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Control of black rot disease in cabbage by integration of mulching, pruning and hot water treatment of seeds

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Abstract

Black rot disease of cabbage caused by *Xanthomonas campestris* pv. *campestris* has been a major hindering factor to cabbage production in Kisii County, Kenya. The conventional technique for controlling this disease has been the use of chemicals. However, this method of control has not been effective as the disease is seed borne. In addition, most chemicals pollute the environment and make it unconducive for the survival of other important organisms such as decomposers. In this paper, the integration of hot water treatment of seeds, mulching, pruning and plant debris management was considered as an approach that can effectively manage this disease. To achieve this objective, seeds of Gloria Hybrid cabbage were inoculated with a bacterial suspension of *X. campestris* pv. *campestris* isolated from leaf segments obtained from plant leaves with characteristics symptoms of black rot disease. A portion of the inoculated seeds were treated with hot water at 50^{o}C for 25 minutes and later planted in the field to evaluate the effects of mulching, pruning and plant debris management on black rot disease. Another portion of the inoculated seeds were not treated with hot water and were planted to serve as a control. Disease was scored on a scale of 1–9 based on the length of the V-shaped lesions developed on the margin of plant leaves. The results obtained show that integration of hot water treatment of seeds, mulching, pruning and plant debris management led to 76.1% less black rot disease and a 78.3% increase in marketable yields. Hence such treatment is recommended as the best approach to manage black rot disease of cabbage in the field.

Key words – disease score – Gloria Hybrid – integration – severity – significant difference – *Xanthomonas campestris*

Introduction

Black rot disease caused by *Xanthomonas campestris* pv. *campestris* is a worldwide problem causing serious damage to all crops of the Brassicaceae family and is of concern to both scientists and farmers (Ryan et al. 2011). According to Lo & Wang (2001), Bila (2008), the loss caused by this disease on cabbage ranges from 30–70%, depending on the prevailing weather conditions. In Kenya, the disease causes severe damage leading to total crop loss during the warm and wet seasons (Anonymous 2000, Varela et al. 2003, Otipa et al. 2013). Various control measures have been adopted both at the farm level and at research centers, but no one method controls the disease

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effectively (Celetti et al. 2002). Some of the practices currently employed by farmers to manage the disease include chemical control, host resistance, biological control, cultural practices and field sanitation.

Chemical control involves the use of sodium hypochlorite, hydrogen peroxide, hot cupric acetate or zinc sulfate to manage the disease (Williams 1980, Miller et al. 1996). Unfortunately, most of the chemicals being used to control black rot disease of cabbage have low efficacies (Massomo et al. 2003, Bila et al. 2013) while others have not been approved. Although Actigard chemical induces resistance in some plants and is labelled for suppressing black rot on commercially grown crucifers, its results in cabbage has been disappointing (Seebold et al. 2008). Moreover, chemical treatment of seeds only disinfects the seed surface and does not kill the pathogens that are found inside the seed (Miller 2002). Copper fungicides previously used to spray cabbage plants were found to cause black spots on leaves and its use has since been outlawed (Averre 2000).

The development and use of black rot resistant cultivars has long been recognized as an important tool for disease control, though the practice has had limited success (Miller et al. 1996). This is because most of the local cultivars are highly susceptible (Massomo et al. 2003, Bila et al. 2013). The challenge of this approach is that some cultivars, which are partially resistant or tolerant to black rot (Seebold et al. 2008), are of poor quality (not sweet tasting) and thus not readily adopted by farmers. Furthermore, the source for major gene (durable) resistance in *Brassica oleracea* is rare and thus new strains of pathogens, capable of infecting resistant varieties may arise with time (Miller et al. 1996, Averre 2000, Seebold et al. 2008). The most common and potentially useful sources of black rot resistance occur in genomes of wild types of *Brassica* (Taylor et al. 2002). Unfortunately, transferring these genes into local cultivars may results in transfer of other undesirable genes such as poor taste (Acquaah 2007). Moreover, development of resistance cultivars takes a long time.

A few biocontrol agents antagonistic to *Xanthomonas campestris* pv. *campestris* have been isolated from various sources and are being used in the control of black rot of *Brassica* crops. Various strains of yeast (Sayonara et al. 1999, Celetti et al. 2002), a *Paenibacillus* isolate (Ghazalibiglar 2014), *Bacillus* strains (Massomo et al. 2003) and plant growth promoting *Rhizobacteria* (PGPR) such as *Bacillus subtilis* R14, *B. pumilus* C116, *B. megaterium* pv. *cerealis* RAB7 and *B. cereus* C210 have being identified as important biocontrol agents due to their antibiotic effect against *X. campestris* pv. *campestris* bacterium (Luna et al. 2002). However, the agrochemicals (fertilizers, pesticides, herbicides and insecticides) that farmers use create an unconducive environment for survival of the biocontrol agents (Huang 1997).

Cultural practices such as mulching (Hunter et al. 1975, Onsando 1992), planting disease-free seeds, maintaining proper field sanitation, elimination of infected plant debris, pruning, rouging of diseased plants, eradication of alternate hosts, and crop rotation have been identified as cultural practices that curb the spread of black rot disease as well as reducing, excluding and eliminating the initial sources of disease (Averre 2000, Smart & Holly 2013). Timing irrigation when plants will dry quickly and restricting field activities until later in the day when fields are dry will help reduce disease spread (Celetti et al. 2002). However, this has not achieved much as the disease is seed-borne (Williams 1980, Celetti et al. 2002). Use of disease-free seeds has not been achieved due to the fact that as few as three infected seeds in 10,000 (0.03% seed infection) can cause black rot epidemics in a field (Celetti et al. 2002). Excessive treatment of seeds reduces the seed germination percentage and vigor. Crop rotation alone has not been effective in controlling black rot disease as infection can be introduced into the farms through infected seeds, surface run off and farm equipment carrying infested soils (Williams 1980, Celetti et al. 2002). In addition, the small sized farms found in Kisii County, due to overpopulation, have limited the practice of crop rotation.

Materials & Methods

Samples of infected leaves of cabbage were randomly picked from KALRO and ATC farms in Kisii County. Three leaves with symptoms characteristic of black rot disease were picked from

randomly selected diseased plants along a transact line in the selected farms. These leaves were then mixed and a total of 10 leaves were randomly picked from the sample. The leaves were then placed in an open woven cotton bag inside a well ventilated box. The box was labelled and transported to the laboratory for isolation of *X. campestris* pv. *campestris*.

The cabbage leaves obtained from the field were washed in running tap water and air-dried at room temperature (27^{o}C). The dried leaves were placed in 1% tween-20 solution to wet them. The leaves were then placed in 10% sodium hypochlorite solution for 5 minutes to disinfect them after which they were rinsed five times using sterile distilled water and then air-dried on a clean disinfected bench. Leaf segments measuring 2 × 3 mm were excised from the lesion margins of the dried and sterilized leaves. The leaf segments were placed in a 50 ml beaker containing 0.85% sterile saline (NaCl) solution and left to stand for 15 minutes in a lamina air-flow chamber to allow the bacteria to ooze out of the plant tissues into the saline solution. The leaf tissue segments were then removed.

Loopfuls of the saline solution were streaked on pre-chilled plates (at -2^{o}C to -4^{o}C) containing nutrient agar. Saprophytic and antagonistic bacteria associated with crucifer tissues grow at a faster rate and prevent the growth of *X. campestris* pv. *campestris*. Thus, to prevent their growth 10 mg/ml of nitrofurantoin and 0.5 mg/ml vancomycin were added to the nutrient agar medium. The plates were then incubated for 48 hours at 28^{o}C after which they were inspected for the presence of pale yellowish and convex mucoid bacterial colonies. Colonies were subcultured on yeast dextrose, calcium carbonate (YDC) agar to purify bacterial colonies. The purified colonies were stored at on porcelain beads in Protect tubes maintained on nutrient agar at 25^{o}C. Cultural characteristics such as the yellowish and convex mucoid colonies, staining characteristics and pathogenicity tests were used to identify the bacterium as *X. campestris* pv. *campestris*.

Relatively clean seeds of Gloria Hybrid seeds were obtained from the Kenya seed company. A 100 ml suspension of the field bacterial isolates (at a concentration of 10^4 CFU/ml) was prepared in 0.85% saline solution. About 50 grams of seeds (10,000 seeds) of Gloria Hybrid were immersed in the cell suspension. The contents were shaken at 125 r.p.m at 25^{o}C for 5 minutes. The liquid was removed with a pipette and the seeds spread on blotting paper to dry overnight in a Bio-Safety cabinet.

The inoculated seeds were divided into two equal portions. The first portion (labelled as I_0) was established in a greenhouse nursery bed without any further treatment to serve as the control. The second portion of inoculated seeds was labeled as I_1. These seeds were subjected to hot water treatment at 50^{o}C for 25 minutes using the ISTA standard procedures of Miller et al. (2005) before being established in greenhouse nursery beds. All seedlings in the greenhouse were transplanted to the field at the age of 4 weeks. In the field, seedlings were established in a complete randomized block design of four replicas. The I_1 seedlings were further subjected to mulching and pruning with all the other field operation practices being carried out as per the recommended standards in cabbage production. This experiment was repeated in two different seasons of 2017.

Disease scoring, on a scale of 1–9, was done on the basis of the level of disease symptoms, which was based on the length of the V-shaped lesions developing on the cabbage leaves. The length of V-shaped lesions, measured in centimeters from the leaf margin inwards, provided the disease score (Table 1). This was done on a weekly basis after crop establishment in the field after transplantation.

Results

In order to ascertain the effect of integrating hot water treatment of seeds, mulching and pruning on black rot disease of cabbage, eight observations were made, which included disease mean scores for integration season 1, no integration season 1, integration season 2, no integration season 2. Disease means scores for integration and no integration across season 1, season 2 and across the seasons are shown in Table 2.

Table 1 Disease score rates

Length of V-shaped lesion	Score rates
< 0.5 cm	1
> 0.5 – 1.0 cm	2
> 1.0 – 1.5 cm	3
> 1.5 – 2.0 cm	4
> 2.0 – 3.0 cm	5
> 3.0 – 4.0 cm	6
> 4.0 – 5.0 cm	7
> 5.0 – 6.0 cm	8
> 6.0 cm to plant death	9

Table 2 Disease mean score for integration across seasons

Weeks after crop establishment	S_1I_1	S_1I_0	S_2I_1	S_2I_0
7			2.75 a	7.625 a
6			2.625 ab	7.25 b
5			2.625 ab	5.875 c
4	3.00 a	7.50 a	2.625 bc	5.75 c
3	2.625 a	7.125 a	1.875 cd	5.75 bc
2	1.25 b	4.75 b	1.625 de	5.0 d
1	1.00 b	2.663 c	1.375 e	3.0 e
Mean	1.969	5.500	2.161	5.750
SE	0.1398	0.184	0.1308	0.1157
CV %	20	9	17	6
P-value	<0.0001	<0.0001	<0.0001	<0.0001

Means figures in the column with different letters are significantly different ($p<0.05$), where S_1I_1 is the disease mean score for season 1 integration, S_1I_0 is the disease mean score for season 1 no integration, S_2I_1 is the disease mean score for season 2 integration, and S_2I_0 is the disease mean score for season 2 no integration.

The scores generally increased with time. There was evidence of significant difference between the times, for example, the score was high (7.625) after 7 weeks compared to after 1 week (3.0) in season 2 where integration was not done. In season 1 the scores were also high at 4 weeks (7.50) as compared to 1 week (2.663) in cases where integration was not done. The score was generally higher in cases where integration was not done compared to cases where integration was done throughout both seasons. Table 3 shows disease mean score for season 1, season 2 and across the seasons.

Table 3 Disease mean score for integration

Treatment	Season 1	Season 2	Across Seasons
No integration	5.500 a	5.750 a	5.659 a
Integration	1.375 b	1.339 b	1.352 b
S.E.±	0.118	0.042	
CV%	19	9	13
P-value	<0.0001	<0.0001	<0.0001

Means figures in the column with different letters are significantly different ($p<0.05$).

This result shows that there was significant difference between the use of integration and no integration within seasons and across seasons. For instance, there was significantly high scores

(5.659) where integration was not used as compared to where integration was used (1.352) across the seasons. A similar trend was observed in seasons 1 and 2. Table 4 shows the effect of integration on black rot disease in terms of percentage disease control.

Table 4 Effects of integration on black rot disease (in percentage disease control)

Treatments	**Score**	**Disease control (%)**	**Cabbage mean weight (kg/head)**	**Marketable yield (tons/ha)**	**Yield increase (%)**
Integration (I_1)	1.353a	76.1	3.250 a	94.791 a	78.30
Control (I_0)	5.659 b		1.823 b	53.163 b	-
S. E.	0.123		0.038	1.112	
CV %	42		5.94	5.94	
P-value	<0.0001		<0.0001	<0.0001	

Means figures in the column with different letters are significantly different ($p<0.05$).

There was evidence of significant difference between I_1 and I_0. Treatment I_1 (which is integration of hot water treatment of seeds, mulching and pruning) had a score of 1.353. On the other hand, I_0 (control) had a disease mean score of 5.659.

The study findings show that at 76.1% disease control, integration had great impacts on black rot disease as far as its control is concerned. Fig. 1a shows the resultant plants from a field where integration was done while 1b gives the plants from a field where integration was not done.

There was severe disease where integration was not done as compared to fields where integration of hot water treatment of seeds, mulching and pruning was carried out. Without integration many plants died while the remaining plants showed servere symptoms of black rot disease (Fig. 1b).

There was significant difference between yields from the cabbage that received treatment and those that never received any treatment (Table 4). Cabbage heads from fields with integration had significantly higher mean weight (3.25 kg) as compared to heads from the control (1.823 kg). A similar trend was observed in terms of marketable yields whereby integration had significantly ($p<0.05$) higher marketable yields (94.791 tons/ha) as compared to control (53.163 tons/ha).

Fig. 1 – a Resultant plants from a field where integration was done. b Resultant plants from a field where integration was not done.

Discussion

This research sought to ascertain the effect of integrating hot water treatments of seeds, mulching and pruning on the control of black rot disease of cabbage. From the research findings, there was significant difference between integration and no integration. Treatment I_1 had a disease mean score of 1.353 while I_0, (control) had a score of 5.659.

The results clearly show that there was evidence of significant difference between where there was integration and no integration. There was a general increase in disease score with time in both seasons 1 and 2. The disease was less severe where integration of hot water treatment of seeds, mulching and pruning was done as compared to where no integration was done. A similar trend was observed in both seasons.

Mulching helped to prevent the spread of disease from the soil to plant leaves through rain splash as well as conserving moisture in the soil. On the other hand, pruning reduced the population of the pathogen in the plants, thus reducing disease spread among the plants. Removal of plant debris from the farm reduced the amount of pathogen present while maintaining field hygiene thus reducing the rate of crop infection. Integrating hot water treatment of seeds, mulching, pruning and proper management of plant debris reduced the severity of black rot disease by 76.1% in fields of cabbage plants. This effect on black rot disease control by integration resulted in higher yields from fields with integration as compared to fields that did not receive any treatment. Cabbage heads from fields with integration had significantly higher mean weight (3.25 kg) as compared to heads from the control (1.823 kg). Similarly, the marketable yields of 94.791 tons/ha from fields with integration was significantly higher as compared to marketable yields from the control (53.163 tons/ha).

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