

Management of *Pectobacterium carotovorum* infections in potatoes (*Solanum tuberosum* L.) using *Tagetes minuta* and *Capsicum frutescens* extracts

Nyamari Jackline Kwamboka* • Wolukau Joseph Ngwela • Gesimba Morwani Robert

Department of Crops, Horticulture and Soil Sciences, Egerton University, Kenya.

*Corresponding author: E-mail: jack19740@yahoo.com.

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Abstract. Potato experiences losses caused by *Pectobacterium carotovorum*, in the field and after harvest. The study was carried out to determine the use of *Tagetes minuta* and *Capsicum frutescens* extracts in the management of blackleg and soft rot in potatoes caused by *P. carotovorum* bacteria. Aqueous extracts of *T. minuta* and *C. frutescens* each at 40, 30 and 20% concentrations were tested against *P. carotovorum* in potatoes with copper oxychloride and water as the positive and negative controls respectively. Data was collected on blackleg incidence and severity on potato plants (in the field), soft rot incidence and severity on tubers (at harvest) and postharvest percent tuber rots. Plants treated with *T. minuta* extracts and copper oxychloride showed significantly low disease incidence and severity compared to those treated with *C. frutescens* and water. The potato plants treated with 40 and 30% *T. minuta*; and copper oxychloride recorded low disease symptom development (2 plants per plot) and severity of 40 to 54% while those treated with water and *C. frutescens* showed high disease symptom development (4 plants per plot) and severity of 57 to 93%. The percent tuber infections significantly differed among the treatments. Copper oxychloride and *T. minuta* recorded the lowest percent postharvest infections of 6.5 to 12.11% while *C. frutescens* had high infections of 40 to 95%. *T. minuta* had antibacterial activity against *P. carotovorum* and therefore it can be used in the management of blackleg and soft rot in potatoes. It is locally available and gets rapidly degraded with no persistence and bio-accumulation in the environment, a major problem associated with synthetic agrochemicals.

Keywords: *Tagetes minuta*, *Capsicum frutescens*, extracts, *P. carotovorum*, potatoes.

INTRODUCTION

Potato is the fourth most important food crop in the world after rice, wheat and maize (Czajkowski *et al.*, 2011) and the second most important food crop in Kenya after maize (Gildemacher, 2012). Yield and quality of potato is affected by diseases, among them are soft rot and blackleg caused by *Pectobacterium carotovorum*. The *Pectobacterium* bacteria are pectinolytic, Gram-negative, facultative anaerobic, non-sporing, motile, straight rods with peritrichous flagellae and they belong to the γ -Proteobacteria subdivision in the Enterobacteriaceae family (Ismael *et al.*, 2012).

P. carotovorum bacteria have a wide range of host that causes soft rot and blackleg diseases in crop plants. In tomato, *P. carotovorum* subsp, *carotovorum* (*Pcc*) causes bacterial soft rot and hollow stem. In potato, it causes blackleg in stems and soft rot in tubers in the field, during transit and storage (Lemma *et al.*, 2014). The bacteria is expressed as soft rot of tubers; and blackleg characterized by blackening of the stem base and an inky black decay of potato plants with the yellowing of foliage that leads to the death of plants. Hydathodes, lenticels, abscission tissues and wounds are points of entry for

Pectobacterium bacteria into the plant tissues (Bibi *et al.*, 2013).

Environmental conditions that may favour soft rot/blackleg development include high humidity, heavy rainfall or irrigation, poor drying conditions and warm temperatures of 22 to 33°C (Cappaert *et al.*, 1988). Extensive contamination may also occur during harvest and grading when the bacteria from rotting tubers spread to fresh wounds on other tubers.

Methods like avoidance of contamination, seed certification, hot water treatment, chemical methods and breeding and genetic modification for resistance (Czajkowski *et al.*, 2011) have been explored to manage the disease. As noted by Wright *et al.* (2005), application of copper-based fungicides was effective in reducing soft rots in Calla lilies. Antibiotics like streptomycin sulphate could also decrease the disease incidences but there are dangers of development of resistance with continued use (McManus *et al.*, 2002).

Use of synthetic agrochemicals has been criticized the world over for their adverse effects on human health and the environment (Hill, 2010). This has led to the current trend of using products with as little synthetic chemicals as possible or no synthetic chemicals at all. Therefore eco-friendly techniques are needed to control crop diseases including control of *P. carotovorum* infections in potatoes (Poudyal and Poudel, 2013).

Onkendi *et al.* (2014) found out that more than 50% of the potato farms across Kenya reported cases of soft rot and blackleg diseases which account for as much as 1/4 of the annual potato losses during the 2012/2013 growing season. There are no reported methods of controlling the disease in Kenya except the avoidance methods that include planting certified seed, avoiding damage during harvesting and sorting diseased potatoes to avoid further contamination.

Many secondary plant products, including flavonoids, steroidal alkaloids and saponins show antibacterial activity against plant pathogenic bacteria (Rahman *et al.*, 2012). Spices and aromatic herbs contain compounds that have been shown to have bactericidal and antifungal properties. Some chilli species contain *capsaicinoids* which have anti-bacterial effects against human bacterial pathogens such as *Staphylococcus* sp., *Escherichia coli*, *Bacillus aureus* and *Bacillus subtilis* (Soetarno *et al.*, 2009). Extracts from three chilli varieties (Habanero, Serrano and bell pepper) showed growth inhibition against *P. carotovorum*. The inhibition was attributed to the compounds in chilli that included meta-coumaric acid, ortho-coumaric acid and trans- cinnamic acid (Ortega *et al.*, 2003).

Tagetes minuta contains essential oils (dihydrotagetone, β -ocimene, terpinolene, piperitone, β -caryophyllene); the major terpenes present in the *Tagetes* plants (Saha *et al.*, 2012). These oils have shown antifungal activity against *Aspergillus niger*, *Aspergillus flavus*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* (Céspedes *et al.*, 2006). A review by Gakuubi *et al.* (2016), also

demonstrated strong antibacterial activity of essential oils of *T. minuta* against three test plant pathogenic bacteria (*Pseudomonas savastanoi* pv. *phaseolicola*, *Xanthomonas axonopodis* pv. *phaseoli*, and *Xanthomonas axonopodis* pv. *manihotis*).

Many studies have been done on the use of *T. minuta* to control human pathogens but with very little on its use against plant pathogens. The objective of this study was to determine the ability of *T. minuta* and *C. frutescens* to manage soft rot of potatoes.

MATERIALS AND METHODS

Research site

The study was conducted in Horticulture Research and Teaching Laboratory and field at Egerton University, Njoro, Kenya. It lies at latitude of 0° 23' South, longitude 35° 35' East and at an altitude of about 2,238 m above sea level (Jaetzold *et al.*, 2005). The potatoes were grown in the open field in two seasons in 2015. The postharvest experiment was performed in the horticulture laboratory.

Preparation of plant extracts

Tagetes minuta plant material grown as weeds was collected from the farm fields at Egerton University, Nakuru, Kenya, and Red ripe fruits of *Capsicum frutescens* were purchased from Nakuru farmers' market. The materials were positively identified and confirmed by a botanist at the Department of Biological Sciences, Egerton University.

The leaves and stems of *T. minuta*, and *C. frutescens* fruits were used to make the two crude extracts that were evaluated. The materials were air dried under shade for three weeks then ground using an electric grinder (SB-808 by SAYONA PPS). They were separately homogenized in distilled water in the ratio of 1:10 (W:V) and steeped for 12 h at 30°C on a rotary shaker (THZ-C-1 Hangzhou, China). The materials were filtered through a muslin cloth and centrifuged (KUBOTA 6800, Japan) at 5,000 rpm for 15 min. The supernatants were collected and concentrated in a water bath at 70°C to make the final volume, one fifth of the original volume (which served as 100% concentration of each extract). The two extracts at 100% concentration were then stored at 4°C until evaluation. The extracts were each diluted with water to 40, 30 and 20% (V:V) concentration to be used for evaluation in the field and postharvest experiments.

Isolation and Identification of the bacteria strains pathogen

P. carotovorum bacteria were obtained from naturally

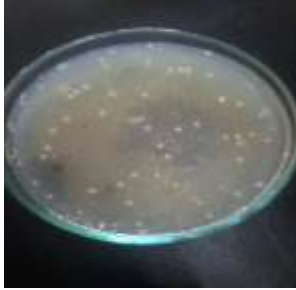


Figure 1. Bacterial colonies on agar.

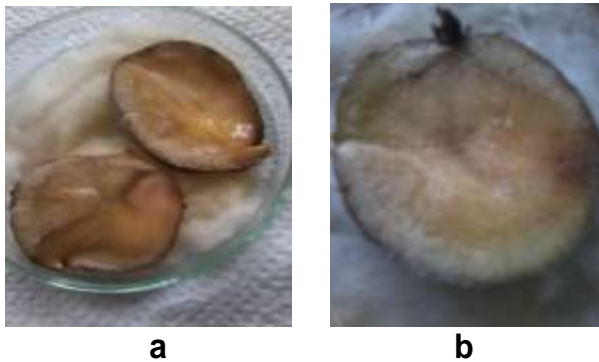


Figure 2. (a) Inoculated potato slices; (b) Control potato slices.

infected potato tubers. After surface sterilization with 70% ethanol solution and washing three times in sterile water, the potato sections were ground in small volumes of sterile water to obtain a potato paste containing the bacteria. A sterile loop was used to pick and streak the bacteria onto nutrient agar plates which were then incubated at 22°C for three days to produce single, round, convex, creamy-translucent, raised and shiny colonies on the nutrient agar (Figure 1). The colonies were sub-cultured three times to obtain pure cultures which were maintained for subsequent uses (Perombelon and Van der Wolf, 2002). The top of single colonies was picked with a sterile loop and inoculated into 250 ml of nutrient broth. The broth culture was then incubated for 12 h to obtain young cultures.

The turbidity of actively growing broth cultures was then adjusted to a 0.5 McFarland standard comparable to a bacterial suspension of 1×10^8 CFUml⁻¹. Later on, the bacterial suspension was used to inoculate the seed stored potato in the postharvest experiment.

Pathogenicity test on potato tubers

Potato tubers were washed thoroughly in running water; air dried then dipped in 70% ethanol for 3 min, washed in sterile water then air dried. Eight, 5 mm thick slices of

potato were cut and placed on wet, sterile serviette in petri dishes. Using a micro pipette, 50 µl of 10^8 CFU ml⁻¹ bacterial suspensions was placed on the surface in the centre of four slices for inoculation with four control slices treated with sterile water. The slices were incubated at room temperature 23°C and examination on the presence of rots was done after 3 days of inoculation. By day 3, the slices treated with the bacterial suspension had soft rotted tissue (Figure 2a), while those treated with sterile water had no rotted tissue (Figure 2b). Re-isolation of bacteria from rotted tissue (Figure 2a) was done and inoculated on nutrient agar (NA) medium (Mikicinski *et al.*, 2010) to ascertain that it was the bacteria in question.

In vivo efficiency of the plant extracts on infected plants

The treatments in these experiments consisted of two plant extracts each at 40, 30 and 20% concentration with water and copper oxychloride as the negative and positive controls, respectively. For the field experiment, inoculated tubers were sprayed with the respective treatments, then left to dry for 24 h in single layers in vented plastic crates to enhance sticking of the compounds before they were planted at a depth of 10 cm. In the field, data was collected on the incidence and severity of blackleg, and soft rot incidence and severity on tubers were collected at the same time of harvesting. Blackleg incidence was obtained by counting the number of infected plants per plot. On the other hand, severity was obtained by calculating the number of infected stems divided by the total number of stems of infected plants multiplied by 100 as shown:

% Soft rots severity = $\frac{n}{N} \times 100$, Where; n is the number of stems with soft rot symptoms and N the total number of stems examined.

For the postharvest experiment, six inoculated potatoes of the same size were picked randomly, sprayed with the respective treatments in three replications, left for 12 h for the chemical to stick and stored in polythene sleeves. Observation for rotted potatoes was made at two-day intervals from 7th day to 11 days after storage. Data was taken on percent postharvest infections (rots).

Data collection and analysis

Data was collected on incidence and severity of blackleg in the field, soft rot incidence and severity on tubers at harvest and percent postharvest rots after storage. The data was subjected to Analysis of Variance (ANOVA), using Genstat Edition 4 and means with significant differences were separated using the Tukey's Honestly Significant Difference Test at $P \leq 0.05$.

Table 1. Effect of *T. minuta* and *C. frutescens* extracts on blackleg incidence at 35, 40 and 44 days after planting (DAP).

Treatment	Season 1			Season 2		
	35 DAP	40 DAP	44 DAP	35 DAP	40 DAP	44 DAP
Copper oxychloride	1.00 ^a	1.00 ^a	1.00 ^a	1.00 ^a	1.00 ^a	1.00 ^a
40% <i>T. minuta</i>	1.00 ^a	1.00 ^a	1.00 ^a	1.00 ^a	1.00 ^a	1.00 ^a
30% <i>T. minuta</i>	1.00 ^a	1.00 ^a	1.00 ^a	1.00 ^a	1.00 ^a	1.00 ^a
20% <i>T. minuta</i>	1.33 ^{ab}	1.67 ^{ab}	1.67 ^{ab}	1.33 ^{ab}	1.67 ^a	2.00 ^b
40% <i>C. frutescens</i>	3.00 ^d	3.00 ^{bc}	3.67 ^c	2.00 ^c	3.00 ^c	3.00 ^c
30% <i>C. frutescens</i>	2.33 ^{bc}	2.67 ^c	3.00 ^c	2.00 ^c	2.67 ^b	2.67 ^c
20% <i>C. frutescens</i>	1.67 ^{ab}	2.33 ^c	2.67 ^{bc}	2.33 ^d	3.00 ^c	3.33 ^c
Water	2.67 ^c	2.67 ^c	2.67 ^{bc}	1.67 ^{bc}	2.33 ^b	2.00 ^b
<i>p</i> -value	<0.001	<0.001	<0.001	<0.001	0.004	0.008

Means followed by the same letter in the same column are not significantly different at $P \leq 0.05$ according to Tukey's HSD test.

RESULTS

Blackleg disease incidence on potatoes plants in the field

Each of the 8 treatments was applied to potatoes in 3 plots giving a total of 24 plots. Plots of potatoes treated with *T. minuta* extracts and copper oxychloride showed a significant difference in blackleg incidence compared to those treated with *C. frutescens* and water in both seasons 1 and 2 (Table 1). The plots treated with 40 and 30% *T. minuta*; and copper oxychloride had very low disease incidents (2 plants per plot) while those treated with water and *C. frutescens* had high disease incidents (4 plants per plot). The plants that had blackleg infections exhibited mushy, watery breakdown of the tissue with a foul pungent odor characteristic of *Pectobacterium* soft rot. The stems also exhibited wilting, yellowing with black discoloration and finally the plant toppled (Figure 3a and b).

Effect of *T. minuta* and *C. frutescens* extracts on blackleg severity at 35, 40 and 44 days after planting (DAP)

The plant extracts showed a significant difference in disease severity on the number of infected stems per plant at $P \leq 0.05$. Potatoes treated with 40 and 30% *T. minuta* showed very few stem infections of 1% infection. The potato plants treated with 20% *T. minuta* showed no significant difference from those treated with 20 and 30% *C. frutescens* of 54.33 to 84.33% infection. The ones treated with 40% *C. frutescens* and water showed very high infections of 92.67 to 100% (Table 2).

Soft rot disease incidence and severity on tubers at harvest

Tubers treated with *C. frutescens* and water had significantly high soft rot incidence (3.3 tubers) compared to those treated with *T. minuta* (1 tuber) at $P \leq 0.05$

(Table 3). Tubers treated with *T. minuta* (40 and 30%) and copper oxychloride also had a significantly low disease severity of 1 to 3.99 % compared to those treated with *C. frutescens* and water that was 10.09 to 57.36% (Table 3).

Percent postharvest soft rot infections

The number of infected (rotted) tubers significantly differed among the treatments. *C. frutescens* had the highest number of infected tubers even compared to water (Table 4). However, *T. minuta* and copper oxychloride had the lowest number of infected (rotted) tubers. The number of infected tubers increased progressively from day 7 to day 11. On day 11, the tubers treated with 40% *C. frutescens* were all rotten oozing slimy liquid with a bad odour (Figure 4e and f). Those treated with *T. minuta* and copper oxychloride were all intact (Figure 4a, b, c and h). Those treated with water were also rotten (Figure 4d) but not as much those treated with *C. frutescens*. The tubers treated with 20% *C. frutescens* (Figure 4g) were not as rotten as those treated with 30 and 40% *C. frutescens* (Figure 4e and f).

DISCUSSION

Tagetes minuta extracts significantly managed blackleg and soft rot as seen from the fewer plants that showed less blackleg symptoms and the reduced infections on harvested and stored potato tubers. These results corroborate with other studies that indicate that plant extracts work in various ways to inhibit microbial growth. Upadhyay *et al.* (2010) showed that essential oils from citrus (*Citrus lemon*), olive (*Olea europaea*), ajwain (*Trachyspirum ammi*), almond (*Amygdalus communis*) and neem (*Azadirachta indica*) have antibacterial activity against both Gram-positive bacteria such as *Lactobacillus acidophilus*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Bacillus cereus* and Gram-negative bacteria such as *Klebsiella pneumoniae* and



Figure 3. Potato plants (a) in the field; (b) a potato plant with blackleg infection.

Table 2. Effect of *T. minuta* and *C. frutescens* on disease severity on stems in at 35, 40 and 44 days after planting (DAP).

Treatment	Season 1			Season 2		
	35 DAP	40 DAP	44 DAP	35 DAP	40 DAP	44 DAP
Copper oxychloride	1.00 ^a	1.00 ^a	1.00 ^a	1.00 ^a	1.00 ^a	1.00 ^a
40% <i>T. minuta</i>	1.00 ^a	1.00 ^a	1.00 ^a	1.00 ^a	1.00 ^a	1.00 ^a
30% <i>T. minuta</i>	1.00 ^a	1.00 ^a	1.00 ^a	1.00 ^a	1.00 ^a	1.00 ^a
20% <i>T. minuta</i>	9.33 ^a	26.00 ^b	42.67 ^{ab}	17.67 ^b	40.00 ^b	54.33 ^b
40% <i>C. frutescens</i>	56.67 ^b	82.67 ^c	69.33 ^{bc}	34.33 ^b	61.00 ^{bc}	92.67 ^d
30% <i>C. frutescens</i>	48.00 ^b	56.33 ^b	69.33 ^{bc}	84.33 ^d	77.67 ^c	84.33 ^{bc}
20% <i>C. frutescens</i>	47.00 ^b	59.33 ^b	72.67 ^{bc}	51.00 ^c	79.00 ^c	78.67 ^{bc}
Distilled water	71.00 ^b	100 ^c	100 ^c	42.67 ^c	37.00 ^b	37.00 ^{ab}
<i>p-value</i>	<0.001	<0.001	0.048	0.005	<0.001	<0.001

Means followed by the same letter in the same column are not significantly different at $P \leq 0.05$ according to Tukey's HSD test

Table 3. Effect of *T. minuta* and *C. frutescens* extracts on soft rot disease incidence and disease severity on tubers at harvest.

Treatment	Season 1		Season 2	
	Incidence	Severity	Incidence	Severity
Copper oxychloride	1.00 ^a	1.00 ^a	1.33 ^a	3.23 ^a
40% <i>T. minuta</i>	1.00 ^a	1.00 ^a	1.00 ^a	1.00 ^a
30% <i>T. minuta</i>	1.00 ^a	1.00 ^a	1.00 ^a	1.00 ^a
20% <i>T. minuta</i>	1.00 ^a	1.00 ^a	1.33 ^a	3.99 ^a
40% <i>C. frutescens</i>	3.33 ^b	37.00 ^b	3.33 ^b	19.85 ^c
30% <i>C. frutescens</i>	2.33 ^b	22.00 ^{ab}	4.67 ^c	17.27 ^c
20% <i>C. frutescens</i>	2.67 ^b	45.09 ^b	3.67 ^c	10.09 ^b
Distilled water	3.33 ^b	57.36 ^c	3.00 ^b	12.10 ^b
<i>p-value</i>	0.007	0.005	0.022	0.029

Means followed by the same letter in the same column are not significantly different at $P \leq 0.05$ according to Tukey's HSD test.

Escherichia coli. Shirazi et al. (2014) demonstrated that *T. minuta* and *Ocimum tenuiflorum* extracts contain phenol-containing monoterpenes which have significant antibacterial activity against both Gram-positive and

Gram-negative bacteria (*Salmonella aureus*, *Salmonella enteritidis*, *Escherichia coli*, *Proteus vulgaris*, *Bacillus subtilis* and *Salmonella paratypha*).

Extracts of *Tagetes* have been found to be active

Table 4. Percent post harvest infections at 7, 9 and 11 days after storage (DAS).

Treatment	Season 1			Season 2		
	7 DAS	9 DAS	11 DAS	7 DAS	9 DAS	11 DAS
Copper oxychloride	1.00 ^a	1.00 ^a	1.00 ^a	1.00 ^a	1.00 ^a	6.56 ^{ab}
40% <i>T. minuta</i>	1.00 ^a	1.00 ^a	1.00 ^a	1.00 ^a	1.00 ^a	1.00 ^a
30% <i>T. minuta</i>	1.00 ^a	6.56 ^a	6.56 ^a	1.00 ^a	6.56 ^a	6.56 ^{ab}
20% <i>T. minuta</i>	1.00 ^a	6.56 ^a	6.56 ^a	6.56 ^a	6.56 ^a	12.11 ^{ab}
40% <i>C. frutescens</i>	23.22 ^c	56.56 ^c	84.33 ^c	39.89 ^c	67.67 ^c	95.44 ^d
30% <i>C. frutescens</i>	6.58 ^b	34.33 ^b	45.45 ^c	28.78 ^b	39.89 ^c	39.89 ^{bc}
20% <i>C. frutescens</i>	6.58 ^b	23.22 ^b	28.78 ^b	17.67 ^b	23.22 ^b	28.78 ^{bc}
Distilled water	6.58 ^b	23.22 ^b	23.22 ^b	17.67 ^b	17.67 ^b	23.22 ^{abc}
<i>p-value</i>	0.015	0.004	<.001	0.006	<.001	<.001

Means followed by the same letter in the same column are not significantly different at $P \leq 0.05$ according to Tukey's HSD test

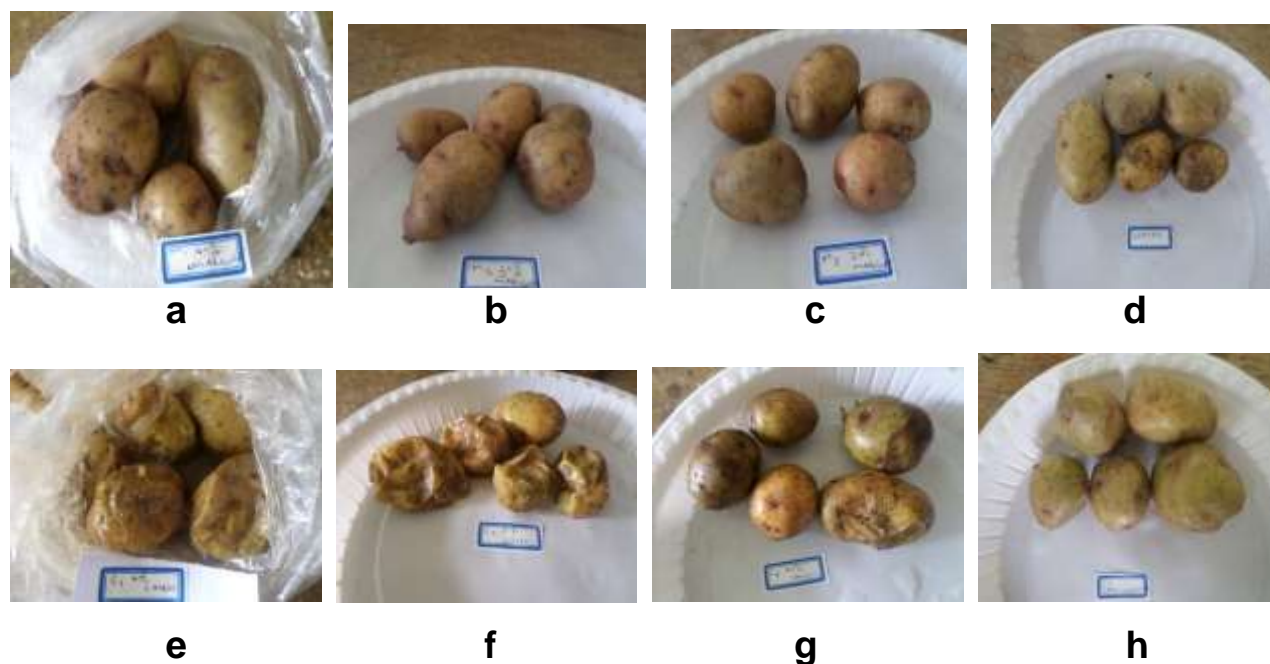


Figure 4. Post harvest infections of potatoes after treatment with the plant extracts. a) 40% *T. minuta*; b) 30% *T. minuta*; c) 20% *T. minuta*; d) Distilled Water; e) 40% *C. frutescens*; f) 30% *C. frutescens*; g) 20% *C. frutescens*; h) Copper oxychloride.

against multi-drug resistant bacteria such as *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae* and *Citrobacter freundii* (Hussain *et al.*, 2014). Studies by Tahir and Khan (2012) attributed the antibacterial activity of *Tagetes* extracts against *Salmonella typhi*, *Escherichia coli* and *Bacillus subtilis due* to the presence of different flavonoids and terpenes especially in the leaf extracts. The same compounds may have antibacterial activity on *P. carotovorum* in the current study.

T. minuta essential oils have been shown to have strong antibacterial activity against *Pseudomonas savastanoi* pv. phaseolicola, *Xanthomonas axonopodis* pv. phaseoli and *Xanthomonas axonopodis* pv. Manihotis

(Gakuubi *et al.*, 2016) all of which are plant pathogens. This agrees with the current results where *T. minuta* showed antibacterial activity against *P. carotovorum* in potatoes both growing in the field and in storage.

The antibacterial activity in the essential oils of several medicinal plants have been related to the attack on the phospholipids in the cell membranes of the microbes, which causes increased permeability and leakage of cytoplasm thereby killing the bacteria (Al Abbasy *et al.*, 2015). This may have been the case with *T. minuta* used in the current study.

The *C. frutescens* extracts used in the current study did not inhibit the *Pectobacterium* bacteria. This may be

because *Capsicum* is a host to the bacteria as indicated by Akbar *et al.* (2015), who isolated *Erwinia carotovora* (*Pectobacterium carotovorum*) from pepper, tomato and potato. The inactivity of *Capsicum* against *P. carotovorum* in the current research may also be attributed to the use of a different species of *Capsicum*. Koffi-Nevry *et al.* (2012) stated that *Capsicum annuum* and *C. frutescens* have antibacterial activity against *Vibrio cholerae*, *Staphylococcus aureus* and *Salmonella typhi* but the extract from *C. annuum* showed a higher antibacterial activity than the one from *C. frutescens*. Previous studies indicate that *C. annuum* inhibited *Erwinia carotovora* in potatoes contrary to the current study. The *C. frutescens* used in the current study may have less amount of the inhibitory compounds like *meta*-coumaric and *trans*-cinnamic acids compared to *Capsicum annuum* that were attributed to the antibacterial activity (Ortega *et al.*, 2003). In the current study, the results may have also been different because the extracts were obtained using the aqueous extraction instead of the Soxhlet extraction method used by Ortega *et al.* (2003). Omolo *et al.* (2014) noted that the extraction methods and inconsistency between analyzed plant materials strongly affect the observed levels of bacterial growth inhibition by chilli. This may also have been the case in the current research in which aqueous extract was used instead of alcohol extract.

However, the current results are in agreement with Amruthraj *et al.* (2013) who reported that acetonitrile and acetone extracts from *Capsicum chinense* were ineffective against *Escherichia coli* and *Erwinia* sp. This shows that the inhibitory components in the species of *Capsicum* significantly vary and this may have been the cause of the different results in the current study.

Afrodet and Salih (2006) showed that the oil extract from *Capsicum annuum* had antimicrobial activity against G-ram positive and Gram-negative bacteria, and attributed it to the presence of capsanthin which is considered as one of the major carotenoids of red pepper fruits. This is however contrary to the current findings where the potatoes treated with *C. frutescens* had almost 100% rots (Figure 4e and f). This could be attributed to the fact that chilli and potato belong to the same family, solanaceae. Therefore, chilli is likely to be affected by the same diseases that affect potato, *Pectobacterium* included (own deductions). Akbar *et al.* (2015), evaluated five isolates of *Pectobacterium* for aggressiveness on tomato fruits, and chilli isolate was found to be the most aggressive followed by tomato and potato isolates producing 22.3, 7.9 and 7.8 mm diameter of soft rot lesions, and on the fruits, respectively. This agrees with the current results where almost all the tubers treated with capsicum were completely macerated. This shows that the *P. carotovorum* infects *Capsicum* sp. and as such the *C. frutescens* extracts in the current experiment may have acted as a source of nutrients instead of killing the bacteria as shown by Oliveira *et al.* (2003) who used *C. annuum* as an enrichment host for *P. carotovorum* during

its isolation before culture.

CONCLUSION AND RECOMMENDATION

Findings from this study show the potential of using *Tagetes minuta* in the management of soft rot and blackleg of potato both in the field and in the store. This is evidenced by the significantly reduced incidences and severity of the disease at 30 and 40% concentrations of *T. minuta*. Besides, *T. minuta* is abundantly available locally and does not pose any environmental hazards. However, further research is required to determine the active ingredients in the *T. minuta* extracts that actually inhibits the growth of the bacteria in the potatoes. Further research is also recommended to establish why *C. frutescens* failed to inhibit the bacteria unlike previous studies by other researchers.

REFERENCES

- Afrodet A, Salih J (2006). Extraction and Identification of Oil Extracts from *Capsicum annuum* L. fruits and study of its antimicrobial activity. Res. Basrah Sci. 32(3):80-87.
- Akbar A, Ahmad M, Khan SZ, Ahmad Z (2015). Characterization of the causal organism of soft rot of tomatoes and other vegetables and evaluation of its most aggressive isolates. Am. J. Plant Sci. 6(04):511.
- Al Abbasy DW, Pathare N, Al-Sabahi JN, Khan SA (2015). Chemical composition and antibacterial activity of essential oil isolated from Omani basil (*Ocimum basilicum* Linn.). Asian Pac. J. trop. dis. 5(8):645-649.
- Amruthraj NJ, Raj JP, Lebel LA (2013). Polar aprotic extraction of capsaicinoids from *Capsicum chinense* (Bhutjolokia) fruit for antimicrobial activity. Int. J. Biol. Pharm. Res. 4(12):959-964.
- Bibi A, Rafi A, Junaid M, Ahamd M (2013). Studies on the characterization and PCR identification of the pathogen of the bacterial blackleg/soft rot of potato. Sarhad J. Agric. 29(2):225-234.
- Cappaert MR, Powelson ML, Franc GD, Harrison MD (1988). Irrigation water as a source of inoculum of soft rot *Erwinias* for aerial stem rot of potatoes. Phytopathol. 78(12):1668-1672.
- Céspedes CL, Avila JG, Martínez A, Serrato B, Calderón-Mugica JC, Salgado-Garciglia R (2006). Antifungal and antibacterial activities of Mexican tarragon (*Tagetes lucida*). J. Agric. Food Chem. 54(10):3521-3527.
- Czajkowski R, Perombelon MC, van Veen JA, van der Wolf JM (2011). A review: Control of blackleg and tuber soft rot of potato caused by *Pectobacterium* and *Dickeya* species. Plant pathol. 60(6):999-1013.
- Gakuubi MM, Wanzala W, Wagacha JM, Dossaji SF (2016). A review: Bioactive properties of *Tagetes minuta* L.(Asteraceae) essential oils. American J. Essent Oils Nat. Prod 4(2):27-36.
- Gildemacher P (2012). Innovation in seed potato systems in Eastern Africa (PHD Thesis) Royal Tropical Institute Amsterdam, Netherlands.
- Hill MK (2010). Understanding environmental pollution. Cambridge University Press.
- Hussain MM, Ahmad B, Rashid E, Hashim S, Marwat KB, Jan ASAD (2014). *In vitro* antibacterial activity of methanol and water extracts of *Adiantum capillus* and *Tagetes patula* against multidrug resistant bacterial strains. Pak. J. Bot. 46(1):363-368.
- Ismael ME, Abdel-Monaim MF, Mostafa YM (2012). Identification and pathogenicity of phytopathogenic bacteria associated with soft rot disease of Girasole tuber in Egypt. Afr. J. Bacterial. Res. 4(1):1-8.
- Jaetzold R, Schmidt H, Hornetz B, Shisanya C (2005). Farm management handbook of Kenya. Vol. II. Natural conditions and farm

- management information. Part B. Central Kenya (Rift Valley and Central Province). Ministry of agriculture. Nairobi, Kenya. pp. 389-413.
- Koffi-Nevry R, Kouassi KC, Nanga ZY, Koussémon M, Loukou GY (2012).** Antibacterial activity of two bell pepper extracts: *Capsicum annum* L. and *Capsicum frutescens*. *Int. J. Food Properties* 15(5):961-971.
- Lemma Z, Dawit W, Negari M, Chaka A, Selvaraj T, Gebresenbet G (2014).** Identification of postharvest rotting microorganisms from tomato fruits (*Solanum esculentum* Mill.) in Toke Kutaye District of West Shoa Zone, Ethiopia. *J. Stored Prod. Postharvest Res.* 5(3):14-19.
- McManus PS, Stockwell VO, Sundin GW, Jones AL (2002).** Antibiotic use in plant agriculture. *Ann. Rev. phytopathol.* 40(1):443-465.
- Mikicinski AJ, Pulawska P, Sobiczewski L, Orlikowski B, (2010).** Pectolytic bacteria associated with soft rot of *Dieffenbachia* (*Dieffenbachia maculata*), *Phytopathologia*, 58:21-32.
- Oliveira AM, Duarte V, Silveira JR, Moraes MG (2003).** Incidence of pectolytic *Erwinias* associated with blackleg of potato in Rio Grande do Sul. *Fitopatologia Brasileira*, 28(1):49-53.
- Omolo MA, Zen-Zi W, Amanda KM, Jennifer CH, Nina CL, Holly AR, Kyle AC, David JB, (2014).** A review: Antimicrobial properties of chili peppers. *J. Infect. Dis. Ther.* 2(4):145-153.
- Onkendi EM, Moleleki LN (2014).** Characterization of *Pectobacterium carotovorum* subsp. *carotovorum* and *brasiliense* from diseased potatoes in Kenya. *European J. Plant Pathol.* 139(3):557-566.
- Ortega CA, Alvarez LD, Flores MEJ, Sánchez HH, Malo AL (2003).** Effect of Chili (*Capsicum annum* L.) extracts and derived compounds on growth of *Erwinia carotovora* subsp. *carotovora* (Jones) Bergey, Harrison, Breed, Hammer and Huntoon. *Revista Mexicana de Fitopatologia*, 21(2):233-237.
- Perombelon MCM, Van der Wolf JM (2002).** Methods for the detection and quantification of *Erwinia carotovora* subsp. *atroseptica* (*Pectobacterium carotovorum* subsp. *atrosepticum*) on potatoes: a laboratory manual. *Dundee, Scotland: Scottish Crop Research Institute Occasional Publication*, (10).
- Poudyal BK, Poudel P (2013).** Beneficial microbes “Jeevatu” for biodegradable waste management and plant disease control. In *Glob. Humanitarian Technol. Conf.* 2013:232-237.
- Rahman MM, Khan AA, Ali ME, Mian IH, Akanda AM, Abdi Hamid SB (2012).** Botanicals to control soft rot bacteria of potato. *Sci. world J.* 79:64-72.
- Saha S, Sijam K, Omar D (2012).** Antibacterial activity of *Psidium guajava* methanol leaf extracts against plant pathogenic bacteria in the genera *Pectobacterium* and *Xanthomonas*. *Int. J. Appl. Biol. Pharm. Technol.* 3:246-252.
- Shirazi MT, Gholami H, Kavooosi G, Rowshan V, Tafsiry A (2014).** Chemical composition, antioxidant, antimicrobial and cytotoxic activities of *Tagetes minuta* and *Ocimum basilicum* essential oils. *Food Sci. Nutr.* 2(2):146-155.
- Soetarno S, Sukrasno S, Yulinah E, Sylvia S (2009).** Antimicrobial activities of the ethanol extracts of *Capsicum* fruits with different levels of pungency. *Jurnal Matematika and Sains*, 2(2):57-63.
- Tahir L, Khan N (2012).** Antibacterial potential of crude leaf, fruit and flower extracts of *Tagetes minuta* L. *J. Public health and Biol. Sci.* 3:74-78.
- Upadhyay RK, Dwivedi P Ahmad S (2010).** Screening of antibacterial activity of six plant essential oils against pathogenic bacterial strains. *Asian J. Med. Sci.* 2(3):152-158.
- Wright PJ, Triggs CM, Burge GK (2005).** Control of bacterial soft rot of calla (*Zantedeschia spp.*) by pathogen exclusion, elimination and removal. *New Zealand J. Crop Hort. Sci.* 33(2):117-123.