EFFICACY OF BOTANICAL EXTRACTS FROM GARLIC AND NEEM ON CONTROLLING POTATO SOFT ROT PATHOGENS

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ABSTRACT

Soft rot of potatoes (*Solanum tuberosum* L.) *caused by Pectobacterium and Dickeya subspecies leads to economic losses in agriculture worldwide including Zimbabwe, where losses range from 20 – 60%. A laboratory experiment was carried out using the potato tuber maceration test to screen the effect of two botanicals extract, neem* (*Azadirachta indica A. Juss.*) *leaf and garlic* (*Allium sativum L.*) *cloves in controlling soft rot disease caused by bacterial pathogens, namely Pectobacterium carotovorum subspecies carotovorum* (*Pcc*), *Pectobacterium atrosepticum* (*Pa*) *and Dickeya dadantii* (*Dd*). *Preparations of two concentrations* [(*10 and 25%* (*w/v*)] *of the aqueous extracts of garlic and neem plants were used in dip and spray applications. Sterile distilled water was used as a negative control. Three tuber halves were used for each treatment. Five 10 mm filter paper discs* (*Whatman's No. 1*) *pre-soaked in 1 x* 10^6 *cfu/mL bacterial cell suspensions of Pcc, Pa and Dd respectively were placed on each tuber half and incubated for 48 h at* 25^oC. *After 48 h the filter paper discs were removed and the rotting zone diameter was measured. The experiment was a 3x2x2x2+1 factorial laid out in a completely randomised design, each treatment replicated three times. The two botanicals significantly inhibited the growth of two bacteria, Pa and Dd but were ineffective against Pcc. Concentration, method of application and their interactions were not significant in inhibiting the bacteria. Neem and garlic extracts can be used to control Pa and Dd infections.*

Key words: Neem, Garlic, Pectobacterium spp., Dickeya dadantii

INTRODUCTION

Bacterial soft rot of potato has become a very important disease world-wide due to the losses it causes during the various stages of crop development and in storage (Reeves *et al*., 1999; Bdiliya and Dahiru, 2006). *Pectobacterium* and *Dickeya* species are known to cause soft rot, blackleg and wilting diseases that cause huge amounts of economic losses (Baghaee-Ravari *et al*., 2011). Sub-species described under *Pectobacterium*: *atroseptica*, *carotovora*, *betavasculorum*, and *wasabie* (Duarte *et al*., 2004; Baghaee-Ravari *et al*., 2011) and sub-species under *Dickeya*: *dadantii*, *zea* and *dianthicola* (van der Merwe *et al*., 2010) are known to cause serious disease on potato. Potato blackleg is caused by *Pectobacterium atroseptica* (*Pa*) in cooler climates and *Pectobacterium carotovora* subsp. *carotovora* (*Pcc*) and *Dickeya dadantii* (*Dd*) cause similar symptoms in higher temperatures (Duarte *et al*., 2004). In Zimbabwe, losses due to *Dickeya dadantii* have been reported to be around 20-60% (Ngadze *et al*., 2010). Under bad handling conditions, losses can reach to about 100% (CAB International, 2000; Manzira, 2010)

Over the years, inappropriate use of agrochemicals, especially fungicides, has resulted in pathogen resistance and undesirable side effects due to their carcinogenic properties (Alkhail, 2005). The promotion of environmentally

sustainable agriculture and organic agriculture has led to alternatives such as the use of natural plant products (Slusarenko *et al*., 2008). The use of plant products is gaining popularity because they have been found to be non-toxic, more systemic with little mammalian toxicity (Bankole, 1996). This work seeks to evaluate the efficacy of extracts from two plants that have been reported to have antimicrobial properties, garlic (*Allium sativum*) (Slusarenko *et al*., 2008) and neem (*Azadirachta indica*) (Bankole, 1996; Alkhail, 2005).

The antimicrobial substance, allicin, which is produced in garlic is active against a wide range of pathogens both *in vitro* and *in vivo* (Portz *et al*., 2008). Garlic has been shown to be effective against *Phytophthora infestans* (Curtis *et al*., 2004; Portz *et al*., 2008) on tomato seedlings. Mycelia growth of *Fusarium solani* (Bowers and Locke, 2000) and *Rhizoctonia solani* (Bianchi *et al*., 1997) was also inhibited by garlic extracts (Bianchi *et al*., 1997). Flavonoids and saponins of red garlic exhibited anti-bacterial properties against *Baccillus subtilis* (Locke, 2006).

Neem products have been used mainly in insect pest management because of their pesticidal and anti-feedant activities (Bdliya and Dahiru, 2006; Slusarenko *et al*., 2008). The most active substance in neem preparations is azadirachtin (Slusarenko *et al*., 2008) and this is active against a wide range of pests (Koul and Walia, 2009). However, neem has been found to have fungicidal (Bankole, 1996; Govindachari *et al*., 1998) and bactericidal (Mahfuzul-Hoque *et al*., 2007; Slusarenko *et al*., 2007) properties.

It is important that more botanicals are explored and evaluated for their efficacy against plant pathogens. With scientific improvement, botanicals might be a low cost solution in plant protection, and this will become a real social value to the subsistence farmer. In this study, the effectiveness of botanical extracts from garlic and neem on controlling potato soft rot was assessed on three soft rot causing bacteria, *Pcc*, *Pa* and *Dd*.

MATERIALS AND METHODS

Preparation of plant extracts

The aqueous plant extracts and their concentrations were prepared according to a method described by Obongoya *et al*., (2010), with minor modifications. The aqueous plant extracts of neem leaves and garlic cloves were prepared separately in two concentrations: 25% and 10% (w/v) by blending 1 kg of garlic cloves and neem leaves in four litres and ten litres of water respectively. The mixture suspension was filtered through a 1 mm sieve. The plant extracts were then stored for later use in a refrigerator at 4˚C for approximately one month.

Inoculum preparation

The pure culture strains of *Pcc* (LMG 2404^{T}, Belgian Coordinated Collection of Microorganisms), *Pa* (LMG 2386^{T}, Belgian Coordinated Collection of Microorganisms) and *Dd* (3937^{T}, Scottish Crop Research Institute), were obtained from the Plant Pathology Laboratory, Crop Science Department at the University of Zimbabwe. The isolates were re-initiated in nutrient broth (NB) and re-streaked on Nutrient Agar (NA) solid media for purity. The cultures were transferred into Luria Bertani (LB) liquid media and bacterial suspensions were adjusted to a concentration of 1 X 10^{6} cells/ml using a spectrophotometer at OD_{600}.

Potato tuber maceration test

The variety Pimpernel was used. Potato tubers of uniform size were washed using tap water, surface sterilised in 10.0% sodium hypochlorite solution for 5 minutes and then rinsed in sterile water. Tubers were allowed to air dry and cut longitudinally into halves. Botanical treatments were applied on the cut surfaces using dip and spray treatment application of neem and garlic extracts at two concentrations [25% (w/v) and 10% (w/v)]. Treatment applications were done

approximately for 5 seconds for each tuber half. Tuber halves treated with the same volume of sterilized distilled water were used as a negative control.

Five filter papers (Whatman's No. 1) discs, 10 mm in diameter, were soaked in individual bacterial suspensions of *Pcc*, *Pa* and *Dd*, all with a concentration of 1 X 10^6 cells/ml. Soon after dipping or spraying application of botanical extracts, the five inoculated filter paper discs were placed on each tuber half and then incubated at 25˚C for 48 hours. The inoculated tubers were placed in transparent plastic bags with a moist absorbent paper at the bottom to maintain humid conditions necessary for disease development. After 48 h, the filter papers discs were removed and the rotting zone diameter was measured in mm using a ruler.

Experimental design and statistical analysis

The experiment was laid out in a completely randomised design (CRD) with a 3x2x2x2+1 factorial treatment structure with a control. Each treatment was replicated three times. Observed data were managed using excel spreadsheets. Data were explored for normality and homogeneity using Anderson-Darling test and Bartlett's and Levene's tests, respectively. The plot of residuals was done using Minitab Release 12.22 (1998) to check assumptions for the analysis of variance (independence, normality, homogeneity). Data were analysed with Genstat Version 13 as a 3x2x2x2+1 factorial using the control as a dummy variable. When F test was significant ($P<0.05$), the means of different treatments were compared to the control using LSD at 5% level.

RESULTS

The analysis of variance, (Table 1), shows that the following factors were significant: control ($P = 0.008$), bacteria ($P < 0.001$) and botanicals ($P = 0.006$). In addition, the interactions bacteria x botanical x concentration and bacteria x botanical x methods were significant with respective P values of 0.003 and 0.008. Concentration, method of application and their interactions were not significant in inhibiting the bacteria.

Table 1: Analysis of variance table showing the effect of three bacterial subspecies, botanical concentration and application method on soft rot.

Source	DF	SS	MS	F	P
Control	1	0.6974	0.6974	7.52	0.008
Bacteria	2	31.7255	15.862	170.94	0.001
Botanical	1	0.7792	0.7792	8.40	0.006
Concentration	1	0.0397	0.0397	0.43	0.516
Method	1	0.183	0.183	1.97	0.166
bacteria*botanical	2	0.2338	0.1169	1.26	0.293
bacteria*concentration	2	0.2186	0.1093	1.18	0.316
bacteria*Method	2	0.0915	0.0458	0.49	0.613
botanical*concentration	1	0.078	0.078	0.84	0.364
botanical*Method	1	0.4156	0.4156	4.48	0.303
concentration*Method	1	0.1005	0.1005	1.08	0.303
bacteria*botanical*conc	2	1.234	0.617	6.65	0.003
bacteria*botanical*Meth	2	0.9978	0.4989	5.38	0.008
bacteria*concentration*Meth	2	0.563	0.2815	3.03	0.057
botanical*concentration*Meth	1	0.0017	0.0017	0.02	0.893
bact*bot*conc*Meth	2	0.3409	0.1705	1.84	0.170
Error	50	4.6421	0.0928		
Total	74	42.3422			

The comparison of the different treatment means with the control is presented in Figures 1 and 2. Garlic and neem extracts were not effective against *Pcc* when compared with the control in all treatments.

In comparison with the control, garlic clove extracts inhibited bacterial growth in *Pa* by 22.3% and 25.2% at 25 and 10% (w/v) respectively. In *Dd*, inhibition was 31.6% and 9.9% at 25 and 10% (w/v) respectively. Neem leaf extracts were also effective against *Pa* and *Dd* at both concentrations. Percentage inhibition by neem was 56.1% and 23.2% for *Pa* at 25 and 10% (w/v) respectively. For *Dd* the corresponding percentages were 13.6% and 36.1%.

Garlic inhibited the growth of *Pa* by 23.6% and 24% in dip and spray treatments, respectively. In addition, the corresponding percentage inhibition by neem was 39.1% and 40.3%, respectively. In treatments with *Dd*, bacterial growth was inhibited by 31.6% and 8.8% when tubers were dipped and sprayed, respectively, with garlic extracts. The inhibition of bacteria due to neem extracts was 100% in dip and 50.6% in spray application methods.

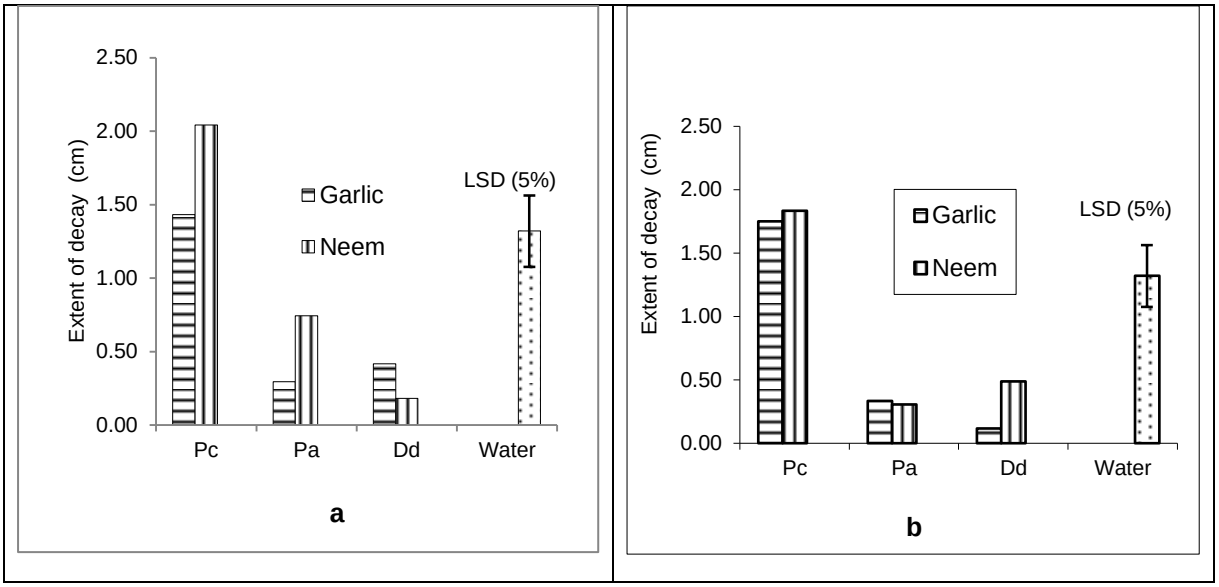

Figure 1. Efficacy of garlic (clove) and neem (leaf) extracts at 25% (a) and 10% (b) concentration in (dip/spray) treatment application against *Pcc*, *Pa* and *Dd* pathogens. Sterilised distilled water used as a control treatment.

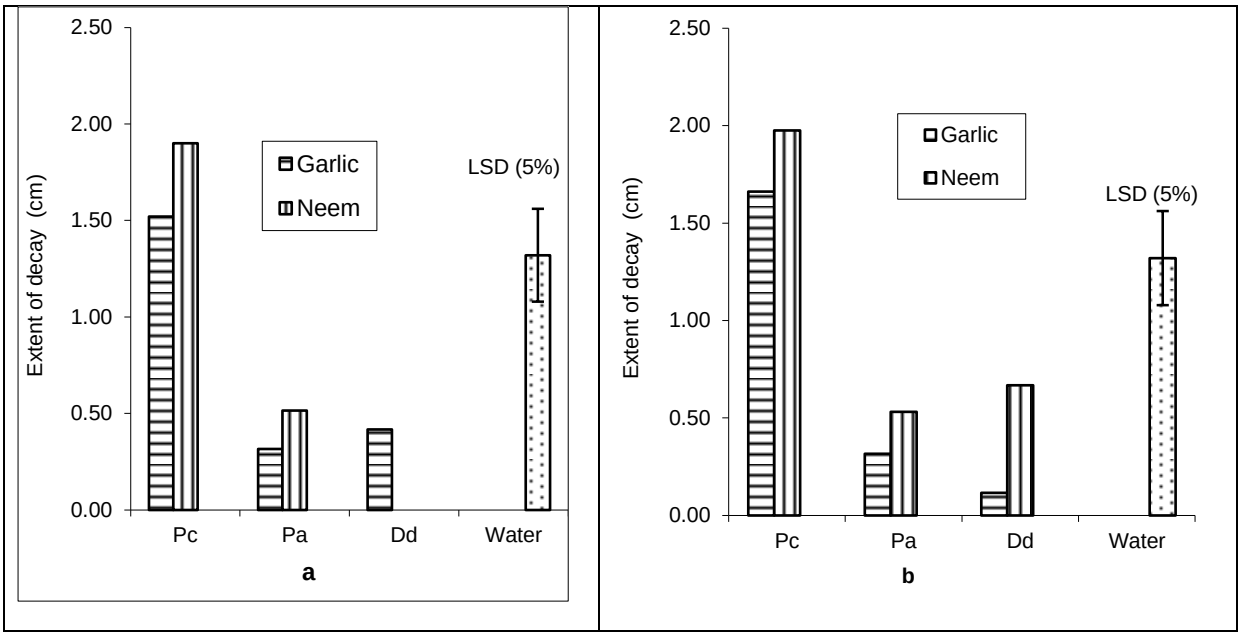

Figure 2. Efficacy of garlic (clove) and neem (leaf) extracts in dip (a) and spray (b) treatment application against *Pcc*, *Pa* and *Dd* pathogens. Sterilized distilled water used as a control treatment.

DISCUSSION

The experiment results showed that the use of botanicals led to a reduction in the severity of soft rot on two bacterial species, *Pa* and *Dd*. When compared to the negative control, inhibition of bacterial growth by garlic was 45.2% and neem provided control by 29.4%. Garlic was, therefore, 15.8% more effective than neem in reducing bacterial maceration of the potato tissue. Similar work that has been done on botanicals has shown that plant extracts from garlic and neem have anti-microbial properties against a wide range of plant pathogens (Amadi and Olusanmi, 2009), though garlic showed to be the most effective against *Rhizoctonia solani* (Alkhail, 2005), when tested with other plants such as half-bar, carnations, craway and neem. Neem contains a compound known as mahmoodin, which has significant antibacterial activity against gram-positive and gram-negative micro-organisms (Hoque *et al*., 2007). The anti-bacterial nature of garlic is widely attributed to allicin, which has inhibitory effects to both gram-positive and gram-negative bacteria (Harris *et al*., 2001). Both botanicals are effective against bacteria such as *Staphylococcus aureus* (Harris *et al*., 2001; Hoque *et al*., 2007).

The results also showed that the three bacterial sub-species behaved differently in response to the botanicals. *Pcc* was the most aggressive and did not respond to the botanical treatments, followed by *Pa*, whose growth was inhibited by 68.3% following botanical treatments. The botanicals proved most effective against *Dd* by inhibiting its growth by 77.3%.

Variation in pathogenicity of different sub-species of soft rot bacteria is related to the different amounts of pectic enzymes (Bhat *et al*., 2010). Pectolytic bacteria cause rotting by producing enzymes such as pectinases, cellulases and proteases which cause tissue maceration (Pitman *et al*., 2008). Pectinases are the main enzymes involved in tissue maceration and they are grouped into pectate lyase (PL), pectin lyase (PnL), polygalacturonase (PGN) and pectin methyl esterase (PME) and they exist as

isoenzymes encoded by independent genes (Perombelon, 1992). The PL enzymes are the main pectinases involved in pathogenesis, and their number varies between species, sub-species and strains (Toth *et al*., 2003). According to Pérombelon (2002), there are five major PL enzymes grouped into two families, PL A, D, E and PL B, C in *Dd,* four major PL enzymes (PL A, B, C and D) in *Pcc* and three major PL enzymes (PL A, B and C) in *Pa*. Although production of these pectinases is important for pathogenicity, not all isoenzymes are required in all situations. In *Dd*, for example, PL A, D and E family plays a larger role in pathogenicity than the PL B and C family. Another difference is in the role of PME. In *Dd*, PME has been shown to play a major role in pathogenesis while PG and PnL appear to contribute more to the pathogenicity of *Pcc* (Perombelon, 2002; Toth *et al*., 2003). The differences in the type and amount of pectinases can therefore be said to account for the variation in the response of the three bacterial species to the same treatment.

Secretion of exoenzymes is also regulated differently amongst different bacterial species. There are three secretion systems (Type I, II and III). The Type I system secretes protease from the cytoplasm into the extracellular space and has a minor role in pathogenicity. The Type II system is essential in pathogenicity and secretes pectinases and cellulases. The Type III system translocates effector proteins into host plant cells to assist in bacterial virulence (De Boer, 2003; Toth *et al*., 2003). All secretion systems are present in the three soft-rot pathogens, but their regulation is different amongst different bacterial species.

Their pathogenicity is also temperature dependent (Pérombelon, 2002). The pectin enzyme, endopolygalacturonic transeliminase (PGTE) production by *Pa* is higher at lower temperatures (< 15˚C) but undetectable at 30˚C. In contrast, production of the enzyme in *Pcc* is equally high at 15˚C and 30˚C. Strains of *Pcc* and *Dd* that would have lost the ability to produce large quantities of PGTE during the course of the experiment lose virulence (Perombelon and Kelman, 1980). Since all the bacteria were exposed to the same temperature of 25˚C, this might have favoured pectic enzyme production in *Pcc* better than in *Pa* and *Dd*. Resultantly, *Pcc* gave the highest tissue maceration followed by *Pa* and *Dd*.

The most commonly used solvents for the extraction of plant are water, ethanol and methanol (Ncube *et al*., 2010). Successful separation of botanical compounds is largely dependent on the type of solvent used. Alcoholic extracts have been shown to provide more antimicrobial activity compared to water (Parekh *et al*., 2005). Active compounds from aqueous extractions lack solubility or are present in insufficient amounts (Parekh and Chanda, 2007). Because of this limitation in aqueous extracts, there is a probability that the botanicals in this experiment were therefore only effective against the less virulent sub-species, *Pa* and *Dd.* Although aqueous extracts of neem and garlic have previously been shown to exhibit moderate control even against *Pcc* (Raju *et al*., 2007), in this study, the botanicals were not effective against *Pcc* and the aqueous extracts of the botanicals did not provide sufficient active compounds to suppress the pathogen.

Dipping or spraying as a method of botanical application had no significant difference on the extent of rotting. A similar study by Bdiliya and Dahiru, (2006), in which unwounded tubers were submerged in or sprayed with neem extracts for longer periods, showed a reduction in the incidence and severity of soft rot regardless of the application method. If the two methods tend to give similar results, it is therefore better to use the spraying method because it is easier and more practical than dipping, and might result in less rotting because it uses less water. Dipping, on the contrary, leaves a film of water on the potato surface which causes anaerobiosis. Anaerobiosis is important for the initiation

of tuber decay because it impairs oxygen dependent host resistance systems, leading to rotting (Perombelon, 1992).

CONCLUSIONS

In this study, garlic and neem provided control on potato soft rot caused by *Pa* and *Dd*. However, both botanicals were not effective against the *Pcc*. The results suggest that garlic and neem have anti-microbial compounds that can be used to control *Pa* and *Dd* infections.

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