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Dieback of Mango and Cutting Method Impact on the Regeneration of Tree in the Sudano-Sahelian Zone

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ABSTRACT

Background and Objective: The dieback of mango (*Mangifera indica*) has become an increasing threat to mango production in Tchad and Cameroon. The present study was conducted to characterize and identify pathogens associated with the mango quick decline tree for the first time in both countries. **Materials and Methods:** Investigations were carried out through field observations of the symptoms. A simple block experimental design was adopted and the scale was used to assess the incidence, severity and prevalence at the two sites during the dry and rainy seasons. Pathogens associated were isolated using a Potato Dextrose Agar (PDA) medium. The control strategy was evaluated. **Results:** During the survey, symptoms of this disease were wilting of leaves and branches which gradually progress into dieback, gummosis, rotting of the stem and vascular discolouration. Six fungi, *Lasiodiplodia theobromae*, *Colletotrichum* sp., *Aspergillus niger* and *Botrytis cinerea* were present in the dry season and the two sites, *Curvularia* sp. and *Schizosaccharomyces pombe* occurring in the rainy season only in Cameroon were isolated. The highest incidence and severity rates were obtained during the rainy season, 43.94 and 14.03%, respectively. Kassai variety (25%) was the most susceptible to dieback. The percentage of tree regeneration from the cutting method depends on the attack percentage of the tree. **Conclusion:** This study suggested that several fungi are associated with this disease in Cameroon and Chad. Molecular analysis and pathogenicity are necessary to upset the control strategy (cutting method) which is efficient when the attack tree is under 50%.

KEYWORDS

Mangifera indica, dieback, incidence, severity, prevalence, cutting method

INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most important fruit crops in tropical and subtropical areas of the world. Mango is the leading fruit crop in the savannas of Cameroon (Adamawa, North and the Far

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North), Chad (Tandjilé and Mayo kebbi) and the Central African Republic with a production of 43.33%[1]. The African continent is the second mango-producing region after Asia. Its production increased by 3.5% per year, from 3.2 million tons in 2006 to just over 3.6 million tons in 2010[2]. Cameroon produces about 15418 tonnes[3,4] and Chad remained the 12th mango-producing country after the ECOWAS region but its production has declined from 2010[2].

The mango yield, in both countries, has decreased due to various biotic and abiotic factors. Mango dieback is the most recent rigorous threat to the Sudano-Sahelian zones[5,6]. *Ceratocystis fimbriata* Ellis & Halst, *Lasiodiplodia theobromae* (Pat.) Griffon, *Fusarium* sp., *Pestalotiopsis microspora*, *Colletotrichum* sp., *Curvularia* sp., *Aspergillus niger*, *Botrytis cinerea* and *Phoma glomerata* are the pathogens often associated with this disease[7-9]. However, *L. theobromae* seems to be the frequently isolated pathogen despite the presence of *Ceratocystis fimbriata* in almost all orchards in Pakistan, Oman and Brazil[7,10-13]. Isolation of *L. theobromae* from different regions ranged from 8-61% and pathogenicity tests always confirm symptoms of dieback with *L. theobromae*.

This disease leads to the sudden death of trees in their numbers. This phenomenon has also been reported in some other parts of the world like Brazil and Oman[8]. The symptoms began with the wilt of leaves and branches. Gummosis and rot occurred in the bark of the stem. The leaves of the trees wither out and remain attached to the dying tree[12,14,15]. These symptoms may be found alone or in a combination of two or more symptoms in different mango orchards in Brazil or Pakistan[16,17]. The mortality of the tree is usually by the blockage of vascular bundles in the proper flow of nutrients[18]. Losses from this disease vary by region and country and can range from 20-60%[19,20].

In Cameroon and Chad, the problem is the lack of data about the dieback of mango trees. Thus, research is needed to confirm the aetiology of the disease in these two countries and to evaluate the incidence of the disease in mango production regions and among the most grown mango varieties for the first time. Focusing on the increasing threat of mango sudden death syndrome in Cameroon and Chad, the present study was conducted to characterize and make isolations of pathogens from the infected tree and evaluated the method of cutting infected trees.

MATERIALS AND METHODS

Experimental design and sampling: This study was carried out in the mango orchards of Far North Cameroon (Diamare Division) and Chad (Mayo Kebbi West Division) during the 2019-2020 production seasons. Maroua (9°10′N, 14°15′E) and Pala (9°10′N, 14°15′E) were selected. At each site, four locations (representing blocks) were selected, Palar, Pitoare, Djarengol and Meskine in the Maroua subdivision, Houa, Wa imbrao, Guewari and Madagascar in Pala (Chad). In each block, three orchards (representing plots) were randomly x chosen. In each plot, 30 trees of each mango variety were randomly surveyed and examined for the incidence and severity of mango dieback. Three leaves, barks and 30 cm of stem or branches were collected in each infected tree for the isolation of pathogens.

Isolation of pathogen associated with mango dieback from the diseased tree: For the isolation of fungal pathogens, standard method[21] and Dianda *et al*.[5] studies were used, Small pieces (about 1 cm) of leaves and small wooden pieces beneath the bark using a sterilized scalpel were removed. These fragments were washed in tap water. Then, sterilized in ethanol (70%) firstly for one minute followed by immersion in 1% of sodium hypochlorite solution for 2 min and then washed three times in Sterile Distilled Water (SDW). Sterilized explants were dried and grown on Petri dishes containing WA and PDA medium. The Petri dishes were incubated at 25°C in 12/12 or 0/12 photoperiod and further subcultured on fresh media plates for further purification. After 7-10 days, the fungal growths were microscopically observed and identified based on culture morphology, spores and conidia of the specific pathogens (fungi)[5,6,22-24].

Assessment of disease incidence: Incidence of mango dieback disease was recorded in each site during the dry season (March and April) and the rainy season (July and August) using formula[12,25]:

$$I(\%) = \Sigma\left(\frac{n}{N}\right)\times 100$$

Where:
I = Mean incidence per locality
N = Number of trees infected per plot and locality
N = Total number of plants assessed

Assessment of disease incidence: For assessment of disease severity, a scale on a visual basis of disease severity symptoms i.e., rotting and drying of leaves in four different portions (North, South, East and West) of the tree (Table 1) were developed[12,26].

The formula to assess disease severity was as follows[5]:

$$S = \Sigma\left(\frac{x_i \times n_i}{N \times Z}\right)\times 100$$

Where:
S = Index of disease severity at the site
x_i = Severity i of the disease on the tree
n_i = Number of trees with severity i
N = Total number of plants observed in each locality and orchard
Z = Highest severity scale[4]

Assessment of the prevalence of dieback of mango: The prevalence assessment was carried out in each variety at the two study sites. An average of 40 trees were surveyed. The following formula[5] was:

$$P = \frac{n}{N}\times 100$$

Where:
n = Number of trees of the various affected
N = Total number of the tree of the variety in the site[5]

Evaluation of the effectiveness of the control method (tree cutting): A survey of all the methods used by the orchards operators to combat dieback was carried out and the effectiveness of this method was evaluated. The evaluation particularly consisted in recording the recovery rates on cut trees that had been affected by 25-50% and 50-100%.

Statistical analysis: Microsoft Excel software was used to enter data. Data collected were analyzed using Analysis of Variance (ANOVA 1) one way. The $p<0.05$ and averages (incidence and severity) were compared through Duncan Multiple Range Test with Statistical Software SPSS 20.0.

RESULTS

Disease symptoms were observed at two sites: Generally, mango dieback symptoms occurred at the end of February at Pala (Chad) and after flowering in Far North Cameroon. Symptoms appear on leaves,

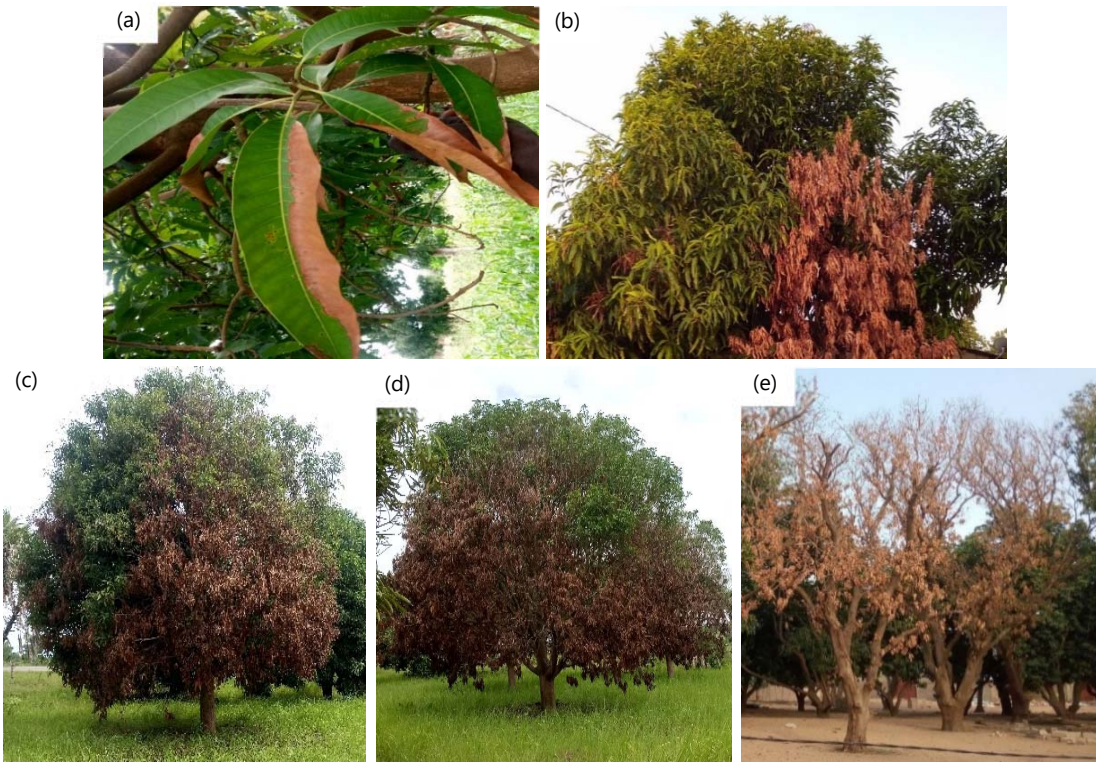


Fig. 1(a-e): Evolution of dieback symptoms on the leaves, (a) Marginal denaturation of leaf colour to brick-red, (b) Evolution of the symptoms of dieback on twigs and branches and (c-e) Partial and complete drying of trees 50, 75 and 100%, respectively

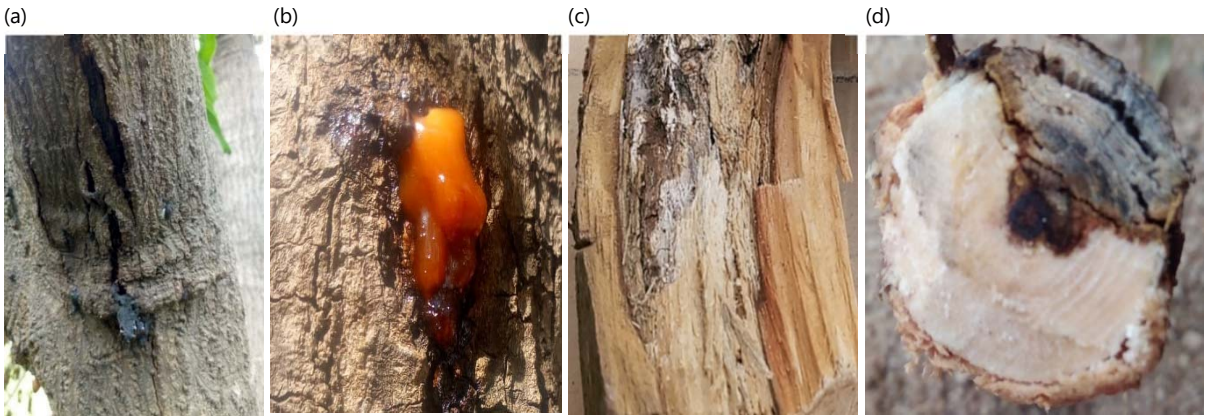


Fig. 2(a-d): Other symptoms of mango dieback on the stem, (a) Bark splitting, oozing of black material from the infected area including bleeding of wood sap, (b) Gummosis on stem, (c) Longitudinal and (d) Cross-section of the stem with rotten tissue

stems, branches and twigs. However, on the leaves, the disease begins with marginal, partial or total denaturation of the leaf from green to brick-red colour (Fig. 1a). This symptom evolves over the whole branch (Fig. 1b-c). Eventually, all the leaves of the tree dry out and cause partial or total wilt of the tree (Fig. 1d-e). The leaves of diseased trees were withered and remained attached to the dying tree.

Other forms of manifestations were observed on the bark such as bark cracking, oozing of black material from the infected area including bleeding of wood sap (Fig. 2a) and gummosis (Fig. 2b). Stem rot or canker is visible after a cross-section (Fig. 2c-d).

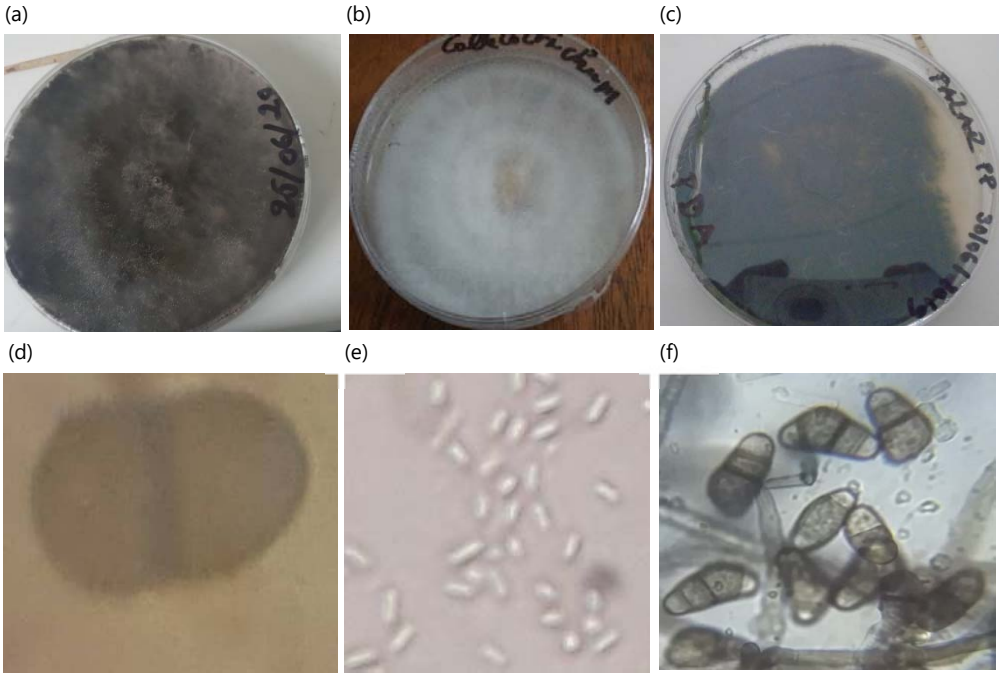


Fig. 3(a-f): Pure cultures and spores of some fungi isolated, (a, d) *Lasiodiplodia theobromae*, (b, e) *Colletotrichum gloeosporioides* and (c, f) *Curvularia lunata*

Table 1: Scale of severity in different areas[12,26]

Scale	Area infected with dieback (%)	Description of symptoms
0	0	Plant with no symptoms
1	25	Appearance of symptoms, brick red necrosis of the leaf blades and browning of the petioles were observed on some leaves
2	50	About half of the tree's branches have dried leaves with or without defoliation
3	75	Dried leaf on the maximum of branches with or without progressive defoliation
4	100	Attachment of leaves after drying of whole plant and death of mango

Table 2: Fungi isolated from disease trees in the sites and during the seasons

	Maroua (Cameroon)		Pala (Chad)	
	Dry season	Rainy season	Dry season	Rainy season
Lasiodiplodia theobromae	+	+	+	+
Colletotrichum gloeosporioides	+	+	+	+
Aspergillus niger	+	+	+	+
Botrytis cinerea	+	+	+	+
Curvularia sp.	-	+	-	-
Schizosaccharomyces pombe	-	+	-	-

-: Absent and +: Present

Pathogens associated with mango dieback in the two sites: Six fungi have been isolated (Table 2). But in Fig. 3a-f, four are present in the two sites during the drying and rainy season, *Lasiodiplodia theobromae* (Fig. 3a, d), *Colletotrichum gloeosporioides* (Fig. 3b, e), *Aspergillus niger* and *Botrytis cinerea*. However, *Curvularia* sp. (Fig. 3c, f) and *Schizosaccharomyces pombe* were only present in Maroua in the rainy season.

Impact of seasons on disease development

Mango dieback disease incidence in the two sites: Disease incidence among the sites was highly significant at the probability level (p = 0.001) in the rainy season. The highest incidence was recorded at the Maroua site, 43.94±6.7 against 33.55±7.1% at Pala. But, no statistical difference (p = 0.241) was observed in the dry season. Incidence was respectively 21.95±4.5 and 20.22±2.4% at Maroua and Pala. Generally, the highest incidence values were recorded during the rainy season (Table 3).

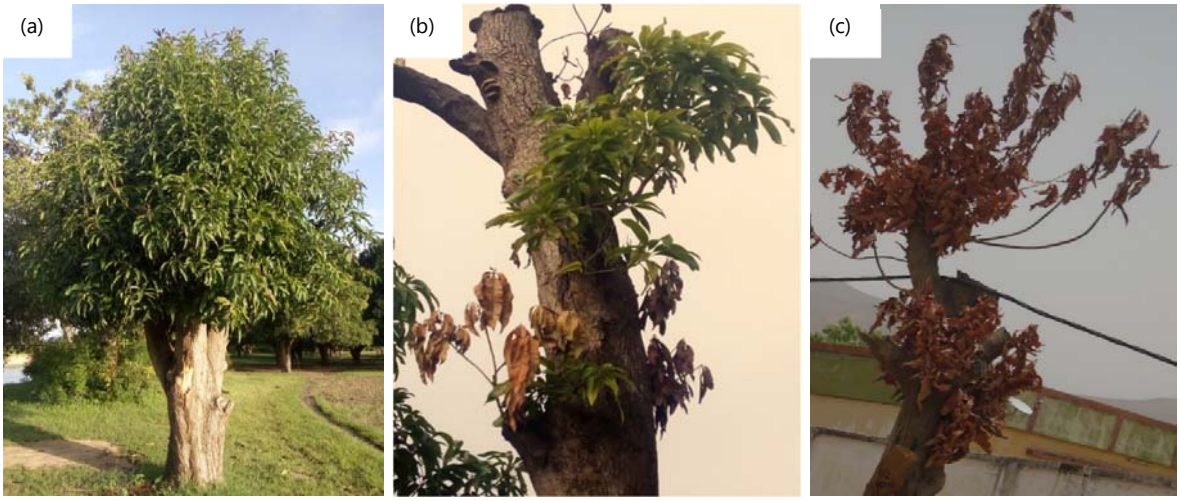


Fig. 4(a-c): Regeneration of mango plants after cutting, (a) Young tree regenerates without the disease, (b) Mango tree regenerates with disease and (c) Dead mango tree after regeneration

Table 3: Incidences of dieback in the two sites

	Mean incidence (%)	
Sites	Dry season	Rainy season
Maroua (Cameroon)	21.95±4.5[a]	43.94±6.7[b]
Palan (Chad)	20.22±2.4[a]	33.55±7.1[a]
Probability	0.24	0.001

Mean values are followed by the same letters in the same column (in the same site) are not significantly different at the 5% threshold according to Duncan's Test

Table 4: Severity of mango dieback in the two sites

	Mean severity (%)	
Sites	Dry season	Rainy season
Maroua	6.80±1.2[a]	14.03±2.4[a]
Pala	4.80±0.8[a]	12.68±1.4[a]
Probabilité	0.22	0.59

Mean values are followed by the same letters in the same column (in the same site) are not significantly different at the 5% threshold according to Duncan's Test

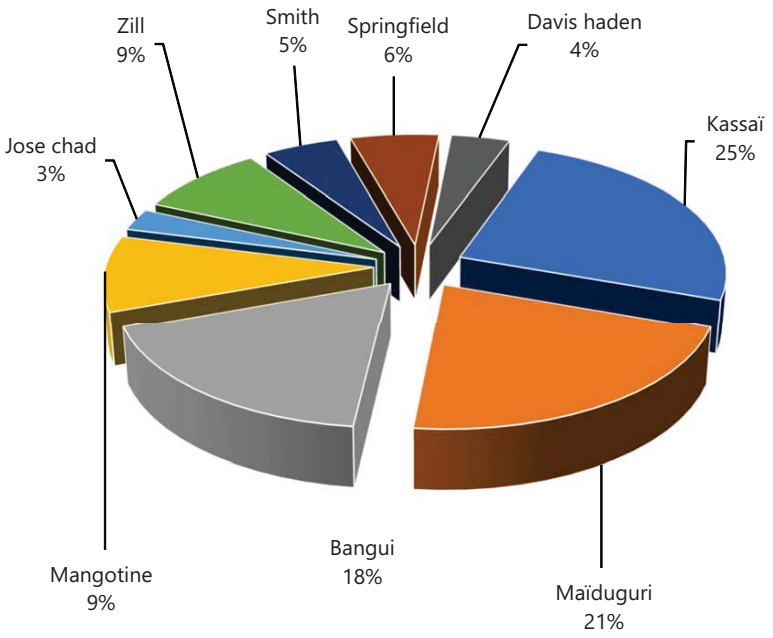
Dieback disease severity in the two sites: No significant difference ($p<0.05$) was observed in disease severity. But severity increased during the rainy season in the two sites. Severity was 6.80 and 14.03% in Maroua and 4.80 and 12.68% in Pala sites, respectively during the dry and rainy seasons (Table 4).

Effectiveness of the method of cutting off the plant affected: The study showed that 75% of the mango trees were affected more than 50% by dieback after cutting. Only 12.95% of mango trees affected at this rate regenerate without manifesting the disease. However, at less than 50% of attacks, 36.39% die (Table 5). This method, as a means of controlling dieback, has proven to be effective only when the degree of disease attack is less than 50% (Fig. 4a-c).

Prevalence of mango tree dieback in the sites

Varietal diversity of cultivated mango trees: Nine varieties grown in Maroua (Cameroon) and Pala (Chad) have been identified. These varieties were divided into two categories. The local varieties (Kassaï, Maïduguri, Bangui and Mangotine) and the improved varieties (José Chad, Zill, Smith, Spring field and Davis Haden).

(a) Prevalence of mango dieback in Maroua



(b) Prevalence of mango dieback in Pala

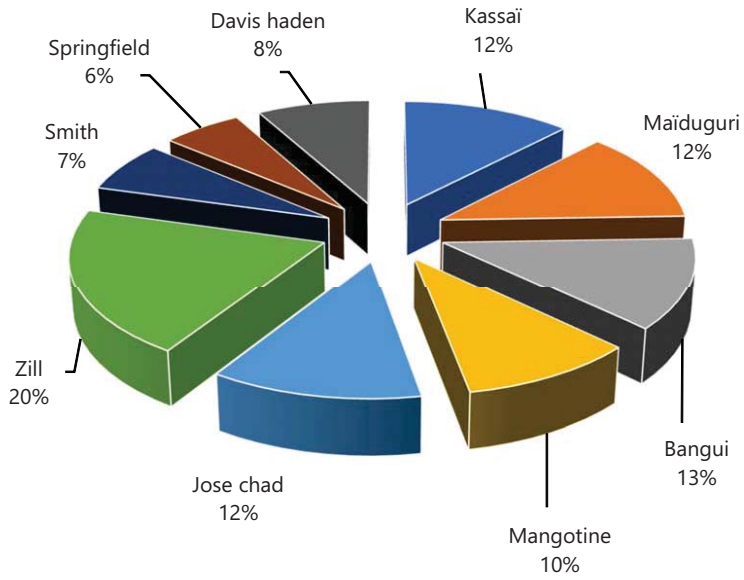


Fig. 5(a-b): Prevalence of mango dieback in the different sites

In the Maroua site, the Kassaï variety is the most sensitive with a high prevalence rate (25%). Maïduguri, Bangui, Mangotine or Mango seeng, Zill, Spring field, Smith and Davis Haden variety follow with average incidences of 21, 18, 9, 6, 5 and 4%, respectively. The lowest prevalence was observed in the variety José Chad with a rate of 3%. (Fig. 5a).

On the other hand, in the study site of Pala, the highest prevalence was obtained on the variety Zill at 20% and the lowest on the variety Spring field at 6%. Average prevalences were observed on the Bangui, Maïduguri, Kassaï, José Chad, Mangotine, Davis Haden and Smith varieties with values of 13, 12, 12, 10, 8 and 7%, respectively (Fig. 5b).

Table 5: Recovery rate of mango trees affected by dieback after cutting

	Scale of severity (%)	
	25-50	50-100
RW_oD	31.43	12.95
RW_hD	40.69	12.04
RD	36.59	75.00

RW_oD: Rate of mango trees regenerated without disease after cutting, RW_hD: Rate of mango trees regenerated with the disease after cutting and RD: Rate of mango trees regenerate and death after cutting

DISCUSSION

Mango dieback is present in all orchards inspected at both sites and in almost all accessions, indicating the serious threat of this disease in the Sahelian areas.

The symptoms of mango dieback which include bark splitting or cracking, oozing of black material, bleeding of wood sap, dried leaf, necrosis, rotting of stems, vascular discolouration and gummosis, appear on various organs such as leaves, stems, bark, twigs and branches. This disease symptom has also been reported from different mango growing areas of the world[5,8,12,16,26]. The mango trees were badly affected by this disease up to 30-40% in India, 80% in Burkina Faso, 55-80% in Niger and Togo and 60% tree mortality in Oman[5-8,27]. All of these symptoms can occur alone or in the combination of two or more in an orchard and cause dieback of mango trees[7,14].

Six pathogenic fungi in Maroua and four in Pala were isolated including *Lasiodiplodia theobromae*, *Colletotrichum gloeosporioides*, *Aspergillus niger*, *Botrytis cinerea*, *Curvularia* sp. and *Schizosaccharomyces pombe*. The diversity of fungal species found in the orchards of the two sites showed that this disease is caused by different species of fungi. Similar results were obtained in Mali and Togo with three species of fungi responsible for dieback mango tree[6,28]. In Niger, four species of fungi have been isolated[27]. However, in Pakistan twelve species of fungi were obtained[19].

Lasiodiplodia theobromae was the most frequently encountered fungus species on all organs and in all study sites (Pala and Maroua). The same result was obtained by Dianda *et al*.[5], with *Lasodiplodia theobromae* frequently found on all organs and in all provinces in Burkina Faso. These results were in disagreement with those obtained by van Wyk *et al*.[10], who demonstrated the presence of *Ceratocystis fimbriata* in all organs in Oman and Pakistan. However, *L. theobromae* is always associated with other fungi such as *Ceratocystis fimbriata*, *Phomopsis* sp., *Fusarium solani* and *Phoma glomerata*[5,11]. The pathogenicity test demonstrated that the most frequently isolated fungi, *Ceratocystis fimbriata* and *Lasiodiplodia theobromae* developed symptoms of dieback of mango after inoculation in healthy plants[29,30]. *Ceratocystis fimbriata* proved to be the more pathogenic in the development of disease symptoms singly or in combination with *L. theobromae*. Although, *L. theobromae* is associated with diseased tree and also give rise to mild symptoms in inoculated plants. *Lasodiplodia theobromae* is an opportunistic fungus and becomes more virulent in combination with other fungi, especially *C. fimbriata*[8]. The fungus, *C. fimbriata,* is more virulent in the development of disease symptoms as it has also caused similar diseases on mango in Brazil and Oman[8] leading to ultimate tree death.

Several varieties of mango trees were encountered in the different orchards at both sites. The local varieties (Kassaï, Maïduguri, Bangui, Mangotine and Shea or Denegaduru) and the improved varieties (José Chad Zill, Smith, Spring field and Davis Haden). Passannet *et al*.[2] Characterized the mango varieties grown in Chad, including four local varieties and ten improved varieties. The prevalence rate varies according to the varieties. Studies have revealed the variability of the disease on certain mango varieties in Togo, Burkina Faso, Mali, the United State and Pakistan[14,26,31]. Local varieties were considered to be the most susceptible to the disease. The highest prevalence in Maroua was observed in the Kassaï variety (25%) and

the lowest was observed in the José Tchad variety (3%). Dianda *et al*.[5] Showed variability in the level of mango tree attack in Burkina Faso on the Amélie variety (77%) which had the highest prevalence while the Brooks variety had a low average incidence (34%). In addition, in Pakistan, Chaunsa (32%) and Almas (0.83%) are varieties with high and low incidences[14]. Some varieties would have the ability to overcome this disease during their evolution linked to their natural resistance.

The incidence and severity of the disease varied by season. The highest incidences were obtained during the rainy season. The water stress and high temperatures generally observed between March and April, in both sites, could weaken the mango trees and lead to the death of the mango trees only two months after the appearance of the first symptoms[5]. In addition, the monthly water requirements of mango trees can reach 200-250 mm during the hottest and driest season. On the other hand, the symptoms of mango tree dieback were more severe in areas with lower rainfall compared to those with higher rainfall[32]. In Pakistan, the highest incidences of mango dieback were recorded in areas with shallow, compact, sandy or loamy textured soils with pH 8.5-9.0[14]. According to scientists[28,30], the mango dieback was due to a combination of all these factors. The drop in the water table causes widespread malnutrition of the mango tree and exposes it to disease. In addition, mean annual temperatures as well as variability in monthly rainfall contribute more to the potential for disease intensification and distribution[33]. Despite pathogens and vectors, mango trees are increasingly vulnerable to infection due to improper irrigation, root injuries either by termites or ploughing and lack of phytosanitary measures in the orchards[7,29].

The appearance of the disease after cutting demonstrated the vascular nature of the disease. It is therefore important to systematically eliminate the affected areas as soon as the first symptoms appear. This may explain why the disease-free recovery rate (RW_oD) is higher when trees are only 25-50% recovered. This rate was 31.43% against 12.95% over 50%. It has been shown that a section of a twig that is more than 15 cm long allows the tree to regenerate without the disease.

The rapid spread of mango dieback across all the mango growing areas of the two sites of Cameroon and Chad suggested the involvement of the disease vector, which necessitates that its role as a putative vector should be addressed for future prospective.

CONCLUSION

Dieback of the mango tree is due to several associated fungi, among them, *Lasiodiplodia theobromae* is widespread. The cutting method is efficient when the area of the infected tree was under 50%. But, Different integrated disease management (worm wash, chemical growth activators, botanical pesticides and micronutrients) options can be interlinked to develop IPM in mango orchards for the farmers and stakeholders to maximize fruit production at both sites.

SIGNIFICANCE STATEMENT

This study reveals for the first time in Cameroon and Chad pathogens responsible for the dieback of mango and the impact of season on the development of the disease. The results of this study, which are databases, can help researchers for implementation of integrated disease management approaches against the impact of pathogens in mango production. However, this work provides a simple agronomic control method, which does not yet exist against this disease. Cutting off branches or stems can help to regenerate mango trees after infection.

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