



Published by

[www.researchtrend.net](http://www.researchtrend.net)

# The efficacy of hot water seed treatment against *Xanthomonas campestris* pv. *campestris* in the control of black rot disease of cabbage under field conditions

Jackson Gitange Ombuna<sup>1</sup>, Johnson Nyangeri<sup>2</sup>, Samson Maobe<sup>3</sup>

<sup>1</sup>Faculty of pure and applied sciences, Kisii University, Kisii-Kenya

<sup>2</sup>Faculty of pure and applied sciences, Kisii University, Kisii-Kenya

<sup>3</sup>Faculty of Agriculture and Natural resources, Kisii University, Kisii-Kenya

**Corresponding author:** [jacksongetange@yahoo.com](mailto:jacksongetange@yahoo.com)

---

| **Received:** 19 April 2019 | **Accepted:** 09 June 2019 |

---

**How to cite:** Ombuna GJ, Nyangeri J, Maobe S. 2019. The Efficacy of hot water seed treatment against *Xanthomonas campestris* pv. *campestris* in the control of black rot disease of cabbage under field conditions. J New Biol Rep 8(2): 125- 133.

---

## ABSTRACT

Several measures have been employed in the control of black rot disease of cabbage, yet none of them has effectively managed the disease since farmers still suffer serious losses. The main objective of this research was to evaluate the efficacy of hot water treatment of seeds against *Xanthomonas campestris* pv. *campestris* in the control of black rot disease of cabbage under field conditions. To achieve this objective, two different varieties of cabbage crop labelled V<sub>1</sub> (Copenhagen market variety) and V<sub>2</sub> (Gloria F1 hybrid) were planted in two different seasons between 2017 and 2018 at the Kenya Agricultural and Livestock Research Organization center (KARLO) – Kisii branch. 50 grams of seeds of each variety were inoculated with 10<sup>4</sup> CFU/ml of bacteria suspension prepared from the field isolates of the bacterium. The artificially inoculated seeds for each variety were then divided into two lots labelled H<sub>1</sub> and H<sub>0</sub>. The H<sub>1</sub> seeds were separately subjected to hot water treatment at 50<sup>0</sup>C for 25 minutes while H<sub>0</sub> seeds were left untreated to serve as control experiment. The seeds were then established in separate nursery beds within a greenhouse before being transplanted into clearly labelled separate field plots, each measuring 4 m by 3 m in a Completely Randomized Block Design. The experiment was repeated in 4 replicas. Data was collected on a scale of 1-9 based on characteristic symptoms of the disease. The data obtained was analyzed by Statistical Analysis System (SAS) using Analysis of Variance (ANOVA) and all tests of significance were conducted at P ≤ 0.05. The research findings obtained indicate that there was significant difference between seeds treated with hot water and those not treated with hot water in terms of field disease score. The use of hot water treatment of seeds at 50<sup>0</sup>C for 25 minutes in the control of black rot disease of cabbage under field conditions was found to be 68.17% effective and thus it is hereby recommended as an alternative method of disease control to chemical control.

**Key words:** Copenhagen Market variety, Gloria F1 Hybrid, Efficacy, Significant difference, Hot water treatment.

## INTRODUCTION

Black rot disease caused by *Xanthomonas campestris* pv. *campestris* has been identified as the major cause for low cabbage yields to farmers all over the world. The disease causes serious crop losses and as such it has become a major concern to many scientific researchers (Alvarez 2000; Christianson 2008 and Ryan et al. 2011). Research findings by Lo & Wang (2001), Miller (2002), Nega et al. (2003), Bila (2008) and RADA (2012) indicate that black rot disease causes serious economic losses on cabbage fields ranging from 30-70%, depending on the prevailing weather conditions. This disease causes severe damages to cabbage crop resulting to total crop loss during the warm and wet weather conditions in Kenya (Anonymous 2000; Varela et al. 2003 and Otipa et al. 2013). A number of measures have been employed to control the disease but none of them has effectively managed the disease (Celetti et al. 2002). The conventional methods currently employed by farmers to manage the disease include chemical control, host resistance, biological control and cultural practices (Celetti et al. 2002; Massomo et al. 2003 and Seebold et al. 2008) which have all failed to manage the disease.

Chemical control has shown very low efficacy given the seed-borne nature of the disease (Massomo et al. 2003 and Bila et al. 2013). Chemical treatment only disinfects the seed surface and does not kill pathogens that are found inside the seed (Miller 2002; Massomo et al. 2003 and Bila et al. 2013). Besides this, chemicals have other side effects such as causing human and animal health problems, environmental pollution, degradation of the ecosystem and killing of useful soil micro-organisms like decomposers and nitrogen fixing bacteria as well as inducing pathogen resistance towards pesticides (Huang 1997 and Massomo et al. 2003) which has further complicated the use of chemicals on disease control. On the other hand, previous researches indicate that there is no durable host-resistance towards the black rot disease pathogen (Massomo et al. 2003; Seebold et al. 2008 and Bila et al. 2013). Not much has been established about the use of biocontrol agents against the pathogen *Xanthomonas campestris* pv. *Campestris*. Furthermore, the use of biological control method has been complicated by the over-dependency on the use Agro-chemicals by Kenyan farmers, which has created an unfavourable soil conditions for the survival of biocontrol agents (Huang 1997; Sayonara et al. 1999 and Massomo et al. 2003).

The most important source of black rot disease of cabbage is infected seeds and transplants (Nega et al. 2003; RADA 2012 and Miller & Ivey 2019). Research findings by Miller (2002), Nega et al. (2003), Bila (2008) and RADA (2012) demonstrate that even one infested seed in a package of 10, 000 seeds could easily results in

total crop loss in the field under favourable field conditions. The best strategy therefore for controlling any seed-borne disease is to eliminate or reduce the amount of pathogen available to start the disease. Only seeds that have been certified as being disease-free should be used for planting (RADA 2012). This can only be achieved through hot water treatment of seeds and other planting materials as the available chemicals can only disinfect the seed surface.

Hot water seed treatment has been found to be a very simple and effective method for controlling bacterial and fungal pathogens spread through seeds and other planting material. This is due the fact that it kills pathogens both from the inside and on the surface of the seed as compared to chemical treatment that disinfects only the seed surface. Hot water treatment of seeds and planting materials is a thermo-physical method of plant protection that has been applied in the management of other plant diseases (Nega et al. 2003).

The usefulness of hot water seed treatment in the control of seed-borne bacterial diseases is rapidly increasing due to the rapid spread of seed-borne bacterial diseases, failure of chemicals and other disease control measures (Jahn et al. 2000; Koch et al. 2000; Linders 2000). Late in the 19<sup>th</sup> Century, the method was used to control loose smut (*Ustilago nuda*) in cereals (Jensen 1888; Nega et al. 2003). In the earlier 1920s, hot water seed treatment was used to treat cabbage seeds against *Phoma lingam* pathogen to manage black leg disease of cabbage. This method was considered as a standard method of disease management throughout the United States of America (Walker 1923; Nega et al. 2003). Hot water was also used to treat oak seeds against the fungus *Ciboria batschiama* during storage of Oak seeds. Nega et al. (2003) used hot water at 50°C for 30 minutes to treat Carrot seeds against *Alternaria* species to manage leaf blight and black spot diseases in carrots with an over 95% efficacy. Nega et al. (2003) found that treating seeds of lamb's Lettuce with hot water at 50°C for 30 minutes against *Phoma* species significantly reduced the disease in the field with 80-95% efficacy. Also treating carrot seeds with hot water at 50°C for 60 minutes against *Xanthomonas campestris* pv. *Carotae* gave good results in the control of bacterial leaf blight disease in fields of carrots (Nega et al. 2003). RADA (2012) used hot water seed treatment to effectively control bacterial leaf spot of Scotch Bonnet pepper without any significant loss in seed germination. Miller & Lewis (2005) and Miller & Ivey (2019) applied hot water at various temperatures to treat various vegetable seeds to eradicate bacterial pathogens in the organic production system. However, the efficacy of hot water treatment of seeds against *Xanthomonas campestris* pv. *campestris* in the control of black rot disease of cabbage under field conditions has not been clearly established hence the need for this research.

## MATERIALS AND METHODS

The following were the methodologies used to achieve the objective of this research;

### Isolation and identification of *Xanthomonas campestris pv. campestris*

Cabbage leaves with characteristic symptoms of black rot disease were randomly collected from selected farms within Kisii County in Kenya. The collected leaves were carefully packed in clean woven cotton bags and transported to the laboratory where they were washed in clean running tap water and air-dried at room temperature (27 °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 5 times using sterile distilled water and then air-dried on a clean disinfected bench. Leaf segments measuring 3 mm x 4 mm were then cut off from leaf margin areas with characteristic V-shaped necrotic lesions, dried and sterilized in 70% alcohol. The leaf segments were then 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 tissue into the saline solution. The leaf tissue segments were then removed.

Loopfuls of the saline solution were streaked on pre-chilled plates (at -2°C to -4°C) containing Nutrient Agar. 10 mg/ml of nitrofurantoin and 0.5 mg/ml vancomycin were added to the nutrient agar medium to prevent the growth of saprophytic and antagonistic bacteria respectively. The plates were then incubated for 48 hours at 28°C after which they were inspected for the presence of pale yellowish and convex mucoid bacterial colonies. Sub-culturing of the colonies was done on yeast dextrose Calcium carbonate (YDC) agar to purify bacterial colonies. The purified colonies were then stored at -80°C on porcelain beads in Protect tubes maintained on nutrient agar at 25°C.

Staining characteristics, cultural characteristics and pathogenicity tests associated with *Xanthomonas campestris pv. campestris* were used to identify the bacterial isolates. Pathogenicity tests were carried out in accordance with Vicente et al. (2001) and Miller & Lewis (2005) International Seed Testing Association standard procedures to confirm the identity of the bacteria isolates. This was achieved through foliar spraying of 4 weeks old seedlings of cabbage with the isolated bacterial inoculum. The artificially inoculated plants were then observed for disease symptom development for a period of 2 weeks.

### Inoculation of Seeds

50g of seeds from each of the two varieties labelled V<sub>1</sub> (Copenhagen market variety) and V<sub>2</sub> (Gloria

hybrid variety) were immersed in separate beakers containing bacterial cell suspension of 10<sup>4</sup> CFU/ml of the field bacterial isolates. About 100 ml cell suspension of the field bacterial isolates (at the concentration of 10<sup>4</sup> CFU/ml) was prepared in 0.85% saline solution containing 1% Tween-20 for each experimental variety. The contents were then shaken at 125 r.p.m (revolution per minute) at 25°C for 5 minutes. The liquid was removed by a pipette and the seeds spread on a blotting paper to dry overnight in a Bio-Safety cabinet.

### 2.3 Land Preparation

Land for field treatments was ploughed three times to a fine tilth and the experimental plots measuring 4 m X 3 m were demarcated and labeled.

### Field Treatments

In this research, a complete randomized block design was used to establish the effect of hot water seed treatment on black rot disease. The artificially inoculated seeds for each experimental variety were divided into 2 lots labeled as V<sub>1</sub>H<sub>0</sub> and V<sub>1</sub>H<sub>1</sub> (for variety 1) and V<sub>2</sub>H<sub>0</sub> and V<sub>2</sub>H<sub>1</sub> (for variety 2). The seeds labelled V<sub>1</sub>H<sub>1</sub> were treated with hot water at 50°C for 25 minutes in accordance with the International Seed Testing Association standard procedures - ISTA (Nega et al. 2003 and Miller et al. 2005) as illustrated below. The seeds labelled V<sub>1</sub>H<sub>0</sub> were not treated with hot water to serve as control for variety 1 (V<sub>1</sub>). The seeds labelled V<sub>2</sub>H<sub>1</sub> were also separately subjected to hot water while those labelled V<sub>2</sub>H<sub>0</sub> were not subjected to hot water to serve as control for variety 2. The treatments were repeated in 4 Replicas and in two different seasons.

### Hot Water Seed Treatment Procedures

The two lots of seeds labelled as V<sub>1</sub>H<sub>1</sub> (for variety 1) and V<sub>2</sub>H<sub>1</sub> (for variety 2) were separately subjected to hot water treatment in accordance with Nega et al. (2003) and Miller et al. (2005) standard procedures. The V<sub>1</sub>H<sub>1</sub> and V<sub>2</sub>H<sub>1</sub> seeds were loosely wrapped in separate woven cotton cheesecloth bags, thoroughly soaked and pre-warmed for 10 minutes in separate water baths, both maintained at 37°C (100°F) to eliminate any air in them. The pre-warmed seeds were then transferred separately into another water bath maintained at 50°C where they were heated for 25 minutes. The water baths were constantly checked by means of clinical thermometers to ensure that the temperatures remained constant at 50°C all through the heating period. After 25 minutes of heating, the bags containing the treated seeds were then removed from the hot water and immediately soaked in cold tap water for 5 minutes to stop the heating action. The treated seeds were then spread in single uniform layers on screens in a Bio-safety cabinet where they were left to dry, after which they were

established in different nursery beds in a greenhouse.

#### Nursery bed and field Establishments.

All the seeds were then established immediately after hot water treatment in separate and well labelled Greenhouse nursery beds using the standard procedures for cabbage crop propagation. The seedlings were left in the nursery beds for a period of 3 weeks after which they were transferred into the field using the standard field operation procedures for the crop.

#### Data collection and analysis

Data was collected on a scale of 1-9 based on the degree of susceptibility of the cabbage plants to black rot disease. Disease scoring was done on the basis of the level of disease symptoms based on the length (in centimeters) of the V-shaped lesions developing on cabbage leaves as described by Guo et al. (1991) and Leonel (2015). Table 1 below shows the rates used to score the disease as the plants were growing in the field. Scoring was done for a period of 4 weeks in season 1 and 7 weeks in season 2 after crop establishment.

**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

The zero rating scale was not applied in this research as it could refer to situations where rating could not be made. Scale 1 & 2 was applied to plants with minimum symptoms therefore showing least disease. Plants with moderate symptoms scored 3, 4 & 5 implying such plants had moderate disease. Scale 6 & 7 was reserved for plants with intense symptoms. The highest scores were 8 & 9



**Plate 2.** Results of pathogenicity test after inoculating young seedlings of cabbage with isolates of *Xanthomonas campestris pv. Campestris*.

reserved for plants with severe symptoms and even death of plants.

The research findings were statistically computed by Statistical analysis system (SAS) using Analysis of Variance (ANOVA). Mean separation was accomplished using the Turkey's multiple range test and all tests of significance were conducted at  $P \leq 0.05$ . The results were then presented using tables and photographs.

## RESULTS

The research findings are presented under the following sub-headings:

#### Isolation and Identification of *Xanthomonas campestris pv. Campestris*

Plate 1 shows the pale yellowish and convex mucoid bacterial colonies observed in the laboratory plates during the isolation of *Xanthomonas campestris pv. Campestris*.



**Plate 1.** Cultures of *Xanthomonas campestris pv. campestris* Colonies

These colonies are characteristic to the bacterium *Xanthomonas campestris pv. Campestris*. The bacterial isolates stained red upon staining with Rugol's Iodine and counter-staining with Safranin red stain which is also characteristic to *Xanthomonas campestris pv. Campestris*, being a Gram negative bacterium.

Plate 2 shows the results of the pathogenicity tests carried out to confirm the identity of *Xanthomonas campestris pv. Campestris* laboratory isolates.

V-shaped lesions seen developing from the leaf margins after 10 days of inoculating one month old cabbage seedlings with  $10^4$  CFU/ml of bacterial suspensions are symptomatic characteristics of the bacterium *Xanthomonas campestris* pv. *Campestris*.

**Results from Field Treatments**

The aim of this research was to evaluate the effect of hot water treatment of seeds in the management of black rot disease of cabbage. To achieve this, a total of 20 observations were made as tabulated in Tables 2 to 5 below.

**Table 2.** Disease Mean Score for Hot Water Treatment of Seeds across varieties.

Time (Weeks)	V <sub>1</sub> S <sub>1</sub> H <sub>1</sub>	V <sub>1</sub> S <sub>1</sub> H <sub>0</sub>	V <sub>1</sub> S <sub>2</sub> H <sub>1</sub>	V <sub>1</sub> S <sub>2</sub> H <sub>0</sub>	V <sub>2</sub> S <sub>1</sub> H <sub>1</sub>	V <sub>2</sub> S <sub>1</sub> H <sub>0</sub>	V <sub>2</sub> S <sub>2</sub> H <sub>1</sub>	V <sub>2</sub> S <sub>2</sub> H <sub>0</sub>
7			3.5 a	8.0 a			3.5 a	6.25 a
6			3.5 a	8.0 a			3.0 ab	5.75 a
5			3.5 a	6.0 b			3.0 ab	4.75 b
4	3.75 a	7.75 a	3.0 a	5.5 b	2.5 a	6.75 a	2.5 bc	4.75 b
3	3.5 a	7.50 a	2.25 b	5.25 bc	2.5 a	6.00 a	2.0 cd	4.25 b
2	1.5 b	4.25 b	2.0 b	4.5 c	1.25 b	4.25 b	1.5 de	4.00 b
1	1.0 b	2.00 c	1.25 c	2.75 d	1.00 b	2.00 c	1.25 e	2.75 c
Mean	2.438	5.375	2.714	5.714	1.813	4.75	2.393	4.643
SE	0.239	0.221	0.207	0.290	0.292	0.312	0.197	0.310
CV %	20	8	15	10	32	13	16	13
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Means figures in the column with different letters are significantly different (p<0.05), where time was measured in weeks after crop establishment in the field, V<sub>1</sub>S<sub>1</sub>H<sub>1</sub> is disease mean score for hot water variety 1 season 1, V<sub>1</sub>S<sub>1</sub>H<sub>0</sub> is disease mean score for no hot water variety 1 season 1, V<sub>1</sub>S<sub>2</sub>H<sub>1</sub> is disease mean score for hot water variety 1 season 2, V<sub>1</sub>S<sub>2</sub>H<sub>0</sub> is disease mean score for no hot water variety 1 season 2, V<sub>2</sub>S<sub>1</sub>H<sub>1</sub> is disease mean score for hot water variety 2 season 1, V<sub>2</sub>S<sub>1</sub>H<sub>0</sub> is disease mean score for no hot water variety 2 season 1, V<sub>2</sub>S<sub>2</sub>H<sub>1</sub> is disease mean score for hot water variety 2 season 2, and V<sub>2</sub>S<sub>2</sub>H<sub>0</sub> is disease mean score for no hot water variety 2 season 2.

In terms of variety, the results show that there was a general increase in disease score with time, that is the highest (6.25) score at the highest time (that is in later stages of crop development) and lowest (2.75) at the initial stages (time 1) for cases where seeds were not treated with hot water variety 2 in season 2. A similar trend was observed for in variety 1 in season 1.

Means figures in the column with different letters are significantly different (p<0.05), where H<sub>1</sub>S<sub>1</sub> is the disease mean score for hot water season 1, H<sub>0</sub>S<sub>1</sub> is the disease mean score for no hot water season 1, H<sub>1</sub>S<sub>2</sub> is the disease mean score for hot water season 2, and H<sub>0</sub>S<sub>2</sub> is the disease mean score for no hot water season 2.

Generally the trend shows that the scores increased with time, that is the highest (7.125) at the highest time (that is at later stages of crop development) and lowest (2.750) at the initial stages (time 1) for cases where seeds were not treated with hot water in season 2. In season 1, the trend was similar to that of season 2 where the highest scores were obtained at time 4 (7.25) as compared to time 1 (2.0) where seeds were not treated with hot water. The scores were generally high in cases where seeds were not treated with hot water as compared to where seeds were treated with hot water in both seasons 1 and 2 indicating hot water treatment of seeds reduced the severity of disease in cabbage fields.

**Table 3.** Disease mean score for hot water treatment of seeds across seasons.

Time in Weeks after crop establishment	H <sub>1</sub> S <sub>1</sub>	H <sub>0</sub> S <sub>1</sub>	H <sub>1</sub> S <sub>2</sub>	H <sub>0</sub> S <sub>2</sub>
7			3.50 a	7.125 a
6			3.25 ab	6.875 a
5			3.25 ab	5.375 b
4	3.125 a	7.25 a	2.75 b	5.12 bc
3	3.0 a	6.75 a	2.125 c	4.75 bc
2	1.375 b	4.25 b	1.75 cd	4.25 c
1	1.0 b	2.0 c	1.25 d	2.75 d
Mean	2.125	5.063	2.554	5.179
SE	0.293	0.331	0.183	0.364
CV	39%	18%	20	20
P-value	<0.0001	<0.0001	<0.0001	<0.0001

**Table 4.** Disease mean score for hot water treatment of seeds.

Treatment	V <sub>1</sub>	V <sub>2</sub>	S <sub>1</sub>	S <sub>2</sub>	Across Seasons
No Hot water	5.591 a	4.682 a	5.063 a	5.185 a	5.136 a
Hot water	2.614 b	2.182 b	2.125 b	2.554 b	2.398 b
S.E	0.154	0.158	0.144	0.117	0.081
CV %	23	28	23	23	23
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Means figures in the column with different letters are significantly different ( $p < 0.05$ ), where V<sub>1</sub> is variety 1, V<sub>2</sub> is variety 2, S<sub>1</sub> is season 1 and S<sub>2</sub> is season 2. There is significant difference between the use of hot water treatment of seeds and no hot water treatment of seeds. The scores were significantly ( $p < 0.05$ ) higher (5.136) where seeds were not treated with hot water as compared to

where seeds were treated with hot water (2.398) across the seasons. In season 1, the trend was similar as to across seasons where by the scores were significantly ( $p < 0.05$ ) higher (5.063) where no hot water was used compared to where hot water was used (2.125). In season 2, the score where no hot water was used was significantly ( $p < 0.05$ ) higher (5.185) as compared to where hot water was used (2.554).

**Table 5.** Disease mean scores for field treatments.

Treatment	Details of treatment	Score	Disease (%)	Score	Disease (%)	control
H <sub>1</sub>	Hot water treatment of seeds for both varieties	2.398 b	31.83			68.17
H <sub>0</sub>	Control (No hot water treatment of seeds)	5.136 a	68.17			
	Total field disease score	7.534				
	S. E.	0.123				
	CV %	42				
	P-value	<0.0001				

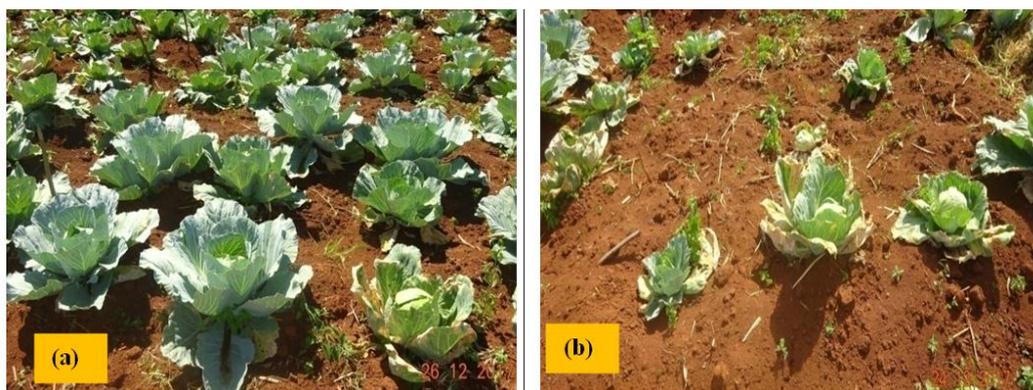
Means figures in the column with different letters are significantly different ( $p < 0.05$ ). There was evidence of significant differences among the treatments. Treatment H<sub>1</sub> (which is hot water treatment of seeds) had significantly ( $p < 0.05$ ) lower scores (2.398) compared to H<sub>0</sub> - the control (5.136). This resulted to 68.17% disease control in the field.

Plate 3 (a) and Plate 3 (b) shows that the disease was more severe in fields of plants where seeds were not treated with hot water as compared to where seeds were treated with hot water. Treating seeds with hot water at temperatures of 50°C for 25 minutes reduced the intensity of black rot disease in fields of cabbage as can be evidenced in Plate 3. The survival of bacteria *Xanthomonas campestris* pv. *Campestris* which is responsible for black rot disease of cabbage is limited at higher temperatures of 50°C.

## DISCUSSION

With regard to the Effect of Hot Water Treatment of Seeds on Black Rot Disease, the results showed that there was significant difference between use of hot water treatment of seeds and no hot water

treatment of seeds. As shown in Table 4, the scores were significantly ( $p < 0.05$ ) higher (5.136) where seeds were not treated with hot water as compared to where seeds were treated with hot water (2.398) across the seasons. From Table 4 again, it is evident that there was significant difference between the use of hot water treatment of seeds and no hot water treatment of seeds for both varieties 1 and 2. In variety 1, the scores for no hot water treatment of seeds (5.591) were significantly higher as compared to the scores for hot water treatment of seeds (2.614). Variety 2 had a similar trend to that of variety 1, whereby the scores for no hot water treatment of seeds (4.682) were significantly higher as compared to the scores for hot water treatment of seeds (2.182). It is also evident that variety 1 had more pronounced high disease scores (5.591) as compared to the disease scores for variety 2 (4.682) for cases where seeds were not treated with hot water at 50°C for 25 minutes. The difference in disease score between the two varieties would only be associated with varietal host pathogen-resistance.



**Plate 3.** (a) plants from inoculated seeds treated with hot water (b) plants from inoculated seeds not treated with hot water.

In season 1, the trend was similar to that of across the seasons. Table 4 shows that the scores were significantly higher (5.063) where seeds were not treated with hot water as compared to where seeds were treated with hot water (2.125). Similarly in season 2, the score where seeds were not treated with hot water was significantly ( $p < 0.05$ ) higher (5.185) as compared to where seeds were treated with hot water (2.554). These results therefore indicate that subjecting cabbage seeds to hot water at 50°C for 25 minutes lowers the intensity of black rot disease in the field. Once more the difference in disease score between seasons would only be linked to the changes in weather conditions that may have affected disease development.

Again this results indicate that the bacterium *Xanthomonas campestris pv. campestris* thrives well in wet and warm climatic conditions which are characteristic of the second planting season in Kisii County where this research was carried out. This research was done using two different varieties and in two different seasons to nullify the error due to host-pathogen resistance and weather conditions which would have adversely affected experimental data.

Generally the scores increased with time, that is, the highest at old stages and lowest at the initial stages of plant growth for both varieties 1 and 2, as shown in Table 2. A similar trend was observed for both seasons 1 and 2 as shown in Table 3. The results also indicate that seeds not treated with hot water had higher disease score compared to those treated with hot water. Plate 3 (b) confirms that the disease was more severe in plants germinating from seeds which were not treated with hot water after inoculation as compared to those plants from seeds treated with hot water after inoculation as illustrated in Plate 3 (a).

From this results, it can be concluded that the survival of the bacterium *Xanthomonas campestris pv. Campestris* at higher temperatures

of 50°C and above is limited. Hot water treatment of seeds at a temperature of 50°C for 25 minutes was able to reduced black rot disease in cabbage by 68.17 % (Table 5). It can therefore be argued that the efficacy of hot water treatment of cabbage seeds against *Xanthomonas campestris pv. Campestris* at 50°C for 25 minutes was 68.17%. These findings thus prove that heat water treatment of cabbage seeds at 50°C for more time than 25 minutes could give better results. However, care should be taken to prevent overheating of seeds as this would results into reduction in germination percentage of seeds which would again make the farmer to suffer a loss.

## CONCLUSION

This research study sought to evaluate the efficacy of hot water seed treatment against *Xanthomonas campestris pv. campestris* in the control of black rot disease of cabbage. This was in realization of the fact that black rot disease, caused by *Xanthomonas campestris pv. campestris*, causes major yield losses to small scale farmers in Kenya. The results obtained indicate that there is evidence of significant difference in disease score between the plants from seeds treated with hot water at 50°C and plants from seeds not treated with hot water after artificial inoculation of seeds with isolates of *Xanthomonas campestris pv. campestris*. There was significantly ( $p < 0.05$ ) high disease score (5.136) for plants germinating from seeds not treated with hot water as compared to those plants from seeds treated with hot water (2.398) across the seasons. The trend was the same for seasons 1 and 2 and across the varieties. The results obtained showed that subjecting cabbage seeds to hot water treatment at 50°C for 25 minutes had an efficacy of 68.17 % thus reducing disease severity in the field by 68.17 %.

## RECOMMENDATION

This research study sought to establish the effect of hot water seed treatment on black rot disease of cabbage. The research findings have indicated that hot water 50°C for 25 minutes had significant effect in the management of black rot disease of cabbage under field conditions. We therefore recommend the use of this technique together with other cultural practices to prevent cabbage losses caused by black rot disease.

## ACKNOWLEDGEMENTS

We wish to acknowledge the Kenya Agricultural and Livestock Research Organization center- Kisii branch for allowing us to use their land and laboratory for this research. Our sincere appreciations to the officers from various departments who willingly provided advice as well as to the manual workers who readily provided labour whenever needed. The security guards did a wonderful job of ensuring our project was safe throughout the research period.

## REFERENCES

- Alvarez AM. 2000. Black rot of crucifers. In: Slusarenko AJ, Fraser RSS, van LLC (Eds.) *Mechanisms of Resistance to Plant Diseases*. Dordrecht, The Netherlands: Kluwer Academic Publishers. Pg 21-52.
- Anonymous. 2000. Plant Protection Manual for Selected Vegetables: French beans, Brassicas and Tomatoes. GTZ/ICIPE CD-ROM. Nairobi, Kenya.
- Bila J. 2008. Status of Bacterial Black rot of Brassicas in Southern Region of Mozambique: Survey, Detection and Identification of the Causal Agent *Xanthomonas campestris pv. campestris*. M.Sc. thesis, University of Copenhagen, Denmark. pg102.
- Bila J, Mortensen CN, Andresen M, Vicente JG, Wulff EG. 2013. *Xanthomonas campestris pv. campestris* Race 1 is the main causal agent of Black rot of Brassicas in Southern Mozambique. University of Copenhagen, Denmark. *African Journal of Biotechnology* Vol. 12 (26): 602-610.
- Celetti M, Kristen C. 2002. Black Rot of Crucifer Crops. Ministry of Agriculture, Food and Rural Affairs. Ontario State. Revised May 2014.
- Christianson J. 2008. Club Root of Crucifers. University of Nebraska-Lincoln.
- Guo H, Dickson MH, Hunter JE. 1991. Brassica napus sources of Resistance to Black Rot in crucifers and inheritance of resistance. New York State of Agricultural Experiment station. Cornell University, Geneva, NY 14456-0462. *Hort Science Journal* Vol. 26 (12) 1547.
- Huang HHC. 1997. Biological Control of Soil-borne Diseases in Canada, In: International Symposium on Clean Agriculture, Sapporo: OECD. Pg 52-59.
- Jahn M, Nega E, Werner S. 2000. Pilzbefall a Gemüsesaatgut: Verträglichkeit und Wirkung der Heißwasserbehandlung. *Gemüse* 36, 17-19.
- Jensen JL. 1888. The propagation and prevention of smut in oats and barley. *Journal of Royal Agricultural Society of England*, series 2, 24, 397-415.
- Koch I, Wahl R, Laun N, Krauthausen HJ, Bauermann W. 2000. Bakterielle Blattflecken a Möhren - Bedeutung und Gegenmaßnahmen. *Gemüse* 36, 28-30.
- Leonel AA. 2015. Evaluation of resistance on Cabbage varieties: Resistance against *Xanthomonas campestris pv. campestris* in Mozambique. College of Agronomy and forest Engineering, Zambezi university. *Internal Journal of Agriculture and Crop Sciences*. Vol. 8 (5) 723-731. 2015.
- Linders R. 2000. Aktueller Wissensstand bei *Xanthomonas campestris* an Kohl. *Gemüse* 36, 31-32.
- Lo CT, Wang KM. 2001. Inoculum sources of Black Rot of Wasabi, caused by *Phoma wasabiae*. *Plant Pathol Bull.* 10: 88- 92.
- Massomo SMS, Mabagala RB, Swai IS, Hockenhull J, Mortensen CN. 2003. Evaluation of varietal resistance in cabbage against the black rot pathogen, *Xanthomonas campestris pv. campestris* in Tanzania. *Crop Protection* 23 (4): 315-325.
- Miller AS. 2002. Disease Management for Conventional and Tomato Growers. New York State Vegetable Conference and Berry Growers Meeting Proceedings: Pg 193-194.
- Miller SA, Lewis IML. 2005. Hot water treatment of vegetable seeds to eradicate bacterial plant pathogens in organic production systems. *Plant Pathology Extension Fact sheet* HYG-3086-05. The Ohio State University.
- Miller S, Ivey M. 2019. Hot Water Treatment of Vegetable Seeds to Eradicate Bacterial Plant Pathogens in Organic Production System.
- Nega Eva, Roswitha Ulrich, Sigrid Werner, Marga Jahn. 2003. Hot water treatment of Vegetable Seed – An alternative Seed Treatment Method to control seed-borne pathogens in organic farming. *Journal of Plant Disease and Protection*. Vol. 110 (3): pp 220234.
- Otipa M, Kamau R, Gekone M. 2013 – Pest management decision guide: green and

- yellow list. Black rot disease- Plantwise. East African pest management innovation lab. The Ohio State University, College of food, Agriculture and Environmental Sciences.
- RADA (Rural Agricultural Development Authority). 2012. Hot Water Seed Treatment for the control of Bacterial Spot of Scotch Bonnet Pepper. Division of Technology, Training and Technical Information, Hope Gardens. Kingston 6, Jamaica, West Indies.
- Ryan RP, Vorhölter FJ, Potnis N, Jones JB, Van SMA et al. 2011. Pathogenomics of *Xanthomonas*: Understanding bacterium-plant interactions. *Nat Rev Microbiol* 9: 344–355.
- Sayonara MP, Mariano RLR, Sami JM, Gil Silva, Elizabeth AAM. 1999. Antagonism of yeasts to *Xanthomonas campestris* pv. *campestris* on cabbage phylloplane in field. *Journal of microbiol.* Vol. 30 (No.3): 375-379. Sao Paulo SP- Brazil 1999. Fitossanidade, Recife, PE, Brasil.
- Seebold K, Bachi P, Beale J. 2008. Black rot of crucifers. UK Cooperative Extension Service. University of Kentucky- College of Agriculture.
- Varela AM, Seif AA, Lohr B. 2003. A guide to Integrated Pest Management in Brassicas production in Eastern and Southern Africa. ICIPE, Nairobi Kenya.
- Vicente JG, Conway J, Roberts SJ, Taylor JD. 2001. Identification and origin of *Xanthomonas campestris* pv. *campestris* races and related pathovars. *Phytopathology* 91: 492-499.
- Walker JC. 1923. The hot water treatment of cabbage seed. *Phytopathology* 13, 251-253