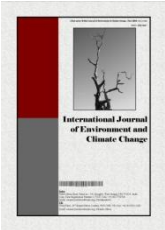

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Efficacy Evaluation of Abiotic Elicitors for the Management of Black Rot Disease of Cauliflower Incited by *Xanthomonas campestris* pv. *campestris*

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To find out the effective abiotic elicitor for management of black rot disease of cauliflower.
Study Design: Completely randomized block design (Pot experiment in glasshouse condition).
Place and Duration of the Study: This experiment was conducted at glasshouse of Division of Plant Pathology, ICAR-IARI, New Delhi during the year 2021-22.
Methodology: Different abiotic elicitors were applied by spraying whole plants (45 days old) with the aid of a fine atomizer onto the upper leaf surfaces. Pathogen *X. campestris* pv. *campestris* was inoculated after 72 h of elicitor treatment, Control plants were treated with the distilled water. The disease assessment was done at 14 and 21 days after inoculation by using six- point scale and Percent Disease severity was calculated.
Results: In case of foliar spray of Salicylic acid before 72 h of inoculation of pathogen, minimum percent diseases severity (25.56) was recorded at the concentration of 3 mM after 21 days of pathogen inoculation. In case of BABA, minimum percent disease severity (18.37) was observed at 800 µg/ml concentration. INA helps to reduce the disease severity at 100 uM concentration. ASM was found effective at 350µg/ml with 14.07% disease severity and 82.91% disease control efficiency. In case of MeJA, minimum percent disease severity (30.32) was observed at 2mM concentration.
Conclusion: Among all the tested abiotic elicitors at different concentration, ASM at the concentration of 350 µg/ml was found very effective to manage the disease.

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Black rot caused by *Xanthomonas campestris* pv. *campestris* (Xcc) is a very important and devastating disease of cauliflower (*Brassica oleracea* var. *botrytis*) crop resulting into 10–50% yield losses every year. So for the management of black rot disease, five abiotic elicitors viz; salicylic acid(SA-0.5, 1.0, 2.0, 3.0 mM), methyl jasmonte (MeJA-0.5, 1.0, 1.5, 2.0mM), β-Aminobutyric acid (BABA-200, 400, 600, 800 µg/ml), acibenzolar-S- methyl (ASM-150, 250, 350, 450 µg/ml) and dichloroisonicotinic acid (INA-50, 100, 150, 200 µM) were tested to induce systemic resistance against the disease. The experiment was conducted under glasshouse condition at Division of Plant Pathology, ICAR-IARI, New Delhi during the year 2021-22. Foliar application of these abiotic elicitors was done on 45 days old plants of susceptible cv. Pusa Sharad of cauliflower. Results indicated that among all the tested chemical elicitors at different concentration, ASM@350 µg/ml was found very effective to manage the disease. It shows 82.91% disease control efficacy with 14.07% disease severity.

Keywords: Cauliflower; black rot; abiotic elicitor; ASM; management.

1. INTRODUCTION

Cauliflower (*Brassica oleracea* var. *botrytis*) is an important vegetable crop, which is widely grown in China, India, Italy, and other Asian and European countries [1,2]. It is originated from North-east of the Mediterranean region. In India, it was introduced from England in 1822 by Dr. Jemson, In-charge of Company Bagh, Saharanpur, U.P. Cauliflower cultivation is done in almost all the states of our country, but the main states are West Bengal, Bihar, U.P., Odisha, Assam, M.P., Rajasthan, Gujarat, Haryana, Himachal Pradesh, Maharashtra and Karnataka. It is rich in minerals like K, Na, Ca, Fe, P and Mg and vitamins like A and C. Globally, India contributes about 13 percent to the world vegetable production and occupies second position in the cauliflower production. In India, it is cultivated on 473 thousand hectares area with annual production of 9225 thousand tonnes accounting the productivity of 19.50 tonnes/ha (Third Advance Estimates of Horticulture Crops 2020-21). This stagnation in yield and productivity over the year is affected by various bacterial, fungal and viral diseases which make serious inroads into cauliflower production in terms of curd yield by affecting seed germination, seedling mortality, killing the plants, rotting of curds and spoiling the quality of curds. Among the various bacterial diseases that affects crucifer crops, black rot caused by *Xanthomonas campestris* pv. *campestris* (Xcc) is one of the most devastating and widespread seed-borne bacterial disease [3,4] and has a widest geographical distribution.

Tropical, subtropical, and other places with warm, humid temperatures are particularly vulnerable to black rot disease. In favourable environmental circumstances like high relative humidity and increased temperature, the bacterium can cause losses of up to 50%. The typical leaf symptoms of black rot disease are V-shaped lesions manifested at leaf margin through hydathodes causing vein blackening and these V-shaped lesions enlarges towards the mid rib of leaf. Disease control is difficult due to systemic nature of pathogen. Management of the disease with chemicals relies upon the use of antibiotics (mostly Streptomycin) and some copper compounds, which prevent bacterial multiplication and further infection in the plant. Unfortunately, the bacterial population develops resistance against the antibiotics. Now a day, use of antibiotics is banned or strictly limited in many countries all over the world. Copper compounds have very limited efficacy when favourable condition coincides with disease development. Thus, induction of plant resistance will be a useful method for management of this devastating disease. Induced protection of plants against various pathogens (bacterial, fungal or viral) by biotic or abiotic agents has been reported. Abiotic inducers of disease resistance play a key role for the integration of the systemic acquired resistance (SAR) concept in modern plant protection strategies [5]. Synthetic compounds are known to induce systemic acquired resistance which is generally effective against a very broad range of pathogens. The low impact of these abiotic elicitors on humans as well as on the environment makes these compounds suitable for use in field or greenhouse plant disease management.The ASM compound was found effective to control bacterial black spot of mango, caused by *Xanthomonas campestris* pv. *mangiferae indicae* (Synonym*: X. citri* pv. *mangiferaeindicae*) [6]. Pre treatment of rose shoots with 50 µM ASM led to resistance against *Agrobacterium tumefaciens* (Synonym: *Rhizobium radiobacter*)

and *Diplocarpon rosae* by significantly reducing the disease severity of crown gall and blackspot [7]. Resistance inducing effects of ASM have been demonstrated in apple against *Erwinia amylovora* in recent studies [8-9]. BABA has shown inhibition of disease development in canker of tomato caused by *Clavibacter michiganensis* [10], bacterial leaf spot in tomato caused by *Pseudomonas syringae* [11], fire blight of apple caused by *Erwinia amylovora* [12], and citrus canker caused by *Xanthomonas citri* subsp*. citri* [13]. Smith et al. [14] reported that free SA can accumulate in plant tissue to levels that inhibit *Ralstonia solanacearum* growth. INA has been tested on many plant species in which it raised resistance against a broad spectrum of pathogens [15-16].

Knowledge about the disease resistance induction against *X. campestris* pv. *campestris* in cauliflower might allow for the development of a novel environmental friendly method for disease protection, which also will improve quality and productivity of the crop. So for the management of black rot disease, efficacy of five abiotic elicitors (SA, BABA, ASM, INA and MeJA) was investigated at different concentration in glasshouse condition with foliar application of these abiotic elicitors on 45 days old plants of susceptible cv. Pusa Sharad of cauliflower.

2. MATERIALS AND METHODS

2.1 Plant Material, Bacterial Culture and Abiotic Elicitor

Seeds of black rot susceptible cv. Pusa Sharad of cauliflower were obtained from the Vegetable Science Division, ICAR-Indian Agricultural Research Institute, New Delhi. Black rot causing bacterial culture of *X. campestris* pv.*campestris* (race-1) was used for inoculation. First seeds were grown in portrays. Twenty-one days old cauliflower seedlings of cv. Pusa Sharad were transplanted in earthen pots of 15cm diameter having autoclaved soil mixture of peat moss, vermiculite and sand in the ratio 2:1:1.Five abiotic elicitors viz; salicylic acid (SA-0.5, 1.0, 2.0, 3.0mM), β-aminobutyric acid (BABA-200, 400, 600, 800 µg/ml), acibenzolar-S- methyl (ASM-150, 250, 350, 450 µg/ml), dichloroisonicotinic acid (INA-50, 100, 150, 200 µM) and methyl jasmonate (MeJA-0.5, 1.0, 1.5, 2.0mM) were used at different concentrations for testing their efficacy against black rot disease.

2.2 Elicitor Application and Pathogen Inoculation

The abiotic elicitors were applied by spraying whole plants (45 days old) with the aid of a fine atomizer onto the upper leaf surfaces. *X. campestris* pv. *campestris* (race-1) was inoculated on to cauliflower seedlings after 72 h of elicitor treatment. Control plants were treated with the distilled water.

2.3 Disease Scoring

The disease assessment was done at 14 and 21 days after inoculation by using six- point scale of 0-9 based on the relative lesion size given by Vicente et al. [17].

Table 1. Relative lesion size

Scale	Relative lesion size
0	No symptoms
1	Small Necrosis or chlorosis surrounding the infection point
3	Typical small V-shaped lesion with black veins
5	Typical lesion half way to the middle vein
7	Typical lesion progressing to the middle vein
9	Lesion reaching the middle vein

Disease severity index (DSI) was calculated by using the formula:

$$\text{DSI}\ (\%) = \frac{[\Sigma(\text{rating no.} \times \text{ no. of plants in rating})]}{(\text{Total no. of plants} \times \text{ highest rating})} \text{X}100$$

Protection against Xcc was assessed by comparing the disease severity values.

Disease control efficacy (DCE) of chemical elicitors was determined as described formula by Guo et al. [18].

$$DCE = [(DC-DT)/DC] \times 100$$

Where DC is disease in control and DT is disease in treatment group.

3. RESULTS AND DISCUSSION

This experiment was conducted to find out the efficacy of five abiotic elicitors at different concentrations against black rot disease of cauliflower cv. Pusa Sharad caused by *X. campestris* pv. *campestris* under glass-house conditions. Results (Table 2) from the study

indicated that in case of foliar spray of Salicylic acid before 72 h of inoculation of pathogen, minimum percent disease severity (25.56) was recorded at the concentration of 3mM after 21 days of pathogen inoculation. In case of BABA, minimum percent disease severity (18.37) was observed at 800µg/ml concentration. INA helped to reduce the disease severity at 100 µM concentration. There was no significant difference in disease control with the higher concentration i.e. 150 and 200uM. ASM was found effective at 350µg/ml with 14.07% disease severity and 82.91 disease control efficiency. There was no significant difference at the ASM concentration 350µg/ml and 450µg/ml. In case of MeJA, minimum percent disease severity (30.32) was observed at 2mM concentration. Among all the tested chemical elicitors, ASM at the concentration of 350µg/ml was found very effective to manage the disease.

In simple terms, plant disease resistance can be defined as the ability of a plant to prevent or restrict the particular pathogen growth and multiplication. The use of resistance inducers to speed up plant responses could give a physiologically, ecologically, and financially viable alternative to current disease management approaches. Some researchers have focused on developing novel synthetic chemical activators with increased efficacy [19]. Exogenous application of biotic and abiotic inducers or elicitors can improve plant resistance to pathogen invasion. Various chemicals have been reported to act at various points in the defense activating networks and mimic whole or parts of the biological activation of resistance, among these, only a few have reached upto commercialization. Several reviews have highlighted the potential of chemical elicitors to activate and enhance natural plant disease resistance [20-23]. Several compounds have been reported to induce SAR in a variety of plants against a wide range of microbial pathogens without having direct antimicrobial activity [24].

Table 2. Evaluation of efficacy of different abiotic elicitors against black rot disease of cauliflower cv. Pusa Sharad under glasshouse conditions

Name of elicitor	Concentration used	Percent Disease Severity			Disease control efficiency (%)
		14 Days	21 days	Mean	
1. SA	Control	48.87± 2.48^{a}	77.42±2.41^{a}	63.14	0.00
	0.5mM	43.35±3.96^{a}	55.27±2.58^{b}	49.31	28.61
	1.0mM	35.58±2.56^{b}	46.13±4.92^{c}	40.85	40.42
	2.0mM	27.78±2.00^{c}	38.41±2.58^{c}	33.09	50.38
	3.0mM	18.82±2.09^{d}	25.56±1.00^{d}	22.19	66.98
2. BABA	Control	48.27± 3.75^{a}	75.46± 4.17^{a}	61.87	0.00
	200µg/ml	32.25±2.79^{b}	48.60±2.36^{b}	40.43	35.59
	400µg/ml	24.21±1.083^{c}	42.19±3.12^{b}	33.20	44.08
	600µg/ml	16.71± 1.99^{d}	21.16± 1.83^{c}	18.94	71.95
	800µg/ml	12.39±1.16^{d}	18.37± 4.05^{c}	15.38	75.65
3. INA	Control	46.64± 3.05^{a}	77.79±4.78^{a}	62.21	0.00
	50µM	41.26± 1.69^{b}	60.44± 2.11^{b}	50.85	22.30
	100µM	17.46± 1.00^{c}	24.34±1.78^{c}	20.90	68.71
	150µM	18.60± 2.25^{c}	25.38±2.57^{c}	21.99	67.37
	200µM	18.25± 0.78^{c}	24.44±2.00^{c}	21.35	68.58
4. ASM	Control	44.17±2.93^{a}	82.37± 2.91^{a}	63.27	0.00
	150 µg/ml	28.09±1.68^{b}	43.16± 1.41^{b}	35.62	47.60
	250 µg/ml	18.18±1.65^{c}	35.47± 4.19^{c}	26.82	56.93
	350 µg/ml	8.78±1.49^{d}	14.07± 2.02^{d}	11.42	82.91
	450 µg/ml	7.75± 2.31^{d}	14.33± 1.66^{d}	11.04	82.60
5. MeJA	Control	46.68± 1.52^{a}	75.64± 6.64^{a}	61.16	0.00
	0.5 mM	43.26± 1.99^{a}	57.31± 3.23^{b}	50.28	24.23
	1.0 mM	24.36± 0.75^{b}	44.44± 2.00^{c}	34.40	41.24
	1.5 mM	24.67± 1.21^{b}	43.59±3.91^{c}	34.13	42.37
	2.0 mM	24.11± 2.04^{b}	30.32± 1.65^{d}	27.22	59.91

**The experimental data are the average of three replicates. Mean with different letters in the same column differ significantly at P ≤ 0.05 (Tukey's test)*

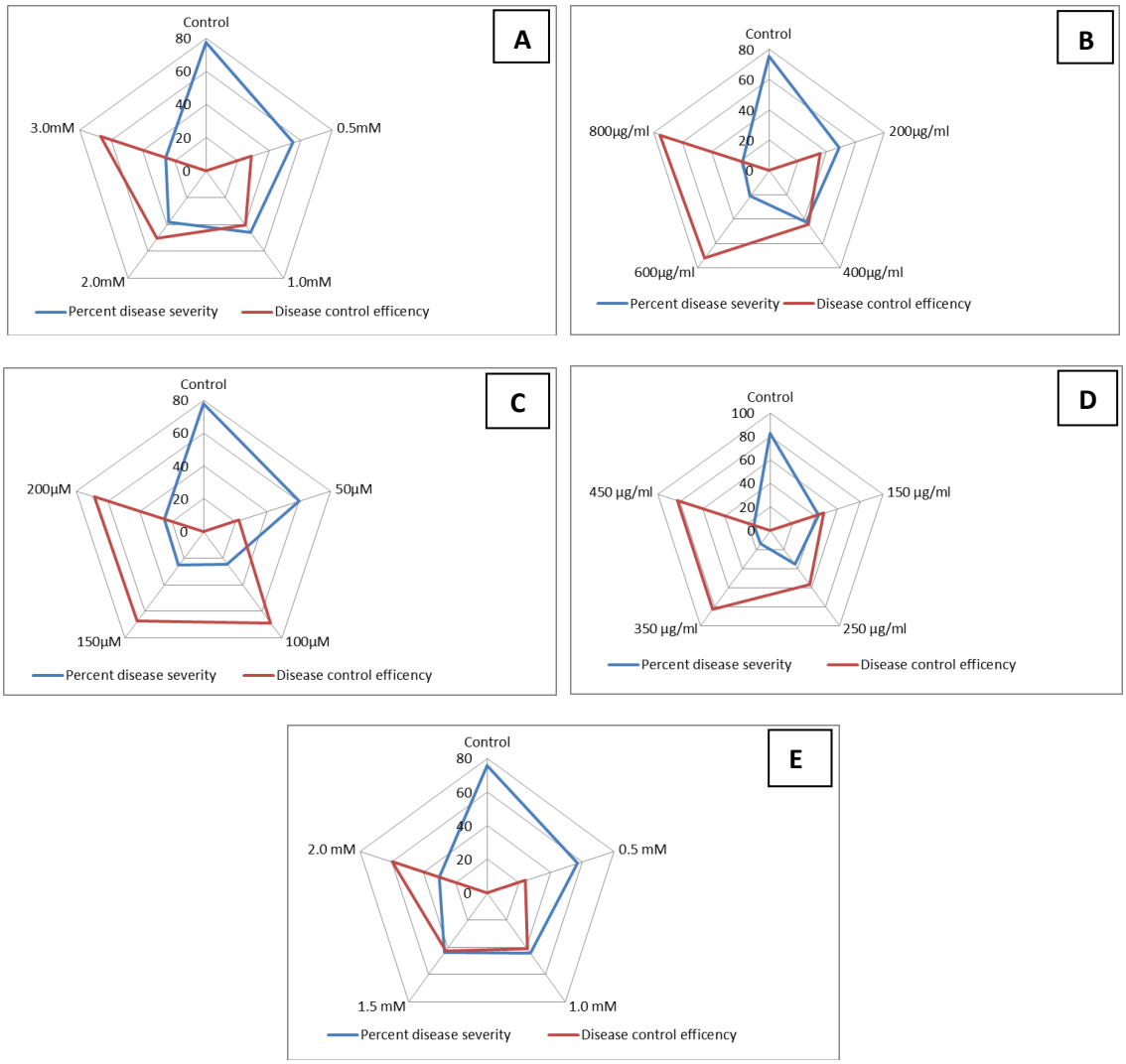

Fig. 1. Correlation between percent disease severity and disease control efficiency; (A) SA, (B) BABA, (C) INA, (D) ASM, (E) MeJA

In the present study, among all the tested abiotic elicitors, ASM@350µg/ml was significantly decreased the black rot disease severity. The results are in agreement with data from the literature describing several plant-pathogen interactions that showed the ability of ASM to induce resistance in plants [25-28]. Results of our study were also supported by the outcomes of Obradovic et al. [29], who have reported the management of bacterial spot in tomato under field conditions by using foliar application of ASM @0.003%. A significant reduction in the severity of tomato bacterial spot was observed in plants treated with ASM [30].

The use of an abiotic inducer to activate the plant's defensive mechanisms is an unique plant protection method. Induction of defense associated proteins makes the plant resistant to pathogen invasion and further invasion. Induction of these defense proteins has been correlated with pathogen invasion in various host-pathogen interactions. The severity of bacterial canker disease was reduced up to 75%, suppression of the bacterial growth was up to 68% with significant manifestation in the defense related enzymes with ASM treatment was reported by Soylu et al. [31]. The abiotic elicitor, ASM-treated seedlings challenged with *Clavibacter michiganensis* subsp. *michiganensis*, support the concept that a triggering signal produced by the pathogen is essential to enhance synthesis and accumulation of various defense-gene products. The study conducted by Boro et al. [32] also supported our results, in which control of

bacterial leaf spot of yellow passion fruit was achieved by using the abiotic resistance inducer, acibenzolar-S-methyl (ASM) as seed immersion and spraying at the concentration of 12.5 µga.i. mL^{-1} with 70% protection.Results of the study conducted by Kuhn [33] was also in close conformity to our results that induction of resistance in bean plants against bacterial blight caused by *X. axonopodis* pv. *phaseoli*, with the use of this SAR inducer was achieved only when low concentrations of ASM were applied. On the other hand, the fact that only the lowest concentration of ASM was active when directly sprayed on leaves in the pathosystem under study, confirmed the statement of Kuć [34] that the protecting effect observed in plants is dependent on several factors, including the concentration of the inducer.Huang et al. [35] also found the significant reduction in the bacterial spot disease severity on tomato plants treated with ASM at 129 µM as compare to the untreated control.

4. CONCLUSION

The use of abiotic inducer to activate the plant's defensive system is a unique and cost effective disease management strategy. Among all the tested abiotic elicitors at different concentration, ASM at the concentration of 350 µg/ml was found very effective to manage the black rot disease in cauliflower. Timely management of this devastating disease will reduces the losses caused and also increase the yield and quality.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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