



# **Efficacy of Neem, Garlic and Aloe Extracts in the Management of Postharvest Potato Soft Rot Caused by *Erwinia carotovora***

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## **Authors' contributions**

*This work was carried out in collaboration between all authors. Author JB designed the study, author EMN wrote the protocol and performed the statistical analysis, author FMN wrote the first draft of the manuscript. Authors EMN and FMN managed the analyses of the study. Author JB managed the literature searches. All authors read and approved the final manuscript.*

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## **ABSTRACT**

Potato (*Solanum tuberosum* L.) production in Kenya is limited by soil borne diseases especially soft rot bacteria caused by *Erwinia carotovora*. Although the use of synthetic pesticides has significantly contributed in the management of *E. carotovora*, their use is often associated with environmental pollution and loss of beneficial soil biodiversity. Here, we investigated the efficacy of neem (*Azadirachta indica* M.), garlic (*Allium sativum* M.) and aloe (*Aloe secundiflora* Engl.) extracts, on the development of soft rot bacteria in three potato varieties; Kenya Mpya, Sherekea and Purple Gold. The treatments were laid out in a completely randomized design with five replicates. Interestingly, all the extracts significantly ( $p < 0.05$ ) reduced the extent of rot on the treated compared to the untreated tubers. Garlic recorded the highest antimicrobial effect as seen from the extent of rot ( $6.50 \pm 0.59\%$ ), while aloe ( $29.00 \pm 1.54\%$ ) gave the least antimicrobial effect. Sherekea potato variety showed the highest tolerance to *E. carotovora* among the studied potato varieties. These results demonstrate potential use of plant extracts in the future development of low cost and environmental friendly biopesticides; a key step towards achieving sustainable global food production.

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## 1. INTRODUCTION

Potato is an economically important cash crop for smallholder farmers and acts as a major source of livelihood for a great population of farmers in Kenya. An estimated 800,000 farmers grow potato in Kenya, while over 2.5 million Kenyans are employed along the potato value chain, either directly or indirectly. As in the rest of the tropical highlands of Africa, potato production in Kenya is dominated by smallholder farmers [1].

However, fungal, viral and bacterial infections in the field and in stores have led to production of poor quality potato tubers. Bacteria soft rot has come out to be a very important disease in the world, due to the losses it causes on potato crop at different stages of development in the field, during harvesting, transport and in stores [2,3]. The soft rot bacteria, secretes pectolytic cell-wall degrading enzymes which digest plant tissues hence causing decay. *Erwinia carotovora* is known to cause soft rot disease that lead to huge amounts of economic losses due to serious potato tuber decay [4,5]. Losses on potato produce in stores at high temperatures and humidity have been reported to be about 20-60%, because the high humidity provides a medium for enzyme action with the high temperatures of about 32°C activating the bacterial pectolytic enzymes which digest the tissues of the potato tubers [6,7]. Poor handling conditions may rise the percentage of losses to about 90-100% due to the wounds inflicted on the potato tubers during harvest and transportation creating an avenue for the bacterium to penetrate and digest the tissues of the tubers [8].

*Erwinia carotovora* attacks a wide host range including tomatoes, carrots, potatoes and onion among other vegetables, but the other species that belong to the genus *Pectobacterium* have a narrow host range [9]. Tubers infected by soft rot bacteria, are rendered unsafe for human consumption and in turn, farmers count on huge losses from their harvests because the quality of infected potato tubers do not meet the market demand.

Currently, synthetic chemical pesticides have been used to control postharvest pathogens such as soft rot bacteria [10]. However, their use has been faced with enormous challenges including chemical residues, non-biodegradability,

phytotoxicity, environmental pollution, development of resistance in target organism, high cost, non availability and hazardous to man and his environment [11]. These negations render the synthetic chemicals either, slow to be adopted by farmers or farmers totally fail to adopt them, hence alternative and environmentally friendly control methods like use of botanicals should be employed to manage *Erwinia carotovora* [12,13].

In this study, we hypothesized that Aloe, Garlic and Neem plant extracts differ in the control of soft rot bacteria in the main potato varieties (Kenya Mpya, Sherekea and Purple Gold). The specific aims of the study were: 1) to determine the level of susceptibility of potato varieties (Kenya mpya, sherekea and Purple gold) to soft rot bacteria. 2) To establish the efficacy levels of plant extract of Neem, Garlic and Aloe in the management of *Erwinia carotovora*.

## 2. MATERIALS AND METHODS

### 2.1 Preparation of Plant Extracts

Fresh healthy leaves of Neem were collected and washed using running tap water after which, they were kept under shed at room temperature for 1-2 weeks to dry in air. The dried leaves were ground into powder form using a sterile electric blender. A 100 g of the powder obtained was soaked in 400ml distilled water for 24 hours in one litre stoppered conical flasks. After 24 hour of soaking, they were filtered through a cheese cloth and the solution extract obtained was refrigerated for use. This solution was used as the stock solution with 100% concentration for use as an anti-microbial solution [14].

Mature Garlic bulbs (*Allium sativum* M.) were washed using sterilized water and then kept in an oven to dry at 40°C for one week. The dried bulbs were then ground using a sterilized electric blender. Garlic bulbs are succulent and air drying could take a longer period than the stipulated period. A 100 g of the powder was soaked in 400 ml distilled water for 24 hours in one litre stoppered conical flasks. After 24 hours of soaking, they were filtered through a cheese cloth and refrigerated for later use as an anti-microbial solution [15].

Fresh mature leaves of *Aloe secundiflora* were collected from the lower portion of the plant and

thoroughly washed with distilled water and air dried at room temperature for 2 hrs in the laboratory. Thereafter they were dipped into 70% ethanol for surface sterilization. The fresh leaves of Aloe were cut into small portions with a flamed sterilized knife and then blend using a sterilized electric blender. The crude extract was dissolved in sterile water and used as the standard solution. For experimental purposes each plant extract was serially diluted using the double fold procedure in presence of sterilized water to obtain concentrations of 100%, 50%, 25% and 12.5% [16].

## 2.2 Bacterial Isolation

Infected potato tubers with soft rot symptoms were first cleaned using flowing tap water followed by surface sterilization in 2% solution of sodium hypochlorite. Using a flamed sharp knife, the tubers were cut to obtain the section between the infected part and the health section [17,18]. The cut section was further broken down into smaller pieces and smashed in about 15 ml of sterilized distilled water in a beaker to form bacterial suspension. A loopful was obtained from the prepared suspension and streaked aseptically in plates containing sterilized nutrient agar. The plates were then incubated at 37°C for 48 hours and observations were made after every 24 hours for colony growth. Once the bacterial growth occurred, a close examination was done on the inoculated plates to identify distinct colonies. Singly formed colonies were re-streaked aseptically and sub-cultured to obtain pure *Erwinia carotovora* cultures. Identification was based on two main levels; colony features and microscopic examination which involved Gram stain, sporulation, body shape and flagellation [19,20]. From the experimental examinations, the bacterium was rod shaped with creamy white, slightly raised and glistening colonies, gram-negative, non-sporing and peritrichously flagellated [21].

## 2.3 Determination of Susceptibility Levels of Potato Varieties

Healthy tubers of the three selected potato varieties (Kenya Mpya, Sherekea and Purple Gold) obtained from farmers in South Kinangop, Nyandarua County, Kenya were dipped in a solution of 2% sodium hypochlorite for surface sterilization. On each of the sterilized potato tubers, a core opening was made using a flamed

5 mm cork borer and about 0.5 ml of the bacterium inocula placed inside the hole using a syringe [22]. The portion removed from the hole was returned back and sealed using a sterilized petroleum jelly. The inoculated potato tubers were kept at room temperature and left to stand undisturbed for 10 days. The treatments were laid in completely randomized design and replicated five times. After ten days, the tubers were cut open longitudinally along the point of inoculation using a sharp knife. The extent of rot in each case was determined by measuring the rotten section in millimeters (mm) using a transparent meter ruler. The extent of rot in length was then converted into percentage by dividing the rotten length by total potato length multiplied by 100% [23]. Following this procedure, the mean percentage extent of rot was determined for each potato variety. The variety with the highest mean percentage of rot was identified as the most susceptible variety to bacteria soft rot and the potato variety with the least mean percentage rot was established as the most tolerant to soft rot bacteria.

## 2.4 Efficacy of the Botanicals on Management of *Erwinia carotovora*

Healthy *Solanum tuberosum* L. tubers of the three potato varieties were surface sterilized and 5 mm core openings made using a flamed cork borer. The core openings were then treated with the plant extracts by flooding the hole and let to stand for about 30 minutes and drained before the bacteria inocula was placed inside the hole [22]. The portion removed from the hole was returned back and the point of inoculation was sealed with sterilized petroleum jelly to make it air tight. A control experiment was set for each treatment, in which tubers were inoculated but not treated with plant extracts. These set ups, were kept at room temperature and observations made after 10 days. Each of the inoculated tubers was cut longitudinally along the point of inoculation using a sharp knife and the extent of rot measured using a transparent meter ruler [23,24]. The extent of rot measured as length (mm) was converted into percentage in relation to averaged length of the potato tubers as calculated in Experiment 1.

## 2.5 Statistical Analyses

Data on susceptibility of the potato varieties to soft rot and efficacy of the selected plant

extracts in management of the disease were tested for homogeneity of variance using Bartlett's test followed by ANOVA using SPSS version 20 computer program. Wherever P was significant, means were separated by Tukey's HSD test at  $P \leq 0.05$ .

### 3. RESULTS

#### 3.1 Susceptibility of the Potato Varieties to Soft Rot

Before the tubers were subjected to any treatment from the plant extracts, the mean percentage of potato rot was determined on inoculated potato tubers. It was established that Kenya Mpya variety had significantly ( $F = 33.74$ ,  $P = 0.001$ ) higher mean percentage rot than

Purple gold, while Sherekea showed the lowest mean percentage rot (Fig. 1).

#### 3.2 Efficacy of Plant Extracts in the Management of *Erwinia carotovora*

In the Kenya mpya potato variety, Garlic treatment showed the lowest extent of rot compared to Neem and Aloe which were significantly higher than the control treatment ( $F = 123.7$ ,  $P = 0.001$ , Table 1). A similar trend was observed in purple gold and Sherekea potato varieties, although aloe and control treatments did not significantly differ in the latter. Generally, the Garlic treated tubers had much reduced extent of rot while control tubers recorded the highest percentage rot followed by Aloe treated tubers (Table 1).

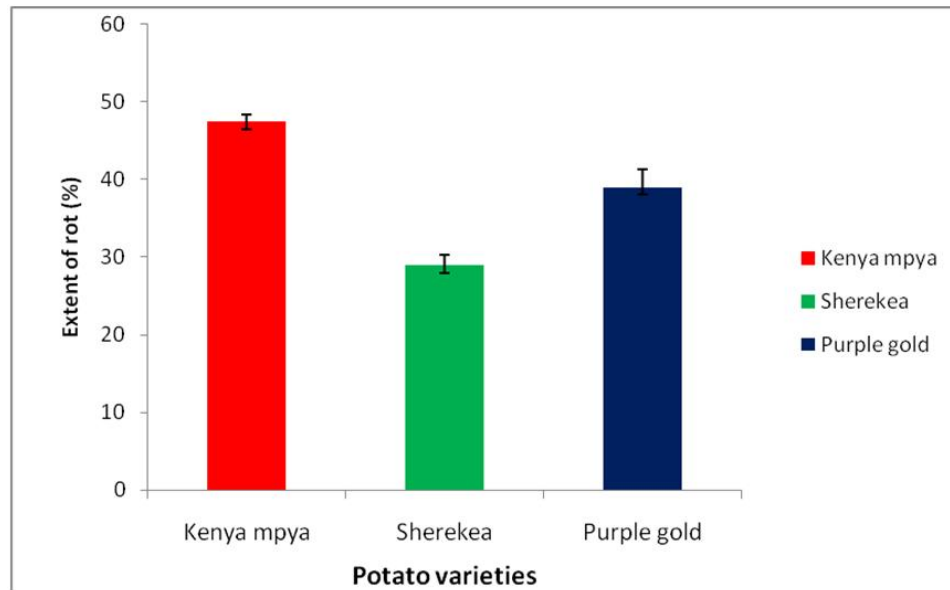
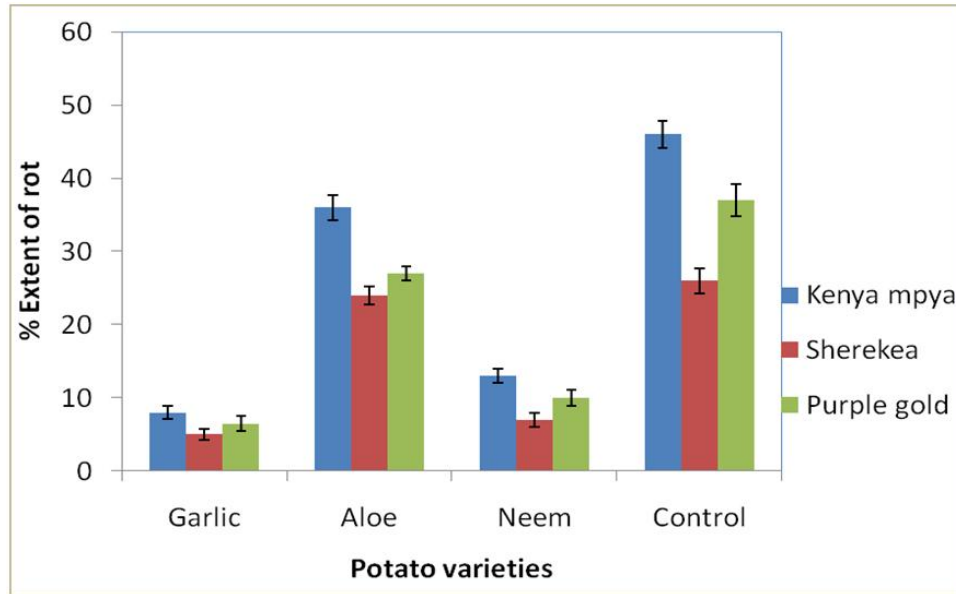


Fig. 1. The mean percentage rot of inoculated and untreated potato tubers of the three potato varieties (Sherekea, Kenya mpya and Purple gold) inoculated with *Erwinia carotovora*

Table 1. Mean % rot of tubers of three potato varieties (Kenya Mpya, Sherekea and Purple Gold) inoculated with *Erwinia carotovora* and treated with plant extracts of Garlic, Neem and Aloe

Treatments	Potato varieties		
	Kenya mpya	Purple gold	Sherekea
Garlic	8.00±0.94a	6.5±1.00a	5.00±0.79a
Aloe	36.00±1.70c	27.00±0.94c	24.00±1.27c
Neem	13.00±0.94b	10.00±1.12b	7.00±0.94b
Control	46.00±1.87d	37.00±2.15d	26.00±1.70cd
P value	<0.001	<0.001	<0.001

Mean values in the same column denoted by similar letters are not significantly different at  $P \leq 0.05$  (Tukeys HSD test)



**Fig. 2. Effects of potato variety and plant extract interaction on mean % extent of rot on tubers**

### 3.3 Effects of Potato Variety and Plant Extract Interaction on Mean % Extent of Rot on Tubers

There was a significant interaction between the potato variety and the kind of plant extract applied on the inoculated tubers. Sherekea showed much reduced mean percentage extent of rot  $15.5 \pm 2.26$  when subjected to treatment of the three plant extracts of Garlic, Neem and Aloe. This was followed by Purple gold and Kenya mpya with greater percentage extent of rot  $20.13 \pm 2.93$  and  $25.75 \pm 3.67$  respectively. Compared to the negative control experiment, the plant extracts revealed varying degree of antimicrobial activities. Garlic was more superior when applied to the three different potato varieties with mean percentage rot of  $6.50 \pm 0.59$  followed by Neem  $10.00 \pm 0.85$  and Aloe  $29.00 \pm 1.54$  giving the least efficacy (Fig. 2).

## 4. DISCUSSION

Our results show that, Kenya mpya potato variety had a higher level of rot than the other two varieties, while Sherekea had the least mean percentage rot. This indicated that the most susceptible potato variety to bacterial soft rot is Kenya mpya, followed by the golden purple. The Sherekea variety showed greater resistance to soft rot bacteria with very much reduced mean extent of rot as compared to Kenya Mpya and Purple gold. These results agreed with an

outcome released by KALRO Tigon hotfresh Journal; (December-February 2012). In this journal, Sherekea potato variety with red skin was reported to be highly tolerant to diseases like late blight and viruses. It also stated that Kenya mpya is very susceptible to soft rot bacteria due to its soft skin as compared to the other potato varieties. Related work by De Boer et al. [25] reported that physiological factors like tuber size, dormancy period and maturity had significant effects on susceptibility to soft rot with larger tuber size, short dormancy and early maturity tubers being more susceptible. Kenya mpya has a short dormancy and early maturity of three months and farmers can start harvesting even when leaves are green for early market [26]. A study by Gudmestad [27] stated that, tubers harvested from green vines are more susceptible to postharvest soft rot because they possess more than 70% moisture creating conducive environment for soft rot to develop. This supports the fact that Kenya mpya is more susceptible to soft rot bacteria and other bacterial diseases. According to Maher et al. [28] tuber soft rot is also promoted by availability of water and low oxygen concentration, presence of wounds which creates an avenue for the bacteria to penetrate the tissues of the potato tubers.

Interestingly our results demonstrated that the selected botanicals had varying levels in managing the bacterium on inoculated potato

tubers [29]. This was established by determining the extent of rot on treated tubers compared to untreated tubers. The three selected plant extracts significantly reduced the extent of rot on treated tubers compared to untreated inoculated tubers. This could have been due to the antimicrobial effect of the chemical components in the plant extracts such as allicin in Garlic, Azadirachtin in Neem and anthraquinones in Aloe among other chemical compounds. Potato tubers treated with Garlic extract showed greater reduced extent of rot in the three potato varieties followed by Neem extract while Aloe extract showed the least antibacterial effectiveness on the development of soft rot bacteria. This outcome agreed with the one experimentally observed by Joshi et al. [30].

## 5. CONCLUSION

In this study we established that, out of the three potato varieties (Kenya mpya, Sherekea and Purple gold), Sherekea was more tolerant to bacterial soft rot by showing greatly reduced extent of rot when inoculated with the bacteria compared to Kenya mpya and Purple gold. Of the three plant extracts of Garlic, Neem and Aloe, garlic gave the highest antimicrobial effect on management of soft rot bacteria in all the three potato varieties followed by Neem and Aloe which gave the least antimicrobial effect against soft rot bacteria. The potato varieties interacted significantly with the treatments where Sherekea-Garlic interaction showed better outcome in inhibiting development of the soft rot bacteria. This promising interaction between Sherekea and Garlic could have been contributed by the high antimicrobial effect of Garlic and high tolerance of Sherekea potato variety to soft rot bacteria as observed in the results. Our results indicate a great potential of the tested plant extracts especially the Garlic extract in the management of potato soft rot which is currently a significant threat to potato production in many parts of the world.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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