# Response of Selected Cabbage Varieties to *Alternaria brassicicola* under Greenhouse Conditions

Mwatabu E., Were J. O. and V. E. Anjichi

University of Eldoret, School of Agriculture and Biotechnology,
Seed Crop and Horticultural Sciences Department, P.O.Box 1125- 30100, Eldoret Email-
edwardmwatabu@gmail.com

**Abstract**

*Dark leaf spot (Alternaria brassicicola) is an important disease of cabbage crop. It is a major constraint in cabbage production in Kenya causing substantial losses if not managed properly. Hybrid varieties are now in the market but there exists little information on their resistance and tolerance to this disease. To undertake breeding for resistance and come up with improved varieties, there is need to identify cabbage genotypes for possible sources of resistant genes to the disease. This study was carried out to assess the response cabbage varieties to Alternaria brassicicola pathogen at the University of Eldoret, School of Agriculture and Biotechnology Plant Pathology Laboratory and in the greenhouse. Isolation and purification of the pathogen was done in appropriate conditions in the laboratory and inoculated on to 10 days old transplanted seedlings. The experiment was laid as completely randomized design (CRD) with each variety replicated four times. Scores were made using a scale of 1-10 on nature of the leaf with leaf spots and percentage disease index per plant was calculated. Data collected was analyzed using Genstat software version 14.1. The study revealed no significant difference in percentage infection among varieties. Using single link method three clusters were formed at 95% similarity coefficient axis. It was concluded that Gloria F1 and Copenhagen were susceptible; Queen F1 was tolerant while Pruktor F1 and Sugarloaf varieties were resistant. Breeding institutions and farmers are recommended to use tolerant varieties for breeding improvements and high yields*.

**Keywords:** Varieties; Response; *Alternaria brassicicola;* Pathology; Tolerance.

## INTRODUCTION

Cabbage is a leafy vegetable which belongs to the genus brassica. It is a major horticultural crop in Kenya grown for both local and export market, mostly to the European market. Cabbage is an important vegetable for the domestic economy, being grown by over 90% small holders. In the year 2007, cabbage was leading in terms of production ahead of tomatoes and kales. Cabbage is an excellent source of vitamin K which has a potential role in bone metabolism. It also contains high amount of dietary fiber, which reduces some form of cancers such as breast cancer in human beings, prevent constipation and it improves digestion. Cabbage production in Kenya has however shown mixed trends over the past years due to disease (Mwangi, 2011). Use of disease susceptible varieties/cultivars and low quality seeds are some of the factors that undermine production (Rop *et al.*, 2009).

Several studies have been carried out to understand and manage dark leaf spot disease in cabbage. Field surveys have been carried out in Kenya to determine the occurrence and incidence of back leaf spot disease (Rop *et al.,* 2009). A lot of breeding is being carried out to develop disease resistant and high yielding varieties in which quite a number are now available in the market. However despite these and many other efforts, little attention has

been given to the study and management of dark leaf spot disease of cabbage crop grown in Kenya.

There is inadequate information on characterization and management of dark leaf spot disease in cabbage. Little focus has been given to the study of commercial varieties with respect to their genetic makeup and their response to the disease. This is a major challenge in planning for the strategic management of the disease (Rop *et al.,* 2009).

Due to the seed borne nature of *Alternaria brassicicola,* the management of dark spot disease should be given priority. This pathogen is known to survive in infected plant debris and affects many plant parts such as leaves, stems, and seeds (Tah *et al.,* 2013). Growing disease resistant varieties besides use of natural products, bio-control agents and alterations in agronomic practices, is a good economic, eco-friendly and safe management practice (Nayyar *et al.,* 2014). Assessment of cabbage varieties for their response to the disease is therefore a necessary step.

## MATERIALS AND METHODS

The research was carried out in the University of Eldoret School of Agriculture Pathology lab and greenhouse. Cabbage (*Brassica oleracea* Var. *capitata*) varieties were obtained from commercial seeds retail shops in Eldoret. Cabbage leaves that had typical symptoms of leaf spot were obtained from the nearest market around University of Eldoret premises.

Preparation of potato dextrose agar (PDA) was made by peeling two hundred grams of potato which were cleaned, cut into small pieces, boiled in distilled water and the extract collected by filtering using a sieve.

Dextrose (20 g) and agar (15 g) were dissolved in the potato extract and the final volume was made up to 1000 ml with distilled water. The flask containing dispensed medium was sterilized at 121 $^{O}$C in an autoclave.

The isolation procedure for the pathogen *Alternaria brassicicola* used, was by following standard tissue isolation method (Tu, 2015). Early symptoms of this disease appear as the presence of small yellow specks on the older leaves and stems. The spots then enlarge to become circular dark brown spots and form alternating light and dark concentric rings. Small pieces of the infected portions of leaves about 1cm$^2$ were cut and surface sterilized in 1% sodium hypochlorite solution for 1 minute. The pieces were washed repeatedly thrice in distilled water and then they were transferred to sterile Petri dishes containing Potato dextrose agar. They were incubated at room temperature of 25-28$^{o}$C in the dark for 7 days and periodic observations were made for fungal growth (Rop *et al*., 2009). The colonies were observed to develop from the bits. Identification of the fungus was done by taking the portions of *Alternaria brassicicola* from the grown culture and mounted on a clear glass slide. Microscopic observation was done by taking mycelia and spore characters. The fungus is known to grow faster in media with high spore formation. It appeared as well developed grayish colony.

Single spore cultures of isolates were purified by scooping small portions of the identified grown fungi on fresh potato dextrose agar (PDA) plates at 25±1 ºC (Swati *et al.,* 2014). The experiment was planted in a completely randomized design replicated four times. Five

weeks old seedlings were transplanted to plastic pots. The varieties used include: Gloria F1, Queen F1, Pruktor F1, Sugar loaf and Copenhagen.

Spore suspensions of the isolate were diluted in sterile distilled water for inoculation of plants. Better adhesion of the water-suspended spores to wax-covered leaf surface of cabbage was enhanced through wiping the upper and lower leaf surfaces with wet cotton. When the plants were 10 days old the inoculums were sprayed on each plant and left to dry so that successful infection could be realized. High humidity was maintained for more than nine hours by application of mist to ensure infection (Tu, 2015).

Disease severity scoring was done by use of a disease severity rating scale of 1-10 of dark leaf spot disease by *Alternaria brassicicola* (Doullah & Okazaki, 2015). Where, 1= no spots and no yellow color on the leaf, 2= a few pin point spots but no yellow, 3= some spots but no large lesions and no yellow, 4= some spots with a few lesions surrounded by light yellow, 5-9=increasing number and size of lesions and yellowing on the leaf and 10=Lesions with yellowing on more than 90% of the leaf. Where: 1-3 highly resistant, 3.1-4 tolerant and 4.1-10 susceptible.

Disease reaction was determined after 8days from the day of inoculation based on disease severity. Infected leaves were detached and kept in the dark. This was to ensure that the disease symptoms were expressed clearly.

Percentage infection was calculated in the experiment using the following formula:

Percentage infection= $\frac{\text{number of infected plants}}{\text{total number of inspected plants}} \times 100$ (Sabry, Ali, Abdel-Kader, & Abou-Zaid, 2015). Percentage of infected leaves for each plant was calculated using the following formula according to (Sabry, *et al*., 2015):

Percentage of infected leaves= $\frac{\text{Number of infected leaves per plant}}{\text{Total number of plant leaves}} \times 100$

Data collected was analyzed by cluster and descriptive analysis using Genstat software version 14.1. Cluster analysis was by forming single link clusters by use of Euclidean test at 95% similarity matrix coefficient.

## RESULTS

The study revealed that Sugarloaf and Pruktor F1 varieties expressed resistance while Copenhagen and Gloria F1 varieties were susceptible to the pathogen. The upper younger leaves expressed resistance while the older lower leaves were susceptible. The total infection was 95%. This was observed when the leaves were detached and kept in the dark with the leaf surfaces kept moist. The results indicated no large lesions were formed but all varieties had pin point spots and some with small lesions with few having a yellow halo.

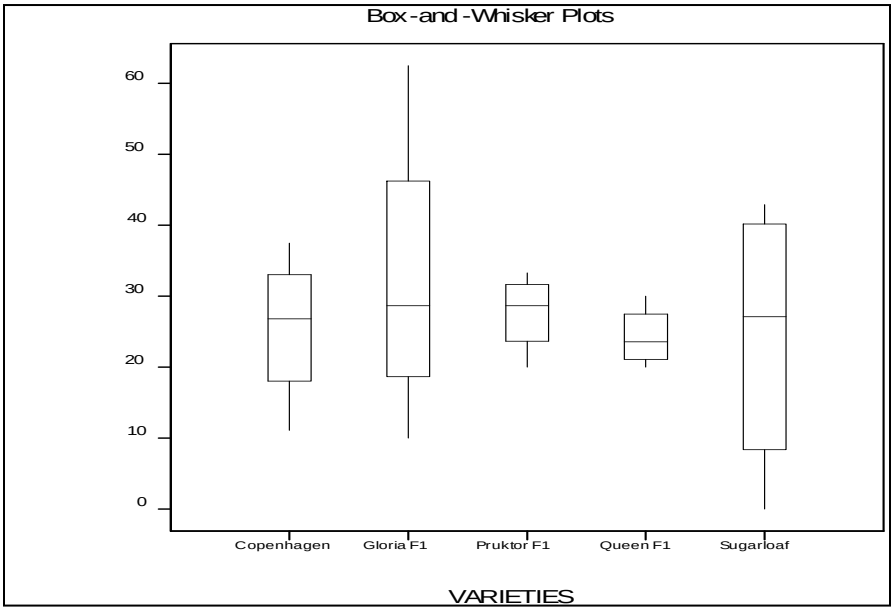


**Figure 1: Disease scale ratings against varieties**

From the above figure, Copenhagen and Gloria F1 variety did not differ significantly but significantly differed with Pruktor F1, Queen F1 and Sugarloaf varieties. Pruktor F1, Queen F1 and Sugarloaf varieties did not differ significantl

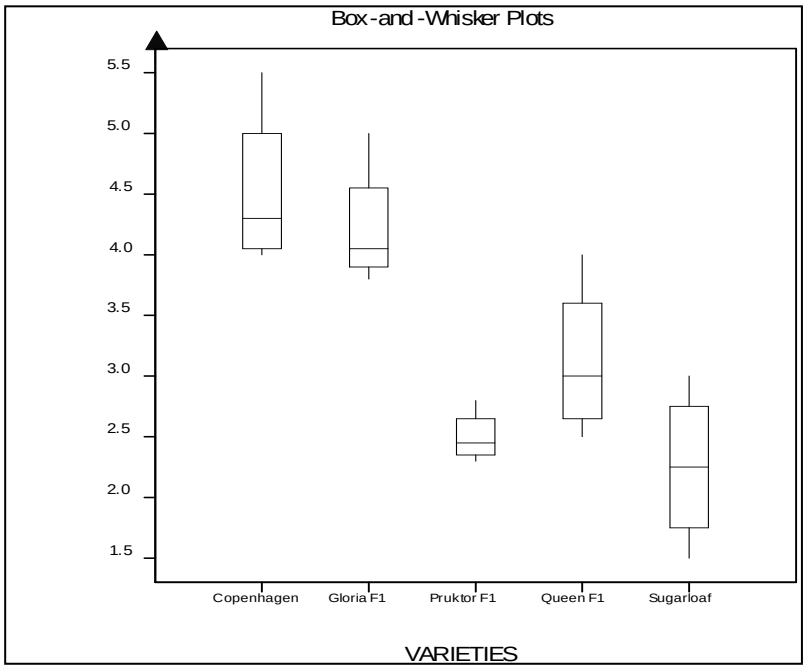


**Figure 2: Percentage infection against Varieties**

From the figure above, there are no significant differences between varieties in terms of percentage leaf infection. It was clear that infection occurred in the first few old leaves of the plants.

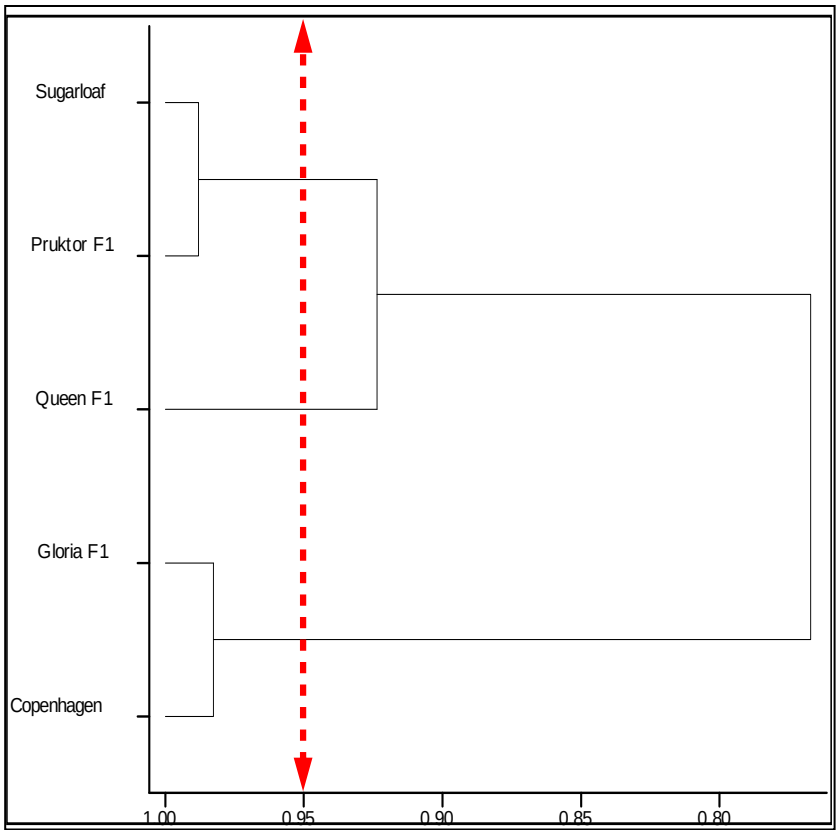


**Figure 3: Clusters at 95% similarity matrix coefficient**

The above figure shows the cluster analysis calculated from scores made by use of the disease severity scale. The clusters were determined by use of Euclidean test at 95% similarity matrix coefficient. The first cluster consists of Pruktor F1 and Sugarloaf varieties while second cluster consists of Copenhagen and Gloria F1 and the third cluster having Queen F1 variety.

## DISCUSSION

Variations in response might be due to physiological and/or genetic variation existing among the tested cabbage varieties which affect the host parasite interaction. Physiological resistance is due to leaf enzymes associated with the phenolic pathway, e.g., polyphenoloxidase, peroxidase and catalase. These enzymes enhance secretion of toxins that inhibit growth of the pathogen. Evaluation of brassica genotypes in existence for physiological factors associated with resistance is essential. Morphological resistance is due to deposition of leaf epicuticular wax thus formation of a hydrophobic coating which reduced the adherence of water-borne inoculum, conidial germination and germ tube

formation. The species and cultivars such as Pruktor F1 which tend to be less sensitive to Alternaria pathogens have relatively more epicuticular wax than others. Older leaves are more susceptible than younger ones to the pathogen; this is probably due to high levels of wax in the young immature leaves which make them resistant against it (Sabry *et al*., 2015).

Production of host specific toxins is a major factor for pathogenicity by the fungus. Most of these host specific toxins are metabolites and are required by the fungi to invade the tissue and cause disease. These toxins cause necrosis on leaves of susceptible varieties even at low concentrations and no necrosis on leaves of resistant varieties even at high concentrations. The exact roles of non host specific toxins in pathogenicity are largely unknown but some are thought to contribute to virulence (symptom development) of the pathogen (Tah *et al.*, 2013).

New developed varieties such as Queen F1 have thick waxy leaves of which this characteristic aid in tolerance to the pathogen. However breeders need to be intentional by developing varieties with characteristics that will enhance tolerance to the disease. This is because the new developed varieties especially the F1 hybrids are cultivated in high rainfall areas with favorable temperatures for disease development. This will aid to combat yield and quality losses caused by the disease. There is need also for further evaluation of genotypes in a wider geographical area and genetic study of wild species to identify sources for tolerance for the disease.

Apart from developing tolerant varieties to *Alternaria brassicicola,* use of various herbal extracts and natural products is being encouraged because they cause no health hazard or pollution. It has been found out that ethanol or methanol extract of speed weed (*Polygonum perfoliatum*) has an inhibitory effect against conidial germination of *A. brassicicola* causing leaf spot of spoon cabbage (Tah *et al*., 2013).

## CONCLUSION AND RECOMMENDATION

In summary assessment is useful to researchers and breeding institutions for the development of better varieties. Pruktor F1 and Sugarloaf varieties were resistant while Queen F1 was tolerant and thus can be used for high yields and further assessment for breeding improvement. From the study, further evaluation of the resistant varieties for the entire growing period is necessary. Extensive studies should be carried out on wild and cultivated species to identify tolerant genes for better varieties.

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