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Research Application Summary

Evaluation of suppressive potential of selected plant species against *Phytophthora infestans* in vitro

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Abstract

Globally, *Phytophthora infestans* causes annual potato losses estimated at over $6 billion with fungicides alone accounting for over $1 billion. In Kenya, potato yield loss estimates reach 119,500 tons annually. Several fungicides recommended for late blight have raised health concerns and have led to emergence of more aggressive strains of the pathogen. These concerns have led to the need for safer alternative which can best be offered by bio-pesticides. Bio-pesticides are classified into biochemical, microbial and plant derivatives derived from natural living organisms. The aim of this study was to source, identify and screen for the bioactive potential of selected plant species against *Phytophthora infestans* in vitro. *Phytophthora infestans* was isolated from infected potato leaves and bioactive plants were sourced from the Kenyatta University surroundings, identified and phytochemical compounds extracted through maceration. Bioassays involved food poisoning technique using the aqueous plant extracts. Data were subjected to analysis of variance using SAS software version 9.1. Tukey's Studentized Range (HSD) at $P<0.05$ was used for mean separations where treatment effects were significant. All the plant extracts showed significant effect ($P<0.05$) on reducing mycelial growth of *P. infestans*. *Azadirachta indica* showed the highest overall percentage mycelia growth suppression with a mean of 54.3% at (P d" 0.05) at ten days after inoculation (DAI) which significantly differed from *Callistermon citrinus* which had the least overall suppressive effect with a mean of 26.75% at ($P< 0.05$). These findings show that potential for managing late blight exist in local plants.

Key words: Bioactive, bioassays, Kenya, late blight management, plant extracts, *Solanum tuberosum*

Résumé

À l'échelle mondiale, *Phytophthora infestans* cause des pertes annuelles de pommes de terre estimées à plus de 6 milliards de dollars, les fongicides représentant à eux seuls plus d'un milliard de dollars. Au Kenya, les estimations de la perte de rendement des pommes de terre atteignent 119 500 tonnes par an. Plusieurs fongicides recommandés pour le mildiou ont soulevé des problèmes de santé et ont conduit à l'émergence de souches plus agressives du pathogène. Ces préoccupations ont conduit à la nécessité d'une alternative plus sûre qui peut être offerte au mieux par les bio-pesticides. Les bio-pesticides sont classés en dérivés biochimiques, microbiens et végétaux dérivés d'organismes vivants naturels. Le but de cette étude était de rechercher, d'identifier et de dépister le potentiel bioactif d'espèces végétales sélectionnées contre *Phytophthora infestans* in vitro. *Phytophthora infestans* a été isolé à

partir de feuilles de pommes de terre infectées et des plantes bioactives ont été extraites des environs de l'Université de Kenyatta, identifiées et des composés phyto-chimiques extraits par macération. Les essais biologiques impliquaient une technique d'intoxication alimentaire utilisant des extraits aqueux de plantes. Les données ont été soumises à une analyse de variance à l'aide du logiciel SAS version 9.1. La petite différence significative de Tukey (HSD) à P <0,05 a été utilisée pour les séparations moyennes où les effets du traitement étaient significatifs. Tous les extraits de plantes ont montré un effet significatif (P <0,05) sur la réduction de la croissance mycélienne de *P. infestans. Azadirachta indica* a montré le pourcentage global de suppression de la croissance des mycéliums le plus élevé avec une moyenne de 54,3% à (P d "0,05) dix jours après l'inoculation (DAI), ce qui différait significativement de *Callistermon citrinus* qui avait le moins d'effet suppressif global avec une moyenne de 26,75% à (P <0,05). Ces résultats montrent qu'il existe un potentiel de lutte contre le mildiou dans les plantes locales.

Mots clés : Bioactif, essais biologiques, Kenya, lutte contre le mildiou, extraits de plantes, *Solanum tuberosum*

Introduction

Potato (*Solanum tuberosum* L.*)* ranks as the third most important food crop in the world after rice and wheat. Global production estimate is about 300 million metric tons consumed by over one billion people (Forbes *et al*., 2014). Kenya is the fifth biggest potato producer in sub-Saharan Africa. Potato remains the second most important staple food crop in Kenya, after maize, and contributes KSh 50 billion annually (NCPK, 2015). *Phytophthora infestans,* causal agent of potato late blight, is one of the world's most damaging plant diseases, with global loses estimated at over $6 billion per year with fungicides alone accounting for over $1 billion (Ristaino and Pfister, 2016). In Kenya, late blight is one of the major potato diseases causing up to 100% yield losses in potato (Ongoro *et al*., 2016).

A research conducted by Kenya Agriculture and Livestock Research Organisation (KALRO) and International Potato Center (CIP) from 1991 to 2007 revealed a reduction in yield from 37,500 to 119,500 tons over the entire period due to the pathogen. Several fungicides have been recommended for use against this disease, but they raise issues of cost, risks from exposure to people and animals, residual compounds on produce and other health and environmental hazards (Riungu, 2011). In addition, increased use of synthetic fungicides has led to the emergence of new and more aggressive biotypes of *P. infestans*. Bio-control products are affordable, safe and effective management options but represent only 1% use in disease management while fungicides, which are expensive chemicals with several side effects on humans and the environment still account for 15% of chemicals used in agriculture (Junaid *et al*., 2013).

There is a global need to increase food productivity, due to the projection that world population will have grown by about 10 billion by 2050. Population increase has heightened the pressure of increasing food production which is faced with several continually emerging challenges (Saravi and Shokrzadeh, 2011). The pressure to produce more food has led to more chemical and synthetic pesticide application. An ideal pesticide should only be target specific, biodegradable and eco-friendly. Unfortunately, most of the pesticides are non-specific and affect or kill beneficial organisms. Only about 0.1% of the pesticides applied reach the target organisms whereas the remaining bulk causes severe environmental contamination. Indeed pesticides have entered into the human food chain causing several acute and chronic human illnesses (Begum and Rajesh, 2015).

There is a rising global shift towards reducing the application of chemical fungicides resulting in a strong public and scientific desire to come up with alternatives guaranteeing safety to people and the environment (Lal *et al*., 2016). This alternative is offered by biological control with the advantages of reduced environmental impacts and greater public acceptance (Begum and Rajesh, 2015; Lal *et al*., 2016). There is therefore need for alternative control methods for late blight. Although there is an increase in the number of bio-control products used in management of plant diseases, fungicides still account for 15% of total chemicals used in agriculture, and bio-control products represent only 1% (Junaid *et al*., 2013). This evidently shows that there is need to increase the production and use of bio-control products and reduce the use of chemical fungicides as a safer management strategy. This study thus aimed to source, identify and screen for bioactive potential of selected plant species against *Phytophthora infestans* in vitro.

Materials and methods

The study was carried out at Kenyatta University (KU), Nairobi County, Kenya. Suspected bioactive plants, Neem (*Azadirachta indica* A. Juss*)*, Mastic (*Pistacia lentiscus*), Mexican sunflower (*Tithonia diversiforlia*) Mexican marigold, (*Tagetes minuta*), European cocklebur (*Xanthium strumarium* L), Sodom apple (*Solanum incanum* L), Jimson weed (*Datura stramonium* L), Lantana (*Lantana camara* L.), Lemon grass (*Cymbopogon citratus*), Devils horsewhip (*Achyranthes aspera* L.), and Lemon bottlebrush (*Callistermon citrinus*) were identified from the available literature, collected from the surrounding environment around Kenyatta University, shade dried and ground. The powder was soaked in two solvents, ethanol and acetone (1:1 vol/vol) at a ratio of 1:10 (wt/vol, dry powder/solvent) and the mixture periodically agitated for 12 hrs then left to stand for 1 hr to settle the plant materials. Afterwards it was filtered through double-layered muslin cloth then subsequent filtration through Whatman filter paper to give a supernatant free from large plant material debris (Kalbende and Dalal, 2013). The supernatant was evaporated at 60^0C until about 10 ml aqueous concentration of the phytochemical compounds was left. This was then kept in glass tubes and stored at room temperature for further use.

Phytophthora infestans was isolated from infected potato leaf tissues then surface sterilized using 0.5% sodium hypochlorite (NaOCl) for a minute. The leaf tissues were placed with the abaxial side up under a surface sterilized potato slice of about 5mm thick in a humid chamber. This was then incubated at at room temperature until fresh sporulation was observed above the potato slice. The sporulated mycelia was then aseptically picked and placed on Petri dishes containing 20ml carrot sucrose agar, and then incubated at room temperature. Multiplication of *P. infestans* was also done on Carrot sucrose agar (Siameto *et al*., 2010). Morphological characteristics of the pathogen were examined under a microscope and identified as *P. infestans* as described by Erwin and Ribeiro (1996).

Poisoned food technique was used for the *in vitro* evaluation of the plant extracts against *P. infestans* as described by Farooq and Nasreen (2015) and Messgo-Moumene *et al*. (2017). Two milliliter of each extract was poured in a conical flask containing 200ml of molten Potato dextrose agar (45^{o}C) then gently swirled to mix uniformly and subsequently dispensed then allowed to solidify on Petri dishes. Media with no plant extracts amendment was dispensed in the control plates. A 5mm mycelial plug from *P. infestans* culture was placed at the centre of every Petri dish containing the treatments and in the control plates and thereafter incubated at room temperature in a Completely Randomised Design (CRD) design with four replicates per treatment. Suppressive activity was recorded on the 4th, 6th, 8th and 10th day after inoculation (DAI) by measuring the colony diameter of the *P. infestans* mycelial growth in the control plate (Dc) compared to growth in the plate with the antagonists (Dt). The readings

were transformed into percentage growth suppression (PGS) by a modified formula from that used by Fulano *et al*. (2016):

$$PGS = \frac{(Dc - Dt)}{Dc} \times 100$$

Where: PGS is the percentage growth suppression, Dc is the diameter of the *P. infestans* colony in the control plate, and Dt is the diameter of the *P. infestans* colony in the plate with the antagonist (Killani *et al*., 2011). The data were analysed using SAS software version 9.1 where means were compared among the treatments using Tukey's Tukey's Studentized Range (HSD) at 95% confidence interval and ANOVA test was used for Analysis of variance.

Results and discussions

Maximum suppressive potential was observed at day ten across all the treatments except for *Callistermon citrinus*. The most promising extracts were from *A. indica, P. lentiscus* and *T. diversiforlia* with PGS values of 59.11%, 52.16% and 50.28%, respectively. These three extracts could be promising for application under field conditions, as field aprays and will be studied for two seasons. *Callistermon citrinus* showed the least suppressive activity with 26.75% in the *in vitro* trials at day ten (DAI).

Little research has been done on the use of plant extracts to control *P. infestans* in vitro (Mizubuti *et al*., 2007) . However, the available findings show mycelial suppression of *P. infestants* by plant extract. *Azadirachta indica* had the maximum suppressive percentage at 59.11%, this was higher than the results reported by Rashid *et al*. (2004). *P. lentiscus* was second most suppressive after *A. indica* with 52.16% this was lower than that reported by Messgo-Moumene *et al*. (2017) at 82%. *Tithonia diversiforlia* recorded 50.28% suppression activity. *Xanthium strumarium* caused 48.18% suppression contradictory to the results reported by Yanar *et al*. (2011) where there was 100% suppressive effect realised. *Datura stramonium,* L. *camara* and *A. aspera* showed 36.11%, 34.54% and 35.11% suppressive potential, respectively, whereas according to the results reported by Nagar *et al*. (2017), *D. stramonium* had 48.47% and *L. camara* had 34.53% suppressive effect, causing slightly higher efficacy than the results from this study.

The varying suppressive effect shown by all these plant extracts could be as a result of various modes or mechanisms of actions possessed by the secondary metabolites contained in varying proportions e.g. phenols, quinones, alkaloids, tannins, flavonoids and sterols being the major compounds among others. For instance, Phenols cause membrane disruption and substrate deprivation while Phenolic acids bind to adhesins, form complex with cell wall, and inactivate enzymes. On the other hand Terpenoids cause perturbation of cytoplasmic permeases, inhibition of ergosterol biosynthesis, and interference with the integrity of the cell membrane. On the other hand Biofilm inhibition through essential oil cause membrane disruption, while Alkaloids intercalate into cell wall causing DNA binding and inhibition, CDR1 cause induction fungal apoptosis; Tannins bind to proteins causing enzyme inhibition and substrate deprivation; Flavonoids bind to adhesins, complex with cell wall and inactivate enzymes, and induce fungal apoptosis; Coumarins cause interaction with eucaryotic DNA, lectins and polypeptides, and form disulfide bridges while Saponin cause disruption of membrane, and induction fungal apoptosis (Gurjar *et al*., 2012; Lee and Lee, 2015).

Table 1. Percentage growth suppression (%GS) of mycelial growth of *Phytophthora infestans* by different plant species extracts

Source of extracts	Family	Days after inoculation (DAI) (Mean±SE)			
		Four	Six	Eight	Ten
A. indica	Maliceae	48. 97±1.68^{a}	53.94±1.39^{a}	55.17±1.76^{a}	59.11±2.27$^{a\ a}$
P. lentiscus	Anacardiaceae	43.86±2.63^{b}	46.84±2.79^{bc}	48.39±2.11^{b}	52.16±1.82^{b}
T. diversiforlia	Asteraceae	40.71±0.52^{b}	47.67±1.65^{b}	45.26±1.27^{b}	50.28±1.95^{b}
T. minuta	Asteraceae	39.05±10.21^{abc}	44.0011.66^{abcdef}	44.73±11.84^{ab}	47.86±12.13^{abc}
S. incanum	Solanaceae	39.95±1.39^{b}	36.63±3.01^{de}	36.43±3.32^{bc}	43.87±2.31^{bc}
X. strumarium	Asteraceae	25.83±1.22^{bc}	40.08±2.35^{d}	42.50±2.87^{b}	48.18±2.17^{bc}
D. stramonium	Solanaceae	29.98±2.53^{c}	35.82±1.39^{e}	35.17±2.03^{c}	36.11±2.37^{d}
L. camara	Verbenaceae	26.27±1.47^{c}	32.71±1.48^{f}	35.35±1.65^{c}	34.53±1.88^{d}
C. citratus	Gramineae	23.69±3.08^{c}	30.32±3.41^{f}	33.12±2.81^{c}	36.01±2.28^{d}
A. aspera	Amaranthaceae	23.09±0.99^{c}	27.81±2.20^{fg}	29.94±2.81^{cd}	35.11±2.87^{d}
C. citrinus	Myrtaceae	24.28±6.64^{c}	26.46±6.68^{fg}	28.73±7.21^{cd}	27.54±7.11^{d}
Control	-	0^{d}	0^{h}	0^{e}	0^{e}
CV(%)		28.49	27.72	27.78	25.63
F-value		11.44	10.25	9.51	11.67
P<0.05		< 0.0001	< 0.0001	< 0.0001	< 0.0001

Means followed with the same letter (s) in each column are not significantly different (Tukey's Studentized Range (HSD),p <0.05)

Conclusion

Generally, all the plant extracts evaluated in this study showed some level of suppressive effects on the mycelial growth of *P. infestans* in vitro. We concluded that there are plant species in the local environment which could be exploited for plant disease management. The results show that selected botanicals could be very useful if the extraction methods are refined and standardized.

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