



## INDUCTION OF SYSTEMIC RESISTANCE BY BIOTIC AND ABIOTIC COMPOUNDS AGAINST *ALTERNARIA BLIGHT* OF CAULIFLOWER CAUSED BY *ALTERNARIA BRASSICAE*

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### Abstract

*Alternaria blight* of cauliflower is initiated by *Alternaria brassicae*, is considered as one of the significant diseases caused in cauliflower crop cultivated in all the growing areas of the world. *Alternaria blight* disease severity in cauliflower occurs after initiation of curd formation and continues till seed pods are set. Several opportunities include improvement of resistant cultivars; crop rotation, biological control, chemical pesticides, and tillage are used to control this particular disease. By using chemical pesticide mostly diseases can be controlled. In present investigation the three abiotic (salicylic acid, oxalic acid, benzothiadiazole) and two biotic compound *Trichoderma harzianum*, and *T.viride* lab formulations were used to control this disease. Treatments were evaluated for their ability to reduce illness and prevent the production of conidia, a hallmark of pathogen proliferation. However, the disease control was maximum in variety Doctor II and under post inoculation technique. The maximum disease control was observed with the treatment of Bion (93.86%) followed by salicylic Acid (90.93%) and oxalic acid (89.06%) @ 50 mg.L concentration. The biotic compound *T. harzianum* (4g/Kg seeds) also showed effective disease control (87.2%) followed by *T.viride* (84.53%). Although, the disease initiation in variety SV40551AC was two days later and the infection index was lower in Doctor II due to its tolerance nature.

**Keywords:** *Alternaria brassicae*, cauliflower, *Alternaria blight*, entry routes, pathogen.

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### Introduction

Cauliflower is the fifth most favorite vegetable crop in India after tomato, brinjal, cabbage and onion; occupies an important place among the fresh vegetables in the world vegetables (Deep and Sharma., 2012). Plants are challenged by a variety of biotic stresses like bacterial, fungal or viral infections. They cause serious loss of crop productivity. There are various options available for the farmers to protect their crop from diseases. Several opportunities include improvement of resistant cultivars; crop rotation, biological control, chemical pesticides, and tillage were used against this disease. By using chemical pesticide mostly diseases can be controlled in which *Alternaria blight* is one of the most important (Abdel et al., 2012). *Alternaria blight* disease severity in cauliflower occurs after initiation of curd formation and continues till seed pods are set. After infection, it is difficult to control the disease by cultural or chemical methods. In cauliflower, symptoms appear after mid-season in the generative phase. It has been seen that the primary symptoms of disease were appeared as some wet soft lesions seen on the cauliflower curd; then these lesions enlarges into somewhat watery rotten mass of tissues and they are covered by a dark appearances (Hossain & Hossain (2010).

Management of *Alternaria blight* is very difficult and requires frequent fungicidal spray. Long term use of the organic pesticides brings harmful impact on people health and it is supposed to be the most important cause to create deleterious effect on environment. One of the most effective methods of fighting plant diseases is to stimulate systemic resistance in plants. Initiation of Systemic Acquired Resistance against disease causing organism is seems to be the important way to control the disease severity under framework of I.P.M. (Prasannath and De Costa, 2015). The present work aims to investigate the induced plant defense system in cauliflower towards *Alternaria brassicae* by the application of abiotic and biotic chemicals.

### Material and Methods

This study was conducted in a laboratory setting to observe the effects of *A.brassicae* on *Brassica oleracea* L. var. botrytis L sub var. cauliflower D.C. in its natural, unaltered state. The lab formulations utilized in the investigation included the biotic compounds *Trichoderma harzianum* and *T.viride* and the abiotic compounds Bion: salicylic acid, oxalic acid, and benzothiadiazole.

Seed priming with biotic and abiotic compounds: For 24 hours, a suspension of (25 and 50mg l<sup>-1</sup>) made composed of sterilized distilled water was used to ingest cauliflower seeds

infused with Bion®, salicylic acid, and oxalic acid. Seeds were soaked in water for 24 hours before being air dried on filter paper at room temperature. The standard seed dressing approach was used to treat seeds with a *T. harzianum* and *T. viride* formulation (@2 and 4g kg<sup>-1</sup> seeds). For purposes of comparison, the seeds soaked in distilled water served as the control group. Throughout the study, SV40551AC and Doctor II cauliflower were used independently.

**Effect of *T. harzianum*, *T. viride*, Bion, salicylic acid, oxalic acid against *Alternaria blight* disease under greenhouse conditions:-** Two days in a row, 1 hour of autoclaving was applied to potting media composed of soil, sand, and farm yard manure (1:1:1 w/w/w). The sorghum grain-based potting medium, in which the virulent *A.brassicae* mass had proliferated, was combined with the sterilized potting medium at a weight-to-weight ratio of 19:1. Containers of 15 cm in diameter and 30 cm in height were filled with soil. Cauliflower seeds (at a rate of 25 per container) were planted densely. The seed was subjected to the earlier indicated method of comparison. Up to 35 days post-pathogen inoculation, plants were frequently watered and the incidence of damping off disease (the proportion of affected plants) was recorded. Conidia production during pathogen development was analyzed for both kinds and inoculation methods. To determine how many conidia were produced by each plant, they were uprooted at regular intervals for up to 40 days following inoculation. Five plants from each pot were chosen at random, and their growth was recorded. Each replication had four containers kept in it. A randomized block design with three replicates was used to set up the experiment. At 15 days post-sowing, we measured the prevalence of illness, the depth of the roots, and the height of the plants.

**Induction of defense mechanisms and challenge inoculation:-** For this experiment, we employed *T. harzianum*, *T. viride*, Bion®, salicylic acid, and oxalic acid to see how well they induced defense reactions in cauliflower. The experimental group had the following procedures: Seeds were either: (1) treated with biotic and abiotic compounds, then challenged with *A.brassicae* (50 g sorghum grain-medium containing 103 cfu g<sup>1</sup> medium in each pot as soil inoculation); or (2) treated with biotic and abiotic compounds, then challenged with the pathogen via the tooth prick method. (3) Untreated plants that were subjected to a pathogen challenge via soil inoculation or a tooth prick. Three replications were maintained in each treatment; each comprised of five pots. The studies were conducted using randomized block design in a greenhouse. The relative humidity (RH) in the greenhouse was kept at roughly 80%. The new day/night temperature setting is 26 degrees Celsius.

Root tissues were not damaged when plants were uprooted at 0, 1, 2, 3, 4, 5, 7, 10, and 15 days after pathogen inoculation. For the purpose of biochemical analysis, four plants were taken from each treatment replication (treatments were maintained in the experimental design) and kept in isolation. The roots were rinsed under running water and then homogenized in a mill and pestle that had been chilled with liquid nitrogen. At -70 degrees Celsius, we kept the homogenized root tissues.

**Estimation of peroxidase (PO) activity:-** One gram of roots was homogenized in 2 milliliters of a phosphate buffer solution at pH 7.0 and 4 degrees Celsius. After centrifuging

the homogenate at 16,000 g for 15 minutes at 4 degrees Celsius, the resulting supernatant was employed as a source of enzymes. The reaction mixture was kept in an incubator at 22.80 degrees Celsius. Absorbance shifts at 420 nm were monitored every 30 seconds for three minutes. Absorbance variations per mg of protein per minute were used to quantify enzyme activity.

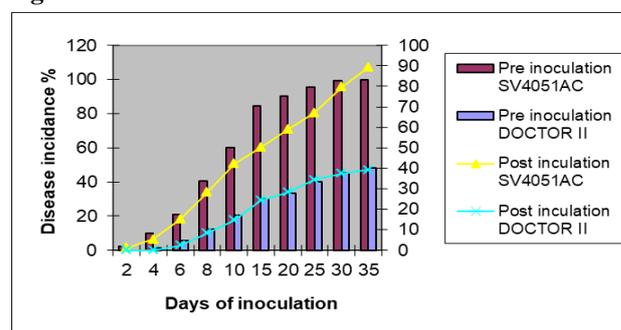
**Estimation of phenylalanine ammonia lyase (PAL) activity:-** Three milliliters of a cold 0.1 M sodium borate buffer, pH 7.0 containing 1.4 mM of 2-mercaptoethanol and 0.1 g of insoluble polyvinyl pyrrolidone were used to homogenize one gram of root samples. After centrifuging the extract at 16,000g for 15 minutes, it was filtered through cheesecloth. The enzymes came from the supernatant. The amount of PAL activity was measured by observing the amount of trans-cinnamic acid produced from L-phenylalanine at a wavelength of 290 nm. Enzyme extract samples (0.4 ml) were treated with 0.1 M borate buffer, pH 8.8 (0.5 ml) and 12 mM L-phenylalanine (0.5 ml) at 30°C for 30 minutes. The production rate of trans-cinnamic acid was determined. The enzyme activity was calculated as the number of moles of trans-cinnamic acid hydrolyzed per minute per 100 milligrams of protein.

**Estimation of poly-phenol oxidase (PPO) activity:-** Homogenized root samples (1g) were centrifuged at 16,000g for 15 minutes at 4°C after being immersed in 2 cc of 0.1 M sodium phosphate buffer (pH 6.5). The enzymes came from the supernatant. 200  $\mu$ l of the enzyme extract and 1.5 ml of a 0.1 M sodium phosphate buffer (pH 6.5) made up the reaction mixture. To initiate the reaction, 200  $\mu$ l of 0.01 mM catechol was added to the reaction mixture containing 200  $\mu$ l of the enzyme extract and 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5) (Mayer et al., 1965). The activity was measured by measuring the rate of change in absorbance at 495 nm per milligram of protein. General statistical methods were used for evaluating the statistical significance of the results.

## Results and Discussion

Effect of biotic and abiotic components on disease control: Application of abiotic compound Bion, salicylic acid and oxalic acid and biotic compound *Trichoderma harzianum* and *T.viride* significantly reduced the infection index severity in both the varieties and inoculation techniques (Kumar., 2015). The application of Bion compound as seed treatment and foliar spray resulted in highest disease reduction in both the varieties and in pre and post inoculation artificial epiphytotic conditions as compared to other treatments and control as evident in figure 1.

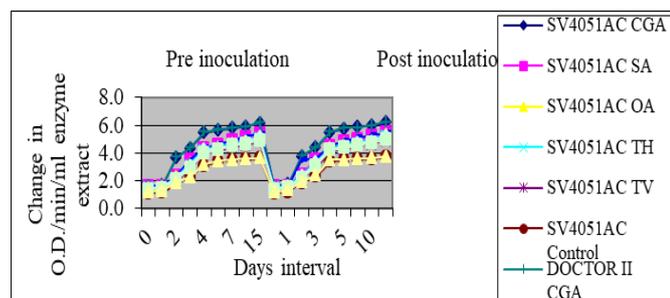
**Figure 1: Effect of seed priming with chemicals and bioagents on disease control**



### Effect of abiotic and biotic compound treatment on Peroxidase (PO) activity.

Perusal of data presented in Figure 2 depicting that peroxidase activity in both the variety SV4051AC and Doctor II and pathogen treated control was found increased. Although the peroxidase activity was remain increased up to 15th days after inoculation but the increase in peroxidase activity was increased in drastic increasing order up to 5th days after inoculation, there after it increased in decreasing order. Kumari and Vengadaramana (2017). The highest increase in peroxidase activity in both the varieties was in the treatment Bion followed by salicylic acid, oxalic acid and *T.harzianum* treatment as compared to control treatment.

**Figure 2: Influence of seed priming with chemicals and bioagents on (PO) activity**

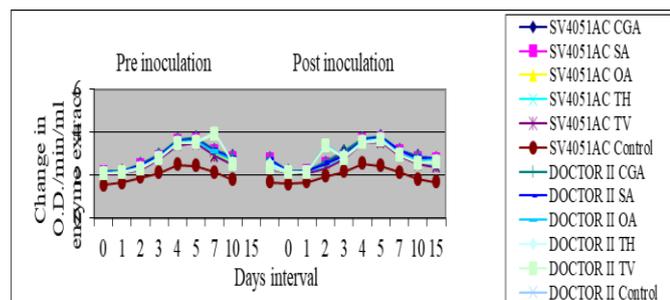


### Effect of abiotic and biotic compounds on PAL activity:-

The data presented in Figure 3 clearly indicated that the rate of trans-cinnamic acid min-1 100 mg protein-1 was increased in all treatments and untreated control challenge inoculated with pathogen upto 15 days after inoculation.

Although, the highest increase in phenylalanine ammonia lyase activity was observed in Bion treatment which was increased with high rate upto 5th days after inoculation. Thereafter the activity was increased with decreasing order. The PAL activity in Bion treatment followed by salicylic acid, oxalic acid and *T. harzianum* (Kumar., 2015). Among the variety treated with above mentioned treatment the significant higher PAL activity was found is variety Doctor II. However, 5th days after inoculation both the varieties were statistically at par in PAL activities with respect of abiotic and biotic treatment and inoculation method.

**Figure 3: Effect of seed priming with chemicals and bioagents on PAL activity**

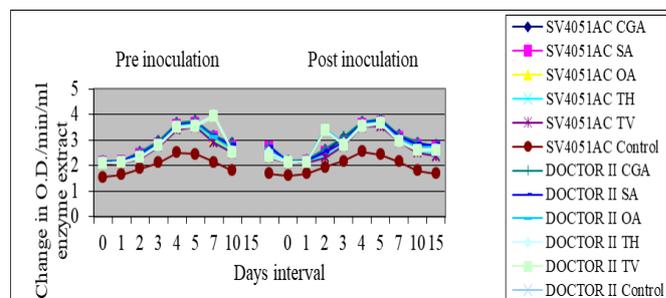


### Effect of biotic and abiotic compound on PolyPhenole Oxidase (PPO) activity:-

The PPO activity in both the varieties SV4051AC and Doctor II with abiotic and biotic compounds treatment and challenge inoculated with *A. brassicae* was found increased up to 5th days after inoculation thereafter, it decreased up to the end of experiment (15th days after inoculation). The highest *J. Sci. Innov. Nat. Earth*

increase in PPO activity among abiotic elicitors was in Bion treatment followed by salicylic acid and oxalic acid (Mahendranathan et al., 2016). Although the difference in PPO activity by abiotic elicitors was statistically at par. *T.harzianum* and *T.viride* treatments were also increased PPO activity in both the varieties up to 5th days after pathogen inoculation control as in same manner as in abiotic elicitor but in control the PPO activity was also increased upto 4th days after inoculated but the rate of increase was very much less and it decreased drastically up to end of observation i.e. 15th days after inoculation and under both the inoculation methods as observed in Figure 4.

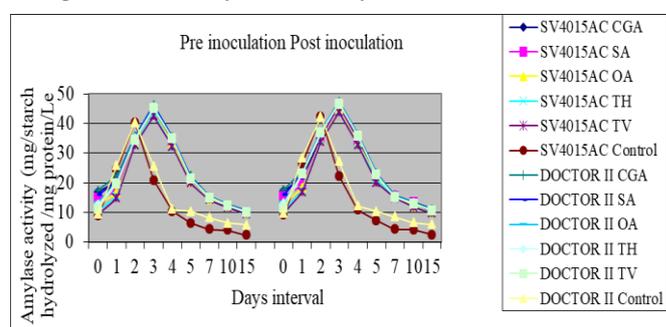
**Figure 4: Effect of seed priming with chemicals and bioagents on PPO activity**



### Effect of biotic and abiotic compounds on α-amylase activity:-

As the data mentioned in Figure 5, the α-amylase activity was found increased in all the treated cauliflower varieties at every days after inoculation. However, the significant increase in amylase activity was upto 3rd days after inoculation in each treatment, thereafter, it decreased drastically and on the 15th days after inoculation it was reached up to 40% of initial rate. The maximum enzyme α-amylase activity was showed by the treatment Bion in variety Doctor II oxalic acid, *T.harzianum* and *T.viride*. (Prasannath et al., 2014)

**Figure 5: Effect of seed priming with chemicals and bioagents on α-amylase activity**

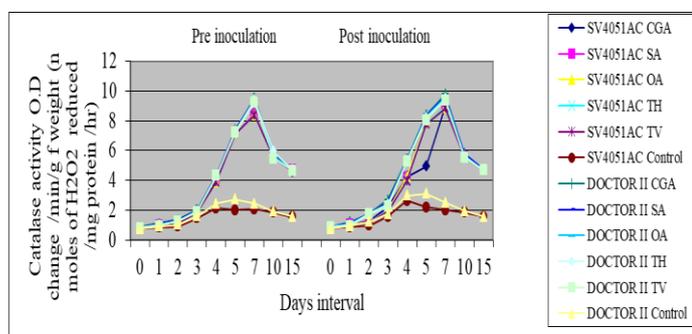


### Effect of biotic and abiotic compounds treatment on catalase activity:-

Fig 6 clearly showed that the peroxidase activity in terms of depletion of  $H_2O_2$  was increased in all the abiotic and biotic treated cauliflower varieties under pre and post inoculation condition. The enzyme activity was found maximum in Bion treated Doctor II variety under post inoculation condition followed by salicylic acid, oxalic acid, *T.harzianum* and *T.viride* treatment as compared to pathogen inoculated control treatment

(Kumari and Vengadaramana., 2017). The increase in enzyme activity was up to 7<sup>th</sup> days after inoculation in all the treatment and in control where it's was maximum level with respect to each treatment thereafter it decrease drastically up to 15<sup>th</sup> days after inoculation.

**Figure 6: Effect of seed priming with chemicals and bioagents on catalase activity**



### Conclusion

It was noticed that each treatment was proven to be successful in lowering infection and inhibiting pathogenic growth in the form of conidial generation inhibition. However, the disease control was maximum in variety Doctor II and under post inoculation technique. The maximum disease control was observed with the treatment of bion (93.86%) followed by salicylic Acid (90.93%) and oxalic acid (89.06%) @ 50 mg.L concentration. The biotic compound *T. harzianum* (4g/Kg seeds) also showed effective disease control (87.2%) followed by *T. viride* (84.53%). Although, the disease initiation in variety SV4051AC was two days later and the infection index was lower in Doctor II due to its tolerance nature.

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