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Study of the Defence response in a cauliflower against *Alternaria brassicae* by using Biotic and Abiotic compounds

Bharat Bhooshan and Devesh Kumar

Department of Botany, R. B. S. College, Agra - 282002 (India)
(Dr. Bhimrao Ambedkar University, Agra - 282004 (India)

Abstract

Alternaria blight of cauliflower initiated by *Alternaria brassicae*, is considered as one of the important 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 (Bion®: benzothiadiazole, salicylic acid, oxalic acid and two biotic compound *T. viride and Trichoderma harzianum*, lab formulations were used to control this disease. It was observed that the all the treatments were found to be effective in reducing disease and inhibiting pathogen growth in the form of conidial formation inhibition. However, the disease control were 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 Aacid (90.93%) and oxalic acid (89.06%) @ 50 mg.L concentration. The biotic compound *T. harziaznum* (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.

Key words : Defence response, Cauliflower, *Alternaria brassicae,* Biotic & abiotic compounds.

Cauliflower (Brassica oleracea L. var. botrytis) the most significant vegetable crop grown in both temperate and tropical regions. It is the most popular crop among the several cole vegetables, including broccoli, cabbage, Chinese cabbage, and kohlrabi. After tomato, brinjal, cabbage, and onion, cauliflower is the fifth favorite vegetable crop in India. It holds a significant place among the world's clean vegetables. In India, cauliflower is grown on massive acreage and the most important productive states of India are Punjab,

Rajasthan, Sikkim, Tamil Nadu, Uttar Pradesh, Uttaranchal and West Bengal.

The cauliflower curd carries about ninety percentage water subsequently it has the tendency to lose humidity in the system of evaporation or transpiration. Water loss in cauliflower curd is excessive due to the fact it is now not blanketed via a tough and waxy pores and skin[9]. High temperatures of the backyard surroundings mixed with low relative humidity (RH) amplify the price of water loss from the curd ensuing in loss of crispiness and therefore browning. It is usually used as cooked vegetable combined with peas, potato and carrot. Its uncooked shape additionally blended with inexperienced salad and portions are plunged into sauces. It is additionally used in the composition of pickle or combined pickle with in addition vegetables. Cauliflower with phytonutrients and antioxidants, offers a herbal detox machine to our body. This crop additionally exhibit the presence of anti-inflammatory effects, digestive and cardiovascular support. Chemically it is viewed that one hundred mg of suitable for eating component of cauliflower curd includes 90.8 percentage water with protein 2.6g, 0.4g fat, 1.9g minerals, 4.0g carbohydrate, 33 mg calcium, 20 mg magnesium, 19 mg oxalic acid, fifty seven mg phosphorus, 1.5mg iron and 0.6mg niacin[19].

The curd in cauliflower is the safe to eat phase which is used as delicious vegetable part. There is rarely any residence the place it is no longer in many instances used single or blended with potato as vegetable due to excessive phosphorus and ascorbic acid content material in the curd. It is both used single or combined with potato as fried or in curry form[14].

Table-1. Nutritional Value of 100 gram Cauliflower edible portion

S.No	Major Constituents	Quantity
1	Water	90.8g
2	Protein	2.6g
3	Fat	0.4g
4	Minerals	1.9g
5	Carbohydrates	4.0g
6	Calcium	33.0mg
7	Magnesium	20.0mg
8	Vitamin C	6.0mg
9	Nicotinic acid	1.00mg
10	Oxalic acid	19.00mg
11	Phosphorus	57.0mg
12	Iron	1.5mg
13	Niacin	0.6mg

During closing three many years efforts are being made to enhance the exceptional of cauliflower in a range of components of the country. The cauliflower curd or head is an undeveloped flower bud consisting of a number of florets. As such, the curd when allowed to developed will showcase 'riciness' with the look of tiny black specks traditional of florets present process opening. 'Riciness' in cauliflower is a signal of over-maturity and bad excellent[1].

The Cole crops have developed from wild cabbage, known as Colewart. The classification of Cauliflower is as follow.

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsidae

Order	: Brassicales
Family	: Brassicaceae
Genus	: Brassica
Species	: Oleracea
Binomial Name	: *Brassica oleracea*

Cauliflower originated on the island of Cyprus and spread to regions as diverse as northwester Europe, Syria, Egypt, Italy, and Spain. Previously, its current structure was unknown in the early middle Ages. Cauliflower may have evolved stepwise from wild cabbage through mutations and changes was probably domesticated by human selection in the Japanese Mediterranean[20]. The use of cauliflower in India was organized by the London botanist Dr. Jameson in 1822 at Kew Gardens at the expense of the Bagh Company (renamed the National Botanical Garden) in Saharanpur, UP. Royal Agricultural and Horticultural Society, Calcutta, West Bengal introduced European vegetable seeds from South Africa in 1824. Seeds brought from England and South Africa were once distributed to farmers in northern India. From 1822 to 1929, these farmers used selective breeding to develop, the modern Indian cauliflower, which can be grown in the high humidity and high temperature conditions of tropical nature and propagated from seed. Plains of northern India cauliflower is grown in plains and hills between 11° and 35° north latitude. It is also commonly grown in the Nilgiri hills and northern southern Himalayas. It is harvested in the hills from March to November, and in the plains of northern India from August or early September to late February or early March. Worldwide, cauliflower grows well between latitudes 11 and 60°N. The normal temperature range is 5-8°C to 25-28°C. Cauliflower might also tolerate temperatures from −10°C to 40°C for a few days at some point of the vegetative increase length[21]. The properly drained moist sandy loam soil with 6 – 7 pH is viewed the excellent soil for production of cauliflower grown in tropical areas in India[11].

Area of production, and productiveness of cauliflower in India and in international are 22,840,000 and 8,573,000 Mt, 1,258,000 ha and 433,000.9 ha, or 18.3 and 19.9 Mt•ha−1, respectively. In world top 10 producing international locations are China, India, Mexico, France, Italy, Poland, the United States, Pakistan, Germany, and Egypt. In India most production of cauliflower takes place in West Bengal, Bihar, Maharashtra, Madhya Pradesh, Odisha, Gujarat, Haryana, Chhattisgarh, Jharkhand, Assam, and Uttar Pradesh[2]. Plants are challenged through a range of biotic stresses like bacterial, fungal or viral infections. They motive serious loss of crop productivity. There are a number of preferences accessible for the farmers to guard their crop from diseases. Several possibilities encompass enhancement of resistant cultivars, crop rotation, organic control, chemical pesticides, and tillage[21].

The *Alternaria* blight or leaf blight disease of cauliflower caused by *Alternaria brassicae* (Berk), Sacc., and *A. brassicicola* (Schw.) Wilt., is one of the most destructive fungal disease causing significant qualitative and quantitative yield loss in cauliflower and cabbage at curd formation and seed setting stage. The disease has a global Presence but more prevalent in subtropical and temperate countries. It is very difficult to manage the disease, due to no proven source of resistance reported till date in any of the hosts. The yield loss due to this pathogen is 5–30 % in the entire cauliflower and cabbage growing areas of India. The disease incidence and severity in Uttar Pradesh was 10–40 % and 26 %, respectively. *Alternaria brassicae* (Berk.) Sacc., is an ubiquitous fungus having a wide host range. It causes black leaf spot in cauliflower leaves and curd[18]. The severity of *Alternaria* blight of cauliflower is seriously affected by variability of temperature and relative humidity. It is considered as one of the most destructive diseases of vegetables in relatively cool and moist areas of the world and causes considerable damage. Various attempts to manage *Alternaria* leaf spot disease with the combination of effective fungicides, botanicals and bioagents are reported[4,5]. The productivity loss due to this microbe is 5–30 % in the whole cauliflower developing areas of India. The disease occurrence and severity in Uttar Pradesh was 10–40 % and 26 %, respectively[17].

Treatment of seeds with biotic and abiotic substances :

Cauliflower seeds treated with Bion®, salicylic acid, and oxalic acid were immersed in a suspension of 25 and 50 mg l^{-1} prepared in distilled water that was sterilized and left for 24 hours. Prior to seeding, the seeds were imbibitiond, dried, and then air dried on filter paper at room temperature for twenty-four hours. Seed treatment with *T. harzianum* and *T.viride* formulation (@2 and 4g kg^{-1} seeds) were treated by normal seed dressing procedure. The seeds treated with distilled water served as control for comparison. The two cauliflower varieties were used separately throughout the experiment *i.e.* SV4051AC and Doctor II.

Efficacy of T. harzianum, T. viride :

Bion, salicylic acid, oxalic acid against *Alternaria* blight disease under greenhouse conditions. Cauliflower seeds treated with Bion®, salicylic acid, and oxalic acid were immersed in a solution of 25 and 50 mg l^{-1} prepared in sterile Two days in a row, the potting medium (soil, sand, and farm yard manure at a ratio of 1:1:1 w/w/w) was autoclaved for one hour. Sterilized potting medium was combined with the virulent strain of A. brassicae mass amplified in sorghum grain-medium at a 19:1 w/w ratio. Pots with a diameter of 15 cm and a height of 30 cm were filled with soil. Cauliflower seeds were evenly distributed across the pots, with 25 seeds per pot. The seed was handled using the previously described procedure for comparison.

Pathogen inoculated and pathogen un-inoculated control (healthy) was maintained earlier in 3.2. Watering was done regularly and damping off disease incidence (the percentage of diseased plants) was recorded at regular time interval up to 35 days after pathogen inoculation.

Induction of defense mechanisms and challenge inoculation :

T. harzianum, T. viride, Bion®, salicylic acid, and oxalic acid used in the experiment for evaluating induction of defense reactions in cauliflower. The following treatments were included in the experiment:

(1) Biotic and abiotic seed treatments, followed by an A. brassicae challenge inoculation (50 g sorghum grain-medium with 10 3cfu g–1 medium in each pot as soil inoculation).

(2) Seeds treated with biotic and abiotic compounds and challenge inoculated with the pathogen by tooth prick method.

(3) Non-treated plants but challenge inoculated with the pathogen as soil inoculation and tooth prick method separately. Three replications were maintained in each treatment; each consisted of five pots.

Estimation of peroxidase (PO) activity :

Peroxidase activity was assayed by following the method of Hammer Schmidt *et al.,*[8].

Preparations of regents :

The following reagents were prepared:

Phosphate buffer :

Using 1000ml of distilled water, 71.18 g of dibasic sodium phosphate (mol.wt.177.99) and 54.42 g of monobasic potassium phosphate (mol.wt.136.09) were dissolved to create 0.1 M phosphate buffer (pH 7.0). Using 1.0 N HC1 and 1.0 N NaOH, the pH meter was used to adjust the pH to 7.0.

Extraction :

A 1g sample of roots was homogenized in 2 ml of a pH 7.0, 0.1 M phosphate buffer at 4°C. Using the supernatant as an enzyme source, the homogenate was centrifuged for 15 minutes at 16,000 g at 4 °C. At room temperature (28±2°C), the reaction mixture was incubated.

Estimation of phenylalanine ammonia lyase (PAL) activity :

Phenylalanin ammonia lyase (PAL) activity was assayed by following the method of Dickerson *et al.,*[6].

Preparations of regents :

The following reagents were prepared:

Sodium borate buffer :

0.1 M borate buffer (PH 7.0) was prepared by dissolving 38.1 g of sodium boreate (mol.wt.381.0) in 1000 ml distilled water. The pH 7.0 was obtained by mixing 0.1 M sodium phosphate (13.0 g/L, (mol.wt. 136.9) solutions and the final volume was adjusted to 1000 ml.

Mercaptaethanole :

1.4 mM of 2 mercaptaehanole was prepared by dissolving in 1000 ml of distilled water.

Extraction :

One g of root samples were homogenized in 3 ml of ice-cold 0.1 M sodium borate buffer (pH 7.0), which also contained 0.1 g of insoluble polyvinylpyrolidone and 1.4 mM of 2-mercaptoethanol. Following a cheesecloth

filtering of the extract, the filtrate was centrifuged for 15 minutes at 16,000 g. The source of the enzyme was the supernatant. The rate at which L-phenylalanine was converted to trans-cinnamic acid at 290 nm was used to calculate the PAL activity.

Assay of enzyme activity :

Samples with 0.4 ml of enzyme extract were incubated for 30 minutes at 30 degrees Celsius in 0.5 ml of 0.1 M borate buffer, pH 8.8, and 0.5 ml of 12 mM L-phenylalanine in the same buffer. It was determined how much trans-cinnamic acid was produced. The unit of expression for enzyme activity was nmol. trans-cinnamic acid minimum –1 100 mg protein –1 or g. f.w.-1

Estimation of poly-phenol oxidase (PPO) activity :

Activity of poly-phenol oxidase was assayed calorimetrically in accordance with the method of Mayer *et al.*,[12].

Preparation of reagents :

The following reagents were prepared:

Phosphate buffer :

0.1 M phosphate buffer (pH 7.5) was prepared as described earlier under
0.2 Peroxidase estimation.

Pyro-catechol :

The 0.01 M catechol reagent was prepared by dissolving 10 mg of Pyro-catechol (mol.wt.110.11g) in 10.0 ml of glass distilled water.

Extraction :

One gram of root samples was homogenized in two milliliters of 0.1 M sodium phosphate buffer (pH 6.5), and the mixture was centrifuged at 16,000 g for fifteen minutes at four degrees Celsius. An enzyme source was obtained from the supernatant. The enzyme extract (200 µl) and 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5) made up the reaction mixture.

Assay of enzymatic activity :

To start the reaction, 200 µl of 0.01-M catechol was added in the reaction mixture consisted of 200 µl of the enzyme extract and 1.5ml of 0.1 M sodium phosphate buffer (pH 6.5) and the activity was expressed as changes in absorbance at 495-nm min^{-1} mg $protein^{-1}$ (Mayer, *et.al.,*1965). For testing of significance of all the results general statistical procedure were adopted.

∝-Amylase activity :

∝-Amylase activity was assayed by measuring the depletion of soluble starch following the method of Shioster *et al.,* (1962).

Preparations of regents :

The following reagents were prepared:

Phosphate buffer :

0.3 M phosphate buffer (pH 7.5) was prepared by dissolving 35.59 g of dibasic sodium phosphate (mol.wt.177.99) in 1000m, and 27.21g monobasic potassium phosphate (mol.wt.136.09) in 1000ml of distilled water. The pH 7.5 was obtained by mixing 80.3 ml of sodium phosphate and 19.7 ml of potassium phosphate solutions. Final pH was adjusted by pH meter using 1.0 N HC1 and 1.0NaOH.

0.4

Starch solution :

67 mg of soluble starch was dissolved in distilled water along with 0.81654 g of monobasic potassium phosphate by mild heating and final volume was made to100ml by glass distilled water.

Iodine solution :

61mg of potassium iodide (mol.wt.166.01) and 6 mg of iodine crystal (mol.wt.126.9) were dissolved in 100 ml of 0.1 N HCL.

Extraction :

Root samples (1g) were collected from field in ice bucket, washed and wiped with filter paper. Weighed sample (2.0g) was macerated in prechilled mortar and pestle with 10.0 ml of 0.2 M phosphate buffer (pH 7.5). Homogenates were centrifuged at 2^0C in Remi refrigerated centrifuge E24 A for 15 minutes at 12,000 rpm. Supernatants were saved and used as a source of enzyme.

Assay of enzyme activity :

0.1 ml of enzyme extract was incubated with incubated with 1.0ml of starch solution for 5 minutes and the reaction was stopped by the addition of 1.0ml of iodine solution. Incubation was done at room temperature. Reagent blank was taken simultaneously with starch (1.0ml) and iodine (1.0ml) .Final volume was made to 6.0ml with distilled water and optical density was taken at 620 mm against reagent blank ((1.0ml of iodine solution + 5.0 ml of water). Amount of starch depleted was calculated through standard curve of starch. Enzyme activity was expressed as mg starch hydrolyzed per mg protein per hour.

Estimation of total phenolic compounds:

Total phenolic compound of the samples was estimated as per the method of Swain and HIllin (1959) with little modification.

Preparation of reagents :

The following reagents were prepared:

Methanol :

80% (v/v) methanol was prepared by adding 80 ml of methanol (mol.wt.32.04) to 20 ml of glass distilled water.

Follin Denis reagent :

To 750 ml of water, 100 g of dehydrated sodium tungstate (mol.wt.329.86), 20g of phosphomolybdic acid (mol.wt. 22557.6) and 50 ml of phosphoric acid (mol.wt.98.9) was added and refluxed for 2 hours. The solution was cooed and diluted to one litter.

Sodium carbonate solution :

35g anhydrous sodium carbonate (mol.wt.105.99) was dissolved in 200 ml of water through heating and final volume was added to 250 ml with glass distilled water.

Extraction :

Weighed root sample (0.5g) was macerated with 10 ml of 80 per cent (v/v) methanol in pestle and mortar, and then centrifuged at 8000 rpm for 10 min. Supernatant was saved. The leftover palate was extracted thrice by 10 ml of 80 per cent (v/v) methanol and all the supernatant was pooled. Final

volume of supernatant was made to 50 ml by 80 percent (v/v) methanol and was used as the sources of total phenolic estimation.

Assay of phenolic estimation :

To 6.5 ml of distilled water, 0.5 ml of extract was taken in duplicate and 0.5 ml of Folin Denis reagent was added. After 5 minutes 2.5 ml of sodium carbonate solution was added. The reaction mixture was allowed to stand for one hour at room temperature and them optical density was taken at 726 nm against reagent blank where 0.5 ml of extract was replaced by 0.5 ml of 80 per alcohol. Total phenolic compound draw by using floro glucinol and were expressed as mg per g fresh weight.

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 varieties. The application of Bion compound as seed treatment and foliar spray resulted in highest disease reduction in both varieties. In pre and post inoculation artificial epiphytotics conditions as compared to other treatments and control as evident in figure 1.

Effect of pre and post inoculation on development of disease				
Infection index (%)				
Days after inoculation	Pre inoculation		Post inoculation	
	SV4051AC	DOCTOR II	SV4051AC	DOCTOR II
2	2.7	0	1.3	0
4	10.3	1.4	5.4	0
6	20.9	5.8	15.3	2.4
8	40.5	12.4	28.3	8.4
10	60.4	20.4	42.4	14.9
15	84.7	30.4	50.3	24.4
20	90.5	33.4	58.8	28.3
25	95.6	40.4	67.3	34.6
30	99.5	45.6	79.8	37.5
35	99.9	48.6	89.4	39.4
CD. Treatment =1.40381	DAF X Var=1.98529	C.D. Treatment=	Var=	0.612
(DAF) = 0.6278		(DAI) 1.36853957211342	DAI X Var	1.9354

Results of Anova : Effect of biotic and abiotic components on disease control

	ANOVA					
	SOV	D F	SS	MSS	F CAL	F TAB
	R	2	4.76	2.3805	1.65014	3.244818
	V	1	20126.35	20126.35	13951.39	4.098172
	D	9	43796.38	4866.265	3373.248	2.137528
	V X D	9	6126.691	680.7435	471.8848	2.137528
	ERROR	38	54.82	1.442605		
		V	D	VXD	T TAB	SQR2
SEM	0.219287	0.490341		0.693447	2.024394	1.414214
CD	0.627803	1.40381	1.985287			

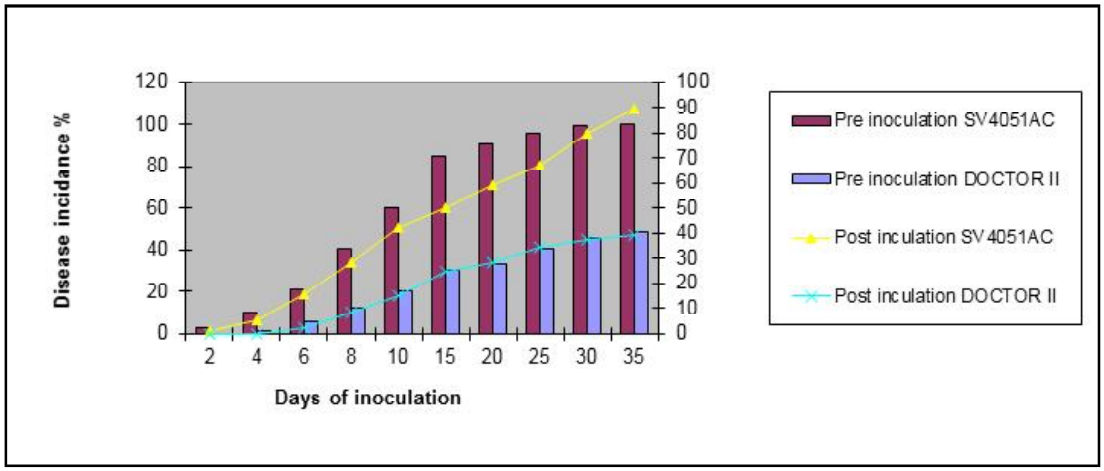

Fig. 1. Effect of biotic and abiotic components on disease control

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

Perusal of data presented is 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[10]. 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.

Effect of abiotic and biotic compounds on peroxidase activity pre and post inoculated cauliflower varieties.																		
Change in O.D./min/ml enzyme extract																		
Pre inoculation										Post inoculation								
Days after inoculation										Days after inoculation								
	0	1	2	3	4	5	7	10	15	0	1	2	3	4	5	7	10	15
SV4051AC										**SV4051AC**								
LGA	1.7	1.66	3.67	4.35	5.43	5.64	5.79	5.89	6.15	1.63	1.72	3.75	4.39	5.49	5.73	5.89	5.96	6.21
SA	1.7	1.62	2.32	3.45	4.32	4.59	4.89	5.1	5.4	1.6	1.63	2.45	3.52	4.46	4.8	4.96	5.24	5.43
OA	1.6	1.56	2.25	3.2	4.25	4.43	4.65	4.82	5.1	1.5	1.58	2.33	3.29	4.32	4.64	4.77	4.89	5.29
TW	1.6	1.51	2.12	3.08	4.18	4.29	4.52	4.73	5.08	1.48	1.55	2.26	3.14	4.26	4.38	4.63	4.78	5.21
TV	1.5	1.4	1.98	2.88	3.92	4.18	4.46	4.58	4.89	1.36	1.42	2.1	2.93	4.12	4.25	4.52	4.67	4.92
Control	1.2	1.16	1.8	2.25	3.1	3.39	3.49	3.54	3.62	1.13	1.19	1.85	2.31	3.37	3.49	3.56	3.61	3.71
DOCTOR II										**DOCTOR II**								
LGA	1.7	1.67	3.71	4.39	5.52	5.69	5.82	5.92	6.19	1.65	1.69	3.79	4.42	5.55	5.81	5.83	5.99	6.25
SA	1.7	1.64	2.35	3.52	4.38	4.61	4.92	5.15	5.42	1.62	1.65	3.49	3.59	4.5	4.88	5.1	5.29	5.46
OA	1.6	1.58	2.31	3.26	4.31	4.48	4.68	4.9	5.12	1.51	1.61	2.42	3.36	4.38	4.69	4.82	4.92	5.32
TW	1.6	1.53	2.19	3.1	4.22	4.32	4.56	4.81	5.1	1.46	1.56	2.31	3.31	4.31	4.45	4.69	4.82	5.25
TV	1.5	1.44	2.08	2.95	3.98	4.23	4.52	4.67	4.92	1.37	1.47	2.15	3.1	4.22	4.37	4.55	4.73	4.95
Control	1.3	1.3	1.85	2.29	3.16	3.42	3.52	3.59	3.64	1.2	1.32	1.92	2.41	3.49	3.52	3.61	3.65	3.74

ANOVA							
SOV	DF	SS	MSS	FCAL	FTAB		
R	2	0.24	0.12	1.569682	3.0380629		
T	5	96.68	19.34	252.9346	2.256255		
V	1	0.15	0.15	2.006613	3.8852794		
D	8	572.9	71.61	936.6879	1.9818546		
T X V	5	0.01	0.00	0.019919	2.256255		
D X V	8	0.02	0.00	0.031266	1.9818546		
T X D	40	20.16	0.50	6.591502	1.4511313		
T X D X V	40	0.04	0.00	0.012954	1.4511313		
ERROR	214	16.36	**0.08**				
	323			FTABU	SQR2	FACTOR	
				1.971111	1.414214	2.787572	
	T	V	D	T x V	D x V	T x D	T x D x V
SEM	0.037626	0.021723	0.046082	0.053211	0.0691233	0.112878	0.159634
CD	0.104885	0.060555	0.128458	0.14833	0.1926863	0.314655	0.44499

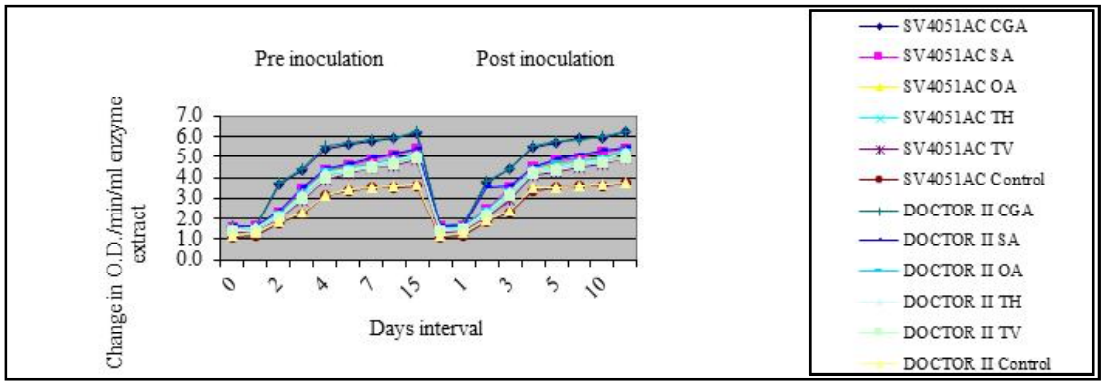

Fig. 2. Effect of abiotic and biotic compound treatment on Peroxidase (PO) activity.

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 amonia lygase 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.* 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.

Effect of abiotic and biotic compounds on Phenyl amonia ligase active in pre and post inoculated cauliflower varieties																		
Change in O.D/min/ml enzyme extract																		
Pre inoculation										Post inoculation								
Days After Inoculation										Days After Inoculation								
	0	1	2	3	4	5	7	10	15	0	1	2	3	4	5	7	10	15
SV4051AC																		
CGA	276.50	280.20	294.30	380.30	484.30	594.20	670.20	750.20	780.20	277.70	280.30	295.30	382.40	489.30	599.30	680.30	759.20	810.30
SA	270.30	271.30	283.40	370.20	472.10	570.30	650.30	730.20	760.40	271.50	272.40	285.40	375.30	495.30	574.20	660.20	745.50	801.50
OA	262.50	263.10	271.30	350.40	440.30	510.20	630.50	710.40	740.20	263.70	268.50	272.50	359.20	446.50	519.30	641.50	729.40	790.30
TH	199.40	199.80	212.30	330.50	418.30	499.20	605.20	695.20	720.40	200.60	203.10	215.40	331.40	422.10	515.40	624.30	712.30	784.50
TV	187.20	189.30	205.30	325.40	410.20	487.30	590.80	681.50	711.50	188.40	197.30	209.30	320.10	419.10	511.20	619.40	709.40	773.20
Control	154.30	158.40	209.40	290.10	312.20	360.20	372.40	410.20	420.20	155.50	156.20	195.20	280.10	320.10	370.10	384.20	415.30	429.10
DOCTOR II																		
CGA	286.30	290.40	310.40	390.50	490.20	596.30	669.30	752.40	782.10	287.70	295.30	310.40	420.30	510.40	612.40	690.40	760.30	811.30
SA	272.50	278.20	305.30	382.40	481.30	565.20	645.20	731.30	770.20	273.90	281.50	295.30	412.40	491.30	596.30	662.30	748.30	805.30
OA	264.20	268.30	293.60	364.50	445.40	505.90	622.50	709.50	745.30	265.60	272.30	286.40	384.20	460.30	576.40	645.30	732.40	792.40
TH	203.10	213.40	225.30	352.30	425.50	492.30	602.40	701.20	722.50	204.50	225.30	280.30	375.40	453.40	550.30	628.30	714.50	786.30
TV	197.40	201.80	211.50	340.40	420.20	481.30	590.10	697.30	715.30	198.80	201.30	273.20	369.30	445.30	541.30	622.10	710.30	725.40
Control	159.30	162.30	215.00	305.10	350.40	410.20	425.50	501.50	440.50	160.70	162.40	210.30	292.40	329.20	372.30	394.10	421.30	435.50

ANOVA							
SOV	DF	SS	MSS	FCAL	FTAB		
R	2	114.41	57.20	0.580776646	3.0380629	NS	
T	5	1261206.01	252241.20	2560.966133	2.256255		
V	1	8642.80	8642.80	87.74903039	3.8852794		
D	8	9954222.9	1244277.86	12632.96174	1.9818546		
T X V	5	7239.07	1447.81	14.69943738	2.256255		
D X V	8	1831.44	228.93	2.32428967	1.9818546		
T X D	40	393425.99	9835.65	99.8598386	1.4511313		
T X D X V	40	12475.78	311.89	3.166616324	1.4511313		
ERROR	214	21077.83	**98.49**				
	323		FTABU	SQR2	FACTOR		
			1.9711112	1.4142136	2.787572		
	T	V	D	T x V	D x V	T x D	T x D x V
SEM	1.350545488	0.7797378	1.654073661	1.909959746	2.4811105	4.0516365	5.7298792
CD	3.764743064	2.17357542	4.61084976	5.3241507	6.9162746	11.294229	15.972452

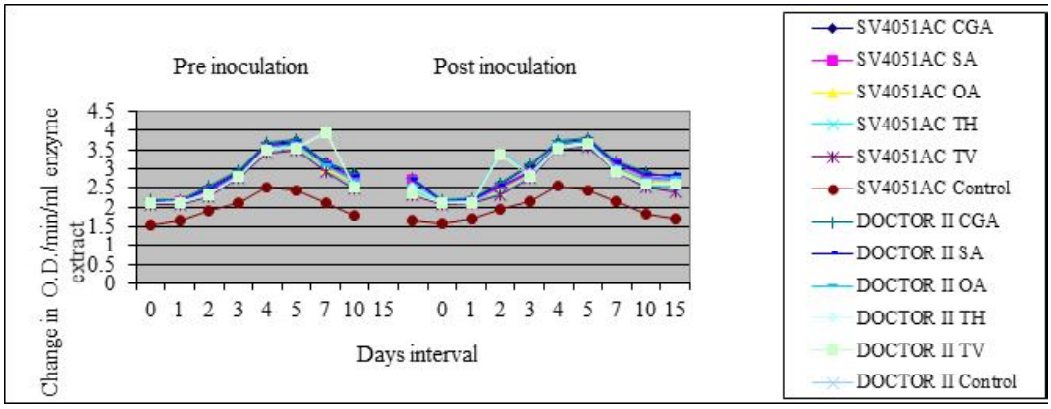

Fig. 3. Effect of abiotic and biotic compounds on PAL activity

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 increase in PPO activity among abiotic elicitors was in Bion treatment followed by salicylic acid and oxalic acid[16]. 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.

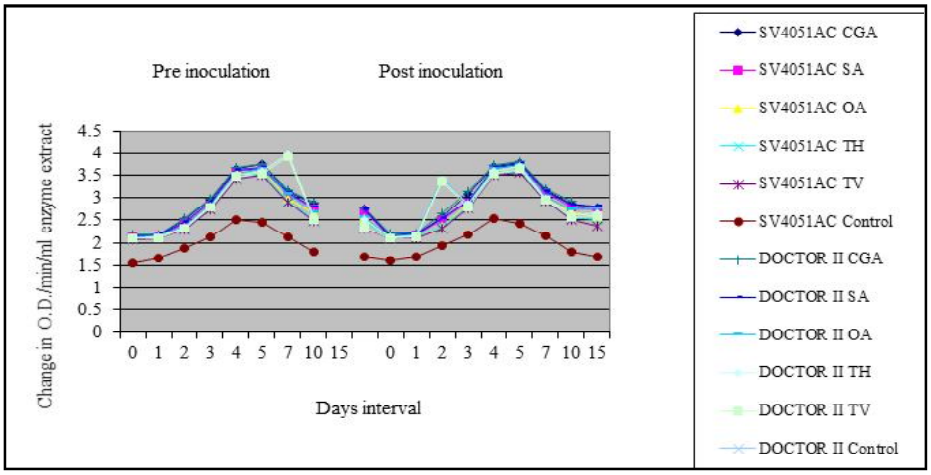

Fig. 4. Effect of biotic and abiotic compound on PolyPhenole Oxidase (PPO) activity

Effect of biotic and abiotic compounds on ∝-amylase activity :

As the data mentioned in Figure 5, the ∝-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 ∝-amylase activity was showed by the treatment Bion in variety Doctor II oxalic acid, *T. harzianum* and *T. viride*[16].

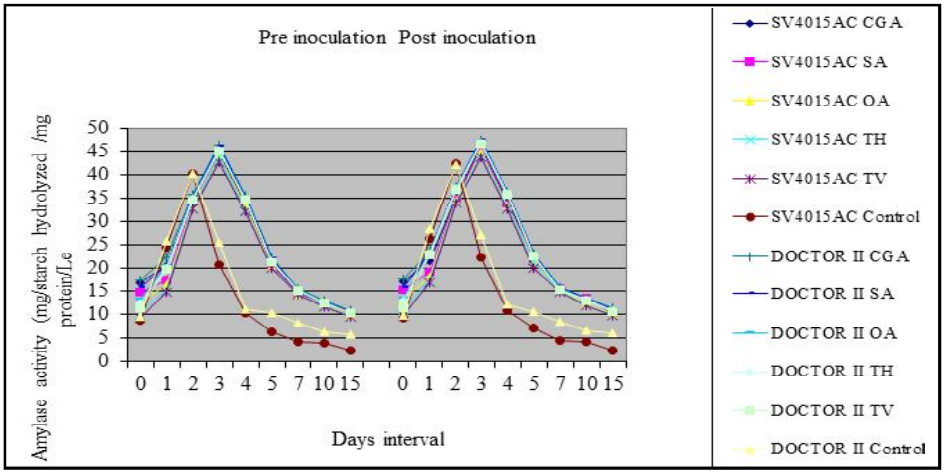

Fig. 5. Effect of biotic and abiotic compounds on ∝-amylase activity

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[10]. The increase in enzyme activity was up to 7th 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 15th days after inoculation.

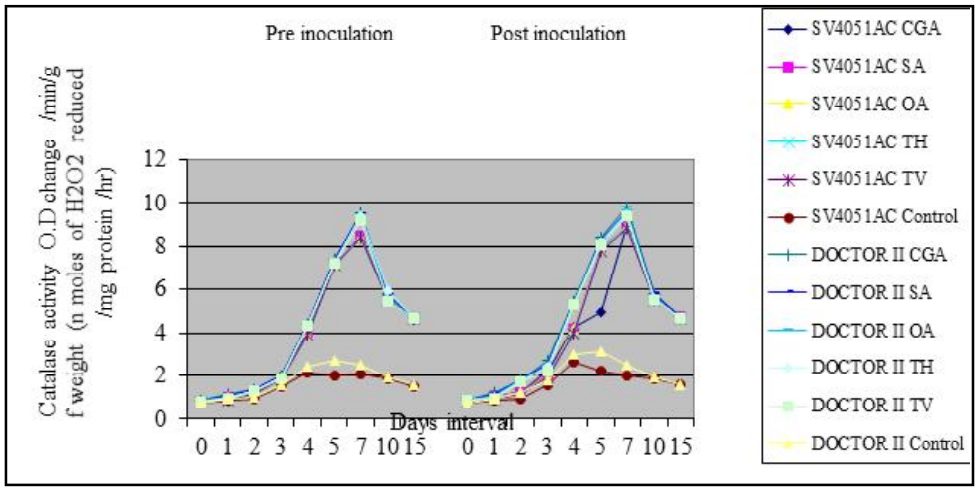

Fig. 6. Effect of biotic and abiotic compounds treatment on catalase activity

It was observed that the all the treatments were found to be effective in reducing disease and inhibiting pathogen growth in the form of conidial formation 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 Aacid (90.93%) and oxalic acid (89.06%) @ 50 mg.L concentration. The biotic compound *T. harziaznum* (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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