

Status and evaluation of systemic acquired resistance (SAR) activators for the management of Alternaria leaf spot of cucumber

ShanazYousuf¹, Farahnaaz Rasool², ShubanaBhat³

Division of Plant Pathology, S. K. University of Agricultural Sciences & Technology of Kashmir, Shalimar, Srinagar - 191 121, Jammu & Kashmir, India

ABSTRACT

Alternaria leaf spot, caused by Alternaria alternata, is an economically important disease of cucumber (Cucumis sativus L.). 250 leaves per location of Alternaria leaf spot bearing symptoms were obtained during the survey in four districts to assess the status of the disease. Amongst the various districts surveyed the highest mean disease incidence and intensity was recorded in Srinagar, closely followed by Baramulla. Least mean disease incidence was recorded in district Bandipora. Greenhouse trials were conducted during the year 2011 and 2012 to assess the efficacy of five SAR activators, 2,6-dichloroisonicotinic acid (INA), benzothiadazole S-methyl ester (BTH), β -aminobutyric acid (BABA), KHCO_3 and NaHCO_3 applied 24 hrs after spore inoculation. The SAR activators were evaluated against Alternaria alternata. Water was sprayed on control plants. All the SAR activators significantly lowered the disease intensity as compared to control. BABA and INA were most effective with least disease intensity after pathogenic inoculation. This induced resistance exploiting natural defense machinery of plants could be proposed as a non-conventional and eco-friendly approach for plant protection.

Key words: Alternaria leaf spot, Cucumber, SAR Activators, Status

I INTRODUCTION

Cucumber (*Cucumis sativus* Linn.) belongs to the family *Cucurbitaceae* and is one of the oldest cultivated vegetable originated from Southern Asia [1,2]. Globally cucumber production is 71.36 million tonnes and is grown on 2.11 million hectare area with yield value of 33.49 tonnes/ha [3]. The cucumber export and import in Jammu and Kashmir is 218.75 and 5.42 thousand tonnes, respectively [4]. In Kashmir valley cucumber is grown on approximately 640 hectare area [5]. India exports about 24,096 metric tons cucumber each year and shares 1.49 per cent vegetable export [6]. Like many cucurbit crops, cucumber is prone to a number of fungal, bacterial and viral diseases which cause serious economic losses to the crop. Mostly fungal diseases which include downy mildew (*Pseudoperonospora cubensis*); powdery mildew (*Sphaerotheca fuliginea*; *Erysiphae cichoracearum*); anthracnose (*Colletotrichum orbiculare* syn. *C. lagenarium*), Cercospora leaf spot (*Cercospora citrullina*); Alternaria leaf spot (*Alternaria alternata*); damping off (*Pythium* spp.) Fusarium wilt (*Fusarium oxysporum* f.sp. *cucumerinum*) and Phytophthora crown and root rot (*Phytophthora capsici*) inflict

huge economic losses worldwide [7,8]. The *Alternaria* leaf of cucumber previously considered to be of minor significance has now attained the status of major disease [9] and is prevalent in almost all cucumber growing fields of Kashmir valley with a potential threat to existing major cucumber growing areas. Leaf spot disease in cucumber, incited by *Alternaria alternata* f. sp. *cucurbitae*, was recorded for the first time in Crete (Greece) and reportedly appeared as necrotic flecks, surrounded by chlorotic halos, on middle and upper leaves of plants, the flecks enlarged and coalesced to form larger lesions of ≥ 2 cm dia. with brown fructifications of pathogen on their surface [10]. [11,12] gave comprehensive account of distinguishing characters of Indian species of *Alternaria* including *A. alternata*. [13] reported *Alternaria* leaf spot caused by *A. alternata* in *Cucumis melo* var. *momordica* (cv. Local) and *Momordica charantia* (cv. Local) from Punjab. Kashmir valley with temperate environmental conditions is a favourable place for the development of fungal diseases [14]. The development of fungicide resistance in pathogenic populations is one of the most serious threat because changes in the populations of several major plant pathogens in their sensitivity to fungicides have been observed. This leads to significant crop damage and forcing either discontinuation or modification in the use of important chemicals. The frequent and successive use of fungi-toxicants is likely to develop resistance among the pathogen [15,16]. There is increasing concern about the ill consequences of synthetic fungicide use on human health and surrounding ecosystem [17]. Therefore, it is imperative to find alternatives to synthetic fungicides. Certain chemicals like salicylic acid (SA), 2,6-dichloroisonicotinic acid (INA), potassium salts, β -amino butyric acid (BABA) and benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH) reportedly induce systemic acquired resistance (SAR) in plants against some plant pathogens [18,19]. The broad-spectrum activity of BTH compounds reportedly protect several plant species against a number of bacterial, fungal and viral diseases suggesting indirect mode of action via activation of plant defense mechanisms [20,21]. SAR chemicals like salicylic acid (SA), 2,6-dichloroisonicotinic acid (INA), β -amino butyric acid (BABA), benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH), etc., have reportedly been used to induce resistance in several host plants but such approach needs thorough investigation prior to their recommendation for field application [22]. Several studies indicated that the SAR compounds are useful in the management of fungal pathogens [23] with the level of pathogen suppression.

Since the crop and disease are of paramount importance to J & K state (India) and no such studies of status of *Alternaria* leaf spot and management through eco-friendly approaches (SAR activators) have been conducted in the State, therefore the present study was undertaken with the objectives:

To study the status of *Alternaria* leaf spot and to evaluate SAR activators against the disease.

II MATERIALS AND METHODS

The present study was conducted during the years 2011 and 2012 in the Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar (J&K). The materials used and the methodology adopted in achieving the set objectives of the study are described as under:

Disease survey

A field survey for the assessment of Alternaria leaf spot disease of cucumber was conducted in four districts of Kashmir valley viz., Srinagar, Budgam, Bandipora and Baramulla in the months of July and August (peak periods of diseases) in the years 2011 and 2012. Five locations from each selected district were selected. The sites selected in district Baramulla were: i) Sopore, ii) Baramulla, iii) Pattan, iv) Janwara and v) Seelu. The sites selected in district Srinagar were: i) Kawoosa, ii) Dal, iii) Shalimar, iv) Shadipora and v) Harwan while the sites chosen in district Bandipora were: i) Patushai, ii) Nadihal, iii) Kemah, iv) Aloosa and v) Ongam. The sites selected from district Budgam were: i) Batpora, ii) Sheikhpura, iii) Gangbug, iv) Kremshora and v) Narkara. From each location three vegetable fields were chosen and in each fields ten random plants and from each plant twenty five leaves were assessed to record disease incidence and intensity. The intensity was recorded on the same leaves used for recording the incidence. The matereological data with respect to temperature, rainfall and relative humidity for the years 2011 and 2012 (from May to July) was collected from the Division of Agronomy, SKUAST-Kashmir, Shalimar.

Disease incidence

The disease incidence of cucumber was recorded by counting the total number of leaves and the number of infected leaves. The per cent disease incidence was calculated as per formula adopted by [24]:

$$\text{Per cent disease incidence} = \frac{\text{Number of infected leaves}}{\text{Total number of leaves examined}} \times 100$$

Disease intensity

The per cent disease intensity was recorded by visual observation using 0-5 scale adopted by [25]. Five disease categories were made on the basis of per cent leaf area diseased as per following key:

<u>Disease rating scale</u>	<u>Leaf area affected (%)</u>
0	No disease
1	1-10
2	11-25
3	26-50
4	51-75
5	≥76

The per cent disease intensity (PDI) was calculated as per the formula:

$$\text{Per cent disease intensity (PDI)} = \frac{\sum (n \times v)}{N \times S} \times 100$$

Where, \sum = Summation, n = Number of leaves in each category

v = Numerical value of each category N = Number of leaves examined, and

S = Maximum numerical value

Management through SAR chemicals

Five systemic acquired resistance (SAR) chemicals were evaluated for their efficacy under controlled conditions of disease development during the year 2011 and 2012. *viz.* **Bion:** [2,6-dichloroisonicotinic acid (INA)], **Actigard:** [benzothiadazol S-methyl ester (BTH)], **Gamma (FH)** [β -aminobutyric acid (BABA)] **Baking Soda:** [Sodium bicarbonate (NaHCO_3)] and **Aerated salt:** [Potassium bicarbonate (KHCO_3)]

The evaluation of SAR chemicals was carried out on cucumber cultivar 'Japanese Long Green' in a polyhouse at $27\pm 1^\circ\text{C}$. Seeds were sown in pots of 30 cm dia. in the 1st week of April. The concentration of each SAR chemical tested was BTH @ 100 ppm, BABA @ 1000 ppm, INA @ 1000 ppm, NaHCO_3 @ 100 ppm and KHCO_3 @ 100 ppm. A check was also maintained where plants were sprayed with water. The experiment was laid in a completely randomized design with each treatment replicated three times. A single plant represented one replication. The plants were categorized into four groups on the bases of spray schedule *viz.*, i) SAR treatment on cotyledon stage (single spray), ii) SAR treatment at cotyledon stage and a single spray of same chemical 15 days later (two sprays), iii) no treatment at cotyledon stage but two sprays of SAR at 15 day intervals, and iv) no treatment at cotyledon stage but two sprays of SAR at 30 day intervals. In each group check plants (water sprayed) were also maintained. In first group, spray of each test SAR compound was given at cotyledon stage (3-4 leaf stage) on each single plant followed by inoculum spray 24 hour later. The disease intensity was recorded after 30 days. In second group, the spray of each test SAR compound was given at cotyledon stage on each single plant followed by inoculum spray 24 hour later and repeating the spray of test SAR chemical 15 days after cotyledon treatment. The disease intensity in this case was recorded 15 days after each spray. In third group, the first spray of test SAR chemical was given to each single plant 15 days after cotyledon stage followed by inoculum spray 24 hour later and repeating the same chemical spray 15 days after first spray. The disease intensity in this case was recorded 15 days after each spray. In last group, the first spray of each test SAR chemical was given to each single plant 30 days after cotyledon stage followed by inoculum spray 24 hour later and repeating the same chemical spray 30 days after first spray. After each spray inoculum spray was given to test plants. Spore suspension of pathogen responsible for diseases was made on the basis of spore count in haemocytometer i.e., 1×10^4 . The plants were sprayed with the help of an automizer. The intensity of each disease was observed after 30 days of in 1st category; while in 2nd and 3rd category disease intensity was observed two times at 15 day intervals. In 4th category the disease intensity was observed two times but at 30 day intervals as per the method described above.

III RESULT AND DISCUSSION

Survey

The Alternaria disease incidence on cucumber leaves, irrespective of years, varied from 13.3 to 68.6 per cent with higher disease incidence in 2012 (45.6%) than in 2011 (35.5%) [Table 1]. The mean disease incidence over the years varied from 18.3 to 66.0 per cent. In the year 2011 highest disease incidence was observed at Baramulla (64.0%) and lowest at Kemah (13.3%). However, in 2012 the highest disease incidence was at Pattan and Shalimar(68.6%) and lowest at Kemah (23.3%). Amongst the various districts

surveyed the highest mean disease incidence of 56.8 per cent was recorded in Srinagar, closely followed by Baramulla (56.0%) [Fig. 1]. Least mean disease incidence of 24.5 per cent was recorded in district Bandipora. The data revealed that the disease intensity on cucumber leaves, irrespective of the years, varied from 5.7 to 29.8 per cent. The disease intensity was higher in 2012 (16.1%) than in 2011 (13.3%). The mean disease intensity over the years varied from 7.3 to 29.5 per cent. In 2011, the highest disease intensity was observed at Baramulla (30.9%) and lowest at Kemah (5.7%). However, in 2012 the highest disease intensity was at Shalimar (29.8%) and lowest at Kemah (9.0%). The highest mean disease intensity was observed in district Srinagar (24.4%) closely followed by district Baramulla (23.6%) [Fig 2]. Least mean disease intensity of 10.4 per cent was noticed in district Bandipora. The preliminary field survey revealed Alternaria leaf spot as one of the important fungal foliar diseases of cucumber in Kashmir valley as it prevailed in all cucumber growing areas surveyed. The high disease severity during 2012 may be ascribed more conducive temperature, more precipitation, more rainy days and high relative humidity. Similar findings have been reported by [26, 27 and 28]. Alternaria leaf spot occurs on cucumber crop worldwide [29]. Of the locations surveyed, higher disease incidence (66.0%) and intensity (29.1%) was recorded at Baramulla and Pattan locations, respectively, which may be attributed to the practice of maintaining less plant spacing, leading to high relative humidity in plant microclimate, and non-disposal of plant debris. The least disease incidence (18.3%) and intensity (7.3%) was recorded in Kemah of district Bandipora which could be attributed to the better field management practices followed by the growers in the area. The moist weather accompanied by conducive temperature conditions favour leaf spot disease in cucumber [30].

SAR Management

The perusal of Table 2 indicated that Alternaria leaf spot intensity was higher (9.49%) in 2012 than in 2011 (8.61%) [Fig. 3]. Spray of SAR chemicals at cotyledon stage significantly lowered disease intensity as compared to water sprayed check. The disease intensity ranged from 6.83 to 8.66 per cent in SAR chemical treatments in comparison to 15.00 per cent in check indicating that all SAR chemicals were effective in lowering disease intensity. Least disease intensity was noticed in BABA sprayed plants which was followed by INA (disease intensity, 7.00%). These were followed by BTH, KHCO_3 and NaHCO_3 with disease intensity of 7.33, 8.16 and 8.66 per cent, respectively. Chemically-induced SAR has been found effective against various pathogens [31,32].

The study on the effect of SAR chemicals at cotyledon stage and 15 days later revealed that the overall disease intensity was higher (6.49%) in 2012 than in 2011 (5.83%) [Table 3, Fig. 4]. SAR chemical sprays significantly lowered disease intensity (4.50-6.33%) as compared to water sprayed check (15.50%) with least disease intensity in BABA sprayed plants. This was followed by INA, BTH, KHCO_3 and NaHCO_3 , with disease intensity of 4.66, 5.33, 5.83 and 6.33 per cent, respectively. BABA, BTH and INA sprays were at par with one another.

The effect of two spray SAR chemicals, 1st spray 15 days after cotyledon stage and 2nd spray 15 days later, indicated that all SAR treatments significantly lowered Alternaria disease intensity as compared to check (Table 4, Fig. 5). The disease intensity ranged from 23.66 to 30.00 per cent in SAR chemical treatments as compared to 39.83 per cent in check. Least disease intensity was noticed in BABA treatment which was at par with INA

having 24.50 per cent disease intensity. These were followed by BTH, KHCO_3 and NaHCO_3 with *Alternaria* leaf spot intensity of 25.66, 28.33 and 30.00 per cent, respectively.

The perusal of Table 5 revealed that all the SAR chemicals sprayed at 30 day intervals starting from 30 days after cotyledon stage significantly lowered *Alternaria* leaf spot disease intensity as compared to check (Fig. 6). The disease intensity was higher (44.05%) in 2012 than in 2011 (41.94%). In SAR spray treatments the disease intensity ranged from 31.66 to 47.33 per cent as compared to 61.33 per cent in check indicating that all SAR activators were effective in lowering disease intensity. BTH spray was most effective in minimizing powdery mildew intensity (31.66%), followed by NaHCO_3 (35.00%), KHCO_3 (39.33%), INA (42.66%) and BABA (47.33%), respectively.

SAR chemicals tested at various growth stages against *Alternaria* leaf spot disease revealed BABA and INA as the most effective SAR inducers which is in agreement with [33] and [34] found BABA as an effective inducer of resistance against *Alternaria* leaf blight pathogen, followed by INA, than other SAR chemicals. Other chemicals viz., BTH, NaHCO_3 and KHCO_3 also effectively minimized disease but were not superior to BABA and INA. Sodium bicarbonate and potassium bicarbonate have curative property and effectively induce plant resistance when in direct contact with pathogen [35, 36]. KHCO_3 was found effective against various fungal diseases including *Alternaria* pathogen [37, 38].

Table 1: Incidence and intensity of *Alternaria* leaf spot (*Alternaria alternata*) on cucumber leaves at various locations of Kashmir during the years 2011 and 2012

District	Location	Disease incidence (%)			Disease intensity (%)		
		2011	2012	Mean	2011	2012	Mean
Baramulla	Sopore	33.3	48.0	40.6	14.4	18.8	16.6
	Baramulla	64.0	68.0	66.0	30.9	27.7	29.3
	Pattan	63.3	68.6	65.9	30.5	28.6	29.5
	Janwara	53.3	66.6	59.9	22.6	25.6	24.1
	Seelu	44.0	51.3	47.6	15.0	22.2	18.6
Mean		51.8	60.5	56.0	22.6	24.5	23.6
Srinagar	Kawoosa	54.6	63.3	58.9	23.2	27.0	25.1
	Dal	51.3	55.3	53.3	22.5	23.0	22.7
	Shalimar	52.0	68.6	60.3	23.3	29.8	26.5
	Shadipora	42.0	63.3	52.6	14.5	28.5	21.5
	Harwan	51.3	66.6	56.8	23.4	28.8	26.1

Mean		50.2	63.4	56.8	21.3	27.4	24.4
Bandipora	Patushai	27.3	34.6	30.9	12.0	13.0	12.5
	Nadihal	22.0	30.0	26.0	8.5	12.6	10.5
	Kemah	13.3	23.3	18.3	5.7	9.0	7.3
	Aloosa	15.3	29.3	22.3	5.8	15.6	10.7
	Ongam	20.6	29.3	24.9	6.0	16.4	11.2
Mean		19.7	29.4	24.5	7.6	13.3	10.4
Budgam	Batpora	28.0	37.3	32.6	12.4	14.6	13.5
	Sheikhpora	22.0	31.3	26.6	11.2	13.0	12.1
	Gangbug	27.3	32.6	29.9	11.8	13.6	12.7
	Kremshore	19.3	31.3	25.3	8.6	13.0	10.8
	Narkara	19.3	24.6	21.9	9.4	11.5	10.4
Mean		23.1	31.4	27.3	10.6	13.1	11.9
Overall mean		35.5	45.6	40.4	13.3	16.1	14.8

Table 2: Effect of single spray of SAR chemicals sprayed at cotyledon stage on disease severity of Alternaria leaf spot

SAR chemicals (ppm)	Disease severity (%)*		
	2011	2012	Mean
BTH @ 100	7.00 (2.64)	7.66 (2.76)	7.33 (2.70)
BABA @ 1000	6.00 (2.44)	7.66 (2.76)	6.83 (2.61)
NaHCO ₃ @ 100	7.66 (2.77)	9.66 (3.10)	8.66 (2.94)
INA @ 1000	6.00 (2.44)	8.00 (2.82)	7.00 (2.65)
KHCO ₃ @ 100	7.66 (2.76)	8.66 (2.94)	8.16 (2.85)
Control (water spray)	14.66 (3.82)	15.33 (3.91)	15.00 (3.87)
Mean	8.16	9.49	8.83
CD(P=0.05)	(0.70)	(0.45)	(0.43)

*Mean of three replication; figures within parenthesis are square root transformed values

Table 3: Effect of two sprays of SAR chemicals (at cotyledon stage and 15 days later) on disease severity of Alternaria leaf spot

SAR chemicals (ppm)	Disease severity (%)*					
	Cotyledon stage			After 15 days		
	2011	2012	Mean	2011	2012	Mean
BTH @ 100	3.33 (1.82)	4.33 (2.08)	3.83 (1.95)	5.00 (2.23)	5.66 (2.37)	5.33 (2.30)
BABA @ 1000	2.33 (1.52)	4.66 (2.15)	3.50 (1.87)	4.33 (2.08)	4.66 (2.15)	4.50 (2.12)
NaHCO ₃ @ 100	3.33 (1.82)	5.66 (2.37)	4.50 (2.12)	6.66 (2.52)	6.00 (2.44)	6.33 (2.51)
INA @ 1000	3.00 (1.73)	4.33 (2.08)	3.67 (1.91)	4.00 (2.00)	5.33 (2.30)	4.66 (2.15)
KHCO ₃ @ 100	3.66 (1.91)	4.66 (2.15)	4.16 (2.03)	5.33 (2.30)	6.33 (2.51)	5.83 (2.41)
Control (water spray)	5.66 (2.37)	6.33 (2.51)	6.00 (2.44)	15.33 (3.91)	15.66 (3.95)	15.50 (3.93)
Mean	3.55	4.99	4.27	6.77	7.27	7.02
CD(P=0.05)	(0.39)	(0.38)	(0.41)	(0.25)	(0.46)	(0.28)

*Mean of three replication; figures within parenthesis are square root transformed values

Table 4: Effect of two sprays of SAR chemicals sprayed at 15 day intervals on disease severity of Alternaria leaf spot

SAR chemicals (ppm)	Disease severity (%)*					
	First spray			Second spray		
	2011	2012	Mean	2011	2012	Mean
BTH @ 100	16.00 (4.00)	18.66 (4.31)	17.33 (4.16)	25.00 (5.00)	26.33 (5.13)	25.66 (5.06)
BABA @ 1000	16.00 (4.00)	17.33 (4.16)	16.66 (4.08)	23.00 (4.79)	24.33 (4.93)	23.66 (4.86)
NaHCO ₃ @ 100	19.00 (4.35)	20.33 (4.50)	19.66 (4.43)	28.66 (5.35)	31.33 (5.59)	30.00 (5.47)
INA @ 1000	15.33 (3.91)	15.33 (4.91)	15.83 (3.97)	24.33 (4.93)	24.66 (4.96)	24.50 (4.94)
KHCO ₃ @ 100	18.00 (4.24)	18.66 (4.31)	18.33 (4.28)	28.00 (5.29)	28.66 (5.35)	28.33 (5.32)
Control (water spray)	26.00	27.33	26.00	39.33	40.33	39.83

	(5.09)	(5.22)	(5.09)	(6.27)	(6.35)	(6.31)
Mean	18.38	19.60	18.96	28.05	29.27	28.66
CD(P=0.05)	(0.33)	(0.32)	(0.632)	(0.39)	(0.31)	(0.23)

*Mean of three replication; figures within parenthesis are square root transformed values

Table 5: Effect of two sprays of SAR chemicals sprayed at 30 day intervals on disease severity of Alternaria leaf spot

SAR chemicals (ppm)	Disease severity (%)*					
	First spray			Second spray		
	2011	2012	Mean	2011	2012	Mean
BTH @ 100	17.00 (4.12)	18.33 (4.28)	17.66 (4.20)	30.00 (5.47)	31.33 (5.59)	30.66 (5.53)
BABA @ 1000	16.00 (4.00)	17.33 (4.16)	16.66 (4.08)	26.66 (5.16)	28.33 (5.32)	27.49 (5.24)
NaHCO ₃ @ 100	19.66 (4.43)	21.00 (4.58)	20.33 (4.50)	35.00 (5.91)	36.33 (6.02)	35.66 (5.97)
INA @ 1000	16.00 (4.00)	16.66 (4.08)	16.33 (4.04)	29.00 (5.38)	29.66 (5.44)	29.33 (5.41)
KHCO ₃ @ 100	17.66 (4.20)	18.33 (4.28)	18.00 (4.24)	31.00 (5.56)	31.66 (5.62)	31.33 (5.59)
Control (water spray)	25.00 (5.00)	26.33 (5.13)	25.66 (5.06)	48.00 (6.92)	48.66 (6.97)	48.33 (6.95)
Mean	18.55	19.66	19.10	33.27	34.32	33.80
CD(P=0.05)	(0.54)	(0.47)	(0.23)	(0.36)	(0.24)	(0.17)

*Mean of three replication; figures within parenthesis are square root transformed values

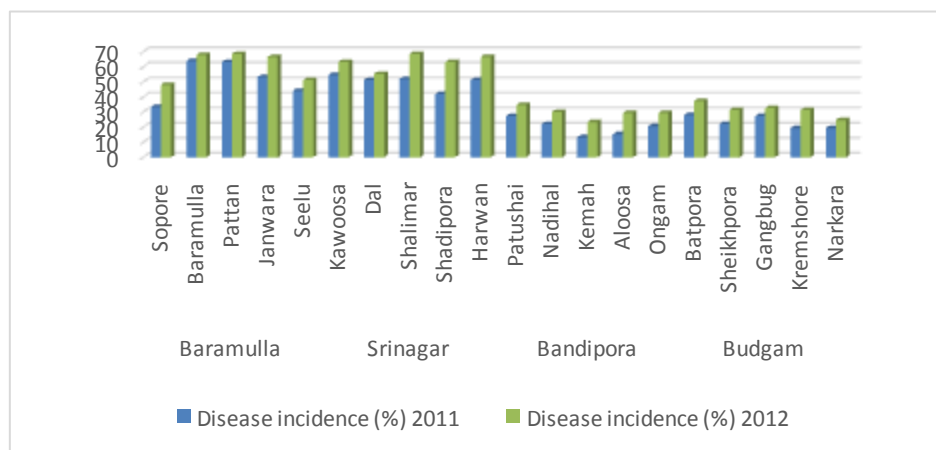


Fig.1: Incidence of Alternaria leaf spot(*Alternaria alternata*)on cucumber leaves at various locations of Kashmir during the years2011 and 2012

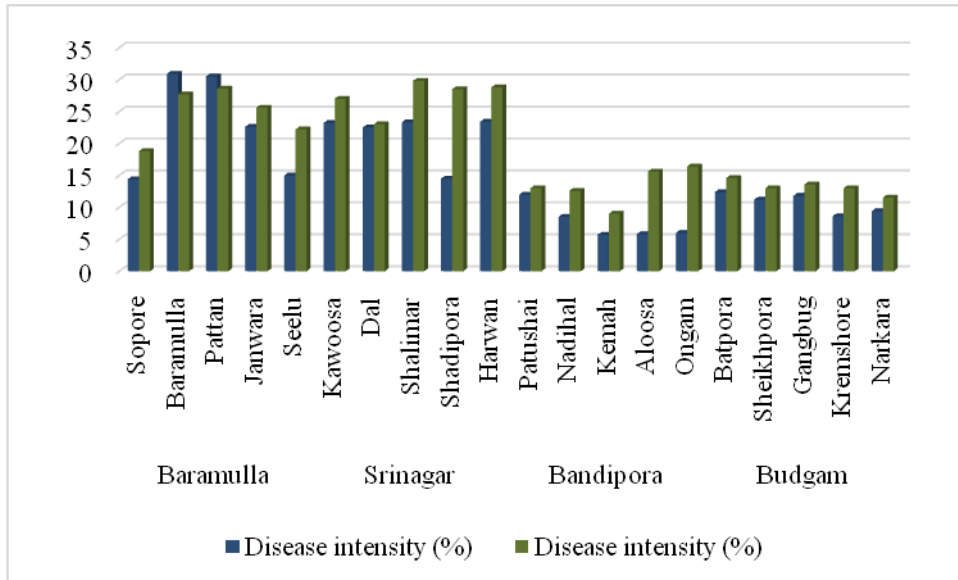


Fig.2: Intensity of Alternaria leaf spot(*Alternaria alternata*)on cucumber leaves at various locations of Kashmir during the years2011 and 2012

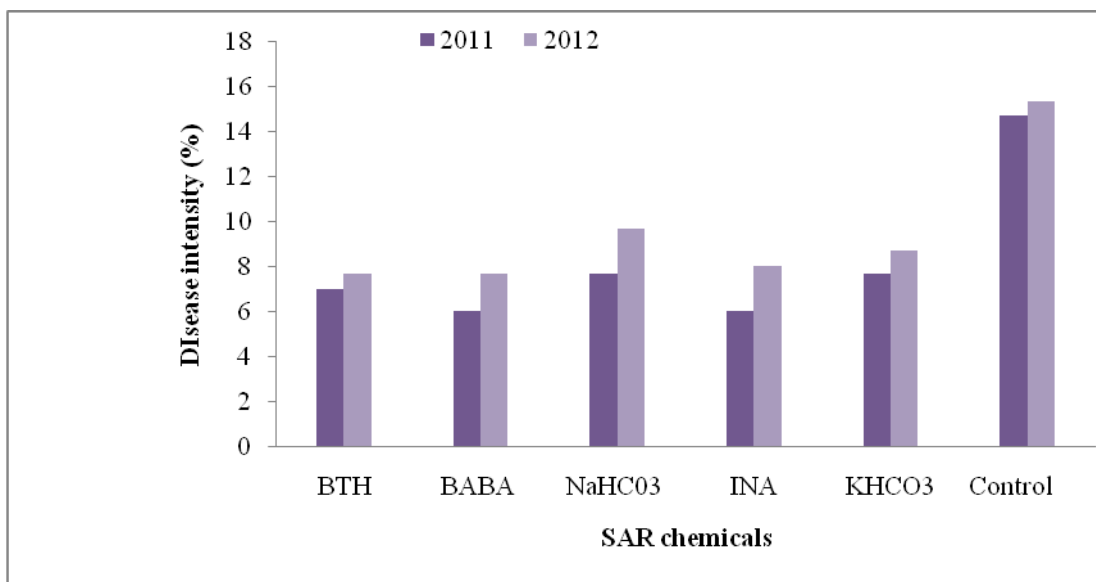


Fig. 3: Effect of single spray of SAR chemical compounds at cotyledon stage on disease severity of Alternaria leaf spot

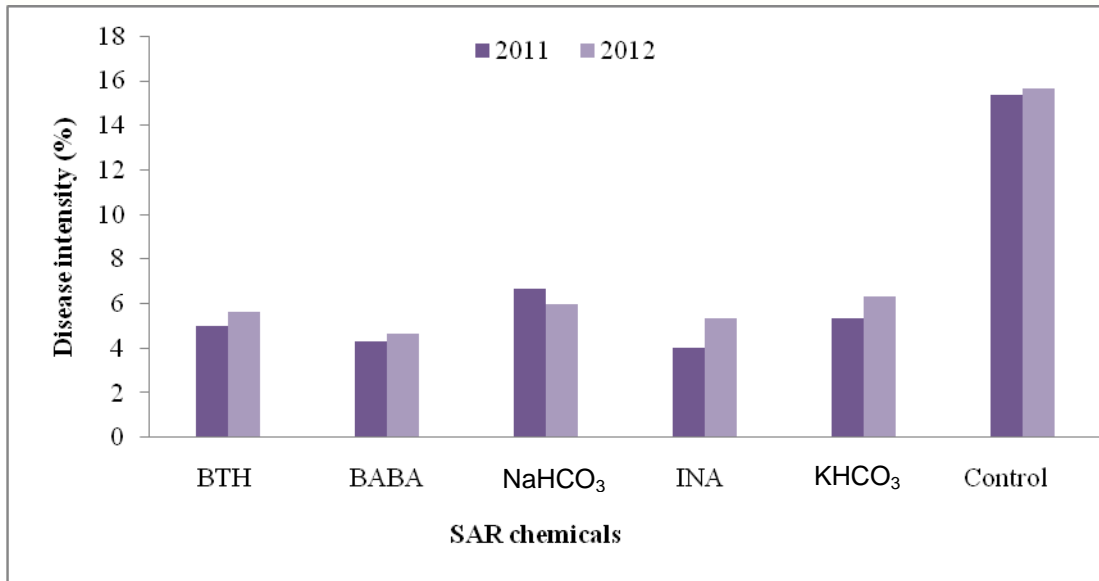


Fig. 4: Effect of two sprays of SAR chemicals (at cotyledon stage and 15 days later) on disease severity of Alternaria leaf spot

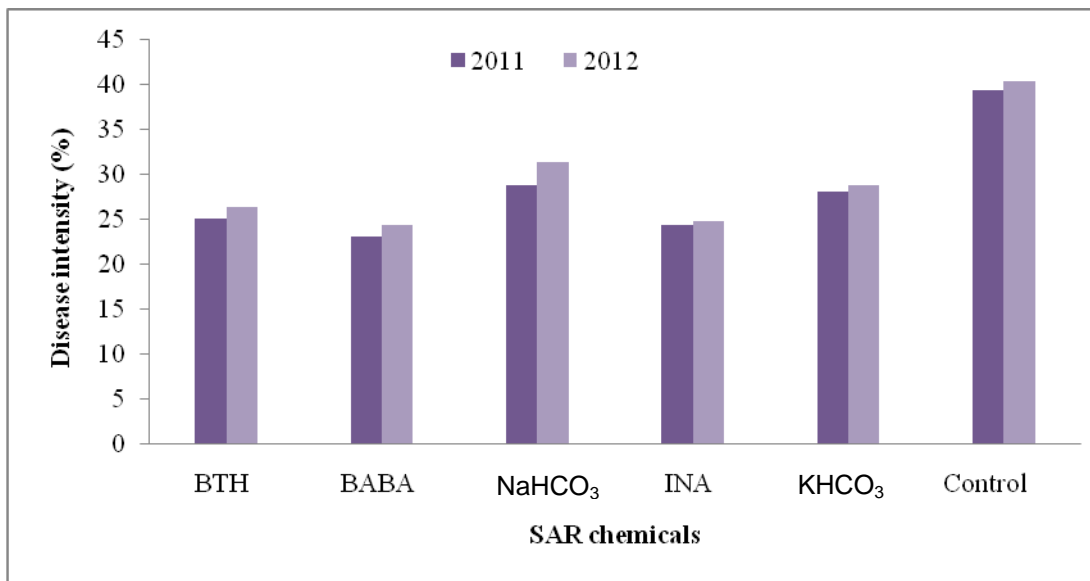


Fig. 5: Effect of two sprays SAR chemical compounds at 15 days interval on disease severity of Alternaria leaf spot

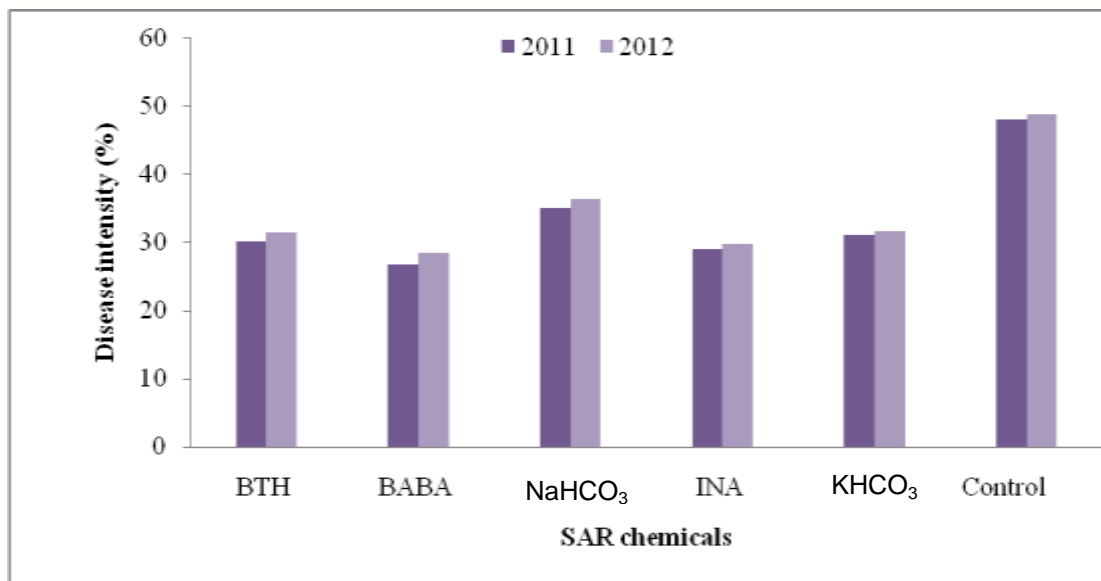


Fig. 6: Effect of two sprays SAR chemical compounds at 30 days interval on disease severity of Alternaria leaf spot

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