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
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.
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. caulesens* (15 mg/ml) effectively increased seedling emergence
**Table 2: Effect of treatment of rape seeds with plant extracts and hot water on black rot disease incidence and severity.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Disease incidence (%)</th>
<th>Black rot index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hot water 50°C for 30 minutes</td>
<td>3.2de</td>
<td>0.5de</td>
</tr>
<tr>
<td><em>Cymbopogon citratus</em></td>
<td>13.9c</td>
<td>2.2c</td>
</tr>
<tr>
<td><em>Agapanthus caulescens</em></td>
<td>4.5d</td>
<td>0.8d</td>
</tr>
<tr>
<td>1% DMSO inoculated</td>
<td>21.3b</td>
<td>3.2b</td>
</tr>
<tr>
<td>Sterile distilled water inoculated</td>
<td>23.9ab</td>
<td>3.3b</td>
</tr>
<tr>
<td>Untreated inoculated control</td>
<td>27.5a</td>
<td>4.0a</td>
</tr>
<tr>
<td>Healthy control</td>
<td>0.0e</td>
<td>0.0e</td>
</tr>
</tbody>
</table>

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**

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

- 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
Acknowledgements

• My supervisors
• Funding bodies
• Fellow postgraduate students
hank you