

# Validation study of alternative seed treatments for *Xanthomonas campestris* pv. *campestris* on *Brassica*

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# Introduction: Black rot disease

- Black rot disease of brassicas : most damaging disease (Wulff *et al.* 2002)
- Caused by the seed-borne bacterium *Xanthomonas campestris* pv. *campestris* (Xcc)
- Major challenge to farmers in South Africa and other African countries (Massomo *et al.* 2004; Bila *et al.* 2009)
- Significant yield losses & reduces the market value of crops
- Disease symptoms of black rot:
  - Chlorotic to necrotic V-shaped lesions
  - Vein blackening



# Introduction: Control of Black rot

- Management of black rot : resistant varieties, cultural practices and chemical control.
- Chemical application as seed treatments/ foliar sprays: inefficient for control
- Need for reduced chemical use
- Increasing demand of vegetables grown organically
- Calls for use of effective alternative seed treatments: eradicate /reduce seed-borne inoculum
  
- Use of **plant extracts, microbial biocontrol agents** and **traditional hot water** as seed treatments has potential
- Alternative methods insufficiently explored against the pathogen *Xcc* on brassica vegetables

# Introduction: *In vitro* tests against *Xcc*

- *In vitro* screening tests : Various plant extracts for antibacterial activity against *Xcc* (MIC)
- Plant extracts tested at three concentrations: 10, 15 and 20 mg as seed treatments
- The best performing extracts selected for the greenhouse trials
  - Acetone extracts of *Cymbopogon citratus* (Lemon grass) at 10 mg/ml
  - Acetone extracts of *Agapanthus caulescens* (Agapanthus) at 15 mg/ml
- Hot water seed treatments tested for activity against *Xcc in vitro*
- Best performing hot water seed treatment selected for the greenhouse
  - 50°C for 30 minutes



# Objectives

To validate selected plant extracts and hot water as seed treatments against *Xanthomonas campestris* pv. *campestris* (black rot disease) in *Brassica* sp. under greenhouse conditions.

# Materials and methods: Greenhouse evaluations

## Artificial inoculation of seed

- *Xcc* bacteria
- $10^8$  cfu/ml

## Seed treatments

### Plant extracts

- Treatments
  - *Cymbopogon citratus* (acet-10mg/ml)
  - *Agapanthus caulescens* (acet-15mg/ml)
- ± 150 seeds per treatment
- Seeds were soaked: 3 hours, 25°C in the dark
- Controls: 1 % dimethyl sulphoxide (DMSO) inoculated, untreated inoculated seeds and healthy seeds
- Seeds dried in a laminar flow

### Hot water (Nega et al. 2003)

- Treatment
  - 50°C for 30 minutes
- ± 150 seeds per treatment
- Thermostatically controlled water bath
- Controls: sterile distilled water (SDW) inoculated-30 minutes, untreated inoculated seeds and healthy seeds
- Seeds dried in a laminar flow

# Materials and methods: Greenhouse validation

## Seed treatments

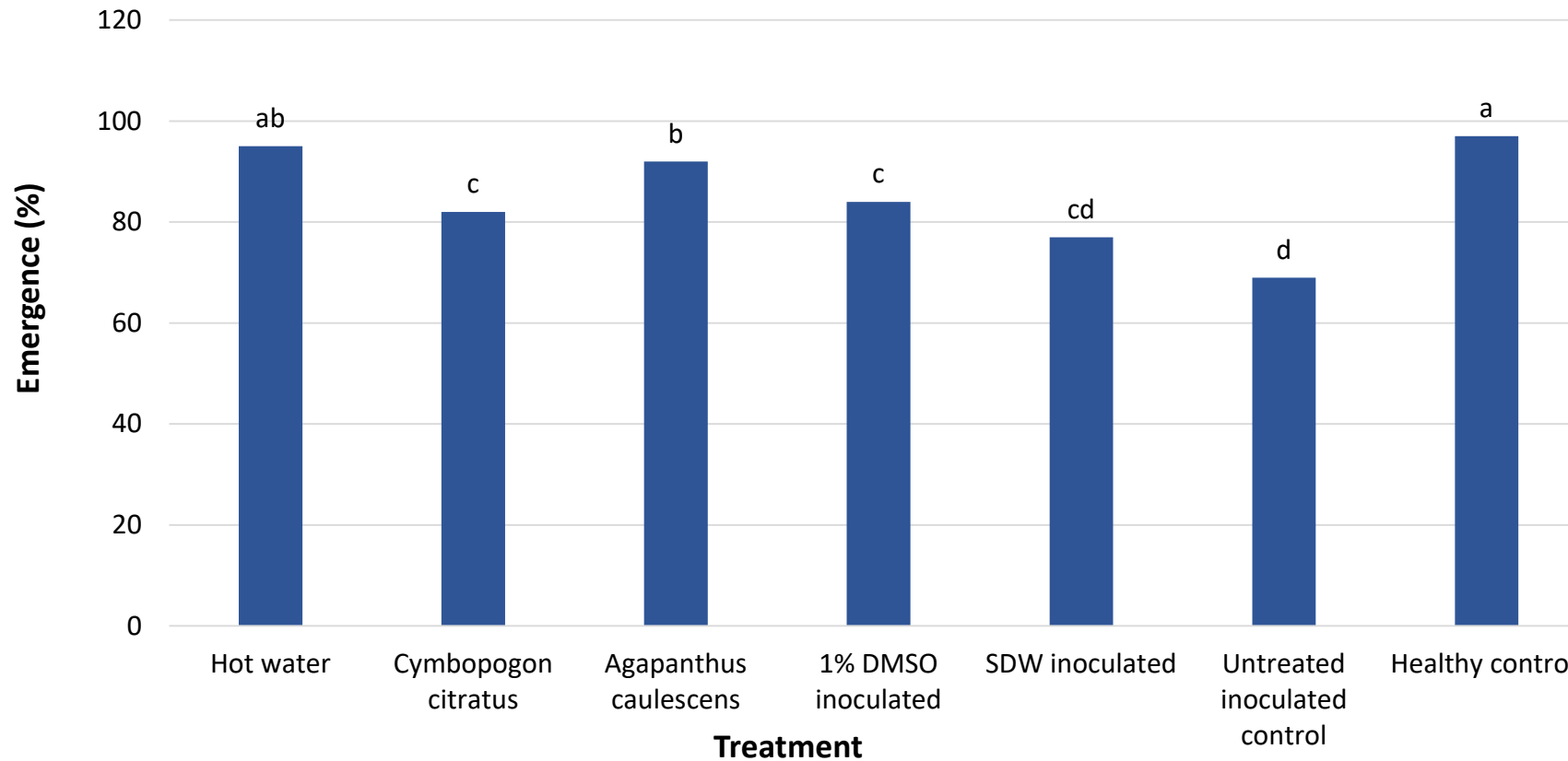
### Trial management and design

- Seeds sown in 128 cell seedling trays
- RCBD : 7 treatments
- Four replicates (4 x 25 seeds)
- Trays kept on bench at 25°C for 4 weeks in the greenhouse
- Experiment repeated
- Two researchers independently monitoring and recording data

### Measurements

- Emergence at 2 weeks after sowing (WAS)
- Healthy seedlings at 2 WAS
- Disease incidence 4WAS
- Disease severity : 4WAS
- Scale used according to Massomo *et al.* 2004 with slight modifications
- The black rot index (BRI) on the leaves calculated :  
$$\frac{\sum(\text{number of plants in class X severity class})}{\text{total number of plants}}$$
 (Alvarez *et al.* 1994; Massomo *et al.* 2004)
- Dry root mass (g) and shoot dry mass (g) at harvest

# Results



**Figure 1:** Effect of treatment of rape seeds with plant extracts and hot water on emergence

Treatment means with the same letters are not significantly different according to Fisher's LSD ( $P=0.05$ ), (LSD= 0.210; CV%= 15,57).

SDW=sterile distilled water, Hot water = 50°C for 30 minutes , 1% DMSO = 1 % Dimethyl sulphoxide

- HW at 50°C for 30 minutes best performing matching the level of healthy control
- *A. caulescens* (15 mg/ ml) effectively increased seedling emergence



# Results

**Table 2:** Effect of treatment of rape seeds with plant extracts and hot water on black rot disease incidence and severity.

Treatment	Disease incidence (%)	Black rot index
Hot water 50°C for 30 minutes	<b>3.2de</b>	<b>0.5de</b>
<i>Cymbopogon citratus</i>	<b>13.9c</b>	<b>2.2c</b>
<i>Agapanthus caulescens</i>	<b>4.5d</b>	<b>0.8d</b>
1% DMSO inoculated	21.3b	3.2b
Sterile distilled water inoculated	23.9ab	3.3b
Untreated inoculated control	<b>27.5a</b>	<b>4.0a</b>
Healthy control	0.0e	0.0e
LSD	0.05	0.755
CV (%)	33.03	25.37

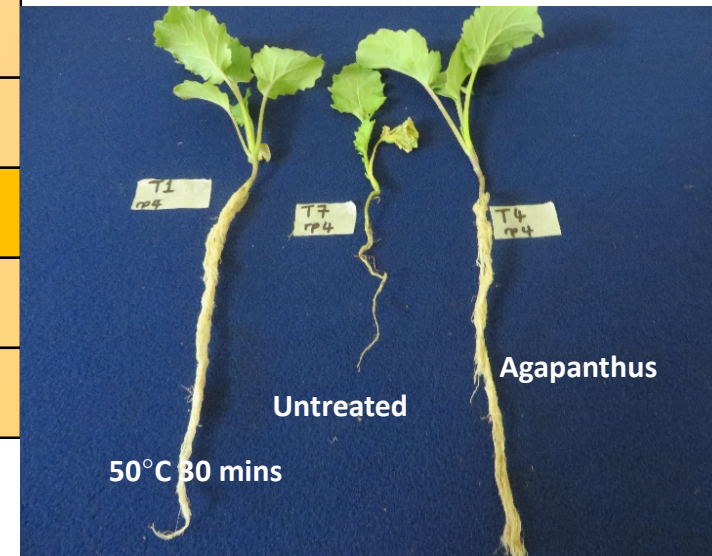
- Hot water (50°C/30 min) recorded the lowest black rot disease incidence and severity index

# Results

**Table 1:** Effects of seed treatments with plant extracts and hot water on growth of rape at harvest

Treatment	Dry mass in grams		
	*Shoot	*Root	*Total
Hot water 50 °C for 30 minutes	<b>0.60a</b>	<b>0.20a</b>	<b>0.80a</b>
<i>Cymbopogon citratus</i>	<b>0.55a</b>	<b>0.20a</b>	<b>0.75a</b>
<i>Agapanthus caulescens</i>	<b>0.58a</b>	<b>0.23a</b>	<b>0.81a</b>
1% DMSO inoculated	0.43b	0.14b	0.57b
Sterile distilled water inoculated	0.43b	0.09c	0.52bc
Untreated inoculated control	0.39b	0.08c	0.47c
Healthy control	<b>0.60a</b>	<b>0.22a</b>	<b>0.82a</b>
LSD	0.078	0.035	0.085
CV (%)	10.32	14.27	8.41

- Non chemical seed treatments increased shoot mass, root mass & total mass
- *A. caulescens* : high total dry mass



# Discussion

- Hot water at 50°C for 30 mins was very effective
  - Use of heat therapy effectively kills pathogens including the deep seated inside the seed
- *A. caulescens* (A) effective against *Xcc* as seed treatments
  - Antibacterial activity of *Agapanthus*: production of saponins: biologically active & they exhibit biochemical effects (Pretorius *et al.* 2002 ; Singh *et al.* 2008)
- *C. citratus* extracts was not as effective as HW & the *Agapanthus* extract but it has potential.
  - Antimicrobial activity of *C. citratus*: terpenoids, alkaloids, phenols, citral, saponins, geraniol and ketones (Santin *et al.* 2009 ; Hindumathy, 2011; Shah *et al.* 2011)
  - Artificial inoculation of seed: high pathogen levels, concealed the effectiveness of extracts

# Conclusion

- HW treatment at 50°C for 30 mins was effective and protected rape plants from black rot disease
- *A. caulescens* extract: produced bioactive compounds that have potential for control of black rot
- Further investigation on *C. citratus*: plant growth promoting effects
- HW at 50°C for 30 min & *A. caulescens* acetone extract at 15 mg/ml : recommended for brassica vegetable production especially in organic farming

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thank you