



Effect of Exogenous application of Alkali Compounds on the Aggressiveness of *Sclerotinia sclerotiorum*

PRESENTATION BY

Prabhjodh Sandhu

Principal Plant Pathologist

Punjab Agricultural University, Punjab,

India

Introduction

- *Sclerotinia sclerotiorum*, necrotrophic pathogen with a wide host range
 - Yield losses up to 40% in Rapeseed-mustard in India (Technical bulletin, NCIPM 2012)
 - *S. sclerotiorum* exhibits some mechanisms to modify the ambient conditions to make extracellular pH more suited for pathogenicity and suppresses the host defense.
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- Some alkali salts were evaluated for their effect on *S. sclerotiorum* pathogenicity and an alternative method of management of the disease.
 - A set of *S. sclerotiorum* isolates for their pathogenic ability, associated pathogenicity factors, and related genes were also studied.



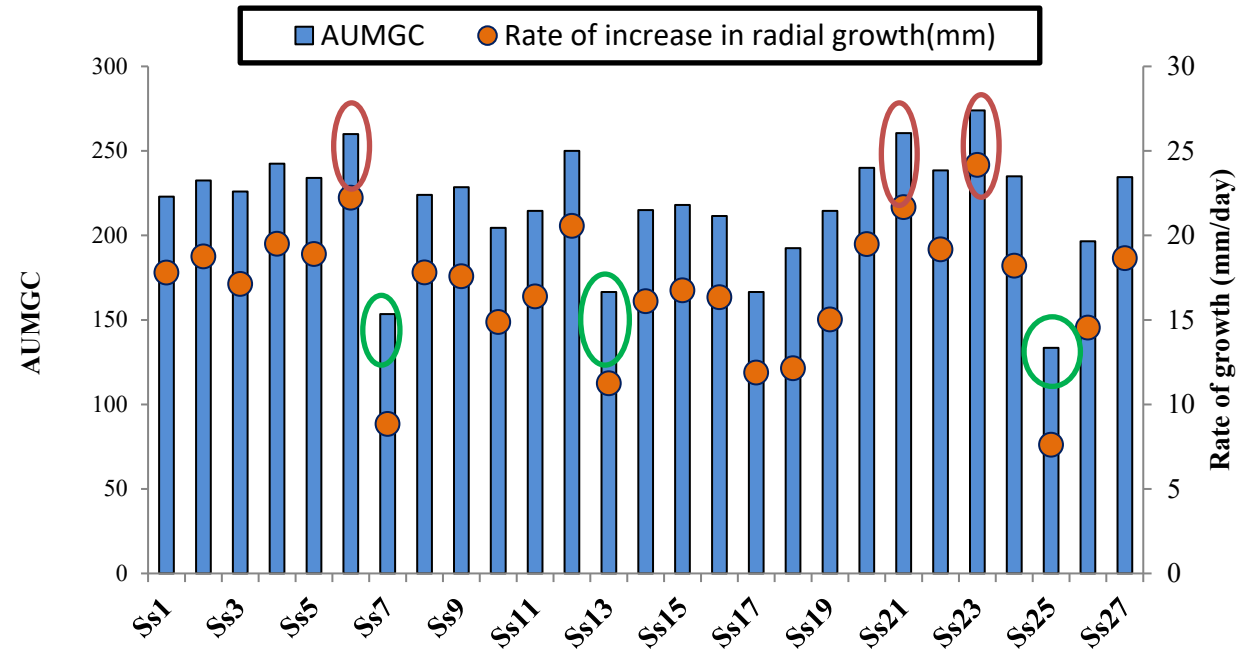
Pathological behaviour of isolates of *Sclerotinia sclerotiorum* under field conditions



State	District	Place	Isolates
Punjab	Ludhiana	PAU	Ss1
		PAU(<i>B.napus</i>)	Ss2
		Rajoana	Ss3
	Bathinda	Bathinda	Ss4
		Maluka	Ss5
	Moga	Malke	Ss6
		Singhawala	Ss7
	Patiala	Sehajpur Kalan	Ss8
	Gurdaspur	Bhumbli	Ss9
		Sathiali	Ss10
	Hoshiarpur	Badesron	Ss11
	Amritsar	Rampur	Ss12
Haryana	Rewari	Bawal	Ss13
	Hisar	Hisar	Ss14
	Jhajjar	Beri	Ss15
Rajasthan	Bharatpur	Bharatpur 1	Ss16
		Bharatpur 2	Ss17
		Hantra	Ss18
		Salabad	Ss19
		Chhonkarwara	Ss20
	Jaipur	Mandha	Ss21
Jodhpur	Shergarh	Ss22	
Jammu	Kathua	Kathua	Ss23
Uttarkhand	Udham Singh Nagar	Pant nagar	Ss24
Uttar Pradesh	Ghaziabad	Bheem nagar	Ss25
West Bengal	Nadia	Habibpur	Ss26
	Murishidabad	Behrampore	Ss27

Isolates from seven mustard growing states, including eighteen districts were surveyed and twenty seven isolates of *Sclerotinia sclerotiorum* were collected

In vitro evaluation of Ss isolates



- ✓ The **high** AUMGC and rate of increase in radial growth was shown by Ss23, Ss21 and Ss6
- ✓ The **low** AUMGC and rate of increase in radial growth was shown by Ss25, Ss7 and Ss13

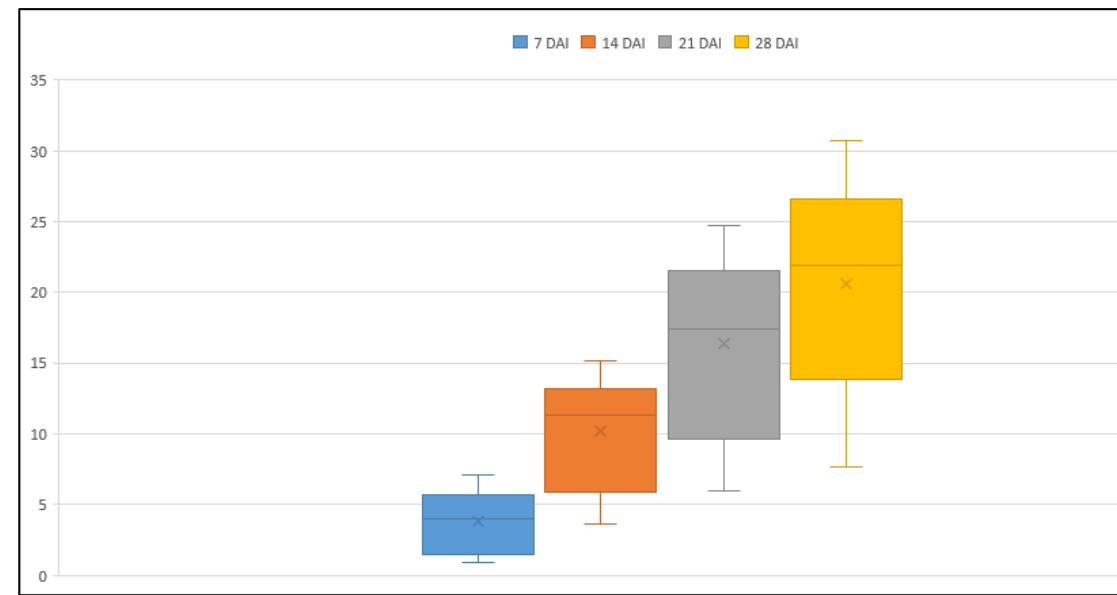
In vivo evaluation

Susceptible *Brassica juncea* variety **RL 1359** was inoculated with different isolates at flowering stage using standard stem inoculation technique.

Lesion length was recorded at 7, 14, 21 and 28 days after inoculation.

Mean lesion length, rate of increase in lesion length and Area under Disease Progression Curve (AUDPC) were calculated to see variability in aggressiveness of isolates.

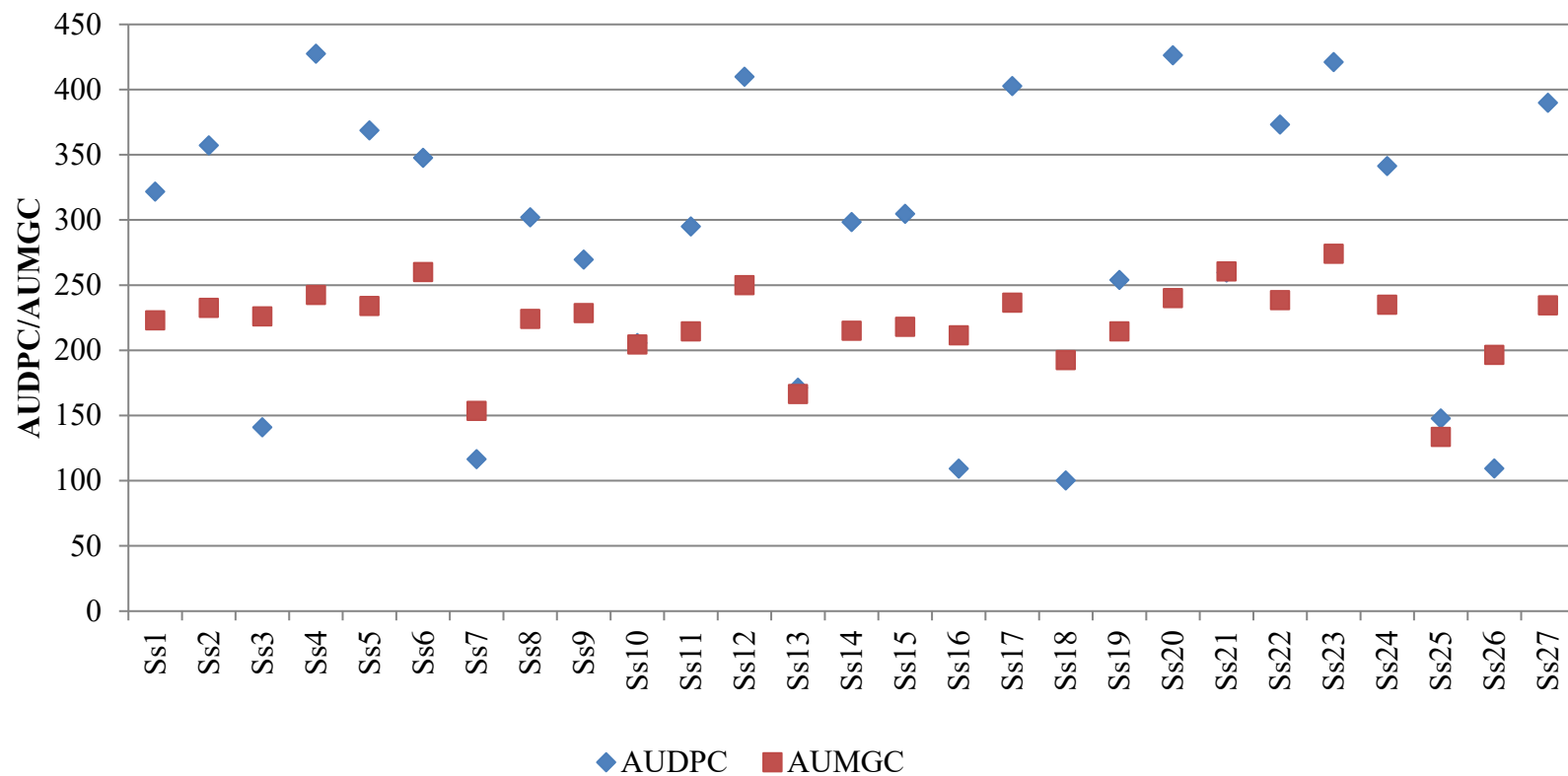
- ✓ The **high** AUDPC and rate of increase in lesion length was shown by **Ss4, Ss20** and **Ss23**
- ✓ The **low** AUDPC and rate of increase in lesion length was shown by **Ss16, Ss18** and **Ss26**



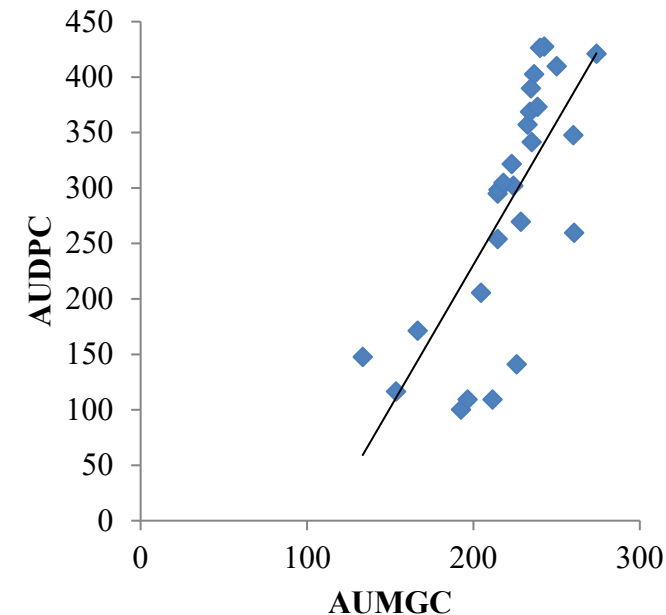
Box plot representation of mean lesion length produced by *S. sclerotiorum* isolates

Summary Statistics	7 DAI (cm)	14 DAI (cm)	21 DAI (cm)	28 DAI (cm)
Minimum	0.88	3.64	5.95	7.64
Maximum	7.06	15.12	24.72	30.75
Range	6.17	11.48	18.76	23.10
Mean	3.82	10.14	16.33	20.55
Median	3.97	11.29	17.39	21.93

AUMGC and AUDPC values shown of *S. sclerotiorum* isolates



Correlation



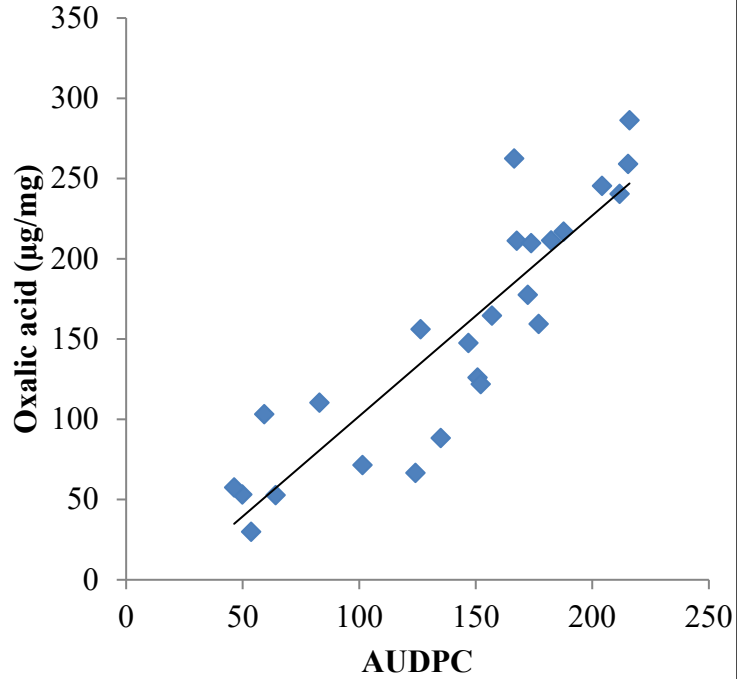
RESULTS

- ✓ The highest and lowest AUMGC and rate of increase in radial growth was shown by **Ss23** and **Ss25** respectively.
- ✓ The highest and lowest AUDPC and rate of increase in lesion length was shown by **Ss4** and **Ss18** respectively
- ✓ Positive correlation (**0.74**) is seen between AUMGC and AUDPC

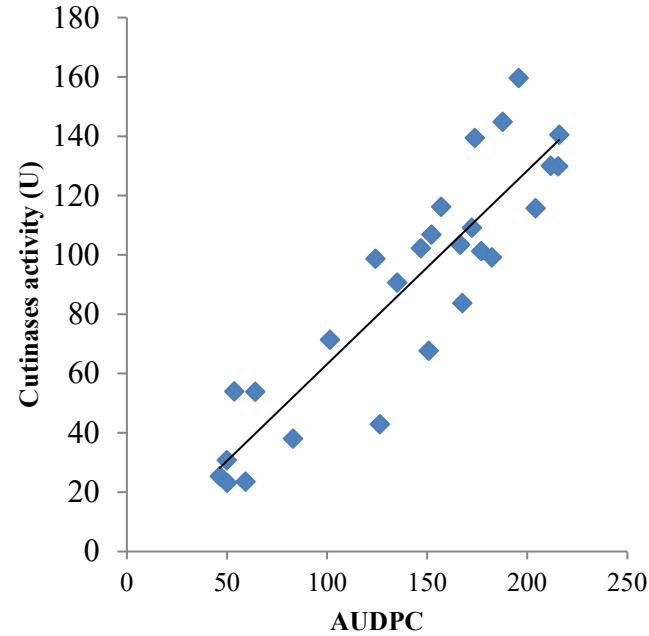
Biochemical factors responsible for aggressiveness of *S. sclerotiorum*



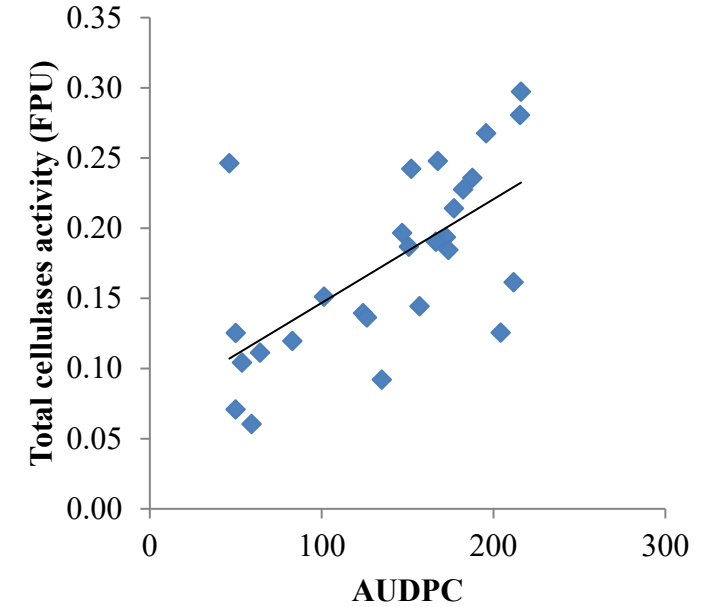
Oxalic acid production by *S. sclerotiorum* isolates



Cutinases production by *S. sclerotiorum* isolates



Total cellulases production by *S. sclerotiorum* isolates



RESULTS

Positive correlation (**0.89**) is seen between oxalic acid production and AUDPC

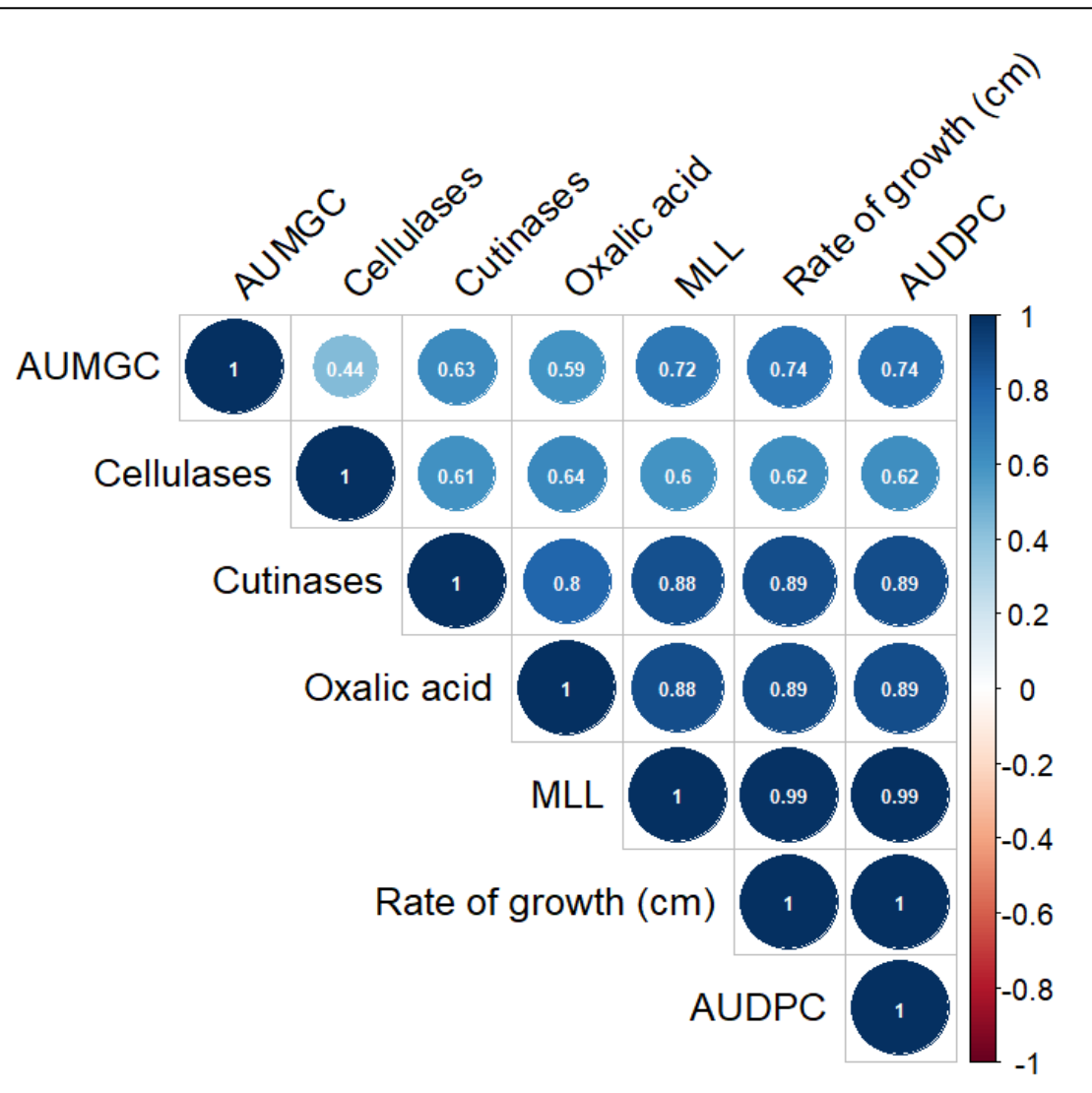
RESULTS

Positive correlation (**0.89**) is seen between total cutinases activity and AUDPC

RESULTS

Positive correlation (**0.62**) is seen between total cellulases activity and AUDPC

Correlation between aggressiveness factors



Cluster analysis using aggressiveness factors

- ✓ k-means clustering was done
- ✓ AUMGC, AUDPC, mean lesion length, Oxalic acid production, total cellulases activity and cutinases activity were the aggressiveness factors considered

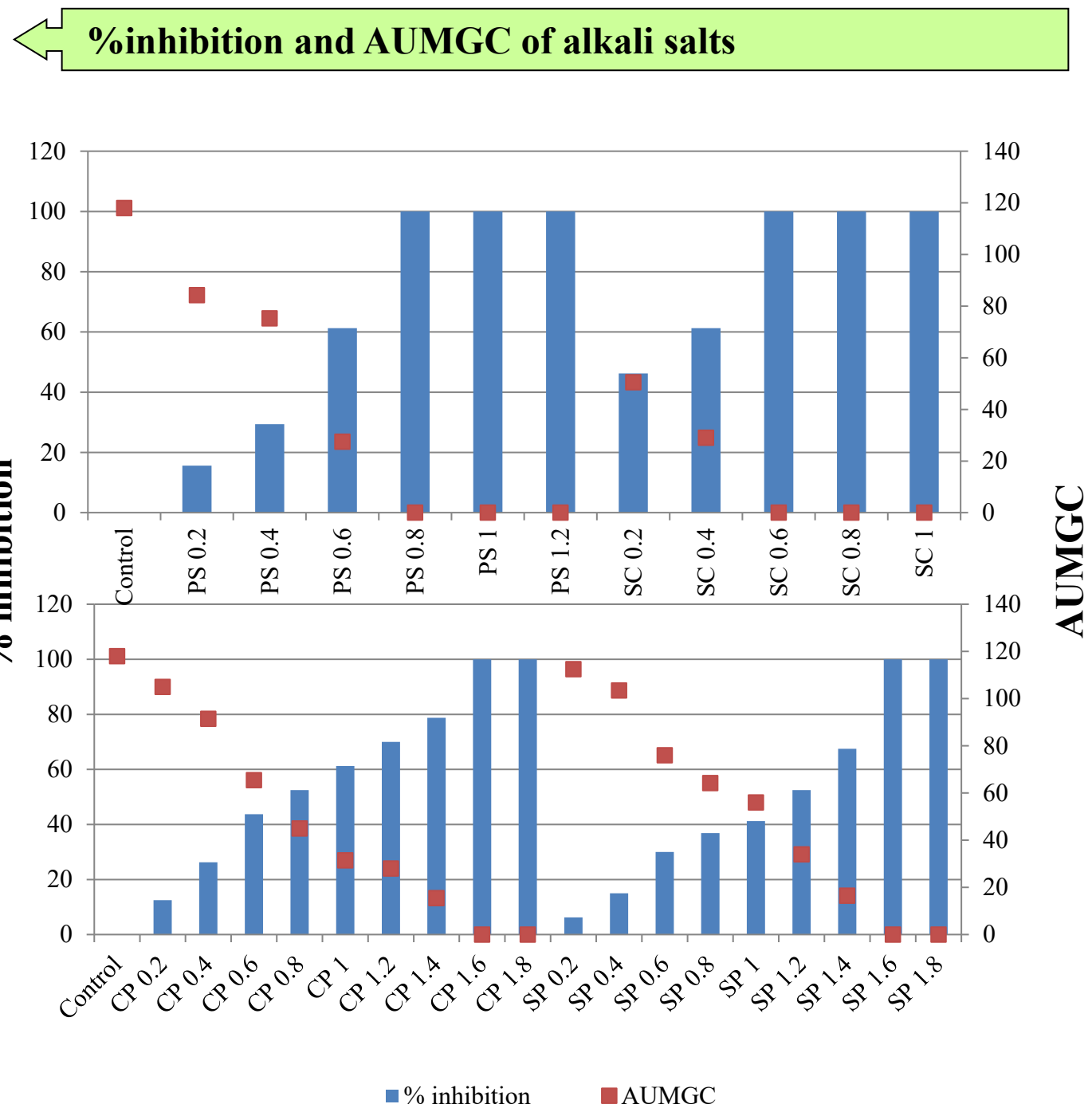
Categories based on cluster analysis

Aggressiveness	Isolates
Low (08)	Ss3, Ss7, Ss10, Ss13, Ss16, Ss18, Ss25, Ss26
Moderate (10)	Ss1, Ss2, Ss6, Ss8, Ss9, Ss11, Ss14, Ss15, Ss19, Ss21
High (09)	Ss4, Ss5, Ss12, Ss17, Ss20, Ss22, Ss23, Ss24, Ss27


















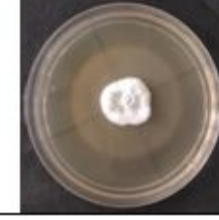



In vitro bio-efficacy of different alkali salts against *S. sclerotiorum*

Treatments		% inhibition	AUMGC
Control		0.00	118.00
Potassium Silicate	0.2%	15.63	84.25
	0.4%	29.38	75.25
	0.6%	61.25	27.50
	0.8%	100.00	0.00
	1.0%	100.00	0.00
	1.2%	100.00	0.00
Sodium Carbonate	0.2%	46.25	50.50
	0.4%	61.25	29.00
	0.6%	100.00	0.00
	0.8%	100.00	0.00
	1.0%	100.00	0.00
Calcium Propionate	0.2%	12.50	105.00
	0.4%	26.25	91.50
	0.6%	43.75	65.50
	0.8%	52.50	45.00
	1.0%	61.25	31.50
	1.2%	70.00	28.00
	1.4%	78.75	15.50
	1.6%	100.00	0.00
Sodium Propionate	0.2%	9.38	112.50
	0.4%	15.00	103.50
	0.6%	30.00	76.00
	0.8%	36.88	64.25
	1.0%	41.25	56.00
	1.2%	52.50	34.00
	1.4%	67.50	16.50
	1.6%	100.00	0.00
	1.8%	100.00	0.00



Poisoned food technique

	0.2%	0.4%	0.6%	0.8%	1.0%	1.2%	1.4%	1.6%
Potassium silicate				Complete Inhibition	Complete Inhibition	Complete Inhibition	Complete Inhibition	Complete Inhibition
Sodium carbonate			Complete Inhibition	Complete Inhibition	Complete Inhibition	Complete Inhibition	Complete Inhibition	Complete Inhibition
Sodium propionate								Complete Inhibition
Calcium propionate								Complete Inhibition



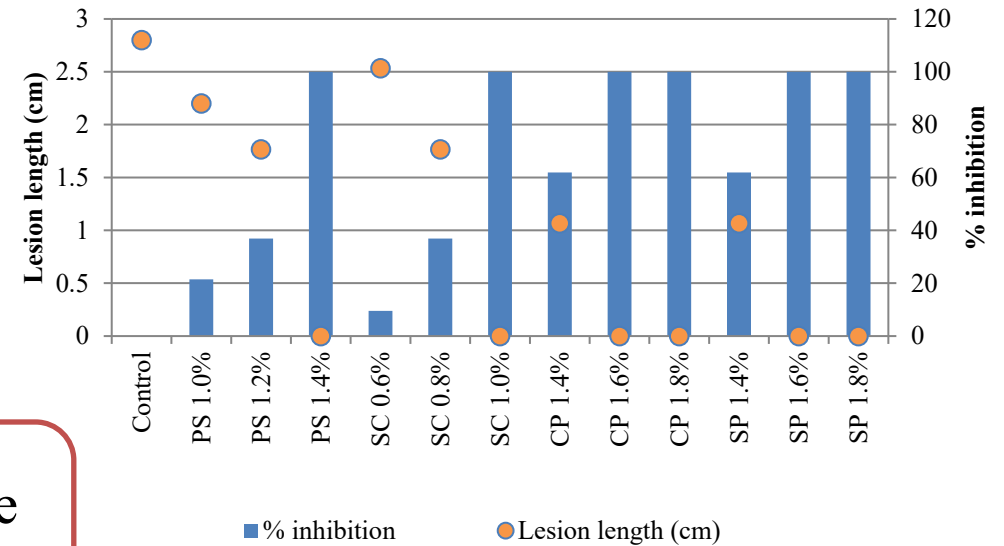
Control

RESULTS

- ✓ The PDA media amended with **sodium carbonate** (0.6% to 1%), **potassium silicate** (0.8% to 1.2%), **calcium propionate** (1.6% and 1.8%), and **sodium propionate** (1.6% and 1.8%) showed **complete mycelial growth inhibition** when compared with unamended PDA medium.

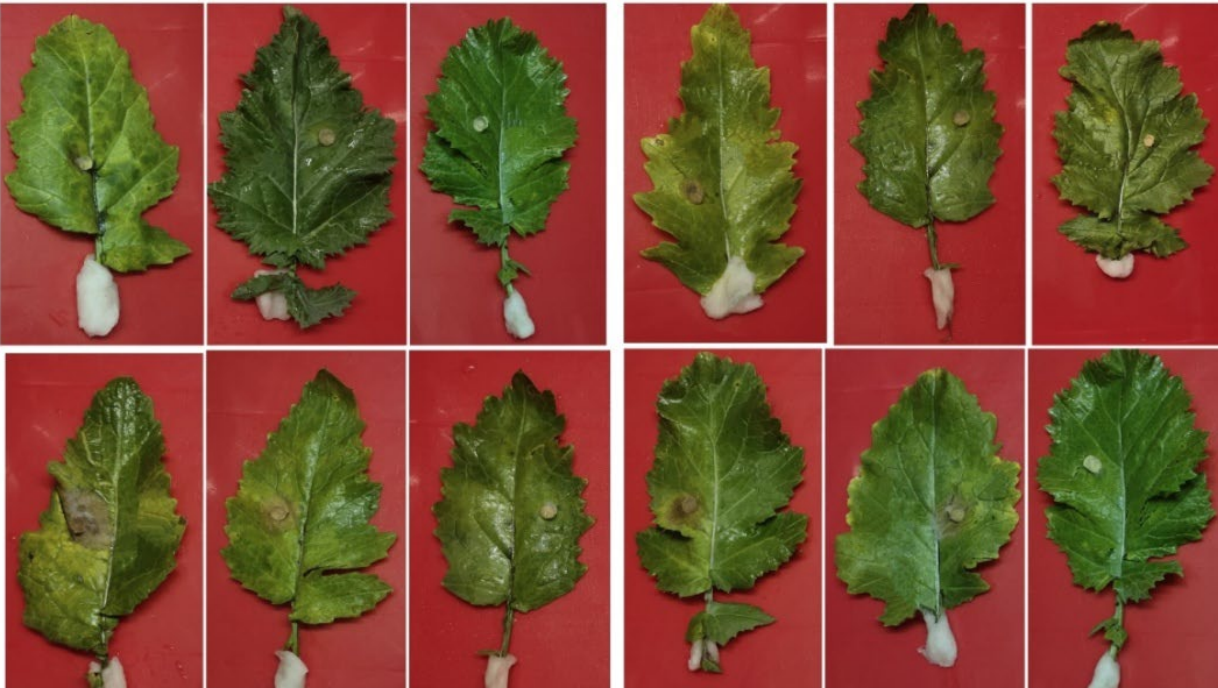
Detached leaf assay

Lesion length and %inhibition of alkali salts on



Effective concentration of alkali salts in poisoned food technique was sprayed on to leaves of *Brassica juncea* cv. **RL1359**

Effect of alkali salts on *S. sclerotiorum* on leaves



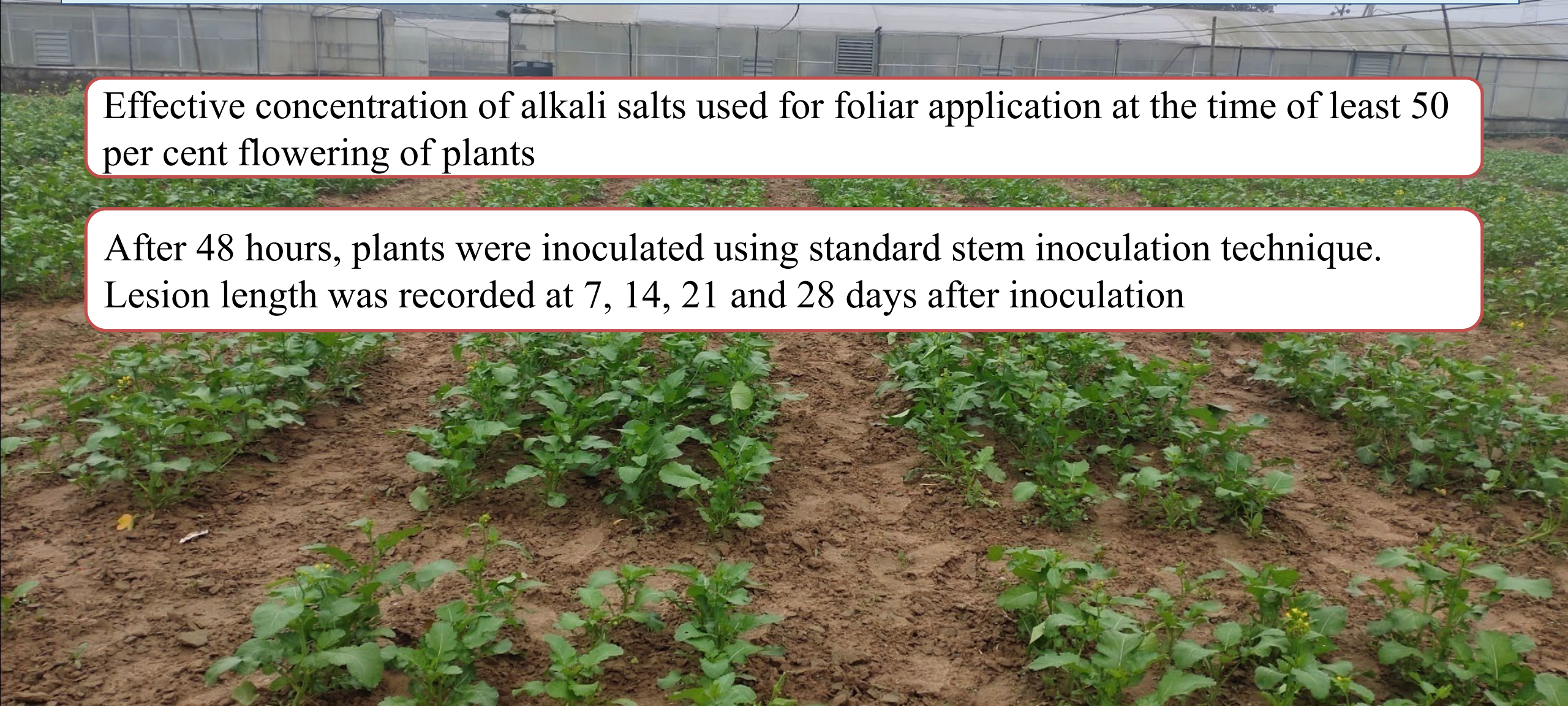
RESULTS

- ✓ Sodium carbonate @ 1%, potassium silicate @ 1.4%, sodium propionate @ 1.8% and calcium propionate @ 1.8% were effective in inhibiting lesion development of pathogen when compared to untreated leaves.

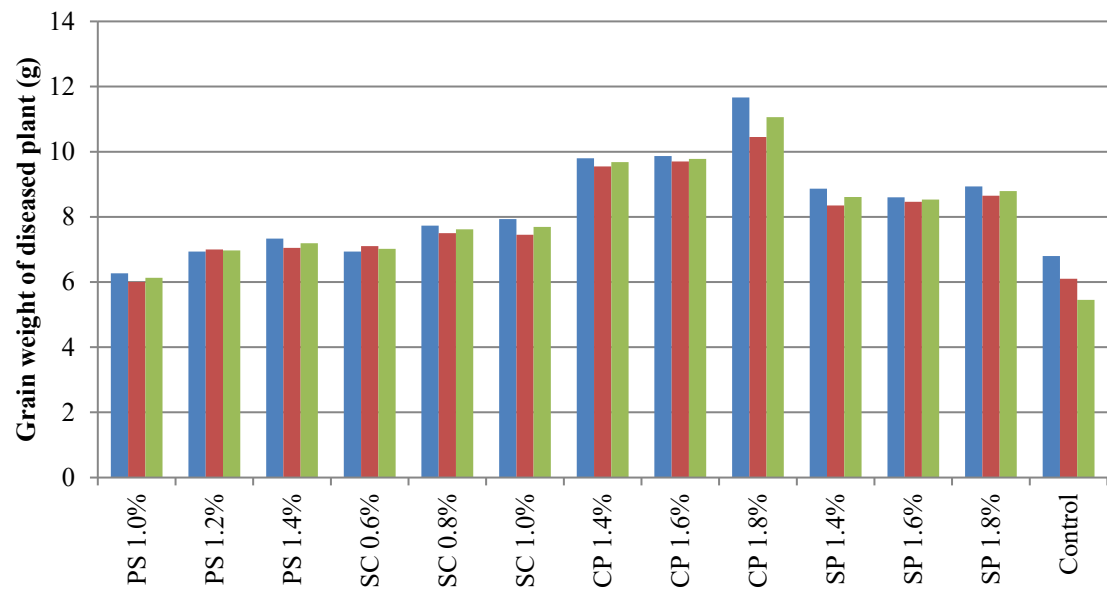
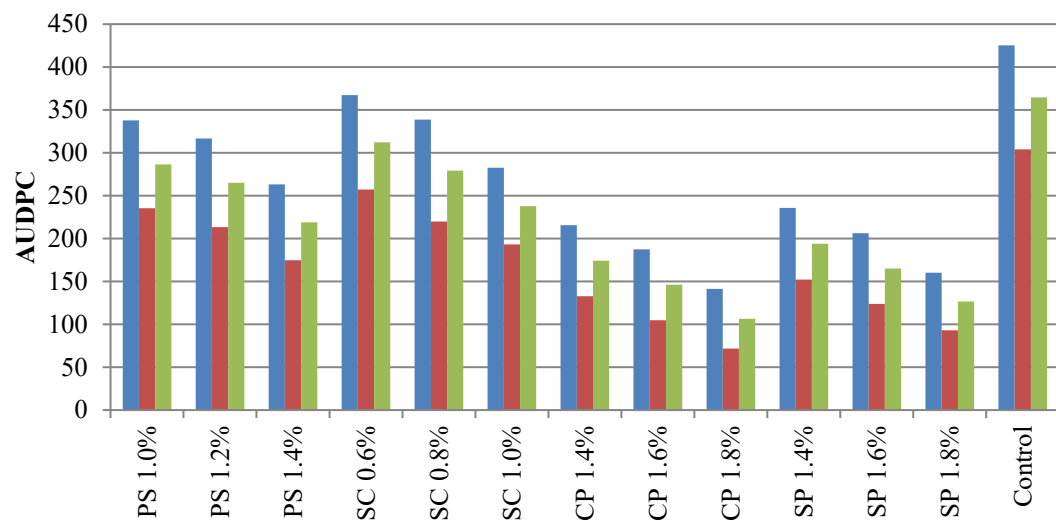
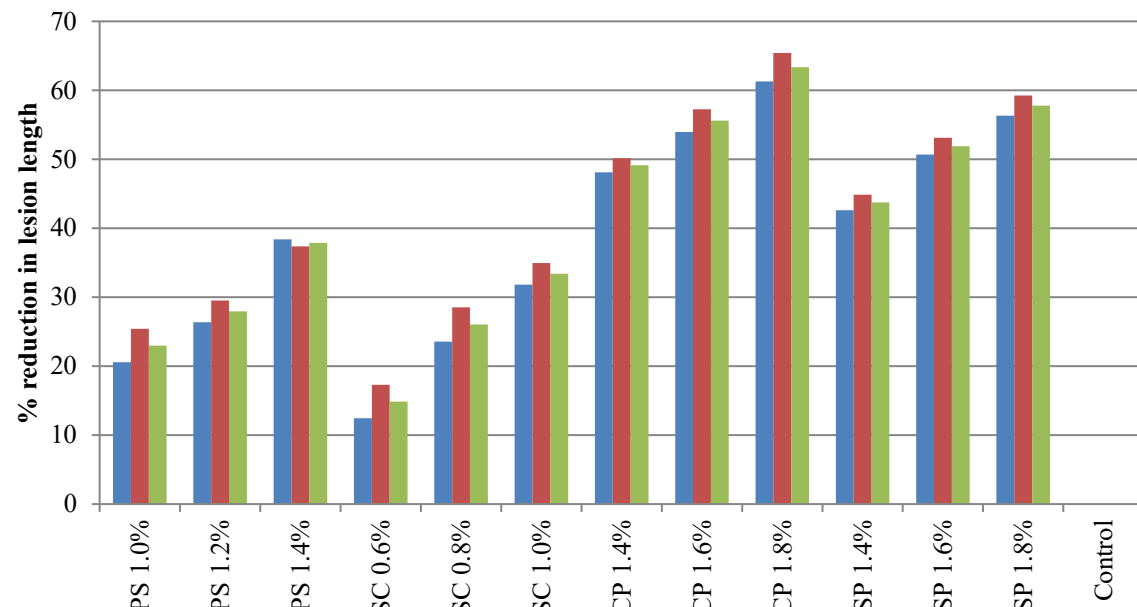
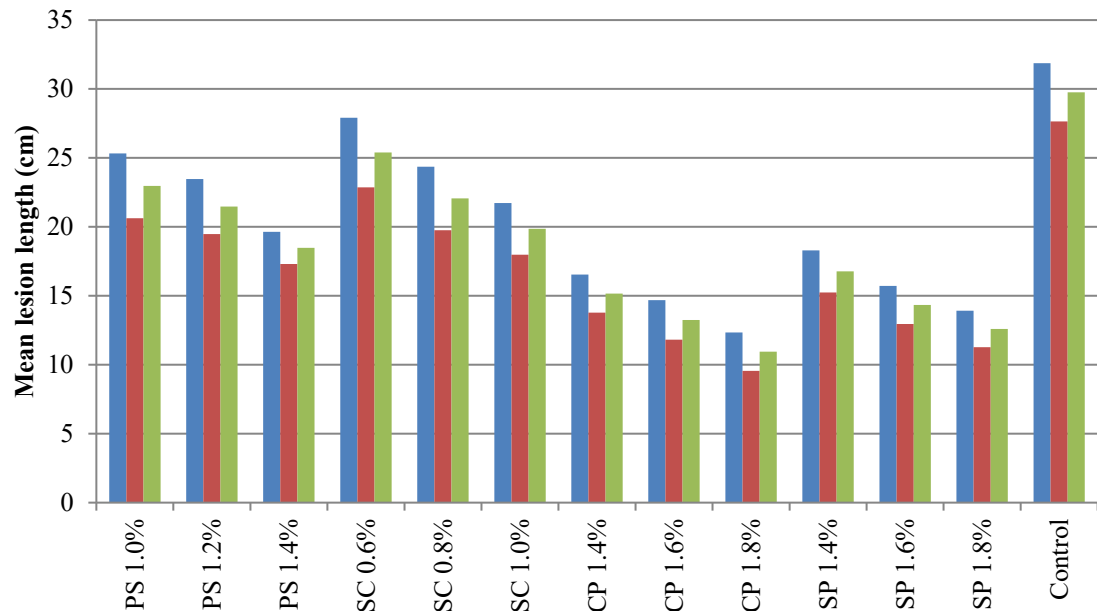
Bio-efficacy of different alkali salts against *S. sclerotiorum* under field conditions

Effective concentration of alkali salts used for foliar application at the time of least 50 per cent flowering of plants

After 48 hours, plants were inoculated using standard stem inoculation technique. Lesion length was recorded at 7, 14, 21 and 28 days after inoculation

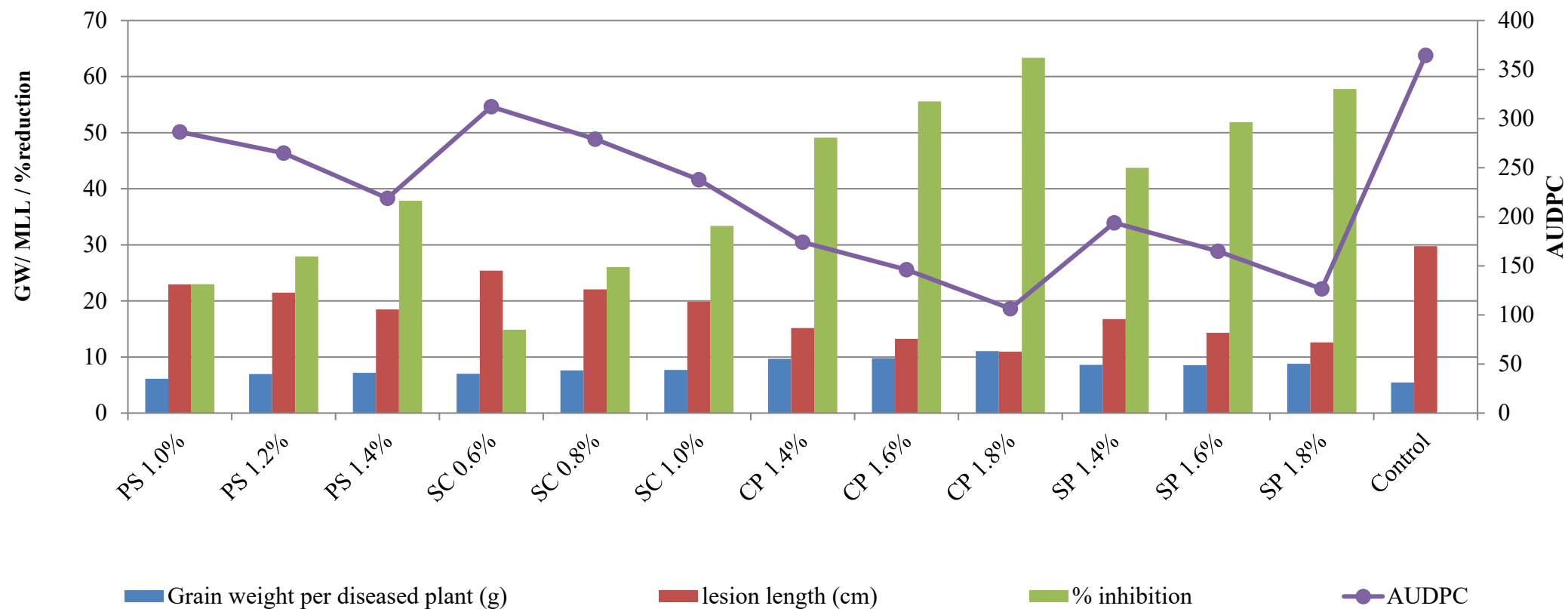


in vivo evaluation of alkali salts at PAU, Ludhiana and RRS, Ballawal Saukheri



■ Ludhiana ■ Ballawal ■ Pooled mean

Pooled mean of all parameters recorded in *in vivo* evaluation of alkali salts

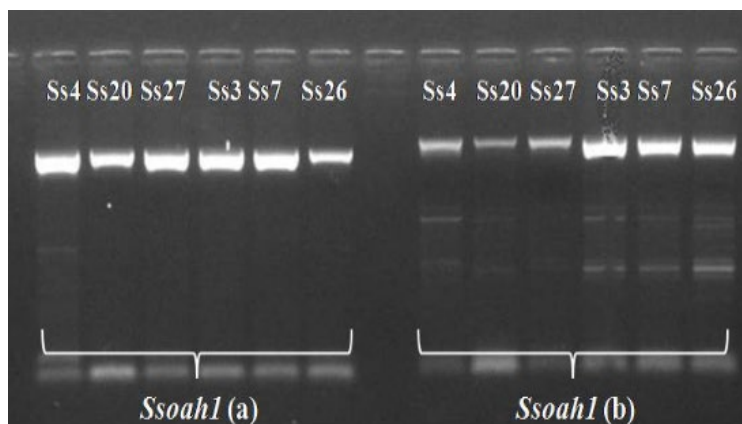


RESULTS

- ✓ Calcium propionate was found effective in controlling stem rot upto 60% inhibition when compared to control (distilled water).
- ✓ Sodium propionate showed similar results as in case of calcium propionate

Variation in *Ssoah1* gene

Gene	Reference	Location	length (bp)	Start	End	Primer	Sequence	Amplicon (bp)
<i>Ssoah1</i>	Lander <i>et al</i> (2017)	Chrom. no. 10	1017	243408		2-F	GATGATAAGGCTGGACGGAG	1022
					244430	2-R	GCATTGCTCATCTTTCCGAC	
				243704		3-f	CAGACCGCATACCTCAAAC	1197
					244901	3-r	CATCAACAACATCAACACCAAC	



Aggressiveness	Isolates
Low	Ss7, Ss26
High	Ss4, Ss20

Location	SNP			
49	G	Ss4, Ss20	A	Ss7, Ss26
229	A	Ss4, Ss20	G	Ss7, Ss26
394	G	Ss4, Ss20	A	Ss7, Ss26
463	G	Ss4, Ss20	A	Ss7, Ss26
562	G	Ss4, Ss20	A	Ss7, Ss26
694	A	Ss4, Ss20	G	Ss7, Ss26
754	G	Ss4, Ss20	A	Ss7, Ss26

Conclusion

- **Acidification**, which is an quintessential mechanism in pathogenesis used by *Sclerotinia sclerotiorum*, can be inhibited by prophylactic spray of alkali salts such as **calcium** and **sodium propionate** which are eco-friendly
- Aggressiveness of isolates studied using **AUDPC** is **significantly correlated** with **AUMGC**, which implies isolates having higher growth rates have more aggressiveness
- **AUDPC** as well as **AUMGC** are **positively correlated** with **oxalic acid** ($r=0.89$), **total cellulases** ($r=0.62$) and **cutinases** ($r=0.89$) which shows prominent role of biochemical parameters in pathogenesis & thus aggressiveness of the isolates

Acknowledgement

- Oilseeds Pathology lab and field staff
- Department of Biotechnology, Govt. of India
- Indian Council of Agricultural Research, Govt. of India
- Punjab Agricultural University, Punjab, India

*Thank
you*

A fountain pen nib is positioned at the end of the word 'you' in the cursive text.