increased by the application of biocontrol agents, plant products and challenge inoculated with *A. chlamydospora* infecting chrysanthemum.

**Induction of phenylalanine ammonia lyase (PAL) activity:**

Like PO and PPO, PAL activity increased significantly upto five days in all the treatments and thereafter declined. The maximum PAL activity was observed in TNAU-AQ101 at 5g $l^{-1}$ two sprays with 0.975 µg of cinnamic acid $min^{-1}$ $g^{-1}$ of fresh leaf sample. The activity of the enzyme increased upto 5th day and declined, thereafter. The next best treatment was TNAU-AQ109 at 5g $l^{-1}$ two sprays with 0.862 µg of cinnamic acid $min^{-1}$ $g^{-1}$ of fresh leaf sample followed by TNAU-AQ103 at 5g $l^{-1}$ two sprays with 0.831 µg of cinnamic acid $min^{-1}$ $g^{-1}$ of fresh leaf sample. Healthy and pathogen inoculated control recorded lesser PAL activity (Table 3).

Umamaheswari *et al.* (2009) reported that watermelon plants pre-treated with bio-agents showed enhanced PAL, PO, PPO, β- 1, 3 glucanase activities upon challenge inoculation with *Alternaria alternata.* Lubaina and Murugan (2015) reported that PAL activity was significantly increased in *Trichoderma* pre-treated

**Table 4 : Induction of -1,3 glucanase activity in okra plants inoculated with pathogen and treated with biocontrol agents under glass house condition**

| Sr. No. | Treatments | Glucanase activity (µg of glucose consumed $min^{-1}$ $g^{-1}$ of sample) at 500 nm | | | | |
|---|---|---|---|---|---|---|
| | | Days after inoculation | | | | |
| | | 1 | 3 | 5 | 7 | 9 |
| $T_1$ | TNAU-AQ101 at 5g $l^{-1}$ one spray | $0.532^{d}$ | $0.635^{c}$ | $0.683^{e}$ | $0.574^{f}$ | $0.487^{f}$ |
| $T_2$ | TNAU-AQ101 at 5g $l^{-1}$ two sprays | $0.647^{a}$ | $0.778^{a}$ | $0.815^{a}$ | $0.795^{a}$ | $0.611^{a}$ |
| $T_3$ | TNAU-AQ103 at 5g $l^{-1}$ one spray | $0.547^{cd}$ | $0.653^{bc}$ | $0.702^{de}$ | $0.599^{e}$ | $0.510^{e}$ |
| $T_4$ | TNAU-AQ103 at 5g $l^{-1}$ two sprays | $0.579^{bc}$ | $0.665^{bc}$ | $0.735^{c}$ | $0.648^{c}$ | $0.565^{c}$ |
| $T_5$ | TNAU-AQ109 at 5g $l^{-1}$ one spray | $0.559^{c}$ | $0.613^{cd}$ | $0.709^{cd}$ | $0.639^{cd}$ | $0.529^{de}$ |
| $T_6$ | TNAU-AQ109 at 5g $l^{-1}$ two sprays | $0.587^{b}$ | $0.681^{b}$ | $0.754^{b}$ | $0.685^{b}$ | $0.597^{b}$ |
| $T_7$ | Dinocap (0.1%) two sprays | $0.527^{e}$ | $0.597^{d}$ | $0.627^{f}$ | $0.614^{d}$ | $0.532^{d}$ |
| $T_8$ | Inoculated control | $0.257^{f}$ | $0.269^{e}$ | $0.451^{g}$ | $0.357^{g}$ | $0.322^{g}$ |
| $T_9$ | Uninoculated control (Healthy) | $0.154^{g}$ | $0.172^{f}$ | $0.191^{h}$ | $0.169^{h}$ | $0.125^{h}$ |

*Values are means of four replications.
Data followed by the same letter in a column are not significantly different from each other according to Duncan's multiple range test at P=0.05

**Table 5 : Induction of chitinase activity in okra plants inoculated with pathogen and treated with biocontrol agents under glass house condition**

| Sr. No. | Treatments | Chitinase activity (µg of glucose consumed $min^{-1}$ $g^{-1}$ of sample) at 585 nm | | | | |
|---|---|---|---|---|---|---|
| | | Days after inoculation | | | | |
| | | 1 | 3 | 5 | 7 | 9 |
| $T_1$ | TNAU-AQ101 at 5g $l^{-1}$ one spray | $0.651^{d}$ | $0.771^{bc}$ | $0.817^{cd}$ | $0.729^{cd}$ | $0.627^{d}$ |
| $T_2$ | TNAU-AQ101 at 5g $l^{-1}$ two sprays | $0.787^{a}$ | $0.852^{a}$ | $0.985^{a}$ | $0.936^{a}$ | $0.911^{a}$ |
| $T_3$ | TNAU-AQ103 at 5g $l^{-1}$ one spray | $0.587^{e}$ | $0.721^{c}$ | $0.783^{d}$ | $0.721^{d}$ | $0.597^{e}$ |
| $T_4$ | TNAU-AQ103 at 5g $l^{-1}$ two sprays | $0.685^{c}$ | $0.797^{ab}$ | $0.825^{c}$ | $0.745^{c}$ | $0.683^{c}$ |
| $T_5$ | TNAU-AQ109 at 5g $l^{-1}$ one spray | $0.467^{f}$ | $0.549^{d}$ | $0.632^{e}$ | $0.571^{e}$ | $0.447^{f}$ |
| $T_6$ | TNAU-AQ109 at 5g $l^{-1}$ two sprays | $0.719^{b}$ | $0.823^{ab}$ | $0.857^{b}$ | $0.811^{b}$ | $0.721^{b}$ |
| $T_7$ | Dinocap (0.1%) two sprays | $0.315^{g}$ | $0.423^{e}$ | $0.617^{ef}$ | $0.537^{f}$ | $0.424^{g}$ |
| $T_8$ | Inoculated control | $0.247^{h}$ | $0.355^{ef}$ | $0.427^{f}$ | $0.531^{g}$ | $0.611^{h}$ |
| $T_9$ | Uninoculated control (Healthy) | $0.212^{i}$ | $0.213^{f}$ | $0.215^{g}$ | $0.191^{h}$ | $0.177^{i}$ |

*Values are means of four replications.
Data followed by the same letter in a column are not significantly different from each other according to Duncan's multiple range test at P=0.05

plants followed by inoculation with *Alternaria sesame.* Bordbar *et al.* (2010) reported a time dependent induction of activities of PAL in apple upon treatment with biocontrol agent *Trichoderma virens*.

**Induction of ß-1, 3-glucanase activity:**

β-1, 3-glucanase activity also increased significantly upto five days in all the treatments and thereafter, declined. The maximum β-1,3-glucanase activity was observed in TNAU-AQ101 at 5g $l^{-1}$ two sprays with 0.815 µg of glucose consumed $min^{-1}$ $g^{-1}$ of fresh leaf sample. The activity of the enzyme increased upto $5^{th}$ day and declined, thereafter. The next best treatment was TNAU-AQ109 at 5 g $l^{-1}$ two sprays with 0.754 µg of glucose consumed $min^{-1}$ $g^{-1}$ of fresh leaf sample followed by TNAU-AQ103 at 5 g $l^{-1}$ two sprays with 0.735 µg of glucose consumed $min^{-1}$ $g^{-1}$ of fresh leaf sample. β-1, 3-glucanase activity remained at a lower level in healthy control leaves (Table 4).

The results are in agreement with the findings of several workers. Amer *et al.* (2015) observed that maximum activity of β-1, 3-glucanase was found in cucumber plants treated with *Ampelomyces* spp. (three times) at 6 hrs and enzyme activity declined, thereafter. Rotem *et al.* (1999) demonstrated that exo-β-1,3 glucanase is excreted both in culture and during mycoparasitism of *Ampelomyces* spp.

**Induction of chitinase activity:**

Chitinase activity increased significantly upto five days in all the treatments and thereafter declined. The maximum chitinase activity was observed in TNAU-AQ101 at 5g $l^{-1}$ two sprays with 0.985 µg of glucose consumed $min^{-1}$ $g^{-1}$ of fresh leaf sample. The activity of the enzyme increased upto $5^{th}$ day and declined thereafter. The next best treatment was TNAU-AQ109 at 5g $l^{-1}$ two sprays with 0.857 µg of glucose consumed $min^{-1}$ $g^{-1}$ of fresh leaf sample followed by TNAU-AQ103 at 5g $l^{-1}$ two sprays with 0.825 µg of glucose consumed $min^{-1}$ $g^{-1}$ of fresh leaf sample. Healthy and pathogen inoculated control recorded lesser chitinase activity (Table 5).

Rotem *et al.* (1999) observed that AQ10 displayed good protease and chitobiase activity. Xue *et al.* (1998) reported that non-pathogenic treatment of binucleate *Rhizoctonia* (BNR) treatment elicited a significant and systemic increase in all cellular fractions of chitinase compared to the diseased and control bean plants.

**Native gel electrophoresis:**

*Peroxidase* (*PO*):

Native PAGE analysis revealed the induction of two isoforms *viz.,* PO1 and PO2 in okra plants inoculated with pathogen and treated with talc formulation of TNAU-AQ101 at 5g $l^{-1}$two sprays. The other treatments showed the isoforms with less intensity. This may be due to an increase in PR- proteins depend on SA pathway.

*Polyphenol oxidase* (*PPO*):

Two isoforms of polyphenol oxidase *viz.,* PPO1 and PPO2 were observed in okra plants inoculated with pathogen and treated with talc formulation of TNAU-AQ101 at 5g $l^{-1}$ two sprays. The other treatments showed the isoforms with less intensity.

**Conclusion:**

The activity of defense enzymes were found to be significantly higher in okra plants inoculated with pathogen and treated with talc formulation of TNAU-AQ101 at 5g $l^{-1}$ two sprays when compared to healthy and inoculated control. Reduction in the incidence of powdery mildew was positively linked to increase of defense enzymes activity in okra treated with talc formulation of TNAU-AQ101. The results reveal that application of *Ampelomyces* have a practical significance on the management of powdery mildew of okra.

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## REFERENCES

**Amer, M.A., El-Abd, S., Deraz., S.F and Zaid, N.A. (2015).** Systemic acquired resistance induced by some biotic agents against downy mildew of cucumber disease. International Conference on Plant, Marine and Environmental Sciences,

Kuala Lumpur (Malaysia).

**Angeli, D., Puopolo, G., Maurhofer, M., Gessler, C. and Pertot, I. (2012).** Is the mycoparasitic activity of *Ampelomyces quisqualis* biocontrol strains related to phylogeny and hydrolytic enzyme production? *Biol. Control*, **63**: 348-358.

**Bharat, N. K. (2013).** Study on the occurrence of perfect stage of okra powdery mildew in Himachal Pradesh. *Internat. J. Farm Sci*., **3**: 52-55.

**Boller, T. and Mauch, F. (1988).** Colorimetric assay for chitinase. *Methods Enzymol*., **161** : 430-435.

**Bordbar, F., Etebarian, H., Sahebani, N and Rohani, H. (2010).** Control of postharvest decay of apple fruit with *Trichoderma virens* isolates and induction of defense responses. *J. Plant Prot. Res.,* **50**: 146-152.

**Dickerson, D., Pascholati, S., Hagerman, A. E., Butler, L. and Nicholson, R. (1984).** Phenylalanine ammonia-lyase and hydroxycinnamate: CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium carbonum. Physiol Plant Pathol*., **25**: 111-123.

**Hammerschmidt, R., Nuckles, E. and Kuc, J. (1982).** Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiol Plant Pathol.,* **20**: 73-82.

**Jayaraman, K., Ramanuja, M., Vijayaraghavan, P. and Vaidyanathan, C. (1987).** Studies on the purification of banana polyphenoloxidase. *Food Chem*., **24** : 203-217.

**Kiss, L. (1997).** Genetic diversity in *Ampelomyces* isolates, hyperparasites of powdery mildew fungi, inferred from RFLP analysis of the rDNA ITS region. *Mycol Res*., **101**: 1073-1080.

**Kiss, L. (2008).** Intracellular mycoparasites in action: interactions between powdery mildew fungi and *Ampelomyces*. British Mycological Society Symposia Series. Elsevier, pp. 37-52.

**Lubaina, A. and Murugan, K. (2015).** Evaluation of biological efficacy of *Trichoderma* species isolates against *Alternaria* leaf spot disease of sesame. *Internat. J. Appl. Biol. Pharm*., **6**: 17-24.

**Mayer, A., Harel, E. and Ben-Shaul, R. (1966).** Assay of catechol oxidase—a critical comparison of methods. *Phytochemistry*, **5**: 783-789.

**Pan, S. Q., Ye, X. S. and Kuc, J. (1991).** A technique for detection of chitinase, β-1, 3-glucanase and protein patterns after a single separation using polyacrylamide gel electrophoresis or isoelectrofocusing. *Phytopathology*, **81**: 970-974.

**Renuka, R. (2003).** Studies on the leaf blight disease on chrysanthemum caused by *Alternaria chlamydospora*. M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, T.N. (India). pp.89.

**Rotem, Y., Yarden, O. and Sztejnberg, A. (1999).** The mycoparasite *Ampelomyces quisqualis* expresses exgA encoding an exo-β-1, 3-glucanase in culture and during mycoparasitism. *Phytopathology*, **89**: 631-638.

**Sidhu, J. S., Ravi, J.S. and Minochal, J. L. (1984).** Peroxidase isozyme patterns in primary trisomics of pearl millet. *Theor. Appl. Genet*., **68**: 179-182.

**Umamaheswari, C., Sankaralingam, A. and Nallathambi, P. (2009).** Induced systemic resistence in watermelon by biocontrol agents against *Alternaria alternata*. *Arch. Phytopathol. Plant Protect.,* **42**: 1187-1195.

**Vimala, R. (2005).** Studies on powdery mildew of bhendi caused by *Erysiphe cichoracearum* (DC) Ph.D. Thesis, Tamil Nadu Agricultural University, Madurai, T. N. (India), pp. 120.

**Xue, L., Charest, P. and Jabaji-Hare, S. (1998).** Systemic induction of peroxidases, 1, 3 -β-glucanases, chitinases and resistance in bean plants by binucleate *Rhizoctonia* species. *Phytopathology,* **88**: 359-365.

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