AJFAND ONLINE Scholarly, Peer Reviewed ISSN 1684 5374
Volume 12 No. 7
December 2012
African Journal of Food, Agriculture, Nutrition and Development
Published by African Scholarly Science Communications Trust

MORPHOLOGICAL CHARACTERIZATION AND IDENTIFICATION OF *PHYTOPHTHORA* SPECIES CAUSING CITRUS GUMMOSIS IN KENYA

Mounde LG[1*], Ateka EM[2], Kihurani AW[2] and L Wasilwa[3]

Leonard Mounde

*Corresponding author email: moundel05@yahoo.com

[1] Pwani University College, P.O Box 195- 80108 Kilifi, Kenya

[2]Department of Horticulture, Jomo Kenyatta University of Agriculture and Technology. P.O Box 62000-00200, Nairobi, Kenya

[3]Kenya Agricultural Research Institute, P.O Box 57811 -00200, Nairobi, Kenya

AJFAND ONLINE Scholarly, Peer Reviewed
ISSN 1684 5374
Volume 12 No. 7
December 2012
African Journal of Food, Agriculture, Nutrition and Development
Published by African Scholarly Science Communications Trust

ABSTRACT

Frequent outbreaks of citrus gummosis in Kenyan citrus orchards have been reported, yet the identity and distribution of the *Phytophthora* species causing the disease are unknown. Work was carried out to (i) characterize and identify *Phytophthora* species associated with citrus gummosis based on cultural and morphological traits and (ii) determine the distribution of these species associated with gummosis in different agroecological zones (AEZ). Some 59 plant and soil samples obtained from symptomatic trees and the rhizosphere were evaluated by direct isolation and baiting, respectively, using *Phytophthora* semi-selective media. *Phytophthora* species were identified on the basis of colony morphology, mycelial characteristics, cardinal growth temperatures, morphology and dimensions of sporangia, oogonia and antheridia. For colony morphology and growth temperature studies, a 5 mm diameter mycelial plug of each isolate was transferred to amended cornmeal agar (ACMA) and incubated at 5, 24 and 35°C for 7 days in the dark. Growth rates were evaluated based on daily records of mycelial growth for 7 days. The occurrence and distribution of these species were determined by recording the number of isolates recovered from samples from each AEZ. *P. citrophthora* was the most prevalent (76.3 %) of all the *Phytophthora* species identified in all the AEZs, followed by *P. nicotianae* (22 %). *P. syringae* was the least (1.7 %) prevalent. *P. citrophthora* was the only species present in all AEZs sampled whereas *P. nicotianae* was confined to the coastal lowlands although also present in other zones in a lower scale. *P. syringae* was present only in low midland zones and was the only species not found in coastal lowland zones. The forty five isolates of *P. citrophthora*, thirteen isolates of *P. nicotianae* and one isolate of *P. syringae* were tested for virulence on fruits of lemon var. rough lemon. The three most virulent isolates of *P. citrophthora*, two most virulent isolates of *P. nicotianae* and the only isolate of *P. syringae* were selected for pathogenicity testing on lemon seedlings. Based on these studies, it may be concluded that *P. citrophthora, P. nicotianae (syn. P. parasitica) and P. syringae* are the *Phytophthora* species associated with citrus gummosis in Kenya. Molecular characterization of the pathogens is recommended to confirm true genetic identity of the species.

Key words: *Phytophthora*, morphological, characterization, virulence, pathogenicity

INTRODUCTION

Citrus gummosis is caused by several *Phytophthora* species [1]. Morphological differences between some of the species are few and variable, making it difficult to classify the species accurately. Misidentification or isolates identified simply as 'unknown *Phytophthora* spp.' can delay recognition of new disease threats and result in economic losses to growers. On the other hand, accurate identification is fundamental for implementation of effective disease control strategies [2].

Phytophthora species have historically been delimited by their morphology, cytology and biochemistry [3, 4, 5]. More than 50 species have been identified based on morphological characteristics [6]. Mycelial growth, oospores and sporangia characteristics, size and shape differences in reproductive structures are used based on taxonomic keys of Waterhouse [7] and Stamps *et al*. [6].

Frequent outbreaks of citrus gummosis in Kenyan citrus orchards have been reported, yet the identity and distribution of the *Phytophthora* species causing the disease are unknown. The objective of this study, therefore, was to identify and characterize the species and determine their distribution in the different ecological zones in Kenya.

MATERIALS AND METHODS

Isolation of *Phytophthora*

A total of 59 bark and 9 soil samples were obtained from 70 affected orchards in 2007 and 2008 and used in the isolation of *Phytophthora*. Bark samples were labeled, placed in brown paper bags and taken to the laboratory. They were washed under running tap water, surface-sterilized in 70% ethanol for 5-10 seconds then dried on filter paper. Bark pieces, about 2-4mm-wide, were cut from the edge of the lesions and placed on cornmeal agar (CMA) (Sigma-Aldrich Chemie GmbH, Germany) amended with 10mg pimaricin, 200mg ampicilin, 10mg rifampicin, 10mg benomyl, 25mg pentachloronitrobenzene and 50mg hymexazol (PARBPH) [8]. Inoculated plates were incubated at 24^{o}C in the dark and examined within 2–3 days. Pure cultures were obtained by subculturing hyphal tips onto amended corn meal agar (ACMA).

About 500 cm^{3} soil samples were collected at 10–20 cm depth from severely affected trees. Four samples were bulked, mixed and small portions placed into wells, 10-mm wide and 15-mm deep, cut into apple fruits. Two wells per fruit and 2 fruits per sample were prepared following the method by Hendrix and Campbell [9] for isolation of *Phytophthora.* After three days, pieces of infected tissue were aseptically removed from the inoculated apples at the junction of the healthy and necrotic tissue and placed on PARBPH medium. A sterile wire loop was used to transfer fungal tips onto to CMA and V8 juice (20% Campbell's Vegetable) agar for pathogen identification.

Identification of isolates

Identification was based on colony morphology, mycelial characteristics, cardinal growth temperatures, morphology and dimensions of sporangia, oogonia and antheridia as follows; Colony morphology and growth temperature: A 5-mm-diameter mycelial plug of each isolate was transferred to ACMA and incubated at 5, 24 and 35 °C for 7 days in the dark. Morphology was recorded as pattern, nature of margin and growth rate of isolates on ACMA. Growth rates were evaluated based on daily records of mycelium growth (mm /day; precision 0.5 mm) for 7 days. Sporangial form and dimensions: Sporangia were produced by cutting 5-mm-diameter disks from the advancing margin of a colony grown on V8 agar and floating these disks on 10 ml of 1·5% sterile soil extract for 4–5 days at 24 °C under fluorescent light as in Mitchell *et al*. [10]. Slide cultures were made by placing a plug of fresh V8 juice agar (13mm) at the centre of a sterile microscope slide placed over a bent glass rod in a Petri dish lined with moist filter paper. Mycelia was aseptically removed from the growing culture with an inoculating needle and smeared around the slide. Another sterile slide was placed on top of the agar block, covered with the top of the Petri dish, sealed with plastic tape to prevent desiccation, and incubated at 25^{o}C under continuous 400 W/m^{2} white light provided by Phillips- Cleo Tubular Fluorescent tubes. After 5 days, the microscope slides were removed, a drop of sterile distilled water and placed on the fungal mycelia and covered with a glass cover slip. Sporangial morphology was examined under a compound microscope and the shape, size, presence or absence of papilla, proliferations and sporangiophore branching recorded. The descriptions of sporangial shape, measurement of papilla length, and length (l) and breadth (b) of 30 sporangia of each isolate were carried out at x400 magnification and their length to breadth (l/b) ratios calculated.

These characteristics were utilized to trace the fungus to the species level through Heffer *et al*. [11] and Newhook *et al*. [12] analytical keys and to species level using specific monographs and publications such as Commonwealth Mycological Institute (CMI) Descriptions of Fungi and Bacteria No.32, 33 and No. 35 [13].

Distribution of *Phytophthora* species

This was determined by recording the number of isolates recovered from samples obtained in each agro-ecological zone (AEZ) as described in Jaetzold and Schmidt [14]. Prevalence of each species was determined by expressing the number of isolates of each species recovered in all AEZs as a percentage of the total number of isolates collected there.

Virulence tests

Forty five isolates of *P. citrophthora*, thirteen isolates of *P. nicotianae* and one isolate of *P. syringae* were initially tested for virulence on lemon (var. rough lemon) fruits. Mature green fruits of uniform size were washed in running tap water and surface disinfected for 1 to 2 min in 75% ethanol. They were inoculated with a mycelial plug (5 mm in diameter) placed aseptically in a hole made with a cork borer. The experiment was replicated four times and laid in a completely randomized design (CRD) in a sterile humid plastic chamber at 20^{o}C and 90% relative humidity. The diameter of the developing lesion was determined 7 days after inoculation.

Pathogenicity tests

With the help of the method described by Agrios [15], 3 most virulent isolates of *P. citrophthora*, (P.CIT1, P.CIT7, P.CIT41), 2 of *P. nicotianae* (P.NIC 11, P.NIC13) one of *P. syringae* (P.SYR) and the susceptible lemon (*Citrus limon* L). cv. Rough lemon [16] was used. The isolates were inoculated onto 1-year-old seedlings grown in a greenhouse in plastic pots (20 cm diameter ×25 cm deep) containing heat sterilized (121^{o}C, 102 kPa, 60 min) field soil.

Five-mm-diameter 2-day old mycelia (CMA) discs containing each isolate were used as inoculum. Healthy plants were surface sterilized using 75% ethanol for 1 to 2 min and the bark removed using a 5-mm cork borer to expose the cambium. Inoculations were made by placing the inoculum onto the wound. The inoculation site was then moistened with a drop of sterile water and sealed with a strip of Parafilm®. Each isolate was inoculated individually onto five seedlings. Control seedlings were inoculated with sterile discs. The experiment was arranged in a complete randomized design. Temperature was 25 to 32^{o}C and relative humidity of approximately 60- 80%.

Symptom development was monitored daily and resultant symptoms were compared with those described on naturally infected plant samples. After 3 weeks, the bark, just above and below the inoculation point, was removed and cultured onto PARBPH. When mycelia growth occurred, its culture characteristics were recorded and the isolate identified. The isolates with similar culture characteristics and disease symptoms on the inoculated plants to those observed originally, were identified as pathogens. Those that failed to produce disease symptoms on seedlings were identified as non-pathogenic to citrus.

Data analysis

The data, mean colony and hyphal diameter, length and breadth of sporangia, length of papilla, mean lesion diameter and length, was subjected to analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) computer software (version 15.0). Significant treatment means were separated by Student Newman Keuls (S-N-K) test at $P<0.05$.

RESULTS

Isolation and identification of isolates

At the species levels, the *Phytophthora* isolates obtained were identified as *P. citrophthora* (76.3%), *P. parastica (*22%) and *P. syringae* (<2%). Colony diameter differed for each species. It was 55mm and 0 mm for *P. citrophthora*, 42mm and 60mm for *P. nicotianae, and* 37.5mm and 0mm for *P. syringae* at 24^{o}C and 35^{o}C, respectively. Fig. 1 shows the growth rates of the three *Phytophthora* species at 24^{o}C.

AJFAND ONLINE Scholarly, Peer Reviewed ISSN 1684 5374
Volume 12 No. 7
December 2012
African Journal of Food, Agriculture, Nutrition and Development
Published by African Scholarly Science Communications Trust

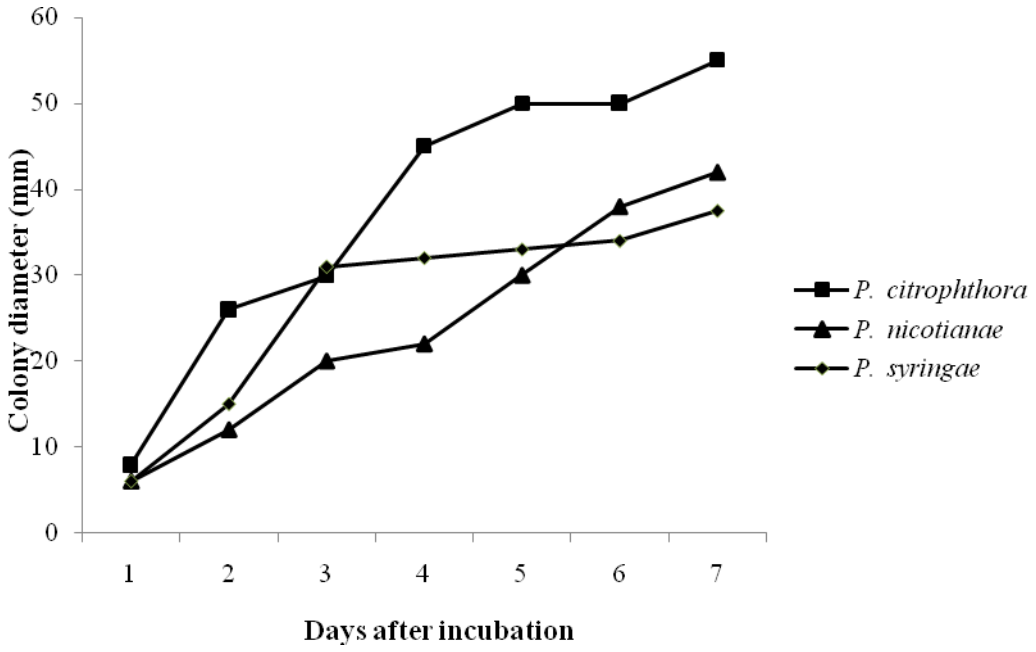

Figure 1: Diameter of *P. citrophthora, P. nicotianae* and *P. syringae* at 24°C Growth rate is expressed as mm colony diameter/day.

As shown in Plate 1A-F, *P. citrophthora* was characterized by a finely radiate, white rosette and slightly cottony colonies (A). Hyphae were 6 to 7nm (B). Sporangia were mostly laterally attached and were about 40 x 30µm. Shapes ranged from spherical, ovoid, obpyriform, obturbinate, to ellipsoidal, but mostly papillate (C and D). Some were asymmetrical (E) and others often had two divergent apices (F). Papilla measured upto 4µm long with an average length-breadth ratio of 1.4:1. Sporangiophores were irregularly branched singly or in a loose sympodium with a swelling at the point of branching. No chlamydospores or sexual structures were produced.

AJFAND ONLINE Scholarly, Peer Reviewed
ISSN 1684 5374
Volume 12 No. 7
December 2012
African Journal of Food, Agriculture, Nutrition and Development
Published by African Scholarly Science Communications Trust

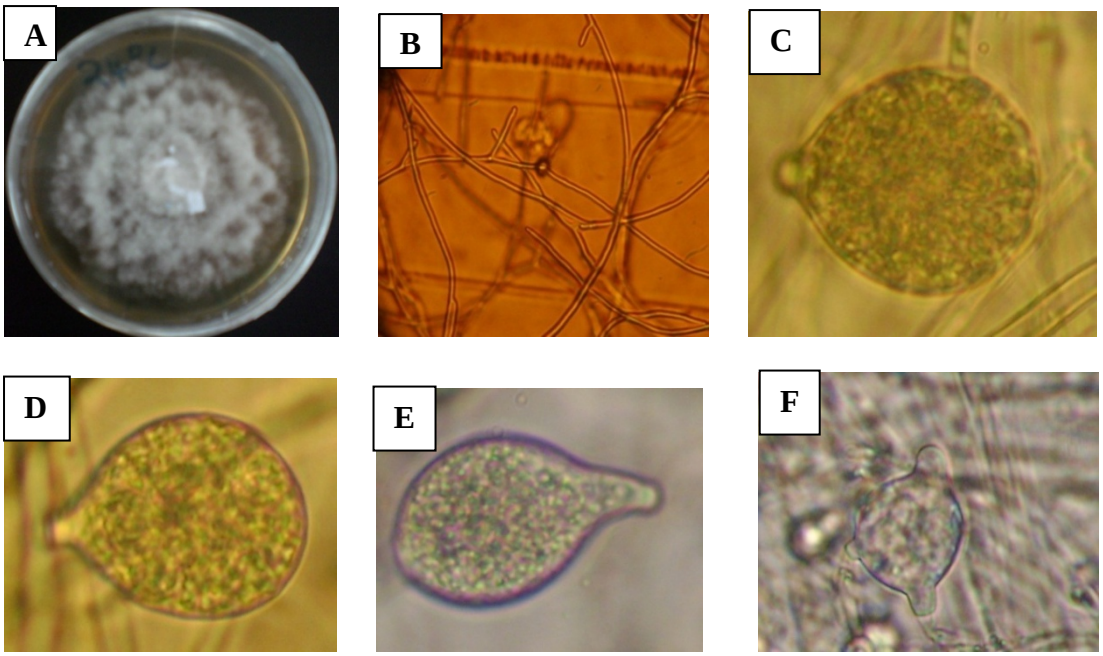

Plate 1: Morphological characteristics of *Phytophthora citrophthora*. Finely radiate cottony mycelium (A), coenocytic hyphae of up to 6-7nm in diameter (B), ovoid (C and D) and asymmetric (E) sporangium with prominent papilla. Sporangium with more than one papilla (F).Bar=10µm

The morphological characteristics of *P. nicotianae* are shown in Plate 2A-E. Mycelium was dense or loose rosette, with no pattern, spreading, and arachnoid aerial (A). Hyphae was coenocytic with average diameter of 7-10nm (B). Sporangial shapes ranged from ellipsoid, ovoid, pyriform, obpyriform, to spherical with a prominent papilla (C). Sporangia size averaged at 36 x 28 µm and length-breadth ratio at 1.34:1. Some isolates produced intercalary sporangia (D). Chlamydospores, 13 to 60 µm in diameter, were produced abundantly intercalary and terminally (E) and no sexual structures in single cultures.

AJFAND ONLINE Scholarly, Peer Reviewed ISSN 1684 5374
Volume 12 No. 7
December 2012
African Journal of Food, Agriculture, Nutrition and Development
Published by African Scholarly Science Communications Trust

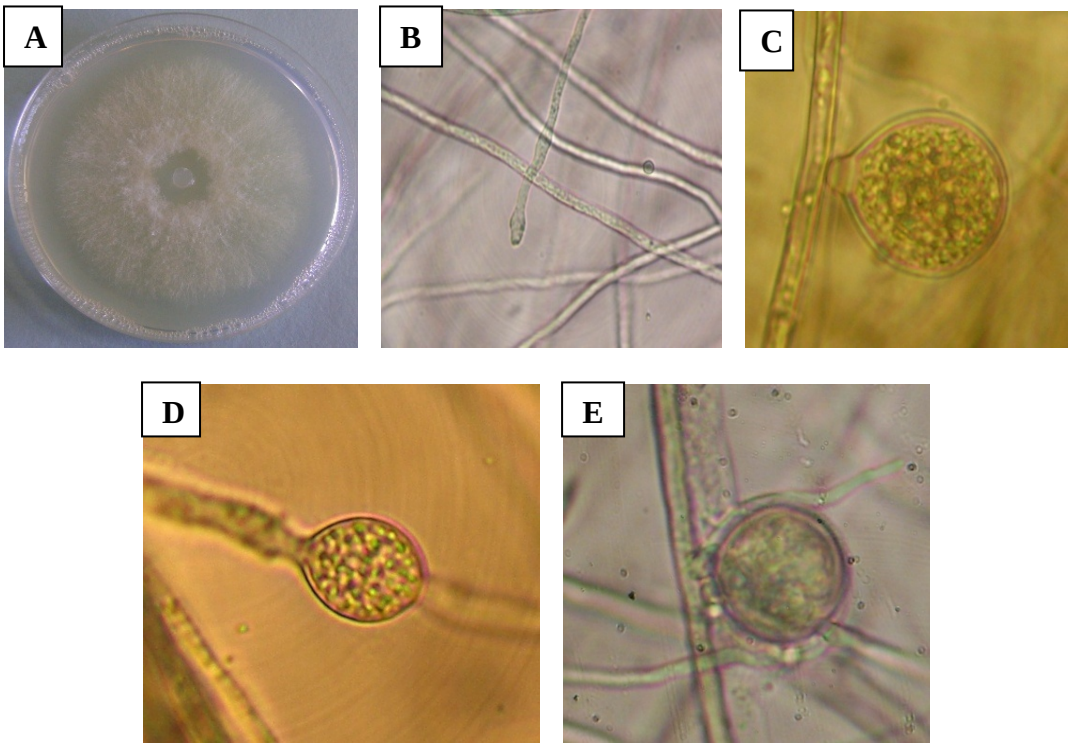

Plate 2: Morphological characteristics of *P. nicotianae* (A) Dense rosette spreading aerial mycelium, (B) coenocytic hyphae of up to 7-10nm in diameter, (C) ovoid terminal sporangia with prominent papilla, (D) intercalary sporangium, (E) chlamydospore. Bar=10µm

Plate 3A-D shows the characteristics of *P. syringae.* Dense or loose rosette spreading aerial mycelium with no pattern (A), slender coenocytic hyphae (B), with rounded or angular hyphal swellings, often in chains (catenulate) and delimited by septa (C). Sporangia were broadly ovoid or obpyriform, semipapillate, and persistent and formed in succession from a single sporangium (D). Average sporangial dimension was 39 x 26.5µm with length-breadth ratio varying from 1.32:1 to 1.85:1. No chlamydospores were produced on agar media.

AJFAND ONLINE Scholarly, Peer Reviewed ISSN 1684 5374
Volume 12 No. 7
December 2012
African Journal of Food, Agriculture, Nutrition and Development
Published by African Scholarly Science Communications Trust

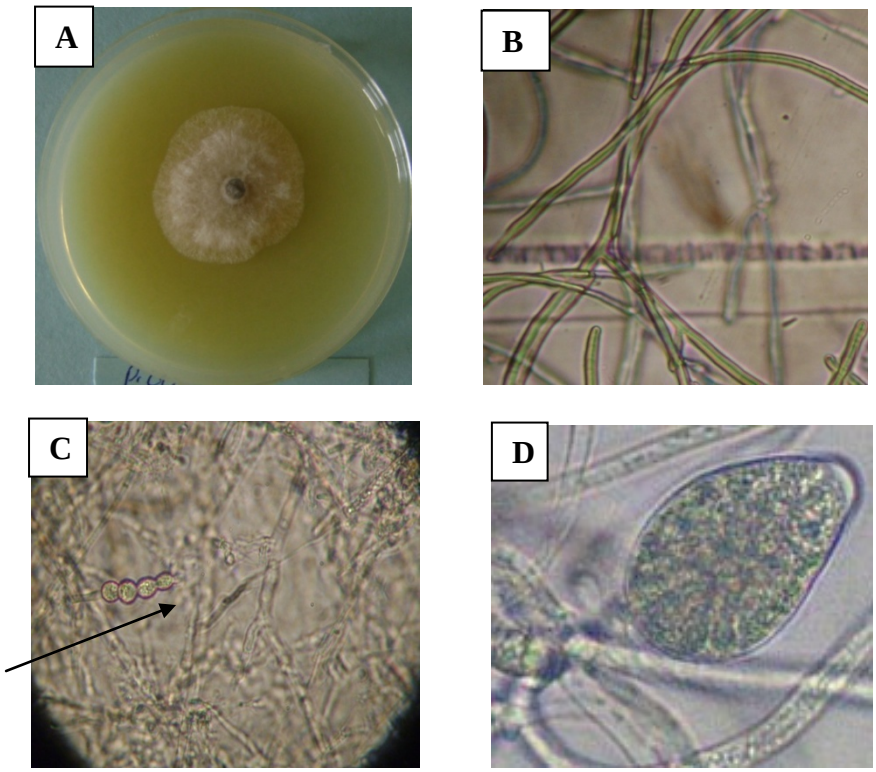

Plate 3: Morphological characteristics of *P. syringae* (A) Loose rosette spreading aerial mycelium, (B) coenocytic hyphae with slender mycelium, (C) rounded hyphal swellings in chains (catenulate, shown with the arrow), (D) ovoid, semipapillate, and persistent sporangia. Bar=10µm.

Distribution of the *Phytophthora* species

The distribution and prevalence of *Phytophthora* species associated with citrus gummosis in Kenya is shown in Table 1. *P. citrophthora* was the only species present in all AEZs sampled. Its distribution was highest in Lower Midland (LM) and lowest in Upper Midland (UM) zones. The distribution of *P. nicotianae* was highest in Coastal Lowlands (CL) zones. *P. nicotianae* and *P. syringae*, were absent in UM and Lower Highland (LH) zones. *P. syringae* was present only in LM zones and the only species not found in CL zones. *P. citrophthora* was the most prevalent in all the AEZs, and *P. syringae* the least prevalent.

Virulence tests

The *Phytophthora* species induced brown to reddish necrotic lesions on lemon fruits (Plate 4). Lesion diameters (LDs) were significantly ($p<0.05$) different among the isolates of *P. citrophthora* and *P. nicotianae* tested. In *P. citrophthora*, isolate P.CIT 1 was the most virulent while P.CIT 18 and P. CIT 35 were the least virulent. *P. nicotianae*, isolate P.NIC 11 was the most virulent and P.NIC 1 the least. The *P. syringae* isolate used (P.SYR) produced small necrotic lesions (Table 2).

AJFAND ONLINE Scholarly, Peer Reviewed ISSN 1684 5374
Volume 12 No. 7
December 2012
African Journal of Food, Agriculture, Nutrition and Development
Published by African Scholarly Science Communications Trust

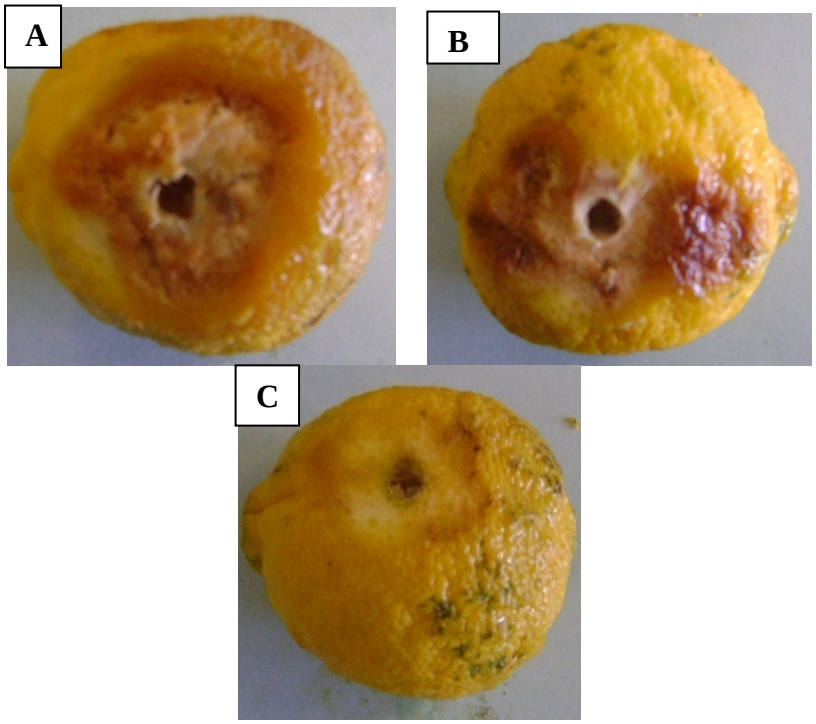

Plate 4: Lesions on lemon fruits caused by *P. citrophthora*, (A), *P. nicotianae* (B) and *P. syringae* (C).

Pathogenicity tests

All the *Phytophthora* test isolates were pathogenic to lemon seedlings and they induced brown to reddish canker lesions and gum exudation (Plate 5B-G). The Lesion sizes were significantly ($p<0.05$) different between *P. citrophthora* and *P. nicotianae* isolates. Isolates developing the largest lesions were P.NIC11 isolate followed by PCIT1. P.SYR, the only *P. syringae* isolate, produced the smallest lesions of 10.5mm (Table 2).

AJFAND ONLINE Scholarly, Peer Reviewed ISSN 1684 5374
Volume 12 No. 7
December 2012
African Journal of Food, Agriculture, Nutrition and Development
Published by African Scholarly Science Communications Trust

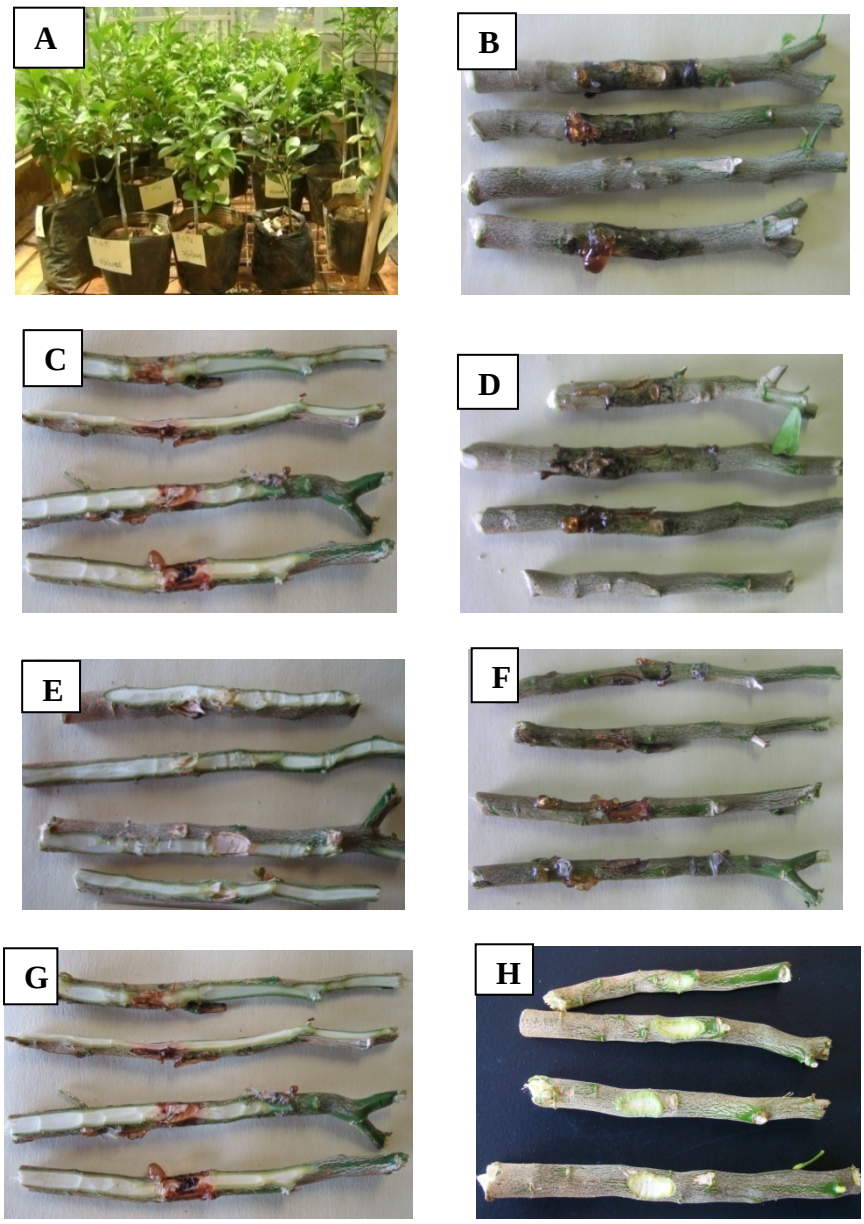

Plate 5: One-year old lemon seedlings one day after inoculation (A), canker lesions caused by different *Phytophthora* test isolates 21 days after inoculation (B-G) and a non-innoculated control (H).

DISCUSSION

Optimum temperature for growth was the main distinguishing feature between the test species with that of *P. citrophthora* being 24-28^{o}C and for *P. syringae* and *P. nicotianae* 20^{o}C and over 30^{o}C, respectively. These results were consistent with those of another study that reported optimum temperature for *P. citrophthora* and *P. nicotianae* to be 24-28^{o}C and 27-35^{o}C, respectively [17]. The diversity in colony

morphology, rate and manner of growth of test isolates on CMA and sporangial size on V8 agar agreed with previous studies [3].

Diversity and similarities between the two test species, *P. citrophthora* and *P. nicotianae*, in the characteristics of their reproductive structures differentiated them from *P. syringae* and confirmed reports from other studies by Hall [18], Mchau and Coffey [19], and Waterhouse and Waterston [13]. The two species exhibited papillated sporangia; the main characteristic that differentiated them from *P. syringae* whose sporangia was semi-papillated. This was earlier reported in a similar study by Waterhouse and Waterston [13]. Similarly, the absence of chlamydospores in the two species *P. syringae* and *P. citrophthora* confirmed reports by Waterhouse and Waterston [13] and Ho and Jong [20], and the presence of intercalary and terminal chlamydospores in *P. nicotianae* was as reported by Hall [18]. The presence of rounded or angular hyphal swellings that were often in chains (catenulate) and delimited by septa is unique to *P. syringae* and facilitated its differentiation from *P. nicotianae* and *P. citrophthora*. The latter two species are heterothallic thus did not produce oospores and or sexual structures in single cultures.

Colony and sporangial measurement combination provided a useful alternative to the characters given by Waterhouse [7] and Stamps *et al*. [6] for large-scale examination of *Phytophthora* species isolates under field and laboratory conditions.

The occurrence of *Phytophthora* species in many citrus fields confirmed earlier studies that these pathogens are present in almost all orchards [21]. The high prevalence of *P. citrophthora* compared to *P. nicotianae* and *P. syringae* in all AEZs could be because citrus is grown in areas with average temperature at < 30^{o}C, which is ideal for the pathogen. Similarly, the ideal temperature for *P. nicotianae* is 28^{o}C and above, hence its high prevalence in AEZ-CL and other high temperature zones. These findings confirm that *P. nicotianae* is most active at high temperatures [17, 22]. Our results also confirm earlier findings by Ricci et al. [22] and Fagoaga et al. [23], which indicated that *Phytophthora citrophthora* is the most widely spread oomycete plant pathogen over all the citrus growing areas. The fact that more *P. citrophthora* species was isolated from soil samples compared to the other two species showed that this species is prevalent in the tropics and agrees with Ricci *et al.* [22] that the species is one of the most destructive citrus gummosis pathogen and predominates in very important regions of citrus production. The tropical regions are major citrus production areas just like the Mediterranean basin.

The test isolates induced characteristic brown rot and gummosis symptoms on lemon fruits and seedlings, thereby confirming *P. citrophthora*, *P. nicotianae, and P. syringae* as the main causal agents of citrus gummosis in Kenya. This is in agreement with reports by Matheron *et al*. [24], Timmer, *et al*. [25] and Timmer [26].

CONCLUSION AND RECOMMENDATIONS

The *Phytophthora* species *citrophthora, nicotianae* and *syringae* are the causal agents of trunk gummosis and brown rot of citrus in Kenya. While *P. citrophthora* is prevalent in all AEZs, *P. nicotianae* is mainly confined within the coastal lowlands occurring only in a limited scale in the other zones. The distribution of *P. syringae* is limited in Kenya. Colony characteristics and growth rates were useful as a first step in identification of *Phytophthora* species by complementing sporangial characteristics in the differentiation of *P. citrophthora, P. nicotianae* and *P. syringae.* This study provided a baseline on the basis of which future studies can build on. The current findings have a practical importance with regard to management of gummosis. Since, the species that cause gummosis have been identified in this study, management approaches can be targeted at these three species. For instance the *Phytophthora* species exhibited different distribution patterns with *P. citrophthora* being prevalent in all AEZs; *P. nicotianae* only confined within the coastal lowlands and only in a limited scale in the other zones and that of *P. syringae* being very limited in Kenya. Thus, control measures such as development of resistance should be deployed with the distribution in mind.

This study recommends further characterization of the three *Phytophthora* species at molecular level since many morphological characteristics of the sporangia, mycelia and colonies used in the differentiation of fungal species are plastic, influenced by environment and often overlap between species. It is also recommended that the species be subjected to selected Oomycete specific fungicides as an initial step in the identification of effective chemicals for the control of citrus gummosis in Kenya.

ACKNOWLEDGEMENTS

We thank the Ministry of Agriculture, Kenya, for financial support through the Agricultural Sector Programme Support (ASPS) and farmers and district agricultural officers in the surveyed area for their assistance. We appreciate the support of director, Kenya Forestry Research Institute (KEFRI), and his staff where part of the laboratory work was done.

Table 1: Distribution and prevalence of *Phytophthora* species associated with citrus gummosis in Kenya.

Species	Distribution*				Prevalence ** (%)
	LM	CL	LH	UM	
P. citrophthora	30	7	5	3	76.3
P. nicotianae	4	9	0	0	22
P. syringae	1	0	0	0	1.7

*Distribution was determined by recording the number of isolates recovered from samples obtained in each AEZ.
**Prevalence of each species was determined by expressing the number of isolates of each species recovered in all AEZs as a percentage of the total number of isolates collected.

Table 2: Lesions induced on lemon fruits and one-year old seedlings inoculated with *Phytophthora* isolates

Isolate	Virulence Test Lesion size (mm)	Pathogenicity Test Lesion size (mm)
P. citrophthora		
PCT1	48.8± 1.76	19.0± 1.71
PCIT7	45.8± 1.76	13.5± 1.71
PCIT41	45.5± 1.76	13.8± 1.71
PCIT18	29.5± 1.76	-
PCIT35	29.5± 1.76	-
P. nicotianae		
PNIC11	30.0± 1.46	26.5± 2.0
PNIC13	28.3± 1.46	12.0± 2.0
PNIC1	17.5± 1.46	-
P. syringae		-
PSYR	16.8	10.5

REFERENCES

1. **Vernière C, Cohen S, Raffanel B, Dubois A, Venard P and F Panabières** Variability in Pathogenicity among *Phytophthora* spp. isolated from Citrus in Corsica. *Phytopathology* 2004; **152(8-9):** 476–483.

2. **CAB International.** Crop protection Compendium CD-ROM Edition. Nosworthy way, Wallingford, United Kingdom, 2005.

3. **Brasier CM and MJ Griffin** Taxonomy of *Phytophthora palmivora* on cocoa. *Transactions of the British Mycological Society* 1979; **72**: 111–43.

4. **Kaosiri T and GA Zentmyer** Protein, esterase, and peroxidase patterns in the *Phytophthora palmivora* complex from cacao. *Mycologia* 1980; **72**: 988–1000.

5. **Appiah AA, Flood J, Bridge PD and SA Archer** Inter- and intraspecific morphometric variation and characterization of *Phytophthora* isolates from cocoa. *Plant Pathol.* 2003; **52 (2**): 168.

6. **Stamps DJ, Waterhouse GM, Newhook F and GS Hall** Revised Tabular Key to the Species of *Phytophthora*. *Mycol. Pap* 1990; No. 162.

7. **Waterhouse GM** Key to the species of *Phytophthora* de Bary. Mycological Papers 92. Kew, Surrey, England: Commonwealth Mycological Institute, 1963.

8. **Jeffers SN and SB Martin** Comparison of two media selective for *Phytophthora* and *Pythium* species. *Plant Dis.* 1986; **70**: 1038–43.

9. **Hendrix FF and WA Campbell** Distribution of *Phytophthora* and *Pythium* species in soils in the continental United States. *Canadian Journal of Botany*,1970; **48**: 377–84.

10. **Mitchell DJ, Kannwischer-Mitchell ME and GA Zentmyer** Isolating, identifying and producing inoculum of *Phytophthora* spp. **In**: Methods for Evaluating Pesticides for Control of Plant Pathogens. Hickey, K. D. (ed.). The American Phytopathological Society, St. Paul, MN.USA, 1986.

11. **Heffer V, Powelson ML and KB Johnson** Oomycetes. The Plant Health Instructor. Laboratory Exercises in Plant Pathology. Oregon State University, USA. *http://www.apsnet.org/education/ Lab Exercises/Oomycetes/Top.hmtl. Accessed in 4th July 2002*

12. **Newhook FJ, Stamps DJ, Waterhouse GM, Stamps DJ and GS Hall** Revised tabular key to the species of *Phytophthora*. Commonw. Agric. Bur. Int. Mycol. Inst. *Mycol. Pap, 1990; 162.*

13. **Waterhouse GM and JM Waterston** *Phytophthora nicotianae* var. *parasitica.* CMI Descriptions of Pathogenic Fungi and Bacteria. No. 35. Common Mycol. Inst. Wallingford, UK, 1964.

14. **Jaetzold R and H Schmidt** Natural conditions and farm management information. In: Farm Management Handbook of Kenya Vol. IIC. Ministry of Agriculture, Kenya and GTZ, Germany, 1983.**15. Agrios GN** Plant Pathology. 4th Edition. Academic press, 1997.

16. **International Plant Protection Congress** Where Chemistry meets Ecology Plant Protection towards the Third Millennium, 15th Edition. Jerusalem, Israel, 1999. *http://www.kenes.co.il/IPPC/mon.htm.* Accessed on 15th July 2007.

17. **Erwin DC and OK Ribeiro** *Phytophthora* Diseases Worldwide. APS Press. St. Paul, Minnesota, USA, 1996.

18. **Hall G** An integrated approach to the analysis of variation in *Phytophthora nicotianae* and a redescription of the species. *Mycological Res.* 1993; **97(5):**559-574.

19. **Mchau GRA and MD Coffey** An integrated study of the morphological and isozyme patterns found within a worldwide collection of *Phytophthora citrophthora* and a redescription of the species. *Mycology Res.* 1994b; **98**:1291-1299.

20. **Ho HH and JC Jong** *P. hibernalis* and *P. syringae*. *Mycotaxon*, 1993; **47**: 439-460.

21. **Ann PJ, Ko WH and HJ Su** Interaction between Lukubin Bacterium and *Phytophthora parastica* in Citrus hosts. *European Journal of Plant Pathology*, 2004; **110**: 1-6.

22. **Ricci P, Pope-de-Vallavieille C, Panabieres F, Marais A and G Auge** Characterization of the *Phytophthora* spp. attacking citrus. *OEPP/EPPO Buletin*, 1990; **20**:19–28.

23. **Fagoaga C, Rodrigo I, Conejero V, Hinarejos C, Tuset JJ, Arnau J, Pina JA, Navarro L and L Peña** Increased tolerance to *Phytophthora citrophthora* in transgenic orange plants constitutively expressing a tomato pathogenesis related PR-5. *Molecular Breeding*, 2001; **7:** 175-185.

24. **Matheron ME, Wright G and M Porchas** Resistance to *Phytophthora citrophthora* and *P.parasitica* and nursery characteristic of several citrus rootstocks. *Plant Dis*. 1998; **82**:1217-1225.

25. **Timmer LW, Garnsey SM and P Broadbent** Diseases of Citrus. In: Diseases of Tropical Fruits Crops. R. C. Ploetz (ed.). CABI Publishing, CABI International, Wallingford, UK, 2003.

26. **Timmer LW and JA Menge** *Phytophthora* induced diseases. Pages 22-24 in: compendium of citrus diseases. Whiteside, J. O., Garnsey, S. M. and. Timmer, L.W. (eds.). APS Press, St.Paul, MN, 1988.