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Malagasy traditional treatments for food crops: A tool to control potato bacterial diseases?

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ABSTRACT

The control and treatment of food crops bacterial diseases remain problematic due to a scarcity of effective phytotreatments. As traditional agricultural practices may represent an attractive venue to explore new treatments, Malagasy traditional practices were investigated for their effectiveness on potato plants. A survey was conducted among Malagasy farmers to collect information on diseases observed on potato crops and on traditional disease control practices. Twelve treatments against potato bacterial diseases were tested on two potato varieties, in experimental plots naturally infected by bacterial wilt disease. The information collected from 52 farmers *(i)* showed that leaf spots and bacterial wilt disease are the most frequently identified threats for potato crops; and *(ii)* allowed to identify 12 traditional treatments among which five exhibited protective properties on potato bacterial wilt disease. Moreover, one recipe (R07) was effective on the two potato varieties after two applications per week for 10 weeks, with 50% and 72% effectiveness rate, respectively. Valorization of traditional practices may provide an effective, safe, economic and standardized phytotreatment against potato bacterial wilt disease.

1. Introduction

Food crops are economically very important in the developing world and represent an essential source of proteins in the diet of the poorest countries (FAO, 2013). In Madagascar notably, 90% of the island central regions crop area are planted to food crops (UPDR, 2003a; UPDR, 2003b).

Food crops are attacked by insects as well as diseases with pathogens; potato crops appear as an attractive target (Horst, 2013b; Kirk et al., 2013; MAEP et al., 2001a; MAEP et al., 2001b). These diseases and insects cause major damages to field crops. Their control and treatment remain particularly problematic due to the scarcity of effective phytotreatments (Horst, 2013a); the major control options reside in crops rotation (Ahmed et al., 2012; Larkin, 2008; Umaerus, 1992), in the use of resistant varieties (AROPA et al., 2011; Fock et al., 2000) and in chemical disinfectants (*e.g.* hydrogen peroxide) (Arora and Khurama, 2004; Bojanowski et al., 2013). The available chemical treatments against insect attacks and fungal diseases are often costly and may pose risks to the ecosystem, farmers and consumers; Moreover, the water and soil can be contaminated by chemical residues

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[1] This article is dedicated to our close colleague and co-author, Jacob Philémon Rajaonarivelo, who died in September 2014.

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(Giroux, 2003) and their accumulation threatening biodiversity (FAO and PAM, 2013).

Biological approaches so far mainly explored the control of insects (Dayan et al., 2009) with neem-based products (Ahmad et al., 2012), spinosads (Gentz et al., 2010), pyrethrins (Clark et al., 2013), rotenone insecticides (Lao et al., 2010), avermectins (Li et al., 2014), milbemycins (Wang et al., 2011), *Ryania speciosa* preparations (Omura, 2011), sabadilla, nicotine and essential oils (Rattan, 2010). Some natural products are also applied for plant pathogen management, including *(i)* in conventional cropping systems, actinomycetes antibiotics (Procopio et al., 2012) and chitin components (Senthilraja et al., 2010); and *(ii)* in organic agriculture, essential oils (Stevic et al., 2014) and extracts of giant knotweed (*Reynoutria sachalinensis* (F. Schmidt) Nakai) (Lalancette et al., 2013).

Further alternative methods based on biological products are certainly worth exploring to control and treat crop diseases (Narayanasamy, 2013). In Madagascar, the majority of farmers rely on both chemical products and traditional practices to solve their culture disease problems. These traditional practices, or "*ady gasy*", are mostly mixtures of plant- and animal-origin materials, based on orally transmitted indigenous knowledge (VOARISOA and EZAKA, 1996).

The present survey has been conducted in 20 localities of two Madagascar farming regions (Analamanga and Vakinankaratra) to collect information on the diseases observed on potato crops and on the recourse to modern and/or traditional practices whenever diseases are detected. The effectiveness of recipes applied against bacterial diseases of potato has been tested in local experimental fields.

2. Methodology

2.1. Survey of traditional practices

2.1.1. Survey area

The survey was carried out in two regions of Madagascar (Analamanga and Vakinankaratra regions) where food crops occupy 90% of growing area (UPDR, 2003a; UPDR, 2003b). Twenty localities were visited in seven districts (one in Analamanga region and six in Vakinankaratra region) (Fig. 1).

2.1.2. Data collection and analysis

A survey of crop diseases and traditional treatment practices was carried out between July and December 2010 through interviews of farmers who are either members or leaders of their local farmer associations. The survey aimed to record the following data: *(i)* background information on the farmers, including name, age, address and GPS coordinates; *(ii)* general information about observed crop disease, including type of crop, observed symptoms, disease outcome and eventual identification; and *(iii)* chemical and/or traditional treatment(s) eventually applied, including composition, preparation, application mode and local availability of components. Relationships between data were graphed as interaction networks using the software Cytoscape 2.7.0 (http://cytoscape.org) with the layout organic (Mukazayire et al., 2011; Shannon et al., 2003). The survey was mainly focused on potato crops, however information about other crops (tomato and rice), was also collected. All data was reflected in the network but were not further explored in terms of treatments.

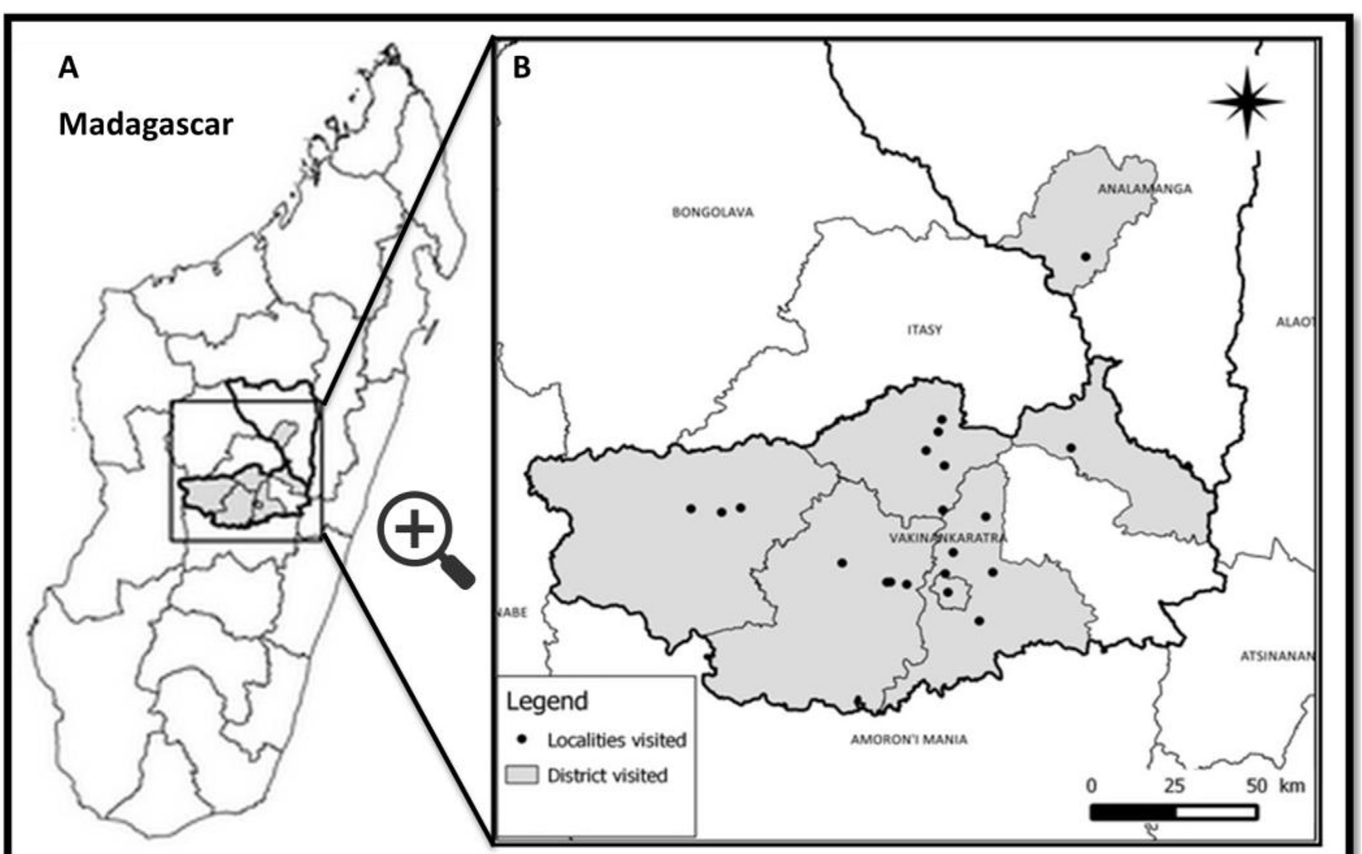

Fig. 1. Map of survey area with visited districts and localities. (A) Map of Madagascar, (B) Map of Vakinankaratra and Analamanga regions (grey color).

2.2. Verification of the effectiveness of selected traditional practices against bacterial diseases of potato

2.2.1. Selection of traditional practices

Twelve traditional practices or recipes, were selected for this study: *(i)* recipes (R01 to R03 and R05 to R12) applied by farmers on their presumed bacterial diseases of potato crops (leaf spots and wilt diseases); and *(ii)* recipes (R04, R07 and R08), the most frequently used, whatever the disease.

2.2.2. Preparation of traditional practices

The recipes components (plant- and animal-origin materials, soap, ashes …) were collected with the help of interviewees in different zones located 10 km around the field test site in Analamanga. Plant materials components are from *Agave vivipara* (L.), *Furcraea foetida* (L.), *Melia azedarack* (L.), *Buddleja madagascariensis* (Lam.), *Tephrosia purpunea* (L.) or *Sida rhombifolia* (L.) and animal-origin materials (cow dung and cow dung manure) are from cow of local breed. Herbs were authenticated by Benja Rakotonirina, botanist of the Department of Botany at the Institut Malgache de Recherches Appliquées (IMRA).

2.2.3. Tests in experimental field

2.2.3.1. Experimental design. The field experiments were conducted at IMRA, Avarabohitra Itaosy, Antananarivo, Madagascar (S 18° 55′33.0″; EO 47°28′44.5″; Elevation 1290 m) in January–May 2012. Trials were performed in two experimental fields, each with a potato variety used by the local farmers and obtained from the local cooperative FIFAMANOR: *(i)* the “Meva” potato that has a short development cycle (12 weeks) and is sensitive to bacterial and fungal diseases; and *(ii)* the “Diamondra” potato that has a longer development cycle (14 weeks) and is resistant to fungal diseases (AROPA et al., 2011). Each experimental field (11.0 m × 6.8 m) consists of four devices (0.50 m between devices) composed of four blocks (Pünterer, 1981) and each block comprises 15 plantlets or plots (Dagnelie, 2003).

As no bacterial or fungal seeding was applied, the experiments relied on natural infections only (Huang, 2002) a quite common occurrence in the test season. A randomized block design was used with three replications of five rows; three rows were treated with three different traditional recipes, one with Mancozeb solution 2.5% (or Dithane, the chemical treatment usually applied by farmers composed by two dithiocarbamates derivative) and last one was not treated (negative control) (Table 1). Concretely, two sprays of traditional recipes or dithane are applied per plantlet using knapsack sprayer. The plant density was 6.25 plants m^{-2}. Trials have been repeated three times: first trial from January to March, second trial from February to April and third trial from March to May.

Table 1
Treatment (recipes and controls) for each device on the field experiment.

Treatment condition	Device			
	I	II	III	IV
Recipe number	01	04	07	10
Recipe number	02	05	08	11
Recipe number	03	06	09	12
Positive Control (Dithane)	Mancozeb 2.5%			
Negative Control	Untread			

2.2.3.2. Treatment application. As per farmer's practices of scouting, monitoring and treating, the initial treatment (traditional recipe or dithane control) was applied on the corresponding infected plots only when the first signs appear and continued until 10 weeks after emergence, two times per week (seven days) (MAEP et al., 2001a; MAEP et al., 2001b).

2.2.3.3. Disease assessment. The disease symptoms observed on naturally infected experimental plot and their evolution under treatment were evaluated. Infection rate for each plot and the recovery rate for each treatment (traditional recipe, dithane control and negative control) (Dagnelie, 2003; Pünterer, 1981) were calculated by recording the numbers of infected plots in every device up to 10 weeks after emergence. Recovery is considered when plantlets are visually free of the disease symptoms initially observed.

$$Infection\ rate\ (\%) = \frac{Number\ of\ plots\ infested}{Total\ number\ of\ plots} \times 100$$

$$Recovery\ rate\ (\%) = \left(1 - \frac{Number\ of\ plots\ infested\ after\ treatment\ application}{Number\ of\ plots\ infested\ before\ treatment\ application}\right) \times 100$$

2.2.3.4. Data analysis. At each time point, the infection and recovery rates were compared by one-way analyses of variance (ANOVA). For each treatment, device and potato variety, the infection and cure rates were compared to the negative control of each block by Dunnett's multiple comparison tests at $P < 0.05$.

3. Results and discussion

3.1. Survey of traditional practices

Vakinankaratra and Analamanga are major cropping regions and food crops occupy 90% of the growing area; Vakinankaratra is the principal Malagasy potato producing region (UPDR, 2003b). The survey has been conducted over 19 localities of Vakinankaratra and one locality of Analamanga, and 52 farmers were interviewed (26 farmers were interviewed individually and 26 farmers distributed in five groups so that final interview number is 31).

3.1.1. Diseases and insects observed by the farmers

Damages from both diseases and insects are regularly observed on crops by the farmers (Table 2). In the absence of diagnostic facilities, the pathogens are practically never formally identified. The bacterial wilt observed on potato crops may correspond to the symptoms caused by well-known phytopathogen bacteria such as *Ralstonia solanacearum* (Horst, 2013a;, 2013b; OEPP/EPPO, 2004) and *Clavibacter michiganense* (Horst, 2013a). The leaf spots can be caused by both fungal and bacterial agents (Arora and Khurama, 2004).

Table 2
Description of symptoms observed by farmers on their infected potato crops.

Recorded symptoms	Identified by the farmers as	Reported by (% of informants; n = 31)
The first day the lower leaves wilt. The second day the aerial parts also wilt; the third day the plant dries out completely and finally dies after 5 days.	"Mandazo"[a] *(Bacterial wilt)*	48.39%
Black spots appear on the leaves of the plant. These spots grow and propagate on the stems of the plant.	"Lagaly" *(Leaf spots)*	64.52%

[a] Vernacular name of the disease.

3.1.2. Inventory of treatments and traditional recipes

The recourse to chemical treatment is frequent (95% of cases); the major part of interviewed farmers apply the same treatment (i.e. Dithane, 2.5% mancozeb, an ethylene bisdithiocarbamate fungicide) on their infected plots, regardless the observed symptoms.

Our survey identified 54 traditional recipes (unpublished data), the majority of which (75%) are no longer used by farmers; the others are either used alone or integrated with chemical treatments (particularly Dithane). Most of identified recipes (80%) were/are used for potato crops (Fig. 2 and S1) leading to the selection of 12 recipes (Table S1).

Although each recipe was only quoted one to two-times, they are simple or elaborate variations based on common components of vegetal- (62%) and animal-origin (24%) with some other materials added (14%). In these treatments, the leaves of 18 plants belonging to 14 families are used; *Melia azedarach* (L.), *Agave vivipara* (L.) and *Furcraea foetida* (L.) are the most frequently cited plants. As for animal-origin material, cow dung, cow dung manure, cow rumen and cow urine are used. Other materials comprise local soap, powder soap and cinder, with local soap as the most cited ingredient. These components vary from recipe to recipe, in nature and proportion (Table S1, Figure S1). All these elements are typically ground, mixed in water or in cow dung manure, macerated during one to fifteen days, filtered and eventually diluted before application. The mixtures are sprayed one or two times per week on the plots, either as preventive or curative measures (Table S1).

Interestingly, all herbs used in cited recipes are part of plants previously inventoried in Madagascar for pests control (VOARISOA, 1998). Other materials, such as cow dung, cow dung manure and soaps, were previously cited by farmers of the Betafo district (Vakinankaratra region) as part of their traditional practices for crops protection and treatment (VOARISOA and EZAKA, 1996). Many of the herbs cited by the farmers have been reported elsewhere for various uses against insects, mites, nematodes and pathogens, in association with other plants or other materials (Gahukar, 2012). The extracts derived from *Agave vivipara* (Ahumada-Santos et al., 2013; McGaw et al., 2008), *Buddleja madagascariensis* (Houghton et al., 2003), cow dung (Krishnamurthy et al., 2008) and mixtures of cow urine with other plants (Gahukar, 2012) have been described for antimicrobial properties. Moreover, the insecticide activities of *Melia azedarach* (Bohnenstengel et al., 1999; Cavoski et al., 2012; Defago et al., 2006, 2011) and *Tephrosia purpurea* (Pugazhvendan et al., 2012) have been already established.

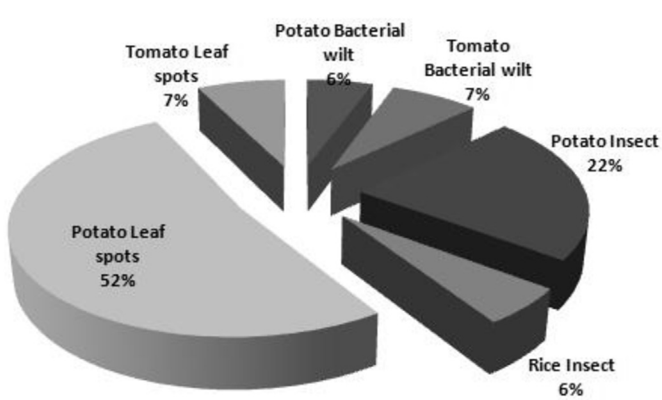

Fig. 2. Use of traditional recipes by the Malagasy farmers to treat diseases and insects infesting their plots.

3.1.3. Symptoms of disease observed on experimental fields

The wilt disease appeared two week after emergence of plantlets on both potato varieties (Meva and Diamondra). Symptoms began with the wilting of leaves and, after two or three days the aerial parts were completely wilted leading the plant death within seven days. These natural infections observed in experimental fields suggest bacterial infection as these signs are symptomatic of potato bacterial wilt disease and correspond to those reported by farmers under the name of "*mandazo*" (Table 2), probably caused by bacterial agents (*R. solanacearum* or *C. michiganense*), that are both known to infect tropical crops (Aliye et al., 2008; Horst, 2013a; N'Guessan et al., 2013; OEPP/EPPO, 2004). Some representative samples of infected plant were investigated by molecular analysis and PCR product according to DNA 16S amplification protocol (Kutin et al., 2009; Massart et al., 2005) confirmed the presence of *R. solanacearum*.

The potato varieties used for this experimental setup are disease-sensitive with similar infection rates, 25–100% and 33–100% for Diamondra and Meva, respectively (Fig. 3). Given the acknowledged resistance of the Diamondra variety to fungal infections, this corroborates the bacterial nature of the observed diseases. For the Diamondra variety, there were no infection differences between all experimental fields (Fig. 3A; $p > 0.05$); for the Meva variety, however, some fields (R01 in device I; R05 and dithane control in device II), close to 100% infection, were significantly different from the negative control ($p < 0.05$) (Fig. 3B).

3.1.4. Effectiveness of selected traditional practices against bacterial diseases of potato

After treatment, the recovery rates, defined as the complete waning of symptoms, range from 0 to 100% of infected plants (Fig. 3C and D). The dithane control was inefficient on all parcels, indicating that the observed diseases are not fungal. For the Diamondra variety, the cure rates of recipes R07 (67% ± 27%), R05 (78% ± 16%) and R09 (56% ± 08%) significantly differ from their respective negative controls ($P < 0.05$) (Fig. 3C) whereas, for the Meva variety, recipes R04 (42% ± 18%), R06 (44% ± 08%) and R07 (72% ± 21%) are significantly active ($P < 0.05$) (Fig. 3D). From all tested recipes, only R07 is efficient to treat the wilt disease on the two potato varieties.

These data are encouraging and could lead to the development of an effective biotreatment method for potato bacterial wilt diseases for which, so far, no effective control method could be developed (Lemessa and Zeller, 2007); indeed, *(i)* only partial biocontrol of *R. solanacearum* could be accomplished by bacterial antagonism with plant growth-promoting *Rhizobacteria* (Aliye et al., 2008; Ghyselinck et al., 2013); *(ii)* soil management (types of soil and amendments) was investigated without much success (Messiha et al., 2009); and *(iii)* plant extracts and microbial

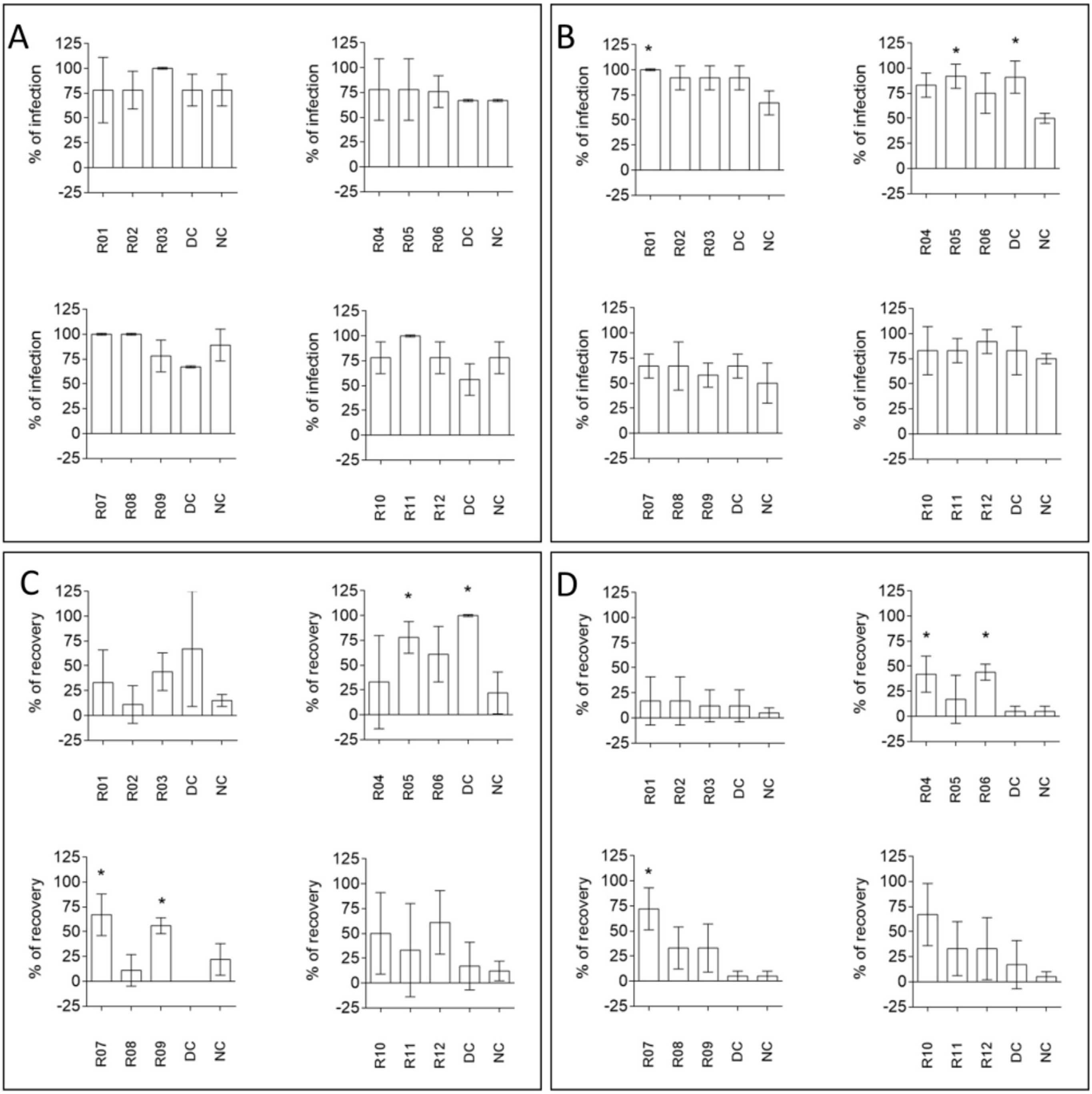

Fig. 3. Infection and recovery rates observed on experimental parcels of two cultivars. (A) Infection rates observed on experimental parcels of the "Diamondra" potato variety. (B) Infection rates observed on experimental parcels of the "Meva" potato variety. (C) Recovery rates observed on experimental parcels of the "Diamondra" potato variety. (D) Recovery rates observed on experimental parcels of the "Meva" potato variety. Data were recorded throughout the 12 or 14 weeks of the vegetation cycle; Three independent experiments, each on four blocks of 15 plantlets; n = 12). Dithane control (DC) represents the positive control. Data were not statistically different compared to negative control (NC) after Dunnett's multiple comparison tests at $P < 0.05$.

antagonists were used to treat wilt fungal diseases with moderate success (Naraghi et al., 2010; Uppal et al., 2008; Yangui et al., 2010).

4. Conclusions

Upon gathering information on the traditional recipes ("*ady gasy*") used by the farmers of surround the capital town of Madagascar to fight insects and diseases of potato, tomato and rice crops, the effectiveness of 12 selected recipes has been investigated on naturally infected potato plants. Despite the lack of adequate diagnostics, the farmers have knowledge on the preparation and utilization of a series of recipes to protect and treat their crops; the present survey has yielded 54 traditional recipes. However, most of them have been abandoned in favor of a so-called "modern" treatment (mancozeb 2.5%) that is used indistinctly, despite its well-known inefficacy on bacterial crop diseases. Strikingly most of recipes consist of complex mixtures, often fermented with cow dungs. This may infer that the fermentation of herbal products yield activated compounds or that the presence of the herbs selectively favors the proliferation of active bacteria that would compete with the phytopathogens. Such aspect deserves further investigation to decipher the precise role of each component of the complex mixtures and the importance of fermentation variables (*e.g.* time, temperature and humidity).

Testing of recipes selected for their high frequency of citation has however yielded some disappointing data; from the 12 investigated recipes, only 5 were active in our experimental setup (based on natural infection of experimental fields) and only one could significantly treat the disease identified as mandazo (*R. solanacearum* bacterial wilt) on the two tested potato varieties (R07). These data certainly warrant research to confirm the interest of this recipe, to understand its chemical and biological properties and to determine its mode of action.

Ancestral knowledge of cropping yields valuable information on agricultural practices in a difficult tropical area; such information should be further investigated to hopefully develop into effective biological products that will improve diseases management in tropical agriculture.

Declaration of interest

All authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cropro.2017.08.011.

References

Ahmad, N., Ansari, M.S., Hasan, F., 2012. Effects of neem based insecticides on *Plutella xylostella* (Linn.). Crop Prot. 34, 18–24.
Ahmed, S., Piggin, C., Haddad, A., Kumar, S., Khalil, Y., Geletu, B., 2012. Nematode and fungal diseases of food legumes under conservation cropping systems in northern Syria. Soil & Tillage Res. 121, 68–73.
Ahumada-Santos, Y.P., Montes-Avila, J., Uribe-Beltran, M., Diaz-Camacho, S.P., Lopez-Angulo, G., Vega-Avina, R., Lopez-Valenzuela, J.A., Heredia, J.B., Delgado-Vargas, F., 2013. Chemical characterization, antioxydant ant antimicrobial activities of six *Agave* species from Sinaloa, Mexico. Industrial Crops Prod. 49, 143–149.
Aliye, N., Fininsa, C., Hiskias, Y., 2008. Evaluation of rhizosphere bacterial antagonist fro their potential to bioprotect potato (*Solanum tuberosum*) against bacterial wilt (*Ralstonia solanacearum*). Biol. Control 47, 282–288.
AROPA, FERT, MAEP, 2011. Etude de la filière pomme de terre dans la région Amoron'i Mania, Rapport final. Département agriculture, Madagascar, pp. 62.
Arora, R.K., Khurama, S.M.P., 2004. Major fungal and bacterial diseases of potato and their management. In: Mukerji, K.G. (Ed.), Disease Management of Fruits and Vegetables. Kluwer Academic Publishers, Netherlands, pp. 189–231.
Bohnenstengel, F.I., Wray, V., Witte, L., Srivastava, R.P., Proksch, P., 1999. Insecticidal melacarpins (C-*seco* limonoids) from *Melia azedarach*. Phytochemistry 50, 977–982.
Bojanowski, A., Avis, T.J., Pelletier, S., Tweddell, R.J., 2013. Management of potato dry rot. Postharvest Biol. Technol. 84, 99–109.
Cavoski, I., Chami, Z.A., Bouzebboudja, F., Sasanelli, N., Simeone, V., Mondelli, D., Miano, T., Sarais, G., Ntalli, N.G., Caboni, P., 2012. *Melia azedarach* controls *Meloidogyme incognita* dan triggers plant defense mechanisms on cucumber. Crop Prot. 35, 85–90.
Clark, J.M., Yoon, K.S., Lee, S.H., Pittendrigh, B.R., 2013. Human lice: past, present and future control. Pesticide Biochem. Physiology 106, 162–171.
Dagnelie, P., 2003. Principes d'expérimentation :Planification des expériences et analyses de leurs résultats. Les Presses Agronomiques de Gembloux, Gembloux (Belgique).
Dayan, F.E., Cantrell, C.L., Duke, S.O., 2009. Natural products in crop protection. Bioorg. Med. Chem. 17, 4022–4034.
Defago, M., Valladares, G., Banchio, E., Carpinella, C., Palacios, S., 2006. Insecticide and antifeedant activity of different plant parts of *Melia azedarach* on *Xanthogaleruca luteola*. Fitoterapia 77, 500–505.
Defago, M.T., Dumon, A., Avalos, D.S., Palacios, S.M., Valladares, G., 2011. Effects of *Melia azedarach* extract on *Cortesia ayerza*, parasitoid of the alfalfa defoliator *Colias lesbia*. Biol. Control 57, 75–78.
FAO, 2013. FAO Statistical Yearbook 2013. World Food and Agriculture, Rome.
FAO, PAM, 2013. Mission FAO/PAM d'évaluation de la securité alimentaire à Madagascar (9 octobre 2013). pp. 75.
Fock, I., Collonnier, C., Purwito, A., Luisetti, J., Souvannavong, V., Vedel, F., Servaes, A., Ambroise, A., Kodja, H., Ducreux, G., Sihachakr, D., 2000. Resistance to bacterial wilt in somatic hybrids between *Solanum tuberosum* and *Solanum phureja*. Plant Sci. 160, 165–176.
Gahukar, R.T., 2012. Evaluation of plant-derived product against pest diseases of medicinal plants: a review. Crop Prot. 42, 202–209.
Gentz, M., Murdoch, G., King, G.F., 2010. Tandem use of selective insecticides and natural enemies for effective, reduced-risk pest management. Biol. Control 52, 208–215.
Ghyselinck, J., Velivelli, S.L.S., Heylen, K., O'Herlihy, E., Franco, J., Rojas, M., De Vos, P., Prestwich, B.D., 2013. Bioprospecting in potato fields in Central Andean Highlands: screening of rhizobacteria for plant growth-promoting properties. Syst. Appl. Microbiol. 36, 116–127.
Giroux, I., 2003. Contamination de l'eau souterraine par les pesticides et les nitrates dans les régions en culture de pomme de terre. In: Direction du suivi de l'état de l'environnement. envirodoq, Québec, p. 23.
Horst, R.K., 2013a. Bacterial Diseases, Westcott's Plant Disease Handbook. Springer Science+Business Media Dordrecht, USA, pp. 69–90.
Horst, R.K., 2013b. Host Plants, Westcott's Plant Disease Handbook. Springer Science+Business Media Dordrecht, USA, pp. 447–699.
Houghton, P.J., Mensah, A.Y., Iessa, N., Hong, L.Y., 2003. Terpenoids in *Buddleja*: revelance to chemosystemetics, chemical ecology and biological activity. Phytochemistry 64, 385–393.
Huang, H., 2002. Screening sunflower for resistance of sclerotinia wilt. Plant Pathol. Bull. 11, 15–18.
Kirk, W.W., Gachango, E., Schafer, R., Wharton, P.S., 2013. Effects of in-season crop-protection combined with postharvest applied fungicide on suppression of potato storage diseases caused by pathogens. Crop Prot. 51, 77–84.
Krishnamurthy, Y.L., Shashikala, J., Shankar Naik, B., 2008. Antifungal potential of some natural products against *Aspergillus flavus* in soybean seed during storage. J. Stored Prod. Res. 44, 305–309.
Kutin, R.K., Alvarez, A., Jenkins, D.M., 2009. Detection of *Ralstonia solanacearum* in natural substrates using phage amplification integrated with real-time PCR assay. J. Microbiol. Methods 76, 241–246.
Lalancette, N., Furman, L.A., White, J.F., 2013. Management of peach rusty spot epidemics with biorational fugicides. Crop Prot. 43, 7–13.
Lao, S., Zhang, Z., Xu, H., Jiang, G., 2010. Novel amphiphilic chitosan derivatives: synthesis, Characterization and micellar solubilization of rotenone. Carbohydr. Polym. 82, 1136–1142.
Larkin, R.P., 2008. Relative effect of biological amendement and crop rotation on soil microbial communities and soilborne diseases of potato. Soil Biol. Biochem. 40, 1341–1351.
Lemessa, F., Zeller, W., 2007. Screening rhizobacteria for biological control of *Ralstonia solanacearum* in Ethiopia. Biol. Control 42, 336–344.
Li, H., Zheng, Y., Sun, D., Wang, J., Du, Y., 2014. Combined effects of temperature and avermectins on life history and stress response of the western flower thrips, *Frankliniella occidentalis*. Pesticide Biochem. Physiology 108, 42–48.
MAEP, FAO, PSDR, 2001a. Fiche techniques de base destinées au techniciens agricoles: Pomme de terre Contre saison, p. 4. PSDR, Madagascar.
MAEP, FAO, PSDR, 2001b. Fiches techniques de base destinées aux techniciens agricoles: Pomme de terre Saison Pluviale, p. 4. PSDR, Madagascar.
Massart, S., De Clercq, D., Salmon, M., Dickburt, C., Jijakli, M.J., 2005. Development of real-time PCR using Minor Groove Binding probe to monitor the biological control agent *Candida oleophila* (strain O). J. Microbiol. Methods 60, 73–82.
McGaw, L.J., Lall, N., Meyer, J.J.M., Eloff, J.N., 2008. The potential of South African plants against Mycobacterium infections. J. Ethnopharmacol. 119, 482–500.
Messiha, N.A.S., van Bruggen, A.H.C., Franz, E., Janse, J.D., Schoeman-Weerdesteijn, M.E., Termorshuizen, A.J., van Diepenigen, A.D., 2009. Effects soil type, management type and soil amendments on the survival of the potato brown rot bacterium *Ralstonia solancearum*. Appl. Soil Ecol. 43, 206–215.
Mukazayire, M.J., Minani, V., Ruffo, C.K., Bizuru, E., Stevigny, C., Duez, P., 2011. Traditional phytotherapy remedies used in Southern Rwanda for the treatment of liver diseases. J. Ethnopharmacol. 138, 415–431.
N'Guessan, C.A., Brisse, S., Le Roux-Nio, A., Poussier, S., Koné, D., Wicker, E., 2013.

Development of variable number of tandem repeats typing schemes for *Raltsonia solanacearum*, the agent of bacterial wilt, banana Moko disease and potato brown rot. J. Microbiol. Methods 92, 366–374.
Naraghi, L., Heydari, A., Rezaee, S., Razavi, M., Jahanifar, H., 2010. Study on antagonistic effects of *Talaromyces flavus* on *Verticillium albo-atrum*, the causal agent of potato wilt disease. Crop Prot. 29, 658–662.
Narayanasamy, P., 2013. Abiotic Biological Control Agents for Crop Disease Management, Biological Management of Disease of Crops, Progress in Biological Control. Springer Science + Business Media Dordrecht, USA, pp. 511–632.
OEPP/EPPO. 2004. Standard PM 7/2(1) Diagnostic protocols for regulated pests - Ralsotnia solancearum, in: Bulletin OEPP/EPPO (Ed.), Bulletin 34. pp. 173–178.
Omura, S., 2011. Microbial metabolites: 45 years of wandering and discovering. Tetrahedron 67, 6420–6459.
Procopio, R.E.L., da Silva, I.R., Martins, M.K., de Azevedo, J.L., de Araujo, J.M., 2012. Antibiotics producer by streptomyces. Braz. J. Infect. Dis. 16, 466–471.
Pugazhvendan, S.R., Ronald Ross, P., Elumalai, K., 2012. Insecticidal and reppellant activities of four indigenous medicinal plants against stored grain pest. Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae). Asian Pac. J. Trop. Dis. 516–520.
Pünterer, W., 1981. Manuel pour essais de plein champ : protection des végétaux, 2 ed. CIBA GEIGY, Paris.
Rattan, R.S., 2010. Mechanism of action of insecticidal secondary metabolites of plant origin. Crop Prot. 29, 913–920.
Senthilraja, G., Anand, T., Durairaj, C., Raguchander, T., Samiyappan, R., 2010. Chitin-based bioformulation of *Beauveria bassiana* and *Pseudomonas fluorescens* for improved control of leafminer and collar rot in groundnut. Crop Prot. 29, 1003–1010.
Shannon, P., Markiel, A., Ozier, O., Baliga, N., Wang, J., Ramage, D., Amin, N., Schwikowski, B., Ideker, T., 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 13, 2498–2504.
Stevic, T., Beric, T., Savikin, K., Sokovic, M., Godevac, D., Dimkic, I., Stankovic, S., 2014. Antifungal activity of selected essential oils against fugi isolated from medicinal plant. Industrial Crops Prod. 55, 116–122.
Umaerus, V., 1992. Crop rotation in relation to crop protection. Neth. J. Pl. Path. 98 (Suppl. 2), 241–249.
UPDR, 2003a. Monographie de la Région d'Antananarivo. Ministère de l'agriculture, de l'élévage et de la pèche, Madagascar.
UPDR, 2003b. Monographie de la Région de Vakinankaratra. Ministère de l'agriculture, de l'élévage et de la pèche, Madagascar.
Uppal, A.K., El Hadrami, E., Adam, L.R., Tenuta, M., Daayf, F., 2008. Biological control of potato *Verticillium* wilt under controlled and field condition using selected bacterial antagonist an plant extracts. Biol. Control 44, 90–100.
VOARISOA, 1998. Un inventaire des pesticides naturels d'origine végétale à Madagascar (Madagascar).
VOARISOA, EZAKA, 1996. Fanomezan-danja Ny Fomba “ADY GASY” Ataon'ny Tantsaha (Mamboly Sy Mpiompy), p. 92. Madagascar.
Wang, X., Zhang, J., Gong, D., Zhang, H., Wang, J., Yan, Y., Xiang, W., 2011. A novel macrocyclic lactone with insecticidal bioactivity from *Steptomyces microflavus* neau3. Bioorg. Med. Chem. Lett. 21, 5145–5148.
Yangui, T., Sayadi, S., Gargoubi, A., Dhouib, A., 2010. Fungicidal effect of hydroxytyrosol-rich preparations from olive mill wastewater against *Verticillium dahliae*. Crop Prot. 29, 1208–1213.