Seed-borne Infection of *Xanthomonas campestris* pv. *Campestris* in Cabbage and its Control through Seed Treatment

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Abstract

Black rot disease of crucifers is one of the economically important diseases of cabbage and cauliflower in Nepal. The causal organism, *Xanthomonas campestris* pv. *campestris* is seed-borne in nature. Experiment has been conducted in the laboratory and screenhouse to find out the role of seed-borne infection in the development of disease in seed and seedling, and to find out the seed dresser for its control. Among the tested varieties, Copenhagen market showed the highest seed and seedling infection. The relationship between seed and seedling infection was found positive ($r = 0.871$, $P = 0.08$). Among the treatments used as seed soaking 0.2% of Copper sulphate and Zinc sulphate were found statistically superior than 0.2% urea and Fluorescing *Pseudomonas*.

*Keywords*: Black rot, Crucifers, Seed dressers, Seed infection

Introduction

Black rot caused by *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson (Xcc) is one of the economically important diseases of brassicaceous vegetable crops of Nepal. The disease is encountered to be major in terai and inner terai regions where cauliflower and other cruciferous vegetables are cultivated. (Manandhar & Thapa 1992). The disease has been reported in cauliflower (*Brassica oleracea* var. *botrytis* L.), cabbage (*B. oleracea* var. *capitata* L.), turnip (*B. rapa* L.), broad leaf mustard (*B. campestris* var. *sugosa* L.) and radish (*Raphanus sativus* L.). However, the incidences were low but observed frequently in different locations of vegetable growing areas. In some of the locations more than 60% yield reduction in cauliflower has been reported (Shrestha 1990). The organism is seed-borne (Richardson 1979) and a single infected seed could cause the epidemics of disease in the field (Walker and Patel 1964). That is, a very low level of seed infection (three in 10,000 seeds) is enough to give rise high disease incidence in the field (Schaad et al. 1980) causing considerable losses. So, seed certification for Xcc has been emphasized by Schaad (1983). A very low tolerance limit has been proposed for foundation and certified seed production for field standard such as, 0.1% and 0.2% respectively (Shrestha & Timila 1997) in Nepal.

Once the bacterium is invaded in soil through infected seed and established the disease, it is very difficult to eliminate. After infecting the lower leaves it gets premature dropping and the symptom may disappear for several weeks. So epidemic is traceable to a given lot of seed or to a seed bed which become infected early from inoculum in seed. Since the organism is established in the vascular system, the expression of the symptom in a plant depends on the ability of the pathogen to multiply in the vascular system of the plant. So, Xcc can produce some other symptoms such as yellowing of cotyledons and deformation of first true leaves on seedlings under common nursery condition (Ignatov et al. 1998). The organism has been detected in seed samples of cauliflower and mustard crops of different parts of Nepal (Shakya & Malla 1988). Seed-borne inoculum plays a significant role in the outbreak of black rot disease.

Taking this into consideration an experiment was carried out to find out the effect of seed infection in the development of disease in the seedling and to find out the seed dresser to reduce the infection.

Methodology

The experiment was carried out in the laboratory as well as in the screenhouse in 1996 at Khumaltar. The seed samples of cabbage of different varieties were collected from local market and Vegetable Development Division, Khumaltar. The varieties included were Copenhagen market 1 and 2, Pride of India, Drum head and Green coronate.
**Seed infection test**

A total of four hundred seeds of each sample were plated in selective medium, SX medium (Mortenson 1989), representing 100 seed per replication. Incubation was given for 2-4 days at 20 °C. Seeds surrounded by grey to purplish mucoid colonies with hydrolyzing zones were counted as infected. The isolation of such bacteria on YDC medium were carried out. Twenty-four hour old culture multiplied from single colony on YDC medium was used for Kovac's oxidase test using tetramethyl paraphenylenediamine hydrochloride, Gram reaction using 3% Potassium chloride (KOH) solubility test and pathogenicity tests.

**Seedling infection test**

For seedling infection test hundred seeds of each varieties were sown in steam sterilized soil on tray in two replications. The seedlings were assessed as infected with typical symptoms on cotyledonary leaves or on true leaves. The infection was confirmed with cutting the symptom bearing leaves and checking under microscope for bacterial streaming through cut surface.

**Seed treatment**

The seed sample of the variety Copenhagen market showing highest infection percent in seed and seedling symptom test was used for seed treatment experiment. Seeds were treated by soaking in the following treatments for 10 minutes and dried under laminar bench.

1. Copper sulphate (CuSO₄), 0.2%
2. Zinc sulphate (ZnSO₄), 0.2%
3. Urea, 0.2%
4. Fluorescing *Pseudomonas* (Turbid suspension)
5. Untreated control.

The treated seed were also tested on SX medium. For seedling infection test, the above mentioned procedures were followed. The data was statistically analysed using M-STAT package.

**Results**

In SX medium, seeds surrounded by grey to purplish bacterial growth with starch hydrolyzing zone were counted as infected. The isolation of such bacteria on YDC medium showed yellow mucoid colonies which were gram negative and Kovac's oxidase negative. Twenty four hours old culture from YDC medium when inoculated on cabbage seedling produced black rot symptom after two weeks of inoculation confirming with bacterial oozing from the cut surface.

Regarding infection level on seed samples of different varieties, Copenhagen market 1 was found to be highly infected and least infection was found in Drum head (Table 1). Similarly seedling infection test showed the same trend of infection showing highest in Copenhagen market 1 with least in Drum head and Green corionate. Copenhagen market 2 also showed the lesser seed and seedling infection. Statistical differences were observed on infection level of seed and seedling among the varieties included in the experiment. Relationship between seed infection and seedling infection was found positive with r=0.871 at P=0.08.

### Table 1. Seed and seedling infection percent of Xcc in different varieties of cabbage

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Varieties</th>
<th>Seed infection (%)</th>
<th>Seedling infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Copenhagen Market 1</td>
<td>8.0 a</td>
<td>27.44 a</td>
</tr>
<tr>
<td>2.</td>
<td>Pride of India</td>
<td>5.5 ab</td>
<td>21.00 a</td>
</tr>
<tr>
<td>3.</td>
<td>Drum head</td>
<td>1.0 c</td>
<td>13.54 ab</td>
</tr>
<tr>
<td>4.</td>
<td>Copenhagen Market 2</td>
<td>2.0 bc</td>
<td>4.4 b</td>
</tr>
<tr>
<td>5.</td>
<td>Green coronate</td>
<td>not included</td>
<td>6.18 b</td>
</tr>
<tr>
<td>CV (%)</td>
<td></td>
<td>47.43</td>
<td>21.81</td>
</tr>
</tbody>
</table>

Means followed by the same letter are not significant at P=0.05 using DMRT

### Table 2. Effect of different seed treatments on the incidence of Xcc in seed and seedling of cabbage (Copenhagen Market)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (%)</th>
<th>Seed infection (%) on SX medium</th>
<th>Seedling infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper sulphate</td>
<td>0.2</td>
<td>0.0 c</td>
<td>6.85</td>
</tr>
<tr>
<td>Zinc sulphate</td>
<td>0.2</td>
<td>0.0 c</td>
<td>6.74</td>
</tr>
<tr>
<td>Urea</td>
<td>0.2</td>
<td>17.0 a</td>
<td>21.95</td>
</tr>
<tr>
<td>Fluorescing pseudomonas</td>
<td>Turbid suspension</td>
<td>7.0 b</td>
<td>21.06</td>
</tr>
<tr>
<td>Untreated Control</td>
<td>18.0 a</td>
<td></td>
<td>33.08</td>
</tr>
<tr>
<td>CV (%)</td>
<td>42.14</td>
<td></td>
<td>22.5</td>
</tr>
</tbody>
</table>

Means followed by the same letter are not significant at P=0.05 using DMRT

Regarding the effect of different treatments on the incidence of seed infection, no infection was found in the treatment, Zinc sulphate and Copper sulphate. The effect of Fluorescing *Pseudomonas*
was statistically different from urea and untreated control as well as Copper sulphate and Zinc sulphate. There was no statistical difference on the effect of different treatments on the incidence of seedling infection. But the differences were obvious with least incidences in Zinc sulphate and Copper sulphate treatments (Table 2). Effect of urea and untreated control were observed to be almost the same. The Effect of Fluorescing *Pseudomonas* was significantly different from urea and untreated control in reducing seedling infection.

**Discussion**

Seed infection on SX medium is lower than in seedling infection. It could be due to interference by saprophytic and antagonistic bacteria associated with seed as mentioned by Schaad and Donaldson (1981). Since a very low percent of seed infection could cause the epidemics in the field (Walker & Patel 1964), seed certification for *Xcc* has been emphasized by Schaad (1983). A very low tolerance limit has been proposed for foundation and certified seed for field standard such as, 0.1% and 0.2% respectively (Shrestha & Timila 1997) in Nepal.

Similarly for the control of the disease seed treatment of cabbage in hot air at 75 °C or 70 °C for 2 or 6 days was effective without the adverse effect on germination (Shiomi 1992). Seed soaking in Sodium hypochlorite (0.525%) for 15 minutes and in streptomycin (500 ppm.) for one hour were effective for the control of *Xcc* as discussed by Babadoost *et al.* (1996) and Napoles *et al.* (1991) respectively. Acidified Zinc sulphate was reported to be better seed dressing agent than Copper acetate, Calcium hypochlorite or hot water treatment without the adverse effect on germination (Huang & Lee 1988). In the present study similar effect of Zinc sulphate and Copper sulphate without acidification was observed without the adverse effect on seed germination.

From the present study it is clear that there is difference in seed infection among the tested varieties. However, to confirm the response of seed infection in different varieties, it needs the testing of more samples. Detection of seed infection on SX medium could be inhibited by the presence of antagonistic and saprophytic bacteria in the seed there by masking the growth of *Xcc*. Either Copper sulphate or Zinc sulphate could be used as the seed soaking agents to reduce the seed and seedling infection. However, field experiment is necessary to verify its effect on transplanted crop.

**Acknowledgements**

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**References**


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